Innovative, Low-Cost Eco-Composite Boards Produced from High-Energy Milled Wood, Plastic Waste, and Bio-Polymer

Petr Pařil,* Jan Baar, and Radim Rousek

Currently, there is a big issue with waste, its processing and subsequent use. While there are many initiatives to replace materials that are poorly biodegradable, it is necessary to process and ideally use the waste that is already produced. In this study, the properties of composite boards made of waste materials and biodegradable polymer were investigated. The composite boards were made from wood and plastic waste using high-energy milling technology. This technology for material preparation is promising, as it includes controlling the morphology of particles, homogenizing the mixture, and drying the material during the milling process. The results showed higher flexural strength of high-density fiberboard (HDF) boards compared with tested composites with one exception. Wood/poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [PHBV] composite exhibited 30% higher modulus of elasticity (MOE) than HDF due to the higher modulus of PHBV. The lowest thickness swelling (3%) and water absorption (12%) were measured for wood/recycled high density polythene (rHDPE) composite. The HDF boards recorded the lowest dimensional stability. The highest water absorption of tested composites was measured for wood/PHBV composite. The resistance to wood-rotting fungi was greatest for wood/PHBV composite containing marble powder, which corresponded to the results of scanning electron microscopy.

DOI: 10.15376/biores.18.3.5635-5654

Keywords: Wood; Polymer; Composite; Physical and mechanical properties; High-energy milling; Wood-rotting fungi; Electron microscopy

*Contact information: Department of Wood Science and Wood Processing Technology, Mendel University in Brno, Zemědělská 3, 613 00 Brno, Czech Republic; *Corresponding author: xparil1@mendelu.cz*

INTRODUCTION

Wood-plastic composite (WPC) is a product made of small wood particles or fibres and virgin or recycled plastic, and it may contain various additives (coupling agents, UV stabilisers, pigments, *etc*.). Composites combine the beneficial characteristics of each constituent and allow its properties to be tailored for a wide variety of specific applications (Ellis 2000). Besides the mechanical properties and hardness, frequently targeted properties are dimensional stability, low moisture absorption, and resistance to decay, weather, and fire.

In 1960, the first composite mixed from wood fibre and thermoplastic was developed and marketed in northern Italy. Further attempts followed, and different kinds of thermoplastic and thermosetting polymers (phenolic resin, polypropylene, low-density polyethylene, acrylonitrile-butadiene-styrene, polyvinyl chloride, and high-density polyethylene) were used as the matrix. Since then, the WPC manufacturing sector has grown rapidly, and in recent years WPC has found applications in decking, automotive parts, cladding, and fencing. Currently, WPC materials most often consist of 60% wood flour and 40% plastic. They typically are based on polyethylene (PE), polypropylene (PP), or polyvinyl chloride (PVC). Other lignocellulosic materials are sometimes used instead of wood include jute, flax, hemp, or plant residues of other agricultural crops (Youngquist *et al.* 1995; Schut 1999; Clemons 2002; Klyosov 2007).

To produce agglomerated materials, primary input raw materials generally have been used, rather than materials from end-of-life products. Wood raw material is the main component for the production of WPC products and is most often a by-product of an industrial process. (Youngquist *et al.* 1995; Schut 1999; Clemons 2002; Klyosov 2007). The EU Waste Framework Directive 2008/98/EC aimed for 70% of construction and demolition waste to be recycled by 2020 and a future recycling rate of 90%. An increasing amount of waste wood is expected from the building sector and from post-consumer wood (Höglmeier *et al.* 2017; Irle *et al.* 2019). A number of European countries have established or are developing procedures and a market for the recovery of waste wood. However, these initiatives are missing in the Czech Republic. One way to improve the circular economy is to use different waste materials and side fractions as part of wood-plastic composites. The advantage of using recycled materials is not only the lower price, but also that it is more reasonable to turn low-value refuse into a useful end product and more environmentally friendly to recycle materials instead of disposing of them (Keskisaari and Kärki 2018). Although WPC extends the lifespan of useless and discarded materials, it is still based on polymeric materials characterised by extremely slow environmental degradation due to its chemical and biological inertness (Chamas *et al.* 2020). The second way to produce eco-friendly materials is to shift from recyclable to compostable products, which can also reduce dependence on petrochemical sources. New bio-based resins such as polylactic acid, cellulose acetate, starch blends, and polyhydroxyalkanoates are used as polymers in biodegradable composites (Teymoorzadeh and Rodrigue 2015).

Chen and Zhong (2011) demonstrated that PHBV can be used as a compatibilizing agent in blends of polylactic acid (PLA) and thermoplastic starch (TPS) to improve their mechanical properties. The study found that the addition of PHBV increased the interfacial adhesion between PLA and TPS, resulting in improved tensile strength and elongation at break. There is limited evidence to suggest that poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) can undergo some chemical reactions with other materials during compounding. However, the extent and significance of these reactions depend on various factors such as the processing conditions, the nature of the other materials, and the composition of PHBV. PHBV typically has a melting point in the range of 170 to 176 °C. This is generally considered to be a favorable characteristic for use as a bioplastic additive, as it is compatible with many processing techniques commonly used in plastics manufacturing. PHBV has favorable solubility parameters, which make it compatible with a wide range of polymers and processing techniques commonly used in the plastics industry. Spierling and Welle (2016) claim that the solubility parameters of PHBV can vary depending on the specific composition of the polymer, but generally fall within the range of 20 to 25 $(\text{MPa})^{0.5}$. This range is similar to that of many other commonly used polymers, including polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET).

Generally, polymers that are used in WPCs are hydrophobic, which results in poor interfacial bonding with the hydrophilic wood particles, and this limits their mechanical characteristics (Rao *et al.* 2018). Bonding can be improved by crosslinking agents and by the chemical pre-treatment of wood fibre or polymer (Zu *et al.* 2000). High-energy milling (HEM) is a material pre-treatment that transfers a large amount of energy to the material. During milling, the specific surface area and thus the surface energy of solids are increased, which changes their chemical reactivity and some of their physico-chemical properties (Austin and Trass 1997; Snow *et al.* 2009). In addition, the disintegration and crumbling of grains of solids, a phenomenon called mechanical or mechanochemical activation, can be observed in most types of high-energy milling. HEM, which is very effective and is characterised by low space, investment, and operational demands can deal with challenging raw materials and can be adjusted to different raw material types and characteristics. (Nayborodenko *et al.* 2002; Fuerstenau and Han 2003; Baláž 2008).

During HEM processing, the materials are subjected to intense mechanical forces, which can lead to a range of physical and chemical changes. It is possible that lignin could come to the outside of the particles during HEM, which could render them more hydrophobic and compatible with typical matrix plastic materials. This is because lignin is a hydrophobic polymer that tends to be located in the outer regions of the cell wall in natural lignocellulosic materials. However, the extent to which this occurs during HEM may depend on a range of factors, including the composition and structure of the lignocellulosic materials, the milling conditions, and the duration of the milling process. In terms of moisture content, HEM can result in a decrease in the moisture content of lignocellulosic materials. This is because the mechanical forces generated during milling can lead to the removal of water from the materials, either through evaporation or by breaking down the hydrogen bonds that hold water molecules in place. Other changes that can occur during HEM include the formation of new surface area and the introduction of defects or imperfections into the materials. These changes can impact the reactivity, mechanical properties, and compatibility of the materials with other matrices or fillers (Alves *et al.* 2015; Mikkonen *et al.* 2016; Ganesan and Velusamy 2017; He *et al.* 2017; Prasad *et al.* 2019).

The milling parameters of HEM (High Energy Milling) include several variables that can affect the morphology and particle size of the milled material. The most important milling parameters include:

- Milling time: The duration of the milling process can affect the degree of deformation, fracturing, and welding of the particles. Longer milling times can lead to finer particle size and more uniform morphology but can also increase the risk of thermal degradation or contamination.
- Milling speed: The rotational speed of the milling media can affect the kinetic energy transferred to the material and the frequency of collisions between the particles. Higher milling speeds can lead to more extensive deformation and fracturing of the particles, resulting in a finer particle size and more homogeneous morphology.
- Type and size of milling media: The type and size of the milling media can affect the degree of deformation and fracturing of the particles, as well as the milling efficiency. Smaller media can lead to finer particle size but may also increase the risk of contamination.
- Milling atmosphere: The milling atmosphere can affect the degree of oxidation, contamination, or thermal degradation of the material during milling. Inert gas atmospheres can reduce the risk of oxidation or contamination, while reactive atmospheres can induce chemical reactions or transformations.

Controlling these milling parameters can help achieve the desired particle size and morphology of the milled material, while minimizing the risk of thermal degradation, contamination, or other undesirable effects (Baláž 2008; Sahu and Bose 2010; Sheykholeslami and Tofighian 2013; Atik *et al.* 2018).

In the production of inorganic hydraulic binder (DASTIT), significant progress and economic and environmental savings are obtained using HEM (Prokšan *et al.* 2017). However, little is known about the use of wood-based input materials in combination with HEM. In a circular bioeconomy, HEM can realise one-step processing of recovered input materials, which results in a homogeneous mixture of different materials with the required particle size and moisture content. In combination with hot-press moulding, which is considered a simple method for producing panels with high fibre content, large dimensions, and lower cost than other methods (Chaharmahali *et al.* 2008), it can be ideal for producing low-cost and eco-friendly boards, which are closely comparable to commercial medium density fibreboard and particleboard.

Objectives

In this work, flat-pressed wood/plastic composites produced from high-energy milled input materials were evaluated for their mechanical properties, water absorption, and decay resistance. The goal of this study was to use recyclable or biodegradable plastic and wood waste for composites, which could substitute for conventional wood-based panels used in floorings, furniture production, sheathing panels, or other applications.

EXPERIMENTAL

Materials

Spruce (*Picea abies* (L.) H. Karst.) sawdust (waste from wood processing industries), industrial plastic waste (recycled high-density polyethylene - rHDPE, recycled low density polyethylene - rLDPE), and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV; ENMAT Y1000P, Tianan Biologic) were used. PHBV was chosen for its a biodegradability and biocompatibility. This polymer has been used in various applications, including as a bioplastic additive. Crushed marble chippings ($CaCO_3 + MgCO_3 \geq 98\%$; 0,2 – 0,5 mm fraction) were used as a filler for PHBV, which also improved the milling process. HDF boards (UNILIN, 12 mm), used as core board for flooring, were chosen for comparison of the physical and mechanical properties. Moisture-resistant wood fibre board (Egger DHF; 15 mm) was used as the reference for the decay test.

Table 1. Composite Board Formulations (Percentage by weight)

Type	Materials	Ratio
A	Wood : PHBV	1:1
B	Wood : rHDPE	6:4
C	Wood : rLDPE	6:4
D	Wood : PHBV : marble	2.4 : 4 : 1.6

Composite Preparation

The materials were prepared using a high-energy mill (FF Servis, Ltd.; Czech Republic). The materials were mixed in the stated ratios (Table 1) and milled and dried in one step. The milling parameters, which affect the morphology and particle size, were set up according to pre-tests.

The particle size distribution was achieved by the sieve analysis: 50 g of composite mixture were sieved by a laboratory sieve shaker (Analysette 3 Pro, Fritsch) for 10 min with 1.5 mm amplitude with two repetitions for each mixture. Three sieves with mesh sizes 1,000, 500, and 200 μm were used.

The homogenised mixture from the mill with initial moisture content under 3% was used for board manufacture. First, it was pre-formed manually in a wooden frame with inner dimensions 600 mm × 600 mm. After the pre-forming process, the frame was removed and the board was pressed in a hydraulic press (Strozatech s.r.o., Czech Republic) to a thickness of 12 or 15 mm with 3.5 MPa of pressure at 200 °C for 10 min (Fig. 1). To prevent distortion after the pressing, the boards were clamped between two plates to allow the composite to cool and harden under pressure. The boards were kept under room conditions (65% relative humidity and a temperature of 20 °C) before testing sample preparation, which followed the rules in the standard ČSN EN 326-1:1997.

Fig. 1. The homogenised mixture after pre-forming (left) and the composite board after pressing (right)

Thickness Swelling and Water Absorption

Thickness swelling (TS) and water absorption (WA) tests were carried out according to ČSN EN 317:1996. Ten replicates (50 × 50 × 12 mm) were cut from each manufactured composite (two boards) and conditioned (relative humidity of 65% and a temperature of 20 °C) until their weight stabilised. The thickness and weight of the samples were measured before and after soaking in water (20 ± 2 °C) for 10 days, and the differences were expressed as percentages.

Density and Mechanical Properties – Flexural Strength (MOR), Modulus of Elasticity in Bending (MOE) and Hardness

Ten conditioned samples (290 × 50 × 12 mm) were cut from each manufactured composite (two boards). A three-point bending strength test was carried out in compliance with ČSN EN 310:1996 to calculate the MOR and MOE using a universal testing machine (Zwick Z050/TH 3A, Zwick Roell AG, Ulm, Germany) with a crosshead speed of 12 mm/min and a span length of 180 mm.

The Janka method, where a steel ball of diameter 11.28 mm is forced into a sample until half of the ball’s diameter is embedded, was used to determine the hardness.

Conditioned hardness test specimens (50 × 50 × 12 mm) were tested on a universal testing device (ZWICK Z050/TH 3A) in compliance with Czech Standard ČSN 49 0136:1984.

Resistance to Wood-Rotting Fungi

The composites' resistance to wood-rotting fungi was determined according to ENV 12038: 2002. Six samples (50 × 45 × 15 mm) from three different boards were used for each testing fungus. Size control specimens (50 × 45 × 15 mm) and virulence specimens (50 × 25 × 15 mm) were prepared from Scots pine sapwood (*Pinus sylvestris* L.) for brown rot fungus and beech (*Fagus sylvatica* L.) for white rot fungus.

All samples were dried (103 °C, 24 h) to obtain oven-dry mass (m_0). Samples were exposed to *Coniophora puteana* BAM 112 (Ebw.15) and *Pleurotus ostreatus* BAM 96 (FPLR 40C). For the test, cultivation vessels with 60 mL of malt-extract agar with mycological peptone were prepared. Mixtures were contained either in Kolle flasks (400 mL) for *C. puteana* or cylindrical glass vessels (480 mL). This enabled the samples to be covered by vermiculite, for *P. ostreatus*.

The steam-sterilised samples were inserted into a vessel on stainless steel wire when the surface was completely covered by fungus mycelium; in the case of *P. ostreatus*, samples were additionally covered by a layer of sterilised wet vermiculite. Samples were incubated for 16 weeks in a stable environment (22 ± 1 °C, 70 ± 5% RH).

At the end of the exposure, the samples were removed, surface mycelium was carefully cleaned, and, after weighing (m_1), the samples were slowly dried (103 ± 2 °C) to obtain their oven-dry mass after exposure (m_2). Sample moisture content after exposure (w), sample mass loss (ML), and decay susceptibility (DS) were calculated by Eqs. 1, 2, and 3, respectively,

$$w = (m_1 - m_2) / m_2 \times 100\ [\%] \tag{1}$$

$$\text{ML} = (m_0 - m_1) / m_0 \times 100\ [\%] \tag{2}$$

$$\text{DS} = (m_0 - m_1) / V_0 \times 100\ [\%] \tag{3}$$

where m_0 is the oven-dry mass before exposure (g), m_1 is the sample oven-dry mass after exposure (g), m_2 is the sample wet mass after exposure (g), and V_0 is the sample oven-dry volume before exposure (cm^3).

Leachate pH Measurement

Two decay-resistance specimens from each group (non-decayed, and brown-rot and white-rot decayed) were compared by pH measurement. Each specimen was ground individually using an MM 400 mixer mill (Retsch GmbH, Germany; fineness ~5 μm) into sawdust. The pH measurement was carried out in leachate prepared by adding 40 mL of demineralised water (t = 23 °C, pH = 7.1) to 2 g of sawdust in a beaker. The mixture was briefly stirred and kept for 90 min at room temperature. After stirring and sedimentation, the pH value of the leachate was measured with the glass electrode of a benchtop pH meter pH8 (XS Instruments, resolution ± 0.01 pH).

Scanning Electron Microscopy Analysis

Small samples (3 × 5 × 5 mm) were cut from the undecayed and decayed test samples – 5 mm from the edge of the samples. The cross section (across the board thickness) was given a smooth surface by hand cutting with a razor blade after softening the samples in water.

Samples dried at room conditions were sputter-coated with gold using a LUXOR™ gold coater (APTCO Group, Germany). The layer thickness was 15 nm. The cross section was observed with a Tescan Vega 4 scanning electron microscope (TESCAN ORSAY HOLDING, a. s., Czech Republic). The scans were prepared in resolution scanning mode in a high vacuum using a detector of secondary electrons. The images were obtained with these settings: landing energy 7 keV, beam current 30 pA, scan speed 3 (1 µs/pixel), image averaging with accumulation of 15 images (to minimise charging of the rough surface) and image resolution of 2,048×1,536 pixels. The magnification of the microscope was set to 1,000× or 5,000×. Images were taken 2 mm from the sample edge, which was 2 mm deep under the board surface.

Statistical Analysis

The data were compared in Excel graphs or *via* a one-way analysis of variance (ANOVA) test and a Tukey honestly significant difference (HSD) test using Statistica software (TIBCO Software, CA).

RESULTS AND DISCUSSION

Particle Size Distribution and Density

The density results of the composites are summarised in Fig. 2. The density varies from 871 to 982 kg/m^3 for all types of composites with no statistically significant differences among them. A lower density compared to composites was measured for HDF board with a mean value of 836 kg/ m^3.

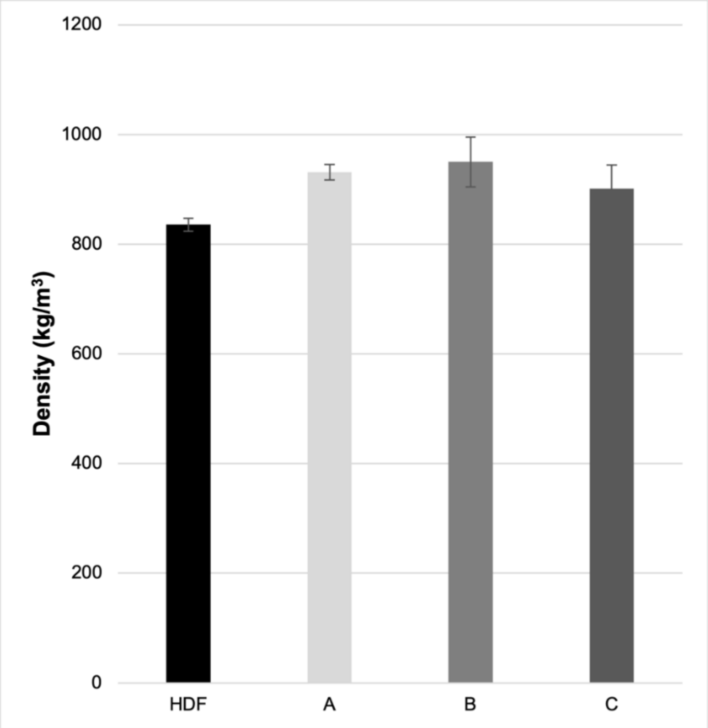

Fig. 2. Density of composites with different composition (A – wood:PHBV, B – wood:rHDPE, C – wood: rLDPE) and reference HDF

The particle size distribution of composites after the high-speed milling is shown in Table 2. The values demonstrated that there were similar particle sizes in milled composite material regardless of the input material. However, the smallest particles were measured for composite C (89% particles <500 µm).

Table 2. The Particle Size Distribution in Different Composite Mixtures

Particle Size (µm)	Percentage (%)			
	A	B	C	D
≥ 1,000	3	4	1	5
500 -1,000	15	16	10	17
200 - 500	40	41	43	39
≤ 200	42	39	46	39

Mechanical Properties

The results indicated that the flexural strength (MOR; Fig. 3A) and modulus of elasticity (MOE; Fig. 3B) of the tested composites were considerably lower than those of high-density fibreboard (HDF), with one exception for the MOE of composite A, which exceeded HDF by about 30%. PHBV is a polymer with greater flexural strength (30 MPa) and modulus (1.3 GPa) than both PE polymers (Persico *et al.* 2011). The addition of the natural fibres leads to a considerable drop in strength, but on the other hand its stiffness increases (Guo *et al.* 2011; Persico *et al.* 2011; Bajracharya *et al.* 2017).

The flexural strength and modulus of composite B were comparable with results of Li (2012), who used a slightly higher ratio (70:30) of poplar wood and rHDPE for production of extruded composite. The determined MOE was in the range of 2.5 to 2.8 MPa and MOR was in the range of 22 to 27 MPa for different particle sizes without an added coupling agent.

Mohammed and Meincken (2021) reported for WPC (rLDPE and *Acacia saligna* wood, 40:60) a flexural strength between 13 and 21 MPa and MOE between 400 and 650 MPa, both affected by different processing factors – particle size, press time, and temperature. Similar values of MOR were found for composite C, but MOE was three times greater, which could be related to the wood particles used in the present study – softwood particles of smaller dimensions. In contrast to PHBV, the flexural strength of composites B and C were similar in both cases to a neat polymer (Fig. 3) – 22 MPa for rHDPE (Adhikary *et al.* 2008) and 12 MPa for rLDPE (Beg *et al.* 2016). Similarly to PHBV, the addition of wood particles increased the stiffness of composite compared to neat polymers (rLDPE ~ 200 MPa, rHDPE ~ 840 MPa).

PHBV is a relatively brittle thermoplastic with greater stiffness and lower elongation to break (Zaidi and Croski 2019) than rHDPE and rLDPE. The differences in the load-displacement curves of HDF and three polymer-wood composites are shown in Fig. 4. The curve for HDF initially has a steep slope followed by a sharp drop in stress after failure, which occurred at an average strain of 6 mm. Composite A showed a similar curve to HDF, but with considerably lower force at breaking point. In contrast, composites B and C reached similar flexural strength after significant deformation, whereas the strain decreased more gradually, indicating the more ductile behaviour of the composite. This behaviour is typical for wood/rHDPE composites without coupling agents, whose addition significantly improves the flexural strength as well as the stiffness of the composite (Adhikary *et al.* 2008).

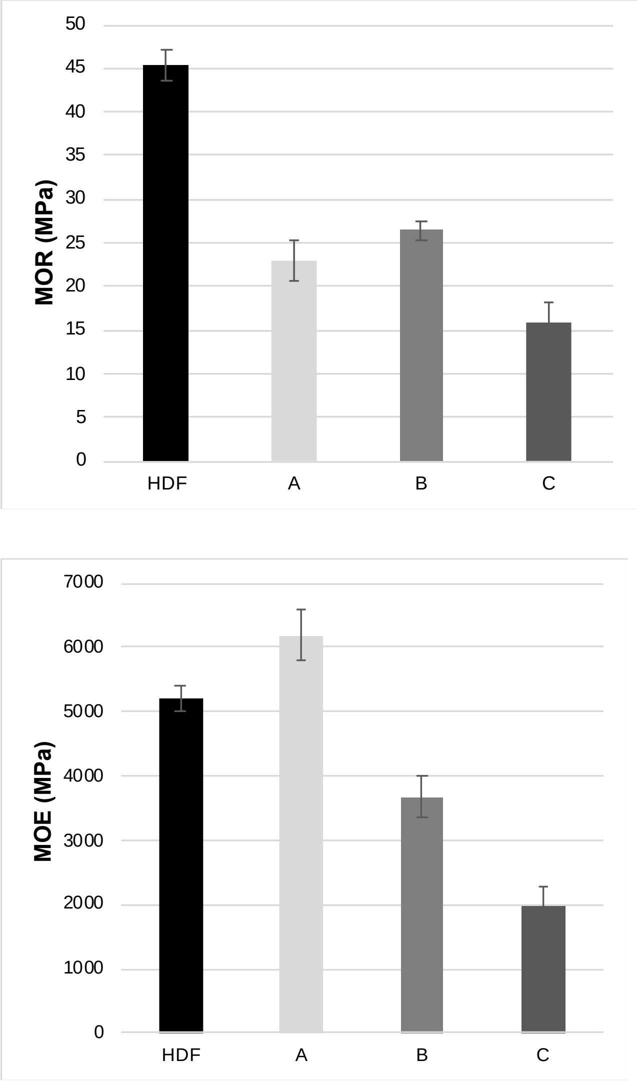

Fig. 3. The flexural strength (top) and modulus of elasticity (bottom) of tested composites (A – wood:PHBV, B – wood:rHDPE, C – wood: rLDPE) and reference HDF

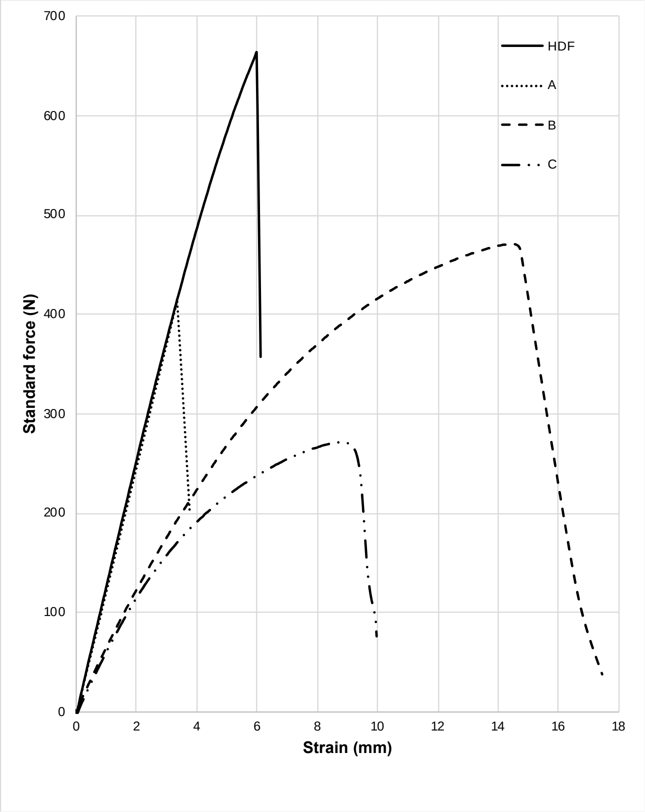

Fig. 4. Load-displacement curves of the typical representative samples of HDF and wood/plastic composites

According to the requirements of EN 312:2015, 10.5 and 11.0 MPa are the minimum values for the flexural strength of particleboard (thickness 6 to 13 mm) for general use and interior equipment (including furniture); the flexural strength for P2 class is 1.8 MPa. All tested composites meet these requirements for use in dry environments.

The greatest hardness was found in HDF samples despite its lowest density (Fig. 5) and the least in composite C. A and B composites showed similar values of hardness with no statistically significant differences.

The hardness of WPC depends on the polymer loading and the hardness of the polymer (Ibach and Rowell 2013), whereas with increasing filler content the hardness of the composite increases (Teymoorzadeh and Rodrigue 2015). At constant wood-particle content, the lesser hardness of composite C responds to the lesser hardness of rLDPE compared to rHDPE (Shebani *et al*. 2018).

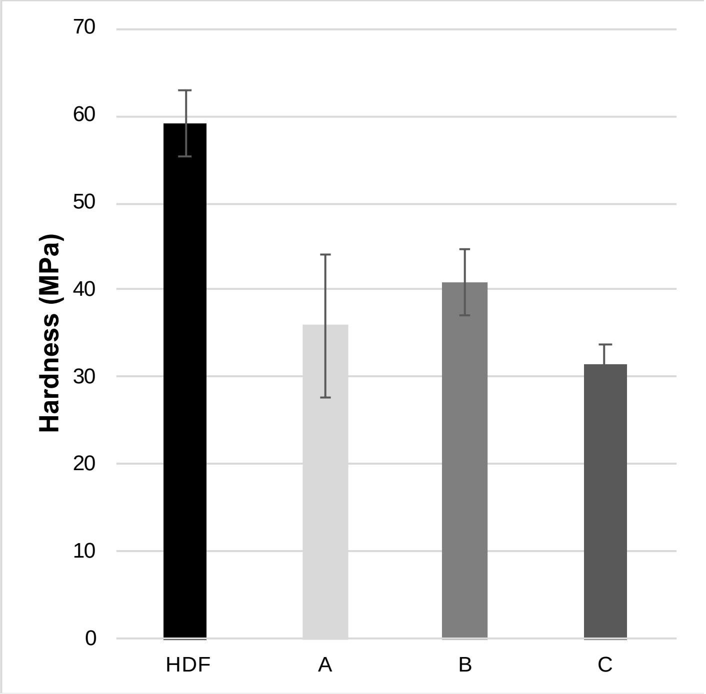

Fig. 5. Hardness of composites (A, wood:PHBV; B, wood:rHDPE; C, wood:rLDPE) and HDF

Physical Properties

One of the important factors of board quality is thickness swelling in contact with moisture. The greatest water absorption and thickness swelling were measured in HDF samples. In general, the wood/plastic composites showed significantly less swelling than HDF, whereas the lowest value was recorded for sample B (3%). The lesser thickness swelling of rHDPE/wood composite (50/50) was reported by Adhikary *et al.* (2008), when the maximum value was not greater than 7% after 63 days. Lesser swelling of composites can be explained by their lower proportion of wood and almost no water absorption of used polymers. The significant thickness swelling, especially in the initial stage of immersion, is usually related to the poor dispersion of particles and their adhesion to the plastic matrix, which allows easy access of water to the wood (Adhikary *et al.* 2008). It can be mentioned that the thickness swelling of all tested composites was less than the maximum value for the 24-hour requirement for some wood-based panels – non-load-bearing particle board used in humid conditions (17%; ČSN EN 312:2011) or general purpose MDF for use in dry conditions (15%; ČSN EN 622-5:2010).

The highest value of WA (54%) was measured for composite A based on PHBV, despite its lowest content of wood particles. When WPC is immersed in water, the weight water absorption is typically between 0.7 and 3% after 24 h; after long-term immersion, WPC absorbs as much as 20 to 30% of water by weight (Klyosov 2007). In contrast to thickness swelling, which was in agreement with former studies, the water absorption of tested composites after long-term immersion was significantly higher. Chan *et al.* (2018) reported a total saturated water absorption value of 18.8% for extruded PHBV/wood composite (50/50). The greatest water absorption of 23.5% was found in moulded composite made of virgin rHDPE with 50 wt.% wood content after 1,512 h (Adhikary *et al.* 2008). Feng *et al.* (2008) showed even slower water absorption in rHDPE/wood composite (40/60), when the moisture content reached only 8% after 32 days of immersion.

The water uptake in WPCs is slower than in solid wood; the rate and amount of absorbed water can vary widely depending on wood content, wood particle size, processing methods, and additives. The water uptake in WPC usually increases with the content and size of wood particles and decreases with the use of coupling agents, which increase the adhesion between wood and polymer (Kaboorani 2017). In composites with well-dispersed wood particles, the polymer matrix should act as a barrier and should prevent the formation of a continuous pathway for rapid water penetration into the material. Based on the results, a combination of manufacturing process and high wood content leads to a porous, interconnected, composite structure that easily absorbs moisture.

The fact that composite C has a lower water absorption than composite B, which however has a greater swelling, can be explained by the difference in the proportion of particle size. LDPE has a 10% larger proportion of small particles, which can be better wrapped and protected from water intake. The difference in absorption is not that significant. The swelling could theoretically be caused by the mechanical properties of both plastics. HDPE is stronger and stiffer in tension, while LDPE has less strength and a significantly lower MOE, which can affect how the matrix withstands swelling pressure.

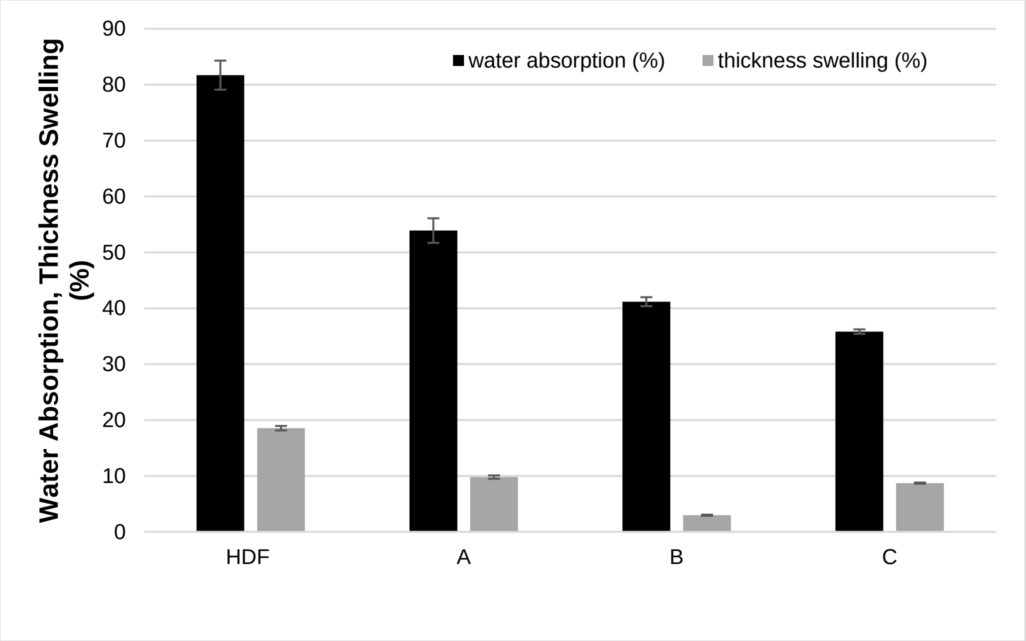

Fig. 6. Water uptake and thickness swelling of tested composites (A – wood:PHBV, B – wood:rHDPE, C – wood:rLDPE) and reference HDF

Decay Test

The mass losses of the tested composites due to fungal degradation are presented in Table 3 for brown-rot fungus, *Coniophora puteana*, and Table 4 for white-rot fungus, *Pleurotus ostreatus*. The resistance of individual composites to both fungi was similar. The lowest mass loss was observed in composite D with added marble and the highest in composite A, where only PHBV and wood were used. The moisture content of composites B and D at the end of exposure was below 25 to 30%, which is necessary for fungal growth and degradation. Considering the composite's greater density, the low water absorption of both matrix polymers and the wood ratio, the moisture content of wood particles should be high enough for fungi degradation.

C. puteana caused about half the mass loss in composite A as in reference pine wood. Because the wood portion in all the tested composites was less than that of solid reference wood, the potential mass loss was proportionally lower. Taking into account the composite's greater density, the absolute mass loss was comparable with reference pine wood and also with HDF board, as was shown by the decay susceptibility index (Table 3). The rHDPE-based composite showed only one-third of the mass loss, although the wood ratio was higher than in the wood/PHBV composite. Composites with higher wood filler content absorb a large amount of water more easily, which makes them more susceptible to microorganism degradation. Most WPCs are not decay resistant because the polymer merely fills the lumens and does not enter the cell walls, which makes the cell walls accessible to moisture and decay organisms (Ellis 2000).

Though PHBV absorbs only a small amount of water (up to 1%), it reacts with water molecules and is slowly hydrolysed over time (Badia *et al.* 2014; Deroiné *et al.* 2014). The molecular weight of biodegradable polymers is reduced by hydrolysis, and the degradation products are considered to be the metabolites for subsequent microbial degradation (Pantani and Sorrentino 2013). Therefore, it is assumed that PHBV hydrolysis contributed to the greater mass loss of composite A. Except for exposure time, pH has a very strong impact on the degradation of bio-degradable polymers, because it acts on both the reaction mechanism and the reaction kinetics (Gorrasi and Pantani 2017; Muhamad *et al.* 2006). The pH of a composite can be influenced by additives (marble) or by the wood itself. The original pH of composite A (5.8) was changed to alkaline 8.3 (D), which provides conditions suitable for PHBV hydrolysis (Muhamad *et al.* 2006). The greater resistance of composite D is related to the presence of marble, which effectively changed the acidic environment to alkaline, thus inhibiting degradation activity, especially in the case of brown-rot fungi (Table 3). Despite its negligible mass loss, composite D showed an incoherent inner structure after exposure. The alkaline environment protects the composite against fungal decay but at the same time supports the hydrolysis of the PHBV matrix, which significantly influences the composite structural stability.

Composite B showed low mass loss, which is in agreement with former experiments (Kamdem *et al.* 2004; Pendleton *et al.* 2002). Generally, the lower mass loss of wood/plastic composites is due to the inaccessibility of degradable wooden particles, which are partly encapsulated by polymer matrix. There are still voids at the interface of plastic and wood due to the weak interface adhesion force caused by differences in molecular polarity, which provides a pathway for water and fungi penetration into the material (Mankowski and Morrell 2000; Pendleton *et al.* 2002). Also, the increased wood portion increased the possibility of the development of a continuous pathway for fungal spread.

Comparable decay susceptibility values of individual composites were found for *P. ostreatus*; in this case the absolute mass loss was found to be almost double for reference beech wood, which is related to the preferential degradation of hardwoods by white-rot fungi as shown for example by Bari *et al.* (2020). A similar effect was observed in HDF board, which is produced solely from softwood fibres.

Table 3. Mean Mass Loss (ML), Moisture Content (w) and Decay Susceptibility (DS) of Tested Composites after Exposure to *Coniophora puteana*

C. puteana				density (kg/m^3)	pH (before/after)
	ML (%)	w (%)	DS (g/cm^3)		
A	16.7 (24.8)a	40.0 (13.6)a	0.15 (24.4)a	909 (4.1)a	5.8/5.3
B	7.3 (11.6)c	18.6 (11.1)b	0.06 (6.5)c	876 (6.2)a	4.9/4.6
D	0.5 (16.2)b	19.5 (31.9)b	0.01 (16.9)b	904 (0.8)a	8.3/7.8
HDF	22.3 (12.8)d	62.6 (7.7)c	0.13 (12.6)a	586 (1.6)b	-
Pine	29.5 (12.0)e	61.0 (21.3)c	0.16 (12.1)a	530 (2.9)b	-

Note: Composite density before exposure to fungus and pH values of water leachate from tested composites before and after exposure. Variation coefficient in brackets.

Table 4. Mean Mass Loss (ML), Moisture Content (w) and Decay Susceptibility (DS) of Tested Composites after Exposure to *Pleurotus ostreatus*

P. ostreatus				density (kg/m^3)	pH (before/after)
	ML (%)	w (%)	DS (g/cm^3)		
A	17.2 (3.1)a	45.2 (4.2)a	0.15 (8.6)a	897 (6.1)a	5.8/5.4
B	7.6 (21.6)c	29.2 (15.7)b	0.07 (18.2)c	930 (4.3)a	4.9/4.9
D	3.4 (20.6)b	22.5 (3.7)b	0.03 (20.4)b	919 (1.5)a	8.3/6.6
HDF	15.1 (3.9)a	78.1 (2.9)c	0.09 (4.1)d	583 (0.8)b	-
Pine	40.3 (7.0)d	120.5 (7.9)d	0.28 (4.1)e	693 (4.4)c	-

Note: Composite density before exposure to fungus and pH values of water leachate from tested composites before and after exposure. Variation coefficient in brackets.

Scanning Electron Microscopy Analysis

Microscopic investigation of the wood-plastic composites showed differences between composites with PHBV and rHDPE. Both composites contained voids and agglomerated wood fibres (Fig. 7), which reduced the mechanical strength of the resulting composite. PHBV was mixed with wood fibres more uniformly (Fig. 7a) than rHDPE (Fig. 7f), probably due to the PHBV polymer being more brittle. However, PHBV was less homogenous (Fig. 7d) and it contained many small voids. rHDPE consisted of large homogenous pieces with large agglomerations of wood fibres among them (Fig. 7f, g). Larger voids in and among rHDPE particles were also observed.

The results also indicated differences in wood-plastic boundaries. Gaps between wood fibres and PHBV were often observed, especially in the case of bigger clusters of tracheids (Fig. 7b, e). rHDPE stuck to wood fibres much better, forming fewer voids and interfacial gaps (Fig. 7g). However, penetration to fibre agglomerations was low, probably due to low pressure during production or the low melt flow index (high viscosity) of molten rHDPE (Cruz and Zanin 2004). Pure PHBV (ENMAT Y1000P) polymer has a much higher melt flow index (Yatigala *et al.* 2018), which could improve penetration into wood fibre aggregates. However, many small voids were observed (Fig. 7a, b, c, d, e). The applied pressure of 3.5 MPa was optimal to produce boards with high density, but probably not high enough to close all the voids in the composite. Berthet *et al.* (2015) showed a much more uniform microstructure in wheat straw/PHBV composite without voids after compression at 15 MPa.

A non-homogenous structure of PHBV with nanoparticles smaller than 2 µm (Fig. 1d) was observed in both composites. This could be due to the presence of boron nitride in the biopolymer as a nucleating agent (Berthet *et al.* 2015). This nanofiller could decrease the smoothness of the sample surface prepared for microscopy.

The microstructure of the wood/PHBV composite with marble powder (Fig. 7c, d, e) was similar to the pure wood/PHBV composite (Fig. 7a, b). It contained visible marble particles of between 5 and 40 µm (Fig. 7e). Many of these were found loose in their place, which indicated low adhesion between the particles and the PHBV matrix. Although fine marble powder in small quantities can help to fill voids in fibre cement boards and thus improve their strength (Khorami and Ganjian 2013), this effect was not observed in wood/PHBV composite.

Microscopic investigation showed that neither PHBV nor rHDPE polymer can prevent wood-rotting fungi from colonising the composite, as is shown for *Coniophora puteana* (Fig. 8a, c). Many hyphae were found in the cell lumina and polymer voids of both samples. However, hyphae were not observed in wood/PHBV composite containing marble powder (Fig. 8b), which had the lowest mass loss of 3.3%.

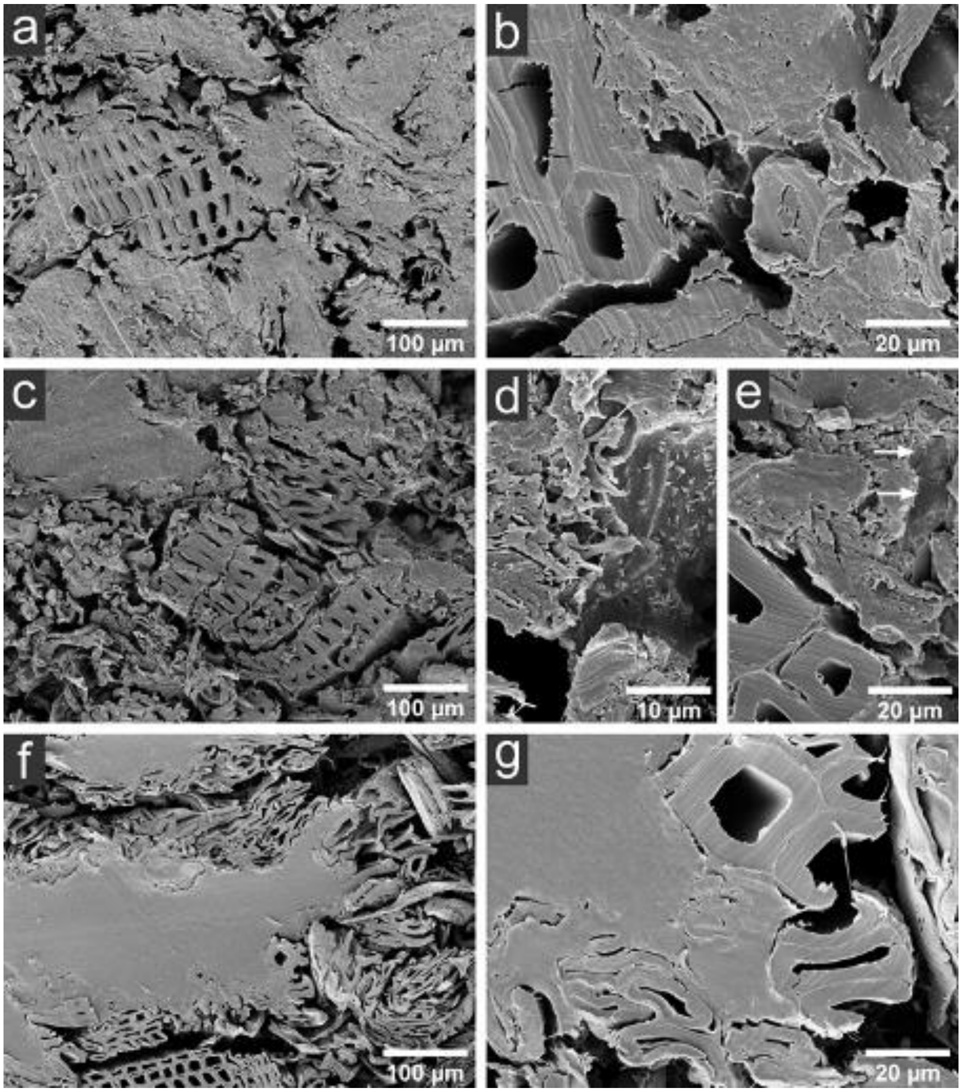

Fig. 7. PHBV and wood composite (a), detail of the wood-PHBV boundary (b), PHBV and wood composite with marble powder (c), PHBV non-homogenous structure on cut surface (left) and natural surface in a void (right) (d), detail of wood-PHBV boundary (e) with marble particles (white arrows), rHDPE and wood composite (f) and detail of wood-rHDPE boundary (g)

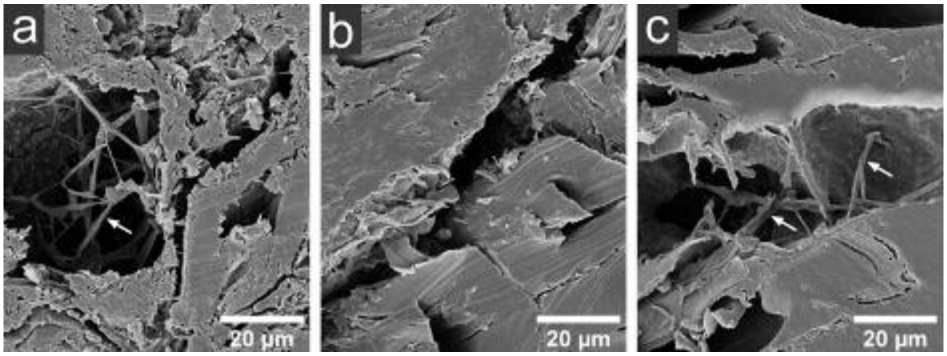

Fig. 8. Samples after decay test with *Coniophora puteana*. PHBV and wood composite (a), with hyphae (white arrow), PHBV and wood composite with marble powder and no visible hyphae (b), rHDPE and wood composite (c) with hyphae growing through voids (white arrows)

The images in Figures 7 and 8 provide evidence of lack of contact at many interfaces between cellulosic material and plastic material. In connection with improving the adhesion between wood and polymers, there are various additives that can be used as compatibilizing agents for next research steps.

- Maleic Anhydride Graft Polymers: Maleic anhydride (MAH) is commonly grafted onto polymer chains to create reactive sites that can form chemical bonds with wood components. Maleic anhydride grafted polypropylene (MAPP) and maleic anhydride grafted polyethylene (MAPE) are often used as compatibilizers for wood-polymer composites.
- Isocyanates: Isocyanate-based compounds such as polyurethane prepolymers can react with the hydroxyl groups present in the wood to form covalent bonds with the polymer matrix. Isocyanates are often used as coupling agents to improve adhesion between wood and polymers.
- Alkoxysilanes: Alkoxysilanes possess functional groups, typically methoxy (CH_3O-), ethoxy (C_2H_5O-), or propoxy (C_3H_7O-), attached to a silicon atom. These functional groups can react with both the hydroxyl groups present in wood and the functional groups in the polymer matrix, forming covalent bonds. The silane coupling agent acts as a bridge between the wood and polymer, improving the interfacial adhesion and compatibility between the two materials.
- Epoxy-based systems: Epoxy resins can be used as compatibilizers due to their ability to react with wood components to form strong adhesive bonds. Epoxy-based systems, including epoxy resins and curing agents, can improve adhesion between wood and polymers.
- Polymeric adhesives: Some polymeric adhesives, such as polyurethane adhesives and epoxy adhesives, can be used as compatibilizers to promote adhesion between wood and polymers. These adhesives often have good adhesion properties and can provide a strong interface bond.

It is important to note that the selection of an appropriate compatibilizer depends on the specific wood and polymer materials used as well as the desired properties of the final composite. Performing compatibility testing and considering Hansen solubility parameters can be helpful in identifying suitable additives to achieve better adhesion between wood and polymers. (Hansen 2007).

CONCLUSIONS

1. High-energy milling has great potential due to its ability to optimize particle size and morphology, homogenize mixtures, and dry them simultaneously.
2. High density fibreboard (HDF) products have greater bending strength than tested wood-plastic composites (WPCs), with the highest hardness values found in HDF and the lowest in composites with recycled low-density polyethylene (rLDPE). Wood-rHDPE composite with 60% wood and 40% rHDPE exhibited the best physico-mechanical properties and met standards for construction board in humid environments.
3. Wood/PHBV composite containing marble powder showed the highest resistance to wood-rotting fungi, potentially due to the alkaline environment created by the presence of marble, inhibiting fungal activity.
4. Microscopic analysis of wood-plastic composites with PHBV and rHDPE showed differences in structure, adhesion, and fungal colonization. PHBV had better fiber mixing but lacked homogeneity and contained small voids. rHDPE exhibited larger pieces with wood fiber agglomerations and voids. Adhesion was better with rHDPE, and limited penetration into fiber clusters was observed. PHBV with marble powder showed similar characteristics but weak adhesion to marble particles. Fungal colonization occurred in both composites, except in wood/PHBV with marble powder, which had the lowest mass loss.

ACKNOWLEDGMENTS

This work was supported by the European Regional Development Fund, project "Valid" reg. no. ATCZ226.

REFERENCES CITED

Adhikary, K. B., Pang, S., and Staiger, M. P. (2008). “Dimensional stability and mechanical behaviour of wood–plastic composites based on recycled and virgin high-density polyethylene (rHDPE),” *Composites Part B: Engineering* 39(5), 807-815. DOI: 10.1016/j.compositesb.2007.10.005

Alves, C., Ferraria, A. M., Botelho do Rego, A. M., and Botelho do Rego, S. (2015). “Lignocellulosic materials as a source of value-added chemicals: A review,” *Biomass Conversion and Biorefinery* 5(2), 141-161.

Atik, M., Al-Qutayri M., and Amara H. (2018). “Effect of ball milling parameters on the synthesis of magnetite nanoparticles by wet milling,” *Materials Science and Engineering: B* 235. DOI: 10.1016/j.mseb.2018.09.011

Austin, L. G., and Trass, O. (1997). “Size reduction of solids crushing and grinding equipment,” in: M. E. Fayed and L. Otten (eds.), *Handbook of Powder Science and Technology*, Springer, Boston. DOI: 10.1007/978-1-4615-6373-0_12

Badia, J. D., Kittikorn, T., Strömberg, E., Santonja-Blasco, L., Martínez-Felipe, A., Ribes-Greus, A., Ek, M., and Karlsson, S. (2014). “Water absorption and hydrothermal performance of PHBV/sisal biocomposites," *Polymer Degradation and Stability* 108, 166-174. DOI: 10.1016/j.polymdegradstab.2014.04.012

Baláž, P. (2008). *Mechanochemistry in Nanoscience and Minerals Engineering,* Springer, Berlin, Heidelberg.

Bari, E., Daniel, G., Yilgor, N., Kim, J. S., Tajick-Ghanbary, M. A., Singh, A. P., and Ribera, J. (2020). "Comparison of the decay behavior of two white-rot fungi in relation to wood type and exposure conditions," *Microorganisms* 8(12), 1931. DOI: 10.3390/microorganisms8121931

Bajracharya, R. M., Bajwa, D. S., and Bajwa, S. G. (2017). "Mechanical properties of polylactic acid composites reinforced with cotton gin waste and flax fibers," *Procedia Engineering* 200, 370-376. DOI: 10.1016/j.proeng.2017.07.052

Beg, M. D., Kormin, S., Bijarimi, M. P., and Zaman, H. U. (2016). Preparation and characterization of low-density polyethylene/thermoplastic starch composites, *Advances in Polymer Technology* 35(1). DOI: 10.1002/adv.21521

Berthet, M. A., Gontard, N., and Angellier-Coussy, H. (2015). "Impact of fibre moisture content on the structure/mechanical properties relationships of PHBV/wheat straw fibres biocomposites," *Composites Science and Technology* 117, 386-391. DOI: 10.1016/j.compscitech.2015.07.015

Chaharmahali, M., Mirbagheri, J., Tajvidi, M., Najafi, S., and Mirbagheri, Y. (2008). "Mechanical and physical properties of wood-plastic composite panels," *Journal of Reinforced Plastics and Composites* 29(2), 310-319. DOI: 10.1177/0731684408093877

Chan, C. M., Vandi, L.-J., Pratt, S., Halley, P., Richardson, D., Werker, A., and Laycock, B. (2018). "Mechanical performance and long-term indoor stability of polyhydroxyalkanoate (PHA)-based wood plastic composites (WPCs) modified by non-reactive additives," *European Polymer Journal* 98, 337-346. DOI: 10.1016/j.eurpolymj.2017.11.041

Chen, L., and Zhong, G. J. (2011). "Effect of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) on the mechanical properties and morphology of polylactic acid/thermoplastic starch blends," *Journal of Applied Polymer Science* 121(1), 89-96. DOI: 10.1002/(SICI)1097-4628(20000705)77:1<89::AID-APP12>3.0.CO;2-5

Clemons, C. (2002). "Wood-plastic composites in the United States: The interfacing of two industries," *Forest Products Journal* 52(6), 10-18.

Cruz, S. A., and Zanin, M. (2004). "Assessment of dielectric behavior of recycled/virgin high density polyethylene blends," *IEEE Transactions on Dielectrics and Electrical Insulation* 11(5), 855-860. DOI: 10.1109/TDEI.2004.1349791

Deroiné, M., Le Duigou, A., Corre, Y.-M., Le Gac, P.-Y., Davies, P., César, G., and Bruzaud, S. (2014). "Accelerated ageing and lifetime prediction of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) in distilled water," *Polymer Testing* 39, 70-78. DOI: 10.1016/j.polymertesting.2014.07.018

Ellis, W. D. (2000). "Wood-polymer composites: Review of processes and properties," *Molecular Crystals and Liquid Crystals* 353(1), 75-84. DOI: 10.1080/10587250008025649

Fuerstenau, M. C., and Han, K. N. (2003). *Principles of Mineral Processing*, Society for Mining, Metalurgy, and Exploration, Inc., Littleton.

Ganesan, K., and Velusamy, V. (2017). "Review on high-energy ball milling (HEBM) and its applications," *Journal of the Mechanical Behavior of Materials* 26(4), 172-184.

Gorrasi, G., and Pantani, R. (2017). "Hydrolysis and biodegradation of poly(lactic acid)," in: *Synthesis, Structure and Properties of Poly(lactic acid)*, Di Lorenzo M., Androsch R. (eds)., *Advances in Polymer Science* 279, Springer, Cham. DOI: 10.1007/12_2016_12

Guo, W. J., Bao, F. C., Wang, Z., Chang, L., and Gao, L. (2011). "Effects of wood fiber content on structure and properties of WF/PLA bio-composites," *Advanced Materials Research* 217-218, 606-613. DOI: 10.4028/www.scientific.net/AMR.217-218.606

Hansen, C. M. (2007). *Hansen Solubility Parameters: A User's Handbook*, 2nd Ed., CRC Press, Boca Raton. ISBN 0-8493-7248-8.

He, J., Zhang, S., Wang, Y., and Liu, Y. (2017). "Changes of cellulose crystalline structure of lignocellulosic biomass in the process of ball milling," *Cellulose* 24(10), 4351-4361.

Höglmeier, K., Weber-Blaschke, G., and Richter, K. (2017). "Erratum: Potentials for cascading of recovered wood from building deconstruction—A case study for south-east Germany (2013) 78(81-91) (S0921344913001444)(10.1016/j.resconrec.2013.07.004)). *Resources, Conservation and Recycling* 117, 304-314. DOI: 10.1016/j.resconrec.2015.10.030

Ibach, R. E., and Rowell, R. M. (2013). "Lumen modification," in: *Handbook of Wood Chemistry and Wood Composites*, R. M. Rowell (ed.), CRC Press, Boca Raton, FL, USA.

Irle, M., Privat, F., Couret, L., Belloncle, C., Déroubaix, G., Bonnin, E., and Cathala B. (2019). "Advanced recycling of post-consumer solid wood and MDF," *Wood Material Science and Engineering* 14(1), 19-23. DOI: 10.1080/17480272.2018.1427144

Kaboorani, A. (2017). "Characterizing water sorption and diffusion properties of wood/plastic composites as a function of formulation design," *Construction and Building Materials* 136, 164-172. DOI: 10.1016/j.conbuildmat.2016.12.120

Kamdem, D. P., Jiang, H., Cui, W., Freed, J., and Matuana, L. M. (2004). "Properties of wood plastic composites made of recycled rHDPE and wood flour from CCA-treated wood removed from service," *Composites Part A: Applied Science and Manufacturing* 35(3), 347-355. DOI: 10.1016/j.compositesa.2003.09.013

Khorami, M., and Ganjian, E. (2013). "The effect of limestone powder, silica fume and fibre content on flexural behaviour of cement composite reinforced by waste kraft pulp," *Construction and Building Materials* 46, 142-149. DOI: 10.1016/j.conbuildmat.2013.03.099

Klyosov, A. A. (2007). *Wood-Plastic Composites,* John Wiley and Sons, Inc., Hoboken, NJ, USA. DOI: 10.1002/9780470165935

Li, Y. (2012). "Effect of coupling agent concentration, fiber content, and size on mechanical properties of wood/rHDPE composites," *International Journal of Polymeric Materials* 61(11), 882-890. DOI: 10.1080/00914037.2011.617338

Mankowski, M., and Morrell, J. J. (2000). "Patterns of fungal attack in wood-plastic composites following exposure in a soil block test," *Wood and Fiber Science* 32, 340-345.

Mikkonen, K. S., Lahtinen, P., Pöhler, T., Oksman, K., and Karppinen, M. (2016). "High-energy ball milling as a tool for nanomaterials synthesis: opportunities and challenges," *Surface Innovations* 4(1), 23-31.

Mohammed, A. S., and Meincken, M. (2021). "Properties of low-cost WPCs made from alien invasive trees and rLDPE for interior use in social housing," *Polymers* 13(15), 2436. DOI: 10.3390/polym13152436

Muhamad, I. I., Joon, L. K., and Noor, M. (2006). "Comparing the degradation of poly-β-(hydroxybutyrate), poly-β-(hydroxybutyrate-co-valerate)(PHBV) and PHBV/cellulose triacetate blend," *Malaysian Polymer Journal* 1(1), 39-46.

Nayborodenko, Y. S., Kasatskiy, N. G., Sergeeva, E. G., and Lepakova, O. K. (2002). "Effect of mechanical activation on high-temperature and phase formation of low-calorie intermetallic compounds," *Chemistry for Sustainable Development* 10, 199-204.

Pantani, R., and Sorrentino, A. (2013). "Influence of crystallinity on the biodegradation rate of injection-moulded poly(lactic acid) samples in controlled composting conditions," *Polymer Degradation and Stability* 98(5), 1089-1096. DOI: 10.1016/j.polymdegradstab.2013.01.005

Pendleton, D. E., Hoffard, T. A., Adcock, T., Woodward, B., and Wolcott, M. P. (2002). "Durability of an extruded rHDPE/wood composite," *Forest Products Journal* 52(6), 21-27.

Persico, P., Acierno, D., Carfagna, C., and Cimino F. (2011). "Mechanical and thermal behavior of ecofriendly composites reinforced by kenaf and caroà fibers," *International Journal of Polymer Science* 2011, 1-7. DOI: 10.1155/2011/841812

Prasad, R., Srinivasan, R., and Ramanujam, K. (2019). "High-energy ball milling of wheat straw: Effects of particle size and milling time on enzymatic hydrolysis," *Journal of Renewable Materials* 7(2), 159-167.

Prokšan, Z., Procházka, M., Faltus, M., Procházka, M., and Valentin, J. (2017). "Use of high-speed milling in waste management," Symposium on waste management 1(9)

Sahu, S., and Bose, S. (2010). "Milling parameters affecting particle size and morphology during high-energy ball milling of nanopowders," *Journal of Nanoparticle Research* 12(2), 2010. DOI: 10.1007/s11051-013-1757-6

Shebani, A., Klash, A., Elhabishi, R., Abdsalam, S., Elbreki, H., and Elhrari, W. (2018). "The influence of rLDPE content on the mechanical properties of rHDPE/rLDPE blends," *Research and Development in Material Science* 7(5), 791-797. DOI: 10.31031/RDMS.2018.07.000672

Sheykholeslami, M., and Tofighian, M. (2013). "Effect of ball milling parameters on the particle size in nanocrystalline powders," *Journal of Nanoparticle Research* 15(6). DOI: 10.1007/s11051-013-1757-6

Snow, R. H., Allen, T., Ennis, B. J., and Litster, J. D. (2009). "Size reduction and size enlargement," http://chc.edu.vn/forum/uploads/file//2009-08-16_092142_Chap20.pdf

Spierling, S., and Welle, A. (2016). "Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) – Properties and applications in biomedicine and biotechnology," *Bioengineering* 3(2), 18.

Teymoorzadeh, H., and Rodrigue, D. (2015). "Biocomposites of wood flour and polylactic acid: Processing and properties," *Journal of Biobased Materials and Bioenergy* 9(2), 252-257. DOI: 10.1166/jbmb.2015.1510

Yatigala, N. S., Bajwa, D. S., and Bajwa, S. G. (2018). "Compatibilization improves physico-mechanical properties of biodegradable biobased polymer composites," *Composites Part A: Applied Science and Manufacturing* 107, 315-325. DOI: 10.1016/j.compositesa.2018.01.011

Youngquist, J. A., Myers, G. E., Muehl, J. H., Krzysik, A. M., and Clemons, C. M. (1995). "Composites from recycled wood and plastics," Report for May 1990-July 1993. United States: N. p., 1995. Web.

Zaidi, Z., and Crosky, A. (2019). "Unidirectional rubber-toughened green composites based on PHBV," *Sustainability* 11(8), 2411. DOI: 10.3390/su11082411

Article submitted: March 14, 2023; Peer review completed: April 8, 2023; Revised version received and accepted: June 16, 2023; Published: July 6, 2023.
DOI: 10.15376/biores.18.3.5635-5654