

Production of Polyhydroxyalkanoates During Bokashi Composting: A Study on Sustainability

Madelyn Brown

Abstract

This study examines how the sustainability of the plastic industry can be improved through a concurrent method of polyhydroxyalkanoate production, specifically that of a bokashi compost media. In studying previous research on the plastic manufacturing processes, the researcher found a gap in knowledge in the affordability and accessibility of bioplastic production, limiting the expansion of the industry. By studying how *Escherichia coli* reacts to a feast famine cycle in a bokashi compost habitat, the study addresses the question: to what extent, if any, does the ability to modify phosphorus, nitrogen, carbon, and oxygen levels in compost bins allow for polyhydroxyalkanoates to be produced in order to determine and expand the accessibility of bioplastics? The researcher collected data of the bacterial body mass containing polyhydroxyalkanoate before and after a feast famine cycle. These data led to the definitive conclusion that polyhydroxyalkanoates can be produced in a concurrent method of production in a bokashi compost bin.

Keywords: polyhydroxyalkanoate, bioplastic, plastic, sustainability

Introduction

Plastic stands as the most discarded single use waste currently. According to *Environmental Health News*, a news source dedicated to

environmental issues, nearly 360 millions of tons of plastic have been discarded every year (Knoblauch, ehcn.org). Problematically, synthetic plastics make up 40% of all manufactured plastics, which derive from fossil fuel sources, primarily coal, natural gas, and crude oil (Parker, nationalgeographic.com). Additionally, synthetic plastics have slow biodegradation rates, resulting in life spans of hundreds or thousands of years. For instance, every single plastic toothbrush you have ever used still exists. Without changing the plastic industry model, researchers can only assume that plastic waste will continue to build up and destroy the environment.

However, when it comes to plastic pollution the immediate halt of production cannot occur due to the necessity of the product in medicine and consumer life. Plastic must be used in some medical practices and equipment, such as IV bags, disposable syringes, and prosthetics (craftechind.com). The heavy integration of synthetic plastics into daily life has hindered researchers abilities to address and find realistic solutions to the waste problem.

It is not impossible though, in *Production of Polyhydroxyalkanoates, a bacterial biodegradable polymers*, published in the African Journal of Biotechnology, researchers TV Ojumu, J Yu, and BO Soloman report on a classification of bioplastic polymers, polyhydroxyalkanoates, that have high

viability in becoming a synthetic plastic substitute. Unlike other bioplastics, polyhydroxyalkanoates, or PHAs, result from the subjection of a feast famine cycle upon microorganisms. This cycle occurs when microorganisms repeatedly undergo the farming process as normal and later become subject to famine. PHAs, much like other bioplastics (polylactides, aliphatic polyesters, polysaccharides, and copolymer blends) serve as alternatives to synthetic plastics given their ability to biodegrade. However, PHAs' distinctive and transformative polymer structures make them unique in the sense that they have similar physical properties to synthetic plastics. These similarities allow the transition from synthetic plastics to bioplastics to become more achievable.

The aforementioned article supports claims suggesting that studying and improving the production of PHAs can lead to less plastic waste and lower the use of nonrenewable resources. Unfortunately, bioplastics have faults of their own. Due to the small amount of time that has been devoted to the industry, PHA production costs can be exorbitantly high. Additionally, the low yield of PHA plastic turns many large corporations away from investing. It can be hypothesized that experimenting and designing ways to sustainably increase PHA market presence, through concurrent production processes, is the key to solving our plastic problem. The following literature review attempts to demonstrate and support this hypothesis.

Literature Review

Polyhydroxyalkanoate Production

One review, titled *Polyhydroxyalkanoates: Characteristics, production, recent developments and applications*, written and researched by Zulfiqar Ali Raza, Sharjeel Abid, and Ibrahim M. Banat explains the methods laboratories use to

produce polyhydroxyalkanoates. PHA production primarily occurs during a feast famine cycle, created through limiting inorganic nutrients in the presence of excess carbon. Before the cycle can occur, a suitable media must be created for the desired microorganism to survive and thrive. The microorganisms, once farmed and collected as normal, become subject to famine by removing the inorganic nutrients they rely upon for food. This cycle forces the microorganisms to store nutrients. Once extracted these nutrients become PHAs pellets that can be transformed into plastic.

However, this article permits the conclusion that the price of production creates the biggest hurdle for this new technology. It can be hypothesized that since manufacturers have been producing synthetic polymers for millennia, they have been able to refine the process to create the highest yield for the highest profit. Bioplastics have not yet had the time and resources to reduce their prices and raise their percent yield. Furthermore, when attempting to increase percent yield while being economically viable current production processes can be viewed as irrelevant. The solution to this would be identifying a method to produce a large quantity of polymer without using a large amount of funding, possibly through having a media already created.

A study titled *Production of Polyhydroxyalkanoate During Treatment of Tomato Cannery Wastewater*, written by a team of researchers lead by Hsin-Ying Liu, divulges the methods used to produce polyhydroxyalkanoates simultaneously with wastewater treatment, exemplifying the aforementioned solution of utilizing medias that have already been formed. The researchers introduce the standard PHA production process and identify the economic weakness with such methods, citing the manufacturing process and its price as a reason against PHA based plastics becoming widespread

and accessible at an affordable price. In pairing the production process with another highly productive system, it becomes more economically feasible. The production process, when utilizing wastewater systems, occurs by using activated sludge, created through wastewater treatment, as the media for the microorganisms. The positive results of utilizing waste from food production include meeting the criteria for PHA production habitat, increasing the sustainability of food waste practices by decreasing the percentage of waste discarded, and decreasing the cost of PHA production by roughly 50%.

Hsin-Ying Liu and their colleagues prove PHA production can not only be successful with concurrent methods, but also highly advantageous. However, comparatively waste water systems lack the ease of location found in other industrial waste facilities, such as composting. This fault lowers the amount of facilities that can manufacture PHA. So, when looking at concurrent production methods it may be highly advantageous to analyze using a compost facility as the established media.

In a video, titled *Bokashi Composting*, host Daphne Lambert, a nutritionist and urban gardener, outlines and explains how to run a Bokashi composting system. Bokashi utilizes microorganisms in a special type of bran to anaerobically ferment food scraps, causing the range of food scraps to expand, allowing meat, fish, and dairy products to be composted. Additionally, the process only takes about two weeks to produce usable compost.

The video guide reasons that, with the correct microorganisms, a Bokashi composting environment can be a suitable media for PHA production. As discussed in *Production of Polyhydroxyalkanoate During Treatment of Tomato Cannery Wastewater*, when PHA production occurs simultaneously with wastewater treatment the main production processes, the feast famine cycle, happens in activated sludge. This activated sludge creates the

perfect habitat for, not only the microorganisms to live, but for nutrients to be modified easily to create the cycle. The compost resulting from the Bokashi process shares multiple similar properties to the activated sludge used in PHA production processed in wastewater treatment facilities. So, a viable experiment would be to research the possibility of PHA production in Bokashi compost, when looking to expand the accessibility and affordability of polyhydroxyalkanoates.

Due to these findings, the researcher identifies a gap in knowledge in the affordability and accessibility of PHA production. This prompts the researcher to ask, to what extent, if any, does the ability to modify phosphorus, nitrogen, carbon, and oxygen levels in compost bins allow for polyhydroxyalkanoates to be produced in order to determine and expand the accessibility of bioplastics?

Method

The method used in this study aimed to answer the question, to what extent, if any, does the ability to modify phosphorus, nitrogen, carbon, and oxygen levels in compost bins allow for polyhydroxyalkanoates to be produced in order to determine and expand the accessibility of bioplastics? To answer this question the researcher manipulated a bokashi compost bin to mimic the polyhydroxyalkanoate manufacturing process, specifically that outlined in the study titled *Factorial Experimental Designs for Enhancement of Concurrent Poly(Hydroxyalkanoate) Production and Brewery Wastewater Treatment*.

In the aforementioned study, the researchers performed and specialized a concurrent method of PHA production outside of lab controlled environments, specifically that of a wastewater plant, and found that two medias needed to be created. The first media served as the base habitat to grow their PHA producing bacteria in. The

second media served as the habitat where the feast famine cycle commences. In the current experiment the researcher created one standard bokashi bin to grow the *Escherichia coli*, a bacteria suited to survive in anaerobic habitats and create PHAs, and one modified bokashi bin to conduct the feast famine cycle, in order to replicate the two medias in *Factorial Experimental Designs for Enhancement of Concurrent Poly(Hydroxyalkanoate) Production and Brewery Wastewater Treatment*. These bins provided an adequate amount of data to support a conclusion concerning the possibility of PHA production in a modified small scale habitat such as bokashi composting bins.

Safety

Due to this study's manipulation of bacteria in compost which can be added to the dirt of crops that will be harvested and eaten, safety must be considered. Certain *Escherichia coli* strains cause severe food poisoning. However, the recombinant strain resides safely within the human intestine. Thus, the conclusion can be reached that if utilizing this strain the bacteria can be added to the fertilizer used in consumer crops without concerns for safety.

Creating Compost Bin #1

In *Factorial Experimental Designs for Enhancement of Concurrent Poly(Hydroxyalkanoate) Production and Brewery Wastewater Treatment* the researchers designed a concurrent polyhydroxyalkanoate manufacturing process in a brewery's wastewater treatment plant. This habitat gave them access to four crucial variables that they concluded should be modified to successfully produce PHAs, the solid retention time (SRT), the hydraulic retention time (HRT), and the carbon to nitrogen ratio (C/N). Through their procedure they found the most important factor in the PHA percent yield to be the length of the SRT and HRT, with a 2 day HRT and 12 day SRT yielding the largest PHA concentration.

In recreating this experiment with the hopes of making it more sustainable and finding a gap in knowledge in the concurrent PHA production processes, utilizing a bokashi compost bin provides a reasonable habitat, due to its modifiable SRT, HRT and C/N ratio not found in other composting methods.

The first stage of experimentation developed a bokashi compost bin modeled after Daphne Lambert's bokashi bin guide. The ease of access of Lambert's guide allows this experiment to be replicated on a large scale, increasing the sustainability of PHA production, as mentioned in the literature review.

The researcher collected food scraps until the bin filled completely. They logged every scrap collected in their scientific journal, the nutrients it contained, its weight, and any additional notes. Once logged, the food scraps were added to a freezer safe container and frozen until added to the compost bin, ensuring no mold would grow on the waste. Due to the nature of the bokashi method which utilizes anaerobic fermentation, the variety of food scraps allowed in the bin becomes much wider, in contrast to other more, well known, composting methods. The food scraps allowed in this bin included, all food waste except large bones, liquids, and food with mold already present.

After collecting an adequate amount of food the researcher added the food scraps to the bokashi bin, the steps of which follow. The researcher added the food scraps one cup at a time. Once the layer measured two inches in height, the researcher sprinkled two tablespoons of Bokashi Bran over the surface area of the visible scraps. After each layer received the bokashi bran, a paper plate and 6 pound weight held the food scraps down for 30 seconds by being placed over the top layer. After 30 seconds passed the researcher would remove both the plate and weight, add another layer of food scraps, and repeat the process. Repeatedly weighing

down the food scraps removes as much oxygen as possible, aiding the anaerobic fermentation process.

The researcher repeated the outlined methods, and added all the scraps to the bin. Before closing the bin the researcher placed the plate and weight over the top layer. The researcher then let the bin sit untouched for 12 days only returning to it to drain every two days. These time frames create the optimized SRT and HRT as previously mentioned, letting the bin remain stationary for 12 days allows it to ferment properly along with achieving the 12 day SRT and draining the bin every 2 days follows the proper Bokashi Bin procedures outlined in Daphne Lambert's bokashi bin guide. To drain the researcher placed a bowl under the spigot and opened it. The bowl then filled with a yellow/orange liquid and the researcher kept the spigot open until the liquid flow stopped. Once stopped, the researcher closed the spigot and if desired diluted the liquid with water at a 1:100 dilution rate to use as a liquid fertilizer.

Creating Compost Bin #2

On the first drainage day of the first bin, the researcher started creating the second compost bin. The collection and addition process mimicked the first bin's, but differed in that the second bin could not have any scraps of food containing high levels of nitrogen and phosphorus. In the study *Factorial Experimental Designs for Enhancement of Concurrent Poly(Hydroxyalkanoate) Production and Brewery Wastewater Treatment*, the feast famine cycle, a controlled pattern of bacterial population and famine designed to force the bacteria into creating emergency energy stores occurred in the second media. This cycle proved to only be possible with the removal of limiting nutrients in the presence of excess amounts of carbon, because PHAs are the energy stores made when the food sources of the *Escherichia coli* become extinguished, and most of the food sources of this bacteria are nitrogen rich.

Allowing Escherichia coli Bacteria to Reproduce

After the first bin completed the 12 day fermentation period and SRT the researcher drained it for a final time and introduced the *Escherichia coli* to the bin through the following methods. After obtaining the freeze dried lyophilized *Escherichia coli* culture the researcher used a sterile pipette to add 1 mL of broth to the *Escherichia coli* palette and let it sit for 30 seconds. Once the 30 seconds ended, the researcher used the pipette to withdraw and expel the broth in order to fully break up the lyophilized bacteria. Having rehydrated the bacterial culture the researcher emptied the contents into the first compost bin. The bin then sat untouched for 2 days to allow the bacteria to grow and begin the feast famine cycle.

Subjecting Escherichia coli to Feast Famine Cycle

After the 2 days of Bin #1 and 12 days of Bin #2 standing untouched, given that Bin #1 gained the *Escherichia coli* when Bin #2 was on its eleventh day of the SRT, the researcher drained Bin #2 for the last time using the same methods as previously outlined. Once the draining process finished they opened Bin #1 and Bin #2 and moved the bacteria from Bin #1 to #2 by removing a cup of the first Bin's compost and transporting it into the second bin. The researcher also took a ten gram sample of the first bin's compost to study at a later date. They then let Bin #2 sit untouched for 1 day in order to allow the feast famine cycle proper time to commence.

Establishing Bacterial Colonies to Sample

Once the feast famine cycle concluded the researcher opened Bin #2 and took a ten gram sample of the compost to observe. In order to obtain a bacterial sample from the compost samples previously collected, the researcher used the dilution plate method outlined in the article *Isolation of*

Bacteria in Soil written by Geetha H. The dilution plate method isolates bacterial colonies, aiding the research by ensuring *Escherichia Coli* can be observed alone. The steps of the dilution plate method follow.

The researcher blended the first bin's compost sample into a soil like consistency. They then sifted the soil through a 2mm sieve and collected three samples, each weighing one gram, into preweighed sterilized containers labeled Sample #1, Sample #2, Sample #3. Three samples provided an adequate amount of data to study. The researcher then added Sample #1 to a 250 mL Erlenmeyer flask labeled "Sample #1 1/100 dilution" and filled with 99 mL of water, and stirred it with a stir stick for 5 minutes. Once the 5 minutes finished the researcher pipetted out 1 mL of the solution and added it and 9 mL of water to a second 250 mL Erlenmeyer flask labeled "Sample #1 1/1000 dilution" and stirred that solution for 5 minutes with a, new sterilized, stir stick. They then pipetted out 1 mL of the 1/1000 dilution and added it to 9 mL of water in a third 250 mL Erlenmeyer flask labeled "Sample #1 1/10000 dilution" and stirred it for 5 minutes with a new sterilized stir stick.

Creating Agar Plates

Additionally, while stirring the different samples the researcher set LB Agar Plates. They mimicked the recipe created by the Bridges Labs protocol site, and the methods are as follows. In a 100 mL beaker the researcher combined 25 mL of LB agar broth, enough water for the broth to dissolve, 15 grams of agar, and a magnetic stir bar. Once everything combined, the beaker was transferred to a hot plate and left until the liquid boiled. After boiling the heat was turned off and the mixture was left untouched until cool to the touch. Then, 15 mL of the LB agar was added to each petri dish and left to cool and set completely.

Once the agar set, the researcher aseptically

transferred 1 mL of each dilution into its own petri dish and labeled it to match the Erlenmeyer flasks, spread them across the agar, and incubated at 37°C for 168 hours, or one week. While incubating the agar dishes were turned upside down in order to avoid any condensation falling into the bacterial colonies and adding an extra unanticipated variable. The methods repeated for each sample, the only change occurring with labels accurately reflecting the sample being prepared for examination. The process resulted in 9 petri dishes of bacteria samples for the first bin. The researcher then repeated the method with the second compost bin's sample, resulting in 18 separate agar plates, 9 for each compost bin.

Obtaining Empirical Data

After incubating for an adequate amount of time, the researcher removed all agar dishes from the incubator. Due to the size of the bacterial colonies, in order to examine the *Escherichia coli*, the researcher needed to use a staining technique, the steps of which follow. Using a small wooden popsicle stick the researcher scraped as much of the bacterial growth off the agar plate as possible and transferred it to a microscopic slide. To ensure each individual cell could be seen the samples were spread as thin as possible across the slide. Then, the slides were each prepared for staining through a procedure known as fixing. Each slide was waved above a bunsen burner repeatedly until the swab of bacteria had dried, on average this took about five seconds. Once fixed a drop of congo red capsule stain was placed on top of the swabs and let sit for five seconds. Then, the researcher used a squirt bottle to gently clean the excess stain off the swab. If they were not given enough time to fix and absorb the stain when removing the excess stain the researcher risks also removing the swab of bacteria from the slide.

Having properly stained all 18 bacterial slides

the researcher moved on to observational data collection through oil immersion. Oil immersion allows for smaller bacterium to be observed due to the higher refraction of light in the concavity of the oil, compared to the unassisted microscope lens. In order to perform an oil immersion the researcher placed a glass cover over each slide. Then, the slides were placed on the microscope and a drop of oil was positioned over the sample. The researcher was then able to lower the microscopic lens until contact was made with the oil droplet creating what looked like a tunnel of oil leading from the slide to the microscope lens. After adequately adjusting the microscope focus and position the researcher was able to notate the bacterial body mass containing PHA in their scientific journal.

Results

In addressing the hypothesis, when added to a bokashi compost habitat and subjected to a feast famine cycle polyhydroxyalkanoates will be produced in recombinant *Escherichia coli*, the researcher conducted the aforementioned procedure and gathered the following results, which can be referenced in Figures 1 and 2. All samples from bin one had a 0% polyhydroxyalkanoate yield. In bin two, "Sample #1 1/100 Dilution" and "Sample #3 1/1000 Dilution" had a 20% polyhydroxyalkanoate yield, "Sample #1 1/1000 Dilution", "Sample #1 1/10000 Dilution", "Sample #2 1/1000 Dilution", "Sample #3 1/100 Dilution", and "Sample #3 1/10000 Dilution" had a 10% polyhydroxyalkanoate yield, "Sample #2 1/100 Dilution" had a 5% polyhydroxyalkanoate yield, and "Sample #2 1/10000 Dilution" had an insufficient number of bacterial colonies to study. The researcher performed a T Test on this data set and obtained a P Value of 0.000003286263521, therefore, the researcher rejects the null hypothesis of, when added to a bokashi compost habitat and subjected to a feast famine cycle polyhydroxyalkanoates will not be

produced in recombinant *Escherichia coli*.

Discussion

Polyhydroxyalkanoate Presence

Based on the data gathered, the researcher concluded that when subjected to a feast famine cycle within a bokashi composting habitat *Escherichia coli* will produce polyhydroxyalkanoates. The bacterial change of habitat, from a nutrient rich media to a nutrient lacking media all within the composting system, produced, on average, a 1,187.5% increase in PHA percent yield, which demonstrates the ability for PHAs to be produced in a bokashi composting bin.

Expansion of Bioplastic Production Processes

The increase in polyhydroxyalkanoate appearance in singular bacterial structures successfully answers the first question posed by the researcher, that being, to what extent, if any, does the ability to modify phosphorus, nitrogen, carbon, and oxygen levels in compost bins allow for polyhydroxyalkanoates to be produced. However, in understanding if the experiment expands the accessibility of the bioplastics derived from PHAs, a much larger discussion must be held. In an article titled *How is Sustainability Measured?* the U.S. Department of Transportation evaluates how different industries, not limited to transportation, must measure sustainability. They devised three principles that must be assessed in order for anything to be considered sustainable. In doing so they acknowledge that, although very important, it is not always possible for all three principles to be met fully, as long as the best attempt is made and the industry is constantly striving to improve the areas they are lacking in.

The first principle devised in the study is social sustainability. Objectively this principle is the hardest to identify parameters around

Percentage of the Bacterial Body Mass Containing PHA		
Sample	Bin #1	Bin #2
Sample #1 1/100 Dilution	0	20
Sample #1 1/1000 Dilution	0	10
Sample #1 1/10000 Dilution	0	10
Sample #2 1/100 Dilution	0	5
Sample #2 1/1000 Dilution	0	10
Sample #2 1/10000 Dilution	0	n/a
Sample #3 1/100 Dilution	0	10
Sample #3 1/1000 Dilution	0	20
Sample #3 1/10000 Dilution	0	10

Figure 1

A collection of all data collected through microscopic observation.

PHA Appearance Before and After Feast Famine Cycle

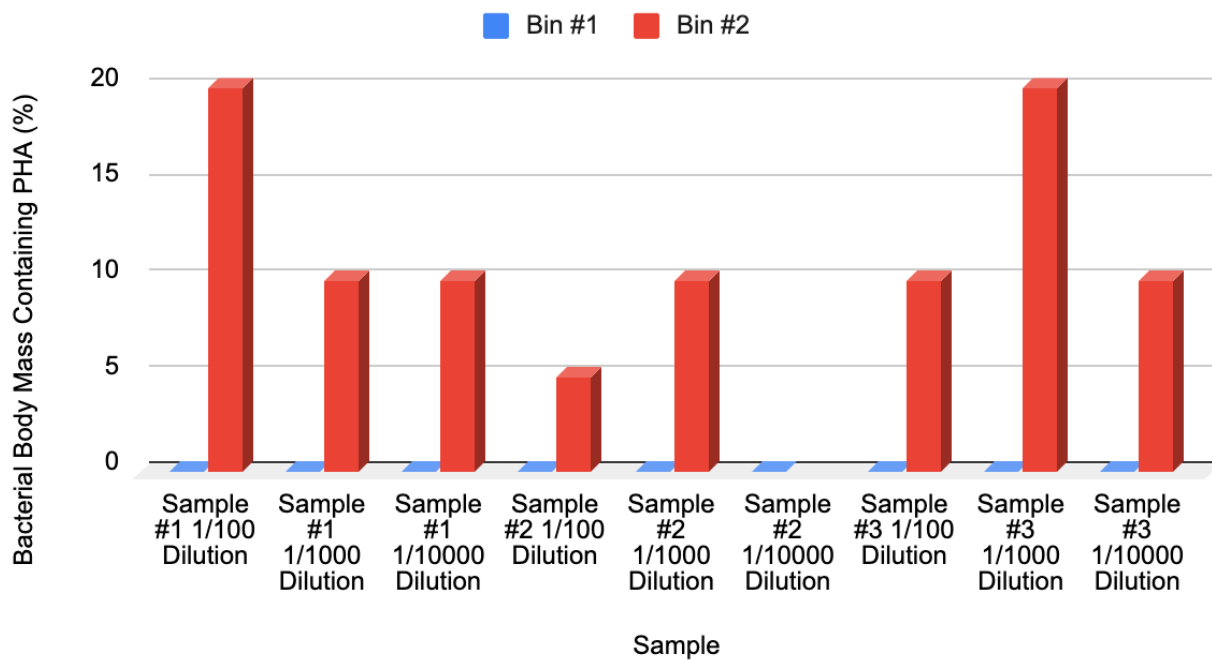


Figure 2

A comparison between the change in the percentage of the bacteria body mass containing PHA after completing the feast famine cycle.

given the current experiment. When applied to PHA production and the use of compost as the production media the best way to analyze the social sustainability aspect would be to ask the question, does the presence of the production process and the associated architecture pose a problem to the inhabitants of the area in which it is located? When this question is asked about compost facilities the common response is yes, it does pose a problem, due to the smell of the composting material. However, bokashi composting's anaerobic nature means no smell is produced during production. So, this experiment can reasonably pass the social test.

In the next two principles of sustainability, environmental and economical, PHAs must be compared to their synthetic options. This allows an industry baseline to be established. So, when evaluating the environmental sustainability of the production process PHAs become easily more sustainable. Synthetic plastic production relies on toxic and non renewable materials, such as coal, natural gas, and crude oil. However, PHA production never uses these materials while also recycling food waste that would otherwise be thrown out and contribute to the waste problem.

Finally, in a study titled *Polyhydroxyalkanoates: Characteristics, Production, Recent Developments and Applications*, researchers Raza, Z. A., Abid, S., & Banat, I. M. assert that in order to serve as an economically viable alternative to standard plastics derived from fossil fuels the PHA plastic production process must have a PHA percent yield above 50%, ideally 80%. This range provides more plastic with the same associated costs compared to synthetic options. Seeing as the current experiment resulted in percent yields ranging from 5-20%, none of the samples collected can be considered economically viable. However, as the manufacturing system devised in this experiment passed the previous two tests, as identified in the reference material The U.S. Department of Transportation acknowledges

that this is still a sustainable practice if further experimentation aims to increase the percent yield. So, in respect to the gap in knowledge found in the affordability and accessibility of PHA production these findings identify a new understanding of the production of biodegradable plastic alternatives.

Limitations

One hypothesis for the low percent yield of PHAs in the *Escherichia coli*'s body is that the biomass selection did not have a high enough difference in nutrient level. The food scraps utilized in experimentation vary greatly based on the household location of the compost bin. For instance, one family may eat out more than another resulting in less nutritional information to be accessible, ultimately lowering the ability to sort the food into the correct bin greatly. This reasoning would lower the C/N ratio, resulting in less PHA production according to *Factorial Experimental Designs for Enhancement of Concurrent Poly(Hydroxyalkanoate) Production and Brewery Wastewater Treatment*. A possible solution to this inquiry would be to conduct the same experiment at a farm, utilizing the unsellable produce in the compost bin, in order to have complete understanding of the food scraps being composted.

Additionally, error in appropriately identifying the PHA in the bacteria structure could have occurred. Due to all lack of microscopic cameras the researcher solely observed and notated all percentages. This brings human error into question. These limitations, however, can be easily addressed in running another experiment identical to the aforementioned one with the addition of a microscopic camera. Despite all of these limitations, the P value of 0.000003286263521 indicates that the difference between the PHA percent yield between both bins is so large that it is very improbable that this data was the result of chance, validating both the data and the experiment.

Conclusion

An increase in overall sustainability of the polyhydroxyalkanoate production process during bokashi composting can increase in the effectiveness and accessibility of bioplastics, leading to a decrease in the need for synthetic plastics. In studying the bioplastic and synthetic plastic industries an inverse correlation is noted between the success and failure of the two. Synthetic plastics have dominated the market due to their ease of creation and reliability in the medical field, so their sales are dependent on the fact that no other substance has the same properties at a lower or similar price range. However, given the current experiment's ability to increase the scope and yield of bioplastic production these factors no longer matter, meaning there are no advantages of using unsustainable plastic options. Ultimately making the production of synthetic plastics null and void and removing the problem of plastic pollution entirely due to PHA's ability to biodegrade fully. Ergo, the results of the experiment addressing the question, to what extent, if any,

does the ability to modify phosphorus, nitrogen, carbon, and oxygen levels in compost bins allow for polyhydroxyalkanoates to be produced in order to determine and expand the accessibility of bioplastics, have larger implications than simply improving the sustainability of bioplastic production.

Future experimentation could be conducted on the expansion of this project on an industrial scale. For example, working with local agricultural farms to utilize their food waste would aim to decrease the amount food waste produced on retail and consumer levels as well as providing easier access to a steady food supply for the composting methods. The future researcher could also look into the simultaneous production of industrial compost and PHAs, mimicking the successful methods used in other studies such as *Production of Polyhydroxyalkanoate During Treatment of Tomato Cannery Wastewater* with the compost media identified as successful in this study.

References

- Bridges Lab. (n.d.). *Making LB Agar Plates*. Making LB Agar Plates - Bridges Lab Protocols. http://bridgeslab.sph.umich.edu/protocols/index.php/Making_LB_Agar_Plates.
- Center for Food Safety and Applied Nutrition. (2021, February 23). *Food Loss and Waste*. U.S. Food and Drug Administration. <https://www.fda.gov/food/consumers/food-loss-and-waste>.
- Craftchind, C. I. I. (2019, November 14). *The Many Uses of Plastic Materials in Medicine - Use of Plastic in Medicine..* <https://www.craftchind.com/the-many-uses-of-plastic-materials-in-medicine/>.
- Federal Highway Administration, U. S. D. of T. (n.d.). *How is Sustainability Measured?* INVEST - Sustainable Highways Self-Evaluation Tool. <https://www.sustainablehighways.org/99/how-is-sustainability-measured.html>.
- H, G. (2017, March 23). *Isolation of Bacteria in Soil: Microbial Biotechnology*. BioTechnology Notes. <https://www.biotechnologynotes.com/bacteria/isolation-of-bacteria-in-soil-microbial-biotechnology/1345>.
- Hao, J., Wang, H., & Wang, X. (2018). Selecting optimal feast-to-famine ratio for a new polyhydroxyalkanoate (PHA) production system fed by valerate-dominant sludge hydrolysate. *Applied Microbiology and Biotechnology*, 102(7), 3133–3143. <https://doi.org/10.1007/s00253-018-8799-6>
- Johnson, T. (2017, July 17). *The Many Uses of Plastics*. ThoughtCo. <https://www.thoughtco.com/uses-of-plastics-820359>.
- Knoblauch, J. A. (2021, March 8). *Environmental toll of plastics*. EHN. <https://www.ehn.org/plastic-manufactured-in-the-first-10-years-of-this-century-eclipses-the-total-produced-in-the-entire-last-century>.
- L. (Director). (2019, February 4). *Biosynthesis of PHA* [Video file]. Retrieved October 20, 2020, from <https://www.youtube.com/watch?v=eeIDqfEzL-E>
- Lipford, D. (2020, September 21). What Nutrients Can Be Found in Compost? Retrieved October 20, 2020, from <https://todayshomeowner.com/what-nutrients-can-be-found-in-compost/>
- Liu, H., Hall, P. V., Darby, J. L., Coats, E. R., Green, P. G., Thompson, D. E., & Loge, F. J. (2008). Production of Polyhydroxyalkanoate

- During Treatment of Tomato Cannery Wastewater. *Water Environment Research*, Vol. 80(No. 4), 367-372.
- Liu, H., VanderGheynst, J. S., Darby, J. L., Thompson, D. E., Green, P. G., & Loge, F. J. (2011). Factorial Experimental Designs for Enhancement of Concurrent Poly(Hydroxyalkanoate) Production and Brewery Wastewater Treatment. *Water Environment Research*, Vol. 83(No. 1), 36-43.
- Nikodinovic-Runic, J., Guzik, M., Kenny, S. T., Babu, R., Werker, A., & O Connor, K. E. (2013). Carbon-Rich Wastes as Feedstocks for Biodegradable Polymer (Polyhydroxyalkanoate) Production Using Bacteria. *Advances in Applied Microbiology*, Vol 84, 139-200.
- Ojumu, T., Yu, J., & Solomon, B. (2004). Production of Polyhydroxyalkanoates, a bacterial biodegradable polymers. *African Journal of Biotechnology*, Vol. 3(No. 1). Retrieved October 20, 2020, from <https://www.ajol.info/index.php/ajb/article/view/14910>
- Parker, L. (2021, February 10). *Plastic pollution facts and information*. Environment. <https://www.nationalgeographic.com/environment/article/plastic-pollution>.
- Raza, Z. A., Abid, S., & Banat, I. M. (2018). Polyhydroxyalkanoates: Characteristics, production, recent developments and applications. *International Biodeterioration & Biodegradation*, Vol. 126, 45-46. Retrieved October 20, 2020, from <https://www.sciencedirect.com/science/article/abs/pii/S0964830517300276#!>
- Rehm, B. H., & Steinbüchel, A. (1999). Biochemical and genetic analysis of PHA synthases and other proteins required for PHA synthesis. *International Journal of Biological Macromolecules*, Vol. 25(Issues 1-3), 3-19.
- Rhu, D., Lee, W., Kim, J., & Choi, E. (2003). Polyhydroxyalkanoate (PHA) production from waste. *Water Science and Technology*, Vol 48(Issue 8), 221-228.
- Scientific, D. (Director). (2016, November 30). *PHA Cycle Animation* [Video file]. Retrieved October 20, 2020, from https://www.youtube.com/watch?v=YWs_VVLNWCo
- Serafim, L. S., Lemos, P. C., Albuquerque, M. G., & Reis, M. A. (2008). Strategies for PHA production by mixed cultures and renewable waste materials. *Applied Microbiology and Biotechnology*, Vol. 81, 615-628.
- Surendran, A., Lakshmanan, M., Chee, J. Y., Sulaiman, A. M., Thuoc, D. V., & Sudesh, K. (2020). Can Polyhydroxyalkanoates Be Produced Efficiently From Waste Plant and Animal Oils? *Frontiers in Bioengineering and Biotechnology*, 8. <https://doi.org/10.3389/fbioe.2020.00169>
- Trust, G. (Director). (2017, August 11). *Bokashi Composting* [Video file]. Retrieved October 20, 2020, from <https://www.youtube.com/watch?v=-w3jajq5-tM>
- Wong, C. H., Barton, G. W., & Barford, J. P. (2003). The nitrogen cycle and its application in wastewater treatment. *Handbook of Water and Wastewater Microbiology*, 427-439.