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Immobilization of fungal biomass with multi-walled carbon nanotubes as biosorbent

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ABSTRACT

Aim: This study was mainly highlighted on a combination of fungal biomass onto MWCNTs in order to enhance the positive integration of impurities removal in aqueous solution.
Methodology and results: The immobilization of fungal biomass and MWCNTs was done in a batch liquid medium with several factors such as agitation speed, dose of MWCNTs, pH and inoculum dosage that were conducted with one factor at one time (OFAT) method. Basically, to verify the functional group of MWCNTs, *Aspergillus 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 showed the best agitation speed, dose of MWCNTs, pH and inoculum dosage were 150 rpm, 0.5 g, 5-6 and 2% respectively. FTIR indicates the presents of the functional groups like –OH (3270 cm^{-1}), C-O (1619 cm^{-1}) and –CH (2915 cm^{-1}) while FESEM illustrates the images of the wrapped MWCNTs on *A. niger* biomass.
Conclusion, significance and impact of study: The conventional technique of adsorption of fungal biomass alone not showing a favorable removal of impurities. Thus, the immobilization of fungal biomass (*A. niger*) with multi-walled carbon nanotubes (MWCNTs) was a good combination since both have potential functional group to accumulate to each other and has a tendency to remove effectively and efficiently the impurities in aqueous solution.

Keywords: Biosorbent, carbon nanotubes, fungal biomass, immobilization

INTRODUCTION

Biosorbent is a combination of two materials which are natural product (biomaterials) and man-made product. The combination of these 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 (Akhtar *et al*., 2007).

Some fungi like *Aspergillus niger* (Kapoor *et al*., 1999), *Penicillium sp*., *Rhizopus arrhizus* (Fourest *et al*., 1994) 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 (Akhtar *et al*., 2007; Kapoor *et al*., 1999; Mamisahebei *et al*., 2007; Tsekova *et al*., 1998). 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 (Kapoor *et al*., 1999). However, fungal biomass has the high efficiency in removing very dilute effluents (Kratochvil and Volesky, 1998). 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 (Lu and Chiu, 2006; Shadbad *et al*., 2011; Allegri *et al*., 2016; Mubarak *et al*., 2016). 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. (Lee *et al*., 2010; Verma *et al*., 2013; Garlet *et al*., 2014; Oliveira *et al*., 2018). 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 (Mamvura *et al*., 2012). This study was focused on the immobilization of MWCNTs with the *A. niger* fungal biomass (whole cell).

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MATERIALS AND METHODS

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 °C) (Willey *et al*., 2009). 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 17 g of malt extract, 17 g of malt extract plus 3 g of peptone. The formation broth contained 3 g of yeast extract, 3 g of malt extract, 10 g of dextrose and 5g of peptone was prepared with 1000mL of distilled water (Procedure and Information, 2008).

Functionalized carbon nanotubes by acid treatment

The 100 mg as-received MWCNTs were dispersed with concentrated nitric acid (HNO_3) and sulphuric acid (H_2SO_4) in ratio 3:1 by 20 mL of volume. Then, the mixture was followed by reflux at 120 °C 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 °C for 8 h (Xu *et al*., 2011).

Immobilization of fungal biomass with carbon nanotubes

A 100mL of the liquid medium (17 g malt extract in 1 L 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 g) 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 °C overnight.

The immobilization percentage was measured by using the formula (Oliveira *et al*., 2018):-

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 (1 mL, 1.5 mL, 2.0 mL and 2.5 mL), agitation speed (100 rpm, 150 rpm and 200 rpm) 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 values 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 methods 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 five 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.

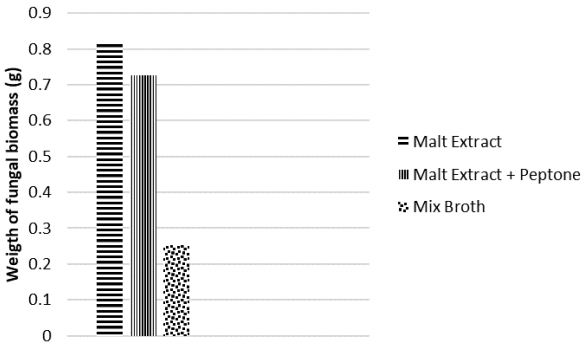

Figure 1: Growth of biomass weight with different media broth in 150 rpm, 5th day fermentation at 25±2 °C

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 were showed in Figure 2 in which graph (A)

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illustrated the amount of fungal biomass produced in different pH. Higher biomass productions occur at pH 5 as for *A. niger* the best pH range was 5-6. This finding was similar with study done by Nair *et al*. (2016), 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) (Nair *et al*., 2016). 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 (Mamisahebei *et al*., 2007; Jangbua *et al*., 2009; Acosta *et al*., 2013). 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.

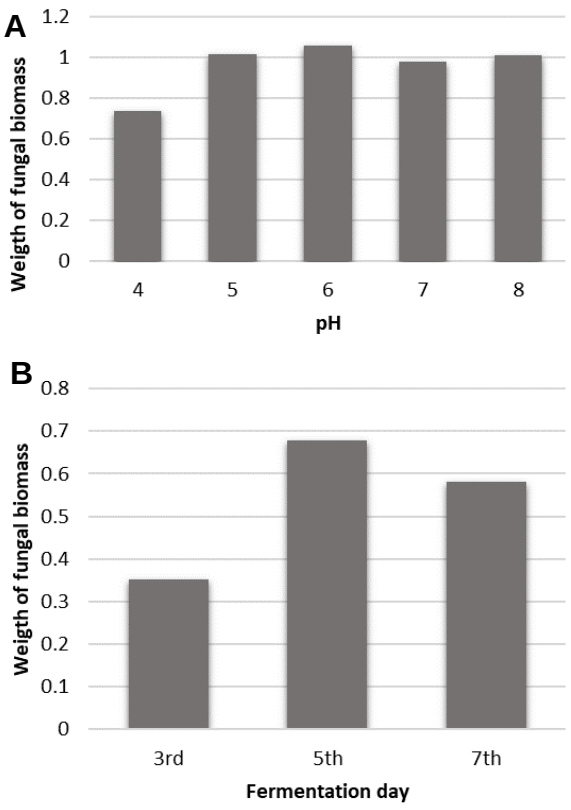

Figure 2: Graph of fungal biomass growth (A) Different pH (B) Different fermentation time (day) with 150 rpm and (25±2 °C).

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, 0.5 g, 2 mL and pH 6 respectively.

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 (Mamvura *et al*., 2012). 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 (Iqbal *et al*., 2005; Peinado *et al*., 2005). Afterwards, the quantity of the MWCNTs used in the immobilization was investigated to fine the best amount of MWCNTs dose in 100 ml of liquid media with 2% of inoculum dosage. Basically, there is a pattern of fungal biomass loading on MWCNTs increasing proportional to the MWCNTs dosage up to 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.

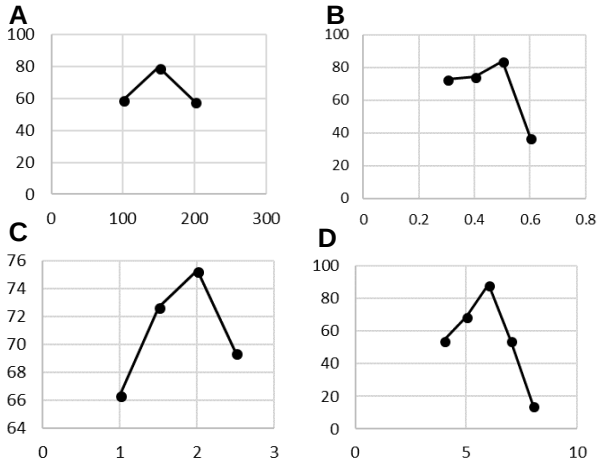

Figure 3: Percentage of immobilization by different (A) agitation speed, (B) MWCNTs dose, (C) inoculum dose and (D) pH at (25±2 °C).

Besides that, the percentage of immobilization was significantly changed with the factor of inoculum dose. There was a trend of fungal biomass loading on MWCNTs increasing proportional to the inoculum dosage as well until it reaches a peak value at 2 mL of inoculum. At the

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beginning, the increase of fungal biomass bounded was predominantly because of the availability many pores and large of open end on the surface of MWCNTs for the attachment of fungal biomass. Eventually, it reached a saturation level as the amount of MWCNTs used in this experiment was fixed (0.5 g/mL) which was the same compared to 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, unfavourable conformation changes occurred 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 decrease in initial reaction rates (Mubarak *et al*., 2014). This result in agreement with a study done by Xie and Ma (Xie & Ma, 2010) where the percentage of immobilization nearly constant as the enzyme/cell loading further increased. It was observed that a significant change 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 in order to find out 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 relates 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. (Gautam *et al*., 2011; Mubarak *et al*., 2014).

FTIR analysis

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

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 (Srivastava *et al*., 2014). On graph Figure 4 (B), the *A. niger* biomass contained band 3265cm^{-1} and this band illustrate the presence of –OH groups (Li *et al*., 2005; Vilar *et al*., 2009; Giri *et al*., 2011; Yu *et al*., 2015; Mubarak *et al*., 2016). It showed a peak at 2924 cm^{-1} that basically attributed to C-H bond while another peak signal of biomass also displayed the band range from 1373 cm^{-1} to 1634 cm^{-1} and this peak was indicative of presence of C=C stretching mode (Pourfayaz *et al*., 2014). Moreover, the sorption of C-C (1020 - 1146 cm^{-1}) was also observed (Soylemez *et al*., 2014). 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 at peaks 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 (Srivastava *et al*., 2014).

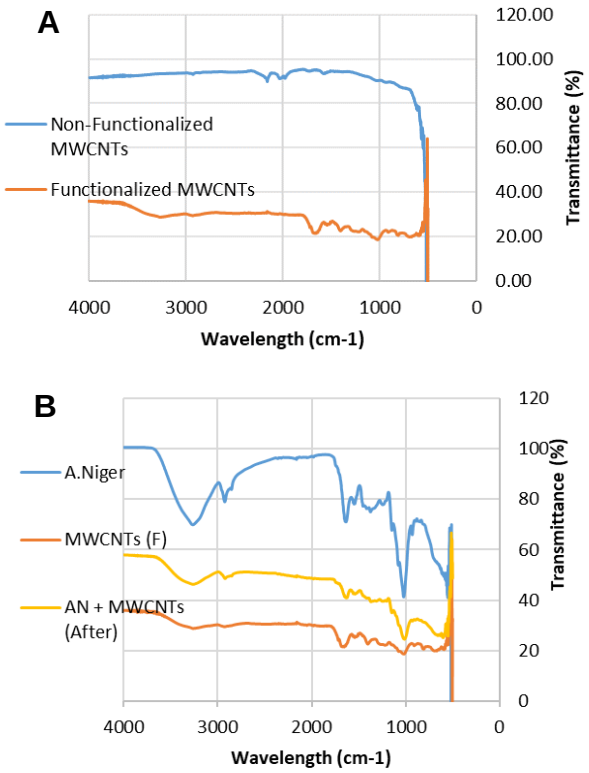

Figure 4: FTIR Spectra (A) Comparison between non-functionalized with functionalized MWCNTs (B) A.niger, MWCNTs, and immobilized MWCNTs graphs.

FESEM analysis

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

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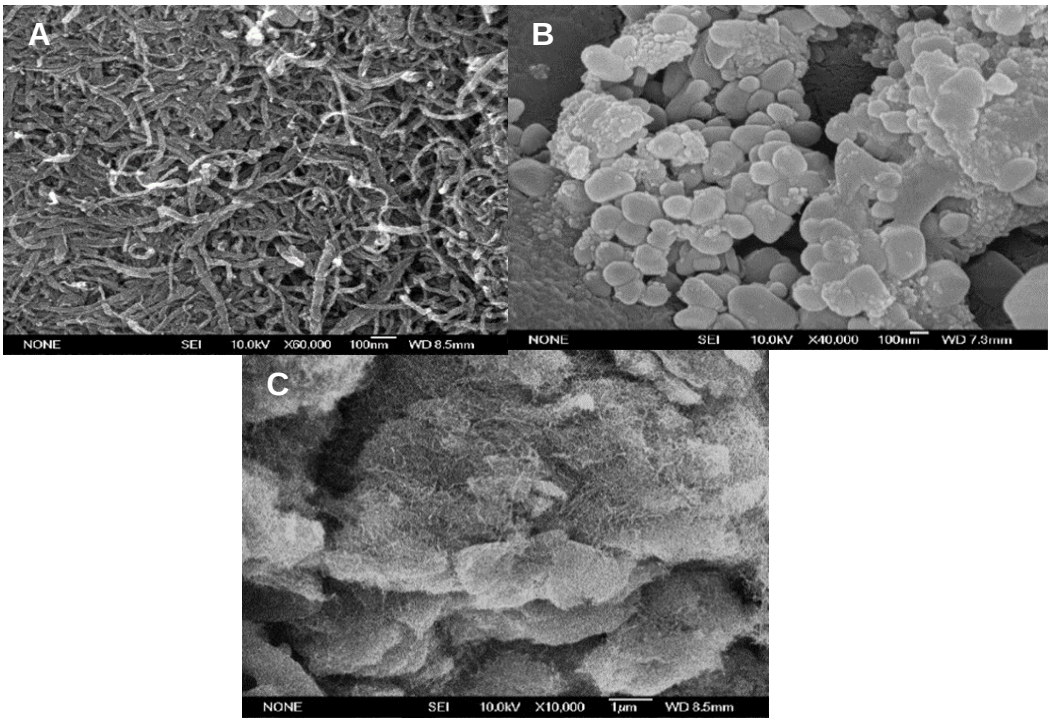

Figure 5: FESEM images (A) A.niger biomass (B) functionalized MWCNTs (C) Immobilized biomass with MWCNTs.

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 (Mubarak *et al*., 2014). 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 (Al-Saadi *et al*., 2016).

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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