Selection of polyolefins-degrading mixed microbial consortia through long-term enrichment

Passanun Lomwongsopon*, Reinhard Wimmer and Cristiano Varrone
Department of Chemistry and Bioscience, Aalborg University, Aalborg, 9220, Denmark
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Presenting author email: paslom@bio.aau.dk

The relentless global utilization of single-use plastics generates a huge amount of post-consumer waste. To change the paradigm of a linear plastic life cycle, new technology should be established to promote the use of plastic waste as a new resource for producing higher- or at least equal-value compounds, instead of being discarded as waste. Biotechnological processes have been explored for plastic degradation and upcycling. Well-known success stories are for example the discovery *Ideonella sakaiensis* 201-F6, able to grow on PET bottles as the only carbon source, or the leaf-branch compost cutinase LCC, able to efficiently hydrolyze polyethylene terephthalate (PET). Within a few years, enzyme engineering techniques allowed the development of thermostable high-activity PET hydrolases that can depolymerize 90% of PET into its monomers within 10 h (Tournier et al., 2020), and a full-scale plant for enzymatic PET recycling is currently under development by the French biotech company Carbios. Such approaches seem to be rather promising when dealing with mixed plastic waste rich in polyesters.

Polyolefins (PO), including polyethylene (PE) and polypropylene (PP), make up approximately half of the plastic production in Europe and are mainly used in the single-use packaging sector (PlasticsEurope, 2021). They are very high molecular weight plastics with C-C bonds that are very recalcitrant toward biodegradation; thus, persisting in the environment for a very long time. Unlike PET, microorganisms and enzymes that completely break down PO have not yet been reported. Previous studies have typically shown a very slow rate of PO biodegradation by single microbial strains, around 1.2-3.8% per year (Goudriaan et al., 2023; Vaksmaa et al., 2023). However, biodegradation can be improved by pre-oxidation; for example, 29.5% weight reduction of PE was found in 126 d at 30ºC with mixed microbial consortia (MMC) degradation of UV-irradiated PE film (Esmaeili et al., 2013).

In this study, the use of MMC was investigated, as the synergistic interactions between members of the consortia are expected to help promoting the utilization of recalcitrant substrates like PO. The functional MMC were developed via long-term enrichment and plastic acclimatization on either PE or PP powder (a mixture between 80 and 750 µm-powders) as the main carbon source. A surfactant, Tween 85, was used to facilitate the accessibility of hydrophobic PO to the bacteria. Subculturing was performed every month by transferring the old culture to the fresh media. Eight cycles of subcultures were done during 9 months enrichment. Development of PO-degrading MMC during enrichment was assessed by measuring the metabolic activity through adenosine triphosphate (ATP) concentration and changes in microbial composition. An increase in ATP concentration over enrichment cycles was observed in some samples, suggesting higher metabolic activity and/or substrate degradation, while half of the samples lost completely their metabolic activity (Fig 1). The ATP concentration reached its peak during the second week of each cycle and then decreased, possibly due to nutrients depletion (nitrogen source and trace elements). The ATP concentrations ranged from 160-263 pmoles/mL in PE enrichment and 47-233 pmoles/mL in PP enrichment. The ATP levels in this study were significantly higher than the best single strains reported in literature (for instance, 20 pmoles/mL from PE film degrading *Rhodococcus rhodochrous* (Fontanella et al., 2010) or 24-166 pmole/mL from PP film degrading *Pseudomonas* and *Bacillus* strains (Aravinthan et al., 2016)), suggesting increased metabolic activity.

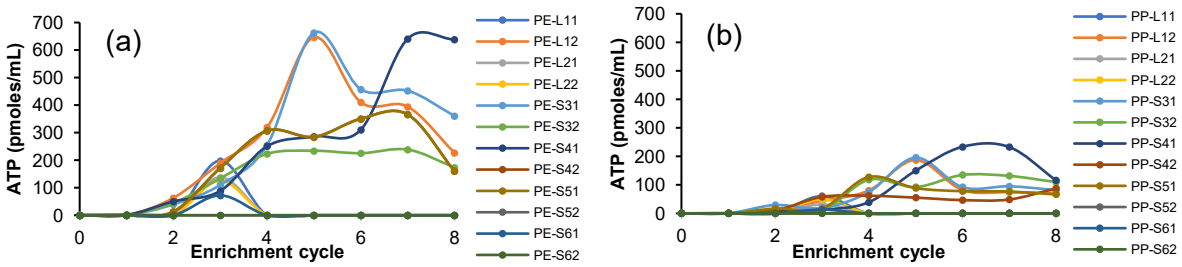

Figure 1 ATP concentration during enrichment cycles of different consortia grown on PE (a) or PP (b).

MMCs in which metabolic activity was still detected after 8 cycles of enrichment were tested for biodegradation efficiency on UVc-treated PE and PP sheets. The highest weight loss of PE and PP sheet observed

was 2.3% and 7.7% after 30 d, respectively. PP was more susceptible to UVc irradiation than PE, evidenced by the formation of new oxidative functional groups including C=O, OH, and C-O, detected by ATR-FTIR. The microbial compositions of the consortia were analyzed by amplicon sequencing. Notably, *Rhodanobacter* became the dominant genus in both PE-S41 and PP-S32 (45.4% and 64.9%, respectively), suggesting its ability to utilize both, PE and PP. This genus has been previously reported for pesticide, herbicide, and hydrocarbon degradation. It was also reported to dominate the MMC enriched on PE mulch film, but the single isolate of *Rhodanobacter* sp. did not degrade PE (Wang et al., 2023). In the current study, physicochemical pretreatment was applied to PE, PP and real mixed plastic waste from various streams, by integrating micronization, UVc oxidation, and Fenton's reagent oxidation, to enhance biodegradability. Fig 2b shows the weight reduction percentage after 30 and 60 d of biodegradation by enriched MMCs (S41 for PE, S32 for PP, and the mixture of both for mixed plastic streams). Maximum 7.7% and 11.7% of weight losses were found on PE and PP micro-powder, respectively. The weight loss stayed constant after 30 d in PE degradation, but improved by 2.8 times in PP when incubating for 60 d. Further increasing of incubation time might improve PP weight reduction even more. The enriched MMC showed good degradation efficiency on real plastic waste streams from Danish municipality, biogas plant, and compost. 16-26% weight loss was found in 30 d, even though extending the incubation time to 60 d did not contribute to higher weight loss.

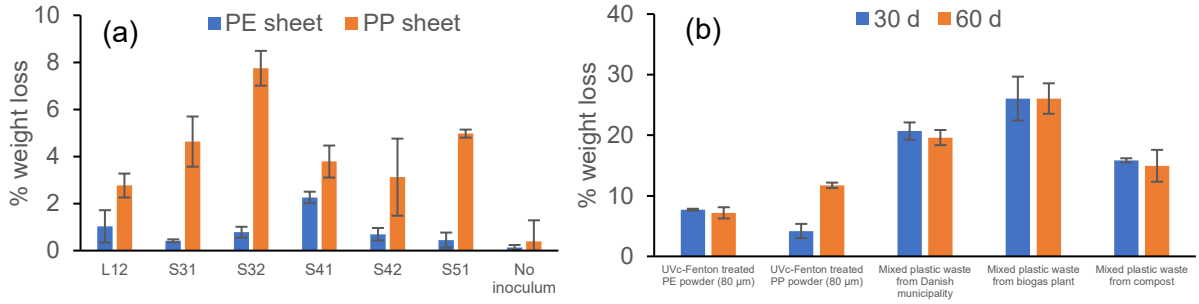

Figure 2 Weight loss percentage of UVc-treated PE and PP sheet degraded by different enriched consortia (as indicated on the x-axis) (a), and weight loss percentage of UVc-Fenton-treated PE and PP micro-powders and micronized mixed plastic waste from different sources (b).

Differential Scanning Colorimetry (DSC) analysis showed that PE crystallinity slightly decreased after soaking in MSM for 30 d (from 39% in original UVC-treated PE to 31%) and slightly increased again after exposure to MMC (33%). This could suggest that the biodegradation started from the amorphous region, leaving the remaining polymer with higher crystallinity. Degradation products were first studied by employing a model substrate, namely octadecane. 1H-NMR, 13C-HSQC, and TOCSY spectra showed the presence of wax-like structure compounds (hydrocarbon chain with ester groups) and branch-chain hydrocarbons in the supernatant, after octadecane biodegradation. If obtained by PO biodegradation, such products could be used as a carbon source for other biotechnological processes as an upcycling strategy to higher value products, such as bioplastics.

In summary, enrichment of environmental samples on PE or PP as the main carbon source allows the selection of PE- or PP-degrading strains, which can survive over a longer period, using PO as sole carbon source. With the integration of physicochemical pretreatment, MMC showed the capability to partially degrade the real mixed plastic waste rich in PO. Further development of this process, for instance through repeated pretreatment-biodegradation cycles and upcycling of released products, could be an interesting approach to deal with contaminated mixed plastic waste streams that are not properly managed by current recycling technologies.

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