Biodegradation of TPS and PHB by *Scenedesmus obliquus* under auto- and mixo-trophic conditions

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1. Introduction

Plastics need to be substituted by biodegradable plastics derived from natural resources due to the environmental impact of plastics' production and disposal, which include landfilling, incineration and recycling (Martinez Villadiego et al., 2022). Incineration and production of plastics emit hazardous particles and gases that impose highly toxic effect on human health and environment, while landfilling and recycling comprise cost-effective methods, thus necessitating not only the turn to bioplastics, but also exploration of new methods for biological degradation of the specific waste (Sharma et al., 2014). Biodegradation constitutes a low-cost, eco-friendly approach to plastic and bioplastic waste management, presenting the advantage of commercial end products' production derived from microbial biomass (Syranidou et al., 2019; Vimal Kumar et al., 2017).

The present study investigated the development of an efficient biodegradation approach for poly-β-hydroxybutyrate (PHB) and thermoplastic starch (TPS) with the use of the green microalgae strain *Scenedesmus obliquus* under autotrophic and mixotrophic conditions (glucose and wastewater). The specific strain holds the capacity to perform elevated biomass productivity and tolerance to climatic variations, as well as rich lipid content, enabling biofuel manufacture (Nicodemou et al., 2023).

2. Materials and methods

2.1. Raw materials and microorganisms

PHB pellets were purchased from Tianan Biologic and TPS (BIOPLAST GF 106/02) was produced by BIOTEC. The size of pellets was about 3 mm and they included cylindrical shape. The freshwater strain *S. obliquus* was obtained by Culture Collection of Algae and Protozoa. All chemicals used were obtained from Sigma-Aldrich Ltd. Company and were of analytical grade.

2.2. Experimental design

S. obliquus CCAP276/3A was cultivated in Bolds Bassal medium and 0.5% w/v PHB or TPS was added in each experiment. Autotrophic growth was performed without addition of organic carbon source, whereas mixotrophic cultivation was conducted by either supplementing the medium with 1% D-glucose or by using sterile wastewater. Cultures were performed under batch conditions using 250 mL glass bottles with 100 mL working volume under continuous shaking at 80 rpm and 30 °C for 35 d. 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. The supplementation of air and CO_2 (approximately 3%) was explored for each bioplastic by adding sterile air in the flasks at 350 mL min^{-1} flow rate. All cultures were conducted in duplicate, while sampling for biomass determination occurred every 7 d and lipids were quantified at the end of cultivation. Control cultures were conducted for each bioplastic without the addition of microorganism.

2.3. Analyses

Dry cell weight and ash-free dry weight (AFDW) was determined according to Borowitzka and Moheimani (2013). Absorbance was determined using a Jenway 7315 spectrophotometer at 650 nm. The extraction of lipids was performed via the Folch method (Folch et al., 1957), while lipid transesterification employing BF_3 and analysis of fatty acid composition were performed as described by Araujo et al. (2008). Prior application in each experiment, bioplastic samples were weighed using a 5-digit laboratory scale. At the end of each trial, bioplastics were gently washed and left overnight to dry. Weight loss was calculated by subtracting the final weight of bioplastics from their initial weight. Moreover, the generation of secondary microplastics (MPs) was estimated in a fluorescence microscope and the type of cells that formed the biofilm were analysed using flow cytometry. Chemical structure alterations of the bioplastics applied were examined using Fourier-transform infrared spectroscopy (FTIR).

3. Results and discussion

Cultivation of *S. obliquus* employing TPS and PHB revealed elevated biomass and lipid production under air and CO_2 in the mixotrophic culture, while results obtained from autotrophy exhibited similar behaviour between the two treatments, for both bioplastics (Table 2). Results on cultivation of microalgae using mixotrophic conditions under glucose supplementation led to increased growth rates and lipid productivity, as opposed to autotrophic cultures. These results are consisted with our previous study, where *S. obliquus* exhibited 3.5-fold increase in the growth rate obtained in autotrophy with the use of mixotrophic cultivation (Nicodemou et al., 2022).

Weight reduction due to biodegradation of TPS and PHB by *S. obliquus* employing mixotrophic and autotrophic cultivation is shown in Table 2. *S. obliquus* efficiently reduced the mass of PHB and TPS without the supplementation of air, in the mixotrophic culture, by 5.8 and 9.8% respectively, while similar results were observed for TPS in autotrophy. However, weight reduction was observed for TPS in control experiments, indicating potential hydrolysis of the specific bioplastic in water. During the experiments, biofilm formation was observed on all bioplastic samples employed, potentially due to their use as a carbon source (Syranidou et al., 2017). Different process parameters should be optimized to enhance bioplastics' biodegradation by *S. obliquus*.

Table 1. Overview of biomass and lipid production as well as bioplastics' weight loss by *S. obliquus*

Trophic mode	Type of bioplastic	Treatment	Bioplastics weight loss (%)	AFDW (g/L)	AFDW (g/L/d)	Lipids (% AFDW)	Lipids (mg/L/d)
Mixotrophic	PHB	Air+5% CO_2	0.0	4.5	0.13	6.0	8.7
		No air	5.8	1.8	0.038	10.1	5.8
		Control	0.0	-	-	-	-
	TPS	Air+5% CO_2	9.0	5.1	0.15	7.9	12.9
		No air	9.8	1.5	0.026	14.2	6.9
		Control	5.5	-	-	-	-
Autotrophic	PHB	Air+5% CO_2	7.3	0.41	0.0024	28.7	3.4
		No air	3.5	0.48	0.0040	8.3	1.1
		Control	0.0	-	-	-	-
	TPS	Air+5% CO_2	8.7	0.46	0.0032	14.5	1.9
		No air	9.9	0.65	0.0091	12.0	2.2
		Control	8.6	-	-	-	-

4. Conclusions

The present work demonstrated that the use of *S. obliquus* for the development of an advanced approach for bioplastics degradation is feasible even without any optimization of process parameters. In addition, the fatty acids profile and the organic carbon reduction in mixotrophic cultures were determined for both bioplastics. Future work will include the co-cultivation of *S. obliquus* with bacterial consortia employing weathered bioplastics.

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