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Degradation of biodegradable products in simulated home compost environments

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Degradation of biodegradable products in simulated home compost environments

by

Jaclyn Neubauer

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Science

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Abstract

Due to the persistent use and disposal of single-use plastics, plastic waste has become the second most prevalent material found in landfills, trailing only food waste. The adoption of biodegradable alternatives to single-use plastics could introduce composting as a sustainable method of disposal. The composting of these materials alongside food waste would effectively reduce the accumulation of plastic and food waste in landfills. Products that are marketed as biodegradable and compostable were evaluated to determine the percentage of weight loss that occurred when they were buried in soil and soil amended with 30% food waste. The impact of forced aeration was also studied. Burial results indicated that among the tested materials, only the cellulose-based products and one starch-based food waste bag met the required composability standards. Microbial culturing and CO₂ evolution data revealed that the addition of food waste enhanced both microbial diversity and biodegradation processes within the compost. However, a significant change in CO₂ production due solely to the biodegradation of polymer samples was not observed.

Chapter 1. Introduction

1.1. Overview

In 1907, the first synthetic plastic was introduced and marketed as the "material of a thousand uses [1]." However, its wide use did not begin until the 1960's when plastic gained popularity as packaging materials [2]. During this time, approximately 15 million tons of plastic per year was produced worldwide, and in 2015, this number has reached over 300 million tons per year [3]. Consequently, this large consumption of plastic has led to a large generation of plastic waste [4]. Plastic accounts for nearly 13% of municipal waste produced in the US and makes up approximately 19% of waste in landfills [5]. This makes plastic the $2nd$ most abundant material in landfills behind food waste [5]. However, unlike organic food waste, plastic will spend centuries on earth before it is remotely gone, leaving behind chemicals that have an unknown long-term effect on our environment [6].

Figure 1. Accumulative plastic waste and their method of disposal [7].

Recycling has been widely promoted as a means of prolonging the lifespan of plastics and reducing the volume of landfills. However, because recycling depends on the actions of individuals and businesses, only a small percentage of plastic waste in the U.S., 9%, actually gets recycled [7] [8]. This means that the vast majority of plastic waste ends up either incinerated (12%) or landfilled (79%), both of which produce high amounts of greenhouse gases [2]. Moreover, when plastic waste is not properly disposed of, it often ends up in the environment where it can degrade in nearby bodies of water [9].

One potential solution to alleviate the environmental impact of plastic disposal is to substitute conventional synthetic polymers with biodegradable polymers. These polymers can be broken down by naturally occurring organisms through chemical means [10]. Such a shift creates an additional avenue for disposal: composting.

1.2. Composting Standards

For a material to be marketed as biodegradable or compostable, it must satisfy a specific set of criteria. Compostable polymers, according to the American Society for Testing and Materials (ASTM), must decompose by the means of naturally occurring aerobic microorganisms [11]. ASTM 6400 also requires that the degradation of the material must yield CO2, water, inorganic compounds, and biomass. They must leave no residue that is visible, distinguishable, or toxic [12]. The BPI Compostable label is verification of composability by ASTM standards in North America [13]. Single polymers are required to degrade 60% by 180 days in compost and polymer blends 90% [14]. These tests however take place in industrial compost, thus do not guarantee their breakdown in a home compost setting.

The European standard (EN 13432) for biodegradable polymers follows the same criteria as ASTM 6400 [15]. Products may be labeled with a "seedling" denoting their certification by European Bioplastics or by TUV Austria labeled with "OK Compost" [15][16]. Similar to the American standard, these certifications only guarantee biodegradability in industrial compost, which takes place at temperatures between 50 \degree C and 60 \degree C [16][15]. However, TUV Austria developed the "OK Compost HOME" certification which ensures that a product can biodegrade in a smaller scale compost with lower temperatures, below 30 $^{\circ}$ C, similar to that of a home compost [16][17]. Biodegradable products may also be labeled as OK Biodegradable SOIL, confirming that material will completely degrade in soil without harm to the environment [18]. Products made sugar cane and paper are certified as commercially compostable and are often not labelled with their approved standards.

Figure 2. Certified compostable and biodegradable labels [12][15][16][17].

1.3. Potential in Home Compost

Promoting the use of biodegradable products to the average consumer and composter would tackle two critical sustainability issues - plastic waste and food waste. Biodegradable and compostable polymers could substitute single-use plastic products, including food containers, waste bags, and mulch films. Incorporating food waste into the composting process along with these biodegradable products would not only decrease the amount of food waste ending up in landfills, but also introduce additional microorganisms into the compost [19].

If the average consumer starts using and composting these biodegradable products, would a typical home compost pile be capable of fully decomposing them in accordance with their certifications? Furthermore, would the inclusion of food waste accelerate their degradation, and would providing additional oxygen in the form of forced aeration have a similar effect? This study aims to answer these questions by investigating the degradability of several biodegradable products under simulated home composting conditions.

Chapter 2. Material and Methods

Three key experiments were carried out to evaluate the degradation of materials: burial experiment, CO² evolution, and microbial culturing and identification. Products marketed as biodegradable and compostable were tested (as listed in *Table 1*). Degradation rates were compared among the products and between burial conditions. The two burial media compared were Soil and Food Waste.

Table 1. Materials tested throughout experiments.

Table 2. Composition of burial media used across all experiments. *Collected food waste included: lettuce, onions, lemons, banana peels, avocado skin, orange peels, asparagus, cabbage, strawberries, and apples.

2.1.1. Initial Burial Experiment

Experiments conducted on biodegradable agricultural mulch films in Soil (*Table 2*) were the first burial tests performed. The experiment monitored the percentage of weight loss of film Dewitt and Dubois Innovations over a period of 137 days following methods of similar studies [21][22].

Nine 7.6 cm² samples were cut of each polymer and their initial masses were recorded. 5gallon bins were filled halfway with soil, followed by laying four strips of mesh over the soil. Triplicate samples of each mulch film were then placed on each strip shown in *Figure 3*. The remaining soil was carefully added to fill the bins, and a sheet of aluminum foil or clear plastic was placed over the top. A total of three bins were prepared, and labeled $T_1 - T_3$, indicating the order in which the samples were removed for weight loss analysis. Bins were stored indoors.

Samples were removed at 30, 61, and 137 days. Samples were removed, carefully, by removing the soil on top of the samples, brushing the samples off from remaining soil, washed with an ethanol solution, and dried for 4 hours at 60 $^{\circ}$ C. The samples were then weighed, and the percent loss for each were calculated.

Following the completion of the mulch film burial, this procedure was repeated with three food scrap bags that are marketed as biodegradable and compostable: BioBag, Unidomum, and Green Earth. The burial of the Dewitt mulch film was repeated as it completely degraded prior to the first removal of the first experiment. In contrast, twelve 7.6 cm^2 samples were cut of each material, triplicate samples distributed in four 5 -gallon bins, labeled T_1 -T₄. Scrap bag and Dewitt samples were removed at 22, 50, 84, and 128 days. Samples were removed from the soil and cleaned in the same manner, and their percent weight loss were calculated.

Figure 3. Initial Burial experimental layout.

Figure 3 illustrates the initial burial set up for one time checkpoint. 5-gallon bins were filled halfway with Soil, mesh strips were laid across, and triplicate 7.6 x 7.6 cm samples of materials were placed on each strip. Soil was then carefully added until the bin was full, and a plastic sheet was placed over the bin (not pictured) to prevent loss of moisture.

2.1.2. Revised Burial Method

To expand the scope of the project, maximize time and minimize materials, the burial method was revised. In addition to the mulch films and scrap bags, the analysis of Egg Cartons and Clamshell food containers were added to the experiment. The burial media tested was expanded to include Food Waste (*Table 2*). Also, to supply additional oxygen to microbes responsible for aerobic degradation, the effect of forced aeration was added to burial experiments.

The percent weight loss BioBag, UniDomum, Green Earth, Dewitt, Dubois, Clamshell, and Egg Carton were monitored in four different compost environments: Non-Aerated Soil, Non-Aerated Food Waste, Aerated Soil, and Aerated Food Waste. For the Non-Aerated Soil experiment, only Clamshell and Egg Carton was monitored as data for the other polymers had already been collected.

To maximize the number of samples in for each experiment, while reducing the number of bins used, mesh bags were made to fit triplicates of each polymer (*Figure 4*). This allows for the vertical placement in compost mixture as well as the quick removal of sample, with minimal loss of material.

Figure 4. Triplicate polymer samples in mesh envelope. (Photo by author)

Twelve samples were prepared for each polymer for each experiment. Wires, approximately half a meter long, were cut and attached to the top corner of the mesh envelopes. The end of the wires were tagged and labeled with the appropriate product title and its designated order of removal (T_1-T_4) .

Non-aerated composts were set up in small bins (5 or 7 gallon). Like the initial burial technique, bins were filled halfway with their respective compost mixture, then rather than placing the mesh bags on top, they were placed in vertically about an inch and a half apart as shown in *Figure 5*. Bags were placed and grouped by product, rather than by time of removal (*Figure 8*). The bin was then filled, with the remainder of their compost mixture, and covered with a sheet of plastic.

Figure 5. Mesh bag placement in Non-Aerated burial experiments. (Photos by author)

As displayed in *Figure 6 A.*, Aerated composts were contained in one 50-gallon tub, that was split down the middle by a sheet of plastic. On either side, PVC pipes, with drilled holes, were placed on the bottom and connected to air pumps. Air pumps were turned on between the hours of 5 pm and 5 am, as to not disturb staff and faculty during working hours. Mulch was

spread over the top on the pipes to prevent the clogging of holes and the even distribution of air flow (*Figure 6 B.*).

Figure 6. Aeration system used in Aerated burial experiments. (Photos by author)

Displayed in *Figure 7*, both sides were then filled approximately halfway with their respective composts, Soil (A.) and Food Waste (B.). Mesh bags containing the triplicate samples were then placed vertically, grouped by polymer (*Figure 8*). The remaining mixture was then placed on top to fill the container.

Figure 7. Mesh bag placement in Aerated burial experimental. (Photos by author)

Figure 8. Experimental set up of revised burial experiments.

Figure 8 illustrates the experimental setup for revised burial experiments, where triplicate polymer samples were placed in mesh envelopes within four different compost environments: Non-Aerated Soil, Non-Aerated Food Waste, Aerated Soil, and Aerated Food Waste. The diagram showcases the placement of four envelopes for each polymer type, with four designated time checkpoints. In Non-Aerated Soil, polymers that were tested in the initial burial experiment only had one envelope of samples to use in microbial culturing.

Upon removal, samples were carefully removed from the mesh envelopes, cleaned, and then dried for 1 day at 30 $^{\circ}$ C.

The percent of decomposition by weight was calculated with the same equation. Used in the Initial Burial experiment:

% decomposition =
$$
\frac{w_0 - w_t}{w_0} X 100
$$

Where *w⁰* is the initial weight of the polymer sample and *w^t* is the weight of the sample following burial, cleaning, and drying.

2.1.3. Soil Sampling

500mL samples of the burial media were sent to Dairy One labs in Ithaca, NY, where it was analyzed for pH, organic matter, phosphorus and potassium [23].

Soil samples were taken of the Soil and Food Waste prior to burial experiments, and the Non-Aerated Soil, Non-Aerated Food Waste, Aerated Soil, and Aerated Food Waste following the conclusion of burial experiments.

2.2. CO² Evolution

To further investigate and compare the rate of biodegradation of polymers in Soil and Food Waste environments, the CO₂ evolution was measured using biometer flasks. This experimental protocol to measure CO₂ production is outlined by EPA guidelines [24].

Triplicate 2.5 cm^2 samples of each polymer were cut, and their initial masses were recorded. The samples were then placed in the Erlenmeyer portion of the flask along with 50g of burial media. A set of controls were prepared, three flasks containing only the Soil or Food Waste compost without a polymer sample. For all flasks, the trap was filled with 10ml of a 0.7 M Potassium Hydroxide (KOH) solution, using a syringe, and stoppered. The tower was filled with ascarite to prevent external CO₂ from entering the system but allowing air flow during titrations. The flasks were incubated at room temperature for the entirety of the experiment.

Periodically, the KOH solution in the trap was removed and replaced with another 10 ml of the 0.7 M KOH using a syringe. The removed solution was transferred to a sealed flask to be titrated. Another 10 ml of the 0.7 M KOH is added to a separate flask to be used as the blank. To all the recovered KOH solutions, a few drops of the pH indicator, phenolphthalein (1%), were added. The KOH solutions are then titrated with a solution of hydrochloric acid at a concentration of 0.25 M. By measuring volume of 0.25M HCl solution required to titrate both solutions, the amount of $CO₂$ by mass (mg) produced can be calculated using the following equation $[24][25]$:

$$
mg\ CO_2 = (V_c - V_E)(M_{CO_2})(M_{HCl})(CF)
$$

Correction Factor = CF = $\frac{M_{HCl}}{M_{KOH}}$
 V_b = vol of HCl to neutralize blank
 V_E = vol of HCl to neutralize experimental

This calculation was performed on all experiments as well as the controls. To calculate the CO² produced solely by the microbial degradation of the samples and not microbial degradation within the compost matrials themselves, the $CO₂$ production of the controls were subtracted. The running total of $CO₂$ produced was then calculated.

One way analysis of variance $(ANOVA)$ was performed on $CO₂$ evolution data for each polymer to determine if difference in burial environments, Soil and Food Waste, yielded significantly different results. The linear slope of each flask's $CO₂$ production over time were calculated, then analysis was performed in the statistical software JMP.

Figure 9. Experimental set up of biometer flask for the CO2 evolution of polymers.

Shown in *Figure 9*, in a biometer flask, 2.5 cm sample of polymers were placed in 50 g of compost mixture and the trap filled with 10 ml of 0.7 M KOH solution. The Erlenmeyer portion of the flask is stoppered along with an ascarite tower and stopcock. The trap is enclosed by a needle and stopper. (Photo by author)

2.3 Microbial Cultures

Microbial culturing was performed to evaluate the microbial composition and diversity of all burial media following the burial of polymer samples. At the conclusion of burial experiments, 1 gram of compost was taken from the surface of all T₄ polymer samples in all composts (Aerated and Non-Aerated Soil and Food Waste). Compost samples were taken by removing compost from directly outside of the mesh envelopes, where the polymer samples were, as well as scraping any compost that reach the polymers inside of the mesh envelopes. The 1-gram compost sample was then placed in a test tube and diluted with 9 ml of a 0.9% saline solution. Serial dilutions of soil were performed (Figure A.1.). Swabs were also taken from the surface of all the T₄ polymer samples.

Fungal cultures were plated on potato dextrose agar (PDA) containing 100ug/ml streptomycin and bacterial cultures on plate count agar (PCA). Soil sample dilutions were plated at $10^{-2} - 10^{-4}$ on PDA and $10^{-4} - 10^{-6}$ on PCA. Food Waste sample dilutions were plated at $10^{-3} 10^{-5}$ on PDA and $10^{-5} - 10^{-7}$ on PCA. All plates were incubated at 23^oC for 6 days. Plate counts were performed by hand and diversity was measured. Diversity was determined by looking at colony morphology, size, and color for both fungi and bacteria. Fungal cultures were identified phenotypically under a microscope [26].

Chapter 3. Results: Burial Decomposition

3.1. Overview

The biodegradation of the chosen polymers was evaluated in four different burial environments. Weight loss of samples were measured throughout their degradation in nonaerated soil, non-aerated food waste, aerated soil, and aerated food waste at timed intervals. Done in triplicates the average percent weight loss were calculated and shown in *figures 10, 11, 12, 13, and 14.*

3.2.1. Initial burial in Non-Aerated Soil

Table 3. Burial experiment set ups.

Table 3 describes the parameters used in each burial experiment. Whether the experiment took place in Soil or Food Waste, the presence of forced aeration, the materials that were tested, the length of the experiment, sampling method, and the size of the samples.

In the initial burial experiment, cellulose based Dewitt mulch film degraded at the fastest rate and had completely broken down by 30 days (*Figure 10*). According to *Figure 10*, BioBag reached a maximum percent weight loss $(21.2%)$ by day 50, but T₄ samples had only lost 14.0% on day 128. Similarly, UniDomum and Green Earth measured a maximum weight loss on day 84 (13.2% and 13.7%), but their final samples removed at 128 days had 11.8% and 8.3% weight losses, respectively. Over 138 days, Dubois has weight loss of 7.5%.

Figure 10. Percent weight loss of polymers in Initial Non-Aerated Soil burial experiment.

Figure 10 displays the percent weight loss over time of BioBag, UniDomum, Green Earth, Dewitt, and Dubois in Non-Aerated Soil environment.

In the revised Non-Aerated Soil burial experiment, only Clamshell and Egg Carton were monitored for percent weight loss. This data is shown in *Figure 11* along with the data collected from the initial burial experiment for comparison. Over 75 days, Clamshell broke down by 93.2% and Egg Carton by 35.2%.

Displayed in *Figure 12*, Dewitt had the greatest percent weight loss by the conclusion of experiment 4, 75 days, losing, on average, 99.2% of its original mass. Following Dewitt, Clamshell had degraded 96.8% by the end of the experiment. In contrast to the Non-Aerated Soil environment, BioBag had lost more mass by the conclusion of the experiment in the Non-Aerated Food Waste. On average, UniDomum (16.7%), Green Earth (27.2%), and Dubois (2.4%), showed the lowest amount of weight loss by day 75. Dubois, although losing weight at the first (T_1) , experienced no loss for T_2 and T_3 samples.

After 73 days, polymers in Aerated Soil followed the same ranking as those in Non-Aerated Soil. As shown in *Figure 13*, Dewitt had lost 55.4% of its original mass by day 53, which decreased to 42.3% on day 73. Following Dewitt, with descending percent weight loss Clamshell (39.2%), Egg Carton (30.6%), BioBag (10.2%), UniDomum (8.5%), Green Earth (8.0%), and Dubois (5.0%).

According to *Figure 14*, By the final day of the experiment, Clamshell samples had completely degraded, and Dewitt had few remnants with an average loss of 99.6%. BioBag (91.7%) had the greatest loss of weight in the Aerated Food Waste than any other burial environments. Egg Carton lost 35.2% by day 73. Green Earth (18.0%) and UniDomum (15.9%) observed similar weight loss throughout experiment #6. Following the same trend as the other burial environments, Dubois lost the least weight with only 4.5%. A summary of these results is provided in *Table 4*.

Figure 11. Percent weight loss of polymers in Non-Aerated Soil burial experiment.

Figure 13. Percent weight loss of polymers in Aerated Soil burial experiment.

Figure 12. Percent weight loss of polymers in Non-Aerated Food Waste burial experiment.

Figure 14. Percent weight loss of polymers in Aerated Food Waste burial experiment.

Figures 11-14 illustrates the percent weight loss over time of polymers in Non-Aerated Soil, Non-Aerated Food Waste, Aerated Soil, and Aerated Food Waste respectively. Each graph shows the loss of all polymers tested in that environment: BioBag, UniDomum, Green Earth, Dewitt, Dubois, Clamshell, and Egg Carton.

Table 4. Maximum percent weight loss of various polymers in all burial environments.

Table 4 displays the maximum percent weight loss observed of BioBag, UniDomum, Green Earth, Dewitt, Dubois, Clamshell, and Egg Carton in burial environments: Non-Aerated Soil, Non-Aerated Food Waste, Aerated Soil, and Aerated Food Waste. The table provides information on both the maximum percent weight loss and the corresponding weight loss in grams. Additionally, the length of burial in days needed to achieve these results is also included. Burial lengths marked with an asterisk (*) indicate that the maximum percent weight loss was observed prior to the final removal of samples, but the final values recorded were lower.

In Soil environments, cellulose based Dewitt, Clamshell, and Egg Carton, had the greatest percent weight loss among all polymer samples. However, in the Food Waste environments, starch based BioBag had surpassed Egg Carton. BioBag in all composts, had the greatest percent loss of mass among the starch-based polymers. In all burials, Dubois exhibited the lowest percent weight loss. In the Aerated Food Waste burial, despite have visible holes in its surface, a negative weight loss was recorded for T₂ and T₃ samples, see *Appendix Figure A5*.

According to *Figures 15-21*, most polymers had greater rates of degradation in an environment containing Food Waste. Green Earth, Clamshell, and Egg Carton showed greatest rates in Non-Aerated Food Waste and BioBag, UniDomum, and Dubois in Aerated Food Waste. Dewitt degraded the quickest in the initial Non-Aerated Soil experiment, however, in the initial burials, polymer samples were not placed in mesh bags, so it had greater surface area exposure to the burial media than in later experiments.

For all polymers, excluding Dubios, the rate of degradation was slowest in the Aerated Soil environment. Although aeration introduces more oxygen to the environment, promoting aerobic degradation, it can also cause the cooling and drying of the compost [27]. It also should be noted that as polymers visually degraded, an increase in weight loss was not recorded. This may be due to an increase in soil adhesion as the experiment progressed.

Figure 15. Percent weight loss of BioBag in all burial environments.

Figure 17. Percent weight loss of Green Earth in all burial environments.

Figure 16. Percent weight loss of UniDomum in all burial environments.

Figure 18. Percent weight loss of Dewitt in all burial environments.

Figure 19. Percent weight loss of Dubois in all burial environments.

Figure 21. Percent weight loss of Egg Carton in all burial environments.

Figure 20. Percent weight loss of Clamshell in all burial environments.

Figures 15-21 present the percent weight loss over time for BioBag, UniDomum, Green Earth, Dewitt, Dubois, Clamshell, and Egg Carton, respectively. Each graph shows the data collected from all four burial environments: Non-Aerated Soil, Non-Aerated Food Waste, Aerated Soil, and Aerated Food Waste. Dotted lines represent linear fit.

Linear trendlines were fit to the percent weight loss over time of the polymers in all burial environments shown in *Figures 15-21*. Using the slope, the predicted time to break down by 100%, 90%, and 60% [13] were calculated and displayed in *Table 5*. Of the tested polymers, Dewitt and Clamshell, both cellulose-based, are predicted to break down by the desired amounts within 180 days. Egg Carton would completely break down in less than 6 months in all environments except for Aerated Soil, for which it would degrade by 60% in 148 days. According to the slope of its weight loss, BioBag would break down completely within 180 days only for samples in Food Waste, not in Soil. By 6 months, Green Earth would only break down by 60% in Non-Aerated Food waste, not in any other environment. Neither UniDomum nor Dubois, was predicted to break down within the allotted time for all burial environments. Based on this study, only Dewitt, Clamshell, Egg Carton, and BioBag meet the standards for composability of which they are certified for.

Table 5. Predicted degradation of polymers in all burial environments.

Table 5 showcases the calculated predicted times for 60%, 90%, and 100% degradation of BioBag, UniDomum, Green Earth, Dewitt, Dubois, Clamshell, and Egg Carton in burial environments: Non-Aerated Soil, Non-Aerated Food Waste, Aerated Soil, and Aerated Food Waste. These predictions are derived using the linear fit lines obtained from *Figures 15-21.* Values with an asterisk (*) indicate that samples were predicted to break down by the desired percentage within 180 days.

3.6. Burial Media Soil Analysis

Single samples of burial media prior to burial and sample following the conclusion of the burial experiments were sent to Dairy One labs for soil analysis. The analysis results revealed that both T_0 Soil and T_0 Food Waste had greater pH values than those following the experiments, 8.29 and 8.36, respectively. Non-Aerated Soil had a pH of 7.02 and Non-Aerated Food waste 6.34, which was the lowest pH measure among samples. Non-Aerated Food Waste also had the only pH that was in the desired range for growing most vegetables, 6.0-7.0, all others were considered high [28].

According to *Table 6*, Soil Organic Matter, which provides Nitrogen to the soil, was greatest in Non-Aerated Soil $(32.56%)$ and lowest in T₀ Soil. The calculation of percent carbon was derived from the assumption that organic matter contains 58% organic carbon [29]. At the beginning of compost, we assume that that carbon nitrogen ratio (C/N) is 30/1 and as this carbon is converted into carbon dioxide, C/N is assumed to decrease to 10/1 [30]. From these ratios, the percent nitrogen content was calculated for the initial burial media and the final compost samples. According to Cornell University, all samples had high levels of Potassium and Phosphorus [28].

Table 6. Results of Soil Analysis from Dairy One of various composting systems.

Table 6 presents the soil analysis results provided by Dairy One Labs for the initial burial media of Soil and Food Waste, as well as samples collected from Non-Aerated Soil, Non-Aerated Food Waste, Aerated Soil, and Aerated Food Waste at the conclusion of the burial experiments. The analysis includes key parameters such as pH, percent Organic Matter, Potassium, and Phosphorus. Percent Carbon and Nitrogen were derived from Organic Matter values.

Chapter 4. Results: Carbon Dioxide Evolution

4.1. Overview

The CO² production from the degradation of polymers was determined by utilizing biometer flasks and a 0.7 M KOH solution. In timed intervals, as the pH of the KOH solution is reduced, the solution is titrated with hydrochloric acid and the mg of $CO₂$ produced during that interval was calculated. Over approximately 2 months, the average $CO₂$ production was determined for each polymer, which was then subtracted by the average of the Controls.

As shown in *Figure 22*, in Soil environment, Clamshell containers saw the greatest production of CO² with an accumulated 65.9 mg by day 56 and plateaued until the remainder of the experiment, day 67. Clamshell was followed by UniDomum and Egg Carton, which produced a total of 51.0 mg and 50.8 mg CO² respectively by day 67. The lowest production was seen by Green Earth (25.8 mg) and BioBag (24.7), both of which plateaued between days 56 and 67. Dewitt had measurable $CO₂$ production after day 29, which accumulated to 17.3 mg by day 71. Dubois did not produce measurable CO² throughout the entirety of the experiment.

Figure 22. CO² Evolution of various polymers in Soil.

Figure 22 illustrates the average accumulative CO2 production over time for BioBag, UniDomum, Green Earth, Dewitt, Dubois, Clamshell, and Egg Carton in Soil environment.

In contrast to the CO² production in the Soil environment, where Green Earth was observed to have near the lowest net production of CO2, Green Earth had the greatest accumulation of CO² (58.9 mg) in the Food Waste environment as shown by *Figure 23*. This value is more than twice that of Green Earth in the Soil environment. Egg Carton and Clamshell, both of which showed no net production until after day 13, produced totals of 44.2 and 37.9 mg CO2, respectively. Over 61 days, Dubois and Dewitt evolved 26.3 and 24.2 mg CO2. BioBag, which began producing following day 9 of the experiment, produced 18.0 mg CO₂. UniDomum produced the lowest amount of $CO₂$ in the Food Waste environment. Its flasks had seen zero production until after day 21, when it rose to 0.6 mg and had remained until at least day 30. Mg CO² gradually rose until the conclusion of the experiment, at which UniDomum reached a net CO² production of 15.7 mg.

Figure 23. CO² Evolution of various polymers in Food Waste.

Figure 23 illustrates the average accumulative CO2 production over time for BioBag, UniDomum, Green Earth, Dewitt, Dubois, Clamshell, and Egg Carton in Food Waste environment.

Comparing the CO² production of the control flasks containing solely 50 mg of burial media, flasks containing Food Waste produced an average total of 229.6 mg CO₂ by day 61 (*Figure 24*). By day 67 Soil controls had only produced an average total of 91.1 mg CO² (*Figure* 24), which is approximately 2.5 times less than the CO₂ evolution of Food Waste. Following an Analysis of Variance (ANOVA) of the slopes of $CO₂$ produced by the control flasks over time. According to *Table 7*, the rates were found to be significantly different.

Figure 24. CO2 evolution of Soil and Food Waste controls.

Figure 24 displays the average accumulative $CO₂$ production in control flasks of the Soil and Food Waste systems, without the presence of any polymer samples.

Table 7. Analysis of variants for CO₂ evolution of Control flasks.

Table 7 displays the Analysis of Variants (ANOVA) results of the CO₂ Evolution of Soil and Food Waste control flasks. The table gives the degrees of freedom (DF) and Sum of Squares for each source of variation along with the calculated Mean Square. The F ratio (87.9) and Prob \geq F (0.0007) support a difference in CO₂ production.

As shown in *Figures 25-31*, in the Food Waste environment, Green Earth, Dewitt, and Dubois were the only polymers that encountered an upsurge in $CO₂$ production, whereas all other polymers (BioBag, UniDomum, Clamshell, and Egg Carton) exhibited a reduction in the measured CO² levels. Green Earth and Dubois both had a greater rate of degradation in Food

Waste than in Soil during burial experiments as well as greater CO₂ production. However, BioBag and UniDomum see the reverse trend, as degradation was higher in Food Waste, yet CO₂ production was lower.

Figure 25. CO² evolution of BioBag in Soil and Food Waste environments.

Figure 27. CO² evolution of Green Earth in Soil and Food Waste environments.

Figure 26. CO² evolution of UniDomum in Soil and Food Waste environments.

Figure 28. CO² evolution of Dewitt in Soil and Food Waste environments.

Figure 29. Percent weight loss of BioBag in all burial environments.

Figure 31. Percent weight loss of Green Earth in all burial environments.

Figure 30. Percent weight loss of UniDomum in all burial environments.

Figures 25-31 display the average accumulative $CO₂$ production over time for BioBag, UniDomum, Green Earth, Dewitt, Dubois, Clamshell, and Egg Carton, in Soil and Food Waste environments.

According to the F Ratios and Prob>F values from *Appendix Tables 9-15*, this analysis did not reveal a significant difference in the $CO₂$ evolution data for BioBag, UniDomum, Green Earth, Dewitt, Clamshell, and Egg Carton between Soil and Food Waste environments. Out of all the polymers, only Dubois displayed a significant difference CO² production between environments with an F ratio greater than 1 and Prob>F less than 0.05 (*Table 8*).

Table 8. Analysis of variants for CO₂ evolution of Dubois.

Table 8 displays the Analysis of Variants (ANOVA) results of the CO₂ Evolution of Dubois in Soil and Food Waste. The table gives the degrees of freedom (DF) and Sum of Squares for each source of variation along with the calculated Mean Square. The F ratio (475.8) and Prob \geq F (0.0002) support a difference in CO₂ production.

Chapter 5. Results: Microbial Cultures

5.1. Colony Counts

Samples taken from the compost mixtures prior to burial of polymers, showed that both fungal and bacterial colony counts were greater in Food Waste than in Soil, as shown by *Figures 32-34*. Following the conclusion of burial experiments, fungal Colony Forming Units (CFU) were found to be greater in Food Waste than in Soil environments for all polymer except Dubois as shown by *Figure 35*. Similarly, as shown in *Figure 36* there were greater amounts of bacterial colonies found in Food Waste environments for polymer samples besides Dubois. Following the conclusion of burial experiments, it was found that fungal colony counts had increased in samples from Soil environments.

Figure 32. Plate counts of fungal and bacterial colonies from compost mixtures prior to burial experiments.

Figure 32 is a bar graph displaying fungal and bacterial colony counts, measured in log(CFU/mL) (Colony Forming Units per mL of plating media), for Soil and Food Waste samples before the burial of polymers.

Figure 33. PDA plates of Soil (left) and Food Waste (right) samples prior to burial experiments.

Figure 33 displays fungal cultures of Soil and Food Waste sample before the burial of polymers. Photo A. shows Soil samples diluted and plated on PDA from 10^{-2} through 10^{-2} ⁵. Photo B. shows Food Waste samples diluted and plated of PCA from 10^{-2} through 10^{-5} . Plates were incubated at 23° C for six days. (Photos by author)

Figure 34. PCA plates of Soil (left) and Food Waste (right) samples prior to burial experiments.

Figure 34 displays bacterial cultures of Soil and Food Waste sample before the burial of polymers. Photo A. shows Soil samples diluted and plated on PCA from 10^-4 through 10^-7. Photo B. shows Food Waste samples diluted and plated of PCA from 10^-4 through 10^-7. Plates were incubated at 23° C for six days. (Photos by author)

Figure 35. Plate counts of fungal colonies from compost samples collected following completion of burial experiments.

Figure 35 shows the fungal colony counts obtained from compost samples taken from the surface of BioBag, UniDomum, Green Earth, Dewitt, Dubois, Clamshell, and Egg Carton in Non-Aerated Soil, Non-Aerated Food Waste, Aerated Soil, and Aerated Food Waste conditions following burial experiments. Counts were measured in log(CFU/mL) where CFU/mL is Colony Forming Units per mL of plating media.

Figure 36. Plate counts of Bacterial colonies from compost samples collected following completion of burial experiments.

Figure 36 shows the bacterial colony counts obtained from compost samples taken from the surface of BioBag, UniDomum, Green Earth, Dewitt, Dubois, Clamshell, and Egg Carton in Non-Aerated Soil, Non-Aerated Food Waste, Aerated Soil, and Aerated Food Waste conditions following burial experiments. Counts were measured in log(CFU/mL) where CFU/mL is Colony Forming Units per mL of plating media.

5.2. Colony Diversity

Based on morphology, size, and color, diversity of fungal and bacterial colonies were determined. Only two different fungal colonies were found in the initial Soil mixture while the initial Food Waste contained 6 different colonies. Diversity was also greater among bacterial colonies found in Food Waste compared to Soil as shown by *Figure 37*.

Figure 37 is a bar graph displaying fungal and bacterial colony diversity for Soil and Food Waste samples before the burial of polymers.

Following the conclusion of burial experiments, fungal colony diversity remained greater in Food Waste environments on almost all polymer samples, except for Dewitt in Non-Aerated Food Waste and Dubois in Aerated Food Waste (*Figure 38*). Bacterial diversity did not follow the same trend according to *Figure 39*. The sample with the greatest bacterial colony diversity was Dewitt in Non-Aerated Soil.

Figure 38. Diversity of fungal colonies from compost samples collected following completion of burial experiments.

Figure 38 shows the fungal diversity of compost samples taken from the surface of BioBag, UniDomum, Green Earth, Dewitt, Dubois, Clamshell, and Egg Carton in Non-Aerated Soil, Non-Aerated Food Waste, Aerated Soil, and Aerated Food Waste conditions following burial experiments.

Figure 39 shows the bacterial diversity of compost samples taken from the surface of BioBag, UniDomum, Green Earth, Dewitt, Dubois, Clamshell, and Egg Carton in Non-Aerated Soil, Non-Aerated Food Waste, Aerated Soil, and Aerated Food Waste conditions following burial experiments.

5.3. Identification of Fungal Cultures

Five unique genera of fungi were identified compost samples following the completion of the burial experiments shown in *Table 9*. Identification was determined by colony morphology and lactophenol cotton blue staining, as shown in *Figures 40-44*. There were two uniquely identified fungal groups in both Non-Aerated and Aerated Soil environments: *Aspergillus* and *Fusarium*. *Aspergillus, Penicillium*, and yeasts *Kluyveromyce*s and *Saccharomyces* were found in both Food Waste composts [31].

	Non- Aerated Soil	Non-Aerated Food Waste	Aerated Soil	Aerated Food Waste
BioBag	Aspergillus	Aspergillus	Aspergillus	Aspergillus
		Penicillium		Penicillium
		Kluyveromyces		Saccharomyces
		Saccharomyces		
UniDomum	Aspergillus	Aspergillus	Aspergillus	Aspergillus
		Penicillium		Penicillium
		Kluyveromyces		Kluyveromyces
		Saccharomyces		Saccharomyces
Green Earth	Aspergillus	Aspergillus	Aspergillus	Aspergillus
		Penicillium		Kluyveromyces
		Kluyveromyces		Saccharomyces
		Saccharomyces		
Dewitt	Aspergillus	Aspergillus	Aspergillus	Aspergillus
	Fusarium	Kluyveromyces	Fusarium	Kluyveromyces
		Saccharomyces		Saccharomyces
Dubois	Aspergillus	Aspergillus	Aspergillus	Aspergillus
		Penicillium		Saccharomyces
		Kluyveromyces		
		Saccharomyces		
Clamshell	Aspergillus	Aspergillus	Aspergillus	Aspergillus
	Fusarium	Penicillium	Fusarium	
		Kluyveromyces		
		Saccharomyces		
Egg Carton	Aspergillus	Aspergillus	Aspergillus	Aspergillus
	Fusarium	Kluyveromyces	Fusarium	Penicillium
		Saccharomyces		Kluyveromyces
				Saccharomyces

Table 9. Identified fungal genera from burial experiment compost samples.

Table 9 displays the fungal genera that were identified on the surface of polymer samples in different burial environments following the completion of burial experiments.

Figure 40. Lactophenol cotton blue stain of Aspergillus.

Figure 41. Lactophenol cotton blue stain of Fusarium.

Figure 42. Lactophenol cotton blue stain of Penicillium.

Figure 43. Lactophenol cotton blue stain of Kluyveromyces.

Figure 44. Lactophenol cotton blue stain of Saccaromyces.

Figures 40-44 show lactophenol cotton blue staining of *Aspergillus*, *Fusarium*, *Penicillium*, *Kluyveromyces*, and *Saccaromyces* under a microscope at a magnification of 400x. (Photos by author)

Chapter 6. Discussion

6.1. Burial Experiments

Based on the burial experiment results, cellulose based Dewitt showed the greatest degradation of all polymers tested. In all environments, except Aerated Food Waste, Dewitt had the greatest percent weight loss by the final removal of samples. In Aerated Food Waste, the percent weight loss of Clamshell had surpassed Dewitt by the end of the experiment. Egg Carton and BioBag broke down the next greatest in burial experiments. In both Soil environments Egg Carton broke down more than BioBag, but in Food Waste environments, BioBag had greater percent weight loss. These mentioned polymers were the only samples to meet the requirements for the composability standards for which they are certified for [13].

Among all the polymers tested, Dubois exhibited the lowest percentage of weight loss at the final removal stage. However, in the presence of Aerated Food Waste, Dubois showed more visible degradation compared to UniDomum and Green Earth, as depicted in *Appendix Figure* A5. Additionally, during the Non-Aerated Food Waste checkpoints T₂ and T₃, Dubois displayed visible signs of fragmentation despite experiencing a net zero weight loss, as shown in *Appendix Figure A3*. This discrepancy could be attributed to an increase in soil adhesion to the polymers as they broke down. Unfortunately, the rise in soil adhesion made it increasingly difficult to remove the burial media from the samples without causing damage.

For all polymers, except Dewitt, degradation rates were greatest in an environment containing Food Waste. Utilization of mesh bags may have impeded the degradation of samples in burial environments. For instance, in the initial burial experiment where samples were positioned on top of mesh strips, the Dewitt mulch film samples had completely decomposed by day 30. However, in all other burial experiments, the Dewitt samples had not fully broken down by the final removal. On the other hand, the Clamshell and Egg Carton samples, enclosed in mesh envelopes across all burial environments, exhibited the fastest breakdown rate in the Non-Aerated Food Waste condition. It is worth noting that in a real home compost setting, the use of mesh is unnecessary and would allow for a greater exposure of composting materials to the surface area, facilitating more efficient decomposition.

The introduction of forced air into the compost pile frequently resulted in the drying out of the pile, requiring regular watering. Initially, the pile required watering at least three times a week to maintain the appropriate moisture levels. However, as the experiment progressed, the frequency of watering gradually decreased. Despite the inclusion of aeration, it was observed that it did not enhance the degradation of all the samples.

Unfortunately, statistical analysis could not be used to support differences in degradation rates between polymers and burial environments. Several variables were not held constant preventing statistical analysis. Due to the varying thickness of the polymers, as indicated in *Table 1*, adjustments to sample sizes should have been made to ensure comparable surface area exposure or volume among samples. Moreover, for future burial removal, it is crucial to conduct it on the same day as the experiment's other samples for that specific checkpoint. Additionally, the removal of samples by envelope was not ideal; instead, one sample should have been extracted from each envelope at every checkpoint. Each envelope represents its own distinct environment, thus requiring a consistent sampling across different environments at each instance.

6.2. CO² Evolution

The inclusion of food waste was shown to largely increase the rate of $CO₂$ production in the control flasks, see *Figure 24.* This evidence of high microbial activity may prove to be problematic. Flasks remained stoppered between KOH extractions throughout the experiment, resulting in a lack of oxygen supply which may have caused the flask systems to become anaerobic. Despite shorter intervals between titrations, $CO₂$ production remained high for the food waste systems. Also, with so much microbial activity in the system, it may be difficult to accurately measure CO² produced solely by the biodegradation of polymers.

The examination of weight loss in polymers did not reveal a clear correlation with greater CO² production. To accurately assess the CO² production along with the physical degradation of polymers, it is crucial to conduct a chemical analysis to determine the carbon content of the materials. This analysis would enable the calculation of the percentage of biodegradation in samples as $CO₂$ is generated. By quantifying the carbon content, a more comprehensive understanding of the relationship between weight loss and CO₂ production can be established.

6.3. Microbial Culturing

Microbial culturing found that addition of food waste to soil increased abundance of both fungi and bacteria in samples prior to burial of polymers. Following burial, microbial counts remained greater on polymers in Food Waste environments. Fungal and bacterial diversity was also greater in soil amended with food waste. As the burial of polymer samples progressed, diversity of both fungi and bacteria increased in the Soil environments. This may be evidence that as it matures, compost can support a more diverse microbial community [32].

Species of *Aspergillus* were found across all burial environments. *Aspergillus* are responsible for the rot of many plants and food and some species have been found in coffee beans [33]. It is among the most common fungi that causes food rot and the biodegradation of other materials [33]. *Fusarium*, only found in Soil compost and only on cellulose-based Dewitt, Clamshell, and Egg Carton, are a common soil fungus known to cause various plant diseases [34]. To improve the quality of compost, *Penicillium spp*., are often added to promote the conversion of organic matter [35]. Yeast groups, *Kluyveromyce*s and *Saccharomyces*, are often associated with the fermentation of grapes and apple [36]. Apples were included in the food waste amended soil. This shows that the inclusion of specific food impacts the microbial composition of the compost and possibly the biodegradation of different materials.

Chapter 7. Conclusion

Based on the findings from burial experiments, several recommendations can be made regarding the choice of biodegradable products. The cellulose-based materials, such as Dewitt mulch film, Stackman clamshell containers, and Goldhen egg cartons, demonstrated commendable degradation performance, aligning with their certified composability standards. Additionally, one starch-based food waste bag, BioBag, also exhibited satisfactory decomposition. These products showcase potential for effective waste management in home composting systems and are recommended for individuals seeking environmentally friendly alternatives to conventional non-biodegradable options.

The results of burial experiments also indicated that the inclusion of food waste, regardless of the presence or absence of added aeration, accelerated the degradation rates of most samples. Furthermore, the CO₂ experiments and microbial plating analysis revealed that the presence of food waste led to increased microbial activity. These findings suggest that incorporating food waste in composting alongside biodegradable materials can stimulate microbial processes and accelerate the overall degradation process.

It is important to address the practical aspects of how a home composter can effectively manage the composting process. Biodegradable products may not break down completely before the maturation of the compost pile. Non-degraded materials could be sieved out manually and placed into a new pile. The degradation of products may also depend on if they are added to the pile as whole items or if they are cut up. The cutting of products can help expedite their degradation rate by increasing surface area exposure. A shredder or grinder could be sed to aid in this process, although this might not be a practical option for the average home composter.

Regarding aeration, there are two potential methods available to home composters. The first is a windrow composting technique, where the pile is manually flipped or turned periodically to ensure proper aeration. The second approach involves utilizing a static aerated pile, where pipes are added to the bottom of the pile and forced air is introduced to enhance aeration, similar to that detailed in this thesis. The cost of implementing these methods may vary, and it is essential to consider the available resources and budget. It is worth mentioning that the height of the compost pile plays a role in the composting process. If forced aeration is utilized, it is important that there is an even distribution of air throughout the pile.

The degradation of polymers in compost is a complex process influenced by numerous variables. This poses many opportunities to further investigation on the degradation of biodegradable polymers in a home compost environment. Future research should focus on investigating additional factors such as the type of food waste, temperature, and moisture. Varying ratios of food waste to soil should be tested as well. It would also be beneficial to study the effects of including yard waste such as lawn clippings on the degradation of polymers and its effect on soil quality. To further mimic a home composting environment, outdoor experiments should be performed as well as the continuous addition of organic waste. With the increasing need for sustainable waste management solutions, exploring the composting of biodegradable polymers in future research offers promising potential for reducing the environmental impact of plastic waste.

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Appendix Additional Figures and Tables

Figure A1. Serial dilution of compost samples and plating for bacterial and fungal cultures.

Figure A2. Clamshell and Egg Carton samples from Non-Aerated Soil burial experiment.

Figure A2 presents a series of photos showcasing the polymer samples extracted from Non-Aerated Soil at different time intervals: 24 days, 39 days, and 75 days. The displayed polymer samples are Clamshell (left) and Egg Caron (right). (Photos by author)

Figure A3. Polymer samples from Non-Aerated Food Waste burial experiment.

Figure A3 presents a series of photos showcasing the polymer samples extracted from Non-Aerated Food Waste at different time intervals: 24 days, 39 days, 56 days, and 75 days. The displayed polymer samples, from left to right, are arranged as follows: BioBag, UniDomum, Green Earth, Dewitt, Dubois, Clamshell, and Egg Carton. (Photos by author)

Figure A4. Polymer samples from Aerated Soil burial experiment removed at 21, 36, 53, and 73 days.

Figure A4 presents a series of photos showcasing the polymer samples extracted from Aerated Soil at different time intervals: 21 days, 36 days, 53 days, and 73 days. The displayed polymer samples, from left to right, are arranged as follows: BioBag, UniDomum, Green Earth, Dewitt, Dubois, Clamshell, and Egg Carton. (Photos by author)

Figure A5. Polymer samples from Aerated Food Waste burial experiment removed at 21, 36, 53, and 73 days.

Figure A5 presents a series of photos showcasing the polymer samples extracted from Non-Aerated Food Waste at different time intervals: 21 days, 36 days, 53 days, and 73 days. The displayed polymer samples, from left to right, are arranged as follows: BioBag, UniDomum, Green Earth, Dewitt, Dubois, Clamshell, and Egg Carton. (Photos by author)

Table A1. Analysis of variants for CO₂ evolution of BioBag.

Table A2. Analysis of variants for CO₂ evolution of UniDomum.

Table A3. Analysis of variants for CO₂ evolution Green Earth.

Table A4. Analysis of variants for CO₂ evolution of Dewitt.

Table A5. Analysis of variants for the CO₂ evolution of Clamshell.

Table A6. Analysis of variants for CO2 evolution of Egg Carton.

Tables A1-A6 display the Analysis of Variants (ANOVA) results of the CO₂ Evolution of polymers in Soil and Food Waste. The tables provide the degrees of freedom (DF) and Sum of Squares for each source of variation along with the calculated Mean Square. The F ratio and Prob>F values do not support a difference in CO² production.