Diverting Bioplastic Waste from the Landfill: Assessing and Optimizing Microbial Degradation of PLA-Based-Plastics in University of Wyoming Compost

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Project Summary:

Since the 1950's mankind has produced an estimated 8.4 billion metric tons of plastic, roughly 30% of which is used by Americans (Dengler, 2017); moreover, up to 98% of plastic waste in the U.S. is simply discarded into landfills rather than recycled (University of Utah, N.D). Certainly, much work is necessary to reduce the accumulation of plastic wastes, but current methods rely heavily on recycling existing plastic waste and phasing out production of traditional plastics in favor of novel, so-called "biodegradable" plastics. In an effort to reduce institutional waste, the University of Wyoming utilizes biodegradable plastics to meet the institution's packaging needs. However, the present University infrastructure at the Agricultural Community Resource for Everyday Sustainability (ACRES) Student Farm is not conducive to composting whole biodegradable-plastic waste. As a result, the bioplastics used at the University of Wyoming are being disposed of in the local landfill, where they will not degrade for more than a century. Further, biodegradable plastics slated for the landfill will contribute upwards of three times the amount of methane gas as compared to bioplastics degraded in compost (Rossi et al., 2015).

Earlier work has shown that mechanical mastication of the biodegradable plastic has augmented the degradation of Poly-lactic-acid (A key constituent of many bio-plastics), but the physio-chemical environment of stage 2 compost has not been adequately examined for its effects on microbial degradation of PLA. Thus, our objectives are to isolate and identify PLA degraders in stage 2 ACRES compost. Further, we intend to quantify the effects of defined environmental conditions -- pH, temperature, and water content -- on the rate of PLA digestion by endogenous microbes. To this end, we expect that a pH of 7.5, a temperature of 37ºC, and a water content of 15% will be most conducive to the microbial hydrolysis of PLA within stage 2 ACRES compost.

In order to assess the aforementioned hypotheses, we will divide the experimental protocol into two distinct phases, both of which will be conducted within the University of Wyoming Microbiology Program infrastructure. During Phase 1, we will utilize selective media and biochemical assays to isolate and identify microorganism present in stage 2 ACRES compost that are capable of degrading PLA. During Phase 2 we will examine the environmental conditions (pH, temperature, and water content) most amenable to microbial degradation of PLA. We will assess the effects of these independent variables by quantifying the extent of PLA degradation across treatment groups. Results will inform our recommendations to ACRES Student Farm on successfully incorporating bioplastics into their compost. This would allow ACRES to utilize much of the University's food service waste and divert large quantities of bioplastics from the landfill. Since the pH and water content of compost can be easily and inexpensively manipulated, our results could be adapted to any small scale composting operation.

Statement of Significance:

According to the Recycling Coalition of Utah, Americans dispose of approximately 35 billion plastic bottles per year (RCU. 2015). As awareness of plastic waste increases, many companies and institutions are attempting to heighten their sustainability through the use of biodegradable plastics, such as those composed of PLA. In theory, these products can be composted and used to enrich soil by increasing nutrient content and water retention. However, due to logistical or financial constraints, many of these materials enter landfills where they may persist for over a century before degrading under suboptimal conditions to produce harmful byproducts like methane, a potent greenhouse gas (Rossi et al., 2015). Ultimately, such greenhouse gasses go on to exacerbate the effects of global warming, negating the point of alternative plastics altogether. However, even in compost, these materials can persist for long periods of time without any ap-

preciable degradation. Prior research has investigated the conditions necessary for efficient microbial digestion of biodegradable plastics in vitro or with the creation of a bioreactor, but these methods are of little use to small-scale compost operations. The proposed research aims to design a cost-effective, field-based method of enriching endogenous, bioplastic-degrading microbial populations by providing conditions optimal for growth. Techniques developed at the University of Wyoming by undergraduate researchers and faculty supervisors will be implemented by the Agricultural Community Resources for Everyday Sustainability (ACRES) Student Farm in conjunction with the Campus Sustainability Office to reduce campus landfill waste and will also be presented at the regional American Society for Microbiology conference. The methods developed by this research could also be adapted to a wide variety of small-scale composting operations.

Relevant Literature:

According to the Recycling Coalition of Utah, enough non-biodegradable plastic is thrown away each year to circle the globe four times. Additionally, these plastics take approximately 1000 years to degrade when disposed of in a landfill (RCU. 2015). In an attempt to reduce such waste, companies have begun manufacturing biodegradable alternatives to plastic. One of the most common constituents of biodegradable plastics is polylactic acid (PLA). PLA is formed entirely from the plant biomass of corn or potatoes, thus eliminating the use of harsh inorganic chemicals (Auras, 2010). While PLA can be safely incinerated with energy recovery, recycled, or composted readily in industrial composting facilities, the requisite infrastructure is often only available in large urban centers. Where these facilities are not accessible, the only remaining options are landfilling and small-scale composting. However, under normal landfill conditions, these plastics are estimated to take more than 100 years to degrade (Kolstad et al, 2016), and research also indicates that bioplastics degrading in a landfill produce 3.76 grams of methane per kg of bioplastic, whereas composting the same plastic only produces approximately 1 gram (Rossi et al, 2015).

Microbiologists have been researching microbial degradation of PLA for several years, and have discovered a wide range of bacterial species, including *Bacillus spp*., *Cryptococcus spp*., and *Pseudomonas spp*. (Mehdi et al, 2016) that are present in compost, and capable of degrading PLA through the use of secreted proteases and cutinases, which convert the PLA polymer into its monomer subunits before metabolizing them (Fukushima et al, 2009). This conversion of PLA polymers to lactic acid subunits is increasingly difficult for microbes as particle size increases. Recent studies have concluded that optimal PLA degradation is achieved with particles of approximately 0.8mm diameter (Ryan et al, 2017).

While much research has been conducted regarding the enzymatic mechanisms of PLA degradation, the role of local environment on microbes' abilities to metabolize PLA remain largely undefined. In any case, optimal PLA degradation in compost will require optimal growth of the organisms responsible for the hydrolysis of PLA. In the case of *Bacillus spp*., degrading organisms are capable of secreting an alkaline serine protease shown capable of converting PLA to lactic acid (Fukushima et al. 2009). Furthermore, prior research into these secreted proteases has shown that they have maximal catalytic activity within a pH range of 7.0 - 11.0 (Gupta et al. 2002). Moreover, *Bacillus spp*. have been demonstrated to exhibit optimal growth within a temperature range of 30ºC and 40ºC (Warth, 1977). Interestingly, other PLA degrading microbes operate under similar conditions. It has been shown that *Cryptococcus spp*. prefer temperatures and pH values similar to those found within mammals, proliferating best within a range of 36ºC-

40ºC and at a pH of approximately 6.5-7.5 (Ravi et al. 2014). Finally, *Pseudomonas spp*. are known to exhibit optimal growth at a pH of 6.5-7 (Todar, 2012). Additional inquiries have shown *Pseudomonas spp*. proliferate best at a temperature between 28ºC-37ºC (Tsuji et al., 1982).

Preliminary Data;

A representative of University of Wyoming's Office of Residence Life and Dining confirmed that the total volume of biodegradable plastics the university purchased for the year 2016-2017 was approximately 5,000 pounds. Moreover, ACRES Student Farm indicated that PLA-based plastic products did not degrade within their composting infrastructure and were therefore sent to the landfill. However, literature regarding PLA degradation culminates in our understanding that microbes already present in ACRES Student Farm can help to reduce the University of Wyoming's landfill contributions. As such, we aim to support the growth of *Bacillus spp*., *Pseudomonas spp*., *Cryptococcus spp*., and other PLA degraders through physical and chemical modifications of stage 2 ACRES compost including pH, temperature, and moisture variation. Providing environmental conditions amenable for the growth of PLA degrading bacteria will optimize their ability to utilize PLA as a carbon source and facilitate its breakdown in compost. Ultimately, the proposed research will serve to augment the PLA composting capacity of ACRES Student Farm and divert nearly 5,000 pounds of bio-plastic waste from the regional landfill each year. Figure 1 offers a conceptual model illustrating the way in which this research endeavor fits into its local context.

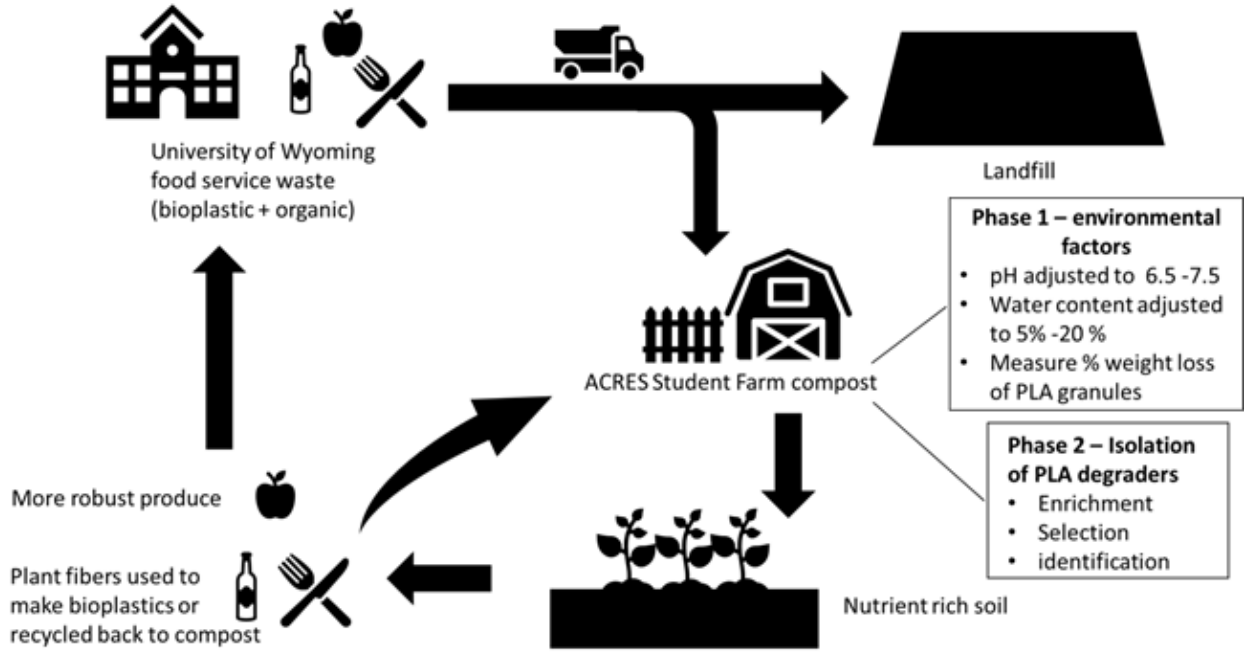


Figure 1. Conceptual model depicting the role of the proposed research in bioplastic composting and campus sustainability.

Justification of Methods:

In order to optimize the environmental conditions necessary

for maximum PLA degradation and identify organisms responsible for this action, we have begun by forging a network with ACRES and our stakeholders at the University of Wyoming. In doing so, we have established an efficient system in which information and resources can be freely exchanged. Additionally, having access to the University of Wyoming Microbiology Program facilities will be paramount to our research, as it will provide us with the tools and equipment necessary to conduct microbiological research in a controlled manner. We also believe that our forced exposure design provides the best chance of ascertaining to what extent the environment may impact PLA hydrolysis, as pH, temperature, and water content can be quantified and adjusted. It is known that microbes and enzymes do have optimal conditions under which they best operate. As such, forcing exposure will allow us to ascertain the best conditions for PLA degrading microbes and their enzymes to hydrolyze PLA. Similarly, we believe that the methods being employed most successfully allow for our hypotheses to be tested. Using lime and citric acid to raise and lower soil pH, respectively, is a practice commonly employed by agricultural facilities nationwide (Williamson. 2012). Temperature controls will be made possible through the use of traditional laboratory incubation. Further, moisture content adjustments will be conducted using sterile water after initial quantification. This will allow us to determine initial water content and precisely adjust it toward levels needed for our samples. As for the isolation of PLA degraders, the use of media with PLA as the sole carbon source will allow us to definitively isolate PLA metabolizers from the compost, as other microbes will be unable to enter the log phase and proliferate (Naiounakis, 2014). The use of Genus-selective media will then allow us to confidently note which PLA degraders are endogenous to the stage 2 compost.

Objectives:

1. Determine which environmental conditions best support resident PLA degrading microbial communities within stage 2 ACRES compost..

2. Make suggestions to ACRES and UW Residence Life and Dining for most effective means of degrading bioplastics within the existing infrastructure in an effort to divert bio-plastic waste from the landfill.

3. To isolate and identify microbes capable of degrading PLA from stage 2 ACRES compost.

Hypotheses:

1. We hypothesize that we will be able to isolate *Bacillus spp*., *Pseudomonas spp*., and *Cryptococcus spp*. capable of degrading PLA from stage 2 ACRES compost.

2. We hypothesize that maintaining a temperature of 37ºC will enhance degradation (Relative to other temperatures tested) of PLA by resident microbes of stage 2 compost.

3. We hypothesize that maintaining a pH of 7.5 in the compost will result in greater PLA degradation (relative to other pHs tested) by endogenous microbes.

4. We hypothesize that maintaining a moisture content of 15% (weight/weight) will result in increased PLA degradation.

Specific Aims:

1. We aim to isolate *Cryptococcus spp*., *Bacillus spp*., *Pseudomonas spp*., and other PLA degrading microbes from stage 2 ACRES compost.

2. We aim to quantify PLA degradation by *Cryptococcus spp*., *Bacillus spp*., and *Pseudomonas spp*. as a function of pH (Across treatment groups: pH 6.5, 7, 7.5) in order to determine what soil treatments may be necessary for maximum PLA degradation.

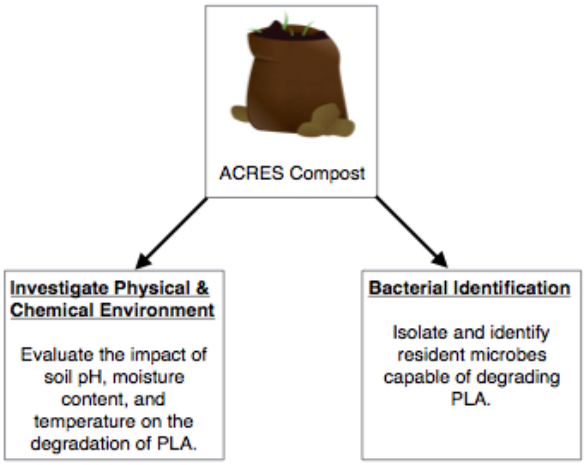
3. We will establish the effects of stage 2 compost moisture content on microbial degradation of PLA (Across treatment groups: 40%, 50%, 60%) in order to inform ACRES watering schedule.

4. We plan to quantify PLA degradation by *Cryptococcus spp*., *Bacillus spp*., and *Pseudomonas spp*. as a function of temperature (Treatment groups: 30ºC and 37ºC) in order to determine what modulations to the composting environment may be necessary.

Research Plan:

In an attempt to address the University of Wyoming's inability to compost biodegradable plastics, we will modulate the physical and chemical environment of stage 2 compost produced at ACRES Student Farm. Broadly, we aim to identify environmental characteristics that can be modulated lending to augmented degradation of bioplastics made from polylactic acid (PLA). In particular, we will examine how the pH of stage 2 ACRES compost impacts microbial degradation of PLA. To do so, we will experimentally alter the stage 2 compost pH to include experimental groups at pHs ranging from 6.5 to 7.5 (Treatment groups: 6.5, 7, 7.5). Moreover, within the experimental groups, we will modulate the water content of the stage 2 compost (Treatment groups: 5%. 7%, 10%, 15%) in order to ascertain the effects of compost moisture content on PLA degradation by endogenous microbes. Additionally, we will elucidate the effects of temperature as a part of the greater physical environment on the degradation of PLA by stage 2 compost microbes. Specifically, we will monitor PLA degradation at two temperatures: 30ºC and 37ºC. Finally, as we work to investigate the effects of the physical and chemical environment on PLA degradation, we will simultaneously work to identify the organisms within stage 2 ACRES compost that are capable of PLA degradation. The culmination of these data(s) will allow us to inform ACRES student farm (And broadly the University of Wyoming) what conditions will have the greatest impact on their ability to enhance PLA degradation within their composting infrastructure. Moreover, the experimental design in which we force exposure to our independent variables (pH, moisture content, and temperature) will allow us to robustly examine these variables within the context of the greater problem. Figure 2, below, communicates the broad experimental design.

Figure 2: Offers an overview of the two experimental aims.

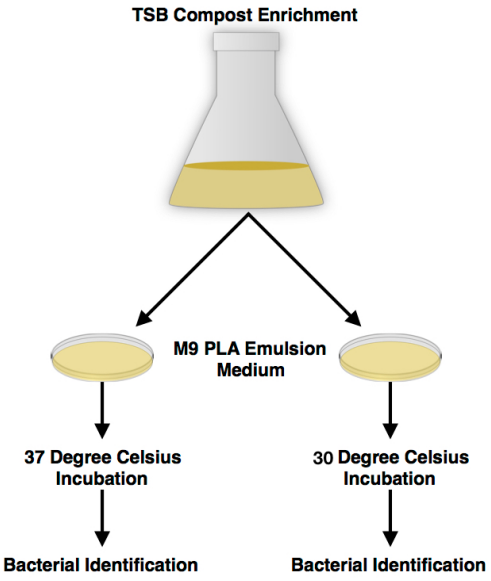


Methods & Materials:

The proposed research will be divided into two phases. The first phase will be devoted to the isolation and identification of PLA degraders from ACRES compost. The second phase will be used to determine the effects of soil and environmental conditions on the degradation of PLA by resident microbes in ACRES soil.

Phase 1:

As Phase 1 will be devoted to the isolation and identification of PLA degrading microbes in ACRES Stage 2 compost, standard soil microbe isolation techniques will be used. We will perform a standard enrichment using stage 2 ACRES compost, combining 10 grams of compost with 500 ml of TSB for a 2% weight/volume enrichment (Becton Dickinson, 2014). We will divide the enrichment into two 250 mL flasks and incubate shaking at 30ºC and 37ºC respectively for 24 hours to induce bacterial growth into the logarithmic phase. Next, we will use an M9 nutritional medium adapted to be selective for PLA degraders. The M9 medium will be made selective for PLA degraders by emulsifying PLA in order to make a film that will serve as the sole carbon source in the medium (Naiounakis, 2014). After plating each enrichment onto two M9 PLA plates, we will incubate the plates at 30ºC and 37ºC according to enrichment temperature to accommodate the growth requirements of Pseudomonas spp., Cryptococcus spp., and Bacillus spp. After growth is observed on the plates, we will perform standard biochemical assays to differentiate microbes within our culture. In particular, we will subject isolates to a saline/chloramphenicol solution and plate on a Niger medium to isolate Cryptococcus spp (Teodoro et al., 2013). We will subject isolates to a catalase test, a gram stain, and a culture on Schaedler Blood Agar (SBA) to identify Bacillus spp. (Paola et al., 2011). We will perform a Gram stain on isolates and culture them on Pseudomonas agar for the identification of Pseudomonas spp. (Sigma-Aldrich, 2017). Species that grow on the PLA selective media but are not further classified as Pseudomonas, Cryptococcus, or Bacillus, will be designated "Other." By accomplishing the above, we will be able to conclusively note the presence of PLA degrading



microbes in stage 2 ACRES compost pursuant to the objectives of this work.

Figure 3: Represents the approach taken to accomplish Phase 1 of our research.

Phase 2:

Phase 2 will be devoted to examining the impact(s) of environmental conditions on the degradation of PLA. Broadly speaking, three independent variables, temperature, compost pH, and compost moisture content (% weight),will be examined for their effects on resident microbes' ability to degrade PLA. We will obtain stage 2 compost from ACRES Student Farm (a

minimum of 2 lbs), 16 oz polypropylene specimen cups, PLA granules, a pH meter, an analytical scale, and parafilm. Ten grams of unsterilized compost will be added to 30 separate polypropylene specimen cups with pH and moisture content adjusted for the appropriate treatment group. Adjustments to pH will be accomplished using citric acid (to lower the pH) and lime (to raise the pH). Adjustments to moisture content will be accomplished using sterile distilled water. Two grams of PLA granules (weighed and counted) will be added to each specimen cup. A control sample will be established for each pH group (maintained at ACRES moisture content) that will consist of 10 grams of sterile compost and 2 grams of PLA granules. Establishing such a control will allow us to note the effects of the pH and temperature on PLA degradation; however, we will not adjust the moisture content of the control group as PLA is an insoluble polymer, so concerns over spontaneous hydrolysis are moot. The resulting treatments will be labeled according to their pH, water content, and PLA addition before being sealed with parafilm to allow gas exchange and maintain moisture content. Next, the samples will be incubated at either 30ºC or 37ºC according to their assigned treatment groups. The incubation period will last exactly four weeks after which the PLA granules will be separated from each treatment, washed, and weighed to assess percent weight loss. Using these data, we will determine which conditions result in the greatest degradation of PLA by resident microbes in order to provide recommendations to ACRES pursuant to our objectives. Figure 4 offers a visual depiction of the experimental setup described for Phase 2.

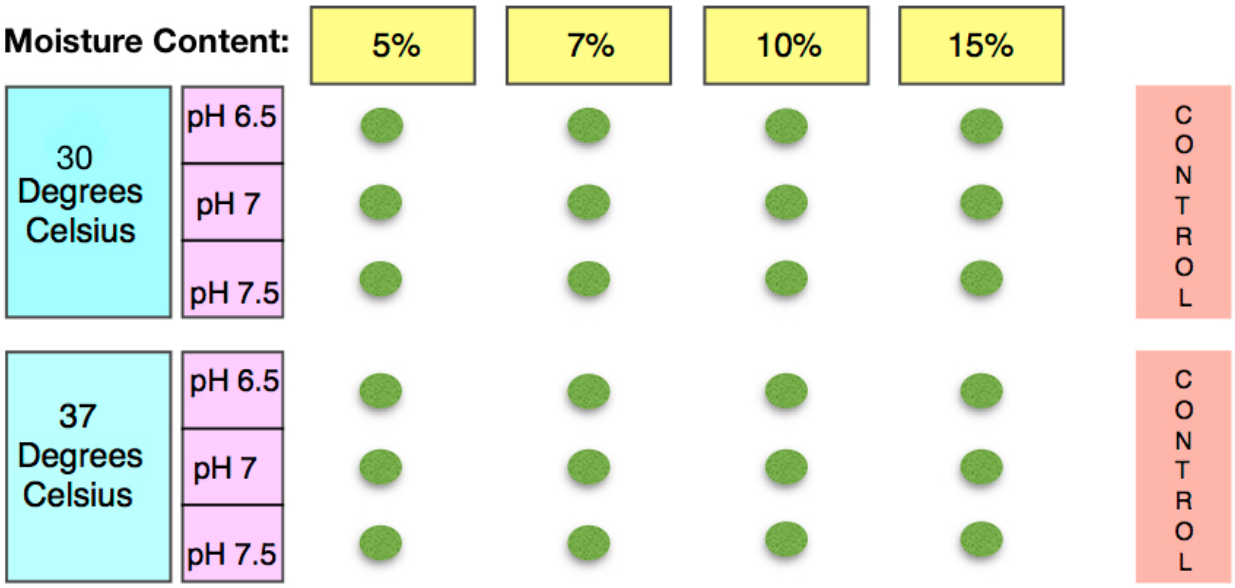


Figure 4: Displays the experimental design that will be utilized to accomplish Phase 2 of the research.

Data Collection & Analysis:

Data regarding the degradation of PLA will be obtained by determining the change in mass of PLA over the 4 weeks of incubation of the compost. Ultimately, this will speak to which environmental conditions best facilitate the degradation of PLA. Additionally, through the use of

the M9/PLA media followed by the selective media, we will be able to effectively collect data on which genera of microbes within the compost can metabolize PLA. All records of this experiment will be kept in the official Capstone Laboratory Notebook, which will remain secure in the University of Wyoming College of Agriculture, room 5028. The numerical data will additionally be stored in a secure, online collective database set up using Google Docs. We will then compare levels of PLA degradation and perform statistical analysis to see if any changes are significant. These tests will include standard deviation, a chi-squared test and p-value determination.

Expected results:

We expect that the ACRES stage 2 compost will contain either *Pseudomonas*, *Cryptococcus*, or *Bacillus* species, if not a combination of the three. As these are common soil microbes known to degrade PLA, we assume that they will be present in the compost and will participate in the degradation of PLA. However, if this is not the case, then we will have to be ready to isolate other microbes that are capable of PLA degradation from the compost bins. We will then have to consider additional biochemical and morphological tests in order to determine which genus this/these species belongs to.

However, operating under the assumption that the compost does have a microbe capable of degrading PLA, we expect that certain conditions will favor PLA degradation more than others. We expect that a temperature of 37 ºC, pH of 7.5, and water content of 15 % will provide the greatest PLA degradation of the treatment groups. This is due to the fact that *Cryptococcus spp*. prefer a slightly basic environment, and *Bacillus spp*. secrete an alkaline protease known to degrade PLA. While possible, it is unexpected that modulations to the environment will fail to have any effect on PLA degradation. Should that be the case, we may elect to reexamine the variables we selected across a larger range in order to better capture variations in PLA degradation.

Based on our findings, we expect to be able to provide suggestions to ACRES Student Farm regarding practical methods which can be used to compost bioplastic waste from the University of Wyoming, thus preventing bioplastic from accumulating in the landfill and providing a fuel source for local food growth. Additionally, we will generate data for bioplastic degradation at a specific particle size and thus speak to the potential of mechanical shredding to that level, which may augment the ability of smaller institutions to compost PLA packaging products.

Timeline:

Activity	**Week 0**	**Week1**	**Week 2**	**Week 3**	**Week 4**
M9 Media Brew	XXX				
PLA Emulsion	XXX				
Gather Compost	XXX				
Enrich Compost	XXX				
Determine Compost Moisture Content	XXX				
Inoculate M9 PLA from enrichment		XXX			
Wash and Weigh PLA		XXX			
Adjust Compost pH		XXX			
Assemble PLA/Compost Treatments		XXX			
Begin Compost/PLA Incubation Cycle		XXX			
Communicate Plan with Stakeholders		XXX			
Plate from M9 PLA onto Niger Agar (+saline/Chloramphenicol)			XXX		

Plate from M9 PLA onto SBA Agar			XXX		
Plate from M9 PLA onto Pseudomonas Agar			XXX		
Conduct Differential Assays from Niger Agar				XXX	XXX
Conduct Differential Assays from SBA Agar				XXX	XXX
Conduct Differential Assays from Pseudomonas Agar				XXX	XXX
Assemble Resident Microbe Report					XXX
Disperse Resident Microbe Report to Communicate with Stakeholders					XXX

Timeline Continued:

Activity	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Week 9**
Liaise with Engineering to Develop Strategic Partnership with ACRES for PLA Grinding.	XXX				
Remove Compost/PLA treatments from incubation.	XXX				
Wash & Weigh PLA granules from treatment groups to determine percent degradation.	XXX	XXX			
Begin Running Chi-Squared analysis for PLA degradation across treatment groups.		XXX	XXX		
Assemble Degradation Report			XXX		
Begin Assembly of Poster to Communicate results to Stakeholders			XXX	XXX	

Preliminary Communication of Results to Stakeholders				XXX	
Dispatch Final Report					XXX
Communicate Results to Stakeholders and UWYO Community.					XXX

References:

Auras, R. Poly(lactic acid). "Encyclopedia of Polymer Science and Technology." *Abstract*. 2010.

Was found using the UW Library database after discovery on Google Scholar. Gave appropriate information on the composition of PLA and how it is manufactured.

Becton Dickinson. "Tryptic Soy Broth (TSB)" *INSTRUCTIONS FOR USE – READY-TO-USE BOTTLED MEDIA* (2014): Available at http://www.bd.com/resource.aspx?IDX=30505

Found through a Google search in an attempt to examine the use of TSB in enrichment. This will be a great resource for us as it describes TSB as being a general purpose medium for enrichment of a wide array of organisms. This confirms our interest in TSB for our enrichment.

Dengler, R. Humans have made 8.3 billion tons of plastic. Where does it all go? PBS. Public Broadcasting Service. 2017.

Found on Google Scholar while searching for information relating to plastic waste. Communicates the shocking reality of plastic waste accrual.

Fukushima, K., Abbate, C., Tabuani, D., Gennari, M., Camino, G. "Biodegradation of poly(lactic acid) and its nanocomposites." Polymer Degradation and Stability, Volume 94, Issue

10, Pages 1646-1655. 2009.

Was found using the UW Library database after discovery on Google Scholar. Gave information on bacteria that may be able to degrade PLA.

Gupta, R., Beg, Q., Lorenz, P. "Bacterial alkaline proteases: molecular approaches and industrial applications. Applied Microbiology and Biotechnology." Issue 59. 2002.

This article was located through a Google scholar search in an effort to identify the optimal pH for secreted alkaline proteases capable of degrading PLA. This article is useful to us because it informs us that a pH greater than 7 will likely produce the greatest PLA degradation.

Kolstad, J., Vink, E., Wilde, B., Debeer, L. "Assessment of anaerobic degradation of Ingeo polylactides under accelerated landfill conditions" Elsevier. 97: 1131–1141. 2012.

This resource was accessed through the Wyoming Web of Knowledge database at the University of Wyoming. It was published in 2012. This will contribute to our research because it helps us understand how the bioplastics degrade in landfills, and how they affect the environment

Niaounakis, Michael. "Biopolymers: Processing and Products." Elesvier Incorporated. Waltham, Massachusetts. pp 188. 2015.

Found upon searching Google Scholar for a method that lends to the creation of PLA films. Will be critical in developing a selective M9 medium for the isolation of PLA degraders.

Ravi, S., Pierce, C., Witt, C., & Wormley, F. L. "Biofilm Formation by Cryptococcus neoformans Under Distinct Environmental Conditions." Mycopathologia, 167(6), 307–314. 2009.

This resource was located through a search of the UWYO libraries database for information relating to PLA degradation by Cryptococcus spp. under various pHs. This is a critical article for our work because it explores the optimal pH for Cryptococcus and informs our treatment groups.

Recycling Coalition of Utah. RCU. 2017. https://utahrecycles.org/get-the-facts/the-facts-plastic/

This article was located via a google search attempting to locate information pertaining to U.S. recycling. It is useful to us in that the resource provides a good "shock-factor" to reel in our readers and underscore the importance of our work.

Rossi, V., Cleeve-Edwards, N., Lundquist, L., Schenker, U., Dubois, C., Humbert, S., Jolliet, O. "Life cycle assessment of end-of-life options for two biodegradable packaging materials: sound application of the European waste hierarchy." Journal of Cleaner Production, Volume 86, Pages 132-145. 2015.

This article was located through a google scholar search in an attempt to identify the impacts on the environment of PLA degradation under various treatments. The resource is useful to us because it relates the stark outcomes associated with PLA ending up in the landfill.

Sigma-Aldrich. "Pseudomonas Media and Tests." Sigma-Aldrich. 2017. http:/www.sigmaaldrich.com/technical-documents/articles/analytix/pseudomonas-media.html

This reference provides information relating to Pseudomonas spp. isolation. It was accessed on the google scholar database. This will contribute to our research because it provides an easy method for Pseudomonas spp isolation

Teodoro, L., Gullo, P., Sardi, J., Torres, M., Fusco-Almeida, M., Mendes-Giannini, M. Jose, S. "Environmental isolation, biochemical identification, and antifungal drug susceptibility of Cryptococcus species." Rev. Soc. Bras. Med. Trop. 46(6): 759-764. 2013.

This reference provides information relating to Pseudomoonas spp. isolation. It was accessed from the google scholar database. This will contribute to our research because it provides an easy method for the isolation of Cryptococcus spp.

Todar, K. Todar's Online Textbook of Bacteriology. Pp 4. 2015.

This resource was located through a Google scholar search. It is useful to us in that it relates the effects of pH on Pseudomonas spp. Growth. In particular, it demonstrates the diversity of optimal pHs amongst Pseudomonas spp., which informs our treatment groups further.

Tsuji, A., Kaneko, Y., Takahashi, K., Ogawa, M., Goto, S. The effects of temperature and pH on the growth of eight enteric and nine glucose non-fermenting species of gram-negative rods. Microbial Immunology. 26(1): 15–24. 1982.

Discovered via a Google scholar search. This article will be useful to us as it relates the optimal temperatures and pHs for Bacillus spp. and Pseudomonas spp. Again, this helps to inform both our temperature and pH treatment groups.

University of Utah. How Much Do Americans Throw Away? Courses: Architecture 4011. University of Utah. N.D.

Discovered through a Google Search aiming to identify the extent to which recycling eases the burden of waste. Provides another good "Shock-Factor."

Warth, A. “Relationship between the heat resistance of spores and the optimum and maximum growth temperatures of Bacillus spp.” Journal of Bacteriology. Volume 134. p 695-705. 1978

Discovered via a Google scholar search. This article is especially relevant to our research as it demonstrates the optimum temperature for growth of Bacillus spp. Ultimately, this informs our temperature treatment groups.

Williamson, Joey. “Changing the pH of Your Soil.” Clemson University, North Carolina. 2012.

Discovered using a Google search aiming to identify methods for altering the pH of soil. This informs our protocol for adjusting the stage 2 compost pH for our treatment groups.