Characterization of Poly-hydroxybutyrate/Luffa Fibers Composite Material

Andrea Melina Avecilla-Ramírez,[a] Ma. del Rocío López-Cuellar,[b] Berenice Vergara-Porras,[c,d] Adriana I. Rodríguez-Hernández,[b] and Edgar Vázquez-Núñez [a,*]

Luffa fibers were evaluated as a reinforcement material in poly-hydroxybutyrate matrix composites. The treatments consisted of varying the incorporation percentage of mercerized and non-mercerized luffa fibers in a poly-hydroxybutyrate (PHB) matrix (5%, 10%, and 20% w/v). Composites made with PHB and reinforced with luffa fibers (treated and non-treated) were mechanically evaluated (tensile strength, Young's modulus, and percentage of elongation at break), the surface morphology was described by using scanning electronic microscopy, and the degradability behavior of composites was obtained. According to the results, mechanical properties decreased when the percentage of fibers increased and no significant effects were observed when compared with mercerized fiber composites. Degradability tests demonstrated that the weight loss increased with increased fiber content in composites, independent of the applied pretreatments. Microscopy images exhibited that mercerization improved the fiber incorporation into the polymeric matrix, diminishing the "pull out" effect; the above-mentioned result was supported by using the Fourier-transform infrared spectroscopy technique, observing the reduction of lignin and hemicellulose peaks in mercerized fibers. Based on the composite mechanical performance and degradability behavior, it was concluded that this material could be used in the packaging sector as biodegradable secondary packaging material.

Keywords: Alkali treatment; Biodegradation; PHB biocomposites; Natural fiber; Sustainable biomaterials

*Contact information: a: Departamento de Ingenierías Química, Electrónica y Biomédica, División de Ciencias e Ingenierías, Campus León, Universidad de Guanajuato, León, Guanajuato, México, Lomas del Bosque 103, Lomas del Campestre, C.P. 37150; b: Cuerpo Académico de Biotecnología Agroalimentaria, Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo, Avenida Universidad Km 1.0, Tulancingo 43600, Hidalgo, México; c: Escuela de Ingeniería y Ciencias, Tecnológico de Monterrey, Campus Estado de México, Carretera Lago de Guadalupe Km 3.5, Atizapán de Zaragoza 52926, Estado de México, México; d: Biology Department, Massachusetts Institute of Technology, Cambridge, MA 02142, USA; *Corresponding author: edgar.vazquez@ugto.mx*

INTRODUCTION

In recent years, interest in the utilization of new renewable materials has rampantly increased, especially interest in those coming from non-valorized vegetable biomass (Popa 2018). These new green materials offer competitive advantages compared with oil-based polymers, such as their biodegradability and low cost (Muniyasamy *et al.* 2019), rendering them a sustainable alternative that can reduce negative environmental impacts (Varma 2019). The gradual substitution of petroleum-based materials with bio-based materials in diverse sectors, such as packaging, biomedicine, and construction, among others, has reduced the dependency on fossil fuel-based materials and decreased plastic solid wastes in landfills (Koronis *et al.* 2013; Luzi *et al.* 2019). Around the world, many research groups

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have centered their interest around developing new material using nature-based raw materials.

The introduction of natural fibers to reinforce bio-based polymers has attracted international interest because it was demonstrated that these blended materials show good physical, chemical, and mechanical properties, making them ideal for multiple applications (Peças *et al.* 2018). Among the most important advantages are the availability of their raw materials, their high tensile strength and relatively high modulus of elasticity, and the well-developed technology for manufacturing these blended composites (Duval and Lawoko 2014; Narayanan *et al.* 2015; Goh *et al.* 2018). Currently, the lignocellulosic residues have become relevant in the development of green materials due to their versatility and availability. Diverse studies have been published recently, reporting that these biopolymers have modifiable properties, and trying to determine why. One important factor in developing this mechanical performance lies in the chemical interactions between the cellulosic fibers and the polymeric matrix, which could be not only chemical in nature but also physical. In most of the reported cases, the interactions were the combination of both, due to the high hydrophobicity of lignin (Ali *et al.* 2018; Yang *et al.* 2019). There have been developed pretreatments, which are mainly chemical, such as mercerization (alkaline solution treatment of fibers), acetylation (treatment of fibers with acetic anhydride and sulfuric acid solutions), *etc*., in order to remove the lignin and facilitate its incorporation into the polymeric matrix and positively impact the properties mentioned above.

Luffa is a genus of subtropical and tropical vines in the cucumber family (Mohana Krishnudu *et al.* 2018; Ittah and Kwon-Ndung 2019). The term "luffa" refers specifically to the fruit of the two species *Luffa aegyptiaca* and *Luffa acutangular*. Luffa is distributed in tropical countries (Fig. 1) and, due to its high fiber content, it has been used as a sponge for cleaning and grooming services. It is produced in a non-industrialized manner, in many cases, by indigenous communities. Therefore, it is important to carry out studies that demonstrate its viability in the market, as it could represent an opportunity to improve the living standards of the producing communities.
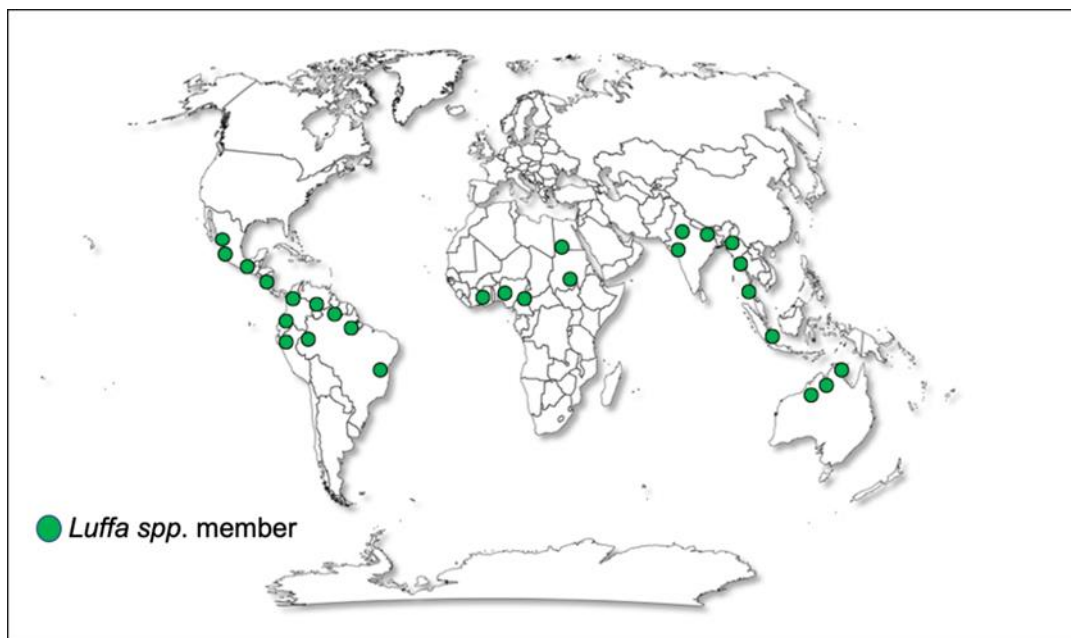

Fig. 1. Geographical distribution of *Luffa* spp. members

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Recently, diverse authors have executed novel research related to the uses of luffa, including, among other studies, Yang *et al.* (2018), who evaluated the photoactivity of Fe_3O_4/Pr-BiOC/luffa composites, and Tanobe *et al.* (2014), who described the process for incorporating sponge gourd into polyester composites and its effect on mechanical properties.

Guo *et al.* (2019) reported the characterization of treated and untreated luffa/poly(hydroxybutyrate-co-valerate) (PHBV) composites. The author observed that the chemical pretreatment (immersion in NaOH and H_2O_2 solutions) of the fibers resulted in a better moisture resistance of composites.

The luffa fiber has a high content of cellulose (60%), hemicellulose (15%), lignin (10%), and other components (Mazali and Alves 2005). Its density is between 0.82 and 0.92 g cm^{-3} (Dairo *et al.* 2007), which is lower than other studied biomass, including sisal (1.26 g cm^{-3}) (Dun *et al.* 2019), hemp (1.48 g cm^{-3}) (Battegazzore *et al.* 2018), and cotton (1.51 g cm^{-3}) (Abbott *et al.* 2010).

In the present study, the effects of carrying out a delignification pretreatment and the incorporation percentage of luffa fibers used as reinforcing material in a polyhydroxybutyrate matrix were investigated. The treated and untreated composites were examined to determine their mechanical performance and biodegradability behavior. Scanning electron microscopy (SEM) was undertaken to characterize the surface topology. To the best of our knowledge, this is the first attempt to develop a composite material based on PHB and luffa fibers, both treated and untreated. Finally, this new biobased composite could be a new class of sustainable material for the packaging industry.

EXPERIMENTAL

Chemical Reagents and Luffa Fibers

Polyhydroxybutyrate (PHB) (M_w = 430 KDa) was supplied by Goodfellow Cambridge Ltd. (#BU396311, Huntingdon, England). According to the provider, the polymer had a density of 1.25 g cm^{-3}, 6% of elongation at break, a tensile modulus of 3.5 GPa, and a tensile strength of 40 MPa. The luffa was obtained at local markets in León, Guanajuato, Mexico. The ripe and dried fruits were superficially cleaned with distilled water. The outer layer was removed, the sponge was cut, and the seeds removed. The sponge was size reduced using physical procedures and the pieces were stored in plastic bags at room temperature until their use.

Pretreatment of Luffa Fibers

The sliced pieces of luffa were washed thoroughly with distilled water; all impurities were removed and then dried in an oven at 70 °C for 24 h.

Luffa fibers were mercerized (immersion in 5% w/v NaOH solution) at constant stirring for 1 h at room temperature. Fibers were then washed repeatedly with distilled water to remove excess NaOH and oven-dried at 70 °C for 24 h. Untreated fibers were immersed in distilled water following the same procedure. Both treated and untreated fibers were ground and sieved; the average size of fibers was between 0.4 and 0.7 mm.

Composite Fabrication

The PHB/luffa fibers composites containing 0%, 5%, 10%, and 20% w/w of treated and untreated fiber were prepared by the solvent casting method according to Pérez-Arauz

et al. (2019). Neat PHB was mixed with chloroform (Sigma Aldrich, St. Louis, MO) at 10% w/v at 150 °C for 2 h. Fibers were then added and mixed for 10 min at 60 °C and 150 rpm to allow the efficient dispersion of fibers in the matrix; the mix was executed in a 50 mL-glass bottle with a polypropylene cap (Pyrex®, Shanghai, China). The dissolved solution was poured into a glass container that had been previously heated to 60 °C and allowed to dry covered at room temperature for 24 h.

Fiber and Composite Characterization
FTIR
The infrared spectrum of untreated and treated composites, as well as for all prepared composites, was recorded using Fourier transform infrared (FTIR) spectroscopy (Agilent Cary 630 FTIR, Santa Clara, CA, USA). Tests were performed at wavenumbers between 4000 cm^{-1} and 700 cm^{-1}.

Surface topology analysis
The morphology of treated and untreated fibers, as well as composites, was analyzed by SEM in a JSM-6510LV microscope (JEOL, Tokyo, Japan). For better resolution, the samples were first covered with gold in a Desk II, Denton Vacuum (Denton Vacuum, Cherry Hill, NJ, USA).

Mechanical characterization
The mechanical characteristics in terms of tensile strength, percentage of elongation, and Young's modulus were evaluated in treated and untreated luffa fibers blended with PHB. The composites were conditioned in a chamber with controlled relative humidity (50 ± 10%), and an average temperature of 25 °C over 48 h according to Kitic *et al.* (1986) and the corresponding ASTM standard (ASTM E104-02 2002). Composite samples (10 cm x 1 cm x 0.6 mm) were cut according to the specifications of the standard ASTM D882-02 (2002). The Young's modulus data were obtained from the force *versus* deformation curves using a universal testing machine (Instron, Model 4500, Canton, MA, USA). Tensile tests were performed at a deformation rate of 12.5 mm/min. For each test, nine specimens per treatment were tested and the average value was reported.

Biodegradation
Biodegradability behavior was determined by measuring weight loss in soil burial according to ASTM G160-03 (2003). Composite samples were cut into 3 x 2 cm pieces and buried at a depth of 10 cm in boxes containing agricultural soil. Soil moisture was monitored weekly and maintained at around 30%. Samples were retrieved at 15, 30, 60, 90, and 120 days. Five specimens were measured at each time period for each sample.
In order to minimize the possibility of any contamination or matrix loss sample, retrieving and washing was done with utmost care.

Statistical Analysis
The statistical analysis was executed using the software IBM® SPSS® Statistic version 21 (IBM Corp., Redmond, WA, USA). The comparison between treatments was executed applying the Welch's ANOVA and Brown-Forsythe (BF) tests, since data did not satisfy variance homogeneity. Weight loss data per day was analyzed by one-way ANOVA.

RESULTS AND DISCUSSION

FTIR Spectroscopy

The FTIR spectra of both treated and untreated *L. cylindrica* fibers are presented in Fig. 2. In the untreated fiber spectrum, typical cellulose peaks can be observed between 4000 and 600 cm^{-1}. The O–H stretching vibrations for cellulose, hemicellulose, and lignin can be assigned at 3335 cm^{-1} (Chen *et al.* 2017), while the peak at 2900 cm^{-1} corresponds to C–H stretching vibrations in both cellulose and hemicellulose (Fiore *et al.* 2014; Chen *et al.* 2017). The absorbance peaks at 1415 and 1033 cm^{-1} are attributed to O–H in-plane bending vibrations and C–O stretching vibrations, respectively (NagarajaGanesh and Muralikannan 2016; Molina-Guerrero *et al.* 2018). The band at 895 cm^{-1} features the characteristic β-glycosidic linkage between anhydroglucose units (Oh *et al.* 2005; Molina-Guerrero *et al.* 2018).
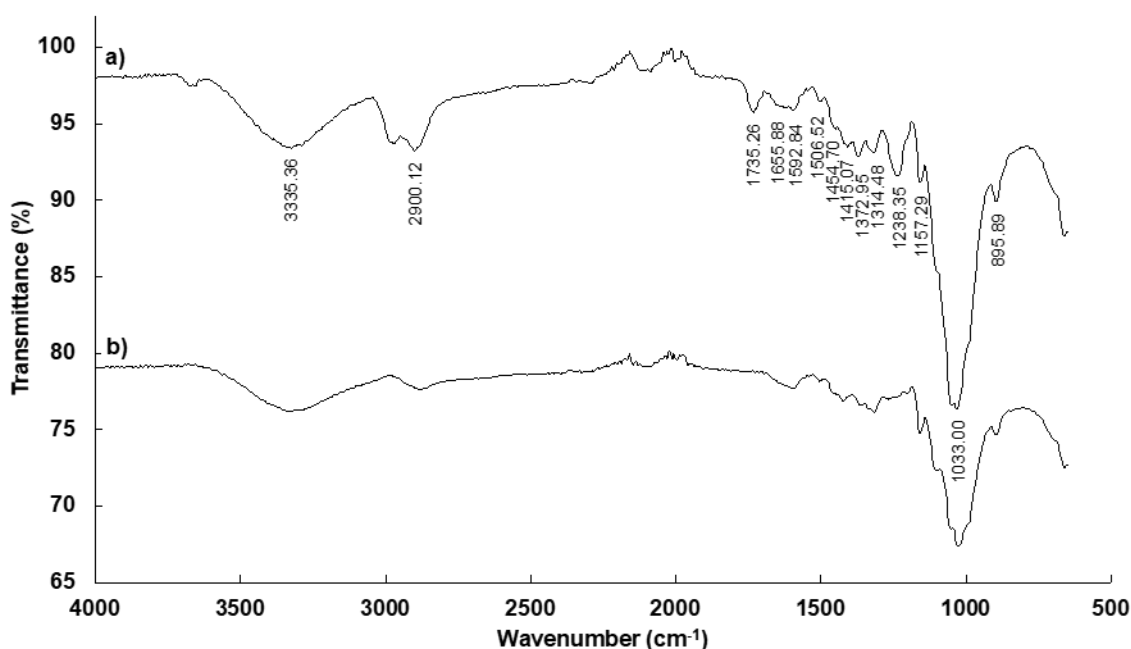

Fig. 2. Infrared spectrum for untreated (a) and treated (b) luffa fibers

Lignin in fibers can be correlated with different bands. Bands at 1655 cm^{-1} were due to stretching of the conjugated carbonyl group (Chen *et al*. 2018). Bands at 1592 cm^{-1} were indicative of the C=C stretching of the aromatic ring (NagarajaGanesh and Muralikannan 2016). Those at 1454 cm^{-1} could be attributed to C–H deformation stretching in either lignin or xylan molecules, the main component of hemicelluloses (Chen *et al.* 2018; Molina-Guerrero *et al.* 2018). At 1372 cm^{-1}, bands indicated C–H bending (Seki *et al.* 2013), and at 1157 cm^{-1} bands corresponded to C–O–C ring vibrational stretching (Kesraoui *et al.* 2016).

Absorbance peaks found at 1735 and 1238 cm^{-1} are associated with C=O and C–O stretching vibrations of the acetyl group in hemicellulose (Wang and Shen 2012; Chen *et al.* 2018). Both peaks were strongly reduced or almost absent after mercerization treatment. Infrared spectra of alkali-treated luffa in several studies have shown clear dampening of these bands (Wang and Shen 2012; Saw *et al*. 2013; Seki *et al*. 2013; Chen *et al.* 2017) because alkali treatment has been proven to accelerate the dissolution of acetyl groups in

hemicelluloses (Chen *et al.* 2017). Furthermore, the xylan peak at 1454 cm^{-1} weakened. This value indicated that hemicelluloses in luffa fibers were partly removed due to alkali treatment.

Absorbance bands corresponding to lignin at 1655, 1454, and 1506 cm^{-1} also weakened to some extent, suggesting a partial removal of lignin in mercerized fibers. The spectra of PHB/luffa composites did not show differences between mercerized and raw fiber composites because the spectrum obtained was primarily that of PHB.
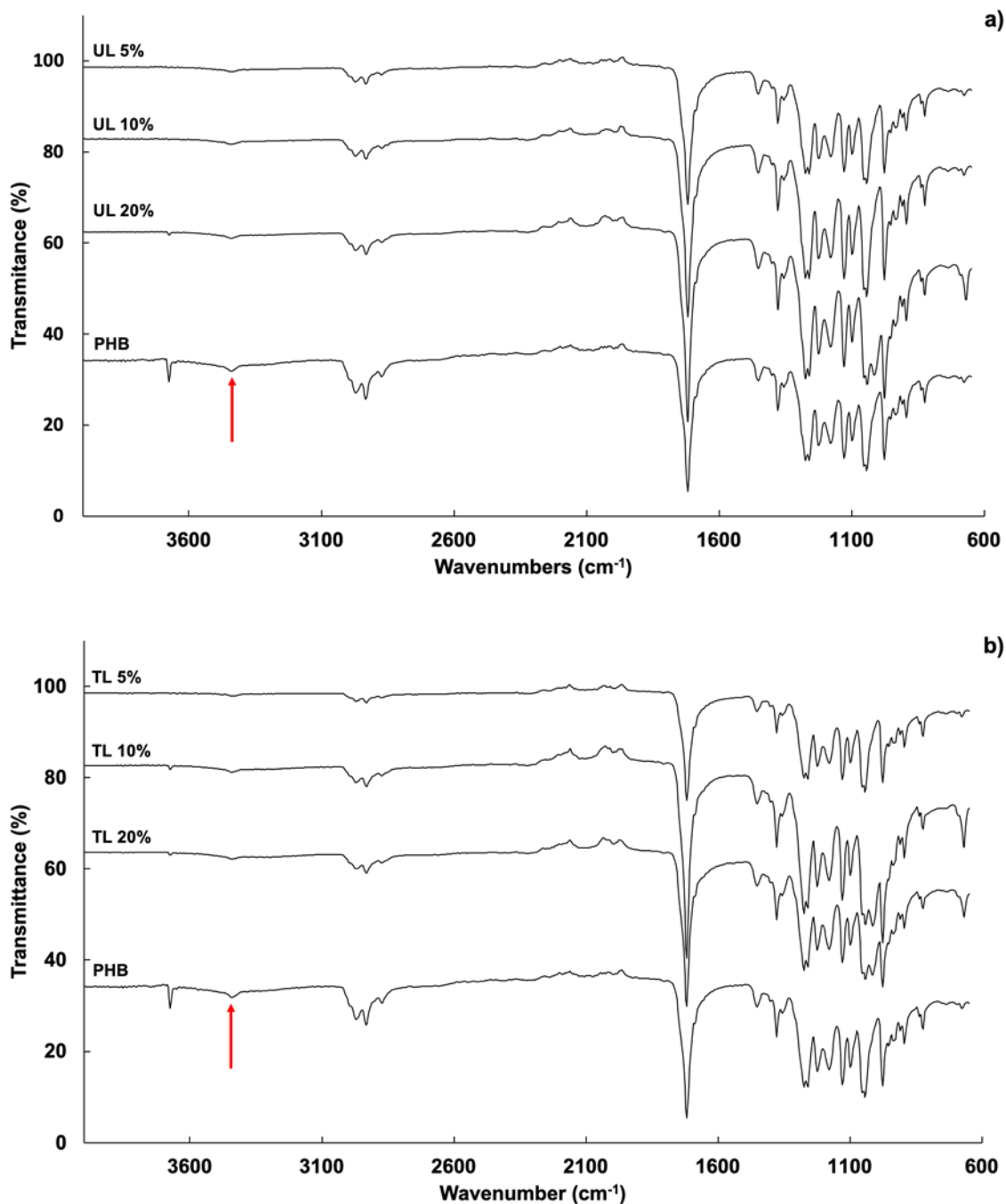

Fig. 3. The infrared spectrum for PHB composites reinforced with untreated (UL) (a) and treated (TL) (b) luffa fibers at different percentages of fiber content

Changes in the intensity of characteristic PHB peaks were evident when comparing fiber content; intensity decreased with increasing fiber content as a result of reduced PHB concentration in the samples. It is relevant to mention that both sides of the films were analyzed, and the resulting spectra presented slight variances on each side, which could be expected as fibers were more exposed on one side of the films.

Hydrogen bonding between the matrix and the fiber is greatly desired because it can allow the transfer of stress to the fiber. The bonding can occur on hydroxyl groups present on the fiber surface, represented by peaks at approximately between 3600 and 3000 cm^{-1}. Changes in these characteristic peaks reveal the formation of hydrogen bonds at the fiber-matrix interface. Increased pronunciation and widening of the bands indicates the formation of new hydrogen bonds from the free hydroxyl groups; if there is a good interaction between the fiber and the matrix, this effect would increase with the increase in the amount of fiber. Furthermore, the displacement of the band at a lower frequency indicates a higher level of hydrogen bonding; while a shift to a higher frequency points to little or no bonding (Mofokeng 2012; Gunning *et al.* 2013). The small peak found at approximately 3400 cm^{-1} for all composites (Fig. 3) suggested that hydrogen bonding was infrequent or nonexistent in the composites analyzed possibly due to a poor fiber-matrix interaction, which could affect mechanical properties.

Surface Topology Analysis

Figure 4 shows the SEM micrographs of raw and mercerized luffa fibers. It can be seen in Fig. 4a that impurities and small particles covering the surface of untreated fibers—expected to be mainly lignin, hemicelluloses, and waxy substances—had been removed due to alkaline treatment (see Fig. 4b).
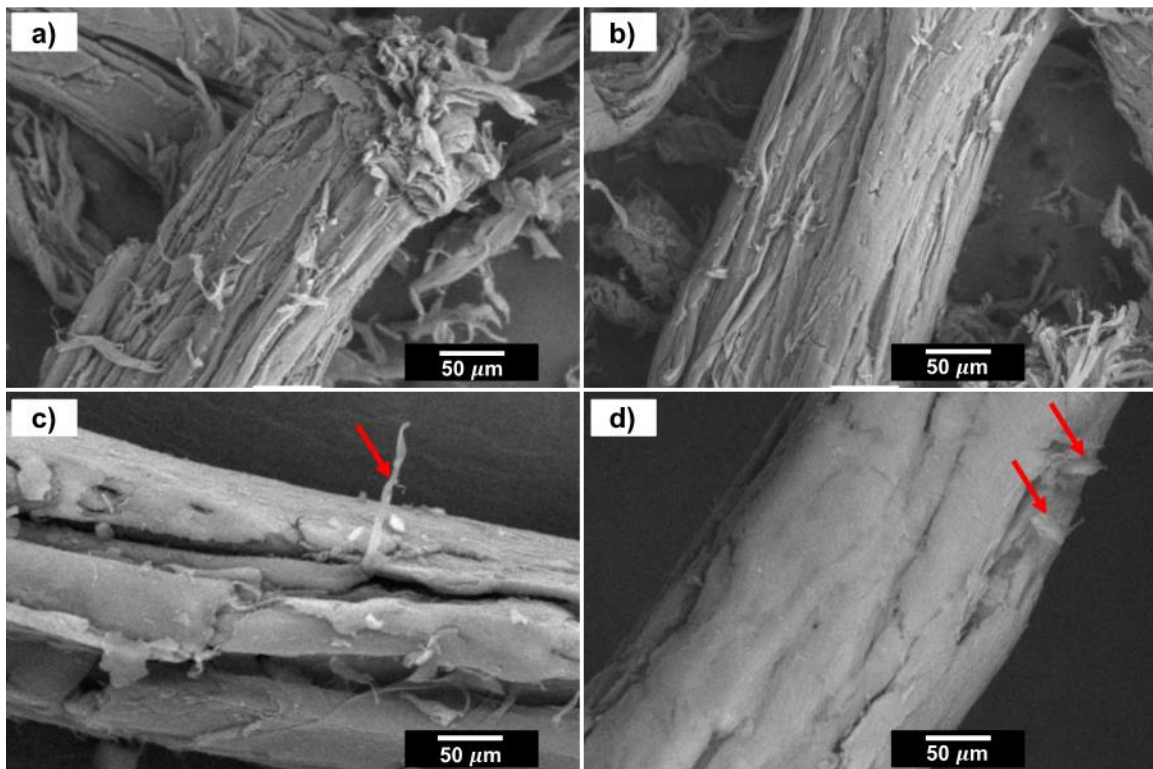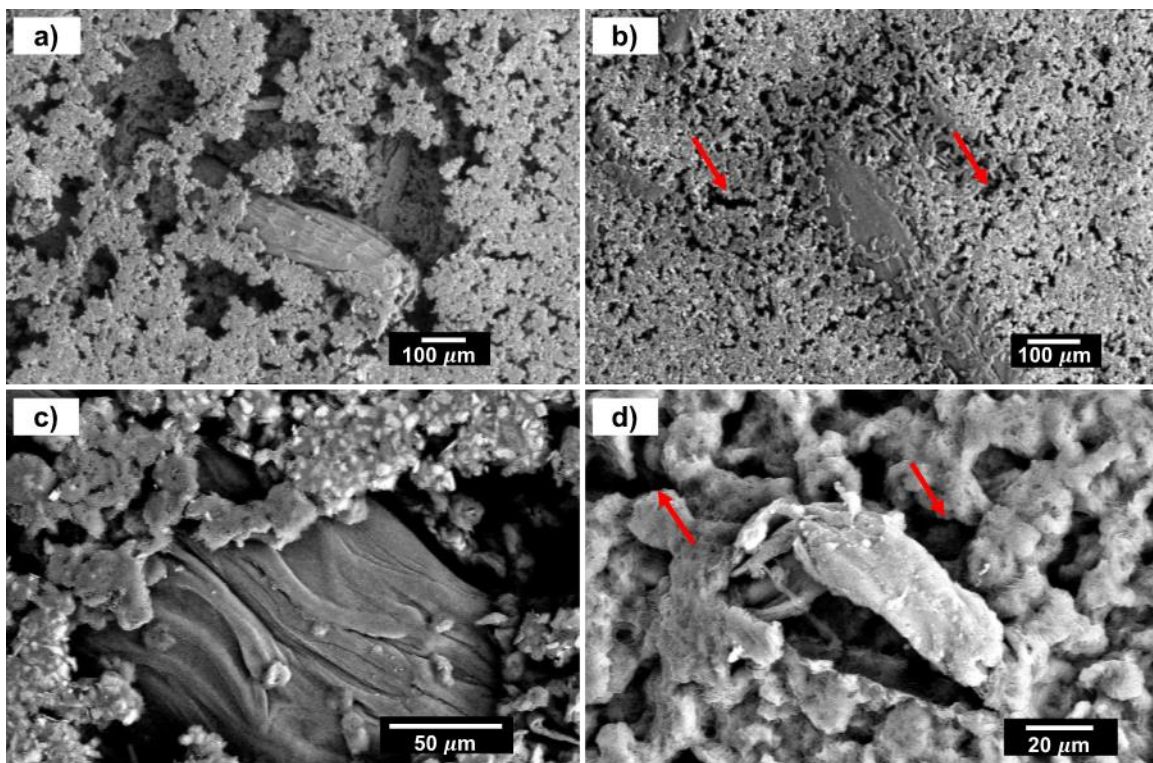

Fig. 4. Micrographs of untreated (a, c) and treated (b, d) luffa fibers

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A size estimation based on SEM images revealed a reduction in single fiber diameter of approximately 22% for treated fibers (images not shown). Additionally, Figs. 4c and 4d show some microfibrils detached from the main body, which could have acted as mechanical anchors to the PHB matrix and therefore improve the fiber-matrix interfacial adhesion. However, contrary to the expected performance, the results of the mechanical tests did not show a positive effect. Similar effects of alkalization were previously reported by Russo *et al.* and Ibrahim *et al.* (Russo *et al.* 2013; Ibrahim *et al.* 2018).

The surface morphology of composite samples is shown in the SEM micrographs of Fig. 5. Poor fiber-matrix adhesion was observed in untreated fiber composites, along with the presence of voids and gaps, indicating low compatibility between the fiber and matrix (Beber *et al.* 2018). Mercerized fibers were better embedded in the matrix (Fig. 5b), and even showed residual resin on the luffa surface. This is shown in Fig. 5d, but it was not observed in the case of untreated fibers. This reflects an improved adhesion between the two materials; however, the composite surface still showed some voids, particularly closer to the fiber.

* Red arrows point to voids in the PHB matrix

Fig. 5. Surface micrographs of PHB composites with untreated (a, c) and treated (b, d) luffa fibers

Cryofractured samples were also observed by SEM. Figure 6 presents fractured surface micrographs of mercerized and non-mercerized composites with 10% fiber content. Untreated fiber composites exhibited several voids created by fiber pullouts (see Fig. 6a), as well as a clear interface gap between fiber and matrix (indicated in Fig. 6c). Literature shows that these characteristics are evidence of weak interfacial interaction between the fiber and the matrix, resulting in reduced performance of the composite material (Muthuraj *et al.* 2017).

After alkali treatment, composites showed signs of improved adhesion, namely, fiber pullouts were markedly less, and the gap between fiber and matrix was visibly reduced, as portrayed in Figs. 6b and 6d. However, the existence of both indicated that the interfacial adhesion was still not the strongest, which could limit the mechanical properties of the composite.
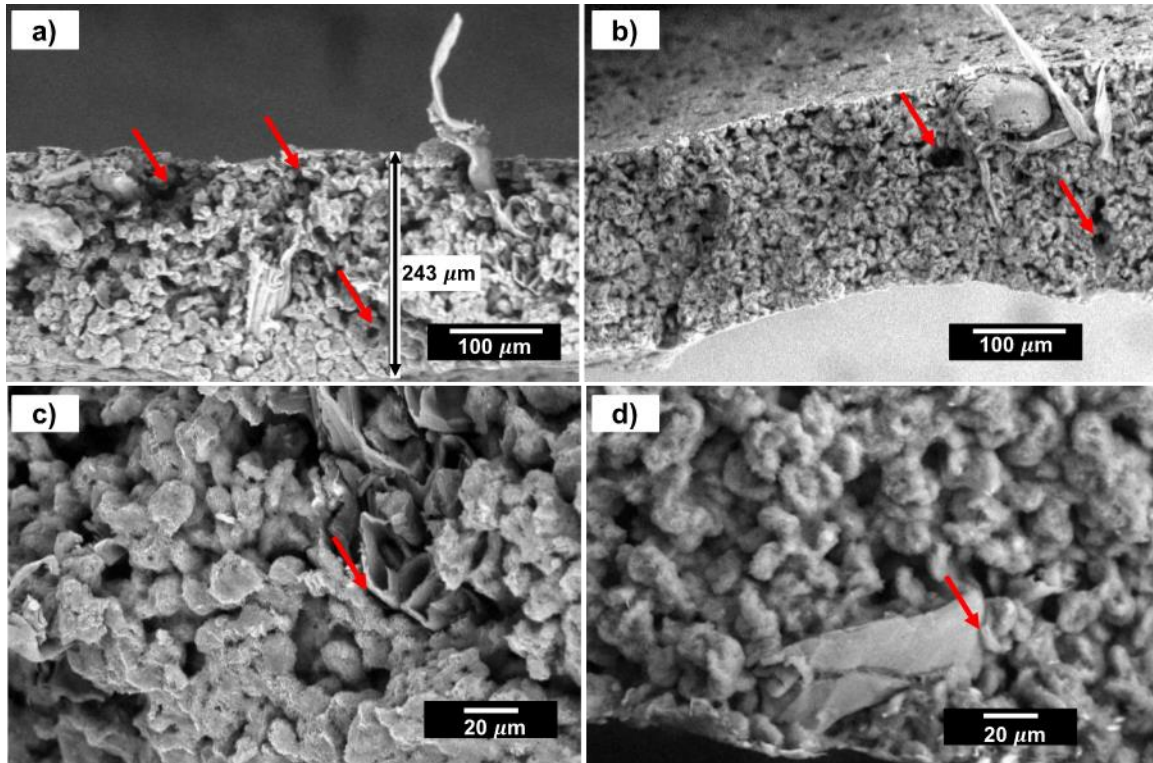

Fig. 6. Transversal micrographs of cryofractured PHB composites with untreated (a, c) and treated (b, d) luffa fibers; in (a) and (b) red arrows point to fiber pullouts, while in (c) and (d), arrows point to interface gaps

Mechanical Properties

The mechanical behavior of PHB/luffa composites was analyzed by tensile tests. The obtained mechanical properties, including Young's modulus, tensile strength, and elongation at break, are summarized in Table 1.

Table 1. Mechanical Performance of PHB/Luffa Composites

Composites (% w/w)	Young's Modulus (MPa)	Tensile Strength (MPa)	Elongation at Break (%)
PHB	412.3 ± 44[a]*	4.3 ± 0.6[a]	2.3 ± 0.5[a]
UL5	343.5 ± 29[b]	3.6 ± 0.5[ab]	1.7 ± 0.4[ac]
UL10	288.1 ± 23[c]	3.1 ± 0.5[b]	1.5 ± 0.3[bc]
UL20	194.6 ± 15[d]	1.9 ± 0.3[c]	1.4 ± 0.3[bc]
TL5	272.9 ± 40[c]	3.1 ± 0.3[b]	1.8 ± 0.3[a]
TL10	260.0 ± 20[c]	2.8 ± 0.3[b]	1.6 ± 0.2[bc]
TL20	168.3± 17[d]	1.7 ± 0.3[c]	1.7 ± 0.3[bc]

*Same letter indicates no statistically significant differences.

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According to both Welch's ANOVA and BF tests, significant differences were found between composites for all mechanical properties tested ($p > 0.05$). Tamhane T2 post hoc testing revealed statistical differences between specific composite formulations, which are reported in Table 1 by means of superscripts of the data.

A clear decrease was found in all tensile properties tested with fiber incorporation, regardless of fiber treatment. Furthermore, the mercerization of luffa fibers was found to have no significant effect on the mechanical behavior of composites. Increasing fiber content showed an inverse correlation to tensile strength and Young's modulus, excluding composites with 5% and 10% fiber, which revealed no statistical difference in tensile strength ($p > 0.05$). In contrast, the elongation at break was not affected by fiber content ($p > 0.05$).

Several studies have found similar responses in the tensile strength of PHAs with the addition of fiber. Batista *et al.* (2010) reported that the incorporation of 10% w/w of peach palm particles in a PHBV matrix produced a 35% decrease in tensile strength. However, when increasing fiber loading to 20% and 25% w/w, there was no further reduction in tensile strength, indicating that the matrix-fiber adhesion was improved. A similar trend was observed for the strain at break. Young's modulus, in contrast, was unaffected by the incorporation of fiber, which was attributed to poor fiber impregnation and an inadequate fiber aspect ratio (length/diameter).

Melo *et al.* (2012) examined the effect of different fiber treatments on PHB/carnauba fiber composites. Tensile strength of all composites decreased compared with neat PHB; nonetheless, and conversely to the results here obtained, fiber alkalization with NaOH 5% did exhibit a positive effect on tensile strength, improving 24% in comparison with untreated fiber composites, due to a better fiber-matrix interface adhesion caused by the amount of exposed cellulose generated by the treatment.

Reduction in tensile strength due to fiber addition was also described in PHB composites with jute and hemp fibers (Gunning *et al.* 2013); crude lignin-rich residues (Angelini *et al.* 2014); PBAT/babassu fibers (Beber *et al.* 2018); and almond shell, rice husk, and seagrass (Sánchez-Safont *et al.* 2018). As reported by Beber *et al.* (2018) and Sánchez-Safont *et al.* (2018) elongation at break dropped when incorporating fibers in all composites analyzed. Furthermore, Sánchez-Safont *et al.* (2018) found a more severe decrease in tensile strength and elongation at break with increasing fiber content, while Young's modulus presented a slight increase also dependent on fiber content.

The worsening of mechanical properties has been primarily attributed to poor compatibility between fiber and matrix, particularly for tensile strength and elongation at break, while Young's modulus is more influenced by fiber wettability and fiber aspect ratio (Batista *et al.* 2010; Nunes *et al.* 2018).

Optimum reinforcement is achieved when stress can be successfully transferred between matrix and fibers across the interface. Tensile strength therefore depends greatly on the interfacial adhesion. Low compatibility signifies limited interaction between the fiber and the matrix, leading to deficient interfacial bonding. Fiber impregnation or wettability can be considered an essential precursor to good fiber-matrix interfacial bonding. Poor fiber wettability generates interfacial defects, which can act as stress concentrators (Gunning *et al.* 2013; Pickering *et al.* 2016). Fiber treatments have been shown to improve wettability and, consequently, interfacial strength. For instance, Ventura *et al.* (2017) achieved a 25% improvement in tensile strength using flax fiber as reinforcement for PHB; however, when using plasma-treated fibers, tensile strength

increased by 75%. Plasma etching produced a rougher fiber surface, which could have contributed to better wettability and thus improved mechanical properties.

As shown in SEM micrographs (Fig. 4), fiber wettability, even after mercerization, appeared to be deficient, and hence the obtained results were not typical.

Fiber size and loading have been identified as major factors influencing the mechanical properties of composites, as both relate directly to fiber dispersion. To allow efficient reinforcement, the average fiber length must be greater than a critical length (l_c). This parameter is determined by the length that allows the complete load transfer from matrix to fiber. At lengths below l_c, complete interfacial debonding is favored, while at lengths above l_c, fiber failure occurs without interfacial debonding (Luz *et al.* 2018).

However, exceedingly long fibers can also reduce the reinforcement efficiency, as fibers might get tangled during mixing and thus hinder fiber dispersion. Composites analyzed by Gunning *et al.* (2013) exhibited the highest tensile properties with the greatest average fiber length, while composites with a fiber length lower than l_c did not surpass neat PHB tensile strength. It was also observed by the same author that the fiber width affects fiber dispersion, and it was proven that lower fiber widths attained better dispersion in the matrix. The best results were achieved with 10 to 20 µm fiber width.

In general, when there is good fiber-matrix interfacial bonding, the strength and stiffness of composite materials have been seen to increase with fiber content. The opposite occurs if interfacial bonding is deficient, decreasing as fiber loading increases. Young's modulus, in contrast, generally increases with increasing fiber content regardless of the fiber-matrix affinity, though it increases more modestly when the interface is not ideal (Pickering *et al.* 2016; Moura *et al.* 2018).

A possible mechanism responsible for limited mechanical behavior is the formation of fiber agglomerates, whose presence becomes more prevalent with higher fiber loadings, causing weak points within the composite (Gunning *et al.* 2013). Porosity has also been known to largely influence these properties, as it increases with fiber loading. It can be caused by poor wettability, the inclusion of air during processing, and hollow features present in fibers (Pickering *et al.* 2016).

As fiber content increased in PHB/luffa composites, luffa fiber agglomerates became more evident and higher porosity was observed. These changes could explain the drop in tensile strength with higher fiber loading.

Good fiber dispersion is crucial to achieving good interfacial bonding, as it ensures that fibers will be completely covered by the matrix and that voids are reduced. Guo *et al.* (2019) achieved uniform fiber dispersion in PHBV/luffa fiber composites of up to 60% fiber content. Even the distribution of fibers favored the formation of an interlocked network structure that was able to withstand external loads, thus enhancing mechanical properties. However, with fiber content above 60%, a large number of fibers were exposed, which reduced the interfacial bonding. In the present study, composites showed an uneven dispersion of fibers, particularly with higher fiber loadings, which most definitely influenced the mechanical properties in a negative way.

The manufacturing process has been reported to affect fiber dispersion. For instance, the use of a twin-screw extruder leads to better fiber dispersion than use of a single screw extruder (Pickering *et al.* 2016). Therefore, a process with more intensive mixing might enhance mechanical properties by improving fiber dispersion.

Biodegradation Behavior

The biodegradation of PHB and PHB/luffa composites was evaluated by weight loss after soil burial for four months, according to ASTM G160-03 (2003), consistent with the procedure explained in the Experimental section.

The visual analysis of composites extracted at different times is displayed in Fig. 7.
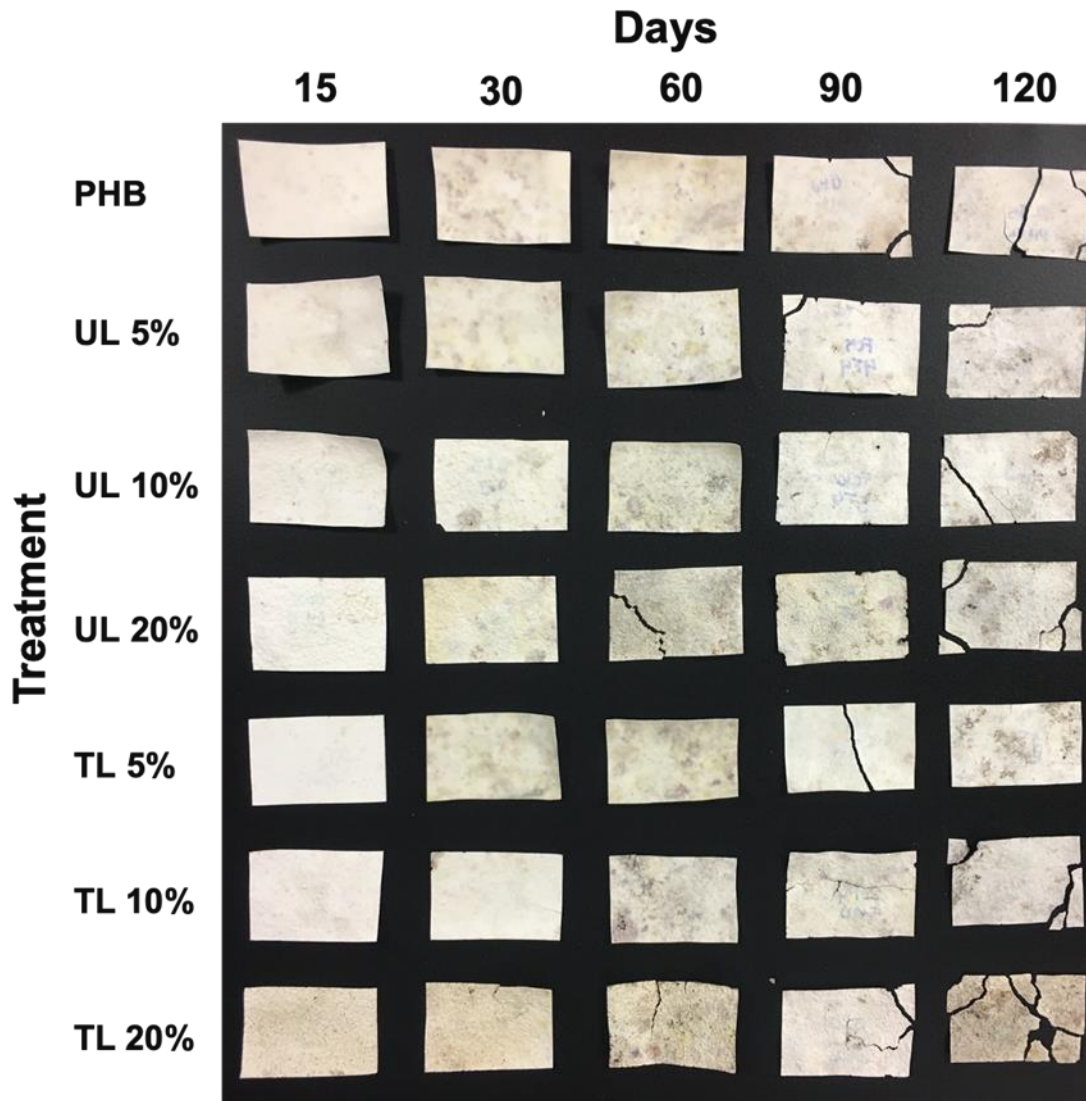

Fig. 7. Pictures of different stages of biodegradation of PHB/luffa fiber (untreated and treated) composites at different w/w ratio after burial soil

In the composites and the neat PHB, decomposition signs were evident after the first month. Samples became fragile, fragmenting easily, and surface roughness increased. According to Gunning *et al.* (2013), the first stage of biodegradation involves the surface attack on the polymer by microorganisms, increasing surface roughness and creating pores that, in turn, act as sites for a biological attack. Colored spots appeared on the surface, which intensified and multiplied with time, thus indicating microbial growth.

Degradation of each composite was confirmed by a steady increase of weight loss as a function of time, which was statistically corroborated with one-way ANOVA testing and Tukey HSD post-hoc.

As shown in Fig. 8, the weight loss in composites for all samples started before the first measurement (15 days) and continued for the entire test duration, with no lag period noticed at the beginning of the process, as has been previously reported by Sánchez-Safont *et al.* (2018) and Seoane *et al.* (2018).
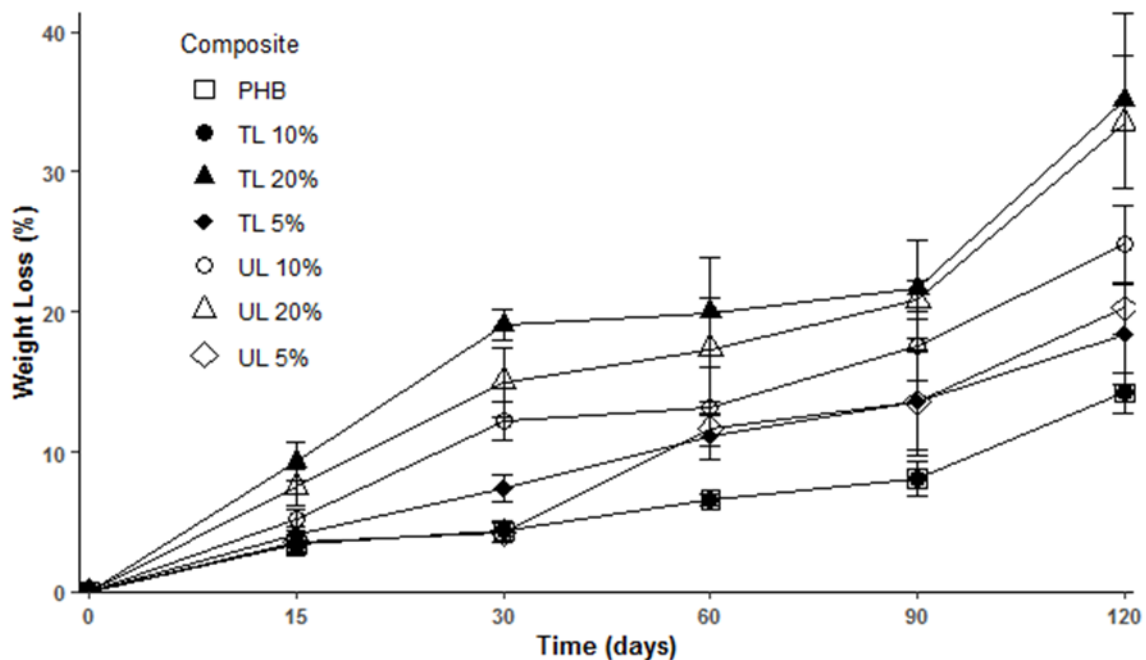

Fig. 8. Weight loss behavior of PHB/luffa fiber (untreated and treated) composites at different w/w ratio after burial soil

Statistically significant differences were observed between composites on each measurement day, as shown by Welch's ANOVA and BF tests ($p < 0.05$). Pairwise comparison by Tamhane T2 post hoc testing verified that all composites exhibited higher degradation rates than PHB alone. Weight loss due to biodegradation increased with increasing fiber loading in both treated and untreated fiber composites. Several studies (Batista *et al.* 2010; Wei *et al.* 2015; Joyyi *et al.* 2017; Cinelli *et al.* 2019) found similar results in PHA/fiber composites, and attributed this effect to the capacity of fibers to increase the water absorption and the accessibility of microorganisms to the composite's inner sites, therefore accelerating biodegradation (Ibrahim *et al.* 2017). Additionally, moisture uptake can increase the surface area for microbial attack, leading to the hydrolysis of ester groups on the PHB backbone, and then the conversion into CO_2 and H_2O by aerobic degradation (Wei *et al.* 2015). No statistical difference was found between treated and untreated fiber composites with the same fiber content percentage ($p > 0.05$).

After 120 days, the PHB matrix reached nearly 15% weight loss, whereas composites with 5%, 10%, and 20% fiber loading exhibited approximately 20%, 25%, and 35% decrease in weight, respectively. It is feasible to presume that biodegradation was the most likely reason for this weight loss, since microbial growth signs were observed in sample surfaces.

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It is worth mentioning that even though soil-burial test results may be affected by soil contamination on samples or loss of matrix during the recovery and cleaning process, the use of a standardized method and the precautions taken when handling samples ensure reliable results. However, as this method only considers the first stages of the biodegradation process, a respirometric analysis is recommended to obtain information on the degree of mineralization.

CONCLUSIONS

1. It was found that the alkali pretreatment of luffa fiber did not affect the mechanical properties of the composites. However, when increasing the luffa fiber content in the matrix, a decrease of the tensile strength and Young's modulus was observed. No differences were found for elongation at break parameters among the evaluated treatments.

2. The elimination of wax compounds was observed for treated fibers, confirming the effectiveness of the alkali treatment.

3. The weight loss analysis confirmed a positive correlation between fiber loading and degradation rate for all treatments; the lowest and highest fiber content percentages were statistically different between untreated and treated luffa.

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