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Degradation of conventional and biodegradable microplastics in the marine environment

Fernández de Villalobos N, Marín-Beltrán I, Costa MC.

Centro de Ciências do Mar do Algarve, Universidade do Algarve, Campus de Gambelas, building 7, 8005-139 Faro, Portugal
E-mail: nuriafvillalobos@gmail.com

Introduction

Microplastics (MPs) (<5 mm)[1] can nowadays be found in every corner of the world. For this reason, and due to the risk they pose to aquatic life[2], they are becoming a huge concern to scientists, policymakers, and the general public. Low-density polyethylene (LDPE) and polyethylene terephthalate (PET) are two of the most produced plastics worldwide[3]. Although several studies have identified different microbial strains capable of colonizing and bio-deteriorating LDPE[(4,5)], its complete biodegradation has not been proved so far. Biodegradable plastics seem to be a partial solution to plastic pollution. However, the conditions at which they degrade are not usually present in the natural environment.

Aims & objectives

In this study, it was evaluated the potential of marine bacterial communities to biodegrade commercially available plastic bags made of LDPE and PET. The PET bag was labelled as "biodegradable", and from now on will be referred as BPET. It was hypothesized that the biodegradation of both polymers will be small, but it was expected to observe higher biodegradation of microplastics coming from the bag labelled as "biodegradable" (BPET), as compared to the microplastics from the LDPE bag.

Methodology

Samples of different marine organisms were collected from two marine caves in Sagres, south-western Portugal, the Cathedral and Queijo Suiço caves (Figure 1). Marine bacterial communities were recovered from these samples, from which one bacterial consortium, inoculum #18 (referred in the figures as I18), was used for the biodegradation experiment. It consisted of 5 assays, with two controls, a negative control (with the MP particles, without the inoculum), and a positive control (with the inoculum)(Figure 2). 3 replicates were used and sampling was carried out at 4 different experimental times (T1-T4), after 7, 14, 31 and 45 days (end of the experiment), with a concentration of 1 microplastic/ml.

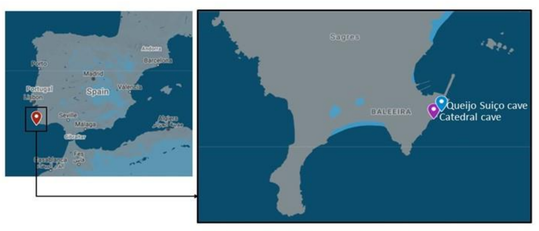

Figure 1. Geographical location of the marine caves (Catedral and Queijo Suiço) in Sagres (southern Portugal), from where bacterial consortiums were recovered for biodegradation experiments.

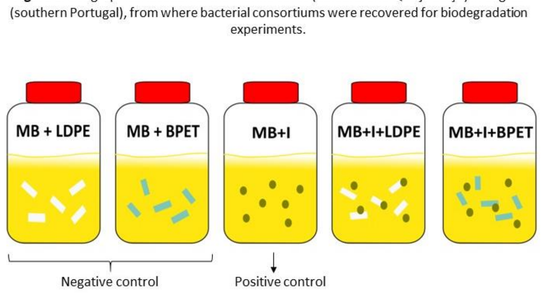

Figure 2. Scheme of the experimental conditions of the biodegradation experiment, in which 10 particles of each polymer (LDPE or BPET), were inoculated (10%) with different marine bacterial consortium. A negative and positive control were used, where either no inoculum, or no MPs were added, respectively. I stands for inoculum; MB for marine broth; LDPE for low-density polyethylene; and BPET for biodegradable polyethylene terephthalate.

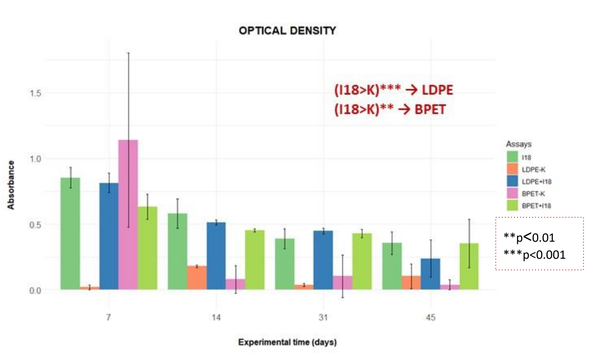

Figure 3. Results of the measurement of the optical density (at 600 nm) – as indicative of bacterial growth – throughout time for both polymers. Experimental time is shown in days. K stands for the controls and I18 corresponds to the bacterial consortium inoculated in the test samples.

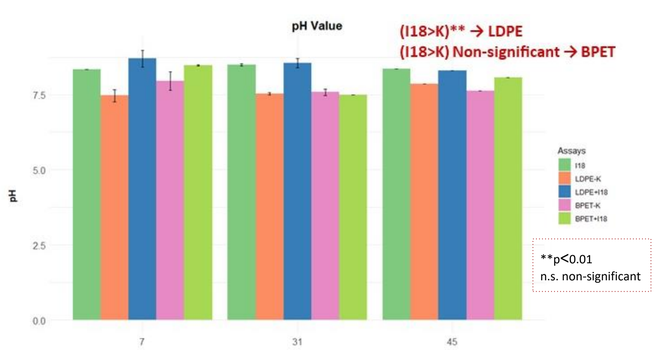

Figure 4. pH of each treatment and polymer at the different sampling times, but for T2. Experimental time is shown in days. K stands for the controls and I18 to the marine bacterial inoculate.

Results & discussion

Significantly higher values were obtained in bacterial growth and **pH** in the samples incubated with I18, compared to the negative controls (Figures 3 and 4). The higher absorbance values in the measurement of the **optical density** were expected since no bacteria was expected to grow in the controls.

In the **FTIR results for LDPE**, a significant decrease in the peaks 2916 and 2819 was observed in the samples incubated with I18 compared to the controls (Figure 5). This is indicative of the polymer being oxidized[6]. Additionally, the appearance of two new peaks at positions 1648 and 871 cm^{-1} was observed, which are the result of the formation of a carbonyl group (e.g., ketone or aldehyde groups) and nitrate ion, respectively[7]. These changes are indicative of the polymer being biodegraded[(5,6)].

In the **FTIR results for BPET**, the most significant differences occurred in the footprint region (Figure 6). While some peaks decreased with incubation time (those characteristic of CH_2 bonds), others increased, such as the peak at position 1649 cm^{-1}. The shifts in these bands could indicate that the polymer is being degraded by the selected bacterial consortium. The bands 1700-1630 cm^{-1} could correspond to the formation of either aldehydes and ketones, whereas the regions 1400-850 cm^{-1} could be related to the presence of aromatic compounds[(7,8)].

The **SEM micrographs** showed changes in the surface features of both LDPE and BPET particles treated with bacterial communities in comparison with the controls (Figure 8). These changes consist of the presence of fractures and holes, as well as the formation of bacterial biofilms. This is indicative of biodegradation by microbial activity[(4,5)].

LDPE

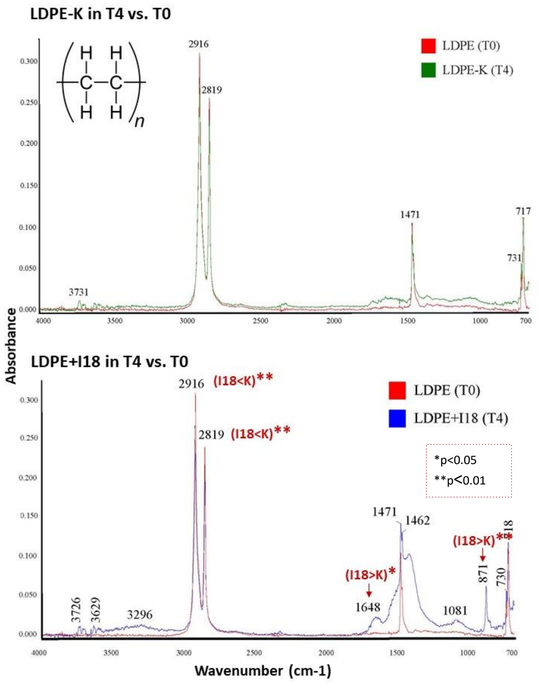

Figure 5. Spectral profile of LDPE, as determined by ATR-FTIR. The plot shows the results of the spectra spectrum of the control (LDPE-K, on the top) and the sample with the bacterial inoculate (LDPE-I18, on the bottom) at T4, superimposed to the spectrum of LDPE microplastics at the beginning of the experiment (T0).

BPET

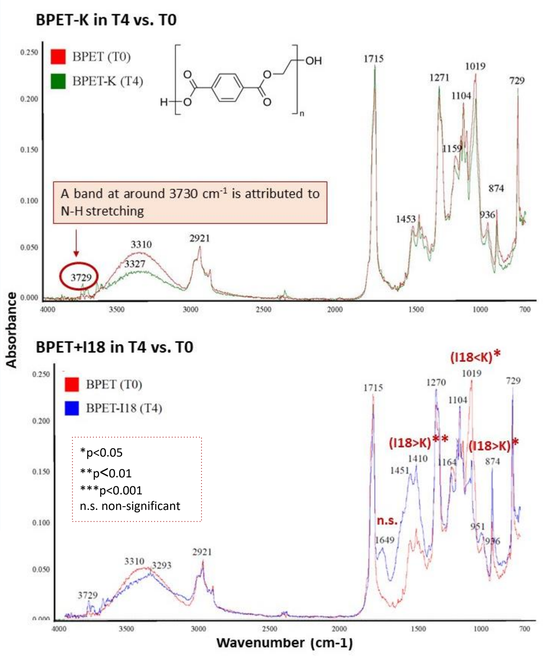

Figure 6. Spectral profile of BPET, as determined by ATR-FTIR. The plot shows the results of the spectra of the control (BPET-K, on the top) and the sample with the bacterial inoculate (BPET-I18, on the bottom) at T4, superimposed to the spectrum of BPET microplastics at the beginning of the experiment (T0).

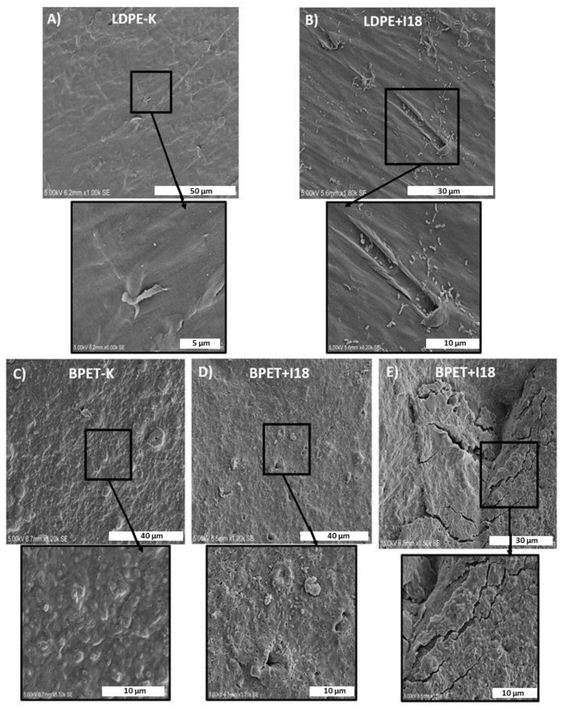

Figure 8. SEM micrographs of LDPE and BPET surface features after 45 days of incubation with the bacterial community (labelled as I18) compared to the controls (labelled as K). A) LDPE surface of the control showing a smooth surface and no bacterial aggregates; B) LDPE surface incubated with I18 showing the attachment of bacterial aggregates into present fractures; C) BPET surface of the control, showing some roughness but not a bacterial biofilm; D) and E) BPET surface after incubation with I18 showing the formation of holes, fractures, and bacterial biofilms. Note the differences in scale.

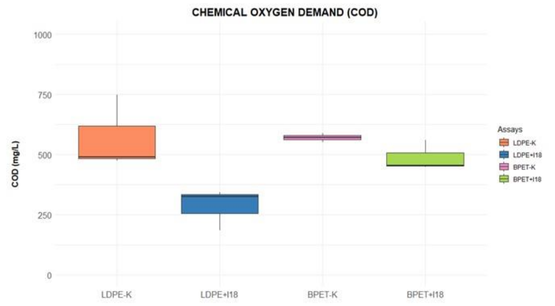

Figure 7. Representation of COD measured at the end of the experiment (after 45 days of incubation) for 5 LDPE and BPET particles. K stands for the controls and I18 for the samples inoculated with marine bacteria (inoculum 18).

The **chemical oxygen demand (COD)** of the MP particles after 45 days of incubation indicated that certain biodegradation had occurred, as the COD values in the controls were higher than in the samples exposed to I18 for both polymers (Figure 7).

Conclusions

Both polymers exhibited some signs of biodegradation when subjected to the selected marine bacterial community. Moderate changes in the chemical structure of the plastics were observed, in comparison to LDPE and BPET microplastics not exposed to bacterial inoculum. These changes were accompanied by an increase in the pH and a reduced oxygen demand, particularly in the case of LDPE. Microplastics subjected to the selected consortium showed modifications in their surface features, such as the formation of fractures and holes, and the formation of bacterial biofilms. The techniques used have been proved satisfactory to assess microplastic biodegradation.

Overall, the obtained results point to the potential of the selected marine bacteria to biodegrade the target microplastics (LDPE and "biodegradable" PET), although additional time may have been necessary to show clearer signs of biodegradation.

Acknowledgements

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