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Research Plan
ME 225EY

Plastic Degrading Toggle Switch

Introduction:

A major problem in the world is the accumulation of plastic objects and particles in the Earth's environment. Much of the plastic pollution can be seen washed up on beaches or floating throughout different waterways and oceans. Scientists estimate about 8 million metric tons of plastic enters the ocean every year with a current estimate of 150 million metric tons circulating our marine environments today [2]. These plastic pollutants could arise due to plastic pack rings (for soda cans), beverage bottles, grocery bags, and straws. These plastic debris accumulate because it does not degrade like other waste, since the chemical structure of plastics leaves them resistant to many natural degradation processes [1]. This resistance to natural degradation leaves these plastic substances susceptible to extremely low decay rates.

Figure 1, below, highlights a tiny fraction of the plastic accumulation in our environment. This image depicts the waters near the Caribbean which is being assaulted by human waste. BBC states that scientists believe the quantity of current ocean plastic litter will triple within the next decade (2015 to 2025) [3]. Figure 2, below, shows the pollution on one of the beaches on Vietnam's coast. This pollution can be seen along the coasts and island, which the inhabitants of these communities have to live with every day. These plastics have the ability to fragment in microscopic pieces, which can possibly decompose, discharging toxic Bisphenol A (BPA) and PS oligomer into water systems [1]. In 2009, researchers from Nihon University in Japan, discovered plastic in warm ocean water can degrade in as little as a year. The plastic degrades into small bits of toxic chemicals where individuals are likely to interact with them through marine life ingestion [4].

Figure 1: Mass of Plastic Waste in Caribbean

Figure 2: Plastic Pollution on Vietnam Beach

The general problem to be addressed is, "How to mitigate the current plastic pollution plaguing the Earth?" This is an issue which heavily affects the environment, where the planet's future depends on how the world handles this plastic pollution. The goal of this paper is to outline a methodology to synthesize a toggle switch that degrades plastic, specifically PET (or

polyethylene terephthalate). The toggle switch will contain two equilibrium states: growth and lysis. PET plastic is common among water & soda bottles, clothing, and carpet fiber. A toggle switch will be designed to break down the polymer structure, because it is uncooperative to the normal degradation process.

Preliminary Findings:

Many plastic items, individuals use daily, are single use so they get thrown out after the plastic item has outlived its usage life. If these items are not properly disposed of, then they can end up in the ocean. Unlike some waste, plastic does not decompose quickly which wreaks havoc on marine ecosystems. Petroleum-based plastics, like PET, does not decompose the same as organic materials [4]. The polymer structure is recalcitrant to the normal polymerization process. In 2016, a bacterium was discovered at a waste dump in Japan which naturally evolved to eat plastic [6]. This mutant enzyme takes a few days to start breaking down the plastic, which is monumentally faster than the current degradation rate to plastics.

An international team of scientists adjusted the enzyme to see how it had evolved, but tests showed an increase in the molecule's ability to breakdown PET plastics [6]. The ability to successfully reduce plastic through this enzyme makes the cleanup of plastic pollution seem feasible. Tests have been conducted to understand the precise structure of the enzyme produced by the Japanese bug. Scientists found the enzyme looked like one evolved by bacteria to break down cutin (polymer used as a protective coating by plants) [6]. Although, tests will need to be done to ensure this enzyme does not solve one environmental problem at the expense of another problem.

Literature Review:

The mutant enzyme, which scientists found by accident, is known as Ideonella sakaiensis. This bacterium's genus is Ideonella and family is Comamonadaceae, which is capable of degrading and assimilating PET. Through the function analysis of the protein encoded in this gene, we were able to find out its ability to swiftly hydrolyze MHET. The bacterium uses two enzymes – PETase and MHETase – to break down PET into its two environmental benign monomers, terephthalic acid (TPA) and ethylene (EG) [8]. These monomers are then broken down further to produce carbon dioxide and water [5]. The bacteria strain uses two types of enzymes to break down PET into monomers, shown below in Figure 3. PET was thought to be immune to biodegradation, but present research has shown there may be a biological solution to introducing PET in synthesis and decomposition of matter within the natural environment [8].

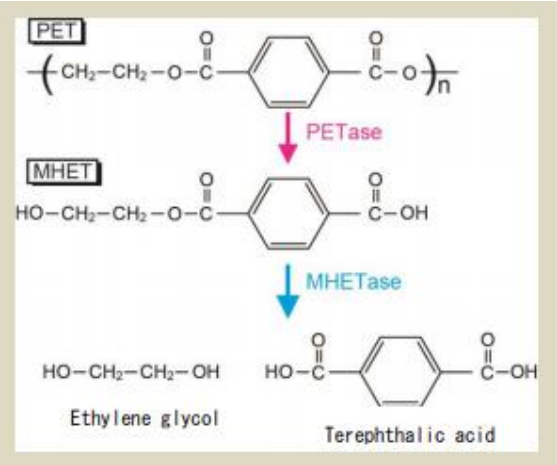
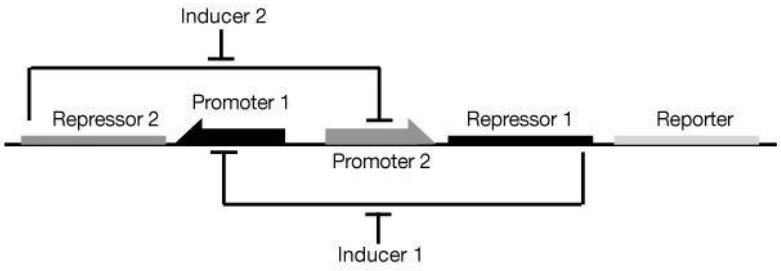


Figure 3: PETase Chemical Reaction

The toggle switch is composed of two repressors and two constitutive promoters, where each promoter is inhibited by the repressor that is transcribed by the opposing promotor [7]. Figure 4, below, shows the toggle switch design which requires the fewest genes and cis-regulatory elements to achieve robust bistable behavior. The robust behavior of the toggle switch exhibits bistabililty over a wide range of parameter values and two states are tolerant of the fluctuations inherent in gene expression [7]. Bistability is possible with any set of promoters and repressors, which arises from the arrangement of the repressor genes. Switching happens by transiently introducing an inducer of the active repressor. The inducer permits the opposing repressor to be maximally transcribed until it stably represses the originally active promotor [7].



Repressor 1 inhibits transcription from Promoter 1 and is induced by Inducer 1. Repressor 2 inhibits transcription from Promoter 2 and is induced by Inducer 2.

Figure 4: Toggle Switch Design

Toggle switches are arranged as a type IV plasmid, which is shown below in Figure 5, where all genes and promoters are contained on a single plasmid. Two classes of toggle switch plasmids were constructed – the pTAK class and the pIKE class, where these classes have two promotor-repressor pairs [7]. In Figure 5, the promoters are marked by arrows and genes are labeled with solid rectangles. The ribosome binding sites and terminators are denoted with outlined boxes. A toggle model qualitative prediction is that a genetic toggle will have nearly ideal switching thresholds. The transition from bistability to monostability occurs in a sharp,

discontinuous fashion due to the existence of a bifurcation [7]. This bifurcation occurs when one of the stable steady states is annihilated by the unstable steady states.

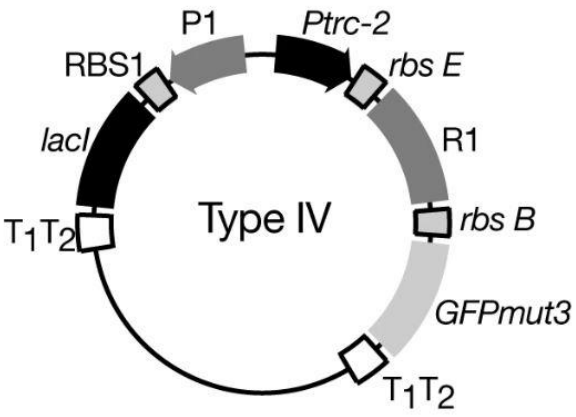


Figure 5: Toggle Switch Plasmid

Method and Approach:

In order to design a plastic degradation toggle switch, it is important to take genetic circuit stability into account. There can be multiple equilibria associated with a circuit, where each equilibrium is a steady state of the genetic circuit. This forms the basis for the "invariance" of the computational behavior in the system. The design problem involves smearing a bacterial streak onto the plastic surface, then the bacteria must grow to colonize the surface. The bacteria lyse when they achieve appreciable density. In this case, we are assuming the ocean provides metabolites of interest for bacterial growth. This toggle switch will contain two equilibrium states: dynamic growth & surface takeover and controlled lysis.

The first mode is dynamic growth and surface takeover via quorum sensing-coupled metabolism. This is after the bacterial streak is first smeared on the plastic surface. There will be some dynamic growth phase for the bacteria to proliferate and, eventually, takeover the plastic's surface. The second mode is the controlled lysis and delivery phase, which breaks down the cell's membrane. This will be the period where the PET plastic will be broken down in a safe manner, rather than the toxic breakdown of plastic due to the sun's rays. This lysis process will be able to improve the environment of marine life, where the PET breakdown will provide new opportunities for biobased plastics.

The toggle switch admits two equilibria, since a genetic circuit with mutual repressive topology admits two stable equilibrium. The repression relationship of the toggle switch can be shown below in Figure 6. There is PETase production during the growth mode and toxin production and lysis gene production during the lysis mode. Lyapunov theory could be used to show the asymptotic stability of an equilibrium. This function can be discovered using the simplified dynamic model for our toggle switch shown above with some assumptions about the system (e.g. $x_L \neq x_T$). If the toggle switch is not Lyapunov stable, then the whole system is unstable

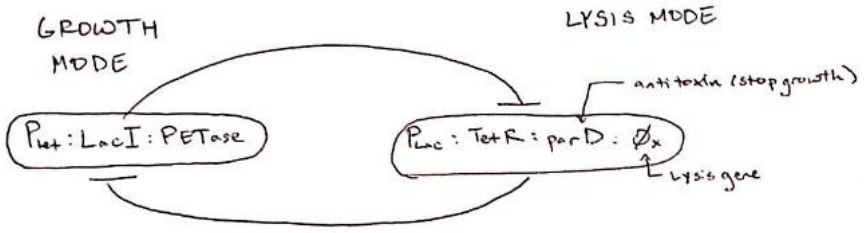


Figure 6: Toggle Switch for Plastic Degradation

Our toggle switch dynamics are modeled with hill functions to describe the relationship of repressed and unrepressed gene expression. If we assume n = 1, the parameters to be tuned will be gamma, K_M, and delta. Gamma is the max hybrid rate of LacI. K_M is the repression threshold for TetR and LacI if the two different proteins have the same constants. Delta is the degradation rate for TetR and LacI if the two different proteins have the same constant. The parameters are assumed to be the same for simplicity but when conducting numerical simulations, the parameters will be different for each protein (e.g. LacI and TetR).

$$\dot{x_L} = \frac{\gamma}{K_M + (x_T)^n} - \delta_p x_L \qquad \dot{x_T} = \frac{\gamma}{K_M + (x_L)^n} - \delta_p x_T$$

The plan of action for this plastic degrading toggle switch would be to try to find a Lyapunov function. After finding a function, you need to select target parameters then tune them to get the desired equilibrium states. After figuring out the desired tuned parameters, it is time to order the DNA sequences. After ordering your DNA sequences, you can use site-directed mutagenesis to make short edits in the sequence. This is a useful technique for making targeted edits at specific genetic locai.

Different methods can be utilized to construct the genetic circuit: Combinatorial circuit design or Golden Gate reaction. Combinatorial circuit uses an assembly workflow that allows for the assembly of multiple combos at the same time. The idea is to design a modular library with ends that interact with each other. The goal of this is to get the binding energy reactions to line up by using a “barcode” to standardize the ends. Golden Gate reaction is done through selection of parts then assembling them into fragments. Use workflow to build genetic circuit, then find standard biological parts from a database.

The parameters of the genetic toggle switch should be able to be tuned, where these parameters will affect the equilibria of the system. The cost of base pair fluorophore tagged DNA is anywhere from $50 - $100. Although, not all sequences can be perfectly synthesized (e.g. hairpin structures). More cost analysis can be done, where a 600 base pair sequence is ~$200 – 300. If there are 100 variants, the price skyrockets to anywhere from $20,000 - $30,000 for a 600 base pair sequence.

The plan for this research proposal is to conduct numerical simulations to figure out the theoretical desired parameters. Biological parts should be ordered in order to construct the genetic toggle switch circuit for PETase. Once the toggle switch is made, experiments should be

run in a controlled environment. There could be many risks involved with this genetic circuit since it is a new topic of research in biology. The primary use for this genetic circuit would be to rid the ocean of all its polluted plastic. There needs to be many experiments run to ensure the toggle switch would not harm the marine life's environment. These experiments can be run in a variety of different conditions which can replicate a portion of the ocean somewhere. The risk mitigation comes from designing your genetic circuit with an appreciable safety factor to ensure there is no failure. Also, risk mitigation comes from running many experiments on the built toggle switch to see if is safe for marine life and the environment.

Novelty & Impact:

Prior to its discovery, the only known degraders of PET were a small number of bacteria and fungi (e.g. Fusarium solani) and no organisms were definitively known to degrade PET as a primary carbon and energy source [8]. The discovery of Ideonella sakaiensis brings up the possibility of PET biodegradation, which can benefit the Earth's environment by helping to get rid of global plastic waste.

Research has been done on this bacteria before, but no one has created a plastic degradation toggle switch. This proposed research solution could optimize plastic waste management by safely disposing of PET plastics using the Ideonella sakaiensis enzyme. This genetic toggle switch would offer solution to a prevalent problem today. A plastic degradation toggle switch with two equilibrium states for dynamic growth & surface takeover and controlled lysis would be a breakthrough in science. This toggle switch would be designed to degrade PET plastics, but this could be a stepping stone for scientists to develop other genetic circuits to tackle other plastic polluting the ocean.

One of the hardest parts of this proposal will be to find a Lyapunov function which offers stability for the toggle switch. This alone, will be a breakthrough in bioengineering since there has not been a Lyapunov function found yet for the toggle switch. This is a new field of research which could inspire more involvement from synthetic biology scientists if a successful toggle switch is created. The biggest impact would be on the environment which is currently suffering from massive plastic pollution. This could be the catalyst for the discovery of new synthetic biology methods to help the environment for the better.

References:

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