UNIVERSITI
MALAYSIA
KELANTAN

# ENZYMATIC-DEGUMMING PROCESS OF KENAF BAST FIBER AND ITS PHYSICAL CHARACTERIZATION

**KIRENTHARA A/L PERUMAL**
**J20A0460**

**A reported submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science Technology of Bioindustry with Honours**

**FACULTY OF BIOENGINEERING AND TECHNOLOGY**
**UMK**

**2023**

**DECLARATION**

I declare that this thesis entitled “title of the thesis” is the results of my own research except as cited in the references.

Signature : _____

Student’s Name : Kirenthara A/L Perumal

Date : 31st January 2024

Verified by:

Signature : _____

Supervisor’s Name : Dr. Mardawani Binti Mohamad

Stamp : _____

Date : 31st January 2024

## ACKNOWLEDGEMENT

I would like to take this opportunity to express my sincere gratitude and appreciation towards my final year project supervisor, Ts. Dr. Mardawani Binti Mohamad for her care, understanding and concern during the entire process of starting, building and completing the final year project. I am really appreciative and thankful to have a supervisor who is so sympathetic, encouraging, and passionate and who has provided me with a tonne of advice, pointers, and support throughout the study paper. I am also fortunate to have a kind supervisor who has consistently inquired about my health and welfare while I've been working on my project paper.

In addition, I want to acknowledge and extend my sincere gratitude to the Faculty of Bioengineering and Technology (FBKT), Universiti Malaysia Kelantan (UMK), and the amazing lecturers and lab assistants who have helped me over the entire process of conducting my experimental study. I've learned a lot and experienced a lot from this chance, which has improved me on many levels.

In addition, I am really indebted to my parents, Mr. Munisparan and Mrs. Guneswari, for their unwavering support and nurturing during the challenges I had with my senior project. It gives me great pleasure to express my gratitude to my closest friends, juniors and fellow classmates, especially Dashalan Daran Nair, for all of his encouragement and support during this research trip. Furthermore, I would especially want to thank my dear buddy Thinesh Kumar. His frank evaluations, insightful remarks, restless evenings and emotional support have undoubtedly assisted me in writing a superb study paper.

## TABLE OF CONTENT

## LIST OF TABLES

**PAGE**

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| Carbon | C |
| Hydrogen | H |
| FTIR | Fourier Transform Infrared Spectroscopy |
| GC-MS | Gas Chromatography Mass Spectrometry |
| LKTN | Malaysian Kenaf and Tobacco |
| MARDI | Malaysia Agricultural Research and Development Institute |
| TGA | Thermogravimetric Analysis |

## LIST OF SYMBOLS

| | |
|---|---|
| α | Alpha |
| g | Gram |
| H | Hour |
| mg | Milligram |
| pH | Aciditiy |
| % | Percentage |

# ABSTRAK

"Degumming" fizikal secara tradisinya digunakan dalam pengeluaran gentian basta kenaf, sebelum pengenalan "degumming" kimia dan enzimatik. Teknik 'degumming" fizikal termasuk hiasan, pembersihan gentian dan pembukaan gentian dengan menggunakan mesin seperti penghias untuk mengurangkan lekatan antara berkas gentian dan mesin shive. Oleh kerana rawatan asid, alkali atau pengoksidaan digunakan semasa proses penyahguman kimia, gentian berkualiti sangat rendah mungkin terhasil, dengan itu menjejaskan kualiti gentian. Sebaliknya, didapati bahawa "degumming" enzimatik boleh memberikan serat berkualiti tinggi dengan lebih cepat. Dalam penyiasatan ini, larutan enzim pektinase sahaja dan gabungan 50:50 pektinase dan enzim selulase digunakan untuk secara enzimatik merebus gentian kulit kenaf. Perubahan ketara dalam tempoh "degumming" reben kenaf hijau (24, 48, dan 72 jam) dan nisbah pepejal kepada air (50 dan 100 ml) dilihat selepas rawatan. Di samping itu, terdapat interaksi antara kedua-dua faktor. Tempoh rehat 48 jam diperlukan untuk proses "degumming", menggunakan gabungan enzim pektinase dan selulase dengan nisbah pepejal kepada air 1:100, untuk menghasilkan gentian kulit putih yang berkualiti tinggi, tulen dan putih daripada kenaf. Dalam pencirian, perbezaan kualiti serat dialami melalui pemerhatian kualitatif. Menggunakan spektroskopi inframerah transformasi Fourier (FTIR), kumpulan berfungsi yang terkandung di dalam gentian telah diperiksa. Selain itu, ia digunakan untuk melihat bagaimana prosedur "degumming" mempengaruhi pengurangan lignin dan hemiselulosa. Hasil penyiasatan penyelidikan ini menunjukkan bahawa serat daripada proses "degumming" yang dicapai dengan menggabungkan enzim pektinase dan selulase berjaya. Oleh itu, "degumming" enzimatik adalah cara yang lebih cepat untuk mencipta gentian berkualiti tinggi daripada rawatan kimia, yang boleh merendahkan kualiti gentian.

Kata kunci: Gentian Basta Kenaf, "Degumming" enzim, Pektinase, Sellulosa, Pencirian

## ABSTRACT

Physical degumming was traditionally employed in the production of kenaf bast fibers, prior to the introduction of chemical and enzymatic degumming. Physical degumming techniques include decortication, fiber cleansing, and fiber opening with the use of machines like decorticators to reduce adhesion between fiber bundles and shives. Because acid, alkali, or oxidation treatment are used during the chemical degumming process, very low quality fibers may be created, thereby compromising the fiber's quality. On the other hand, it was discovered that enzymatic degumming can provide high-quality fiber faster. In this investigation, pectinase-only enzyme solutions and 50:50 combinations of pectinase and cellulase enzyme were used to enzymatically degum kenaf bast fibers. Substantial changes in the green kenaf ribbon's degumming duration (24, 48, and 72 hours) and solid-to-water ratio (50 and 100 ml) were seen after treatment. Additionally, there was an interaction between the two factors. A 48-hour retting period was required for the degumming process, using enzyme combinations of pectinase and cellulase with a 1:100 solid to water ratio, to create high-quality, pure, and white bast fiber from kenaf. In characterization, the difference in fiber quality was experienced through qualitative observation. Utilising Fourier transform infrared (FTIR) spectroscopy, the functional groups contained inside the fiber were examined. Additionally, it was utilised to look at how the degumming procedure affected the reduction of lignin and hemicellulose.The results of this research's investigation demonstrate that fiber from the degumming process achieved by combining the enzymes pectinase and cellulase is successful. Therefore, enzymatic degumming is a faster way to create high-quality fiber than chemical treatment, which can degrade fiber quality.

Keywords: Kenaf Bast Fiber, Enzymatic Degumming, Pectinase, Cellulase, Characterization

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of Study

The annual herbaceous plant known as kenaf (Hibiscus cannabinus L.) has a short photo-period and high levels of cellulose. More than 300 species of the genus Hibiscus exist around the world. Kenaf is categorized under Malvaceae. In the kingdom of plants, it is remarkable to see such variation in the number of chromosomes and genomes. Large, bell-shaped flowers of kenaf range in colour from pale yellow to purple and have an open corolla. The blooms have five petals and a diameter of 8 to 10 cm (Y.Zhang et al.,2018). Kenaf has been utilized as a cattle feed and a cordage crop for more than 6000 years. It also used as a source of textile fiber for goods including carpets, rope and twine. Since World War II, kenaf has been introduced in China, Russia, Thailand, South Africa, Egypt, Mexico and Cuba as a reliable supply of raw material fiber for pulp, paper and other fiber products. Kenaf was initially domesticated in northern Africa and it was introduced to India 200 years ago, Russia in 1902 and China in 1908.

The primary reason for cultivating kenaf is for its fiber, namely for use as a raw material in the production of particle board and fiber board, two types of wood-based panels. Kenaf has been chosen as the future's source of fiber material for three primary reasons which is quick development, ecological flexibility and zero waste (Rowell RM et al.,1995). Nonetheless, kenaf fiber's characteristics differ according on the region,

species, age of the tree, climate and soil. The early 1970s saw the introduction of kenaf in Malaysia and by the late 1990s, the 7th Malaysian Plan 1996-2000 had identified it as a viable substitute fiber material for the pulp, paper and wood composite sectors. The majority of kenaf plant uses were based on its fibroid shape, thus the first step in converting bast plants into high-value items is to remove the fibers from the plant's bark (Jiantang Xu et al.,2020).

Age, source and the circumstances surrounding of the fiber extraction all affect the chemical composition of the kenaf bast fibers. But cellulose makes up the majority of all fibers, followed by hemicellulose, lignin, pectin, and trace quantities of fat and wax. Microfibrils are formed when long polymers of glucose molecules are joined to form cellulose. Microfibrils can have anywhere from 30 to 100 chains. The main component that gives fibers their strength, stiffness, and stability is cellulose. A type of complex polysaccharides known as hemicellulose is made up of beta-glucans, mannans, xyloglucans, and glucomannans. Despite being extremely hydrophilic, hemicelluloses lack a common chemical structure. The cellulose microfibrils' matrix is made up of hemicellulose and lignin. Hemicellulose has a lower molecular weight than cellulose. Lignin is a three-dimensional polymer with an amorphous structure and a high molecular weight. Because it has a high carbon content and a moderate hydrogen content, it gives plants more stiffness as they age (Ying Chen et al.,2018)

Most industries use the degumming technique to obtain the fiber from the bast of kenaf. Degumming is the process of releasing bonds between the cellulosic fibers and non-cellulose substrate. It may also be called retting.The four types of fiber degumming techniques are chemical, biological and physical. To improve the properties of bast fiber, most degumming procedures actually combine two or three of these categories. Applying mechanical pressures to the straw to separate the fiber bundles and shives is

known as physical extraction. The goal of the chemical process is to dissolve the non-cellulose components. When used with the right experimental parameters, chemical treatments are more effective and simpler to obtain perfect samples than physical or semi-physical extraction. This therapy nevertheless has several drawbacks, most notably environmental pollution from the byproducts' disposal. The hunt for chemical degumming solutions that are more ecologically friendly is still ongoing. These new reagents have simpler experimental procedures and are comparatively more ecologically friendly. The two main techniques for biologically separating bast fibers are enzymes and fungi. In general, these approaches are considered to be more ecologically friendly than others (Pei Lyu el at.,2021). Furthermore, pectinases, cellulases and xylanases are among the enzymes that catalyse the enzymatic degumming process of kenaf bast (Ding, Zhang, and Yu 2014). Perhaps, the degumming of kenaf bast should be implicated in the simple enzymatic interactions between enzymes and gum.

In this study, enzymatic degumming is more focused than the other methods to produce the bast fiber from kenaf. Pectinase, cellulase, xylanase and ligninase are the main enzymes needed for degumming, according to the chemical composition of bast fibers. Since enzymes have a high degree of specificity, mixtures of several enzymes are frequently used in this degumming method. The degumming duration, pH level and solid to water ratio have a significant impact on the enzymatic activity during the degumming process.

## 1.2 Problem Statement

Bast fibers, particularly kenaf are favourable because to their abundance in nature and biodegradability, making them excellent prospects for the automotive sector, structural composites, pulping and textile applications. However, the degumming techniques for kenaf bast fiber are still out of date, requiring a lot of time and labour with just average results of bast fiber. The dew degumming technique is only applicable in places where the climate is conducive to the growth of fungus. In addition, compared to other techniques, the quality of the fiber generated is sometimes poor and variable. The fibers made by water retting are often of greater quality than those made by dew retting, but the method has an environmental effect since it uses a lot of water and energy which causes enviromental pollution (Sisti et al.,2018). Chemical retting produces hazardous waste and uses energy. The problem of weather influences in dew degumming and wastewater formation in water degumming are addressed by enzymatic degumming.

One of the degumming method that can advance the kenaf bast fiber sector is artificial biological method which using enzymes. Enzymatic mixtures are used for the quicker and simultaneous degumming of fiber from the woody core and the pectin dispersion between the bundles of the fiber. This shortens the degumming process and improves quality, primarily because the fiber strands are more easily divided and have a better fineness (Ryszard Kozlowski et al.,2006). The key objective of this study is to develop the enzymatic degumming process that produces fiber fast and of high quality while taking into account the effects of enzyme concentration and degumming time.

The study's findings helped identify the ideal conditions that guarantee the degumming process on kenaf can be achieved. In addition to creating high-quality kenaf bast fiber, the goal of this study is to identify the best combinations of enzymes to help lower the cost of degumming processing.

## 1.3 Objectives

The research objectives are :

- To produce bast fiber from kenaf using enzymatic degumming
- To study the effect of parameters (pH,solid to water ratio,degumming duration) on degumming process
- To physically characterize the produced kenaf bast fiber

## 1.4 Scope of Study

The goal of this study is to identify the ideal parameters for the kenaf industry's enzymatic degumming process. During the kenaf degumming process, the solid to water volume ratio, pH and degumming duration were taken into consideration while optimizing the enzyme combination. Higher-quality fiber will be produced and environmental pollution will be reduced by employing the enzymatic degumming process. For this investigation, two sets of enzyme combinations were employed, one

of which included 100% of the enzyme pectinase and the other a 50:50 mixture of enzymes with a percentage ratio of cellulase to pectinase.

Using qualitative observation, optical microscopy, and Fourier transform infrared spectroscopy (FTIR), the physical characteristics of the kenaf bast fiber formed by enzymatic degumming were determined.

## 1.5 Significant of study

The outcomes of this study will serve as the foundation for improving the kenaf bast fiber degumming process, resulting in higher-quality fibers with less potential for pollution. This study is significant, particularly in light of the focus on the green environment. As a result, fewer chemical products that might harm the environment will be used. The study's findings demonstrate the necessity of enzymatic degumming, which is more sustainable and under control than chemical or water degumming. This work offers a promising enzymatic degumming technique for producing high-grade fiber. This study is a component of a larger effort to disseminate information on kenaf and degumming techniques. As a result, this study will help and serve as a reference for researchers as they look for further data and enhance the existing study in the future.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Kenaf

A dicotyledon plant with roots in ancient Africa, kenaf is a 6000-year-old crop that has been successfully cultivated around the world. It is a member of the Malvaceae family and belongs to the genus Hibiscus (Jiantang Xu et al.,2020). Kenaf is a tough plant with a fibrous stalk that is resistant to insect damage and is able to thrive under a broad range of climatic conditions while requiring little fertiliser, water and pesticides. Since kenaf is a dicotyledonous plant, its stalk contains three layers. An outer cortical layer, also known as bast tissue, called phloem, an inner woody core tissue layer, called xylem and a thin central pith layer, made up primarily of non-ferrous cells and sponge-like tissue. Since kenaf needs a lengthy growing season with sufficient heat and moisture, it is usually grown in tropical and subtropical locations. It is a crop that is appealing for both small-scale and large-scale agriculture due to its quick development and ability to adapt to various soil types (V.Sadrmanesh et al.,2019). The kenaf plant's bast fibers have a number of advantageous qualities, including a high tensile strength, superior absorbency and low density. Kenaf may grow well in a variety of soil types and needs just a single application of herbicide in terms of chemical treatment (Y.Zhang et al.,2018).

Kenaf fibers are useful for a variety of applications due to their characteristics. Kenaf fibers are used in a variety of sectors, including textile, automotive, building, packaging, and paper manufacturing. To create fabrics and textiles with increased strength, breathability and environmental friendliness, kenaf fibers are combined with other natural or synthetic fibers in the textile industry (Jiantang Xu et al.,2020). Kenaf fibers are used as reinforcement in composite materials for lighter and more environmentally friendly car parts in the automotive industry. In addition, kenaf fibers are employed in the creation of paper and biodegradable packaging (Ankitha Kakoty et al.,2019). To improve fiber quality and produce fibers with specified properties for various applications, many fiber extraction and processing procedures have been researched. It is well recognized as a cellulose source with both economic and ecological advantages (M.Ramesh et al.,2016).

In conclusion, the kenaf plant has become known as an adaptable and sustainable fiber crop with a wide range of industrial applications. Current research efforts are concentrated on strengthening fiber characteristics, researching new uses and evaluating the plant's environmental advantages.

## 2.2 Bast fiber

Bast fibers are strong, long-lasting and versatile natural fibers that are obtained from the stem or outer bark of certain plants. Bast fibers have a number of noteworthy qualities that support their broad range of uses. High tensile strength is a characteristic of bast fiber that makes them ideal for use in textiles, ropes and composites. Their adaptability and durability make them simple to process and manipulate. Bast fibers are

useful for textiles and insulating materials because they absorb moisture well and have strong thermal conductivity (Jiantang Xu et al.,2020).

However, cellulose makes up the majority of all fibers with hemicellulose, lignin, pectin and minor quantities of wax and fat. A lengthy polymer of glucose molecules, cellulose is linked together form microfibrils. Microfibrils can have anywhere from 30 to 100 chains. The main component that gives fibers their strength, stiffness, and stability is cellulose. The financial advantage of fiber extraction is increased by higher fiber cellulose content (V.Sadrmanesh et al.,2019).

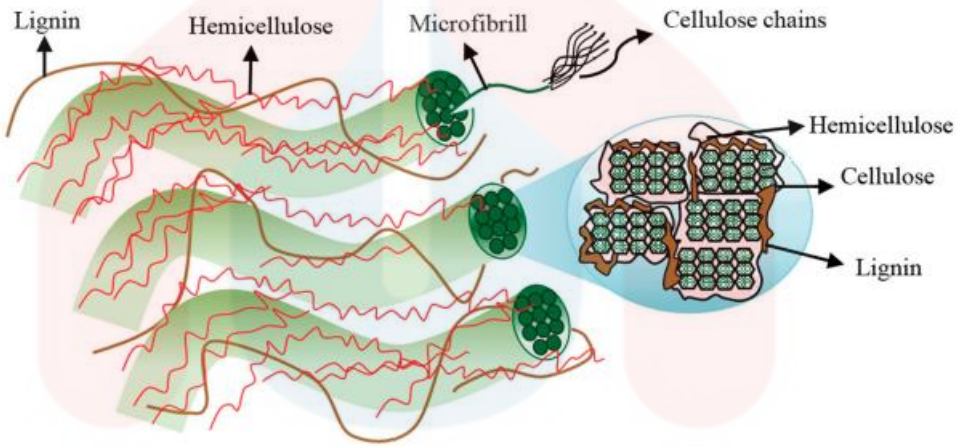


Figure 2.2: Main compositions of bast fiber and microfibril cross-section

Source : (V. Sadrmanesh et al,.2019)

This lignocellulosic fiber called has been used to make fiber boards, particle boards, textiles, fuel, and reinforcing materials for composites. The structure of lignocellulosic fibers is intricate. To create the technical fiber bundles that make up kenaf bast fiber, elementary fibers are bound together by a pectin interface (B.Ahmed Amel et al.,2012). Other than that, cellulose, noncrystalline cellulose, lignin and surface of crystalline cellulose all play a part in moisture absorption, although hemicellulose account for the majority of it. A plant's many components each have unique chemical

and physical characteristics. That is, different periods of the growth season result in distinct chemical compositions and fiber characteristics of plant tissue collected from the roots, stem, trunk and leaves (M. Ramesh et al.,2016).

In conclusion natural fibers with a variety of uses, bast fibers are adaptable and sustainable. Through continued study, researcher's understanding of their characteristics, manufacturing processes and possible applications is growing. The performance of materials based on bast fibers has to be improved, new applications need to be investigated and processing efficiency need to be increased.

## 2.3 Physical Degumming

Fibers are degummed physically by employing just mechanical processing tools to remove the bark and shavings. Physical extraction frequently requires huge machinery and a multi-step procedure, making it a factory-based operation. fibers that have been physically removed are frequently not separated further chemically or biologically. Nonetheless, they could have undergone pre-treatment in the form of field retting or pre-treatment using chemical or biological techniques. Shearing, pushing or ripping forces alone are what cause fiber bundles and shives to separate from one another. For physical degumming, there are primarily three sequential steps which decortication, fiber cleaning and fiber opening. The adhesion between fiber bundles and shives is decreased by decortication. To accomplish further separation and dust removal, the partially detached fiber-shive samples and the dusty fiber bundles must go through additional fiber cleaning procedures. To obtain fine fibers or even single fibers, the cleaned fiber bundles that have been separated will proceed through fiber opening.

The majority of physical extractions reduce time, but the fiber quality is only appropriate for specific uses, and sample findings are inconsistent. fiber breakage results in short fiber length and poor separation, which complicates fiber spinning.

The blade crusher is an antiquated technology that primarily consists of a set of blades and a high-speed motor. The type of blade, the spacing between the blades, and the angle at which the blade slides all affect the decortication effect. Hammer mills grind fiber more gently than blade crushers, producing a stunning effect in the process. A high speed rotor is housed inside a chamber that makes up a hammer mill. Walking hammers are hung on a disc that makes up the rotor. Centrifugal forces cause the rotor to revolve quickly while the hammers are operating, spreading them outward. Next, one or more pairs of rollers moving in the direction of one another make up a roll crusher. Friction then causes the stalks to move into the intended space between the rollers. The shive is split into short lengths, yet the bark may move freely between the rollers without being crushed. Moreover, ball mills can aid in the separation of fibers and shives. The two main parts of a ball mill are the horizontal barrel that contains rotating grinding balls and the feeding shaft. As stalks are fed into the feeding shaft, the barrel begins to rotate, causing centrifugal force and inertia to cause grinder balls to tumble. The stalks in the barrel are then broken up by the descending abrasives, resulting in fiber separation.

In field decortication, modified machines are typically more effective. Adrian Clarke's D8 Static Decorticator machine, a modified roller crusher, was able to remove the fiber from hemp plants in a quarter of the time it took using traditional methods. This is due to the fact that most decoration takes place in a factory, but the invention works in the field during harvest. A modified forage harvester is an additional in-field decorticator. A regular harvester has twelve knives, but this one has nine scutching bars

and three knives. Shorter fibers are produced by fewer cuts; additional scutching bars strengthen shearing pressures and enhance fiber-core separation (Y.Zhang et al.,2021)

In conclusion, physical degumming of fibers using mechanical processing instruments is a common procedure carried out in factory-based activities. The main procedures, which are meant to lessen adhesion between fiber bundles and shives, are decortication, fiber cleaning, and fiber opening. Physical degumming is effective and time-saving, but the resulting fiber quality is only appropriate for certain uses, and sample results are sometimes contradictory. The ensuing spinning process is made more difficult by issues including fiber breakage, short fiber length, and poor separation that arise from the employment of outdated technology, such as blade crushers. Modern alternatives to conventional factory-based methods, however, such as Adrian Clarke's D8 Static Decorticator and adapted forage harvesters, have shown improved efficiency in field decortication. These developments highlight the possibilities for better fiber extraction, faster processing times, and higher-quality fiber in the rapidly developing field of fiber degumming technology.

## 2.4 Chemical Degumming

The goal of chemical degumming is to remove waste from each individual fiber. Chemical therapy remains a very common procedure due to its great effectiveness and fast treatment duration, despite the fact that it may include a high oxygen demand. Reaction reagents must be carefully regulated during chemical treatment to prevent excessive or inadequate degumming and improve fiber quality. This chemical degumming consists of alkali treatment with alternative of oxidation and organic

solvents treatment. Alkali is important for degumming because it can cause fiber cells to enlarge and react with the hydrogen bonds inside the fibers. The majority of the crystalline region, which is predominantly made up of cellulose, is largely untouched by the reaction, which mostly occurs in the amorphous portions, which are generally made up of gums. In the degumming technique, fibers are helped to separate from one another while alkali concentrates on removing gum. Gums are further divided into smaller component parts and distributed throughout the liquid used for treatment, preserving the fibers. The fiber texture may be harmed by a rise in alkali concentration, resulting in a fine and brittle fiber quality. The length of the immersion mostly affects the bast fibers' tensile characteristics (Christopher Hurren et al.,2021).

An alternative to alkali treatment that can be used to prevent refractory by-products is oxidative degumming. Hydrogen peroxide ($H_2O_2$) and sodium percarbonate ($2Na_2CO_3 \cdot 3H_2O_2$) are two of the oxidants employed in degumming. Since the sole breakdown products are oxygen and water, it is a green oxide. Gum's low degree of polymerization makes it nearly always faster to depolymerize than cellulose in oxidation degumming. The primary variables are the reaction temperature, the length of the treatment, and the oxidant concentration. Because of the great oxidation ability and potential for intense reactions, it is imperative to stabilise the concentration. In light of this, oxidising stabilisers are added in order to keep the hydroxyl radical level constant (Xungai Whang et al.,2021).

Another alternative method for chemical degumming of organic solvents comes from their role in separating biomass used to make paper pulp. The organic solvents and catalysts are used in a water matrix for the reaction to occur. Sodium chloride ($NaCl$), calcium chloride ($CaCl_2$), magnesium sulphate ($MgSO_4$), ferric chloride ($FeCl_3$), ferric sulphate ($Fe_2(SO_4)_3$), cupric chloride ($CuCl_2$), aluminium chloride

(AlCl3), acetic acid (CH3COOH), formic acid (HCOOH), and sulfuric acid (H2SO4) are examples of salts and acids that are commonly used as catalysts. In an environment with elevated temperatures and pressure, organic solvents have the ability to seep into the spaces between individual fibers, which are home to gums. Aryl ether bonds and ether bonds inside lignin, as well as those between lignin and activated carbohydrates, are further broken by the solvent molecules. Subsequently, catalysts quicken the pace at which residual and broken gums dissolve in the solution. Rinsing yields fine fibers, and any leftover solution can be distilled and used again. The solubility variations of gums in different organic solvents may make gum recovery by degumming with organic solvents a viable option. However, using strong organic solvents may provide challenges (Pei Lyu et al.,2021).

To sum up, chemical degumming accomplishes the important goal of eliminating waste from individual fibers, and because of its efficiency and short treatment time, it is still a commonly used technique. By expanding the fiber cells and interacting with the hydrogen bonds inside the fibers, alkali treatment mainly targets the amorphous areas that contain gums. Although alkali makes fiber separation easier, an overabundance of it can result in tiny, brittle fibers, which affects tensile properties. Oxidative degumming, which yields only oxygen and water as byproducts and uses chemicals like sodium percarbonate and hydrogen peroxide, provides a greener option. In the last technique, lignin and activated carbohydrates' aryl and ether linkages are broken by solvents and catalysts in a water matrix. All things considered, chemical degumming processes provide a variety of approaches, each with pros and downsides that underscore the continuous search for efficient and long-lasting degumming procedures in the fiber processing industry.

## 2.5 Enzymatic Degumming

Enzymatic degumming is a promising approach for the processing of natural fibers, including kenaf bast fiber. Enzymes are used in the enzymatic degumming of kenaf bast fiber to take out non-cellulosic elements such pectins, waxes and hemicelluloses. The quality and effectiveness of the fiber might be adversely affected by these contaminants in a variety of applications (R.Kozlowski et al.,2006). These non-cellulosic components are selectively degraded and solubilized using enzymes with specialized activity, such as pectinases, xylanases and cellulases, leaving behind pure bast fibers. Pectins, a significant portion of the gum-like compounds found in bast fibers, are hydrolyzed by pectinases, such as polygalacturonases and pectate lyases. The hemicelluloses in the fiber are the target of xylanases, which convert them into soluble sugars (Y.Zhang et al.,2018). Additionally, amorphous cellulose can be eliminated by cellulases, improving the quality of the fiber.

Several parameters involved in the enzymatic degumming of kenaf bast fiber may be optimized to improve efficiency and fiber quality. The effectiveness of degumming can be affected by variables such enzyme concentration, reaction duration, temperature, pH and the presence of additives. These parameters' influence on the level of impurity removal, fiber shape, mechanical characteristics and process economics have all been studied through optimization studies (Pei Lyu et al.,2021).

A prior research found that while the temperature is a major component in enzymatic retting, the temperature also has a considerable effect on the reaction of the enzyme to the temperature. It is recommended that the enzyme activities be activated at a temperature of 45 °C. Next, a constant pH of 3.0 was employed, which was the ideal pH. In this experiment, the optimal enzyme combinations were also examined in order to determine which combination would yield the highest-quality bast fiber.

The characteristics and uses of kenaf bast fiber can be dramatically impacted by enzymatic degumming. The purity, cleanliness and homogeneity of the fiber are improved by the removal of non-cellulosic contaminants, which results in improved mechanical qualities and greater fiber strength. Enzymatic treatment of fiber surfaces can also alter the fiber's wettability, dye-ability and compatibility with different matrices in composite materials (Qi Yang et al.,2019).

In conclusion, the purification and processing of kenaf bast fiber may be done using an effective and environmentally friendly technology called enzymatic degumming. Utilizing certain enzymes enables the selective elimination of contaminants while retaining the fiber's desired characteristics. Enzyme optimization, process engineering and the assessment of enzymatic degumming in industrial-scale kenaf bast fiber applications are the main areas of ongoing study. Finding the ideal circumstances that maximize degumming effectiveness while preserving the consistency and calibre of the bast fiber is the objective.

## 2.6 Pectinase

Pectinases are a class of enzymes that catalyse the hydrolysis, transelimination, and deesterification events that break down pectin, a polysaccharide present in plant cell walls. It is helpful because other components of the cell wall, such cellulose fibrils, are embedded in pectin, the jelly-like matrix that helps hold plant cells together. Pectinase enzymes are therefore frequently employed in procedures requiring the breakdown of plant materials, such as accelerating the process of extracting fruit juice from fruits, such as apples and sapota. Since the 1960s, pectinases have also been utilised in the

manufacture of wine. It is possible to extract pectinases from fungus like Aspergillus niger.

These enzymes are produced by the fungus to dissolve the middle lamella in plants, allowing the fungus to insert fungal hyphae and take nutrients from the plant tissues. Boiling pectinase causes it to become denatured or unfolded, which hinders its ability to bind to pectin at the active site and reduces its juice production. Current pectinase enzymes are not synthesised by animal or human cells instead, they are naturally created by fungus and yeasts (50%), insects, bacteria and microbes (35%), and diverse plants (15%). Pectinase enzymes in plants hydrolyze the pectin present in their cell walls, facilitating new development and modifications. Pectinases break down pectin throughout the fungal developmental stage, in a manner similar to their function in plants.

## 2.7 Cellulase

The 1, 4 glucosyl links found in cellulose, an insoluble linear glucose homopolymer, are hydrolyzed by cellulases. Important industrial enzymes, cellulases are essential to the global carbon cycle. There is great variation in the structures and modes of action of cellulases. All plants, numerous fungi, certain bacteria and a few mammals generate them. Due to its use in juice extraction, paper recycling, detergent enzyme production, cotton processing (stonewashing denim) and animal feed additives, cellulases are presently the third-largest industrial enzyme in terms of commercial value. On the other hand, if ethanol, butanol, or any other fermentation product of sugars, derived from biomass, becomes a major transportation fuel, as appears

inevitable, cellulases will become the greatest volume industrial enzyme (D B Wilson et al.,2009).

Cellulose that has been combined with hemicellulose and lignin makes up the majority of lignocellulose compounds. Pretreatment is necessary to separate cellulose, hemicellulose, and lignin precisely from the reaction of enzyme cellulases with pure cellulose. Cellulases and xylanases together have been shown to improve fiber strength, which may boost cellulase accessibility, by removing xylan and its substituents as well as any low molecular weight lignin fragments associated with xylanases. However, cellulase has demonstrated the accessibility of xylanases to softwood as mild cellulase pretreatments by raising the apparent median pore size of the pulp, hence simplifying the subsequent pre-bleaching process by xylanases (R. Bura et al.,2007).

## 2.8 Physical Characterization

The mechanical and processing characteristics of kenaf bast fiber are greatly influenced by its morphological characteristics, including fiber length, diameter and aspect ratio. To measure and analyse the fiber size and shape, research investigations have used methods including microscopy (optical, scanning electron microscopy) and image analysis (Karim, S.Tahir et al.,2014).

The performance of kenaf bast fiber in applications including textiles, composites and reinforcements is largely determined by its tensile strength and mechanical characteristics. To assess the tensile strength and elasticity of kenaf fibers, researchers have used procedures such single fiber tensile testing, bundle testing and fiber fragmentation analysis (Faisal Islam et al.2019).

Thermal conductivity and heat resistance of kenaf bast fiber are crucial factors to take into account for applications in insulation, composites, and thermal management. To evaluate the thermal behaviour of kenaf fiber, researchers have used methods such as thermal conductivity tests, differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), (Yi Cui, Shuyi Gao et al.,2020).

Kenaf bast fiber's surface properties, including as surface roughness, topography, and chemical composition, affect how it interacts with matrices in composite materials and impact the strength of the interfacial bonding. The surface characteristics and chemical makeup of kenaf fibers have been studied using surface analysis technique such as Fourier-transform infrared spectroscopy (FTIR), (Da Silva et al.,2021).

Understanding kenaf bast fiber's physical characteristics is essential for maximization its performance in a variety of applications. Researchers have examined the fiber's shape, size, tensile strength, moisture absorption, thermal conductivity and surface properties using a variety of approaches. The knowledge and application of kenaf bast fiber in many sectors will continue to progress with further investigation into physical characterization techniques and their relationship to the fiber's performance.

# CHAPTER 3

# MATERIAL AND METHODS

## 3.1 Materials

Lembaga Kenaf dan Tembakau Negara, Kubang Kerian, Kelantan is where the fiber for kenaf bast will be harvested. From kenaf plants that were three to four months old, green ribbons of kenaf will be obtained.

## 3.2 Chemical and Reagents

Chemicals were used in this experiment were glacial acetate acid (100%), EDTA surfactant, pectinase, cellulase, distilled water. Those chemicals were obtained from faculty laboratory.

## 3.3 Apparatus and Equipment

The apparatus were used in this experiment are media bottle (100 ml and 250 ml), beakers (100 ml and 250 ml), measuring cylinder (10 ml and 50ml), forceps and caliper.

The equipment were used in this experiment are water bath, weighing balance, pH meter, drying oven, fourier-transform infrared spectroscopy (FTIR) and digital microscope.

## 3.4 Methodology

### 3.2.1 Preparation of sample

From kenaf plantations in Kubang Kerian, Kelantan's Lembaga Kenaf Dan Tembakau Negara (LKTN), kenaf stalks will be harvested and gathered. The kenaf tree will be picked because it was mature and ready for harvest. Based on the kenaf tree's size and length, the maturity of kenaf will be calculated. As a sample size, green ribbon kenaf weighing 400 g or more will be used. The number of samples needed for this investigation is 50 samples. To prevent bias in the samples, the centre of the kenaf plant, where the 0.5 metre from bottom and 0.5 metre tips will be cut and used for all sampling. The total sample was divided into 10 cm and approximately 10 g of samples were required for the degumming procedure.

To enter the plant bast fibers and extract the water soluble substance or heavy metal, all samples will be treated with catalyst solution using EDTA-surfactant. Glacial acetic acid is used to adjust the pH of the solution. The samples will be then

supplemented with enzyme into the solution to begin the enzymatic degumming process.

### 3.2.2 Enzymatic degumming process

To determine the best response to employ pectinase in the bio retting process, pectinase is used independently without combining with any other enzyme. Different concentration of pectinase is represented by the usage of 0.1 g. fibers from kenaf bast placed in a 100 ml beaker. Make sure the kenaf bast fibers are completely immersed in the pectinase solution. To aid the enzymatic process, the solution stirred periodically. The kenaf bast fibers left for allocated time for the degumming process to occur. The continuous working settings for this experiment will be 45 °C and anaerobic conditions. Kenaf bast fiber also will be produced from water retting method which is a conventional method which act as an control in this experiment. The physical characterization of the produced fiber also will be compared.

It will be investigated how pH, degumming duration, solid to water ration affect as parameter in degumming process. Three range pH have used in this experiment which is 2.0, 2.5 and 3.0. The pH adjusted by glacial acetic acid and Henderson-Hasselbatch equation. Initial pH, interval pH and final pH were recorded. The degumming duration was measured over of 24, 48 and 72 hours. Different solid to water ratio implemented which is 1:50 and 1:100. For example, a ratio of 1 to 100 of solid to volume of water means 0.1 g of pectinase with 100 g of water. 100 g of water is equivalent to 100 mL of water used to dilute the enzyme powder.

Table 3.1 : The range of various parameters used for enzymatic degumming process

| **Parameters** | **pH range (1-14)** | **Degumming duration (hours)** | **Solid to water ratio (Weight of kenaf : weight of water)** |
|---|---|---|---|
| Pectinase | 2.0<br>2.5<br>3.0 | 24<br>48<br>72 | 1 : 50<br>1 : 100 |
| Pectinase / Cellulase (50 : 50) | 2.0<br>2.5<br>3.0 | 24<br>48<br>72 | 1 : 50<br>1 : 100 |

Table 3.2 : The constant parameters used for enzymatic degumming process

| **Parameters** | **Description** |
|---|---|
| Temperature | 45℃ |
| Aeration | Anaerobic |

### 3.2.3 Fiber opening and drying process

The fibers removed from the pectinase solution after the enzymatic treatment. The fibers rinsed well with distilled water to get rid of any leftover enzyme solution or degumming byproducts. Filtration method used to filter the degummed kenaf bast fibers. The fiber opening were done using forceps. The fibers placed in drying oven for drying process. Repeat this procedure for other enzyme and enzyme combination.

### 3.2.4 Physical characterization of kenaf bast fiber

Physical properties such as fiber morphology and surface characteristics will be analyzed in this research.

A microscope slide should be prepared by lightly covering it with kenaf bast fiber before doing the fiber morphology investigation. Utilizing the proper magnification of 30x and 50x, the fiber morphology is examined with an optical microscope (SEM). With the use of imaging software or a digital camera, pictures of the fiber taken. The pictures analysed to determine the fibers' length, diameter and aspect ratio.

Finally for surface characteristics analysis, a Fourier transform infrared spectroscopy (FTIR) analysis was carried out. Prior to this examination, all fibers were

crushed to a diameter of around 100 µm, mixed with KBr to make homogeneous suspensions. Then pressed into clear pellets and analyzed in transmittance mode in the 3500 - 500 cm^-1 range. Samples were tested at Universti Malaysia Terengganu (UMT).

## 3.5 RESEARCH FLOW CHART

The research flow chart is used to analyse, assess and compare the experimental data that was acquired and is displayed in Figure 3.1.

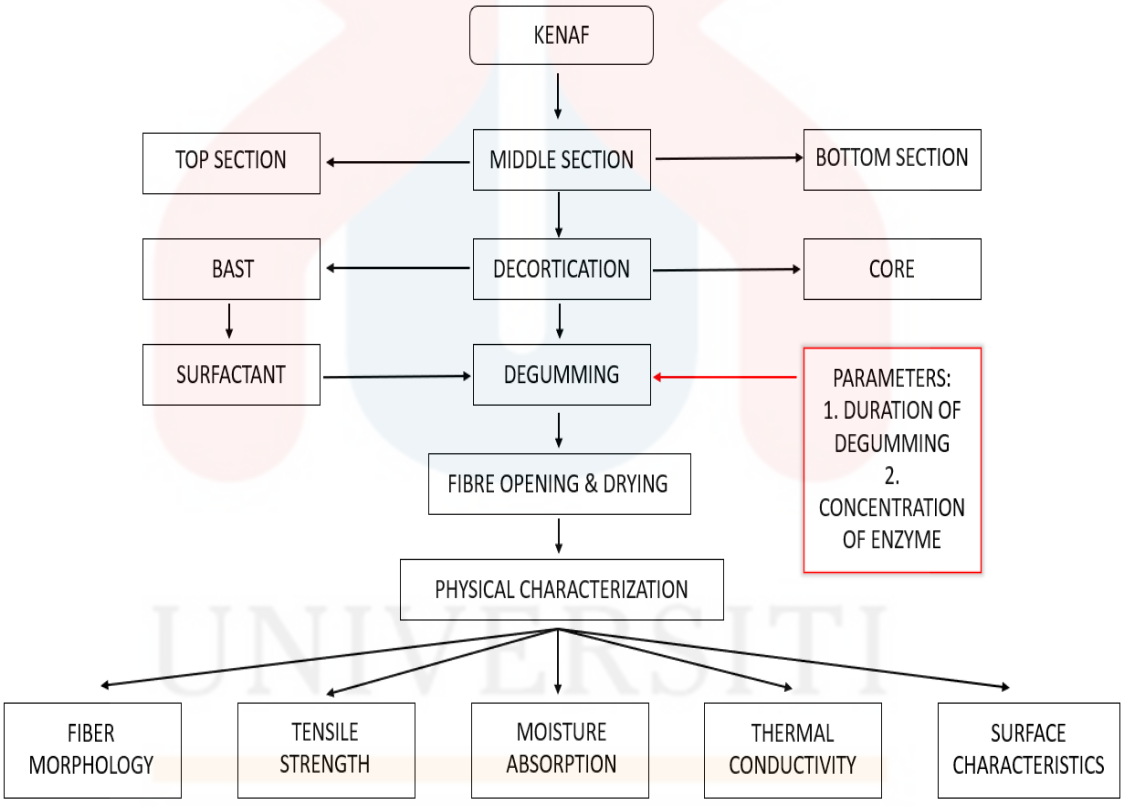


Figure 3.1: Research flow for enzymatic degumming process and physical characterization of kenaf fiber.

# CHAPTER 4

# RESULTS AND DISCUSSIONS

## 4.1 Qualitative observation of kenaf bast fiber

### 4.1.1 Observation results on pectinase (100%) with degumming duration of 24 hours

The largest volume of enzyme solution with a ratio of 1:100 of solid to volume of water was found to more readily remove pectin from the bast fiber based on the qualitative observation findings of the kenaf bast fiber for degumming process at 24 hours. In comparison to an enzyme solution with a high concentration, the fiber is also cleaner. Figure 4.1 (a) and Figure 4.1 (b) below shows the green kenaf ribbon before and after degumming for 24 hours.

a)

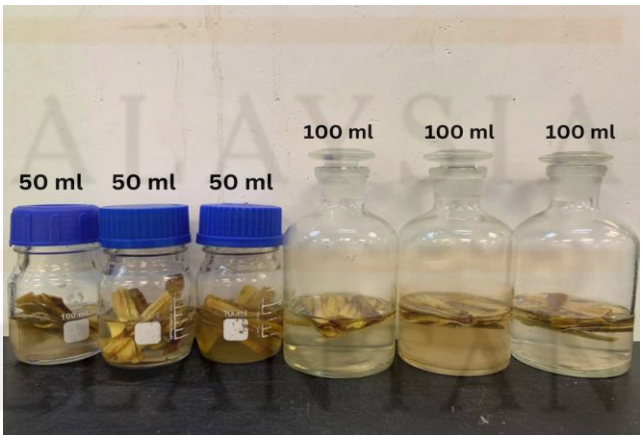


Figure 4.1 (a) : Green kenaf ribbon before degumming (0 hour)

b)

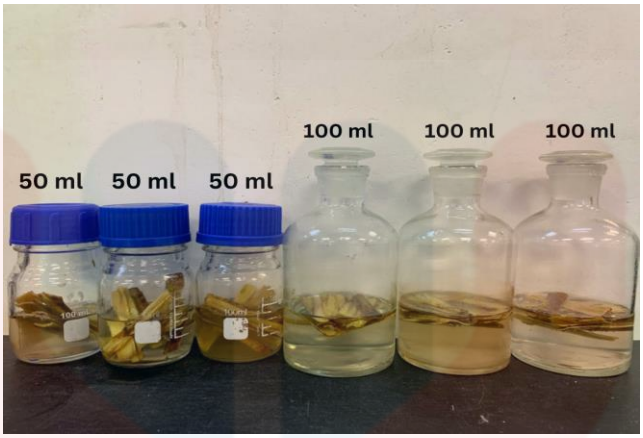


Figure 4.1 (b) : Green kenaf ribbon after degumming process (24 hour)

The green kenaf ribbon is not entirely degummed at the ratio of 1:50 of solid to volume of water because, despite the high concentration of enzymes, the volume of the enzyme solution is smaller. The solution completely encapsulates one of the fundamental tenets of the degumming process. Making sure the fiber is completely degummed is crucial. However, a larger volume of acetic acid is employed to guarantee the pH range of the solution. The strength of the fiber may be impacted when a large volume of acetic acid is employed during the degumming process. This is due to the fact that the study will be conducted on enzyme activity in three distinct pH ranges which is 2.0, 2.5 and 3.0.

It was also noted that the color of the enzyme solution changed. It is demonstrated that more particles are released into the enzyme solution and that the color of the solution changes to hazy. The color variations of the enzyme solution are visible in Figures 4.1(a) and 4.1(b), which display the color of the solution before and after the enzymatic degumming process throughout a 24 hour period.

Table 4.1 : Observation experimental results on kenaf bast fiber produced for degumming duration 24 hours

| Solid to volume of water ratio | Initial pH of the enzyme solution | Interval pH of the enzyme solution | Final pH of the enzyme solution | Acetic acid used (ml) | Observation (Texture, Fiber whiteness) |
|---|---|---|---|---|---|
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 2.0 | 1.96 | 1.88 | 45 | Rough, Less furry, Brown |
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 2.5 | 2.4 | 2.33 | 30 | Rough, Hardest, Brown |
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 3.0 | 2.91 | 2.83 | 15 | Rough, Hardest, Brown |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 2.0 | 1.82 | 1.79 | 90 | Rough, Furry, Yellowish |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 2.5 | 2.39 | 2.2 | 60 | Rough, Less furry, Yellowish |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 3.0 | 2.85 | 2.66 | 30 | Rough, Less furry, Yellowish |

The experimental results for a 24 hour degumming process are displayed in Table 4.1. The ratio 1: 50 enzyme solution's pH values have somewhat decreased. This is because not all of the kenaf ribbon was adequately submerged, which prevented the degumming process to complete. In comparison to other pH values, the fiber generated at a 1:100 solid to water ratio is yellowish and furrier at pH 2.0. When 10g of kenaf ribbon and 50ml of water are used, the fiber generated at 1:100 ratios is noticeably

superior since the ribbon is not fully immersed and degummed. The kenaf ribbon has firmer, rougher fiber as a result and has less pectin overall. In comparison to the toughest, roughest and yellowish-colored fiber generated from 50 mL of enzyme solution, the fiber produced from 100 mL of enzyme solution is yellowish and furrier.

### 4.1.2 Observation results on pectinase (100%) with degumming duration of 48 hours

All of the fibers are degummed and formed from varying volumes of enzyme solution, and they are all furrier than the fibers produced from a 24-hour retting period, as demonstrated by the results of the 48-hour retting period. The discrepancy arises from comparing the fibers' pH levels and solid-water ratios. Where pectin is generated on the kenaf ribbon, it turns jelly-like and more sticky. The fiber becomes more white and cleaner and the pectin washed away easily during the washing process.

The state of the enzyme solution is identical to what was seen throughout the 24-hour degumming procedure. When the degumming process produces solutions from kenaf ribbon, the colour of the enzyme solutions changes to unclear, with more particles and a dump of pectin.

To ensure that the kenaf ribbon is completely submerged in the 1:50 solid to water ratio enzyme solution, the process was modified and a larger media container was employed. Compared to the fiber generated by the 1:50 solid to water ratio enzyme solution, the fiber produced by the 1:100 solid to water ratio is whiter in appearance and has a finer texture. This is because the fiber still has rough spots where pectin is still attached to the fiber. The fiber at pH level of 2.5 and 3.0 still has a noticeable yellow

tint since it still includes pectin. When the gum has fully degummed, the colour of the fiber changes from brown to yellowish to white, indicating the highest grade of fiber. The experimental results for a 48 hour degumming process are displayed in Table 4.2.

Table 4.2 : Observation experimental results on kenaf bast fiber produced for degumming duration 48 hours

| **Solid to volume of water ratio** | **Initial pH of the enzyme solution** | **Interval pH of the enzyme solution** | **Final pH of the enzyme solution** | **Acetic acid used (ml)** | **Observation (Texture, Fiber whiteness)** |
|---|---|---|---|---|---|
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 2.0 | 1.91 | 1.79 | 45 | Smooth, Furry, White |
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 2.5 | 2.39 | 2.26 | 30 | Smooth, Less furry, White |
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 3.0 | 2.9 | 2.79 | 15 | Smooth, Less furry, White |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 2.0 | 1.86 | 1.81 | 90 | Smooth, Furry, White |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 2.5 | 2.41 | 2.29 | 60 | Smooth, Furry, White |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 3.0 | 2.88 | 2.75 | 30 | Smooth, Furry, White |

### 4.1.3 Observation results on pectinase (100%) with degumming duration of 72 hours

The fiber quality from the 72-hour degumming procedure demonstrates that the fiber quality is worse than that of the 48-hour degumming process. Compared to the fiber that was degumming for 48 hours, the resulting fiber is somewhat smoother and more yellowish in colour. Figures 4.3(a) and 4.3(b) compare the conditions before and after the 72 hour degumming procedure.

a)


Figure 4.3 (a) : Green kenaf ribbon before degumming (0 hour)

b)

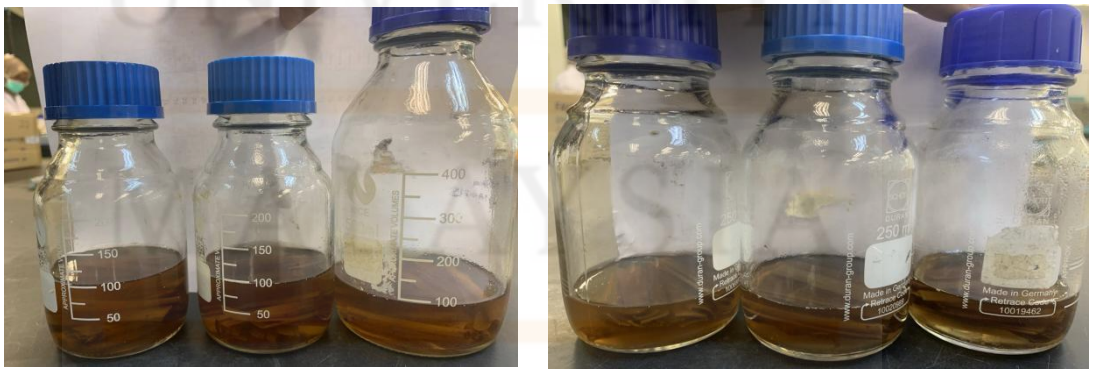

Figure 4.3 (b) : Green kenaf ribbon after degumming (72 hour)

The observation on kenaf fiber produced from the degumming method during 72 hours is displayed in Table 4.3. The fiber extracted from the 100 mL enzyme solution has a whiter, smoother, and furrier texture. Although the fiber extracted from 50 mL of enzyme solution has a little coarse texture and a yellowish hue. It is obliquely demonstrating that kenaf ribbon fully submerged in an enzyme solution and yields the highest grade fiber.

Table 4.3 : Observation experimental results on kenaf bast fiber produced for degumming duration 72 hours

| **Solid to volume of water ratio** | **Initial pH of the enzyme solution** | **Interval pH of the enzyme solution** | **Final pH of the enzyme solution** | **Acetic acid used (ml)** | **Observation (Texture, Fiber whiteness)** |
|---|---|---|---|---|---|
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 2.0 | 1.94 | 1.83 | 45 | Smooth, Less Furry, White |
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 2.5 | 2.42 | 2.33 | 30 | Less Smooth, Less furry, Yellowish |
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 3.0 | 2.91 | 2.85 | 15 | Less smooth, Less furry, Yellowish |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 2.0 | 1.89 | 1.84 | 90 | Smooth, Furry, White |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 2.5 | 2.39 | 2.31 | 60 | Less smooth, Furry, White |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 3.0 | 2.89 | 2.72 | 30 | Less smooth, Less furry, Yellowish |

### 4.1.4 Observation results on enzyme combination pectinase and cellulase (50:50) with degumming duration of 24 hours

Pectinase, xylanases and cellulases are three combinations of enzymes that have been used to analyse the quality of fiber. Nonetheless, the same amount of pectinase and cellulase enzyme is employed because the goal is to eliminate lignin and pectin as well as break down plant cell cellulose to increase the lustre and brightness of the fiber. The enzyme solution is in the same condition as it was during the whole 24-hour degumming process. The colour of the enzyme solutions turns cloudy with more particles and a pectin dump, when the degumming procedure yields solutions from kenaf ribbon. Figures 4.4(a) and 4.4(b) compare the conditions before and after the 24 hour degumming procedure.

a)

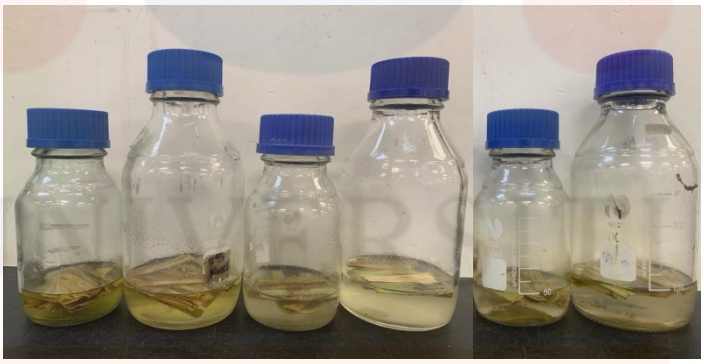

Figure 4.4 (a) : Green kenaf ribbon before degumming (0 hour)

b)

Figure 4.4 (b) : Green kenaf ribbon after degumming (24 hour)

The 24 hour degumming duration demonstrates that every fiber generated is in a hairy state. Nevertheless, the fiber generated from 1:50 solid to water ratio enzyme solution has a rougher texture than the largest capacity, a 1:100 solid to water ratio enzyme solution. Next, compared to other fibers, the 1:100 solid to water ratio amount of enzyme solution also yields fiber with more lustre and brightness. The fiber from the 1:100 solid to water ratio enzyme solution removes pectin and lignin the easiest during the washing process. The experimental results for a 24 hour degumming process are displayed in Table 4.4.

Table 4.4 : Observation experimental results on kenaf bast fiber produced from enzyme combination of pectinase and cellulase for degumming duration of 24 hours

| Solid to volume of water ratio | Initial pH of the enzyme solution | Interval pH of the enzyme solution | Final pH of the enzyme solution | Acetic acid used (ml) | Observation (Texture, Fiber whiteness) |
|---|---|---|---|---|---|
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 2.0 | 1.89 | 1.67 | 45 | Smooth, Furry, White |
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 2.5 | 2.39 | 2.24 | 30 | Smooth, Less furry, White |
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 3.0 | 2.86 | 2.73 | 15 | Less smooth, Less furry, White |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 2.0 | 1.84 | 1.79 | 90 | Smooth, Furry, White |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 2.5 | 2.31 | 2.23 | 60 | Smooth, Furry, White |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 3.0 | 2.87 | 2.61 | 30 | Smooth, Furry, White |

### 4.1.5 Observation results on enzyme combination pectinase and cellulase (50:50) with degumming duration of 48 hours

The colour of the fiber produced by the various volumes of enzyme solution was used to examine the retting time of 48 hours. The fiber that is displayed has a yellower hue than the fiber that was degummed after a 24 hour degumming process. The colour, fineness, and lustre of an enzyme indicate its quality, which is influenced by both its volume and concentration. Based on observations, kenaf bast fiber made using an enzyme solution with a solid to water ratio of 1:100 seems more superficial due to increased smoothness, fineness, and pectin loss. On the other hand, the fiber's hue is yellowish in contrast to the whiter fiber that is produced in a day. This is because the effects of the immersion process take longer to manifest.

The enzyme solution is in the same condition as it was during the whole 24-hour degumming process. The colour of the enzyme solutions turns cloudy with more particles and a pectin dump, when the degumming procedure yields solutions from kenaf ribbon. The experimental results for a 48 hour degumming process are displayed in Table 4.5.

Table 4.5 : Observation experimental results on kenaf bast fiber produced from enzyme combination of pectinase and cellulase for degumming duration of 48 hours

| Solid to volume of water ratio | Initial pH of the enzyme solution | Interval pH of the enzyme solution | Final pH of the enzyme solution | Acetic acid used (ml) | Observation (Texture, Fiber whiteness) |
|---|---|---|---|---|---|
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 2.0 | 1.84 | 1.62 | 45 | Less smooth, Less furry, White |
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 2.5 | 2.33 | 2.20 | 30 | Less smooth, Less furry, Yellowish |
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 3.0 | 2.78 | 2.66 | 15 | Less smooth, Less furry, Yellowish |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 2.0 | 1.75 | 1.59 | 90 | Smooth, Furry, White |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 2.5 | 2.28 | 2.19 | 60 | Smooth, Less furry, White |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 3.0 | 2.83 | 2.69 | 30 | Smooth, Furry, Yellowish |

### 4.1.6 Observation results on enzyme combination pectinase and cellulase (50:50) with degumming duration of 72 hours

After a 72-hour retting time, the quality of the fiber is nearly identical to that after 48 hours. Every fiber created is cleaner and furrier. The 1:100 solid to water volume ratio of the enzyme solution yields the optimum fiber quality for the 72-hour parameter retting period. The fiber is yellowish-colored, hairy and silky. However, the fiber made from a 1:50 solid to water enzyme solution is less shiny, brighter, and less smooth. When compared to fiber made from an enzyme solution with a 1:100 solid to water ratio, the furry state is not as soft. This study's parameter of 72 hours on two distinct enzyme combinations demonstrates that, for every enzyme concentration, a different volume of acetic acid was utilised for every ratio of water volume. Figures 4.6(a) and 4.6(b) compare the conditions before and after the 72 hour degumming procedure.

a)

Figure 4.6 (a) : Green kenaf ribbon before degumming (72 hour)

b)

Figure 4.6 (b) : Green kenaf ribbon after degumming (72 hour)

The experimental results for a 72 hour degumming process are displayed in Table 4.6. Following the green kenaf ribbon degumming procedure, the pectin was eliminated by rinsing with tap water and air drying. Since pectin dissolves in an enzyme solution that might alter its colour, some pectin from fiber cannot be extracted directly. The amount of acid employed in the degumming process also had an impact on the removal of lignin and pectin.

Table 4.6 : Observation experimental results on kenaf bast fiber produced from enzyme combination of pectinase and cellulase for degumming duration of 72 hours

| Solid to volume of water ratio | Initial pH of the enzyme solution | Interval pH of the enzyme solution | Final pH of the enzyme solution | Acetic acid used (ml) | Observation (Texture, Fiber whiteness) |
|---|---|---|---|---|---|
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 2.0 | 1.89 | 1.67 | 45 | Less smooth, Less furry, Yellowish |
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 2.5 | 2.39 | 2.24 | 30 | Less smooth, Less furry, Yellowish |
| 1 : 50 (10 g of kenaf ribbon with 50 ml of water) | 3.0 | 2.86 | 2.73 | 15 | Less smooth, Less furry, Yellowish |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 2.0 | 1.84 | 1.79 | 90 | Smooth, Furry, White |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 2.5 | 2.31 | 2.23 | 60 | Smooth, Less furry, Yellowish |
| 1 : 100 (10 g of kenaf ribbon with 100 ml of water) | 3.0 | 2.87 | 2.61 | 30 | Smooth, Furry, Yellowish |

## 4.2 Physical characterization of kenaf bast fiber

### 4.2.1 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Particular functional groups found in the kenaf bast fiber may be identified using FTIR. It is possible to distinguish between different functional groups in a fiber by their ability to absorb infrared light at distinctive frequencies. These components include cellulose, hemicellulose, lignin and pectin. From the qualatitative observation, 5 samples including raw kenaf were selected for FTIR analysis. All extracted sample FTIR spectra were obtained and are displayed in Figure 4.7.

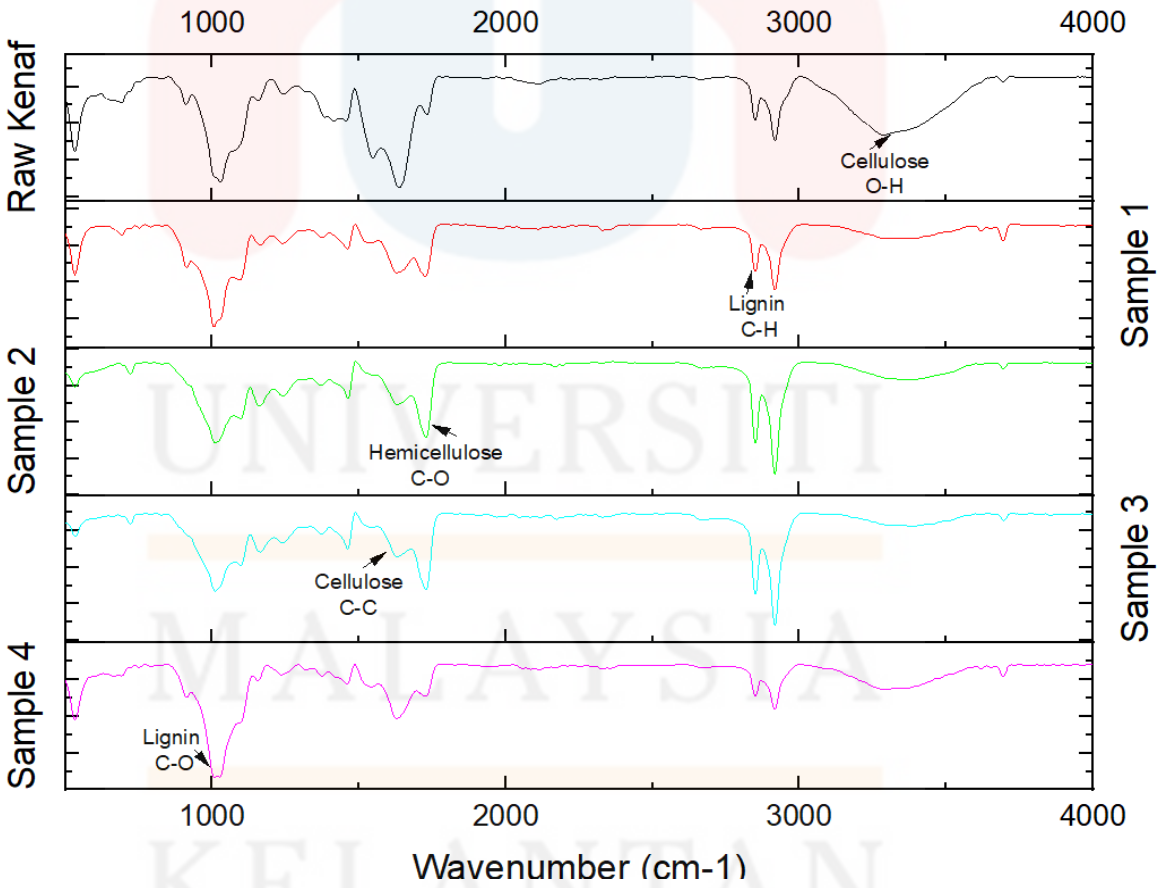


Figure 4.7 : Spectra of the Raw Kenaf, Sample 1, Sample 2, Sample 3 and Sample 4

Sample 1 is fiber obtained from enzyme combination pectinase and cellulase (50:50) with degumming duration of 72 hours at pH of 2.0. Sample 2 is fiber obtained from enzyme combination pectinase and cellulase (50:50) with degumming duration of 48 hours at pH of 2.5. Sample 3 is fiber obtained from enzyme combination pectinase and cellulase (50:50) with degumming duration of 48 hours at pH of 3.0. Sample 4 is fiber obtained from enzyme combination pectinase and cellulase (50:50) with degumming duration of 24 hours at pH of 2.0.

From the raw kenaf, the peaks are beginning to be observed for comparisons at absorbance peaks 3285.69 cm-1. OH groups are associated with the area 3330-3400 cm' for both treated and untreated kenaf bast fibers. OH groups were also found in other samples, but at a different transmittance level and at the same range. The wide absorption band at 3285.69 cm-1 is caused by cellulose's O-H stretching vibrations. On the other hand, the absorbance peaks that were found between 2800 and 3000 cm'were caused by the C-H groups stretching, as this stretch is present in every fiber. When sample 4 was compared to sample 1, the transmittance level was greater. The C-H groups linked to the chemicals that make up lignin.

The acetyl group of hemicellulose from the xylan sources was identified as the cause of the C=O stretching seen from the peak at 1731.11 cm-1 in raw kenaf green ribbon. This peak acquires lower frequency following the enzymatic degumming treatment. This disappearance is a result of the retting process removing the hemicelluloses from the fiber.

The peak positions of 1242.22 cm-1 and 1028.42 cm-1 in the raw kenaf green ribbon were ascribed to the lignin's aryl group's C-O stretching. This peak illustrates the wavenumber frequency drop following enzymatic degumming, as seen in Figure 4.7. The frequency decrease indicates the elimination of cellulose, lignin and hemicellulose with use of pectinase and cellulase in the degumming process was more successful. Table 4.7 displays the FTIR analysis's summary of the usual functional groups as well as the IR signal including the potential chemical.

Table 4.7 : Functional groups in raw kenaf and other samples

| **Wave Numbers (cm^-1)** | **Functional Groups** | **Compounds** |
|---|---|---|
| 3285.69 | O - H stretching | Cellulose |
| 2917.04 | C - H stretching | Lignin |
| 1731.11 | C - O stretching | Hemicellulose |
| 1637.39 | C - C stretching | Cellulose |
| 1242.22 | C - O stretching | Lignin |

# CHAPTER 5

# CONCLUSION AND RECOMMENDATIONS

## 5.1 Conclusion

In order to create high-quality fiber, this study looked into the enzymatic retting process at various degumming durations, enzyme combinations, and solid to water ratios. The best outcomes from the experiment show that, as compared to 24 hours, kenaf bast fiber generated for 48 and 72 hours provided high-quality fiber based on observations of its whiteness and cleanliness. In terms of furriness and whiteness, the enzymatic combination of pectinase alone or pectinase plus cellulase (50:50) produced different fiber quality.

Qualitative observation demonstrates that enzymatic degumming is successful in the first place by yielding fiber of varying grade. According to observations, the smoothest and whitest fiber is obtained after a 48-hour degumming procedure, making it appropriate for industrial use. Apart from that, fiber in the pH range between 2.0 and 2.5 is of higher quality than fiber in the pH 3.0 range. Acidity facilitates a successful degumming procedure. Excessive acidity also causes fiber breakage, as demonstrated by a pH of 2.0 after a 72-hour degumming procedure. When it comes to qualitative observations, the combination of enzymes presents a doubtful picture of the fiber. The

fiber's fineness may be one of them, however FTIR analysis can provide reliable findings.

To identify changes in the functional group throughout the 24-, 48-, and 72-hour treatments of the enzyme combination pectinase and cellulase for the purpose of enzymatic degumming of kenaf bast fiber, Fourier transform infrared (FTIR) investigation is employed. This study demonstrated that enzymatic degumming reduces the amounts of hemicellulose, pectin, waxes, and lignin that were in the fibers prior to retting at 1731.11 cm-1. When pectinase and cellulase are used together for enzymatic degumming, the fiber reduces more hemicellulose than when pectinase is used alone. Therefore, employing pectinase for enzymatic degumming to remove lignin content is more successful than utilising a combination of pectinase and cellulase enzymes due to the increased pectinase concentration.

## 5.2 Recommendations

First of all, because it is the severe rainy season and the plantation will be closed, getting kenaf from September to December might be difficult. It is advised to obtain the kenaf throughout the month of August. It is advised that the sample be pre-treated for upcoming research on the kenaf bast fiber's ability to produce fiber. To ensure the production of high-quality fiber, consideration must be given to the quality of the green kenaf ribbon throughout the enzymatic degumming process. For example, there are no other fungi on the green kenaf ribbon that may contaminate it and react with the enzyme during the retting process, so reducing the fiber's quality.

More combinations can be tested to find out which enzyme combinations will produce the greatest results from the enzymatic degumming process.For example, xylanase and pectinase together can be utilised. Finally, although not in-depth, characterisation techniques like TGA and GC-MS as well as basic characterization methods like moisture absorption aid in our findings. To get deeper insights, chemical characterisation is another suggestion that may be made.

## REFERENCES


1. Nie, K., Liu, B., Zhao, T., Wang, H., Song, Y., Ben, H., Ragauskas, A. J., Han, G., & Jiang, W. (2020). A Facile Degumming Method of Kenaf Fibers Using Deep Eutectic Solution. *Journal of Natural Fibers*, *19*(3), 1115–1125. https://doi.org/10.1080/15440478.2020.1795778
2. Kozlowski, R., Batog, J., Konczewicz, W., Mackiewicz-Talarczyk, M., Muzyczek, M., Sedelnik, N., & Tanska, B. (2006). Enzymes in Bast Fibrous Plant Processing. *Biotechnology Letters*, *28*(10), 761–765. https://doi.org/10.1007/s10529-006-9044-4
3. Zhang, Y., Song, Y., Jiang, W., & Han, G. (2019). A novel degumming process of kenaf. *Textile Research Journal*, *89*(7), 1258–1266. https://doi.org/10.1177/0040517518767153
4. Sadrmanesh, V., & Chen, Y. (2019). Bast fibers: structure, processing, properties, and applications. *International Materials Reviews*, *64*(7), 381–406. https://doi.org/10.1080/09506608.2018.1501171
5. Lyu, P., Zhang, Y., Wang, X., & Hurren, C. (2021). Degumming methods for bast fibers—A mini review. *Industrial Crops and Products*, *174*, 114158. https://doi.org/10.1016/j.indcrop.2021.114158
6. Amel, B. A., Tahir, P. M., Sudin, R., Anwar, U. M. K., & Hussein, A. (2013). Effect of fiber extraction methods on some properties of kenaf bast fiber. *Industrial Crops and Products*, *46*, 117–123. https://doi.org/10.1016/j.indcrop.2012.12.015
7. Ramesh, M. (2016). Kenaf (Hibiscus cannabinus L.) fiber based bio-materials: A review on processing and properties. *Progress in Materials Science*, *78–79*, 1–92. https://doi.org/10.1016/j.pmatsci.2015.11.001

8. Yu, H., Cheng, L., Feng, X., Zheng, K., Liu, Z., Shengwen, D., & Peng, Y. (2021). Analysis of the Relationship Between Enzymatic Activity and Microbial Degumming Effect of Kenaf Bast. *Journal of Natural Fibers*, *18*(9), 1217–1228. https://doi.org/10.1080/15440478.2019.1688750

9. Duan Shengwen, Cheng, L., Liu, Z., Feng, X., Zheng, K., & Peng, Y. (2018). *Diversity and Characteristics of Kenaf Bast Degumming Microbial Resources*. *15*(6), 799–807. https://doi.org/10.1080/15440478.2017.1369206

10. Jiang W;Han G;Zhang Y;Liu S;Zhou C;Song Y;Zhang X;Xia Y; (n.d.). *Monitoring chemical changes on the surface of kenaf fiber during degumming process using infrared microspectroscopy*. Scientific reports. https://pubmed.ncbi.nlm.nih.gov/28450712/

11. Hossain, M. M., Subbiah, V. K., & Siddiquee, S. (2022). Augmented Retting Effect on Kenaf Fibers Using Alkalophilic Pectinase-Producing Bacteria in Combination with Water Solvents. *Applied Sciences*, *12*(14), 7136. https://doi.org/10.3390/app12147136

12. Sapuan, S. M., Ishak, Sahari, J., & Sanyang, M. L. (2018). *Kenaf Fibers and Composites*. CRC Press.

13. Kakoty, A., Phukan, A. R., Binita, B., & Sangma, W. S. (2019). Extraction of kenaf fiber and its physico-chemical properties for various end uses. *ResearchGate*. https://www.researchgate.net/publication/341286447_Extraction_of_kenaf_fiber_and_its_physico-chemical_properties_for_various_end_uses

14. Song, Y., Jiang, W., Zhang, Y., Ben, H., Han, G., & Ragauskas, A. J. (2018). Isolation and characterization of cellulosic fibers from kenaf bast using steam explosion and Fenton oxidation treatment. *Cellulose*, *25*(9), 4979–4992. https://doi.org/10.1007/s10570-018-1916-y

15. Hamidon, M. a. B., Sultan, M. T. H., Ariffin, A. K., & Shah, A. U. M. (2019). Effects of fiber treatment on mechanical properties of kenaf fiber reinforced composites: a review. *Journal of Materials Research and Technology*, *8*(3), 3327–3337. https://doi.org/10.1016/j.jmrt.2019.04.012

16. Rozyanty, A. R., Zhafer, S., Shayfull, Z., Nainggolan, I., Musa, L., & Zheing, L. (2021). Effect of water and mechanical retting process on mechanical and physical properties of kenaf bast fiber reinforced unsaturated polyester composites. *Composite Structures*, *257*, 113384. https://doi.org/10.1016/j.compstruct.2020.113384

17. Sisti, L., Totaro, G., Vannini, M., & Celli, A. (2017). Retting Process as a Pretreatment of Natural Fibers for the Development of Polymer Composites. In *Springer series on polymer and composite materials* (pp. 97–135). Springer International Publishing. https://doi.org/10.1007/978-3-319-68696-7_2

18. Karimi, S., Tahir, P. M., Karimi, A. R., Dufresne, A., & Abdulkhani, A. (2014). Kenaf bast cellulosic fibers hierarchy: A comprehensive approach from micro to nano. *Carbohydrate Polymers*, *101*, 878–885. https://doi.org/10.1016/j.carbpol.2013.09.106

19. Islam, F., Joannès, S., & Laiarinandrasana, L. (2019). Evaluation of Critical Parameters in Tensile Strength Measurement of Single fibers. *Evaluation of Critical Parameters in Tensile Strength Measurement of Single fibers*, *3*(3), 69. https://doi.org/10.3390/jcs3030069

20. Cui, Y., Shuyi, G., Wang, W., Cheng, L., & Yu, J. (2020). Study on the Moisture Absorption and Thermal Properties of Hygroscopic Exothermic Fibers and Related Interactions with Water Molecules. *Polymers*, *12*(1), 98. https://doi.org/10.3390/polym12010098

21. Da Silva, T. T., Da Silveira, P. M., Ribeiro, M. G., Lemos, M. F., Da Silva, A. R. V., Monteiro, S. N., & Nascimento, L. F. C. (2021). Thermal and Chemical Characterization of Kenaf Fiber (Hibiscus cannabinus) Reinforced Epoxy Matrix Composites. *Polymers*, *13*(12), 2016. https://doi.org/10.3390/polym13122016

22. Ku, H. S., Wang, H., Pattarachaiyakoop, N., & Trada, M. (2011). A review on the tensile properties of natural fiber reinforced polymer composites. *Composites Part B: Engineering*, *42*(4), 856–873. https://doi.org/10.1016/j.compositesb.2011.01.010

23. Akin, D. E., Henriksson, G., Evans, J. D., Adamsen, A. P. S., Foulk, J. A., & Dodd, R. B. (2004). Progress in Enzyme-Retting of flax. *Journal of Natural Fibers*, *1*(1), 21–47. https://doi.org/10.1300/j395v01n01_03