Green Biodegradation: Analysis of Potential Polyurethane-degrading Enzymes and Their Secretion in *Chlamydomonas reinhardtii*

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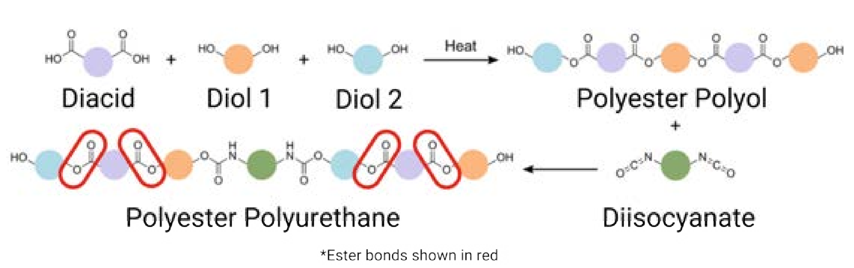
INTRODUCTION

Currently, only 5% of plastics are recycled. One way to combat plastic pollution is through **enzymatic biodegradation.** This form of degradation breaks plastics into individual molecules rather than just creating microplastics. One of the most versatile and prevalent plastics is polyester polyurethane (PU). Work by Gunawan et al. has shown that PU foam can biodegrade in compost and organisms capable of surviving on it have been identified. Here we examine a potential PU-degrading enzyme, found to be secreted by one of these organisms, known as PS5. PS5 was found to have esterase activity that is capable of cleaving ester bonds, which are one of the bonds in PUs. Since the green microalgae *C. reinhardtii* can be used to make PU precursors such as lipids and diacids, in addition to sequestering carbon, it has the potential to serve as a more sustainable platform for the production of these PU-degrading enzymes. This platform would allow for contributions to the production of new PU products as well as the complete biodegradation of PU products that have reached the end of their lifespan.

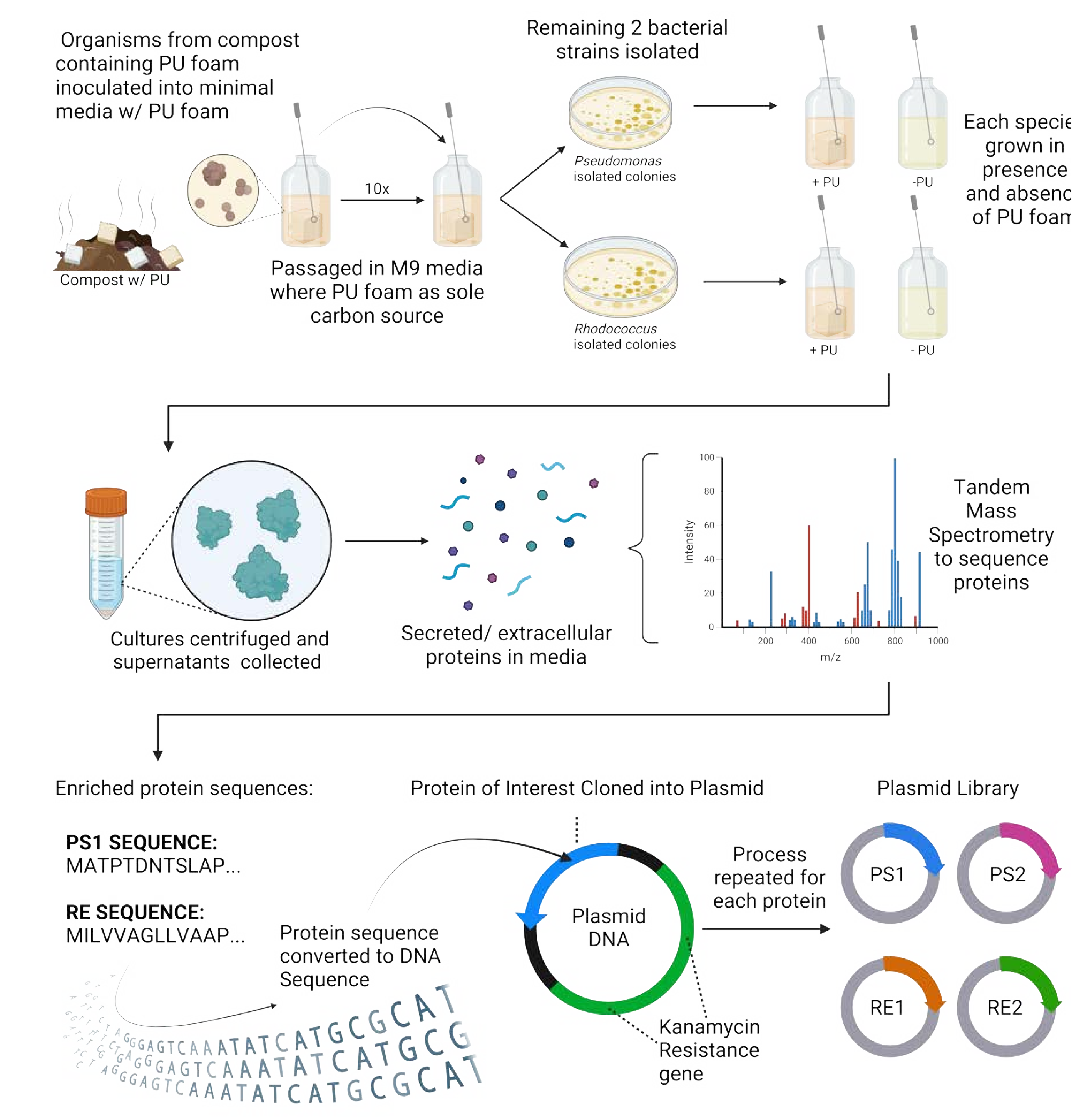
Microplastics on a human fingertip

Plastic pollution
Kamilo Beach, Hawaii, 2020

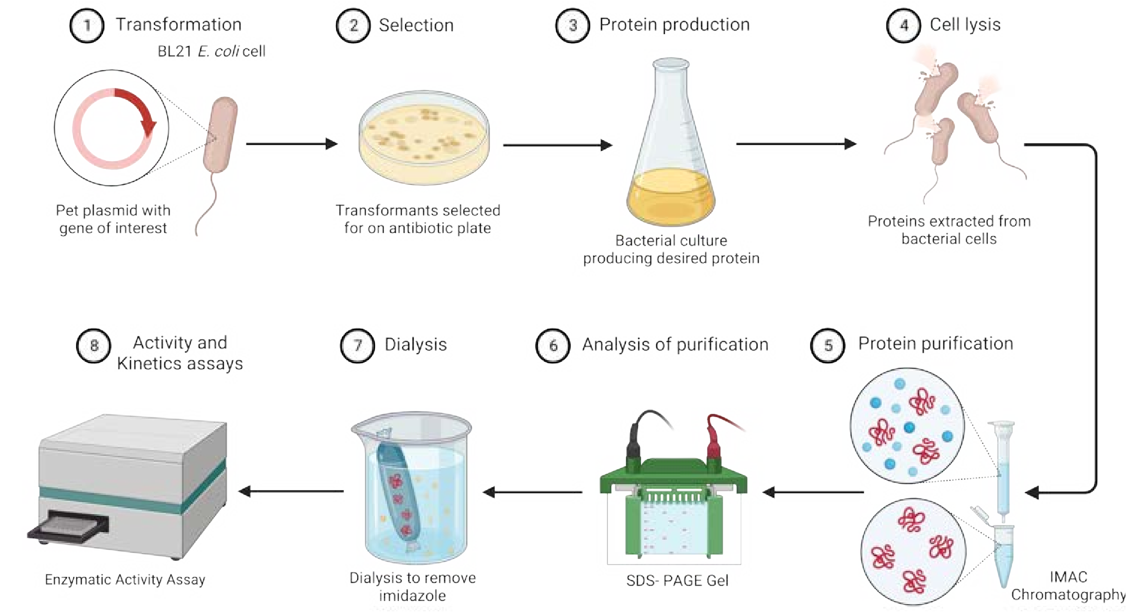
Polyester Polyurethane (PU)



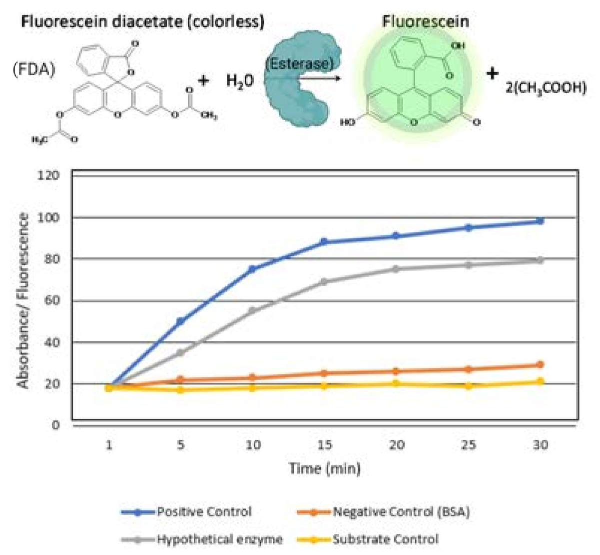
PREVIOUS WORK



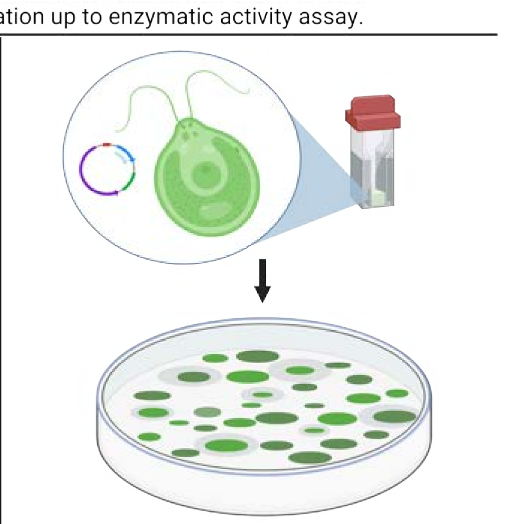
METHODS



Workflow for enzyme expression and purification up to enzymatic activity assay.

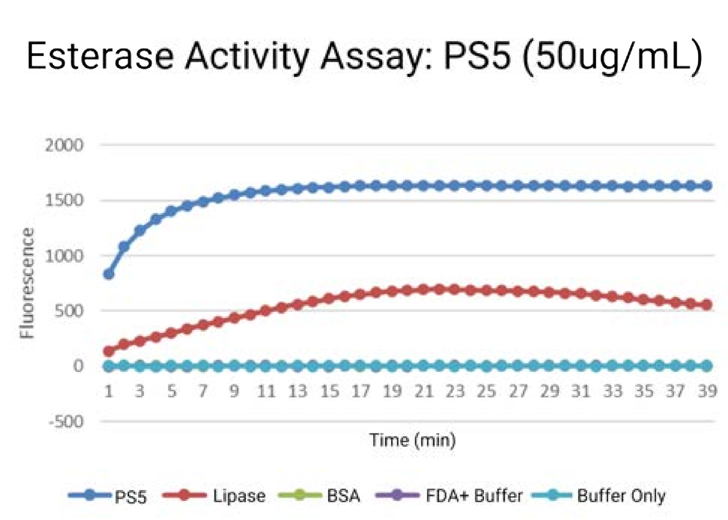


Enzyme Activity Assay: Cleavage of ester bonds in FDA yields a fluorescent molecule indicative of esterase activity

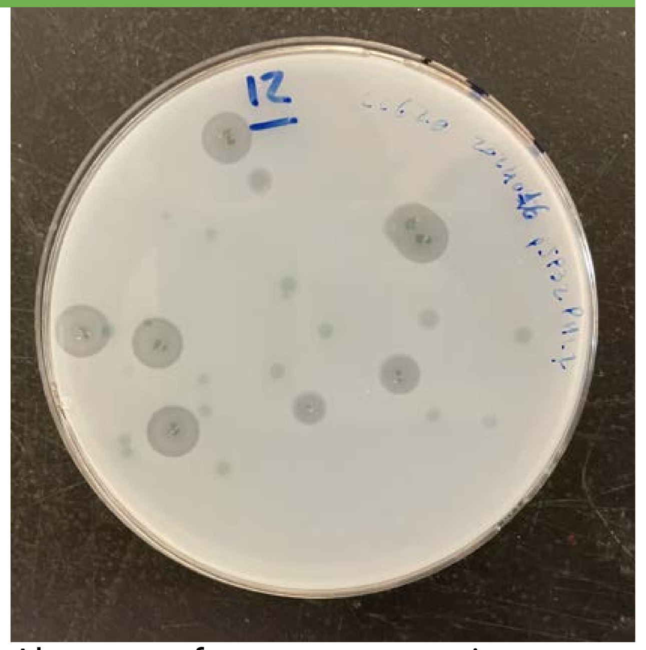


Transformation of C reinhardtii with secretion plasmid containing PS5 esterase. Transformants plated on plates containing Impranil -DLN. Transformants secreting PS5 will have "halos: surrounding them which is an indicator of successful transformation and protein expression

RESULTS



In the presence of PS5, the FDA substrate is cleaved to release a fluorescent molecule. The increase in fluorescence over time served as an indication that PS5 does have esterase activity . (unpublished). The activity of this enzyme was much higher than that of a commercial lipase that served as a positive control.

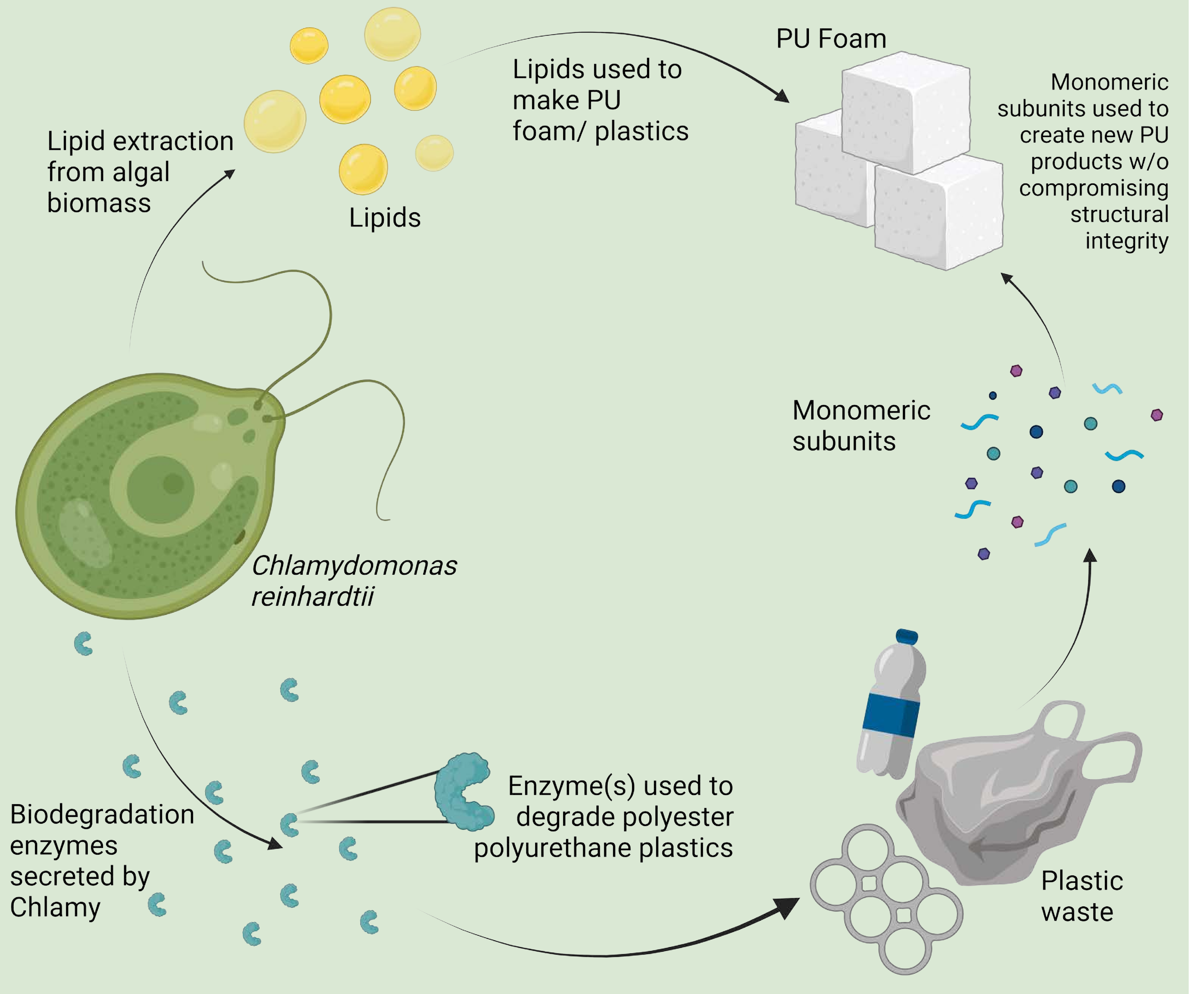


Algae transformants expressing a modified PETase (PS5 Results In progess) that is creating halos on and Impanil-Agar plate. impranil is a PU dispersion (unpublished).
This image serves as proof of concept. Crediu: Joao Dutra Molino

CONCLUSIONS

PS5 has esterase activity and is therefore a candidate for enzymatic degradation of PU products and subsequent transformation into *C. reinhardtii.* The green microalgae C. reinhardtii are capable of expressing and secreting enzymes that are capable of degrading polyester polyurethane dispersions.

Enzymatic degradation and sustainable processing have the potential transform the face of recycling and our environment.



In order to eventually produce these enzymes at an industrial scale, we propose the use of the green microalgae *C. reinhardtii* as a more sustainable platform for enzyme production. This is due to the ability of microalgae to serve a source of plastics precursors . This could eventually allow individual molecules to be up-cycled into new products without compromising structural integrity.

REFERENCES:
1. Hai TAP, Neelakantan N, Tessman M, Sherman SD, Griffin G, Pomeroy R, et al. Flexible polyurethanes, renewable fuels, and flavorings from a microalgae oil waste stream. Green Chemistry. 2020;22(10):3088-94.
2. Gunawan NR, Tessman M, Zhen D, Johnson L, Evans P, Clements SM, et al. Biodegradation of renewable polyurethane foams in marine environments occurs through depolymerization by marine microorganisms. Science of The Total Environment. 2022;850:158761.12.
3. Natasha R. Gunawan MT, Ariel C. Schreiman, Ryan Simkovsky, Anton A. Samoylov, Nitin K. Neelakantan, Troy A. Bemis, Michael D. Burkart, Robert S. Pomeroy, Stephen P. Mayfield. Rapid biodegradation of renewable polyurethane foams with identification of associated microorganisms and decomposition products. Bioresource Technology Reports.