Study of biodegradation of Poly(butylene adipate co-terephthalate) (PBAT) by maritime microorganisms from the Atlantic Coast of Recife-PE (Brazil)

Estudo da biodegradação do Poli(butileno adipato co-tereftalato) (PBAT) por microrganismos marítimos do Litoral Atlântico do Recife-PE (Brasil)

Estudio de biodegradación de Poli(co-tereftalato de adipato de butileno) (PBAT) por microorganismos marítimos La Costa Atlántica de Recife-PE (Brasil)

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Abstract

Biodegradable polymers undergo a degradation process resulting from the action of microorganisms such as bacteria, fungi and algae. Poly(butylene adipate co-terephthalate) (PBAT) is considered a biodegradable synthetic polymer, even if its degradation has been confirmed under industrial composting conditions, the investigation of its degradation in the marine environment is still limited. Therefore, this work aims to study the biodegradation in the marine environment, of the biodegradable polymer (PBAT), and for that, it was submerged in a static system, using seawater from the coastal region of Pernambuco/Brazil as a fluid. The samples were studied by chemical, thermal and microbiological analyses, after 7, 14, 30, 90, 120 and 180 days of immersion. Microbiological analyzes indicated that aerobic heterotrophic bacteria (AHB), anaerobic heterotrophic bacteria (AnHB) and iron precipitating bacteria (IPB) were quantified in the system at all times at high concentrations, with the exception of Sulfate reducing bacteria (SRB), fungi and Pseudomonas that showed lower concentrations compared to other bacterial groups. Biodegradation was observed by the percentage of mass loss of approximately 2.25%. In the DSC, the expansion of melting peaks after exposure to the marine environment was noted, while the TGA did not show changes in the curve trends. The FTIR showed that no new band appeared, nor displacement, since the vibrations of the covalent bonds of the groups are present regardless of the biodegradation. Indicating that no significant microbiological degradation of PBAT was observed.

Keywords: Biodegradation; Polymers; PBAT; Microorganisms; Marine environment.
1. Introduction

The growing concern with the disposal of synthetic polymers, observed in recent decades, due to their enormous quantity and environmental impact, has motivated the search for the development of biodegradable polymers (Andrade et al., 2021). Biodegradable polymers can offer ideal solutions in many applications, they are found in several market segments: packaging, horticulture, agriculture, automotive, consumer goods, and others, in order to reduce environmental impacts (Barreto et al., 2020).

PBAT is an aliphatic-aromatic, thermoplastic, synthetic copolyester and has an accelerated degradation process, which can degrade in a few weeks in contact with a favorable environment, through the action of natural enzymes, because its chemical structure is composed of an aliphatic fraction (adipate butadiene), responsible for its biodegradability, and an aromatic part (terephthalate), which provides good mechanical properties, having a maximum elongation of 700%, as well as maintaining integrity of the polymer, making its degradation difficult (Shahlari & Lee, 2012). PBAT has characteristics and properties that are similar to Low Density Polyethylene (LDPE), representing 29% of biodegradable plastic production (Munhoz et al., 2021).

Its main characteristic, degradability, is due to the presence of enzymes that cause PBAT to completely degrade in a few weeks. This process has been studied for some years, and investigations are focused on degradation under variable...
environmental conditions. Kijchavengkul et al., (2010) reported the microbial activities in PBAT in a compost environment at high temperatures and pH. Witt et al., (2001) observed that a thermophilic bacterium, Thermomonospora fusca, could monomerize PBAT at high temperature within 3-4 weeks. Some microorganisms have already been identified as PBAT degraders, Kasuya et al., (2009) tested the action of soil microorganisms from three different regions of Japan. The results showed that after 124 days 95% of the PBAT content had been mineralized to carbon dioxide and that during the initial periods the PBAT strips were in contact with soil microorganisms were covered with microbial biofilms.

Among studies found on the biodegradation of polymers, few were found in the literature using the aquatic environment as a biological fluid for the analysis of the biodegradation process. Given the above, the aim of this study was to evaluate the biodegradation of the polymer, PBAT, in a static system, using seawater from the Coastal Region of the State of Pernambuco in Brazil as a fluid.

2. Methodology and Methods

2.1 Materials

The investigation was carried out with PBAT – poly[(butylene adipate)-co-(butylene terephthalate)]. The analysis fluid used in the experiment was seawater located at ground zero, Recife.

PBAT, a modified polyester, was acquired by the company BASF under the name Ecoflex-F BLEND C1200. Some PBAT properties are shown in Table 1.

| Properties                             | PBAT            |
|----------------------------------------|-----------------|
| Density (ASTM D 1238, 190 °C / 2.160g) (g/cm³) | 1.26            |
| Melting temperature (°C)               | 106-120         |
| Elastic Module (MPa)                   | 90              |
| Tensile Strength (MPa)                 | 15              |
| Elongation at break (%)                | 580-820         |
| Tg(°C)                                 | -30             |
| Fluidity index (MFI) (g/10 min)        | 2.5-4.5         |

Source: Yamamoto et al. (2002).

2.1.1 Bioreactors

The experiments were carried out in a static system, using 3-liter glass bioreactors. The specimens were suspended by Nylon wires and attached to the lids of the bioreactors (Figure 1), so that they were exposed at the same depth in the fluid, for a period of up to 180 days.
Every 7, 14, 30, 60, 90, 120 and 180 days of experiments, counted from the date of assembly of the bioreactors, ten specimens, previously numbered and weighed, were removed for analysis. Planktonic microorganisms from seawater samples were also quantified.

2.2 Methods
2.2.1 Sample preparation

The PBAT was processed and injected at SENAI - CIMATEC, in an IMACON modular, twin-screw, co-rotating extruder, with L/D ratio = 30 and screw speed equal to 250 RPM, and in an injector with a capacity of 100 tons of closing force, ROMI brand, Primax model. According to ISO 527 standards for tensile testing (ISO, 2019). Processing temperature conditions and injection conditions are shown in Tables 2 and 3, respectively.

Table 2 - PBAT processing temperature conditions in the extruder (Flow = 3.0 kg/h).

| Sample | Z1 (°C) | Z2 (°C) | Z3 (°C) | Z4 (°C) | Z5 (°C) | Z6 (°C) | Z7 (°C) | Head (°C) | Melted (°C) |
|--------|---------|---------|---------|---------|---------|---------|---------|-----------|------------|
| PBAT   | 130     | 135     | 135     | 140     | 150     | 160     | 170     | 170       | 175        |

Source: Authors (2021).

Table 3 - Injection conditions.

| Sample | Z1 (°C) | Z2 (°C) | Z3 (°C) | Beak |
|--------|---------|---------|---------|------|
| PBAT   | 160     | 150     | 155     | 150  |

Source: Authors (2021).

2.2.2 Characterization of Samples

The PBAT before and after the biodegradation tests were evaluated by Microbiological Analysis of the surface of the samples, Mass loss, Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectrometry (FTIR) and Thermogravimetric Analysis (TGA).

2.2.2.1 Microbiological Analysis

The quantification of the cellular concentration of microbial groups in the biofilm was carried out in the periods of 7, 14, 30, 90, 120 and 180 days of the experiment. The following groups of microorganisms were quantified: aerobic
heterotrophic bacteria (AHB), anaerobic heterotrophic bacteria (AnHB) and Pseudomonas sp. according to the methodology described by Silva et al., 2005; Iron precipitating bacteria (IPB) according to the methodology described by CETESB, 1988; sulfate-reducing bacteria (SRB) and filamentous fungi (FG) according to the methodology described by Postgate, 1984.

The specimens were removed from the bioreactor and placed in containers containing 30 mL of saline solution for aerobic microorganisms (AHB, IPB, fungi and Pseudomonas), and 30 mL of reducing solution for anaerobes (AnHB and SRB) and then, submitted to ultrasound for 15 seconds. The surface of the specimens was scraped with a sterile spatula in saline or reducing solution, from which 1 mL aliquots were removed for inoculations and dilutions. For quantification, the Most Probable Number (MPN) technique was used with dilutions up to $10^{-7}$ and counting of Colony Forming Units (CFU). All procedures were performed according to aseptic technique standards.

### 2.2.2.2 Weight loss (Biodegradation Rate)

The mass of the specimens was measured before and after each immersion period. The samples were weighed before and after each period on a METTLER TOLEDO XS 105 Dual Range analytical balance, with 0.01mg sensitivity. Mass loss was determined according to Equation 1 (Roy et al., 2008):

$$ R(\%) = \frac{(M_0 - M_t)}{(M_0)} $$

Where: $M_0$ the mass before biodegradation; $M_t$ is the mass after the $t$ biodegradation period and $R$ is the percentage reduction.

### 2.2.2.3 Differential scanning calorimetry (DSC)

The DSC analyzes were performed in a METTLER TOLETO DSC 1 Star System equipment, at the Petrochemical Laboratory (LPQ) at the Federal University of Pernambuco. About 5 to 10 mg of samples were placed in standard aluminum crucibles. The tests were carried out in three stages: heating – cooling – reheating. All ramps took place under a N$_2$ atmosphere with a flow rate of 50 mL/min.

### 2.2.2.4 Fourier Transform Infrared spectroscopy (FTIR)

The spectra of the PBAT samples were obtained by infrared spectroscopy (FTIR), using a Thermo Scientific Nicolet model 32 spectrometer IS10, carried out at the State University of Santa Cruz-Ilheus-BA. Spectra with a resolution of 4 cm$^{-1}$ and wavenumber range from 4000 to 400 cm$^{-1}$ were obtained. Data were translated using the Origin 8.5 software, adjusting the baseline and normalizing. To calculate the carbonyl index (CI) Equation 2 was used.

$$ IC = \frac{Abs_{C=O}}{Abs_{C-H}} $$

IC being the carbonyl index, $Abs_{C=O}$ the absorbance of the carbonyl band and $Abs_{C-H}$ of the C-H band chosen as reference.

### 2.2.2.5 Thermogravimetric Analysis (TGA)

The thermogravimetric analysis was carried out in a METTLER TOLETO TGA 2 Star System equipment, the test was carried out at the Petrochemical Laboratory (LPQ) at the Federal University of Pernambuco. The heating ramp was from 30 to 600 °C with a heating rate of 10 °C/min, nitrogen atmosphere with a gas flow of 50 ml/min. Approximately 5 mg of the material was used in a 40 μL alumina crucible.
3. Results and Discussion

3.1. Microbiological Analysis

3.1.1 Seawater Plankton Bacteria

Cellular concentrations of planktonic heterotrophic aerobic (AHB), iron precipitant (IPB), anaerobic heterotrophic (AnHB) and sulfate reducing (SRB) bacteria present in seawater were quantified and shown in Figure 2.

**Figure 2 – Quantification of plankton bacteria in seawater used in the experiment.**

The highest concentrations found were of AHB, AnHB and SRB, all in the order of 10^8 cells/ml. In smaller quantities are the IPB, with a concentration of around 10^3 cells/ml. In the literature, other studies were found for the concentration of microorganisms in seawater at the port of Recife-PE: Argolo et al., (2015) quantified the same microbial groups and obtained values of around 10^6 cells/ml for AHB and IPB, 10^5 cells/ml for AnHB and 10^3 cells/ml for SHB. Ferreira et al., (2016) found concentrations in the order of 10^4 cells/mL for AHB, 10^3 cells/mL for IPB and AnHB, and 10^1 cells/mL for SRB. The presence of these bacteria in seawater evidences a potential environment for the occurrence of biodegradation. These analyzes can generate different results, as it is influenced by location, season, weather conditions and other factors (Videla, 2003; Dutra, 2017).

3.1.2 Sessile Bacteria in Different Systems

Figure 3 shows the results of the microbiological analyzes of the biofilms formed on the surfaces of the specimens, in the periods of 7, 14, 30, 90, 120 and 180 days for PBAT.

**Figura 3 - Sessile microbial quantification of specimens of the system at all times studied a) AHB, IPB, AnHB and SRB and b) Filamentous fungi and Pseudomonas sp.**

Source: Authors (2021).
According to Figure 3a, it is possible to observe that the sessile AHB and AnHB were quantified in the system at all times of analysis, however, there was a reduction in the concentration of these microorganisms, from the 30° and 90° days of exposure of the specimens to the medium. Returning to quantify in 120° days and 180° days in the order of 10⁸ cells/cm². This behavior can be explained by the exchange of water that occurred in the system every 14 days, which may have caused displacing and renewal of the biofilm, changing the nutrients and concentrations of microorganisms (Dutra, 2017).

The concentrations found at all times for IPB were in the order of 10⁸ cells/cm². SRB, on the other hand, had lower concentrations compared to other bacterial groups, only being detected in high concentrations on the 7° day, not being quantified on the 14° day, suffering a small increase on the 30°, 90° and 120° day. Requantifying around 10⁶ cells/cm² at the end of 180 days of the experiment, probably because there was an increase in bacterial biomass and the biological demand for oxygen favored the appearance of anaerobic reducing zones, which are associated with the sulfate reduction process and the presence of BRS (Characklis & Marshall, 1990; Wimpenney, 2000).

According to Figure 3b there was the quantification of fungi and Pseudomonas at all times studied, observing a concentration in the order of 10³ cells/cm² from the 90° day for Pseudomonas.

Many bacterial species are important for the degradation of polymers of the polyester class because they produce lipase enzymes, in particular the Pseudomonas. These enzymes play a fundamental role in the hydrolysis reactions of the ester groups present in the chemical structure of PBAT, the amorphous part of the polymer structure is more susceptible to the action of enzymes, initiating the process of breaking the bond in the initial stage of polymer biodegradation (Wu & Gan, 1998; Tserki et al., 2006).

3.2 Weight loss

Figure 4 shows the evolution of PBAT mass loss over 180 days of the experiment.

At the end of 180 days of the specimens submerged in sea water, the PBAT presented a mass loss of approximately 2.3%. With regard to PBAT, only a few studies have been carried out to assess its biodegradability in seawater at room temperature conditions. However, a similar behavior was found by Wang et al., (2018) where a loss of less than 2.5% of its weight was observed in more than 56 weeks of immersion in tanks with natural sea water. Delacuverlierie et al., (2021) identified a loss of 1.5% after 82 days of immersion where PBAT was submerged in situ in the sediment and water column of the Mediterranean Sea. In soil Moraes, (2020), reported that at the end of the test, 168 days of the films buried in the soil, the pure PBAT films presented a mass loss of 33%.
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 the polymers.

3.3 Differential Scanning Calorimetry (DSC)

DSC analyzes were used to investigate PBAT crystallization and melting events 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, then rapidly cooled and heated again.

PBAT (Figure 5 and Table 4) crystallizes around 89.5°C and melts at approximately 126.08°C and it is possible to visualize a broad peak. According to Kuwabara et al., (2002), the broad melting peaks may indicate that the crystalline regions of PBAT are neither ordered nor rigid, as confirmed in their studies of atomic force microscopy, solid state carbon nuclear magnetic resonance and XRD. Magnification of melting peaks after exposure to the marine environment can be seen, in some cases it is probably caused by changing the size distribution of the crystallite.

![Figure 5 - PBAT DSC curve before and after biodegradation.](source: Authors (2021)).

According to Wang et al., (2011), microbial attack on the PBAT adipate butylene units makes the crystals present in the polymer more orderly, since PBAT forms mixed crystal structures of butylene adipate butylene terephthalate units. The oscillation of the degree of crystallinity may indicate that the crystalline regions were also reorganized by the enzymatic attacks of microorganisms.
Table 4 - Melting temperature (Tm) and crystallization (Tc), degree of crystalline (Xc) and fusion enthalpy (ΔHf), and crystallization (ΔHc) for all polymers obtained through DSC.

| Days | Tc (ºC) | ΔHc (J/g) | Tm (ºC) | ΔHm (J/g) | ΔXm (%) |
|------|---------|-----------|---------|-----------|--------|
| PBAT | 89.56   | 17.43     | 126.1   | 12.52     | 10.99  |
| 7    | 118.99/91.6 | 46.07     | 129.5   | 41.54     | 36.44  |
| 14   | 118.7/91.91 | 18.04     | 127.0   | 13.17     | 11.55  |
| 30   | 91.80    | 18.66     | 126.6   | 13.30     | 11.67  |
| 60   | 118.10/95.6 | 22.69     | 128.2   | 17.29     | 15.16  |
| 90   | 93.45    | 18.34     | 126.5   | 12.85     | 11.27  |
| 120  | 119.6/94.1 | 55.17     | 130.5   | 46.69     | 40.96  |
| 180  | 93.54    | 17.39     | 126.2/141.8 | 12.56  | 11.02  |

Source: Authors (2021).

3.4 FTIR

The PBAT spectra (Figure 6) indicate the FTIR measurements of the polymer before and after the biodegradation test. Note the presence of vibrational stretches at 2954 cm⁻¹, which is attributed to the axial deformation of the C-H bond of aliphatic carbon. The characteristic band of the C-O group in the ester bond at 1270 cm⁻¹, furthermore, it is possible to observe characteristic bands of C=O stretching at 1710 cm⁻¹ and the CH₂ group at 728 cm⁻¹, as previously reported (Yanming et al., 2012; Shankar & Rhim, 2016; Palsikowski et al., 2018; Nikolić et al., 2017; Venkatesan & Rajeswari, 2017; Jiang et al., 2020).

Figure 6 - Initial PBAT FTIR spectrum, PBAT 7 days, PBAT 14 days, PBAT 30 days, PBAT 60 days, PBAT 90 days, PBAT 120 days, and PBAT 180 days in seawater. In the range of 4000 - 400 cm⁻¹.

Comparing the PBAT spectra before and after the biodegradation test, it can be seen, from Figure 6, that no new band appears, nor shifts, since the vibrations of the covalent bonds of the groups are present regardless of the biodegradation. In a similar study of structural characterization by FTIR was carried out by Palsikowski et al., (2018) and Moraes, (2020), who also did not detect significant changes in the PBAT absorption bands.

Therefore, to characterize the change in molecular structure, carbonyl indices were calculated through equation 2 and the results are shown in Table 5.
Analyzing the evolution of the carbonyl index, it is noted that there was no significant change in the material under study. Usually, the structural change occurs by monitoring the terminal carboxylic groups, however this measure can be difficult to identify if these new structures reorganize, masking the chemical changes in the polymer. As PBAT has carbonyl in its main chain, if the microbiological attack hydrolyses the ester-type bonds, the total number of carbonyl will not change, however alcohol will be produced by hydrolysis. But if hydrolysis occurs at another bond, new carbonyls will form. In addition, there may be formation of carboxylic acids during hydrolysis, which can be assimilated by microorganisms, resulting in a decrease in the carbonyl index (Moraes, 2020).

Therefore, the increase or decrease in the carbonyl index is related to the chemical environment, the stage of degradation of the material and the biota of the sea water to which the polymer was exposed.

### 3.5 Thermogravicmetric Analysis (TGA)

Figure 7(a) shows the thermal decomposition curve of the PBAT polymer during 180 days.

The degradation of PBAT, as shown in Figure 7(a) happened abruptly. The 180-day exposure time in seawater did not change the trend of the curves: all of them had near maximum degradation temperatures. This behavior shows that most bonds present in the chemical structure of the polymer are present. However, larger splits in the PBAT main chain could be favored.

| Sample          | C=O (1710) | C-H (1410) | IC   |
|-----------------|------------|------------|------|
| PBAT            | 6,096      | 6,296      | 0.968|
| PBAT 7 days     | 6,243      | 6,543      | 0.954|
| PBAT 14 days    | 6,247      | 6,535      | 0.956|
| PBAT 30 days    | 6,295      | 6,536      | 0.963|
| PBAT 60 days    | 6,264      | 6,536      | 0.958|
| PBAT 90 days    | 6,171      | 6,489      | 0.951|
| PBAT 120 days   | 6,151      | 6,538      | 0.941|
| PBAT 180 days   | 6,375      | 6,551      | 0.973|

Source: Authors (2021).
probably, if the production of extracellular lipases were required. In this context, the addition of nutritional supplements such as fats or fatty acids would be valuable for inducing lipase excretion and, consequently, more pronounced degradation in PBAT.

Table 6 shows data on thermal degradation that can be obtained from the curves in Figure 7. Temperature onset (TONSET) and end (TENDSET) of degradation were obtained by the intersection of tangents to the curves in the respective region of interest (Figure 7a). The maximum degradation temperature was obtained by reading the peak obtained in Figure 5b.

| Amostras       | TONSET (ºC) | TENDSET (ºC) | TMÁX (ºC) | Massa residual (%) |
|----------------|-------------|--------------|-----------|------------------|
| PBAT           | 381,8       | 419,9        | 406,7     | 12,4             |
| PBAT - 7 dias  | 382,2       | 418,2        | 406,2     | 11,6             |
| PBAT - 14 dias | 383,6       | 419,6        | 406,2     | 11,6             |
| PBAT - 30 dias | 384,1       | 420,7        | 406,3     | 15,4             |
| PBAT - 60 dias | 381,7       | 419,8        | 405,7     | 12,8             |
| PBAT - 90 dias | 384,2       | 419,1        | 406,0     | 9,0              |
| PBAT - 120 dias| 381,9       | 418,1        | 406,0     | 14,2             |
| PBAT - 180 dias| 381,8       | 417,5        | 406,7     | 12,3             |

Source: Authors (2021).

It can be said that degradation started around 382 ºC and ended around 419 ºC for most samples. The maximum degradation rate was obtained around 406 ºC and the residue ranged from 9 to 15%.

Zehetmeyer et al., (2016) found two stages of thermal degradation. The first, which coincides with the one found in this work, which may be due to the maximum decomposition of the adipic acid aliphatic copolyester and 1,4-butanediol, and the other stage around 600 ºC with the decomposition of the terephthalate aromatic copolyester. However, other authors found only one stage of degradation, from 350 to 430 ºC (Cobo et al., 2021; Andrade, et al., 2020; Andrade et al., 2018; Arpaporn et al., 2013).

According to Morais (2020), the amount of stage in degradation can be linked to the amount of each monomer in the polymer, being those with more aliphatic units degraded in a single stage.

4. Conclusion

The biodegradability of PBAT in a marine environment was evaluated. The 180 days immersed in sea water influenced the composition of the microorganism community developed in the polymer. However, the signs of degradation observed were very low with no significant degradation. It was observed that the bacterial community developed on the surface of PBAT was insufficient for biodegradation and they use this polymer mainly as physical growth support, probably due to the large availability of carbon in seawater collected in the zero point region in Recife-PE. Consequently, the microorganisms did not use the polymer as a carbon source and did not assist in degradation.

It was observed through the FTIR there was no significant change in the carbonyl index, there was no appearance of any new band, or displacement, since the vibrations of the covalent bonds of the groups are present regardless of biodegradation. In relation to the TGA in 180 days of exposure to seawater, it did not change the trend of the curves: all of them had a maximum degradation temperature close to each other. And the mass loss test showed a result of approximately 2.3%, which indicates that there was no significant degradation of PBAT. The current standards and norms used are insufficient to predict biodegradability in an aquatic environment.
As referências não foram fornecidas no corpo do texto. No entanto, o trabalho de Andrade, M. F. Filho, L. E. P. T. de M. Silva, I. D. de L. Lima, J. C. da C. Carvalho, L. H. Almeida, Y. M. B., e Vinhas, G. M. (2020) é mencionado em vários contextos, destacando a influência de radiação gamma na biodegradação de PBAT - Poly(butylene adipate-co-terephthalate) - filme ativo com óleo de laranja e querosene. A pesquisa de Companhia de Tecnologia de Saneamento Ambiental, CETESB, sobre a formação de bactérias que precipitam o ferro em colônias de bactérias em meio aquoso é também destacada. A preocupação com a biocorrupção de aço carbono exposto ao meio marinho e o papel dos querosene e óleo de laranja na sua prevenção é abordada em vários artigos. A biodegradação de filmes ativos de PBAT, como filmes de poliéster polivinílicos e biodegradáveis, também é uma questão central em vários trabalhos mencionados.

Os conceitos de polímeros compostáveis e filmes ativos de PBAT são relevantes para a indústria de embalagens e sustentabilidade ambiental. A pesquisa na área de biologia marinha é também destacada, com a formação de bactérias que precipitam o ferro em meio aquoso e a biocorrupção de aço carbono exposto ao meio marinho.

As referências bibliográficas são todas em português e são utilizadas como fontes de pesquisa para as questões discutidas no trabalho.

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