THE IMPACT AND INTERACTION OF UNICELLULAR GREEN ALGAE WITH BIO-BASED PLASTICS

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In this experiment, the attachment of two strains of freshwater unicellular green algae, *Chlamydomonas reinhardtii* and *Raphidocelis subcapitata* on three types of starch-based polyethylene terephthalate (PET) plastic bags PET-C made from corn starch, PET-CB (made from corn starch and beetroot), and PET-CPW made from corn starch, potato and wheat, was studied as the initial phase of plastic degradation.

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

INTRODUCTION

Polyethylene terephthalate (PET) is one of the most significant plastics produced and used worldwide. The annual production of PET has reached 70 million tons and the numbers keep increasing throughout the years (Tournier et al., 2020). Coming together with their benefits, plastic waste management has also become a global concern. The plastic waste is 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 and became pollutant (www.plasticseurope.org).

Following the growing concern about fossil-based plastics pollution, the fast growth of bio-based plastics as its alternativ is also rising. The bio-based plastics derived from renewable biological sources, are believed to be more environmentally friendly. However, their biodegradation and fate in the environment are not fully understood. Therefore, the aim of this study was to investigate the impact and interaction of natural microbes, unicellular green algae, with bio-based plastics.

METHODS

Characterization of plastics

The components of three bioplastics were analyzed by measuring their spectra by attenuated total reflectance Fourier transform infrared (ATR-FTIR) technique on a diamond crystal using Nicolet iZ10 (Thermo Fisher Scientific, USA). The atmospheric contaminants were corrected and the baseline was normalized to the same intensity of the highest band.

The morphology of plastics surface was analyzed using a Zeiss Ultra Plus field-emission SEM (Zeiss, Germany). The samples were 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.

Algal Culture and Plastics Exposure

The green algae *Chlamydomonas reinhardtii* (CCALA 928) and *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.

For plastics exposure, algae were cultured in 250 mL flask with total volume of 100 mL and initial optical density of 0.01 at 680 nm (OD680) measured using AquaPen AP-100 (PSI Ltd., Czech Republic). 40 pieces of PET plastics (1 cm x 1 cm) were sterilized by 1 h incubation in 70% EtOH followed by 30min UV exposure and added into each flask. The exposure culture was incubated at the same conditioned as maintenance culture until the sign of degradation was observed.

The algal attachment was observed under epifluorescence microscope and their growth on bio-based plastics were counted as cell numbers per cm2.

RESULTS AND DISCUSSION

The FTIR analysis showed that the three bio-based PET plastics have different components as shown in Figure 1. Besides polyester and therepthalic 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 components may come from difference source of raw materials used for plastic production and from the additives added during the plastic polymerization process to improve its performance (Hadladakis et al., 2018).

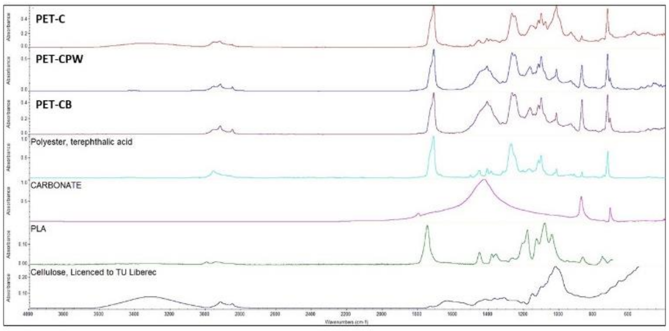

Fig 1: FTIR analysis of PET-C, PET-CB, and PET CPW

Algal cells attachment and growth were shown in Figure 2. and 3. The results showed that both algae, *Chlamydomonas reinhardtii* and *Rapidocelis subcapitata*, were able to attach and colonize all three bio-based plastics in which the PET-C showed the highest support for the attachment and cell growth.

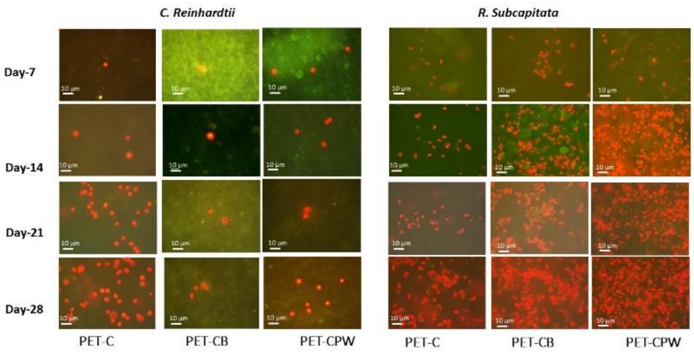

Fig 2: Algal attachment on the surface of bio-based plastics

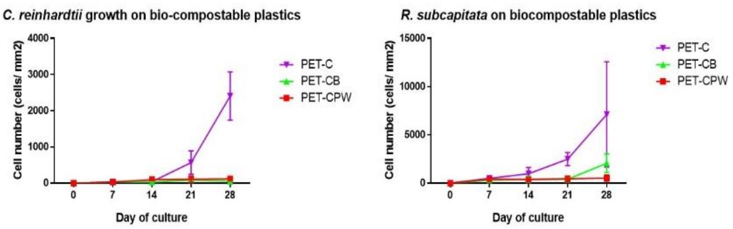

Fig 3: Algal growth on the surface of bio-based plastics

The process of algal attachment involved two steps, initial adhesion when algal cells are transported to the surface of material by gravitational or hydrodynamic force and the thickening of biofilm formed by algal (Wang et al., 2018). *Raphidocelis subcapitata* as non-axenic culture produced high biofilm and extra polymeric substrated (EPS) that support stronger attachement and colonization on the membrane surface (Tong et al., 2022).

Study by Kumar et al., (2017) in Amobonye etall., (2021) showed that algae including *Scenedesmus dimorphus*, *Anabaena spiroides* and *Navicula pupula* are able to degrade high density and low-density PE. The SEM results showed that Chlamydomonas reinhardtii able to change the morphology of the bio-based plastic surface at day-72 (Figure 4.)

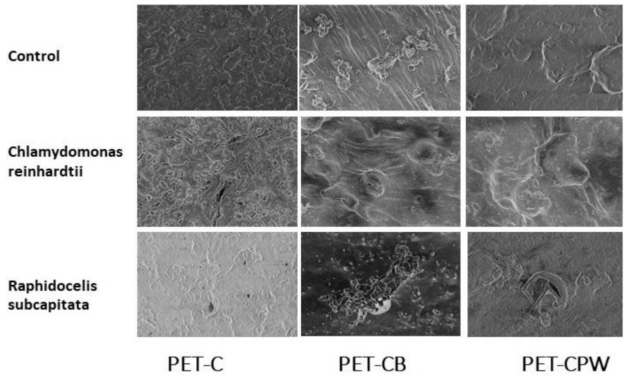

Fig 4: Morphology of the bio-based plastic surface

CONCLUSIONS

Unicellular green algae, *Chlamydomonas reinhardtii* and *Raphidocelis subcapitata*, able to attach and colonized PET bio-based plastics, especially PET-C made from corn starch which contains cellulose. Further study must be conducted to investigate the mechanisms of algae to involve in plastic bio-degradation.

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