ADSORPTION STUDY OF FRUCTOSYLTRANSFERASE IMMOBILIZATION BY USING VARIOUS ADSORBENT

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SUPERVISOR DECLARATION

I hereby declare that we have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering (Biotechnology).

Signature :

Name of Supervisor : PROFESSOR MADYA DR. MIMI SAKINAH ABD MUNAIM

Date : 1 FEBRUARY 2013

STUDENT DECLARATION

I declare that this thesis entitled "*Adsorption study of Fructosyltransferase immobilization by using various adsorbent*" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature :
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This thesis is dedicated to my father (Azmi Bin Hussain), who always gives support and encouragement and to my mother (Raja Eshah Binti Raja Musa), who taught me that even the largest task can be accomplished if it is done one step at a time. It is also dedicated to my beloved sister (NoorHaizun Binti Azmi) and brothers (Ahmad Syafiq Firdaus & Amir Syukrillah) who are very supportive and caring. Thanks to all of you for being with me at good and bad times.

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ADSORPTION STUDY OF FRUCTOSYLTRANSFERASE IMMOBILIZATION BY USING VARIOUS ADSORBENT

ABSTRACT

Immobilized enzyme are widely used in the industry because of its stability and economic. Because of the high demand from the industry, the research is done in order to enhance the quality of immobilized enzyme. This study discusses about the adsorption in the enzyme immobilization process. The purposes of this study are to find out the species of sawdust (*Keruing*, *Meranti* and *Kempas*) which have the excellent adsorption capability, to investigate the effect of various initial enzyme concentrations and the time required for the adsorption of adsorbate onto adsorbent reach equilibrium. For the immobilization process, it was occurred in water bath shaker for 26 h at room temperature and at pH 5.5. The enzyme activity was analyzed by using DNS method. To determine the equilibrium time, the solutions was being agitated in water bath shaker at 100 rpm and the samples were taken out at a given time intervals for analyses (0-40 hours). The concentration of FTase was analyzed by using UV-Vis Spectrometer. The effect of various initial enzyme concentrations (0, 2, 4, 6, 8, 10, 12, and 14 g/L) in the adsorption process was analyzed by using UV-Vis Spectrometer and employing Langmuir and Freundlich models in order to find out the isotherm which fit with the data obtained.SEM and FTIR was done to identify the structures of the untreated, treated sawdust and immobilize enzyme. All the three adsorbents had achieved the equilibrium at time 25 to 30 hours and it is showed that *Keruing* adsorbent comes out with the highest loading capacity (0.945g of FTase/L) due to the size of pores itself. Enzyme concentration residue increases after the addition of 6 g/L of FTase due to the amount of enzyme were exceeds the amount of adsorbent. Freundlich isotherms fitted the data while *Keruing* showed the highest capability of adsorption followed by *Meranti* as adsorbent.

KAJIAN TENTANG PENJERAPAN FRUCTOSYLTRANSFERASE ENZIM TERPEGUN MENGGUNAKAN PELBAGAI JENIS BAHAN PENJERAP

ABSTRAK

Enzim terpegun digunakan secara meluas dalam industri kerana kestabilannya dan harganya yang berpatutan.Kerana permintaan yang tinggi daripada industri, penyelidikan dilakukan dalam usaha untuk meningkatkan kualiti enzim terpegun. Kajian ini membincangkan tentang penjerapan dalam proses memegunkan enzim. Tujuan kajian ini adalah untuk mengetahui spesies habuk kayu (Keruing, Meranti dan Kempas) yang mempunyai keupayaan penjerapan yang tinggi, untuk mengkaji kesan kepelbagaian kepekatan enzim pada permulaan proses dan masa yang diperlukan untuk proses penjerapan mencapai tahap keseimbangan. Bagi proses untuk memegunkan enzim, ia telah berlaku dalam mesin penggoncang selama 26 jam pada suhu bilik dan pada pH 5.5. Aktiviti enzim telah dianalisis dengan menggunakan kaedah DNS. Untuk menentukan masa keseimbangan, larutan sampel digoncang dalam mesin penggoncang pada had laju 100 rpm dan sampel telah diambil pada selang masa yang tertentu (0-40 jam) untuk analisis. Kepekatan FTase dianalisis dengan menggunakan Spektrometer UV-Vis. Kesan kepelbagaian awal kepekatan enzim (0, 2, 4, 6, 8, 10, 12, dan 14 g/L) dalam proses penjerapan telah dianalisis dengan menggunakan Spektrometer UV-Vis dan menggunakan Langmuir dan Freundlich model untuk mengetahui isoterma yang sesuai dengan data yang diperolehi. Analisis dengan menggunakan SEM dan FTIR telah dilakukan untuk mengenal pasti struktur habuk kayu yang tidak dirawat, habuk kayu yang dirawat dan habuk kayu yang telah mengandungi enzim. Kesemua tiga jenis bahan penjerap telah mencapai keseimbangan pada masa 25 hingga 30 jam dan ia menunjukkan bahawa Keruing mempunyai kapasiti muatan tertinggi (0.945g FTase / L) dipercayai berpunca daripada saiz liangnya yang besar. Kepekatan sisa enzim meningkat selepas penambahan FTase sebanyak 6 g/L, disebabkan jumlah enzim telah melebihi jumlah bahan penjerap. Model Freundlich menunjukkan kesesuaian pada data manakala Keruing menunjukkan keupayaan penjerapan tertinggi diikuti oleh bahan penjerap Meranti.

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LIST OF ABBREVIATIONS

A	Quantity of adsorbent
S	Quantity of feed solution
A	Enzyme activity
t_i	Incubation time
D_f	Dilution factor
OD	Adsorbance
K_L	Affinity constant for Langmuir isotherm
K_F	Freundlich adsorption constant
UV	Ultraviolet
MW	Molecular weight
C_U	Unbound solute concentration
C_B	Solute bound per unit amount of adsorbent
C_{U0}	Initial solute concentration in the feed solution
C_{B0}	Initial solute concentration in the adsorbent
C_{Bmax}	Saturation constant
FOS	Fructooligosacharide
SEM	Scanning electron microscope
NMR	Nuclear magnetic resonance
DMF	Dimethyl formamide
FTase	Fructosyltransferase
SDAC	Sawdust activated carbon
RHAC	Rice husk activated carbon
SBAC	Sugarcane baggase activated carbon

HPLC	High performance liquid chromatography
FTIR	Fourier transform infrared spectroscopy
NaCl	Sodium chloride

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Enzymes are biological catalysts that increase the rate of chemical reactions taking place within living cells by lowering the activation energy. Nowadays, immobilization of enzyme is undertaken either for the purpose of basic research or for use in technical processes of commercial interests. Immobilised enzyme has a high potential as a catalyst in chemical processes in a wide range of industries and medicine. They offer more advantages over the classical catalyst due to their specificity, high catalytic efficiency at low temperatures and, being biodegradable, present fewer disposable problems (Bullock, 1995) as cited in (Vujcic et al.,2011). There are three techniques in enzyme immobilization which are carrier binding, cross-linking and entrapment. In this study, the technique use is carrier binding which one of it is adsorption.

Adsorption is a process where one or more components of a gas or liquid stream are adsorbed on the surface of a solid adsorbent and a separation is accomplished (Geankoplis., 2003). Compared to another techniques such as cross linking, adsorption method are simple and cheap. It is also not change the active site of the enzyme. While, the cross linking method may cause significant changes in the active site of the enzyme (Goel, 1994).

In this study, the adsorbent used are *Keruing*, *Meranti* and *Kempas*. This three species of hardwood are very famous in furniture manufacturing because of high demand from customer. Since it gets a very high demand from the customer, there are many sawdust wastes from these types of wood. From this study, the waste from this species of wood can be reuse in another industry and it is one of the new finding, which can help to reduce the material cost in industry.

Fructosyltransferase (FTase) is an enzyme used in this study and also known as adsorbate. Immobilized enzyme is used because from the previous studies, it shows that compared to the free enzyme, the immobilized enzyme form revealed a higher optimal reaction temperature, low activation energy, higher Km (Michaelis constant) and lowers V_{max} (maximal reaction rate) (Faten et al., 2009).

1.2 Problem Statement

Sawdust from the hardwood trees as such *Keruing, Meranti* and *Kempas* which have a high demand in forestry industry. Since it gets a high demand from the customer, there are many sawdust wastes from these types of woods. This waste can be use as adsorbent and can be marketable.

Nowadays, there are some researchers who did the study about immobilization process by using sawdust but there is nobody has study about the capability of this new adsorbent in terms of equilibrium time and adsorption isotherm mainly from these three types of hardwoods.

In other words, there is lack of research been done about these types of adsorbents. There is no previous study prove that this species of adsorbent have an adsorption capability and which species of hardwood have the most excellent adsorption capability.

1.3 Research Objectives

This study is carried out:

(a) To investigate the species of sawdust that has the most excellent adsorption capability.

(b) To find out the effect of various initial enzyme concentration on the adsorption process.

(c) To obtain the time required for the adsorption of FTase reach equilibrium.

1.4 Scope of Study

Absorbent is very important in the adsorption process especially in the enzyme immobilization in order to maintain the stability of enzyme and to ensure that the enzyme can be reused for many times. So that, the scope of this study is to find:

i. The species of sawdust adsorbent are collected from Seng Peng Sawmill, Gambang (*Keruing, Meranti* and *Kempas* which sieving to get the size 0.4μm-0.63μm) where has the most excellent adsorption capability when incubate in 30 ^{0}C for 26 hours.

ii. The effect of various initial enzyme concentrations (2, 4, 6, 8, 10, 12, and 14 g/L) in the adsorption process by using UV-Vis Spectrophotometer and employing Langmuir and Freundlich models.

iii. The equilibrium time (0-40h) for the sawdust and enzyme achieved as it is complete the reactions and UV-Vis Spectrophotometer will be used to analyze the concentrations of FTase at a given time intervals.

1.5 Significance of The Proposed Study

This study is conducted in order to find out the species of sawdust adsorbent whether from *Keruing, Kempas,* or *Meranti*, which have the higher capability of adsorption. It is also conducted to investigate the effect of various initial enzyme concentrations, which shows the capability of the sawdust in the adsorption, the

equilibrium times achieved by the reaction between adsorbent and adsorbate, and the enzyme activities.

From the theory, the molecular size of adsorbate and the size of micropore of adsorbent play an important role in the adsorption process. In the previous study, the adsorption capability is higher when the adsorbate is small in molecular size react with the larger micropore region of adsorbent (Zhang et al., 2006). This theory can be use for the further study.

This study is conducted in order to find out the species of sawdust that have an excellent adsorption capability for the immobilization of FTase. The finding from this study hopefully will be useful for the industrial purpose since most of the adsorbent used in the industry nowadays are very expensive (Hamdaoui, 2006). This raw material can help the industries to minimize their operation cost and contribute to the environment safety since it is waste from nature and not contains any chemicals. In addition, from this finding, the waste problem in the forestry industry can be solving by use it as adsorbent in the adsorption process. Furthermore, the problem in the adsorption process also can be solves.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Sawdust or also known as wood dust is a by-product or waste, a lignocellulosic material (Rafiqul et al., 2011), come from cutting, grinding, sanding, or otherwise pulverizing wood with a saw or other tool. It is composed of fine particles of wood. In this study, the sawdust from hardwood trees was used. *Keruing, Meranti* and *Kempas* are the types of hardwood which have a very high demand in furniture industry in Malaysia. Since it is marketable, large quantities of the sawdust from these species was produced throughout the year. It causes serious economical disposal problems to the wood industries. Some of researchers had tried to overcome this problem by using this waste as a raw material to produce xylose solution. The wood sawdust biomass contains cellulose, hemicelluloses and lignin as the major of biopolymers (Santos et al., 2011).

Figure 2.1 Meranti wood sawdust

2.1.1 The Application of Sawdust (Lignocellulosic Material)

Recently, biodegradable and eco-friendly wastes were used as a raw material for the production of certain products or for the other usage in the industry. The lignocellulosic waste materials residue can produce valuable product since it is renewable, easy to get, and cheap sources of raw material in nature. It is also one of the way for reducing the generated of waste (Bozlur et al., 2010). Currently, sawdust was widely used in the production of activated carbon and also for the production of xylose.

(a) Production of Activated Carbon from Lignocellulosic Material

Activated carbon is one of the most important microporous adsorbents due to its large adsorptive capacity, ability to adsorb variety of dissolved organic, and ability to be costume specific application (Ismadji et al., 2005). The most commonly used raw materials are coal (anthracite, bituminous and lignite), coconut shells, wood (both soft and hard), peat and petroleum based residue. Wood and peat based carbons are mostly meso/macropore structures and are, therefore, usually suitable for the adsorption of large molecular species. Such properties are used to advantage in decolourization processes (Cameron Carbon Incorporated, 2006). Mostly, the activated carbon was used in the waste water treatment, water purification, gas purification, desulphurization and mercury removal (Lam et al., 2008).

Scientist and researchers have been working on synthesis of activated carbon from agricultural waste materials for removal of dyes from the solution. Pandharipande et al., (2012) was done the research about the ability of raw material which are rice husk, sugarcane baggase and sawdust for removal of methylene blue from aqueous solution and removal of potassium dichromate from aqueous solution. All of these raw materials were undergo thermal and chemical treatment for the preparation as adsorbent. Rice Husk Activated carbon (RHAC), Saw Dust Activated carbon (SDAC) & Sugarcane Baggasse Activated carbon (SBAC) have different surface area and porous structures which make them differ in ability of removal heavy metal above. From the Pandharipande et al., (2012) study, it showed that, SDAC and RHAC were more suitable for removal of potassium dichromate from aqueous solution. While for the removal of methylene blue from aqueous solution, SDAC and SBAC were more suitable than RHAC. In conclusion, the structure of

activated carbon sawdust is suitable for the adsorption of large molecular species and this advantage give the ability for SDAC to be used in the decolourization process and heavy metal removal. However, the highly cost of lignocellulosic material pre-treatment must be carried out before it can be used as activated carbon (adsorbent).

(b) Production of Xylose from Wood Sawdust (Lignocellulosic Material)

Wood sawdust biomass contains cellulose, hemicellulose and lignin as the major biopolymers. Hemicellulose is the easiest component to be converted. Xylose was produced from the hydrolysis of hemicellulosic fraction of lignocellulosic material. The hemicelluloses fraction of lignosellulosic material generally hydrolyzed by an acidic process, autohydrolysis or enzymatic process (Rafiqul et al., 2011). On hydrolysis, cellulose yield glucose and the non-cellulosic polysaccharides yields xylose, mannose, galactose and arabinose (Mussato et al., 2006). Further, xylose was used to produce xylitol which is sweetener in chewing gum and pastilles. In oreder to produce this specialty chemical, the high technology of pre-treatment and enzymatic reaction is needed to be performed; therefore this application of sawdust is costly and complicated.

Other than its application in the production of activated carbon and xylose, sawdust also can be used as an adsorbent in the immobilization of enzyme. It was proved by Mahmoud (2007) where he was used sawdust as an adsorbent to immobilize invertase enzyme. From his study, he found that immobilize invertase more stable at high pH and temperature and sawdust was a good adsorbent.

2.2 Fructosyltransferase (Ftase).

Fructosyltransferase (FTase) is an enzyme transforming sucrose into fructooligosaccharides (FOS). FOS have a great potential to improve the quality of many foods because they have functional properties, low caloric value, are not carcinogenic, and can be used for diabetic people (Mussato et al., 2009). Since this product is marketable, many researchers try to find out the solution for the production of this product which can help the industries to minimize their cost. As cited in Antosova et al., (2007), Yun (1996) stated that, Ftase can be found in many higher plants and microorganisms, but than, the most important industrial sources are strains of *Aspergillus niger*, *Aspergillus japonicas*, and *Auriobasidium pullulans.* Sunjay and Sugunan (2006) found the optimum pH and temperature for Ftase activity are at pH 6 and 60 ^{0}C respectively, while Tanriseven & Aslan (2005), stated that the optimum pH and temperature for Ftase activity are at 5.5-6.5 and 65^{0}C respectively. A higher purity and specific activity of the purified FTase preparation could be advantageous not only for laboratory scale experimentation but higher immobilization efficiency and volumetric activity of immobilized biocatalyst could be achieved as well. In this study, Ftase was used as an adsorbate which immobilize into sawdust (adsorbent).

2.3 Enzyme Immobilization

Enzymes which are intent or immobilize in a membrane support known as immobilize enzyme. This technique is used to selection the cells for the synthesis naturally with the specific enzyme (Ramaswami, 2009). There are three techniques used in the enzyme immobilization which are carrier binding (physical adsorption, covalent bonding and ionic bonding), cross linking and entrapment (occlusion within a cross linked gel and microencapsulation) (Guisan, 2008).

Enzymes are able to catalyze the most complex chemical processes under the mildest experimental and environmental conditions. Enzymes could be excellent industrial catalyst for a sustainable development. However, in general, enzymes do not fulfill the requirements for industry. Guisan (2008) stated that, enzymes are instable, they are soluble, they undergo inhibitions, and they may be poorly selective on non-natural substrates. In order to solve this problem, immobilization process was introduced. During the immobilization process, the enzymes are not present in the products, so no need of purification. The enzymes also are immediately available for reuse, so that, a continuous process can be carried out. The enzymes are able to process large amount of substrate since it can easily separate from the mixture of substrate and product (Kotwal & Shankar, 2009). The immobilization enzymes also more stable. The main goal of immobilization is the re-use of enzymes for many times (Tischer & Kascher., 1999). Sungur et al., (2006) stated that, the immobilized enzyme could be use for 20 times over a period of 2 months without considerable of loss activity.

2.3.1 Technique Used In the Enzyme Immobilization

There are three techniques which can be apply in the immobilization of enzyme which are carrier binding, cross linking and entrapment.

(1) Carrier Binding

(a) Adsorption

Adsorption (**Figure 2.2**) method is based on the adsorption of enzyme protein on the surface of water-insoluble carriers. There are some of the suitable adsorbents commonly used which are ion-exchange matrices, porous carbon, clay, hydrous metal oxides, glasses and polymeric aromatic resins. There is a bond between the enzyme and carrier molecule. The bond may be ionic, covalent or even combination of these. Immobilization can be brought about by coupling an enzyme either to external or internal surface of the carrier. The external surface binding method is advantageous as it does not involve conditions like pore diffusion (Amarjeet, 2011).

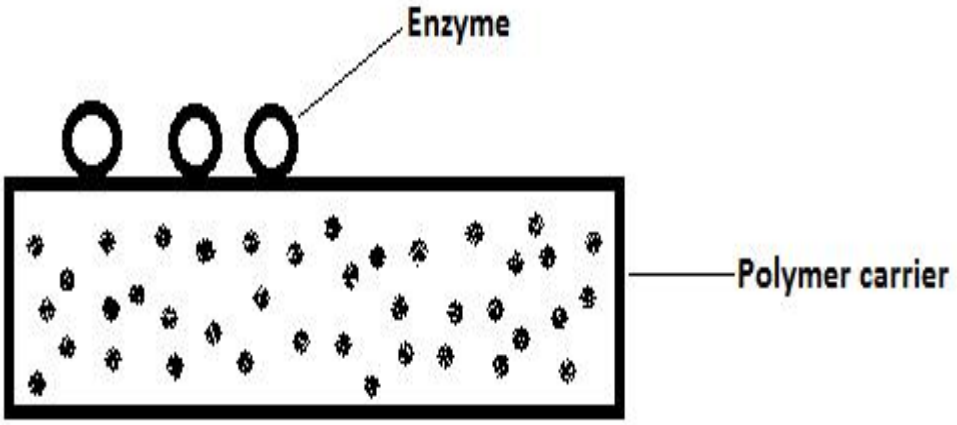


Figure 2.2 Adsorption of enzyme onto the carrier (Source: Guisan, 2008)

(b) Covalent Bonding

Covalent bond (**Figure 2.3**) is formed between the chemical groups of enzyme and chemical groups on surface of carrier. Covalent bonding is thus utilized under a broad range of pH, ionic strength and other variable conditions. Immobilization steps are attachment of coupling agent followed by an activation process, or attachment of a functional group and finally attachment of the enzyme. The most common technique is to activate a cellulose-based support with cyanogens bromide, which is then mixed with the enzyme. The protein functional groups which could be utilized in covalent coupling include amino group, carboxylic group, phenol ring, indole group and imidazole group (Khoshnevisan et al., 2011). However, this method has a weak point such as chemically modified the enzyme and many of the enzymes are denatured during immobilization, and also only small amount of enzyme will be immobilized.

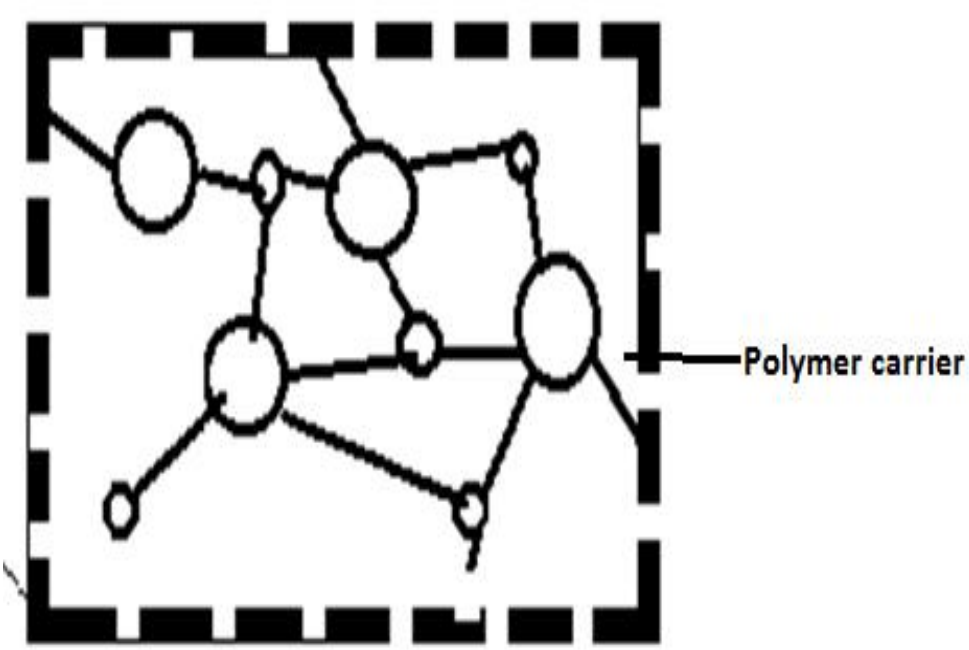


Figure 2.3 Covalent Bonding (Source: Guisan, 2008)

(2) Cross Linking

This method is based on the formation of covalent bonds between the enzyme molecules (**Figure 2.4**), by means of multifunctional reagents, leading to three dimensional cross linked aggregates. The most common reagent used for cross-linking are glutaraldehyde and diazonium salt. The demerit of using polyfunctional reagents is that they can denature the enzyme. This technique is cheap and simple but not often used with pure proteins because it produces very little of immobilized enzyme that has very high intrinsic activity and it is also cause a significant changes in the active site (Yeo et al., 2006).

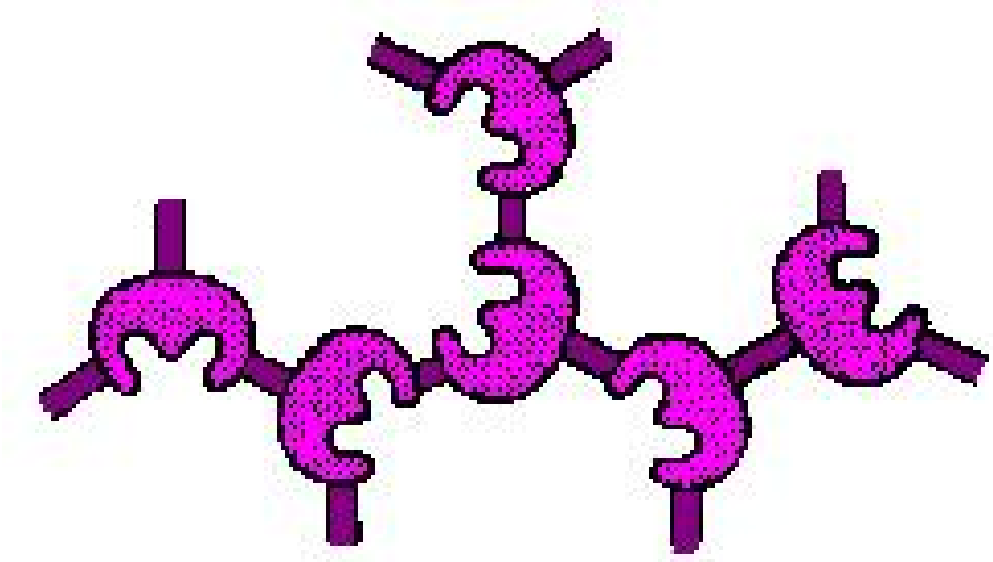


Figure 2.4 Cross-linking (Source: Biotech-environ)

(3) Entrapment

In entrapment, the enzymes or cells are not directly attached to the support surface, but simply trapped inside the polymer matrix. Entrapment is carried out by mixing the biocatalyst into a monomer solution, followed by polymerization initiated by a change in temperature or by a chemical reaction. Polymers like polyacrylamide, collagen, cellulose acetate, calcium alginate or carrageenan are used as the matrices (Amarjeet, 2011). This method has some disadvantages such as the enzyme can leak into the surrounding medium and substrate cannot diffuse deep into the gel matrix. **Figure 2.5** and **Figure 2.6** shows the enzyme entrapped in matrix and droplets, respectively.

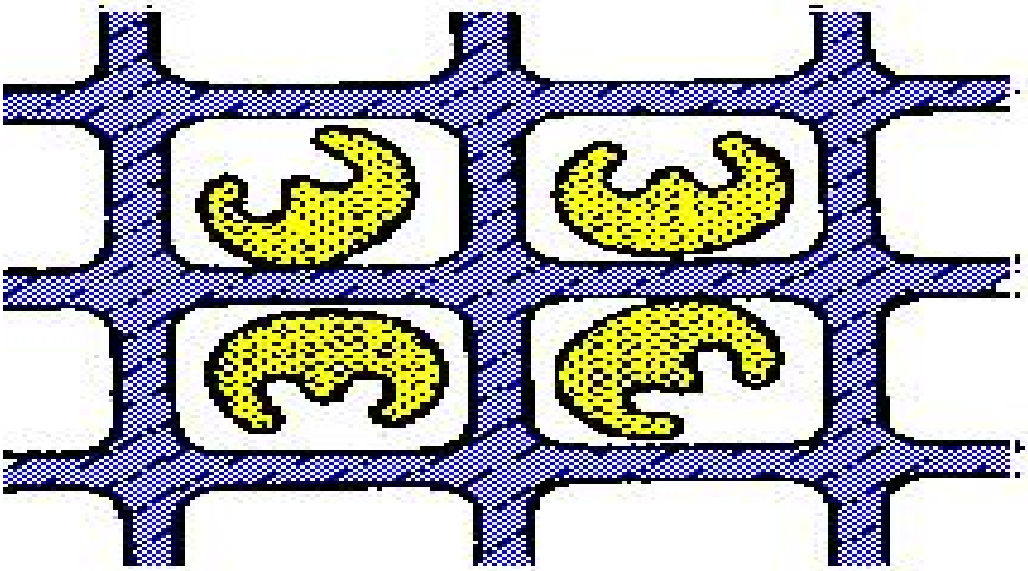


Figure 2.5 Enzyme entrapped in matrix (Source: Biotech-environ)

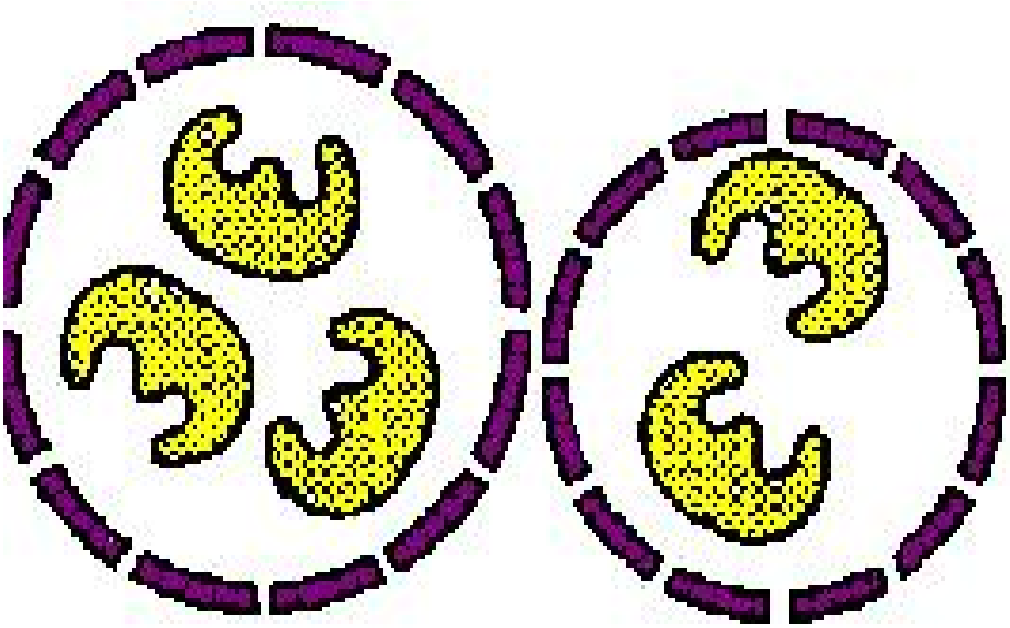


Figure 2.6 Enzyme entrapped in droplets (Source: Biotech-environ)

(a) Occlusion within a Cross Linked Gel

In this entrapment method, a highly cross-linked gel is formed as a result of the polymerization which has a fine “wire mesh” structure and can more effectively hold smaller enzymes in its cages. Amounts in excess of 1 g of enzyme per gram of gel or fibre may be entrapped. Some synthetic polymers such as polyarylamide and polyvinylalcohol have been used to immobilize enzymes using this technique. This technique usually not being choose since the substrate cannot diffuse deep into the gel matrix (Amarjeet, 2011). **Table 2.1** below shows the methods used by the other researchers for the immobilization of enzyme, respectively.

Table 2.1 The methods used in the previous study of enzyme immobilization.

METHOD	ADSORBENT	ADSORBATE	PARAMETERS	REFERENCES
Carrier-binding	Clay	Horseradish peroxidase	• Adsorption capacity • pH and temperature stability	Kim et al., 2012
	Silica aerogels	Rhizopus oryzae lipase (ROL)	• pH and temperature stability • Effect of organic solvent on enzyme stability	Kharrat et al., 2011
	Superparamagnetic nanoparticles	Cellulase	• pH and temperature stability • Binding efficiency	Khoshnevisan et al., 2011
Cross-linking	SBA-15	Candida rugosa lipase (CRL)	• Enzyme activity • Adsorption capacity • Effect of pore diameter	Gao et al., 2010

Table 2.1 Continued

METHOD	ADSORBENT	ADSORBATE	PARAMETERS	REFERENCES
	Poly(styrene-co-DVB) resin,	Microbial nuclease P1	• pH and temperature stability • Kinetic parameters (Km and Vmax)	Li et al., 2012
	Polypyrrole–polyvinyl sulphonate (Ppy–PVS) composite film	Glucose oxidase (GOD)	• Effect of pH • Kinetic parameters (Km	Gade et al., 2006
Entrapment	Sodium silicate sol–gel	Methanotrophic bacteria Methylomonas sp. strain GYJ3	• Effect of loading amount on the activity of cells • Effect of temperature and pH	Chen et al., 2004
	Acrylamide gel	Alkaline phosphatase	• Effect of pH and temperature on the enzyme activity • Activity of the free and immobilized enzyme • Measurement of the swelling ratio	Gonzalez & Pizzaro, 2001

2.4 Adsorption Study

Adsorption refers to the binding of molecules (adsorbate) on the surface of solid material (adsorbent). Adsorption can be used to separate a molecule from a complex mixture of molecules, or simply separate a solute from its solvent. This is achieved by contacting the solution with the solid material. The solute binding usually takes place on specific locations on the surface of the adsorbent (Ghosh, 2006).

2.4.1 Adsorbents

Adsorbent can be made from natural or synthetic material. Most adsorbents have amorphous or microcrystalline structure. These physical forms result in very high specific surface areas (Ghosh, 2006). Each adsorbent has their own uniqueness such as porosity, pore structure and nature of its adsorbing surfaces. Pore sizes in adsorbents may be distributed throughout the solid. Pore sizes are classified generally into 3 ranges: macropores have diameters in excess of 50 nm, mesopores have diameters in the range 2-50 nm, and micropores have diameters which are smaller than 2 nm (Cameron Carbon Incorporated, 2006). Most of the adsorbents used nowadays are non eco-friendly materials and it can cause harmful to environment. Natural materials as such sawdust is environmental friendly and inexpensive. It is also has a high ability to be used as an adsorbent in the adsorption process due to its structures. **Table 2.2** shows the types of adsorbents commonly used in industry and its application.

Table 2.2 Typical applications of commercial adsorbents

Adsorbent	Applications
Silica gel	Drying of gases, refrigerants, organic solvents, transformer oils Desiccant in packing and double glazing Dew point control of natural gas
Activated alumina	Drying of gases, organic solvents, transformer oils Removal of HCl from hydrogen Removal of fluorine in alkylation process
Carbons	Nitrogen from air Hydrogen from syngas Ethene from methane and hydrogen Vinyl chloride monomer (VCM) from air Removal of odours from gases Recovery of solvent vapours Removal of SOX and NOX Purification of helium Clean-up of nuclear off-gases Water purification
Zeolites	Oxygen from air Drying of gasses Removing water from azeotropes Sweetening sour gases and liquids Purification of hydrogen Separation of ammonia and hydrogen Recovery of carbon dioxide Separation of oxygen and argon Removal of acetylene, propane and butane from air Separation of xylenes and ethyl benzene Separation of normal from branched paraffins Separation of olefins and aromatics from paraffins Recovery of carbon monoxide from methane and hydrogen Drying of refrigerants and organic liquids Pollution control, including removal of Hg, NOX and SOX Recovery of fructose from corn syrup

Table 2.2 Continued

Adsorbent	Applications
Polymers & resins	Water purification Recovery and purification of steroids, amino acids Separation of fatty acids from water and toluene Separation of aromatics from aliphatics Recovery of proteins and enzymes Removal of colours from syrups Removal of organics from Hydrogen peroxide
Clay	Treatment of edible oils Removal of organic pigments Refining of mineral oils Removal of polychlorinated biphenyls (PCBs)

(Source: Bhatnagar & Minocha, 2006)

2.4.2 Pre-treatment of Adsorbent for the Preparation of Adsorption

Pre-treatment is very important to ensure that the adsorbent can give a good performance during the adsorption process. The adsorbent which undergo pre-treatment process will have a change in the surface morphology where the size of pore bigger than the untreated adsorbent. This is very important to make sure a highest loading capacity achieved during adsorption. There are two methods commonly used for the treatment of lignocellulosic material which are autohydrolysis and acid hydrolysis. The choices of methods are based on the focus of the study. Autohydrolysis is an alternative technology for the solubilization of hemicelluloses, with various advantages over the dilute acid hydrolysis. There is no chemicals used other than water during the treatment. Normally, autohydrolysis is

used as a pre-treatment without modifying the structure of cellulose and lignin (Rafiqul et al., 2011). Both of these two methods were done due to the desired to obtain hemicelluloses from the material used, but, in this study, distilled water was used as a medium since the purpose of doing the treatment is just to enlarge the size of pore without destroying the structure of the sawdust itself, and the treatment is based on the effect of temperature.

2.4.3 Unit Operation of Adsorption

Commonly, there are two types of unit operation used in the adsorption process which are packed bed and batch.

(a) Packed Bed Adsorption

Particulate adsorbent material is frequently used in the form of a porous packed bed within a cylindrical column. The column holds the adsorbent in place and facilitates its reuse. The feed solution is passed through the packed bed for selective adsorption of the target molecule. The feed is usually pumped into the packed bed but where flow-rate and pressure requirements are not very critical, flow due to gravity could also be utilized. The exit or effluent stream from the packed bed is usually monitored for the target molecules as well as the impurities (Ghosh, 2006). However, this system usually not being used in the industry due to the higher operation cost and long operation time (Sridhar, 1996)

(b) Batch Adsorption

During the study, batch adsorption process was applied. This system was choose due to its effectiveness for the adsorption process and requires less time for the operation compared to the packed bed system (Sridhar, 1996). In a batch adsorption process the feed solution and adsorbent particles (sawdust) are brought into contact until equilibrium binding is achieved. The spent solution (or raffinate) which also contains impurities is then separated from the adsorbent by using an appropriate solid-liquid separation technique such as centrifugation and filtration. The solid material obtained (the adsorbent with the bound enzyme) is frequently washed for complete removal of impurities. An analytical solution of batch adsorption is possible when the bound enzyme follows a linear adsorption isotherm (Ghosh, 2006). With Freundlich and Langmuir adsorption isotherm, graphical solutions are preferred. The general form of the isotherm, which is also called as equilibrium line is as Eq. 2.1 below (Ghosh, 2006).

$$C_B = \not{\emptyset} C_U \qquad \text{(Eq. 2.1)}$$

The solute material balance is of the form shown below:

$$C_{U0}S + C_{B0}A = C_US + C_BA \qquad \text{(Eq. 2.2)}$$

Where,

C_{U0} = initial solute concentration in the feed solution (e.g. kg/m^3)

C_{B0} = initial solute concentration in the adsorbent (e.g. kg/m^3)

C_B = solute bound per unit amount of adsorbent (e.g. kg/m^3)

C_U = unbound solute concentration (in solution) (e.g. kg/m^3)

S = quantity of feed solution (e.g. m^3)

A = quantity of adsorbent (e.g. kg)

The operating line was obtained by rearranging equation (2.2).

$$C_B = C_{B0} + \frac{S}{A}(C_{U0} - C_U) \qquad \text{(Eq. 2.3)}$$

The point of intersection of the equilibrium line and the operating line gives the equilibrium concentrations.

2.4.4 Adsorption Isotherm Models.

The equilibrium adsorption of Ftase enzyme onto sawdust was analyzed using Langmuir and Freundlich isotherms. **Table 2.3** below showed the isotherms used in the previous studies.

Table 2.3 List of previous studies using isotherm

Title	Isotherm	References
Enhancement of chromium (VI)removal by pre-treatments of cocolumber (*cocos nucifera*) sawdust: vacuum drying and plasma treatments	• Langmuir • Freundlich	Ting et al., 2010
Adsorption of dyes and phenol from water on resin adsorbents: effect of adsorbate size and pore size distribution	• Langmuir • Freundlich	Zhang et al., 2006
Production of activated carbon from sawdust using fluidized bed reactor	• Langmuir • Freundlich	Lam and Zakaria, 2008
Regeneration and reuse of spent NaOH – treated oil palm frond for copper and zinc removal from wastewater	• Langmuir • Freundlich	Salamatinia et al., 2010
Effects of pH on isotherms modeling for Cu(II) ions adsorption using maple wood sawdust	• Langmuir • Freundlich	Safiur and Rafiqul, 2009
Hexavalent chromium removal from aqueous solution by adsorption on treated sawdust	• Langmuir	Baral et al., 2006
Removal of malachite green from dye wastewater using neem sawdust by adsorption	• Langmuir	Khattri and Singh, 2009

(a) Langmuir Isotherm

Langmuir model is the simplest theoretical model for monolayer adsorption onto a surface with fixed number of identical sites. It is originally developed to represent chemisorptions on a set of distinctive, localized adsorption sites. Langmuir has developed a theoretical equilibrium isotherm concerning the amount of gas adsorbed on a surface due to the pressure of the gas (Ghosh, 2006). The equation is relevant to homogeneous adsorption where adsorption process has equal activation energy, based on the following basic assumptions (Abdullah et al., 2009):

(i) Molecules are adsorbed at a fixed number of well-defined localized sites.

(ii) Each site can hold one adsorbate molecule.

(iii) All sites are energetically equivalent.

(iv) There is no interaction between molecules adsorbed on neighbouring sites.

The linear Langmuir equation is as follows (Ghosh, 2006):

$$\frac{1}{C_B} = \frac{1}{C_{Bmax}} + \frac{k_l}{C_{Bmax}C_U} \qquad \text{(Eq. 2.4)}$$

C_B = solute bound per unit amount of adsorbent (e.g. kg/m^3)

C_U = unbound solute concentration (in solution) (e.g. kg/m^3)

C_{Bmax} = saturation constant (e.g. kg/m^3)

K_L = affinity constant for Langmuir isotherm (e.g. kg/m^3)

K_L and C_{Bmax} are the equilibrium constant of Langmuir equation. Plotting $\frac{1}{C_B}$ against $\frac{1}{C_U}$ yields a straight line with slope (Figure 2.7), $\frac{k_l}{C_{Bmax}}$ and intercept $\frac{1}{C_{Bmax}}$ indicates the monolayer saturation capacity Q_o. The shape of the isotherm can be determined whether the adsorption is favorable or not (Abdullah et al., 2009).

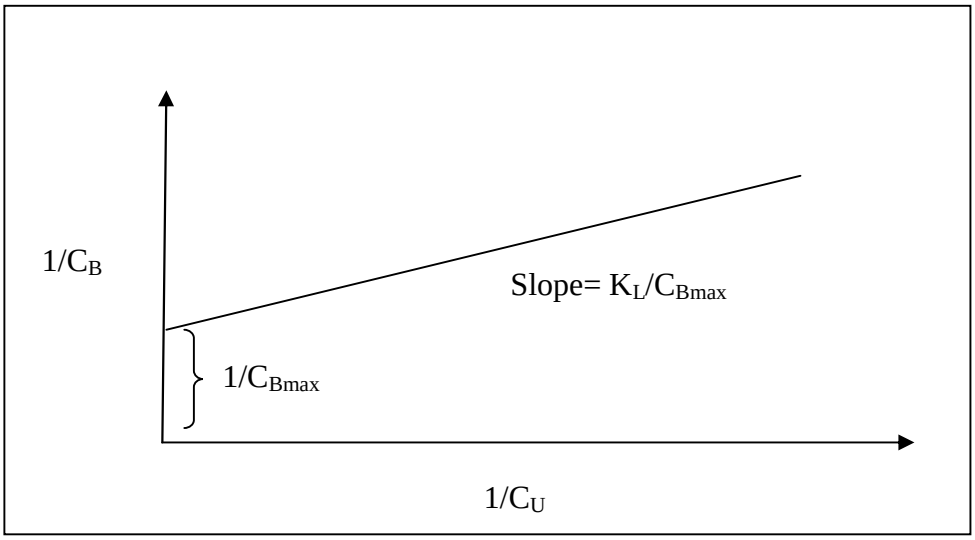


Figure 2.7 Determination of Langmuir Isotherm constant

(b) Freundlich Isotherm

Freundlich expression is an empirical equation applicable to non-ideal sorption on heterogeneous surface as well as multilayer sorption. The model is given as Eq. (2.5) below (Ghosh, 2006).

$C_B = K_f C_U^n$ (Eq. 2.5)

C_B = solute bound per unit amount of adsorbent (e.g. kg/m^3)

C_U = unbound solute concentration (in solution) (e.g. kg/m^3)

K_f = Freundlich adsorption constant

If the concentration of solute in the solution at equilibrium, C_U is raised to the power of n, with the amount of solute adsorbed being C_B, then C_U^n / C_B are constant at a given temperature. K_f indicates virtual indicator of adsorption capacity, while the dimensionless, n, is investigative of the energy or intensity of the reaction and propose the favorability and capacity of the adsorbent/adsorbate system. According to the theory, n > 1 represents favorable adsorption conditions. Eq. (2.6) is linearized into logarithmic form for data fitting and parameter evaluation as follows (Abdullah et al., 2009):

$$\log C_B = \log K_f + n \log C_U \qquad \text{(Eq. 2.6)}$$

By plotting log C_B versus log C_U (Figure 2.8), constant K_f and exponent n can be calculated.

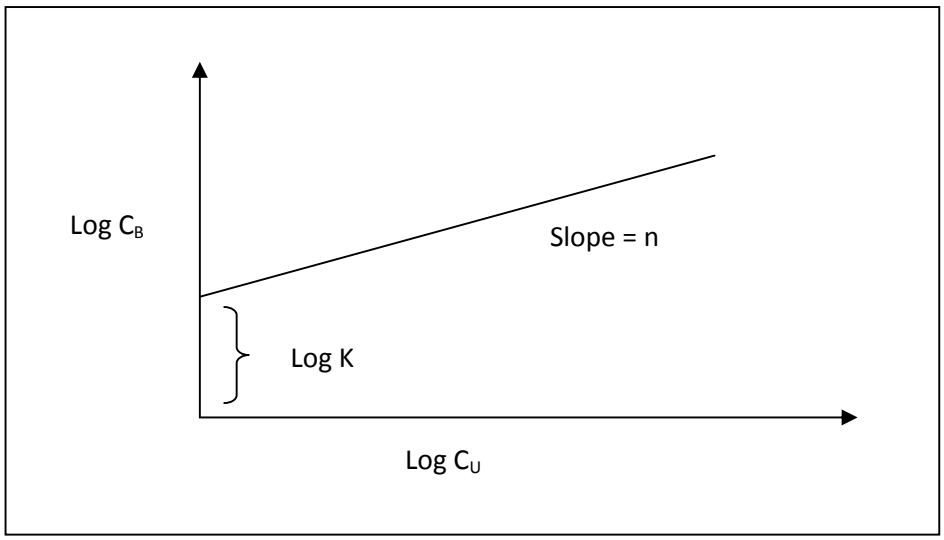


Figure 2.8 Determination of Freundlich Isotherm constant

2.4.5 The Advantages of Adsorption Method.

Adsorption is the adhesion of atoms, ions, biomolecules or molecules of gas, liquid, or dissolved solids to a surface. This process creates a film of the adsorbate (the molecules or atoms being accumulated) on the surface of the adsorbent. Many of the researchers are using this method in their study of immobilization process but than they are using a different types of adsorbent as such chelating resin (Lin et al., 2000), and gelatin (Tanriseven & Olcer, 2008). Although there are another method can be used in the immobilization process, adsorption is the one that always chosen by the researcher cause it is simple and cheap. Besides that, the adsorbed materials are not chemically altered.

2.4.6 The Previous Study of Sawdust by using the Adsorption Method.

Adsorbent are very important materials in the adsorption process. Typically, adsorbents are in the form of small pellets, beads, or granules ranging from about 0.45mm to 2mm in size (Genisheva et al., 2011). Nowadays, there are lots of adsorbents used in the industry but than mostly, it is expensive. The use of low-cost adsorbents has been investigated as a replacement for costly current methods. On 2006, Yasemin and Zeki were studied about removal of heavy metals from aqueous solution by sawdust adsorption. They are using the sawdust of walnut. From their studies, they concluded that sawdust of walnut could be a good adsorbent for the metal ions from aqueous solutions. From this finding, it is proved that the waste as sawdust also can be use as an adsorbent and it can replace another adsorbent used in industry which is high in cost. **Table 2.4** showed the usage of various species of sawdust in the adsorption study.

Table 2.4 Adsorption study by using various species of sawdust

Types of Sawdust	Topic Study	References
Coniferous tree species	Sawdust pellets from coniferous species as adsorbent for NO_2 removal	Pietrzak, 2009
Softwood–poplar, and coniferous softwood–fir	Modified softwood sawdust as adsorbent of heavy metal ions from water	Sciban et al., 2006
Beech sawdust	Simulation of methylene blue adsorption by salts-treated beech sawdust in batch and fixed-bed systems	Batzias & Sidiras, 2007
Neem sawdust (Azadirachta indica	Removal of malachite green from dye wastewater using neem sawdust by adsorption	Khattri & Singh, 2009
S. robusta (Sal)	Hexavalent chromium removal from aqueous solution by adsorption on treated sawdust	Baral et al., 2006
Pine sawdust	Carbonaceous adsorbents prepared by physical activation of pine sawdust and their application for removal of NO_2 in dry and wet conditions	Nowicki & Pietrzak, 2010

2.5 Analyses

Immobilize enzyme can be analyzed by qualitative and quantitative analysis. The qualitative analysis that can be used such as SEM, FTIR, NMR and XRS, while, the quantitative analysis that can be used such as HPLC and Uv-Vis Spectrometer.

2.5.1 Quantitative Analysis

(a) High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres (Clark, 2007). **Figure 2.9** showed the whole process for the HPLC.

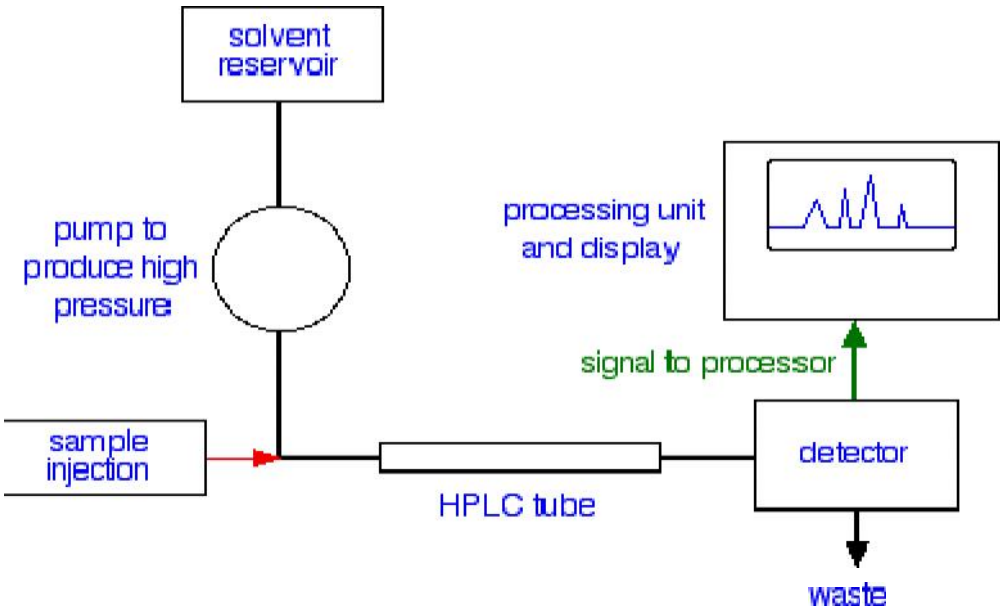


Figure 2.9 Flow scheme for HPLC (Source: Clark, 2007)

(1) Injection of the sample

Injection of the sample is entirely automated.

(2) Retention time

The time taken for a particular compound to travel through the column to the detector is known as its retention time. This time is measured from the time at which the sample is injected to the point at which the display shows a maximum peak height for that compound.

Different compounds have different retention times. For a particular compound, the retention time will vary depending on:

(a) The pressure used (because that affects the flow rate of the solvent)

(b) The nature of the stationary phase (not only what material it is made, but also particle size)

(c) The exact composition of the solvent

(d) The temperature of the column

(3) The detector

There are several ways of detecting when a substance has passed through the column. A common method which is easy to explain uses ultra-violet absorption. Many organic compounds absorb UV light of various wavelengths. The amount of light absorbed will depend on the amount of a particular compound that is passing through the beam at the time. **Figure 2.10** showed the UV detector.

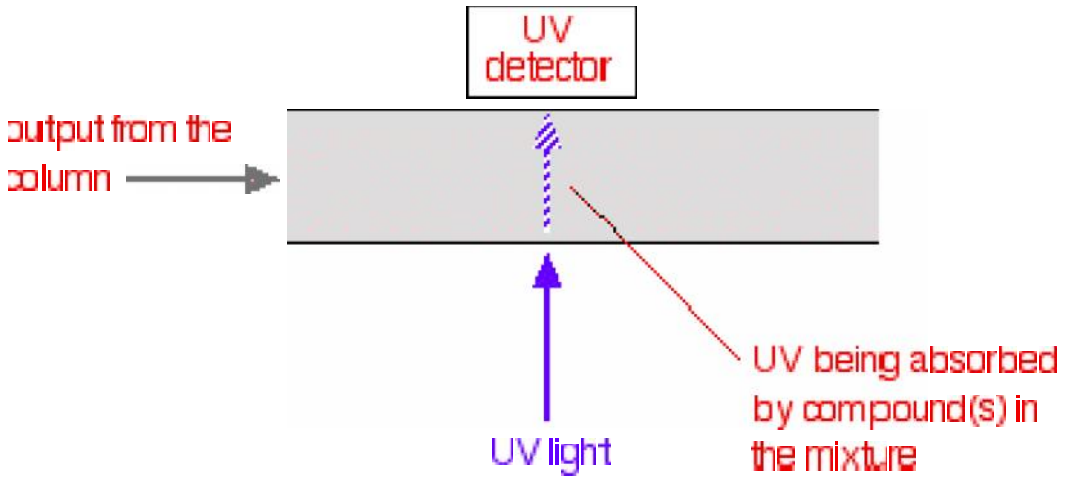


Figure 2.10 UV Detectors (Source: Clark, 2007)

However, HPLC have some disadvantages such as it is difficult to detect coelution (two compounds escaping from the tubing at once), which may lend to inaccurate compound categorization, there is a high cost for equipment needed to conduct HPLC, its operation can be complex and the equipment has low sensitivity to some compounds due to the speed of the process (Clark, 2007).

(b) UV-Vis Spectrometer

Ultraviolet (UV)-visible spectrometers are used to analyze the interactions between radiation and matter in the UV-visible region of the electromagnetic spectrum. The principle involved is the measurable absorption of energy by chemical compounds when certain electronic transitions are excited between their molecular orbitals (Agilent Technologies, 2000).

(1) Spectrometer Principle

A source of polychromatic light with a continuous UV spectrum is separated by a prism or diffraction grating into component UV wavelengths between 200 and 400 nm (nanometers) and visible wavelengths between 400 and 800 nm. Each wavelength is split into two equal-intensity beams by a half-mirrored device. The sample beam passes through a container of the compound being studied. A reference beam passes through an identical container of sample solvent (Agilent Technologies, 2000).

(2) Scanning and Detection

The spectrometer automatically scans all the component wavelengths passing through the containers. The intensity of each wavelength is measured by electronic detectors and compared. The reference beam intensities are subtracted to determine the absorption of energy by the sample at each wavelength (Agilent Technologies, 2000).

(3) Absorption Spectrum

The spectrometer records all the wavelengths at which absorption occurs together with the amount of absorption at each wavelength. The instrument then

creates a graph, called a spectrum, of absorbance versus wavelength (Agilent Technologies, 2000)

Sample analysis using UV-Vis is a very quick process compared to other methods of sample detection, such as HPLC. The UV-Vis technique also cause non-destructive to the sample and has a high sensitivity for detecting organic compounds (Agilent Technologies, 2000). In this study, UV-Vis spectrometer was used to analyze the samples during the experiment.

2.5.2 Qualitative Analysis

The structure of untreated, treated sawdust and also immobilize FTase can be analyzed by using scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR).

(1) Scanning Electron Microscope (SEM)

The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to create a diversity of signals at the surface of solid specimens. Electron-sample interactions derive the signals which expose information about external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample. Commonly, the data are collected over a selected area of the surface of the sample. Then, a 2-dimensional image is generated. It displayed spatial variations. Area comprising about 1 cm to 5 microns in width can imaging in scanning mode using a conventional SEM techniques

(magnification from 20X to about 30,000 X, the spatial resolution of 50 to 100 nm). SEM is also able to perform analysis on the sample location of the point selected; this approach is very useful in a qualitative or semi-quantitatively determines the chemical composition, crystal structure, and crystal orientation (Swapp, 2012). In this study, SEM was used in order to determine the surface morphology of the adsorbent before and after the treatment, and also to identify the accessible of enzyme in the pore of adsorbent after the immobilization occurred.

There is lots of advantages of a scanning electron microscope include its wide-array of applications, the detailed three-dimensional and topographical imaging and the versatile information garnered from different detectors. SEM is also easy to operate with the proper training and advances in computer technology and associated software make operation user-friendly. This instrument works fast, often completing SEI, BSE and EDS analyses in less than five minutes. In addition, the technological advances in modern SEMs allow for the generation of data in digital form (Anderson, 2010). SEM was chosen to analyze the structure of the adsorbents in this study due to the well performing system and high quality of image obtained.

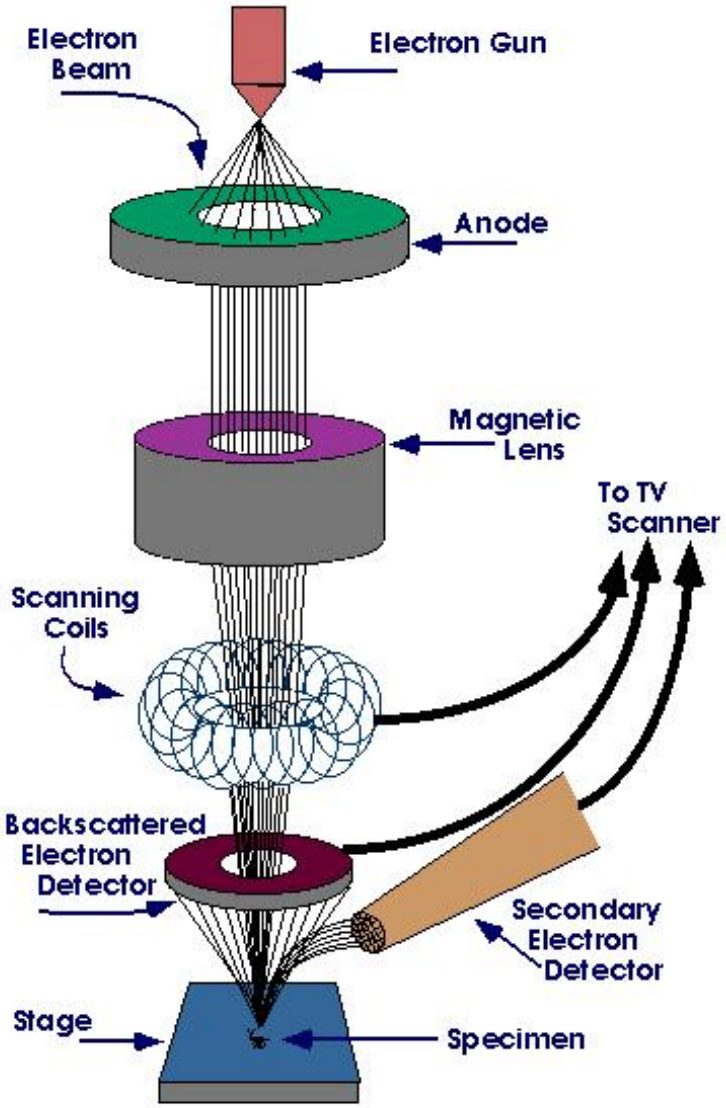


Figure 2.11 Principle of Scanning Electron Microscope
(Source: Swapp, 2012)

(2) Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectroscopy has become a workhorse technique for materials analysis in the laboratory. The infrared spectrum representing a fingerprint sample with absorption peaks suits the frequency of vibration of the atomic bonds making the material. Because each different material is a unique combination of atoms, no two compounds produce the exact same infrared spectrum. Therefore, infrared

spectroscopy can result in a positive identification (qualitative analysis) of every different kind of material. In addition, the size of the peak in the spectrum is a directly indication of the amount of material that are present. The normal instrumental process is as follows (Thermo Nicolet Corporation, 2001):

1. The Source: Infrared energy is emitted from a black body glowing source. The beam is through the aperture which controls amount of energy that presented to sample (and, eventually, the detector).

2. The Interferometer: The beam enters the interferometer where the "spectral encoding" takes place. The consequential interferogram signal then exits the interferometer.

3. The Sample: The beam enters the sample compartment where it is transmitted through or rejected off of the surface of the sample, depending on the type of analysis being consummate. This is where specific frequencies of energy, which are uniquely characteristic of the sample, are absorbed.

4. The Detector: The beam finally passes to the detector for final measurement. The detectors used are expressly designed to measure the special interferogram signal.

5. The Computer: The deliberate signal is digitized and sent to the computer where the Fourier transformation takes place. The final infrared spectrum is then presented to the user for interpretation and any further manipulation.

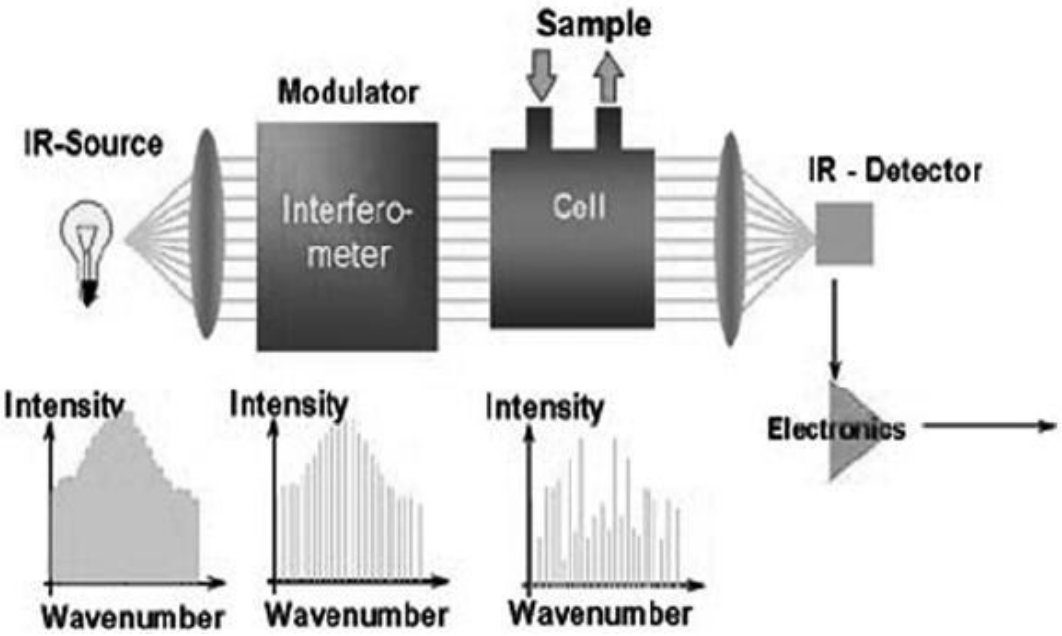


Figure 2.12 Principle of Fourier Transform Infrared Spectroscopy
(Source: Thermo Nicolet Corporation, 2001)

One of the primary advantages is that infrared spectroscopy causes no damage. Several other forms of mechanical sight can detect particles through other spectrum, but many of their methods use radiation. For example, X-ray technology requires precautions so that the radiation does not cause damage to people in the area. However, infrared radiation is harmless and would not damage the environment or the area being viewed. A major advantage of infrared spectroscopy is the samples being viewed do not require any sort of special preparation. Some test may require a subject to be bathed in radiation or have radioactive dye put on it, but infrared spectroscopy does not require that (Litherland, 2012). This equipment was used in this study in order to identify the functional groups occurred in the samples and to study the differences between the structures of untreated and treated sawdust.

(2) Nuclear Magnetic Resonance (NMR)

The NMR phenomenon is based on the fact that nuclei of atoms have magnetic properties that can be utilized to yield chemical information. Quantum mechanically subatomic particles (protons, neutrons and electrons) have spin. In some atoms (eg ^{12}C, ^{16}O and ^{32}S) these spins are paired and cancel each other out so that the nucleus of the atom has no overall spin. To determine the spin of a given nucleus one can use the following rules (Murali, 2010):

(a) If the number of neutrons and the number of protons are both even, the nucleus has no spin.

(b) If the number of neutrons plus the number of protons is odd, then the nucleus has a half-integer spin (i.e. 1/2, 3/2, 5/2).

(c) If the number of neutrons and the number of protons are both odd, then the nucleus has an integer sp

The instrumental process is as follow (Edwards, 2010):

1. Temperature, Tuning, Locking, Shimming

The sample for an NMR experiment should not contain any particulate matter that may affect the field homogeneity within the sample. After the sample is stopped in the magnet it is necessary to tune the probe to get the most effective power transferred to the sample, and the most effective detection of the signal. Tuning the probe involves altering the complex impedance of the coil to minimize the reflected power. (This needs to be performed once during installation). After the probe is tuned it is necessary to "lock" the spectrometer on the external LiCl sample.

2. Adjusting the Transmitter

The next step is set the frequency of the pulses and to adjust the sweep (or spectral width). In general, spectrometer frequencies are specified using two parameters, a fixed number that depends on the magnetic field strength of the instrument and the observed nuclei, and a user adjustable offset that is added to the fixed number to give the frequency of the transmitted pulse. At the beginning the user defined offset is set to zero and a very large spectral width is used (e.g. 20 KHz). With such a wide window all of the resonance lines should fall in this frequency range. Since the time required for the 90^o pulse is not yet known, the first spectrum is obtained using a short (6 μs) pulse. Once the position of the resonance peaks has been determined, the offset is moved to the middle of the spectrum and the spectral width is adjusted to be just large enough to span all of the resonance lines in the spectrum. The 90^o pulse length is determined by observing the effect of the pulse length on the spectral intensity or on the FID signal intensity (RMS – root mean signal). The spectrometer frequency is set to be 300-500 Hz from the resonance frequency of the main resonances in the spectrum. The pulse length is set to 4 μs (e.g. $<< 90^o$) and a spectrum is obtained. The spectrum is phased to produce a positive absorption line. The pulse length is increased until the line goes through a maximum value of RMS or signal intensity (90^o) and then reaches zero (180^o). The pulse length is then doubled to produce a 360^o pulse. The pulse length is slightly modified to produce a null signal. Final adjustment of the pulse length using 360o pulses avoids the necessity of waiting for the spins to relax.

3. Shim System

The shim system consists of a number of small coils that surround the area of the sample, which is contained within the most homogeneous region of the magnet. The purpose of the shim coils is to provide a means to make slight adjustments to the static magnetic field to increase its homogeneity. There can be a large number of coils, each of which generates a small magnetic field, which is shaped like a spherical harmonic. Since these functions are orthogonal the field generated by each shim coil is, in theory, independent - 14 - of the fields generated by the other shim coils. However, in practice there can be considerable interaction and it is usually necessary to adjust several coils at the same time. All magnets show drift or a change in field strength, over time. These changes are usually small enough that they can be compensated by adjusting the ^{1}H transmitter frequency to match the magnetic field / shim changes.

4. The Lock

Changes in the magnetic field strength are detected by measuring the resonance position of Li^{7} in the external lock reference. Both the absorptive and dispersive components of the Li^{7} resonance line are used in adjusting for field inhomogeneity. Based on the absolute frequency of the Li^{7} resonance the transmitter frequency is adjusted to compensate for changes in magnet, sample, or shim.

5. Generating the Pulse

The actual frequency of the RF pulse is generated by mixing two frequencies together. The first, ωsyn is adjustable and is generated from the frequency synthesizer under computer control. The second is an internal constant frequency called the intermediate frequency, IF. After mixing of these two frequencies and amplification the signal is sent to the probe. Since the same sample coil is used to both send the RF pulse and receive the FID it is necessary to route the RF pulse to the coil and not to the pre-amplifier. Otherwise the rather intense RF power may damage sensitive components in the preamplifier. This routing is accomplished by grounding the circuit (using diodes) 1/4λ from the junction point. Under these conditions the pre-amplifier side of the circuit appears as an infinite resistance and most of the RF pulse goes to the sample coil.

6. Receiving the NMR Signal

The induced transverse magnetization is detected using the same coil that carried the RF pulse to the sample. However, since the induced EMF in the coil is quite weak, the grounded point in the pre-amplifier is not seen as a ground and the signal can pass the 1/4λ coil. The frequency of the induced RF is quite high (e.g. 58 MHz). This is an extremely fast rate (i.e. 58 MHz) and there are practical problems associated with operating analog to digital converters at this frequency. Instead of trying to sample the magnetization at 58 MHz, the frequency of the signal is reduced to that in the rotating frame (audio range) by mixing the signal first with ωsyn and then with the IF.

However, this instrument is very costly for the operation and it is also consume long time to interpret the spectra. Due to their disadvantages, this instrument was not chosen in this study.

CHAPTER 3

METHODOLOGY

3.1 Introduction

In chapter 3, it discusses about the methodology with a specific focus on the immobilization of Ftase into sawdust by using adsorption method. The experiment was conducted in a sequence as below:

Phase 1: Preparation of raw material

Phase 2: Pre-treatment process

Phase 3: Immobilization enzyme by using adsorption method

Phase 4: Glucose analysis

Phase 5: Characterization of immobilize enzyme

3.2 Materials Used In This Study

The FTase enzyme was obtained commercially from Sigma and used as adsorbate. The sawdusts of *Keruing, Kempas* and *Meranti* were taken from Seng Peng Sawmill, Gambang and were used as adsorbent.

Phase 1: Preparation of raw material

The sawdust taken from sawmills need to be dry first and undergo grinding by using grinder model ZM 200 and sieving process by using sieve shaker (Analysette 3 Pro, Fritsch, Germany) in order to obtain the desired size which is 0.4µm-0.63µm (Mussato et al., 2009).

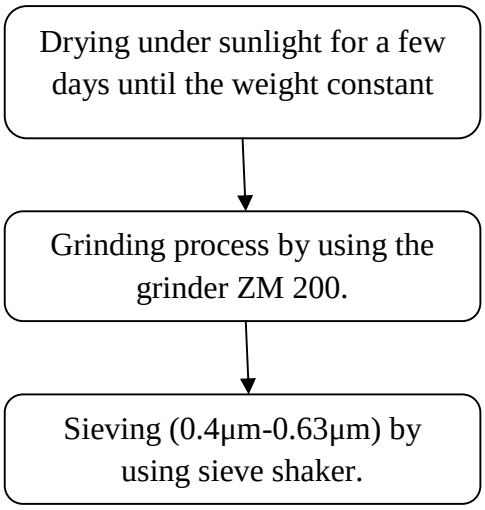


Figure 3.1 Process flow for the preparation of sawdust

3.3 Preparation of raw material

3.3.1 Drying process

Primarily, the sawdust as shown in **Figure 3.2** was drying under sunlight for a few days until get the constant weight. Before that, the weight of sawdust was measured by using weighing balance in the FKKSA lab. To ensure that it was fully dried, the moist concentration was measured for three times until the weight constant. The moist concentration was measured by using equation 3.1 (Reeb & Milota, 1999).

$$\text{Moist concentration (\%)} = \frac{M_{initial} - M_{dried}}{M_{initial}} \times 100\% \qquad \text{(Eq. 3.1)}$$

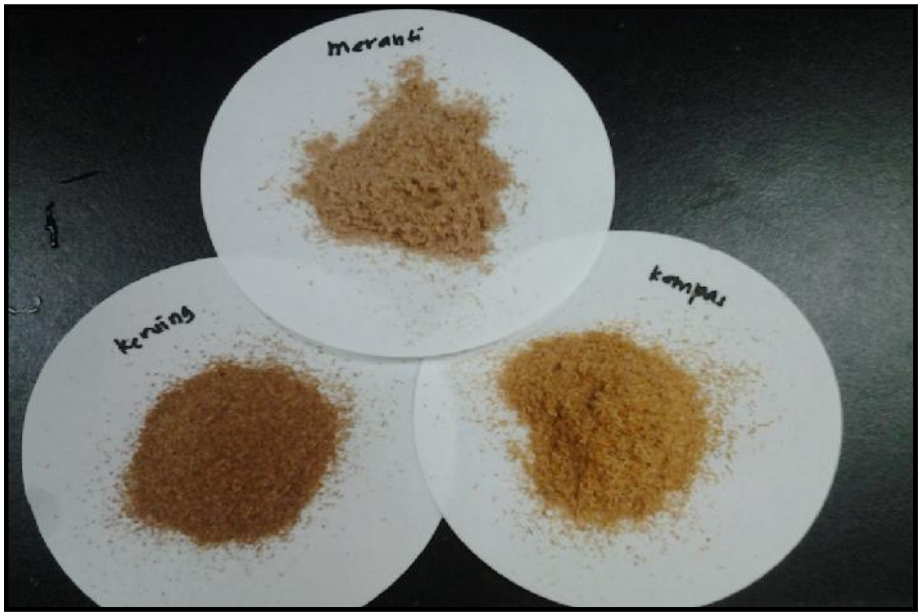


Figure 3.2 Different types of sawdust

3.3.2 Adsorbent Sizing

In order to obtain the desired size, the sawdust was first being grinding by using the grinder model ZM 200 (**Figure 3.3**). Then, the sieving process was done by using sieve shaker (Analysette 3 Pro, Fritsch, Germany) as showed in **Figure 3.4** in order to get particle size in range of 0.4µm-0.63µm. **Figure 3.5** and **Figure 3.6** below shows the sawdust before and after the grinding process.

Figure 3.3 Grinder model ZM 200

Figure 3.4 Sieve shaker machine (Analysette 3 Pro, Fritsch, Germany)

Figure 3.5 Sawdust before grind

Figure 3.6 The sawdust after grind

Figure 3.7 The sawdust after sieved

Phase 2: Pre-treatment process

Pre-treatment process was done in order to ensure that the size of pore of the sawdust will enlarge for the preparation of adsorption process (Mussato et al., 2009).

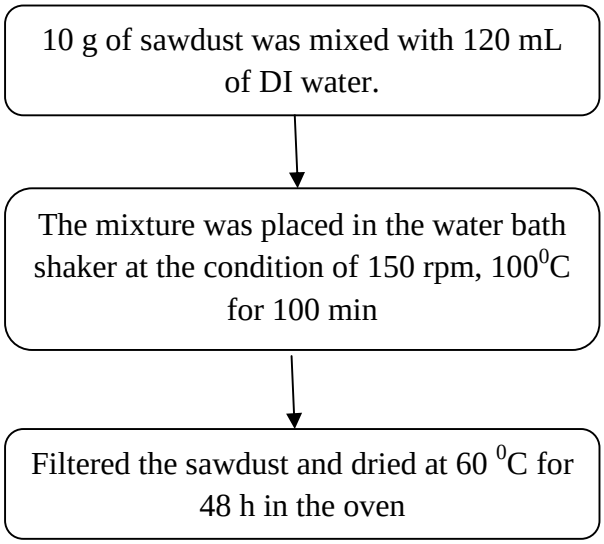


Figure 3.8 The process flow for the pre-treatment of sawdust

3.4 Pre-treatment of Sawdust

The adsorbent was undergoing the pre-treatment process in order to open and enlarge the pore size for the preparation of adsorption process. Distilled water was used as a medium due to its characteristic which not destroying the structure of the sawdust itself. 10 g of sawdust was mixed with 120 ml of distilled water in an Erlenmyer flask. Then, the mixture was placed in the water bath shaker at 150 rpm, 100^{0}C for 100 min. After finished the treatment process (**Figure 3.9**), the sawdust was then being filtered by Whatman no. 2 filter paper, (**Figure 3.10** and **Figure 3.11**)

and dried again at 60 ^{0}C for 48 h in the oven as shown in **Figure 3.12** (Mussato et al., 2009).

Figure 3.9 The mixture before filter

Figure 3.10 Filtration process

Figure 3.11 The sawdust after filtered

Figure 3.12 The sawdust in the oven for drying process at 60 ^{o}C

Phase 3: Immobilization of enzyme by using adsorption method

The immobilization of enzyme was done by using adsorption method. There are two parameters were focused in this study which are equilibrium time and adsorption isotherm.

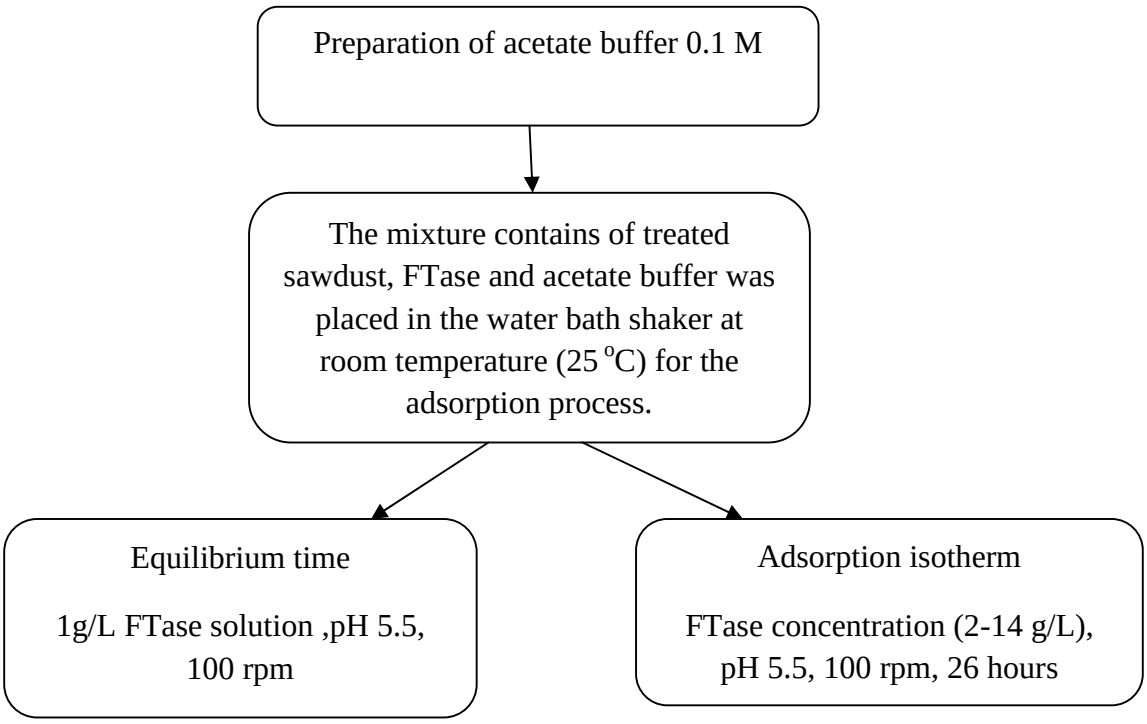


Figure 3.13 The process flow for the adsorption study

3.5 Immobilization of FTase onto Sawdust

3.5.1 Preparation of sodium acetate buffer, 0.1 M

(a) Solution A: 11.55 mL glacial acetic acid per litre (0.1 M)

(b) Solution B: 16.4 g sodium acetate per litre (0.1 M)

Solution A and B were mixed by following the indicated volumes as shown in Table 3.1, then it was diluted with distilled water to a total volume of 100 mL. Solution B was added drop by drop until reached the desired pH 5.5. The pH value was measured by using pH meter as shown in **Figure 3.14**.

Table 3.1 Preparation of buffer solution

Desired pH	Solution A(mL)	Solution B (mL)
4.0	41.0	9.0
5.0	14.8	35.2

Figure 3.14 Preparation of acetate buffer

3.5.2 Equilibrium Time

Experiments to determine the time required for the adsorption of FTase reach equilibrium was performed by batch technique in room temperature (25 ^{o}C). 20 ml of 1 g/L FTase solution in 100 ml glass flask was used. Each of the flasks was filled with 100 ml of acetate buffer pH 5.5. Then, 5 g of adsorbent, which is treated sawdust, was loaded into the solutions. The conical flask were then agitated in incubator shaker KS 4000i as shown in **Figure 3.15** at 100 rpm and the liquid samples was taking out at a given time intervals for analyses. The concentration of FTase was being analyzed by using UV-Vis Spectrophotometer. (Azlina et al., 2010).

Figure 3.15 The Mixture Being Shaken at 100 rpm in The Incubator Shaker

3.5.3 Adsorption Isotherm

The adsorption isotherm study was conducted through batch adsorption of FTase at various initial concentrations. Various initial enzyme concentrations were prepared by serial dilution in the range of (2-14 g/L) of stock solution of FTase. Then, 3 mL from each concentration was taken and was mixed with 1 g of treated sawdust and 5 mL of acetate buffer as shown in Figure 3.16 and Figure 3.17. The reaction was occurred in the water bath shaker (100 rpm) (**Figure 3.18** and **3.19**) at room temperature for 26 hours. The adsorption isotherm studies were performs on sawdust with maximum adsorption capacity and at the optimum pH. Langmuir and Freundlich isotherms adsorption models are employee to fit the adsorption characteristics (Azlina et al., 2010).

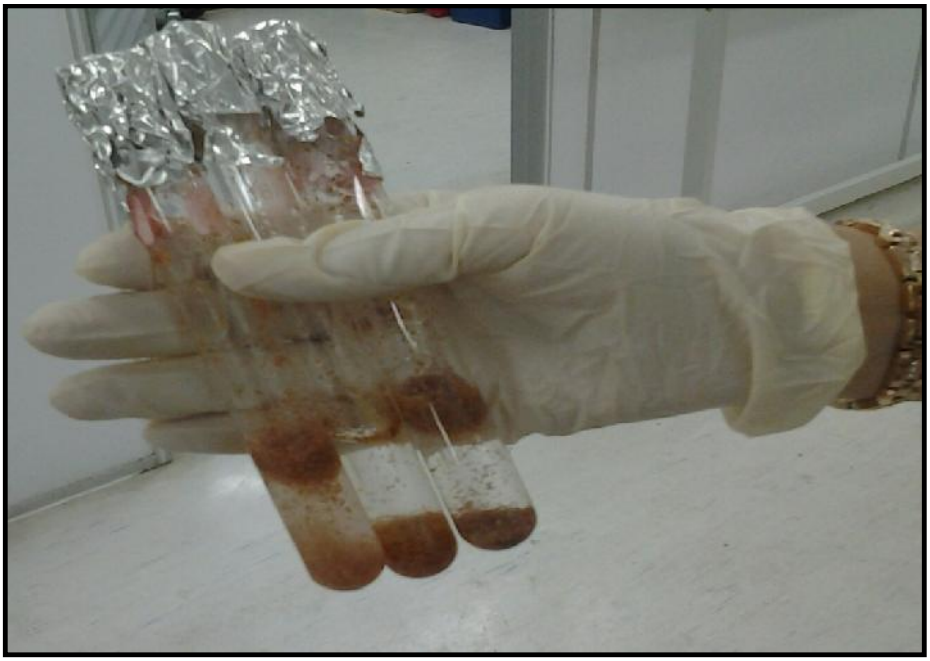


Figure 3.16 Prepared Samples for Immobilization Process

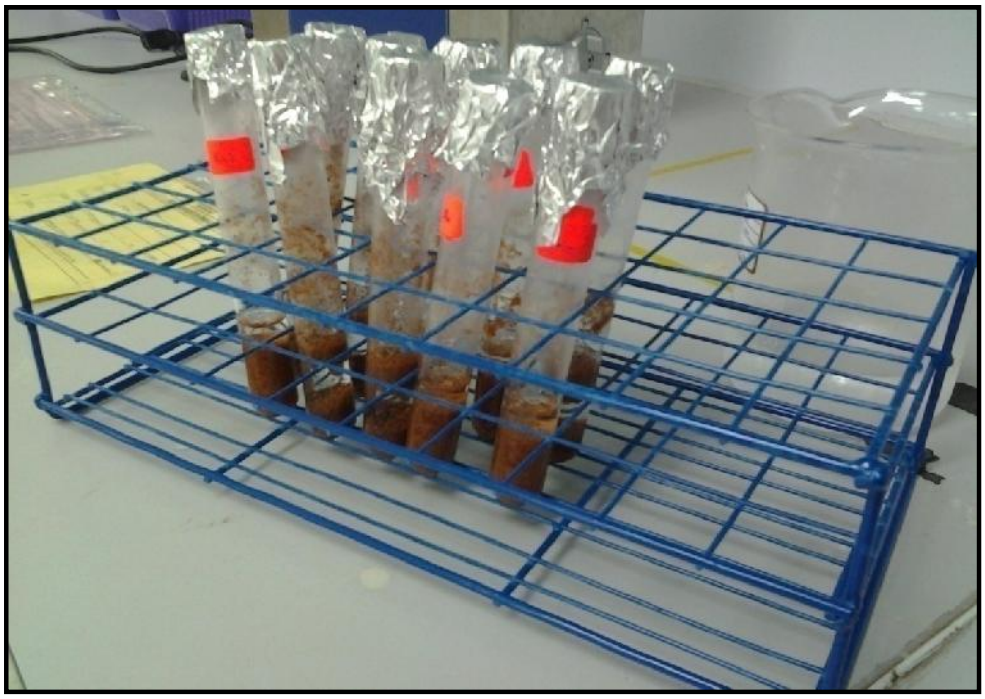


Figure 3.17 Prepared Samples with Various Enzyme Concentrations

Figure 3.18 Shaking water bath (Model U 1800 Spectrometer)

Figure 3.19 Prepared Samples Being Shaken at 25 ^{o}C in the Water Bath Shaker

Adsorption Isotherm Analysis

(a) Langmuir Isotherm

Langmuir isotherm equation has been widely applied to describe experimental adsorption data based on the assumptions that adsorption energy is constant and independent of surface coverage, and the maximum adsorption occurs when the surface is covered by monolayer adsorbate. The linear Langmuir isotherm is given by the following equation (Ghosh, 2006):

$$\frac{1}{C_B} = \frac{1}{C_{Bmax}} + \frac{k_l}{C_{Bmax}C_U} \qquad \text{(Eq. 3.2)}$$

C_B = solute bound per unit amount of adsorbent (e.g. kg/m^3)

C_U = unbound solute concentration (in solution) (e.g. kg/m^3)

C_{Bmax} = saturation constant (e.g. kg/m^3)

K_L = affinity constant for Langmuir isotherm (e.g. kg/m^3)

(b) Freundlich Isotherm

Freundlich isotherm is the earliest known relationship describing the sorption equation. This empirical isotherm can be used for non-ideal sorption that involves heterogeneous surface energy system and is expressed by the following equation (Ghosh, 2006):

$$\log C_B = \log K_f + n \log C_U \qquad \text{(Eq. 3.3)}$$

C_B = solute bound per unit amount of adsorbent (e.g. kg/m^3)

C_U = unbound solute concentration (in solution) (e.g. kg/m^3)

K_f = Freundlich adsorption constant

Phase 4: Glucose analysis

Glucose analysis was done by using DNS method and all the samples were analyzed by using UV-Vis Spectrometer.

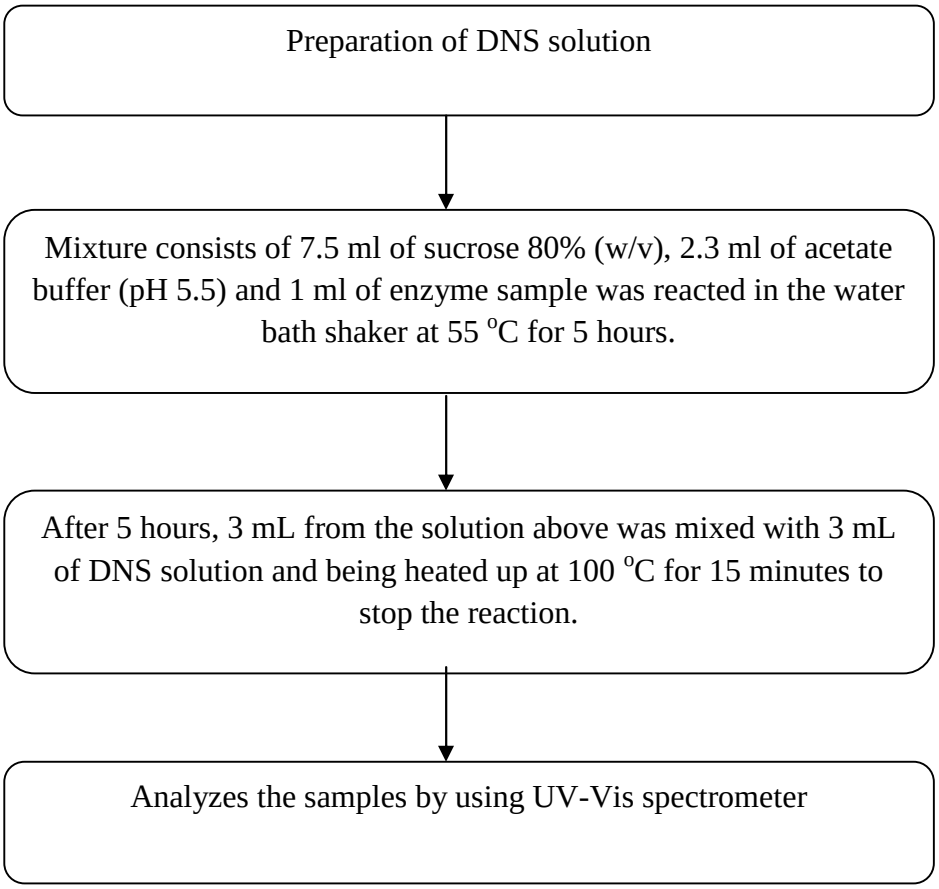


Figure 3.20 The process flow for glucose analysis by using DNS method.

3.6 Enzyme Assay by using DNS method

DNS solution was used to detect the concentration of glucose. To prepared 1% of the DNS solution, 10 g of dinitrosalisylic acid, 2 g of phenol, 0.5 g of sodium sulphite, and 10 g of sodium hydroxide were mixed with 1L of deionised water. One unit (IU) was defined as the amount of enzyme activity required to produce 1µmol of FOS/min under the following conditions: (a) pH 5.5, (b) temperature 55 ^{o}C, (c) reaction time 5 hour, (d) reaction mixture consisting of the following composition: 7.5 ml of sucrose 80% (w/v), 2.3 ml of acetate buffer (pH 5.5) and 1 ml of enzyme sample. After the reaction, 3 ml of DNS solution (1%) was added into 3 ml of the solution above. The solution was heated up at 100 ^{o}C for 15 min to stop the reaction. The glucose released from enzyme reaction was measured by using UV-Vis Spectrophotometer (Park et al., 2003). Equation (3.4) is the calculation method of Ftase activity (Khoshnevisan et al., 2011).

$$A = \frac{\%OD_{\,decrease} \times Y_p \times D_{f \times 10^3}}{MW \times t_i} \qquad \text{(Eq. 3.4)}$$

A = Enzyme activity (U/ml or µmol/ml) of unknown sample

$\%\ OD_{decrease} = \frac{OD_{\,decrease} - OD_{\,sample}}{OD_{\,control}} \times 100\ \%$

Y_p = mg of FOS equivalent to 100% $OD_{decrease}$ of the standard curve

D_f = dilution factor

t_i = incubation time

MW = molecular weight of FOS, 180.1559 g/mol

Figure 3.21 Positioning the Cuvette in the UV-Vis Spectrometer

Phase 5: Characterization of Immobilize Enzyme

The characters of immobilized enzyme were observed by using two methods which are scanning electron micrograph (SEM) and Fourier Transformed Infra Red (FTIR).

3.7 Characterization of Immobilize Enzyme

3.7.1 Scanning Electron Microscop (SEM) Analysis

The samples raw sawdust, pretreated sawdust, and sawdust, where is reacted with Ftase, was put on the stage of SEM (EVO 50) called STUV. A little amount of the samples attached on the STUV by using carbon tape and blower. It is then be irradiated with platinum in the Autofine Coater to make it conducive as preparation for the electron scanning to avoid charging to obtain three-dimensional images (Singh et al., 2011).

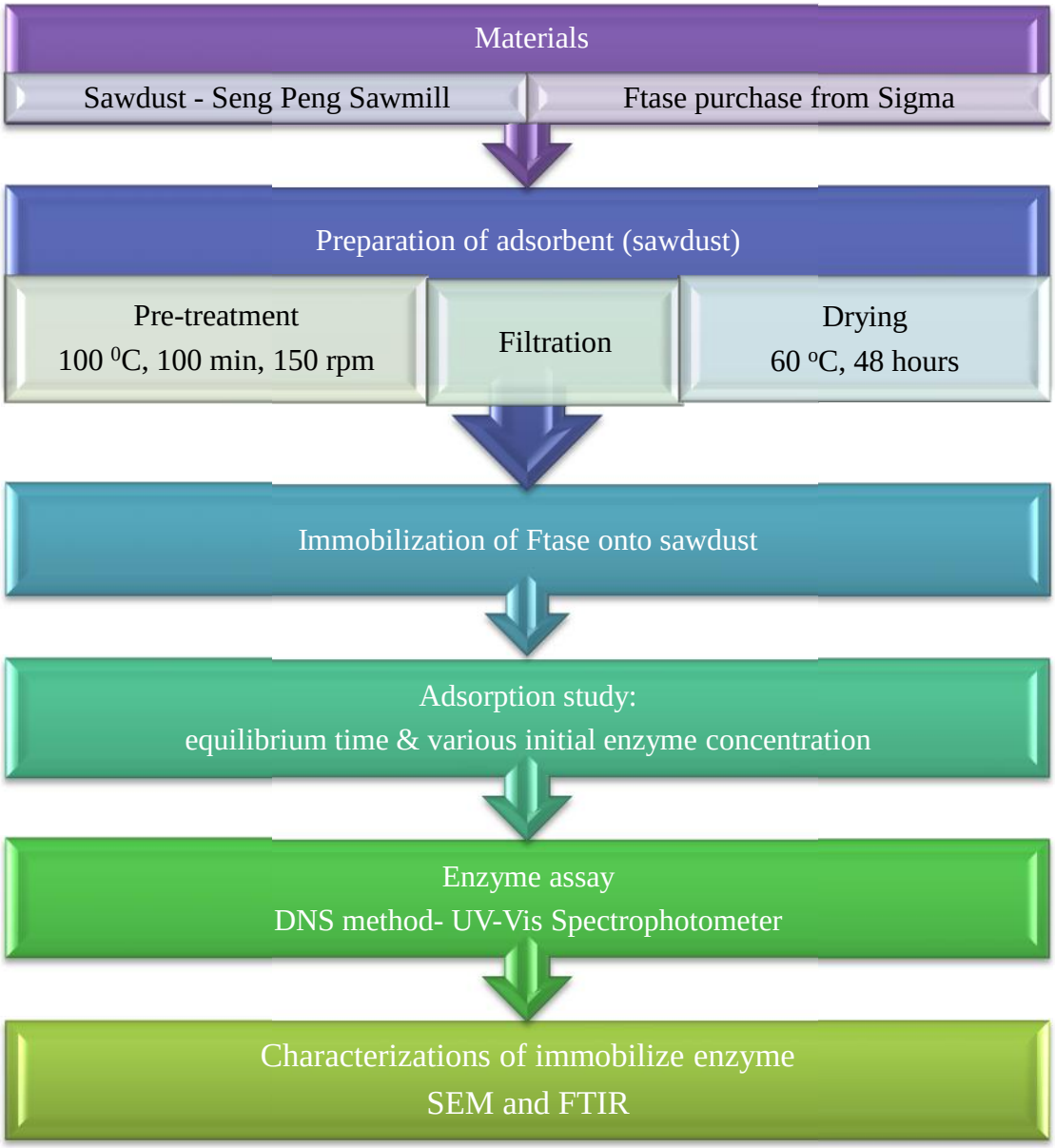
Figure 3.22 Scanning Electron Microscop (SEM)

3.7.2 Spectral Analysis

The final character that has been studied is the functional group of Ftase. It has been determined using Fourier Transformed Infra Red (FTIR). System 2000 FTIR from Perkin Elmer, USA (**Figure 3.22**) has been used in the analysis. The film casting technique on sodium chloride (NaCl) disks was adopted in this study. The samples were prepared in dimethyl formamide (DMF). The concentration was maintained around 0.5%. The film thickness is not so critical in this type of analysis; however, too thick or too thin a film may generate bad quality spectra with poor resolution. To avoid this type of a problem, a lead spacer of 0.01 mm was placed on the NaCl disk. The FTIR spectrum was collected after background correction. The peak intensity was measured from the adsorbance value after base line correction (valley to valley). The corrected height in absorbance units was noted (Singh et al., 2011).

Figure 3.23 Analyses of Samples by Using FTIR

3.8 Flowchart of Adsorption Study of FTase Immobilization Using Various Adsorbent



CHAPTER 4

RESULT AND DISCUSSION

4.1 Introduction

In this chapter, result obtained from the experiment will be discussed. The experiment was performed to investigate the species of sawdust that has the most excellent adsorption capability, to find out the effect of various initial enzyme concentrations on the adsorption process and to obtain the time required for the adsorption of FTase to reach equilibrium. In order to achieve the objectives, the qualitative analysis (SEM and FTIR) and quantitative analysis (UV-Vis Spectrometer) were done.

4.2 Pre-treatment Process

As mentioned in the previous chapter, the purposed of doing the pre-treatment is to enlarge the pores of the adsorbent for the preparation of adsorption. By using deionised water, the sawdust from three species which are *Keruing*,

Meranti and *Kempas* was boiled for 10 minutes in the water bath shaker at 100 rpm. The surface morphologies of the sawdust before and after the treatment were observed by using scanning electron micrograph (SEM). **Table 4.1** shows the size of pores after the treatment. **Figure 4.1 (a)** and **(b)** shows the SEM photograph of untreated and treated *Kempas* at 500x magnifications, respectively. **Figure 4.2 (a)** and **(b)** shows the SEM photograph of untreated and treated *Keruing* at 500x magnifications, respectively. **Figure 4.3 (a)** and **(b)** shows the SEM photograph of untreated and treated *Meranti* at 500x magnifications, respectively.

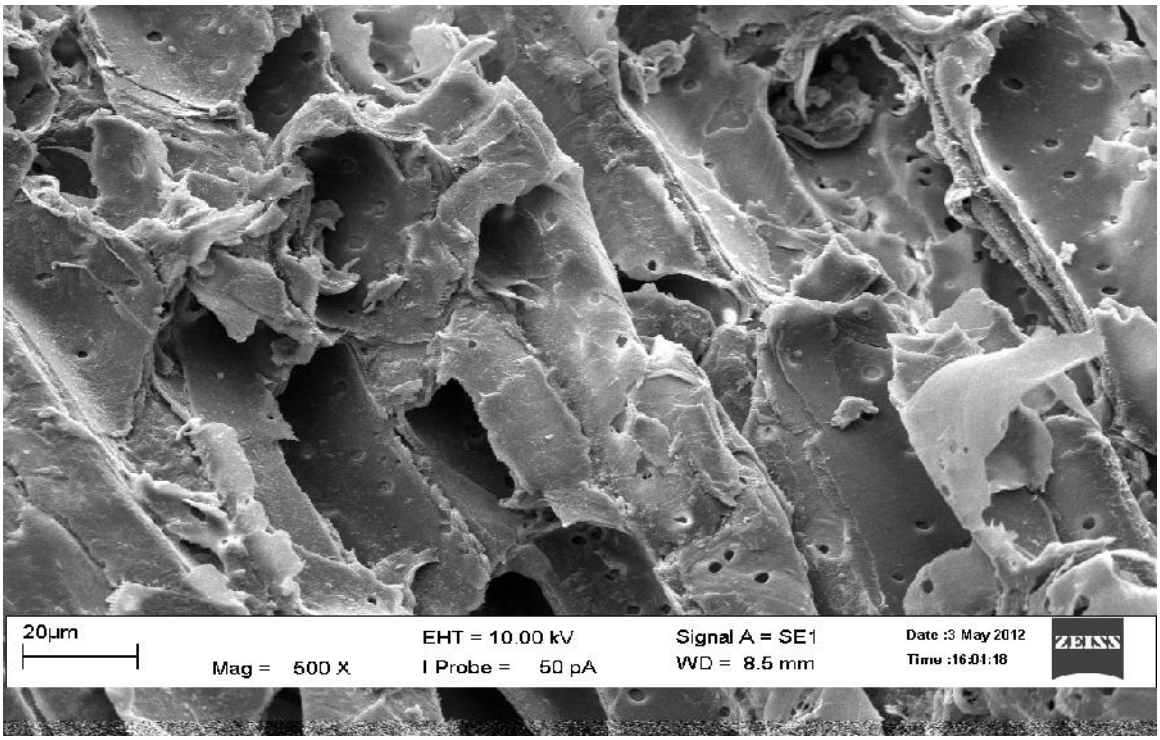


Figure 4.1 (a) Untreated *Kempas*

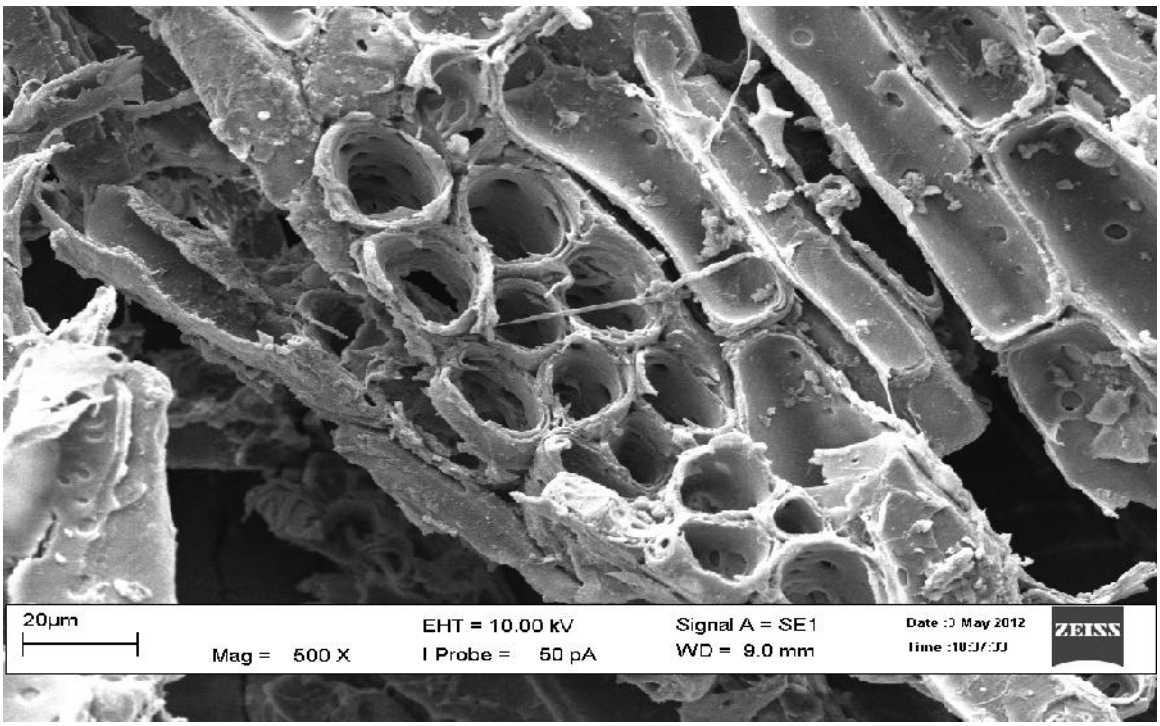


Figure 4.1 (b) Treated *Kempas*

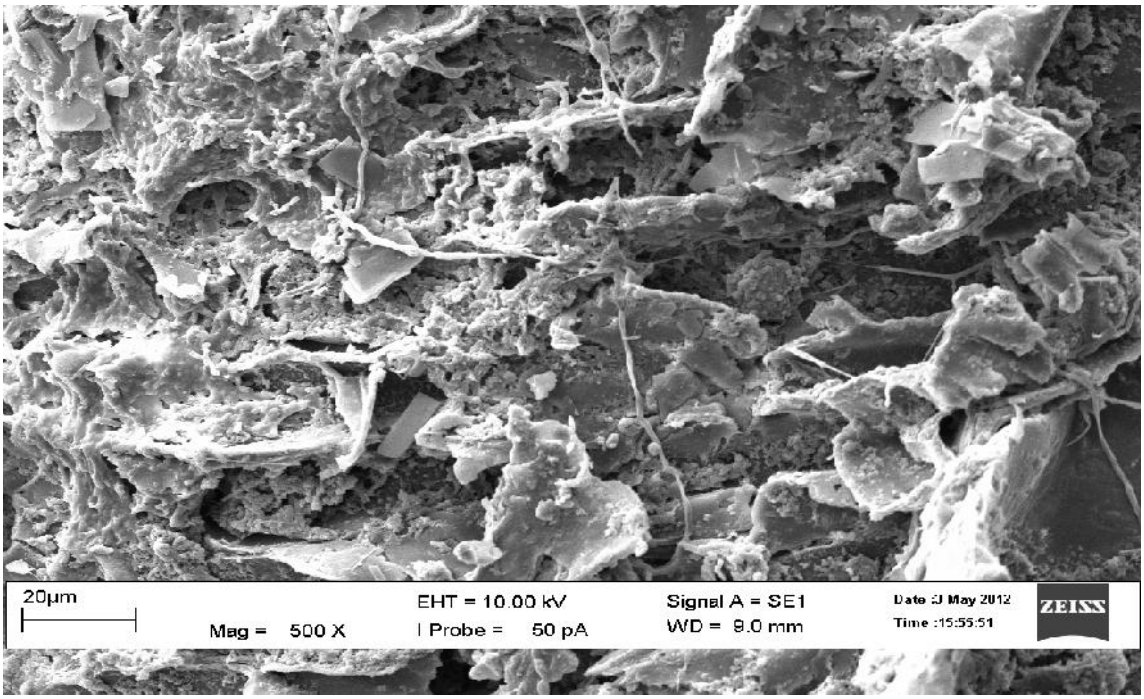


Figure 4.2 (a) Untreated *Keruing*

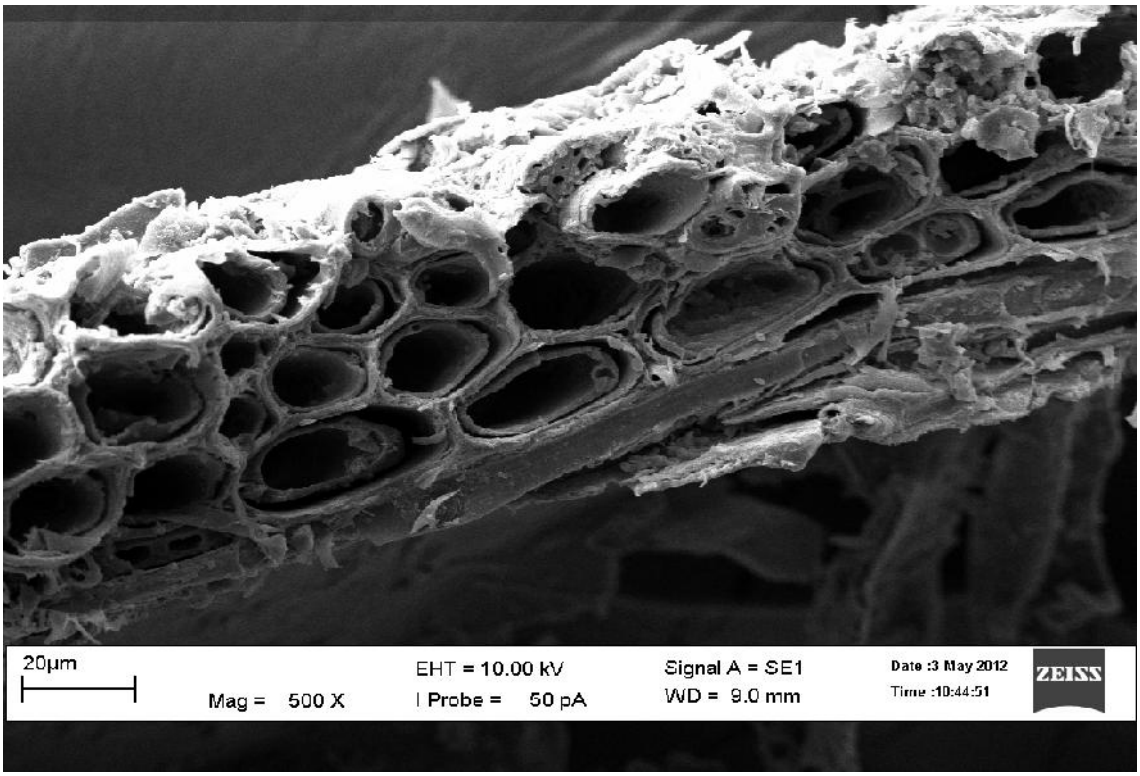


Figure 4.2 (b) Treated *Keruing*

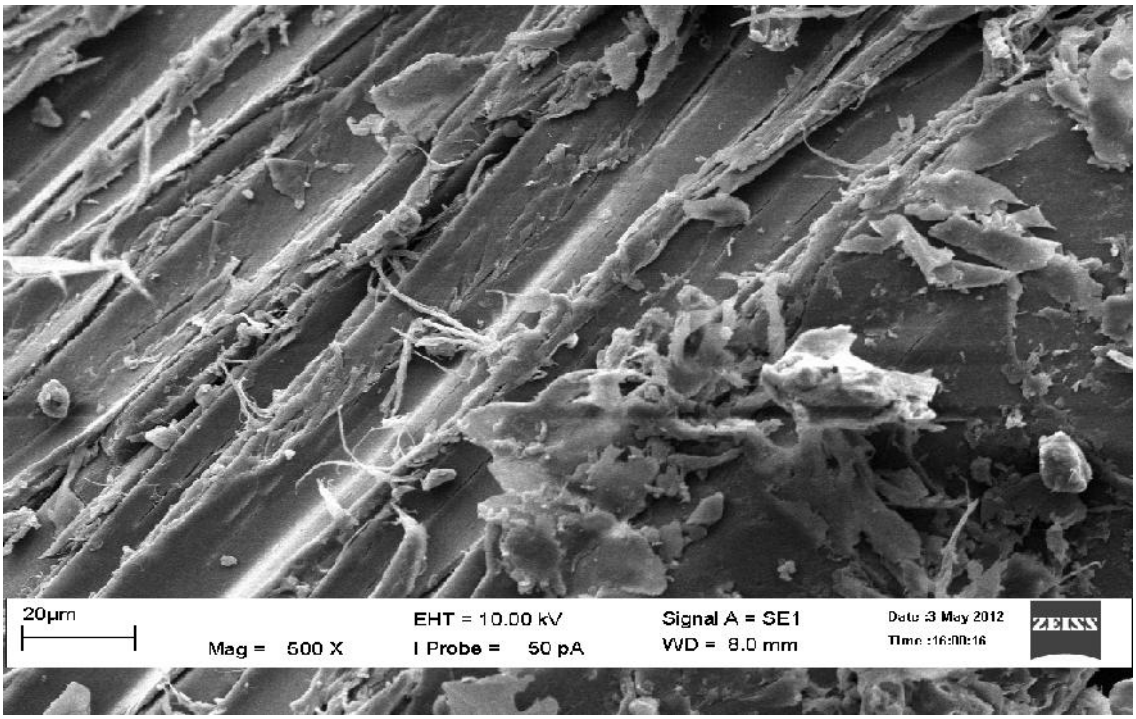


Figure 4.3 (a) Untreated *Meranti*

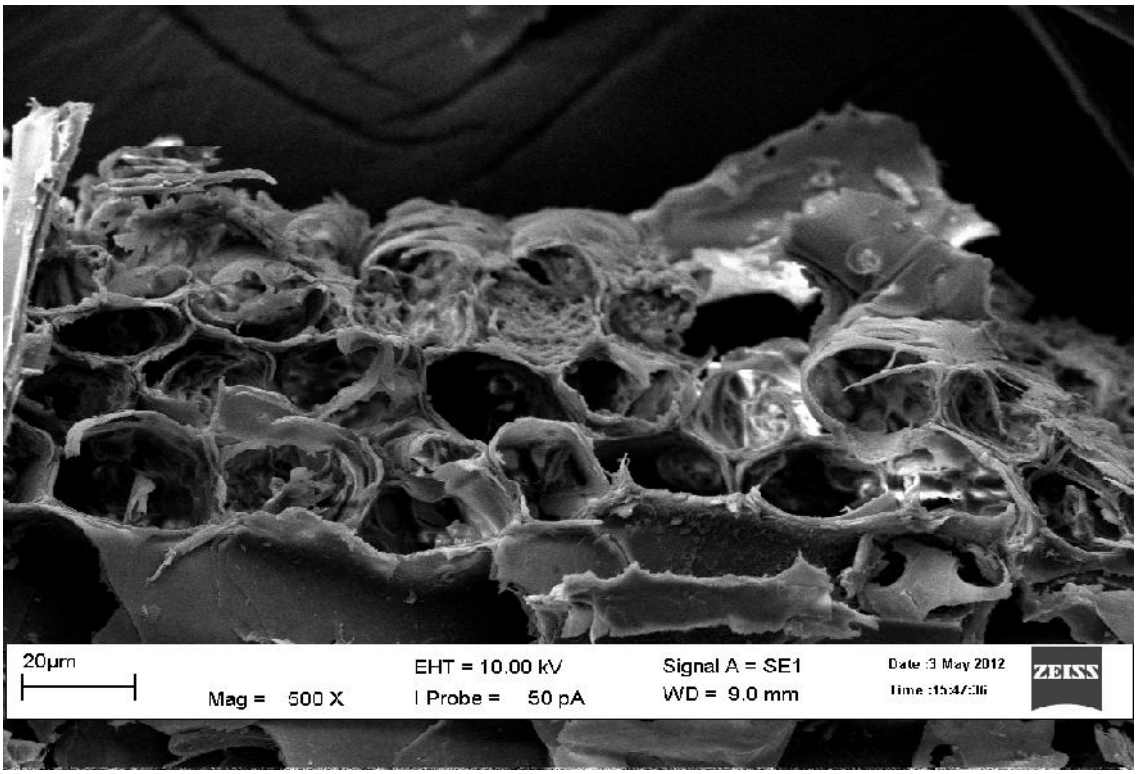


Figure 4.3 (b) Treated *Meranti*

Table 4.1 Size of pores of adsorbents

Adsorbent	Size of pore (μm)
Kempas	14.11 ± 0.09
Keruing	26.33 ± 0.09
Meranti	17.78 ± 0.2

4.3 Equilibrium Time

The equilibrium time achieved by the adsorbent to adsorb the adsorbate was studied. In this study, the purposes are to identify the equilibrium time achieved for the maximum loading of adsorbent and to compare the effectiveness of the adsorption of the adsorbate by using three types of adsorbents. The concentration-time profiles of the adsorbate onto three types of adsorbents are depicted in **Figure 4.4 (a-c)**. It is indicate that Qt is the amount adsorbed at time t.

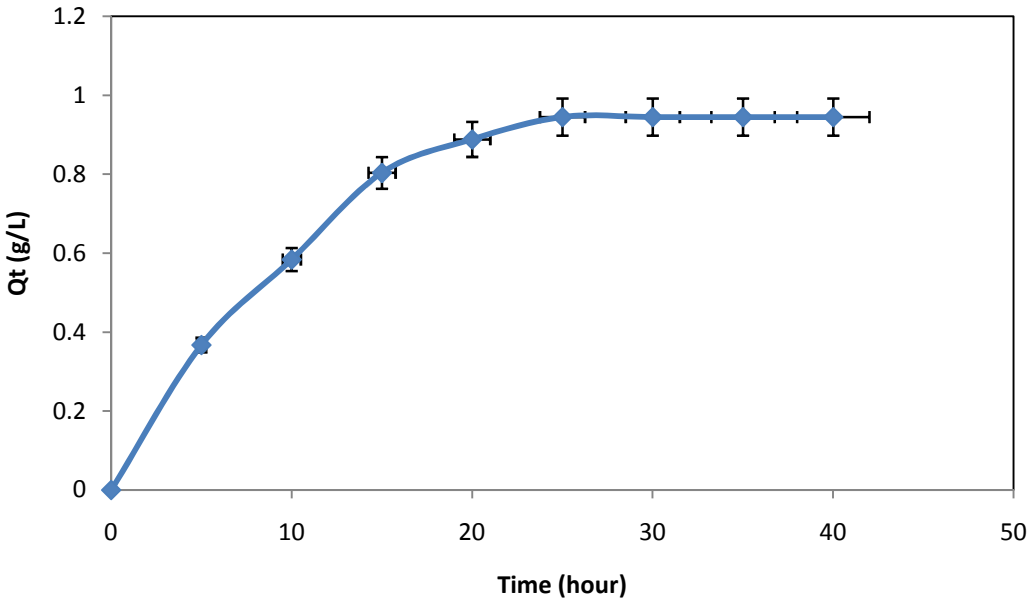


Figure 4.4(a) Concentration-Time profile of FTase adsorption onto *Keruing* adsorbent

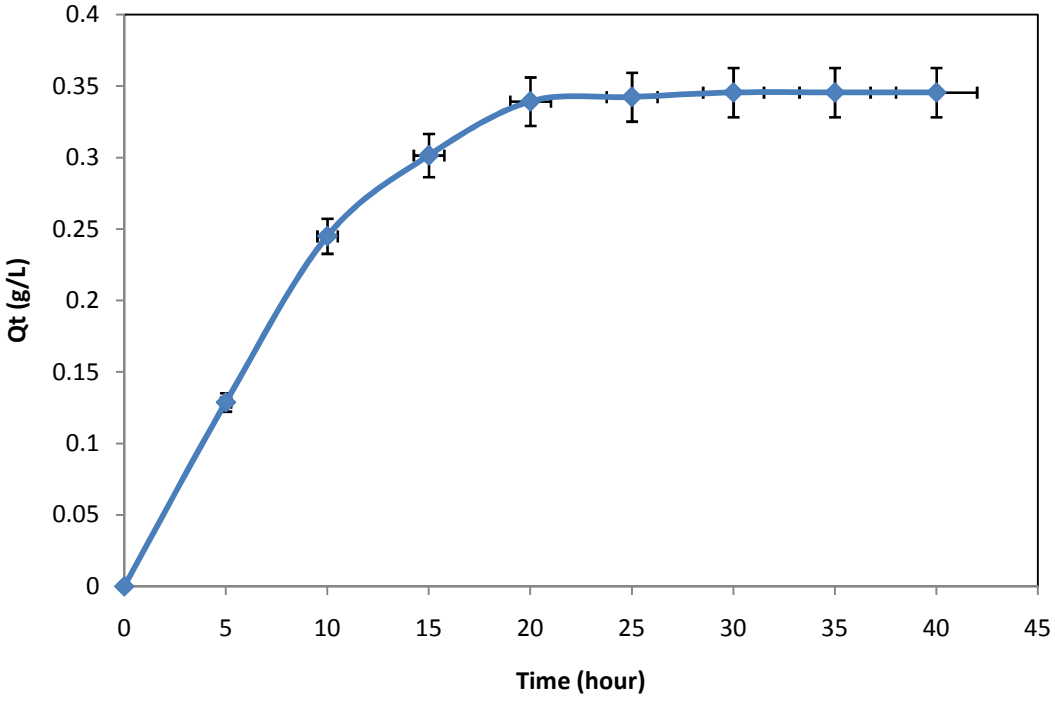


Figure 4.4(b) Concentration-Time profile of FTase adsorption onto *Kempas* adsorbent

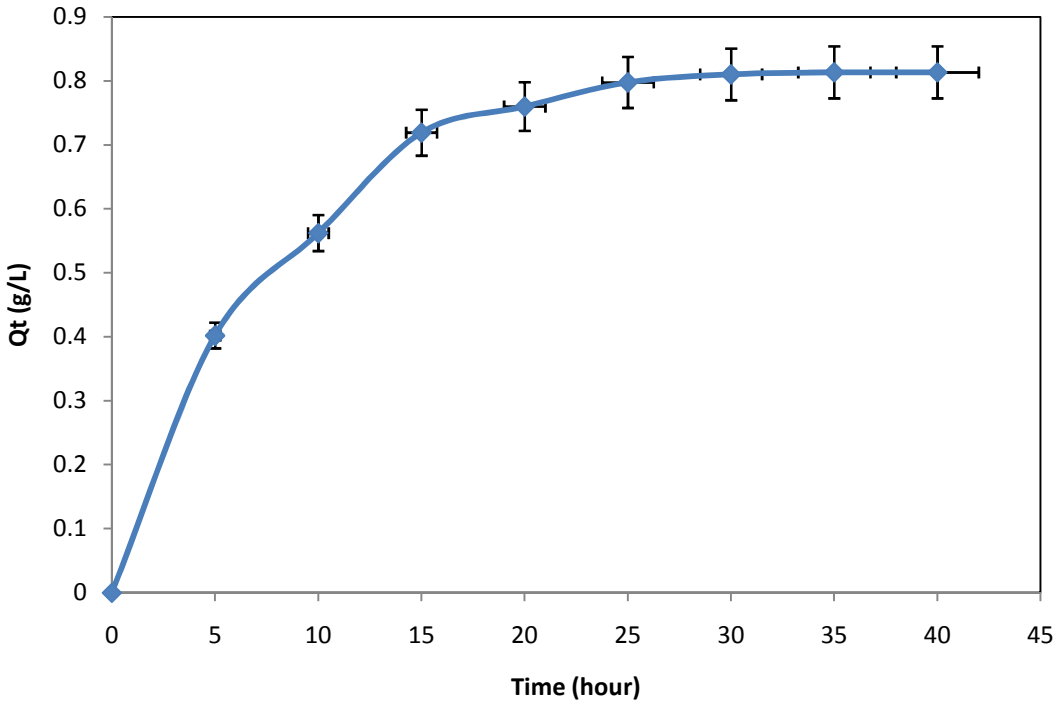


Figure 4.4(c) Concentration-Time profile of FTase adsorption onto *Meranti* adsorbent

From the results obtained in the **Figure 4.4(a-c)**, it showed that all the three types of adsorbents reach their equilibrium time at a range of 25 to 30 hours with different amount of loading capacity. *Keruing* adsorbent reached the equilibrium time at 25 hours with the amount adsorbed of 0.945g of FTase/L. For the *Kempas* and *Meranti* adsorbents, they reached the equilibrium at time 30 hours with the amount adsorbed of 0.345 g FTase/L and 0.810 g FTase/L, respectively.

The different equilibrium time reach and amount adsorbed are due to the size of pore of adsorbent and also the particle size of the adsorbate. It can be said that, the adsorbate particle size are smaller than the pore size of the *Keruing* adsorbent so that it can adsorb more adsorbate and reach the equilibrium time faster than the others two. **Figure 4.5** showed clearly the comparison of the equilibrium time reached by these three adsorbents. At phase I, the trend was increased due to the large number of

empty pores which can adsorb the adsorbate. But, at phase II, the adsorbate concentrations adsorbed by the adsorbent become constant because there were no more empty pores which can adsorb the remains adsorbate.

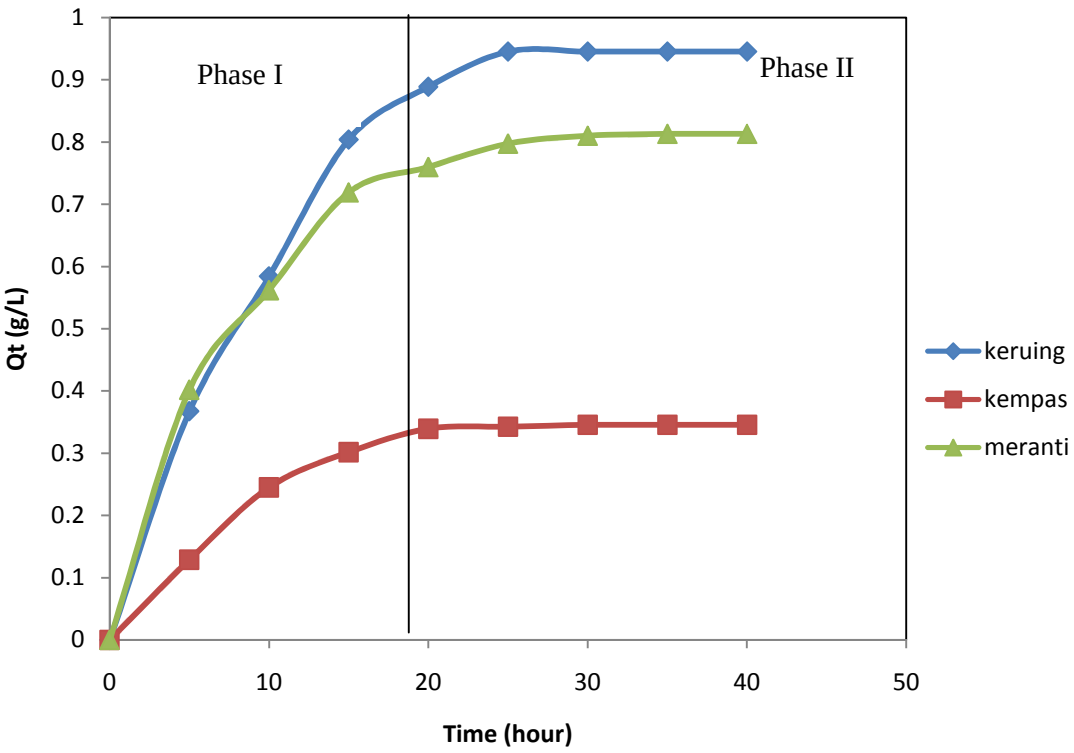


Figure 4.5 The comparison of equilibrium time reached by the three adsorbents (*Keruing*, *Kempas* and *Meranti*)

Figures 4.6 and **Figure 4.7** below shows the conditions of the *Keruing* and *Kempas* adsorbents (SEM 1.5 K x magnification) at the beginning of the immobilization process which is at time 5 hours. It shows the adsorbate start to adsorb onto the pores of the adsorbent. At this time, there are still lots of empty pores which can be fulfilling by the adsorbate.

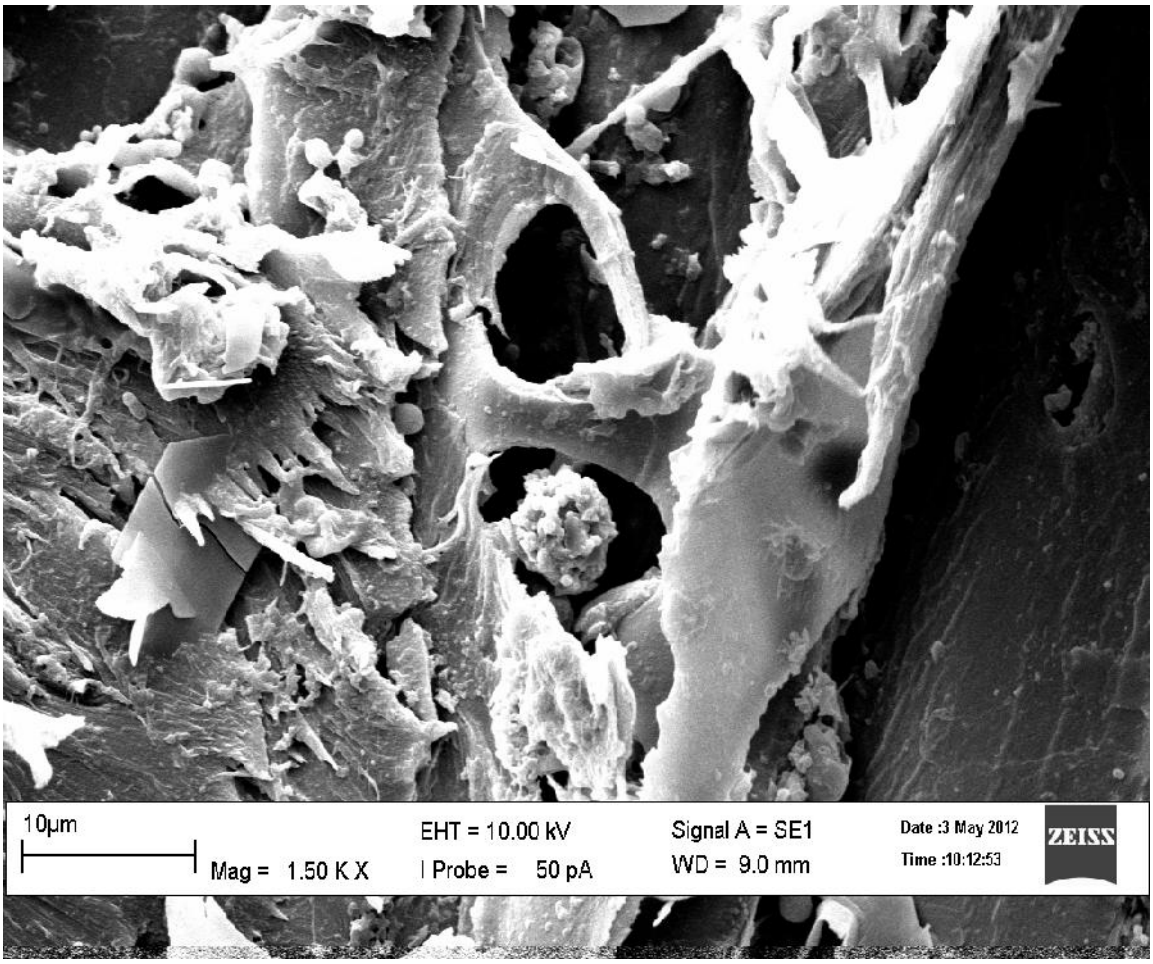


Figure 4.6 Immobilization of enzyme onto *Keruing* adsorbent at time 5 hours

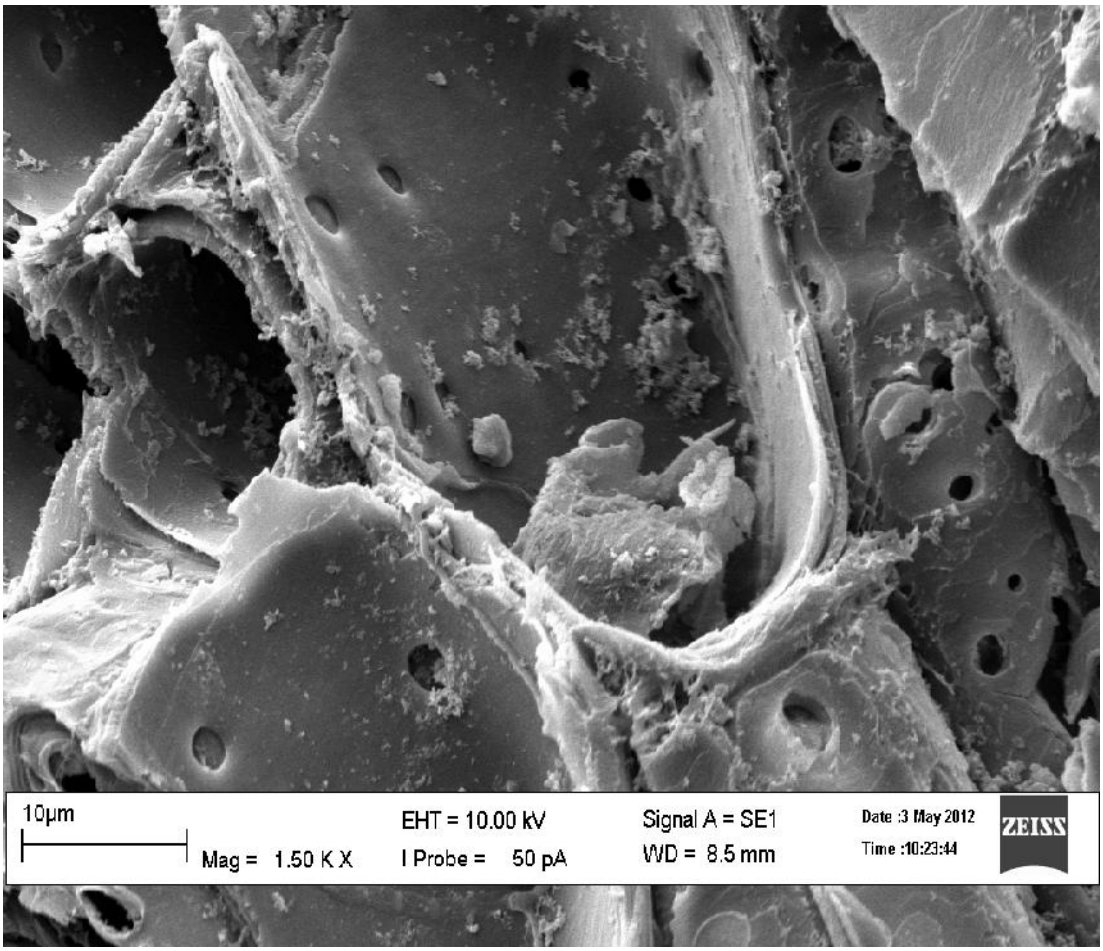


Figure 4.7 Immobilization of enzyme onto *Kempas* adsorbent at time 5 hours

The following SEM Figure 4.8 (2.50 K x magnification) shows the conditions of Meranti adsorbent when the adsorption tends to reached equilibrium at time 30 hours, where the concentration of adsorbate exceeds the number of pores of the adsorbent. It shows that the adsorbate was covering up the pores due to over loaded.

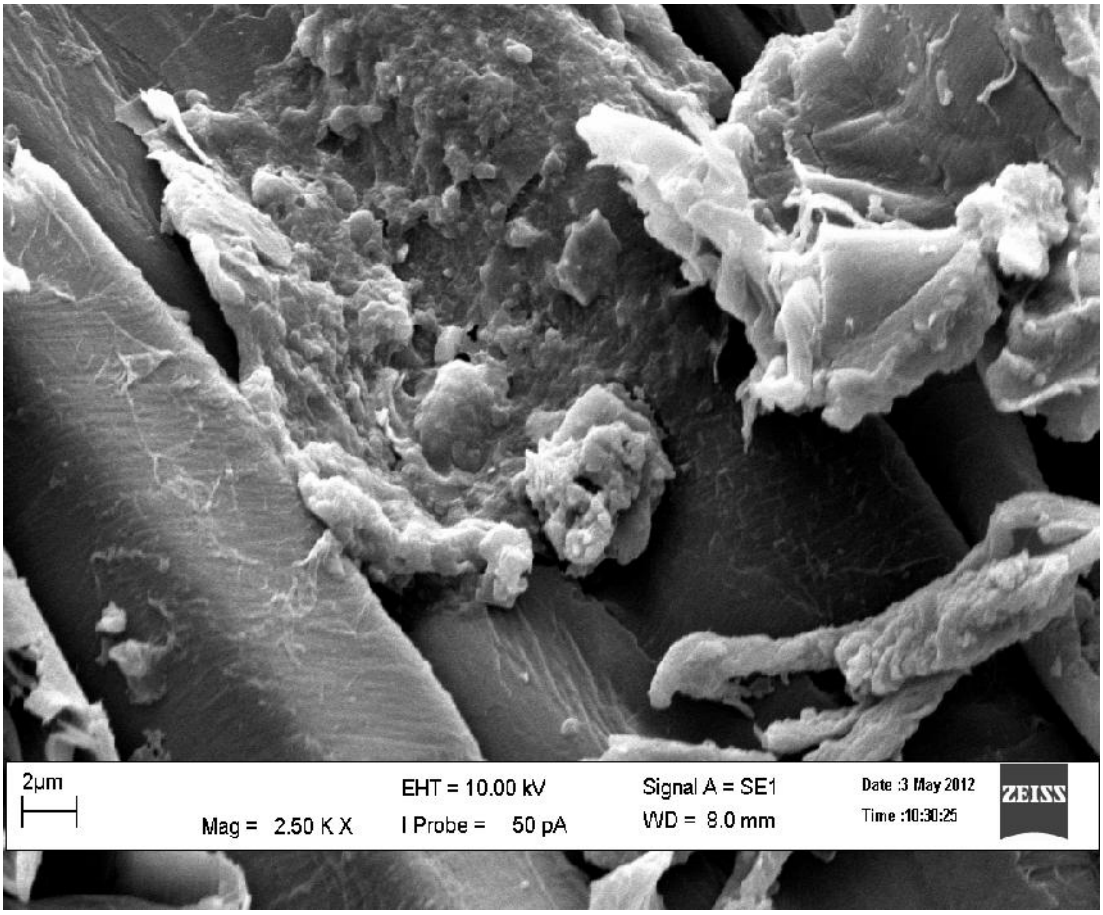


Figure 4.8 Immobilization of enzyme onto *Meranti* adsorbent at time 30 hours

4.4 Effect of Various Initial Adsorbate Concentrations

In **Figure 4.9, 4.10** and **4.11**, the effect on adsorption of initial adsorbate concentration, for fixed amount of adsorbent and pH are presented. It can be observed that, there are 3 phases occurred in the process. It can be said, at the first phase, the concentration of adsorbate are lower than the amount of pores of the adsorbent, so that all the adsorbate can be adsorb onto the adsorbent and there is almost no adsorbate residue obtained in this phase. While at the second phase, the adsorbate concentration residue was increase because the concentrations of adsorbate are higher or same as the amount of the adsorbent. Then, at the third phase, the adsorbate concentrations residue kept increasing because the adsorbate added were already become saturated and the adsorbate were repel to each other.

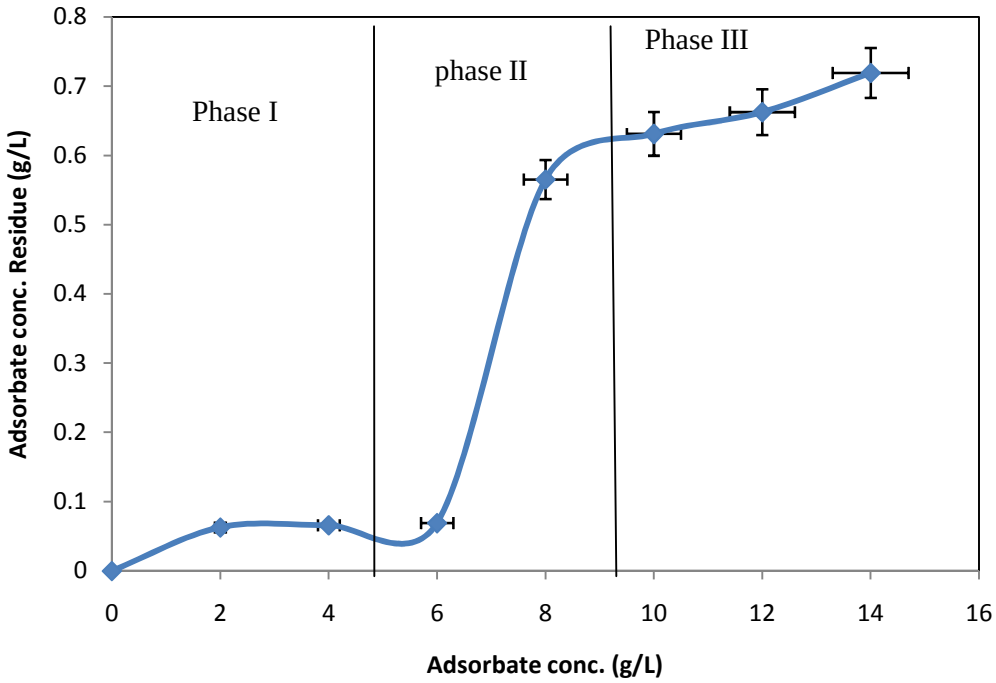


Figure 4.9 Effect of increasing initial adsorbate concentration to the *Keruing* adsorbent

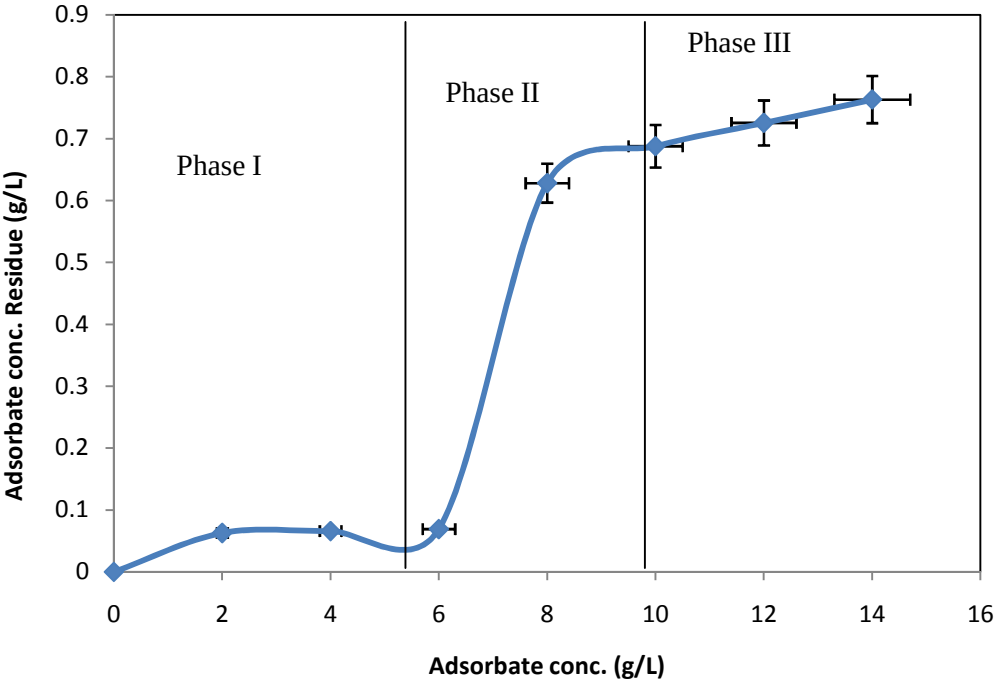


Figure 4.10 Effect of increasing initial adsorbate concentration to the *Meranti* adsorbent

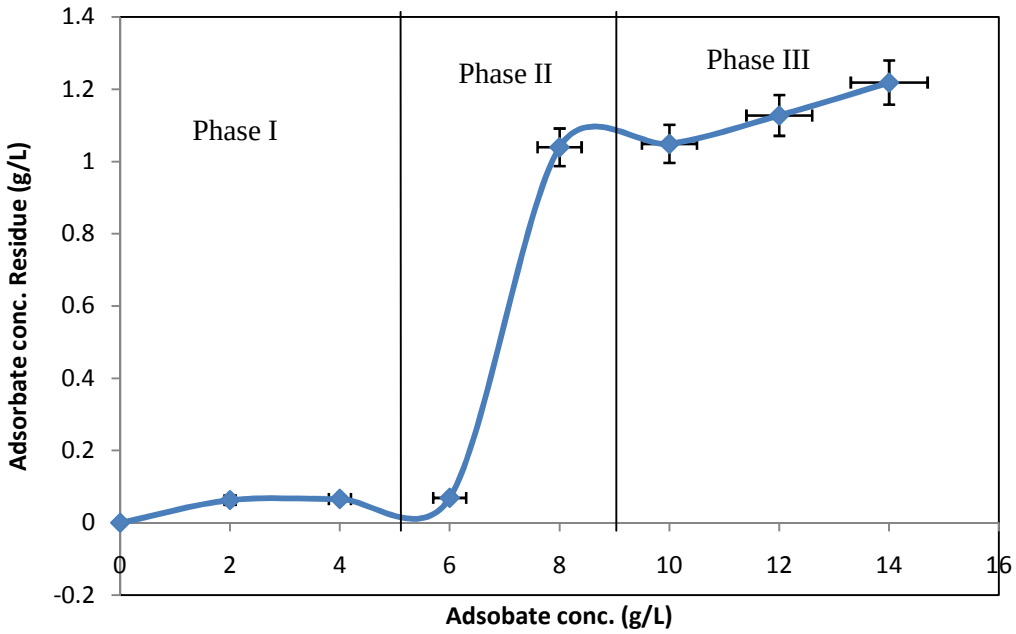


Figure 4.11 Effect of increasing initial adsorbate concentration to the *Kempas* adsorbent

Figure 4.12 below shows the comparison of the adsorbate concentration residue obtained between three adsorbents which are *Keruing*, *Kempas* and *Meranti*. The figure shows that *Kempas* adsorbent gained highest adsorbate concentration residue. It is because of the size of pore of that adsorbent is smaller than the others, so that the amount of adsorbate which can be adsorb are lower than the other two adsorbents.

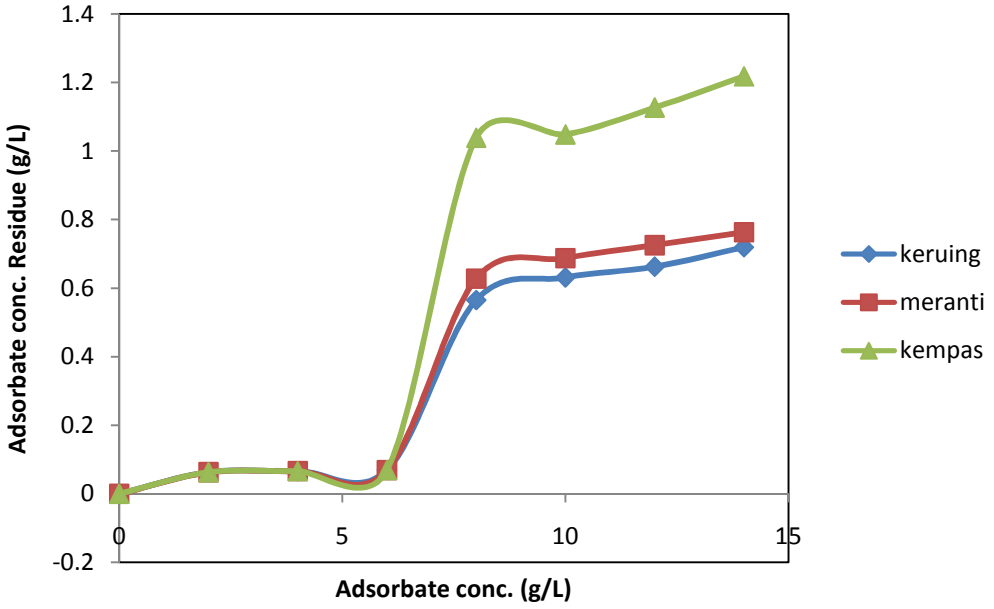


Figure 4.12 Comparison of the adsorbate concentration residue between three adsorbents

In order to identify the conditions of the adsorbent during the first and second stage of the process, SEM analysis was done to the *Keruing* adsorbent**. Figure 4.13 (a & b)** and **Figure 4.14 (a & b)** shows the conditions at the first and second stages, respectively. It shows that, in the **Figure 4.13**, there are still a lot of empty pores while in the **Figure 4.14**; it shows that the adsorbate was overloaded.

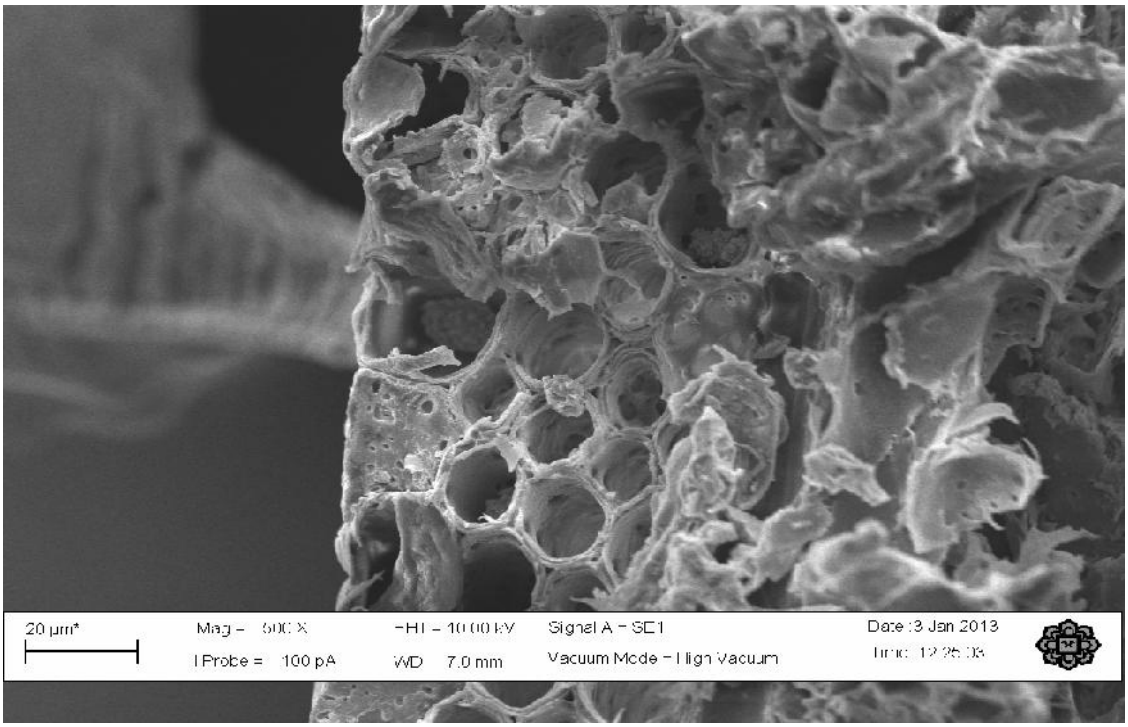


Figure 4.13 (a) *Keruing* adsorbent at the initial stage (500 x magnifications)

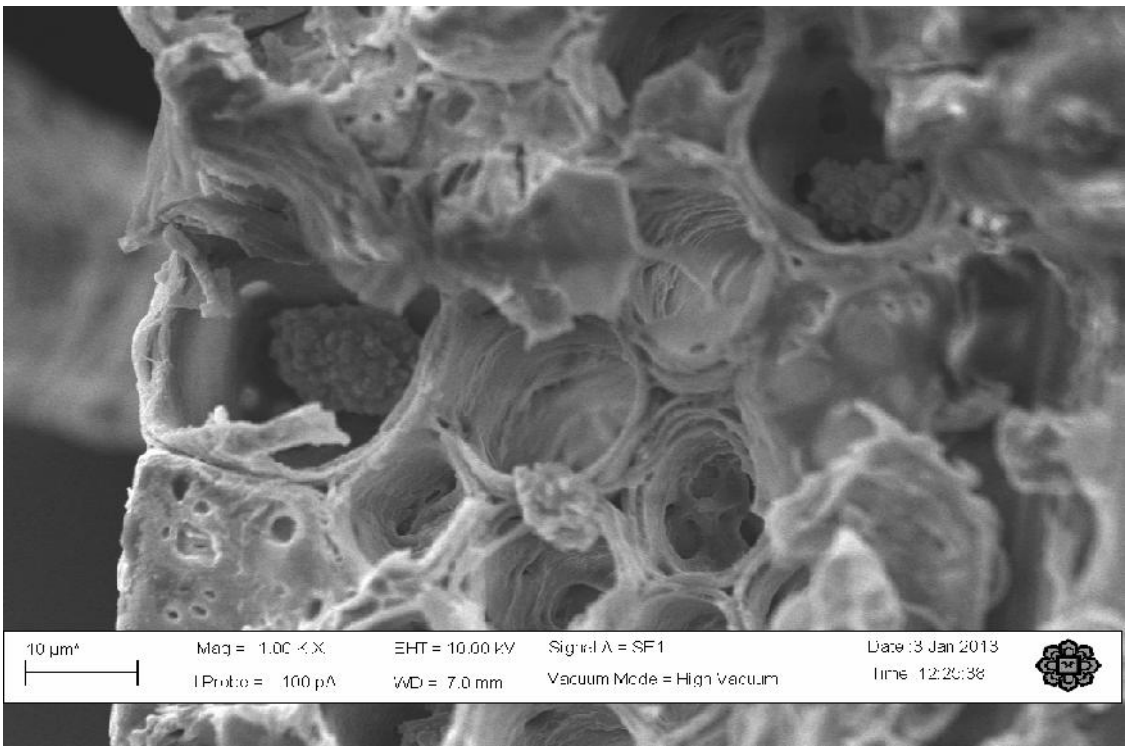


Figure 4.13 (b) *Keruing* adsorbent at the initial stage (1000 x magnifications)

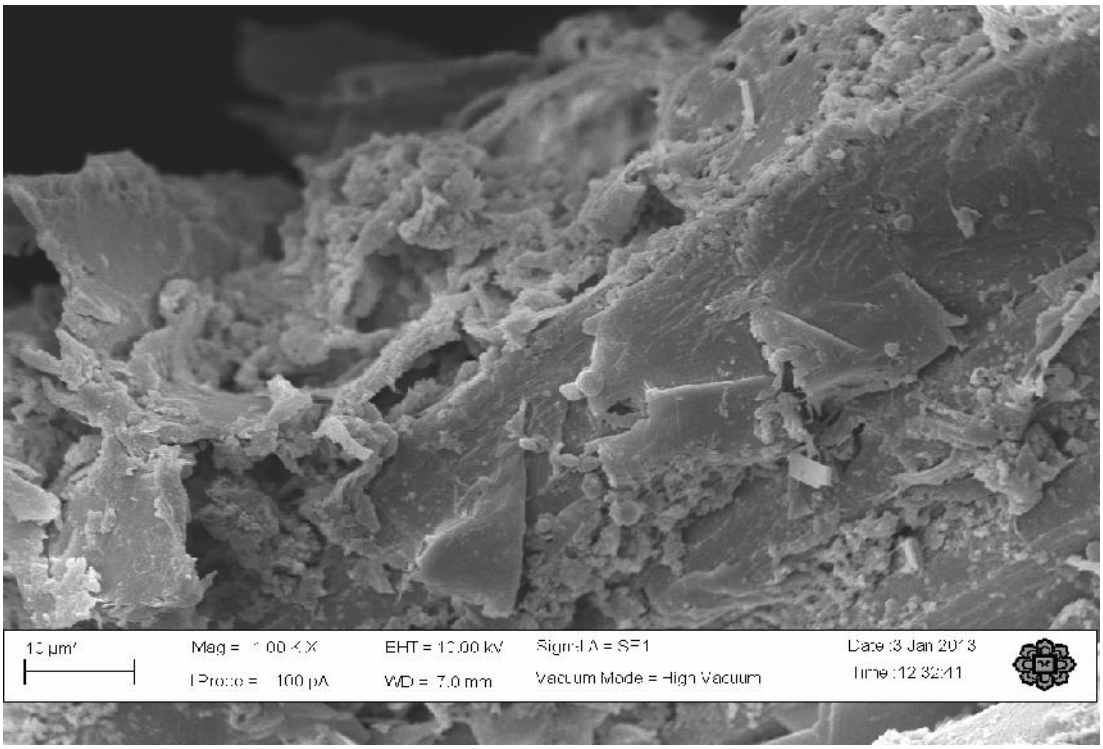


Figure 4.14 (a) *Keruing* adsorbent at the final stage (1000 x magnifications)

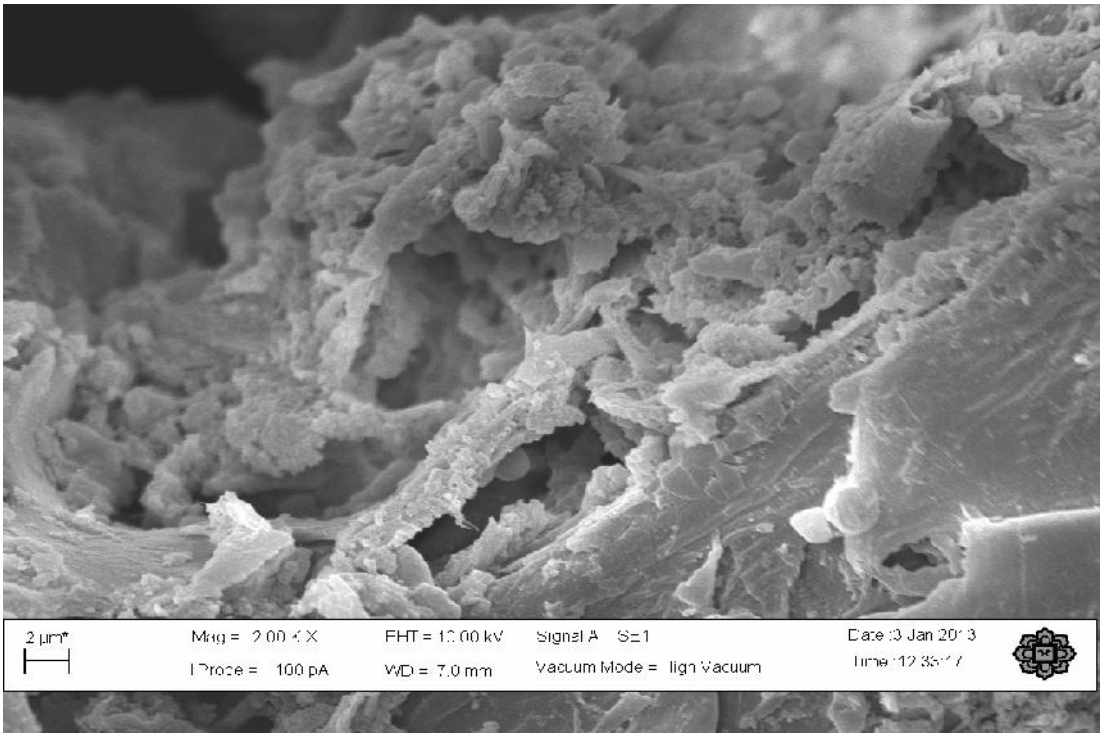


Figure 4.14 (b) *Keruing* adsorbent at the final stage (2000 x magnifications)

4.5 Adsorption Isotherm

The adsorption mechanism of a system can be described using adsorption isotherm model. Two types of adsorption isotherms are presented in this study: Langmuir and Freundlich isotherm models.

The Langmuir model assumes that the uptake of metal ions occurs on a homogenous surface by monolayer adsorption without any interaction between adsorbed ions. Therefore, all adsorption sites are equivalent and can accommodate, at most, one adsorbed atom (Ting et al., 2010). **Figure 4.15, 4.16** and **4.17** shows the plots for FTase adsorption by adsorbents by following Langmuir model.

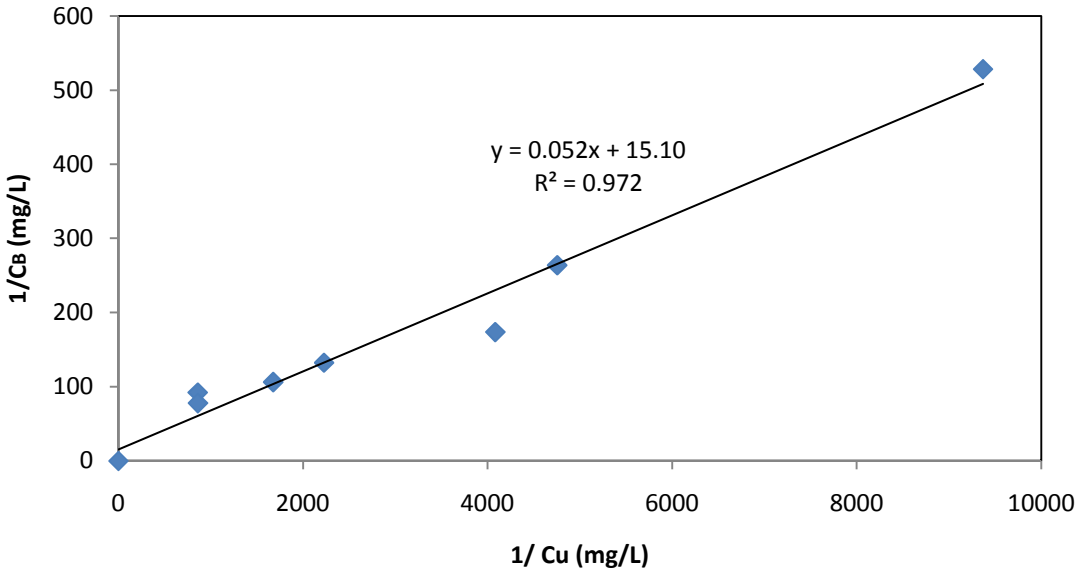


Figure 4.15 Langmuir isotherm plots for FTase adsorption by *Keruing* sawdust

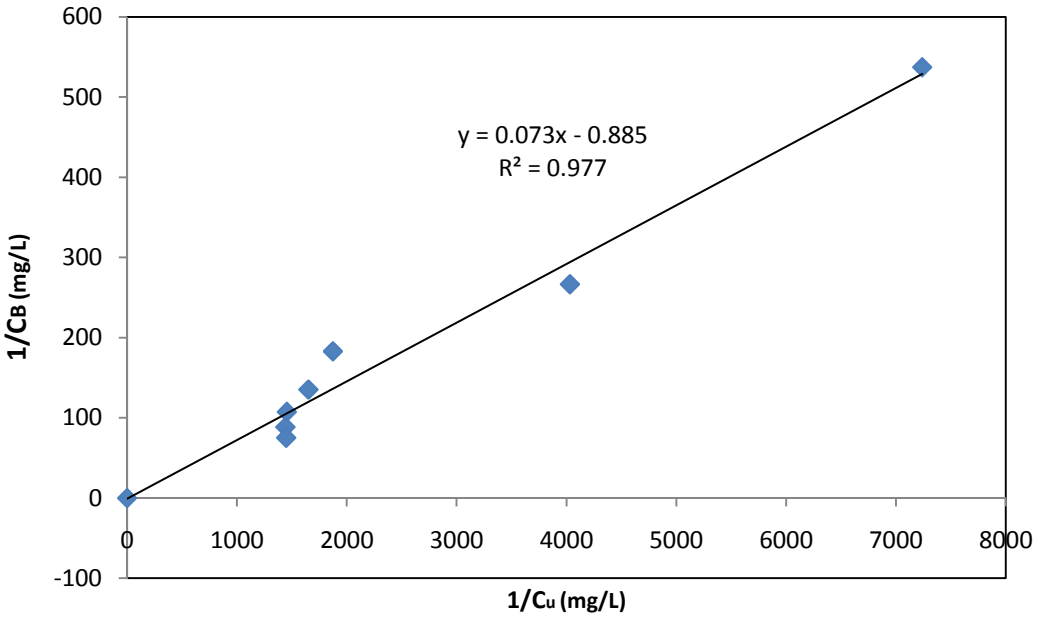


Figure 4.16 Langmuir isotherm plots for FTase adsorption by *Meranti* sawdust

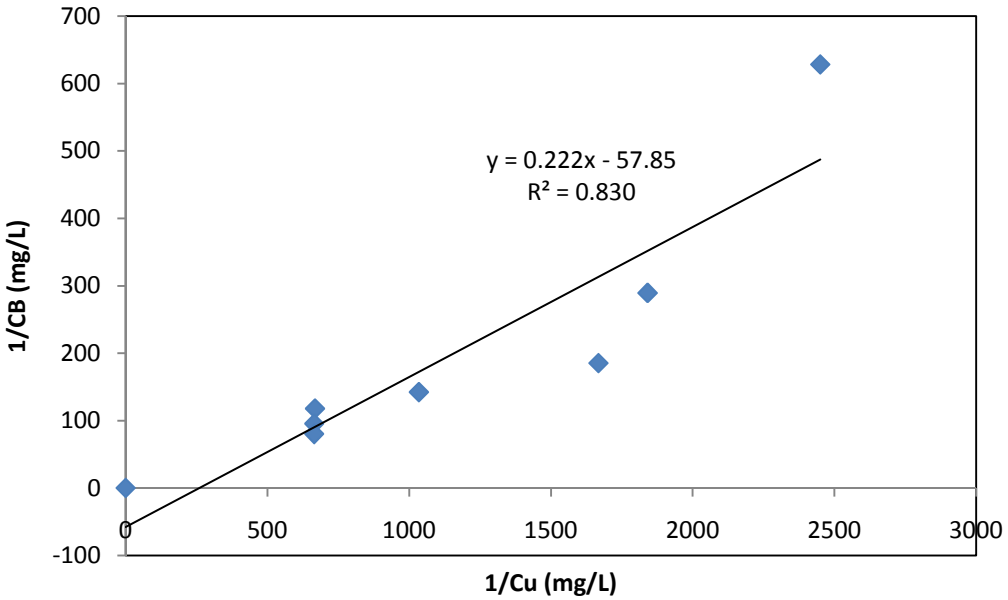


Figure 4.17 Langmuir isotherm plots for FTase adsorption by *Kempas* sawdust

The Freundlich model assumes that the uptake of metal ions occurs on a heterogenous surface by multilayer adsorption (Ting et al., 2010). **Figure 4.18, 4.19** and **4.20** shows the plots for FTase adsorption by adsorbents by following Freundlich model.

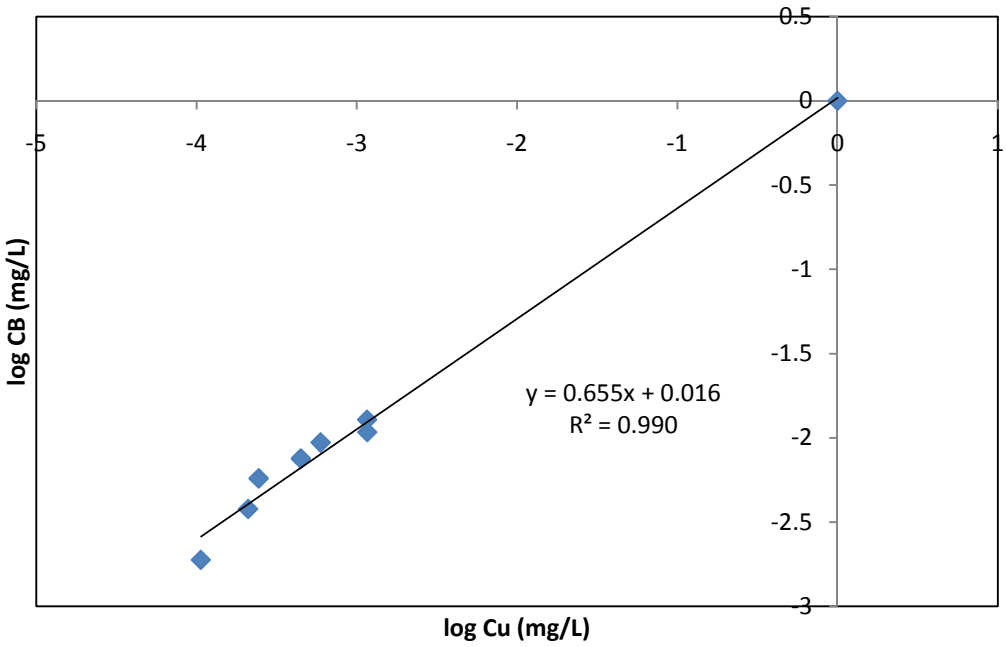


Figure 4.18 Freundlich isotherm plots for FTase adsorption by *Keruing* sawdust

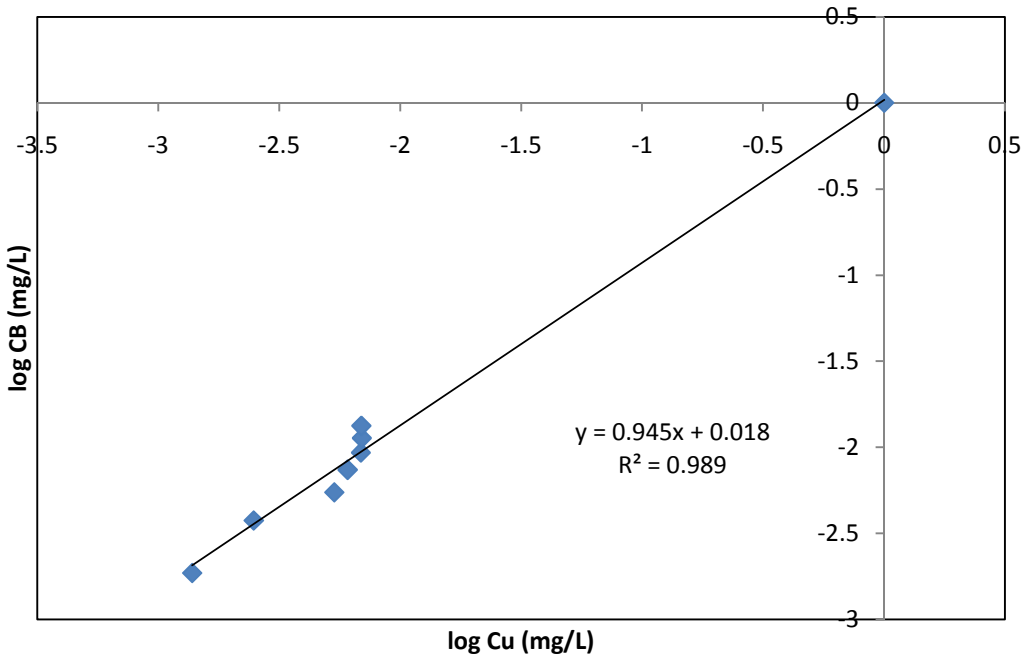


Figure 4.19 Freundlich isotherm plots for FTase adsorption by *Meranti* sawdust

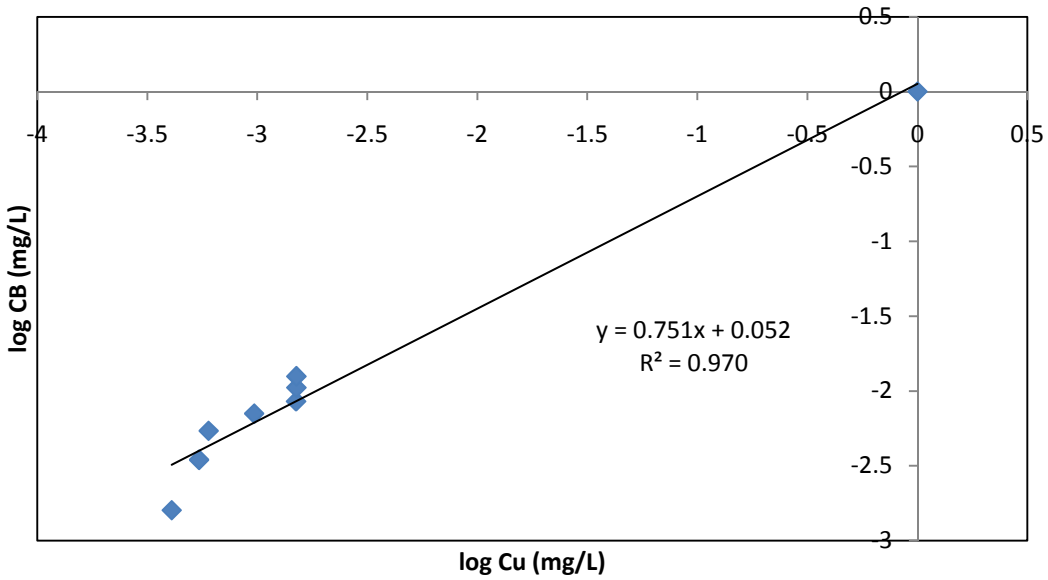


Figure 4.20 Freundlich isotherm plots for FTase adsorption by *Kempas* sawdust

Table 4.2 Langmuir and Freundlich isotherm constants for FTase adsorption

Adsorbent	Langmuir model			Freundlich model		
	K_L (mg/L)	$C_{B\ MAX}$ (mg/L)	R^2	K_F (mg/L)	n	R^2
Keruing	3.4E-3	0.0662	0.972	1.0375	1.5267	0.990
Meranti	8.2E-2	1.1299	0.977	1.0423	1.0582	0.989
Kempas	3.8E-3	0.0173	0.830	1.1272	1.3316	0.970

The isotherm constants and the correlation coefficient (R^2) are listed in the **Table 4.2.** The Langmuir constant K_L and $C_{B\ MAX}$ are calculated using the slope and intercept of the line, obtained from the plot of $1/C_B$ vs $1/C_{U,}$ and whereas, Freundlich constants K_F and n are determined from the slope and intercept of the line obtained from the plot of log C_B vs log $C_{U.}$ It can be observed that both Langmuir and Freundlich isotherm models showed good fits with the adsorption data. The R^2 value obtained for the both isotherms showed that both model may be applicable in demonstrating FTase adsorption by the sawdust. The steepness of the isotherms indicated highest affinity of enzymes to the freely exposed binding sites. So that, from the results obtained, it can be said that Freundlich model is more fitted with the data since this isotherm showed the highest steepness. From this model also, the best adsorbent can be choose which are *Keruing* and *Meranti.*

4.6 Fourier Transform Infrared Spectroscopy (FTIR)

The formation of functional group in the hardwood species of untreated and treated sawdust was conducted using FTIR spectroscopic analysis. The FTIR spectrums of the untreated and treated wood sawdust from three species were come out with the same trend of spectrum. This is due to the all the samples are come from same functional group. **Figure 4.21, Figure 4.22** and **Figure 4.23** showed the spectrum of untreated and treated wood sawdust.

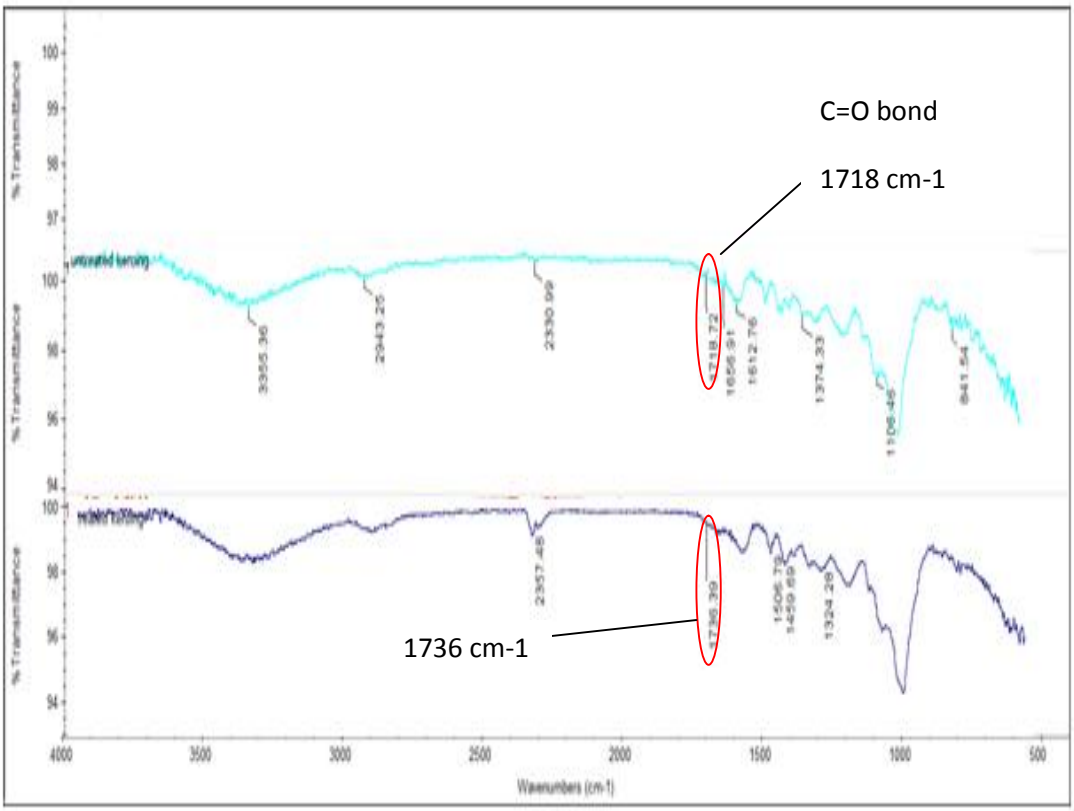


Figure 4.21 FTIR spectra of untreated and treated *Keruing* sawdust

From the results of *Keruing* species, the FTIR spectrum of untreated wood sawdust shows the absorption bands in the region of 3355 cm–1, 2943 cm-1 and1718 cm–1 due to O-H stretching vibration, C-H stretching vibration, and C=O stretching vibration, respectively. These absorption bands are due to hydroxyl group in cellulose, carbonyl group of acetyl ester in hemicellulose, and carbonyl aldehyde in lignin.

Then, the absorption band at treated sawdust show at the carbonyl peak C=O at 1718 cm_{-1} was slightly shifted towards 1736 cm_{-1} in the spectra of treated sawdust because the ester carbonyl bonds in the hemicellulose was break due to the water treatment. All the difference happens due to the treatment using water based on temperature effect.

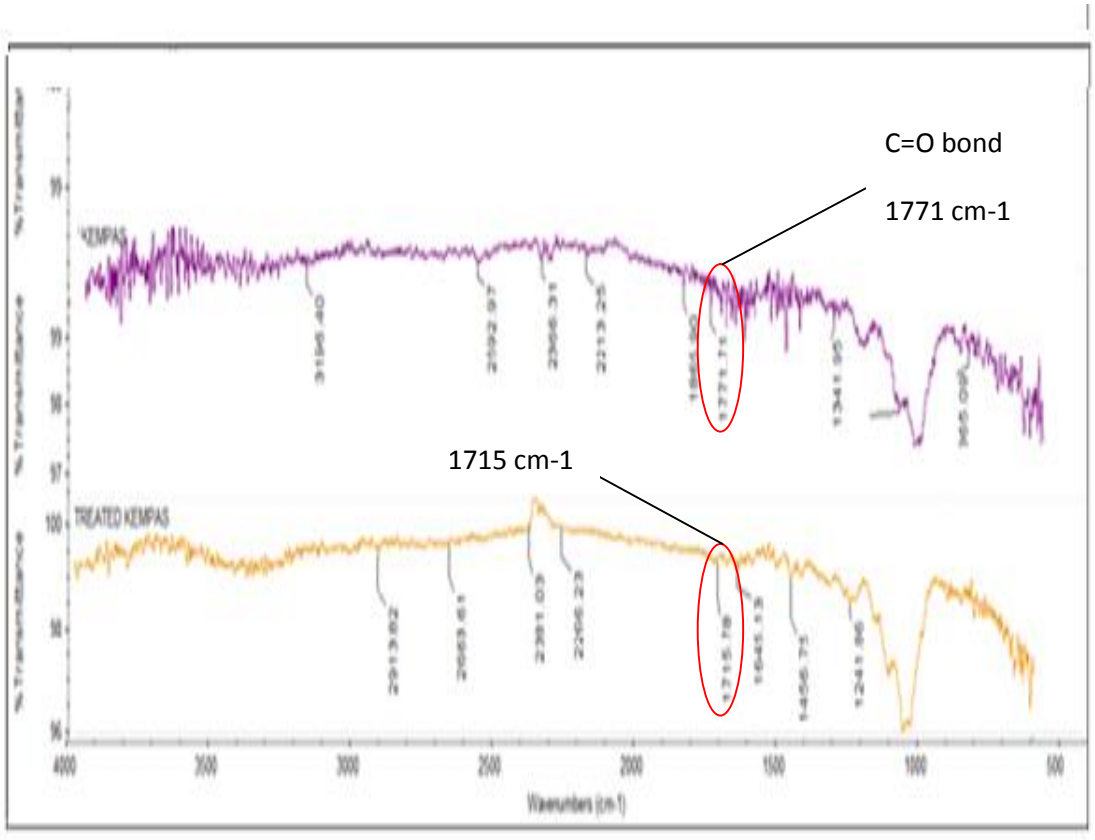


Figure 4.22 FTIR spectra of untreated and treated *Kempas* sawdust

From the results of *Kempas* species, the FTIR spectrum of untreated wood sawdust shows the absorption bands in the region of 2592 cm–1 and1771 cm–1 due to O-H stretching vibration and C=O stretching vibration, respectively. These absorption bands are due to hydroxyl group in cellulose, carbonyl group of acetyl ester in hemicellulose, and carbonyl aldehyde in lignin.

Then, the absorption band at treated sawdust show that O-H which shifted towards 2553 cm–1. From the observation, there are higher absorption bond produce at O-H groups due to the moister contained in the sawdust. It can be seen that, the carbonyl peak C=O at 1771 cm–1 was slightly shifted towards 1715 cm–1 in the spectra of treated sawdust because the ester carbonyl bonds in the hemicellulose was break due to the water treatment. All the difference happens due to the treatment using water based on temperature effect.

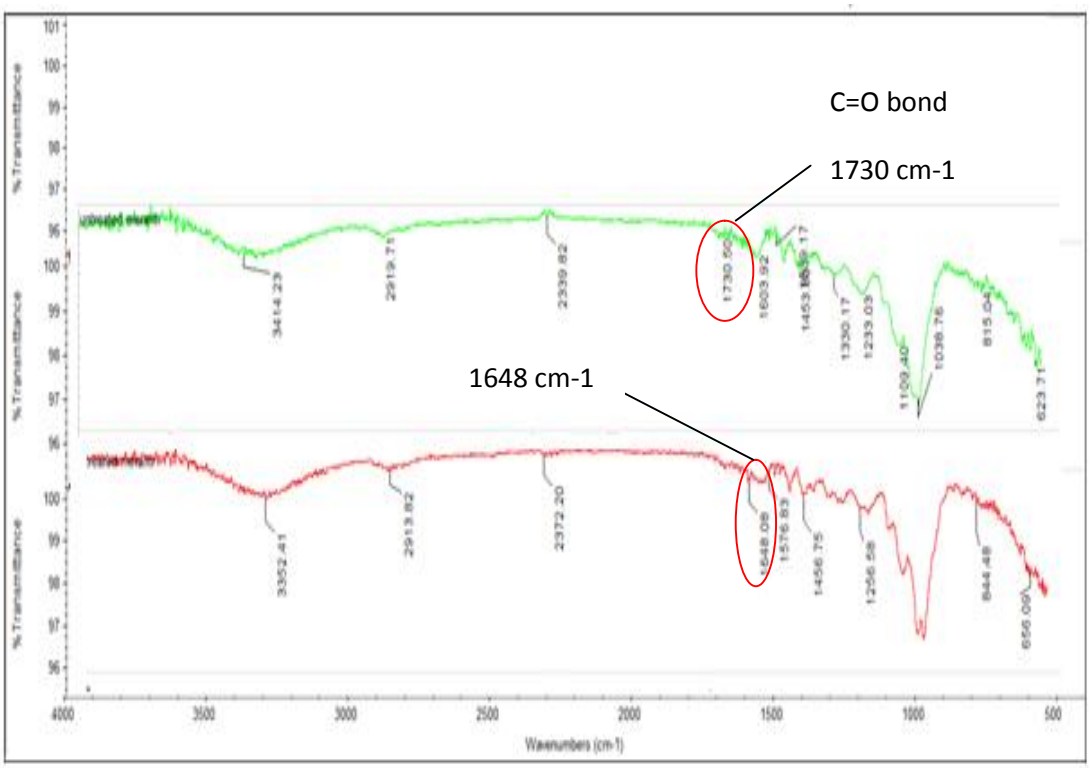


Figure 4.23 FTIR spectra of untreated and treated *Meranti* sawdust

From the results of *Meranti* species, the FTIR spectrum of untreated wood sawdust shows the absorption bands in the region of 3414 cm–1 and 2919 cm–1 and 1730 cm-1 due to O-H stretching vibration, C-H stretching vibration, and C=O stretching vibration, respectively. These absorption bands are due to hydroxyl group in cellulose, carbonyl group of acetyl ester in hemicellulose, and carbonyl aldehyde in lignin.

Then, the absorption band at treated sawdust show at O-H which shifted towards 3352 cm–1 and at C-H the absorbance shifted towards into 2913 cm–1 respectively. From the observation, there are higher absorption bond produce at O-H groups due to the moister contained in the sawdust. It can be seen that, the carbonyl peak C=O at 1730 cm–1 was slightly shifted towards 1648 cm–1 in the spectra of treated sawdust because the ester carbonyl bonds in the hemicellulose was break due to the water treatment based on the temperature effect.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Lignocellulosic material which is sawdust has an adsorption capability like the other commercial adsorbents used in the industry nowadays. From the three hardwood species used, *Keruing* showed the raising size of pores after the treatment process which is 26.33µm which lead to the highest amount adsorbed by FTase which is 0.945g of FTase/L during the equilibrium time study. It is also showed the fastest adsorbent which achieved the equilibrium which is at time 25 hours. It showed that the enzyme molecules were easily adsorbed onto the surface of the *Keruing* adsorbent due to the large pore of that adsorbent. It is showed that when the enzyme concentration increases, the concentration of enzyme residue also increases due to the overloading and it can be said that the amount of enzyme are exceeds the amount of adsorbents. The enzyme residue concentration increases after 6 g/L of enzyme concentration was added to the fixed amount of adsorbent for all the three species. Freundlich isotherm fitted the data to indicate the presence of heterogenous metal sorption sites.

5.2 RECOMMENDATION

In order to improve the immobilize enzyme, some recommendations are required to make sure this study more effectively:

i. Studies on the structure of hardwood species should be done before continue to the treatment process.

ii. Studies on the active sites of the enzyme after the adsorption process in order to ensure that all the active sites are active.

iii. Studies the parameters and chemicals that can cause the desorption of the enzyme.

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APPENDIX A

Table A.1 Preparation of standard glucose

Concentration (g/L)	Stock solution (mL)	Water (mL)
0.2	2	8
0.4	4	6
0.6	6	4
0.8	8	2
1.0	10	0

0.01 g of glucose was diluted in 100 mL of H_2O (1g/L): 10 mL

$C_1V_1 = C_2V_2$

(0.01 g) V_1 = (0.02 g) (10 mL)

V_1 = 2 mL

To get 10 mL of solution (0.2 g/L), 2 mL of stock solution will be add with 8 mL of water.

APPENDIX B

Table B.1 Result of equilibrium time for *Meranti* adsorbent

Time (hr)	OD sample	OD decrease	Glucose concentration (g/L)	Enzyme activity (U/L)	Enzyme concentration, Qt (g/L)
0	0.632±0.06	0	0	0	0
5	0.741±0.02	0.109	0.0446148	0.446148	0.401880972
10	0.743±0.07	0.111	0.07668	0.7668	0.690717721
15	0.842±0.04	0.21	0.0798188	0.798188	0.718991388
20	0.855±0.02	0.223	0.0843499	0.843499	0.759806607
25	0.867±0.06	0.235	0.0885326	0.885326	0.797483512
30	0.871±0.03	0.239	0.0899268	0.899268	0.810042179
35	0.872±0.04	0.24	0.0902754	0.902754	0.813182297
40	0.872±0.04	0.24	0.0902754	0.902754	0.813182297

Table B.2 Result of equilibrium time for *Keruing* adsorbent

Time (hr)	OD sample	OD decrease	glucose concentration (g/L)	Enzyme activity (U/L)	Enzyme concentration, Qt (g/L)
0	0.435±0.04	0	0	0	0
5	0.533±0.02	0.098	0.040780759	0.40780759	0.367344717
10	0.602±0.02	0.167	0.064830951	0.64830951	0.583983916
15	0.672±0.04	0.237	0.0892297	0.892297	0.803762846
20	0.699±0.03	0.264	0.0986406	0.986406	0.888534303
25	0.717±0.02	0.282	0.104914604	1.04914604	0.945049245
30	0.717±0.07	0.282	0.104914604	1.04914604	0.945049245
35	0.717±0.03	0.282	0.104914604	1.04914604	0.945049245
40	0.717±0.06	0.282	0.104914604	1.04914604	0.945049245

Table B.3 Result of equilibrium time for *Kempas* adsorbent

Time	OD sample	OD decrease	Glucose concentration (g/L)	Enzyme activity (U/L)	Enzyme concentration,Qt (g/L)
0	0.893±0.05	0	0	0	0
5	0.915±0.06	0.022	0.014290693	0.14290693	0.128727633
10	0.952±0.03	0.059	0.027187173	0.27187173	0.244896481
15	0.97±0.03	0.077	0.033461136	0.33461136	0.301411054
20	0.982±0.03	0.089	0.037643778	0.37643778	0.339087435
25	0.983±0.05	0.09	0.037992331	0.37992331	0.342227129
30	0.984±0.02	0.091	0.038340885	0.38340885	0.345366832
35	0.984±0.02	0.091	0.038340885	0.38340885	0.345366832
40	0.984±0.04	0.091	0.038340885	0.38340885	0.345366832

APPENDIX C

Table C.1 Effect of various initial enzyme concentrations by using *Keruing* adsorbent

Conc. (g/L)	OD sample	OD decrease	Glucose concentration (g/L)	Enzyme activity (U/L)	Enzyme concentration (g/L)
0	0.54±0.04	0	0	0	0
2	0.541±0.05	0.001	0.00697107	0.0697107	0.062793969
4	0.542±0.06	0.002	0.00731963	0.0731963	0.065933727
6	0.543±0.03	0.003	0.007668177	0.07668177	0.069073366
8	0.701±0.07	0.161	0.06273963	0.6273963	0.565145725
10	0.722±0.04	0.182	0.070059	0.70059	0.63107711
12	0.732±0.04	0.192	0.073544789	0.73544789	0.662476383
14	0.75±0.06	0.21	0.0798187	0.798187	0.718990487

Table C.2 Effect of various initial enzyme concentrations by using *Meranti* adsorbent

Conc. (g/L)	OD sample	OD decrease	Glucose concentration (g/L)	Enzyme activity (U/L)	Enzyme concentration (g/L)
0	0.72±0.03	0	0	0	0
2	0.721±0.06	0.001	0.00697107	0.0697107	0.062793969
4	0.722±0.07	0.002	0.00731962	0.0731962	0.065933636
6	0.723±0.07	0.003	0.007668177	0.07668177	0.069073366
8	0.901±0.04	0.181	0.0697107	0.697107	0.627939695
10	0.92±0.06	0.2	0.076333217	0.76333217	0.68759397
12	0.932±0.06	0.212	0.080515859	0.80515859	0.725270352
14	0.944±0.04	0.224	0.084698501	0.84698501	0.762946734

Table C.3 Effect of various initial enzyme concentrations by using *Kempas* adsorbent

Conc. (g/L)	OD sample	OD decrease	Glucose concentration (g/L)	Enzyme activity (U/L)	Enzyme concentration (g/L)
0	0.532±0.07	0	0	0	0
2	0.533±0.03	0.001	0.00697107	0.0697107	0.062793969
4	0.534±0.04	0.002	0.00731962	0.0731962	0.065933636
6	0.535±0.06	0.003	0.007668177	0.07668177	0.069073366
8	0.844±0.06	0.312	0.115371209	1.15371209	1.0392402
10	0.847±0.02	0.315	0.11641687	1.1641687	1.0486593
12	0.872±0.05	0.34	0.125130707	1.25130707	1.127151757
14	0.901±0.02	0.369	0.1352387	1.352387	1.218202486

APPENDIX D

(A) LANGMUIR ISOTHERM

Table D.1 Data for Langmuir isotherm (*Keruing* adsorbent)

Conc. (g/L)	OD sample	OD decrease	glucose conc (g/L)	enzyme activity (U/L)	CU(g/L)	CB (g/L)	CU (mg/L)	CB (mg/L)	1/CU	1/CB
0	0.54±0.04	0	0	0	0	0	0	0	0	0
2	0.555±0.04	0.015	0.011851	0.118508	0.10675	1.89325	0.000107	0.001893	9367.704	528.1922
4	0.588±0.04	0.048	0.023353	0.233531	0.21036	3.78964	0.00021	0.00379	4753.76	263.8773
6	0.599±0.06	0.059	0.027187	0.271872	0.244896	5.755104	0.000245	0.005755	4083.358	173.7588
8	0.664±0.05	0.124	0.049843	0.498432	0.448977	7.551023	0.000449	0.007551	2227.286	132.4324
10	0.711±0.02	0.171	0.066225	0.662252	0.596543	9.403457	0.000597	0.009403	1676.326	106.3439
12	0.892±0.04	0.352	0.129313	1.293133	1.164828	10.83517	0.001165	0.010835	858.4957	92.29203
14	0.891±0.02	0.351	0.128965	1.289648	1.161688	12.83831	0.001162	0.012838	860.816	77.89186

Table D.2 Data for Langmuir isotherm (*Meranti* adsorbent)

Conc. (g/L)	OD sample	OD decrease	glucose conc (g/L)	enzyme activity (U/L)	CU (g/L)	CB (g/L)	CU (mg/L)	CB (mg/L)	1/CU	1/CB
0	0.72±0.05	0	0	0	0	0	0	0	0	0
2	0.745±0.05	0.025	0.015336	0.153364	0.138147	1.861853	0.001381	0.001862	723.868	537.0993
4	0.78±0.02	0.06	0.027536	0.275357	0.248036	3.751964	0.00248	0.003752	403.167	266.5271
6	0.871±0.07	0.151	0.059254	0.592541	0.533749	5.466251	0.005337	0.005466	187.3541	182.9407
8	0.894±0.04	0.174	0.067271	0.672708	0.605962	7.394038	0.00606	0.007394	165.0269	135.2441
10	0.92±0.03	0.2	0.076333	0.763332	0.687594	9.312406	0.006876	0.009312	145.4347	107.3836
12	0.922±0.03	0.202	0.07703	0.770303	0.693873	11.30613	0.006939	0.011306	144.1185	88.44762
14	0.921±0.02	0.201	0.076682	0.766818	0.690734	13.30927	0.006907	0.013309	144.7736	75.13562

Table D.3 Data for Langmuir isotherm (*Kempas* adsorbent)

Conc. (g/L)	OD sample	OD decrease	glucose conc. (g/L)	enzyme activity (U/L)	CU (g/L)	CB (g/L)	CU (mg/L)	CB (mg/L)	1/CU	1/CB
0	0.532±0.03	0	0	0	0	0	0	0	0	0
2	0.643±0.03	0.111	0.045312	0.45312	0.408161	1.591839	0.000408	0.001592	2450.015	628.2042
4	0.686±0.02	0.154	0.0603	0.602998	0.543168	3.456832	0.000543	0.003457	1841.052	289.2822
6	0.704±0.03	0.172	0.066574	0.665737	0.599682	5.400318	0.0006	0.0054	1667.549	185.1743
8	0.821±0.04	0.289	0.107354	1.073545	0.967027	7.032973	0.000967	0.007033	1034.097	142.1874
10	0.99±0.08	0.458	0.16626	1.6626	1.497636	8.502364	0.001498	0.008502	667.7189	117.6144
12	0.992±0.05	0.46	0.166957	1.669571	1.503916	10.49608	0.001504	0.010496	664.9309	95.27362
14	0.992±0.05	0.46	0.166957	1.669571	1.503916	12.49608	0.001504	0.012496	664.9309	80.02507

(B) FREUNDLICH ISOTHERM

Table D.4 Data for Freundlich isotherm (*Keruing* adsorbent)

Conc (g/L)	OD sample	OD decrease	glucose conc. (g/L)	enzyme activity (U/L)	CU (g/L)	CB (g/L)	CU (mg/L)	CB (mg/L)	Log CU	Log CB
0	0.54±0.0.4	0	0	0	0	0	0	0	0	0
2	0.555±0.04	0.015	0.011851	0.118508	0.10675	1.89325	0.000107	0.001893	-3.97163	-2.72279
4	0.588±0.04	0.048	0.023353	0.233531	0.21036	3.78964	0.00021	0.00379	-3.67704	-2.4214
6	0.599±0.06	0.059	0.027187	0.271872	0.244896	5.755104	0.000245	0.005755	-3.61102	-2.23995
8	0.664±0.05	0.124	0.049843	0.498432	0.448977	7.551023	0.000449	0.007551	-3.34778	-2.12199
10	0.711±0.02	0.171	0.066225	0.662252	0.596543	9.403457	0.000597	0.009403	-3.22436	-2.02671
12	0.892±0.04	0.352	0.129313	1.293133	1.164828	10.83517	0.001165	0.010835	-2.93374	-1.96516
14	0.891±0.02	0.351	0.128965	1.289648	1.161688	12.83831	0.001162	0.012838	-2.93491	-1.89149

Table D.5 Data for Freundlich isotherm (*Meranti* adsorbrnt)

Conc. (g/L)	OD sample	OD decrease	glucose conc. (g/L)	enzyme activity (U/L)	CU (g/L)	CB (g/L)	CU (mg/L)	CB (mg/L)	Log CU	Log CB
0	0.72±0.05	0	0	0	0	0	0	0	0	0
2	0.745±0.05	0.025	0.015336	0.153364	0.138147	1.861853	0.001381	0.001862	-2.85966	-2.73005
4	0.78±0.02	0.06	0.027536	0.275357	0.248036	3.751964	0.00248	0.003752	-2.60548	-2.42574
6	0.871±0.07	0.151	0.059254	0.592541	0.533749	5.466251	0.005337	0.005466	-2.27266	-2.26231
8	0.894±0.04	0.174	0.067271	0.672708	0.605962	7.394038	0.00606	0.007394	-2.21755	-2.13112
10	0.92±0.03	0.2	0.076333	0.763332	0.687594	9.312406	0.006876	0.009312	-2.16267	-2.03094
12	0.922±0.03	0.202	0.07703	0.770303	0.693873	11.30613	0.006939	0.011306	-2.15872	-1.94669
14	0.921±0.02	0.201	0.076682	0.766818	0.690734	13.30927	0.006907	0.013309	-2.16069	-1.87585

Table D.6 Data for Freundlich isotherm (*Kempas* adsorbent)

Conc. (g/L)	OD sample	OD decrease	glucose conc. (g/L)	enzyme activity (U/L)	CU (g/L)	CB (g/L)	CU (mg/L)	CB (mg/L)	Log CU	Log CB
0	0.532±0.03	0	0	0	0	0	0	0	0	0
2	0.643±0.03	0.111	0.045312	0.45312	0.408161	1.591839	0.000408	0.001592	-3.38917	-2.7981
4	0.686±0.02	0.154	0.0603	0.602998	0.543168	3.456832	0.000543	0.003457	-3.26507	-2.46132
6	0.704±0.03	0.172	0.066574	0.665737	0.599682	5.400318	0.0006	0.0054	-3.22208	-2.26758
8	0.821±0.04	0.289	0.107354	1.073545	0.967027	7.032973	0.000967	0.007033	-3.01456	-2.15286
10	0.99±0.08	0.458	0.16626	1.6626	1.497636	8.502364	0.001498	0.008502	-2.82459	-2.07046
12	0.992±0.05	0.46	0.166957	1.669571	1.503916	10.49608	0.001504	0.010496	-2.82278	-1.97897
14	0.992±0.05	0.46	0.166957	1.669571	1.503916	12.49608	0.001504	0.012496	-2.82278	-1.90323