Hong Kong Student Science Project Competition 2022
Template of Extended Abstract (Invention Design Proposal)
(Word Limit: 1,000 words, Pages: 2 pages only)

Team Number: SCBC108

Project Title: fil-PLEST

Project Type: Invention Design Proposal

To our best knowledge and after thorough literature research, as at 31/8/2021 , there are / ~~are no~~* similar works. If there are, the reference links are as below:

https://2019.igem.org/Team:Exeter/Model

The enhancement our project has made for the existing related products or research is summarized as below:

We have tried to enhance the filter to digest the microplastics from the clothes. We also tried to dehydrate the enzyme and pack it into a small package to facilitate the use.

Please delete if not applicable. HKSSPC values the originality of works. Students must conduct literature research thoroughly to ensure that their works are unique, and to list relevant reference materials to complement the research or invention.

I. Background

It is estimated that every synthetic fabric washed may produce 1,900 pieces of microplastic fibres. Washing clothes, meanwhile, releases 500,000 tons of microfibers into the ocean each year. Many of those fibers are polyester, which does not break down in the ocean. A 2017 report from the International Union for Conservation Nature (IUCN) estimated that 35% of all microplastics in the ocean came from the laundering of synthetic textiles like polyester.

Due to hygienic reasons under the pandemic, the high spending power of Hongkongers and the popularity of fast fashion brands, whose products are made of synthetic rather than natural fibre. There is a large amount of microplastic waste from laundry is to be drained into the ocean.

So reducing micro plastic to the sea starting from laundry is urgent and essential in Hong Kong.

Our invention is a two-step solution called fil-PLEST, which stands for "filtration-PLastic digEST". The first step of our solution is to use a filter to trap the synthetic fibers. Our second step is to add enzyme to degrade the microplastics.

II. Objective(s)

We would like to use our research product to decrease the amount of microplastic originated from clothes to be polluted into the environment and to remove the possible microplastic pollution as much as possible.

To achieve the above objective, we have three sequential goals in our research project.

(1) To insert the DNA of PETase- MHETase and nylonase into E. coli so that the GM E. coli carries information to make the two enzymes.

(2) To prove the enzymes are correctly expressed by the E. coli.

(3) To test the ability of the enzymes to digest microplastics collected from the washing machine discharge.

III. Methodology

1. Plasmid ordering

We ordered the plasmid which contains PETase-MHETase from the biotechnology company Addgene.

2. Miniprep and transformation

The plasmid is first extracted out from a culture developed from a single colony isolated from the NEB Stable bacterial stab. The extracted plasmid is then introduced back to the cloning strain E. coli BL21 via

heat shock transformation. The success of transformation is reconfirmed by colony PCR, which visualize the size of the designated area framed by custom DNA primers.

3. Expression

Single colonies from transformation were then inoculated into a starter culture of Luria Broth (LB) media containing 100 μg/mL ampicillin and grown at 37°C overnight. Protein expression was then induced by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. Cells Harvested cells were resuspended in a lysis buffer. Lysate was clarified by centrifugation at 10,000 x g for 45 minutes.

4. Protein purification

A C-terminal Histidine-tag sequence is included in our ordered plasmid so that the protein can be easily recognised as it is fused with a His-tag.

5. Bradford assay

During the whole experiment, bradford assay will be completed using samples of before E.coli induct protein and after inducting protein and also purified protein we kept.

6. SDS-PAGE

SDS-PAGE will be run to confirm correct expression of PETase-MHETase.

7. Functional assay

Functionality assay will be conduct for PETase enzymatic degradation.

IV. Design of Invention

Our invention is a two-step solution called fil-PLEST, which stands for "filtration-PLastic digEST". It aims to decrease the amount of microplastic originated from clothes to be polluted into the environment and to remove the possible microplastic pollution as much as possible. The first step of our solution is to use a filter to trap the synthetic fibers. Our second step is to add enzyme solution in powder form (with PETase-MHETase and Nylonase B) to the residue of the filter. The solution can degrade the PET, MHET and nylon. To accommodate the working pH of all of the above enzymes, the pH will be controlled at pH 8 using a buffer, which is freeze-dried together with the enzymes. After the degradation of microplastics, the solution can be poured into the sink or flushed into the toilet.

V. Application / Market Need

Due to hygienic reasons under the pandemic, the high spending power of Hongkongers and the popularity of fast fashion brands, whose products are made of synthetic rather than natural fibre. There is a large amount of microplastic waste from laundry is to be drained into the ocean. So, we expected result are implementing with the help of education and supervision to improve Hong Kong's pollution, the world microplastic pollution.

VI. Conclusion

In conclusion, we believe that our design project is feasible once we overcome the difficulties which we are facing. For example, we cannot verify the existence of protein in our induced samples due to the low concentration. In fact, once we have obtained the protein, we will do functionality assay for PETase enzymatic degradation.

Also, in the future study, we would try to insert the gene of both PETase and Nylonase (which can digest Nylon) in the plasmid in order to digest more smicroplastics in the clothes. Also, we would like to undergo the dehydration in the experiment

☐ **Our project is developed based on our school's previous project and the enhancement is as below:**

No.