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Biodegradation of TPS and PHB by *Scenedesmus obliquus* under autotrophic and mixotrophic conditions

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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 [1]. 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 shift to bioplastics, but also exploration of new methods for biological degradation of the specific waste [2]. 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 [3,4].

Objectives

The main objectives of the present work comprise:

- 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.

Fig. 1 Biofilm formed by *S. obliquus* autotrophically on TPS after 7 d (left) and 35 d (right) of incubation

Materials and methods

S. obliquus CCAP276/3A was cultivated in Bold's Basal medium and 0.5% w/v PHB or TPS pellets was added in each experiment. Autotrophic growth was performed without addition of organic carbon source, whereas mixotrophic cultivation was conducted by supplementing the medium with 1% D-glucose. 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 (12:12 h ligh:dark 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.

Analyses:

- The growth of each culture was monitored by measuring the ash-free dry weight (AFDW) and optical density (680 nm).
- Reducing sugars in mixotrophic algae cultures were determined by HPLC.
- Weight loss of bioplastics was calculated gravimetrically.
- Lipids were extracted from algae cells using the Folch method.
- Secondary microplastics (MPs) content was estimated in a fluorescence microscope.
- Chemical structure alterations of the bioplastics applied were examined using FTIR.

Results and discussion

Biomass and lipid production by *S. obliquus*

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 behavior between the two treatments, for both bioplastics (Table 1). Results on cultivation of microalgae using mixotrophic conditions under glucose supplementation led to increased growth rates and lipid productivity, as opposed to autotrophic cultures. The results on glucose consumption during the time course of mixotrophic growth indicate that *S. obliquus* consumed the whole contend of the carbohydrate provided.

Bioplastics' weight reduction

Weight loss due to biodegradation of TPS and PHB by *S. obliquus* employing mixotrophic and autotrophic cultivation is shown in Table 1.

- *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.
- Weight reduction was observed for TPS in control experiments, indicating potential hydrolysis of the specific bioplastic in water.
- Biofilm formation was observed on all bioplastic samples employed, potentially due to their use as a carbon source.

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)
Mixo-	PHB	Air	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	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	-	-	-	-
Auto-	PHB	Air	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	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	-	-	-	-

MPs generation

MPs generation observed at the end of mixotrophic cultivation for both bioplastics was lower compared to autotrophy. MPs content formed ranged between 77-7433 MPs μL-1 and 1-43 MPs μL-1 employing autotrophy and mixotrophy respectively. Interestingly, despite the difference in weight loss of PHB between cultures supplemented with air and without air in mixotrophy, MPs concentration was similar.

Chemical structure alternations

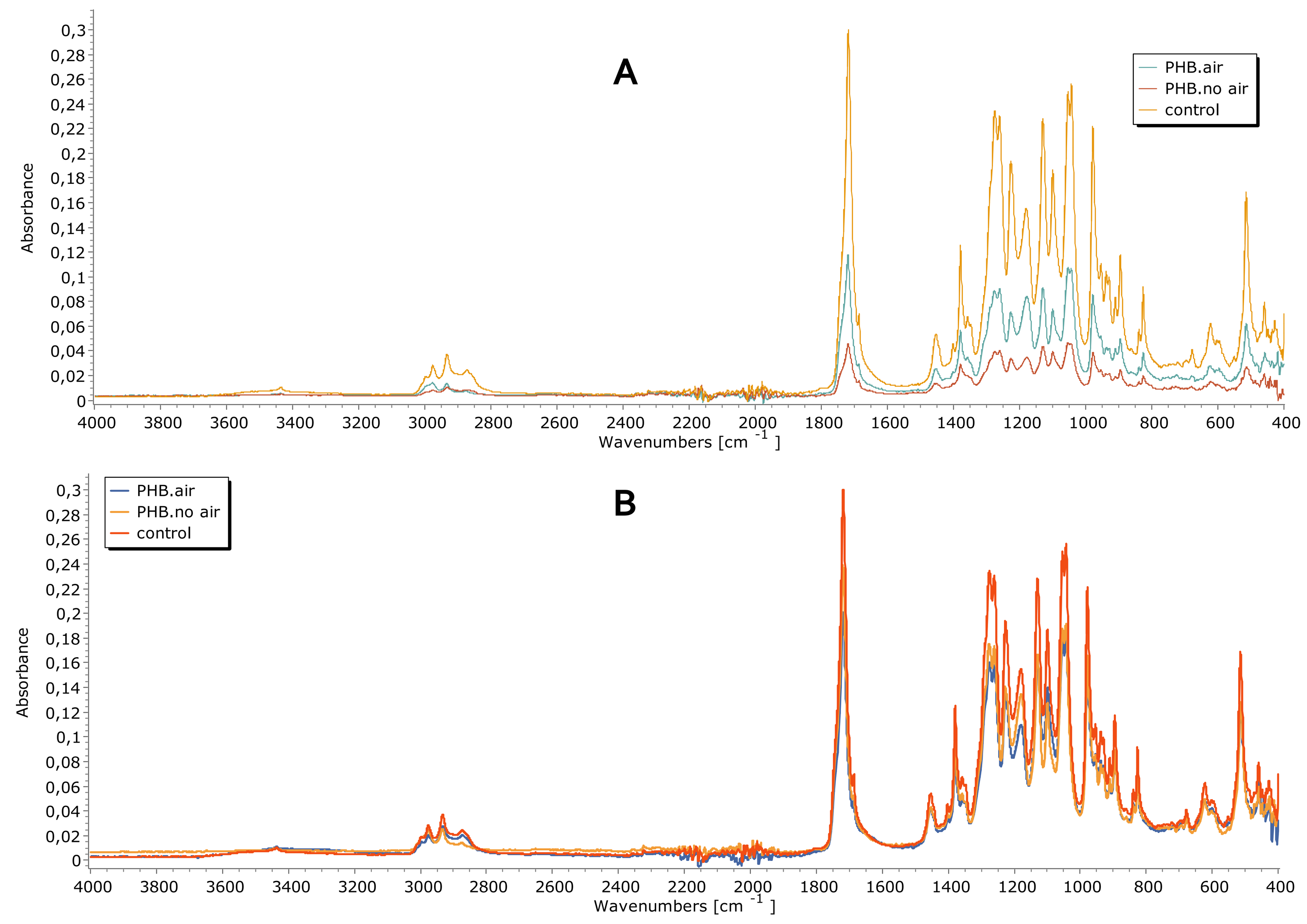

Fig.2 ATR profile of the PHB pellets after incubation with *S. obliquus* under mixo- (A) and autotrophic (B) nutrition

The ATR profiles of PHB pellets employing mixotrophic and autotrophic conditions are presented in Fig. 2. A decrease in the intention of the peaks was observed after exposure of PHB pellets to *S. obliquus* cultures under mixotrophic nutrition without air supplementation, in accordance with weight reduction. Moreover, a decrease in the intensity of peaks occurred in cultures provided with air, as opposed to weight reduction of PHB pellets. Similar results were detected upon PHB pellets application in autotrophic conditions using S. obliquus. The intensity of peaks was decreased employing both cultivation methods, in accordance to weight loss. Higher peaks were observed using mixotrophic nutrition.

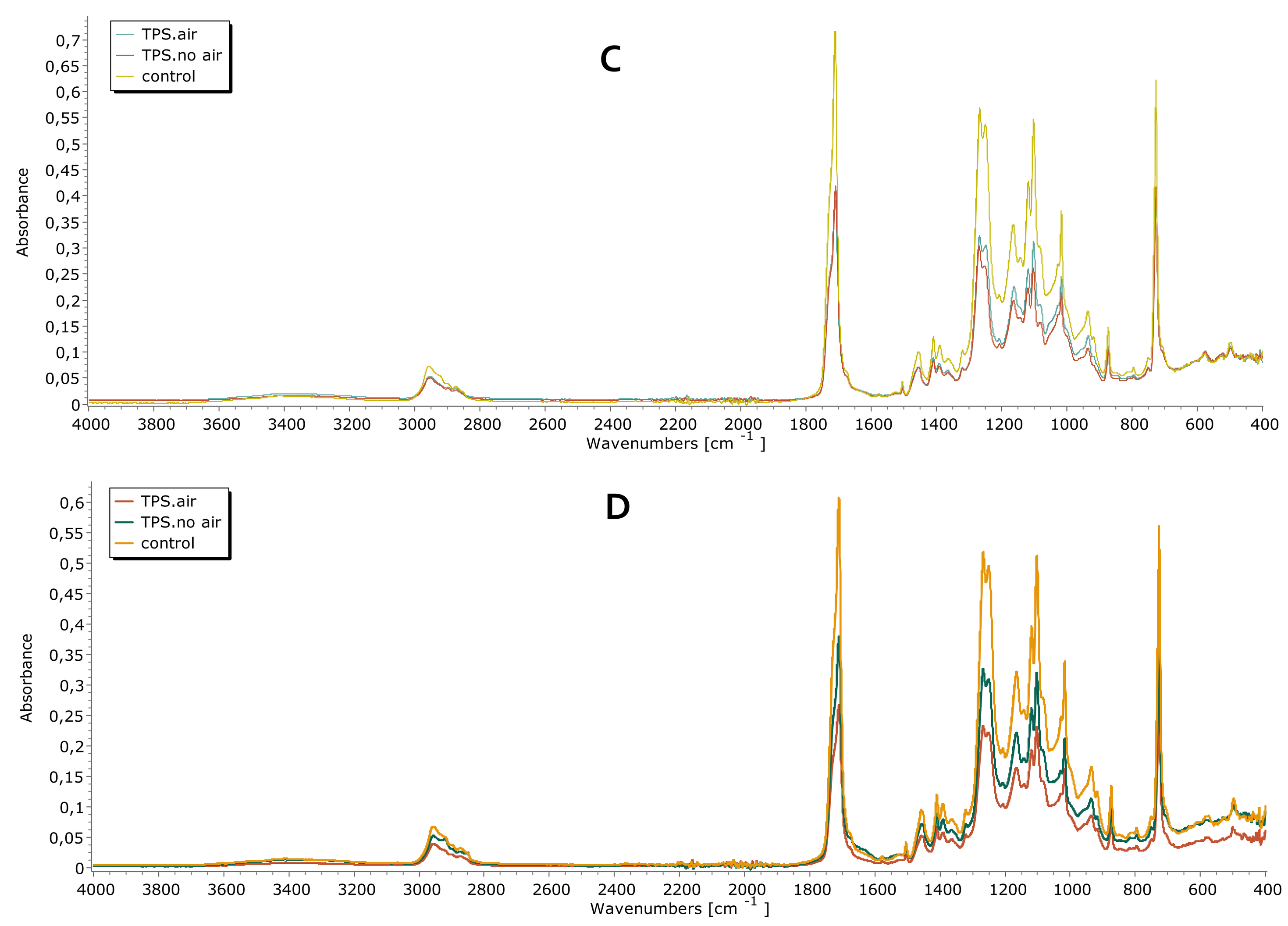

Fig.3 ATR profile of the TPS pellets after incubation with *S. obliquus* under mixo- (C) and auto-trophic (D) nutrition

The ATR profiles of TPS pellets under mixotrophy and autotrophy are shown in Fig. 3. Results on TPS pellets incubated with *S. obliquus* indicate a reduction in the intensity of peaks after cultivation with mixotrophic and autotrophic growth. The observed decrease in peak intensity and weight reduction of TPS pellets after exposure to the specific microalgal strain suggest that the biodegradation of TPS can be achieved employing a sustainable approach. *S. obliquus* showed higher ability to degrade TPS pellets compared to PHB. Similar extent of biodegradation (weight reduction, FTIR profiles) was observed for both cultivation modes.

References

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CONCLUDING REMARKS

- The biodegradation of bioplastics is feasible even without any optimization of process parameters.
- Results on the weight loss of PHB suggest autotrophy supplemented with air as the optimal culture method for *S. obliquus* (7.3% weight reduction). TPS weight loss did not exhibit significant differences between cultivation methods and nutrition.
- Based on lipid production, *S. obliquus* constitutes an excellent candidate for the production of biodiesel through use of algal biomass.

FUTURE WORK

- Future work involves the biodegradation of PHB and TPS pellets utilizing wastewater as culture media for *S. obliquus*.
- Co-cultivation of *S. obliquus* with bacterial consortia employing weathered and non-weathered bioplastics.

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