Study of The Biodegradation of Poly (3-Hydroxybutyrate) (PHB) and High-Density Polyethylene (HDPE) by Microorganisms from the Sea waters of the Atlantic Coast of Brazil

Lhaira Souza Barreto

Federal University of Pernambuco: Universidade Federal de Pernambuco

Erika Emanuele Gomes Silva

Universidade Federal de Pernambuco

Luiz Emílio Pessôa Timeni Moraes Filho

University of Utah

Anna Raffaella Matos Costa

University of Pernambuco: Universidade de Pernambuco

Josiane Dantas Viana Barbosa

Instituto de Pesquisas Tecnológicas: Instituto de Pesquisas Tecnológicas

Sara Horacio de Oliveira Maciel

Universidade Federal de Pernambuco

Yêda Medeiros Bastos de Almeida

Universidade Federal de Pernambuco

Gloria Maria Vinhas (✉ gloria.vinhas@ufpe.br)

Universidade Federal de Pernambuco <https://orcid.org/0000-0001-5073-609X>

Research Article

Keywords: Biodegradation, Polymers, PHB, HDPE, Marine environment

Posted Date: December 20th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1166847/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The objective of this study was to evaluate the biodegradation of Poly (hydroxybutyrate) (PHB) and high-density polyethylene (HDPE) in static systems, using as fluid the seawater of the Coastal Region of the State of Pernambuco (Brazil). The physical and chemical modifications of the polymers, as a function of biodegradation, were evaluated by Fourier transform infrared spectroscopy (FTIR), mechanical tensile assay, differential scanning calorimetry (DSC), gravimetric test, and microbiological analysis. Through the FTIR, it was possible to observe in the PHB a decrease of 23.22% in the carbonyl index for the crystalline phase and 32.30% in the amorphous phase after 180 days, which evidences the effect of the biodegradation present. The mechanical properties of PHB were altered with biodegradation, but the thermal properties remained. During the gravimetric tests, there was a reduction in mass and consequently higher degradation rates for PHB, which is corroborated by the microbiological tests of the system. All characterizations demonstrated that the surface of the HDPE is less susceptible to biofilm formation and, consequently, to the enzymatic action of microorganisms. After 180 days of immersion, no significant microbiological degradation was observed in the HDPE, except for some abiotic alterations.

1. Introduction

Polymeric materials play a fundamental role in society, having their origins at the end of the 19th century, but only became known and in the mid-twentieth century [1]. They're used for various applications, due to their high versatility, low cost, and good mechanical, chemical and physical properties [2].

However, the environmental impact caused by its rapid disposal, associated with the low rate of degradation, has caused a large accumulation, both in terrestrial and aquatic ecosystems. Becoming a target of interest from scientists who recognize the need for urgent management measures for the sustainability of marine ecosystem services in the future [3-9].

Due to industrial activity, consumption habits, and mainly poor waste management, it is foreseen that up to 26 billion tons of plastic waste will be produced by 2050. More than half will be thrown away in landfills and finally enter ecospheres, such as oceans and lakes, leading to severe environmental pollution [10-12].

Although many polymers present as a notorious characteristic resistance to degradation, they are not exempt from the rupture of their bonds, when exposed to environments favorable to their deterioration. Therefore, industry and research groups conduct studies with the aim of reducing the rate of degradation in many applications [13,14].

The main mechanisms of plastic degradation in the environment are photodegradation, thermo-oxidative degradation, hydrolytic degradation, and biodegradation by microorganisms [13]. The importance of these mechanisms varies according to environmental conditions, being, for example, photodegradation significantly decreased in seawater due to low temperature and oxygen levels [15].

Recently, studies have tested the degradation capacity of different polymers. Naranci et al., (2018) [16], observed that PLA is not biodegradable in composting or in marine environment simulator reactors. PHBV (poly(3-hydroxybutyrate-co-3-hydroxyvalerate)) colonization was monitored in an aquarium in natural seawater for 6 weeks, showing that colonization density is higher in this plastic than in non-biodegradable ones (Dussud et al., 2018) [17] but the bacterial colonization profile of biodegradable plastics has not been described. Finally, Delacuvellerie et al. (2021) [18] studied biodegradation in the natural marine environment, of compostable polymers (PBAT and semicrystalline and amorphous PLA) and non-compostable polymers (polyethylene, polystyrene, polyethylene terephthalate, polyvinyl chloride) submerged *in situ* in sediment and in the water column in Mediterranean seawater. After 82 days of immersion, no significant degradation of the different polymers was observed, except for some abiotic changes of PBAT and HDPE probably due to a photo-oxidation process.

The marine environment comprises a wide variety of habitats under different environmental conditions. At the bottom of the sea, sediments are significant areas of organic matter with large concentrations of microorganisms compared to a column of water where polymers near the surface will receive light favoring photodegradation in relation to those present in the depths, and therefore the degradation of polymers is expected to be different due to the extra accumulation of organic matter [19].

The objective of this study was to evaluate the biodegradation of polymers, Poly (3-hydroxybutyrate) - PHB and High-density Polyethylene - HDPE, in static systems, using seawater as fluid. To monitor and evaluate the degradation process of plastic materials, the techniques were used by Fourier transform infrared spectroscopy (FTIR), mechanical tensile test, differential scanning calorimetry (DSC), gravimetric test, and microbiological analysis.

2. Materials And Methods

2.1 MATERIALS

The investigation was carried out with PHB - poly (hydroxybutyrate), and HDPE – high-density poly (ethylene). The analysis fluid used in the experiment was collected at a point in the Region of Marco Zero, Recife-PE, specifically at latitude 8°3'15" and longitude 34°52'53".

The PHB is naturally produced by a fermentative process of some bacteria, and depending on the species, there may be characteristics that influence properties from one batch to another. The PHB used in this study was produced by the *bacterium Burkholderia sacchari*. The material was supplied in powder form by PHB Industrial S/A (Serrana, SP). And high-density polyethylene (HDPE) was obtained in the form of small grains (pellets), and supplied by the thermoplastic company of Nordeste Eteno LTDA. Table 1 presents the main properties of these polymers.

Table 1. Comparison of the properties of the polymers under study.

Properties	PHB	HDPE
Molar Mass (g.mol ⁻¹)	600.000	5 x 10 ⁵ – 5 x 10 ⁶
Density (ASTM D 1238, 190 °C / 2.160g) (g/cm ³)	1.23	0.968
Melting temperature (°C)	140-180	245-265
Elastic Module (MPa)	4000	2800-4100
Tensile strength (MPa)	25-40	48-72
Elongation at break (%)	6	300-600
T _g (°C)	0	73-80
Fluidity index (MFI) (g/10 min)	40.0	0.35

Cast iron: AGARWAL (2012) [20]

2.1.1 Bioreactors

The experiments were conducted in static systems using glass bioreactors. The specimens were suspended by nylon wires and attached to the bioreactor lid (Figure 1), in order for the test bodies to be exposed at the same depth in the fluid, for a period of up to 180 days.

For the collection of samples, intervals ranging from 7, 14, 30, 60, 90, 120, and 180 days of experiments were used, counted from the date of assembly of the bioreactors. For each analysis in the aforementioned interval, ten previously numbered and weighed specimens were removed.

2.2 METHODS

2.2.1 Extruder processing

The polymers were processed in SENAI - CIMATEC, in modular IMACON extruder, with double thread, co-rotating, with ratio L/D = 30 and thread speed equal to 250 RPM. These materials were processed in the form of threads that passed through a stainless-steel vat, containing water at room temperature, in order to reduce the temperature gradient. Table 2 shows the processing temperature conditions for the polymers processed in the extruder.

Table 2 - Temperature conditions of the mixtures in the extruder (Flow = 3.0 kg/h).

Samples	Z1 (°C)	Z2 (°C)	Z3 (°C)	Z4 (°C)	Z5 (°C)	Z6 (°C)	Z7 (°C)	Head (°C)	Melted (°C)
PHB	145	145	145	150	155	155	155	160	175
HDPE	165	165	170	170	175	175	180	185	185

Source: Author, 2021

The next step was pre-drying by a compressed air collimator jet. Following, the formation of pellets the SAGEC equipment, model SG70, 220 v, with rotating knives, at a speed adjusted with the production of the thread.

2.2.2 Injection

After granulation and pellet formation, the materials were placed in trays in an air-circulation oven (PALLEY) at a temperature of 100 °C and kept for a period of 4 hours, in order to improve drying and thus mitigate the electrolytic degradation of these materials during processing [21]. The specimens were molded by the injection process in SENAI - CIMATEC, according to ISO 527 standards for tensile testing [22]. For this stage, a Primax ROMI injector with a capacity of 100 tons of closing force was used. Table 3 shows the injection conditions of the polymers under study.

Table 3 - Injection conditions.

Samples	Z1 (°C)	Z2 (°C)	Z3 (°C)	Beak
PHB	155	150	160	170
HDPE	170	180	190	200

Source: Author, 2021

2.2.3 CHARACTERIZATION OF SAMPLES

The raw materials were submitted to the following characterization techniques: Fourier Transform Infrared Spectrometry (FTIR), Differential Scanning Calorimetry (DSC), Mechanical test (Traction), Gravimetric Assays and Analysis of Sessile Microorganisms.

2.2.3.1 Infrared spectroscopy (FTIR)

The chemical characterization of polymers was obtained by infrared spectroscopy (FTIR), using a Thermo Scientific Nicolet model 32 IS10 spectrometer, performed at the State University of Santa Cruz-Ilhéus-BA. The spectra were recorded at room temperature in the wavenumber range 4000-400 cm⁻¹, with a resolution of 4 cm⁻¹. After the acquisition of the spectra, they were treated using origin 8.5 software,

through the process of baseline adjustment, normalization, and deconvolution. The Carbonyl Index (CI) was calculated according to Equation 1.

$$CI = \frac{Abs_{C=O}}{Abs_{C-H}} \quad (1)$$

Where CI is the Carbonyl index, $Abs_{C=O}$ is the absorbance of the carbonyl band, and Abs_{C-H} is the absorbance of the C-H band chosen as reference.

2.2.3.2 Differential scanning calorimetry (DSC)

Differential scanning calorimetry was performed in METTLER TOLEDO DSC 1 Star System equipment at the Petrochemical Laboratory (LPQ) at the Federal University of Pernambuco. The tests were performed with samples from 5 to 10 mg, in three stages: heating - cooling - reheating. All ramps happened under a nitrogen atmosphere with a flow of 50 mL/min.

2.2.3.3 Analyses of sessile-organisms

The cell concentrations of the microbial groups present in the biofilms were determined according to the periods of removal of the specimens to evaluate the biodegradation. The water inside the bioreactors was replaced every fourteen days, which is the time to exhaust the nutrients present in the seawater [23]. To quantify the sessile microorganisms, the specimens removed were placed in containers with 30 ml of saline solution and/or 30 ml of the reducing solution for the analysis of aerobic and anaerobic heterotrophic microorganisms, respectively. Then, these samples were submitted to the ultrasonic bath for 15 seconds to ensure the removal and dispersion of sessile microorganisms. The biofilms formed on the surface of the polymeric test bodies were removed by scraping the surface with a sterile spatula in the appropriate solutions. All procedures were performed in aseptic conditions.

Microorganisms were quantified using the following methods: a) aerobic heterotrophic bacteria (AHB) and *Pseudomonas spp.*: according to the methodology described by Silva *et al.*, 2005 [24]; b) iron precipitating bacteria (IPB): according to the methodology described by CETESB, 1992 [25]; c) anaerobic heterotrophic bacteria (AnHB): according to the methodology described by Silva *et al.*, 2005 [24] and Vieira *et al.*, 2008 [26]; d) Sulfate Reducing Bacteria (SRB): according to the methodology described by Postgate, 1984 [27]; e) Filamentous fungi: according to the methodology described by Silva *et al.*, 2005 [24].

2.2.3.4 Gravimetric Assay (Biodegradation Rate)

In the mass loss tests, the specimens were weighed before the start of the process and at the end of the process. Mass loss was quantified by the difference between its original weight and its weight after the test. For the calculation of the biodegradation rate of polymers, the average of 5 specimens was

considered. When removed from the systems, the biofilms were removed from the specimens, then washed with distilled water, ethyl alcohol, and acetone, and were subsequently dried with warm air.

2.2.3.5 Mechanical tensile test

The tensile tests, before and after the biodegradation process, were performed at SENAI - CIMATEC, in a universal MACHINE EMIC, model DL 2000 with a load cell of 20 kN. Each test used 5 test bodies, with procedures and dimensions specified by ISO 527. The data of the mechanical test were analyzed through analysis of variance (ANOVA) using the software Statistica, version 10.0.228.8.

3. Results

3.3 FTIR

Fourier transform infrared spectroscopy was used to evaluate the chemical modifications that occurred on the surface of polymers. The PHB (Figures 2 and 3) and HDPE (Figures 4 and 5) spectra indicate the FTIR measurements of polymers before biodegradation compared to polymers after biodegradation ones.

Figure 2 shows the infrared spectra of PHB samples at the beginning and after the biodegradation period.

Fig. 2 Initial PHB FTIR spectrum, PHB 7 days, PHB 14 days, PHB 30 days, PHB 60 days, PHB 90 days, PHB 120 days, and PHB 180 days in seawater

The PHB spectra (Figure 2) presented a band at 1720 cm^{-1} attributed to the stretch of the group C=O of the crystalline phase and around 1748 cm^{-1} attributed to the stretching of group C=O of the amorphous phase. The band at 1380 cm^{-1} is attributed to the deformation of CH_3 groups [28].

In the intensity of absorbance at 1748 cm^{-1} , corresponding to the carbonyl group (C=O), there was a difference when the specimens were submitted to seawater, similar to that occurred in the literature [28], which reports the decrease in the intensity of the carbonyl band with the test time in the water of the Atibaia River.

In general, the spectra found are similar (Figure 2), but the form used to quantify the degradation of polyesters is the determination of the presence of terminal carboxylic groups in the polymer. This can be used to quantify degradation because the formation of these groups is due to the degradation of polyester by the microorganism producers of extracellular esterase. For a better resolution of the bands, a deconvolution was performed, through the Lorentzian function, of the PHB spectra (Figure 3) for the normalization of the absorbance intensity of a band considered as an internal standard at 1380 cm^{-1} , attributed to the deformation of CH groups [29,30].

From the values obtained with deconvolution performed on the graphs in Figure 2, it was possible to calculate (Equation 1), analyze, and compare the carbonyl indices of the amorphous phase and the

crystalline phase of PHB initial and after biodegradation (Table 4)

Table 4 - Amorphous phase carbonyl indices 1749 cm^{-1} and crystalline 1720 cm^{-1} , calculated for PHB.

Sample	C=O (1720)	C=O (1749)	C-H (1380)	CI (1720)	CI (1749)
PHB	0.2513	0.869	0.0860	2.920	1.010
PHB 7 days	0.070	0.020	0.027	2.547	0.752
PHB 14 days	0.139	0.040	0.055	2.512	0.724
PHB 30 days	0.059	0.017	0.023	2.537	0.732
PHB 60 days	0.053	0.016	0.021	2.477	0.754
PHB 90 days	0.067	0.020	0.027	2.448	0.743
PHB 120 days	0.104	0.030	0.039	2.648	0.763
PHB 180 days	0.147	0.016	0.065	2.241	0.684

Source: Author, 2021

It was observed that the indexes of terminal carboxylic groups for all samples showed a decrease in this functional group during the degradation time. The results showed a decrease of 23.22% in the carbonyl index for the crystalline phase and 32.30% in the amorphous phase after 180 days. The decrease in absorbances related to carbonyl indices, both in the amorphous and crystalline phase, evidences the effect of present biodegradation, resulting from the action of esterase that probably occurred in carbonyl carbon of the ester bond.

Thus, PHB samples submitted to microbial treatment in seawater were biodegraded by the action of microorganisms in this biome, occurring hydrolytic reactions of ester groups and oxidative breaks of the polymer chain.

Observing Figure 4, we notice the presence of three absorption bands in the HDPE, these three bands refer to the different vibrations of the C-H bond.

The presence of a band at 1464 cm^{-1} , can be attributed to angular deformation outside the plane of CH_2 , which does not vary depending on the degradation time. Figure 5 shows the expanded FTIR spectra in the region from 1800 to 1400 cm^{-1} , which is the region of interest.

Comparing Figures 4 and 5, it is possible to observe that the periods of immersion in seawater did not cause changes in the formation of carbonyl groups or double bonds that indicate the process of biodegradation of the HDPE [31].

One of the characteristics of this polymer is hydrophobicity, which can hinder enzymatic attack, evidencing a great resistance to biodegradation with microorganisms present in the studied fluid. But

Liyoshi et al. (1998) [32] submitted three species of lignin-degrading fungi, including *P. chrysosporium*, to degradation tests with the HDPE and concluded that manganese peroxidase enzymes caused significant degradation of this polymer, suggesting that this enzymatic complex may be the key to polyethylene degradation. This is because the biodegradation of the material is linked to the type of enzyme, microorganism, temperature, and exposure time [18].

3.2 Differential Scanning Calorimetry (DSC)

DSC analyses were used to investigate the crystallization and fusion events of PHB and HDPE before and after exposure to the marine environment for periods of 7, 14, 30, 60, 90, 120, and 180 days. The samples were initially heated to erase the thermal history, and then cooled quickly and heated again. Table 6 presents the parameters of crystallization temperature (T_c), melting (T_m), and the most endothermal heat (ΔH) and crystallinity (ΔX).

For the PHB sample (Figure 6), during cooling, a peak of cold crystallization (C1) was detected for the PHB/0 days. From 7 days of exposure to the marine environment, a very subtle peak appears around 49 °C and remains with more advanced exposure times, resulting from a possible reordering of the crystalline structure of the PHB due to the attack of microorganisms. Some authors raise the hypothesis of inter-spherulitic segregation [33].

Then, during the second PHB/0 day heating, two thermal events are observed: (1) an exothermic peak associated with a crystalline reordering of PHB or secondary crystallization (C2) [33]; and, (2) the fusion of crystals, which occurs in the range of (159 °C). The fusion of these structures is characterized by a double peak, which could be explained in terms of fusion/recrystallization processes [33]: peak I is characterized by a shoulder around 159°C, corresponds to fusion of imperfect crystals, while peak II, peak itself, around 166.3°C, corresponds to the fusion of perfect crystals produced by crystallization during cooling.

A subtle decrease in crystallinity was observed in the first 60 days, and an increase in the crystallinity of the samples from 90 days on. Due to the hydrolytic scissions of the chain segments, increases in the degree of crystallinity and melting temperature indicate reorganization of the remaining polymer chains after the consumption of the amorphous phase and increased thickness of the lamellae.

With regard to crystallization in cooling, it can be seen in Figure 7 that the HDPE presents a narrow and well-defined peak around 118, characteristic of the formation of well-uniform crystals. Table 4 shows that crystallization temperatures did not change with increased exposure to the marine environment, and only a discrete shift of T_c after 180 days of exposure can be seen.

In reheating, only a single endothermic peak is detected, close to 130 °C, the melting temperatures of the polymers after exposure remained almost equal to the melting temperatures of the HDPE without exposure. For the HDPE, there was an increase in its crystallinity of 81.6%, with 60 days of exposure to the marine environment. The crystallinity of polymeric materials is often one of the factors that hinder the

access of microorganisms to biodegradation, that is, amorphous parts are preferably attacked, consequently the crystallinity of the HDPE increases by the division of its chains, followed by subsequent recrystallization of smaller chains.

It can also be observed a decrease in crystallinity after 60 days of exposure to the marine environment, the reason for this could be the lack of homogeneity introduced from the formation of the biofilm during exposure that can act as obstacles during the growth of crystalline lamellae. Crystalline lamellae have to overcome the obstacle that slows the general crystallization process [34].

Biofilms are materials composed of extracellular polymeric substances excreted by microorganisms, favoring the adhesion of cells on the surface of the material and on other cells [35]. During the stages of biodegradation evaluation, it is common to observe the formation of a biofilm on the surface of the exposed materials. With the formation of biofilm, microorganisms, the metabolism of the beings involved, produce several enzymes and substances such as acids that interact with the surface of the polymer promoting chemical reactions responsible for degradation [36, 37].

Table 4 - Melting temperature (T_f) and crystallization (T_c), degree of crystalline (X_c) and fusion enthalpy (ΔH_f), and crystallization (ΔH_c) for all polymers obtained through DSC.

	Days	T _c (°C)	ΔH _c (J/g)	T _m (°C)	ΔH _m (J/g)	ΔX _m (%)
PHB	0	77.09	62.18	159/166.3	97.69	66.9
	7	68.65/118.44	60.35	165.94	98.69	67.59
	14	81.59/118.38	66.68	166.70	92.71	63.50
	30	74.45/118.48	47.89	158.87/166.83	91.85	62.91
	60	81.30/118.21	60.21	158.5/166.44	94.02	63.40
	90	80.67/118.87	48.21	156.91/164.80	103.38	70.81
	120	80.93/118.26	70.81	166.20	97.49	66.78
	180	81.08/118.26	64.21	166.23	96.92	66.39
HDPE	0	118.0	197.8	130.5	205.6	70.1
	7	117.7/89.0	201.3	130.7	203.7	69.5
	14	117.7/72.8	211.7	130.8	202.9	69.3
	30	118.0	211.2	130.9	199.6	68.1
	60	118.5	221.9	129.7	237.4	81.3
	90	117.6	202.2	132.4	198.8	67.9
	120	117.6	209.5	130.9	202.7	69.2
	180	115.4	202.5	134.4	192.5	65.7

Source: Author, 2021

3.4 MICROBIOLOGICAL ANALYSES

3.4.1 Seawater Plankton Bacteria

The cellular concentrations of aerobic heterotrophic plankton bacteria (AHB), iron precipitants (IPB), anaerobic heterotrophic bacteria (AnHB), and sulfate reducers (SRB) quantified in seawater, studied in this study, were quantified and presented in Figure 8.

The highest concentrations found were from AHB, AnHB, and SRB, all in the order of 10^8 cells/ml. In a smaller amount is the IPB, with a concentration of 10^3 cells/ml. In the literature, other studies were found for the concentration of microorganisms in seawater in the port of Recife-PE: Argolo *et al.*, (2015) [38] quantified the same microbial groups and obtained values of the order of 10^6 cells/ml for AHB and IPB, 10^5 cells/ml for AnHB and 10^3 cells/ml for SRB. Medeiros *et al.*, (2016) [39] found values of the order of 10^5 cells/mL for AHB and AnHB, 10^4 cells/mL for IPB and 10^2 cells/mL for SRB. In the studies

mentioned, the concentrations obtained were lower than those found in the present study for all plankton groups, except for IPB that presented lower concentrations.

The presence of these bacteria in seawater evidences a medium with potential for the occurrence of biodegradation. These analyses can generate different results, as it is influenced by the location, time, climatic conditions, and other factors [40].

3.4.2 Sessile Bacteria in Different Systems

Figures 9, 10, 11, 12, 13, 14, and 15 show, respectively, the results of microbiological analyses in the periods of 7, 14, 30, 60, 90, 120, and 180 days in both systems, PHB and HDPE.

An analysis in the graphs allows us to observe that, at 7, 14 and 30 days of the experiment (Figures 9, 10, and 11) the aerobic microbiota (AHB, IPB, and filamentous fungi) had higher sessile concentrations for the PHB polymer. Only at 7 days, the filamentous fungi had similar sessile concentrations for the two polymers. *Pseudomonas spp.* were only quantified at 7 days for PHB polymer. The sessile concentrations of anaerobic microorganisms (AnHB and SRB) were also benefited on the surfaces of PHB polymers in these experiment times. With 14 days, the AnHB had similar concentrations for the two polymers. These higher concentrations of the microbiota for PHB demonstrate that this polymer is more susceptible to the adherence of microorganisms, with biodegradation initiated within fewer days than the HDPE, as shown in the graph in Figure 11.

It is also important to highlight that aerobic bacteria (AHB and IPB) showed a reduction in their growth from 7 to 14 days, probably due to nutrient deficiency and/or oxygenation of the medium. This reduction in the concentration of these microbial groups may also be related to the increase in sessile exopolysaccharides (SEP), because the IPB aggregate iron precipitates in the biofilm, decreasing oxygenation and leading to the growth of anaerobic heterotrophic bacteria, as shown in Figures 9, 10 and 11 [41]. From 30 days of the experiment, the concentrations of anaerobic microorganisms increased continuously up to 180 days. In the final times analyzed, the aerobic microbiota (AHB and IPB) also had high concentrations, *Pseudomonas spp.* were again quantified in the systems and the filament fungi maintained a low concentration in the two polymers (Figure 12, 13, and 14).

As already discussed in the literature, PHB is a natural polyester, that is, it is produced by bacteria as an intracellular energy reserve. Thus, this material is compatible with the mechanisms of bio assimilation of smaller chains, generated from hydrolysis and biological oxidation promoted by enzymes produced by bacteria and fungi [42].

However, for the HDPE, its hydrophobicity limits the interaction between the polymer and enzymes, hindering their attack and, consequently, biodegradation (Figure 4) [43]. Therefore, the count in the initial periods, 7, 14, and 30 days of the experiment were lower in the HDPE, since this polymer did not serve as a source of carbon to microorganisms. Over time, microorganisms start to develop new enzymes, which facilitate biodegradation, and the microbial population increases concentration [28].

3.5 GRAVIMETRIC BIODEGRADATION ASSAYS

The gravimetric assay is the most commonly applied and relatively sensitive method for determining changes caused by microbial attack in polymers [44].

Figure 15 shows the profile of the degradation rate of PHB and HDPE over 180 days of the experiment.

Two different degradation profiles are observed, comparing polymers, higher degradation rates for PHB are shown. This is corroborated by the microbiota of the systems, shown in Figures 9, 10, and 11, which demonstrated that the surface of the HDPE is less susceptible to the formation of biofilms and, consequently, to the enzymatic action of microorganisms.

This can be explained by the chemical composition of polymers, PHB contains in its structure carboxylic groups, which are hydrophilic. The HDPE, because it is formed by hydrophobic chemical groups, hinders the enzymatic action of microorganisms on the polymer surface [45] confirming what was presented by the analysis of the chemical structure of polymers by FTIR.

Through mass loss analyses, it was observed that biodegradation can be favored, depending on the microbiota of the fluid, exposure to abiotic agents, and the structure of polymers.

3.6 Traction

Table 5 presents the results of the stress at rupture, modulus of elasticity and specific strain for the PHB before and after the inoculation process.

Table 5 shows that after the periods of exposure of the specimens to the marine environment, there were changes in all mechanical properties analyzed as a function of time. However, from the exposure time of 30 days to 90 days, the rupture stress, the elastic modulus, and the specific strain did not undergo significant changes in their values. During the biodegradation process, the surface and interior of the specimens are filled with voids, facilitating the propagation of cracks during the tensile test, resulting in the decrease of mechanical properties [46]. For this reason, it was not possible to perform the tensile tests of the specimens of 120 and 180 days.

Table 5- Mechanical properties before and after PHB inoculation

Samples	Tension at break (MPa)	Elastic module (N/m ²)	Specific deformation (u.a)
PHB	6.90 ^a ± 0.55	2969.6 ^a ± 46.07	0.68 ^a ± 0.166
PHB 7 days	5.21 ^a ± 1.96	2168.7 ^b ± 187.25	0.24 ^b ± 0.18
PHB 14 days	4.49 ^b ± 0.95	2168.86.7 ^b ± 49.70	0.32 ^c ± 0.32
PHB 30 days	2.44 ^c ± 0.44	1186.62 ^c ± 199.32	0.39 ^a ± 0.12
PHB 60 days	2.35 ^c ± 0.38	1358.10 ^c ± 130.85	0.85 ^a ± 0.06
PHB 90 days	0.72 ^c ± 0.51	670.79 ^c ± 340.02	0.86 ^a ± 0.18
PHB 120 days	-	-	-
PHB 180 days	-	-	-

^{a,b,c} shows that they are significantly different with a $p < 0.05$.

Source: Author, 2021

PHB is a very hard and fragile material, has a low viscosity, and is a polymer with a high modulus of elasticity [47]. Evaluating the modulus of elasticity in traction of the initial PHB and after the biodegradation process, it is observed, in general, that with the time of exposure to the marine environment this property is decreased.

The modulus of elasticity is a measure of the stiffness of the material, that is, it is directly related to the material's ability to resist deformations [48]. For better visualization of the behavior of the samples, the elastic module graphs (Figure 16a) and Rupture Stress (Figure 16b) were plotted:

Analyzing the modulus of elasticity (Figure 16a), there was a decrease of 26.9% after 7 days. In the 14-day period, the modulus of elasticity showed no significant difference in relation to the previous point. Behavior that corroborates the results of mass loss (Figure 15) and counting of microorganisms (Figure 10), which did not present alterations at these times. In the times of 30, 60, and 90 days, the elasticity modules showed non-significant decreases over time.

Figure 16b indicates the behavior of the maximum tensile stress found for the studied times. It can be observed that the maximum tensile stress values gradually decreased over time. This property corresponds to the greatest tension that the material can resist, if this tension is applied and maintained, the result will be the fracture.

Through the data obtained in the tensile tests, it is verified that the PHB presents fragile behavior. Elastic deformation in the polymer occurs due to its crystalline part, which provides resistance to the material and still provides elasticity due to the relative mobility of crystalline chains [49]. As most of the PHB structure is amorphous, it breaks before the crystalline portion of the material, causing irreversible deformations, which lead to decreased material resistance and consequently rupture of polymer chains.

It is noted that the mechanical properties of PHB samples were influenced by the conditions of the biodegradation tests. It can be explained by a possible decrease in the molecular mass of PHB. According to Montoro (2010) [50], molar mass is an extremely important factor, because it directly affects the mechanical resistance of the polymer, the ability to swell, the ease of being hydrolyzed, and, consequently, the rate of biodegradation, which is related to its crystallinity.

Table 6 and Figure 17 show the results of the stress at rupture, modulus of elasticity, and specific deformation obtained for the HDPE before and after the biodegradation process.

Table 6 - Mechanical properties before and after THE PEA biodegradation.

Samples	Maximum tension under traction	Tension at break (MPa)	Elastic module (Nm ²)	Specific deformation (u.a)
HDPE	17.36 ± 2.16	2.44 ^a ± 0.88	1083.52 ^a ± 29.62	525.83 ^a ± 21.42
HDPE 7 days	17.30 ± 0.38	6.93 ^b ± 1.21	1077.53 ^a ± 65.23	635.27 ^{a,b} ± 8.79
HDPE 14 days	15.57 ± 4.02	6.78 ^b ± 2.29	1072.13 ^a ± 48.04	684.09 ^b ± 2.66
HDPE 30 days	15.12 ± 1.68	3.44 ^a ± 1.20	1071.59 ^a ± 8.94	567.81 ^{a,b} ± 54.66
HDPE 60 days	14.85 ± 1.81	2.27 ^a ± 1.17	1070.16 ^a ± 7.64	337.36 ^c ± 127.36
HDPE 90 days	14.82 ± 1.81	3.01 ^a ± 1.83	1071.21 ^a ± 8.03	361.88 ^c ± 92.67
HDPE 120 days	14.39 ± 1.85	5.11 ^b ± 1.75	1075.1 ^a ± 5.49	338.72 ^c ± 142.9
HDPE 180 days	14.25 ± 1.44	3.88 ^a ± 2.65	1069.0 ^a ± 31.53	639.3 ^b ± 25.29

^{a,b,c} shows that they are significantly different with a $p < 0.05$

Source: Author, 2021

It is observed that the HDPE is highly tenacious, that is, it is capable of suffering a high degree of deformation before breaking, and presents low tensile strength and low modulus of elasticity (Figure 17a).

The modulus of elasticity and maximum tensile stress did not undergo significant changes after 7 and 14 days. The elasticity modules for the HDPE after 30, 60, and 90 days immersed in seawater differed by 3.5%, 3.54, and 4.2%, respectively, when compared to HDPE before the experiment. A fact probably resulting from the increase in population density of microorganisms present on the surface of the material, since the compositions are practically similar to those of the pure material. The results show that the HDPE presented behavior similar to those reported in the literature by Elleuch and Taktak (2006) [51].

4. Conclusion

For the period of time studied, the microorganisms present in the water collected in the Marco Zero region in Recife-PE were able to biodegrade the PHB. The HDPE remained resistant to microbial action. PHB biodegradation occurs proportionally in the amorphous and crystalline phases and for the HDPE there were no changes in the bands that indicate the biodegradation process. The PHB showed higher rates of degradation when compared to HDPE, demonstrating that the surface of PHB is more susceptible to the formation of biofilms and, consequently, to enzymatic actions of microorganisms. Analyzing the results obtained and comparing with the HDPE, it was observed that after the inoculation periods there were changes in all mechanical properties analyzed for PHB. The HDPE, on the other hand, did not undergo significant changes. Impacted environments such as the Marco Zero region of Recife-PE are significant in prospecting microorganisms capable of biodegrading polymers.

References

- [1] Bergmann M, Gutow L, Klages M (2015) *Marine Anthropogenic Litter*. 1st ed. London, Springer.
- [2] Yuan J, Ma J, Sun Y, Zhou T, Zhao Y, Yu F (2020) Microbial degradation and other environmental aspects of microplastics/plastics. *Sci. Total Environ* 715:136968.
- [3] Eriksen M, Mason S, Wilson S, Box C, Zellers A, Edwards W, Farley H, Amato S (2013) Microplastic pollution in the surface waters of the Laurentian Great Lakes. *Mar. Pollut Bull* 77 (1–2):177–182
- [4] Rochman C, Browne M, Halpern B, Hentschel B, Hoh E (2013) Classify plastic waste as hazardous. *Nature* 494:169–171
- [5] Vegter AC, Barletta M, Beck C, Borrero J, Burton H, Campbell ML, Costa MF, Eriksen M, Eriksson C, Estrades A, GILARDI KVK (2014) Global research priorities to mitigate plastic pollution impacts on marine wildlife. *Endangered Species Research* 25: 225–247. <http://dx.doi.org/10.3354/esr00623>.

- [6] Perkins S (2015) Nearly every seabird may be eating plastic by 2050. Retrieved from, <http://www.sciencemag.org/news/2015/08/nearly-every-seabird-may-be-eatingplastic2050>. Accessed September 21, 2021.
- [7] Álvarez-Hernández C, Cairós C, López-Darias J, Mazzetti E, Hernández-Sánchez C, González-Sálamo J, Hernández-Borges J (2019) Microplastic debris in beaches of Tenerife (Canary Islands, Spain). *Marine Pollution Bulletin* 146: 26–32
- [8] Bergmann M, Mützel S, Primpke S, Tekman MB, Trachsel J, Gerdtz G (2019) White and wonderful? Microplastics prevail in snow from the Alps to the Arctic. *Science Advances*. <https://doi.org/10.1126/sciadv.aax1157>
- [9] Danso D, Chow J, Streit WR (2019) Plastics: environmental and biotechnological perspectives on microbial degradation. *Appl Environ Microbiol* 85: e01095-19.
- [10] Jambeck JR, Geyer R, Wilcox C, Siegler TR, Perryman M, Andrady A, Narayan R, Law, KL (2015) Plastic waste inputs from land into the ocean. *Science* 347: 768– 771
- [11] Lönnstedt OM, Eklöv P (2016) Environmentally relevant concentrations of microplastic particles influence larval fish ecology, ecotoxicology. *Science* 352(6290): 1213-1216
- [12] Geyer R, Jambeck J, Law KL (2017) Production, use, and fate of all plastics ever made. *Sci Adv* 3(7):e1700782.
- [13] ANDRADY AL (2011) Microplastics in the marine environment. *Marine Pollution Bulletin* 62:1596–1605
- [14] Azevedo JB, Viana JD, Carvalho, LH, Canedo LE (2016) Caracterização de compósitos obtidos a partir de polímero biodegradável e casca de arroz utilizando duas técnicas de processamento. *Revista Matéria* 1517-7076: 391-406
- [15] Webb HK, Arnott J, Crawford RJ, Ivanova EP (2013) Plastic degradation and its 692 environmental implications with special reference to poly (ethylene terephthalate). 693 *Polymers* 5: 1-18
- [16] Naranci T, Verstichel S, Chaganti SR, Morales-Gamez L, Kenny ST, Wilde B, Padamati RB, O’connor KE (2018) Biodegradable plastic blends create new possibilities for end-of-life management of plastics but they are not a Panacea for plastic pollution. *Environ. Sci. Technol* 52 (18): 10441–10452.
- [17] Dussud C, Hudec C, George M, Fabre P, Higgs P, Bruzaud S, Delort AM, Eyheraguibel B, Meistertzheim AL, Jacquin J, Cheng J, Callac N, Odobel C, Rabouille, S, Ghiglione JF (2018) Colonization of non-biodegradable and biodegradable plastics by marine microorganisms. *Front. Microbiol* 9: 1571
- [18] Delacuvellerie A, Benali S, Cyriaque V, Moins S, Raquez JM, Gobert S, Wattiez R (2021) Composição do biofilme microbiano e degradação do polímero de plásticos compostáveis e não compostáveis

imersos no meio marinho. *Journal of Hazardous Materials* 419: 126526

[19] Middelburg JJ, Vlug T, Jaco F, Vandernat WA (1993) Organic matter mineralization 635 in marine systems. *Glob Planet Change* 8(1-2): 47-58

[20] Agarwal S (2012) Biodegradable Polyesters. In *Polymer Science: A Comprehensive Reference*. Elsevier, 333–361.

[21] Pachekoski WM (2001) Desenvolvimento de blendas poliméricas do polímero biodegradável poli(hidroxibutirato) (PHB) com Polipropileno (PP), São Carlos, SP. 134 p., Dissertação (Mestrado) – Programa de Pós-Graduação do Departamento de Ciências e Engenharia de Materiais (PPG-CEM), Universidade de São Carlos.

[22] ISO 527-1:2019. Plastics – Determination of tensile properties – Part 1: General principles/ Também no catálogo da ABMT.

[23] França FP, Ferreira CA, Lutterbach MTS (2000) Effect of different salinities of a dynamic water system on biofilm formation. *Journal of Industrial Microbiology & Biotechnology* 25: 45-48

[24] Silva N, Neto RC, Junqueira VCA, Silveira NFA (2005) Manual de métodos de análise microbiológica da água. Varela Editora e Livraria Ltda, São Paulo

[25] CETESB (1992) Contagens de colônias de bactérias que precipitam o ferro. Norma L5.207:1-11

[26] Vieira DM, De França FP (2008) *Corrosion Review* 26

[27] Postgate JR (1984) *The Sulphate-reducing bacteria*. 2nd ed. London: Cambridge University Press

[28] Faria AU, Franchetti SMM (2010) Biodegradação de Filmes de Polipropileno (PP), Poli(3-hidroxibutirato) (PHB) e Blenda de PP/PHB por Micro-organismos das Algas do Rio Atibaia. *Polímeros* 20: 141-147

[29] Xu J, Gou BH, Yang R, Wu Q, Chen GQ, Zang ZM (2002) *Polymer* 43: 6893.

[30] Furukawa T, Sato H, Murakami R, Zhang J, Noda I, Ochiai S, Ozaki Y (2007) *Polymer* 48: 1749

[31] Hadad D, Geresh S, Sivan A (2005) Biodegradation of polyethylene by the termophilic bacterium *Brevibacillus borstelensis*. *J. App. Microbiol* 98: 1093-1100

[32] Liyoshi Y, Tsutsumi Y, Nishida T (1998) Polyethylene degradation by lignin-degrading fungi and manganese peroxidase. *Journal of Wood Science* 44: 222-229

[33] Costa AR, Santos RM, Ito, EN, Carvalho, LH, Canedo, EL (2019) Melt and cold crystallization in a poly(3-hydroxybutyrate) poly(butylene adipato-co-terephthate) blend. *J Therm Anal Calorim* 137:1341-1346

- [34] Musuc AM, Badea-Doni M, Jecu L, Rusu A, Popa VT (2013) FTIR, XRD, and DSC analysis of the rosemary extract effect on polyethylene structure and biodegradability. *Journal of Thermal Analysis and Calorimetry* 114(1):169–177
- [35] Kokare CR, Chakraborty S, Khopade, AN, Mahadik KR (2009) Biofilm: Importance and applications. *Indian Journal of Biotechnology* 8:159-168
- [36] Gu JD (2003) Microbiological deterioration and degradation of synthetic polymeric materials: Recent research advances. *International Biodeterioration and Biodegradation* 52: 69-91
- [37] Marchand S, De Block J, De Jonghe J, Coorevits A, Heyndrickx M, Herman L (2012) Biofilm Formation in Milk Production and Processing Environments; Influence on Milk Quality and Safety. *Comprehensive Reviews in Food Science and Food Safety* 11: 133-147
- [38] Argolo E, França F, Oliveira S, Silva R, Cruz T, Queiroz V, Urtiga Filho S, Lima M (2015) Biocorrosão em cupons de aço carbono AISI 1020 expostos à água do mar e óleo diesel S10/Biodiesel B6. In: Conferência sobre Tecnologia dos Equipamentos, 13, Cabo de Santo Agostinho, Anais. São Paulo: Abendi
- [39] Medeiros LBS; Argolo EJ, Da Silva RGC, Maciel SHO, De França FP, Lima MAGA (2016) Processo de Corrosão Microbiológica do Aço Carbono AISI 1020 imerso em Óleo Diesel B30 com Água do Mar. In: ANAIS DO CONGRESSO BRASILEIRO DE ENGENHARIA QUÍMICA
- [40] Videla, HA (2003) Biocorrosão, biofouling e biodegradação de materiais. 1 ed. São Paulo: Editora Eggard Blucher Ltda, 148p.
- [41] Boari CA, Alves MP, Tebaldi VMR, Savian TV, Piccoli RH (2009) Formação de biofilme em aço inoxidável por *Aeromonas hydrophila* e *Staphylococcus aureus* usando leite e diferentes condições de cultivo. *Ciência e Tecnologia. Alimentos. Campinas*, v. 29(4): 886-895
- [42] Franchetti SMM, Marconato JC (2006) Polímeros Biodegradáveis - Uma solução parcial para diminuir a quantidade de resíduos plásticos. *Química Nova* 29: 811–816
- [43] Arcana IM, Sulaeman A, Padiangan KD, Handoko A, Ledyastuti M (2006) Synthesis of polyblends from polypropylene na poly(R,S)- β -hydroxybutyrate, and their characterization. *Polym. Interact* 55: 435-440
- [44] Flemming HC (1998) Relevance of Biofilms for the biodeterioration of Surfaces of Polymeric Materials. *Polym. Degrad, Stab* 59: 309
- [45] Costa CZ, Albuquerque MCC, Brum MC, Castro, AM (2015) Microbial and Enzymatic Degradation of Polymers: A Review. *Química Nova (Impresso)* 38: 259-267

- [46] Luo S, Netravali AN (2003) A study of physical and mechanical properties of poly(hydroxybutyrate-co-hydroxyvalerate) during composting. *Polymer degradation and stability* 80: 59-66.
- [47] Abdelwahab MA, Flynn A, Chiou BS, Imam S, Orts W, Chiellini E (2012) Thermal, mechanical and morphological characterization of plasticized PLA-PHB blends. *Polymer Degradation and Stability* 97: 1822-1828
- [48] Callister Jr WD (2016) *Ciência e engenharia de materiais: Uma introdução*, LTC; p 705
- [49] Canevarolo Jr SV (2010) *Ciência dos polímeros: Um texto básico para tecnólogos e engenheiros*, Artliber; p. 280
- [50] Montoro SR (2005) *Redução da Massa Molar do Poli (3-Hidroxibutirato-co-3-Hidroxivalerato) (PHBHV) para sua Posterior Utilização no Desenvolvimento de Sistemas de Liberação Controlada*", Dissertação de Mestrado, Universidade de São Paulo, Brasil
- [51] Elleuch R, Taktak W (2006) Viscoelastic Behavior of HDPE Polymer using Tensile and Compressive Loading. *Journal of Materials and Engineering Performance* 15: 111–116

Figures

Figure 1

Figure 2

Initial PHB FTIR spectrum, PHB 7 days, PHB 14 days, PHB 30 days, PHB 60 days, PHB 90 days, PHB 120 days, and PHB 180 days in seawater

Figure 4

FTIR spectrum of the initial HDPE, HDPE 7 days, HDPE14 days and HDPE 30 days, HDPE 60 days, HDPE 90 days, HDPE 120 days, and HDPE 180 days in seawater. In the range of 4000 - 400 cm⁻¹

Figure 8

Quantification of plankton bacteria in seawater used in the experiment

Figure 9

Sessile microbial quantification of the specimens of the four systems in 7 days of the experiment: a) AHB, IPB, AnHB, and SRB and b) Filamentous fungi and Pseudomonas sp

Figure 12

Sessile microbial quantification of the specimens of the PHB and HDPE systems in 90 days of the experiment: a) AHB, IPB, AnHB, and SRB and b) Filamentous fungi and Pseudomonas sp

Figure 17

Values for elastic modulus (a) and rupture stress (b) obtained before and after HDPE biodegradation