Enzymatic Retting of Kenaf Bast Fiber: Effect of Enzyme Combination, Retting Time and Solid to Volume Ratio

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research except for the quotations and citations which have been properly acknowledged. I also declare that it has not been previously, and is not concurrently submitted in candidature of any other degree to any universities or institutions.

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LIST OF ABBREVIATIONS

DTG	Derivative Thermogravimetric Analysis
EDTA	Ethylenediaminetetraacetic acid
FTIR	Fourier Transform Infrared Spectroscopy
G	Guaiacyl
GC-MS	Gas Chromatography Mass Spectrometry
LKTN	Malaysian Kenaf and Tobacco
NEAC	National Economic Advisory Council
NP9	Nonylphenol Ethoxylate
MARDI	Malaysia Agricultural Research and Development Institute
RT	Retention Time
S	Syringyl
TGA	Thermogravimetric Analysis

LIST OF SYMBOLS

g	Gram
h	Hour
mg	Milligram
mL	Milliliter
pH	Acidity
ºC	Celsius
%	Percentage

Pelembutan Enzim Gentian Basta Kenaf: Kesan Kombinasi Enzim, Masa Pelembutan dan Nisbah Kenaf kepada Ispadu Air

ABSTRAK

Secara tradisinya dalam menghasilkan gentian basta kenaf telah menggunakan proses pelembutan air, pelembutan kimia dan pelembutan menggunakan mekanikal sebelum pelembutan enzim diperkenalkan. Proses pelembutan air memerlukan 14 hingga 28 hari untuk meleraikan bahan pektik, hemiselulosa, dan lignin. Untuk proses pelembutan kimia dan mekanikal, gentian serat yang dihasilkan berkualiti rendah di mana proses itu boleh merosakkan kualiti serat kenaf. Manakala, pelembutan enzim didapati dapat menghasilkan fiber yang berkualiti tinggi dalam masa yang singkat. Dengan menggunakan proses pelembutan enzim, gentian serat kenaf telah direndam dalam larutan enzim 100% pektinase dan gabungan enzim pektinase, xylanse, dan selulosa dengan munggunakan nisbah peratusan sebanyak 60: 20: 20. Ribbon kenaf hijau menghasilkan variasi yang ketara berikutan rawatan pada kepekatan larutan enzim yang berlainan (10, 30, 50, 70, dan 100 mL), masa rendaman (24, 48, 72 dan 96 jam), dan dengan interaksi antara kedua-dua parameter. Hasil rendaman pada 24 jam bagi proses rendaman enzim menggunakan kombinasi enzim pektinase, xylanase, dan selulosa menghasilkan fiber kenaf yang berkualiti dengan lebih putih dan bersih. Spektroskopi transformasi infra merah Fourier (FTIR) digunakan untuk menyiasat penurunan lignin dan hemiselulosa dalam proses rendam enzim. Manakala, Thermogravimetric (TGA) menunjukkan bahawa serat kenaf yang diperoleh daripada hasil rendaman gabungan enzim pektinase, xylanase, dan selulosa selama 24 jam menunjukan keunggulan dalam kestabilan terma. Selain itu, analisa sebatian residu dalam serat kenaf selepas proses rendaman enzim dijalankan menggunakan spektrometri jisim-kromatografi gas (GC-MS). Dua sampel residu daripada rendaman gabungan enzim yang berbeza telah di analisis menggunakan GC-MS untuk mencari kandungan lignin tertinggi yang dikeluarkan yang membuktikan sebatian tersebut mengeluarkan lignin dan pektin. Anilisis menunjukan bahawa serat kenaf daripada proses rendaman gabungan enzim pektinase, xylanase, dan selulosa adalah penyingkiran lignin tertinggi. Oleh itu, proses rendaman enzimatik ini boleh menghasilakan fiber dengan serat berkualiti tinggi dalam masa yang singkat berbanding menggunakan rawatan kimia yang boleh merosakkan kualiti fiber.

Kata kunci: Gentian Basta Kenaf, Pelembutan enzim, Pencirian gentian, Bahan pektik, Enzim

Enzymatic Retting of Kenaf Bast Fiber: Effect of Enzyme Combination, Retting Time and Solid to Water Volume Ratio

ABSTRACT

Traditionally in producing kenaf bast fibers was used water retting, chemical retting, mechanical retting before enzymatic retting are introduced. Water retting requires 14 to 28 days to degrade pectic materials, hemicellulose, and lignin. For chemical and mechanical retting, the fibers produced are very low quality fiber where the process can harm the quality of fiber. While, for enzymatic retting was found that can produce high quality fiber in shorter time.For this study, by using enzymatic retting, kenaf bast fibers were retted from enzyme solution of 100% pectinase and combination of enzyme pectinase, xylanase, and cellulase by using ratio of 60: 20: 20. The green kenaf ribbon retted significant variations following treatment at different enzyme solution concentration (10, 30, 50, 70, and 100 mL), retting time (24, 48, 72 and 96 hours), and with interaction between both parameters. It was that a retting time of 24 hours from retting process in enzyme combinations of pectinase, xylanase, and cellulase with enzyme concentration of 100 mL are produced good quality kenaf bast fiber in term of whiteness and clean fiber. Fourier transform infrared (FTIR) spectroscopy were used to investigate the decreased of lignin and hemicellulose in the retting process. While, Thermogravimetric (TGA) indicated that kenaf bast fiber that retted from enzyme combination of pectinase, xylanases, and cellulase for 24 hours displayed a superior thermal stability. Moreover, chemical anylsis of kenaf bast fibers after enzymatic retting process are conducted using gas chromatography-mass spectrometry (GC-MS). Two sample of residue from different combination enzyme was analyzed by using GC-MS to investigate the highest compound in residue are found that removed that prove on removing of lignin and pectin. The analysis was shown that fiber from retted process by using enzyme combination of pectinase, xylanases, and cellulase is highest compound of residue removal. Thus, the enzymatic retting process can produce fiber with high quality fiber in shorter time compared to chemical treatment that can harm the quality of fiber.

Keyword: Kenaf Bast Fiber, Enzymatic Retting, Fiber characterizations, Pectic materials, Enzyme

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Kenaf plant or scientifically known as *Hibiscus cannabinus L.* is essentially exist to Africa where it has been planted for use in ropes and animal consumption for around 4,000 years. Kenaf was founded from scientific reseacrh is a plant that was categorized in the *Hibiscus* group in the family of *Malvacea.* The kenaf nature of the tropical plants is good in water-holding capacity for the best progress on land and is drought. Kenaf is ready for harvesting after reach the height between ranges of 3.7 to 4.3 meters within 4 weeks. This kenaf species only produce flower for one day. The kenaf stem having separated into two parts which are the bark or bast and the stem. In comparison, the long fibers can be obtained from the bark or bast meanwhile short fibers are obtained from the stem of kenaf or known as core. The ratio of the bast that can be produced from the whole stem weight is 40% and the length of the bast is usually 2.6 mm after processed. Kenaf is suitable for planted on various types of soil but the best producing of yield is

grown during the rainy season (Sisti, Totaro, Vannini and Celli, 2018). Kenaf also can produce yields in the range of 8 to 12 metric tons of dry stem per hectare and large-scale cultivation is needed in industrial operation from seeding to harvesting kenaf. Nowadays, in real life, kenaf bast fibers get high demand and mainly having achievable use as commercial fiber crop for the manufacture such as automotive panels, composite boards, geotextiles and insulation mats attributable to the high quality of fiber and the fast growth of kenaf plant (Harusmas, 2011; Khalil, Yusra, Bhat, & Jawaid, 2010).

Kenaf can be cooperated as main economic activity in the agricultural sector, which will be the main axis of the East Coast Economic Region (ECER). According to the ECER master plan, 10 000 hectares of kenaf will be planted in Pahang, Kelantan, and Terengganu by 2020. At present, kenaf has been planted in the district of Bachok and Pasir Puteh in Kelantan where the BRIS (beach ridges interspersed with swales) soil is abundant. Kelantan and Terengganu get seeds that were supplied from kenaf plantation in Kedah and Perlis to be planted. Table 1.1 shows that the plantation of kenaf for Kedah, Perlis, Kelantan and Terengganu are increasing until year 2008 and drop on 2009 but there is still achievable for the development of the kenaf industry in Malaysia (Kamaruddin & Othman, 2012).

Table 1.1: Kenaf planted area by states-hectare

State/ Year	2006	2007	2008	2009
Kelantan	41	200	283	228
Terengganu	70	85	180	115
Kedah	51	84.5	126	885
Perlis	8	67.5	101	46.5
TOTAL	170	437	690	478

(Kamaruddin & Othman, 2012)

Kenaf is non-food crop or known as industrial crop where the crops have high potential for cultivation in a tropical climate. Considerable research has been conducted on the kenaf retting process for the last 10 years and still no bio-retting process for bast fiber has been commercialized in Malaysia. In 1999, NEAC with the full name is National Economic Action Council and now known as National Economic Advisory Council was initiated way back Kenaf project in Malaysia that was under leadership of the previous Sabah Chief Minister YAB Datuk Musa Aman. NEAC was formed a govern committee to find out of any potential on growing kenaf in Malaysia as another industrial crop (Basri, Abdu, Junejo, Hamid, & Ahmed, 2014).

Kenaf has been identified as a potential crop to replace tobacco growing area located where a reduction in import duties for tobacco. NEAC was collaborating with MARDI which is Malaysia Agricultural Research and Development Institute on year 2000 to coordinate a fast track research and development kenaf project. From the collaboration, MARDI had successfully found variety screening, agronomic practices for kenaf cultivation, retting, fiber processing, harvesting and mechanization, and some downstream applications in production of animal feed and bio composite (Basri et al., 2014).

Besides that, in the year 2000, kenaf project has been introduced to the HASB which is Harusmas Agro Sdn. Bhd based in Sabah, Malaysia. This kenaf project was aimed on the spreading out of agriculture industry and their effort on developing the green economy that was presented to the leadership of previous Sabah Chief Minister. By the year 2020, Malaysia itself has committed to reduce 40% of its greenhouse gas

emission. Malaysia was launched a Green Technology program with the objective on greenhouse issues by developing biodegradable of kenaf since 1999 (Harusmas, 2011).

Research on the use of enzymes for the fiber retting process has been carried out by several researchers but it is not ready yet for the commercialization due to cost factors and impractical to be implemented at farmer level. The investigation and evaluation of enzymes combination and improvement of chemical process was highlighted in this study. Industry of kenaf still struggle with the quality of kenaf fiber produce by midstream industry using mechanical processing and water retting technique even though almost 15 years kenaf was introduced in Malaysia.

A combination of enzyme in retting process is the other method that has been reported to give excellent result as well as water retting process that seems to be the best method to produce high quality fiber in retting process. However, method of enzymatic retting requires high cost in production and complicated in production that cannot be applied in the rural areas. With the advancement of technology, it is expected that lower cost and more practical methods can be developed to generate high quality of fiber with less environmental pollution (Paridah et al., 2011).

1.2 Problem Statement

In production of long and high quality of fiber, retting process is the best method used for processing. However, retting is the major problem in applying high grade kenaf bast fiber that separate kenaf bast and core fibers. Some conventional methods are used

in removing the long bast fiber from the core by using dew method, water retting method and enzymatic retting method. Both dew and water retting methods require a long time period between 14 to 28 days to degrade the pectic materials, lignin, and hemicellulases. Besides taking long time period on retting process, it is also causing water pollution despite the fibers that are produced by using water retting method can be of superiority fiber. In water retting process, the outermost layer of bast fiber are breaking down, and thus provoking an increased absorption of moisture and the development of a pectinolytical bacterial community. Type of water and the temperature of water will affect the duration of the treatment as well as any bacterial inoculum (Sisti, Totaro, Vannnini, & Celli, 2018).

In chemical retting process for kenaf bast fiber production will produce high quality fiber where the fibers are much cleaner. However, it will effect on the strength of fiber where is chemical retting process will cause low tensile strength on fiber. This is because chemical retting processes dealing with chemical product as chemical product is strong solution that can cause harmful to fiber. Enzymatic retting obviously shown its benefits by having considerably shorter retting time and acceptable quality fibers differ to other retting methods. However, it is quite costly (Paridah, Amel, Syeed, Saiful, & Zakiah, 2011).

From this study, the optimum parameters for bio-retting process were determined to ensure the achievability of the retting process on kenaf. The aims of this study is to develop the optimum enzymes combinations that can reduce the retting and processing cost besides producing high quality of kenaf bast fiber.

1.4 Objectives

The objectives of this study are:

i. To investigate the optimum parameters of bio-retting process which are enzymes combination, retting time and solid to water volume ratio.

ii. To study the quality of fiber produced from kenaf bio-retting process.

iii. To characterize the fiber produced from enzymatic retting.

1.5 Scope of Study

This study is designed to determine the optimum parameters for bio-retting process on kenaf industry. The optimization of the enzymes combination were determined during kenaf retting process on initial pH, retting time and solid to water volume ratio. By using enzymatic retting method, it will help on less environmental pollution as well as will producing higher fiber quality. Kenaf samples were harvested from Pasir Putih, Kelantan. The middle part of kenaf plant was chosen for the experimental works. There are two combinations of enzymes that were used for this study based on the percentage ratio of enzyme such as pectinase that are 100% used and combination of enzymes between pectinase, xylanase, and cellulase with percentage ratio are 60: 20: 20. These entire enzymes were tested on constant parameter which are pH 3.5 at 50 ºC with aerobic condition. While, for various parameter were tested on

retting time and solid to water volume ratio or enzyme solution concentration. Physical characteristic of kenaf bast fiber produced from enzymatic retting were determined using Fourier transforms infrared spectroscopy (FTIR), Thermogravimetric (TGA), and Gas chromatography-mass spectrometry (GC-MS).

1.6 Significant of Study

The result of this study will provide the basis for developing better retting of kenaf bast fiber to help improve the eco-efficiency of Malaysia where is certified organic and 100% natural to prevent pollution. This study is important especially to emphasize on green technology. So, it will reduce the uses of chemical product that can cause harmful to the environment.

The result from this study is able to prove that there is a need for the enzymatic retting to reduce chemical use besides to produce high grade with low cost of bast fiber to the industrial of kenaf. This study provides potential bio-retting method to produce high quality fiber.

CHAPTER 2

LITERITURE REVIEW

2.1 Kenaf

Kenaf is an *herbaceous* or *hibiscus cannabinus* annual dicotyledonous plant of the family Malvaceae that can be described as part of the hibiscus family that is suitable growth in warm-temperature, subtropical, and tropical areas. Kenaf is sustainable and environmentally safe because it is biodegradable and renewable crop that can be the main source of fiber for composites and different commercial utility. In addition, the production of yield is increasing because of the description of kenaf on effective growing that allows two harvest times annually (Majid et al., 2013; Wong, Zuhainis, Rosfarizan, & Paridah, 2016). The specialty of kenaf differ to the traditional reinforcing fibers such as carbon and glass that have low density, affordable, very little abrasion during processing, high toughness, and acceptable specific mechanical properties made it has valuable properties for medium density fiber board (MDF) production, pulp and paper production and other composites (Majid et al., 2013; Paridah et al., 2011).

Kenaf is a fibrous plant where the stem part of kenaf is composed of an outer bast fiber (25-40%) and inner core fiber (60-75%). An outer bast fiber will produce high quality pulp differ to an inner bast fiber that will produce low quality pulp (Khalil et al., 2010; Majid et al., 2013). Khalil (2010) also proved that inner core fiber is produced low quality pulps differ to outer bast fiber. The outer bast fiber is producing high quality pulp from the kenaf stem. Kenaf bast fiber can be obtained from the retting process where it goes a biochemical process that disconnected biopolymers such as cellulose, hemicelluloses, lignin, pectin, and other mucilaginous substances with an apparently liquid state of gummy matter that are found in the cuticle and epidermis of the plant itself. All the substances grip the adjacent bundles together and the retting process will remove the non-fibrous substances then the fibers can be produced. From the retting method either water retting, chemical retting or by using enzymatic retting it will be easier on separating the stem into bark and core (Wong et al., 2016).

Kenaf also used in processing of auto parts, ethanol production, potting soils, decorative plates, and adsorbent (Duan et al., 2018). Researchers have found that kenaf properties of woody core material with high absorbency can be used as an absorbent. With the higher absorbency, kenaf also can serve as animal bedding and broiler litter as well as possible to be a bulking agent for sewage sludge composting. In paper and pulp industrial, fibers from kenaf also useful as a virgin fiber for increasing recycled paper strength and paper quality (Hossain, Hanafi, Jol, & Hazandy, 2011).

2.2 Bast fiber

Fiber quality is very important in many industries, especially textile industry. Quality of fiber will be affected from the non-cellulosic substances that will also influence to the recovery, isolation, purification and polishing process in production of product. For that reasons, non-cellulosic materials need to be removed in order to produce superiority of kenaf bast fiber (Wong et al., 2016). Bast fibers are the fiber getting from the outer cell layers of the stems of various plants. In various proportions, bast fibers usually associated with of lignin, hemicellulose and cellulase. Kenaf is a plant fiber collected from the phloem or bast surrounding the stem of certain dicotyledonous plants containing of 53% - 66% cellulose, 8 – 16% lignin, and 23 - 25% pectin (Duan et al., 2018).

Figure 2.1 shows the kenaf stem contains the bast fiber. The outer fiber is called as bast. Kenaf core fiber will be coiled by the pectin-bound bast fiber that was exposed kenaf bast from the furry part. Wax, pectin, water soluble, and mineral compounds are other compounds are shown in expansion to the primary factors. Pectin benefits as glue to form a bundle of fiber to non-fibrous tissues (Sisti et al., 2018). Bast fibers are suitable to be used in manufacturing such as carpets and mats, brushes, textiles, mattresses, ropes and nets, as well as on paper and board materials. Bast fiber have their own advantages such as low density, low cost, acceptable specific strength properties, high toughness, improved energy recovery, carbon dioxide sequestration, and biodegradability differ to the fibers reinforced traditionally such as carbon and glass

(Paridah et al., 2011). In the production of eco-friendly textiles products, the bast fibers provide the gain of biodegradability and renewability raw material. Kenaf fibers have a long staple that is suitable for production of high quality textiles with very fine and strong yarn can be spun. Bast fibers are simple process on producing fiber (Ayadi et al., 2016).

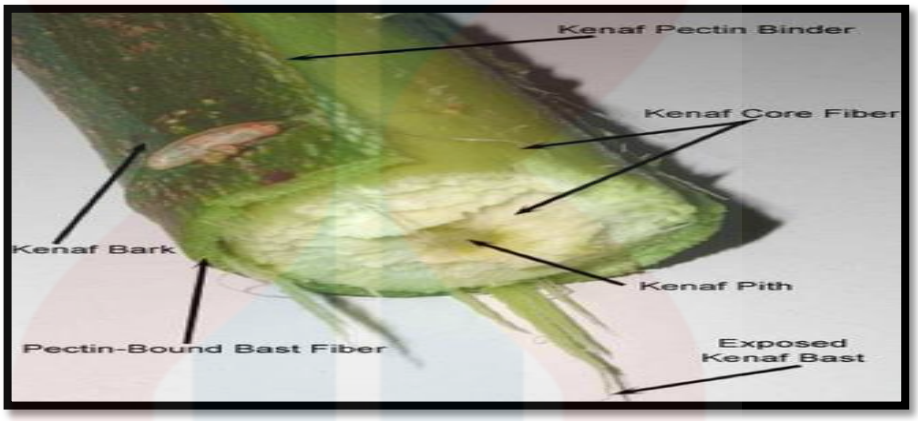


Figure 2.1: Kenaf stem (Industri, 2014)

2.3 Retting process

Retting process is a process involving extraction process of fiber bundles from the non-fibrous tissues on the harvested stem. This extraction process also known as degumming process. It is a microbial process that burst apart the chemical bonds such as non-cellulosic material that attached the stem together to fibers and allows releasing and detachment of the individual bast fibers from the core fiber (Paridah et al., 2011). Several retting methods are still applied and widely used such as dew retting and water retting (Sisti et al., 2018). The fleshy part of the stem that is exposed to water will be

rotted during retting process. The chemical bond is breakdown the pectinase substances that bind the fiber together with the stems and allows detachment of the bast fiber from the core fiber by a microbial process or known as retting process.

The fiber that was bind by the pectinase substances with different plant tissues such as non-fibrous tissues will be forming jelly and degenerated by microorganism when the stem are immersed into the water for 2 to 3 weeks after harvesting process. The retting condition and retting time during retting process is greatly influencing a quality of fiber (Paridah et al., 2011). Figure 2.2 shows a rationalization of all the retting treatments. There are five methods that are usually used including microbiological such (dew retting and water retting), mechanical retting, chemical retting, physical retting and enzymatic retting. All these techniques are still not yet practiced on an industrial scale even though is proven that more obviously benefits from these techniques.

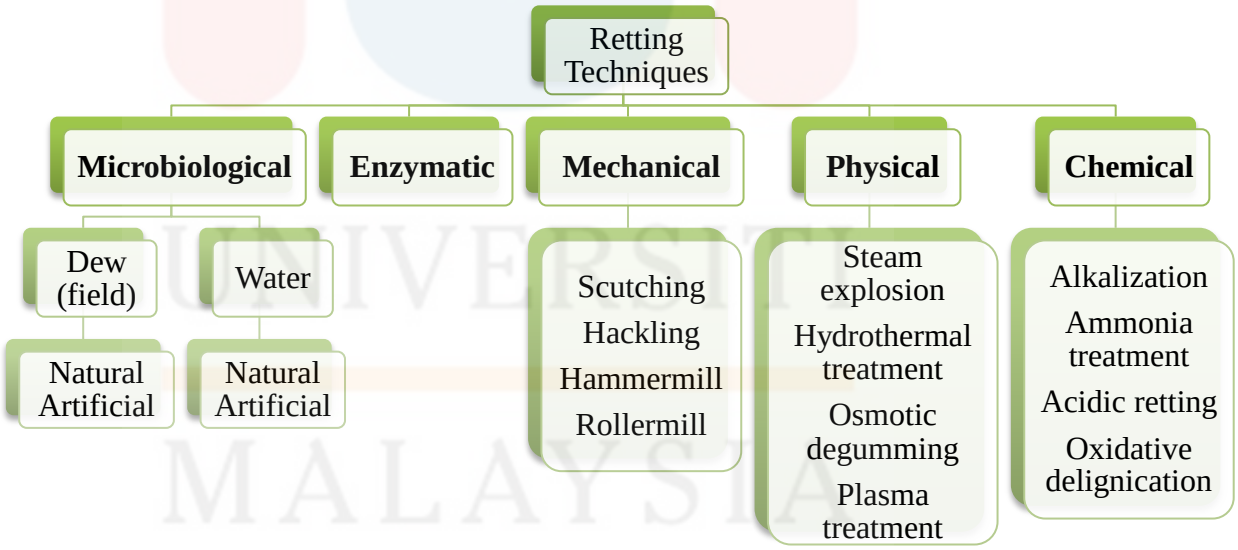


Figure 2.2: Retting Technique (Sisti et al., 2018)

In the chemical degumming or known as chemical retting process will produce of high temperature, high pressure and caustic soda that will give negative impact to the environmental pollution and corrupted fiber (Duan et al., 2018). In the production of long fiber the duration of production is a dominant problem where long period of natural degradation is needed for 14 to 28 days to complete the retting process (Paridah et al., 2011).

2.4 Water retting

Water retting method is part of the conventional methods that are widely being used for 50 years ago to produce high-quality fibers by soaking in freshwater such as rivers or pond and nowadays in large tanks (Sisti et al., 2018; Wong et al., 2016). The most fiber crop straws need 7 to 14 days to fulfill the reaction of this retting process. The central stalk portion will be absorbing water to dissolve the outer layer of bast fiber as well as to extend absorption of moisture and the improvement or pectinolytical bacterial community. The proper movements of water carry away the bacteria and thus affect the fermentation process by slowing down that process. All the bundles of stem will be fully immersed and covered with straw and stone to make sure all the bundles of stem are fully immersed. Within 2 weeks, the outer layer of the stem will start unconnected from the fiber and called as stocks. Then, the stocks from the bundles are taken out of the water and ready for drying process. This retting method uses large volumes of water. Then, the used of water must be treated before being discharged (Sisti et al., 2018).The

major imperfection of this water retting process are water pollution and strong odor resulting from this retting process (Wong et al., 2016). The alternative on the retting method by using on enzymatic retting that is more environmental friendly will be quite potential matter to overcome water pollution.

2.5 Enzymatic Retting

Enzymatic retting is the fastest and safest process of retting where the process of separating fibers from the stem can be done within few hours. This enzymatic retting technique has been exhibited as promising method on replacement for traditional retting techniques for producing high-quality fibers in easily controllable condition, time-saving, ecology friendliness, and convenient function (Sisti et al., 2018; Wong et al., 2016). This technique is especially achieved by the pectic enzymes made by bacterium. In the process of retting, the bacteria reproduce and generate extracellular pectinases that will dissolve the pectin to release bast fiber from the surrounding cortex. Enzymes can be commercially produced by the increasingly sophisticated biotechnology devices and enzymatic retting process as more prevalent choice in producing long fibers (Paridah et al., 2011). By using the enzymatic retting technique, flax stems or bast fiber stem are kept at suitable temperature of enzyme where the enzyme used will degrade the cell wall of plant stem. In the best possible way, enzymatic retting will break down the pectin and hemicellulose of plant cell due to reaction with water without harm the cellulose fibers.

Furthermore, in producing of bast fiber by using specific enzymes or a specific enzymes combination, systematically retted fibers will produce (Evans, Akin, & Foulk, 2002).

Enzymes can help in different stages of bast fiber processing like retting, cottonization, improving pretreatment and others. Many intentions have been described towards by considering the degradation of hemicellulose, lignin, and a group of polysaccharides in plant cell walls through enzymatic degradation due to the long retting time issues. Many researchers found that pectinase has high possibility in effectiveness of enzymatic retting without damage to the fibers and producing high grade fiber quality.

A conventional feature of enzymes is their selectivity. Enzyme bio catalysis effective on low concentration and is characterized by using moderate conditions of process such as temperature, pH and humidity. The effectiveness of enzymes appearing in retting method is pectinases, xylanases and cellulases. Pectinase are the principle of enzymes hired for retting process, with a view to free the fibers from different tissues (Kozlowski et al., 2006).

Enzymatic retting is applied method at the moment on removing and release fibers from kenaf bast fiber that can be credible alternative to water retting on reducing the water pollution as well as getting high quality of fiber. Enzyme technology has been recognized by the world for its environmental friendly nature with a focused and specific performance. By using enzymatic retting method, it can be more controllable as well as can reduce the production of effluents during retting process. The synergistic effect between enzyme from different extracellular enzymes are used in this retting process has

caused biodegradation of pectin (Wong et al., 2016). From the previous research, enzymatic retting are prove that can produce high quality fiber with respect to the observation on high increased strength, yarn count and can released number of imperfections of the yarn after spinning rather than traditional retting process (Kozlowski et al., 2006).

According to the previous study, the temperature plays important factor in enzymatic retting whereas the enzyme significantly reflect and response to the temperature level. The optimum temperature is 50 ºC suggested to activate the enzyme activities. Then, the optimum pH was used constant pH on 3.5. The best combinations of enzymes also were study in this experiment to find the best combination of enzyme in producing high quality of bast fiber.

By the combination of enzyme was shown the effectiveness on retting time and quality of fiber. Enzymes are extremely efficient and highly specific biocatalysts (Hoondal, Tiwari, Tewari, Dahiya, & Beg, 2002). Microbial pectolysis is important in the decomposition of plant by breaking the pectin polymer. During degradation, the plant polysaccharides can be attacked by several enzymes. However, this process is being initiated by pectic enzymes, as it is the most readily available. Hence this type of enzyme has been used by many researchers for retting of plant fibers without damage to the fibers (Hongqin & Chongwen, 2010; Hoondal et al., 2002)

2.6 Pectinase

Pectic substances are a group of polysaccharides in plants cell wall with high molecular weight that are widespread in the plant kingdom. This polysaccharide consists of large number of glucose units covalently linked by glycosidic bonds. A group of polysaccharide is found in the constituents of middle lamella and in the primary and secondary cell walls of plants that made up of galactourinic acid units bound in a long chain. In this pectic produce four main types of substances which are pectinic acids, pectins, protopectins, and pectic acid. Protopectins is considered as water soluble properties and the other three pectic substances are considered either totally or partially soluble in water (Alkorta, Garbisu, Maria, & Serra, 1998). To make it hydrolyzed, a specific enzyme is needed even though these compounds are closely related. *Pseudomonas Bacillus, Erwinia, Clostridium,* and several fungi such as *Streptomyces* are the examples of microorganisms that are involved in pectin degradation. Pectinase is an enzyme involved in collective pectin digestion (Chaundry, 2016).

In the retting process, polygalacturonase and pectin lyase are two types of pectinase enzyme that involved in this process. By using pectinase in enzymatic retting method high strength fiber with consistent quality and varying fineness can be produced then further uses in novel resins or developed for natural fiber agricultural feedstock composites. Hectic polysaccharide of plant tissues are breaking down by using of pectinase that is part of a big group of enzyme (Wong et al., 2016). The use of enzymes with non-toxic and eco-friendly characteristics is rapidly gaining globally recognition as

well as increasingly important requirement for fiber production in reducing pollution (Bernava, Reihmane, & Strazds, 2015). Xylanase is function as on effective reaction for pectinase enzyme reaction on retting process (Yinghua, Xiaolan, Xiqun, & Lu, 2013).

2.7 Xylanase

The enzymatic hydrolysis of plant cell component xylan is called as xylanase. The role of enzymes in the breakdown of xylan was observed by Hopper – Seyler over 100 years ago (Burlacu, Cornea, & Israel-Roming, 2016). Most xylanase from fungi have the optimum pH in range between 4.5 and 5.5. While, it is different to xylanase from actinobacteria are active at pH 6.0 – 7.0. The optimal temperature of 40 – 60 ^{o}C is the active reaction for this single chain glycoprotein. Besides that, the substrate used as xylan affects the optimum temperature and different pH and has its diversity in its structure. Xylan shows a large of polydiversity and polymolecularity as well as polysaccharides of plant origin. Xylan is the main of hemicellulose polymer in hardwood and a cereal of plant cell wall that is corresponds to their aspect in a variety of plant species (Pérez, Muñoz-Dorado, de la Rubia, & Martínez, 2002; Ronald, de Varies, & Visser, 2001). All land plant xylans are characterized by a β-1,4 – linked D – xylopyranosyl main chain that carries a variable number of neutral or uronic monosaccharide substituents or short oligosaccharide side chains (Nayak, 2014).

Xylanase are the xylosidic hydrolase usually found in microorganisms like bacteria, fungi, actinomycetes, marine algae, seeds, crustaceans, snails, insects,

protozoans and intestine of termites, which cleave the glycoside bond of xylans forming hemi acetyls and glycans but the main sources for these enzymes are fungi and bacteria. From the studies, xylanases have different characteristics that will give benefits on the further applications (Burlacu et al., 2016; Motta, Andrade, & Santana, 2013). Many useful and commercial product can be produced from xylanases for industrial application where it is high capability on hydrolyze xylan, agro wastes and lignocellulosic. Many products can be produced from xylanases including paper pulp bleaching, bio-ethanol production, baking industries, fruit juice clarifications, beer manufacturing industries, cellophane production, textiles bioprocessing such as rayon, animal fodder industries, chemicals manufacturing such as cellulose ethers and cellulose esters and many others production. By 1980, xylanases was reported the property of bio-bleaching such as bio-bleaching paper pulp (Martins et al., 2018).

Otherwise, from other research it was found that xylanases produced by bacteria and actinomycetes such as *Bacillus sp.*, *Pseudomonas sp.*, and *Streptomyces sp.* that are effective in a comprehensive pH range of 5 – 9 where is xylanases activity occur with the optimum temperature in range of 35ºC to 60ºC (Burlacu et al., 2016). Xylanases also have their own specialty in improving the process of retting of flax fibers before further processed to form linen(Nayak, 2014). In producing bast fiber, also need same concept on retting process of flax fiber. So, xylanases also can be the best enzyme that can be used on retting process of bast fiber from kenaf stem on producing linen.

2.8 Celllulase

During 1970s and 1980s, research has proven that the enzyme is induced bioconversion of lignocellulose to sugars can solved but face with exceedingly challenging. Plant cell wall polysaccharides are the most abundant organic compounds are founded in nature where is covered 90% of the plant cell wall and can be divided into three different group of proteins such as cellulose, hemicellulose and pectin. Cellulose was shown that it was a major constituent of cell wall polysaccharides and contain of linear polymer of β – 1, 4 – linked D – glucose residues. The cellulose polymers are present as ordered structures or known as fibers, and their main function is to ensure the rigidity of the plant cell wall (Ronald et al., 2001). Cellulose makes up about 45% of the dry weight of wood.

However, research on pectinase, cellulase and hemicellulase shown the biotechnological applications in the industry is can be varied such as brewery, animal feed, textile and laundry, food, agriculture, pulp and paper, and wine (Barati & Amiri, 2015). The major component of lignocellulose materials is cellulose that was bind with lignin and hemicellulose. From the reaction of enzyme cellulases with the pure cellulose perfectly the pretreatment should fractionate the cellulose, hemicellulose and lignin. The cell wall structure of kenaf can be described from Figure 2.3. The combinations of cellulases and xylanases was reported that by eliminating xylan and its substituents as well as any low molecular weight lignin fragments related to xylanases can improve the properties of fiber strength which may increase accessibility to cellulase. Whereas, in

facilitating the next pre-bleaching process by xylanases, cellulase has shown the accessibility of xylanases to softwood as mild cellulase pretreatments by increasing the apparent median pore size of the pulp (Chandra et al., 2007).

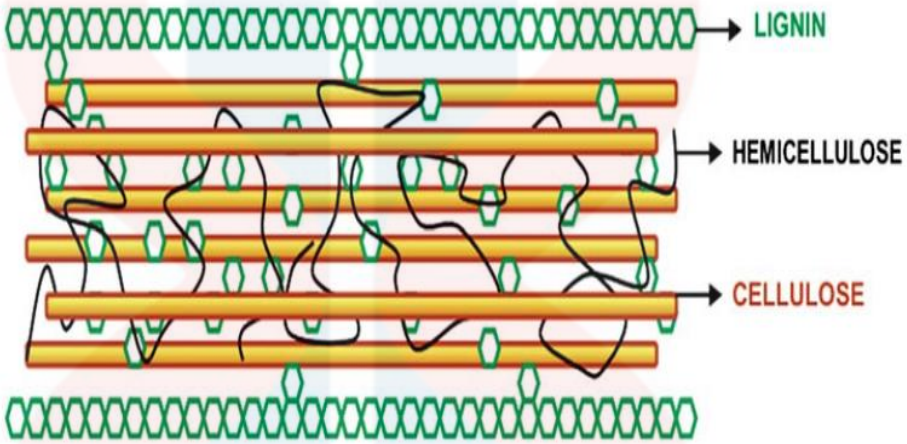


Figure 2.3: Portrayal of cellular complex of lignocellulosic material mainly consisting of lignin, hemicellulose, and cellulose microfibrils. Sources from (Fu et al., 2012).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Kenaf bast fibres were taken from kenaf plantation at Bukit Bunga, Kelantan. Kenaf green ribbons were harvested from kenaf plant with the age range of three to four month.

3.2 Chemicals

Chemicals that were used in this study are acetate acid (1%), EDTA-Surfactant (0.01%), Nonylphenol Ethoxylate (NP-9) as wetting agent (0.01%) and enzyme including pectinase, xylanases and cellulases. Those chemicals were obtained from Malaysian Kenaf and Tobacco (LKTN) and Kenaf Adsorbent Sdn. Bhd.

3.3 Apparatus and equipment

The apparatus that were used in this study are media bottle (100 mL and 250 mL), forceps, beakers (250 mL), beakers (100 mL), dropper, measuring cylinder (100 mL), HPLC vial (1 mL) and pipette.

While, the equipments are water bath, spectrophotometer, weighing balance, pH meter, gas chromatography-mass spectrometry (GC-MS), Fourier transforms infrared spectroscopy (FTIR) and Thermogravimetric analysis (TGA).

3.4 Methodology

3.4.1 Preparation of sample

Stalks of kenaf were harvested and collected from kenaf plantation in Bukit Bunga, Kelantan. Kenaf tree that already achieved the maturity and suitable for harvesting was chosen. The maturity of kenaf was determined based on length and size of kenaf tree. 400 g and above of green ribbon kenaf were taken as a sample size. The samples required for this study are 40 samples. All samples were taken from middle part of kenaf plant where the 0.5 meter from bottom and 0.5 meter tips were cut to avoid bias sample. The entire samples were cut into 10 cm and around 10 g of samples needed for

retting process. All samples were treated with catalyst solution such as acetic acid 1%, EDTA-surfactant and nonylphenol ethoxylate (NP-9) that was used as wetting agent to penetrate the plant bast fibers and remove the water soluble material or heavy metal. Then, the enzymes were added into the solution for enzymatic retting process.

3.4.2 Enzyme combinations study for optimum parameter in bio-retting process

Pectinase is individually used with 100% pectinase without any combination with other enzyme to test the optimum reaction using pectinase in bio retting process. 100% pectinase is representing as 0.1 g of pectinase are used. Second enzyme combinations that were used pectinase, xylanases, and cellulase with the ratio of 60: 20: 20. These ratios represent as 0.06 g pectinase, 0.02 g xylanase, and 0.02 g cellulase. The constant operating conditions used for this study are temperature 50 ºC, pH of 3.5 and anaerobic condition. Different variables on retting time and solid to water volume ratio were studied. For the retting time, ranges that were used are 24, 48, 72, and 96 hours. While, for the solid to water volume ratio parameter, the range that were used are 1:10, 1:30, 1:50, 1:70 and 1:100. It means that, 10g of kenaf samples were tested by immersion process with different amount of volumes of water as well as different enzyme solution concentration. For example, for the ratio 1 to 100 of solid to volume of water ratio represents as 10 g of kenaf with 100 g weight of water. 100 g weight of water is equal to 100 mL volume of water that was used to dilute the enzyme powder. Table

3.1 shows of the range of parameters that were used in this study various parameter. Table 3.2 shows the constant parameters that were used in this study.

Table 3.1: The range of various parameters used to investigate the optimum parameters for bio-retting process

Parameters	**Retting Time (hours)**	**Solid to volume of water ratio (Weight of kenaf : weight of H_2O)**
Pectinase (100%)	24, 48, 72, 96	1:10, 1:30, 1:50, 1:70, 1:100
Pectinase/ xylanase/ cellulase (60%, 20%, 20%)	24, 48, 72, 96	1:10, 1:30, 1:50, 1:70, 1:100

Table 3.2: The constant parameter used to investigate bio-retting process

Parameters	**Description**
Temperature (^oC)	50
pH	3.5
Aeration	Anaerobic

3.4.3 Physical properties and characteristic of kenaf bast fiber

The physical characteristics on kenaf bast fiber were investigated from the colour changes on kenaf bast fiber from the fresh ribbon kenaf after retting process. Besides that, the colour changes on enzyme solution that were used in retting process also investigated. The colour and luster, and fineness and cleanliness of kenaf bast fiber have

been observed. The best quality of kenaf bast fiber by the observation from colour of fiber is the white yellowish colour. The finesses of fiber were observed during the process of washing on separating the peptin from the kenaf green ribbon. The cleanliness of kenaf was proved when the fiber was easily obtained during washing process. The strength of the fiber was also evaluated by stretching a few strands by hand.

The compound present in residue after retting process was measured using gas chromatography-mass spectrometry (GC-MS). Areas of the following peak are shown the different compositions are present in the residue. The amounts of compound from the residue were measured from the GC-MS data (Song & Obendorf, 2006). Otherwise, the Fourier transforms infrared spectroscopy (FTIR) was used to determine the changes in functional groups that caused by treatment. Thermogravimetric (TGA) was used to analyze the thermal stability of kenaf bast fiber were produced.

3.5 Research Flowchart

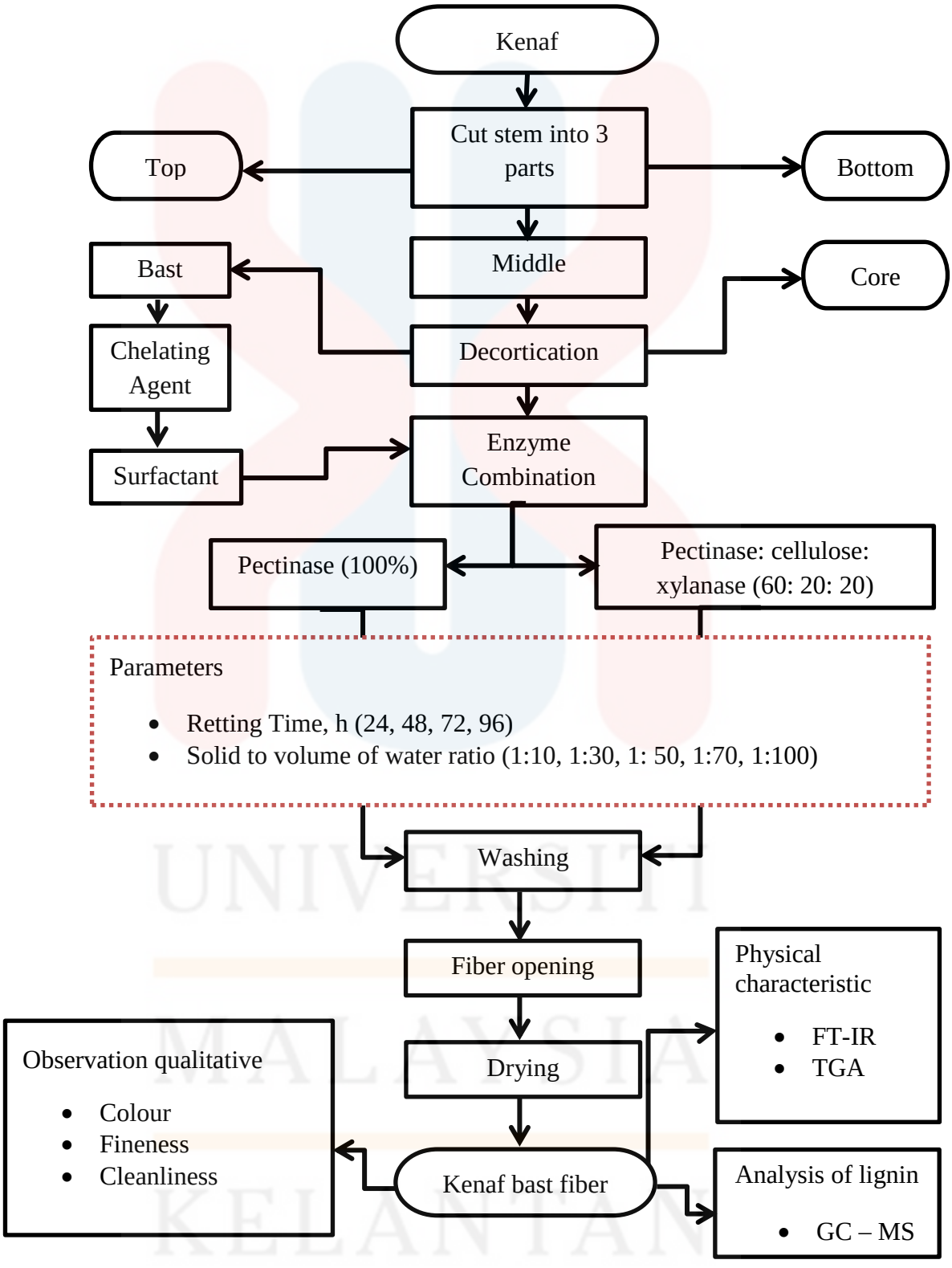


Figure 3.1: Research flow chart of bio-retting process

CHAPTER 4

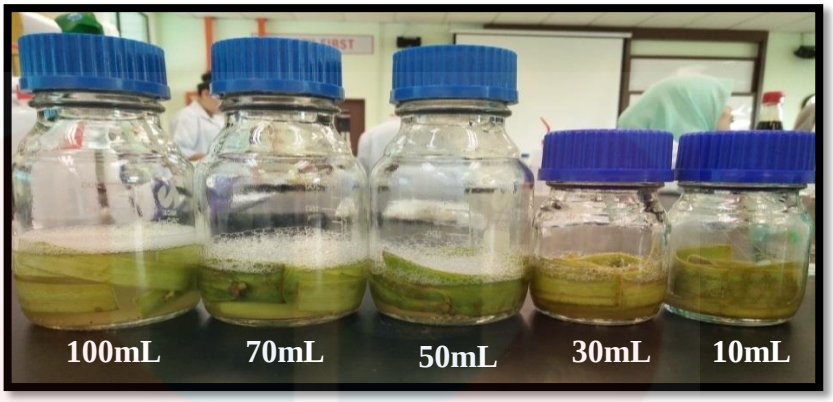
RESULTS AND DISCUSSION

4.1 Physical properties of kenaf bast fiber

4.1.1 Observation results on pectinase (100%) with retting time of 24 hours

From the observation results on physical properties of kenaf bast fiber for retting time process at 24 hours, it was found that the highest volume of enzyme solution with ratio 1:100 of solid to volume of water more easily in removing pectin from the bast fiber. The fiber also is cleaner than the high concentration of enzyme solution. The comparison between both green kenaf ribbon before retted and fiber after retted are shown in Figure 4.1(a) and Figure 4.1(b).

a)



b)

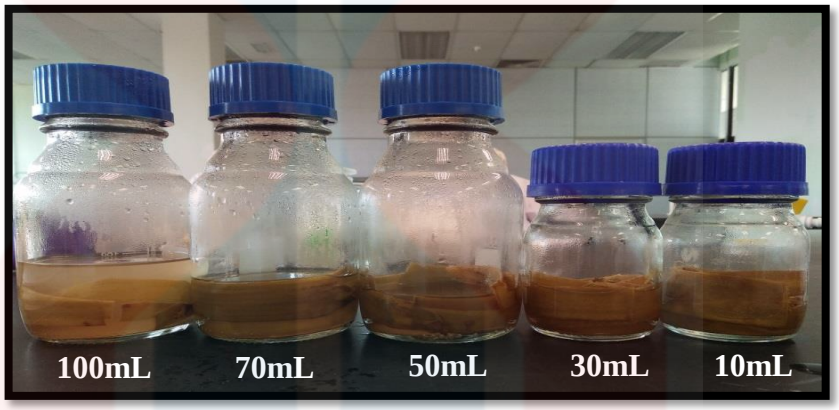


Figure 4.1: Green kenaf ribbon (a) before retted (0 hour) and (b) after retted (24 hours)

Even the enzyme concentration is high but the volume of enzyme solution is lower and the green kenaf ribbon cannot totally immerse in the solution and thus not fully retted. One of the main principles on retting process is totally immersed in the solution. It is important to ensure that the fiber is totally retted. However, in order to ensure the pH of the solution is 3.5, higher volume of acetic acid is used. This is because the enzyme activity is active in condition of pH 3.5. When high volume of acetic acid is used in retting process this can affect the strength of fiber.

Table 4.1 shows the experimental data for retting time of 24 hours. Weight of fiber getting from the ratio 10g of kenaf ribbon with 100mL volume of distilled water

used shown as the lowest weight of fiber are produced. This is because the fiber are produced is more furry and superficial differ to others. Compared to by using of 10g of kenaf ribbon with 10mL of distilled water are used shown that weight of fiber is very high because of the ribbon is not totally immersed and retted. So, less pectin are removed from the kenaf ribbon and the fiber is hardest and roughness.

Table 4.1: Observation experimental result on kenaf bast fiber obtained for retting time of 24 hours

Solid to volume of water ratio	**Weight of kenaf fiber - after retting (g)**	**Final pH of enzyme solution**	**Acetic acid used (mL)**	**Observation (Texture, Fiber whiteness)**
1: 10 (10g of kenaf riben with 10 mL of H_2O)	2.74	3.2	6	Rough, Hardest, Not clean, Brown
1: 30 (10g of kenaf riben with 30 mL of H_2O)	2.52	3.8	11	Rough, Hardest, Does not clean, Brown
1: 50 (10g of kenaf riben with 50mL of H_2O)	2.47	3.1	20	Rough, Hardest, Does not clean, Brown
1: 70 (10g of kenaf riben with 70mL of H_2O)	2.44	2.8	29	Rough, Hardest, Clean, White
1: 100 (10g of kenaf riben with 100mL of H_2O)	2.30	2.8	31	Rough, Furry, Clean, White

For the changing of colour for enzyme solution also was observed. It is shown that the enzyme solution colour changes to cloudy and more particles are released in the solution. The colour changes of enzyme solution can be seen from the Figure 4.1(a) and Figure 4.1(b) that shows the colour of enzyme solution before retting after 24 hours through enzymatic retting process. Figure 4.2 shows the fibers are produced after retting process after washed and dried. From the figure, the quality if fiber produced was observed for the volume of water 10mL, 30mL, 50mL, 70mL, and 100mL by observing their hardest, roughness and furry condition. The fiber produced from 100mL of enzyme solution is furrier, clean and white compared to the fiber that produced from 10mL of enzyme solution that is hardest, roughness, does not clean and brown colour of fiber.

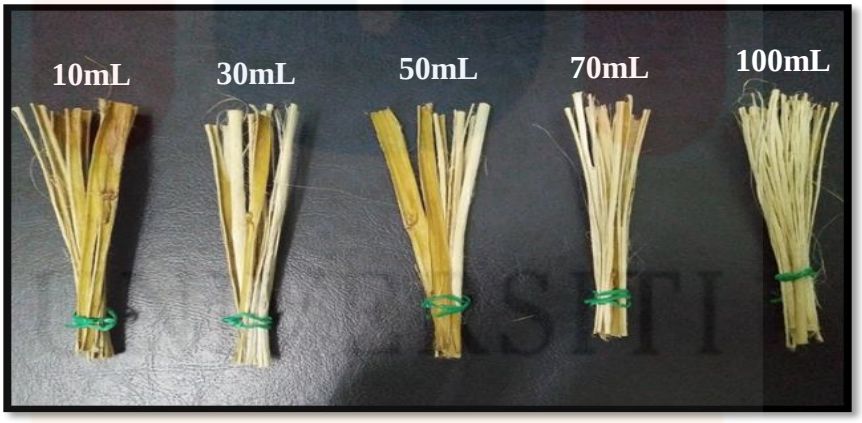


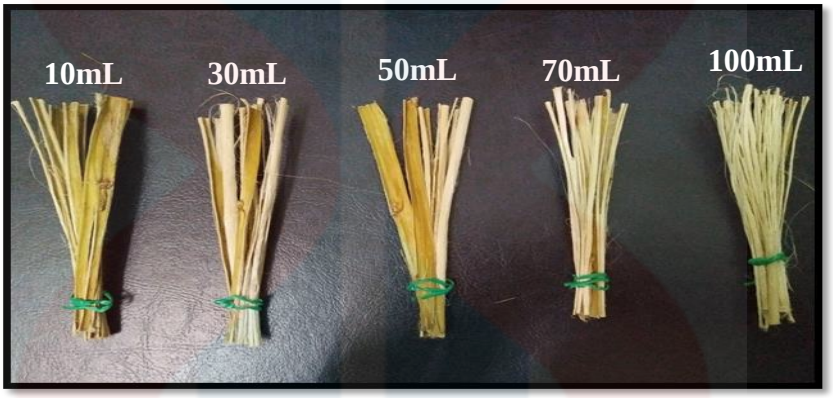
Figure 4.2: Kenaf bast fiber from enzyme combination of 100 % pectinase for 24 hours

4.1.2 Observation result on pectinase (100%) with retting time of 48 hours

For the retting time of 48 hours shown that all the fibers are retted and all the fiber produce from different volume of enzyme solution are more furrier than the fiber

are produced from retting time on 24 hours. The kenaf ribbon becomes jelly and gummy where pectin are produced. When kenaf ribbon producing high amount of jelly and gummy pectin and it is easily to remove pectin from the fiber during washing process and the fiber is more cleaner and whiteness. The comparison of quality of fiber for two retting time 24h and 48h can be observed from the Figure 4.3(a) and (b).

a)



b)

Figure 4.3: (a): Fiber retted for 24h (b) Fiber retted for 48h

Table 4.2 shown that fiber from 100 mL of enzyme solution is lost more pectin and become the lowest weight of fiber. These bast fibers are more fineness, cleanliness and white condition. While the fiber from the volume of enzyme solution 10 mL is shown that the fiber produce is highest weight of fiber. This is because where the fiber is still roughness and still having pectin that still bind with the fiber. Cause of still having

pectin on the fiber the colour of fiber also still brown. The best quality of fiber that can be observe from the colour fiber is starting with the white colour followed by yellowish, light gray, and last is brown.

For the enzyme solution condition observation is similar with the process occurred for 24 hours retting time. The colour of enzyme solutions are changed to cloudy and having more particles and dump of pectin in the solutions that are produce from kenaf ribbon during retting process.

Table 4.2 Observation experimental results on kenaf bast fiber obtained for retting time of 48 hours

Solid to volume of water ratio	Weight of kenaf fiber - after retting (g)	Final pH of enzyme solution	Acetic acid used (mL)	Observation (Texture, Fiber whiteness)
1: 10 (10g of kenaf riben with 10 mL of H_2O)	4.07	3.8	3.0	Rough, Furry, Clean, Brown
1: 30 (10g of kenaf riben with 30 mL of H_2O)	3.39	3.6	3.2	Rough, Furry, Clean, Brown
1: 50 (10g of kenaf riben with 50mL of H_2O)	3.34	3.6	3.6	Smooth, Furry, Clean, Yellowish
1: 70 (10g of kenaf riben with 70mL of H_2O)	2.94	3.6	4.0	Smooth, Furry, Clean, White
1: 100 (10g of kenaf riben with 100mL of H_2O)	2.27	3.5	4.8	Smooth, Furry, Clean, White

4.1.3 Observation on pectinase (100%) with retting time of 72 hours

The quality of fiber from retting process occur within 72 hours is shown that the quality of fiber is lower than 48 hours process retting time. The fiber produced is more rough and hardest compared the fiber produced from 48 hours process of retting time that is smooth and finesses. Figure 4.3 shows the comparison on quality of fiber between

retting time of 48 hours and 72 hours. This is might be happen because of long time taken for retting process can affect the quality of fiber.

a)

b)

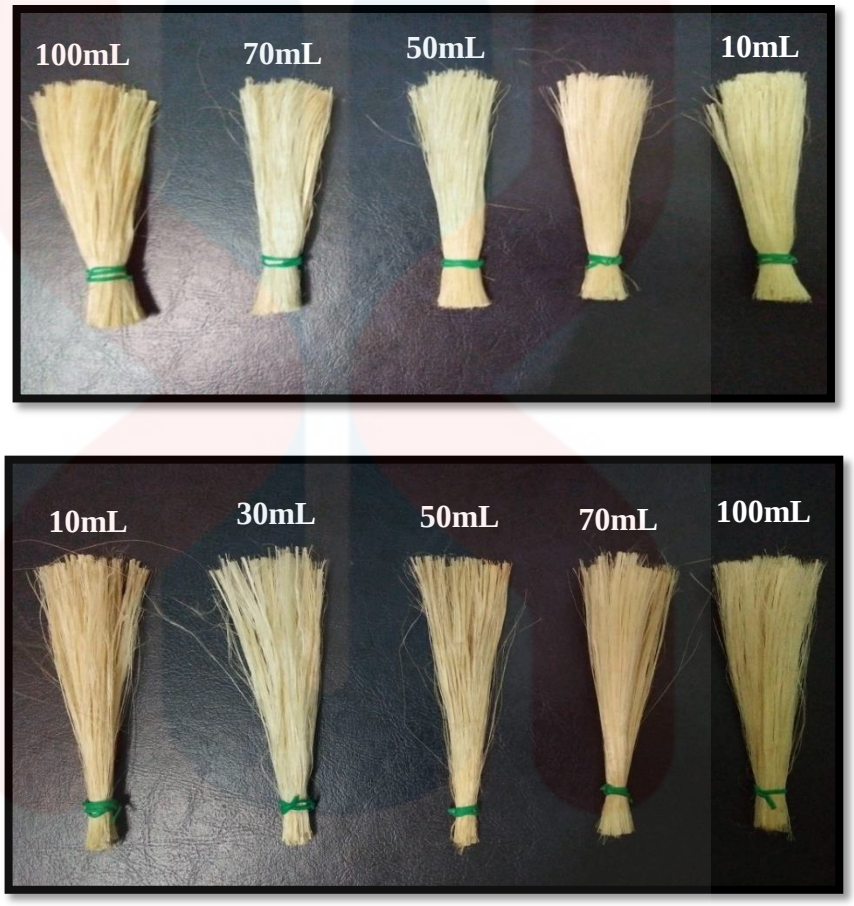


Figure 4.3: (a): Fiber retted for 48h (b) Fiber retted for 72h

Table 4.3 shows the observation on kenaf fiber that obtained from retting process within 72 hours. The fiber obtained from the enzyme solution of 100 mL is furrier, smooth, clean and white colour condition. While the fiber is obtained from 10 mL of volume enzyme solution is rough, hardest, does not clean and brown colour condition. It is indirectly proving that the kenaf ribbon that is totally immersed into enzyme solution is more retted and produced best quality of fiber.

Table 4.3: Observation experimental result on kenaf bast fiber for retting time process of 72 hours

Solid to volume of water ratio	Weight of kenaf fiber - after retting (g)	Final pH of enzyme solution	Acetic acid used (mL)	Observation (Texture, Fiber whiteness)
1: 10 (10g of kenaf riben with 10 mL of H_2O)	2.55	3.7	8.0	Rough, Hardest, Does not clean, Brown
1: 30 (10g of kenaf riben with 30 mL of H_2O)	2.47	3.5	11.5	Rough, Furry, Does not clean, Light gray
1: 50 (10g of kenaf riben with 50mL of H_2O)	1.91	3.5	15.5	Smooth, Furry, Clean, Yellowish
1: 70 (10g of kenaf riben with 70mL of H_2O)	1.90	3.5	18.0	Smooth, Furry, Clean, White
1: 100 (10g of kenaf riben with 100mL of H_2O)	1.67	3.5	20	Smooth, Furry, Clean, White

4.1.4 Observation result on pectinase (100%) with retting time of 96 hours

The fibers produced were finer, clean and smoother, compared with fiber from retting time on 72 hours that can be observed from the Figure 4.5. All the fibers produced from various concentrations and volume of enzyme solution shown that easily to remove pectin and lignin from the bast fiber. All the fiber produced from the enzyme

retting process by using 100% pectinase with retting time 96 hours form furry condition of fiber. The colour of fiber also shows white color. No grading standard has been developed to date to specify kenaf fiber's quality. In India, the grading of jute is considered good in terms of colour specification where the colour of fiber is light cream to white (Wong et al., 2016).

However, the fiber from the volume enzyme solution of 50mL is shown the colour of fiber is brownish colour. This is might be the cause of the kenaf ribbon itself where the ribbon is not in good quality. The colour of fiber is still brownish because lower amount of lignin is removing. The colour of the fiber was satisfactory where the brightness index increased with retting time of retting process. Researcher was mentioned that natural bast fiber are classified and graded according to their lusture, colour, strength cleanliness, and freedom from the retting defects (Mahbubul, 2013). Figure 4.5 shows the condition of retted kenaf bast fiber by using 100% pectinase for 96 hours.

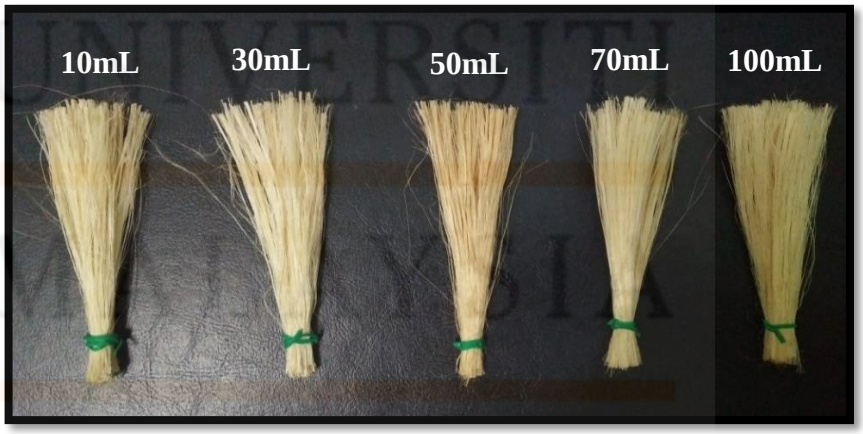


Figure 4.5: Fiber retted kenaf bast fiber by using 100% pectinase for 96 hours

As mention before the jelly and gummy are formed after retting process make it easiest on removing pectin and lignin from the fiber bundle that hold together in kenaf ribbon. Then, the fiber quality are produced is more whitening and luster. Fiber are produced from volume of enzyme solution 100 mL is the best quality fiber that satisfied on brightness index and very smooth on furry condition differ to others especially on 10 mL volume of enzyme solution. Table 4.4 shows the range of quality fiber that was observed in term of weight of fiber, colour and quality of fiber produced during retting process.

Table 4.4: Observation experimental results on kenaf bast fiber for retting time process of 96 hours

Solid to volume of water ratio	Weight of kenaf fiber - after retting (g)	Final pH of enzyme solution	Acetic acid used (mL)	Observation
1: 10 (10g of kenaf riben with 10 mL of H_2O)	2.55	3.7	8.0	Rough, Hardest, Does not clean, Brown
1: 30 (10g of kenaf riben with 30 mL of H_2O)	2.47	3.5	11.5	Rough, Furry, Does not clean, Light gray
1: 50 (10g of kenaf riben with 50mL of H_2O)	1.91	3.5	15.5	Smooth, Furry, Clean, Yellowish
1: 70 (10g of kenaf riben with 70mL of H_2O)	1.90	3.5	18.0	Smooth, Furry, Clean, White
1: 100 (10g of kenaf riben with 100mL of H_2O)	1.67	3.5	20	Smooth, Furry, Clean, White

4.1.5 Observation results on enzyme combination of pectinase, xylanases, and cellulases (60: 20: 20) with retting time of 24 hours

The quality of fiber also has been tested by using three combinations of enzyme such as pectinase, xylanases, and cellulases. However, the ratio of enzyme pectinase used is highest because the aim is on removing pectin and lignin to produce high quality

of fiber. Xylanase were used to make the fiber is more fineness and smooth as well as become furry condition by removal the lignin content. Otherwise, cellulases enzyme is functioned as to degrade the cellulose of plant cell and make the fiber is more luster and brightness.

For the retting period of 24 hours shows that all the fiber produced is in furry condition. However, for the fiber is produced from 10 mL volume of enzyme solution is roughness than the highest volume 100 mL of enzyme solution. Then, for 100 mL volume of enzyme solution also produces fiber that is more luster and brightness than others. During washing process, fiber from 100 mL enzyme solution is the easiest on removing pectin and lignin. While the weight of fiber can produced from 100 mL of enzyme solution is lowest with 2.12 g of fiber than 10 mL of enzyme solution production with the weight is 2.68 g of fiber.

For the volume of 10 mL enzyme solution 0.6 mL of acetic acid is needed compared to 100 mL of enzyme solution need 1.4 mL of acetic acid to control the pH 3.5. From this observation, this combination of three enzymes shows that use of acetic acid is lowest than the uses of enzyme 100% pectinase. The highest the acetic acid is used the hardest on removing pectin during washing process make the fiber become harder and more acidic.

Table 4.5 are shows the result of enzymatic retting of 24 hours using combination enzyme of pectinase, xylanase, and cellulase. The enzyme solution reaction was observed from the colour changes of enzyme solution where the colour changes from clear colour to cloudy. While, on kenaf ribbon is changed from green colour to

brown colour after retting process same as the reaction happens to retting process by using on 100% pectinase.

Table 4.5: Observation experimental result on kenaf bast fiber obtained from enzyme combination of pectinase, xylanases, and cellulase for retting time process of 24 hours

Solid to volume of water ratio	**Weight of kenaf fiber - after retting (g)**	**Final pH of enzyme solution**	**Acetic acid used (mL)**	**Observation**
1: 10 (10g of kenaf riben with 10 mL of H_2O)	2.68	4.1	0.6	Rough, Furry, Clean, Yellowish
1: 30 (10g of kenaf riben with 30 mL of H_2O)	2.43	4.2	0.8	Rough, Furry, Clean, Yellowish
1: 50 (10g of kenaf riben with 50mL of H_2O)	2.42	4.2	0.8	Smooth, Furry, Clean, Yellowish
1: 70 (10g of kenaf riben with 70mL of H_2O)	2.28	4.0	1.2	Smooth, Furry, Clean, White
1: 100 (10g of kenaf riben with 100mL of H_2O)	2.12	4.1	1.4	Smooth, Furry, Clean, White and luster

4.1.6 Observation on enzyme combination of pectinase, xylanases, cellulases (60: 20: 20) with retting time of 48 hours

The observation on retting time of 48 hours was observed from the colour of fiber produce from the different volume of enzyme solution. The colour of fiber shown is yellowish than fiber retted from retting process on 24 hours. The volume and the concentration of enzyme is affected the quality of enzyme from their colour, fineness, smoothness, brightness and luster. Some fiber becoming in brownish colour because of too high acid are used and the kenaf ribbon itself is not good quality where is have some fungi or others organisms on the ribbon of kenaf.

From the observation, kenaf bast fiber produced from 100 mL of enzyme solution is more superficial with weight 2.02 g because of the fiber is more smoothness, fineness and lost more pectin. However the colour of fiber is yellowish compared to fiber produces in 24 hours that is more whiteness. This is due to effect from the process of immerse are taken longer time. Figure 4.6 shows the comparison between of fiber produces for 24 hours and 48 hours.

a)

b)

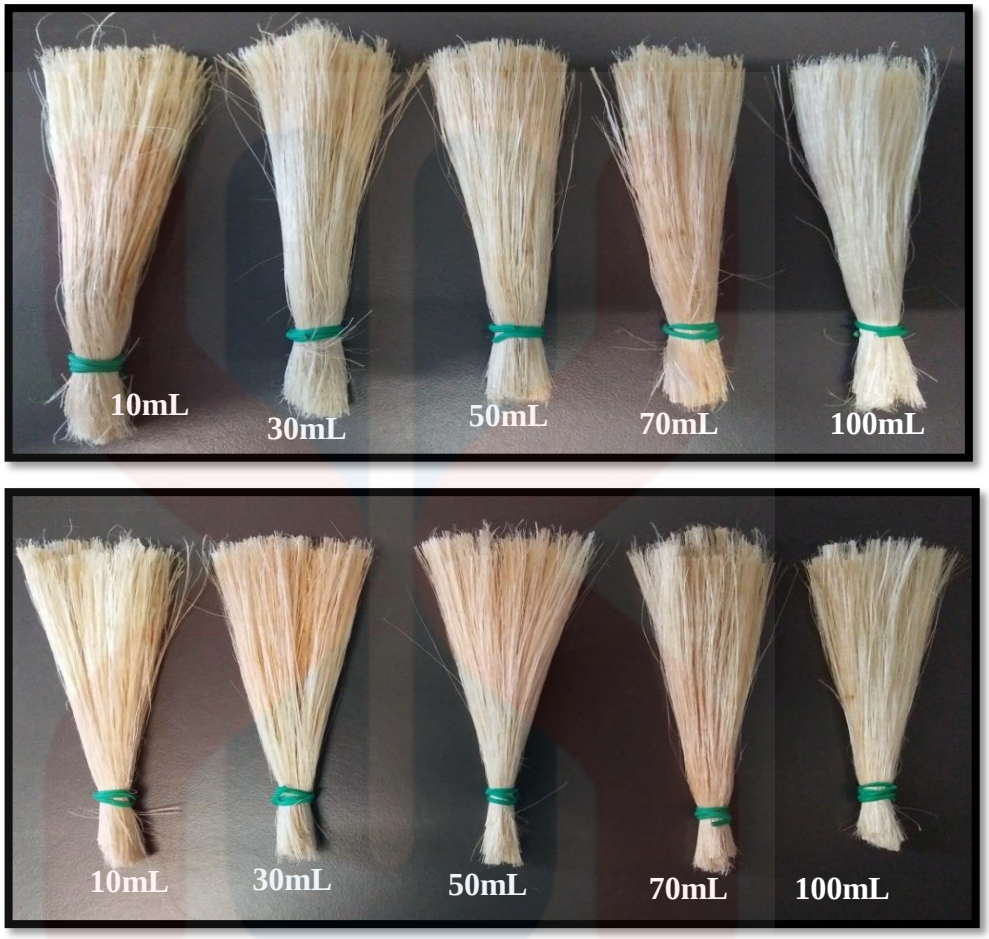


Figure 4.6: Kenaf bast fiber from (a) retting process 24 hours and (b) retting process 48 hours from enzyme combination of pectinase, xylanase, and cellulase

Table 4.6 shows the observation on kenaf bast fiber for retting process of 48 hours that shows the physical quality of fiber is lowest than fiber produced in 24 hours that are observed from the weight of kenaf bast fiber were produced and the texture and fiber whiteness.

Table 4.6: Observation experimental result on kenaf bast fiber obtained from enzyme combination of pectinase, xylanases, and cellulase for retting time process of 48 hours

Solid to volume of water ratio	Weight of kenaf fiber - after retting (g)	Final pH of enzyme solution	Acetic acid used (mL)	Observation (Texture, fiber whiteness)
1: 10 (10g of kenaf riben with 10 mL of H_2O)	2.43	3.9	0.8	Rough, Furry, Clean, Yellowish
1: 30 (10g of kenaf riben with 30 mL of H_2O)	2.16	3.6	1.4	Rough, Furry, Clean, Yellowish
1: 50 (10g of kenaf riben with 50mL of H_2O)	2.11	3.7	2.0	Smooth, Furry, Clean, Yellowish
1: 70 (10g of kenaf riben with 70mL of H_2O)	2.10	3.7	2.6	Smooth, Furry, Clean, Yellowish
1: 100 (10g of kenaf riben with 100mL of H_2O)	2.02	3.6	2.8	Smooth, Furry, Clean, Yellowish

4.1.7 Observation on enzyme combinations of pectinase, xylanases, and cellulases (60: 20: 20) with retting time of 72 hours

The quality of fiber that can be observed from retting period of 72 hours is almost the same with quality fiber on 48 hours. All the fibers produced are furrier and clean. The best fiber quality for parameter retting time of 72 hours is on 100 mL of volume

enzyme solution. The fiber is smooth, furry, clean and yellowish. While fiber produced from 10 mL enzyme solution is rougher, not too brightness and not luster. The furry condition is not too soft compared to fiber that produced from 100 mL of enzyme solution.

After retting process is reacted to the green kenaf ribbon, the pectin was removed by washing with tap water and dried under direct sun. So, the weight of green kenaf lost from 10 g until 2.84 g. Some pectin is not directly removed from fiber because it is dissolved in enzyme solution that can change the colour of enzyme solution.

The removal of pectin and lignin also affected from quantity of acid used in retting process. Acid is the best method used to remove pectin from fiber. But high volume of acid are used can make the enzyme died and no enzyme activity is occurred during retting process. In this study, for the parameter of 72 hours on three combinations of enzyme shows that all the enzyme concentration was used only 0.6 mL of acetic acid and the final pH is 4.3 for all the ratio of volume of water. This method for this experiment is the best controlling on parameter where it is synchronize. Table 4.7 shows the data collected from the observation of kenaf bast fiber on the observation of texture and fiber whiteness as well as the volume of acetic acid used in retting process.

Table 4.7: Observation experimental result on kenaf bast fiber obtained from enzyme combination of pectinase, xylanases, and cellulase for retting time process of 72 hours

Solid to volume of water ratio	Weight of kenaf fiber after retting (g)	Final pH of enzyme solution	Acetic acid used (mL)	Observation
1: 10 (10g of kenaf riben with 10 mL of H_2O)	2.84	4.3	0.6	Rough, Furry, Clean, Yellowish
1: 30 (10g of kenaf riben with 30 mL of H_2O)	2.70	4.3	0.6	Soft, Furry, Clean, Yellowish
1: 50 (10g of kenaf riben with 50mL of H_2O)	2.46	4.3	0.6	Soft, Furry, Clean, Yellowish
1: 70 (10g of kenaf riben with 70mL of H_2O)	2.45	4.3	0.6	Soft, Furry, Clean, Yellowish
1: 100 (10g of kenaf riben with 100mL of H_2O)	2.30	4.3	0.6	Soft, Furry, Clean, and yellowish

4.1.8 Observation on enzyme combination of pectinase, xylanases, cellulases (60: 20: 20) with retting time 96 hours

For retting time of 96 hours shows that the quality fiber is lower than the quality fiber from 24, 48 and 72 hours as shown in Figure 4.6 (a), (b), (c) and (d). The colour fiber produced is in brownish colour. The fibers also quite rougher compared to fibers

are produced from retting process with retting time of 72 hours where it is considered the long period of immersed into the acid. By the combination of different three enzymes should be taking short period for retting process. Longer time taken to immerse the green kenaf bast fiber will affect the colour of fiber. This is because the acid will react to the fiber and changes the colour of fiber when it is no enzyme activity occurs. The colour of fiber also can be affected from the quality of green kenaf ribbon. If the bast fiber is harvested from the old kenaf tree, the fiber are produced is lower quality. The quality of fiber can be effect on strength of fiber, brightness and luster of fiber.

The lowest weight of fiber is produced from 100 mL of enzyme solution with 2.42 g. The highest weight of fiber is produced from 10 mL of enzyme solution with 2.86 g. Fiber that produced from 10mL of enzyme solution is heavier cause of amount of removal pectin. The fiber also rougher and does not clean compared to fiber from 100 mL of enzyme solution. The colour is more yellowish compared to the fiber from 100 mL of enzyme solution that can be seen from Figure 4.6 (c). Table 4.8 shows the summarization of observation on kenaf bast fiber for retting period of 96 hours.

Table 4.8: Observation experimental result on kenaf bast fiber obtained from enzyme combination of pectinase, xylanases, and cellulase for retting time process of 96 hours

Solid to volume of water ratio	**Weight of kenaf fiber - after retting (g)**	**Final pH of enzyme solution**	**Acetic acid used (mL)**	**Observation**
1: 10 (10g of kenaf riben with 10 mL of H_2O)	2.84	4.3	0.6	Rough, Furry, Clean, Yellowish
1: 30 (10g of kenaf riben with 30 mL of H_2O)	2.70	4.3	0.6	Soft, Furry, Clean, Yellowish
1: 50 (10g of kenaf riben with 50mL of H_2O)	2.46	4.3	0.6	Soft, Furry, Clean, Yellowish
1: 70 (10g of kenaf riben with 70mL of H_2O)	2.45	4.3	0.6	Soft, Furry, Clean, Yellowish
1: 100 (10g of kenaf riben with 100mL of H_2O)	2.30	4.3	0.6	Soft, Furry, Clean, Yellowish

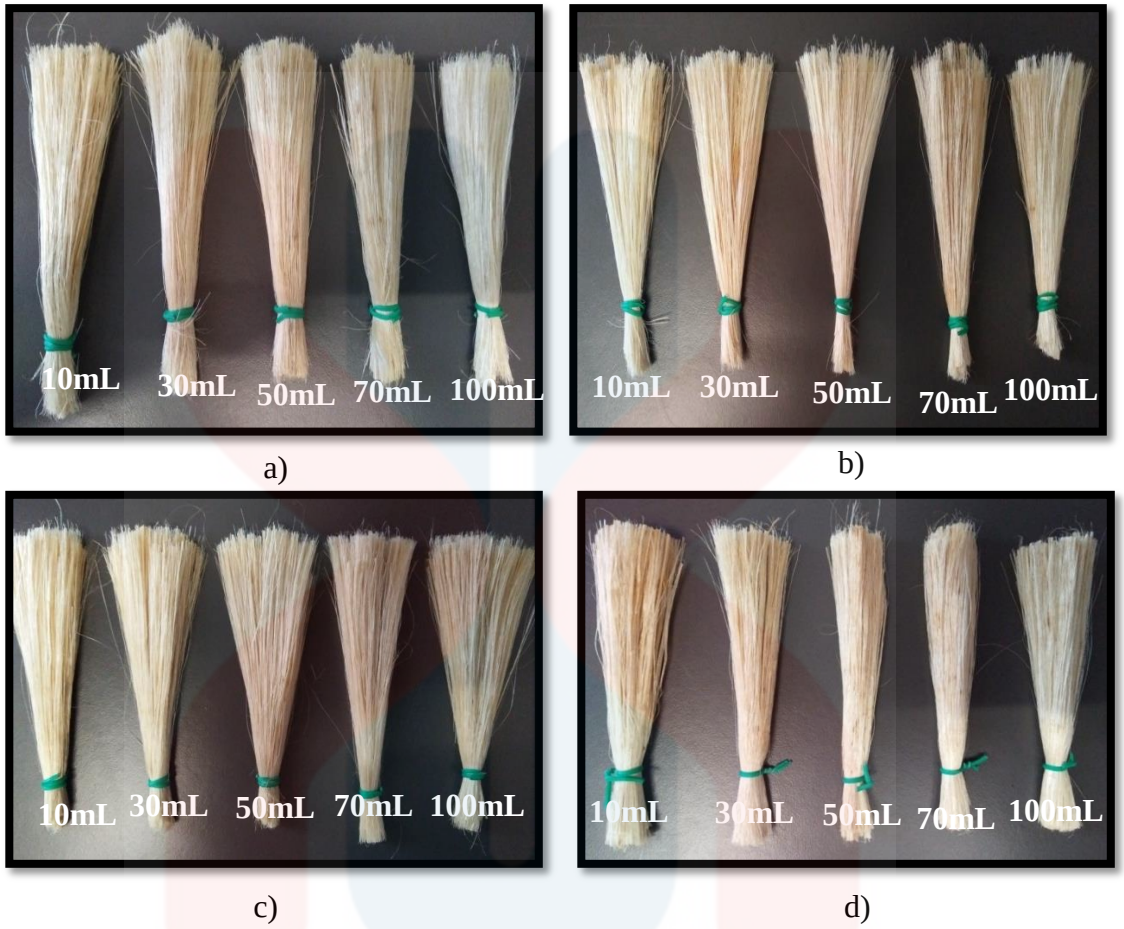


Figure 4.7: Kenaf bast fiber from retting time (a) 24 hours, (b) 48 hours, (c) 72 hours, and (d) 96 hours

4.2 Characterization of kenaf bast fiber

4.2.1 Fourier Transform Infrared Spectroscopy (FTIR)Analysis of Kenaf Bast Fiber

FTIR spectroscopy was used to show the physical structure and functional groups of the lignocellulosic components as well as to investigate the efficiency of the

different combination of enzyme treatments in production of fibers. The FTIR spectra of all samples extracts were taken and compared shown in Figure 4.8.

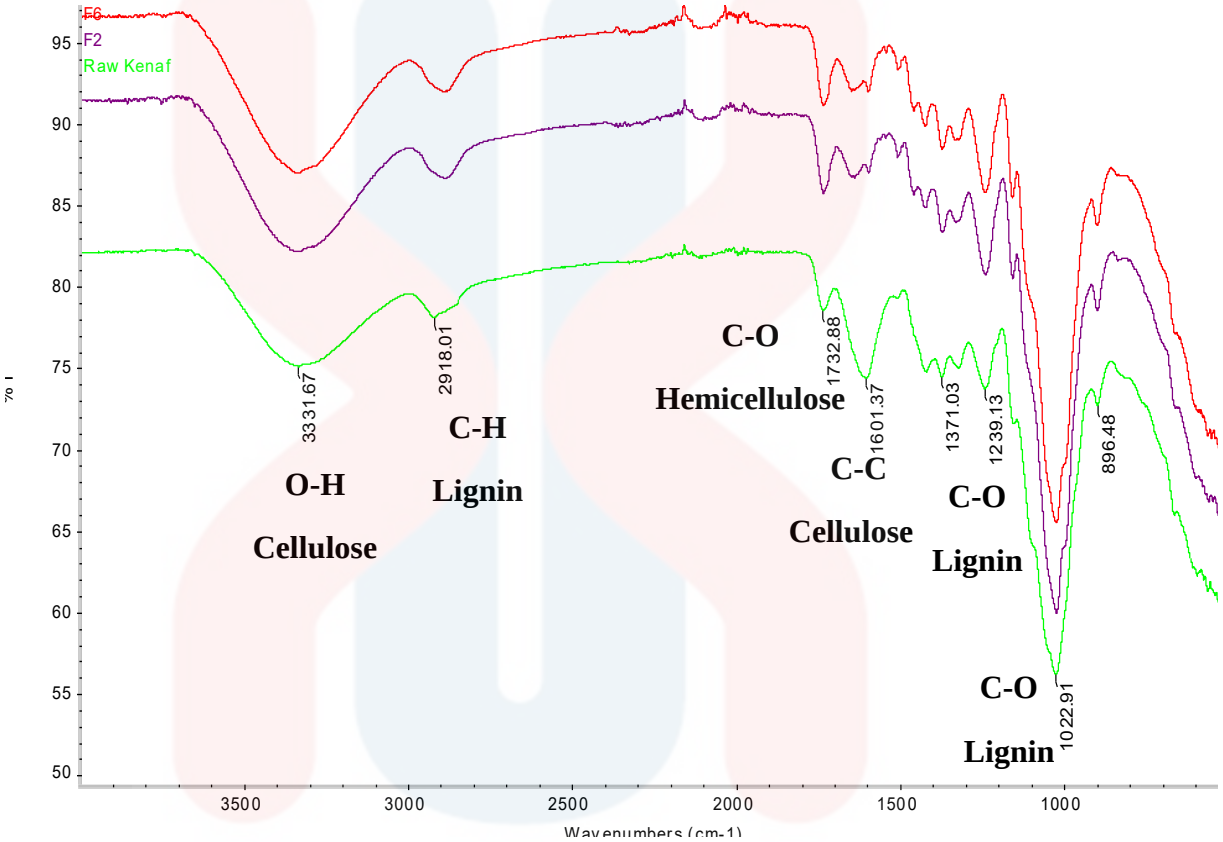


Figure 4.8: Spectrum of untreated and treated green kenaf bast fibers.

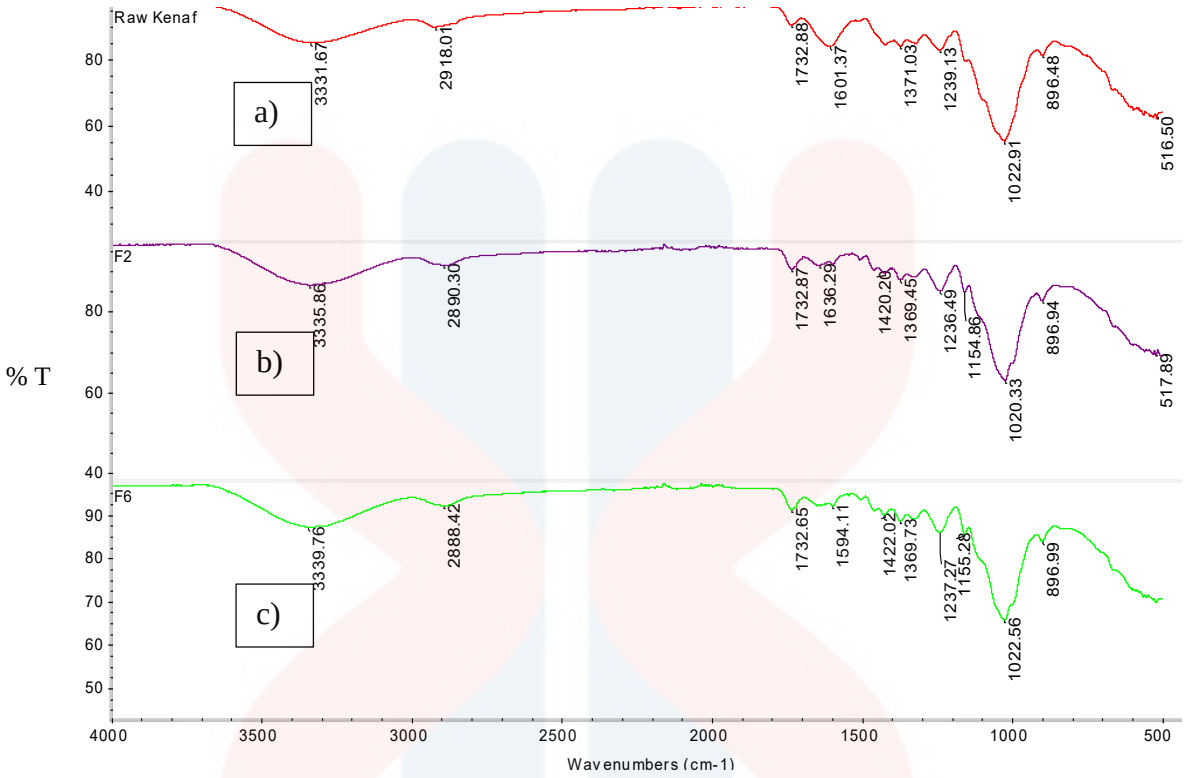


Figure 4.9: FTIR spectra of kenaf fiber (a) raw kenaf green ribbon and combination enzyme retting process in (b) 48 hours for pectinase (100%), (c) 24 hours for pectinase, xylanases, cellulase (60%, 20% and 20%)

Figure 4.8 shows FTIR spectra of untreated and enzymatic retting treated of kenaf bast fiber for two enzyme combination by using of 100% of pectinase and combination of 60% of pectinase, 20% of xylanases and 20% of cellulase (F2 and F6, respectively) in the region of 4000 – 500cm^{-1}. The peaks are starting for observation for comparisons at absorbance peaks 3331.67cm^{-1} from the raw kenaf green ribbon. The region 3330-3400cm^{-1} for the untreated and treated kenaf bast fibers is related to OH groups. A broad absorption band at 3331.67cm^{-1} is due to O-H stretching vibrations of cellulose (Jiang et al., 2017). In contrast, those absorbance peaks located around 2800-3000cm^{-1} were due to the stretching of C-H groups where the C-H stretch is present in all fibers (Jonoobi, Jalaluddin, Alireza, Manjusri, & Kristiina, 2009). The C-H groups

attributed to the lignin compounds. The C=O stretching is observed from the peak located at 1732.88cm^{-1} in raw kenaf green ribbon was attributed to the acetyl group of hemicellulose from the sources of xylan (Troedec et al., 2008). This peak becomes lower frequency after the treatment of enzymatic retting. This disappearance is consequences of the hemicelluloses are removed from the fiber after retting process.

In the raw kenaf green ribbon, the peak located at 1239.13 cm^{-1} and 1022.91 cm^{-1} were attributed to the C-O stretching of aryl group in lignin. This peak shows the decrease of frequency of wavenumbers after enzymatic retting that shows in Figure 4.9. The decreasing of frequency proves that lignin was removed and more effective on retting process using of 100% pectinase. The summarization of the typical functional groups and the IR signal with the possible compound are found from the FTIR analysis are shows in Table 4.9.

Table 4.9: Summary for the main functional groups of the raw kenaf, kenaf retted from 100% pectinase, and kenaf retted from combination enzyme of pectinase, xylanases and cellulase.

Wave number (cm^{-1})	**Functional groups**	**Compounds**
3331.67 (s, b)	O – H stretching	Cellulose
2918.01 (m)	C – H stretching	Lignin
1732.88 (m)	C – O stretching	Hemicellulose
1601.37 (w)	C – C stretching	Cellulose
1239.13 (s), 1022.91 (s)	C – O stretching	Lignin

4.2.2 Thermal stability of Kenaf Bast Fiber retted from enzyme combination of pectnise, xylanases, and cellulase (60: 20: 20) for retting time 24h.

The thermal stability of kenaf bast fiber was studied by TGA under nitrogen to a maximum temperature 600 ºC heating rate of 10 ºC/ min. Figure 4.2 shows the TGA and DTG curves obtained from the runs Thermogravimetric analyzer (TGA). The peak degradation temperature of retted kenaf bast fiber starting occurs around 250 ˚C and finishes at 420 ˚C as shown in Table 4.10. Cellulosic components tended to decompose between 200 and 400 ˚C (Jonoobi et al., 2009). The thermal stability of the residues increases with enrichment in cellulose where the cellulose content increases as non-cellulosic sub-components are gradually removed (Sango et al., 2018). From Figure 4.3(a) the TGA curves that observe the first weight loss in range of temperature 50 ˚C and 150 ˚C that generally respond to a loss of water in the composites (Arrakhiz et al., 2012). 11.8999 mg of kenaf bast fiber are burn for 57.50 min and 93.80% was burned. From Table 4.10, also shows that at temperature 420 ˚C with 19.23% residue and 15.04% residue at 600 ˚C. Then, Figure 4.3(a) shows the curves pattern of TGA and DTG for kenaf bast fiber from the enzymatic retting process.

Table 4.10: Thermal degradation and residue data of kenaf bast fiber by TGA

Sample	$T_{initial}$ (ºC)	T_{final} (˚C)	Midpoint (˚C)	Residue (%)
				600˚C
F_{pxc} (24h)	250	420	350.84	15.04

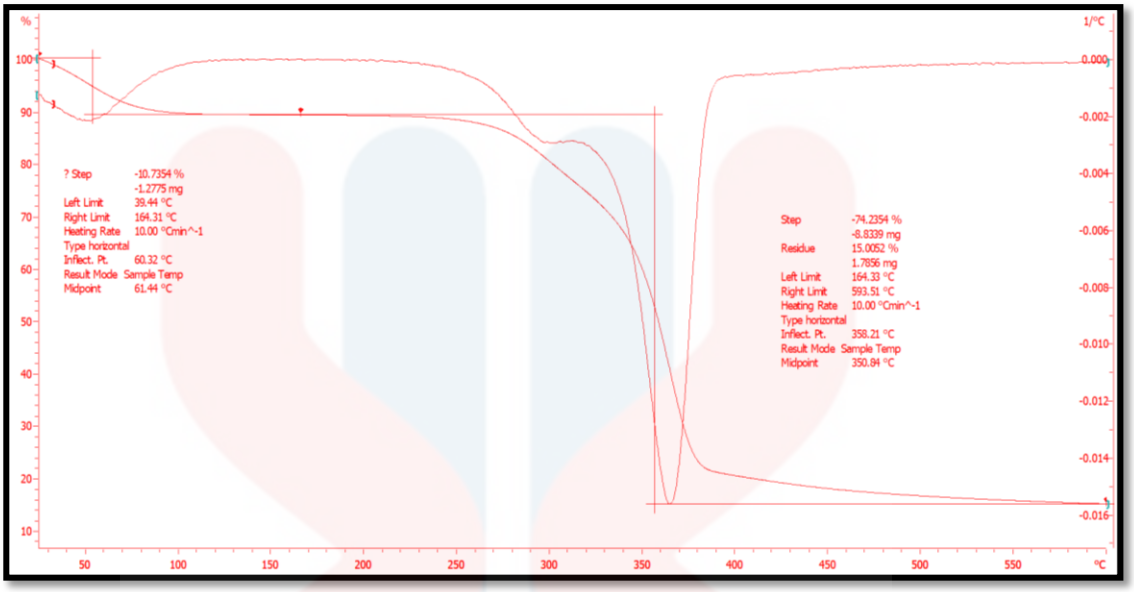
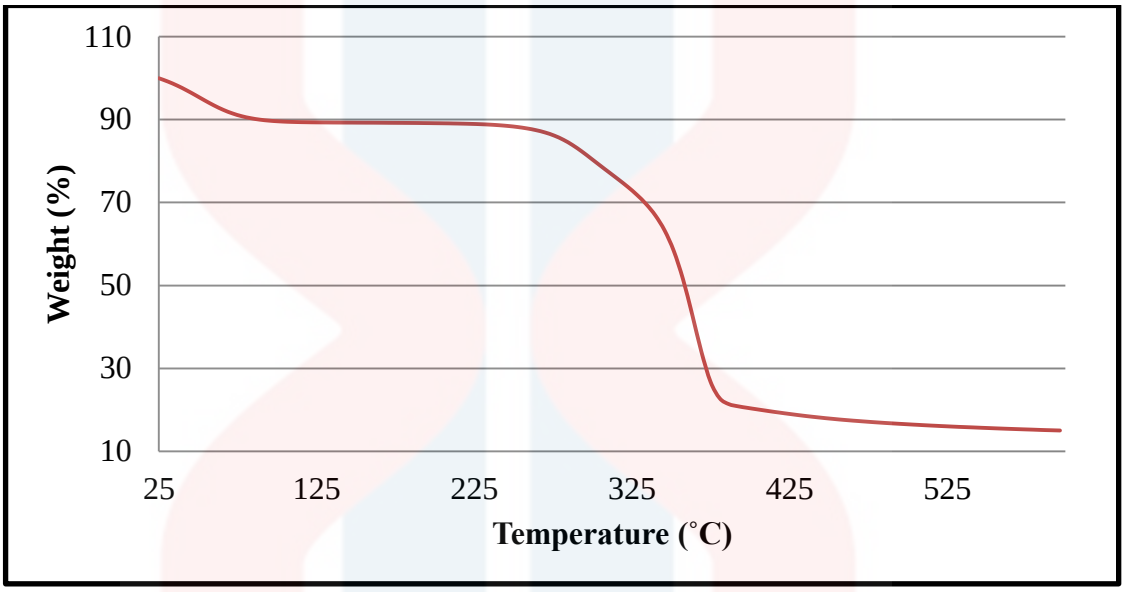


Figure 4.10: TGA and DTG thermograms of kenaf bast fiber retted from enzyme combination of pectinase, xylanases, and cellulase for 24h.

Furthermore, Figure 4.11 (a) presented the curves of retted kenaf bast fiber must move to the right which suggested there was significant trend of increasing thermal stability. The main degradation step (T_{max}) was found at the midpoint 350. 84 ˚C from the curves of kenaf bast fiber retted. The peak of (T_{max}) was attributed to the decomposition of cellulose (Jonoobi et al., 2009). Based on these results, the thermal stability of cellulosic components was increased by the enzymatic retting process. This could be attributed to the removal of hemicellulose and lignin during enzymatic retting process. The lowest the amounts of residue prove that retted kenaf bast fiber undergoes fastest degradation. As shown in Table 4.10, the amount residue for retted kenaf bast fiber from the enzyme combination of pectinase, xylanases, and cellulase for 24 hours is 15 wt% at 600 ˚C. Figure 4.5 (b) shows the derivative Thermogravimetric curves (DTG) of retted kenaf bast fiber from the parameters use of enzyme combination pectinase,

xylanase, and cellulase for 24 hours. TGA data indicate the enzymatic retting processes removed non-cellulose substances from fiber.

a)



b)

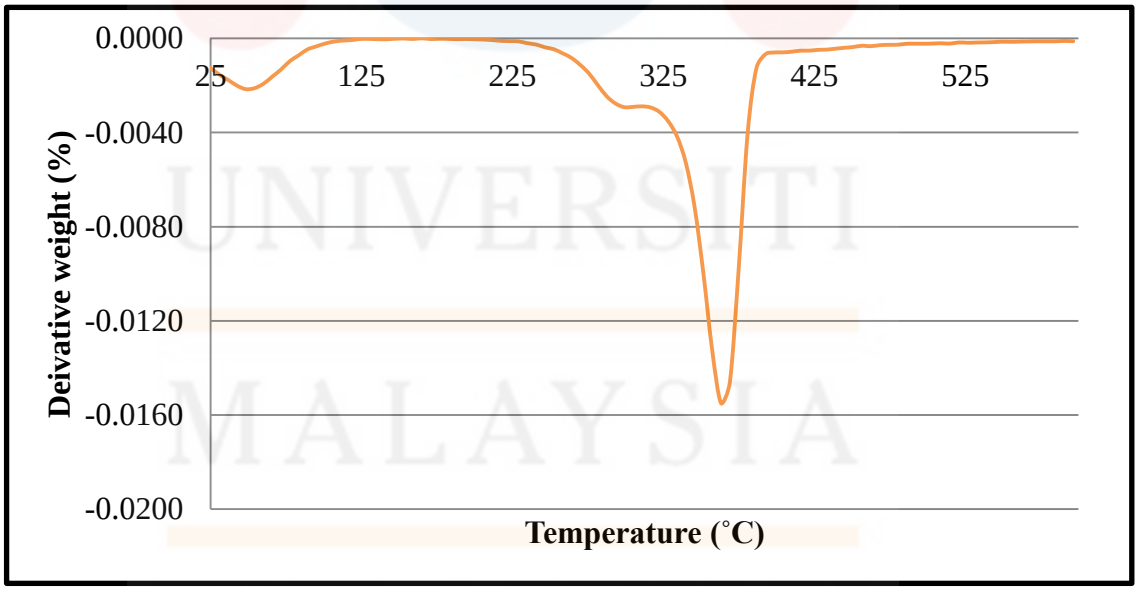
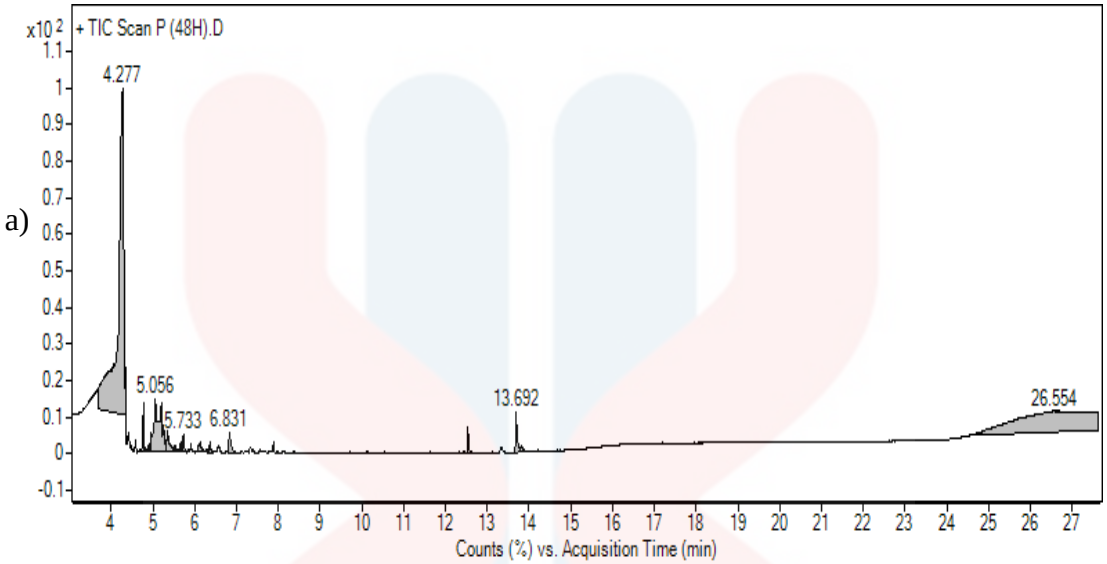


Figure 4.11: (a) Thermogravimetric analysis (TGA) and (b) Derivative Thermogravimetric analysis (DTG) analysis Kenaf Bast Fiber retted from enzyme combination of pectinase, xylanase and cellulase for 24hours.

4.2.3 GC-MS analysis for kenaf bast fiber from 100% pectinase enzymatic retting process for 48h and enzyme combination of pectinase, xylanases, and cellulase for 24h.

Gas chromatography-mass spectrometry (GC-MS) was used for the identification of compound presence in the residue of kenaf bast fiber extraction. The compound that was found in the residue of kenaf bast fiber from the retting process by using 100% pectinase are Oleic acid ($C_{18}H_{34}O_2$) at the retention time (RT) 13.692 with the highest score of similarity 87.94. Then, at RT of 4.277 it was found that compound of Ammonium acetate ($C_2H_7NO_2$) with score of similarity 84.23. At the RT of 6.831 was found compound Catechol ($C_6H_6O_2$) with score similarity of 72.62. Catechol is part of compound that are contain in lignin (Haz, Jablonsky, Orgasova, & Surina, 2013). By finding of compound catechol in the residue was proved that lignin content was removed. The chromatogram for analysis of residue presence from enzyme combination of 100% pectinase was shown in Figure 4.12 (a). Analysis also was done from the residue of enzyme combination of pectinase, xylanases, and cellulase to find out the compound is presence in the residue. The compounds were found out in residue extract of kenaf bast fiber such as Ammonium actetate ($C_2H_7NO_2$) at the RT of 4.287 with the similarity score of 93.62. The second highest of similarity was found at RT of 4.779 is compound Paromomycin ($C_{23}H_{45}N_5O_{14}$) with score 82. While, Glycerine ($C_3H_8O_3$) with the similarity score of 81.64 was found at the RT of 5.333. The compound is valid in range of score between 80 and above. The peaks of the compound are shown in the chromatograms in Figure 4.12 (b).

a)



b)

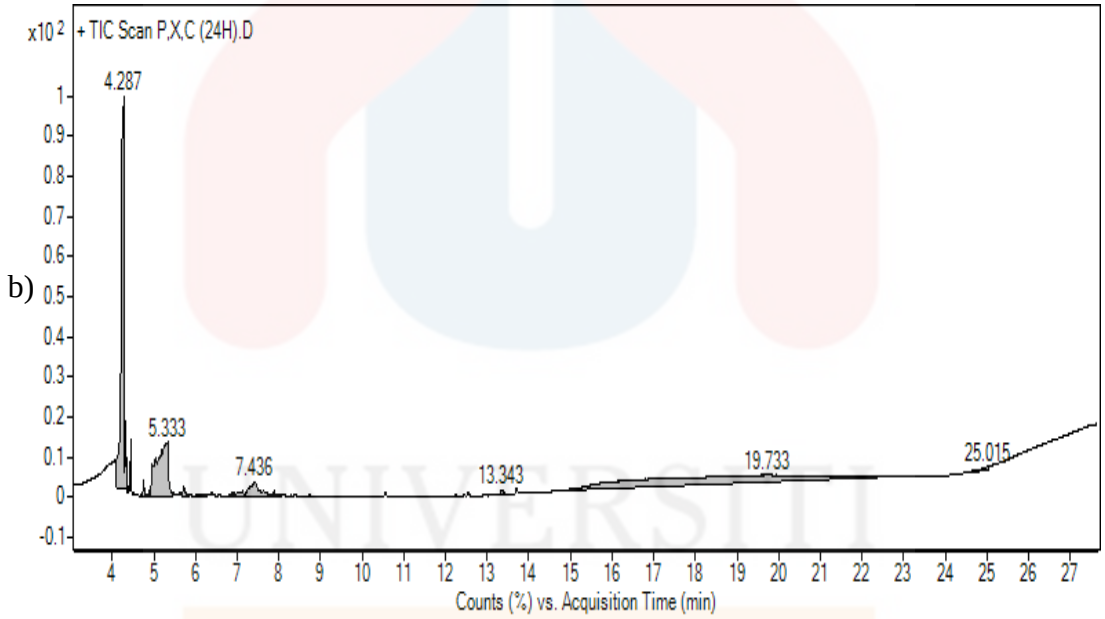


Figure 4.12: GC-MS analysis of residue from kenaf bast fiber extraction from (a) 100% pectinase enzymatic retting process for 48h and (b) enzyme combination of pectinase, xylanases, and cellulase for 24 hours

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study was conducted to investigate enzymatic retting process at different of retting time, enzyme combination and enzyme concentration to produce high quality fiber.

The optimum results obtained from the experimental work is found that kenaf bast fiber produced from enzyme combination of pectinase, xylanases, and cellulase for 24 hours has produced high quality of fiber by observation on its whiteness and cleanliness. While, for enzymatic retting process using 100% pectinase shown that the optimum result of kenaf bast fiber is from 48 hours retted with 100 mL enzyme concentration. From these optimum observations, the optimum samples were then proceeding with FTIR analysis and GC-MS analysis. FTIR study is used for the optimum retted fiber for enzyme combination of pectinase, xylanases and cellulase for 24 hours and retted fiber for enzyme combination of 100% pectinase for 48 hours

treatment to detect changes of functional group in enzymatic retting kenaf bast fiber. This study proved that the hemicellulose, pectin, waxes and lignin present in the fibers before retted at 1732.88cm^{-1} are decrease after enzymatic retting. The fiber from enzymatic retting by using enzyme combination of pectinase, xylanases and cellulase are reducing highest hemicellulose than using 100%pectinase. Then, for the lignin content removal more effective on enzymatic retting by using 100% pectinase compared to enzyme combination of pectinase, xylanases, and cellulase. For TGA study has confirmed the themal stability of kenaf bast fiber after enzymatic retting by using enzyme combination of pectinase, xylanases, and cellulase are increase where the curves presented move to the right which suggested there was significant trend.

This study was further to the analysis of compound presence were removed from the kenaf green ribbon. GC – MS analysis was shown several compounds were found in the residue of enzyme solution of 100% pectinase and enzyme solution with combination of enzyme pectinase, xylanases, and cellulase. The compounds were found is part compound found in lignin and pectin. So, it was proved that the enzymatic retting method is successful on removing lignin and pectin.

5.2 Recommendation

For further research, the preparation sample is important on producing fiber from kenaf bast fiber. The quality of green kenaf ribbon need to be considered for enzymatic retting process to ensure the quality of fiber produce is in good quality. For example, the green kenaf ribbon does not contaminated with other fungi where it is can react to enzyme during retting process and can cause reducing on quality fiber. Furthermore, the other part of kenaf stem can be chosen for retting process such as the top and the bottom part of kenaf stem. The use of specific green kenaf ribbon part for retting process also needs to be considered to ensure the quality of fiber produced. Other combinations of enzyme also can be tested to find out the optimum result from the enzymatic retting process. For example, the combination of pectinase and cellulose can be used.

Besides that, to get more shining and luster of fiber can be further with the process of bleaching. Process of bleaching is the process to remove the excess of pectin on green kenaf ribbon. From the optimum result of experimental data can, be proceed to the development of pilot scale for the integrated system for bio-retting process on kenaf bast fiber.

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