Biodegradability of Polyethylene/Hydrolyzed Collagen Blends in Terrestrial and Marine Environmental Conditions

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Received Novenber 14, 2016; Accepted April 03, 2017

ABSTRACT: In this study, blends of low-density polyethylene (PE) containing 20 wt% of hydrolyzed collagen (HC) from the leather industry were processed by the film blowing technique. A biodegradation study of these innovative materials was performed by two different biodegradation tests, one in terrestrial environment, the other one under aquatic conditions. Degradation rates were determined for both systems and an environmental degradability parameter was calculated. The results proved the positive influence of hydrolyzed collagen on degradation of polyethylene, but also showed a relatively low biological degradability of PE/HC blends under the applied test conditions.

KEYWORDS: Biodegradability, hydrolyzed collagen, polyethylene

1. INTRODUCTION

Polyolefins constitute the majority of plastics currently used as packaging materials. Plastics currently account for about 20% by volume of municipal solid waste [1]. Since the world production of plastics has been continuously increasing over the last decades [2] and reached 322 million tonnes in 2015 [3], post-consumer plastic waste has become an important issue for economic and environmental reasons. The huge amount of polymers which originate from fossil resources has caused many social problems, such as global warming, environmental pollution and the oil crisis, motivating academia and industry to devote considerable efforts to the development of polymers from renewable resources. Thus, in the last few years, there has been an increased interest in the production and use of bioplastics [4] with the main goal of replacing non-biodegradable plastics [5]. Biobased polymers are produced from natural materials, such as starch and oils, or by fermentation processes, like polylactic acid (PLA), polyhydroxyalkanoate (PHA), and polyhydroxybutyrate (PHB). Although these pure biodegradable polymers possess the required properties and can be used for the production of blown film and injection-molded materials, they are not widely used because of their high cost.

To overcome the disposal issues associated with polyolefins, environmental (triggered) degradation of polyolefins has been explored for the last few decades [6]. The methods proposed to enhance the potential biodegradability of polyolefins have been focused on the introduction of functional groups and substances (pro-oxidants) within the backbone [7] that are capable of promoting their decomposition, in other words, the direct incorporation of natural biodegradable polymers such as proteins of vegetal and animal origin [8].

Among biobased polymers, hydrolyzed collagen (HC) from the tanning process is a natural biopolymer derived from the solid wastes of the fleshing/shaving phases of the tanning process. Fleshings and shavings represent one of the most important by-products of the tannery compartment, and are mainly composed of raw collagen. The high content of salts of collagen hydrolysate, whose separation is rather expensive, represents the main obstacle to its actual recovery and reutilization. Thus, collagen hydrolysate from the leather industry is easily available at low cost and its use is not in competition with food industries or other main applications.

Many biodegradation tests have been developed and standardized in the last 25 years [9]. Biodegradation of polymers may be monitored in respirometric tests by using soil as substrate [10]. In comparison with

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DOI: 10.7569/JRM.2017.634138

other environments, the behavior of plastics in the marine environment has been less studied because the expected fate of bioplastics is to be treated in solid waste treatment facilities or in soil (for agricultural applications). However, recently there has been great interest regarding the characterization of marine biodegradation of bioplastic products [11]. It is imperative to know the marine biodegradability of plastic materials in case of accidental or uncontrolled release.

In previous works [12–14], blends of hydrolyzed collagen and low-density polyethylene (LDPE) have been shown to be melt processable and provide cohesive and flexible films using a heated hydraulic press and the film blowing technique, producing biofragmentable thermoplastic films for packing applications. In the present study, LDPE/HC blends with 20 wt% of HCs characterized by different molecular weight and salinity were produced by using film blowing technique. The biodegradation behavior of the films was investigated in two different natural environments, in soil and in seawater. The effective biodegradation in both tests was assessed by monitoring the amount of CO_2 developed over time using standardized procedures.

2. EXPERIMENTAL SECTION

2.1 Materials

The low-density polyethylene (PE) Riblene FC 20 was supplied by Polimeri Europa, as uniform granules, having the following characteristics: melting temperature 113 °C; density 0.922 g/cm^3; melting flow index (MFI) 0.25 g/10 min (190 °C/2.16 kg). Riblene FC 20 is a high molecular weight low-density polyethylene resin suitable for blown film extrusion, characterized by a high melt strength leading to a good bubble stability during extrusion.

Two kinds of hydrolyzed collagen (HC) in powder form were used: derived from fleshings (HC1) and from shavings (HC2) by alkali hydrolysis, supplied by SGS SpA (Santa Croce sull'Arno, Italy). Before use, the powdered hydrolysates were dried under vacuum at 40 °C for 12 hours. The properties of HCs are reported in Table 1.

2.2 Extruded Film Preparation

The blends of PE with 20 wt% of collagen hydrolysate of the total blend weight, were produced by a Thermo PRISM double screw extruder, Model TSE-16/28 TC, using the following temperature profile: 120/130/140/150 °C. The extrudate was air-cooled and then reduced to pellets by an automatic cutter.

Table 1 Composition and physical-chemical properties of HC.

Properties	HC1	HC2
Dry matter (wt%)	96.7	96.0
Ashes (800 °C wt%)	23.4	2.9
Water solubility	Total	Total
Average molecular weight (kDa)	1.53	4.08
Ultimate analysis (dry basis):		
Carbon (wt%)	33.38	42.21
Hydrogen (wt%)	5.31	6.37
Nitrogen (wt%)	10.29	15.71
Oxygen (wt%)	51.02	35.71

Table 2 Extrusion and blowing parameters.

Die diameter	24 mm
Die gap	0.8 mm
Screw diameter	19 mm
Screw length/diameter ratio	25
Screw speed	20 rpm
Temperature profile	130/140/150/150 °C

Blown films of the blends were manufactured from pellets using a blow film line Thermo Scientific Rheomex 19/25 QC equipped with a single screw extruder and a blown film take-off. The extrusion and blowing parameters are shown in Table 2.

2.3 Characterization of Materials

The PE/HC films were characterized by using infrared spectrometry and scanning electron microscopy (SEM).

Fourier transform infrared (FTIR) spectra of PE/HC film samples, as well as of the HCs, were obtained from 4000 to 650 cm^{-1} regions using an attenuated total reflectance (ATR) mode with a zinc-selenide crystal JASCO 4100 spectrometer. A total of 16 scans were acquired per image at a resolution of 4 cm^{-1}.

For SEM analysis the film surface of the samples were observed by using a JEOL 5600LV microscope. Before the observation, the surfaces were coated with Au on an SEM coating device (Edward Sputter Coater) to induce electroconductivity. A homogeneous layer of metal of 5–6 nm thickness coated the entire sample surface.

2.4 Terrestrial Biodegradation Tests

Respirometric biodegradation tests were performed in flasks (300 ml capacity) following the method

developed by Chiellini *et al.* [4]. Each vessel contains a multilayer substrate in which defined amounts of forest sandy soil (10–15 g), sieved at 0.6 mm and mixed with 20–25 g perlite, were placed. The samples, in the form of films, were placed into the mixed soil and sandwiched between two layers of 10 g perlite wetted with 30 ml distilled water (Figure 1). This arrangement guarantees favorable and reliable signal-to-noise ratio in the assessment of the substrate mineralization and hence an improved test accuracy, particularly when limited CO_2 emissions are expected from the test samples.

The vessels were kept in the dark and incubated at room temperature. For trapping the CO_2 evolved from samples, each test vessel was equipped with a beaker containing 40–50 ml of 0.05 KOH solution, which was substituted every 3–14 days and back titrated with 0.1N HCl. The biodegradation extent of each test material was calculated as a percentage (corrected for the inoculum endogenous emissions – blank flask) of the overall theoretical CO_2 production calculated on the basis of the determined carbon content of the samples. Each test was carried out in triplicate. Filter paper (Whatman 50) was used as positive control as suggested by standardization prescriptions.

2.5 Aquatic Biodegradation Tests

The biodegradability of the films in marine environment was evaluated by measuring the amount of carbon dioxide evolved, applying a method for the determination of the degree of aerobic biodegradability of plastic materials based on ISO 14852. The test was carried out using the device shown in Figure 2, where the films settled on marine sandy sediment were exposed to the seawater/sediment interface.

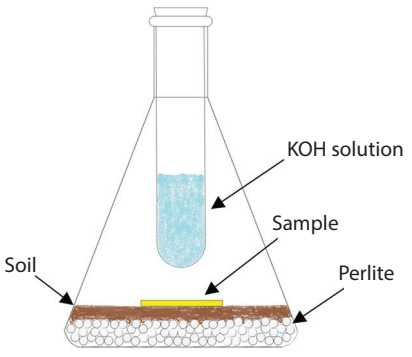

Figure 1 Flask for terrestrial biodegradation test.

The biodegradation test was performed in 300 ml glass flasks connected to each other with tubes in which atmospheric air flowed at about 100–120 ml/min. Flasks *a* and *b* were filled with 250 ml NaOH solution (5 M) to remove CO_2 from pumped air by pump. Flask *c* was filled with 200 ml $Ba(OH)_2$ solution (0.04 M) to indicate complete removal of CO_2 in pumped air. Flask *d* was the bioreactor filled with 100 g of marine sandy sediment and 150 ml of seawater, and portions of film (about 300 mg) placed on the sediment. Flask *e* was filled with 20 ml KOH solution (0.08–0.1 M) to absorb the CO_2 released in the bioreactor during the biodegradation process and it was substituted at variable time ranges. Seawater and sediment were collected from a littoral zone of the Ligurian Sea (Italy) at a depth of 30–35 cm near a sea meadow of *Cymodocea nodosa* (Ucria) Ascherson. The sediment was kept with the seawater during transport; it consisted of fine sand and, before being placed in flask *d*, coarser materials were removed. The pH of the seawater was 7.8, the salinity 37.8 and the temperature 20 °C.

The test was carried out at room temperature. Filter paper (cellulose) was used as reference material as suggested by standardization prescriptions. The biodegradability of the paper demonstrates the growth of microorganisms that are able to implement degradation processes. A blank control (just seawater and marine sediment) was also prepared. Since marine sediment is a good substrate for microorganisms and produces CO_2, it is necessary to estimate the average amount of CO_2 evolved and then to calculate by difference the effective CO_2 produced by sample. The test was carried out in triplicate for each sample and the average values were considered. The amount of released CO_2 in the biodegradation test was calculated from the consumption of KOH determined by titration with 0.1 M HCl using phenolphthalein as indicator.

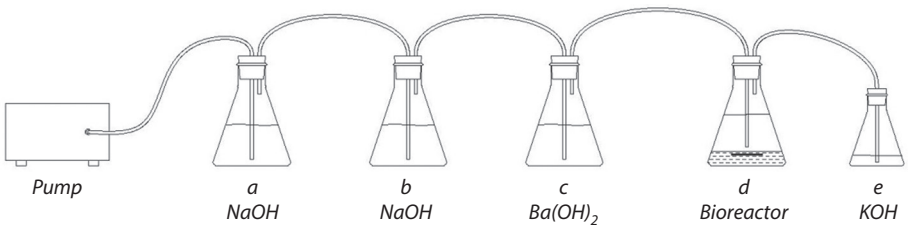

Figure 2 Device for the biodegradation test in marine environment.

2.6 Biodegradation Parameters

The biodegradation extent of each material was evaluated as neat percentage (corrected for emission from blank samples) of the overall theoretical CO_2 production calculated on the basis of the relevant carbon content in the testing sample. The biodegradation rate of materials (D_m) in the environments was then calculated from the ratio of the amount of CO_2 released to the maximum theoretical amount of CO_2 that could be released ($ThCO_2$), as in Equation 1:

$$D_m\,(\%) = \frac{\sum (CO_2)_{material} - \sum (CO_2)_{blank}}{(ThCO_2)_{material}} \cdot 100 \quad (1)$$

where D_m is the biodegradation rate of the test material, $\sum(CO_2)_{material}$ is the total amount of CO_2 released by the material, $\sum(CO_2)_{blank}$ is the amount of CO_2 released in the blank bottle and $(ThCO_2)_{material}$ is the maximum theoretical amount of CO_2 that could be released.

From D_m of tested materials, the environmentally degradable parameter was calculated following Equation 2:

$$B_d\,(\%) = \frac{D_m - D_{PE}}{D_{cellulose} - D_{PE}} \cdot 100 \quad (2)$$

where B_d is the environmental degradability parameter of the tested material, $D_{cellulose}$ is the biodegradation rate of cellulose, D_{PE} is the biodegradation rate of polyethylene, and D_m is the biodegradation rate of material.

3 RESULTS AND DISCUSSION

3.1 Terrestrial Biodegradation Tests

Cumulative CO_2 emissions for the terrestrial respirometric test detected from the test flasks during 48 weeks of incubation are reported in Figure 3. The effectiveness of the test conditions was demonstrated by CO_2 productions from blank. Moreover, the performance of filter paper (positive control) showed the presence of appropriate test conditions able to ensure soil microorganism growth. The amounts of CO_2 released by PE was similar to that of the blank controls, showing its well-known resistance to biological attack because of its hydrophobicity, high molecular weight and its lack of functional groups recognized by microbial enzymatic systems [15, 16]. The blends of PE containing 20 wt% of collagen hydrolysate from fleshings (sample PE/20HC1) showed a slightly greater tendency to be degraded compared to blends with 20 wt% of HC from shavings (sample PE/20HC2). Therefore, the beginning of biodegradation was observed in PE/HC blends compared to PE: HC contributes to the fragmentation of PE matrix with a similar effect of starch in starch/polyethylene blends [17]. Indeed, addition of readily biodegradable compounds, such as hydrolyzed collagen, to a polyethylene matrix may enhance the degradation of the carbon-carbon backbone.

Biodegradation extents after 48 weeks of incubation for PE/HC samples, PE and filter paper are reported in Table 3. As shown, filter paper reached about 64.1% of biodegradation, whereas the obtained degradation extents for PE/HC blends were rather low: 3.6% for PE/20HC1 (HC from fleshings) and about 5.4% for

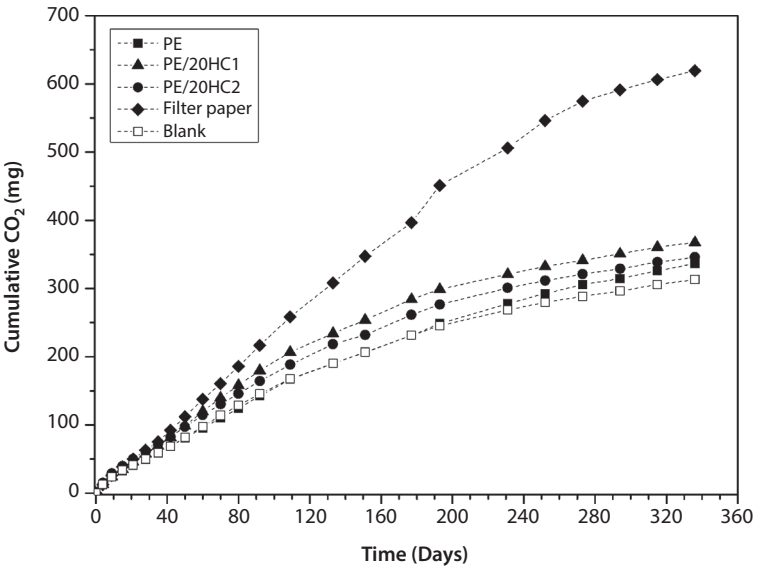

Figure 3 Cumulative CO_2 emissions of PE/HCs, PE, filter paper and blank in terrestrial respirometric test.

PE/20HC2 (HC from shavings). The biodegradation rate of the sample PE/20HC1 in soil was lower than that of the sample PE/20HC2. This can be attributed to the higher NaCl content of HC1 (Table 3) compared to HC2; high salt concentrations in soil can stress or even kill soil microorganisms [18], reducing their microbial activity and, consequently, their capacity to degrade the sample.

At 48 weeks of incubation, test flasks were opened and PE/HC film samples were collected and characterized by SEM analysis. As shown in Figure 4, the film surface of PE/20HC1 was covered with a microbial colonization attributable to filamentous microorganisms such as fungi: the hydrophilic characteristic of collagen hydrolysate allows the formation of an environment favorable to the growth of microbial colonies.

The infrared spectra of the original and soil retrieved sample after 48 weeks of incubation (Figure 5) show a reduction in the typical carbonyl absorption, around 1640 cm^{-1}, as a consequence of the preferential assimilation of oxidized polymer chains by soil microorganisms. On the basis of the relative intensities of the carbonyl band (at 1635 cm^{-1}) to that of methylene scissoring band (at 1465 cm^{-1}), the carbonyl bond indexes were calculated for original and soil retrieved samples. The decrease of carbonyl index confirms that macromolecular cleavage is promoted by biotic scission.

Table 3 Biological degradation of PE/HCs, PE and filter paper in terrestrial environment (see Eq. 1).

Sample in test flask	D_m (after 48 weeks) (%)
Filter paper	64.1
PE/20HC1 (fleshings)	3.6
PE/20HC2 (shavings)	5.4
PE	2.1

3.2 Aquatic Biodegradation Tests

The results of cumulative CO_2 emissions of samples during 54 weeks of incubation in marine environment are reported in Figure 6. Aquatic test conditions appeared to be satisfactory in terms of microorganism growth, as revealed by the high level of CO_2 production from filter paper. The blends of PE containing 20 wt% of collagen hydrolysate from fleshings (PE/20HC1) and shavings (PE/20HC2) showed no significant differences in terms of CO_2 production in seawater.

The obtained degradation extents of samples calculated by using Equation 1 after 54 weeks of incubation in marine environment (Table 4) were about 78% for

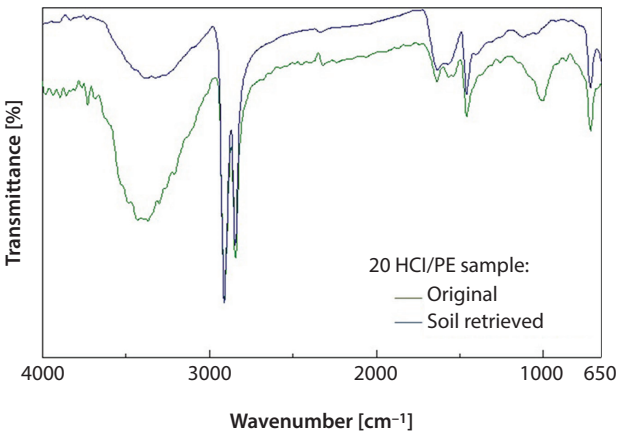

Figure 5 FTIR spectra of PE/20HC1.

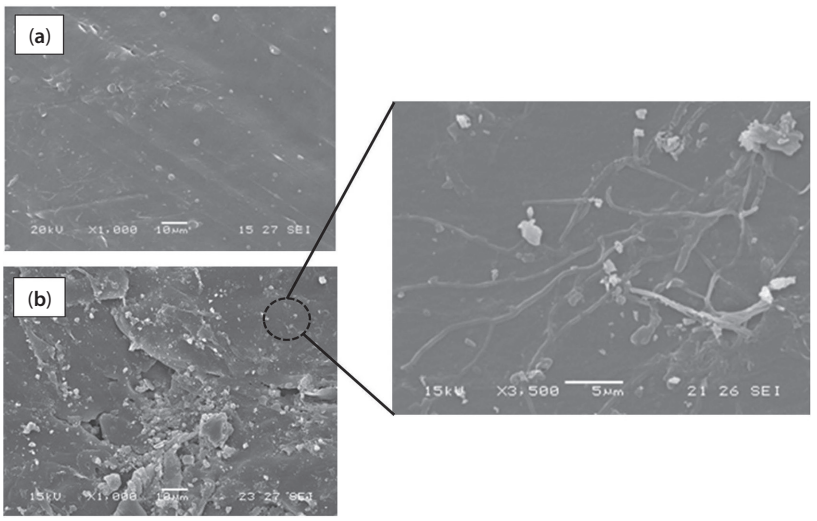

Figure 4 SEM micrographs of film surface of PE/20HC1 (**a**) and PE/20HC2 (**b**) retrieved from terrestrial respirometric test flasks after 48 weeks of incubation.

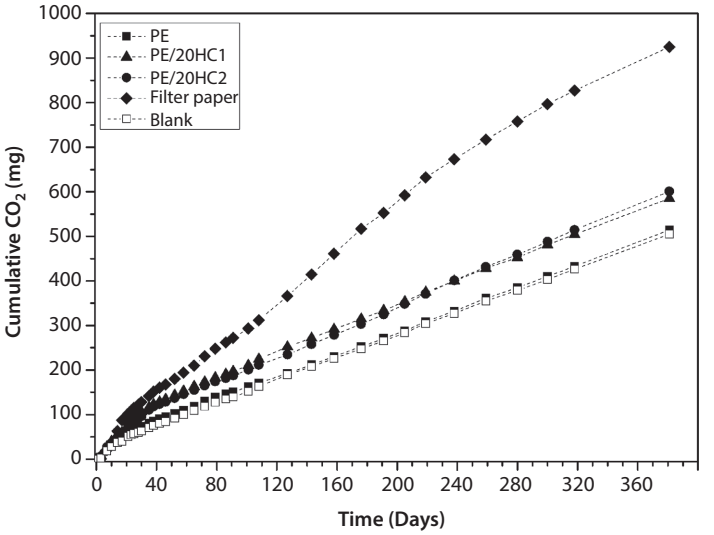

Figure 6 Cumulative CO_2 emissions of PE/HCs, PE, filter paper and blank in marine environment test.

Table 4 Biological degradation of PE/HCs, PE and filter paper in marine environment; % values calculated on base of theoretical amount of CO_2 from sample carbon content (see Eq. 1).

Sample in test flask	D_m (after 54 weeks) (%)
Filter paper	77.99
PE/20HC1 (fleshings)	9.57
PE/20HC2 (shavings)	12.31
PE	0.97

filter paper, 10% for PE/20HC1 (HC from fleshings) and 12% for PE/20HC2 (HC from shavings), while there was no significant biodegradation for pure PE.

To investigate the degradation process, at the end of marine environment testing the film samples were collected and characterized by SEM analysis (Figure 7). Figure 4a shows the film surface of PE sample after 54 weeks of incubation; it can be observed that the surface did not present significant degradative processes. Whereas, PE/20HC1 and PE/20HC2 (Figure 4b–d) presented a biofilm formation on the surface attributable to a microbial colonization.

The values of environmental degradability parameter, reported in Table 5, highlight an increase in biodegradability of PE/HC films in seawater with respect to the terrestrial terrestrial environment. The results indicated that the different salt content of hydrolysates did not affect the rate of degradation of the samples in marine environment, observing similar behavior for both samples (PE/20HC1 and PE/20HC2). In the end, the biodegradation rates of PE/HC blends were strickingly low in both test systems.

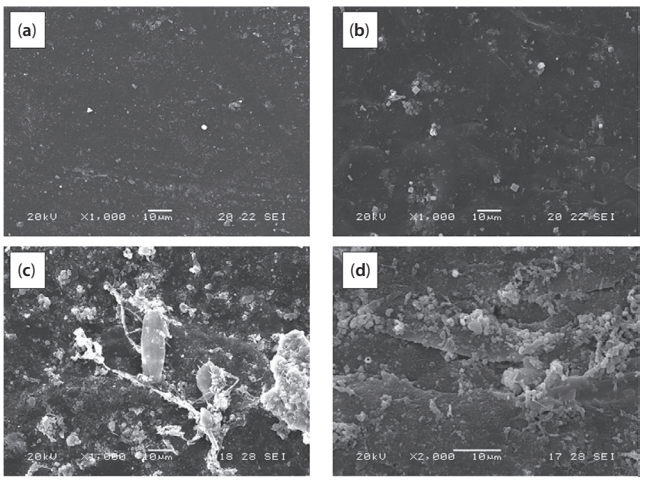

Figure 7 SEM micrographs of film surface of PE (**a**), PE/20HC1 (**b**) and PE/20HC2 (**c,d**) retrieved from marine environmental test flasks after 54 weeks of incubation.

4 CONCLUSIONS

The biodegradability of blends of low-density polyethylene (PE) containing 20 wt% of hydrolyzed collagen (HC) from the leather industry was studied in terrestrial and marine environmental conditions. The biodegradation behavior of the developed composites was evaluated by the CO_2 emissions from systems, and an environmental degradability parameter was calculated.

The obtained degradation extents for PE/HC blends were 3.6% for PE/20HC1 (HC from fleshings) and about 5.4% for PE/20HC2 (HC from shavings) in soil, and 10% for PE/20HC1 and 12% for PE/20HC2 in seawater. Therefore, the results of respirometric tests

Table 5 Environmental degradability parameter (B_d) of the tested materials.

Sample	D_m, terrestrial environment (%)	D_m, marine environment (%)
Cellulose	100	100
PE/20HC1	8.5	11.1
PE/20HC2	5.1	14.7
PE	0	0

highlight low degrees of biodegradability of the investigated PE/HC blends under two different conditions and environments. Further studies are in progress on hybrid blends based on oxo-biodegradable polyethylene samples and hydrolyzed collagen aimed at enhancing the biodegradability of materials.

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