Exploitation of microbial consortia and mixed cultures with microalgal species for the treatment of bioplastics

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1. Introduction

Bioplastics that are biobased polymers, are currently produced at a scale of ~1.5 million t per year. They are considered as integral part of future circular economies to help achieve some of the United Nations' (UN) Sustainable Development Goals, such as diversion from fossil resources, introduction of new recycling or degradation pathways and use of less toxic reagents and solvents in production processes, while their application is fostered by the European Union (Karan et al. 2019; Di Bartolo et al. 2021). Compared with fossil-based plastics, bioplastics can impose a lower carbon footprint and exhibit advantageous material properties; moreover, some offer biodegradation as an EOL scenario if performed under controlled or predictable environments (Rosenboom et al. 2022).

However, biopolymers should overcome certain limitations such as reduction in manufacturing costs, and improved biodegradability in order to completely replace traditional petro-polymers (Awasthi et al. 2022). In this context, the present study investigated the potential of microbial consortia alone and in mixed cultures with microalgal species aiming to degrade the bioplastics poly-β-hydroxybutyrate (PHB) and thermoplastic starch (TPS).

2. Materials and methods

2.1. Raw materials and microorganisms

The TPS pellets used (BIOPLAST GF 106/02) in the experiments are produced by BIOTEC. They are plasticizer-free and GMO-free thermoplastic containing natural potato starch. Their density is 1.25 kg/m^3, they have a cylindrical shape with a height of about 3 mm. PHB polymer was purchased from Tianan Biologic and it is a thermoplastic resin that can be used in injection molding, thermoforming and extrusion. PHB pellets are made by bacterial fermentation and is 100% biobased and 100% biodegradable.

Agricultural soil communities able to develop biofilm on the surface of the pellets were used. In parallel, the freshwater algal strain *Chlorella vulgaris* (*C. vulgaris*, numbered as CCAP211/51) and the green microalgae strain *Scenedesmus obliquus* CCAP276/3A were provided by SAMS Limited.

2.2. Experimental setup

Before use, both polymers were surface sterilized by immersion in ethanol solution and then they were dried for 24 h at 30 ◦C. Biodegradation experiments were carried out under mesophilic (30 ◦C) conditions in 250 mL flasks using mineral minimum medium, the microbial community and the pellets (0.5g). The mineral minimum medium (MMM) consisted of 2g/L NaH_2PO_4, 0.5g/L $MgSO_4 \cdot 7H_2O$, 0.2g/L KH_2PO_4 and 1g/L yeast extract. The communities exhibiting the highest biofilm development on the surface of the pellets and growth were also co-cultured with the microalgal species. In this case, the medium BG-11 or the Bold's Basal Medium was used together with MMM at 1:1 ratio. Cultures were maintained under white fluorescent light (12 h light followed by 12 h darkness cycle) at 60 μmol s^{-1} m^{-2} of light intensity.

2.3. Analyses

At the end of the experiment, dry cell weight and ash-free dry weight (AFDW) was determined according to Borowitzka and Moheimani (2013). The extraction of lipids was performed via the Folch method (Folch et al., 1957), while lipid transesterification employing BF3 and analysis of fatty acid composition were performed as described by Araujo et al. (2008). The number of cells in the plastisphere was studied using flow cytometry, the chemical alterations on the surface of the pellets were examined using Fourier-transform infrared spectroscopy (FTIR) and the generation of secondary microplastics (MPs) was estimated in a fluorescence microscope.

3. Results and discussion

Several soil communities were found able to decrease the weight of the pellets after 1 month cultivation, while higher weight decrease was observed for TPS in comparison with PHB. For example, the TPS2 community reduced the weight by 24.5% and the highest weight decrease observed for PHB was 18%. The modifications on the surface after incubation with the microbial communities are presented in Figure 1&2.

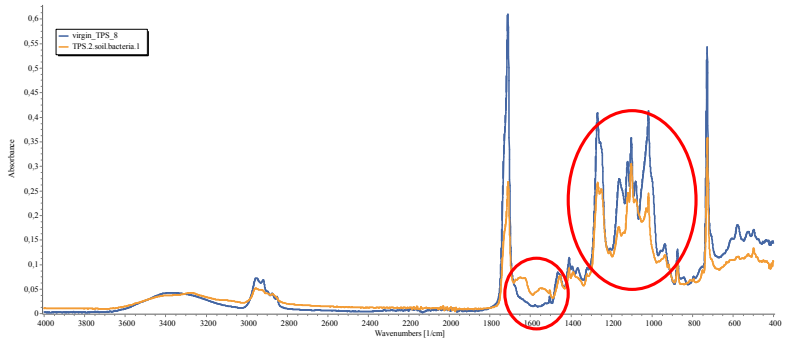

Figure 1. ATR profile of the TPS pellets after incubation with the TPS2 soil community.

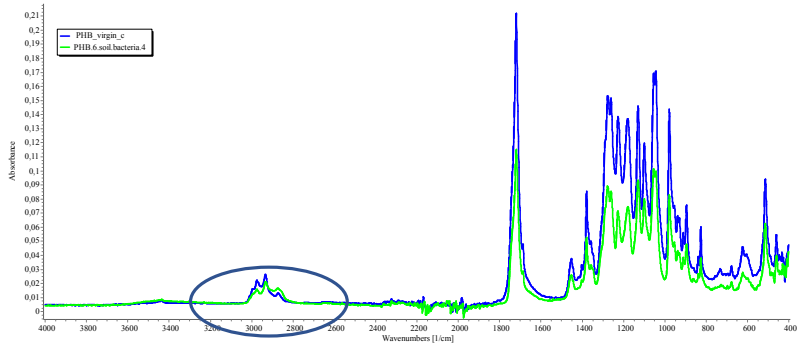

Figure 2. ATR profile of the PHB pellets after incubation with the PHB6 soil community.

A decrease in the intention of the peaks was observed after exposure of both pellets to the microbial communities in accordance with weight reduction. New peaks can also be detected on the surface of TPS pellets. When the algal strain *Chlorella vulgaris* was added in the cultures with the biofilm covered pellets, no growth inhibition of the strain was observed. Instead the mixed cultures displayed increase in the concentration of the cells and two separate layers were observed on the surface of pellets. To conclude, mixed cultures of microbial communities and microalgal species can be considered as a sustainable and efficient approach for the degradation of bioplastics.

4. Acknowledgements

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