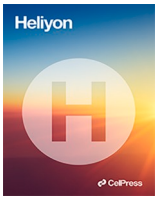
Heliyon 10 (2024) e29149

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Optimizing cellulose fraction for enhanced utility: Comparative pre-treatment of *Agave tequilana* Weber var. blue bagasse fiber for sustainable applications

A. Lazaro-Romero [a], S.M. Contreras-Ramos [a,**], M. Dehonor-Gómez [c], J.M. Rojas-García [c], L. Amaya-Delgado [b,*]

[a] *Unidad de Tecnología Ambiental, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco A.C. (CIATEJ), Av. Normalistas #800, Col. Colinas de la Normal, Guadalajara, Jalisco, Mexico*

[b] *Unidad de Biotecnología Industrial, CIATEJ, Camino Arenero 1227, Col. El Bajío, Zapopan, Jalisco, México*

[c] *Centro de Tecnología Avanzada A.C. (CIATEQ), Circuito de la Industria Poniente Lote 11, Manzana 3, No. 11, Col. Parque Industrial Exhacienda Doña Rosa, Lerma, Estado de México, Mexico*

ARTICLE INFO

Keywords:
Agave bagasse
Cellulose
Chemical analysis
Surface treatments

ABSTRACT

In recent decades, natural fibers have emerged as an alternative to synthetic fibers due to their renewable nature, lower environmental impact, and comparable strength properties. Agave bagasse, a byproduct of agave juice extraction in Mexico, stands out for its potential in various industrial applications, notably biocomposite production. Bagasse is rich in cellulose, along with hemicellulose and lignin. Cellulose is the most suitable to be converted into valuable products, and it is versatile, renewable, and biodegradable. An effective pre-treatment is crucial to enrich its fraction. This study aims to determine the optimal pre-treatment conditions for the agave bagasse. Three different pre-treatments were tested, acid (H_2SO_4), enzymatic (Cellic® HTec2 enzymatic preparation), and sequence of acid-enzymatic (sulfuric acid and Cellic® HTec2), to determine which pre-treatment got the optimal cellulose fraction increase. The acid pre-treatment was conducted over three time ranges (5, 10, and 15 min) at different acid concentrations (1%, 1.5%, and 2%). Enzymatic reactions were conducted over 24 h, testing three different enzyme concentrations (1.5%, 3%, 4.5%). The sequential pre-treatment utilized the optimal conditions derived from the acid experiments (1.5% H_2SO_4 for 10 min), followed by enzymatic reactions carried out over three different durations (6, 12, and 24 h). The findings revealed that a 1.5% acid concentration applied for 10 min was the most efficient pre-treatment method. This pre-treatment resulted in a 1.9-fold increase in the cellulose fraction while reducing hemicellulose content by 30%. The hemicellulose reduction was confirmed through Fourier Transform IR spectroscopy (FTIR) analysis, complemented by scanning electron microscopy (SEM) observations highlighting physical alterations in the fiber structure. Furthermore, thermogravimetric analysis (TGA) demonstrated improved thermal stability, suggesting potential use in biocomposites. Future research should evaluate the environmental impact of optimized pre-treatment methods for agave bagasse.

* Corresponding author
** Corresponding author
E-mail addresses: smcontreras@ciatej.mx (S.M. Contreras-Ramos), lamaya@ciatej.mx (L. Amaya-Delgado).

https://doi.org/10.1016/j.heliyon.2024.e29149
Received 28 November 2023; Received in revised form 23 February 2024; Accepted 2 April 2024
Available online 6 April 2024

1. Introduction

Lignocellulosic fibers, derived from plant sources, offer an opportunity to develop biomaterials as they present a renewable alternative to synthetic fibers [1]. These fibers possess advantages: they are low-cost, biodegradable, and have a low density, making them suitable for meeting the mechanical needs of polymers [2]. Moreover, when they are sourced from agricultural waste, lignocellulosic fibers not only alleviate the pressure on food resources but also promote circular economy principles [3].

Researchers have explored various fibers that can potentially be biopolymer reinforcements due to their chemical structure; some examples are coir, hemp, sugarcane bagasse, flax, and sisal [4]. Of particular interest for this study is agave bagasse, a lignocellulosic fiber, produced as a waste a byproduct of the tequila production process in Mexico. The bagasse represents 40% of the total weight of the agave stem [5]. Tequila, a distilled alcoholic beverage distinguished by its protected designation of origin, relies on the blue variety of *Agave tequilana* Weber (ATW), which is primarily cultivated in Mexico. The state of Jalisco is the biggest producer of this kind of agave, producing 75% of the total annual crop in Mexico [6]. In 2022, 2,036,000 tons of agave were harvested for tequila production, increasing by 9.14% compared to the previous year [7]. The substantial amount of bagasse generated poses an environmental challenge due to its significant proportion and the impact of its disposal. Mexico has no economically profitable use for these agricultural wastes, exacerbating the environmental concerns surrounding their disposal.

Agave bagasse primarily consists of cellulose, hemicellulose, lignin, and other compounds. Studies have shown its composition to include cellulose (42%–44.5%), hemicellulose (20%–25.3%), lignin (15%–20.1%), and other components (7.3%–23%) [5,8,9]. Its high cellulose content makes agave bagasse a promising resource for biomaterial development. However, its full potential remains largely untapped, highlighting the need for research into methods to optimize its cellulose fraction to transform it into a valuable material for biopolymers.

Studies assert that increasing the cellulose fraction cannot be overstated [10,11]. Cellulose is a polysaccharide that plays a crucial role in determining the strength of the fiber and its potential performance as part of a biopolymer. According to Fitch-Vargas (2019) and Zhou (2019), higher cellulose content correlates with enhanced biomass strength, highlighting the importance of defining an effective method to increase the cellulose fraction in the fiber. Another critical consideration in biopolymer performance is the fiber-matrix interphase, affected by the amount of hemicellulose present in lignocellulosic fibers. The hydrophilic nature of hemicellulose, characterized by the presence of hydroxyl (-OH) groups, increases the fiber's susceptibility to water absorption through hydrogen bonding, particularly when exposed to moisture in the environment [12–14].

Various pre-treatment methodologies have been proposed to address natural fiber limitations, such as spanning physical, chemical, biological, and thermophysical approaches [15]. Effective pre-treatments should separate the biomass components into useable fractions while potentially altering the fiber morphology [16]. These alterations can increase porosity and surface roughness, enhancing fiber-matrix adhesion in biopolymer composites and reducing their susceptibility to high moisture absorption. Additionally, they may improve the fiber's resistance to high temperatures, making them more suitable for a wider range of applications [4,17,18]. Acid hydrolysis is an effective method for depolymerizing the hemicellulose fraction in lignocellulosic waste [19]. Furthermore, combining acid and enzymatic pre-treatments has been shown to increase the amount of dissolved hemicellulose, consequently enhancing the porosity of the plant cell wall [20].

Central to this investigation is optimizing the pre-treatments, which aimed to maximize the efficacy of acid, enzymatic, and sequential acid-enzymatic pre-treatments on ATW bagasse. Through structured experimental design, the research aims to observe the impact of the pre-treatment variables on the cellulose fraction of agave bagasse. Various parameters were explored; for the acid pre-treatment using sulfuric acid, concentration and duration were varied to define optimal conditions for hemicellulose dissolution. Similarly, the enzymatic pre-treatment protocol employing Cellic® HTec2 enzymes was optimized, focusing on enzyme dosage and incubation period for maximal cellulose increase and hemicellulose degradation. Furthermore, the sequential acid-enzymatic pre-treatment approach was designed to leverage the synergic effect of both methodologies to enhance cellulose enrichment.

Chemical composition analysis, including cellulose, hemicellulose, and lignin, was conducted using the National Renewable Energy Laboratory Methodology (NREL) [21] and Fourier Transform IR spectroscopy (FTIR). Thermal degradation and surface morphology were assessed via thermogravimetric analysis (TGA) and scanning electron microscopy (SEM).

The optimization of pre-treatment methodologies holds significant importance for the valorization of agave bagasse as a sustainable feedstock for biopolymer production. Despite advancements in pre-treatment strategies for various lignocellulosic fibers, including agave bagasse [4,10,14,22], there remains a critical need for further investigation to define the optimal conditions specifically tailored to enhance its natural characteristics and transform it into a valuable resource for biopolymer production. This study endeavors to address this gap and unlock the full potential of agave bagasse as a renewable source by defining the optimal pre-treatment conditions. The research aims to promote innovation in the biomaterials area and advance the principles of circular economy and sustainability.

2. Materials and methods

2.1. Agave tequilana weber bagasse fiber (ATW)

The ATW bagasse was sourced from an Amatitan factory in Jalisco, Mexico. The agave cooking process utilized a diffuser, where hot water was sprayed onto a bed of agave that had been torn and placed on a transporting chain. This method facilitated the dissolution of the sugar in the agave, preventing the fiber from becoming recalcitrant.

Subsequently, the collected bagasse was left to dry in the sun for two days. Following the drying process, it underwent partial grinding using a mill (Pulvex® 200, Mexico). This milling action resulted in smaller fibers with a particle size measuring 841 μm (mesh 20).

2.2. Pre-treatment bagasse fiber

The agave bagasse fiber was involved in a series of three experiments. In the first experiment, the fibers were subjected to an acid pre-treatment with sulfuric acid and exposed to different durations and concentrations of the acid solutions.

In the second experiment, the bagasse fibers were pretreated with an enzymatic solution (Cellic® HTec2) containing endoxylanase with high specificity toward hemicellulose [23]. The fibers were exposed to different dosages of the enzymatic solution at the same incubation time range.

Finally, in the third experiment, a sequential pre-treatment approach was employed. The most effective pre-treatment methods determined from the first and second experiments were combined. Specifically, the optimal conditions defined from the acid pre-treatment in the first experiment and the enzymatic solution pre-treatment in the second experiment were utilized sequentially. This approach aimed to exploit the strengths of both pre-treatment methods. For a visual representation of the sequential pre-treatment approach, please refer to the process diagram in Fig. 1.

2.2.1. Acid pre-treatment

In the first experiment, different concentrations of sulfuric acid (H_2SO_4) (Fermont PA Cert) were applied to bagasse fibers at different times (see Fig. 2). Concentrations and time ranges were according to Table 1. The dried bagasse fibers were submerged in an acid solution to 10% solids (W/V) and placed in an autoclave (Sterilizer SM510 Yamato, United States) at 121 °C. Five repetitions of each pre-treatment were performed (n = 5). Posteriorly, the pre-treated fibers were washed with tap water and dried at 60 °C in a convection oven for 24 h. Once the bagasse was completely dry, it was ground in a blender (Krups® model gx410011, Germany) to mesh number 80 (177 μm) to characterize the sample by the National Renewable Energy Laboratory Methodology (NREL) [21].

2.2.2. Enzymatic pre-treatment

The ATW dried and, with constant weight, was ground to a size of 125 mm (mesh 20). The enzymatic pre-treatment was performed at 10% solids with Cellic® HTec2 enzyme solution (Novozymes®) containing mainly endoxylanase with high specificity toward hemicellulose and cellulase background [23]. Different concentrations of enzymes were applied Cellic® HTec2 to bagasse fibers. The concentrations were evaluated according to Table 2. The bagasse fiber samples were submerged in the different enzymatic solutions for 24 h with a pH of 5 and a constant temperature of 45 °C in an incubator (INFORS HT Multitron Pro shaker, Switzerland). This treatment was tested with five repetitions (n = 5). After pre-treatment, the fibers were washed with tap water and dried at 60 °C in a convection oven for 24 h. Dry bagasse was ground in a blender (Krups® model gx410011, Germany) up to mesh number 80 (177 mm) and characterized by the NREL methodology (Sluiter et al., 2012).

2.2.3. Acid-enzymatic pre-treatment combination

The acid and enzymatic concentrations that presented the highest fraction of cellulose in earlier acid and enzymatic experiments were selected for a sequential experiment, which combined both pre-treatments. The pre-treatments were evaluated according to Table 3. ATW dry fibers were submerged at 10% solids (W/V) in a 1.5% H_2SO_4 (sulfuric acid) solution and were placed in a Yamato SM510 Sterilizer (United States) autoclave at 121 °C. Once the first pre-treatment was finished, the fibers were washed with tap water and dried at 60 °C in a convection oven for 24 h. Subsequently, they were adjusted to a pH of 5 and immersed at 10% solids (W/V) in Cellic® HTec2 solution (Novozymes® enzymes) at 1.5% and a constant temperature of 45 °C in an incubator (INFORS HT Multitron Pro shaker, Swiss). This treatment was tested with five replicates (n = 5). After pre-treatment, the fibers were washed and dried as described above, and they were ground into a blender (Krups® model gx410011, Germany) to mesh number 80 (177 mm) to characterize the sample by the NREL methodology [21].

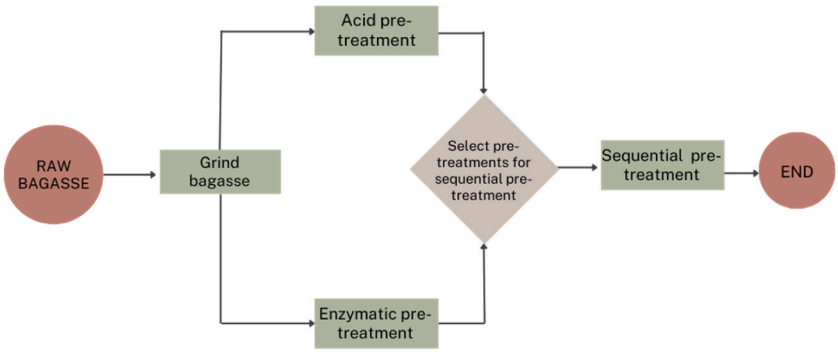


Fig. 1. Pre-treatment process diagram.

Fig. 2. a) Acid pre-treatment of agave bagasse. b) Agave bagasse after acid pre-treatment.

Table 1
Experiments for the acid pre-treatment. Code, time (min), and acid concentration (%).

Code	Time in autoclave (min)	Sulfuric acid concentration (%)
A5-1	5	1
A5-1.5	5	1.5
A5-2	5	2
A10-1	10	1
A10–1.5	10	1.5
A10-2	10	2
A15-1	15	1
A15–1.5	15	1.5
A15-2	15	2

Table 2
Experiments for the enzymatic pre-treatment. Code, time (h) reaction in the shaker, and enzyme (Cellic® HTec2) concentration (%) in the enzymatic pre-treatments.

Code	Time in the shaker (h)	Cellic® HTec2 concentration (%)
E24–1.5	24	1.5
E24-3	24	3
E24–4.5	24	4.5

Table 3
Experiments for the sequential pre-treatment. Code, acid concentration (%), time (h) reaction, enzyme (Cellic® HTec2) concentration (%) in the acid-enzymatic sequential pre-treatments.

Code	Acid pre-treatment		Enzymatic pre-treatment	
	Sulfuric acid concentration (%)	Time (min)	Time in the shaker (h)	Cellic® HTec2 concentration
AE6-1.5	1.5	10	6	1.5
AE12–1.5	1.5	10	12	1.5
AE24–1.5	1.5	10	24	1.5

2.3. Characterization

2.3.1. Chemical characterization

The structural carbohydrates (cellulose and hemicellulose) and lignin of the bagasse fiber were measured using the National Renewable Energy Laboratory methodology (Sluiter et al., 2012). This process utilizes two-step hydrolysis to fraction the biomass and make it easier to measure. Lignin is fractionated into acid-insoluble and acid-soluble materials. During hydrolysis, polymeric carbohydrates hydrolyze into monomeric forms, which are soluble in the hydrolysis liquid (Naik et al., 2010).

2.3.2. Fourier Transform IR spectroscopy (FTIR)

A spectrometer from ©Agilent Technologies 4500 Series (Germany) FTIR was employed for the infrared analysis. The methodology

followed was the standard practice for general techniques for obtaining infrared spectra for qualitative analysis (ASTM E1252–98 (2007). The analysis interval was 650–4000 cm^{-1}, with 32 scans. The software used to run the model was R version 4.2.1, and the samples were in a solid state.

2.3.3. Scanning electron microscopy (SEM)

After pre-treatments, the bagasse's surface structure modification was evaluated in a scanning electron microscopy operating at 20 kV (Jeol, IT100, Benelux). The agave bagasse samples were placed in a carbon patch on an aluminum pin. The raw fiber and the treated were observed in detail with high-resolution images.

2.3.4. Thermogravimetric analysis (TGA)

The thermogravimetric analysis was carried out with a TGA TA-Instruments Q500 (United States) advantage Software v5.5.24. The standard method utilized was the standard test method for compositional analysis by thermogravimetry (ASTM E 1131-08). Samples of approximately 10 mg were placed in platinum pans; they were heated from 20 to 900 °C at a 10 °C min^{-1}, under a nitrogen atmosphere from 20 to 650 °C (100 mL min^{-1}), after 650 °C there was an atmosphere change to oxygen.

2.3.5. Statistical analysis

The data analysis was carried out with GraphPad Prism software®, version 8. The experiments were performed with an $n = 5$, and the results are expressed as mean ± standard error of the mean. The normality of the data was assessed with the Shapiro-Wilk test. When the data lacked normality, the statistical differences were evaluated with Kruskal-Wallis with a 95% confidence interval. In the cases where the data was in a normal distribution, the differences were assessed with a two-way ANOVA followed by a Tuckey ad hoc test with a 95% confidence interval.

Specifically, comparisons were made between the control and each pre-treatment group and between different pre-treatments. In cases where the data followed a normal distribution, differences between the control and pre-treatments were assessed using a two-way ANOVA followed by a Tukey ad hoc test with a 95% confidence interval. Statistical significance was set at $p < 0.05$. Please refer to Tables 1–3 in the methodology section for detailed information about the pre-treatment groups.

Table 4 summarizes the key factors and their corresponding levels for the three pre-treatment experiments. Each experiment focuses on investigating specific pretreatment conditions to understand their effects on the outcome of the process. By systematically varying the levels of these factors, valuable insights into optimizing pretreatment methods for various applications are intended to be gained.

3. Results and discussion

The characterization of the raw agave bagasse revealed a significant insight into its composition, with 20.12 ± 0.5% of cellulose fraction, 11.54 ± 0.31% of hemicellulose fraction, and 17.45 ± 1.31% of lignin. These results align with the lower range of values reported in existing literature (Cellulose 20.85%–41.90%; hemicellulose 12.24%–18%, lignin 17.31%–21.10%), which highlights the consistency of the results with established data [5,24,25]. Even though the cellulose, hemicellulose, and lignin contents of this sample

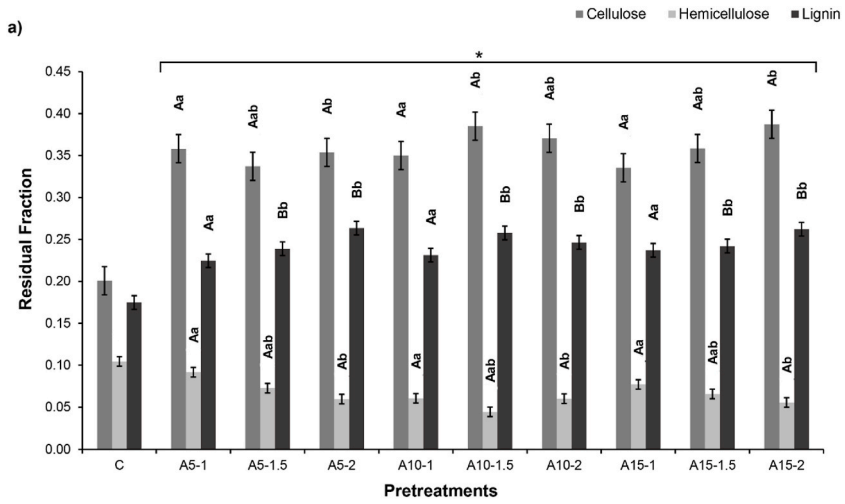


Fig. 3. Residual fraction of acid pre-treatments with different times in the autoclave and concentrations of sulfuric acid. The asterisk shows a statistical difference between the control and the pre-treatments. Different capital letters mean significant differences comparing pre-treatments, between three times evaluated (5, 10, 15 min), and the same acid concentration. Lower letters show significant differences between three concentrations (1, 1.5, and 2%) at the same reaction time. Residual fraction only considers cellulose, hemicellulose, and lignin. Bars indicate standard error.

fall within the range exposed by the authors, the present variability in the fiber's composition can be attributed to factors such as geographical location, harvesting practices, and plant genetics [26,27]. Furthermore, as agave bagasse is a byproduct of an agro-industrial process, its composition may be subjected to additional variation [28]. As we advance in the discussion of the pre-treatments, it becomes apparent that these initial characteristics play an essential role in the results of the subsequent experiments.

3.1. Acid pre-treatment

The acid pre-treatment of agave bagasse resulted in significant changes in its composition, notably affecting the cellulose, hemicellulose, and Lignin fraction. On average, the cellulose fraction increased by 78.8% ± 1.9%, while the hemicellulose content decreased by 30% ± 1.4% compared to the untreated bagasse (control). The significant increase in the cellulose portion within the agave bagasse can be attributed to the role of acid pre-treatment in hydrolyzing hemicellulose. These results are comparable to observations in other biomass sources such as sugarcane bagasse (32–45%) and hemp (36.5–75.6%) have been reported [29–33].

Within Fig. 3, pre-treatments A10–1.5 and A15-2 emerge as the most effective methods for augmenting the cellulose fraction. Pre-treatment A15-2 yields a significant 1.92-fold increase compared to control, followed by A10–1.5 with an increase in the proportion of 1.91 times. Furthermore, compared to the control, the A15-2 treatment resulted in a notable 46% reduction in hemicellulose content compared to the untreated sample. The A10–1.5 treatment exhibited the most substantial decrease, with a hemicellulose content 67% lower than that of the untreated sample.

The observed results reveal a complex dynamic in biomass deconstruction. Despite using lower acid concentration and shorter duration, the hemicellulose reduction was more significant in the A10–1.5 pre-treatment (1.5% of acid concentration for 10 min) compared to the pre-treatment with higher acid concertation and longer duration A15-2 (2% of acid concentration for 15 min). These results highlight lower acid concertation's selective degradations and preservation effects for a shorter time [34]. The shorter reaction time may limit the extent of cellulose degradation, contributing to a higher cellulose fraction despite the reduction in hemicellulose content.

In Fig. 3 it is observed that the statistical difference between the cellulose fraction of pre-treatment A10–1.5 and A15-2 is not significant. This suggests comparable outcomes despite variations in acid concentration and duration. Previous studies have demonstrated similar findings. They demonstrated significant hemicellulose hydrolysis using diluted sulfuric acid at 1%–2%. They also noted insignificant hemicellulose hydrolysis, resulting in an increase in cellulose fraction due to hemicellulose dissolution in the acid in similar lignocellulosic materials such as sugarcane bagasse [20,35]. The study may not account for potential variability in biomass samples, which could influence the outcomes of pre-treatment methods. Factors such as moisture content and chemical composition can vary and may affect the reproducibility of results.

3.2. Enzymatic pre-treatment

The enzymatic pre-treatment showed a noticeable increase in the cellulose fraction, averaging 17% compared to the control, as illustrated in Fig. 4. However, this statistically significant increase is 43% lower than achieved through the acid pre-treatment. In contrast, enzymatic pre-treatments led to an average decrease of 10% ± 0.4% in hemicellulose compared to the control. Additionally, there was a substantial increase of 65% ± 0.9% in lignin content. In Fig. 4, it can also be observed that there were no significant differences in the cellulose fraction among the enzymatic pre-treatments. These findings suggest that the enzymatic pre-treatment

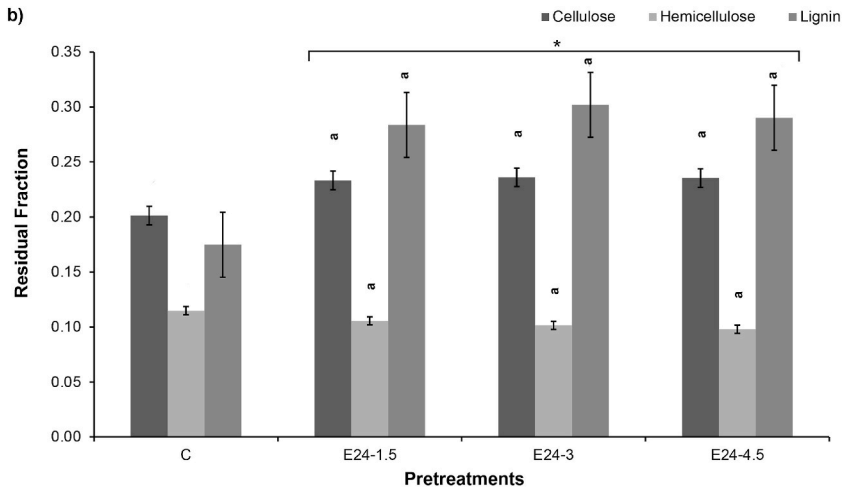


Fig. 4. Residual fraction of enzyme *pre-treatment* at different concentrations. The asterisk shows a statistical difference between the control and the pre-treatments. Different lowercase letters indicate a significant difference between pre-treatments at different enzymatic concentrations (1.5%, 3%, 4.5%). Bars indicate standard error.

alone may not be sufficient to overcome the barrier posed by the structure of the agave bagasse fiber. The resistance could be due to crystalline cellulose and lignin barriers, which may be challenging for the hemicellulose to overcome [8].

Another potential reason could be attributed to the industrial processing undergone by the agave bagasse. This process may alter the fiber's complexity and increase its recalcitrant characteristics, making it more resistant to breakdown [36,37]. Additionally, the substantial increase in lignin observed after enzymatic pre-treatment could be due to the enzymatic solution's composition, which mainly contained endoxylanase, specifically targeting hemicellulose [14]. Consequently, this enzymatic activity may have led to a higher lignin concentration within the fiber.

These results align with findings from other studies [14,38], which confirm the effectiveness of biological pre-treatments involving enzymes, bacteria, or fungi in targeting hemicellulose. However, the efficacy of these treatments is often hindered by biomass composition, including factors like lignin content and cellulose crystallinity, which can significantly impact the effectiveness of enzymatic hydrolysis.

3.3. Acid-enzymatic pre-treatment

In the concluding phase of the experiments, we implemented acid-enzymatic pre-treatments to augment the cellulose fraction. The acid pre-treatment utilized a 1.5% concentration for 10 min in an autoclave, yielding a notable 91.4% increase in the cellulose fraction. A 1.5% concentration of the enzymatic solution was used for the sequential enzymatic pre-treatment because earlier stages revealed no statistically significant differences among the pre-treatments. Various time frames (6, 12, and 24 h) were explored during this pre-treatment phase. After the sequential pre-treatment, the cellulose fraction saw a significant increase of 1.59 times compared to the control, while the hemicellulose content decreased by approximately 33%.

Additionally, the lignin content showed a moderate increase of around 39%. As shown in Fig. 5, there is a significant difference between the control and the pre-treatment results. However, no significant differences were observed among the different pre-treatment combinations.

The study findings indicate that the reduction in the hemicellulose fraction was not as significant as anticipated in the sequential pre-treatment designed to target hemicellulose. The difference in the cellulose and hemicellulose fractions observed after the enzymatic pre-treatment and after the acid pre-treatment indicates a complex interaction of factors. While the enzymatic pre-treatment might have contributed to the hydrolysis of hemicellulose, it also played a role in the hydrolysis of a portion of the cellulose. While the acid pre-treatment typically involves breaking down the rigid structure of cellulose fibrils to increase enzyme accessibility, it is plausible that the pre-treatment generated furan aldehydes, such as 5-hydroxymethylfurfural (HMF) and furfurals [19]. These compounds may have acted as inhibitors to the endoxylanase enzyme responsible for hemicellulose degradation [38,39]. It is suggested that the severity of the acid pre-treatment conditions be moderated to mitigate the formation of inhibitory compounds by lowering the temperature [14]. This adjustment could potentially enhance the efficiency of hemicellulose degradation in subsequent processes.

Furthermore, the duration of the pre-treatments did not significantly affect the cellulose or hemicellulose fractions, as can be observed in Fig. 5. Other authors have suggested that the time required for successful enzymatic hydrolysis can vary depending on factors such as substrate type, specific enzymes used, enzyme source, and pre-treatment conditions [35,38]. These highlights underscore the need for further investigation to define the specific conditions required to reduce the hemicellulose fraction effectively. Moreover, it highlights the importance of optimizing enzyme mixtures and concentrations to ensure desired outcomes in cellulose preservation during sequential pre-treatment processes. Further exploration and adjustment of the enzymatic solution may be necessary to achieve more favorable results in future experiments.

The successful pre-treatments were compared with the control, as illustrated in Fig. 6. Across all pre-treatment methods (acid,

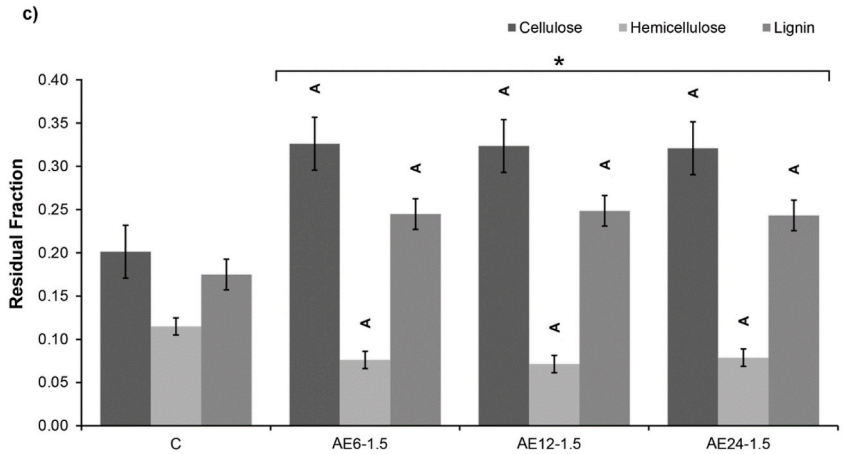


Fig. 5. Acid-enzymatic pre-treatment with different intervals of time in the shaker. The asterisk represents the statistical difference between the control and the pre-treatments. Lower letters show a significant statistical difference between time intervals (6, 12, and 24 h). Bars indicate standard error.

enzymatic, or acid-enzymatic), a significant increase in the cellulose fraction was observed compared to the control. Notably, the acid pre-treatment outperformed the enzymatic pre-treatment, yielding a 65% higher cellulose fraction and an 18% higher cellulose fraction than the sequential pre-treatment (acid-enzymatic). Furthermore, concerning hemicellulose reduction, the acid pre-treatment demonstrated the most favorable outcomes, with a 57% lower hemicellulose fraction than the enzymatic pre-treatment and a 41% lower fraction than the acid-enzymatic pre-treatment. In contrast, the enzymatic pre-treatment exhibited a lower increase in the cellulose fraction (17 $\pm$ 0.1%) and a reduction in hemicellulose (10 $\pm$ 0.9%) compared to the control.

The comparative analysis of pre-treatment methods highlights the effectiveness of the acid pre-treatment in enhancing cellulose fraction and reducing hemicellulose content in agave bagasse. However, while combined pre-treatments have been reported to offer advantages over single methods, it is essential to acknowledge that they entail additional processing steps and necessitate further research to optimize the process [40]. Future research endeavors should prioritize fine-tuning the combined pre-treatment conditions for agave bagasse and exploring innovative approaches to maximize biomass conversion efficiency and sustainability.

The impact of the factors employed for the pre-treatments was assessed using the data presented in Table 4. Both variables are statistically significant in the acid pre-treatment process. However, these results suggest that to specifically target hemicellulose, adjustments in the pre-treatment parameters could be beneficial. Those parameters include reducing the acid concentration and extending the hydrolysis time. Such adjustments could result in comparable hemicellulose reduction while minimizing acid usage, thus reducing environmental impact and reducing the resources required for the pre-treatment process. Moreover, existing literature emphasizes the importance of the time factor in hemicellulose hydrolysis, which is consistent with the findings of this study [40]. However, these parameters may not apply to other biomass or pre-treatment processes.

There is a significant impact of enzyme concentration in the enzymatic pre-treatment on cellulose, hemicellulose, and lignin content. These findings underscore the importance of adjusting enzymatic concentration as a crucial factor in controlling the composition of the material under study, such as agave bagasse or similar biomass materials. Specifically, increasing the enzymatic dosage for agave bagasse could potentially lead to a more substantial reduction in the hemicellulose fraction during enzymatic pre-treatment. The literature suggests that the optimal enzyme dosage or concentration required for hydrolysis varies significantly depending on the type of biomass. Factors such as lignin content and cellulose crystallinity influence the composition and structure of biomass, impacting enzymatic hydrolysis efficiency [41]. Hence, future studies on the enzymatic pre-treatment of agave bagasse should consider increasing enzyme concentration to optimize hydrolysis outcomes.

Table 5 reveals that time significantly influences combined pre-treatment outcomes. The results suggest that extending the duration of enzymatic hydrolysis in combined pre-treatments may enhance efficacy. Literature highlights the importance of defining optimal enzymatic hydrolysis times based on substrate characteristics, processing conditions, and enzymatic cocktail composition [41,42]. The interplay between time and concentration is critical for effective enzymatic hydrolysis. However, additional considerations arise for combined pre-treatment, including the potential presence of inhibitors [19]. This introduces more variables to consider for enzymatic pre-treatment optimization, necessitating further investigation.

3.4. Spectrophotometry analysis

The Fourier Transform IR spectroscopy (FTIR) analysis was conducted to identify functional groups in the samples of pre-treated agave bagasse (acid pre-treatment 1.5% for 10 min, enzymatic pre-treatment 1.5%, and acid-enzymatic pre-treatment 1.5% for 6 h). The FTIR spectra of the agave bagasse are presented in Fig. 7.

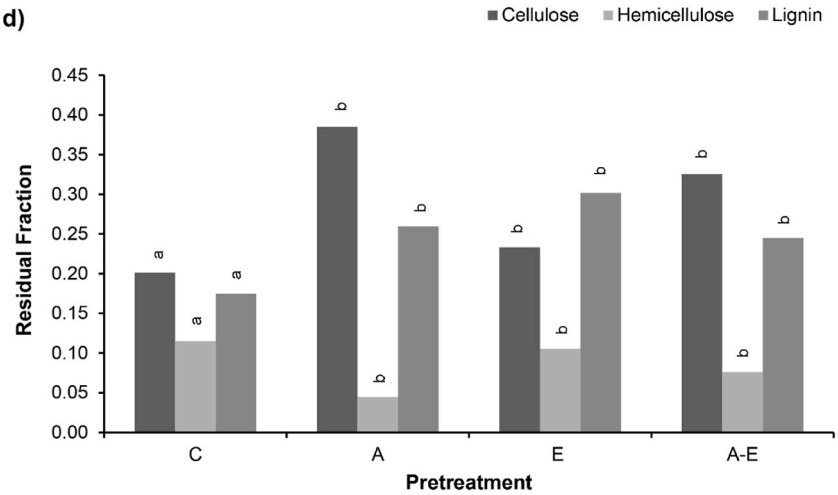


Fig. 6. Residual fraction of chemical composition of the ATW fibers from (C) control and after better pre-treatments: (A) Acid pre-treatment, (E) Enzymatic pre-treatment, and (A–E) acid enzymatic. Lower letters show significant statistical differences between the control and the pre-treatments. Bars indicate standard error.

Table 4
Factors and levels for each experiment.

Experiment	Factors	Levels	Experimental design
Acid pre-treatment	Time	5 min, 10 min, 15 min	Two factors in three levels
	Acid concentration	1%, 1.5%, 2%	
Enzymatic pre-treatment	Enzymatic Concentration	1.5%, 3%, 4.5%	One factor in three levels
Sequential pre-treatment	Sequential Pretreatment Time	6 h, 12 h, 24 h	One factor in three levels

Table 5
Effect of acid concentration, time reaction, enzyme concentration, and sequential pre-treatments on cellulose, hemicellulose, and lignin fractions. It was determined by one-way analysis of variance with GraphPad Prism software® version 8, followed by a Tuckey ad hoc test with a 95% confidence interval.

Pre-treatment and interactions	*P-value* (type III)		
	Cellulose	Hemicellulose	Lignin
Acid pre-treatment			
Time	0.0900	<0.0001	0.9402
Acid Concentration	<0.0001	<0.0001	<0.0001
Enzymatic pre-treatment			
Enzymatic Concentration	0.0017	<0.0001	0.0005
Combined pre-treatment			
Time	<0.0001	<0.0001	<0.0001

In the FTIR spectrum, several distinct peaks can be observed. The first prominent and broad peak was observed at 3321 cm^{-1}, according to Sidi-Yacoub et al. [43], associated with the O–H stretching vibrations of the hydrogen-bonded hydroxyl group (OH) present in cellulose. According to Zhuang et al. [44], these results indicate decreased water content due to the pre-treatments. The intensity of this peak decreased in all the pre-treatments, with the enzymatic pre-treatment showing the most significant reduction.

The peaks at 2920 cm^{-1} could correspond to the aliphatic saturated C–H stretching vibration on polysaccharides (cellulose and hemicelluloses) [43,45]. A decrease in absorbance at this peak was observed in all the pre-treatments, with the enzymatic pre-treatment demonstrating the most pronounced decrease, followed by the acid-enzymatic pre-treatment, consistent with research by Ref. [46]. The decrease indicated that there was cellulose degradation. These results also agree with the chemical characterization results [46,47].

According to the literature [48], the peak around 1761 cm^{-1} is attributed to the stretching vibration of the C=O carbonyl group in the xylem component of hemicellulose and the lignin chemical group. The peak at 1618 cm^{-1}, close to the 1600 cm^{-1} band, represents the O–H–O scissor bending and conferring to Kubovský et al. [47]. It confirms the hydrophilic nature of lignocellulosic fibers, reducing water content with the pre-treatments [44]. It also indicates that the applied pre-treatments led to a decrease in the number of OH groups. The strong peak at 1316 cm^{-1} corresponds to the C–H bending in hemicellulose, suggesting a significant reduction in its composition after the pre-treatment [47]. Finally, the peak at 1030 cm^{-1}, indicating the C–O stretching, suggests the slightly degrading

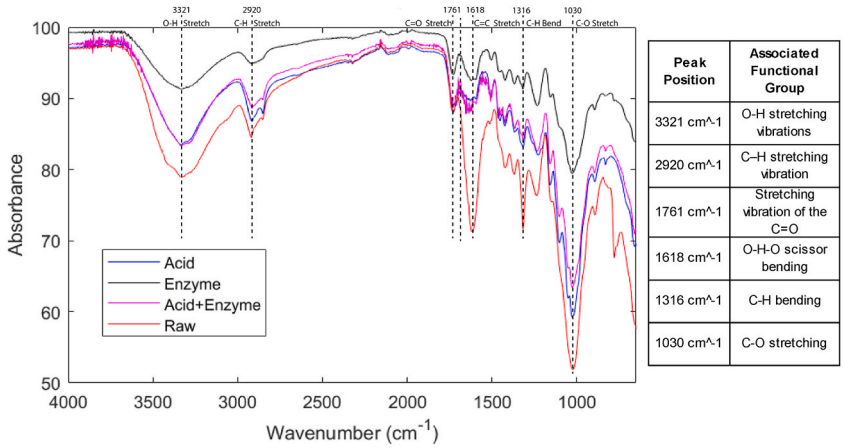


Fig. 7. FTIR spectra of ATW untreated (red), 1.5% concentration acid pre-treatment for 10 min (blue), 1.5% concentration enzymatic pre-treatment for 6 h (black), and acid-enzymatic pre-treatment (1.5% acid concentration for 10 min, 1.5% enzymatic pre-treatment for 6 h (black), and acid-enzymatic pre-treatment (1.5% acid concentration for 10 min, 1.5% enzymatic concentration for 6 h) (pink). Peaks are shown in the table to the right. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

portion of the cellulose [47].

3.5. Scanning electron microscopy analysis (SEM)

During the analysis using scanning electron microscopy (SEM), it was observed that the untreated surface of the agave bagasse resembled a honeycomb structure (Fig. 8a). The analyzed sample was further examined using Energy Dispersive X-ray Spectroscopy (EDX), which detected the presence of the following elements: carbon (51.98%), oxygen (45.13%), calcium (1.94%), potassium (0.25%), magnesium (0.31%), aluminum (0.17%), and phosphorus (0.13%). The SEM morphology observation also revealed the presence of "bright bars" in the agave bagasse, consistent with the findings reported by Delgadillo Ruíz et al. [49], who suggested the formation of calcium oxalate crystals in agave bagasse. These mineral crystals can develop in specific plant tissues through biomineralization, a process involving inorganic materials, often crystalline, forming on the external cell wall, within the cell wall, or surrounding tissue areas [50]. Therefore, the "bright bars" observed in Fig. 8a and 6c could represent calcium oxalate crystals [49].

In the acid pre-treatment, there appeared to be more filaments at the edges of the fiber walls, increasing the surface roughness and resulting in a less defined honeycomb appearance (Fig. 8b). This outcome is probably attributed to the effect of H_2SO_4 on the fiber structure [40]. The EDX analysis detected the presence of the following elements: carbon (49.16%), oxygen (41.61%), calcium (3.03%), magnesium (0.12%), and aluminum (0.08%). Another possible indicator that the crystals present in the agave bagasse could be calcium oxalate is their absence in the micrograph of the bagasse pretreated with sulfuric acid, as these crystals are soluble in sulfuric acid without effervescence [51].

In the enzymatic pre-treatment, the fiber walls seemed to have little effect as they appeared to maintain their original shape (Fig. 8c). The EDX analysis detected the presence of the following elements: carbon (43.20%), oxygen (45.05%), calcium (3.10%), magnesium (0.01%), and aluminum (0.20%). Crystals that could be calcium oxalate were observed in micrograph 6c, which appeared unaffected by the enzymatic pre-treatment.

Finally, the acid-enzymatic pre-treatment (Fig. 8d) affected the fiber's cellular walls. After the acid pre-treatment, the more exposed structure of the cell wall allowed greater accessibility to hydrolytic enzymes, facilitating the hydrolysis of lignocellulosic biomass [52]. The EDX analysis detected the presence of the following elements: carbon (43.07%), oxygen (41.61%), calcium (1.70%), magnesium (0.12%), and aluminum (0.11%). In this case, no formations of calcium oxalate could be observed, possibly because sulfuric acid, which can dissolve calcium oxalate, was used in this pre-treatment. So, a lower calcium percentage was detected by EDX.

The elemental composition obtained by SEM-EDX analysis of the agave bagasse samples was found to be quite similar in all particles/structures, regardless of their origin (natural) or the chemical or biological treatment they underwent.

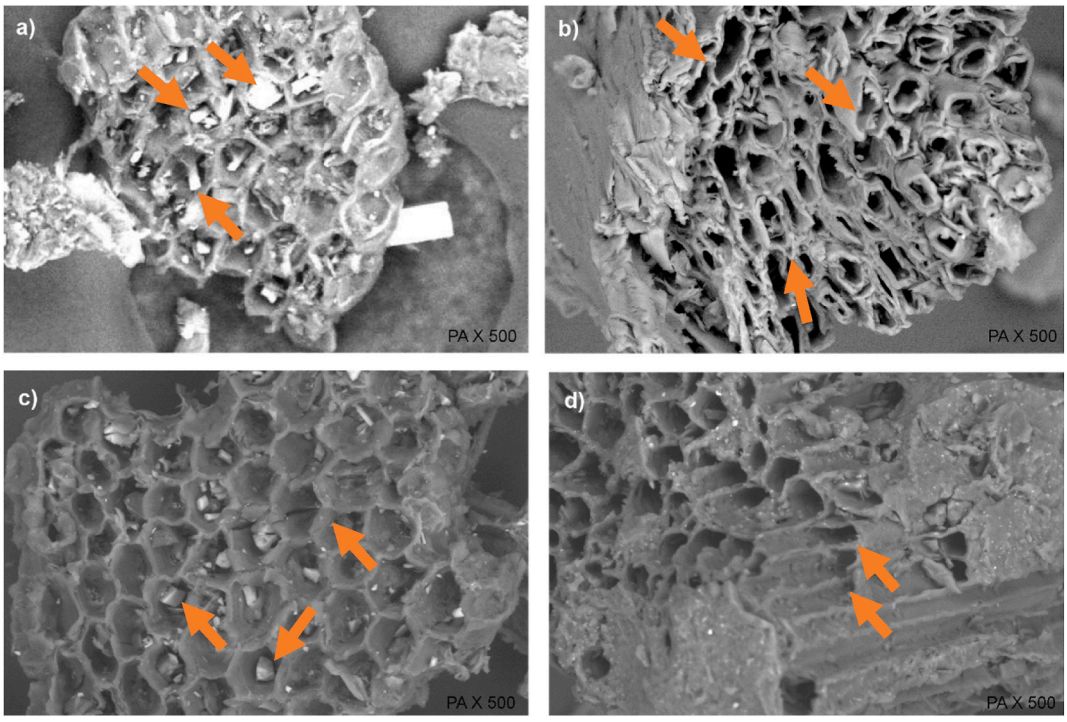


Fig. 8. SEM micrographics. a) Control bagasse, b) bagasse with acid pre-treatment (A10–1.5), c) bagasse with enzymatic pre-treatment (E24–1.5), d) bagasse with acid-enzymatic pre-treatment (E6-1.5).

3.6. Thermogravimetric analysis

The thermogravimetric (TG) and the differential thermogravimetric (DTG) curves for the four biomass materials are presented in Fig. 9 a-b, illustrating the thermal degradation process. The degradation can be divided into three phases: moisture loss, devolatilization, and the formation or removal of char and secondary gases. Phase I, which corresponds to moisture loss, occurs between room temperature (around 30 °C) and 160 °C. This phase is evident in all DTG curves (Fig. 9 a-b), characterized by the first peak within this temperature range. After removing the moisture, phase II, devolatilization occurs between 180 °C and 420 °C. This phase represents the primary pyrolysis region where most of the sample mass is lost. It can be further subdivided into two subsections, with the first subsection showing a shoulder indicating the decomposition of hemicellulose [53,54]. This shoulder, observed in the DTG curve, occurs between 220 °C and 310 °C for the pretreated and untreated bagasse. One notable observation is that, in the case of bagasse with acid pre-treatment, there is no shoulder before the cellulose decomposition, further confirming that the pre-treatment was successful in the hemicellulose hydrolysis.

The second part of phase II corresponds to the decomposition of cellulose, which typically occurs within the temperature range of 300–410 °C [53]. This behavior is observed in all four biomass samples, as their significant peaks are located within this temperature range. If we move to phase III, the curve's tailing section indicates lignin decomposition and the formation of final char residues. It is worth noting that lignin decomposition has been reported to occur gradually over a wide temperature range of 200–600 °C [4].

In summary, the bagasse with the enzymatic pre-treatment resulted in higher susceptibility to thermal degradation at 250 °C and faster degradation at 400 °C than other materials. The enzymatically pretreated sample also showed a lower char residue after 400 °C, indicating a more rapid thermal degradation. This result suggests that the enzymatically pretreated bagasse may decrease the thermal stability of composite materials [55]. On the other hand, the untreated bagasse exhibited higher thermal stability compared to the pretreated bagasse. The thermogram demonstrates that the pre-treatments applied to the bagasse accelerate its thermal degradation, with the enzymatic pre-treatment showing the highest acceleration, followed by the acid-enzymatic pre-treatment. In contrast, the acid pre-treatment exhibits the least acceleration. These findings highlight the importance of considering materials' thermal behavior and stability in future composite applications.

Agave bagasse holds significant potential for various industrial applications. Concerns regarding the excessive accumulation and improper disposal of agave bagasse underscore the need for sustainable utilization practices to mitigate environmental impact. One promising avenue is its use in producing natural fiber polymer composites, where its fibrous composition lends itself as a reinforcement material. These composites exhibit excellent mechanical properties, making them ideal for automotive, lightweight, and structural applications [56]. By harnessing agave bagasse in manufacturing processes, waste reduction can be achieved, and environmental sustainability can be promoted while yielding value-added products across multiple sectors.

A combination of analytical and experimental methodologies is essential to accurately determine the optimal amount of agave bagasse required in a composite material [56]. However, there is variability in the suggested cellulose fiber content for polylactic acid (PLA) composites. While Kai Li et al. (2021) proposed a 5%–30% range, Binoj et al. (2018) suggested a 40% fiber content. This discrepancy underscores the need for a comprehensive approach to determine the appropriate fiber quantity, taking into account various parameters such as the morphology and volume fraction of inclusions, the distribution of inclusion orientations, and the elastic characteristics of both the composite and its matrix [56,57]. Adaptation of the formulation is necessary to align with the specific requirements of the composite material [58,59]. By considering these factors comprehensively, the formulation can be tailored to meet the composite's desired characteristics and performance criteria.

4. Conclusion

The study identified the acid hydrolysis with 1.5% H2SO4 for 10 min at 121 °C as the most effective method for augmenting the cellulose fraction in agave bagasse, and it resulted in a 1.9-fold increase in cellulose content and a 30% reduction in hemicellulose, highlighting the effectiveness of this pre-treatment in enhancing the cellulose fraction of agave bagasse. This treatment yielded the highest cellulose fraction among the tested conditions. Moreover, the pre-treatments induced a reduction in hydroxyl groups present in the biomass, confirmed through compositional analyses, thereby decreasing the biomass's hydrophilicity. This reduction in hydroxyl groups potentially enhances the biomass's resistance to moisture uptake and may increase its durability for various applications.

The agave bagasse exhibited a complex composition with diverse elements and functional groups, as revealed by the analytical techniques. SEM-EDX analysis indicated the presence of calcium oxalate crystals, potentially formed through biomineralization processes, in the untreated bagasse, which displayed a honeycomb-like surface morphology. Acid pre-treatment induced structural changes and increased surface roughness, which helps improve the adhesion of the fiber to a polymer matrix. The enzymatic pre-treatment had a lesser effect on the surface morphology of the biomass. In contrast, the combined acid-enzymatic pre-treatment enhanced fiber accessibility and promoted biomass breakdown, facilitating further processing.

Interestingly, enzymatically pretreated bagasse exhibited higher susceptibility to temperature, degrading faster than raw and acid-pretreated bagasse in thermogravimetric analysis. These changes may affect its thermal stability and suitability as a composite material.

These findings contribute to a better understanding of optimal agave bagasse pre-treatment methods to increase the cellulose fraction for potential applications across various industries. By elucidating the effects of different pre-treatments on biomass composition, morphology, and thermal behavior, this research informs the development of sustainable and efficient utilization strategies for agave bagasse. The study may have used a limited number of samples and may not fully represent the variability inherent in agave bagasse from different sources. Future studies should aim to incorporate a more diverse range of samples to account for potential

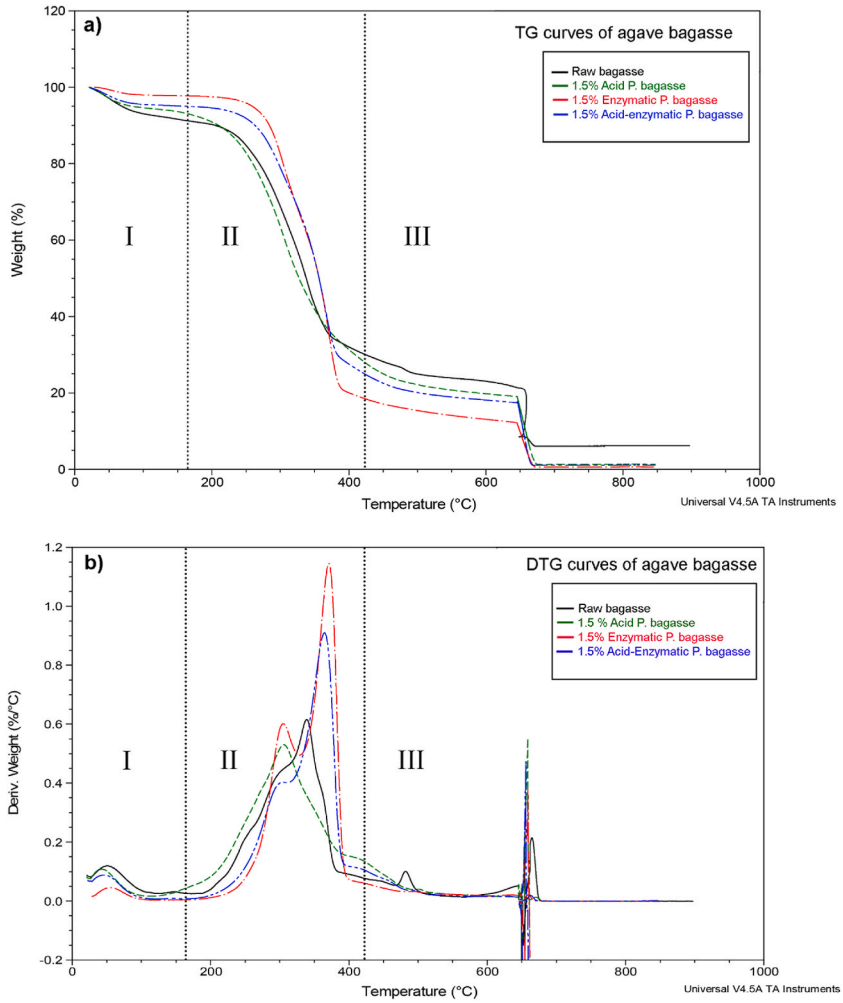


Fig. 9. a) Thermogravimetric curves of agave bagasse. b) thermal decomposition characteristics curves of ATW. Raw bagasse (black), with a 1.5% acid pre-treatment for 10 min (green), with a 1.5% enzymatic pre-treatment for 6 h (red), and with a 1.5% acid and enzymatic pre-treatment (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

variations in composition and properties. Further optimization of pre-treatment conditions, including acid concentration, reaction time, enzymatic dosage, and temperature, could enhance the efficiency of cellulose extraction and hemicellulose hydrolysis while minimizing energy consumption and environmental impact.

CRediT authorship contribution statement

A. Lazaro-Romero: Formal analysis, Methodology, Writing – original draft. **S.M. Contreras-Ramos:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis. **M. Dehonor-Gómez:** Validation, Supervision, Methodology, Investigation, Conceptualization. **J.M. Rojas-García:** Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **L. Amaya-Delgado:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors thank FODECIJAL Project 7890-2019, CIATEJ, and CONAHCYT for their financial support of A.L. with fellowship

840342.

References

[1] N. Karthi, K. Kumaresan, S. Sathish, S. Gokulkumar, L. Prabhu, N. Vigneshkumar, An overview: natural fiber reinforced hybrid composites, chemical treatments and application areas, Mater. Today Proc. 27 (2019) 2828–2834, https://doi.org/10.1016/j.matpr.2020.01.011.

[2] R. Vijay, A. Vinod, D. Lenin Singaravelu, M.R. Sanjay, S. Siengchin, Characterization of chemical treated and untreated natural fibers from Pennisetum orientale grass- A potential reinforcement for lightweight polymeric applications, Int. J. Light. Mater. Manuf 4 (2021) 43–49, https://doi.org/10.1016/j.ijlmm.2020.06.008.

[3] M.P. Harikrishnan, V. Vishnu, A. Kothakota, R. Pandiselvam, T. Venkatesh, S. Pillai, M.R. Manikantan, Impact of integrated ultra violet-ozone treatment on textural and structural properties of dough made of natural fiber based agro residues. J. Nat. Fibers 20. https://doi.org/10.1080/15440478.2022.2161690, 2023.

[4] P. Madhu, M.R. Sanjay, M. Jawaid, S. Siengchin, A. Khan, C.I. Pruncu, A new study on effect of various chemical treatments on Agave Americana fiber for composite reinforcement: physico-chemical, thermal, mechanical and morphological properties. Polym, Test 85 (106437) (2020), https://doi.org/10.1016/j.polymertesting.2020.106437.

[5] G.S. Kestur, T.H.S. Flores-Sahagun, L.P. Dos Santos, J. Dos Santos, I. Mazzaro, A. Mikowski, Characterization of blue agave bagasse fibers of Mexico, Compos. Part A Appl. Sci. Manuf 45 (2013) 153–161, https://doi.org/10.1016/j.compositesa.2012.09.001.

[6] INEGI, Colección de Estudios Sectoriales y Regionales, Conociendo la Industria del Tequila y Mezcal, Julio (2019) 1–37.

[7] Sagarpa-Siap, Info siap-sagarpa [WWW document], URL, http://infosiap.siap.gob.mx/gobmx/datosAbiertos.php, 2023, 77.23.

[8] H. Palacios Hinestroza, J.A. Hernández Diaz, M. Esquivel Alfaro, G. Toriz, O.J. Rojas, B.C. Sulbarán-Rangel, Isolation and characterization of nanofibrillar cellulose from agave tequilana weber bagasse, Adv. Mater. Sci. Eng. 2019 (2019), https://doi.org/10.1155/2019/1342547.

[9] L. Yang, M. Lu, S. Carl, J.A. Mayer, J.C. Cushman, E. Tian, H. Lin, Biomass characterization of Agave and Opuntia as potential biofuel feedstocks, Biomass Bioenergy 76 (2015) 43–53, https://doi.org/10.1016/j.biombioe.2015.03.004.

[10] P.R. Fitch-Vargas, I.L. Camacho-Hernández, F. Martínez-Bustos, A.R. Islas-Rubio, K.I. Carrillo-Cañedo, A. Calderón-Castro, N. Jacobo-Valenzuela, A. Carrillo-López, C.I. Delgado-Nieblas, E. Aguilar-Palazuelos, Mechanical, physical and microstructural properties of acetylated starch-based biocomposites reinforced with acetylated sugarcane fiber, Carbohydr. Polym. 219 (2019) 378–386, https://doi.org/10.1016/j.carbpol.2019.05.043.

[11] S. Zhou, L. Nyholm, M. Strømme, Z. Wang, Cladophora cellulose: unique biopolymer nanofibrils for emerging energy, environmental, and life science applications, Acc. Chem. Res. 52 (2019) 2232–2243, https://doi.org/10.1021/acs.accounts.9b00215.

[12] V. Bhuvaneswari, B. Devarajan, B. Arulmurugan, R. Mahendran, S. Rajkumar, S. Sharma, K. Mausam, C. Li, E.T. Eldin, A critical review on hygrothermal and sound absorption behavior of natural-fiber-reinforced polymer composites, Polymers 14 (2022), https://doi.org/10.3390/polym14214727.

[13] N.M. Nurazzi, M.M. Harussani, H.A. Aisyah, R.A. Ilyas, M.N.F. Norrrahim, A. Khalina, N. Abdullah, Treatments of natural fiber as reinforcement in polymer composites-a short review, Funct. Compos. Struct. 3 (2021), https://doi.org/10.1088/2631-6331/abff36.

[14] H. Zhang, L. Han, H. Dong, An insight to pretreatment, enzyme adsorption and enzymatic hydrolysis of lignocellulosic biomass: experimental and modeling studies, Renew. Sustain. Energy Rev. 140 (110758) (2021), https://doi.org/10.1016/j.rser.2021.110758.

[15] E. Hosseini Koupaie, S. Dahadha, A.A. Bazyar Lakeh, A. Azizi, E. Elbeshbishy, Enzymatic pretreatment of lignocellulosic biomass for enhanced biomethane production-A review, J. Environ. Manag. 233 (2019) 774–784, https://doi.org/10.1016/j.jenvman.2018.09.106.

[16] K. Merkel, H. Rydarowski, J. Kazimierczak, A. Bloda, Processing and characterization of reinforced polyethylene composites made with lignocellulosic fibres isolated from waste plant biomass such as hemp, Composites, Part B 67 (2014) 138–144, https://doi.org/10.1016/j.compositesb.2014.06.007.

[17] L.C. Hao, S.M. Sapuan, M.R. Hassan, R.M. Sheltami, Natural Fiber Reinforced Vinyl Polymer Composites, Natural Fibre Reinforced Vinyl Ester and Vinyl Polymer Composites, Elsevier Ltd, 2018, https://doi.org/10.1016/b978-0-08-102160-6.00002-0.

[18] L. Prabhu, V. Krishnaraj, S. Sathish, S. Gokulkumar, N. Karthi, L. Rajeshkumar, D. Balaji, N. Vigneshkumar, K.S. Elango, A review on natural fiber reinforced hybrid composites: chemical treatments, manufacturing methods and potential applications. Mater, Today Proc. 45 (2021) 8080–8085, https://doi.org/10.1016/j.matpr.2021.01.280.

[19] B.E. Valdez-Guzmán, E.E. Rios-Del Toro, R.L. Cardenas-López, H.O. Méndez-Acosta, V. González-Álvarez, J. Arreola-Vargas, Enhancing biohydrogen production from Agave tequilana bagasse: detoxified vs. Undetoxified acid hydrolysates, Bioresour. Technol. 276 (2019) 74–80, https://doi.org/10.1016/j.biortech.2018.12.101.

[20] N. Sritrakul, S. Nitisinprasert, S. Keawsompong, Evaluation of dilute acid pretreatment for bioethanol fermentation from sugarcane bagasse pith, Agric. Nat. Resour 51 (2017) 512–519, https://doi.org/10.1016/j.anres.2017.12.006.

[21] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D.C. Nrel, Determination of Structural Carbohydrates and Lignin in Biomass Determination of Structural Carbohydrates and Lignin in Biomass, Biomass Anal. Technol. Team Lab. Anal. Proced. (2011) 1–14.

[22] R. Sindhu, P. Binod, A. Pandey, Biological pretreatment of lignocellulosic biomass - an overview, Bioresour. Technol. 199 (2016) 76–82, https://doi.org/10.1016/j.biortech.2015.08.030.

[23] Novozymes, FUEL ETHANOL APPLICATION SHEET Cellic® CTec2 and HTec2-Enzymes for hydrolysis of lignocellulosic materials (2010) 1–9.

[24] M.S. Pino, R.M. Rodríguez-Jasso, M. Michelin, H.A. Ruiz, Enhancement and modeling of enzymatic hydrolysis on cellulose from agave bagasse hydrothermally pretreated in a horizontal bioreactor, Carbohydr. Polym. 211 (2019) 349–359, https://doi.org/10.1016/j.carbpol.2019.01.111.

[25] M.Á. Robles-García, C.L. Del-Toro-Sánchez, E. Márquez-Ríos, A. Barrera-Rodríguez, J. Aguilar, J.A. Aguilar, F.J. Reynoso-Marín, I. Ceja, R. Dórame-Miranda, F. Rodríguez-Félix, Nanofibers of cellulose bagasse from Agave tequilana Weber var. azul by electrospinning: preparation and characterization, Carbohydr. Polym. 192 (2018) 69–74, https://doi.org/10.1016/j.carbpol.2018.03.058.

[26] P. Bajpai, Structure of lignocellulosic biomass 7–12. https://doi.org/10.1007/978-981-10-0687-6_2, 2016.

[27] K. Kucharska, P. Rybarczyk, I. Hołowacz, R. Łukajtis, M. Glinka, M. Kamiński, Pretreatment of lignocellulosic materials as substrates for fermentation processes, Molecules 23 (2018) 1–32, https://doi.org/10.3390/molecules23112937.

[28] J.S. Lupoi, S. Singh, B.A. Simmons, R.J. Henry, Assessment of lignocellulosic biomass using analytical spectroscopy: an evolution to high-throughput techniques, Bioenergy Res. 7 (2014) 1–23, https://doi.org/10.1007/s12155-013-9352-1.

[29] Anu Alokika, A. Kumar, V. Kumar, B. Singh, Cellulosic and hemicellulosic fractions of sugarcane bagasse: potential, challenges and future perspective, Int. J. Biol. Macromol. 169 (2021) 564–582, https://doi.org/10.1016/j.ijbiomac.2020.12.175.

[30] H. Jariwala, P. Jain, A review on mechanical behavior of natural fiber reinforced polymer composites and its applications, J. Reinforc. Plast. Compos. 38 (2019) 441–453, https://doi.org/10.1177/0731684419828524.

[31] S. Pirah, X. Wang, M. Javed, K. Simair, B. Wang, X. Sui, C. Lu, Lignocellulose extraction from sisal fiber and its use in green emulsions: a novel method, Polymers 14 (2022), https://doi.org/10.3390/polym14112299.

[32] S. Radoor, J. Karayil, S.M. Rangappa, S. Siengchin, J. Parameswaranpillai, A review on the extraction of pineapple, sisal and abaca fibers and their use as reinforcement in polymer matrix, Express Polym. Lett. 14 (2020) 309–335, https://doi.org/10.3144/expresspolymlett.2020.27.

[33] J. Zhao, Y. Xu, W. Wang, J. Griffin, K. Roozeboom, D. Wang, Bioconversion of industrial hemp biomass for bioethanol production: a review. Fuel 281. https://doi.org/10.1016/j.fuel.2020.118725, 2020.

[34] I.B. Soares, K.C.S. Mendes, M. Benachour, C.A.M. Abreu, Evaluation of the effects of operational parameters in the pretreatment of sugarcane bagasse with diluted sulfuric acid using analysis of variance, Chem. Eng. Commun. 204 (2017) 1369–1390, https://doi.org/10.1080/00986445.2017.1365061.

[35] J.C. Solarte-Toro, J.M. Romero-García, J.C. Martínez-Patiño, E. Ruiz-Ramos, E. Castro-Galiano, C.A. Cardona-Alzate, Acid pretreatment of lignocellulosic biomass for energy vectors production: a review focused on operational conditions and techno-economic assessment for bioethanol production. Renew. Sustain, Energy Rev. 107 (2019) 587–601, https://doi.org/10.1016/j.rser.2019.02.024.

[36] R. Palomo-Briones, I. López-Gutiérrez, F. Islas-Lugo, K.L. Galindo-Hernández, D. Munguía-Aguilar, J.A. Rincón-Pérez, M.Á. Cortés-Carmona, F. Alatriste-Mondragón, E. Razo-Flores, Agave bagasse biorefinery: processing and perspectives, Clean Technol. Environ. Policy 20 (2018) 1423–1441, https://doi.org/10.1007/s10098-017-1421-2.
[37] J.C. Solarte-Toro, Y. Chacón-Pérez, S. Piedrahita-Rodríguez, J.A. Poveda Giraldo, J.A. Teixeira, K. Moustakas, C.A.C. Alzate, Effect of dilute sulfuric acid pretreatment on the physicochemical properties and enzymatic hydrolysis of coffee cut-stems. Energy 195. https://doi.org/10.1016/j.energy.2020.116986, 2020.
[38] E. Hosseini Koupaie, S. Dahadha, A.A. Bazyar Lakeh, A. Azizi, E. Elbeshbishy, Enzymatic pretreatment of lignocellulosic biomass for enhanced biomethane production-A review, J. Environ. Manag. 233 (2019) 774–784, https://doi.org/10.1016/j.jenvman.2018.09.106.
[39] M. Sriariyanun, P. Amnuaycheewa, W. Rodiahwati, P. Sanvarinda, K. Cheenkachorn, A. Tawai, Effect of Organic Acid Pretreatment on Napier Grass (Pennisetum Purpureum) Straw Biomass Conversion, King Mongkut's Univ. Technol, North Bangkok, 2017, https://doi.org/10.14416/j.ijast.2017.05.005.
[40] Lorenci Woiciechowski, A., Dalmas Neto, C.J., Porto de Souza Vandenberghe, L., de Carvalho Neto, D.P., Novak Sydney, A.C., Letti, L.A.J., Karp, S.G., Zevallos Torres, L.A., Soccol, C.R, Lignocellulosic biomass: acid and alkaline pretreatments and their effects on biomass recalcitrance – conventional processing and recent advances, Bioresour. Technol. 304 (122848) (2020), https://doi.org/10.1016/j.biortech.2020.122848.
[41] A. Zoghlami, G. Paës, Lignocellulosic biomass: understanding recalcitrance and predicting hydrolysis, Front. Chem. 7 (2019), https://doi.org/10.3389/fchem.2019.00874.
[42] R. Łukajtis, P. Rybarczyk, K. Kucharska, D. Konopacka-Łyskawa, E. Słupek, K. Wychodnik, M. Kamiński, Optimization of saccharification conditions of lignocellulosic biomass under alkaline pre-treatment and enzymatic hydrolysis. Energies 11. https://doi.org/10.3390/en11040886, 2018.
[43] B. Sidi-Yacoub, F. Oudghiri, M. Belkadi, R. Rodríguez-Barroso, Characterization of lignocellulosic components in exhausted sugar beet pulp waste by TG/FTIR analysis, J. Therm. Anal. Calorim. 138 (2019) 1801–1809, https://doi.org/10.1007/s10973-019-08179-8.
[44] J. Zhuang, M. Li, Y. Pu, A.J. Ragauskas, C.G. Yoo, Observation of potential contaminants in processed biomass using fourier transform infrared spectroscopy, Appl. Sci. 10 (2020) 1–13, https://doi.org/10.3390/app10124345.
[45] G. Bekiaris, J. Lindedam, C. Peltre, S.R. Decker, G.B. Turner, J. Magid, S. Bruun, Rapid estimation of sugar release from winter wheat straw during bioethanol production using FTIR-photoacoustic spectroscopy, Biotechnol. Biofuels 8 (2015) 1–12, https://doi.org/10.1186/s13068-015-0267-2.
[46] C.D.D. Naranjo, L. Alamilla-beltrán, G.F. Gutiérrez-lopez, E. Terres-rojas, J. Solorza, Aislamiento y caracterización de celulosas obtenidas de fibras de Agave salmiana aplicando dos métodos de extracción ácido-alcali * Isolation and characterization of cellulose obtained from Agave salmiana fibers using two acid-alkali extraction methods Re, Rev. Mex. ciencias agrícolas 7 (2016) 31–43.
[47] I. Kubovský, D. Kačíková, F. Kačík, Structural changes of oak wood main components caused by thermal modification, Polymers 12 (2020), https://doi.org/10.3390/polym12020485.
[48] M.N. Mahamad, M.A.A. Zaini, Z.A. Zakaria, Preparation and characterization of activated carbon from pineapple waste biomass for dye removal, Int. Biodeterior. Biodegrad. 102 (2015) 274–280, https://doi.org/10.1016/j.ibiod.2015.03.009.
[49] L. Delgadillo Ruíz, R. Bañuelos Valenzuela, E.L. Esparza Ibarra, H. Gutiérrez Bañuelos, F.J. Cabral Arellano, A. Muro Reyes, Evaluación del perfil de nutrientes de bagazo de agave como alternativa de alimento para rumiantes. Rev. Mex. Ciencias Agrícolas 2099–2103. https://doi.org/10.29312/remexca.v0i11.778, 2018.
[50] M. Cuéllar-Cruz, K.S. Pérez, M.E. Mendoza, A. Moreno, Biocrystals in plants: a short review on biomineralization processes and the role of phototropins into the uptake of calcium, Crystals 10 (2020) 1–23, https://doi.org/10.3390/cryst10070591.
[51] P.K. Mukherjee, Morphological and microscopical evaluations, quality control and evaluation of herbal drugs. https://doi.org/10.1016/b978-0-12-813374-3.00005-3, 2019.
[52] L.Q. Wang, L.Y. Cai, Y.L. Ma, Study on inhibitors from acid pretreatment of corn stalk on ethanol fermentation by alcohol yeast, RSC Adv. 10 (2020) 38409–38415, https://doi.org/10.1039/d0ra04965d.
[53] T. Emiola-Sadiq, L. Zhang, A.K. Dalai, Thermal and kinetic studies on biomass degradation via thermogravimetric analysis: a combination of model-fitting and model-free approach, ACS Omega 6 (2021) 22233–22247, https://doi.org/10.1021/acsomega.1c02937.
[54] M. Ji, F. Li, Li Jianyong, Li Jianfeng, C. Zhang, K. Sun, Z. Guo, Enhanced mechanical properties, water resistance, thermal stability, and biodegradation of the starch-sisal fibre composites with various fillers, Mater. Des. 198 (2021) 109373, https://doi.org/10.1016/j.matdes.2020.109373.
[55] S. Shahinur, M. Hasan, Jute/coir/banana fiber reinforced bio-composites: critical review of design, fabrication, properties and applications, encyclopedia of renewable and sustainable materials, Elsevier Ltd, 2020, https://doi.org/10.1016/b978-0-12-803581-8.10987-7.
[56] J.S. Binoj, J.S. Bibin, Failure analysis of discarded Agave tequilana fiber polymer composites, Eng. Fail. Anal. 95 (2019) 379–391, https://doi.org/10.1016/j.engfailanal.2018.09.019.
[57] K. Li, D. Mcgrady, X. Zhao, D. Ker, H. Tekinalp, X. He, J. Qu, T. Aytug, E. Cakmak, J. Phipps, S. Ireland, V. Kunc, S. Ozcan, Surface-modified and oven-dried microfibrillated cellulose reinforced biocomposites: cellulose network enabled high performance, Carbohydr. Polym. 256 (117525) (2021), https://doi.org/10.1016/j.carbpol.2020.117525.
[58] G. Josefsson, F. Berthold, E.K. Gamstedt, Stiffness contribution of cellulose nanofibrils to composite materials, Int. J. Solid Struct. 51 (2014) 945–953, https://doi.org/10.1016/j.ijsolstr.2013.11.018.
[59] E.K. Uusi-Tarkka, M. Skrifvars, A. Haapala, Fabricating sustainable all-cellulose composites, Appl. Sci. 11 (2021) 1–21, https://doi.org/10.3390/app112110069.