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PREFACE

Some of the greatest moments in scientific history were documented not in the most popular of textbooks or the most renowned of journals, but within the conscience of explorers who dedicated their lives unravelling the mysteries of the universe. As the flame of curiosity descended through apprentices and students, their burning desire for knowledge soon gave rise to a culture of exploration that transcended generations.

With that, the road towards research and development on science and sustainability was mapped; it is now up to us to nurture this flame by taking steps to explore the horizon for the benefit of mankind. And it was with this spirit that the International Conference on Research and Technology or ICERT was founded, with the aim of bringing together a diverse set of minds that would contribute to the frontiers of research in sustainability and environmental technology. Naturally, this tradition evolved to encompass researchers from across the spectrum, coming under one roof to deliberate on these frontiers.

We are proud to host participants who profess a multitude of disciplines from both the arts and sciences. Our approach towards research at the division of Environmental Technology, School of Industrial Technology, Universiti Sains Malaysia, places emphasis on multi-disciplinary endeavours, with many of our researchers actively involved in fundamental, exploratory and community development programs with an emphasis on sustainable research development.

The following pages contain the compiled papers presented and discussed during the 5th International Conference on Environmental Research and Technology (ICERT) 2017 held from 23rd to 25th August 2017 at Penang, Malaysia. With the guiding principle being sustainability in research and development, it seems apt for this year's ICERT 2017 to be themed 'Synergizing Environmental Science Research & Technology for A Sustainable Tomorrow '. We would like to highlight that all of the papers published in this proceeding were peer-reviewed.

With that, it gives us great pleasure to unveil the proceedings of the 5th ICERT 2017, and to extend our earnest appreciation for your collective effort in making this proceeding a successful one. We would like to thank all the authors for contributing their work to this proceeding and reviewers for providing constructive feedback to the authors.

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IMMOBILIZATION OF FUNGAL BIOMASS WITH MULTI-WALLED CARBON NANOTUBES AS BIOSORBENT

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ABSTRACT

An efficient and effective biosorbent with high performance is needed to remove impurities in solution and the conventional method of adsorption of fungal biomass alone not showing a promising removal of impurities. The immobilization of multi-walled carbon nanotubes (MWCNTs) with fungal biomass (A. niger) was good combination since both has potential functional group to bind to each other. This research was basically focused on combination of fungal biomass with MWCNTs to enhance the positive integration of impurities removal in solution. The immobilization of both elements was done in a batch liquid medium with several parameters like pH, agitation speed, dose of MWCNTs and inoculum dosage that were conducted with one factor at one time (OFAT) method. In order to verify the functional group of MWCNTs, A. niger biomass and immobilized A. niger biomass, the FTIR was applied and FESEM was done to demonstrate and compare the image of the immobilized A. niger biomass with MWCNTs and fungal biomass alone. The finding illustrated the best pH, agitation speed, dose of MWCNTs and inoculum dosage were 5-6, 150 rpm, 0.5 grams and 2% respectively. FTIR indicates the presents of the functional groups in before and after immobilization while FESEM showed the images of the wrapped MWCNTs on A.niger biomass.

Keywords: biosorbent, carbon nanotubes, fungal biomass, immobilization.

INTRODUCTION

Biosorbent is basically a combination of two materials which are natural product (biomaterials) and man-made product. The combination of this two materials can be used as absorbent to remove the impurities in solution such as heavy metals, dye and etc. Many studies have reported the ability of many types of fungal possess metal-binding properties in removing selected heavy metals. Numerous studies on removing heavy metals by carbon nanotubes have been done as well. The most important factors for selecting the biosorbent are maximum loading capacity, rapid rate of metal uptake and high affinity [1].

Some fungi like *Aspergillus niger* [2], *Penicillium sp, Rhizopus arrhizus* [3] have been proved that could help in biosorption in removing selected heavy metals such as nickel, lead, cadmium and etc. Normally the fungal organisms have a negative surface charge in a pH range 3 to 10. Many research have been done that they found out that fungal organisms had an excellent potential for cationic heavy metal sorption [1-5]. Biosorption process involved fungal biomass has a good advantages over conventional separation techniques due to their low operating costs, minimization of the chemical or biological sludge to be disposed [2]. However, fungal biomass has the high efficiency in removing very dilute effluents [6]. Therefore, there is demand for a new biosorbent capable to detoxify the concentrated effluents effectively and efficiently.

The multi-walled carbon nanotubes (MWCNTs) have showed the removal of the heavy metals effectively in a short adsorption time due to their high adsorption capacity, highly porous and hollow structure, light mass density, strong interaction between carbon and hydrogen molecules and large surface area [7-10]. Many research have been done and showed that instead of being an appropriate absorbent, MWCNTs can be a good carrier to immobilize with enzymes like lipase, amino acid, inulinase etc. [11]–[14]. MWCNTs have showed a good result in immobilized with yeast cell due to the present of negative charge and attracted to positive charge of MWCNTs [15]. This study was focused on the immobilization of MWCNTs with the *A.niger* fungal biomass (whole cell).

METHODOLOGY

Microorganisms and Growth Condition

Aspergillus niger was obtained from the lab stock at Bioenvironmental Engineering lab IIUM, Gombak and sub-culture on PDA agar. The strain was incubated for 3 days at room temperature (25 ± 2^0C) [16]. Cultures were incubated on the rotary shaker for 1 day at 150 rpm. The spore concentration was determined by counting the numbers of spores using haemocytometer to maintain its uniform strength (1×10^8 to 2.5×10^8). By using the 250 mL of shake flask medium, the *A.niger* spores was cultivated in the three different liquid medium that contain 17g of malt extract, 17g of malt extract plus 3g of peptone. The formation broth contained 3g of yeast extract, 3g of malt extract, 10g of dextrose and 5g of peptone was prepared with 1000mL of distilled water [17].

Functionalized Carbon Nanotubes by Acid Treatment

The 100mg as-received MWCNTs were dispersed with concentrated nitric acid (HNO_3) and sulphuric acid (H_2SO_4) in ratio 3:1 by 20mL of volume. Then, the mixture was followed by reflux at 120^oC for 30 min. The functionalized MWCNTs were filtered with millipore membrane filter paper and rinsed with distilled water until pH of the running water reach to original and dry in the oven at 80^oC for 8 hours [18].

Immobilization of Fungal Biomass with Carbon Nanotubes

A 100mL of the liquid medium (17g malt extract in 1L of distilled water) was placed in a 250mL Erlenmeyer conical flask, and the optimum dose of MWCNT was found by added different amounts (0.30, 0.40, 0.50 and 0.6 grams) of MWCNT. 2% of the fungal inoculum was inoculated and transferred to the incubator shaker at 150 rpm for five days. The unbound MWCNT was separated with supernatant using PTFE membrane and dried in the oven for 80^0C overnight.
The immobilization percentage was measured by using the formula [13]:

Immobilization percentage = (total mass of MWCNT - mass of unbound MWCNT) / total mass of MWCNT x 100% (1)

The one factor at one time like inoculum dose (1mL, 1.5mL, 2.0mL and 2.5mL), agitation speed (100rpm, 150rpm and 200rpm) and pH (4, 5, 6, 7 and 8) were conducted accordingly by following the same procedure.

Experimental Set-up

The *A.niger* strain grown in the selected liquid media was fermented on two different parameters which were fermentation days and pH to get the optimum biomass production. The optimum value of selected parameters were used to immobilize the fungal biomass with functionalized MWCNTs. The immobilized *A.niger* fungal biomass with functionalized MWCNTs was characterized by using two analytical method which were FTIR and FESEM.

Fourier Transform Infrared Spectroscopy (FTIR)

The analysis was done to investigate the functional group of as-received MWCNTs, oxidized MWCNTs, fungal biomass and the immobilized fungal biomass with MWCNTs.

Field Emission Scanning Electronic Microscopy (FESEM)

The scanning was done by FESEM JEOL JSM-6700F, Japanese manufacturer (JEOL Ltd) with a magnification range from 15× to 300,000× and a resolution of 5 nanometers. Prior to investigate the immobilization of fungal biomass with multi-walled carbon nanotubes.

RESULTS AND DISCUSSION

Microorganisms and Growth Condition

The amount of fungal biomass production in three different liquid media which were malt extract, malt extract with peptone and mix broth contained yeast extract, malt extract, dextrose/glucose and peptone. The most favorable liquid media for *A.niger* growth was malt extract as shown in Figure 1. Figure 2 showed *A.niger* was grown well in pH 6 and after the fifth day of fermentation.

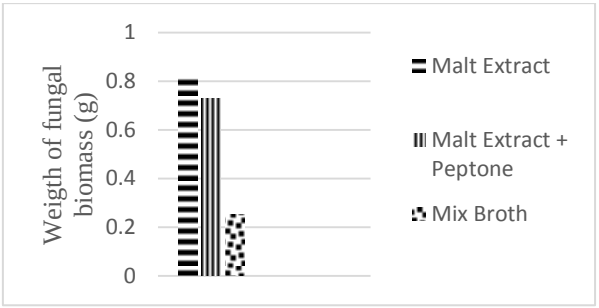
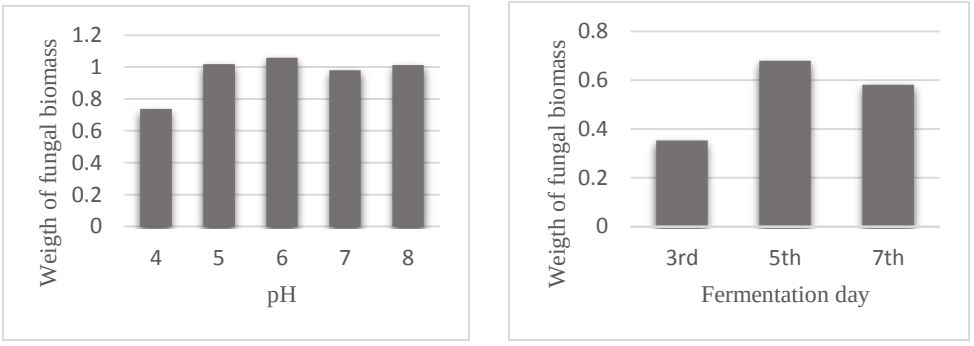


Figure 1. Growth of biomass weight with different media broth in 150 rpm, 5th day fermentation at 25±2°C



(a) (b)

Figure 2. Graph of fungal biomass growth (a) Different pH (b) Different fermentation time (day) with 150 rpm and (*25±2*°C)

An appropriate liquid media was needed in order to produce the highest amount of fungal biomass. This was as important factor to improve the effectiveness of immobilization between fungal biomass with MWCNTs due to the highest

chances of bounded those two materials. As shown in Figure 1 *A.niger* grown well and produced highest amount of fungal biomass in malt extract.

The capabilities of *A.niger* to produce more fungal biomass was showed in Figure 2 in which graph (a) illustrated the amount of fungal biomass produced in different pH. Higher biomass production occur in pH 5 as for *A.niger* the best pH range was 5-6. This finding was similar with study done by Nair et al [19]., where he found out that the optimum biomass formation in the form of biomass yield and mycelia clump was at pH 5.5 (acidic condition) [19]. Next, demonstrated in graph (b), the fermentation days was investigated by harvesting the fungal biomass on various days which were 3rd, 5th and 7th day of fermentation. This finding was similar to other studies conducted by many researchers where the best day to harvest the fungal biomass was the 5th day of fermentation because the fungal cell reached the stationary phase and the prominent amount of fungal biomass obtained [4, 20, 21]. This factor is important since the immobilization between fungal biomass with MWCNTs should be done in five days in order to have the optimum amount of immobilized biosorbent.

Immobilization of fungal biomass with MWCNTs

The percentage of immobilization by selected parameters which were agitation speed, MWCNTs dose, inoculum dose and pH were conducted by OFET method and result as present in Figure 3. The optimum value for agitation speed, MWCNTs dose, inoculum dose and pH were 150 rpm, 0.5g, 2ml and pH 6 respectively.

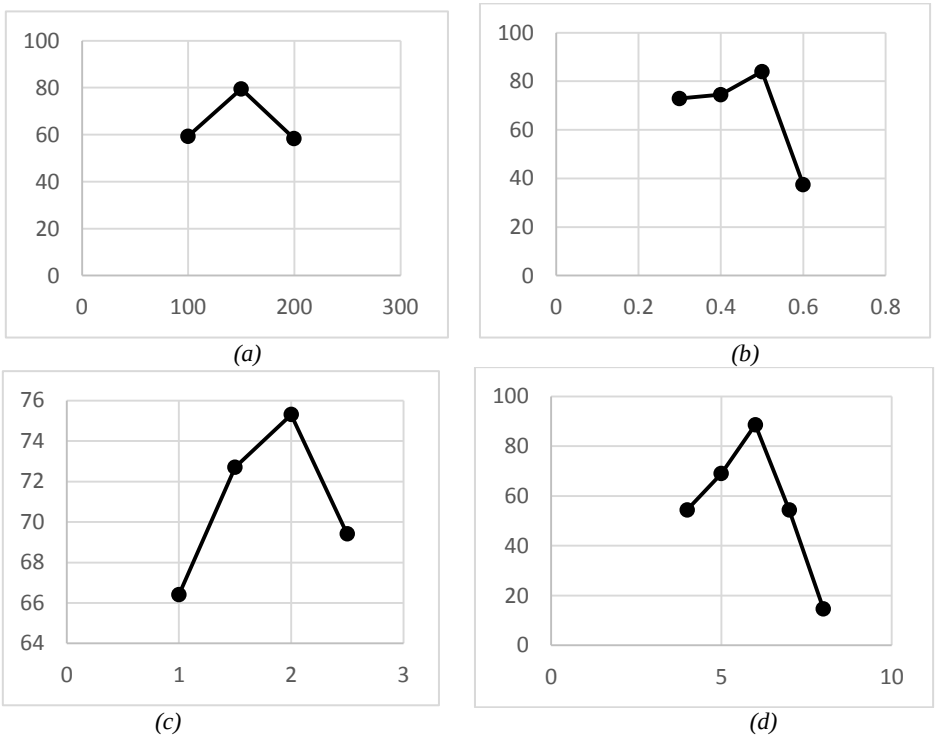


Figure 3. Percentage of immobilization by different (a) agitation speed, (b) MWCNTs dose, (c) inoculum dose and (d) pH at (*25*±*2*^{o}C)

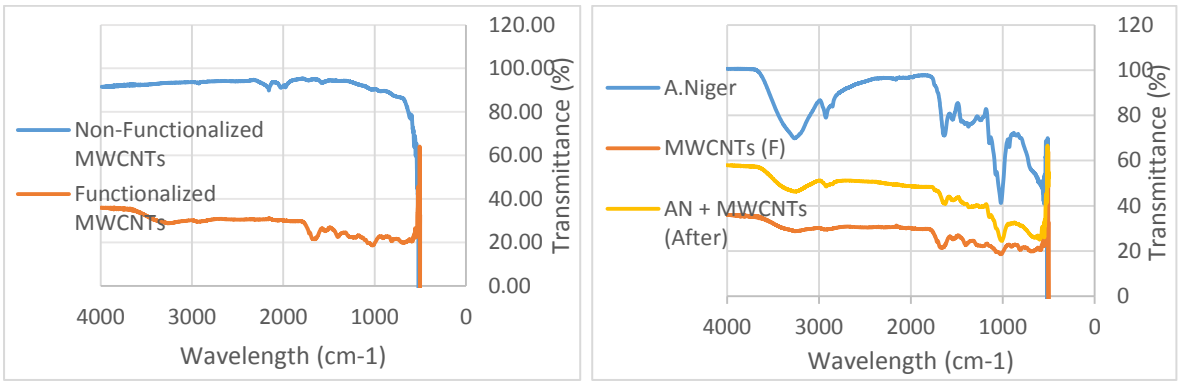
The productiveness of the immobilization was investigated with two important parameters which were agitation speed and the concentration of MWCNTs. The percentage of immobilization was calculated from the equation (1) and the result was displayed in the Figure 3. An appropriate agitation speed was important in immobilization since it would help the efficiency of the process. Not strong enough agitation speed may lead to clump of biomass and this reduced the surface area for the MWCNTs to attach to the biomass, nevertheless, too strong agitation speed create another problem as the biomass and immobilization facing too much shear stress and hard for them to bound to each other [15]. As shown in the graph (a), the percentage of immobilization was highest in 150 rpm agitation speed. This result was in line with other studies that applied 150 rpm to immobilize carbon nanotubes with loading material [22-23]. Afterwards, the quantity of the MWCNTs used in the immobilization was investigated to fine the best amount of MWCNTs dose in 100ml of liquid media with 2% of inoculum dosage. Initially, there is a trend of fungal biomass loading on MWCNTs increasing proportional to the MWCNTs dosage until it reaches an optimum value at 0.50 g. The percentage was significantly dropped in more than 0.50 g of MWCNTs because the unused MWCNTs was too much since the amount of fungal biomass produced was less than the available MWCNTs to bind with. Hence, generates higher remaining MWCNTs and cause lower percentage of immobilization.

Besides that, the percentage of immobilization was significantly changed with another factor which is inoculum dose. There was a pattern of fungal biomass loading on MWCNTs increasing proportional to the inoculum dosage until it reaches an optimum value at 2ml of inoculum. The increase of fungal biomass bounded at the beginning was mainly due to the availability of large of open end or pores on the surface of MWCNTs for the attachment of fungal biomass. However, it eventually reached a saturation level as the amount of MWCNTs used in this experiment was fixed (0.5g/ml) which supported by previous experiment. The pore site on the surface of MWCNTs was fully covered by the fungal biomass with

the concentration of 2 ml of fungal inoculum. Thus, with further increase in fungal inoculum over the optimal amount of inoculum dosage, as those extra fungal biomass would try to maximize its contact with hydrophilic surface of the MWCNTs, by squeezing into pore site that have covered with fungal biomass molecule which could eventually lead to undesirable conformation changes and thus decrease in initial reaction rates was observed [24]. This result in agreement with a study done by Xie and Ma [25] where the percentage of immobilization nearly constant as the enzyme/cell loading further increased. It is observed that a significant changes of the immobilization efficiency since the growth of inoculum was disturbed by limited nutrient supply from media. Thus, 2 ml of inoculum dose with 0.5 g of MWCNTs dose was selected for further studied to determine the effect of pH on the immobilization process. As shown in the graph (d), the highest of immobilization percentage was at pH 6. This can be relate to the optimum pH for fungal inoculum growth in range 5 - 6. Results also demonstrated there was dramatically decline of immobilization percentage in alkaline pH condition. This is because the fungal inoculum cannot survived in alkaline condition which is similar to other studies. [24, 26].

FTIR Analysis

The spectra showed the functional group of non-functionalized MWCNTs, functionalized MWCNTs, *A.niger* biomass and immobilized MWCNTs present in Figure 4.



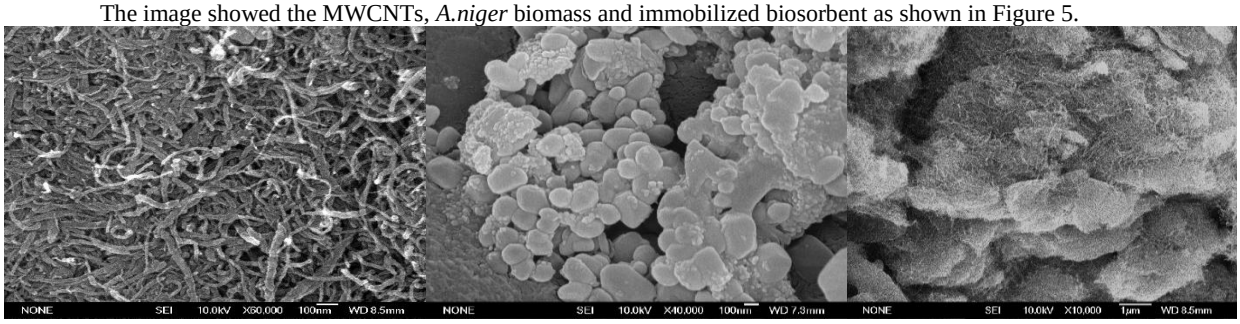
(a) *(b)*

Figure 4. FTIR Spectra (a) Comparison between non-functionalized with functionalized MWCNTs (b) A.niger, MWCNTs, and immobilized MWCNTs graphs

After the immobilization and physical observation, a validation and confirmation of the immobilization conducted. Both analysis were done by having three samples which were MWCNTs, *A.niger* biomass and immobilized fungal biomass. An analysis of FTIR to compare the functional group of non-functionalized MWCNTs and functionalized MWCNTs have been done as well. As demonstrated in graph, Figure 4(a), the FTIR analysis showed that the non-functionalized MWCNTs have a band at 1560 that represents the carbon nanotubes backbone stretching mode. After undergone an acid treatment, the functionalized MWCNTs spectrum shows one weak peak appeared at 3281 (3200-3400 cm^{-1}) which ascribed to the present of –OH stretching mode of hydroxyl group [27]. Besides that, after execution of oxidation process the peak of 1560 cm^{-1} changed to 1680 cm^{-1}, this represents the C-O stretch mode [28]. On the other graph (Figure 4 (b)), the *A.niger* biomass contained band 3265cm^{-1} and this band illustrate the presence of –OH groups. [9, 29-32]. It showed a peak at 2924cm^{-1} that basically attributed to C-H bond while another peak signal of biomass also displayed the band range from 1373cm^{-1} to 1634cm^{-1} and this peak was indicative of presence of C=C stretching mode [33]. Moreover, the sorption of C-C (1020-1146 cm^{-1}) was also observed [34]. These presence groups on both materials indicate the chemical binding of fungal biomass with MWCNTs. The small peak at functionalized MWCNTs (3280 cm^{-1}) has bind to the –OH group presents in *A.niger* biomass (3282 cm^{-1}). This band has changed to 3270 cm^{-1} in the line showed the immobilization of both materials. An increase relative intensity between peaks at 1628 cm^{-1} and 1645 cm^{-1} for *A.niger* biomass and functionalized MWCNTs respectively, signifies the increase in the intensity of C-O stretch mode and suggests that immobilization between *A.niger* biomass and functionalized MWCNTs has taken place [28].

FESEM Analysis

The image showed the MWCNTs, *A.niger* biomass and immobilized biosorbent as shown in Figure 5.



(a) *(b)* *(c)*

Figure 5. FESEM images (a) A.niger biomass (b) functionalized MWCNTs (c) Immobilized biomass with MWCNTs

From the FESEM analysis, the immobilization can be confirmed as successful owing to the microscopic image formed. The topography and composition of the biosorbent was observed Figure 5 (c). The image of fungal biomass and MWCNTs alone were illustrated (Figure 5 (a) and (b)) as well in order to compare their feature. Obviously, compared to (a) and (b), (c) showed that the immobilization was occurred as MWCNTs is wrapping on the fungal biomass. The surface morphology of functionalized multi-walled carbon nanotubes (MWCNTs) can still be observed clearly by FESEM in 100k magnification and showed the typical fibrous shapes. However, the coated *A.niger* biomass can be observed in 40k as it was non-conductive elements. The combined MWCNTs with the *A.niger* biomass (biosorbent) can possibly be clearly seen in less than 40k magnification. This could be explained by the disability of the uncoated *A.niger* biomass to transfer the electron. This fact also leads to a higher conductivity and improved electrochemical properties for the biosorbent. The MWCNTs uniformly cover the electrode surface and provide porosity and a network. *A.niger* biomass molecules were well-adhered on the coated electrode surface with the help of covalent binding due to the functional groups. However, once the magnification went larger, the wrapped fungal biomass could not support the electron. This may lead to charging of the electron, the state where the electrons are accumulated at one place, thus, produced bright and unclear imaged [24]. The biosorbent (c) should have higher tendency to removes the impurities since it contained unaligned and not clumped MWCNTs together with completely covered A.niger biomass surface that could increase the opportunity of absorption [35].

CONCLUSION

The immobilization of fungal biomass onto MWCNTs was showed to be successful since both of materials contained functional group that chemically combined. This new biosorbent could be a new good biosorbent to remove all impurities in a solution. A proper strain to produce well-shaped and abundant amount of fungal biomass was needed. The effectiveness of immobilization might be increased with optimum MWCNTs dosage, inoculum dose, pH value and appropriate agitation speed.

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