1. ENHANCING THE MOISTURE RESISTANCE OF FLAX FIBRES VIA ENZYMATIC TREATMENT

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ABSTRACT

As a sustainable and eco-friendly material, flax fibres offer a viable alternative to glass fibres in composite applications due to their good specific mechanical properties. However, addressing their moisture sensitivity is crucial to expanding their use in various applications. This study investigates the impact of enzymatic treatment on improving the moisture resistance of flax fibres. FlaxTape^{TM} 200 was treated with two types of polygalacturonase enzymes to selectively remove pectin. The moisture resistance of the treated fibres and their composites was compared with that of untreated samples. The results revealed a significant reduction in moisture uptake at high relative humidity conditions and a decrease in percentage water uptake in both longitudinal and transverse composites after enzymatic treatment. FTIR spectra and contact angle measurement results supported the observed improvement in the moisture resistance of flax fibres. This study highlights the effectiveness of enzymatic treatment in enhancing the moisture durability of flax fibres which further broadens their potential for structural and lightweight composite materials.

2. INTRODUCTION

Natural fibre-reinforced composites have emerged as promising sustainable alternatives to synthetic fibre-based systems across various structural, functional, and environmentally driven applications [1–4]. Among the range of available natural fibres, flax has attracted growing interest due to its valuable combination of mechanical performance and environmental benefits. Flax fibres exhibit high specific tensile strength and stiffness rendering them competitive with conventional glass fibres in terms of load-bearing capacity [4]. As sustainability becomes a central focus in materials research, flax fibres stand out for being biodegradable derived from renewable resources and associated with a substantially lower environmental footprint. Despite their numerous advantages, the widespread application of natural fibre composites is often constrained by their sensitivity to moisture [1,2,5]. Flax fibres are inherently hydrophilic due to the presence of pectin, hemicellulose, and other polar constituents within their structure. When exposed to humid conditions or direct water contact, these fibres absorb moisture which lead to fibre swelling. This behaviour can compromise the

fibre-matrix interface and negatively impact the mechanical performance and long-term durability of the resulting composites.

To reduce the moisture sensitivity of flax fibres, traditional retting methods such as water retting and dew retting are often employed to degrade binding components like pectin. While these techniques can partially remove hydrophilic substances, they also have several drawbacks. This includes limited control over microbial activity, dependence on environmental conditions, and inconsistent treatment outcomes. As a result, these conventional methods can lead to variability in fibre quality, compromising the performance and reliability of the final composite materials [3,6]. In this context, enzymatic treatment offers a promising alternative to conventional retting techniques due to its high specificity and controllability. Additionally, it is time-efficient which requires significantly less time compared to the weeks or months needed for traditional methods [3]. Among the enzymes assessed for flax fibre processing, polygalacturonase has demonstrated significant effectiveness [4,7]. In a study by Zhang et al. (2000), seven commercial enzyme mixtures were tested for their retting efficiency, with polygalacturonase emerging as the key enzymatic component responsible for efficient flax fibre separation [8]. This enzyme catalyzes the hydrolysis of α-1,4-glycosidic bonds in pectin, a major hydrophilic component in flax fibres [1,3,7]. By targeting pectin, polygalacturonase promotes fibre separation and reduces the fibres' moisture sensitivity.

In this study, commercially processed flax fibres (FlaxTape™ 200) will be treated with two polygalacturonase enzymes to evaluate their effectiveness in modifying the moisture-related properties of both the fibres and their epoxy-based composites. By comparing untreated and enzymatically treated fibres, this research aims to gain deeper insights into how enzymatic treatment impacts moisture resistance, mechanical performance and durability of flax fibres in composite applications.

3. RESEARCH TESTS & EXPERIMENTS

3.1 Materials

FlaxTape™ 200 supplied by EcoTechnilin (France) was utilized in this study. This material is a unidirectional mat of flax fibers with an areal weight of 200 g/m². It was processed to deliver enhanced mechanical properties while maintaining a reduced weight which makes it suitable for applications where both structural performance and aesthetic purposes are important [9]. For the enzymatic treatment, two types of polygacturonases were used. The first pectin-degrading enzyme, available in powdered form, was obtained from Tokyo Chemical Industry (TCI Japan) Europe N.V. and derived from *Aspergillus niger*. The second polygalacturonase, Sustine® 450 (Novonesis, Greece) was in liquid form. Prior to their application, enzyme activity assays were conducted using the 3,5-dinitrosalicylic acid (DNS) method, following Miller's protocol. This is to standardize the activity levels of both enzymes [4,10] for the two polygalacturonase treatments.

3.2 Polygalacturonase Treatment

The starting material for each polygalacturonase treatment was 400g of FlaxTape™ 200 fibres (25cm × 30cm per ply) enough for all the tests and characterizations to be done. The fibres

were immersed in 5L of enzyme solution containing 25mM of ethylenediaminetetraacetic acid (EDTA, VWR International, Belgium) and 0.6% (v/v) enzyme adjusted to pH 6.5. Shaking incubation of the s-oaked fibres were done for 24 hours at an optimal temperature of 40℃ at 32rpm. Following incubation, the enzymatically-treated fibres were then rinsed twice with cold water, and then dried at 105 ℃ [4] until constant weight. For the two enzymatic treatments, treated fibres from Sustine® 450 enzyme will be referred to as E1Flax, and the treated fibres with polygacturonase from *Aspergillus niger* will be called as E2Flax.

3.3 Composite Production

Composite fabrication was carried out using vacuum-assisted resin infusion (VARI) method with a target volume fraction of 40% and laminate thickness of 2mm. An epoxy resin (Epikote™ 828, Belgium) served as the matrix to impregnate both untreated and enzymatically treated fibres. Curing was [achieved] by mixing the resin with 1,2-diaminocyclohexane (Sigma-Aldrich, Germany) as a hardener, in a ratio of 100 parts resin to 15.2 parts hardener by weight. The flax–epoxy laminates were cured for 1 hour at 70 °C, and then followed by a post-curing step at 150 °C to ensure complete crosslinking. Composites were cut to dimensions of 80mm × 10mm for mechanical tests and water immersion.

3.4 Dynamic Vapor Sorption of Fibres

The moisture sorption behaviour of untreated and treated E1 and E2 flax fibres was assessed using a Dynamic Vapor Sorption (DVS) analyzer (Surface Measurement Systems, United Kingdom) operating under isothermal conditions at 21 °C. This gravimetric technique enables accurate determination of moisture uptake by maintaining strict control over relative humidity (RH) and temperature [1]. Fibre samples weighing approximately 3 – 5 mg (cut to lengths of approximately 2 mm) were analyzed using an ultra-sensitive microbalance with a mass resolution of ±0.1 µg. The RH was incrementally adjusted in both sorption and desorption phases through a series of steps: 0%, 20%, 50%, 70%, and 95%. Each relative humidity step was initiated once the sample reached quasi-equilibrium indicated by a mass variation rate below 0.02% per minute. The percentage moisture uptake $\%\, M$ per RH was calculated based on Equation 1 as shown below:

$$\%\, M = \frac{m_{eq} - m_0}{m_{eq}}\, x\, 100 \qquad \text{(Eq. 1)}$$

where m_{eq} is the fibre mass after equilibrium RH step, and m_0 is the initial dry mass of the fibre obtained at 0% RH.

3.5 Water Immersion of Composites

The moisture durability of the produced composites was evaluated by immersing the samples in distilled water for 30 days at ambient conditions. Changes in water mass uptake and thickness were measured from triplicate samples periodically. The weight of the samples were taken using a precision balance (Mettler Toledo, Belgium) with ±0.1µg sensitivity. Using Equation 2, the percentage water uptake ($\%\, W$) was calculated, where m_i is the weight of the sample for each immersion time, and m_0 is the initial dry mass of the composite.

$$\%\, W = \frac{m_i - m_0}{m_0}\, x\, 100 \qquad \text{(Eq. 2)}$$

The thickness swelling of the specimens after each sampling time was also determined using a digital micrometre (Mitutoyo, Japan) with a precision of ±1µm. Equation 3 below shows the computation of percentage thickness swelling ($\%\,T$), where t_i is the thickness of the sample for each immersion time, and t_0 is the initial thickness of the dry composite. Three measurements along the length of each composite were averaged to get the thickness. Prior to mass and thickness measurements, the samples were gently blotted dry using a clean towel to remove surface water.

$$\%\,T = \frac{t_i - t_0}{t_0}\, x\, 100 \qquad \text{(Eq. 3)}$$

Lastly, six specimens were taken out from the immersion chamber after 3 days, 10 days, and 30 days to monitor changes in flexural properties after exposure to water.

3.6 Flexural Testing of Composites

The mechanical performance of pristine, enzymatically-treated, and water-immersed composites was assessed through three-point bending tests in accordance with ASTM D790. Testing was performed using a universal testing machine (Instron 5943, Belgium) equipped with a 1 kN load cell, a support span of 48 mm, and a crosshead speed of 2 mm/min. Six specimens from each composite type were tested in both longitudinal and transverse directions. Water-immersed samples were first oven-dried at 60 °C until constant mass was achieved. This was followed by equilibration at ambient conditions prior to flexural testing to ensure measurement consistency.

3.7 Contact Angle Measurements of Fibres

The wettability of the surface of the fibres was determined by measuring the formed contact angle with water. A single fibre tensiometer (K100SF, Krűss, Germany) equipped with Motic SMZ-171 microscope and Moticam (CMOS) digital microscopy camera was used to obtain advancing contact angles of the pristine and enzymatically-treated fibres. This method is based on Wilhemy technique which follows the equation:

$$F = P\gamma cos\theta \qquad \text{(Eq. 4)}$$

where F is the force measured on the fibre due to the surface tension of water, P is the wetted length or perimeter of the fibre, γ is the surface tension of water and θ is the contact angle formed between the fibre and water. Hexane (Chem-lab Analytical, Belgium) was the liquid used to determine the perimeter of the fibre as it gives perfect wetting ($\theta = 0$) [11].

3.8 Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR)

Chemical characterization was conducted to evaluate the efficiency of the enzymatic treatment on FlaxTape™ 200. FTIR-ATR spectra of the specimens were recorded using a Nicolet™ iS50 FTIR spectrometer (the Netherlands), equipped with an ATR accessory. A resolution of 4 cm^{-1} over the range 4000 – 450 cm^{-1} with 100 scans was set for each specimen. The spectra of the untreated and treated fibres were compared to identify changes in the intensity of specific functional groups before and after enzymatic treatment.

3.9 Scanning Electron Microscopy (SEM)

The surface morphology and structural changes of pristine and enzymatically treated FlaxTape^{TM} 200 fibres were examined using scanning electron microscopy (SEM). Both treated and untreated fibres were analyzed with a Philips XL-30 FEG SEM (Netherlands). Prior to imaging, samples were degassed and coated with a 10-nm layer of platinum/palladium (Pt/Pd) via plasma sputtering to enhance electrical conductivity. SEM imaging was conducted at an accelerating voltage of 10 kV using a backscattered electron (BSE) detector to obtain high-resolution images.

4. RESULTS

4.1 Moisture Uptake of Fibres

Figure 1 presents the moisture sorption and desorption profiles of FlaxTape^{TM}200 fibres subjected to polygalacturonase treatment, and the control sample. The results clearly demonstrate that enzymatic modification significantly alters the moisture uptake characteristics of the fibres. The application of polygalacturonase, which targets and breaks down pectin components within the fibre matrix, appears to reduce the overall hydrophilicity. This is reflected in the decreased sorption and desorption levels observed in the treated samples, E1Flax and E2Flax, relative to the control sample. Notably, the difference in moisture uptake between E1Flax and E2Flax is minimal. This is likely due to the controlled and uniform enzymatic activity applied to both enzymes upon standardization of their polygalacturonase activity prior to fibre treatment.

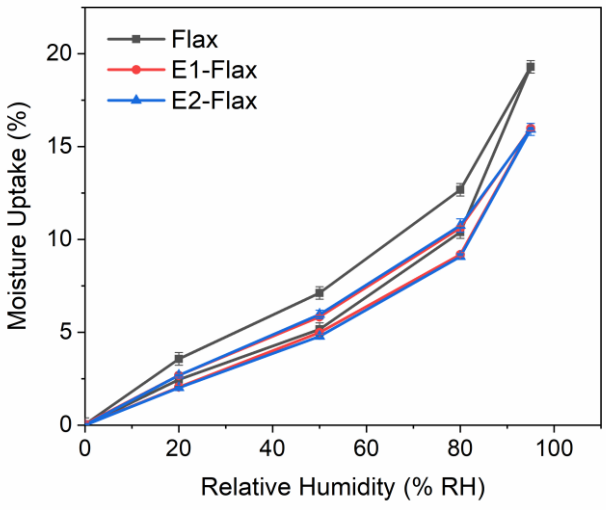

Figure 1. Moisture uptake of untreated and treated FlaxTapeTM200 fibres.

Moreover, the difference in moisture uptake between treated and untreated fibres becomes more pronounced at higher %RH levels within the DVS chamber. This can be attributed to the higher pectin content in the untreated fibres which enhances their moisture affinity at elevated RH. In addition, all fibre samples exhibited hysteresis with desorption values exceeding those

recorded during sorption. The non-overlapping sorption and desorption curves reflect this hysteresis effect which likely resulted from fibre swelling upon moisture uptake. Upon desorption, the fibre structure does not fully revert to its original state further contributing to this behaviour [12]. This effect is especially evident in the untreated FlaxTape^{TM}200 fibres, indicating a stronger structural response to moisture exposure.

4.2 Water Immersion of Composites

The moisture resistance of the composites produced from epoxy with untreated and treated fibres is shown in Figure 2. Both water uptake (%W) and thickness swelling (%T) exhibited comparable time-dependent trends. However, higher values were consistently observed in composites containing untreated fibres. This observation aligns with the previously established moisture absorption characteristics of the fibres in Figure 1. The increase in composite thickness is attributed to fibre swelling induced by water ingress. The reduction in water uptake is more evident in composites incorporating enzymatically treated fibres. This observation highlights the effectiveness of the treatment in enhancing moisture resistance.

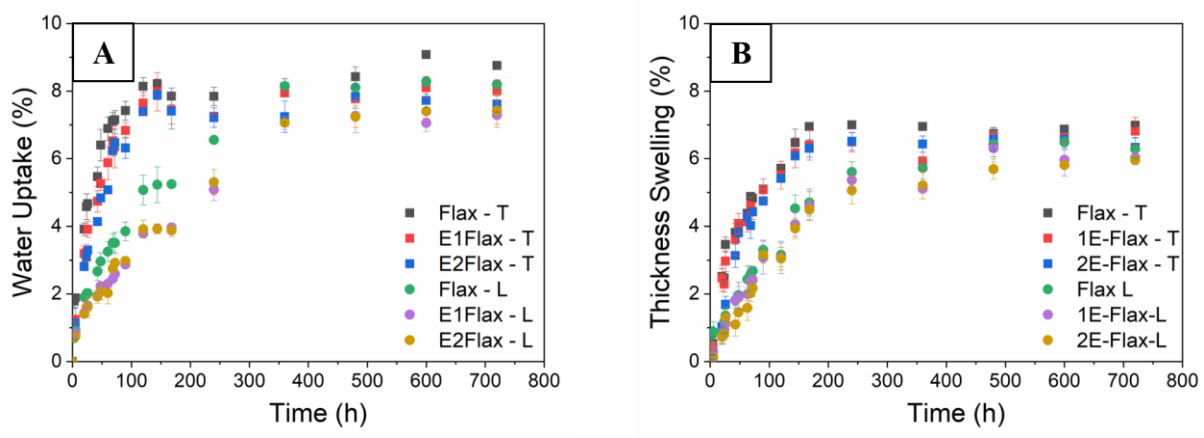

Figure 2. Water uptake (a) and thickness swelling (b) of flax-epoxy composites.

Moisture diffusion was evaluated in both longitudinal (L) and transverse (T) composite orientations. The diffusion profiles for water uptake and thickness change revealed a more rapid moisture ingress in the transverse direction during the early stages of immersion. Nevertheless, the values gradually converged with those of the longitudinal samples as equilibrium was approached[12]. This initial difference is likely due to a greater number of exposed fibre ends and a shorter diffusion path in the transverse configuration which facilitated faster water transport.

4.3 Flexural Properties of Flax-Epoxy Composites

Figure 3 summarizes the flexural performance of pristine, enzymatically treated, and water-immersed composites based on the results from three-point bending tests. Initial measurements (prior to immersion) revealed no significant differences in mechanical properties among the different composite types. This indicates that the enzymatic removal of pectin does not compromise the structural integrity or load-bearing capacity of the composites, despite pectin's known role in stress transfer at the fibre–matrix interface [3,5]. Another possible reason is that enzymatic retting does not add further damage to the fibres unlike in traditional processing[13].

After three days of water immersion, composites containing enzymatically-treated fibres demonstrated enhanced resistance to moisture-induced degradation. This maintained better flexural properties relative to untreated samples. This trend continued even after 30 days of immersion, particularly evident in the longitudinal strength and modulus. This suggests improved long-term moisture durability due to enzymatic treatment. In contrast, the transverse flexural properties showed no notable improvement. This may be attributed to the extensive leaching of pectin and subsequent weakening of the fibre–matrix bonding in that orientation [2].

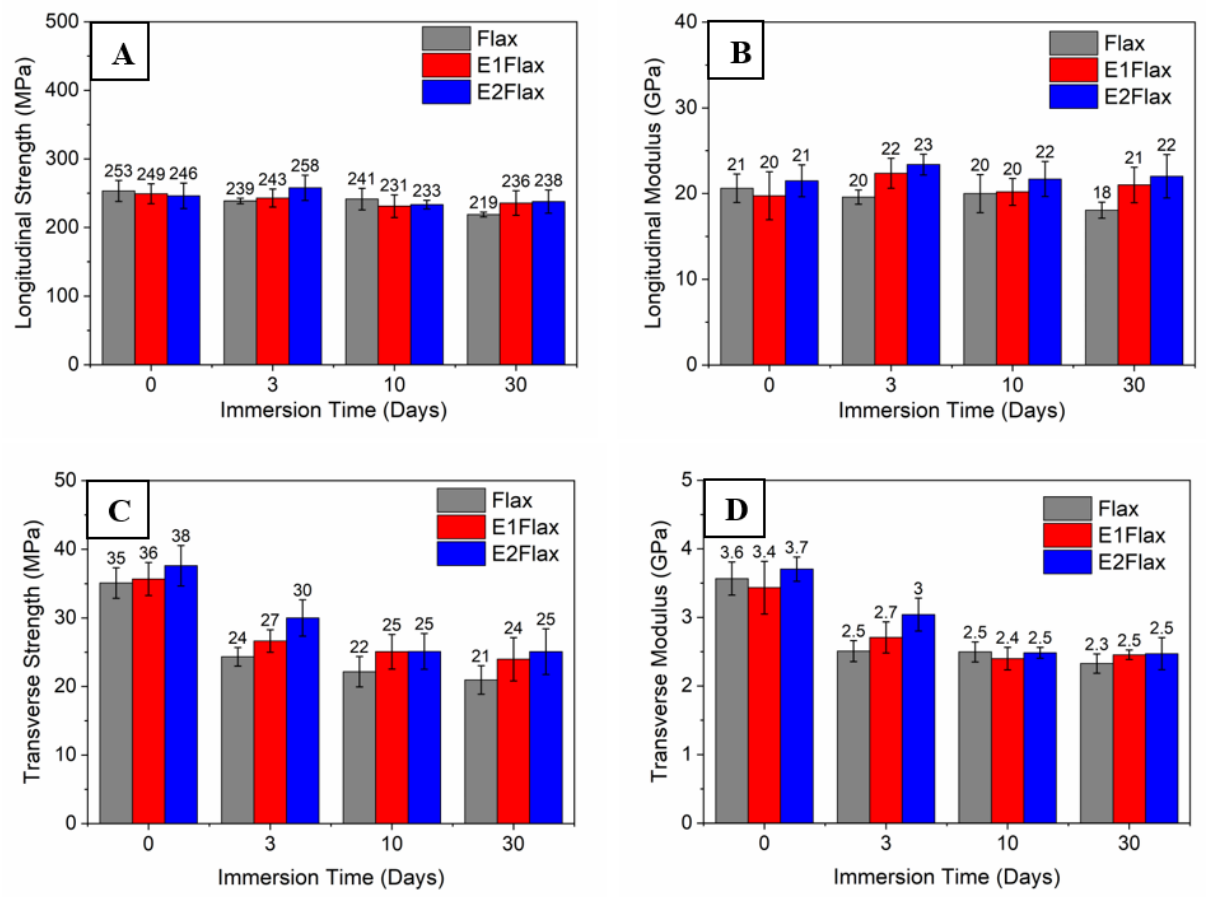

Figure 3. Flexural properties of flax-epoxy composites: longitudinal strength (a), longitudinal modulus (b), transverse strength (c), and transverse modulus (d).

4.4 Contact Angle Measurements of Fibres

The results of fibre tensiometry shown in Figure 4 provide evidence of the changes in the fibres' surface hydrophilicity resulting from enzymatic treatment. Consistent with the previously observed improvements in moisture resistance both in individual fibres and in composite materials, the treated fibres (E1Flax and E2Flax) exhibited higher dynamic advancing contact angles of 85° and 83°, respectively. In contrast, untreated flax fibres showed a lower contact angle which indicates a more hydrophilic surface. This change in surface hydrophilicity is likely due to more pectin [3,7] retained on the surface of the untreated technical fibres. Moreover, the untreated flax fibre samples showed greater variability in contact angle

measurements compared to the enzymatically-treated fibres. This variation may be attributed to the presence of a binder in the untreated flax which could have influenced the surface characteristics and wettability of the fibres.

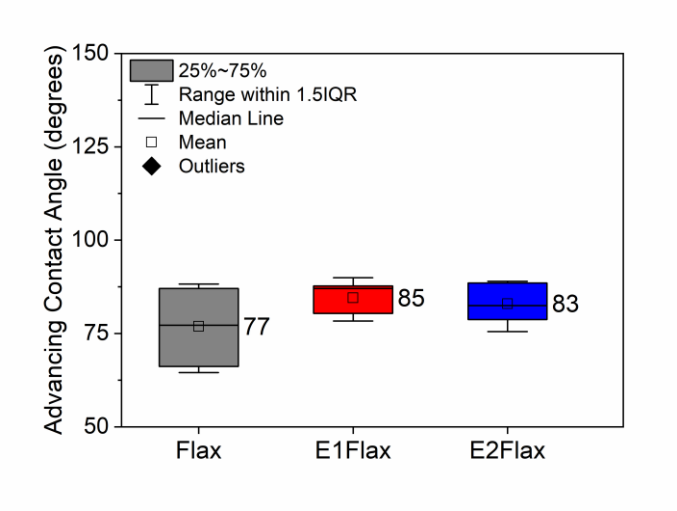

Figure 4. Contact angle measurements from untreated and treated FlaxTapeTM 200 fibres.

4.5 Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR)

Both untreated and enzymatically treated flax fibres were analysed using FTIR-ATR to assess changes in the intensity of hydrophilic functional group peaks following treatment. A decrease in the peak at 3304 cm^{-1} associated with the stretching vibrations of hydroxyl (–OH) groups [14], was observed and may be attributed to the reduction in pectin content. Additionally, variations in the peak around 1630 cm^{-1} which is linked to the asymmetric stretching of carboxylate (COO^-) groups were noted. This aligns with the findings of Raj et al. (2009) who reported similar spectral changes after pectinase treatment [15].

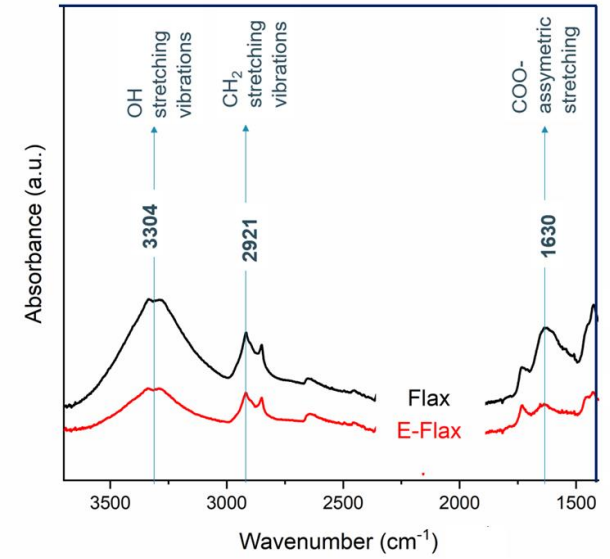

Figure 5. FTIR-ATR spectra of untreated and treated fibres.

4.6 Scanning Electron Microscopy (SEM)

The microstructural differences between untreated and enzymatically treated fibres were examined to evaluate the effectiveness of the polygalacturonase treatment. SEM micrographs clearly demonstrate that fibre separation was successfully achieved as a result of pectin degradation. In addition to the disruption of pectin, the treatment also facilitated the removal of the binder responsible for holding technical fibres together. The surfaces of the enzymatically-treated fibres appeared smoother compared to those of the untreated FlaxTape™200, further indicating effective surface modification.

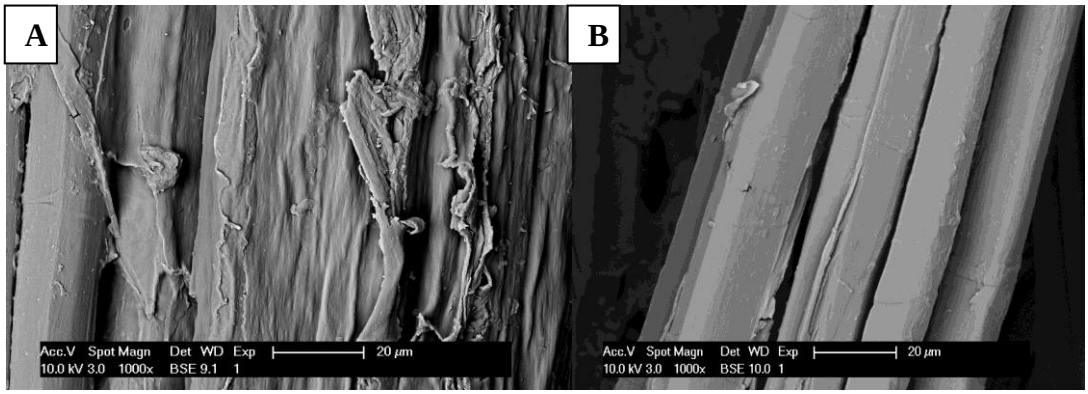

Figure 6. SEM images of untreated (a) and enzymatically-treated fibres (b).

5. CONCLUSIONS

The findings of this study indicate that enzymatic treatment using polygalacturonase significantly improves the moisture resistance of flax fibres by selectively removing pectin. Both dynamic vapour sorption and water immersion tests confirmed reduced water uptake in treated fibres (E1Flax and E2Flax) compared to untreated FlaxTape™200. Contact angle measurements revealed decreased surface hydrophilicity, consistent with FTIR-ATR spectra showing reduced intensities of hydrophilic functional groups. SEM micrographs further supported the effective separation of fibre bundles following enzymatic treatment.

Importantly, this surface modification did not compromise the mechanical integrity of the fibres and even enhanced their moisture durability after prolonged exposure. These results demonstrate that enzymatic retting presents a sustainable and effective strategy for improving the long-term performance and broadening the practical applications of flax fibre-reinforced composites.

6. REFERENCES

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