INTERACTION BETWEEN UNICELLULAR GREEN ALGA RAPHIDOCELIS SUBCAPITATA WITH BIO-COMPOSTABLE PLASTIC BAGS

[1, 2] Marlita MARLITA, [2]Nhung H. A. NGUYEN, [2]Alena ŠEVCŮ

[1]*Faculty of Mechatronics, Informatics and Interdisciplinary Studies, Technical University of Liberec, Liberec, Czech Republic, EU, marlita.marlita@tul.cz*

[2]*Institute for Nanomaterials, Advanced Technologies and Innovation, Technical University of Liberec, Liberec, Czech Republic, EU, nhung.nguyen@tul.cz, alena.sevcu@tul.cz*

https://doi.org/10.37904/nanocon.2023.4760

Abstract

Plastic waste has been a growing threat to the environment and society. As most plastics are made from petroleum, eco-friendlier material, named bio-based plastics, made fully or partially from renewable sources, have been introduced and expected to be more biodegradable. However, their biodegradation and fate in the environment are not fully understood. Here, we studied the initial phase of plastic biodegradation, which was an attachment of freshwater microalga *Raphidocelis subcapitata*, on three types of starch-based bio-compostable plastic bags with the main component of polyethylene terephthalate (PET). PET-C was made from corn starch, PET-CB made from cornstarch and beetroot, and PET-CPW made from corn, potato and wheat starch. The plastic pieces (1 x 1 cm^2) were added into the algal culture and incubated at laboratory temperature with low light density. The algal attachment and growth were then monitored and the changes of plastic properties were analyzed by a scanning electron microscope (SEM) for morphology, a confocal microscope (CM) for surface roughness and a contact angle for hydrophobicity. After the first week, algal cells were already attached on the plastic surface and formed colonies. The highest cell density was found in samples with PET-CPW following by PET-C and PET-CB. The surface morphology and surface roughness changed to be less rough, and the hydrophobicity of the plastics was lower.

Keywords: *Raphidocelis subcapitata*, PET, bio-based plastics

1. INTRODUCTION

Plastics have been widely used for a century due to their versatility and durability as well as light weight. Most plastics are made from petroleum that are not degradable and generally disposed off through recycling, incineration and landfilling. Conversio Market & Strategy in 2018 reported that around 25% of plastic post-customer waste in Europe (EU28+NO/CH) was still sent to landfill (www.plasticseurope.org), as a result, plastics waste is accumulated in the environment and become a global pollutant threat to the environment and society. To overcome the problem, an eco-friendlier material, named bio-based plastics, made fully or partially from renewable sources, have been introduced and expected to be more suitable for biodegradation. However, their biodegradation and fate in the environment are not fully understood, favorable conditions and time for biodegradation are vary [1].

Biodegradation of plastic waste is an economically cheaper process, more efficient than any other conventional methods of plastic waste disposal, but it is complex, involves steps including biodeterioration, depolymerization and mineralization. Algae are able to colonize plastic surface and degrade some plastics [2,3]. In this paper, the impact and interaction of unicellular green algae, *Raphidocelis subcapitata*, with bio-compostable bio-based plastics was investigated.

2. MATERIALS AND METHODS

2.1 Materials

Three different commercial bio-compostable polyethylene terephthalate (PET) plastic bags were purchased from three companies in Czech Republic. First, PET-C plastic, made from cornstarch was purchased from EKO-Plasty Company. Second, PET-CB plastic, made from cornstarch and beetroot was purchased from ECOFOL Company. Third, PET-CPW plastic, made from cornstarch, potato, and wheat was purchased from AJprodukty Company.

2.2 Characterization of Plastics

2.2.1 Components on bio-compostable plastics

The components of three bio-compostable plastics were analyzed by measuring their spectra by fourier transform infrared (FTIR) technique (Thermo Fisher Scientific, USA).

2.2.2 Contact Angle

The hydrophobicity of bio-compostable plastics was determined by contact angle measurement with dropping water method using drop shape analyzer (Kruss, Germany).

2.2.3 Surface Roughness

The surface roughness of bio-compostable plastics was determined based on two roughness parameters, the average surface roughness Sa (μm) and maximum height Sz (μm) (based on ISO 25178). The measurement was conducted by a confocal microscopy (Sensofar metrology, Spain) with a S neox high-performance 3D optical profiler and an EPI 50X v35 objective that allows non-contact optical 3D profiling. The neox uses a high-resolution charge-coupled sensor (0.69 μm/pixel) of up to 1226 × 1022 pixels. A smaller area of 100 x 100 μm was selected using the appropriate software in a scanned area of 338.38 × 282.07 μm.

2.2.4 Surface morphology under Scanning Electron Microscope

The surface morphology of bio-compostable plastics was analyzed using a Zeiss Ultra Plus field-emission SEM (Zeiss, Germany). The plastic samples were soaked with 2% SDS for 10 minutes followed by rinsing with water to remove all biofilm and algal biomass, dried at room temperature and then fixed onto aluminum stubs using double-sided carbon tape, and cleaned with RF plasma (Evactron) for 10 min before SEM analysis. SEM images were acquired at an accelerating voltage of 5 kV at low probe current (about 15 pA) using an InLens secondary electron detector with SmartSEM software.

2.3 Algal Culture and Exposure to Plastics

2.3.1 Alga Maintenance

The green algae *Raphidocelis subcapitata* (CCALA 433) were obtained from Culture Collection of Autotrophic Organism (CCALA, Czech Republic). The algae were maintained in BBM medium with constant white light at 5.6 μE in static condition at room temperature.

2.3.2 Exposure to Plastics

Algae were cultured in 250 mL flask with total volume of 100 mL and initial optical density of 0.01 at 680 nm (OD_{680}) measured using AquaPen AP-100 (PSI Ltd., Czech Republic). 40 pieces of PET plastics were sterilized by 1 h incubation in 70% EtOH followed by 30min UV exposure and added into each flask. The exposure culture was incubated in the same conditions as maintenance culture up to 28 days and sample plastics were taken every week.

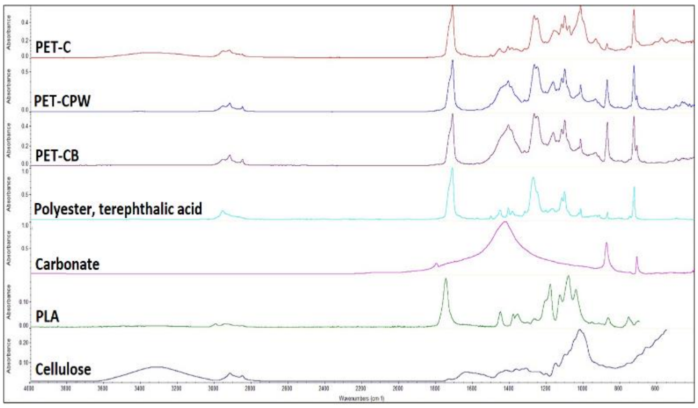
2.3.3 Algal growth

The algal attachment was observed under epifluorescence microscope AxioVision (Zeiss, Germany) after careful washing using distilled water. Attached cells were counted on the surface of bio-compostable plastics as cells per mm^2 from the captured image. The number of free-living cells was determined using Burker counting chamber as cells per milliliter.

3. RESULTS AND DISCUSSION

3.1 The components of bio-compostable plastics

The FTIR analysis revealed that the three bio-based PET plastics have different components as shown in **Figure 1**. Besides polyester and terephthalic acid as the main components of PET, PET-C contains cellulose, PET-CB contains some polyethylene and carbonate, and PET-CPW contains some carbonate. The difference in components may come from difference source of raw materials used for plastic production and from the additives added during the plastics polymerization process to improve their performance [4].



Sample ID	Compositions based on FTIR result				
	Poly-ester	tere-phthalic acid,	cellulose	carbonate	poly-ethylene
PET-C	V	V	V		
PET-CB	V	V		V	V
PET-CPW	V	V		V	

Figure 1 FTIR analysis of three different bio-compostable plastic PET-C, PET-CP and PET-CPW

3.2 Algal attachment and growth on bio-compostable plastics

Raphidocelis subcapitata is one of the representative models of freshwater ecosystem. It belongs to Chlorophyceae, order Sphaeropleales, and was recommended by international guidelines for ecotoxicological bioassays due to their high growth rates [4]. Our results demonstrated that *R. subcapitata* was able to attach and colonize all three bio-based plastics in which the PET-CPW showed the best support for the attachment (**Figure 2**). The process of algal attachment includes two steps, initial adhesion when algal cells are transported to the surface of material by gravitational or hydrodynamic force and the forming of biofilm [5]. It has been reported that *R. subcapitata* as non-axenic culture produced high biofilm and extra polymeric substrates (EPS) that support stronger attachment and colonization on the membrane surface [6].

The result of cell growth (**Figure 3**) showed that PET-CPW could support the highest cell number of both free-living cells in the supernatant and the attached cells on the surface of plastics, followed by PET-CB and PET-C. PET-CPW and PET-CB both contain carbonate. The study of Tong et al. [7] showed that the carbonate could capture more CO_2 to be used as feedstock or carbon source for algae culture.

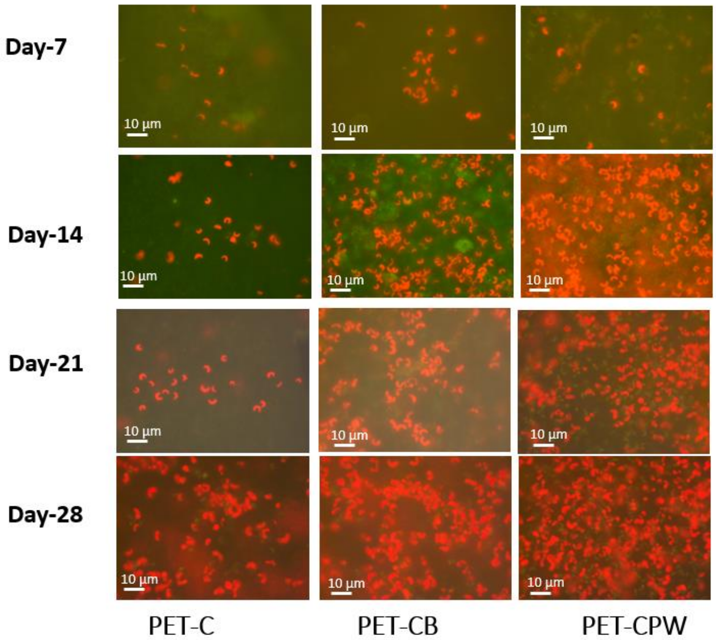


Figure 2 Algal cells attachment on bio-compostable plastics

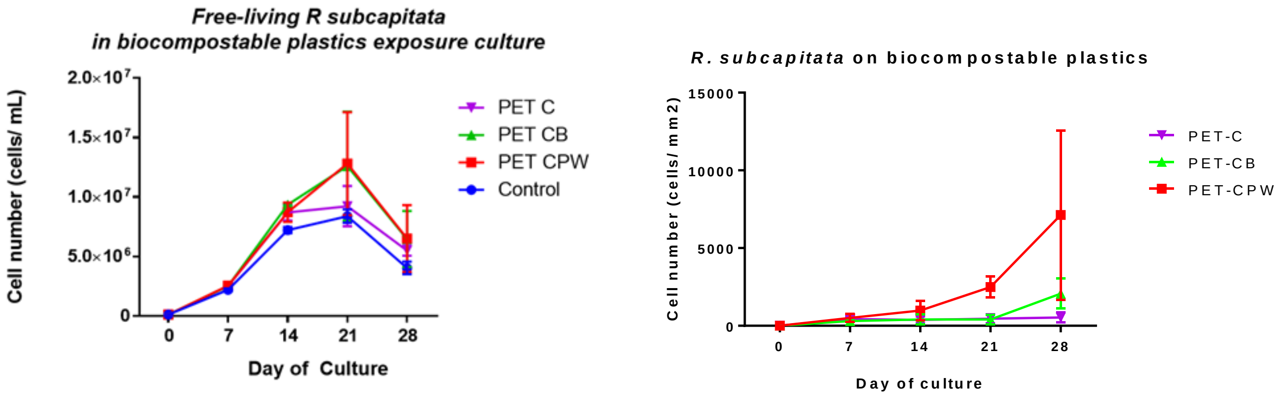


Figure 3 Algal growth in medium (left) and on bio-compostable plastics (right)

3.3 Plastic surface characteristics

Study by Kumar et al. (2017) in Amobonye et al. [8] proved that algae including *Scenedesmus dimorphus*, *Anabaena spiroides* and *Navicula pupula* are able to degrade high density and low-density PE. Our study showed that alga *Raphidocelis subcapitata* able to change the surface morphology and decrease surface roughness under scanning electron microscope (**Figure 4**.) and confocal microscopy analysis (Figure 5a.). Moreover, the contact angle analysis showed that the plastic become less hydrophobic after the exposure to the algal culture compared to the control plastic (**Figure 5b**.).

Cell attachment and biofilm formation is irreversible process, in which it deeply anchored through the membrane [9]. The scanning electron microscopy showed the cells still remained on the surface of PET-CPW and PET-CB after the washing steps, therefore covered the surface of plastics. Surface roughness has an effect on biofilm formation as a major factor for microbial adhesion [10]. PET-CPW has the highest surface roughness compared to PET-CB and PET-C, to support algal attachment. The hydrophobicity of the plastics was not significantly different, but the PET-CPW and PET-CB materials were harder compared to PET-C, that could support better cells attachment as well.

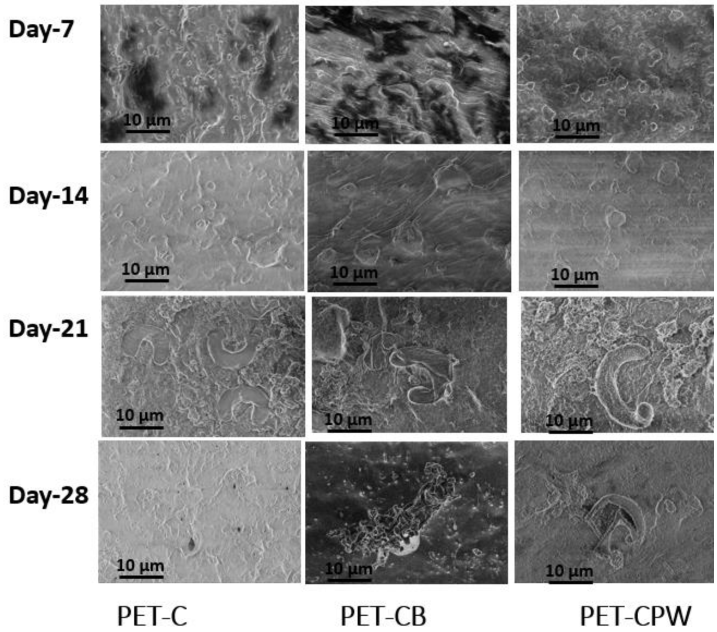


Figure 4 Plastic surface morphology under scanning electron microscope

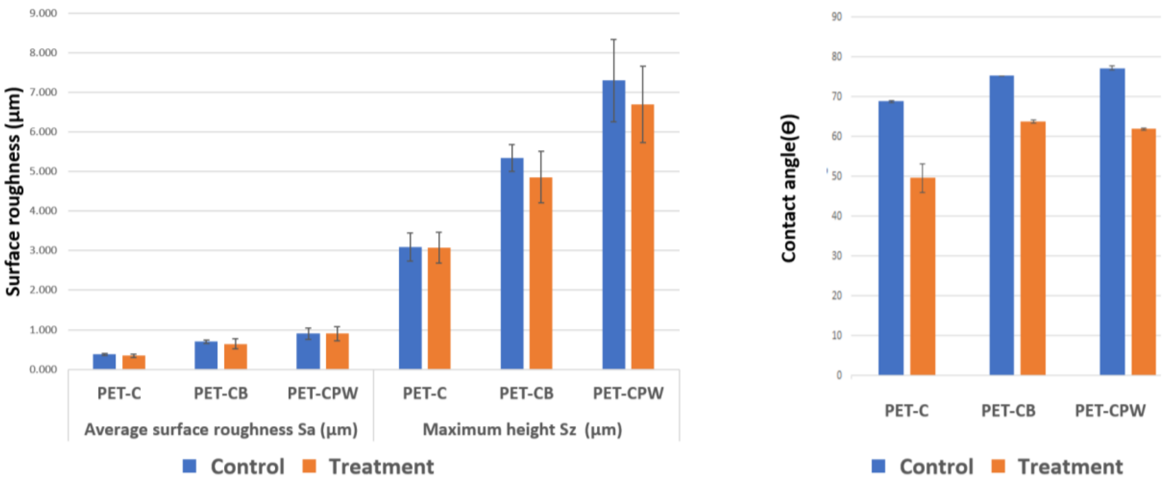


Figure 5 a Surface roughness and **b.** Contact angle analysis of the three bio-compostable plastics

4. CONCLUSION

Unicellular green algae, *Raphidocelis subcapitata*, was able to attach and colonize PET bio-compostable plastics, especially PET-CPW made from corn starch, potato and wheat. The surface morphology and surface roughness changed to be less rough, and the hydrophobicity was lower, indicated the effect of algal culture on bio-compostable plastic. Further study must be conducted to reveal the potential and mechanisms of the algae in plastic bio-degradation processes.

ACKNOWLEDGEMENTS

This work was supported by the Student Grant Competition (SGS) project at the Technical University of Liberec in 2023 and by the Ministry of Education, Youth and Sports of the Czech Republic within the Research Infrastructures NanoEnviCz.

REFERENCES

[1] SHEN, M.; SONG, B.; ZENG, G.; ZHANG, Y.; HUANG, W.; WEN, X. and TANG, W. Are biodegradable plastics a promising solution to solve the global plastic pollution? *Environmental Pollution*. 2020, vol. 263, pp: 114469. Available from: https://doi.org/10.1016/j.envpol.

[2] CHIA, W. Y.; TANG, D. Y. Y.; KHAO, K. S.; LUP, A. N. K. and CHEW, K. W. Nature's fight against plastic pollution: Algae for plastic biodegradation and bioplastic production. *Environmental Science and Ecotechnology*. 2020, vol. 4., pp: 100065

[3] OBERBECKMANN, S.; LABRENZ, M. Marine microbial assemblages on microplastics: diversity,adaptation, and role in degradation. *Annual Review of Marine Science*. 2020, vol. 12, pp: 209–232.

[4] HAHLADAKIS, J. N., VELIS, C. A., WEBER, R., IACOVIDOU, E., and PURNELL, P. An overview of chemical additives present in plastics: Migration, release, fate and environmentl impat during their use, disposal, and recycling. *Journal of Hazardous Materials*. 2018, vol. 334, pp: 179-199.

[5] SUZUKI, S.; YAMAGUCHI, H.; NAKAJIMA, N. and KAWACHI, M. Raphidocelis subcapitata (=Pseudokirchneriella subcapitata) provides an insight into genome evolution and environmental adaptations in the Sphaeropleales. *Scientific Report* [online]. 2018, vol. 8, no. 1, pp. 1–13

[6] WANG, J. H.; ZHUANG, L. L.; XU, X. Q.; DEANTES-ESPINOSA, V.M.; WANG, X. X. and HU, H.Y. Microalgal attachment and attached systems for biomass production and wastewater treatment. *Renewable and Sustainable Energy Reviews*. 2018, vol. 92, pp: 331-342.

[7] TONG, C. Y.; LEW, J. K.; DEREK, C. J. C. Algal extracellular organic matter pre-treatment enhances microalgal biofilm adhesion onto microporous substrate. *Chemosphere*. 2022, vol. 307, pp: 135740

[8] AMOBONYE, A.; BHAGWAT, P.; SINGH, S. and PILLAI, S. Plastic biodegrdation: Frontlline microbes and their enzymes. *Science of the Total Environment*. 2020, vol. 759, pp: 14536. Available from: https://doi.org/10.1016/j.scitotenv.2020.143536

[9] DE-LA PINTA, I.; COBOS, M.; IBARRETXE, J.; MONTOYA, E.; ERASO, E.; GURAYO, T. and QUINDOS, G. Effect of biomaterials hydrophobicity and roughness on biofilm development. *Journal of Material Science: Materials in Medicine*. 2019, vol. 30, pp: 77

[10] MUHAMMAD, M. H.; IDRIS, A. L.; FAN, X.; GUO, Y., YU, Y.; JIN, X.; QIU, J.; GUAN, X. and HUANG, T. Beyond Risk: Bacterial biofilms and their regulating approaches. *Front Microbiol*. 2020, vol. 11, pp: 929