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Abstract. Lipase is an enzyme used widely in many major industries. Immobilization of enzymes will help to enhance its sustainability as enzymes are more resistance to changes in environment and can be reused. This experiment examines effects the immobilization of lipase with adsorption technique through carbon nanotubes. The paper investigates the enzyme activity and efficiency of immobilized enzyme lipase by using assay solution. It also presents the effects of pH on immobilized enzyme and the characterization of the immobilized enzyme lipase with FTIR spectrum and FESEM technique. The results showed that there are as enzyme concentration increases, the enzymatic activity increases too. However, this lowers the immobilization efficiency due to saturation of binding pores on functionalized MWCNTs. Meanwhile, the optimum pH for maximum immobilization activity of enzyme lipase is at pH 6. Based on the characterization by FTIR spectrum and FESEM, it is confirmed by the presence of functional group in FTIR spectrography. On the other side, FESEM also confirms that immobilization of enzyme has occurred.

1. Introduction

As the chemical industry progresses, sustainable and effective practices with technology infused applications are preferable now as they do not only fulfill the demands of the industry, but also generate better by products, usually more cost effective and more environmental friendly. Biotechnology is one of such industries that was revolutionized in which this industry is gaining more importance and prominence in many fields such as the pharmaceutical, food processing, textile, cosmetic

industry, medical science and many more. One of the highly sought after tools in biotechnology is the use of enzymes. Enzymes act as catalysts and play a crucial role in various applications and are seen as a tool for future medical and therapeutic advancements [1]

Previously, recycling enzyme and renewal of enzymes is not possible as isolation of enzyme with solutes is a very hard task. Meanwhile, another concern with the use of enzymes is due to its low tolerance towards the change in environment that may entirely hinder the biochemical processes. This causes wastage when conditions of the solute is manipulated, thus leading to enzymes no longer usable [2]

With the current technological advancements in the biomedical and biochemical field, enzymes immobilization was discovered and introduced in which the immobilization process significantly enhances stability and reusability of enzymes [3]. Such improvement is not only cost efficient but opens a whole new platform of venturing more into enzyme handling and enzyme products. Meanwhile, another revolutionary technology applied in enzyme immobilization is the usage of nano materials. Carbon nanotubes (CNT) are gaining importance in biotechnology field in which two or three dimensional nano sized materials are preferred in performing enzyme immobilization [26]. Nanomaterials are used because they act as potential carriers that possess stabilizing effects to certain enzyme molecules without hindering their functionality. Besides, there is also a high potential and possibility of its application in the development of biosensors and industrial biotransformation [26].

The discovery of Carbon nanotubes (CNTs) in 1985 was a result of an unexpected finding from the synthesis of buckminsterfullerene. Researchers found out that besides graphite and diamond, carbon can also form stable and ordered structures which have sparked much interest in finding other carbon allotropes. Carbon nanotubes are carbon molecules cylindrical in shape and are constructed via rolling graphenes sheets into a cylinder [28]. Similar to graphite, the nanotubes are aligned naturally by van de Waals force and are composed of sp2 bonds. As such, they possess excellent properties, either physical or chemical. As their name suggest, nanotubes are 50000 smaller compared to a human hair, and they are member of the fullerene structure family [4]. As such, CNTs have high tensile strength in which they are 100 times stronger than steel, possess high conductivity and compared to diamond, higher thermal conductivity, and high thermal stability [26]. This enables CNTs to be applied widely in many forms and in various industries, such as nano electronics, nano optics. CNTs have demonstrated exceptional characteristics and have high potential to be applied as one of an advanced tool. Thus, the main application areas for CNTs are such as conductive reinforced plastic, CNT based transistor, CNT based ceramics, molecular technology, energy storage, field emitters and so forth [5].

The usage of enzyme has been long existent since ancient civilization for various purposes. Up to date, there are almost 4000 enzymes known and 200 types of enzymes used commercially in different industries [27]. In the current decade, an exponential growth of enzyme usage is seen with total sales of a few million dollars annually indicating the growing importance of industrial enzymes that are of microbial origins [6]. Meanwhile, the advancement of biochemistry that assists in many enzyme related processes such as fermentation, catalyzation and pharmaceutical field has encouraged more mass production of affordable enzymes, leading to an expansion of usage of enzymes in many industries [28].

So far, there are 12 major producers and 400 minor suppliers in the enzyme industry globally with more than 60% of the total global supply based in Europe. With 75% of the industrial enzyme that majors in hydrolytic action, protease dominates the market with more than 40% of all enzyme sale [7]. Meanwhile, lipase is also used in various applications as shown in Table 1.

Lipase also known as triacylglycerol acylhydrolase is ubiquitous that possesses significant physiological importance and high industry potential [27]. Lipase is mainly used to catalyze the

hydrolysis process of triacylglycerols to glycerol and free fatty acids. When being absorbed into an oil-water interface, lipase will be activated and does not hydrolyse substrates that are dissolved in bulk fluids [8]. Lipases are also serine hydrolases, which means it shows minimal activity in aqueous solutions that has soluble substrates; instead, it splits emulsified esters of glycerine and long chain fatty acids. Lipases are involved in the process of lipid metabolism in eukaryotes such as fat digestion, absorption, and lipoprotein metabolism. As for plants, lipases are stored in energy reserve tissues [9].

Up to today, lipase remains as one of the most studied enzyme due to its wide-ranging significance. The main focus of research on lipase concerns its general performance characterization, structural characterization, action mechanisms, kinetic sequencing and lipase gene cloning [10]. Nevertheless, ironically, not much research has been conducted in examining the development of commercially used lipase bioreactor systems [11].

Lipase is heavily used various industries such as fat and oil processes, detergents and degreasing applications, food processing, pharmaceutical and cosmetic manufacturing, to name a few [12]. Lipase is preferred as it is a catalyst and it accelerates the process in degrading polyurethane and fatty waste. In the commercial industry, two types of lipases are used- native or immobilized [27]. Lipase that are native are generally found in laundry detergents while immobilized lipase is used in various synthesis and biotransformation where these processes require biocatalyst to be immobilized in order to increase efficiency as immobilized enzymes has higher stability and activity. Meanwhile, as immobilized enzymes are recyclable, therefore it is more cost efficient.

Due to the benefits of immobilization, many methods have been developed, in which the non-covalent bonding is considered to be one of the techniques with the highest efficacy. This is because this approach retains the conformational structure of the immobilized enzyme [26]. As enzymes are absorbed onto the surface of CNTs, inactivation can be avoided when there is a high surface loading of enzyme. With the adsorption technique, it was reported that there is a higher activity. Since adsorption is a non-covalent method of immobilization, enzymes are adsorbed physically and attached onto carbon nanotubes (CNTs) (refer to Fig 1) [26]. The CNTs are bathed in a solution of enzyme and the solution is shaken as this allows physical adsorption to occur while clearing off enzymes that are unadsorbed. When enzymes are immobilized, this improves enzyme's resistance towards thermal denaturation [28].

With the direct physical adsorption method, the interacting force between the enzyme and CNT is predominantly a hydrophobic interaction [29]. An enzyme including hydrophobic regions on its exterior can interact with the wall of a CNT through hydrophobic interactions, as illustrated by Fig. 1. The π–π stacking interaction between the sidewalls of CNTs and the aromatic rings also contributes to the adsorption [30]. These hydrophobic and π–π interactions have been widely used to explain the driving force of the direct adsorption of enzyme on CNTs.

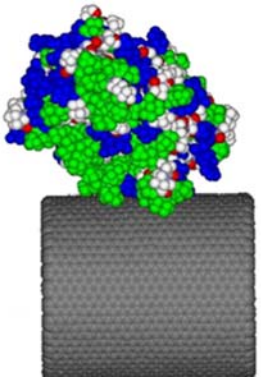

Fig. 1. Schematic illustration of lipase adsorbed on carbon nanotubes by molecular dynamics simulation. The hydrophobic parts are in green, hydrophilic parts in blue

In this research, immobilization of enzyme with carbon nanotubes via adsorption is being examined. This experiment aims to perform a biocatalyst that is stable, reusable and is easy to handle in the meantime ensuring that it can also be utilized in other batch or continuous flow mode of biotransformation [27]. The experiment investigates the immobilization process of enzyme lipase where lipase is a biocatalyst that is important in the enantioselective synthesis process especially for industries such as the pharmaceutical and biomedical field [28].

2. Materials and Methods

2.1. Materials

All studies were performed using commercial lipase that was purchased from Sigma Chemical Co. (St. Louis, USA). Commercial Functionalized multi-walled carbon nanotubes (F-MWCNTs) (95% pure, 1.5 m in length and with a 15–30 nm outer diameter). Virgin olive oil was purchased from one of the local trading company to make assay solution. PTFE membrane filter, NaOh and KH2PO4 was provided by the University (Curtin Malaysia). All the other chemicals are used without any further purification.

2.2. Functionalization of MWCNTs

The functionalization of MWCNT is achieved via acid oxidation treatment method. 1000mg of pristine MWCNT is mixed with 100mL of concentrated acid of HNO3 and H2SO4 at the ratio of 1:3 [15]. The mixturewas put into a 200mL conical beaker and put in an ultrasonic bath for 4.5 hours at 40 degree Celcius. During this period of time, functionalization will take place. Meanwhile, preparation of the hydrophilized membrane can be performed for the further steps. The PTFE membrane is pre-wetted with ethanol solution that is of 95% where the color will change from white to transparent. When the period of bath finishes, the mixture is diluted with distilled water. With a vacuum filtration system, the mixture is filtered with a 0.45m hydrophilized PTFE membrane prepared just now. The filter products are washed with distilled water until the pH of the filter cake becomes pH 7. The functionalized MWCNT is ready to be collected and is dried in a vacuum oven for 48 hours at 80 degree Celcius. After dried, the functionalized MWCNT is cooled for 24 hours before using.

2.3. Immobilization of lipase enzyme on functionalized MWCNT

Immobilization of lipase enzyme was achieved via physical absorption method. A total eight samples of functionalized MWCNT solutions (2,3 mg/mL) are prepared by dispersing 20,30 mg into 10

mL 0.05 M phosphate buffer solution (pH = 7.0). After that, various concentration of lipase solution (2, 4, 6, 8 mg/mL) are prepared by dispersing 2, 4, 6, 8 mg of lipase into 1 mL 0.05 M phosphate buffer (pH = 7.0) solution respectively. Afterwards, 2 and 3mg/mL of functionalized MWCNT samples are mixed with 2, 4, 6, 8 mg/ml of lipase concentration samples respectively. In result, there will be a total of eight solution samples. The prepared solutions samples are mixed in a container properly, followed by incubation in an incubator shaker at a temperature of 30 ◦C, 200 rpm speed for 2.5 h. After incubation, the functionalized MWCNT–lipase composite mixture is centrifuged at 3800 rpm for 15 min to settle the functionalized MWCNT–lipase conjugate. Then, the supernatant is carefully decanted without loss of any conjugate. Next, the functionalized MWCNT–lipase composite is re-dispersed into fresh phosphate buffer solution (pH 7) to wash the supernatant. The sequential process of washing and centrifugation are repeated at least 3–4 times to wash and remove the unbounded functionalized MWCNT. Eventually, functionalized MWCNT–lipase supernatant is left to air dry for 24 hours prior conducting the analysis on the sample [15].

2.4 Characterization of functionalized MWCNT and immobilized enzyme

The chemical composition of functionalized MWCNT–lipase supernatant was characterized by FTIR (Brand: Bruker, Model: IFS66v/S). FTIR spectra was recorded using KBr pellets in the frequency range (4000–400 cm−1) and the optical properties of the samples were identified by using a laser that emits red light with wavelength of 410 nm. In contrast, the physical structures of the above samples are observed by using FESEM (Brand: Zeiss, Model: Auriga) under two different magnification scales: 1 m and 300 nm; the presence of the functional group or enzyme on carbon nanotube can be confirmed as well throughout this analysis.

2.5 Olive oil assay

Olive oil assay was used as a substrate solution to determine the lipase activity after the immobilization. To produce olive oil assay, 15mL virgin olive oil was diluted with 10 mL of 0.05M phosphate buffer (pH = 7) in a 25 mL test tube. First, the diluted olive oil is pre-warmed at 65◦C for 8 min; followed by the addition of 75 mL of distilled water into the sample, then incubated in a water bath at 70◦C for 40 min. After incubation, the olive oil dilution assay of 100mL is immediately transferred to an ice bath for cooling. After that, the olive oil assay is ready for determination of lipase activity.

2.6 Determination of lipase activity

The hydrolytic activity of lipase was measured using olive oil assay as the substrate. The substrate solution was prepared as stated in section 2.5 in which the samples are mixed vigorously with the olive oil assay and heated at 85 ◦C for 20 minutes. The assay mixture consisted of 1.5 mL of substrate, 1.0 mL of 0.05M of phosphate buffer (pH 8.3) and 0.5mL of test solution. The absorbance was read at 410 nm at 25◦C for every sample. One unit of activity was expressed as 1 μmol of olive oil assay released per minute under assay condition.

2.7 Characterization of functionalized MWCNT

After finding out all the immobilization efficiency of different supernatants, further characterization has been performed which is mainly based on FTIR and FESEM. FTIR spectrum analysis is analysed through KBr pellets and then photographed images is obtained. Whereas FESEM technique will be applied in order to analyse the morphology structure of immobilized enzyme of that supernatant under the range of 1μm - 300nm. The chemical composition of functionalized MWCNT–lipase composite is characterized by FTIR (Brand: Bruker, Model: IFS66v/S). FTIR spectra was recorded using KBr pellets in the frequency range (4000–400 cm−1) at a resolution of 4 cm-1 and the optical properties of the

samples were identified using He–Ne (helium–neon) laser that emits red light with wavelength of 410 nm. In contrast, the physical structures of the above samples are observed by using FESEM (Brand: Zeiss, Model: Auriga) under two different magnification scales: 1 m and 300 nm; the presence of the functional group or enzyme on carbon nanotube can be confirmed as well throughout this analysis.

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3. Result and Discussion

A. Enzyme activity on functionalized MWCNTs

In determining the enzyme activity during immobilization process with f-MWCNTs, it was observed that the lowest enzyme activity occurs when 2mg/ml of f-MWCNT is dispersed in 2 mg/ml of enzyme lipase whereby the activity was recorded with the value of 11,537,414. Meanwhile, the maximum enzyme activity occurs with 8mg/ml of lipase, which gives a value of 16,987,012 as shown in Fig. 11.

On the other hand, while observing the enzyme activity when enzyme is immobilized with 3mg/ml of f-MWCNT, the lowest activity occurs during the dispersion of 2mg/ml enzyme lipase, in which a value of 7,026,038 is obtained. The number is the greatest, with a value of 15,500,600 when 8mg/ml of enzyme is used (refer to Fig. 2). As seen in the Fig. 1 and 2, both graphs show that the concentration of enzyme and enzyme activity is directly proportionate. This means that as the concentration of enzyme increases, the enzyme activity also increases which follows the same trend as shown in studies [16].

When 2mg/ml of f-MWCNT is used to immobilized lipase enzyme, it was observed that the lowest value of enzymatic activity is almost twice as much compared to the usage of 3mg/ml of f-MWCNT, with a difference of 4,511,376. Similarly, the highest value of enzymatic activity is observed in 2mg/ml of f-MWCNT dispersed in 8mg/ml of enzyme lipase, in which it gives the highest value of 16,987,012, compared to 3mg/ml of f-MWCNT dispersed in 8mg/ml of lipase, which gives only a value of 15,500,600, a difference of 1,486,412.

The immobilization capacity towards enzyme lipase for both concentrations of f-MWCNT (2mg/ml or 3mg/ml) is relatively similar regardless of the f-MWCNTs' concentration. In this case, the values of immobilization show an increasing trend and the numbers show lesser difference as the concentration of enzyme lipase dispersed increases. As 2mg/ml of f-MWCNT has a smaller surface area compared to 3mg/ml of f-MWCNTs, the attachment of enzyme lipase on the pore surface of 2mg/ml of f-MWCNT is much wider and therefore, consume less time to be immobilized compared to 3mg/ml of f-MWCNT in all lipase concentration despite the fact that the efficiency of immobilization of f-MWCNT is the same [17].

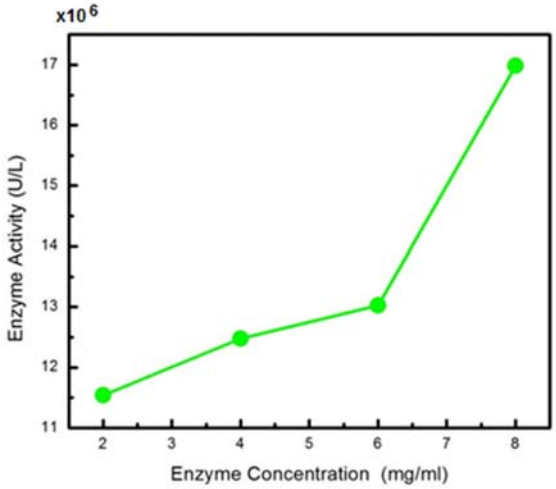

Fig. 2 Enzyme activity when dispersed in 2mg/ml F-MWCNTs

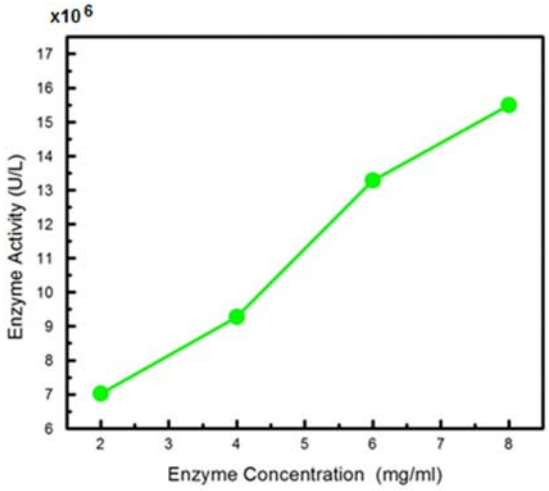

Fig. 3: Enzyme activity when dispersed in 2mg/ml F-MWCNTs.

B. Immobilization efficiency for enzyme lipase in functionalized MWCNTs

The effect of immobilization activity was determined while performing two different concentrations of f-MWCNTs - 2mg/ml and 3 mg/ml with an increasing amount of lipase enzyme concentration of 2 mg/ml, 4 mg/ml, 6mg/ml and 8mg/ml respectively. The lowest immobilization activity in the supernatant while performing with 2mg/ml of f-MWCNT is 11,537,414 U/l when 2 mg/mL of enzyme dosage is used for the immobilization process. Meanwhile, the highest immobilization activity in supernatant was achieved with 8mg/ml which is 16,987,012 U/l. On the other hand, the lowest immobilization activity in the supernatant while performing with 3mg/ml of f-MWCNT is 7,026,038 U/l when 2 mg/mL of enzyme dosage is used for the immobilization process. Meanwhile, the highest immobilization activity in supernatant was achieved with 8mg/ml which is 15,500,600 U/l.

Meanwhile, in terms of immobilization efficiency, the numbers are decreasing as the lipase enzyme increases for both 2mg/ml and 3mg/ml f-MWCNTs. The highest recorded percentage of immobilization

efficiency with 2mg/ml f-MWCNT is 87% with 2mg/ml lipase enzyme and the lowest is 66% with 8mg/ml of lipase enzyme.

Based on the graphs for both 2mg/ml and 3mg/ml of f-MWCNT, the immobilization efficiency rate is showing a downward trend which shows a decrease. This indicates that as the lipase concentration increases, the immobilization efficiency is inversely proportionate as it decreases instead which is similar to other studies [19], in the discussion about enzyme concentration with enzyme activity and immobilization efficiency. Similar pattern of graph is obtained which indicates that the immobilization efficiency indeed decreases when the enzyme concentration increases [18]. The initial increase in activity of supernatant is due to the high availability of open end pores found on the surface of f-MWCNTs which allow a high amount of lipase enzyme to be attached. However, as the lipase enzyme concentration increases, the pore saturation level for the fixed amount of f-MWCNTs (2mg/ml and 3 mg/ml) reaches its limit and reaches a plateau. This is also an indication that the pore sites on the surface of f-MWCNTs are saturated with lipase.

As enzymes squeezes into the pores of f-MWCNT as the enzyme concentration increases, this will affect the surface of the MWCNT, in which there will be undesirable changes in the surface. Such changes lead to the loss of enzyme immobilization activity. Nevertheless, the activity for supernatant is increasing because the amount of lipase increases as the physical adsorption relies on hydrophilic interactions between enzy

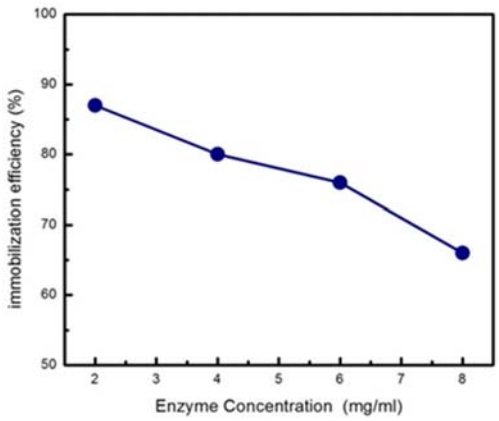

Fig. 4 Immobilization efficiency when done with 2(mg/ml) F-MWCNTs

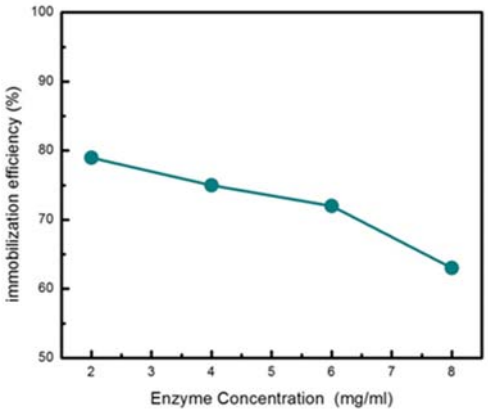

Fig. 5 Immobilization efficiency when done with 3(mg/ml) F-MWCNTs

C. Effect of pH on enzyme activity

The variation of pH has significant impact on the enzyme stability due to the value of pH would affect the protein dissociation and net charge [10]. From Fig. 5, immobilized enzyme has the highest enzyme activity at pH 6 for both concentrations of f-MWCNT which shows that this pH condition is the optimum working condition for the lipase enzyme to obtain its maximum enzyme activity. In 2mg/ml of f-MWCNT that is dispersed with 2mg/ml of lipase enzyme, the result obtained is 11,530,864 U/l. On the other hand, in 3mg/ml of f-MWCNT that is dispersed with 2mg/ml of lipase enzyme, the result obtained is 9,716,475 U/l.

The result shows that lipase enzyme activity increases between pH 4-6 while there is a sudden decline when it reaches pH 7. As lipase's working condition is most optimum in acidic conditions, this explains its sharp decrease in efficiency when the solution gradually becomes alkaline in pH [19]. The decrease in enzyme activity is due to a strong electrostatic repulsion, leading to the stretching of protein molecule, destruction and degeneration of the enzyme active center, resulting in the loss of lipase enzymatic activity [20]. Other studies have also reported that the crystalline style of lipase enzyme is stable at acidic pH (pH= 3.0–6.0), but it will dissolve at higher pH in which the crystalline style remains intact for several days when it was kept at pH 3.5–6.0 [21]; however, it will completely dissolve within 1 h when subjected to a pH of 9.0. Furthermore, acidic condition is the natural habitat for the growth of lipase enzyme and it favors the enzymatic activity as similar to the findings in our study. The immobilized enzyme showed a significant higher activity in both concentrations of f-MWCNTs (2mg/ml and 3mg/ml) and immobilized lipase activity possess high pH stability at pH 6 but then decreases after pH 6. This is mainly due to the restriction of lipase enzyme molecules in the pore site of functionalized MWCNTs and the stretching of the enzyme molecules was blocked after the immobilization. Thus, the pH stability of the immobilized enzyme is higher.

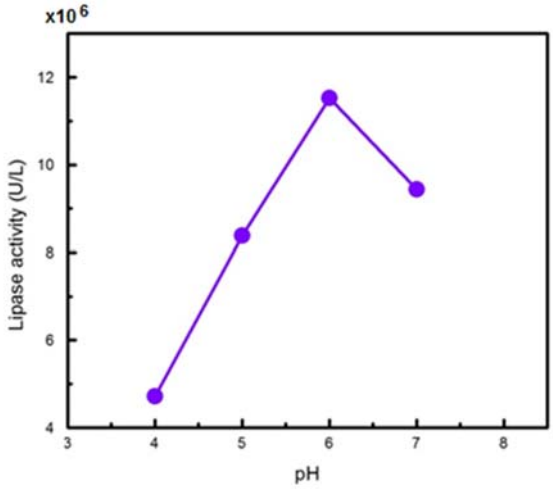

Fig. 6 Effect of pH on enzyme activity when dispersed with 2 mg/ml F-MWCNT

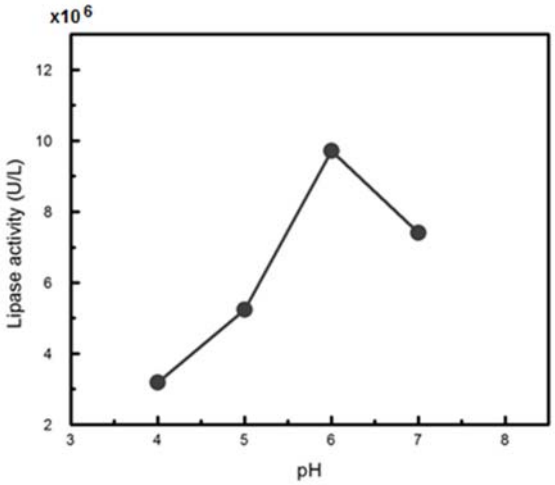

Fig. 7 Effect of PH on enzyme activity when done with 3 mg/ml F-MWCNTs

D. Characterization of immobilized lipase

There are two main methods to determine characterization- FESEM and FTIR. Field emission scanning electron microscopy (FESEM) is a device that examines the topographical and elemental information of a composite in which its magnifications ranges from 10x to 300,000x. Unlike the traditional SEM (Scanning Electron Microscopy), FESEM is has an unlimited depth of field and it is able to generate images that are not influenced by electrostatic influence, thus producing clearer images. Fourier Transform Infrared (FTIR) Spectroscopy on the other hand is used to generate qualitative and quantitative analysis that helps to examine the chemical structure of the composite and the presence of other organic compound. FTIR helps to perform analysis of samples even to the minutest ones up to few microns [22].

E. FESEM Characterization of the immobilized enzyme

FESEM (Brand: Zeiss, Model: Auriga) technique was used to examine the morphology process that occurs on the physical surface of the MWCNTs before and after functionalization. The FESEM characterization is performed on functionalized MWCNTs and f-MWCNT lipase. The magnification scales that are being used are 1µm and 300nm. The selection was made on the basis of immobilization efficiency as it can be observed earlier that on the concentration of 2mg/ml of lipase, the immobilization was the highest. Therefore, it has been selected to undergo FESEM to examine the morphology on its surface. Meanwhile, the other samples of different concentrations that underwent FESEM analysis is attached in the appendix for reference

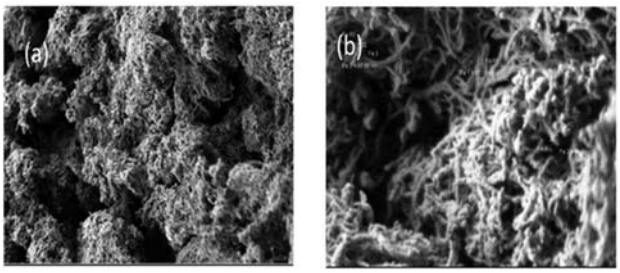

Fig. 8 FESEM image of functionalized MWCNTs in different magnification scales: (a) 1µm and (b) 300nm

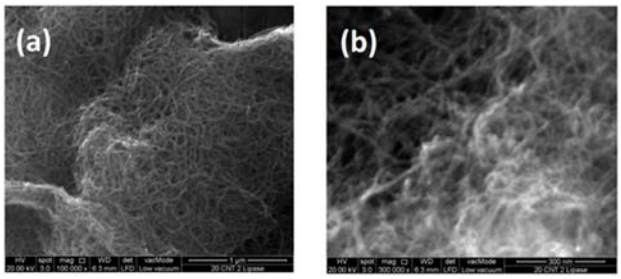

Fig. 9 FESEM image of immobilized f-MWCNT lipase composite in different magnification scale (a) 1µm and (b) 300nm

As shown in Fig. 7(a) and (b), it can be seen that there are tangled tubes presented in bundles with an order of micrometer and impurities can also be seen on these bundles. These impurities are agglomerated and are irregular in shape. When the tubes are immobilized, as shown in Fig. 19(a), it can be seen the tubes are no longer clumped together, instead they are untangled and appears to have more order. The tubes are also more visible and are elongated in shape. The orderly manner of the tubes therefore allows more pores on its surface to be exposed to other particles that are dispersed; hence this confirms that the immobilization process has been achieved. Meanwhile, on other samples, similar patterns are also shown in which the tangled tubes are no longer agglomerated in which this is also shown by other studies [18].

F. FTIR Characterization of the immobilized enzyme

In the following graphs, a comparison is made between two different samples which were gleaned after the immobilization process. Fig. 9 is the FTIR result of 2mg/ml of f-MWCNT with 2 mg/ml of lipase. Meanwhile, Fig. 10 is the FTIR result of 3mg/ml of f-MWCNT with 6 mg/ml of lipase. In the

FTIR spectrum, there are four regions in the graph. The first region has a range between 4,000 cm-1 to 2,500 cm-1 while the second region has a range between 2,500 cm-1 to 2,000 cm-1. The third region is ranged from 2,000 to 1,500 cm-1 and the fourth region is ranged from 1,500 cm-1 to 400cm-1. In the peaks that emerge in first region, it indicates the presence of the functional groups that are being absorbed as these peaks corresponds and indicates that absorption has occurred and is caused by N-H, C-H and O-H single bonds [23].

From Fig. 9, it can be seen that the three major initial peaks in the first region (4000 to 2500cm-1) are at 3748cm-1, 3632.4cm-1, and 3530 cm-1. There is a side range of spectrum that is observed, in which this show that there is stretch between N-H and O-H in which vibration occurs. The presence of N-H and O-H indicates the presence of lipase in the sample.

Meanwhile, in Fig. 10, in the first region, three major peaks are noted with 3750.5cm-1, 2973 cm-1, and 2668.7cm-1. The three peaks indicate the presence of aliphatic amide bond (C-N) which is also a sign that amidination is detected and immobilization of lipase is confirmed via the adsorption process [24].

In the second region for Fig. 9, there is a single peak of 2327.4cm-1 indicating the adsorption process that is caused by triple bonds, and in this case C≡N [25]. The presence of the triple bonds is due to the stretching of the bonds which is a result of side reaction that occurs during the functionalization process. It is the effect of interaction between the phosphate buffer and the f-MWCNT. Meanwhile, in the third region that ranges from 1,500 cm-1 to 400cm-1, two peaks can be seen- 1739.2 cm-1 and 1542.2 cm-1, in which double bonds are presented [26].

In Fig. 10, there are two main peaks in the second region which are 2309 cm-1 and 1991.2 cm-1. The third and fourth region continues to have multiple peaks. The peaks are an indication that the functionalized MWCNT has maintained its structure even after being immobilized. In comparing Fig. 9 and Fig. 10 in the third region, it can be seen that the region in Fig. 10 has a higher and broader spectrum. This indicates that the f-MWCNT-lipase composite possesses a higher amount of molecules, thus causing a higher range in the FTIR spectrum.

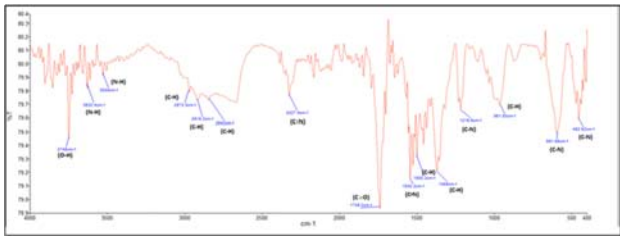

Fig. 10 FTIR spectra: (a) functionalized MWCNT–lipase composite

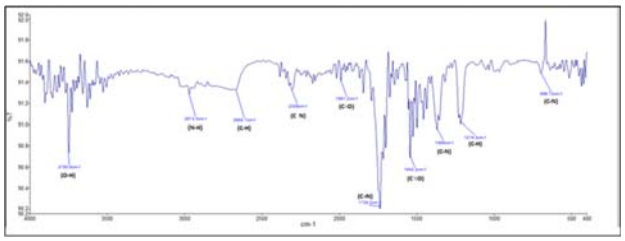

Fig. 11 FTIR spectra: (b) functionalized MWCNT–lipase composite

4.0 Conclusion

The overall objective of this research is performing immobilization of enzyme lipase on Carbon Nanotubes via physical adsorption process in which it is successfully achieved. As confirmed by images from FTIR and FESEM, f-MWCNT has successfully immobilized enzyme lipase. This is shown by the changes in the morphological surface structure of f-MWCNT as they are no longer agglomerated but are arranged in a more orderly manner. All the 8 samples are observed, and it was confirmed from the data as enzyme concentration increases, the enzyme activity also increases. While performing 2mg/ml of f-MWCNT with 2mg/ml of lipase, the lowest activity was recorded at 11,537,414 U/l. The highest value of enzyme activity is achieved while conducting 2mg/ml of f-MWCNT with 8mg/ml of enzyme lipase that gives a value of 16,987,012 U/l. On the other hand, a similar pattern is observed when the concentration of f-MWCNT is increased to 3mg/ml. While performing 3mg/ml of f-MWCNT with 2mg/ml lipase, it shows the lowest enzyme activity that is 7,026,038 U/l. The highest number is achieved with 3mg/ml of f-MWCNT and 8 mg/ml lipase that produces a value of 15,500,600 U/l. In immobilization efficiency, with 2mg/ml of f-MWCNT and 2mg/ml of lipase, the immobilization efficiency is found to be decreasing and this pattern continues with the remaining samples of different enzyme lipase concentration. The highest enzyme immobilization efficiency is achieved with 2mg/ml f-MWCNT with 2mg/ml of enzyme lipase which is 87%. Meanwhile, the lowest immobilization efficiency was recorded at 2mg/ml of f-MWCNT with 8mg/ml of enzyme lipase which gives a 66%. A similar pattern has been observed with 3mg/ml of f-MWCNT when the concentration of enzyme lipase is increased. The highest immobilization efficiency percentage is recorded at 79%, while the lowest efficiency is recorded at 63%. In the pH optimization process of the experiment, it was observed that the pH profile of enzyme lipase is stretched after immobilization, in which the optimum pH is 6.

In the results obtained from the experiment, when enzyme concentration increases, lesser lipase enzyme can be attached, thus, the efficiency of immobilization decreases. Nevertheless, this does not indicate that CNTs are unsuitable for immobilization. Such results were achieved due to the condition of the CNTs. In this experiment, the CNTs used is in powder form, hence, the results show lower immobilization efficiency. It is suggested that CNTs can be made into granulated form which enhances attachment of enzymes as there are more pores available. Meanwhile, modifications can also be made onto the CNT materials, such as adding enzyme binder or co-solvent to ensure that enzymes are covalently bond onto the CNTs' surface.

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