

Article

New Bio-Composites Based on Polyhydroxyalkanoates and *Posidonia oceanica* Fibres for Applications in a Marine Environment

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Abstract: Bio-composites based on polyhydroxyalkanoates (PHAs) and fibres of *Posidonia oceanica* (PO) were investigated to assess their processability by extrusion, mechanical properties, and potential biodegradability in a natural marine environment. PHAs were successfully compounded with PO fibres up to 20 wt % while, at 30 wt % of fibres, the addition of 10 wt % of polyethylene glycol (PEG 400) was necessary to improve their processability. Thermal, rheological, mechanical, and morphological characterizations of the developed composites were conducted and the degradation of composite films in a natural marine habitat was evaluated in a mesocosm by weight loss measure during an incubation period of six months. The addition of PO fibres led to an increase in stiffness of the composites with tensile modulus values about 80% higher for composites with 30 wt % fibre (2.3 GPa) compared to unfilled material (1.24 GPa). Furthermore, the impact energy markedly increased with the addition of the PO fibres, from 1.63 (unfilled material) to 3.8 kJ/m² for the composites with 30 wt % PO. The rate of degradation was markedly influenced by seawater temperature and significantly promoted by the presence of PO fibres leading to the total degradation of the film with 30 wt % PO in less than six months. The obtained results showed that the developed composites can be suitable to manufacture items usable in marine environments, for example, in natural engineering interventions, and represent an interesting valorisation of the PO fibrous wastes accumulated in large amounts on coastal beaches.

Keywords: polyhydroxyalkanoates; composites; *Posidonia oceanica*; marine degradation

1. Introduction

Bio-based and biodegradable polymers derived from renewable resources (in particular agricultural and biomass feedstocks) have attracted increasing attention over the last two decades due to their low environmental impact and no dependence on fossil resources [1–4]. The biodegradability of bio-based materials in different natural environments, not only in composting systems, is an important property for their sustainability and reduction of environmental impact associated with the petrolchemical-based plastics. It has been estimated that several hundred thousand tons of plastics are directly or indirectly discarded into the marine environment every year with a consequent

negative impact on the marine pelagic and benthic habitats [5–7]. Consequently, the development of plastics biodegradable in marine environment is the key to prevent or mitigate in the future the problems caused by marine plastic debris [8]. To date, few biopolymers are biodegradable in the marine environment; among these there are the polyhydroxyalkanoates (PHAs) [3]. PHAs are a family of microbial polyesters, synthesized by a wide range of microorganisms under conditions of nutrient stress and have similar thermoplastic properties of conventional plastics [9,10]. One of the main limitations in the application of PHAs, for the production of single use items, is based on their relatively high cost (5–7 €/kg) compared to other polymers [11], restricting their use to high-value applications, such as those in medical and pharmaceutical sectors [3]. However, a reduction in the cost of the PHAs is expected due to the increase of their production in emerging countries. In addition, the use of highly-available and low-cost natural fillers can allow the production of PHA-based composites more economically suited for applications in, for example, packaging and agriculture. A wide variety of lignocellulosic fibres and natural fibres coming from agricultural and industrial crops, such as corn, wheat, bagasse, orange and apple peel [12,13], abaca, kenaf, hemp, flax, and jute are widely used in the production of composites in various industrial sectors, such as the automotive industry, packaging, and building [14,15]. Such a success is derived by their environmentally-friendly character, their low-cost, and interesting mechanical and physical properties comparable properties to traditional fibres, such as glass fibre [16].

In recent years, some authors have also investigated the use of fibres of the seagrass *Posidonia oceanica* (PO) as a filler in potato starch-based films [17] and as a reinforcement in films based on a bio-based high density polyethylene (HDPE) [18], maleic anhydride grafted polyethylene/HDPE blend [19], polypropylene (PP), polyvinyl alcohol (PVA) [20,21], and wheat gluten [16], obtaining interesting results in terms of mechanical performance.

Posidonia oceanica is a Mediterranean endemic species that covers 60% of the seabed from 0 to 40 m depth [22]. The chemical composition of its fibres is similar to that of other lignocellulosic materials consisting mainly of cellulose, hemicellulose, and lignin. *P. oceanica* fragments accumulate in large amounts in the form of balls (*egagropili*) along many coastal beaches as a consequence of storms that tear off leaves, stems and, in some cases, the whole plant [23]. Although stranded *P. oceanica* residues play an important ecological role for the protection of coasts from erosion, the presence of such biomass along some stretches of the coast represents a complex problem for the coastal municipalities: they cause odours due to their decomposition and have negative visual impact. In Italy, every summer, the coastal municipalities are forced to remove seagrass residues that, in the absence of alternative solutions, are disposed of in landfills as municipal wastes with significant economic costs. Italian legislation (Legislative Decree no. 75 of 29 April 2010) allowed, fairly recently, the use of these *Posidonia* wastes in the production of compost, although at a maximum rate of 20 wt % by fresh weight of the composting mixture. However, the high presence of sand and high salinity levels represent the main obstacles to the large use of this biomass for composting. Consequently, the search for alternatives to landfilling or composting is strongly encouraged.

To our knowledge, no published research reports the use of PO fibres in PHA-based composites for potential applications in marine environment, such as natural engineering interventions, including restoration of seagrass habitats. Most transplanting systems currently used in restoration programs to anchor plants to the substrate are made with non-biodegradable materials (polyethylene and polypropylene) [24]. Only recently, have structures made in Mater-Bi[®], a bioplastic obtained by corn starch, vegetable oils, and compostable esters, been developed to transplant seagrass rhizomes [25].

In the present study, fibres of PO up to 30 wt % were used in combination with PHAs as a thermoplastic matrix to obtain, by extrusion, “green” composites with good processability, mechanical properties, and biodegradability in a marine environment. The influence of the PO fibre content on rheological behaviour of the developed composites was investigated by a micro-compounder, monitoring and recording the torque momentum. Their morphology was investigated by scanning electron microscopy (SEM), whereas their thermal stability and mechanical properties were evaluated

by thermogravimetric analysis (TGA), tensile, and Charpy's impact tests, respectively. In view of their use in the marine environment, a mesocosm study was also carried out to investigate the fragmentation/degradation of composite films in natural marine sediments. The degradation of these composites in marine habitats is expected to have no negative impact, as the PO fibres released during the PHA degradation process will return in their original habitat. In addition, the use of these fibres in composites may represent their eco-sustainable valorisation and, at the same time, a solution to the present-day management problem associated with their accumulation on the coasts.

2. Materials and Methods

2.1. Materials

P. oceanica (PO) balls (*egagropili*) were collected along a sand beach at Rosignano Solvay, Livorno (43°19'01.75" N, 10°27'52.76" E; Northwestern Mediterranean, Italy) during the winter months. Both the site and the sampling period were chosen on the basis of the seasonal availability of PO balls. The collected balls were washed with tap water to eliminate sand and other contaminants and dried at 50 °C in an electric oven for 24 h. The resultant fibrous material was milled using a lab-scale mill, the final length of the fibres was in 1.5–2 mm range, and the aspect ratio was in the 7–10 range. Figure 1 shows the dried fibres obtained after the milling process. As a thermoplastic matrix, two PHAs were selected: PHI002™ supplied in pellets by Naturplast® (Caen, France), hereinafter referred to as PHA_np, and Mirel M4100™ supplied in pellets by Metabolix® (Lowell, MA, USA) hereinafter referred to as PHA_m. PHA_np is a Poly(3-hydroxybutyrate-3-hydroxyvalerate) (PHBV) with 5 wt % valerate content, characterized by melting point of 145–150 °C, a density of 1.25 g/cm³, melt flow index (190 °C, 2.16 kg) of 10–20 g/10 min. PHAm is a copolymer of 84% [R]-3-hydroxybutyrate (3HB) and 16% 4-hydroxybutyrate (4HB) with molecular weight of 380,000 Da and a density of 0.4 g/cm³.



Figure 1. (a) Photo and (b) SEM image of *P. oceanica* fibres obtained after milling.

PHA_np is easily processable by extrusion, but it is a stiff material (elongation at break 2.1%, tensile strength 23.0 MPa, Young's modulus 2.3 GPa), while PHA_m is a rubbery polymer (elongation at break 100%, tensile strength 13.8 MPa, Young's modulus 0.6 GPa). Thus, addition of PHA_m to PHA_np leads to an increase in flexibility avoiding the addition of excessive amounts of plasticizers in the polymeric matrix that often leads to leaching/migration phase separation from the polymer matrix during aging. In the present study, a blend with a weight ratio PHA_np/PHA_m of 90/10 was selected as polymeric matrix with suitable mechanical properties and processability. In order to improve the processability of the composites at high loading of fibres, Polyethylene glycol (PEG), PEG 400 from Fluka, was used as a plasticizer in consideration of its good miscibility with PHB (Polyhydroxybutyrate) [26], proved capacity to reduce the melting temperature of PHB in PHB/PEG400 blends [27], and non-toxicity and biodegradability. Moreover, it has been reported that PEG addition allows for easier processing of

melted PHB/wood fibre compounds, having a lubricating effect due to the adhesion of PEG to the natural fibres [3].

2.2. Composite Preparation

Formulations with 0, 10, 20, and 30 wt % fibres, with respect to the total weight, were produced using the 90/10 *w/w* PHAnp/PHAm blend as a polymeric matrix. PHA_{np}/PHA_m and PO fibre composites were prepared by melt blending method using a Thermo Scientific Haake Minilab Micro-compounder (Minilab), a co-rotating conical twin-screw extruder. The extruder operating conditions adopted for all the formulations are reported in Table 1. The nomenclature of the samples was designed as follows: PHA for the unfilled material and PHA10, PHA20, and PHA30 for the composites containing, respectively, the different fibre loadings. As the mixture of components passed through the screws of the twin-screw extruder, melting and mixing took place and a homogenized extrudate exited through the flush orifice. The extrudate was collected and cut into small pellets used for the production of films by compression moulding. During the extrusion process in the Minilab the torque momentum and the pressure were monitored and recorded in order to evaluate the effect of the fibre loading on the melt rheological behaviour.

For each formulation, ten specimens for the tensile test (Haake III type dog-bone tensile bars 10 mm × 4.8 mm × 1.35 mm) and ten specimens for the Charpy's impact test (bars 80 mm × 10 mm × 4 mm) were produced feeding the molten material, produced in the Minilab, in a Thermo Scientific HAAKE MiniJet II. The adopted processing parameters are summarized in the Table 1. In addition, composite films were prepared by compression moulding using a hot press (Polystat 200 T). About 5 g of composite pellets, produced from the Minilab extruder, were positioned between two metal plates of dimensions 200 mm × 200 mm × 2 mm, with two interposed Teflon sheets to allow the detachment of the film. The pellets were subjected to a temperature of 170 °C and a pressure of 200 bar for 2 min. Then, the mould plates were cooled down by a cold-water flow and the pressure was maintained for an additional 15 min. When the temperature was about 40 °C, the composite films with an average thickness of 400 µm were released and used for characterizations.

Table 1. Processing parameters.

Extrusion Temperature (°C)	Screw Speed (rpm)	Cycle Time (s)	Injection Temperature (°C)	Injection Pressure (bar)	Moulding Time (s)	Mould Temperature (°C)
170	100	60	170	300	20	60

2.3. Composite Characterization

Thermal properties of the raw materials and the composites in form of pellets were evaluated by thermogravimetric analysis (TGA). Thermogravimetric measurements were carried out on about 20 mg of sample using a Q500 TGA (TA Instruments; New Castle, DE, USA), under nitrogen flow (100 mL/min), at a heating speed of 10 °C/min from room temperature to 600 °C. The TGA measurements were carried out in duplicate.

Tensile tests were performed on the injection moulded Haake Type 3 (557-2290) dog-bone tensile bars of composites in accordance with ASTM D 638. Stress-strain tests were carried out at room temperature, at a crosshead speed of 10 mm/min, by an Instron 5500R universal testing machine (Canton, MA, USA) equipped with a 10 kN load cell and interfaced with a computer running the Testworks 4.0 software (MTS Systems Corporation, Eden Prairie, MN, USA). An impact test was carried out on V-notched specimens using a 15 J Charpy pendulum (CEAST 9050, Instron, Canton, MA, USA) following the standard method ISO 179:1993. For each mechanical test, at least five replicates were carried out at room temperature for each sample.

The morphology of the composite tensile specimens (dog-bones), intact and fractured from tensile tests, was investigated by scanning electron microscopy (SEM) using a JEOL JSM-5600LV (Tokyo, Japan). The intact samples were frozen under liquid nitrogen and then fractured. Prior to the analysis, the surfaces of all samples were coated with a gold layer.

Given the potential application of the developed composites in marine environment, a preliminary degradability/fragmentation test was conducted on the composite films in a semi-controlled marine environmental conditions at the Mariculture Center of Rosignano Solvay (Livorno, Italy) situated close to the sea (Ligurian Sea). At the beginning of February 2015, PHA and PHA30 films were cut into small pieces (3 cm × 1 cm) and placed into nylon mesh bags (mesh size 2 mm). Each bag containing three pieces of PHA or PHA30 samples were randomly placed into commercial plastic pots (30 cm × 15 cm × 10 cm) filled with natural marine sediment collected at a depth of 30 cm along the coast near the Centre. The bags were covered with a layer of sediment (5 cm) and the pots were then allocated into a mesocosm consisting of an open tank (5000 m³) alimented with running natural seawater collected offshore, which was proven to be suitable for growing marine organisms [28]. There were two pots, each containing four bags, for each type of composite film. The test lasted six months, from February to July 2015. During this period, the height of the seawater column above the bags was maintained constant (50 cm) and the main environmental parameters (salinity, pH, and temperature of seawater) were measured weekly. Prior to the test, each specimen was dried overnight at 40 °C and weighed using a high-precision balance. For each type of film, a bag was collected at random every month and the three samples contained in the bag were washed in distilled water, dried overnight at 40 °C and weighed. The weight loss of each specimen, calculated as a ratio of the difference between the final and initial weight to the initial weight and expressed as percentage, was considered as the percentage of degraded material. The degradation process was also documented with a series of photos taken before and during incubation test. The samples collected after five and six months of incubation were observed by SEM.

3. Results and Discussion

The thermal stability of PO fibres, PHA_{np}, PHA_m, PEG400, and the developed composites in terms of thermogravimetric (TG) and their derivative (DTG) curves, is shown in Figure 2. As shown, PO fibres show an initial limited weight loss (about 4%) up to 100 °C attributable to the residual humidity, then a 50 wt % weight loss is recorded from 250 °C to 450 °C corresponding to the main thermal degradation process. The onset temperature higher than 250 °C attests the suitability of these natural fibres to be processed with thermoplastic polymer matrices, such as PHAs, without incurring thermal degradation. The broad peak of degradation of PO fibres is attributed to the overlapping of the degradation steps of the different components (hemicellulose, cellulose and lignin) of the fibres [19]. For PHA_{np} and PHA_m, the degradation onset temperature was about 260 °C and 250 °C and the maximum rate decomposition temperatures of 305 °C and 295 °C, respectively, typical values for the PHAs. For both the PHAs no residue was recorded above 350 °C. PEG400 showed lower thermal stability than PO fibres and PHAs, but it was still suitable for processing in the melt at 170 °C. The thermal stability of the produced composites was due to the concomitant effect of all of the components, the lowest values of onset temperature were due to the presence of PEG400 for the sample PHA30 + PEG and of the PO fibres. For all of the produced composites the thermal degradation started over 200 °C, with the main degradation peak over 250 °C.

The torque momentum measured after 60 s at 170 °C for the different composites is a useful parameter from a manufacturing point of view in order to evaluate the effect of the fibre addition on their processability by injection moulding. Figure 3 shows the values of the torque in terms of the percentage of fibre content. As observed, the torque momentum increased with increasing the fibre content. As reported in the literature [29], short fibre polymer-based composites may exhibit a higher viscosity than the pure polymer, when a hindered mobility of polymer chain segments in flow is induced by fibres presence. In this case, increasing the fibre content increases also the probability

of fibre-fibre collision, with consequent difficulty of alignment and distribution along the direction of flow that favours the fibre entanglement phenomena, leading to fibre packing [30,31]. However, this reduction of fluidity did not create problems in the processing of the composites up to 20 wt % of fibres, as reported also for PHA-np and wheat straw fibre composites [32]. While for the sample with 30 wt % of PO fibres, the addition of 10 wt % of PEG 400 as a plasticizer was necessary to increase the fluidity (Figure 3) and improve its processability by extrusion.

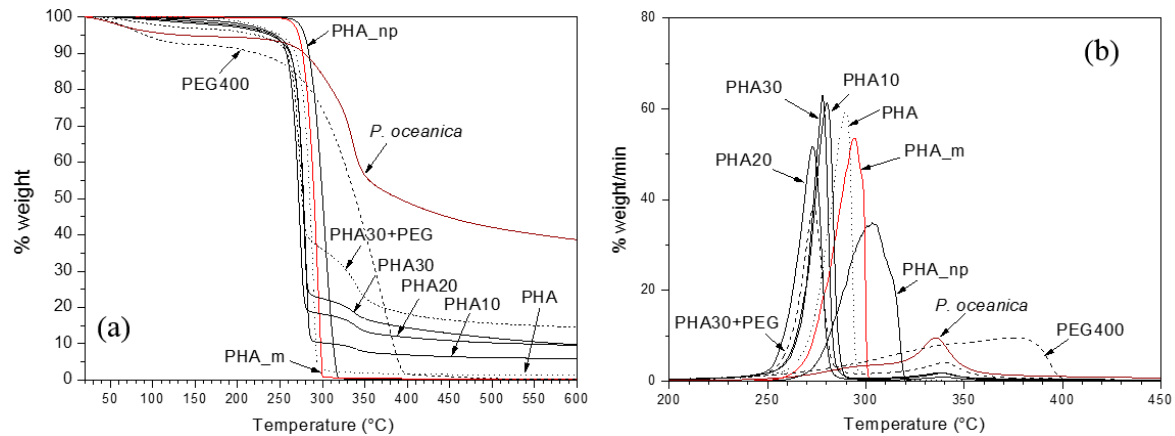


Figure 2. (a) TG and (b) DTG curves of PO fibres, PHA_{np}, PHA_m and the developed composites.

The SEM morphological analysis was carried out both on the cross-section of the intact dog-bone-shaped specimens and on the fractured surface of the specimens after the tensile tests in order to evaluate the dispersion of the fibres into the polymeric matrix and the fibre/matrix interactions, respectively. The SEM images of the composites with different PO loadings are reported in Figure 4. As shown, in the unbroken samples, the PO fibres are quite homogeneously distributed within the thermoplastic matrix, and a regular surface is observed for PHA10 (10 wt % of PO fibres), while the emergence of fibres is observed with PHA20, even if they are still well embedded in the polymeric matrix. For PHA30, some irregularities and small voids are observed that are reduced by the addition of the plasticizer that improved the fibre dispersion in the matrix. The interfacial interactions between the fibres and the matrix were not sufficiently strong to maintain the cohesion during the tensile tests, as shown in the fractured samples (Figure 4) where a significant fibre pull-out is shown in all of the samples. These weak interfacial interactions are typical of the wood flour filled composites, because the surface free energy of both the filler and the polymer is very small [26].

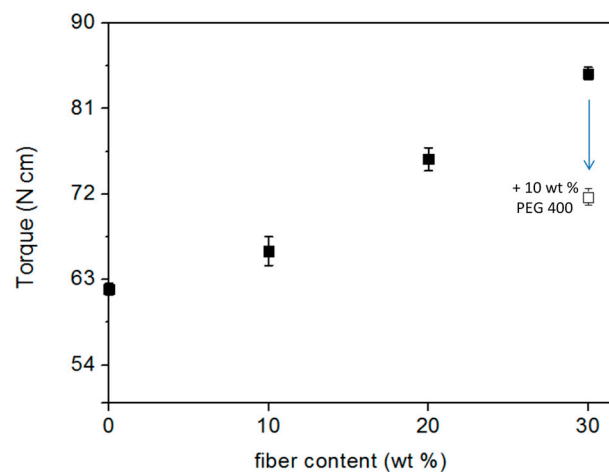


Figure 3. Variation of torque momentum (\pm SE) measured at 170 °C in terms of the wt % of PO.

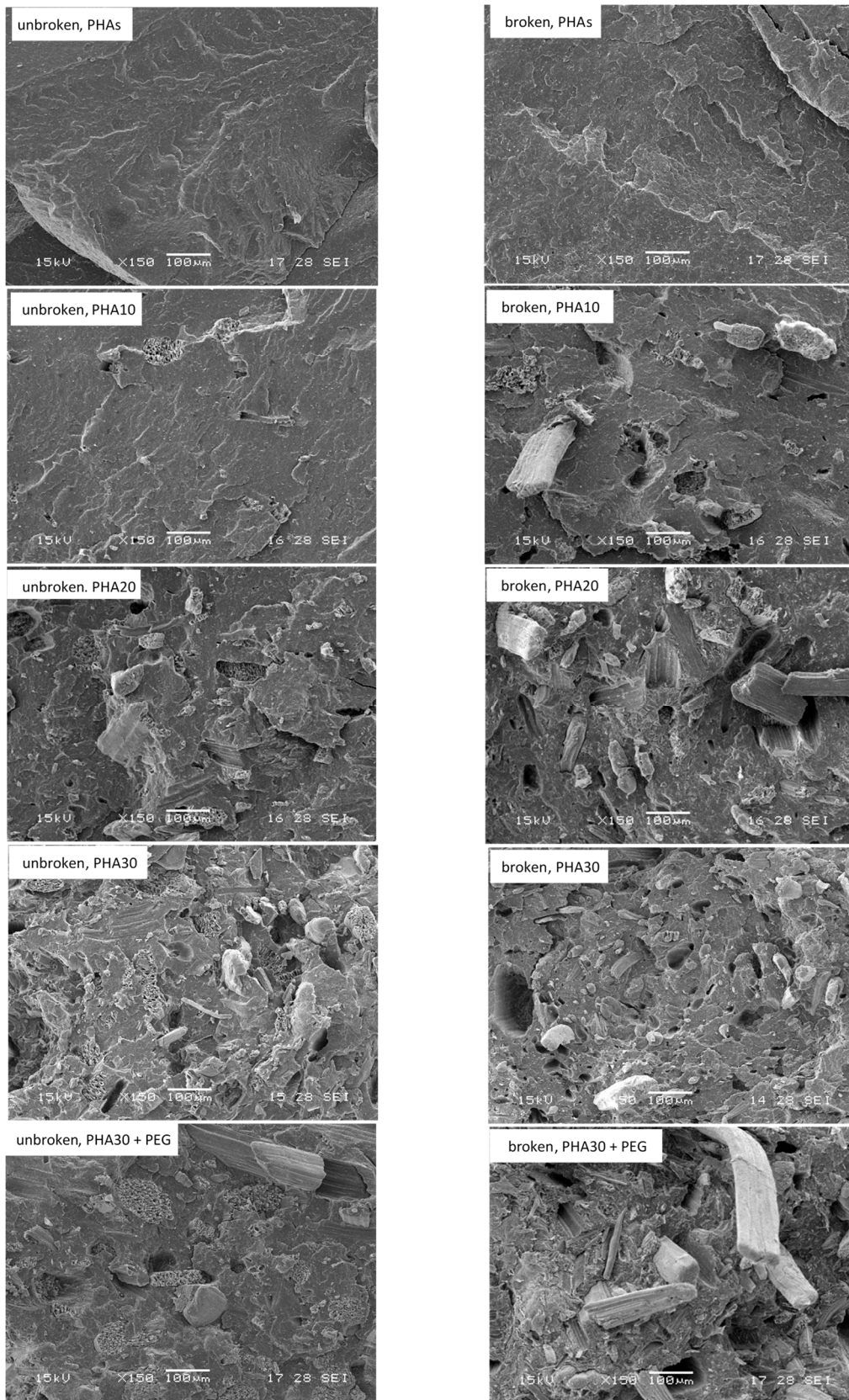


Figure 4. SEM images of the cross-sections of the samples before (unbroken samples) and after (broken samples) tensile tests.

Table 2 shows the mechanical properties of the composites obtained by tensile and Charpy's impact tests. As expected, the elongation at break decreased significantly with increasing fibre loading, due to the stiffening effect induced by lignocellulosic fillers [33], while the stress at break decreased slightly, remaining at interesting values of about 22 MPa. Additionally, the Young's modulus increased with increasing the fibre loading, the modulus of the composite PHA30 is about twice that of the matrix. These results are in agreement with those of other authors [17,18] relating to composites containing *P. oceanica* fibres. This behaviour is typical of particle-filled polymeric matrices with poor or no compatibility between the components so that stress transfer phenomena cannot occur and the filler particle becomes a stress concentrator leading to early fracture [34]. When PEG400 was added, a reduction in tensile strength was observed due to both the plasticizing effect of PEG on PHA and to the higher affinity of PEG for the hydrophilic natural fibres that further reduced the fibre/matrix adhesion.

The results of the Charpy's impact test showed that the presence of PO fibres improved the capacity of the composites to absorb impact energy compared to the unfilled matrix. The unfilled matrix, PHA, showed an impact energy of about 1.6 kJ/m² while, for the samples with 30 wt % of PO, with or without plasticizer, the impact energy increased to about 3.8 kJ/m². This marked increase of the impact resistance with increasing fibre loading can be attributed to the poor fibre/matrix interaction since the impact failure of a composite occurs by factors such as fibre/matrix debonding, fibre and/or matrix fracture and fibre pull out. Fibre fracture dissipates less energy compared to fibre pull out, and this is common in composites with strong interfacial bonds, while the occurrence of the latter is a sign of a weak bond [35–37]. The applied load transferred by shear to fibres may exceed the fibre/matrix interfacial bond strength and the composites fracture in a brittle mode, which is in agreement with the experimental results.

Table 2. Tensile properties of the composites with different PO content.

Sample	Tensile Properties		Impact Properties	
	Young's Modulus (GPa)	Tensile Strength (MPa)	Elongation (%)	Charpy's Impact Energy (kJ/m ²)
PHA	1.24 ± 0.09	24.80 ± 0.42	6.47 ± 0.69	1.63 ± 0.10
PHA10	1.57 ± 0.21	22.89 ± 2.12	4.18 ± 0.90	3.42 ± 0.69
PHA20	1.82 ± 0.19	22.78 ± 0.15	3.20 ± 0.45	3.66 ± 0.42
PHA30	2.32 ± 0.15	21.88 ± 2.67	2.37 ± 0.45	3.76 ± 0.30
PHA30 + 10 wt % PEG400	2.25 ± 0.20	18.70 ± 0.98	1.80 ± 0.22	3.82 ± 0.32

The values are the mean ± SD of five determinations.

Figure 5a shows the film specimens used for the degradation test. The degradation was evaluated by weighting and visual observation of the samples incubated in natural sediments in the mesocosm. During the test, salinity was 38–38.2, pH 7.8–8, and temperature ranged from 13.5 to 21 °C in winter-late spring (February–May) and from 23–27 °C in summer months (June and July). The weekly seawater temperature and the percentage of the weight losses of the samples recorded during incubation are presented in Figure 6. During winter–spring, no relevant visual macroscopic changes and weight loss were observed both for the unfilled films and for those with 30 wt % of PO fibres, while in the summer months (June and July) the rate of degradation increased considerably, especially for the PHA30 samples, when the seawater temperature was above 23 °C (Figures 5b and 6). After six months of incubation, only a few small fragments of the PHA films were still visible (final average weight loss of 93%), while the PHA30 films were totally disintegrated, with no visible fragments (Figure 5c). These results are consistent with data of a previous study [8] suggesting that the sea water temperature played an important role in the degradation process of the PHA-based films. They also showed that the presence of fibres favoured the fragmentation of the films making the PHA matrix more susceptible to microbial attacks and, consequently, accelerating the film degradation rate, as evidenced also by the SEM analysis.

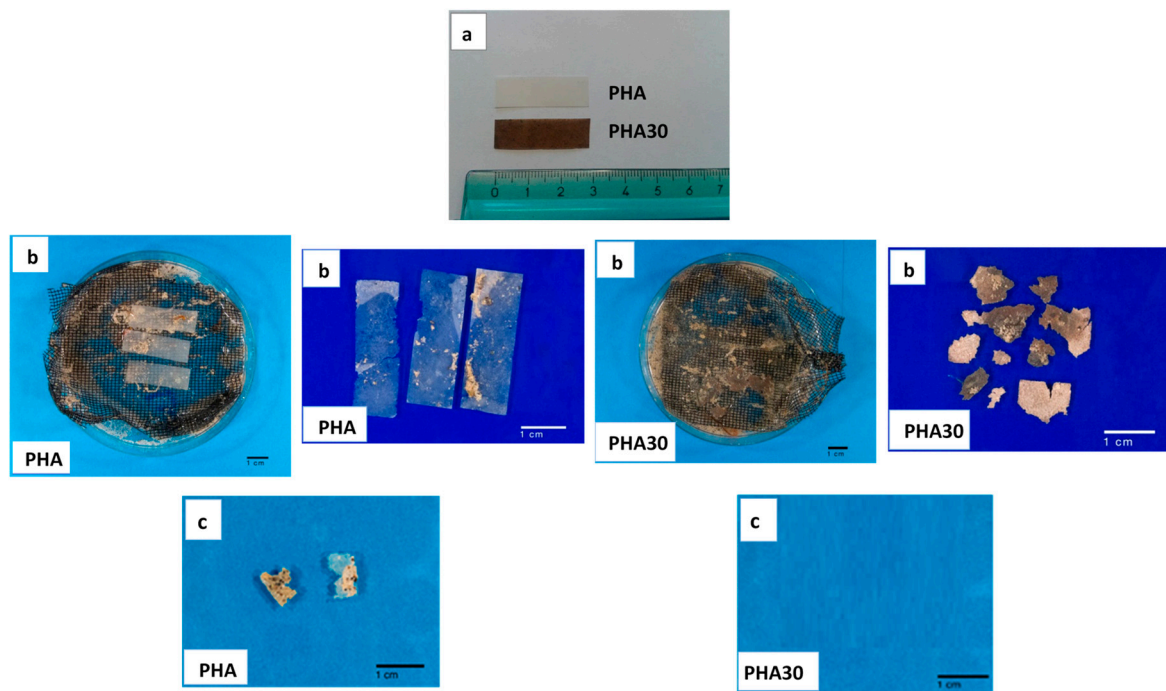


Figure 5. PHA and PHA30 film specimens before (a); after five (b) and six months (c) of incubation in marine sediment.

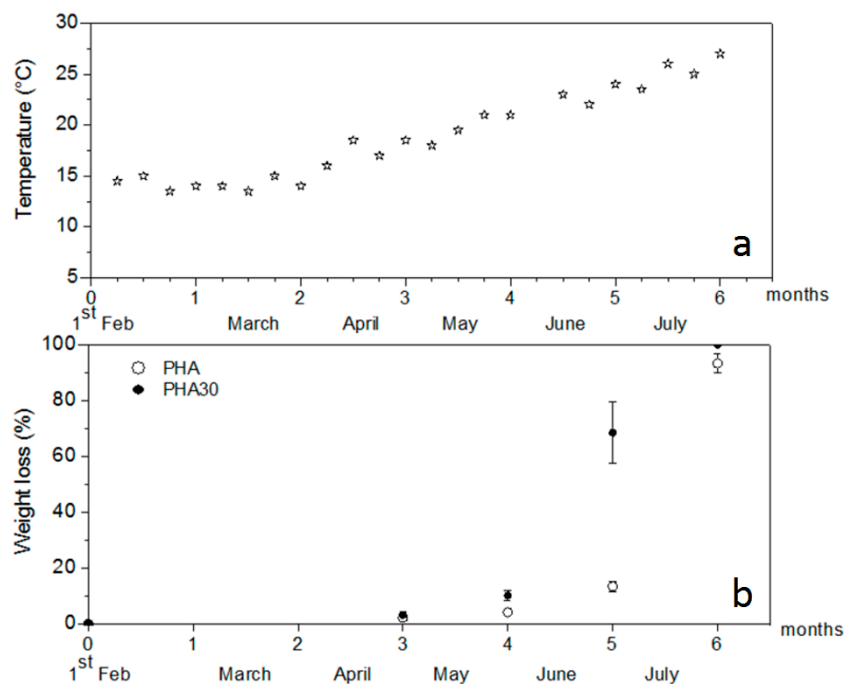


Figure 6. (a) Sea water temperature (weekly value) and (b) weight loss percentage (mean \pm SE) of PHA and PHA30 films during incubation in marine sediment.

Figure 7 shows the SEM images of the surface of PHA films before and after five and six months of incubation and of PHA30 films before and after five months. Images of PHA30 samples after six months are not shown since no fragment remained after this period. For both samples, the images of the film surface after five months show clear evidence of degradation with pits, surface roughening, grooves, cavities. More apparent physical changes took place in the PHA30 film compared to the ones

that occurred in the unfilled PHA-based film supporting the obtained weight loss results. For the PHA sample, the numbers of cracks and small holes were becoming deeper with increasing exposure time. Studies on the biodegradability of PHA films in soil and seawater [38] demonstrated that the PHA degradation is a concerted effect of a microbial consortium colonizing the film surface, including fungi, bacteria, and actinomycetes. In fact, SEM images show that the PHA and PHA30 films were largely colonised by numerous bacteria and fungi, providing support for their possible role in the degradation of the polymeric matrix. In addition, microalgae, such as benthic diatoms (see Figure 7), are frequently observed, but they appeared to use the PHA film as a growth support rather than degrading it [39].

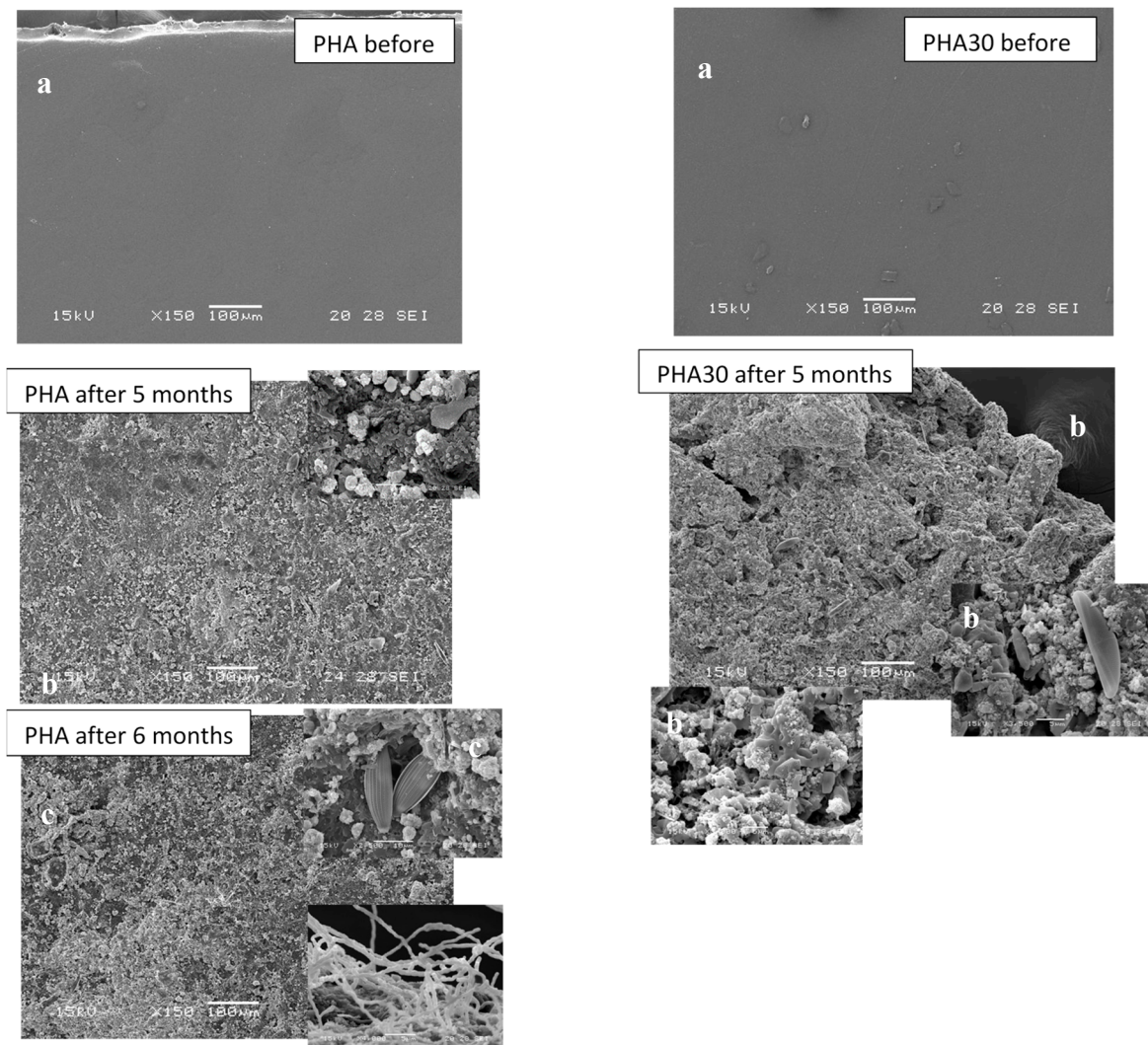


Figure 7. SEM images of surfaces of the PHA and PHA30 films before (a) and after 5 (b) and 6 months (c) of incubation in natural marine sediment.

4. Conclusions

Composites based on PHAs and different *Posidonia oceanica* fibre loadings (0, 10, 20, 30 wt %) were prepared and characterised by rheological, thermal, morphological, and mechanical analyses. Preliminary degradation test of the composites in a natural marine sediment was also carried out. The processing by extrusion of the composites did not show any difficulty up to 20 wt % PO fibres, while at 30 wt % PO fibres the use of PEG 400 as a plasticizer-lubricant was necessary to reduce the melt viscosity, improving the processability of the composites. The tensile modulus of the composites was increased as the fibre content was increased. The modulus of the unfilled material was 1.24 GPa, and

this value increased up to values of 2.25 GPa for composites containing 30 wt % PO fibres. The impact energy-absorbing capability was markedly increased with increasing the fibre loading. The impact energy remarkably increased from 1.6 kJ/m² (unfilled material) to 3.6 kJ/m² for composites with 30 wt % PO. More rapid degradation in marine sediment was recorded for films with 30 wt % PO fibres, indicating that the presence of *P. oceanica* fibres facilitated the disintegration of the films and, consequently, accelerated the degradation of the polymeric matrix, allowing total disintegration of the films in less than six months.

In conclusion, the good results obtained show that the manufacture of composites based on PHAs and *P. oceanica* fibres is technically feasible and it represents a valuable solution to the present-day management problem associated to the accumulation of seagrass wastes on the coastal beaches providing an eco-sustainable alternative to their disposal in a landfill. More importantly, the developed composites, fully based on renewable resources and biodegradable in the marine environment, in a relatively short time, could find application in the production of items usable in the sea and sand dunes, such as transplanting tools and structures for restoration or protection of coastal habitats, replacing the non-biodegradable commodity plastics traditionally used for such purposes.

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Conflicts of Interest: The authors declare no conflict of interest.

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