Designing protocol for maximizing Polyhydroxy butyrate (PHB), a biodegradable plastic, production in engineered E.coli in order to relieve the shortcomings of biodegradable plastic.

(Technical Paper)

Investigating the economic hindrances and social barriers of advancements in the bioplastic industry.

(STS Paper)

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On my honor as a University Student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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Introduction

Plastic pollution has become a major problem for our environment, and it is increasingly worsening as the years are coming. Through recent studies it has been emphasized more of the seriousness and implications of plastic pollution. In a study in 2015 it was concluded that the plastic pollution problem is actually way worse than what people know or think it is. It is estimated that there are 150 million tons of plastic in the ocean and adding around 8 million more tons of plastic every year. Another study in 2016 by MacArthur Foundation predicted that by 2050 if nothing is done to reduce plastics flow into waterways there will be more plastic than fish (Ocean Plastics, n.d.). Plastic is something that can be seen used everywhere day to day. There are more than 480 billion plastic bottles sold in 2016 worldwide and just a decade ago that number was 180 billion. About 269,000 US tons of plastic waste that goes into the ocean are from takeout orders (*Fact Sheet*, 2018). Sadly, bioplastic only composes one percent of about 360 million tonnes of plastic that is produced annually (EUBIO_Admin, n.d.) .

Recently there has been a slight turn in the usage of bio-based plastic in order to help reduce the amount of plastic adding to the environment. Bio-based plastics are plastics based off of or at least partly based off of biological matter and will reduce the dependence on fossil fuels. There is also a biodegradable component to bioplastics where the plastic can actually be degraded by microbes in a reasonable amount of time ("Are Bioplastics Better for the Environment than Conventional Plastics?," n.d.).

Technical Project Description

Plastic usage is a big part of human day to day life. It can be found pretty much anywhere in the world. Plastics are mostly non-biodegradable because it is made to be something low cost and durable. Therefore, once the non-biodegradable plastics are out in nature and are not handled properly it becomes a harm to our environment and health. It is almost impossible to take plastic completely out of our daily life because it is widely used everywhere. However, it is possible to slowly transition to bio-based plastic and or biodegradable plastic. The major problem with biobased and biodegradable plastic is the cost and time that it requires which hinders the cheap and mass production requirement that the current plastic industry satisfies.

PHB which is a biodegradable plastic that can degrade within a reasonable amount of time when it is in contact with microorganisms in the biologically active environments. This is included when they are in soil, fresh water, and aerobic and anaerobic composting. This allows their trait of being eco-friendly and alternative for synthetic plastic. PHBs is commonly produced from rice bran, glucose-based carbon sources, and other agro-industrial based sources. This is also a reason why PHB production is expensive (McAdam et al., 2020). The problem with the current design of producing PHB is due to the carbon source used. E.coli has shown to grow best on glucose (Frontiers | Can Polyhydroxyalkanoates Be Produced Efficiently From Waste Plant and Animal Oils? | Bioengineering and Biotechnology, n.d.). Many methods have been trying to utilize the maximization of PHB production. A prior study has looked into the pta-acka pathway in E.coli. The pta-acka pathway is responsible for controlling excess acetate and activating the switch to consumption of acetate instead of acetyl CoA. Acetate is responsible for regulating glucose uptake, glycolysis, and the TCA cycle at the transcriptional level. This prior work can be used as a validation of establishing the relationships between the role acetate in E.coli. It also serves as a reference for how the deletion of acKa gene can effect changes to acetate flux and cell metabolism and increased accumulation of acetyl-CoA in E.coli as an intermediate metabolite for ultimate goal of production of PHB (Enjalbert et al., 2017).

My capstone team and I will approach the problem by designing a protocol and procedure that will allow E.coli to grow on phenylacetic acid (paa) and maximizing PHB production by maximizing acetyl CoA production. We will be working with Transform which is a startup company based in Charlottesville. I had the honor to join Transform in August, 2021.Their main research goal is instead of using agro-industrial sources as the main carbon source for E.coli, use another waste. This waste that they are focusing their research and product on is styrene which is another non-biodegradable waste. The sty plasmid in E.coli is responsible for breaking styrene monomers into paa. Then, E.coli will use paa as its sole carbon source in order to produce PHB. PHB is produced through the paa pathway which is responsible for converting paa to acetyl CoA. The paa pathway is connected to the PHA plasmid which converts acetyl CoA into PHB (Lee & Lee, 2003). We will not be working for sty plasmid. However, our design will verify that acetyl CoA can be maximized when using paa as sole carbon source. A visual of the plasmid can be seen in figure 1 below. Additionally, our design will be designing a metabolic model that will show the expected response of relevant metabolic fluxes to the different growth mediums and objective function.

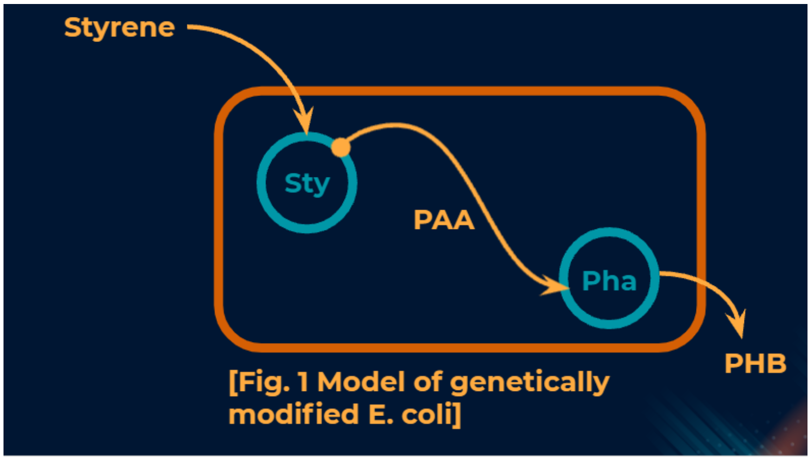

Figure 1: Model of genetically modified E.coli. This figure shows the sty and pha plasmid.

Our overarching aim is to maximize biosynthesis of poly-3-hydroxybutyrate (P3HB) for future scale-up of P3HB industrial production in order to offer a sustainable bioplastic alternative that can compete with current petrochemical based plastics. The main hindrance to increasing production of P3HB with recombinant *E. coli* consists in raising the availability of the precursor acetyl-CoA and the cofactor NADPH (Ku et al., 2020). The biosynthesis of P3HB is performed by the concerted catalysis of three enzymes found within the operon *phbBAC* that is part of the pha plasmid that will then be transfected into chassis E.coli (Centeno-Leija et al., 2014). The enzyme B-ketothiolase (*PhbA*) is inhibited by high levels of CoA when glucose is being metabolized at a high rate and not enough oxygen to support aerobic growth; CoA is then increased that triggers the response of intense dissimilation of acetyl-CoA through the TCA cycle(Centeno-Leija et al., 2014). The production of P3HB will be in two stages: the first designed for cellular proliferation (growth in glucose medium), and the second (growth in phenylacetic acid) with decreased oxygen and nutrients to promote P3HB accumulation(Centeno-Leija et al., 2014). Glucose will be changed out phenylacetic acid as the carbon source in the second stage to verify that the byproduct of sty plasmid that turns styrene into phenylacetic acid is able to survive on it and doing that without transfecting the bacteria.

A coupled enzyme assay(*Acetyl-Coenzyme A Assay Kit Sufficient for 100 Fluorometric Tests | Sigma-Aldrich*, n.d.) is responsible in figuring out enzyme activity by coupling two enzymatic reactions together (*Coupled-Enzyme Assay - Terminology of Molecular Biology for*

Coupled-Enzyme Assay – GenScript, n.d.) and verifies the intracellular concentration of acetyl-CoA. A coupled enzyme will measure the acetyl-coA concentration, resulting in a fluorometric product, proportional to the acetyl-CoA present. The acetyl-CoA of the parent strain will be evaluated and compared with the modified strain to make sure that the modified strain has a higher acetyl-CoA level. The surveyor assay will be used to determine the efficiency of genome edits and cell viability and proliferation rate to ensure that the bacteria growth and the PHB accumulation.

In order to simulate the metabolic reactions of the genetically engineered *E. coli* in response to different constraints and gene deletions, a genome-scale metabolic model (GEM) is needed. An existing and commonly used GEM model for E.coli strains of TG1 and W will be modified to fit sty and pha plasmid. The flux balance analysis (FBA) has to perform appropriately to verify that the GEM model is following laws of mass and energy. The increase of the carbon sources phenylacetic acid and glucose will be inputted into the model should show relative increases in metabolite fluxes between the two strains of E.coli. Where the ideal value would be greater than zero verifying that there was a flux change (Ebrahim et al., 2013).

STS Project Details

Though advancing to bioplastics allows for a very promising future and better for our environment, there are still factors that are hindering the growth of that field. The economic and social barriers are two major factors that are hindering the advancement and growth in the bioplastic industry. This will also include how economic and social barriers hinder each other and the combination of two hinders the development of biobased plastic. I hope to answer the question of what implications each barrier causes and what leads to these hindrances.

The cost of carbon source metabolized by microorganisms is the major constraint for the scale-up production of PHA. Therefore, instead of paying the same price for the product you are using you would have to pay at least double or triple the price of the same product. Currently PHB is 16 times the price of polypropylene (PE) (Hankermeyer & Tjeerdema, 1999). This is very economically not favorable, and consumers would definitely choose the cheaper option. For most people, affordability and cost is a major determining factor for most of their purchases. Economic growth dominates over people's rights and environmental factors. In order to make a shift it would require the mindset of thinking of environmental factors as part of economic factors. Additionally, decisions and determining factors would need to adapt more towards environmental targets and goals. It is a hard concept and mindset to change but the realization of the amount of impact the environment has is the key. Without nature and resources given by our environment then the economy is far away. This type of mindset is not embedded in most people. In a lot of peoples' minds, economic growth of their country and convenience of their daily life is more of the concern.

Regarding the social barriers, the more people there are then the more demand is required to sustain a population. For parts of the world that are not wealthy and overpopulated their main concern would not be the environment. There is not really an incentive to choose to be sustainable or choose a more sustainable option for most people. Some more developed and wealthier countries may have the ability to switch to more expensive and sustainable plastic. However, third world countries do not have the ability or resources to do so. The concept of sustainability and awareness is also not spread to third world countries because that cannot be their main concern. Additionally, because there is not a significant or drastic change in human behavior it makes sustainability not a main concern in most people.

In order to explore the socio-technical dimension of the barriers, surveys, interviews, and polls can be conducted and reviewed in order to better understand how the current companies are managing this situation. It is important to evaluate how the situation is currently managed in order to make the step to coming closer to solving the problem. The current biotech and bioplastic companies and research groups can be interviewed to understand what may be other barriers that are hindering them in order to better analyze the problems. Some interview questions that can be considered to ask the biotech and bioplastic companies and research groups can be:

1. What are the biggest challenges and hindrances the group is encountering?
2. What are some ways that these challenges and hindrances can be overcome or improved?
3. What is the company or research group currently doing to overcome the challenges?
4. Would you say the progress is hindered more than by social or economical aspects?
5. What are the target carbon source and why is that chosen?

Even though both economic and social barriers hinder growth, analyzing the interaction between people and their choices can give us some answers to other possible factors that are also hindering the growth. Taking into consideration the different hindering factors and trying to tackle them, really understanding the roots of them is an important part in the transition and research process of the STS study to truly investigate the social-technical dimension.

Conclusion

It is clear and well known the pollution and the environmental impact the plastic industry has brought to the world. Everyday there is more and more plastic added to our nature and ocean that is harming wildlife and eventually to humans. However, many teams including Transfoam are aiming to find a solution to reduce the plastic pollution using more sustainable energy and more degradable methods. In order to truly allow the bioplastic industry to fully grow it is not only important to approach the technical approaches of how to actually perform the methods but to explore the social and economic aspects as well.

Citation:

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