Proposal Development

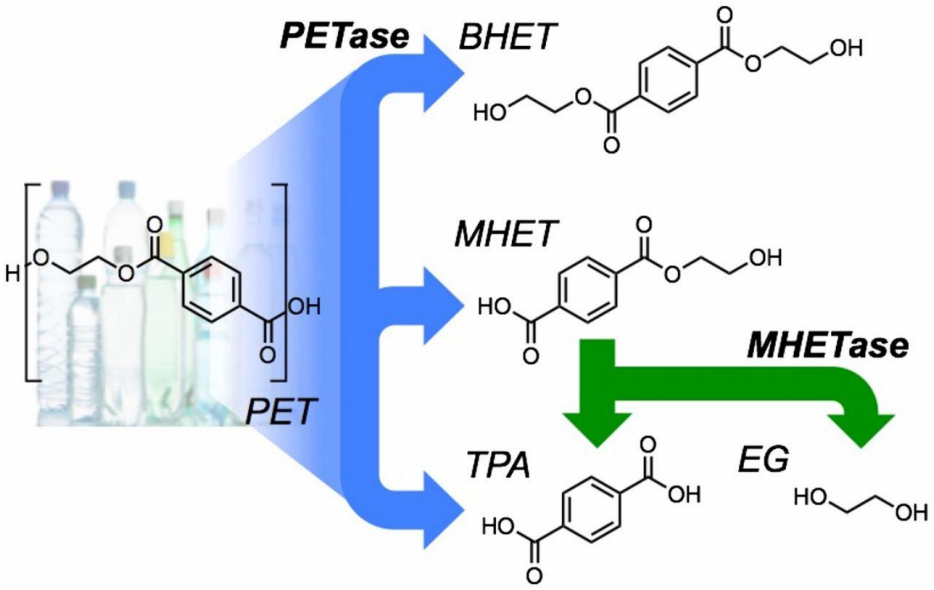
Questions and Technical Challenges

- What parts and devices could be used to ultimately hydrolyze these polymers to simpler monomers? This requires finding and selecting enzymes that can carry out chemical hydrolysis of these polymers.
- Should we/can we deal with multiple types of polymers (polyamides/polyesters) all at once?
- What enzymes/device concentration is needed to efficiently hydrolyze these polymers?
- Is there some type of “kill-switch” for the chassis?
- Should the chassis live off the monomers to maintain the cell population?
- Should the chassis utilize chemotaxis proteins such as a synthetically engineered Methyl-Accepting Chemotaxis Protein (MCP) so that the chassis moves towards higher concentrations of synthetic fibers?
- Should we utilize an inducible promoter?
- Should we use the synthetically engineered organism at the step in the water filtration status whereby the freshwater is about to re-enter the public water supply? If this is the case, what temperature is the water kept at?

This progress report will look specifically at polyester hydrolysis and will not focus on polyamides.

Progress and Potential Parts

The major polyester that is used in clothing is polyethylene terephthalate (PET). This polymer can be broken into monomers by many processes including hydrolysis[1]. In 2016, researchers in Japan actually found a bacteria, known as *Ideonella sakaiensis* 201-F6, that was able to live on thin PET film. It utilizes two enzymes, PETase and MHETase, and uses the products (terephthalic acid/ethylene glycol) as an energy source[2]. The products are ultimately converted to CO_2 and H_2O. UniProt IDs for PETase and MHETase are A0A0K8P6T7 and A0A0K8P8E7, respectively[3,4]. The following is a schematic to better understand PETase and MHETase activity[5]:



The novel species of bacteria that can live on PET film, *Ideonella sakaiensis*, has not been studied or utilized extensively. However, the protein products of interest have been investigated and characterized. PETase specifically has been revealed using crystallography and was codon optimized for high expression using a plasmid in *E. coli*[5,6]. The specific amino acid sequence of PETase is also available online[7].

Considering the rapid division rate and the strong understanding we have of *E. coli*, they are the best option for a chassis. A good idea would be to insert plasmids into these bacteria with the PETase and MHETase genes and select for those that are able to live exclusively off PET film. This would mean they metabolize MHETase products, TPA and EG to survive. If no *E. coli* are found to evolve metabolic pathways for this, we

can still utilize *Ideonella sakaiensis* 201-F6 in the freshwater medium to take in the MHETase products.

Synthetic engineering of MCP receptor proteins has been accomplished[8,9]. More specifically, a general mechanism of genetic modification of cells coupled with engineered G-Protein Coupled Receptors (GPCR) allowed redirection of bacteria to small molecule drugs[9].

Hence, an important opportunity is the chemotactic manipulation of *E. coli* via synthetically engineered Methyl-Accepting Chemotaxis Protein (MCP) receptor. An example would be precise engineering of the MCP to specifically recognize PET and move towards concentrations of high PET. As ideal as this would be, even utilizing an H+ negative chemotaxis protein would be useful. This is since terephthalic acid is produced when the reaction is fully completed. It's a potentially simplistic way of moving bacteria away from an area of completed reaction.

Using the *E. coli* chassis, here are the parts I intend to have.

PETase (combine the 3 below to make a new "part"):

1. BBa_J23119
   Strongest constitutive promoter available. This is useful to have all other parts constantly expressed in *E. coli*.
2. BBa_K1921019
   Means of anchoring PETase to outer membrane of *E. coli*. Lpp is a lipoprotein whereas OmpA is Outer Membrane Protein A.
3. BBa_K1921003
   Use this mutant form of PETase to replace the PETase in the previous part. It shows much more activity in lower temperatures which is useful.

MHETase:

1. BBa_K2651002
   Utilize strong promoter, strong RBS and MHETase with PelB tag. Get rid of the PETase/PelB tag and the AmilCp reporter gene.

Chemotaxis:

1. BBa_K1639003

   This part gives a negative chemotactic response to H+ ions. This is useful since MHETase yields terephthalic acid. This part could be a signal so bacteria realize that they have done their job in the full hydrolysis of PET.

References

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