*Review*

# A Comprehensive Review on Bast Fiber Retting Process for Optimal Performance in Fibers Reinforced Polymer Composites

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**Abstract:** Natural fibers are a gift from nature that we yet fully utilized until now. It can be classified into several groups and bast fibers are the group having the most promising performance when reinforced in polymer composites. However, numerous factors have been reported that influences mechanical properties of fiber reinforcements in the composite. In this review, bast fiber retting process and the effect of enzymatic retting on fiber and fibers reinforced polymer composites have been discussed and reviewed for the latest researches. Retting precedes mechanical processing (i.e. scutching) of the fiber from the stem and is essential for reduction of fiber breakage. All retting methods except chemical retting process are involving secretes of enzymes by bacteria or fungi under controlled (enzymatic retting) or random conditions (water and dew retting). Besides, enzymatic retting is claimed to have more environmentally friendly wastewater products, shorter retting period and controllable fiber biochemical components under mild incubation conditions. This review comprehensively assesses the enzymatic retting process for producing high-quality bast fiber and will become a reference for future development on bast fiber reinforced polymer composites.

**Keywords:** enzyme; Bast Fibers; Enzymatic retting; characterizations

## 1. Introduction

Due to the alarming rise of global warming issues and perish of marine living organisms caused by accidentally swollen of non-degradable plastic products, the awareness of plastic disposal issue (difficulties to recycle, environmental burdens, high recycle costing) had been heightened. As a result, bioplastics have gradually substituted conventional plastics in many applications [1, 2]. However, many users are still struggling on finding suitable replacements as bioplastic has inconsistency and low performances profile. On the other hand, reinforcement of natural fibers on plastic was reported to strengthen the product with better/or maintaining biodegradability [3, 4]. The natural fibers are renewable resources because they are produced as part of the plant from photosynthesis, where $O_2$ is released by absorbing $CO_2$ gases. Therefore, it decomposes naturally, consequently imposes lesser burdens to our environment.

Natural fibers can be extracted from three sources (plants, minerals and animals), as shown in **Figure 1.** The main component in mineral and animal fibers is asbestos or basalt and protein, respectively. Plant fiber itself can be recognized as a composite material since it is composed mainly by cellulose, hemicelluloses, lignin and other components. Performance of natural fiber is often influenced by its chemical composition and physical properties [5]. However, noticeable differences in performances were found in every single natural fiber even though the fibers were taken from the same plant [6]. Climatic variations, plant variations and geographical were reported to have influenced the chemical composition (cellulose, hemicellulose and lignin) of natural fibers [7, 8]. Fortunately, its properties can be enhanced by chemical surface treatment as detailly discussed in previous work [9]. The natural fibers possess greater characteristics than conventional fibers, such as environmental friendliness, renewability, price and performances-per-unit-mass. Hence, natural fibers become an emerging filler reinforcement in composites, where glass and carbon fibers are being used traditionally [10-13].

Approximately 2,000 species of plants have been used as natural fiber reinforcements, but only a few fibers are dominating by holding 90% of natural plant fiber's market [14]. The plant fibers have been further classified into more details, according to the location of fiber obtained on the plant, as shown in Figure 1. Bast fibers are the most widely used among other groups (bast fibers, fruit fibers, grass fibers, root fibers, seed fiber, and leaf fibers) [15-17]. Retting is the first extraction process to obtains high quality fibers. Several retting processes have been introduced in previous time and enzyme retting is found the most environmentally friendly due to mild retting parameters yet obtaining high grade bast fibers. In this review, the focus is putting on the retting process of bast plant fiber, with emphasis on enzyme retting process. Numerous studies have been done on bast fibers, but there is a lack of discussion on the overview of bast fiber retting methods. Besides, the effects of enzyme retting on bast fiber and its polymer composites are also discussed in this paper.

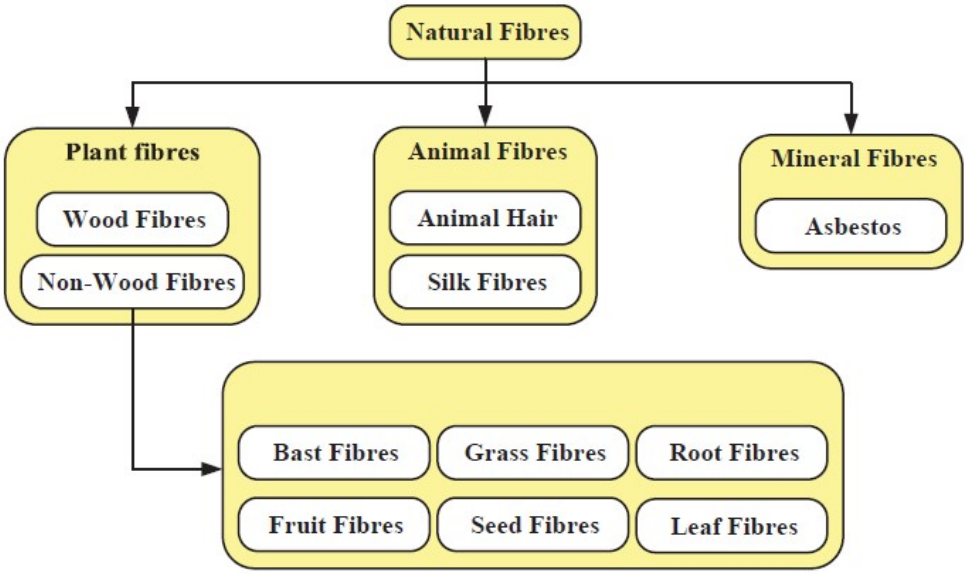


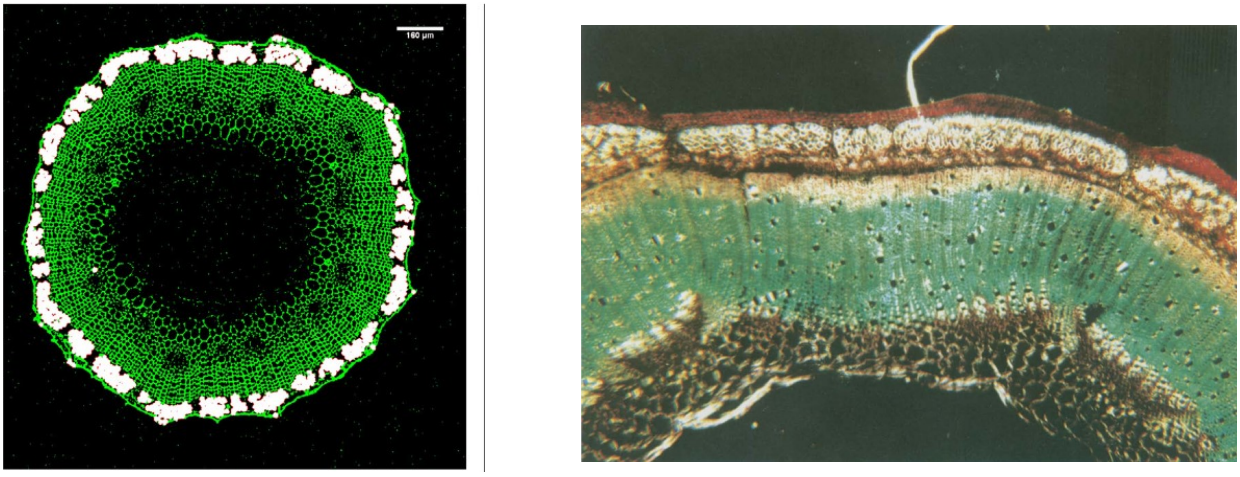

Figure 1: Classification of natural fibers [14].

## 2. Structural and chemical composition of bast fiber

Bast fibers are cellulosic fibers that extracted from phloem or outer back of bast fiber plants (Figure 2). Since bast fiber plants are annual crops, continuous supply of fibers is one of the attractive strong points for gaining interest in non-wood composite manufacturing. Table 1 shows the details of the main five bast fiber contributors.

Bast fibers are extracted from the phloem which located at the stem of the fibrous plant. Epidermis, shives, woody core and a combination of xylem must be removed in order to obtain the bast fiber (Figure 3). The epidermis (bark) is used to prevent the plant from moisture evaporation and resist moderate mechanical damage while xylem, woody core and shives help to transfer water and nutrition from the roots to the whole plant [18]. The fibers located in phloem appeared as fiber bundles and providing strength and stiffness to the plant [19]. Fiber bundle consists of numerous single fiber and each fiber is connected by middle lamella to act as glue, composed by pectin component [2] and lignin [20]. The major task of retting process is to remove the pectin components and releases the fibers from bundle attachment.

Every single fiber constructed by two layers of the wall (primary and secondary wall) and a hollow lumen (Figure 4). The primary wall is built by a network of hemicellulose, pectin and glycoproteins to protects cellulose microfibrils. On the contrary, the middle layer from three layers of secondary walls (S1, S2 and S3) contributes about 70-80% of fiber's mass [21]. Therefore, S2 layer has predominantly varied the properties of fiber by the factors of cellulose content, microfibril angle and thickness [22]. Besides, it has been protected from excessive radial expansion and rotation prevention or sideway collapse avoided by S1 and S3 layers, respectively [23].



(a) (b)

Figure 2: X-ray μCT image and optical micrograph of flax and hemp stem, respectively [24, 25].

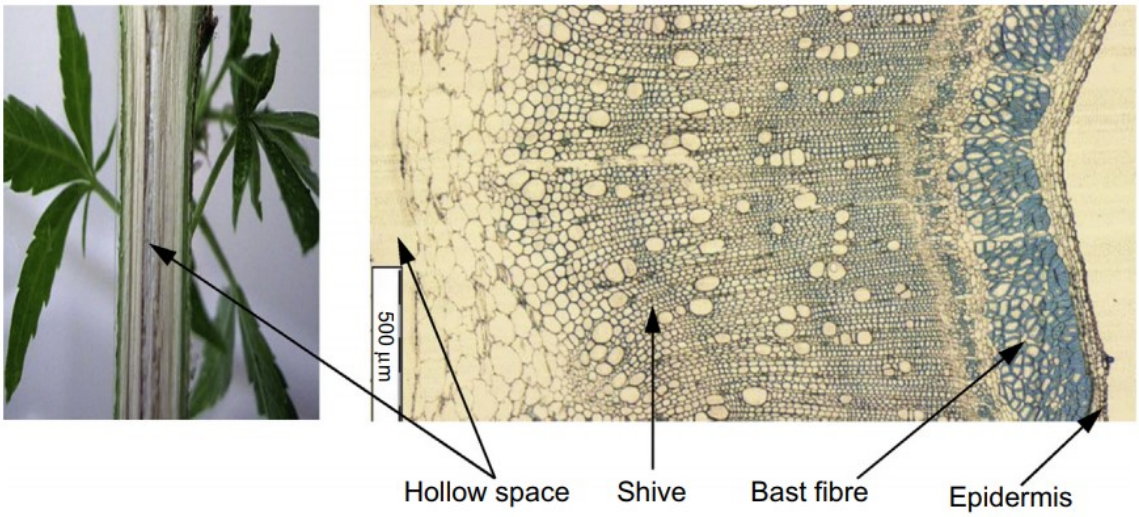
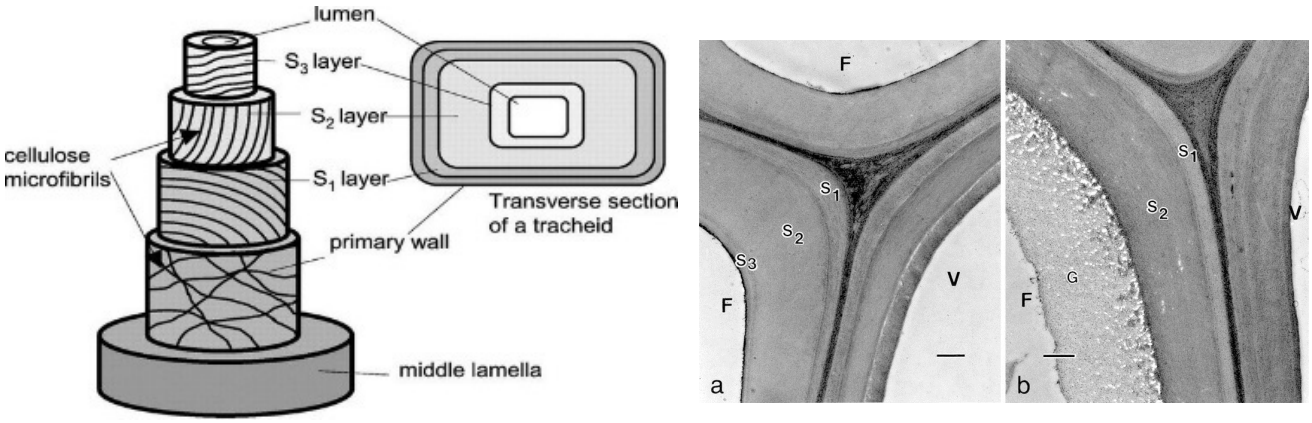



Figure 3: Cross-section of hemp stem and location of bast fiber [26].




(a) (b)

Figure 4: (a) Schematic of single fiber structure [27] and (b) TEM micrography of cell wall layer showing middle lamella (darkest shaded color between fibers), S1, S2 and S3 [28].

One of the biggest drawbacks for natural fiber is its inconsistence performance from fiber to fiber, due to the different biochemical profile of every single fiber. Plant age, fiber sources and retting methods are reported to be influencing chemical composition significantly [29]. Among the bast fibers, hemp fiber has the highest

cellulose content, and therefore the highest tensile strength is expected. Cellulose is the major component that provides stiffness, stability and strength to the fiber. Hemicellulose is highly hydrophilic with lower molecular weights. It acts as a matrix for cellulose microfibrils. However, it is very susceptible to thermal degradation, biodegradation and moisture absorption. On the other, lignin is a high molecular weight, highly branched amorphous component. It used as the cement in microfibrils, to provide rigidity to the plant. On the other hand, the fiber with high contents of pectin (found mostly at middle lamella) is generally high in flexibility [1]. However, easy degradation of pectin affects the stability of fiber's performance. Therefore, retting process tends to remove pectin components as well as releasing the bast fibers from the fiber bundle.

1 Table 1: Details of main bast fiber contributor (Flax, Hemp, Jute, Kenaf and Ramie).

| Bast fiber | Flax | Hemp | Jute | Kenaf | Ramie |
|---|---|---|---|---|---|
| General information [30-34] | | | | | |
| Scientific name | *Linum usitatissimum* | *Cannabis sativa* | *Corchorus Capsularis* and *Corchorus olitorius* | *Hibiscus cannabinus* | *Boehmeria nivea* |
| Plant outlook | Can grow to a height of 80-150 cm in less than 110 days<br>Fiber flax plants are very tall, with few branches and low seed production | Plant stalks can grow in 1.5-2.5 m tall and 6-16 mm thick.<br>It has smooth and hollow stems, rough foliage at the top | Can grow to a height of 2-3.5m with high lignin content during their lifespan of 120 days.<br>Able to absorb 15 tons of CO2 and release 11 tons of O2 for One hectare of jute plants | Relatively easy to grow with high yields, grow to 5 m tall in 5 months.<br>Produces about 6–10 tons of dry matter per acre in a year. | Plant stalks can grow in 1-2.5 m tall and 8-16 mm thick. |
| Grow climate | Grow in moderately moist climates | Grow in a mild, humid atmosphere, and 625-750 mm/year of rainfall is needed. | Hot and humid climate | tropical and subtropical regions | - |
| Country | Europe and Asia | Central Asia, eastern Europe and equatorial countries. | India, Bangladesh, China, and Uzbekistan. | Northern Africa, India, Russia and China | China, Philippines, Japan, Brazil, and Europe |
| Fiber quality | The fine long flax fibers are usually spun into yarns for linen textiles. | Hemp fibers are less flexible and coarser than flax fibers | Long Jute fibers are ranging from 1-4 m with the polygonal section of various sizes with a wide lumen, resulting in a high deviation of fiber diameter, which in turn causes variations in strength.<br>The fiber has moderate moisture retention, good resistance to microorganisms, but not to photochemical and | A potential substitute fiber for jute fibers<br>Preferred over other fibers because of its homogeneity, uniform fiber orientation, and good carbon footprint due to kenaf's high CO2 absorption | Retting is not possible due to high gum contents (xylan and araban content up to 35%); the degumming process is more preferred. |

| | | | | | |
|---|---|---|---|---|---|
| | chemical attack. | | | | |
| Chemical Properties [35, 36] | | | | | |
| Cellulose, % | 62-71 | 67-75 | 59-71 | 45-57 | 68-76 |
| Hemicellulose, % | 16-18 | 16-18 | 12-13 | 21.5 | 13-14 |
| Pectin, % | 1.8-2.0 | 0.8 | 0.2-4.4 | 3.0-5.0 | 1.9-2.1 |
| Lignin, % | 3.0-4.5 | 3.0-5.0 | 11.8-12.9 | 12.0-13.0 | 0.6-2.0 |
| Wax, % | 1.5 | 0.7 | 0.5 | - | 0.5 |
| Fiber properties [36-38] | | | | | |
| Moisture content, wt% | 8-12 | 6.2-12 | 12.5-13.7 | - | 7.5-17 |
| Angle microfibril | 5-10 | 2-6.2 | 8.1 | 9-15 | 7.5 |
| Average diameter, μm | 15-30 | 10-40 | - | - | 34 |
| Density, kg/m$^3$ | 1530 | 1520 | 1520 | 1450 | 1500 |
| Mechanical properties[37-39] | | | | | |
| Tensile modulus, GPa | 58 | 70 | 60 | 14-38 | 18.3 |
| Tensile Strength, MPa | 500-1500 | 920 | 860 | 240-930 | 399 |
| Elongation at break, % | 3.27 | 1.7 | 2 | 1.6 | - |
| Moisture absorption, % | 7 | 8 | 12 | - | - |

*2.1. Climatic, plant and geographical variations on bast fiber*

In addition to the bast fiber harvesting strategies reviewed by Pari, et al. [40], some important cultivation technologies in Europe and China to obtain the best quality of hemp fiber bundles have also been discussed in previous works [41, 42]. Liu, et al. [43] has introduced a new continuous harvest technology for better quality fibers. Average fiber diameter, fiber breaking strength, and elongation rate were varied due to different harvest mode. Beyond these, plant growth parameters are affecting the chemical composition of bast fiber naturally. One study has investigated changes in cell wall thickness, lumen dimension and cell breath according to plant age for two varieties of jute plant [44]. If the plant is harvested too early, low yield with soft and immature fibers was obtained and if they are harvested too late, though the yield is higher, the fibers are less flexible.

However, Liu, et al. [45] have claimed that not much difference on biochemical properties for early and late harvest fibers. Bennett, et al. [46] have studied a few growth parameters on dew-retted hemp fibers. According to statistical analysis, hemp variety, seeding rate and harvest time are the most significant on influencing fiber yield and its properties. Generally, dioecious hemp varieties producing higher fiber yields than monoecious hemp. Under the high seedling rate (seeds/m2), thinner stem and lower stem volume of hemp plants were observed.

Besides, a huge variation of the bast fiber's chemical compositions was reported due to the climate and soil condition. Jankauskienė, et al. [47] have studied the weather conditions of Lithuania for the year 2010 and 2011. Both years are suitable for hemp plantation except higher relative humidity with richer organic matter in the soil in year 2010. Hence, more fertilizers were applied in the latter year. As results, hemp fibers with higher cellulose contents (81.7%) but lower lignin contents (9.91%) were obtained. Haag, et al. [48] also conducted a similar study for flax fibers in the year 2012 and 2013. The results have confirmed that the plantation weather during flax plantation influences the fiber's finesses and consequently its mechanical properties.

Kenaf plants are mostly produced in China and India, Bourguignon, et al. [49] migrated the kenaf plantation to Iowa and Kentucky, US, to supply local needs. Relatively warmer and wetter of Kentucky has higher kenaf productivity and better fiber's performance. However, a low amount of kenaf yielded in the year 2015 at Kentucky was due to the infestation of Japanese beetle pest that harmed the kenaf plants. Besides, the plantation of "Tainung 2" kenaf variety is suggested by the author in US southern states to compete in the bio-fiber market [49]. On the other hand, another study has outlined a zoning model for agro-ecological and agro-climatic to analyses potential growing areas for kenaf plantations in Argentina [50].

On the other hand, Liu and Labuschagne [51] have studied the effects of nine kenaf variations plantation on cultivars yields. The findings show El Shlvador has high and stable dry stalk yields among other varieties due to good adaptation to the environment and irrigated condition in South Africa. Besides, warm-season species of kenaf has successfully migrated into the semi-arid Mediterranean area [52]. The dry yield has strongly affected by the amount of water applied but nitrogen concentration in the soil observed little or no changes to the dry yield. Every 2-24tonnes/hectare of kenaf yield under no water limitations but reduced irrigation system saved 42-45% of water with reduction of crop yield of 23-36%, showing more cost-effective of plantation method [52].

Angelini and Tavarini [53] have investigated the variation of ramie fiber's chemical components on caused by plant densities, harvest time and crop stand durations. The results concluded that the harvest time has significantly affected the fiber's chemical contents while the other factors found little interaction on basal stem diameter and stem development.

## 3. Retting Process

Fiber extraction from straw is the very first step in fiber processing. At this moment, the outer layer of fiber bundles must be separated from the plant by breaking off the bonds between stem cores and fiber bundles. Réquilé, et al. [54] has studied the peeling effect on hemp fibers during the retting process. A detail of peeling fracture mechanisms helps to understand the conditions of fiber bundle cohesion during retting.

A schematic diagram of bast fibers processing was shown in Figure 5. There are two main methods that have been applied to extract bast fibers (mechanical extraction, retting). A comparison between retting methods is shown in Table 2. This review only focused on retting processes. Previous researchers found that mechanical extraction provides a simple but rapid process with a high quantity of fiber yield when compared to the retting processes. Mechanical extraction uses mechanical forces to tear bonds between fiber and its core.

Plant stalks are fed into a decorticator to break into pieces via compressive, shear and/or impact forces. Different types of decorticator have been used for fiber processing such as crushing roller, hammer mill, ball mill and drop weight. On the other hand, post-decortication cleaning separates detached fibers from the mixture of fiber/core bounded components and fine particles. However, it is hard to control the mechanical forces applied [55]. Also, a wide range of fiber lengths produced and high in cost are disadvantages of the mechanical extraction [34].

Fiber retting is a complex process and its properties are highly dependent on the type of retting methods and parameters. Under-retted and over-retted fiber makes inefficiency fiber separation and weakening of the fiber, respectively [56]. During the retting process, phloem-derived fiber bundles are loosened from hemicellulose, lignin and pectin. Left-over fibers are rich in cellulose contents and exerting high strength properties. Sisti, et al. [57] have justified the effectiveness of retting process on pectin, waves, and lignin removal, thus breaking up the bonding bounded between hemp fibers, thereby improving adhesion between fibers and matrix during composite fabrication.

Retting is a biological process, which removes non-cellulosic materials attached on the fiber bundle by enzymatic activities, consequently yielding detached cellulosic fibers. All retting processes except chemical retting use enzymatic activities to extract fibers from bundles. Water- and dew-retting applying anaerobic bacteria fermentation and fungi colonization method, respectively, on fiber bundles, to produces enzymes that hydrolyze fiber binding components. *Clostridium* sp. that lives in lakes, rivers and ponds is an anaerobic bacterium. It rets bast fibers by producing pectinases enzymes during water retting process. However, the anaerobic fermentation has found severe water pollution, contaminated wastewater, putrid odor and resulted in a shift to dew retting process [58]. Dew retting uses colonization of fungi presented in soil on bast stems [59]. Unfortunately, it is a time-consuming process with inconsistence outcomes, which fiber quality depending on geographical conditions even though this method found cheaper, higher fiber yields and creating fewer pollutions.

On the other hand, chemical retting produces a more controllable bast fiber quality within a short retting duration. High processing cost, unfavorable color and deteriorated tensile strength of retted bast fibers have driven scientists to discover better retting method. Enzyme retting has been so famous due to its mild process conditions, specificity and high selectivity with no chemical presents. The characterization of enzyme retted bast fiber shows comparable quality as water retted fibers. However, some studied found lower fiber strength because of the continued activity of cellulases in the enzyme mixtures. Therefore, controlling retting duration is essential to avoid over-retting. Song and Obendorf [60] have compared water-, chemical- and enzyme retting process on kenaf fibers. The authors commented that enzyme retting process has the highest lignin removal activity based on the evidence of GCMS and FT-IR results. Besides, it also allows greater process control under shorter retting duration, which highly recommended for large-scale production [61].

Pandey [62] has studied a variety of retting methods to analysis suitable end uses according to the fiber retted properties (line and tow fibers percentages, line and short fiber length, fiber tenacity, fineness and elongation at break). Figure 6 depicts the differences in fiber yield from varies of retting methods, ranging from 8.8 to 30.0%. The methods are sorted in descending sequences based on fiber yield as following: enzyme pectinase > gel > EDTA disodium > double retting > mixture of cellulases and/or $\alpha$-amylase > chemical retting with hydrogen peroxide > water retting > sodium hydroxide chemical retting. Angelini, et al. [63] have compared the biochemical composition of ramie fibers obtained from chemical and microbial retting. A higher percentage of cellulose contents and effective removal of hemicellulose and lignin observed on chemical retted-ramie fiber. Liu, et al. [64] have studied the effects of EDTA chelator on hemp fibers retting process. The epidermis of hemp fiber, which rich in Ca2+ ions, has bounded to EDTA through proton displacement. Hence, the structure became unstable and resulted in pectin and low-methoxyl pectin removal from hemp bast fiber. The outcomes were show similar to another study [65]. Further details of chelators will be discussed in later, at enzymatic retting section.

Amel, et al. [66] have studied the effects of retting methods on morphologies, physical and mechanical properties of kenaf fiber. Water- and NaOH-retted kenaf fiber have comparable tensile strength values, which is 426 and 393 MPa, respectively. However, both methods contribute to high strength from different perspectives. Water retting remains fiber length as crude fiber but transferring higher load as long fiber. NaOH retted fiber was observed a severe reduction of fiber length, yet improved cellulose chain packing contains more crystalline cellulose and thereby higher tensile strength.

On the other hand, water retted flax fibers show higher strength properties than dew-retted fibers due to the conservation of cross-linking fractions [67]. These findings agreed by van der Westhuizen [68] who reported water retting produces the highest fiber strength for all kenaf varieties (Tainung-1, T15, Cuba-108, Everglades 71 and BG52 except Cubano) among six retting processes (enzyme, dew, water retting, NaCl, urea and NaOH). On the contrary, Yu and Yu [69] have the opposite opinion, suggesting water retting process produces weak, poor quality of kenaf fiber, evidence of highest gum residual content and lowest tenacity of fiber.

Mazian, et al. [70] have study influences of retting period on hemp fiber biochemical contents. The results show the development of cellulose over the period from X-ray diffractogram, higher intensity of crystalline peak on R9 (specimen retted in nine weeks). Similar results found in the previous study, increases of crystallinity on retted flax fibers with the lower amorphous region [71]. However, different cellulose, lignin and pectin content throughout different retting period, is due to the different degradation rate. The highest rate found on Pectin removal, followed by cellulose and lignin [45].

Besides, the color changes of fibers also found varying due to the different of retting periods [70]. The colonization of fungi during retting causing the flax fiber to change its color from light green-yellow to dark grey is observed [72]. Superior tensile performance on 19 days retted flax fibers (1036 ± 270 MPa) is recorded due to a low degree of impurities.

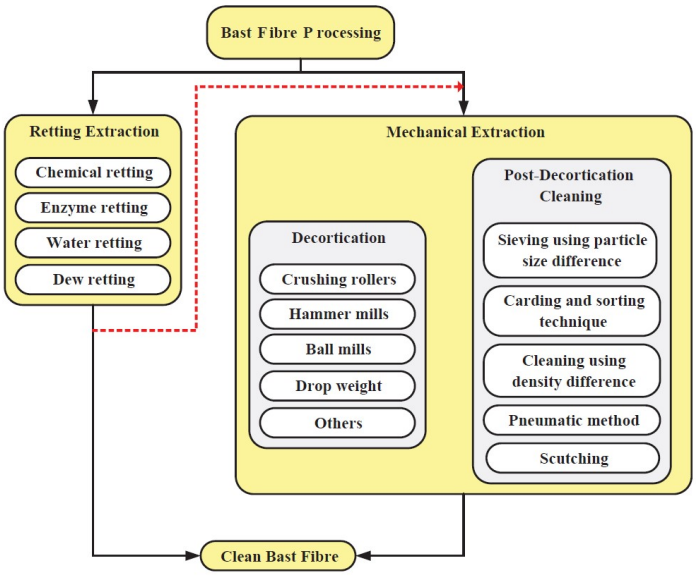



Figure 5: Schematic diagram of bast fiber processing [14].

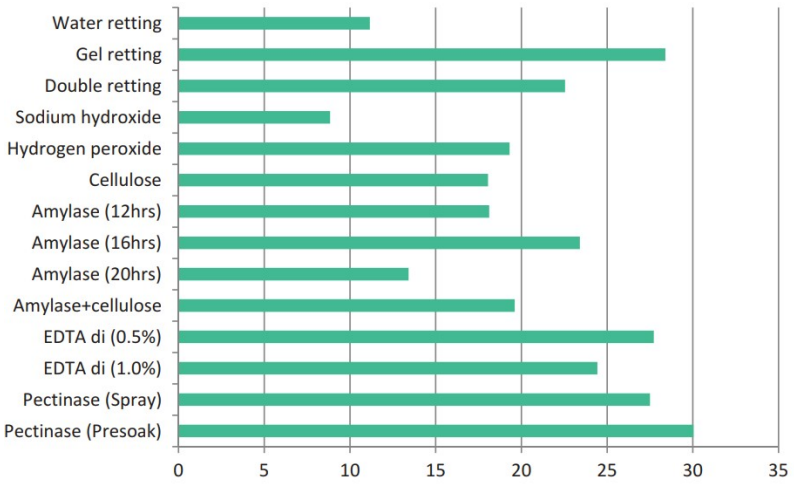



Figure 6: Shikha flax Fiber yield from varies retting methods [62].

*3.1. Steam explosion (STEX) pre-treatment prior to retting*

Pre-treatments prior to the bast fiber retting process are commonly applied to enhances retting efficiency. Pre-treatment allows the penetration of enzymes during retting process, hence producing better fiber's quality. Among varies of pre-treatment techniques, STEX process is the most widely use due to its high-efficiency-to-low cost. STEX is a thermo-mechanical-chemical defibration method. It widely used as pre-treatment to enhances retting efficiency. During the process, fibers are both chemically modified and mechanically defibrated. STEX process breakdowns lignocellulosic structural components by heating, formation of organic acids, and creates shear forces to the fibers. The hemicellulosic components hydrolysis processes, chemical structure of lignin and cellulose crystallinity index alteration allow the opening of lignocellulosic structures and increase the retting efficiency. At the end of the process, the instantaneous release of pressure stops the reaction and eventually separating fibers from bundles. The STEX process is suitable for woody bast core, which can be purified to provide chemical grade cellulose, or quality fibers for textiles and composites [73]. One study shows this process removes amorphous materials from the inner part of the hemp fiber via depolymerization and defibrillation [74].

Ramie provides the longest and strongest natural fiber material in the textile industry, but its traditional degumming processes are costly and requires a large amount of alkali, which causes serious environmental concerns. STEX treatment is an efficient and environment-friendly method for degumming of various of natural fibers but the treatment alone has very low retting efficiency. Subsequent chemical degumming treatment is necessary. Jiang (2018) has reduced the gum contents on ramie fiber to below 5% and 11.65% for STEX process with and without environment-friendly and economically feasible reagent, sodium percarbonate (SP) bleaching agent [75]. Besides, more than 50% of chemical was recycled with only 35% of the pollution levels as compared to traditional retting process. Lower tenacity and fineness ramie fibers were recorded from this STEX-SP retting method. However, the retted products are achieving refined dried ramie fiber requirements from Chinese National Standard (GB/T 20793-2006) [75].

The STEX process is also certified as an appropriate extracting process for bast hemp fiber. STEX has found a noticeable xylan contents reduction [76]. Table 3 shows the ratio content of xylan, lignin and pectin acid to glucan in various hemp samples. On the other hand, the best condition to separates and purifies woody fibers is steam treatment of acid-impregnated process with 180 seconds duration at 200-230oC [25]. Higher temperature will induce fiber damages.

The STEX has been widely used as a pre-treatment technology for lignocellulosic materials to improve enzyme catalyzed cellulose degradation [77]. Pakarinen [76] have studied STEX and alkalization pre-treatment prior of enzyme retting process to enhance hemp fibers properties. The most significant increase in the enzymatic hydrolysis was observed with supplemented pectinases in the conversion of anaerobically preserved hemp fibers. The pre-treatment inducing swelling of microfibrils thereby increasing substrate availability to hydrolyses enzymes. Delignification has enhanced the enzymatic efficiency since lignin provide structural rigidity to the fibers, preventing swelling. Besides, removing xylan components also allowing swelling of the fibers to increases surface area and eventually cleaves some lignin components. After the pre-treatment process, removal of pectin showed a strong correlation with the enzymatic hydrolysis, due to the high accessibility of enzymes between the substrate cell wall surface.

A study has applied STEX pre-treatment on dried hemp stem by using three different water vapor pressures for 30 minutes before enzymatic retting process [78]. A reduction of arabinan, galactan, xylan and lignin contents were recorded while a gradual decrease in pH value was responsible for the liberation of acetic acids and galacturonic acids at elevated temperature. Therefore, an observation of better accessibility of pectinases during enzymatic retting was enhanced by hydrothermal pre-treatment. The major contribution was come from higher water retention and creates large macro-pores for improved enzyme penetration. Besides, clear synergistic action between cellulases and xylanase was observed in the hydrolysis of steam-exploded hemp [79]

Microbial contamination is one of the retting processes disadvantages. Further enzymatic hydrolyzations secreted by microorganisms have caused over-retting on fiber, thereby reducing the fiber strength. The microbial quality has been investigated on enzyme retted hemp fibers with and without STEX treatments [80]. The enzyme retting process promoted 300-900-folds of microorganism growth on the hemp fibers. However, a tenfold fungal contamination reduction on hemp fibers was observed after the pre-treatment process.

The findings show that the STEX pre-treatment process is significant to increase retting efficiency due to better penetration of chemicals or enzymes into the inner part of bast fibers. Nonetheless, the STEX process after enzymatic retting process helps to maintain the quality of retted fibers. Reduced numbers of enzyme

activities on retted fiber have avoided unintentional retting process to happen, which may deteriorate fiber quality.

Table 2: The comparison between bast fiber retting and extraction process [34].

| Retting Methods | Description | Advantages | Disadvantages | Duration of retting |
|---|---|---|---|---|
| Water Retting | Plant stems need to be submerged in water and checked periodically | Produces retted fiber with great uniformity and high quality | Severe pollution issue arising from anaerobic bacterial fermentation, putrid odor, environmental problems and high cost. Requires intense treatment on wastewater. | 7-14 days |
| Dew Retting | The Plant stems are spread evenly on fields to receives sufficient sunlight, atmospheric air and dew for fungal colonization and thereby break down cellular stem tissues and adhesive substances to release single fiber | Pectin materials could easily be removed | product contaminated with soil, restriction to certain climatic change, inconsistent quality, and reduced strength | 2-3 weeks |
| Enzymatic retting | Enzymes hydrolyze gum and pectin material in the stem. Controllable retting conditions is allowed to maximize retting efficiency. | Specific properties can be achieved for different application by varying retting period and type of enzymes used. The process is cleaner and faster. | Low fiber strength | 12-24 hours |
| Chemical retting | Hydrogen peroxide, sodium benzoate or sodium hydroxide is normally used in chemical retting. | The smooth and clean surface can be obtained inconsistency within a short period | Deterioration of fiber strength when the concentration of NaOH more 1% is being used High processing cost and unfavorable color | 60-75 minutes |
| Mechanical extraction | Force applied on the fed stem to separate fibers then post cleaning and further filter impurities. | High quantities of short fiber shall yield in a short period. | Lower fiber quality and high cost | - |

Table 3: Ratio content of xylan to glucan, lignin to glucan and pectin acid to glucan in various hemp samples [76]

| Hemp Fiber Pre-treatment Processes | Xylan/Glucan | Lignin/Glucan | Pectin Acid/Glucan |
|---|---|---|---|
| Untreated | 0.21 | 0.39 | 0.13 |
| Steam Exploded | 0.08 | 0.23 | 0.02 |
| Chemical Treated | 0.10 | 0.09 | 0.00 |
| Acid Ensiled | 0.19 | 0.33 | 0.13 |
| Alkali Preserved | 0.19 | 0.29 | 0.13 |

*3.2. Water Retting*

Water retting is the oldest historical retting method. This process once is the famous on producing quality retted bast fibers. However, generating huge amounts of wastewater is a major issue, which must not be ignored [81]. The discussions of water retting parameters and most importantly, wastewater management, including water recycling or non-freshwater retting process has been done in this review paper. Besides, some innovative modifications on bast fiber water retting process also included at the end of this section.

Magnusson and Svennerstedt [82] investigated the effects of temperature on water-retted hemp fibers. However, the findings showed that the temperature has little influences on final yield, yet temperature higher than 45 °C would not suitable for enzymatic activities. A synergetic effect for temperature (37.5 °C), pH value (4.4) and retting duration (192 hours) has observed a dramatic reduction of pectin contents [82].

Zhang, et al. [83] have compared the seawater retting process with freshwater-retted hemp fiber. And it demonstrated a slightly lower quality fiber. However, the reduction of freshwater consumption would make the method in flavor. On the contrary, Boukhoulda, et al. [84] found better mechanical properties for seawater retted alfa fibers. The morphology micrograph shows smoother fiber surface, and this is because saltwater help to remove waxes. Previous researchers observed similar findings, water retting produces finer fibers and higher mechanical properties [85, 86]. Yet water retting is a time-consuming process.

To check the time factor on the retting process, Fatma and Jahan [87] have retted Kydia bast fiber by using stagnant water at room temperature for 10-25 days. Highest fiber tenacity and elongation were observed for 20 days retting specimen. The results have comparable mechanical and physical properties with other bast fibers (flax and jute fibers) and SEM micrographic for Kydia bast fiber (figure 7), as an evidence of successful water retting process.

Freshwater retting has been used to produces high-quality bast fiber for many years. Contamination of freshwater during process always is an environmental debate topic. The high concentration of organic materials and bad unpleasant smell produced during the process. A case study on river water retted kenaf fibers in Malaysia have found most of the contamination compounds after retting process exceeding the standard that regulated by the department of environmental Malaysia, which not suitable to release into main water stream [88]. This finding has revealed the major pollution of water retting.

Abou-Elela, et al. [89] have investigated the effectiveness of Fenton oxidation process with granular activated carbon on the treatment of retted wastewater (schematic flow shown in figure 8). Effective results on removing organic and inorganic pollutants were recorded and the treated water allowed to reuse in next retting cycle. Apart of this, in India and Bangladesh, pondwater is used for retting jute fibers, but this has caused high arsenic (toxic metalloid) contents and contaminated the retted pondwater by four-to-40-folds according to the WHO safe limit (0.05 mg/L) [90]. However, bacterial growth population during retting transforming the arsenic-III to less toxic arsenic-V. Therefore, jute fiber cultivation after summer is a great option to stabilize the arsenic content.

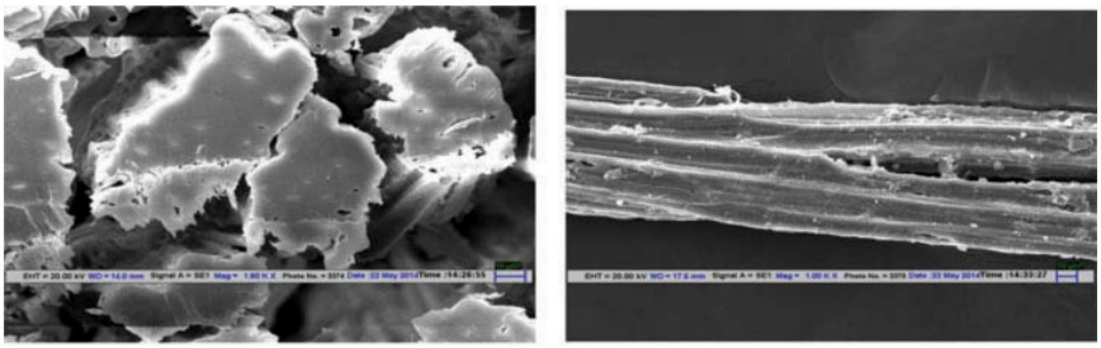
On the other hand, solar photo-Fenton oxidation post-treatment was introduced on water-retted pond wastewater. Highly effective of COD (97.5%) and phenol compounds (98.4%) removal was being recorded under optimum parameters [91]. High phenol concentration in wastewater was due to the retting process on phenolic compounds presented in husks or coir, generating dark colored compounds to the wastewater by oxidation. Therefore, phenol is an important removing role in the treatment.

Apart from this, undeniable efforts have been made by innovation modifications on bast fiber water retting process to increase retting efficiency and reduce freshwater usage as well as shorten retting period. Konczewicz, et al. [92] has applied a new water retting method with multiple water change or continuous water flow, named osmotic retting. Water diffuses into the stem where the fiber swelled after absorbed the water, causes pectin to expand in several folds. Besides, increased hydrostatic pressure causes tensions exerted on the epidermis from longitudinal and peripheral directions. Resulted in a dramatic decrease of pectin strength and then being diluted and dissolved in the flowing water. Generally, all soluble substances containing dyes, bacteria, pectin and mineral salts are removed from the stem, where high-quality fiber is obtained. The finest fiber was obtained after 96 hours of osmotic retting with 40 °C working temperature. This fiber retting method claimed to be more suitable for fiber reinforcement applications [93].

On the other hand, Ruan, et al. [94] have introduced radiofrequency treatment to improve retting efficiency in varies of water retting temperature and durations. The absorption of radio frequency energy has caused the cellulose molecules to vibrate violently with pectin molecules, referring to weakened cellulose

molecular chain. However, maintaining treatment temperature for a longer period observes less vigorous vibration of cellulose molecules, consequences increased the crystallinity index.

Jahan, et al. [95] have introduced ribbon retting in Bangladesh for jute retting process. In the ribbon retting, the barks are removed from jute in the form of ribbon. The ribbons are coiled and then allowed for retting in water. This method claimed to use a lesser amount of water in a shorter time, more environmentally friendly and the paper product show no differences in term of properties between conventional water and retted ribbon materials.



(a) (b)

Figure 7: SEM images of (a) cross-sectional view and (b) longitudinal view of Kydia fiber [87].

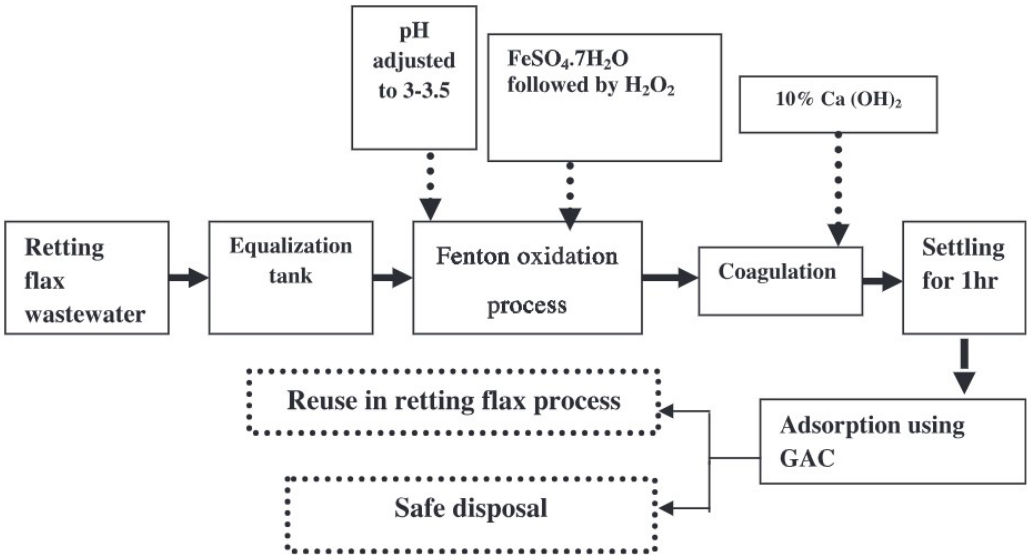



Figure 8: Schematic diagram of retting flax by using wastewater [89]

*3.3. Dew Retting*

In the dew retting method, stems of the plant were being cut and evenly distributed in the fields, where the presence of bacteria, sunlight, atmospheric air and dew causes the breakdown of stem cellular tissues and adhesive substances that surrounded the fibers [96]. The place that has a warm day and heavy might dew are preferred for dew retting process to promote colonization of fungi.

Bleuze, et al. [97] have studied the changes in flax fiber during dew retting process. Microbial colonization has directly related to the cell wall chemical compositions. Fungal hyphae and parenchyma were observed on the epidermis and around fiber bundles, respectively after seven days of retting. It shows partially damaged and fiber bundle decohesion. Higher enzymatic activities further decrease the primary cell wall of polysaccharides on 14th days [97]. Spreading of microbial colonization towards the inner core of the stems until the end of the retting process (42 days), evidence with the degradation of parenchyma and fiber bundle decohesion.

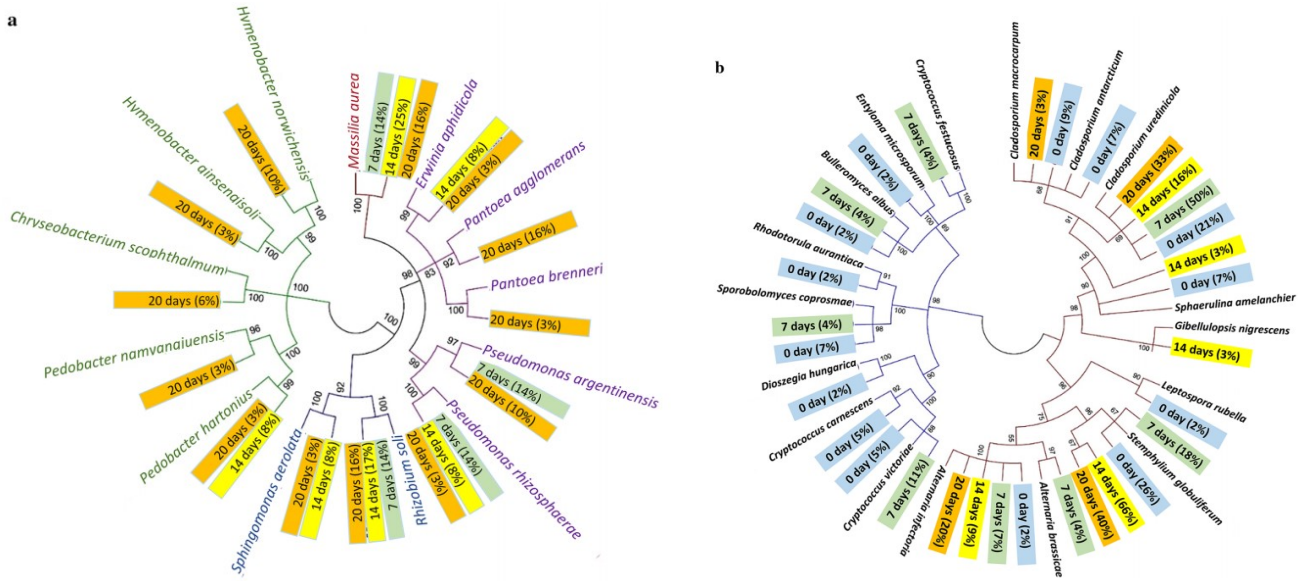
FILA, et al. [98] have isolated 23 types of dew retting agent fungi from southern Europe. He has confirmed that all Aspergillus and Penicillium strains produce high quality retted flax fibers. On the other hand, the worst

retting results were obtained with M. *verrucaria* and E. *nigrum* which are celluloses, degrading cellulosic fiber causing over-retting and reducing fiber quality. Repečkien and Jankauskiene [99] studied the effects of fungal complexes on the flax dew-retting accelerating under the field conditions. High colonization of Cladosporium species variants (25-29%) reported as best fungal for fiber separation. The largest amount of fungi persisted on flax treated with fungal complex N-3 containing six fungal strains.

Jankauskiene, et al. [100] have optimized the dew retting process on a commercial scale. Two fungal mixtures were developed and applied on the straw once after pulling on swathe and once just after returning of swathe, and twice after pulling and after later turning over the swathe. Besides, exceptional high of fiber separation was observed after spraying of suspension of *Cladosporium herbarum* during fiber harvesting.

3.3.1. Bacterial and Fungi Interaction (BFI)

Fungi colonization were considered the major enzymatic active responsible for dew retting. However, recent studies have interested on the interaction of bacterial and fungi community during dew retting, for better knowledge on dew retting mechanisms occurred in bast fibers. Liu, et al. [101] have studied the relationship between chemical compositions of hemp fibers and microbial community variation throughout the retting process. Fungal colonization was found in the first seven days with very few bacteria were observed. Gradually increase of bacterial attachments on the fiber surface with fewer fungal hyphae were recorded after 20 days. The location for high bacterial intensity found highly degraded. Figure 9 shows the phylogenetic tree for the bacterial and fungal community presented in dew retting hemp fibers. Table 4 listed the highlights of ultrastructural changes on hemp stem and fibers with microbial activities along retting process.




(a) (b)

Figure 9: Phylogenetic tree of (a) bacterial community and (b) fungal community present in the hemp fiber samples. The color of the branches shows a different type of proteobacteria while the color of the tag shows the number of bacteria/fungal in different days. [101].

Table 4: Highlights of ultrastructural changes on hemp stem and fibers with microbial activities along retting process [102].

| *Retting Period* | ***0 days*** | ***7 days*** | ***14-20 days*** | ***After 50 days*** |
|---|---|---|---|---|
| *Changes in the ultrastructure of hemp stem and fibers.* | • Stem with intact layered structure<br>• Un-collapsed, intact cells with native cell geometry<br>• Cytoplasm-filled living cells<br>• Clear surface with undamaged cuticle and trichomes<br>• Abundant chloroplasts in the upper epidermis | • Overall structure intact<br>• Fungal presence on the surface and inside of the stems<br>• Cellular anatomy is less stable with deformed epidermis and parenchyma | • Cuticle severely decayed<br>• Changes to the cellular anatomy and major destruction of the living cells<br>• Fiber bundles separated from the epidermis and each other<br>• Thick-walled cells seldom colonized-Parenchyma totally degraded, but chlorenchyma has less damage<br>• Bast fibers with infrequent mild attack<br>• Fiber morphology affected the characteristics of colonization and the decay morphology | • Hemp structure severely affected and disintegrated<br>• Extensively colonized epidermis and cambium with dominant bacteria<br>• Complete destruction of the parenchyma cells and loss of structural integrity in the bast regions<br>• Hyphae inside of the lumina of all the cell types, including the fibers<br>• Intensified BFIs inside of the stem<br>• Major loss to the anatomy and ultrastructure<br>• Thick-walled bast fibers with decay characteristics<br>• Effects on the fiber wall ultrastructure<br>• Loosening /degradation of the CML, which led to delamination and defibration<br>• Loosening and decay of the S3 layer<br>• Prominent effect on the S2 layer with delamination within the s2transwall, and intrawall fractures in the S2<br>• Direct removal of S2 materials<br>• (e.g., S2 thinning, broken S2, and disintegration into nano-sized cellulose fibrillar structures) |
| Microbial Dynamics and Activities | **Fungi**<br>• Rarely seen<br>**Bacteria**<br>• Not observed | **Fungi**<br>• Sparsely growing mycelia<br>• Less diverse<br>• Colonization outside of the cortical layers, primarily in living cells<br>• Dense | **Fungi**<br>• Extensive and abundant<br>• Dense mycelia over cuticle<br>• Diverse population<br>• A variety of abundant spores<br>• Intense activities and interactions<br>**Bacteria**<br>• Abundant | **Fungi**<br>• Less abundant on the outside of the stem<br>• Surface mycelia in non-living state, but active hyphae inside of the stem<br>• Mycelia an exclusive source of nutrients for the invading bacteria, which reflected bacterial mycophagy (i.e., extracellular and endocellular biotrophic and extracellular necrotrophic activities)<br>**Bacteria** |

| | | | | |
|---|---|---|---|---|
| | | colonization close to the surface trichomes<br>• Dependence on readily available foods<br>• Less damage to the cell walls<br>**Bacteria**<br>• Less abundant | • Diverse population<br>• Colonies over cuticle<br>• Associated with hyphae and fungal spores<br>• More pronounced activities after 20 days<br>• Highly degraded cuticle | • Highly abundant inside and outside of the stems<br>• Highly diverse and dominant role<br>• Visible as dense overlay representing<br>• biofilms<br>• morphologically different colonies<br>• randomly scattered cells<br>• Showed strong BFIs<br>• Bacterial motility occurred over and inside of the hemp stem using fungal highways<br>• Showed enhanced cutinolytic and cellulolytic activities |

### 3.4. Chemical retting

Chemical retting degrades non-cellulose components, but cellulose degradation happens when over-retting. Retting duration, chemical concentration and retting temperature have been reported that affecting the quality of retted bast fibers [14]. Besides, the uses of chemical and production of wastewater have increased the cost as well as polluting environmental. One study has reused the wastewater for continues chemical retting process. An addition of 50% of chemicals is needed to obtain comparable physic-mechanical properties of retted fiber as the first bath retted [103]. This method has essentially reduced the cost of chemical retting process as well as the wastewater generated.

Basu, et al. [104] have used a combination of sodium carbonate, sodium hydroxide and sodium sulphide for chemical fiber retting. Effective chemical retting reduces retting time to two hours. The chemical retted fiber showed positive results toward mechanical properties with reducing flexural rigidity, linear density and diameter. Softer fibers were obtained at the end of the study by removing impurities. However, contaminated water from chemical retting show exorbitant chemical oxygen demand, biological oxygen demand, total dissolved solids, sulphide content and having a blackish brown color. Therefore, a new treatment (electrocoagulation) has been introduced to reduce the contamination of wastewater and the outcome was satisfied. It provides quicker, simpler and economically to treat the water by using electrocoagulation [105]. A treatment of 90 mins give a noticeable reduction on contamination compounds. Besides, cheaper treatment cost was found as compared to the traditional method.

Yu and Yu [69] concluded that chemical retting is the most effective method to remove pectin and releases fibers from bundles. Acidic souring and alkaline boiling were used in the study. A concentration of 0.8-1.5 g/L of sulphuric acid was used for acid souring at 50 °C for an hour. A concentration of 10-15 g/L of sodium hydroxide applied in alkali boiling with an assistant of sodium phosphate chemical at 100 °C for three hours. Parikh, et al. [106] have studied the caustic soda retting process on kenaf fiber and the effects of additional chemical used. The additional 0.1-0.2% of anthraquinone, 1% of sodium bisulphite or higher contents of caustic soda only increase 0.77-8.00% of fiber weight loss, which does not justify any significant economic or environmental benefits. However, short retting period has been achieved and the retted fibers are recommended for automotive nonwovens applications.

### 3.5. Other retting methods

There are some other retting methods, have been introduced by researchers in hoping to have higher retting efficiency. However, such innovative retting methods may not reach to upstream due to multiple factors like cost issues, not user-friendly etc. One interesting retting method, microwave retting process being applied on flax fibers by Raveendran Nair, et al. [107]. The microwave energy applied to breaks strong pectin bonds between flax fiber and increases relative cellulose ratio in the fibers. On the other hand, the short pre-soaking period increases moisture and decreases the glass transition temperature of pectin. Therefore, the higher efficiency of pectin removal can be done by microwave energy. The 24 hours-soaked samples treated at 2 W/g power for 20 min showed the maximum retting efficiency of 100%. A mathematical model for compositional changes (rate of change of lignin content, hemicellulose content and cellulose content) during the microwave-assisted retting of flax stem [108] and method optimization [109, 110] were established in previous papers.

Another gel-retting method has been introduced with four hours of retting period, yet high fiber yielded [62]. It has high capability of absorbing and retaining liquid, by hundreds of times its own weight. On the other hand, Gel retting using three-folds lesser water as compared to the water retting since freshwater environment is needed for every 24 hours to prevent fiber damage. Apart from the aforementioned retting techniques, there is also a well-known and traditional retting process called microbial retting [111]. Microbial retting could be conducted using bacteria or fungi in order to attain shorter retting time and better fiber quality. In microbial retting, some microorganisms are capable to generate pectic-digesting enzymes that play an important role in breaking down the pectic substances of the fibers. Various species of Clostridium, Pseudomonas and Bacillus have been identified as retting agents [112]. Ali [113] reported in his study that jute fibers were completely retted within 9 days by a bacterium, *Bacillus polymyxa*. On the other hand, Visi, et al. [114] found that bacteria from the order of Clostridiales was the most dominant species during the retting process of kenaf fibers. As for fungal retting, white rot fungi, namely *Dratronia* sp. and *Oligoporous* sp. were used in retting *Hibiscus sabdariffa*

L. fibers [115]. Increased solubility of pectic was observed after the fungal treatment and subsequently improve the retting efficiency. Besides, enzyme retting considered as one of the most potential environment-friendly retting methods as an alternative to above-discussed retting methods, to produce high-quality fibers as reinforcements for composites will be discussed in detail in the following section.

**4. Enzyme Retting**

Enzyme retting process has been introduced for some years back as a potential substitution to above discussed retting methods. Dew retting process is often constrained by the poor and inconsistent fiber quality as well as geographical region, which require optimum temperature and moisture to promote microbial growth. Therefore, it is less efficient in countries with dry climate [58]. On the other hand, enzyme retting showed promising results in Europe when commercial pectinase-rich enzyme was used in retting of flax fiber. The retted flax fiber has higher yield and comparable quality to that of the water retted fiber [116]. Heller et al., 2015 concluded that substrate species, initial pH of culture medium, cultivation temperature, retting time and inoculum size are important parameters for enzyme retting. The parameters involved in microbes retting has been reviewed once previously [117]. Figure 10 shown the customization of enzyme retting process. Source of enzymes, type of enzymes and retting parameters are flexible to customize in order to obtain optimum fiber's properties for specific applications. Yu and Yu [118] have recorded 85.54% and 91.31% removal of gum and pectin, respectively on microbe retted kenaf fiber. The author suggested the optimal retting conditions are held on 32 $^{o}$C with pH of 6.0 for 24 hours cultivation time and 21 hours of retting periods. However, gum removal dissatisfied when compared to about 3% of gum residual, observed from chemical retting method [69]. Higher fiber tenacity from enzyme retting due to mild situations minimize the damage to cellulose.

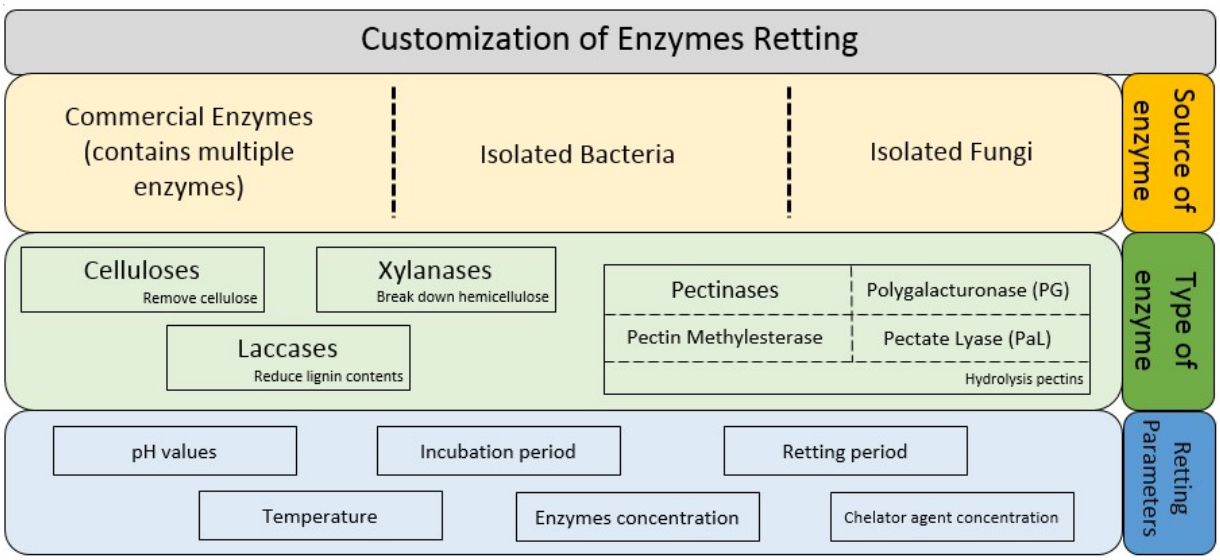



Figure 10: Customization of enzymes retting

Many of the enzyme mixtures contain multiple enzyme activities against plant cell walls, including cellulases. Yılmaz [86] has confirmed that enzyme retting process produces the finest fiber as compared water retting or NaOH extraction. The concentration of xylanase has observed a significant reduction of linear density and breaking force. Besides, higher celluloses concentrations help to enhance breaking tenacity. High crystallinity fiber reported as more non-load bearing contents being removed by celluloses [119]. On the other hand, kenaf fiber has the highest amount of lignin content among bast fibers, lignin decomposition is relatively slow and therefore over-retting is not critical as in other bast fibers [120]. Higher laccases enzyme may apply on kenaf enzymatic retting.

Zhao, et al. [121] have successfully employed *Bacillus licheniformis HDYM-04* microbe for enzyme retting on flax. The composite enzymes consisted of 587.5 U/mL pectinase and 140.1 U/mL xylanase, which produced an effective fiber degumming after 48 hours. Most importantly, the incubation medium maintained at pH 4.0-6.0, demonstrating a stable retting process. Significant reduction of gum components has been observed after 120 hours of retting, higher fiber strength, productivity and long fiber yield rate compared to water retted fiber. On the other hand, Akin, et al. [122] has suggested pre-soaked flax fiber with distilled water before

enzyme retting to produce finer fiber with better fiber yields yet sacrificing a portion of fiber strength. Besides, the author found that enzyme retting was as efficient at 4 hours as 24 hours for fiber yield.

The right dosages of response enzyme are crucial to obtain an efficient enzyme retting process. The use of chelators into enzyme formulation have shown a magnificent effect on retting efficiency. Chelators are small molecules that bind very tightly to calcium ion, $Ca^{2+}$. Removal of epidermis and cuticle can be done easily since calcium is highly presented in the epidermis. Easier degradation of the plant cell wall was found with the appearance of chelators [123]. Among all types of chelators, ethylenediaminetetraacetic acid (EDTA) is considered as the best agent in facilitating enzyme retting [124]. The efficiency of chelator is highly affected by temperature and pH. Most of the chelators worked on alkaline medium but EDTA work effectively even under acid medium. Figure 11 shows the level concentration of free calcium dissolved by various chelators under different pH conditions. The lesser the detection of the $Ca^{2+}$ ions, the more effective the chelator agent is.

Besides, there was a strong dependence of depectinization selectivity on the stem section, decreased from bottom to top presumably due to higher lignin content at the bottom stem. The thinner bast fiber layer at the top section has a lower amount of wax substances and lignin in fibers, allowing easier entry of microbes and their secreted enzymes. Hence, easier depectinization resulted in over-retting and lower in cellulose content and tensile strength. Furthermore, the fiber responded differently to P. *radiata Cel 26* and C. *subvermispore*, causing a variation in mechanical properties [20]. However, an observation of significant differences for properties of untreated hemp fiber in different locations.

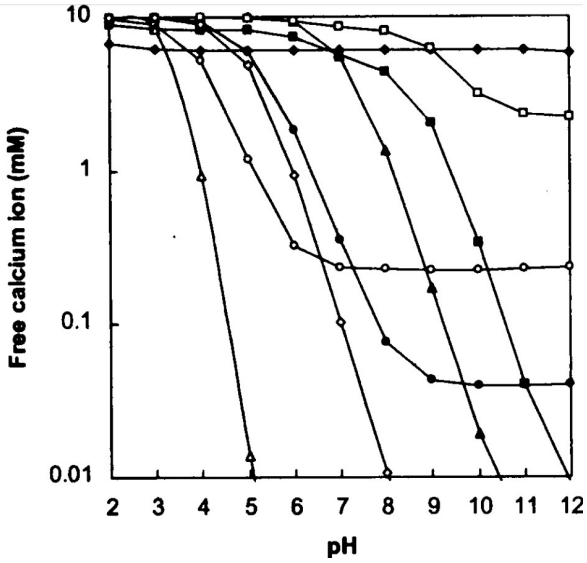



Figure 11: Concentration of free calcium dissolved by various of chelators under different pH conditions Symbols: sulfate (◆), nitrilotriacetic acid (◊), EDTA (⌂), carbonate (□), citrate (○).orthophosphate (▉), diphosphate (●), and tripolyphosphate (▲) [124].

*4.1. Enzymatic retting mechanisms*

Enzymatic hydrolyzations can be done on every lignocellulosic fiber component by different enzymes. Cellulose, hemicellulose, lignin and pectin components are hydrolyzed by cellulases, xylanases, laccases and pectinases, respectively.

Cellulases hydrolyze β-1,4-glucosidic bonds in the cellulose polymer. It degrades amorphous cellulose before crystalline stage cellulose. Therefore, appropriate cellulases enzymes enhanced fiber crystallinity and yet, deteriorated performances found under long retting period [125]. On the other hand, xylanases break down hemicellulosic components on fiber bundles by hydrolyzing β-1,4- bonds in xylan chains. The most important enzymes for degrading arabinoxylan are endo-1,4- β-xylanase and β-xylosidase [126]. Endo-1,4-β-xylanase responses to the xylan backbone arbitrarily which forms xylo-oligo- saccharides while β-xylosidase yields xylose by removing terminal monosaccharide at the non-reducing end of the oligosaccharides. Another enzyme that can affect the retting process of bast fibers is laccases enzyme, which can degrade lignin structure in the fiber.

Pectinases is the most important retting enzyme to separate fiber from fiber bundles. Pectin methylesterase removes the methyl groups to give access to the depolymerizing enzymes like

polygalacturonase (PG) or pectate lyase (PaL). PG randomly hydrolyses $\alpha$-1,4 galactosiduronic bonds in homogalacturonans, while PaL activity resulted in eliminative cleavage to give oligosaccharides with 4-deoxy-$\alpha$-D-galact-4-enuronosyl groups at their non-reducing ends and $\alpha$-D-glucuronic acid [127, 128]. Table 5 shows pectic enzymes production by numerous microorganisms and its substrate and fermentation methods (Solid-state fermentation, SSF and submerged fermentation, SmF).

Table 5: Pectic enzymes production by numerous microorganisms and its substrate and fermentation methods [129].

| Microorganisms | Enzyme | Carbon sources | Fermentation methods |
|---|---|---|---|
| *Aspergillus spp.* | Pectinolytic enzymes | wheat bran (WB) and orange-peel waste (OPW) | SSF |
| *A. niger* | Pectinase and cellulase | WB and OPW | SSF and SmF |
| *A. giganteus* | Polygalacturonase (PG) | OPW and pectin | SmF |
| *A. awamori* | Exo-PG and xylanase | OPW and grape pomace | SSF |
| *A. japonicus* | Pectinase and CMCase | OPW | SmF |
| *A. foetidus* | Pectinase | OPW | SmF |
| *A. fumigatus* | Pectinase | OPW | SSF |
| *A. sojae* | PG | OPW | SmF |
| *A. sojae* | PG | WB | SSF |
| *Penicillium oxalicum* | Pectinase and CMCase | OPW | SmF |
| *P. oxalicum* | PaL | OPW | SSF |
| *P. viridicatum* | Pectin lyase and PG | OPW and WB | SSF |
| *Aspergillus & Penicillium* | PG | OPW | SSF |
| *Trichoderma sp.* | PG | OPW | SSF |
| *Eupenicillium javanicum* | Cellulase, pectinase, xylanase | Citrus processing waste | SSF |
| *Pseudozyma sp.* | Pectinase | OPW | SSF |
| *Bacillus licheniformis* | PG | Pectin | SmF |
| *Thermoascus aurantiacus* | PG | OPW and WB | SmF |
| *Rhizopus oryzae* | PaL | OPW | SSF |
| *Fusarium solani* | Exo- PG | OPW | SSF |

*4.2. Commercial enzyme*

There are always commercialize enzymes available in the market. However, very high in cost and fixed contents of enzymatic formulations discouraged the use of commercial enzymes. Currently, not many researchers are preferred to use commercial enzymes due to economical consideration. Therefore, the commercial enzymes discussion in this paper is limited to the studies that have been done for a long time.

Retting temperature is an essential parameter as enzymatic activities are sensitive to the surrounding temperature. Novozymes 249 was worked optimized at 60 °C while Pectinol AC and Ultrazym maximized their enzymatic activities at 45 °C [130]. Two commercial enzymes Lyvelin (containing 11,000 units U/g of PG) and Peclyve (containing 375 U/g of PaL) being applied on flax fiber retting process and compared with dew retting (6 weeks of dew retting followed by mechanical scutching) [131]. PaL-enzymatic retted flax fiber shows similar strength performance and chemical compositions as dew-retted flax fiber. This was expected since PaL specifically eliminates pectin on fiber bundles.

To maximize enzyme retting efficiency, Akin, et al. [132] applied 0.05% v/v of Viscozyme L with 50mM EDTA chelator in water at pH 5.0 under three pressure conditions (pressurized, vacuum or atmospheric condition) and two enzymatic applications mode (enzyme applies before or after pressurized conditions being applied) [133]. The prior of enzyme application before pressurized conditions have improved enzymatic absorption in flax fibers while no statistical differences observed for prior pressurized and vacuum conditions before enzyme application. On the other hand, retting formulations have produced a fiber with different properties in term of fiber fineness and strength. The effect of chelator (EDTA) and Viscozyme found influencing the fiber strength and fiber yield, respectively [132].

Evans, et al. [134] have used four high PG content enzymes to study the effect of PG activity for retting process. Enzymatic activities of all enzymatic solutions used have shown in figure 12. A. *niger* was concluded as the best retting agent among others. It produces the strongest and finest flax fibers. This finding was agreed by Zhang, et al. [135], who studies the value of PG in enzymatic flax retting. The use of chelator reported further enhance the degree of flax retting by disrupting and weakening middle lamella. However, the importance of the use of non-cellulolytic enzyme solution to produce strong fibers shall not be ignored [134].

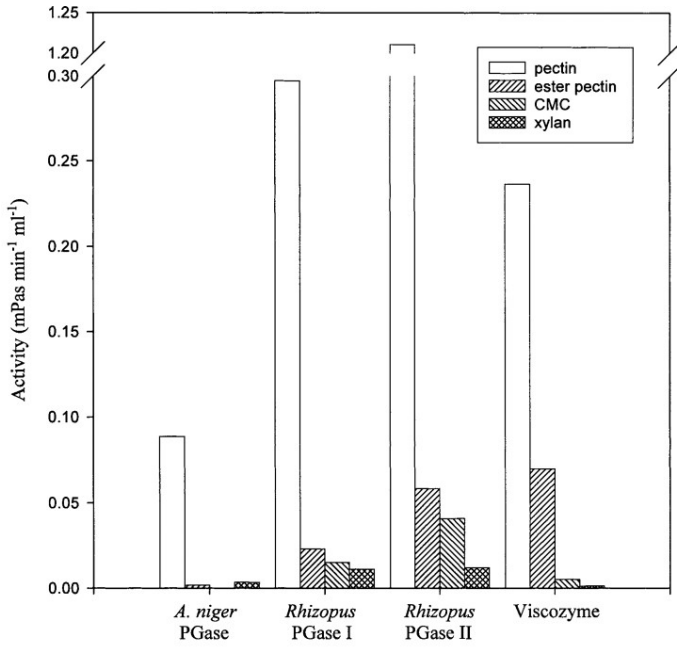



Figure 12: PG activities of enzymatic solutions [134].

*4.3. Bacteria isolated enzyme*

To reduce the cost of enzyme retting process, bacteria or fungi isolation often being done to allow secretion of suitable enzyme(s) for retting. Wang, et al. [136] have isolated two bacterial strains (X12 and P05) that producing promising levels of xylanase and pectinase from the soil of a ramie garden. A synergic retting effect was observed on retted bast fiber under the formulation of two bacteria with equal ratio. A bioaugmentation concept is being applied by the author, showing better gum removal and breaking strength with shorter retting duration. Bioaugmentation is a practice of adding cultured microorganisms for biodegrading specific soil and groundwater contaminants originally. However, it works perfectly fine for bast fiber enzyme retting process. The author has confirmed that the composition of two bacterial strains (P05 and X12) change rapidly in the first 12 hours after bioaugmentation (Figure 13a). The amount of Clostridium, Bacillus and Paenibacillaceae increased significantly on 36th hours, which giving the highest pectinase activity in the retting process (Figure 13b). On the other hand, *Bacillus pumilus* strain, DKS1, was isolated from soil to study a combination of enzymatic and chemical retting process [137]. The microbial intervention followed by mild alkali treatment showed a high percentage of weight loss from retted fibers.

A thermo-alkaline PAL gene from an alkaliphilic *Bacillus clausii* strain S10 was cloned and overexpressed in Escherichia coli [138]. It shows the highest specific activities of 936.2 U/mg on methylated pectin at pH 10.5 and 70oC, presenting its high cleavage capability on methylated pectin. Another Bacillus strain, B. *cereus* was identified in the previous study, which able to produce the highest pectin hydrolysis activities in a selective culture medium among 153 single bacterial colonies [139]. Dramatically reduction of sugar contents in kenaf carbon source at 2-6 hours, indicating the growth and reproduction of bacterial strains. After that, relatively constant of sugar content giving evidence that reproduction of B. *cereus* bacteria via consuming non-cellulolytic components from bast fibers. The outcomes of retted kenaf fibers were reported close to B. *tequilensis* SV11-UV37 bacteria retted kenaf and Sunn hemp fibers, which reported a good efficiency-to-cost ratio under eco-friendly manner [140].

Four bacterial strains from Bacillus strains with high PG, PaL and xylanase activities with minimal cellulase activity were used in jute retting [141]. A highlighted synergetic effect of combining microorganisms resulting in better PG (35.52-46.61 IU/g), pectin lyase (39.79-72.12 U/ml) and xylanase 0.705-0.840 μmol/ml/min) activities. As expected, retting jute fibers produced remarkable enhancement on tenacity and fineness. On the contrary, contaminated post-retted water increases in hardness, acidic value, chemical oxygen demand (COD).

On the other hand, the comparison has been made between anaerobic strain *Clostridium* sp. L1/6 and aerobic strain *Bacillus* sp. ROO40B which isolated from raw flax and hemp ret liquor, respectively [142]. The selection was based on the highest PG activity secreted by bacteria. One very distinct retting condition is the acidity of the incubation medium. Anaerobic strains found highest PG activity (100 IU/g) at pH 4.8 medium while aerobic strains displayed a pectinolytic activity (169 IU/g) at pH 8.0. Besides, absent of celluloses activity in ROO40B bacteria preserved the fiber strength but all pectinolytic anaerobic strains observed a significant amount of celluloses activity [143]. There are no significant retting differences observed between both bacteria in sugar hydrolysis process, even though aerobic bacteria were predominant in the first phase of the process while anaerobic strains became predominant at the latter process. The reason being this is because the growth of aerobic bacteria creates anaerobic condition that are suitable for anaerobic bacterial spore germination [142]. Another study has also confirmed that highest enzymatic activity for anaerobic strains and aerobic strains belongs to Clostridium sp. and Bacillus sp., respectively which showed a PG activity more than 100 IU/g [143]. High PG activity is well correlated with retting efficiency. Inoculation of water tanks with the highest PG activity strains reported reduced the time required by half (12 days to six days) [144].

As the retting process via microbial isolation is significantly reducing the cost of fiber production. Fan, et al. [145] have designed a retting process by using *Bacillus* sp. (HG-28) for ramie fibers in commercialize scale. Detailed retting schematic for in-situ microbial retting process shown in figure 14. An in-situ microbial retting process with the direct involvement of bacterium on the

ramie fiber as a carbon source, rather than treating fiber bundles with enzymes secreted by bacteria, was highlighted. This method could increase retting efficiency as well as reducing the costs. Lower gum contents on ramie fiber and higher breaking tenacity were achieved and additionally, consumption of chemicals, water and energy were significantly reduced. Another rapid ramie degumming by using *Pactobacterium* sp. CXJZU-120 was conducted and 90% of gum removal have been evaluated in just 6 hours of retting period [146]. Furthermore, it could reduce production cost up to 20.5%, resources optimized more than 50% as well as reducing 80% of pollution level. The study also recorded the schematic for traditional chemical and rapid enzymatic retting process, to provides a repeatable methodology for further researches.

Fungi always shares a common substrate with Bacteria, and their co-existing in many situations have gave us synergistic or antagonistic interactions. In the discussion of previous section, 3.3.1-Bacterial and Fungi Interaction, fungal colonization will come before growth of bacteria. An inversely relationship between fungal growth and tolerance towards bacteria was observed in aquatic solutions. The fungi growth is suppressed by the presences of bacteria. If fungi incubations done under controlled environmental. Established fungi medium producing higher biomass was reported [147].

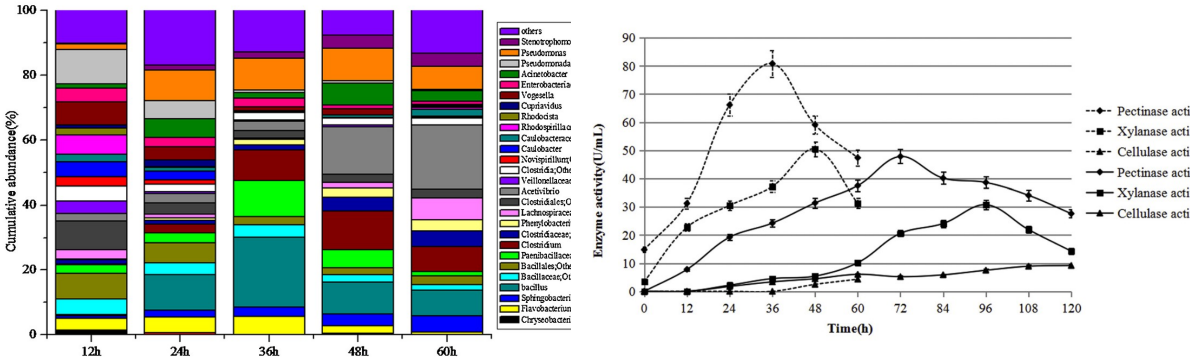



Figure 13: (a) The changes in bacterial composition during bioaugmentation retting throughout 60hours and (b) Enzyme activity levels (U/mL) of water retting, C and P05/X12 microbe retting, PX during bioaugmentation retting [136].

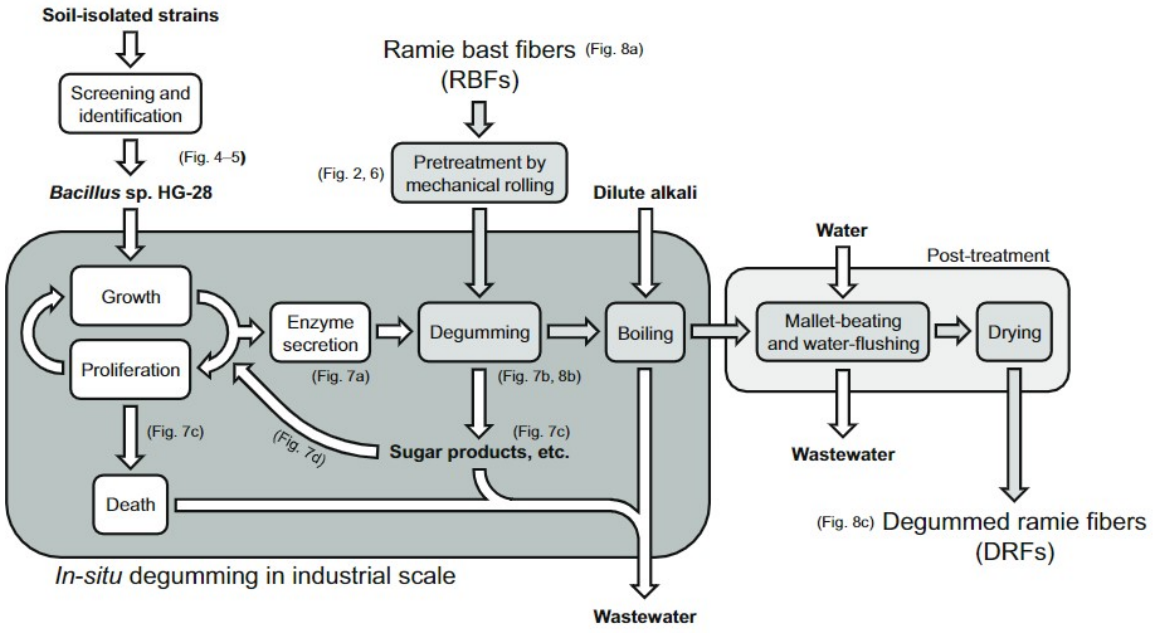



Figure 14: Detailed retting schematic for *in-situ* microbial retting process [145].

*4.4. Fungi isolated enzyme*

Under controlled environment, fungi isolated enzymes worked very well as compared to dew retting process. Maheshwari, et al. [148] have studied the effects of controlled colonization environment (spore concentration of 107 U/ml, 35 °C, four days of retting period and relative humidity of 45%) on Sunn hemp fiber. Traditional water- and dew-retting method have been used as controlled sets. The results showed all fungi retted fibers under a controlled environment having higher strength properties.

Musialak, et al. [149] have improved the retting process by using Aspergillus aculeatus (pectinases) to reduce pectin contents in tissue-cultured and field-grown plants. The fungus was contributing a significantly high retting efficiency without alteration of lignin or cellulose contents throughout the process. These have indicated that the over-expression of enzymes does not affect flax fiber chemical compositions. The growth rate and soluble sugar and starch contents were in the range of the control levels

Apart of this, Henriksson, et al. [150] have isolated *Rhizomucor pusillus, Fusarium equiseti sw, Trichoderma 6irens sg, Alternaria alternata sog, F. oxysporum fw, Epicoccum nigrum hp, F. lateritium sr* fungus from dew-retted flax, to produce enzyme filtrates that highly effective in flax retting process. However, only the R. *pusillus* enzyme with the use of chelator (oxalic acid) recorded a higher retting score than commercial enzyme (Flaxyzme). The fungus can be characterized as thermotolerant since it can be cultivated at higher temperature. Besides, the culture of R. *pusillus* did not show any significant xylanases and manages activities, suggesting hemicelluloses and cellulase may not be required to ret flax.

Zeni, et al. [151] have studied the PG activities from 107 microorganisms (92 newly isolated and 15 pre-identified), which collected from soil, leaves, fruits, teas, processed products and argo-industrial wastes. Among all, there are 20 strains able to synthesize PG activities above 3 U/ml. Furthermore, five isolated fungi (A. *niger* ATCC 9642, *Penicillium* sp. W23, *Penicillium* sp. W42 and *Penicillium* sp. D2) undergo 24 hours fermentation and led to PG activities of 30, 41, 43 and 45 U/ml, respectively. Throughout the kinetic study of PG activity, pH variation can be used to predict enzyme production, as the release of galacturonic acid strongly affects the acidity of fermentation medium [152].

The enzymatic activities of A. *niger* HYA4 incubation by solid-state fermentation (SSF) in the absence or near-absence of free water has been studied previously [153]. The SSF requires lower initial capital and operating cost, producing lesser wastewater and energy needed with higher productivity. The outcomes revealed that the HYA4 is a mesophilic fungus where grows best in moderate temperature (32-36 °C). Besides, highest PG and xylanase activities found at 72 hours of incubation period with low-cost wheat bran as carbon source. The proposed methodology has resulted in a significant reduction of flax fiber retting cost.

Wong, et al. [154] have discovered a newly isolated A. *fumigatus* R6 fungus using rice bran as a substrate in SSF condition. Optimized conditions (initial moisture level of 49.6%, 33 °C and 129 hours of incubation time) produces a 2.65-fold increment of PG enzyme activities (565 U/g) and consequently yielding satisfying mechanical properties. The author also investigates the effect between initial moisture level, temperature and incubation time on enzyme activities (Figure 15).

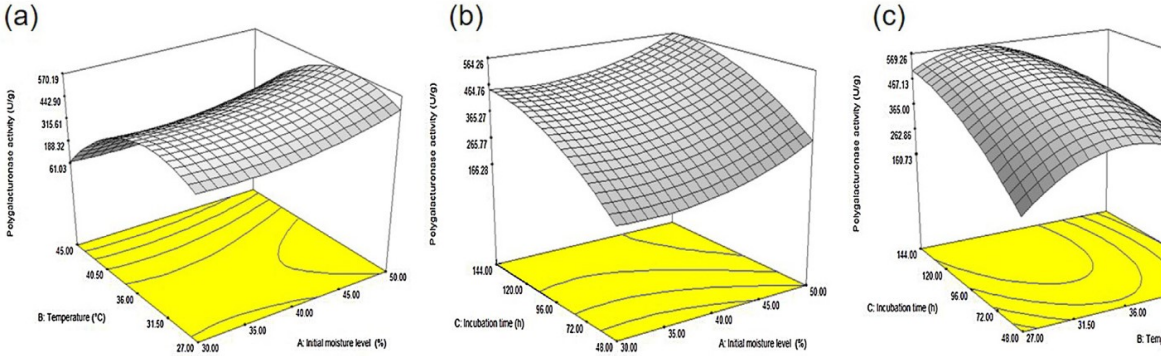


Figure 15: Response surface plot on the effect of (a) initial moisture level vs temperature, (b) initial moisture level vs incubation time and (c) temperature vs incubation time [154].

**5. Properties of enzymatic retted fibers and its composites.**

One of the useful applications of bast fibers are to serve as reinforcement in composites. Bast fibers reinforced in the petroleum-based matrix could provide a certain degree of biodegradability to the composite, in order to meet current global market needs. Besides, bast fibers reinforcement could enhance the strength properties of composites by regulating better load transfer mechanism. However, hydrophilic nature of bast fibers is not compatible with hydrophobic polymers, making the composite low in performances. The main reason behind this is the poor interfacial bonding between fiber and matrix. Therefore, enzymatic retting process allowed complete bast fiber separation from fiber bundles and removing non-cellulosic components on fiber surface to provides more active sides for stronger interface bonding, thereby improving strength performance of bast fibers reinforced polymer composites. Table 6 shows the recent study on enzyme retted fibers reinforced composites.

Kenaf bast fibers were retted under pectinase with chelators or NaOH retting process [155]. The retted fibers were then used in composite fiber reinforcements. All mechanical properties show chemical retting provides better enhancement to the fibers. However, enzyme retted fiber composites showed higher biodegradability. This is because the enzyme acts as a catalyst to boost up microorganic activities in the soil, thereby composite has higher biodegradability.

Optimum enzyme retting formulation was reported to improve the quality of fiber, thereby performance of composites. Smoother fiber surface and better separation from fiber bundles are the main reason to have better strength of enzyme retted bast fiber reinforced polymer composites. A study of the effectiveness of controlled water retting, enzyme retting with and without EDTA chelator was performed on flax fiber reinforced epoxy [156]. The untreated flax fibers were, on average, having 30-35% stronger than retted flax while no obvious difference in the strength properties among different retting methods. However, reinforcement of untreated flax fibers in epoxy matrix showed the lowest tensile value due to most of the reinforcement fibers are presented in bundle forms which have the brittle manner and significant amounts of debris. Thus, affecting the degree of adhesion and poor interfacial bonding between matrix and fiber was found. Besides, fiber bundled reinforcements would restrict effective surface area by covering the fiber surfaces and hindering active bonding between fiber and matrix [157].

On the contrary, EDTA treated flax fibers reinforced epoxy composite shows the highest tensile value because chelator contains $Ca^{2+}$ ions that allows the fibers to separate from epidermis easily, resulted in clearer and smoother retted flax fibers. Over-retting was suspected for enzyme retting process with EDTA since relatively poorer bonding between matrix and fiber was observed. Excellent fiber separation with ethylene diamine tetramethylene phosphonic acid (EDTMPA) yet more environmentally friendly has been reported [158]. Relative environmental, economic and strength performance have been listed in table 7 for chelator, and combined chelator and enzyme-treated hemp reinforced polypropylene composite.

The pectinolytic enzymes retted hemp bast fiber strips were aligned and untangled to process the fibers unidirectionally in epoxy composites followed by vacuum degassed. A 100 kPa hydrothermal pre-treatment before enzymatic retting produces the highest fiber ultimate tensile strength [78]. This is because macro-pores observed on the cell walls have been prevented during hydrothermal pre-treatment and hence reduces the probability of over-retting due to the penetration of enzymes. However, some researchers commented that individual pectinase retting show lack of fiber separation. Neither the laccase nor pectinase able to breakdown the waxy layer and remove lignin and pectin to release fibers from fiber bundles [157]. On the other hand. Effective of removal pectin by enzymes have reported with evidence of superior mechanical properties of hemp fibers reinforced epoxy composites. An increment of 31% and 41% of tensile strength and stiffness was recorded, respectively [78]. Similar results observed on enzyme retted flax fibers reinforced epoxy composites [159, 160]. Besides, SEM micrographic in the study show the lowest porosity factor for

enzyme retted hemp fibers due to parenchyma cells elimination on the fiber surface, indicating good impregnation of the hemp fibers by epoxy matrix. There was no strong correlation being observed between pectinase retting process with composite's impact strength and elongation at break of [161].

High effective of enzyme retting on impurities removal being confirmed in previous work [57]. Surface chemical modifications have induced by enzyme retting was evident by ATR-FTIR spectroscopy. Different density of spectrum peaks has been recorded in figure 16 when comparing between raw and retted hemp fibers. Disappearances of 2850 $cm^{-1}$ peak responsible to -CH symmetrical stretching of polysaccharides, for waxes and oils. The absence of 1640, 1550-1400 and 1244 $cm^{-1}$ peak observed in retted fibers responsible for effective removal of carboxylate ions for pectin and hemicellulose and aromatic ring in lignin by enzyme hydrolysis. Besides, a higher amount of cellulose and hemicellulose contents for retted fibers can be viewed by the sharper peak of 3300 $cm^{-1}$. Apart from this, synchronized information from XRD spectra shows a higher crystallinity index for retted hemp fibers. When these retted fibers inserted into PBS matrix, better tensile and flexural strength values were observed, regardless of any fiber volume contents in the composites (Table 8).

Besides, enzyme retted kenaf fibers and modified starch biocomposites were fabricated with additional plasticizers (glycerol, poly(ethylene glycol) and poly(vinyl alcohol)(PVA)), for the purpose of starch fluidity [155]. The author claimed that PVA plasticizer is most compatible with enzyme retted kenaf fibers reinforced starch composites. At the same time, this composite formulation given highest biodegradability since the enzyme acts as a catalyst for microorganic activity in the soil, accelerating biodegradation rate. Two bacterial strains of *Clostridium felsineum* L. (MIC10690 and MIC 9539) have been selected for ramie microbe retting process in a previous study [63]. The results show chemical retting is more effective than both microbial retting process from the biochemical contents of retted ramie fibers. However, both microbe retted ramie fibers reinforced PHA composites found better mechanical properties than chemical retted fibers composite, suggesting higher suitability of microbial retting process for composite application.

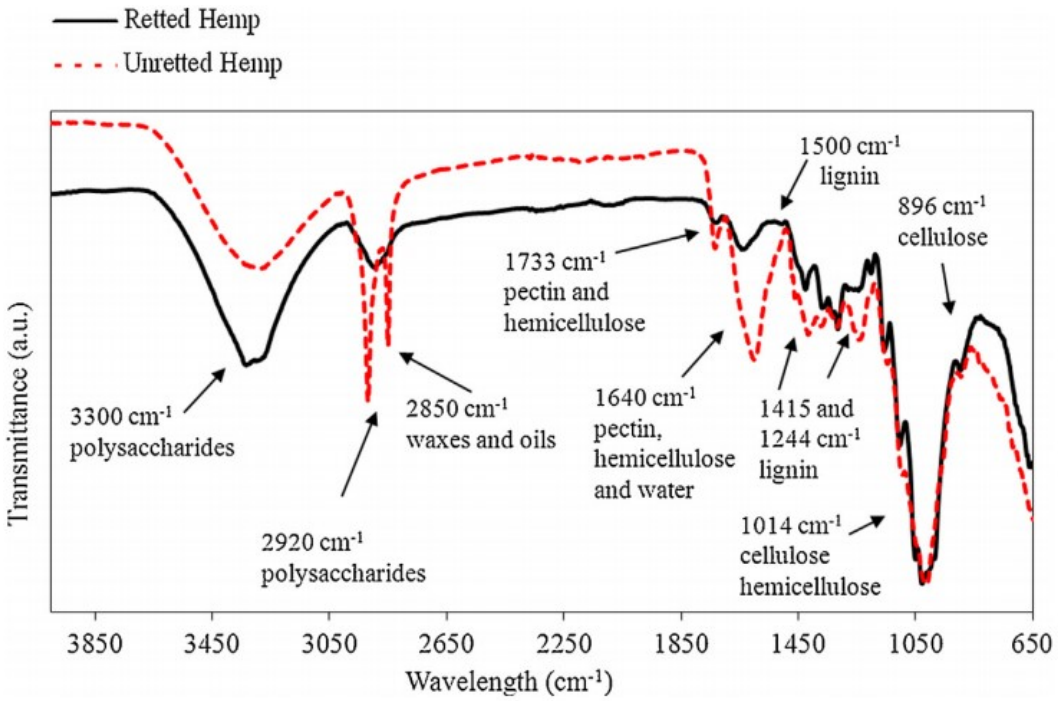



Figure 16: ATR-FTIR spectroscopy for enzymatic retted and un-retted hemp fibers [57].

To enhance composite performances, interfacial bonding between matrix and fibers is an important key factor. Three white root fungi have been applied to investigate the strength of hemp fibers reinforced polypropylene composite affected by the condition of interfacial bonding [162]. White rot fungi reported to have high degradation rate of non-cellulosic components and producing

micro-holes on the fiber surface, which can roughen the surface of hemp fibers [163]. FTIR spectra observed a reduction of the 1736 cm-1 peak for white rot fungi retted hemp fibers, which can be attributed to effective pectin and wax removal, resulted in high crystallinity index of retted hemp fibers (84-88%) although the over-retted process was being blamed to the fine holes that roughen fiber surface and caused structural degradation of cellulose to the fiber, thereby deteriorated tensile strength of fibers [162]. The fine holes that made roughen surface have improved the interfacial bonding at the same time, increasing mechanical interlocking and showing the importance of interfacial bonding than single fiber strength performances. The previous study found similar findings, enzyme-treated flax fibers pulled out from the matrix, were more intensely coated with the PLA matrix [164]. Improved adhesion between PLA and enzyme retted fiber was shown in figure 17 (a) due to the different surface morphology of untreated (figure 17(b)) and enzyme retted fibers (figure 17(c)). Figure 17(b) show waxy materials covering the fibers and hence hindering effective adhesion while figure 17(c) revealing a smooth flax fiber surface which promoting good interfacial bonding. On the contrary, Foulk, et al. [165] claimed that smoother fiber surface by long enzyme retting periods found reduced mechanical interlocking between flax fiber and thermoset vinyl ester resin but somehow 22 hours and 46 hours of enzyme exposure recorded significant higher tensile value than short period of enzyme retted fibers composite, without any specific explanations [165]. Apart from this, strong interfacial bonding having a positive effect on shrinkage- and fire-resistant properties of enzyme retted bast fibers reinforced polymer composites [166].

Liu, Thygesen, Summerscales and Meyer [32] have confirmed that 50 wt% of laccase retted hemp fiber reinforced epoxy composite having 33% and 56% increment of stiffness and strength, respectively. Besides, higher thermal stability of retted hemp fibers was observed due to oxidized of lignin components by laccase enzyme. However, flax fibers are generally having more hemicellulose than hemp fibers. Removal of non-cellulosic components resulted in weaker structure since lignin act as an adhesive holding the fibrils network together [126]. On the other hand, ineffective retting by laccase enzyme alone was reported in previous [167]. This is because of most of the lignin components in hemp fiber confined at inner regions of primary cell wall and lumen. Hybrid treatment (alkaline treatment with laccase retting) is a solution to increases fiber adhesion force, surface polarity and smoother surface [167, 168]. George, et al. [169] have proven that mercerization before enzymatic fiber retting did enhance fiber thermal stability, regardless of the type of enzymes (table 9). The alkaline treatment has found to disrupt hydrogen bonding and caused swelling on fiber macrostructure, resulted in better enzyme penetration to hydrolyze non-cellulosic components that low in thermal stability. Nevertheless, the thermal stability of matrix playing a dominant role in thermal stability properties of biocomposites. The insertion of reinforcement bast fibers with or without retting process into highly thermal stability matrix will only observed a slight differences in thermal stability [57].

Table 6: Recent study on enzymatic retted fibers reinforced composites.

| Year of study | Enzymes used | Incubation conditions/ retting conditions | Target fibers | Matrix | Composite strength, MPa | Reference |
|---|---|---|---|---|---|---|
| 2019 | Eethylenediaminetetraacetic acid | Treated with an aqueous solution of pectinase (1%) containing ethylenediaminetetraacetic acid at 50 °C for 24 h | Kenaf | Starch | [a] | [155] |
| 2019 | pectate lyase, pectin methylesterase, PG, hemicellulases and xylanase | 25 mM EDTA, pH 6.5, 40 °C, 24 h | Flax | Epoxy | - | [159] |
| 2018 | - | Approximately 30 kg of hemp fibers were treated at 39–42 °C for 24–30 h | Hemp | Epoxy | 46 | [170] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | depending on the conditions. The pH of the solution was 4.1–4.5 throughout the process. The treated fibers were rinsed with water until the water dripping from the fibers reached a pH of at least 6.0 | | | | |
| 2017 | P. *radiata Cel 26* and pectinase | The retting was conducted for 7, 14 and 20 days in 1 L Erlenmeyer flasks at 28 °C. | Hemp | Epoxy | 280-300 | [101, 171] |
| 2016 | Endo-polygalacturonase (EC 3.2.1.15) and Pectin lyase (EC 4.2.2.10) | Hydrothermal pretreatment with three different water vapour pressure at 40oC followed by 0, 30, 90, 150, 240 and300 min at a pH of 6.0 using a 25 mmol dm3 citrate buffer, a temperature of 40°C, and an agitation of 100 rpm | Hemp | Epoxy | 325 | [78] |
| 2008 | SIHA- Panzym ® DF (EC 3.2.1.15, Novozyme A/S, Bagsvaerd, Denmark) | 1M sodium hydrogen carbonate, 1M citric acid, 10 mM EDTA at 35 °C and pH 4.5 | Hemp | Polypropylene, PP | 34.0-47.6 | [161] |
| 2014 | cellobiohydrolases and endoglucanases | 50 mM Na–citrate buffer, pH 5.0, with a fiber consistency of 4%. The treatment time was two hours and the temperature were 45 °C | Flax | PLA | - | [164] |
| 2012 | pectinase, laccase, cellulase and xylanase enzyme | Jute fabrics were treated with 2 wt% non-ionic detergent solution at 70 °C for 1 h before enzymatic treatment. The liquid ratio was 1:40 for all the treatments. Enzymatic treatments were performed with various enzyme solutions for 90 and 180 min | Jute | polyester | 35.86-50.19 | [172, 173] |
| 2012 | cellulase enzyme | A bath ratio of 20:1, pH 4.8, and 50 °C in acetic acid and sodium acetate buffer solution | flax | PLA | a | [166] |
| 2011 | bacterial pectinolytic enzyme with lyase activity | HCl 50 mM with Ca buffer, pH 8.5, 42 °C and 50rpm in lab scale reactors for 10-46 hours | flax | vinyl ester | 48.54-71.46 | [165] |
| 2009 | White rot fungi *Phanerochaete sordida* (D2B), Pycnoporus species (Pyc) and Schizophyllum commune (S.com) | Dried non-retted hemp fibers were sterilized using gamma radiation of 26.0 kGy (kilogray) in sealed sterilization bags. Irradiated hemp fibers were then inoculated with white rot fungi (D2B, Pyc and S.com) | Hemp | PP | 37.54-45.33 | [162] |

| | | |
|---|---|---|
| | for 2 weeks. Water was added for all fungal treatments and bag retting to give a moisture content of 60 wt.% | |

a Values are shown in the manuscript chart which unable to defines accurate value.

Table 7: Relative environmental, economic and strength performance for chelator, and combined chelator and enzyme-treated hemp reinforced polypropylene composite [157].

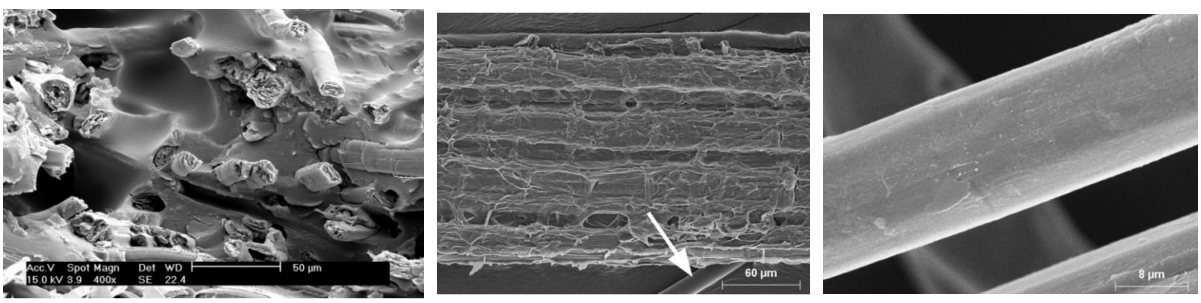
| Treatment | Environmental impact | Treatment cost | | | | Composite strength, MPa |
|---|---|---|---|---|---|---|
| | | Chemical | Energy | Equipment | processing | |
| EDTMP treatment, 5g/l | Low | Lowest | Lowest | Lowest | Lowest | 41.55 |
| EDTMP treatment, 10g/l | Low | Low | Lowest | Lowest | Lowest | 42.3 |
| EDTMP treatment, 5g/l followed by pectinase retting | Low | High | High | High | High | 41.73 |
| EDTMP treatment, 5g/l followed by laccase retting | Low | High | High | High | High | 41.41 |

Table 8: Tensile and flexural properties of hemp fibers reinforced PBS composites [57].

| Sample | Tensile properties | | | Flexural properties | | |
|---|---|---|---|---|---|---|
| | $\sigma_{max}$, MPa | $\varepsilon^b$, % | E, MPa | $\sigma_{max}$,MPa | $\varepsilon^b$,% | E, MPa |
| PBS | 17±1 | 3.8±0.3 | 599±25 | 11±1 | 1.4±0.2 | 854±150 |
| 10UR | 23±3 | 2.4±0.3 | 1094±200 | 27±0 | 2.8±0.3 | 1500±132 |
| 10R | 25±4 | 2.8±0.5 | 968±180 | 26±2 | 2.6±0.1 | 1433±196 |
| 20UR | 29±3 | 1.9±0.2 | 2221±210 | 31±2 | 2.7±0.1 | 1884±150 |
| 20R | 32±3 | 2.5±0.3 | 2414±241 | 40±5 | 2.8±0.8 | 2523±165 |
| 30UR | 22±4 | 1.7±0.2 | 2232±255 | 32±5 | 2.4±0.3 | 2045±175 |
| 30R | 28±3 | 2.0±0.4 | 2295±250 | 35±3 | 2.4±1.0 | 2259±170 |

Table 9: Maximum decomposition temperature for the different system [169].

| System | Maximum decomposition temperature, $^oC$ | |
|---|---|---|
| | Enzyme | NaOH+ Enzyme |
| Control | 354 ± 0.52 | 350 ± 2.34 |
| Xylanase | 346 ± 1.04 | 360 ± 1.24 |
| Xylanase + cellulase | 351 ± 0.31 | 364 ± 1.03 |
| PG | 365 ± 0.50 | 367 ± 1.39 |
| Pectinmethylesterase | 354 ± 0.53 | 362 ± 1.03 |
| Laccase | 348 ± 1.24 | 364 ± 1.35 |




(a) (b) (c)

Figure 17: (a) Improved adhesion between PLA and enzyme retted fiber due to the different surface morphology of (b) untreated and (c) enzyme retted fibers [164].

## 6. Conclusion

As more and more innovative researches being conducted on natural bast fiber reinforced composites in advanced sectors, single bast fiber with high performance must be achieved. Bast fiber retting process is the first and the most important process of getting promising strength of the fiber. Water retting process were used to be the most recommended retting process for quality bast fibers production. Nevertheless, generation of a large amounts of wastewater has it prohibited by most countries. Chemical and dew retting was then applied to substitute water retting process. However, high chemical cost and low retted fiber quality, of chemical and dew retting process, respectively, has driven people to look for another suitable process.

Enzyme retting is claimed to have more environmentally friendly wastewater products, shorter retting period and controllable fiber biochemical components under mild incubation conditions. The right dosages of response enzyme are crucial to obtain an efficient enzymatic retting process. On the other hand, higher enzymatic retting efficiency could be done by additional chelators which containing a large amount of Ca2+ ions that can remove epidermis and cuticle easily to release bast fiber from fiber bundles. EDTA reported as the best chelator agent. However, commercial enzymes are not preferred due to not selective-friendly of enzyme ratios and high in cost. Fungi always shares a common substrate with Bacteria, and their co-existing in many situations have gave us synergistic or antagonistic interactions. However, an inversely relationship between fungal growth and tolerance towards bacteria was observed in aquatic solutions. The fungi growth is suppressed by the presences of bacteria. Established fungi medium producing higher biomass was reported. Therefore, isolation of bacteria and fungi show highly flexible on retted bast fiber's properties. Pectinase is the main enzyme for retting process, hydrolyzing pectin components in middle lamella to release single fiber. However, celluloses, xylanases and laccases shall apply depending on the type of bast fiber or the applications.

To fabricates a promising material for advanced sector applications, enzyme retted bast fiber reinforced polymer composite was found to meet the criteria often. High effectiveness of enzymatic retting on impurities removal produces a high cellulosic fiber which has high strength and crystallinity. Besides, roughen the surface of microbial retted bast fiber have improved interfacial bonding with matrix, thereby increasing strength performance and positive effects on product shrinkage- and thermal-resistance properties. Retting process, especially enzyme retting could offer a tremendous benefit to bast fibers as green composite reinforcements, at the same time increases the value of non-food crops by optimizing its potential as advanced materials.

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