AN INVESTIGATION OF SUSTAINABLE AND BIODEGRADABLE PLASTIC PRODUCTION WITHIN THE BIOMANUFACTURING INDUSTRY TO REDUCE SINGLE USE PLASTIC POLLUTION

A Research Paper submitted to the

Faculty of the School of Engineering and Applied Science
University of Virginia | Charlottesville, Virginia

In partial fulfillment of the requirements of the degree
Bachelor of Science, School of Engineering

Tammy Tran
Technical Portion written in collaboration with
Xin Chen, Thomas Nguyen, Sang-Hoon Park, Hannah Towler, and Julia Yao
November 2021

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.

Approved ____ Date 08 May 2022
Richard D. Jacques, Ph.D., Department of Engineering and Society

Introduction

In recent years, the progression and transformation of our society has resulted in a widespread accumulation of plastic waste. Globally, researchers estimate that more than 8.3 billion tons of plastics have been produced since the early 1950s, but only 9% of the plastics produced have been recycled or reused. The remaining majority of the plastic is abandoned in the environment where it can take up to 500 years to decompose while simultaneously leaching out toxic chemicals into the environment, increasing public exposure to carcinogenic components. In an attempt to preserve the environment, there has been growing interest in alternative bioplastics that are both natural and biodegradable. Although there are currently many different types of plastic that are in the market, the majority of them are not biodegradable and can hang around in the environment for hundreds of years and can cause you to be exposed to chemicals such as BPA. Therefore, a solution to this is to move towards a more sustainable option such as polyhydroxybutyrate (PHB). Biodegradable bioplastics, such as PHB, offer a sustainable alternative as they are natural byproducts of facilitated degradation of these synthetic plastics, such as styrene. PHB is a bio-based plastic that offers a more efficient and fully circular solution to plastic pollution. The biodegradability of these bioplastics provides promising hope against the extensive duration of time that petroleum based plastics spend in the environment. Among the most promising bioplastics being developed is polyhydroxybutyrate (PHB), a biodegradable and non-toxic microbial synthesized polymer, which possesses favorable mechanical properties similar to petroleum-derived plastics. However, high production costs and minimal yields are hindering its expansion into the marketplace as a competitive alternative to petroleum-based plastics. In order to reduce production costs and maintain competitive viability in a market saturated with cheap standard plastics, we improved upon a current microbial factory design based in *Escherichia coli* (*E. coli*) to maximize metabolic flux towards acetyl-CoA production, a key metabolic precursor of PHB that feeds directly into PHB production.

To facilitate this process, we identified and performed gene modifications on a bioengineered strain of *E. coli* designed by Transfoam LLC. Transfoam LLC, is a biomanufacturing platform that tackles single-use plastic pollution at the beginning and end of the product lifestyle. Transfoam has three main priorities they are focusing on: remediating global plastic pollution, manufacturing fully biodegradable plastic, and harnessing the power of microorganisms. Specifically, Transfoam employs an engineered strain of *E. coli* to turn waste into PHB to make healthier consumer goods and packaging. This modified strain of bacteria consists of two exogenous plasmids, the sty and the pha plasmids, that are bridged together by the endogenous phenylacetic acid degradation pathway. In order to increase PHB yield, we increased the intracellular concentration of acetyl-CoA, by inhibiting nonessential pathways that consume acetyl-CoA and increasing the availability of CoA, the precursor for acetyl-CoA. In addition, we created a metabolic model to evaluate the impacts of the gene modifications on metabolic fluxes involving acetyl-CoA production and consumption, which allowed us to confirm the efficacy of our proposed changes. The genetic modifications that we performed were the gene deletions of acetyl-CoA kinase (*acka*) and phosphate acetyltransferase (*pta*), also known as the Pta-AckA pathway in order to inhibit the conversion of acetyl-CoA into acetate thereby increasing intracellular concentrations of acetyl-CoA available for PHB production. The primary protocol to perform the gene deletions uses a recombineering approach with phage-derived lambda Red system and CRISPR-Cas9 as the expression system.

Literature Review

The ability of biodegradable plastics to break down means that it has several advantages over traditional petroleum plastics. Biodegradable plastics decrease the waste sent to landfills or incinerators. Typically, when petroleum plastics are thrown into the trash, the majority of the negative environmental impact derives from their end destination in landfills, where they can potentially sit for hundreds of years. On the other hand, incinerators burn plastics, which can release harmful chemicals into the natural environment. However, biodegradable plastics resolve this problem by breaking down in a landfill and do not need to be burned. An additional benefit of biodegradable plastics is that they require less energy to manufacture, using fewer fossil fuels and decreasing the amount of greenhouse gas emissions that harm the planet. Corn-based plastics represent approximately 40% of the biodegradable source materials in the United States. When comparing the polymers made from these corn crops to those produced from petroleum, the corn based ones require 65% less energy to create a similar quality biodegradable product, and reduces the greenhouse gasses that occur during the manufacturing process by 68% (Chief, 2019). Typical petroleum plastics can also leach toxic chemicals into the environment, harming our surroundings. Instead, with the transition to biodegradable plastics, fewer harmful byproducts would be released. Biodegradable plastics release a combination of water, carbon dioxide, and biomass which are not harmful to the environment (*Biodegradable Plastic Guide: Explore the Pros, Cons, and Uses - 2021 - MasterClass*, n.d.). The transition to bioplastics does not come without ethical complications. The most common method for bioplastic production is through the cultivation of starch-based feedstocks. However, this creates competition for arable land, contributing to the global food crisis, and results in a greater carbon footprint due to agricultural emissions. In response, companies have turned to upcycling, which is the repurposing of waste into new materials or products of better quality and environmental value, to circumvent the issues associated with agriculturally derived bioplastics and reduce production expenses hindering bioplastic market expansion.

Methodology

In order to examine the societal implications that this new technology will have, I examined the actions of large corporations and their primary plastic type consumption, especially those who are under constant scrutiny by the public for their role in environmental destruction on a global level because of their high consumption rate of single use plastics. These large corporations are where the majority of single use plastic waste comes from, especially for companies that use plastic for their food/drink containers or storage. Many consumers purchase and throw away these disposable plastics without even realizing, such as plastic straws, cups, containers, cutlery and bags. Food packaging accounts for approximately one third of the 3.5 million tons of biodegradable plastics produced globally each year, and over 60% of its revenue. Bioplastics serve as excellent materials for food packaging because it does not require cleaning given that the food and packaging can be incinerated or composed together. Although there is no definite method to define corporate sustainability performance, in general it can be thought of as finding a balance between economic profit, environmental and social responsibility and other stakeholders. Large corporations should undertake environmental and social responsibility in their business activities in order to improve their sustainability performance. To determine whether or not these large corporations are acknowledging the grand challenge of sustainability, the primary research method I will be using is an assessment that examines the analytical sustainability claims that a company makes and comparing that to specific case studies of the type of plastic used. The assessment I will be using is the Life Cycle Assessment (LCA). The LCA is an established methodology for assessing environmental impacts associated with all the stages of the life cycle of a commercial product, process or service. It is a cradle-to-grave or a cradle-to-cradle analysis. A cradle-to-grave is a full life cycle assessment that includes all the stages of a life cycle. A cradle to cradle analysis is typically a cradle to grave assessment, however, the end-of-life stage of a product is a recycling process, and therefore the product will not be sent to the "grave" or discarded after the end of life (*Life Cycle Assessment - an Overview | ScienceDirect Topics*, n.d.). In a nutshell, the LCA attempts to answer the question "What environmental impact does one object have on the world". The four phases of a LCA are defined in the ISO standards 14040 and 14044. The assessment consists of four steps: definition of goal and scope, inventory analysis, impact assessment, and interpretation. In the first phase of the LCA, specific definitions and details on what needs to be analyzed are placed, including defining a functional unit, the system, and the limits of analysis. In other words, the first step defines what needs to be analyzed, how it should be analyzed, and how in-depth the analysis should be. Following that step is the inventory analysis using the Life Cycle Inventory Analysis (LCI), which looks at the environmental inputs and outputs of a product or service. Essentially, this step is to primarily collect data for the LCA. Examples of inputs and outputs could be raw materials or resources, different types of energy, water, emissions to air, land, or water by substance. This analytical process is extremely complex since it involves complications of the production processes and supply chain. Following the LCI is the impact assessment. This phase involves evaluating how significant the impacts are using the LCI flows from phase 2. The three tasks in phase 3 are: selection of indicators and models, classification, and impact measurement. The selection of indicators and models involve further specifying the impact categories that were loosely defined in phase 1. An impact category groups different emissions into one effect on the environment. There are infinite types of emissions and they can come in different shapes and formats. However, during stage 2 or the LCA phase, different emissions that cause the same impact on the environment are converted into one unit that translates into one impact category. Common impact categories include human toxicity, global warming potential, ecotoxicity, acidification, and eutrophication. After defining the impact categories, the next step is to classify, or sort through the LCI and actually assign them to the previously defined impact categories. The last task in phase 3 is to actually calculate the equivalents and summing them up in overall impact category totals. Phase 4 and the final phase in the LCA, is to interpret the results of the LCA. How the results should be determined are defined in the ISO norms defining the Life Cycle Assessment. According to ISO 14044:2006, the interpretations of a LCA should include: identifying significant issues based on our LCI AND LCIA phase, evaluating the study itself, how complete it is, if it's done sensitively and consistently, conclusions, limitations and recommendations. Using these results and interpretations, I can then draw conclusions from it such as: how high are the emissions of the product or service, how does it compare to other products in the portfolio, what are the biggest leverages to reduce the impact of the product, and can the manufacturing process be made more efficient ("Life Cycle Assessment (LCA) - Complete Beginner's Guide," 2019). The aim of using these LCA is to assist the decision makers within an organization and policy makers to increase general awareness of the public regarding the packaging industry and its contribution and impact on the environment.

Body of the Paper

Some of the companies that consume the most single-use plastic products in the food industry are Coca-Cola, Nestle, Marriott International, and Evian. Coca-Cola had by far the largest plastic footprint in 2020, with approximately 3 million tons of plastic packaging produced globally each year- equivalent to about 200,000 bottles a minute (*• Chart: The Companies With the Largest Plastic Footprint | Statista*, n.d.). The Coca-Cola company first used a form of LCA in 1969 to examine the environmental impact of switching from glass to plastic bottles. In this particular study, Coca-Cola quantified the raw materials and fuels used, along with the environmental impact of the manufacturing process. As a result, their brand is forever connected to the first attempts of environmental footprinting ("Coca Cola's First LCA in 1969 - A Brief History of LCA," 2020). The life cycle of a Coca-Cola bottle consists of raw material extraction, manufacturing, distribution and transportation, construction and use, and then reuse, recycling and disposal. The plastic bottles are made of polyethylene terephthalate (PET) plastic. With their annual plastic production, Coca-cola contributes to about a fifth of the world's PET bottle production. The production of these plastics relies on petroleum and fossil fuels, leading to significant CO2 emissions. The estimated California PET market is 60% bottled water, 16%

carbonated sodas, and 24% juice/sports/other drinks. At present, Coca-Cola is constantly improving and innovating in the material and packaging of bottles, and reducing the damage to the environment. The LCA of a PET bottle provides the environmental impact associated with the production, consumption, and recycling of a PET bottle. The impact categories that I primarily looked at were greenhouse gas emissions and global warming potential, with the functional unit of kg CO2, in order to accurately quantify the impact. One functional unit was defined as the delivery of beverages packaged in single-use bottles made from 1 kg PET resin. After performing the LCA, most environmental indicators regarding air pollution and air quality directly reflect the combustion of fossil fuels to produce energy. The results of the LCA assessment on PET plastics showed that the usage of 1 kg of polymer has a primary energy demand of 119.6 megajoules (MJ), requires 20,500 kg*km of freight services, and generates 0.727 kg of solid waste. Regarding the impact categories mentioned earlier, this leads to 5.79 CO2-eq of global warming potential, 57.5 g SO^2-eq of acidification potential, and 10.9 g P-eq of eutrophication potential. Therefore, the contribution of each state to these impact scores is largely proportional to that stage's relative demand for delivered energy. On a volume of beverage basis, one liter of beverage delivered to the consumer has a primary energy demand of 4.80 MJ, requires 825 kg*km of freight services, and produces 23 g of secondary PET and 29 g of waste. To put these units into perspective, one megajoule of energy is approximately the kinetic energy required for a one tonne vehicle moving at 161 km/h or 100 mph ("Joule," 2022). Kg*km is used to measure freight and is measured in mass-distance. One unit of freight is the moving of one kilogram of payload a distance of one kilometer ("Units of Transportation Measurement," 2022). Many of the environmental indications that correlate with air pollution and air quality, including global warming potential, acidification potential, human health criteria and smog, directly reflect the effects of combusting fossil fuels to produce energy. As a result, the capacity for improvement in these impact areas is dependent on the ability to reduce demand. Therefore, the majority of environmental impacts come from energy-intensive pre-consumer stages of production (*Publication Summary*, n.d.).

The second largest consumer of single use plastic in the food industry is Nestle. The company was also named the worst plastic polluter following 2017 and 2019 waste and brand audits in the Philippines. However, Nestle has pledged to make 100% of all its products packaging recyclable or reusable by 2025. As part of their pledge, they are also transitioning

Nesquik drinks from plastic to paper containers while Nestle Waters will increase the recycled PET (rPET) content in its bottles to 50% in the United States (*Big Companies That Are Getting Rid of Plastic for Good | Reader's Digest*, n.d.). An LCA comparing rPET and regular virgin PET (vPET), demonstrates the many quantifiable environmental benefits of rPET. Each unit of rPET that replaces vPET results in: 40% less process and transport expended energy, 60% reduction in greenhouse gas emissions, and 75% lower total energy demand. Each year, the equivalent environmental savings from rPET usage in products in the US and Canada add up to enough electricity to power more than 760,000 US homes in terms of energy, and removes more than 200,000 cars from the road in terms of greenhouse gas emissions (*NAPCOR Releases Updated PET Life Cycle Analysis and Calculator - Recycling Today*, n.d.). Based on the results of the LCA, rPET scored lower than the regular PET plastics in all categories, proving that rPET is more beneficial for the environment. According to this LCA, the total global warming potential of rPET is 1.6 CO2-eq and has an energy demand of 2.5 MJ. Compared to the values that the researchers received for PET in this LCA, using rPET lowers the global warming potential by 42% and lowers energy demand by 63% (*RPET_LCA_whitepaper.Pdf*, n.d.). Currently, Nestle has made significant progress towards their pledge of more sustainable packaging. 85.4% of their total packaging is recyclable or reusable, 74.9% of their plastic packaging is designed for recycling, and there has been an 8.1% reduction in vPET plastics since 2018 (*Waste Reduction*, n.d.) (*What Is Nestlé Doing to Tackle Plastic Packaging Waste?*, n.d.).

There is strong evidence that the past and current plastic production and management industries are unsustainable and have already caused irreparable damage to the environment. For comparison, an LCA study was conducted on PHB plastics. The results of the LCA showed that the values of the impact categories depend strongly on the specific renewable raw material used and the allocation methodology adopted. For example, the amount of greenhouse gasses emitted for corn sugar was 3.95 kg CO2-eq/kg polymer, whereas sugarcase released -2.58 kgCO2-eq/kg polymer. However, the LCA showed that the production of PHB is advantageous compared to polymer production from non-renewable resources, especially when specific raw materials are combined with the use of the most appropriate energy allocation methodology resulting in significantly lower greenhouse gas emissions than the values previously found for petroleum-derived plastics (Kookos et al., 2019). Therefore, it is concluded that PHB production is advantageous compared to its petroleum-derived counterparts.

Conclusion

Through these results, it is clear that consumers and corporations should both begin deviating from the usage of PET plastics, whether they are regular or rPET, and begin the transition to PHB instead. Doing so will greatly benefit the environment and ensure the ethical sustainability that everyone should be held accountable for. Each year, 380 million tons of plastic waste is produced globally with an estimated half of that waste being for single use purposes only. Single-use plastic is manufactured to last forever, however is often only used for a few minutes before being thrown away, and not even recycled (“Plastic Pollution Issues | The Problems With Plastic,” n.d.). The reliance on synthetic plastics prolongs the release of toxic emissions into the environment, not only polluting the environment but exposing all living organisms to carcinogens. For example, bisphenol A (BPA), found in polycarbonate bottles and the linings of food and beverage cans, can leach into food and drinks. The U.C Centers for Disease Control and Prevention reported that 93% of people had detectable levels of BPA in their urine. Exposure to high BPA levels in premature infants is an area of great concern and research suggests that BPA may cause cancer in people. BPA is a weak synthetic estrogen found in many rigid plastic products, food and formula can linings and cashier receipts. It has an estrogen-like activity that makes it a hormone disruptor, which is a commonly shared trait among other chemicals in plastics. Hormone disruptors can affect how estrogen and other hormones act in the body, by blocking or mimicking them, which can throw off the body’s hormonal balance (*Exposure to Chemicals in Plastic*, 2020). The transition of bioplastics into current plastic infrastructure requires a concerted effort on part of individuals, companies and governments to make change, including our work with the optimized strain of *E. coli* for PHB production. Public advocacy for corporate and governmental responsibility and recognition that these changes must be accompanied by simultaneous improvements in bioplastic producing infrastructure to enact the most comprehensive and ethical solution to the global plastic waste issue.

References

• *Chart: The Companies With the Largest Plastic Footprint | Statista*. (n.d.). Retrieved April 19, 2022, from https://www.statista.com/chart/23744/branded-plastic-waste-found-in-global-cleanups/

Big Companies That Are Getting Rid of Plastic for Good | Reader's Digest. (n.d.). Retrieved April 19, 2022, from https://www.rd.com/article/companies-getting-rid-plastic/

Biodegradable Plastic Guide: Explore the Pros, Cons, and Uses—2021—MasterClass. (n.d.). Retrieved October 13, 2021, from https://www.masterclass.com/articles/biodegradable-plastic-guide

Chief, E. in. (2019, March 27). 22 Advantages and Disadvantages of Biodegradable Plastics. *ConnectUS*. https://connectusfund.org/7-advantages-and-disadvantages-of-biodegradable-plastics

Coca Cola's first LCA in 1969—A brief history of LCA. (2020, June 10). *Ecochain*. https://ecochain.com/knowledge/coca-colas-first-lca-in-1969-a-brief-history-of-lca/

Exposure to Chemicals in Plastic. (2020, September 11). Breastcancer.Org. https://www.breastcancer.org/risk/factors/plastic

Joule. (2022). In *Wikipedia*. https://en.wikipedia.org/w/index.php?title=Joule&oldid=1079670832

Kookos, I. K., Koutinas, A., & Vlysidis, A. (2019). Life cycle assessment of bioprocessing schemes for poly(3-hydroxybutyrate) production using soybean oil and sucrose as carbon sources. *Resources, Conservation and Recycling*, *141*, 317–328. https://doi.org/10.1016/j.resconrec.2018.10.025

Life Cycle Assessment (LCA)—Complete Beginner's Guide. (2019, May 3). *Ecochain*. https://ecochain.com/knowledge/life-cycle-assessment-lca-guide/

Life Cycle Assessment—An overview | ScienceDirect Topics. (n.d.). Retrieved April 19, 2022, from https://www.sciencedirect.com/topics/earth-and-planetary-sciences/life-cycle-assessment

NAPCOR releases updated PET life cycle analysis and calculator—Recycling Today. (n.d.). Retrieved April 19, 2022, from https://www.recyclingtoday.com/article/napcor-pet-life-cycle-analysis-calculator/

Plastic Pollution Issues | The Problems With Plastic. (n.d.). *Plastic Free July*. Retrieved October 13, 2021, from https://www.plasticfreejuly.org/the-plastic-pollution-issues/

Publication Summary. (n.d.). Retrieved April 19, 2022, from https://www2.calrecycle.ca.gov/Publications/Details/1487

RPET_LCA_whitepaper.pdf. (n.d.). Retrieved April 19, 2022, from http://www.hp.com/hpinfo/newsroom/press_kits/2010/ecoachievement/RPET_LCA_whitepaper.pdf

Units of transportation measurement. (2022). In *Wikipedia*. https://en.wikipedia.org/w/index.php?title=Units_of_transportation_measurement&oldid=1083556000

Waste reduction. (n.d.). Nestlé Global. Retrieved April 19, 2022, from https://www.nestle.com/sustainability/waste-reduction

What is Nestlé doing to tackle plastic packaging waste? (n.d.). Nestlé Global. Retrieved April 19, 2022, from https://www.nestle.com/ask-nestle/environment/answers/tackling-packaging-waste-plastic-bottles