Biorecycling and bioremediation of plastics and plasticizers by thermophilic enzymes from the Great Australian Artesian Basin

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Introduction

Plastics are an important, and versatile material we have been using for a centuries. However, over the past few decades we have seen a dramatic increase in their use which has caused both sustainability and environmental concerns. A majority of plastic waste ends up in landfills, and over time it spills over into ecosystems[1]. These plastics can harm endemic life as they can break down into microplastics and be consumed, incapacitating fauna. Alternatively they leech toxins into the surrounding environment. Virgin plastics are a finite resource as they are sourced from fossil fuels which are diminishing. So, plastic recycling will be inevitable in sourcing plastics[2]. Our current methods of recycling are physical or chemical recycling in which plastics are melted or dissolved respectively, to recycle the plastic. However, a prerequisite for these methods to be economically viable is that they require a pure plastic feedstock[3]. Sorting to make a pure feedstock remains a large challenge as there are a numerous polymers and polymer blends which make it difficult to sort by hand or automation. Enzymatic biorecycling could be a complimentary recycling technology as it offers a selective method of depolymerisation, specific to the desired polymer. This can circumvent the need to sort plastics, or catch what traditional methods miss. For feasible economical use of enzymes for recycling they need to be effective, robust, and easy to produce. In this research we utilize thermophiles from the Great Australian Artesian Basin (GAB) for their thermophilic enzymes to degrade plastics as it is believed heat tolerance is a useful in developing robust enzymes

Methodology

The genome of a *Bacillus* sp. collected from the GAB was blasted against a list of known plastic degrading enzymes from literature. Candidates were chosen and rank ordered based off peptide sequence identity Fig. 2A. Candidates were then cloned into pETDuet-1 vectors with 6xHis-tag motifs Fig. 2B. Vectors were then transformed into Tuner (DE3) cells. Paranitro benzyl (PNB) esterase was expressed using IPTG (0.5mM). Cells were then lysed and purified Fig. 2 C. Plastics/ plasticizers were then degraded under optimal conditions (Fig. 2D). Samples were then analysed through HPLC (Fig. 2E).

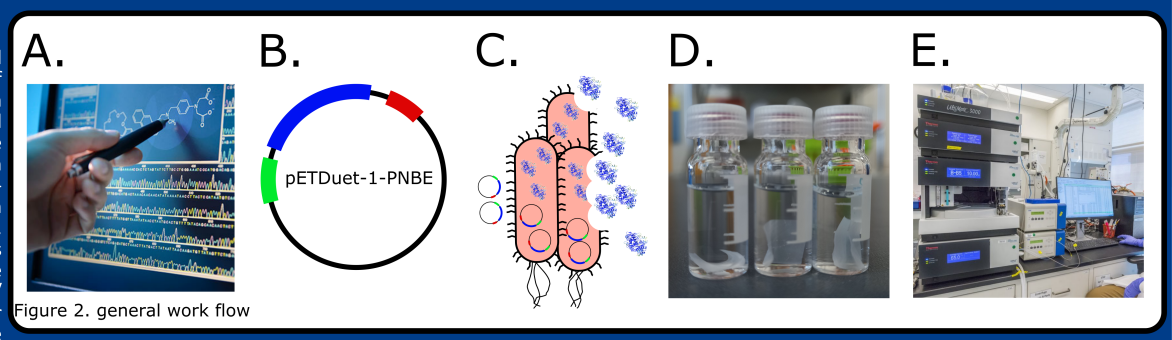

Figure 2. general work flow

Results 1

Polybutylene adipate terephthate (PBAT) a plastic film was able to be enzymatically hydrolysed by PNB esterase to its monomers or Terephalic acid (TPA), Adipic acid (AA), and 1-4 Butanediol (BDO). TPA was used to quantitate enzymatic hydrolysis and it can be seen in figure 3 that the detection is significant to control groups. This suggest PNB esterase is a possible candidate for plastic recycling. The monomers from this enzymatic degradation are able to be fully recycled into the parent polymer PBAT or other similar polymers (Figure 4)

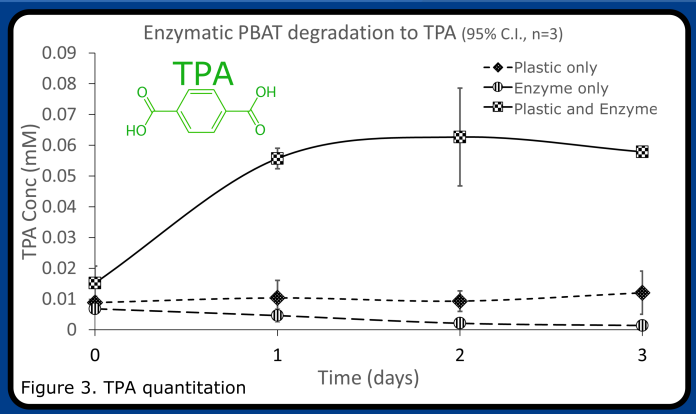

Figure 3. TPA quantitation

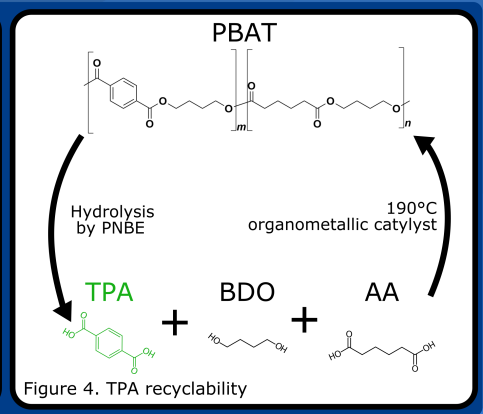

Figure 4. TPA recyclability

Results 2

Di-isobutylphthate (DiBP) a plasticizer was able to be enzymatically hydrolysed into its products Mono-isobutylphalate (MiBP), and Phthalic acid (PTH). In figure 6 this can be visually assessed as DiBP is not miscible with water but its products MiBP and PTH are miscible with water. So overtime the emulsion transforms into a solution. If PNB esterase can be bound to a substrate it could have potential bioremediation application as seen in figure 7.

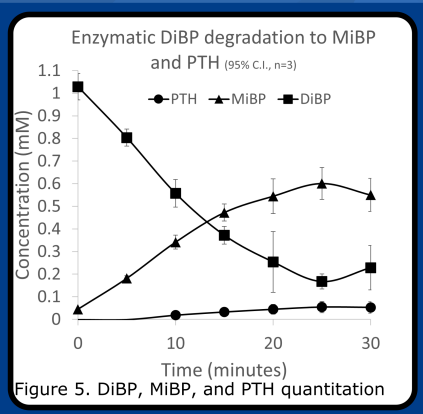

Figure 5. DiBP, MiBP, and PTH quantitation

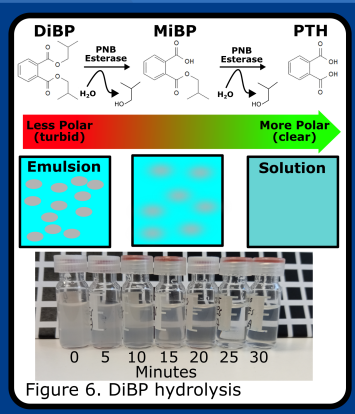

Figure 6. DiBP hydrolysis

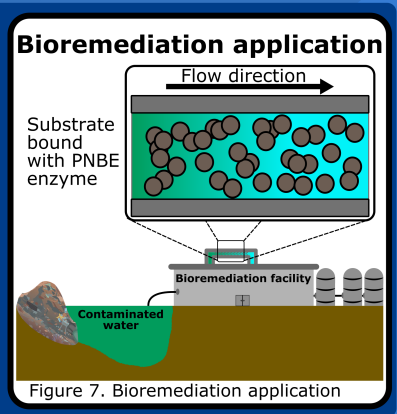

Figure 7. Bioremediation application

Conclusion

PNB esterase is a promising candidate for enzymatic biorecycling and has additional bioremediation functionality. It shows that there is possibility of a enzymatic biorecycling industry. However, this is only the foot in the door and there is still much further work to do. Future work involves futher developing the PNB esterase by adding binding domains, optimizing expression, and upscaling production.

Acknowledgments

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References

1. Bennett, E. M., & Alexandridis, P. (2021). Informing the Public and Educating Students on Plastic Recycling. Recycling, 6(4). doi:10.3390/recycling6040069

2. Van Eygen, E., Laner, D., & Fellner, J. (2018). Circular economy of plastic packaging: Current practice and perspectives in Austria. Waste Management, 72, 55-64. doi:10.1016/j.wasman.2017.11.040

3. Ragaert, K., Delva, L., & Van Geem, K. (2017). Mechanical and chemical recycling of solid plastic waste. Waste Management, 69, 24-58. doi:https://doi.org/10.1016/j.wasman.2017.07.044