Breaking Down the Plastic Crisis

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The world is currently facing a major plastic crisis. About 4.8 to 12.7 million tons of plastic enter the oceans each year, yet only 9% of the world's plastics are recycled. One of the most widely-used plastics in packaging of food and beverages, polyethylene terephthalate (PET), has surpassed 50 million tons produced yearly. PET can break down into the environmentally-friendly compounds ethylene glycol and terephthalic acid, but this process can take several decades We have designed a product that can significantly shorten the time of degradation of PET. It can be used on both small-scale or large-scale applications, such as households or commercial landfills, respectively.

The conceptualization of the product was based on the recently discovered ability of the wax moth (*Galleria mellonella*) to degrade plastic. However, the gene from this organism is not compatible with our desired chassis, baker's yeast. To make our product compatible with yeast, we chose a different gene,the ISF6_4831, which degrades and assimilates PET. Although this gene can be derived from the *Ideonella sakaiensis,* we decided against simply using the bacterium because its extremely expensive cost would prevent our product from being universally accessible. Instead we chose to insert the ISF6_4831 gene into yeast. GFP (Green Fluorescent Protein) will be inserted along with the isolated gene as an indicator of when the gene is successfully working. This product can be used in a variety of situations but we are planning on focusing on commercial areas, specifically community receptacles.

Keywords: Plastic, degradation, PET, chassis, yeast

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Watch a video introduction by the authors at http://bit.ly/2TfQVnT

Background

The plastic crisis has grown exponentially over the past few years. The harmful effects of our continued dependence on synthetically-made plastic products range from the annoying pieces of litter scattered on roadsides to the tons of plastic waste polluting our oceans. Some of the chemicals associated with plastic have been shown to have negative effects on reproduction, impairing the growth of amphibians and crustaceans (Thomson 2009). Researchers across the world are collaborating to achieve a sustainable global method of eliminating the plastic on Earth currently and preventing this problem from persisting.

Although PET (polyethylene terephthalate) is the most widely recycled plastic, the time for plastic water bottles to break down is a striking 450 years minimum (Kari 2017). Many studies and experiments have been conducted on methods to reuse PET or to speed up the PET degradation process. One such experiment was the "Use of plastic waste (poly-ethylene terephthalate) in asphalt concrete mixture as aggregate replacement" conducted by Hassani et al. (2005). investigated how PET waste could be added into asphalt concrete mixes. The final product was an aggregate replacement named Plastiphalt, the use of which could reduce the overall negative effects associated with PET waste (Hassani et al. 2005).

Another method that has been discovered to alleviate the issues caused by PET waste is by using biotechnology to accelerate the degradation process. In the article "Feeding on Plastic" (Bornscheuer 2016), the authors discovered the *Ideonella sakaiensis* strain as the sole microorganism that can break down PET.

Upon finding this research, we decided that we wanted to utilize this gene and the enzymes in this bacteria on a larger scale. We hoped to create a product that would allow for the degradation of plastic when it first enters the recycling system. Ideally, the product will be implemented into our target area of supermarket water bottle collection facilities where the process of PET breakdown can start at the earliest possible stage of the recycling process. We also decided on adding a Green Fluorescent Protein (GFP), to ensure that we can confirm that the degradation process is occurring. A bright green fluorescent glow can be a visual indicator for the consumer to confirm that the gene is being expressed.

Systems Level

Our goal is to utilize a chassis to produce the gene, ISF6_4831.The ISF6_4831 gene, forming the protein PETase will catalyze the hydrolysis of PET plastic to produce mono(2-hydroxyethyl) terephthalate (MHET). A second protein known as MHETase also works to further break down MHET into the relatively simple chemicals ethylene glycol and terephthalic acid. Ethylene glycol can be used in a variety of industries because of its significant solvent capabilities and competitive cost. Similarly, terephthalic acid, has no harmful environmental effects.

Our system is designed to quicken the degradation of PET plastic. We will use our chosen chassis to Device Level

We chose *Saccharomyces cerevisiae*, simply known as baker's yeast, as our chassis because of its versatile and cost-efficient qualities. We also wanted to ensure that the ISF6_4831 gene would function equally well in our chosen chassis as it does in the *I. sakaiensis* microorganism. Although *I. sakaiensis* is a gram-negative proteobacterium, and yeast is a eukaryotic unicellular fungus, it is not difficult to insert a gene from a prokaryotic cell into that of a eukaryotic cell. Furthermore, there are many benefits to using yeast as it is often used in synthetic biology because of its versatility and rapid growth.

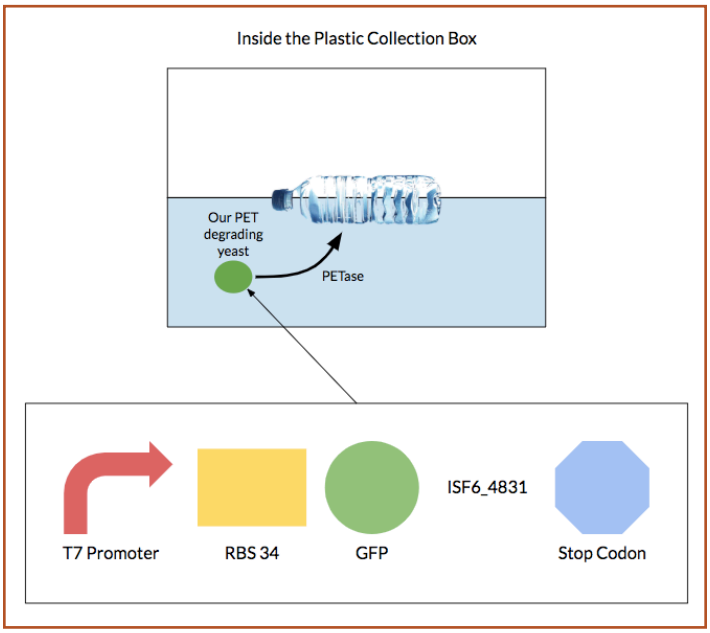

Figure 1. *Visual representation of plastic collection box*

Parts Level

In this project, we chose a promoter, a ribosome binding site, and the coding sequence. For our promoter, we chose the BBa_I766556 promoter as it is believed to be a strong promoter in yeast. For our ribosome binding site, we decided on the part BBa_K165002 which starts the translation for eukaryotic mRNA. Part BBa_K2010000, the gene ISF6_4831, along with a green fluorescent protein will be expressed to create PETase to break down the plastic and to alert users that the gene is being produced.

Safety

A liquid solution will be the medium for this product. It will be sold in the form of a machine-like box (Figure 1) containing a large touch-sensitive hole for the plastic to enter through. To prevent the chemicals in the plastic-degrading solution from direct skin contact, one will drop plastic into the hole without making direct contact with the yeast solution or any chemicals released as products. The box will mechanically release the solution containing the yeast when a chosen number of bottles

has entered the box. This solution will surround the plastic, beginning the process of degradation. The products formed, ethylene glycol and terephthalic acid, are environmentally-friendly, so there is little concern over their effects on our surroundings.

Discussions

Our project will begin the process of degradation at an early stage, when it first enters the recycling system, thus drastically decreasing the amount of undegraded PET that finds its way into the world's oceans or landfills. In addition, we anticipate that this product can be sold on a larger scale, and will be easier to commercialize because it is relatively safe and easy to use.

However, with each new project comes challenges. One of the main challenges of our product is how the products created from plastic degradation will be transported, and where they will be placed. We are also looking into how we can improve on the materials of the physical box. Designing a biodegradable box would ensure that we are not contributing to the worldwide waste problem with the creation of new boxes.

Another important aspect to consider is the creation of a designated area to quicken the process. Instead of individual boxes, a chosen area could be formed to do the same task but on a larger scale. Furthermore, cost is always a significant consideration when thinking of possible applications of our product. Although yeast is a relatively inexpensive chassis, constant reproduction of enzymes to maintain the environment of the box would be expensive.

In addition, we hope to incorporate a receptor that will alert the chassis to produce the PETase enzyme only when plastic is present, rather than the enzyme being constantly produced. With a few changes that can make the process more cost-efficient and accessible, the project can be more practically and realistically implemented in the world today.

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References

Bornscheuer UT. Feeding on plastic [Internet]. Science. American Association for the Advancement of Science; 2016 [cited 2019 May28]. Available from: http://bit.ly/2M7VMHc

European Bioinformatics InstituteProtein Information ResourceSIB Swiss Institute of Bioinformatics. Poly(ethylene terephthalate) hydrolase [Internet]. European Bioinformatics InstituteProtein Information ResourceSIB Swiss Institute of Bioinformatics. 2019 [cited 2019 May28]. Available from: http://bit.ly/2yRaSrH

Free Image on Pixabay - Water Bottle, Plastic Bottle, Water [Internet]. Water Bottle Plastic - Free photo on Pixabay. [cited 2019 May31]. Available from: http://bit.ly/2OMl8wj

Fuller HC. Ethylene Glycol—Its Properties and Uses. Industrial & Engineering Chemistry. 1924; 16(6):624–6.

Hassani A, Ganjidoust H, Maghanaki AA. Use of plastic waste (poly-ethylene terephthalate) in asphalt concrete mixture as aggregate replacement. Waste Management & Research. 2005; 23(4):322–7.

Kari O. How Long Does It Take a Plastic Bottle to Biodegrade? [Internet]. Postconsumers. 2017 [cited 2019 May28]. Available from: http://bit.ly/33hLhGw

Moore C. Plastic Pollution [Internet]. Encyclopædia Britannica. Encyclopædia Britannica, inc.; 2019 [cited 2019May28]. Available from: http://bit.ly/33cTJXw

PET Safety & Use [Internet]. Safety and Uses | PETRA: Information on the Use, Benefits & Safety of PET Plastic. [cited 2019 May28]. Available from: http://bit.ly/2Tkz7bl

Thompson RC, Moore CJ, vom Saal FS, Swan SH. Plastics, the environment and human health: current consensus and future trends. *Philos Trans R Soc Lond B Biol Sci*. 2009 Jul 27; 364(1526):2153-66. doi: 10.1098/rstb.2009.0053. PubMed PMID: 19528062; PubMed Central PMCID: PMC2873021.

2016 MGM. Plastic-eating bacteria show way to recycle plastic bottles sustainably [Internet]. Chemistry World. 2016 [cited 2019 May28]. Available from: http://bit.ly/33kzU0q