RIT

Biodegradation and microbial CO_2 evolution of blended polymers in food-waste and coffee supplemented disposal environments

by

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

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July 30, 2021

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Acknowledgments

Firstly, I would like to thank my committee member and advisor, Dr. Jeff Lodge, for his support, guidance, and knowledge he shared with me while writing this thesis. I also thank my other committee members, Dr. Carrie McCalley and Dr. Carlos Diaz-Acosta for their expertise, insight, and advise.

Many thanks go to my fellow RIT student Harshal Jayesh Kansara, for his continual collaboration, especially his guidance with SEM imaging. Also, thank you to another RIT student, Marissa Schroeter for her help with genomic sequencing of microbial isolates.

Lastly I thank my friends and family for their support and words of encouragement along the way.

Abstract

The dependency of many industries on single-use materials, has led to an accumulation of plastic waste and subsequent harm to the planet. Since avoidance of plastic is often not viable and the current processes of recycling have their own environmental impact, many have looked toward development of sustainable replacements for plastics. Homogenous blended polymers made from varied ratios of polycaprolactone (PCL), starch, and biochar were made in the Rochester Institute of Technology (RIT) Packaging Science Department, tested for percent weight loss and for carbon dioxide (CO_2) evolution in soils. Testing was done across three burial matrices: soil amended with compost starter, soil with 30% pulverized food waste, and soil with 30% spent coffee grounds. Percent weight loss derived from burial experiments food waste and soil with compost starter show a positive correlation between material degradation and starch content. However, burial experiments with a coffee amended environment did not lead to as high of percent weight loss. CO_2 evolution showed samples of 45% starch/ 45% PCL/ 10% biochar producing the highest cumulative amount (346.9mg) over 120 days. Fungal and bacterial isolates from the burial experiments show the most diversity in food and soil environments, as well as more species of interest (used in bioremediation or found to degrade polymers) compared to the coffee environment.

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Chapter 1. Introduction

1.1. Overview

The increased demand for plastics in industrial and domestic applications has amplified the accumulation of plastic waste over time[1], [2] . Fifteen to thirty million tons of plastic waste is generated annually in the United States and Western Europe [3] and fifty-seven million tons is generated globally [1]. The presence of these discarded non-biodegradable polymeric materials in terrestrial and aquatic habitats poses a substantial threat to the various species that reside in these affected ecosystems, as well as overall ecosystem function [4]. In addition to directly impacting ecosystem health after consumer use, plastic manufacturing is solely petroleum based which leads to greenhouse gas emissions. These emissions are problematic when considering the drastic greenhouse gas reductions needed to combat climate change [5]. Current manufacturing, use, and disposal of plastic material is consistently unsustainable, and to lessen further anthropogenic harm to the planet there needs to be a disruption in our current plastic usage.

To lessen the accumulation of plastic waste, we want to think beyond current methods. Reduce, reuse, and recycle is a widely known slogan meant to promote sustainable navigation through plastic consumption [6]. However, reducing plastic consumption is often unavoidable because of how deeply plastic is engrained in modern society. Reuse of plastic materials may extend the lifetime of a single-use plastic, but disposal is inevitable. Recycling can reutilize non-virgin plastics, but within the recycling process are steps that are environmentally detrimental. Also, the potential impact of recycling is often greater in the minds of consumers versus what is achieved or possible.

The recycling rate of plastic is low compared to the high rate of plastic use. Also, of the small percent of plastics sent to be recycled, some materials are unable to be recycled and then discarded. The processing and chemical treatment of recyclables creates its own environmental impact. Fillers and additives routinely used within mechanical recycling processes are often pollutants, eventually making their way into water supplies [6]. Public ideations of recycling often depict a cyclic flow of virgin material production, use, then recycling, where recycled plastic has the same potential as virgin plastic. However, this is not the case. Plastic materials are often “downcycled” into materials that are not able to be recycled again, therefore virgin plastic is still needed to maintain the anthropogenic demand of plastic products [7]. As for recycling rates, from 2015 to 2017 total plastic recycling fell from 9.1% to 8.4% of total plastic waste in the U.S., according to the EPA [8].

At our current rate of plastic use and inevitable disposal, we need to make more substantial changes to how we interact and produce plastic [9]. Instead of looking at ways we can sustainably clean up conventional plastic waste, we should be considering how we can change plastic itself to be more environmentally friendly [9]. Development of biodegradable plastic alternatives sourced partially or fully from renewable resources has the potential to change the environmental impact of single and short-term use packaging.

When developing biodegradable packaging, the many industries that utilize plastic material need to be considered. To maximize the environmental benefits gained from the adoption of

biodegradable plastics, it's necessary to identify a target industry that heavily utilizes plastics and has widespread impact. The food industry, specifically food packaging, is an ideal target for implementation of biodegradable packaging material [10]. This industry is convenience-driven with individually portioned and ready-to-eat food packaging used daily by a large population of individuals. If the plastic utilized for food packaging were more sustainable, the positive environmental impact of this change could be massive.

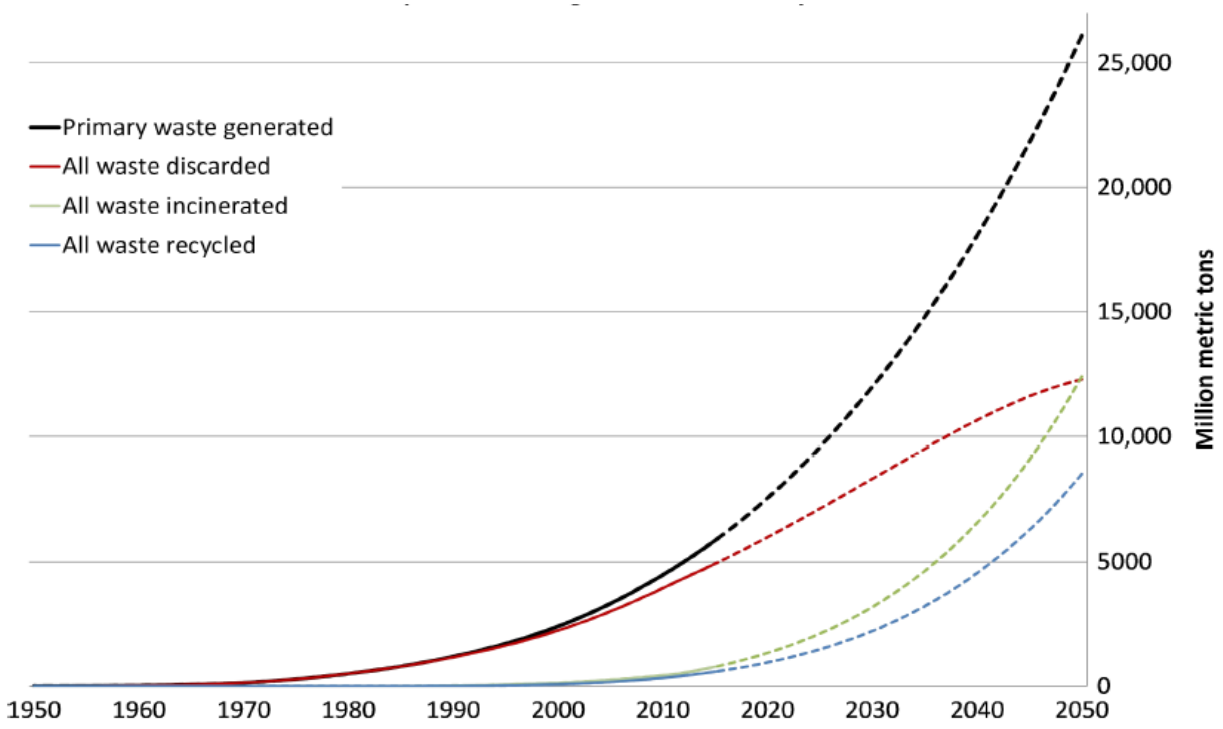

Figure 1. Cumulative plastic waste generation and disposal. Graph source: [10]

1.2. Materials of interest

1.2.1. Polycaprolactone (PCL)

The Packaging Science Department at R.I.T. has identified polycaprolactone (PCL) and starch based blended polymers as having high potential for utilization as biodegradable packaging. Polycaprolactone is a hetero-chain biodegradable polymer, once primarily utilized for medical applications like drug delivery and tissue engineering [11], [12]. Biodegradation is an enzymatic process driven by microbial activity. The inclusion of oxygen atoms in the molecular backbone of PCL characterizes the aliphatic polyester as a hetero-chain biodegradable polymer. Unlike conventional plastic which is comprised solely of carbon-carbon bonds, PCL contains an ester functional group, making the material more susceptible to enzymatic microbial processes. Polycaprolactone has also been shown to biodegrade in sea water via hydrolysis and enzymatic microbial activity. The degradation of PCL in salt water is also greatly influenced by mechanical

stress and light exposure present in ocean environments [13]. The presence of the ester functional group within the PCL backbone also results in polymer flexibility which is necessary for the polymer to fit correctly into the enzymatic active site allowing for microbial attack of the polymer [14], [15]. However, PCL has a slow rate of hydrolytic degradation, ranging from two to three years [16]. To successfully utilize PCL as a biodegradable packaging material in efforts to reduce packaging waste buildup, the rate of degradation as a function of time must be increased. [17]

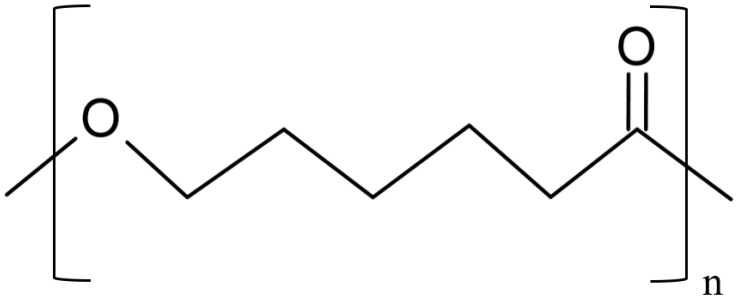

Figure 2. Polycaprolactone (PCL).

Chemical structures generated using ACD/ChemSketch [18].

1.2.2. Starch

Starch is a natural polymer that is highly biodegradable, however the hydrophilicity of starch proves problematic when trying to develop a mechanically sound packaging material. Incorporation of starch with PCL in a homogenous blended polymer may have the potential to yield a blended polymer with the benefits of PCL's mechanical function, and increased degradation rates due to starch content. The inclusion of starch with more mechanically sound polymers has been identified as having potential for development of promising biodegradable blended polymers [19], [20]. Starch is comprised of two isomers: amylose and amylopectin. The specific ratio of these two components depend on the botanical source of the starch [21].

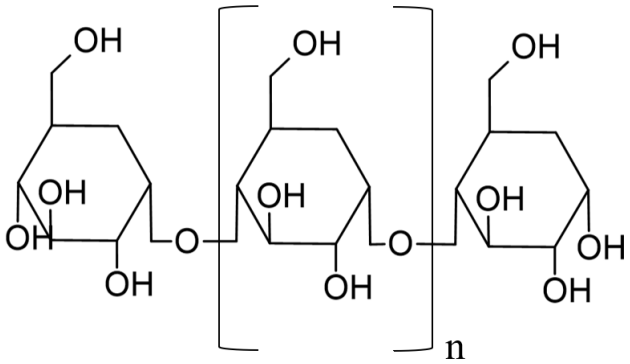

Figure 3. Structure of the starch isomer: amylose.

Chemical structures generated using ACD/ChemSketch [18].

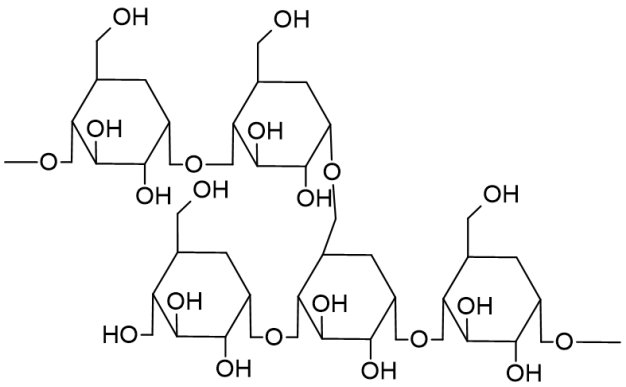

Figure 4. Structure of the starch isomer: amylopectin.

Chemical structures generated using ACD/ChemSketch [18].

1.2.3. Biochar

The Intergovernmental Panel on Climate Change (IPCC) has identified biochar as having potential for carbon sequestration, making it a desirable material to utilize for sustainable practices [22] Biochar is a high carbon content material produced via pyrolysis of various organic feedstocks under anaerobic conditions, molecular morphology of biochar can vary depending on feedstock and pyrolysis methods [23]. Many potential feedstocks of biochar are food waste based. Pyrolysis of food waste to form a new material would divert food waste from landfills, thereby reducing methane emissions. High porosity makes biochar an attractive candidate for biodegradable material synthesis because microbial surface colonization is needed for microbial decomposition. The use of biochar based degradable materials may benefit the burial environment post-material degradation, since biochar has been shown to improve soil quality [24], [25]

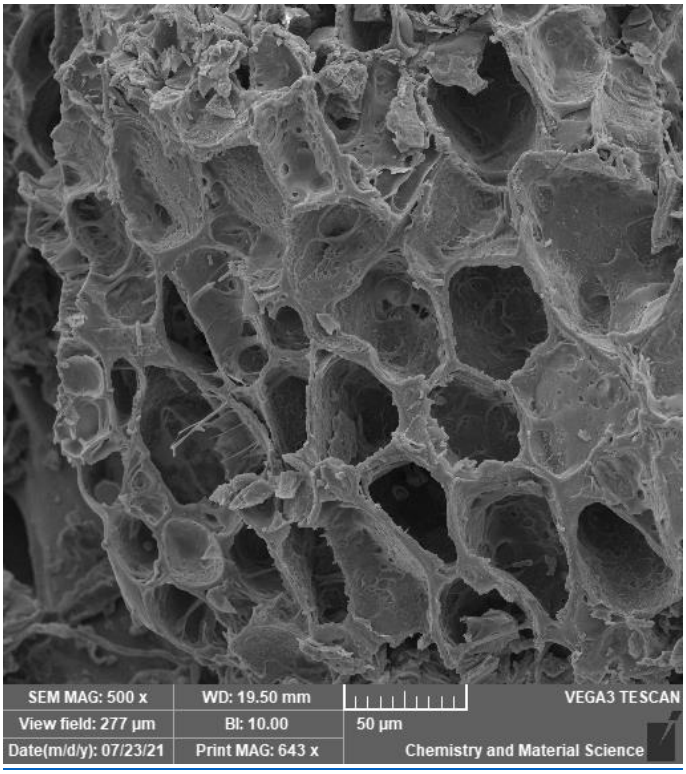

Figure 5. SEM image of pores seen on food waste biochar at 500X magnification.

1.3. Potential within food industry

Targeting the food industry for the adoption of biodegradable packaging material poses a unique opportunity to find a multi-dimensional solution to two major sustainability issues in the industry: plastic waste and food waste [26]. When looking to implement a biodegradable material into consumer use, it is vital to determine if the potential disposal environment of the material will facilitate microbial decomposition. Decomposition is a microbial process, so if the disposal environment is not hospitable for the necessary or ideal microbial community the materials may not degrade. Food waste can serve as a powerful agricultural fertilizer due to microbial richness [27]. If biodegradable materials were adopted within the food industry, could food waste be utilized as a disposal environment to help degradation? If an end-of-life plan was utilized for the disposal of a biodegradable food packaging that incorporated food waste, would the rate of decomposition increase over time? This would avoid a shortfall seen in many currently available biodegradable materials where their disposal environment post-consumer use does not facilitate decomposition. Consequently, a slow rate of degradation would lead to a buildup of packaging waste.

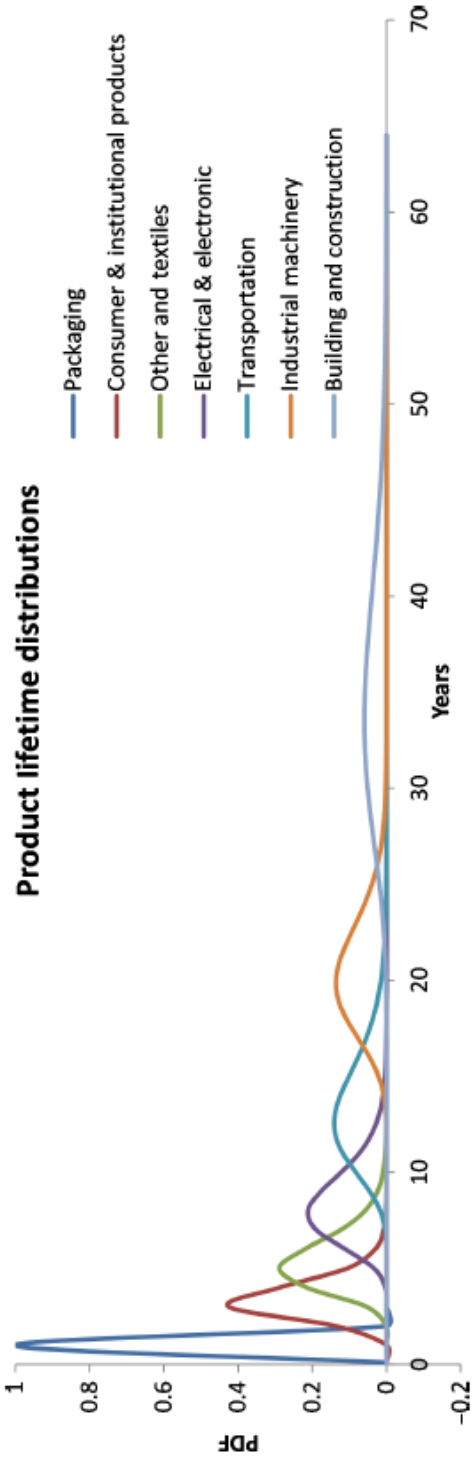

Figure 6. Product lifetime distributions. Graph source: [28]

Chapter 2 . Materials and methods

2.1. Polymers

Material name (used in this paper)	Material composition	Material preparation
PCL60	60% polycaprolactone (PCL) 40% starch	Pressed homogenous polymer ~0.5mm
ST60	60% starch 40% PCL	Pressed homogenous polymer ~0.5mm
BC	45% starch 45% PCL 10% biochar	Pressed homogenous polymer ~0.5mm
CELL	100% cellulose	Paper (<0.5mm)
LDPE	low density polyethylene	<1.0mm
PET	Polyethylene terephthalate	Film
PP	Polypropylene	Film
BOPP	Biaxially Oriented Polypropylene	Film
PLA	Polylactic Acid	Film
BIOBAGS	MATER-BI	Film

Table 1. Overview of all materials tested throughout study.

2.2. Methods

Three primary experimental methods were performed: burial experiment, carbon dioxide capture, and microbial culture and identification. The primary polymer blends being tested are designated by: PCL60, ST60, BC, and CELL (*table 1*). Other polymers (PP, PET, BOPP, PLA, BIOBAGS) were tested for percent weight loss in a soil environment to serve as reference to currently used packaging materials. BIOBAGS and PLA are both marketed as biodegradable. However, products marketed as biodegradable may require specific post-consumer treatment and burial matrix to uphold their marketed biodegradability. These necessary treatments (such as shredding) of biodegradable material create a false consumer illusion of the end-of-life fate when a biodegradable material is disposed of.

Comparison of degradation rates was made between polymer type, as well as burial matrices. Three burial matrices, or disposal environments, were applied across all testing of the four main polymers: PCL60, ST60, BC, CELL. These matrices are designated in this paper as: soil, coffee, and food. Their specific composition is detailed in *table 2*.

Burial Matrix Designation	Matrix composition
Soil	100% Miracle-Grow® All Purpose Potting Soil Amended with Espoma® compost starter (35-40 grams per bin)
Food Waste	30% pulverized food waste (64% potato peels and 36% tomato scraps (by weight))

	70% Miracle-Grow® All Purpose Potting Soil (compost started omitted)
Coffee	30% spent coffee grounds (caffeine extracted) 70% Miracle-Grow® All Purpose Potting Soil (compost starter omitted)

Table 2. Composition of burial matrices used across all methods of testing.

2.2.1. Initial Burial Experiment

The percent weight loss of polymers was measured in soil environments over 122 days following the methods of similar burial studies [29]. Two novel polymer blends were tested: PCL60 and ST60. These blended polymer samples were supplied by the Diaz-Acosta lab group from the Packaging Science Department at RIT and were prepared as thin malleable pressed polymers (~0.5mm). In addition, BIOBAGS and CELL were tested to provide comparison. CELL is a known biodegradable material, and BIOBAGS are a commercially available product marketed as fully compostable. Ten samples of PCL60 and ST60, and 6 samples each of BIOBAG and CELL were tested for percent weight loss via decomposition within a burial environment. All samples were cut into $3cm^2$ pieces.

Miracle-Grow® All Purpose Potting Soil was used as a burial matrix. Espoma® Compost Starter was used as a microbial source for the soil. The compost starter was added according to the manufacturers' recommendations with 35-40grams being added based on the surface area of the bins. Bins were filled with the soil and compost mixture approximately 11.5cm deep. This blended burial matrix of potting soil and compost starter will be designated as a 'soil' burial environment as detailed in *table 2*. All bins were watered routinely and monitored with a moisture meter throughout the experiment. Bins were also given drainage holes and lined with mesh to avoid oversaturation of soil. Arrangement of samples within bins is shown in *figure 7.* with sample buried approximately 6cm beneath the soil surface.

The burial experiment ran for 122 days. At days 0, 10, 20, 30, 42, 55, 65, 77, 88, 98, 112, and 122 each sample was weighed. At these checkpoints, samples were cleaned of debris, washed with alcohol, and weighed to track weight loss via decomposition. After weight assessment, samples were placed back into soil as they previously were. Photos were also taken to visually monitor the breakdown of materials. Routine water application to bins was done to maintain a consistent soil moisture content, without saturation of soil. At the conclusion of the experiment, weights were analyzed and percent weight loss was calculated to easily compare degradation as a function of weight loss, since weight of initial samples varied due to material specific density.

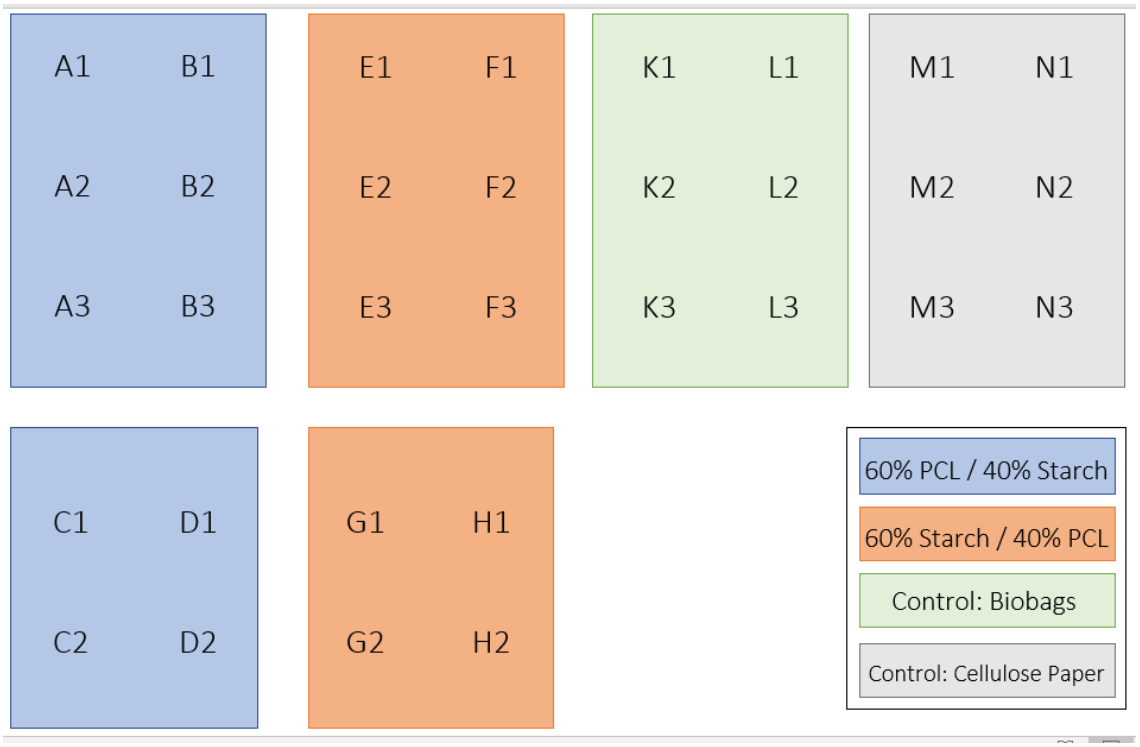

Figure 7. Initial burial experiment layout.

Shown in *figure 7*, samples were labeled with an alpha-numeric system for easier data collection to follow the decomposition of individual samples. Soil was uniform across all bins and each bin contained duplicates of the same sample type. Sample groups A, B, E, F, K, L, M, and N were in bins the larger bins (45.7cm × 38cm) and sample groups C, D, G, and H were in the smaller bins (39.4cm × 28cm).

2.2.2. Revised Burial Experiments

Methods were slightly revised for the remaining burial experiments. Revisions were made to improve and further standardize the general methods used to collect preliminary burial data. The table below shows the experimental variables for all revised burial experiments performed:

Exp #	Burial Matrix	Tested Polymers	Duration	Data Sampling	Polymer size
2	Soil	PCL60, ST60, BC, CELL, LDPE	120d	Initial weight as average of samples, experimental weight as individual sample not reused for later checkpoints	2.5cm^2– PLC60, ST60, BC, LDPE 3.8cm^2 -CELL
3	Coffee	PCL60, ST60, BC,	120d	Initial weight as average of samples, experimental weight as	2.5cm^2– PLC60, ST60,

		CELL, LDPE		individual sample not reused for later checkpoints	BC, LDPE 3.8cm^2 -CELL
4	Food	PCL60, ST60, BC, CELL	120d	Initial weight as each individual samples' initial weight, experimental weight as individual sample not reused for later checkpoint	7.6cm^2- All samples
5	Soil	PP, PET, BOPP, PLA	120d	Initial weight as average of samples, experimental weight as individual sample not reused for later checkpoints	7.6cm^2- All samples

Table 3. Overview of revised burial experiment setups.

The percent weight loss of PCL60, ST60, CELL, and BC was monitored in three burial environments: soil, coffee, and food waste, over 120 days. The placement and sample collection of the three revised burial experiments differed compared to the initial burial experiment, as illustrated in *figure 7*. To better regulate data collected, triplicates of each sample type was placed into each bin. Shown in *figure 8* for coffee and soil environments, each bin represented a different timepoint within each experiment. All bins were watered routinely and monitored with a moisture meter throughout the experiment. At each timepoint samples within one bin are unearthed, cleaned, dried in an oven at 60 ºC for 4 hours, cleaned again, and weighed. No samples were placed back into the burial environments for further data collection. This allowed for the samples to be completely untouched until their respective checkpoint day, unlike how sample data was collected within the first burial experiment.

Percent weight loss was calculated with the same formula in the initial and revised burial experiment:

$$Weight\ Loss\ (\%) = \frac{w_i - w_e}{w_i} \times 100$$

Where w_i is the initial dried weight of sample and w_e is the experimental weight of sample after being cleaned and dried.

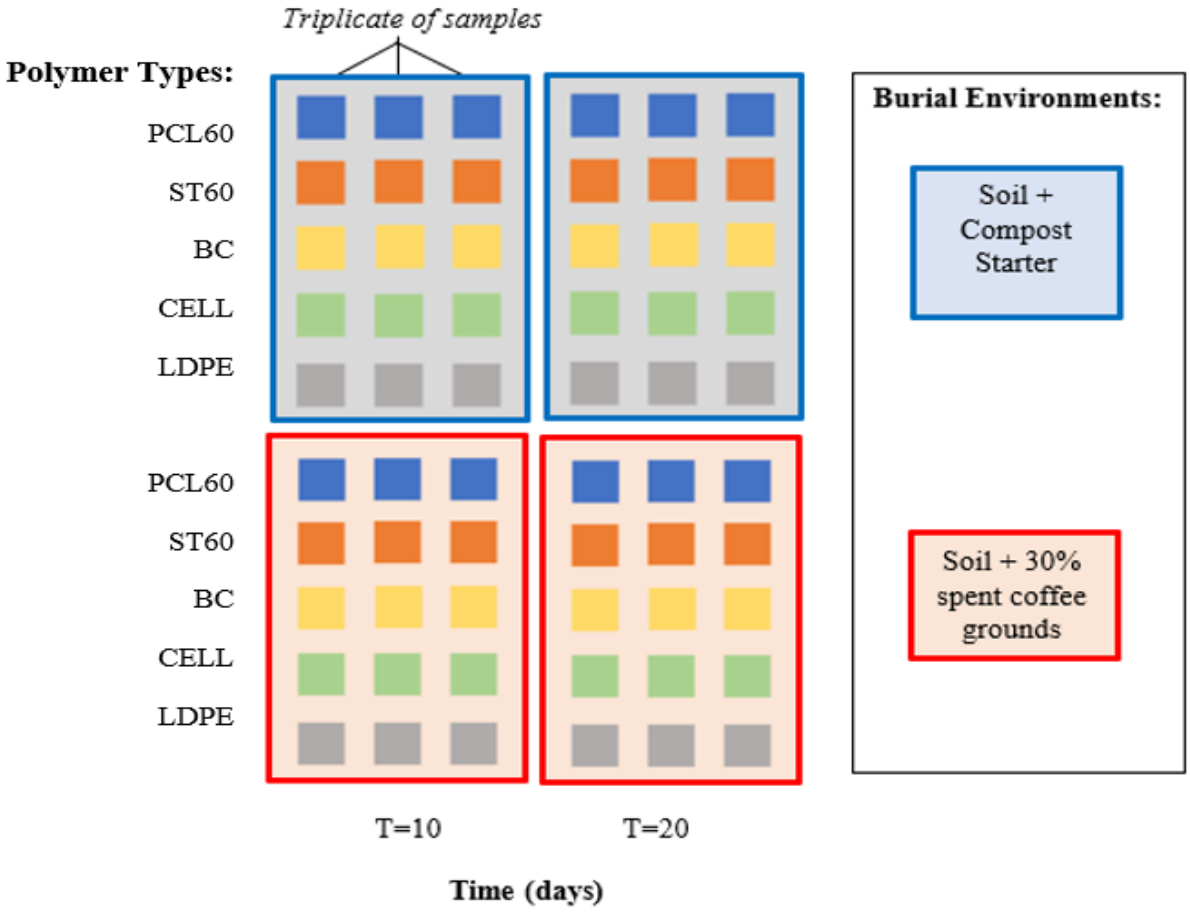

Figure 8. Revised burial experiment layout

Figure 8 illustrates the revised burial experimental setup for two checkpoints, t=10 and t=20, for two burial environments: coffee and soil. For all revised burial experiments, the number of bins was reflective of the number of checkpoints. At each timepoint, the samples within one bin for both environments was unearthed. The food burial experiment was setup in the same manner as shown in the figure.

2.2.3. Carbon Dioxide Evolution

Biometer flasks were used to measure the microbially driven CO_2 evolution of polymers in each burial environment: soil, food, and coffee. This experimental protocol to measure CO_2 production is outlined by EPA guidelines [30]. A 2.5cm^2 sample of polymer in 50g of burial matrix (soil, food, or coffee) was placed in the Erlenmeyer portion of a biometer flask. The sidearm contained 10mL KOH, the concentration of KOH used varied due to differing CO_2 production across experiments. An Ascarite tower was used to absorb atmospheric CO_2 when opening the system to remove the spent KOH at 10-day intervals. Polymers tested across the three burial matrices include: PCL60, ST60, BC, and CELL. The described biometer flask setup was done in duplicate for each polymer type, as well as the respective burial matrix alone. The burial matrix-only setups were done to obtain approximate measures of endogenous microbial

activity of each disposal environment. Flasks were kept at room temperature and incubated for 120 days. Data was collected in duplicate, with two flasks labeled A and B for each polymer type plus the control setup.

The 10mL KOH was drawn from the side arm via syringe for titration and replenished every 10 days. Titration of KOH was done using 0.1mL of a 1% phenolphthalein solution as a pH indicator and HCl as the acid. A sample of control KOH was also neutralized each week to get the V_c value. Molarity of KOH and HCl was adjusted based on absorption of CO_2. Changes in reagent molarity is accounted for within the equation by the *CF*. The equation below was used to determine the gross amount of CO_2 production per flask [31] [32]:

$$Mg\ CO_2 = (V_c - V_E)(^{M_{CO_2}}/_2)(M_{HCl})(CF)$$

$$Correction\ Factor = CF = \frac{M_{HCl}}{M_{KOH}}$$

$$V_c = vol\ of\ HCl\ to\ neutralize\ control$$

$$V_E = vol\ of\ HCl\ to\ neutralize\ experimental$$

Calculations were done to measure the CO_2 production for both experimental setups and control setups (burial matrix only). To get the amount of CO_2 produced by the microbial breakdown of materials, the CO_2 production of the soil-only controls were subtracted to account for endogenous microbial activity within the soil.

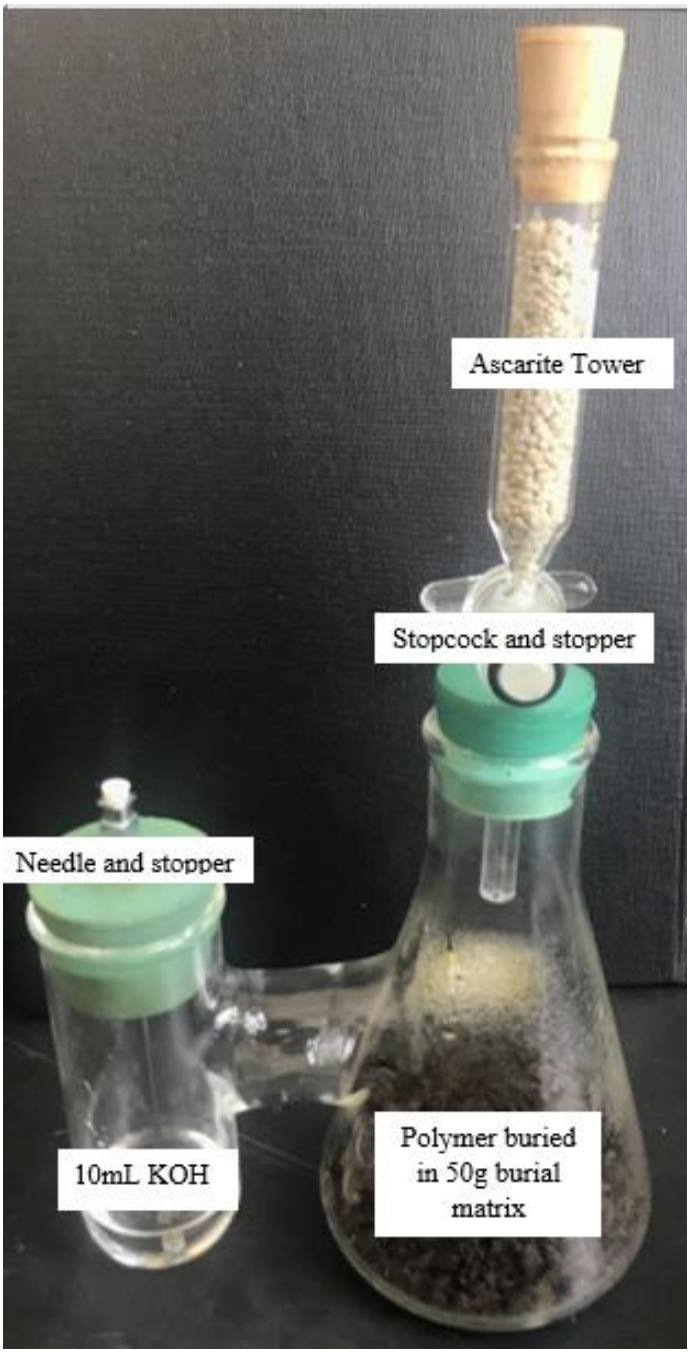

Figure 9. Experimental setup of biometer flasks used to collect CO_2 evolution data.

2.2.4. Microbial Culturing and Identification

Soil samples were taken off the surface of polymers: PCL60, ST60, CELL, and BC for burial experiments 2, 3, and 4. Samples were also taken off LDPE, which was tested in soil and coffee environments. One-gram samples of the burial matrix was scraped off the surface of each polymer type, then diluted in a 0.9%NaCl solution for serial dilutions and plating. Potato dextrose agar (PDA) containing 100ug/ml streptomycin was used for fungal cultures and plate count agar (PCA) was used for bacterial cultures. The dilutions of fungal cultures were plated

from 10^{-2}-10^{-5} and the dilutions of bacterial cultures were plated from 10^{-3}-10^{-6}. Plate counts were done at day 3 of incubation at room temperature. Day 3 was chosen because it showed the best distribution of countable colonies (30-300) for cultures across all environments. The soil samples were collected at day 100 to select from the most prevalent colonies remaining at the end of the experiment. Plate counts were done using an image mapping software (ImageJ)[33].

DNA sequencing of bacterial isolations was done at the genomics lab at RIT, under the supervision of Dr. Andre Hudson. Following dilution, plating, and incubation of samples, bacterial colonies of interest were isolated for purity and the 16S rRNA segment was amplified via polymerase chain reactions (PCR). PCR is used to amplify the genomic area of interest, which for bacterial samples is the 16S rRNA segment. For each isolated bacterial colony sample, a PCR tube was inoculated with the sample, 1µL forward and reverse V3/V4 primers, 12.5µL master mix, and 10.5µL nuclease-free H_2O. Samples are then centrifuged and processed in the thermo cycler. Gels were made with 50mL TAE 1X and 1.2g agarose, heated until fully dissolved. After cooling slightly, 3µL of ETBR is added prior to pouring in the gel box for solidification. Larger gels were made with triple the amounts given. After the gel is solidified and the comb removed, TAE 1X buffer is added to the box to completely cover the gel, channels are filled with 5µL PCR samples, and one lane is designated to hold the DNA ladder as reference. Once the gel box is connected to a current with the channels closest to the negative node, the gel electrophoresis is run until the visible dye travels past the end of where the ladder extends.

PCR samples that successfully show bands at the 16S point on the DNA ladder are then cleaned up and sent to GENEWIZ for analysis. Bands present at the 16S point in the DNA ladder show amplification of the necessary 500bp section of DNA needed for identification. Samples that show replication of the 16S DNA segment are then tested for DNA concentration (ng/µL). Based on DNA concentrations, amounts totaling at least 20ng DNA are combined with 1ng Forward primer and brought to 15µL with nuclease-free H_2O.

Sequencing data was then run through the BLAST NCIB to analyze for similarity against the current database of bacterial sequences. [34] Specifically nucleotide BLAST was used to find percent match of 16S rRNA sequences. After species were identified, a cladogram of species found was created with MUSCLE and NCIB Genomic Workbench programs shown in *figure A. 1.* in the appendix [35][36].

Genomic sequencing was not possible for fungal specimens, due to the necessary primers not being available. So, identification of fungal colonies was done based on colony morphology and microscopy staining. Lactophenol cotton blue was used to stain fungal slides. This allowed visualization of hyphae variations (septate or non-septate) as well as spore morphology. [37]

2.2.5. SEM imaging

Samples of polymers retrieved at days 40 and 100 of burial experiments 2, 4, and 5 were analyzed via SEM imaging. Scanning electron microscope (SEM) images were taken off the surface of polymers to visualize the progression of material decomposition at a microscopic level. Prior to taking the images, samples roughly $5mm^2$ were sputter coated with a metal on their

surface. Sputter coating is a necessary pre-treatment when SEM imaging surfaces. The thin layer of a conducting material allows the electrons that are bombarding the material during imaging to evenly disperse over the entire surface- resulting in an accurate SEM depiction of the material surface[38]. Images were taken at 500X and 2000X. These magnifications were chosen based on related literature [39]. Images were also taken on the polymer samples in their initial state to provide a starting point comparison.

Chapter 3 . Results: Burial Decomposition

3.1. Overview

Multiple burial experiments were performed to follow the progression of blended polymer decomposition with variant soil environments. Soil amended with food waste, coffee grounds, or soil amended with compost starter (details of burial matrix composition in *table 2)* were used as variable environments for the novel blended polymer samples that were tested for decomposition within soil.

3.2. Soil burial experiment

Exp #	Burial Matrix	Tested Polymers	Duration	Data Sampling	Polymer size
1	Soil	PCL60, ST60, CELL, BIOBAGS	122d	Removal, clean of debris, weigh, then re-bury for addition data collection	2.5cm^2

Table 4. Overview of experimental details for burial experiment 1.

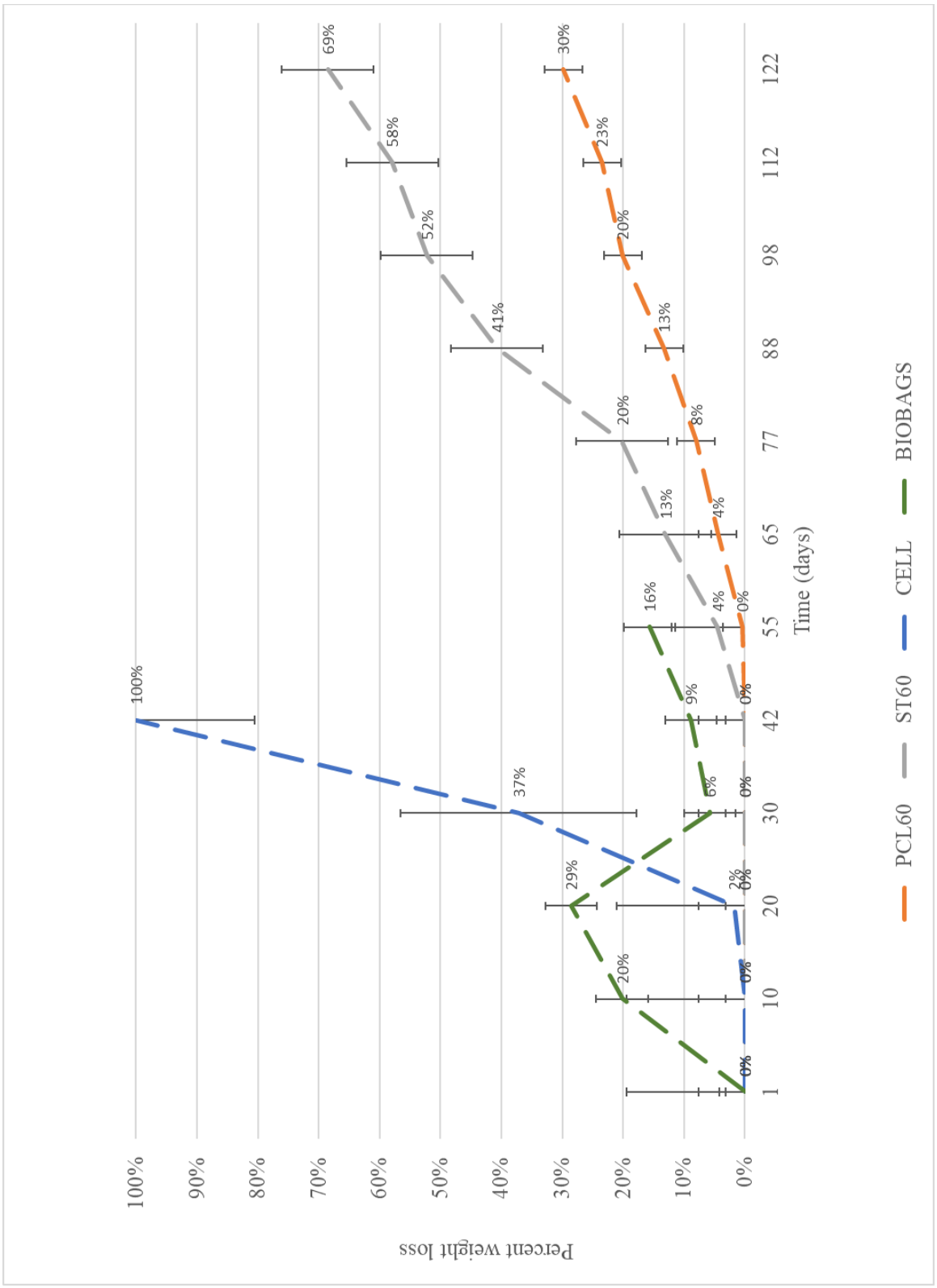

Figure 10. Percent weight loss of polymer samples in soil burial experiment 1.

The starch-heavy blended polymer ST60 degraded at a faster rate compared to PCL60, . Cellulose paper degraded as expected, functioning well as a positive control. On day 42, no distinguishable pieces of the cellulose material were found in the soil or able to be weighed. BioBag samples proved difficult to accurately weigh due to their low weight, leading to their data collection to be stopped on day 55.

When collecting data for BIOBAG decomposition, the low initial weight of the material (<0.1g) led to difficulties weighing the material as the experiment progressed. The light-weight material was not able to be cleaned of soil adhering its' surface without damaging the sample. Weights measured after day 20 showed innacuracies because the weight of soil unabled to be cleaned off heavily impacted the weighed mass of the BioBag material. This led to data collection on BioBag decomposition to be haulted after day 55, shown in *figure 10.* However, it should be noted that visually there was no major physical deterioration of the material within the 55 days data was collected.

3.3. Revised soil burial experiment

Exp #	Burial Matrix	Tested Polymers	Duration	Data Sampling	Polymer size
2	Soil	PCL60, ST60, BC, CELL, LDPE	120d	Initial weight as average of samples, experimental weight as individual sample not reused for later checkpoints	$2.5cm^2$ – PLC60, ST60, BC, LDPE $3.8cm^2$ -CELL

Table 5. Overview of experimental details for burial experiment 2

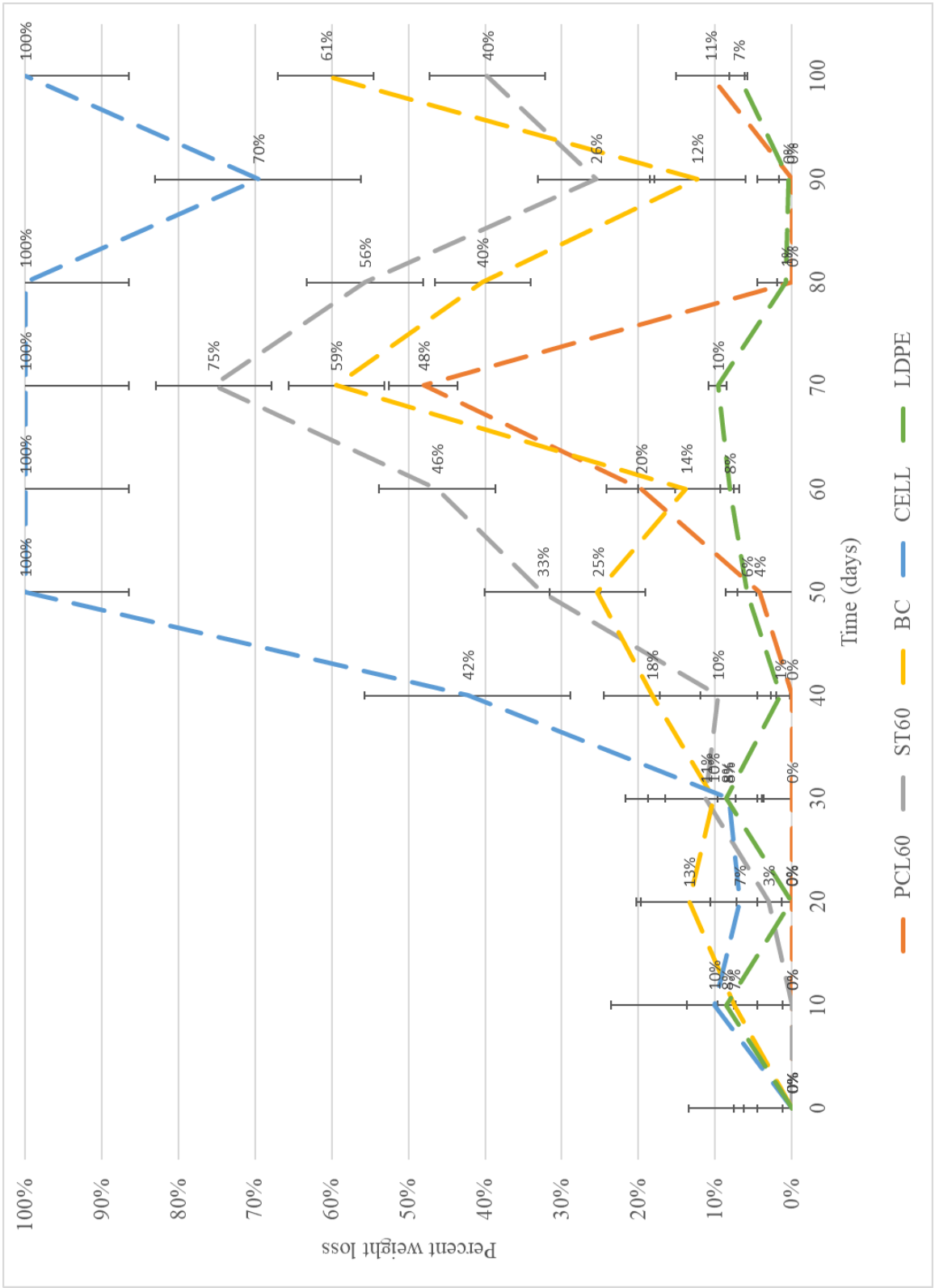

Figure 11. Percent weight loss of polymers in soil burial experiment 2.

Prior to day 60, bins of samples were kept in a temperature-controlled lab at RIT, however due to COVID-19 school closures in March of 2020, the bins were relocated to my apartment for the remainder of the experiment. The temperature variance from an unregulated residential heating system may be to blame for the irregularities in data after day 70.

Decomposition of materials followed similar trends displayed by the preliminary burial experiments. Days 0-120 showed samples of ST60 had higher degradation trend compared to PCL60. In this time span the ST60 samples also showed a slightly higher degradation trend when compared with BC. The three experimental blended polymer samples showed a positive correlation between degradation and starch content for days 0-120. For days 0-70, prior to possible temperature variations, CELL functioned as a positive control with the highest degradation trend line, and LDPE functioned as a negative control with the lowest degradation rate.

3.4. Coffee burial experiment

Exp #	Burial Matrix	Tested Polymers	Duration	Data Sampling	Polymer size
3	Coffee	PCL60, ST60, BC, CELL, LDPE	120d	Initial weight as average of samples, experimental weight as individual sample not reused for later checkpoints	2.5cm^2 – PLC60, ST60, BC, LDPE 3.8cm^2 -CELL

Table 6. Overview of experimental details for burial experiment 3.

As stated earlier for experiment 2, burial bins for experiment 3 were also relocated after COVID closures in March 2020. Data preceding day 50 was kept under temperature conditions aligning with the other experimental setups. However past day 50, the watering schedule of the bins did not maintain soil moisture at the higher room temperature they were being kept at. Despite the shortened period of time where temperature and moisture levels were uniform, data collected prior to day 50 showed low rates (<10%) of percent weight loss for the three novel polymers besides BC at day 10 and the control: CELL. The sporadic jumps in CELL percent weight loss, paired with the generally low percent weight loss for the remaining polymers do not support coffee amended soil as an ideal or improved degradation environment for the polymers being tested. Although generally coffee grounds are accepted as a food waste type in composting, this may negatively impact the microbial community needed for degradation of materials. One study found that amending plant material-based composts with coffee grounds led to compost acidification and reduced biological activity. Reduced biological activity as defined by reduced germination capacity of seeds compared to control compost.[40]

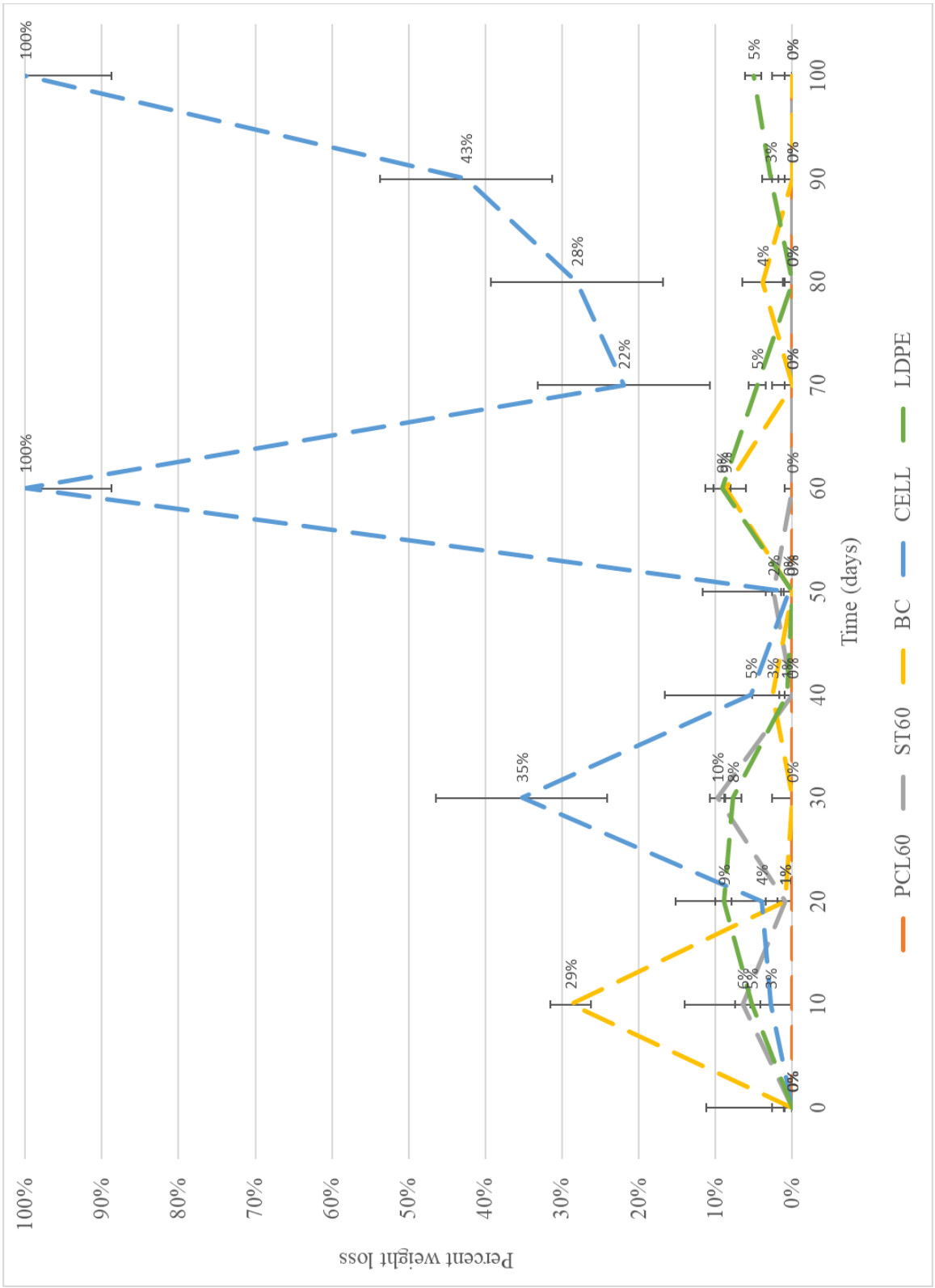

Figure 12. Percent weight loss of polymers in coffee burial experiment 3.

3.5. Food burial experiment

Exp #	Burial Matrix	Tested Polymers	Duration	Data Sampling	Polymer size
4	Food	PCL60, ST60, BC, CELL	120d	Initial weight as each individual samples' initial weight, experimental weight as individual sample not reused for later checkpoint	7.6cm^2- All samples

Table 7. Overview of experimental details for burial experiment 4.

In burial experiment 4, CELL was fully degraded by day 40, and continued to be fully degraded at all check points following day 40. Similar to burial experiments 1 and 2 in soil, ST60 showed higher percent weight loss overtime when compared to the other novel polymer blends. Interestingly, at day 80 BC and PCL60 followed similar downward trends, while ST60 peaked in degradation amount. This could indicate microbial preference to the ST60 material within the day 80 bin. The ending percent weight loss for the novel polymers were also within 10% of one another. When thinking about curating the ideal decomposition environment, having samples clustered closely in an upward degradation trend is ideal.

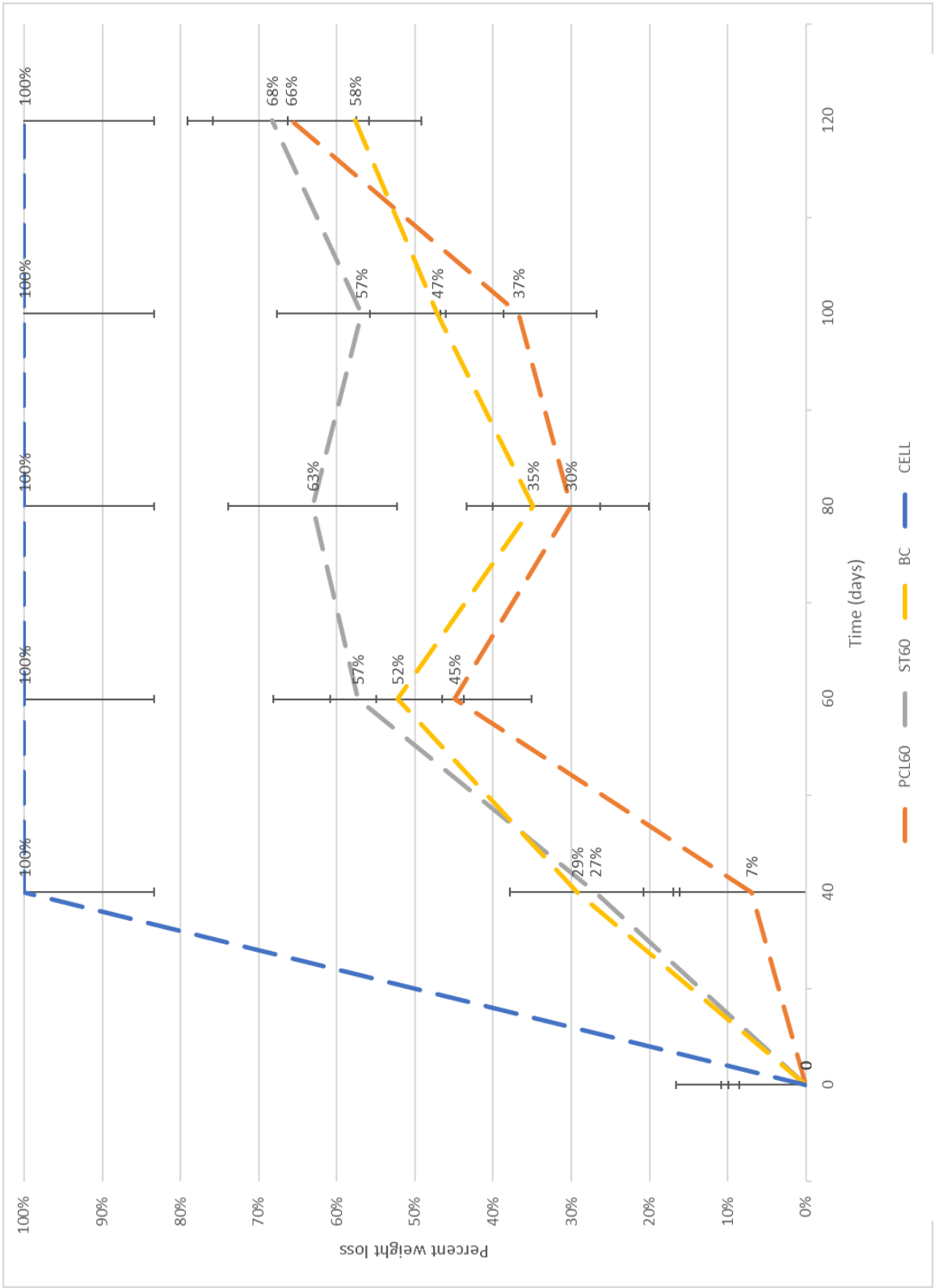

Figure 13. Percent weight loss of polymers in food burial experiment 4.

3.6. Soil burial experiment with PP, PET, BOPP, and PLA

Exp #	Burial Matrix	Tested Polymers	Duration	Data Sampling	Polymer size
5	Soil	PP, PET, BOPP, PLA	120d	Initial weight as average of samples, experimental weight as individual sample not reused for later checkpoints	7.6cm^2- All samples

Table 8. Overview of experimental details for burial experiment 5

Decomposition of commercially available films over 120 days are shown in *figure 14.* BOPP samples were found to average 31% weight loss by day 120. Film samples tested showed soil adhesion and weathering as the burial experiment progressed, which lead to the increasing and decreasing levels of decomposition seen at the progressing time points. Compared to the novel polymers (PCL60, ST60, and BC) tested in the same burial environment, PP, PET, PLA, and BOPP did not show a general upward trend in percent degradation.

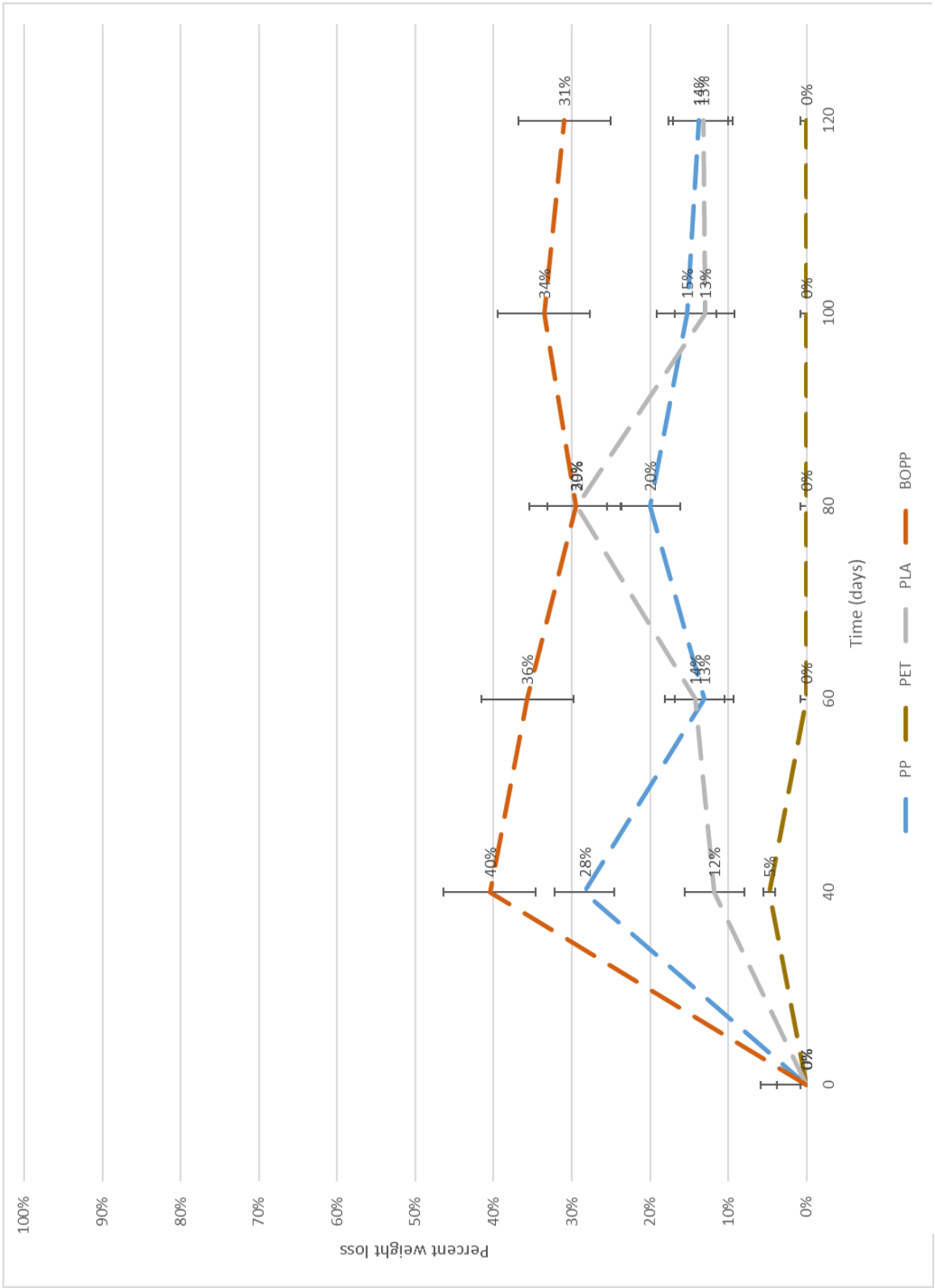

Figure 14. Percent weight loss of commercially available polymers in soil burial experiment 5.

3.7. SEM imaging

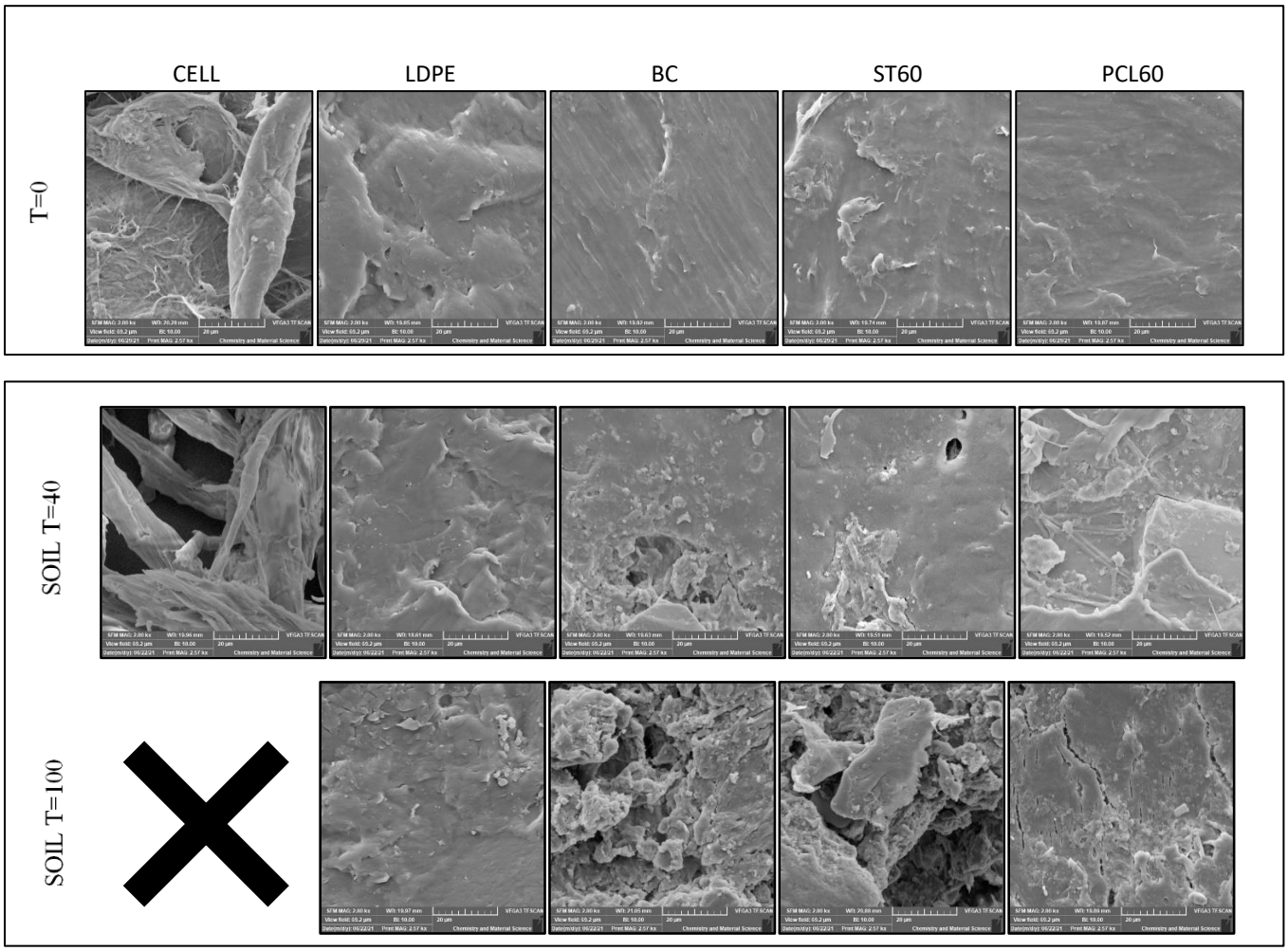

Figure 15. SEM images of polymers post-burial at 2000X magnification.

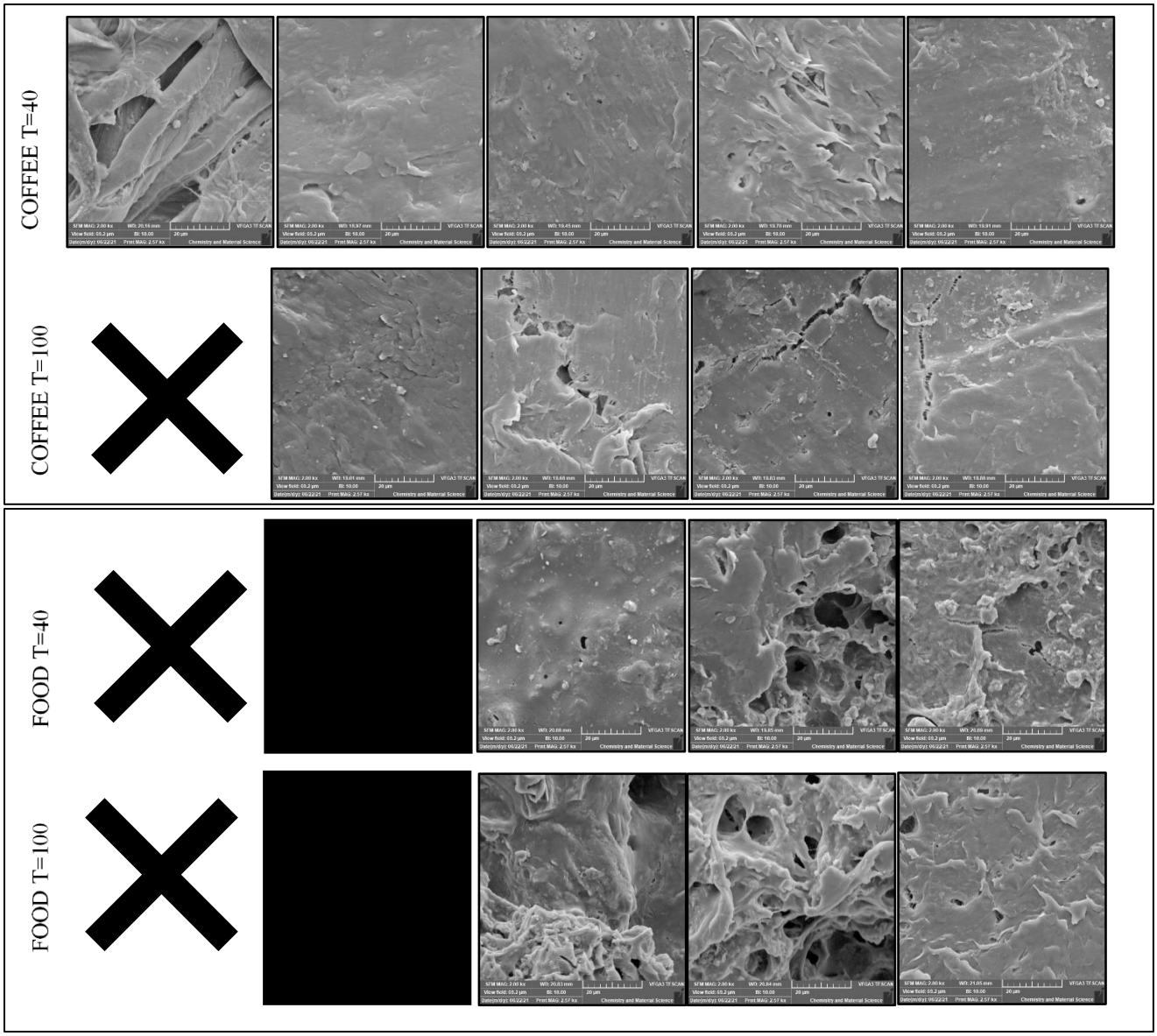

Figure 16. SEM images of polymers post-burial at 2000X magnification.

2000X magnification scanning electron microscope (SEM) imaging of polymer samples from burial experiments 3, 4, and 5 at days 40 and 100. From left to right: CELL, LDPE. BC, ST60, PCL60. Images of untreated polymers included for reference (T=0). 'X' represents complete degradation of material, and the black boxes are for polymers that were not tested in all environments.

3.8. Burial experiment statistics

Percent degradation data gathered from burial experiments 2, 3, and 4 were compared at days 40 and 100. A two-way Analysis of variance (ANOVA) was performed on each set of data: T=40 and T=100 using the JMP program[41]. The ANOVA method was used to test if the null hypotheses was supported or rejected by the data. Meaning, if the null hypothesis is accepted,

there is no difference in means. This hypothesis testing looks at comparisons withing polymer type, burial environment, and the interactions between those two factors. For both T=40 and T=100 all null hypotheses were rejected.

3.8.1. T=40 statistics

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	11	2.5642384	0.233113	9.1525
Error	24	0.6112736	0.025470	**Prob > F**
C. Total	35	3.1755120		<.0001*

Table 9. Analysis of variance for percent weight loss data from burial experiments 2, 3, and 4 at day 40.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
POLYMER	3	3	1.0275907	13.4485	<.0001*
ENVIRONMENT	2	2	0.6299486	12.3666	0.0002*
POLYMER*ENVIRONMENT	6	6	0.9066991	5.9332	0.0007*

Table 10. Effects Tests for percent weight loss data from burial experiments 2, 3, and 4 at day 40.

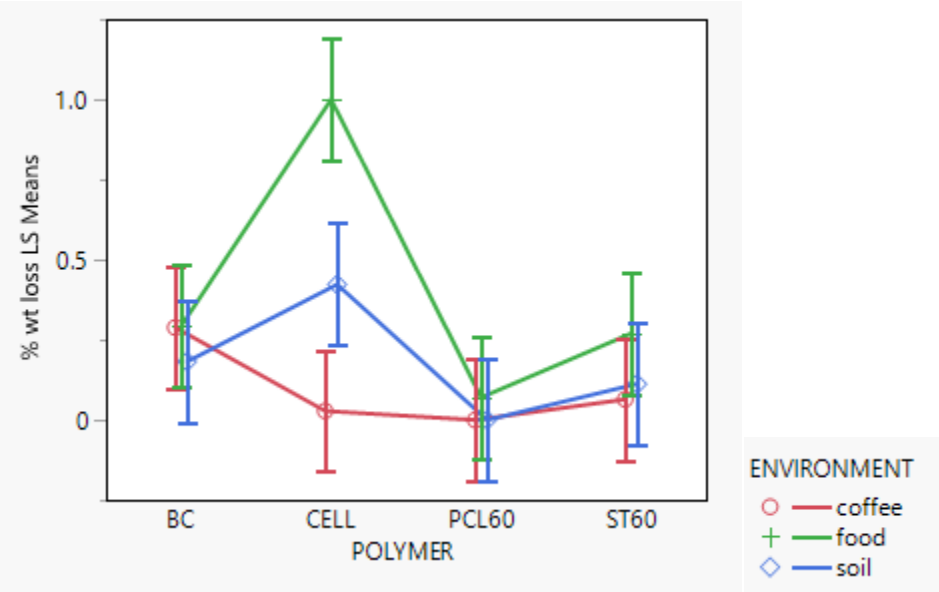

Figure 17. Least square means plot for T=40 burial experiment data.

3.8.2. T=100 statistics

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	11	3.5624167	0.323856	25.6498
Error	24	0.3030261	0.012626	**Prob > F**
C. Total	35	3.8654428		<.0001*

Table 11. Analysis of variance for percent weight loss data from burial experiments 2, 3, and 4 at day 100.

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
POLYMER	3	3	2.9491721	77.8592	<.0001*
ENVIRONMENT	2	2	0.2733563	10.8251	0.0004*
POLYMER*ENVIRONMENT	6	6	0.3398883	4.4866	0.0035*

Table 12. Effects Tests for percent weight loss data from burial experiments 2, 3, and 4 at day 100.

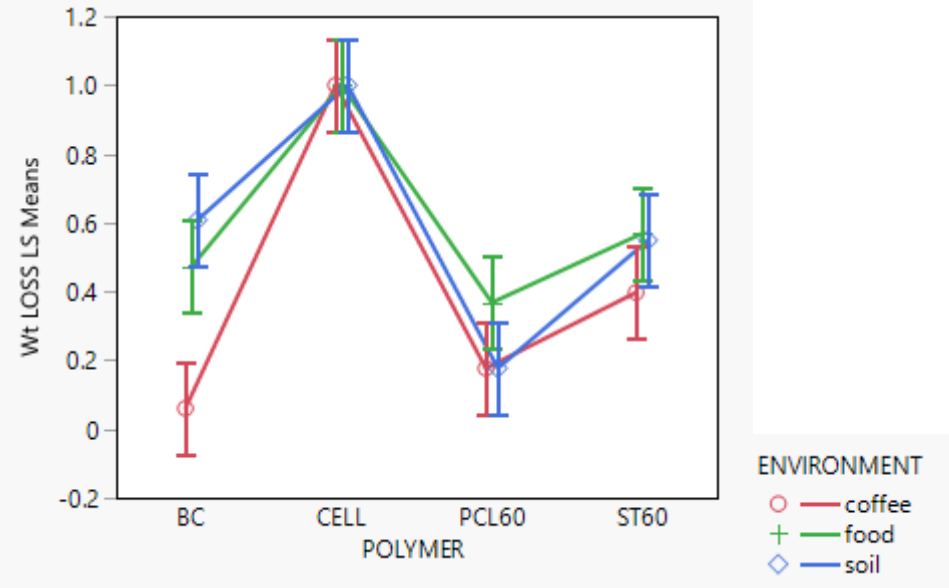

Figure 18. Least square means plot for T=100 burial experiment data.

Chapter 4 . Results: Carbon Dioxide Evolution

4.1. Overview

Carbon dioxide production was determined by the pH change of KOH within biometer flasks, and the subsequent titration every 10 days of the experimental KOH. Mg CO_2 shown on

the *figures 17, 18,* and *19* below are the net totals CO_2, where the each set of data is averaged and the average of the control flask containing only the respective burial matrix is deducted. The plotted lines represent the cumulative totals and reflect the total of the data gathered at and prior to the timepoint given.

4.2. CO_2 evolution in soil environment

CO_2 evolution in soil burial matrix mostly followed the ranking of percent weight loss of the same samples in the soil burial experiment, minus CELL. Plateaus of CO_2 production were seen for all materials except BC by day 110. In the soil biometer flasks, polymers with higher starch content produced the most CO_2. Over the 120 days, the net production of CO_2 for ST60 was 99.5mg, BC evolved 93.5mg, and PCL60 produced 43mg. CELL produced 29.8mg and the lowest production was seen from LDPE (22.6mg). There was also an initial plateau in net CO_2 production seen for all polymers until day 30.

4.3. CO_2 evolution in coffee environment

Unlike the percent weight loss data from the coffee burial experiment, the CO_2 evolution of polymers all followed a positive trend in coffee, and by day 120 ST60 produced more CO_2 (119.6mg) versus CO_2 evolution seen with soil. PCL60 produced 60.2mg CO_2 in coffee, almost 1.5 times the amount seen in soil. CELL also produced almost twice as much CO_2 in coffee (57.8mg) compared to soil. BC produced the least CO_2 (25.3mg) versus other polymers in coffee. Both BC and PCL60 saw plateaus of net production from days 80 to 120.

4.4. CO_2 evolution in food environment

CO_2 evolution in the food burial matrix showed BC, CELL and PCL60 with higher production when compared to the other CO_2 experiments: coffee and soil. BC produced the highest average of CO_2 across all burial matrices (346.9mg) which was over three times the amount of the second highest CO_2 evolution across all experiments: ST60 in coffee (119.6mg) shown in *figure 20*. ST60 only produced an average of 25.0 mg CO_2 across 120 days, the lowest CO_2 evolution of ST60 across all burial matrices. Fungal growth in individual flasks of CELL and PCL60 was seen to increase CO_2 production, but was not seen throughout all flasks.

4.5. CO_2 statistics

A two-way ANOVA was done on the T=120 CO_2 evolution data for each biometer flask within all burial matrices using JMP[41]. Based on the F ratio and P>F value, there was no significant difference between means between polymer type, burial environment, and their interactions. Although no significant statistical difference was found, the trends seen from the averaged duplicates will be further discussed. CO_2 experimental setups were done in duplicate due to availability of biometer flasks, but to obtain better statistical results methods should be increased to triplicate or more.

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	11	170826.13	15529.6	0.7009
Error	12	265875.05	22156.3	**Prob > F**
C. Total	23	436701.18		0.7182

Table 13. Analysis of variance for carbon dioxide evolution experiments.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
POLYMER	3	3	19457.54	0.2927	0.8299
ENVIRONMENT	2	2	50528.95	1.1403	0.3521
POLYMER*ENVIRONMENT	6	6	100839.65	0.7585	0.6155

Table 14. Effects test for carbon dioxide evolution experiments.

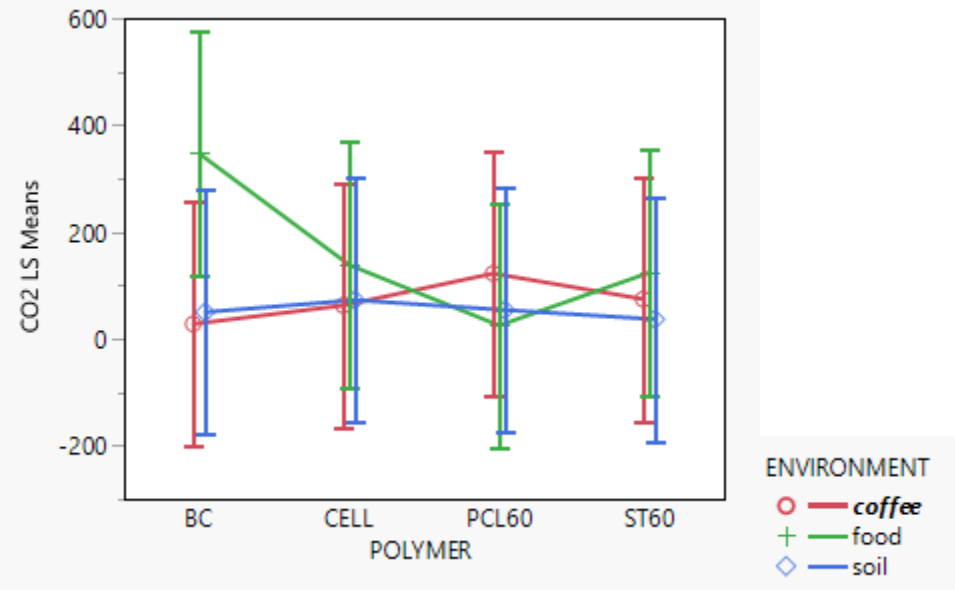

Table 15. Least square means plot for carbon dioxide evolution experiments.

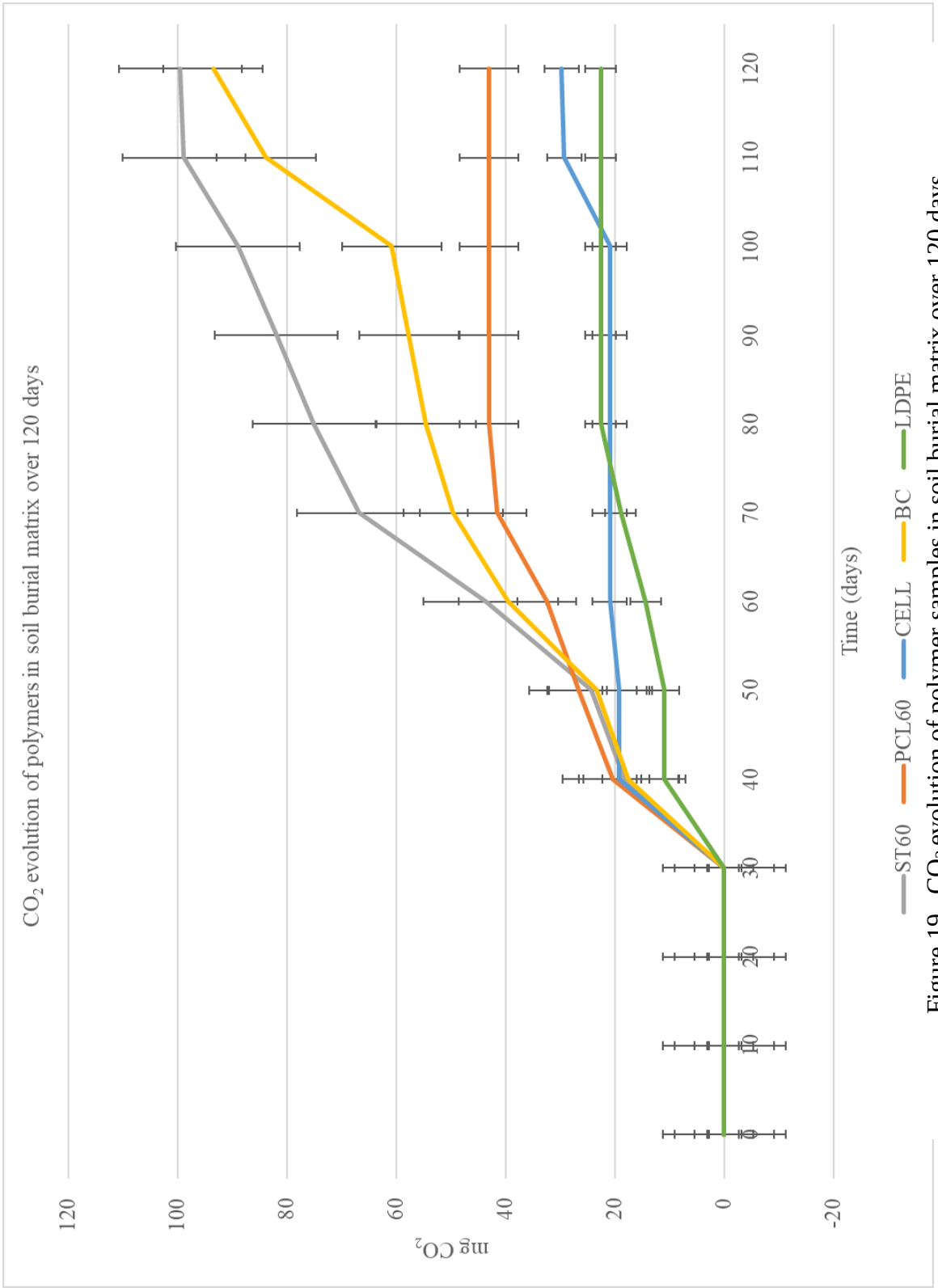

Figure 19. CO_2 evolution of polymer samples in soil burial matrix over 120 days.

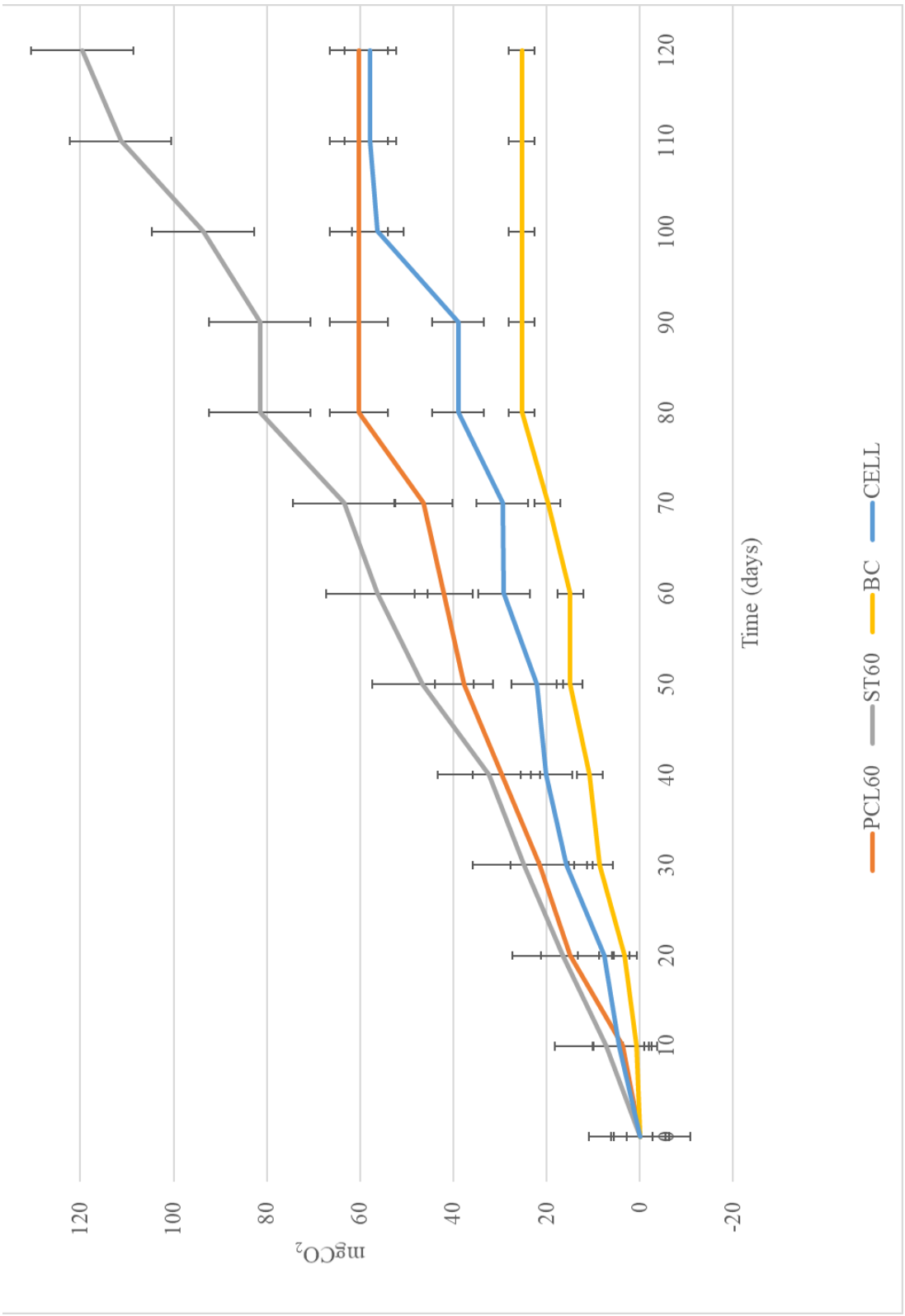

Figure 20. CO_2 evolution of polymer samples in coffee burial matrix over 120 days.

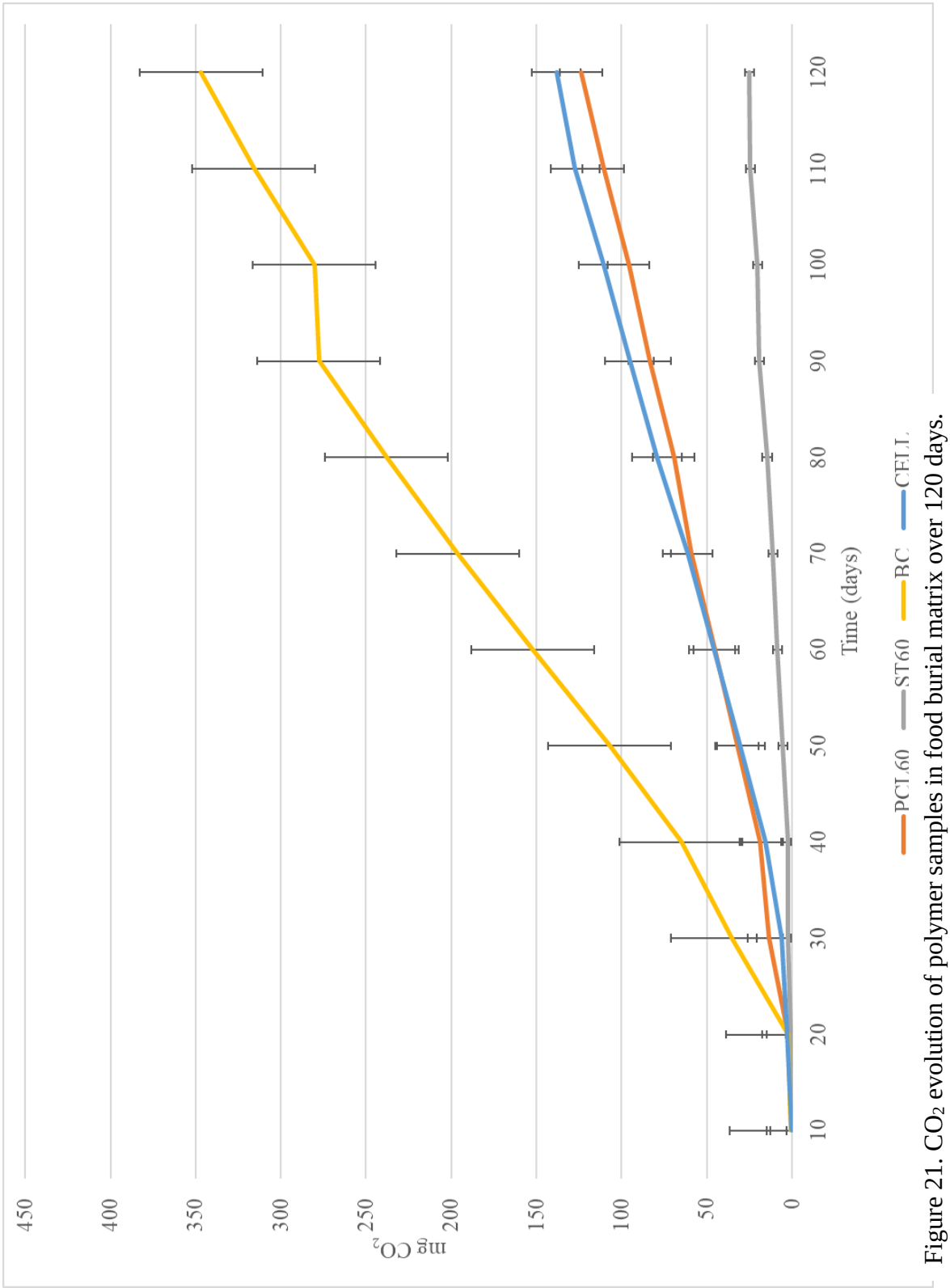

Figure 21. CO_2 evolution of polymer samples in food burial matrix over 120 days.

Chapter 5 . Results: Microbial Communities

5.1. Bacterial

Polymer Type	Burial Environment		
PCL60	FOOD	SOIL	COFFEE
	Microbacterium sp. Gram-positive, non-spore forming rod shaped. Isolated from soil, insects, human specimens, dairy productions and more [41] Has been found to produce enzymes that degrade Polystryene (PS) films when buried in soil [42]. ***Rhodococcus globerulus*** Present in soils, some being pathogenic, some harmless. Well known taxa for their biodegradation and bioremediation [43]. Has been shown to form biofilms along with Aeromonas sp. in study investigating the biodegradability of films (including PCL) in activated sludge (a wastewater treatment process)[44]. ***Chryseobacterium cucumeris*** This genus is rod shaped, occurring in soil, water, rhizospheres, chicken, fish, and raw milk. Many isolated from plants have part in the plants defense of pathogens[42].	***Ochrobactrum sp*** Gram-negative bacillus, oxidase producing, non-lactose fermenting with environmental origins that has been shown to be pathogenic in immunocompromised people. [43] *Ochrobactrum anthropi* was found in high frequency in activated sludge. [44] Microbes that exist within anthropogenic-derived waste conditions are often considered for bioremediation potential [45].	unable to sequence

ST60	FOOD	SOIL	COFFEE
	Pedobacter sp. Rapidly growing genus. One species, *P. heparinus,* produces enzymes that degrade mucopolysaccharides.[46] ***Stenotrophomonas maltophilia*** an environmentally ubiquitous bacteria used in bioremediation that has also been found to be an opportunistic pathogen to immuno-compromised persons [47].	***Enterobacter cloacae*** Gram-negative, rod-shaped, facultatively anaerobic bacterium of clinical significance. Research using *E. cloacae* in microbial fuel cells showed its degradation of cellulose. Highlighting the bacterium's cellulolytic and exoelectrogenic activity [48] *E. cloacae* strain AKS7 has been shown to develop biofilm over LDPE – leading to enhanced degradation of the plastic [49]. ***Enterobacter sp.*** Common gram negative bacteria, with some species acting as opportunistic pathogens [50] Found in water, soil, sewage, and intestinal tracts of animals. Capable of nitrogen fixation and are have been isolated from the rhizospheres of crops like wheat and rice. [51].	***Bacillus subtilis*** A rhizobacterium species that is non-pathogenic, gram-positive, rod shaped bacteria, that feeds on decayed organic matter that can form biofilms. *B. subtilis* is also endospore forming [52], [53].

BC	FOOD	SOIL	COFFEE
	Bacillus megaterium Gram positive, endospore forming rod shaped bacteria, aerobic. Large variety of enzymes produced-leading to its use in bioremediation[54]. ***Brucella melitensis*** Soil microbe that causes reproductive losses and illness in some ruminant species [55].	***Pseudomonas moorei*** Specific strain (KB4) of this species was shown to degrade paracetamol (an emerging medicine-derived pollutant). [56] ***Enterobacter cloacae*** gram-negative, rod-shaped, facultatively anaerobic bacterium of clinical significance. Research using *E. cloacae* in microbial fuel cells showed its degradation of cellulose. Highlighting the bacterium's cellulolytic and exoelectrogenic activity [48] *E. cloacae* strain AKS7 has been shown to develop biofilm over LDPE – leading to enhanced degradation of the plastic [49].	***Pseudomonas sp.*** Broad genus of gram-negative bacterium. Many species are used for bioremediation of various anthropogenic pollutants, due to their metabolic diversity [57]. ***Brucella melitensis*** Soil microbe that causes reproductive losses and illness in some ruminant species [55].

CELL	FOOD	SOIL	COFFEE
	Pseudomonas sp. Broad genus of gram-negative bacterium. Many species are used for bioremediation of various anthropogenic pollutants, due to their metabolic diverstiy [57]. ***Paenibacillus sp.*** Rhizobacterium species with at least 16 strains known to be nitrogen-fixing. Other bacterial characteristics to enhance plant health and soil health are seen within this species [58].	***Paenarthrobacter ureafaciens*** a gram-positive aerobic bacterium shown to degrade sulfamethazine[59] ***Sphingobacterium faecium*** A species of *Sphingobacterium* isolated from cattle feces. The genus is comprised of gram-negative bacilli that are positive for catalase and oxidase. Commonly isolated from soil and compost [60].	***Brucella melitensis*** Soil microbe that causes reproductive losses and illness in some ruminant species.[55]

LDPE	FOOD	SOIL	COFFEE
		Rhodococcus sp. Environmental bacterium commonly found in soils. Large group of diverse bacteria that have shown great degradation properties. This is due to their ability to amass many catabolic genes [61]	***Pedobacter ginsengisoli*** This species was first isolated from a ginseng field soil sample in South Korea. Members of the *Pedobacter* genus are obligate aerobes, gram-negative, and positive for oxidase, catalase, and heparinase.[46], [64]
		***Pseudoxanthomonas sp*.** **G**ram-negative bacteria with some specific sub-species showing potential for the bioremediation of diesel oil [62] and antibiotics in the environment [63].	

Table 16. Bacterial species identified from burial experiment soil samples via 16S rRNA genomic sequencing.

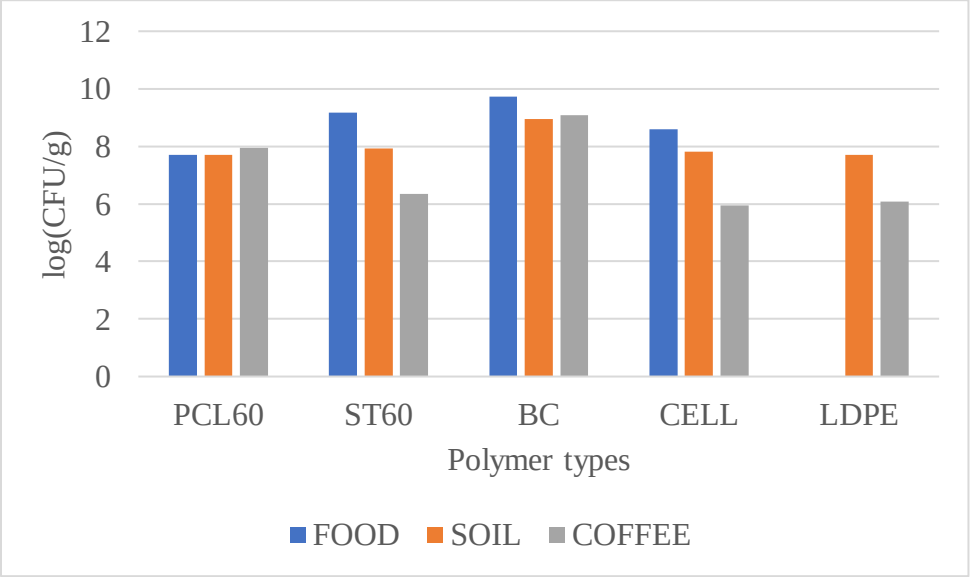

Figure 22. log(CFU/g) of bacterial colonies isolated from polymer surfaces at T=100 of burial experiments 2,3 and 4

5.2. Fungal

Polymer Type	Burial Environment		
PCL60	FOOD	SOIL	COFFEE
	Trichophyton	unidentified	***Trichoderma***
	Genus of fungi that can be pathogenic (ex. ringworm). Present in natural environments. Some species are wood degrading and have been shown to be successful for the bioremediation of textile azo dyes through fungal biodegradation and bioadsorption [65]		Used for industrial production of cellulase, specifically β-glucosidases which break down cellulose into monomers of glucose. [66] Trichoderma species have also been shown to degrade PLA [67]
ST60	FOOD	SOIL	COFFEE
	Deuteromycetes	***Trichoderma***	***Trichoderma***
	Grouping of mold fungi. Broad category, but different species strains have been shown to degrade lignin in composts [68]. As well as Polycyclic aromatic hydrocarbons or PAHs which naturally occur in crude oil and gasoline [69]	Used for industrial production of cellulase, specifically β-glucosidases which break down cellulose into monomers of glucose. [66] Trichoderma species have also been shown to degrade PLA [67]	Used for industrial production of cellulase, specifically β-glucosidases which break down cellulose into monomers of glucose. [66] Trichoderma species have also been shown to degrade PLA [67]
	Fusarium		** unidentified yeast species also present
	Common soil fungi that is also found in mycorrhizae of plants. Strain *F. proliferatum* CF2 has		

	been shown to degrade the pesticide allethrin.[70] Fusarium sp were also in a fungal community that led to depredation of polythene[71]		
BC	FOOD	SOIL	COFFEE
	Deuteromycetes Grouping of mold fungi. Broad category, but different species strains have been shown to degrade lignin in composts [68]. As well as Polycyclic aromatic hydrocarbons or PAHs which naturally occur in crude oil and gasoline [69]	***Fusarium*** Common soil fungi that is also found in mycorrhizae of plants. Strain *F. proliferatum* CF2 has been shown to degrade the pesticide allethrin.[70] *Fusarium sp* were also in a fungal community that led to depredation of polythene[71]	**unidentified yeast species ***Aspergillus*** Aspergillus strains were found in abundance on the surface of polymers (PCL, PHB, PLA, and PBS) in a biodegradation study[72]
CELL	FOOD	SOIL	COFFEE
	Trichoderma Used for industrial production of cellulase, specifically β-glucosidases which break down cellulose into monomers of glucose. [66] Trichoderma species have also been shown to degrade PLA [67]	***Trichoderma*** Used for industrial production of cellulase, specifically β-glucosidases which break down cellulose into monomers of glucose. [66] Trichoderma species have also been shown to degrade PLA [67]	***Trichoderma*** Used for industrial production of cellulase, specifically β-glucosidases which break down cellulose into monomers of glucose. [66] Trichoderma species have also been shown to degrade PLA [67]

LDPE	FOOD	SOIL	COFFEE
		Trichophyton	***Aspergillus***
		Genus of fungi that can be pathogenic (ex. ringworm). Present in natural environments. Some species are wood degrading and have been shown to be successful for the bioremediation of textile azo dyes through fungal biodegradation and bioadsorption [65]	Aspergillus strains were found in abundance on the surface of polymers (PCL, PHB, PLA, and PBS) in a biodegradation study[72]

Table 17. Fungal isolates from burial experiment soil samples identified by colony morphology and microscopy.

Soil samples were collected off the surface of polymers at T=100 from burial experiments 2, 3, and 4. Identification of fungal colonies was based off lactophenol cotton blue staining and colony morphology.

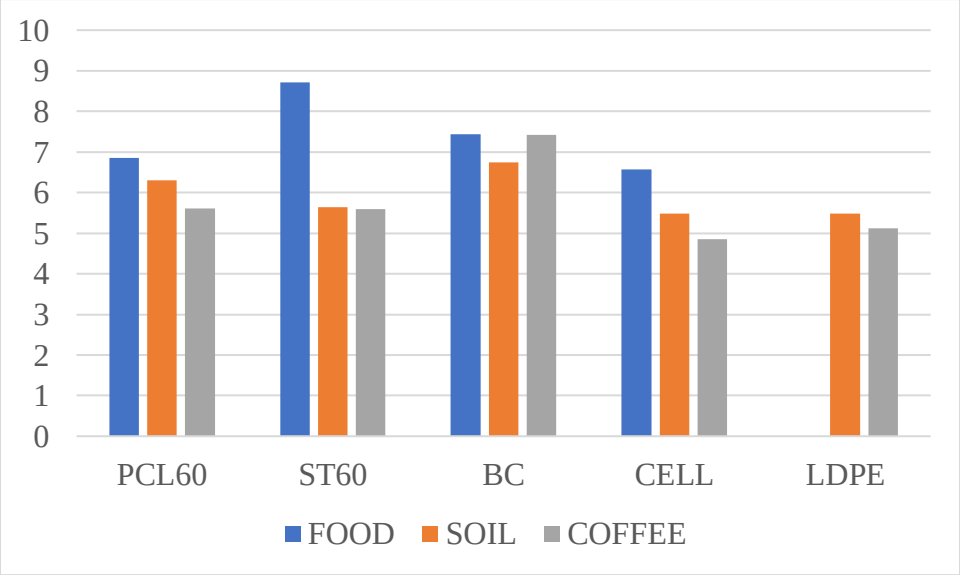

Figure 23. log(CFU/g) of fungal colonies isolated from polymer surfaces at T=100 of burial experiments 2,3 and 4

Chapter 6 . Discussion

6.1. Burial Experiments

As supported by the ANOVA findings, the inclusion of food waste shows a favorable increase in percent weight loss for ST60, PCL60, and partially BC. The soil environment showed higher rates of BC percent weight loss (61%) versus food burial BC (47%) only at T=100. Prior to T=100, the BC buried with food waste outperformed the soil-only environment. Across all novel blended polymers (PCL60, ST60, and BC) the coffee burial environment resulted in lower percent weight loss. Cellulose paper functioned well as a control in all environments, with all three reaching 100% degradation by day 60. However, within the coffee burial environment, there was a drop below 30% degradation which may be attributed to the temperature variations that were previously mentioned.

Utilization of food and soil burial environments both resulted in polymers with higher starch content (ST60 and BC) reaching higher percent weight loss that PCL60. Higher percent inclusion of starch was shown to reflect more degradation, as measured by percent weigh loss, in food and soil burial environments. These findings show that PCL and starch blended polymers should be considered as having potential for use as biodegradable materials. In addition, increasing starch content is shown to increase percent weight loss in burial environments with favorable microbial communities. Also, the inclusion of biochar was not inhibitory to degradation.

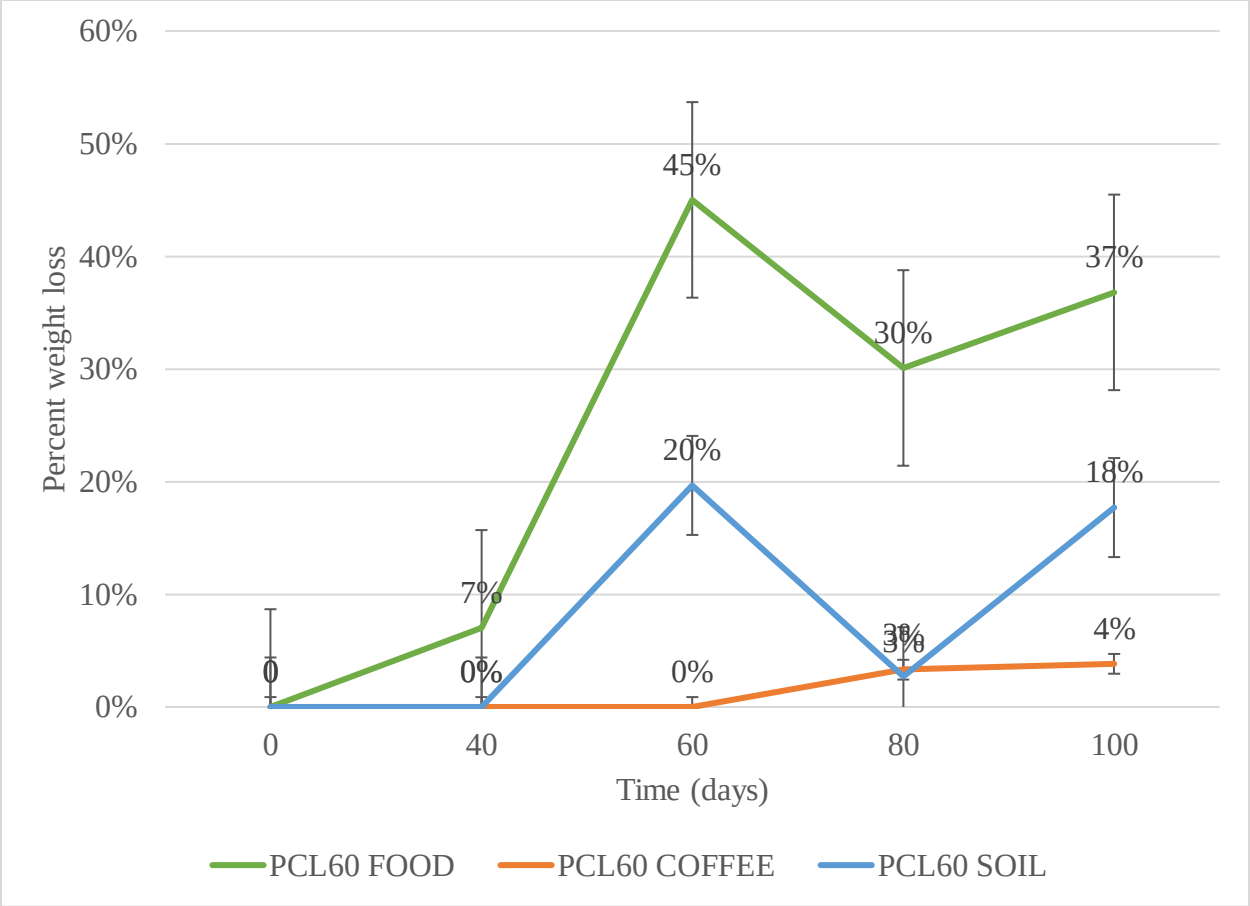

Figure 24. Percent weight loss of PCL60 in three burial environments over 100 days.

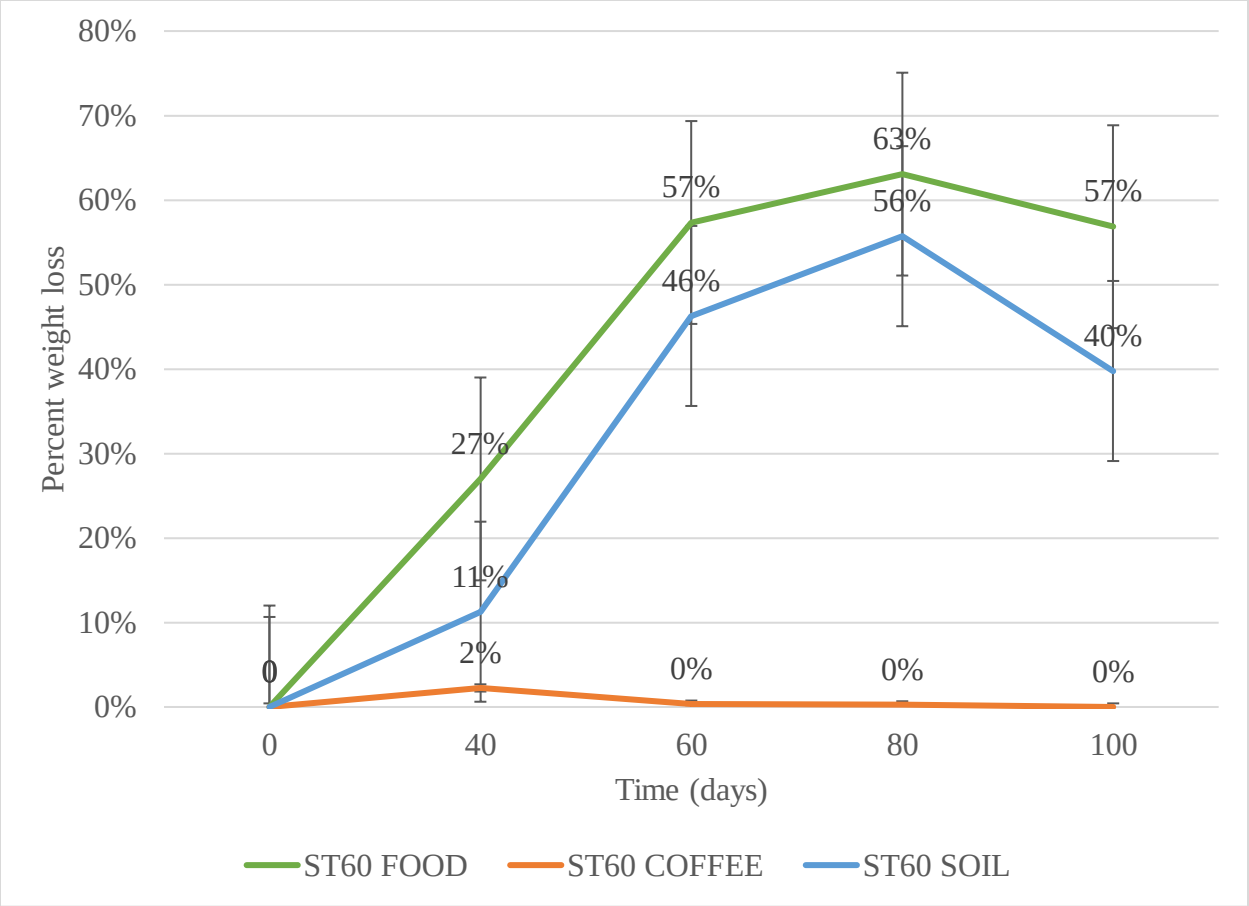

Figure 25. Percent weight loss of ST60 in three burial environments over 100 days.

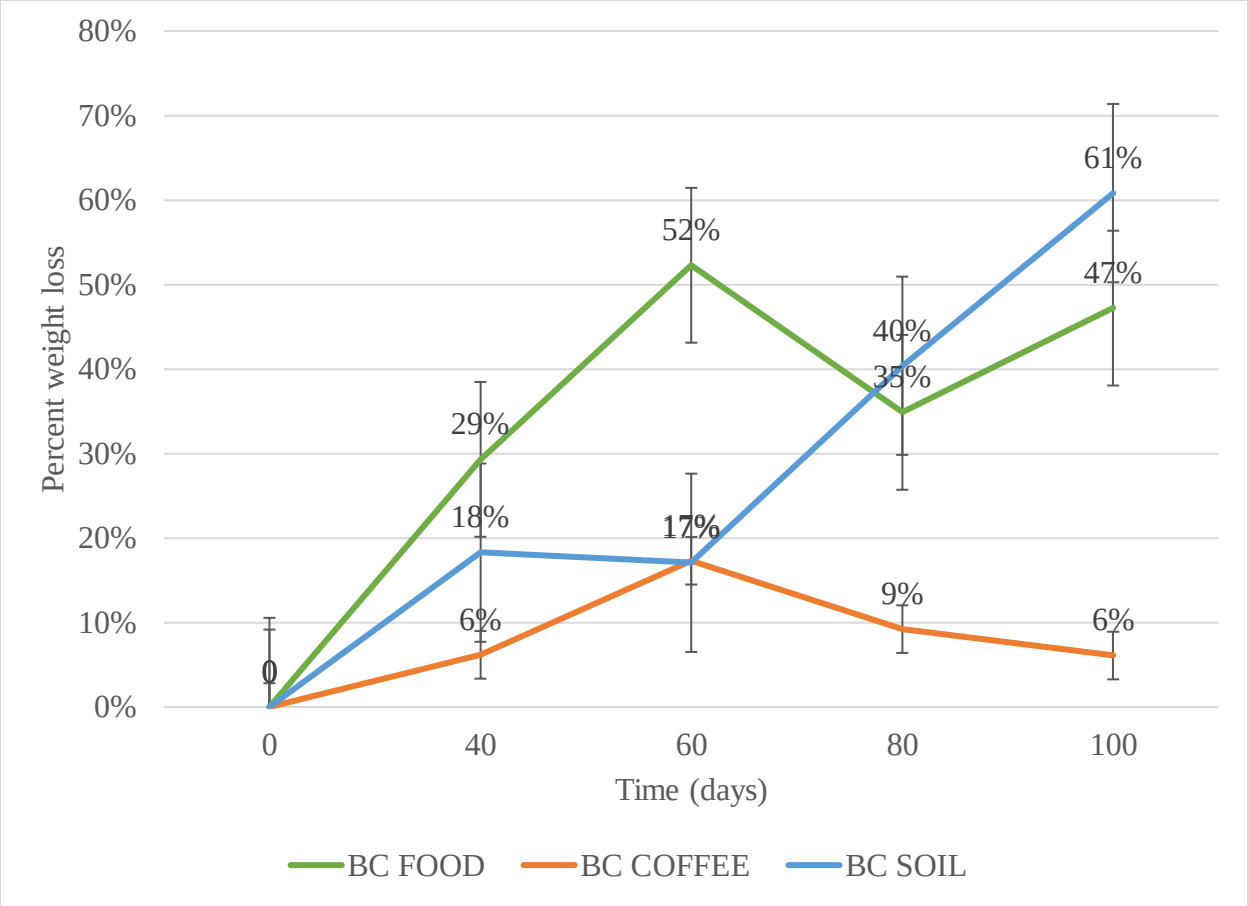

Figure 26. Percent weight loss of BC in three burial environments over 100 days.

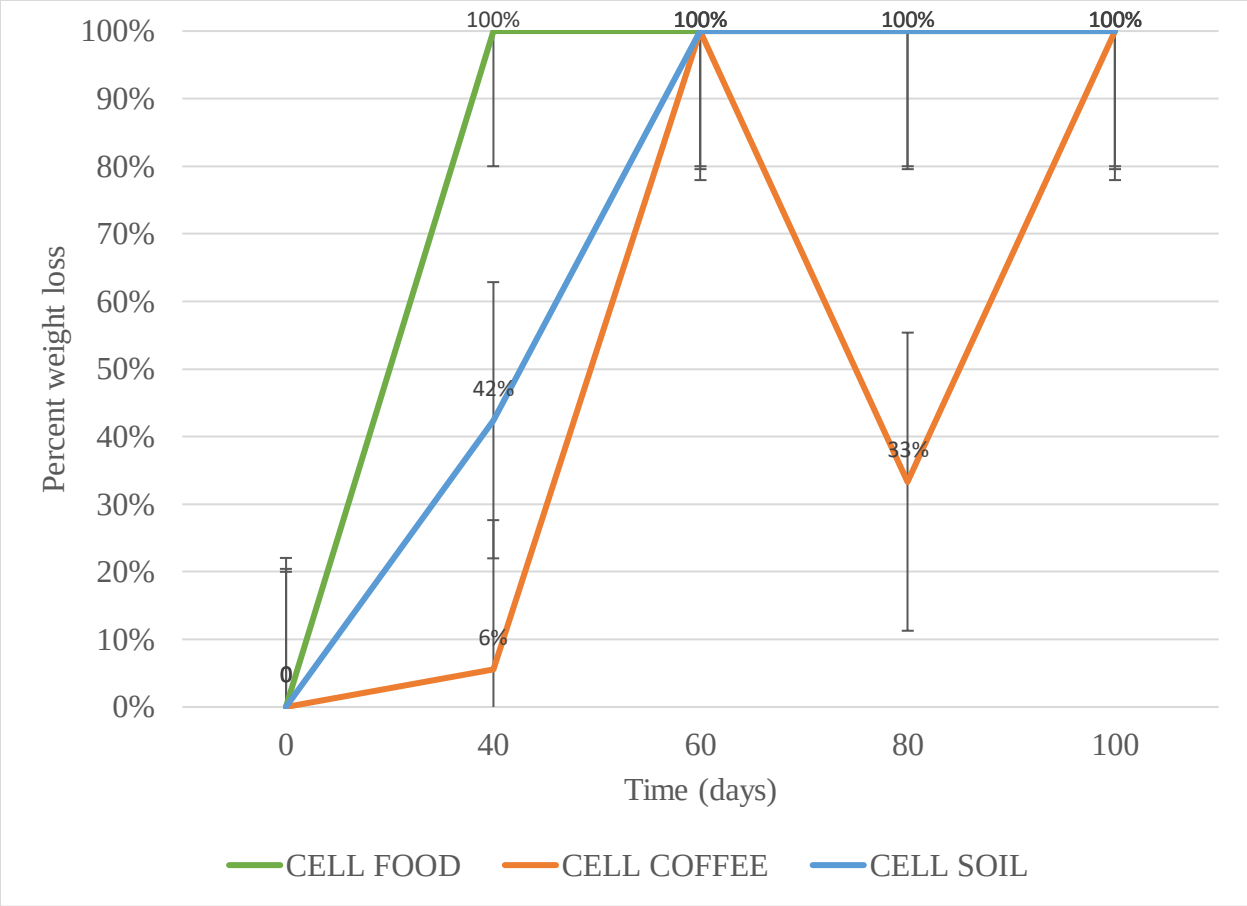

Figure 27. Percent weight loss of CELL in three burial environments over 100 days.

6.1.1. SEM imaging

SEM imaging done at 500X and 2000X magnification showed progression of polymer surface degradation in all three burial environments for days 0, 40 and 100. Comparison between polymer types showed materials with higher starch content ST60 (60% starch) and BC (45% starch) showed more pore formation compared to PCL60 and LDPE.

Looking between burial environments, samples unearthed from coffee show the least amount of surface breakdown for both timepoints 40 and 100. However, the control CELL was seen to fully degrade by day 100. Soil burial environment showed breakdown of CELL and minimal pore formation for BC at day 40. At day 100 in soil there was noticeable degradation for BC, ST60, and PCL60, as well as full CELL degradation. The food burial environment showed the most noticeable surface breakdown for BC, ST60, and PCL60 at both timepoints. Also, in the food burial matrix was the only CELL reached full degradation by day 40.

6.2. Microbial Identification

Both bacterial and fungal species identified were chosen due to their majority colonization on either PCA or PDA with streptomycin plates. The microbial source of the plated

cultures was the polymer surfaces at T=100 from the three burial environments. Although the microbial source are the polymer surfaces, it is necessary to note that selection of the best growing colony on plates may favor species that simply grow well on the selected medias. Although general growth medias were selected to best grow all fungal or bacterial species present in soil samples, it cannot be assumed that the colonization of plate growth is perfectly reflective of polymer surface colonization.

6.2.1. Bacterial 16S rRNA sequencing

Nine unique species were identified across all polymers in both soil and food environments. Within the coffee environment, four unique species were identified. Food and soil burial environments saw the most diversity of isolates, there was also more bacteria of interest for these burial matrices. These bacteria of interest are the same species or closely related to specific strains that have shown material degradation properties in literature cited. Isolated from PCL60 in the food burial environment, *Rhodococcus globerulus* is a bacterial species that has been shown by other research to form biofilms with *Aermonas sp.* and degrade PCL films. [73]. In soil, *Enterobacter cloacae* was isolated off ST60. Interestingly, a specific strain (AKS7) of *E, cloacae* has been shown to enhance the degradation of LDPE via the formation of a biofilm [49]

Beyond bacteria that has been researched specifically for material degradation properties, many species isolated have been cited for having potential bioremediation applications. *Bacillus megaterium, Pseudomonas sp.,Pseudomonas moorei, Ochrobactrum sp., Stenotrophomonas maltophilia, Rhodococcus globerulus,* and *Pseudoxanthomonas sp.* are all bacterium cited within literature for their potential use for bioremediation [39] [43] [45] [52] [54] [55] [60]. Out of the bacterium of interest for bioremediation, only *Pseudomonas sp.* were isolated from coffee environments.

6.2.2. Fungal identification

Fungal identification was done based off isolated colony morphology and staining with lactophenol cotton blue (LPCB). Between disposal environments, there were four uniquely identified fungal species or groups in the food burial matrix: *Trichophyton*, *Deuteromycetes, Fusarium* and *Trichoderma*. Three unique classifications for soil environments*: Trichoderma, Fusarium,* and *Trichophyton*. And in the coffee burial experiment only *Aspergillus* and *Trichoderma* species were found. The only sample without an identified fungal species was PCL60 in soil burial. The LPCB stain for this unidentified species is shown in *figure 28.*

In both soil and coffee environments, *Trichoderma* was isolated off ST60 samples. In literature cited, *Trichoderma* was shown to break down cellulose and PLA. This is of interest because PLA has an ester functional group, just like PCL. Also, cellulose is similar structurally to starch due their shared repeating monomer unit: glucose. Though a *Trichoderma* species was not isolated from the food environments: a *Deuteromycetes* species was which is the group of fungi *Trichoderma* belong to. *Trichoderma* was also seen on CELL samples across all environments.

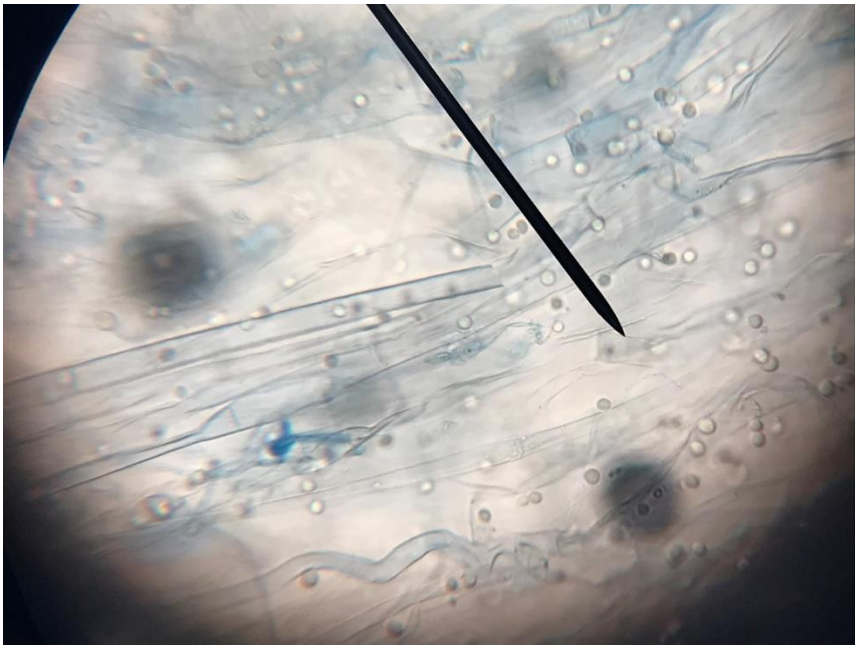

Figure 28. Lactophenol cotton blue stain of unidentified fungi.

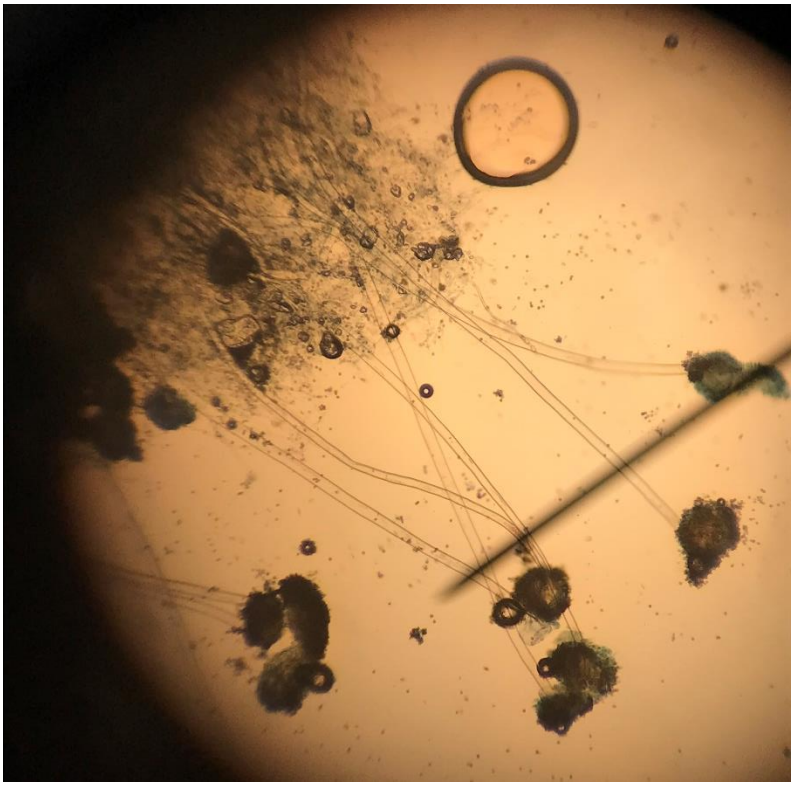

Figure 29. Lactophenol cotton blue stain of *Aspergillus.*

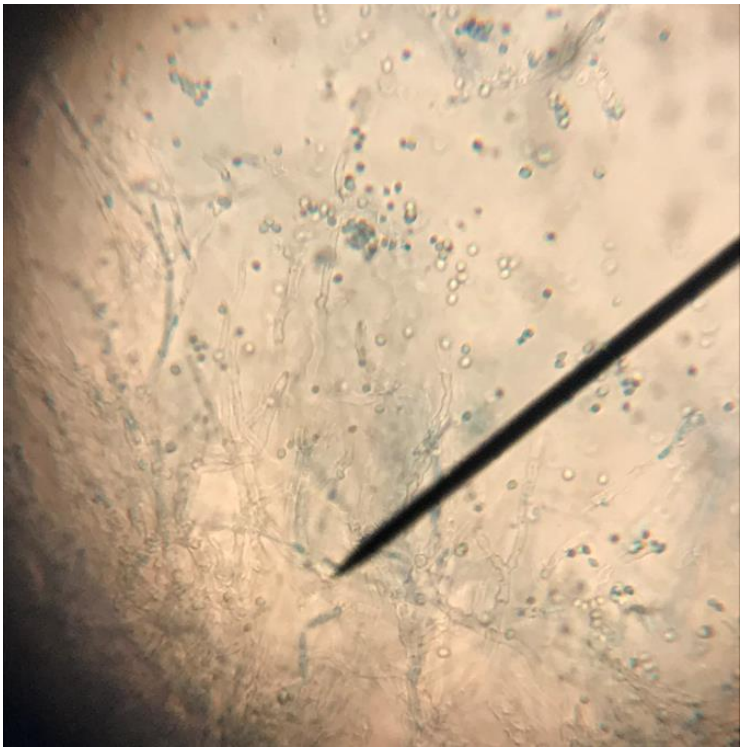

Figure 30. Lactophenol cotton blue stain of *Trichoderma.*

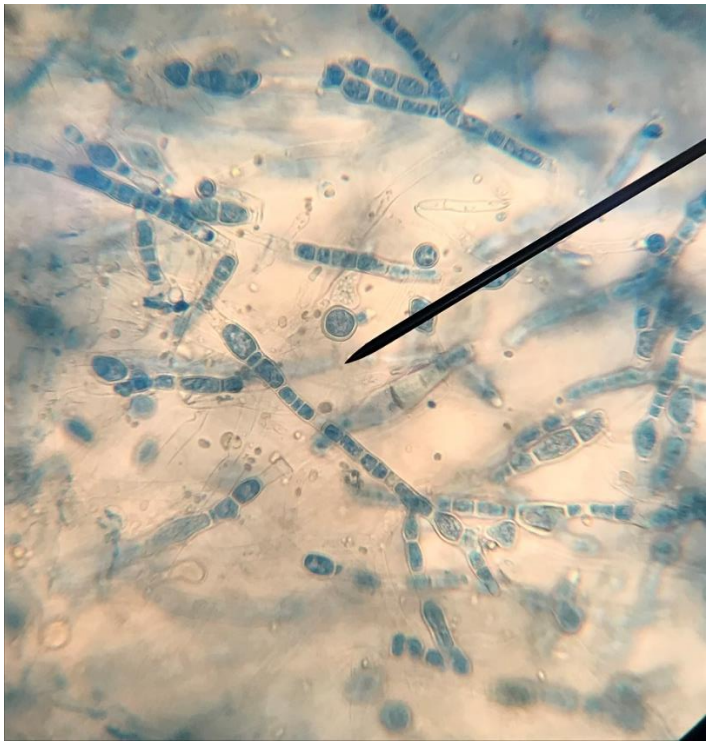

Figure 31. Lactophenol cotton blue stain of *Trichophyton.*

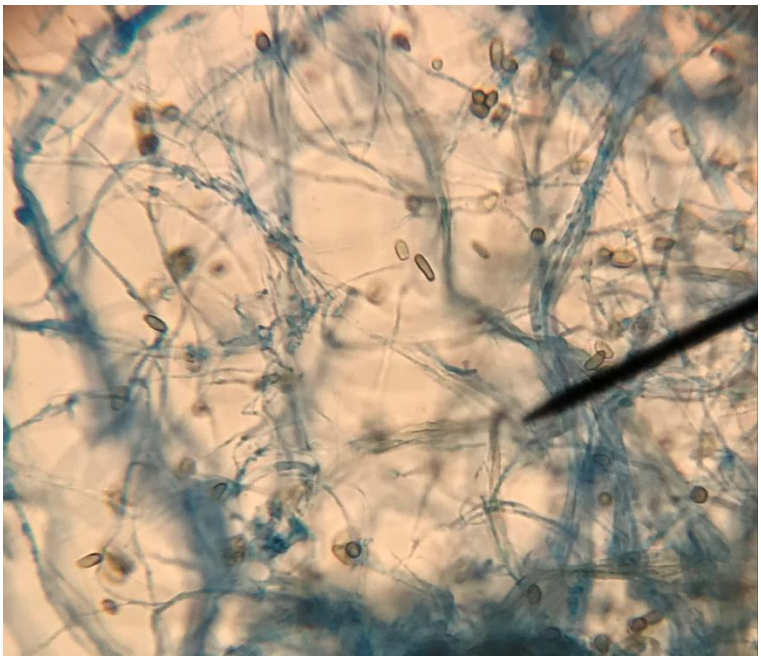

Figure 32. Lactophenol cotton blue stain of *Fusarium.*

6.3. CO_2 Evolution

CO_2 evolution data across all three burial environments for polymers PCL60, ST60, BC, and CELL were not shown to have statistically significant differences. This may be attributed to only duplicates being used to collect data. Any replication of these methods are recommended to use triplicates for data collection. Although shown to be statistically insignificant, observations from the CO_2 experiment will be discussed further. Comparisons of cumulative CO_2 production of polymers within the three environments are shown in *figures 33, 34,* and *35*.

From the CO_2 data gathered from all burial matrices, BC in the food burial environment/matrix showed the highest evolution of CO_2 (346.9mg) over 120 days amongst all polymer types. Polymers PCL60 and CELL showed higher CO_2 emissions in the food burial environment compared to coffee and soil. However, ST60, which showed higher percent weight loss for 3 out of 4 burial experiments (1,2,4) of the novel blended polymers produced the least amount of CO_2 in the food burial environment.

It is important to note that beyond the time when the ascarite tower is being vented on the biometer flasks and the KOH is taken via syringe from the side arm, the system is closed. This lack of oxygen influx during CO_2 capture may have proven problematic when using a burial matrix with high endogenous microbial activity, like food waste. It has also been cited that soils rich in nitrogen may turn biometer flask systems anaerobic [75].

All CO_2 evolution shown is theoretically sourced only from the breakdown of polymer samples, since the CO_2 evolution of the respective burial matrix is deducted from the experimental CO_2 evolution that involves both the burial matrix and the polymer. When

comparing the CO_2 evolution of polymers in varied burial matrices, nutrient availability could dictate the microbial breakdown of materials. Microbial favored burial environments may favor breaking down the burial material over the polymer material in a closed system like a biometer flask. Conversely, a microbially unfavorable burial environment may lead microbial breakdown of the polymer. Because CO_2 evolution is microbially driven, and we are purely monitoring the output of CO_2 based on pH change and titrations, it is necessary to acknowledge the complexities of microbial activity and consider all potential feedstocks that are present and may be used by both fungi and bacteria to produce CO_2.

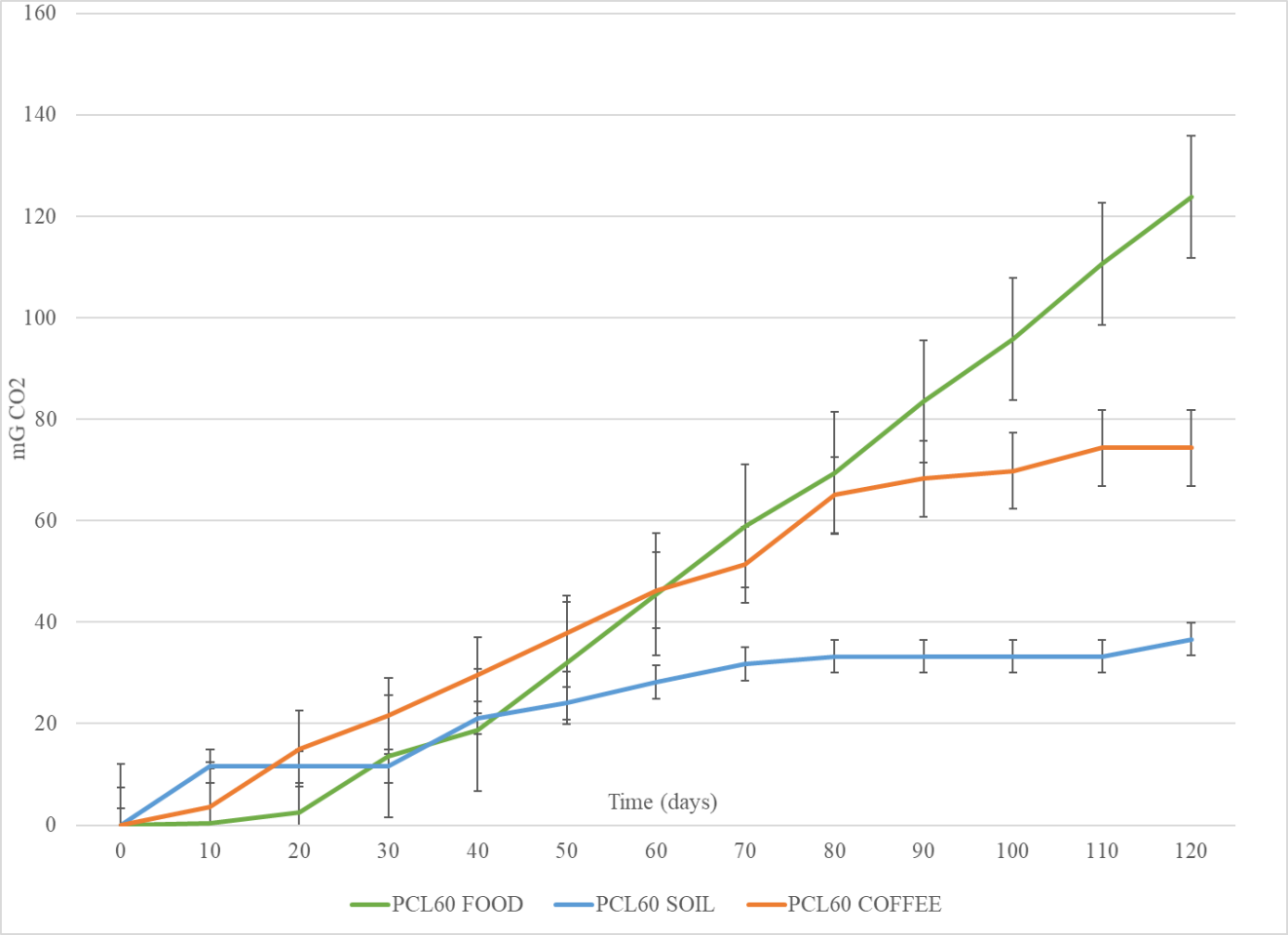

Figure 33. CO_2 evolution of PCL60 polymer samples in food, coffee, and soil burial matrices.

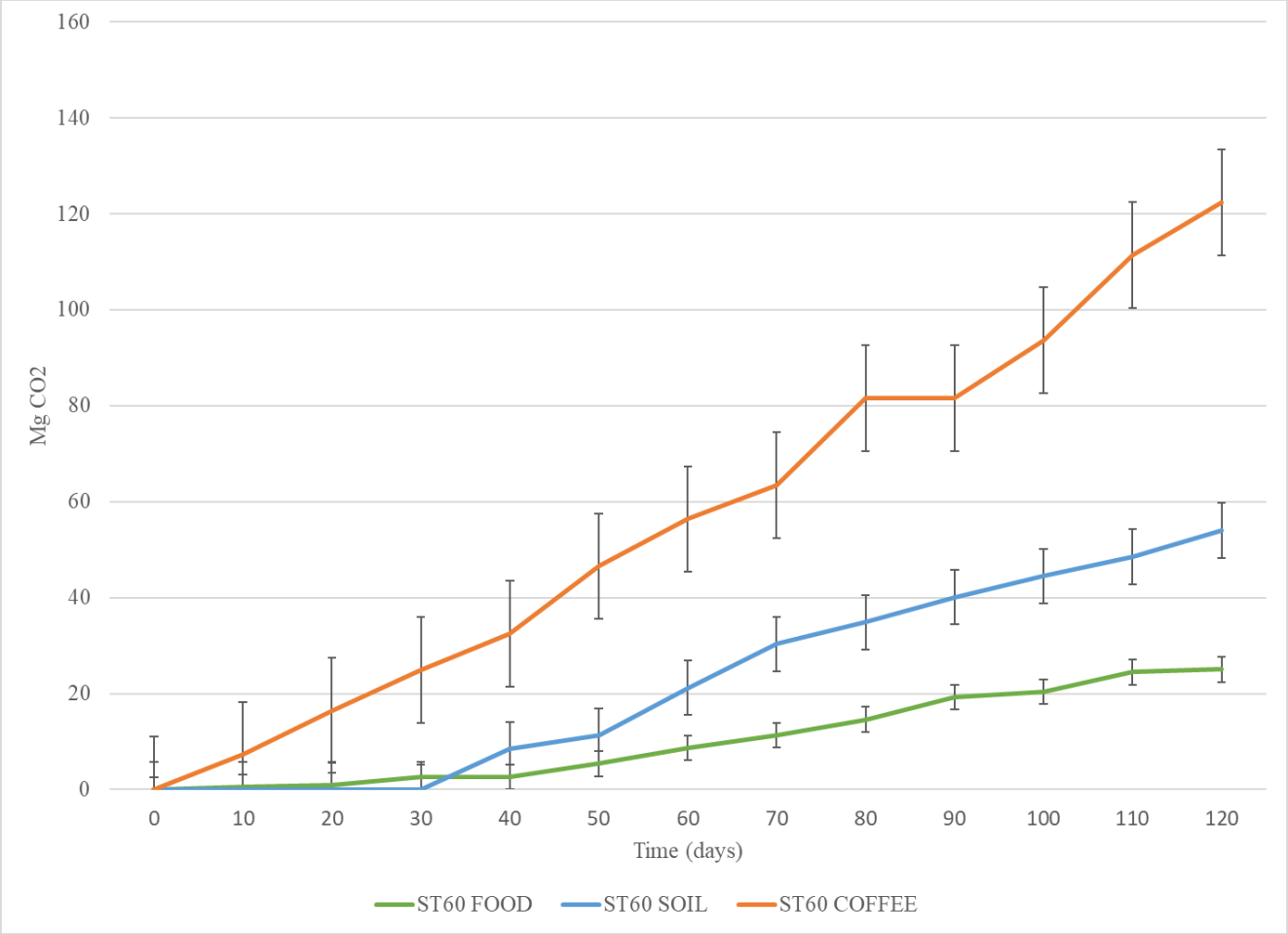

Figure 34. CO2 evolution of ST60 polymer samples in food, coffee, and soil burial matrices.

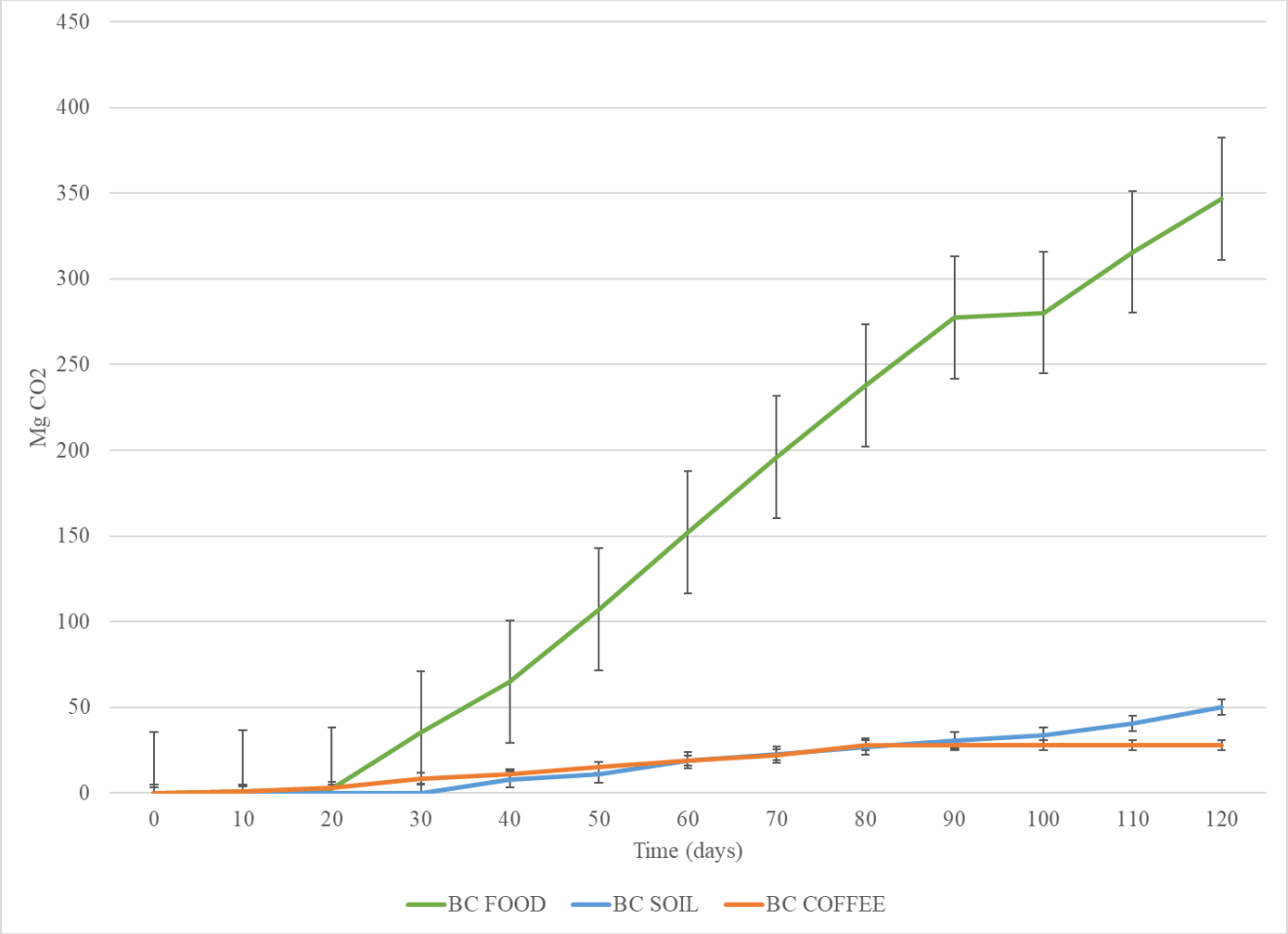

Figure 35. CO2 evolution of BC polymer samples in food, coffee, and soil burial matrices.

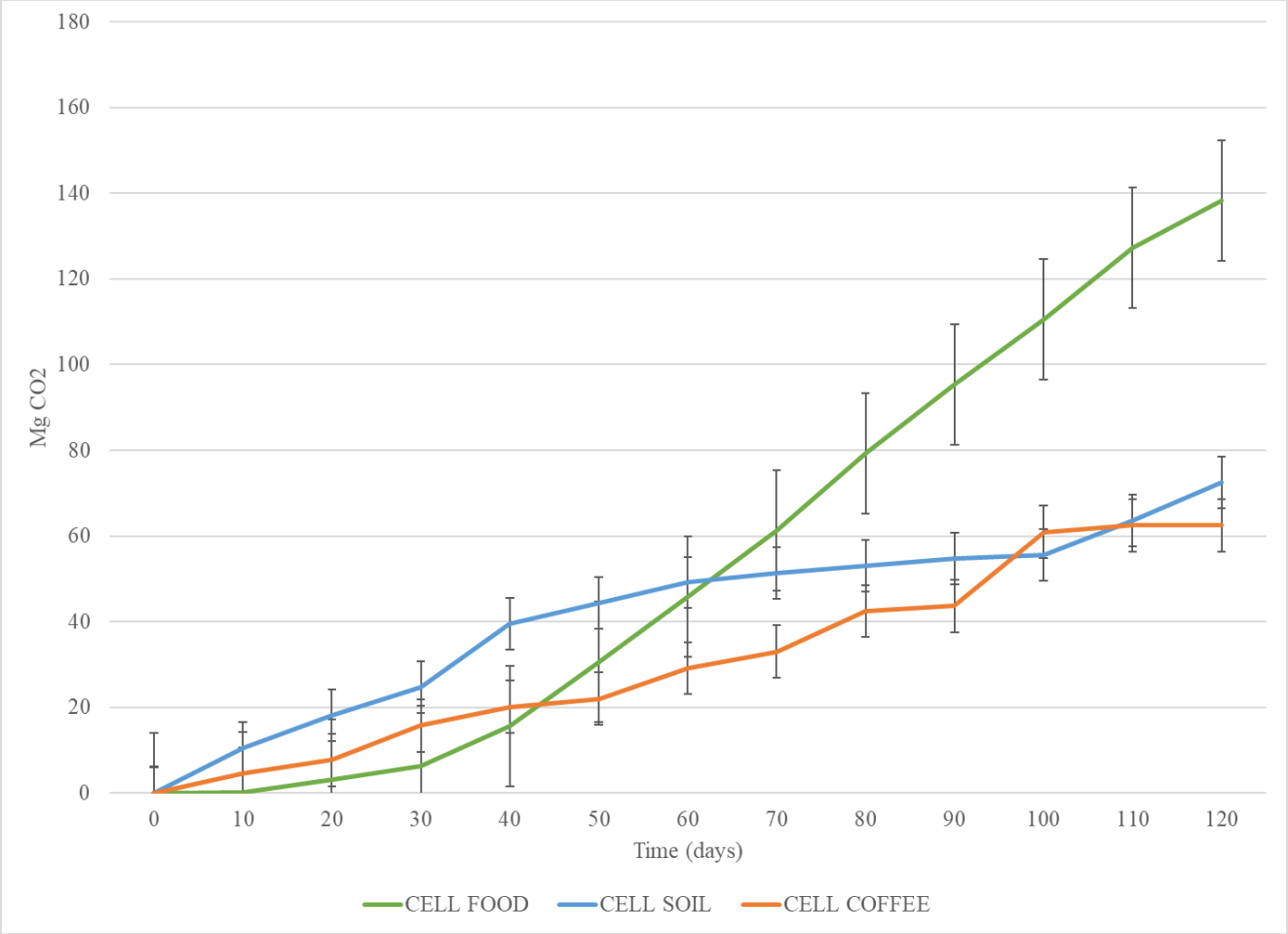

Figure 36. CO2 evolution of CELL polymer samples in food, coffee, and soil burial matrices.

Chapter 7 . Future research

To successfully implement biodegradable materials that rely on enzymatic activity for breakdown, the microbiology of disposal environments needs to be understood. However, soil-based disposal environments with or without food waste amendments, are extraordinarily complex. The interactions within the microbial flora, available nutrients, and polymers of interest cannot be oversimplified. Based on findings in this research, future research should address:

- Impact of nitrogen-rich disposal environments on CO_2 evolution within biometer flasks.
- Does inclusion of specific food waste impact polymer degradation? For example, did the starch heavy polymers degrade with the food waste used because it contained starchy food waste (potato peels)?
- Do microscopic fragments of PCL, biochar, or starch remain in the disposal environment, or are they fully utilized and broken down

by microbes? Would these materials be considered fully compostable?

- How would a disposal environment with both food waste and coffee interact with polymer degradation?

Blended polymers made from PCL and starch show promise as potential biodegradable polymers for utilization in food packaging. Inclusion of biochar has been shown to not inhibit biodegradation and should also be considered in further studies of biodegradable packaging. Inclusion of food waste generally led to a more diverse and numerous microbial community and resulted in more desirable percent weight loss trends seen in the food burial environment.

Chapter 8 . References

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Appendix- Supplemental figures

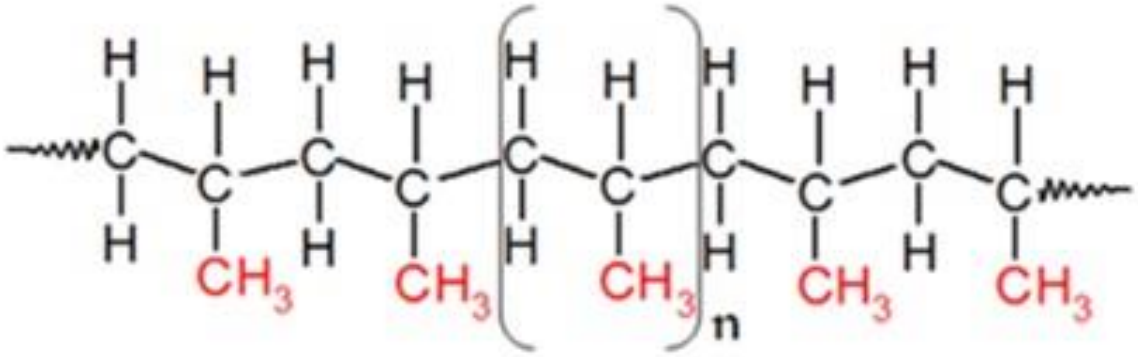

Figure A. 1. Structure of polypropylene (PP) *[76]*

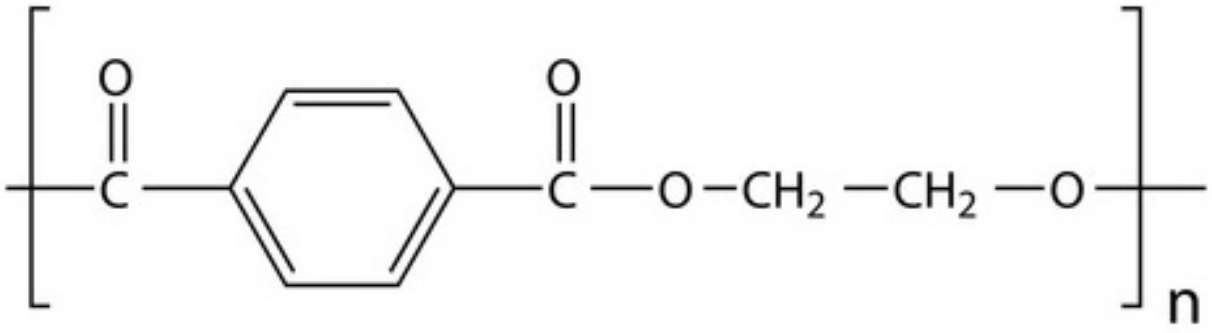

Figure A. 2. Structure of repeating monomer unit of polyethylene terephthalate (PET) *[77]*

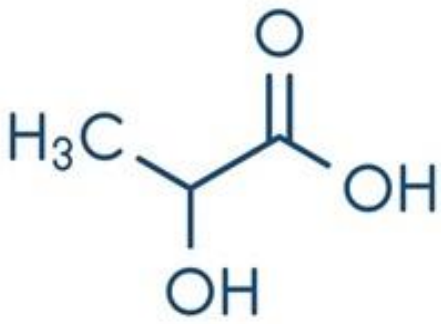

Figure A. 3. Structure of repeating monomer unit for polylactic acid (PLA) *[78]*

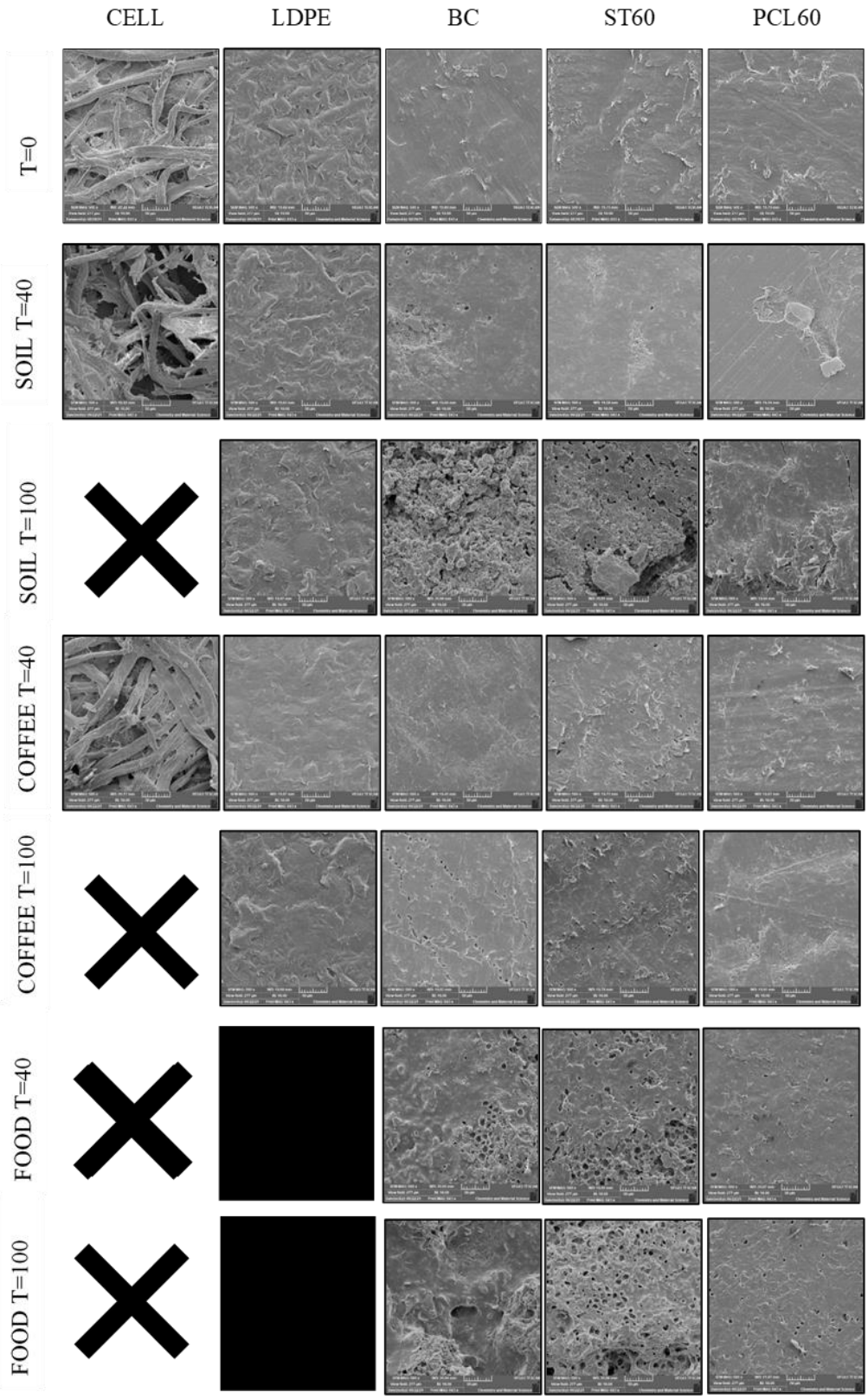

Figure A. 4. SEM imaging at 500X of polymers from burial experiments.

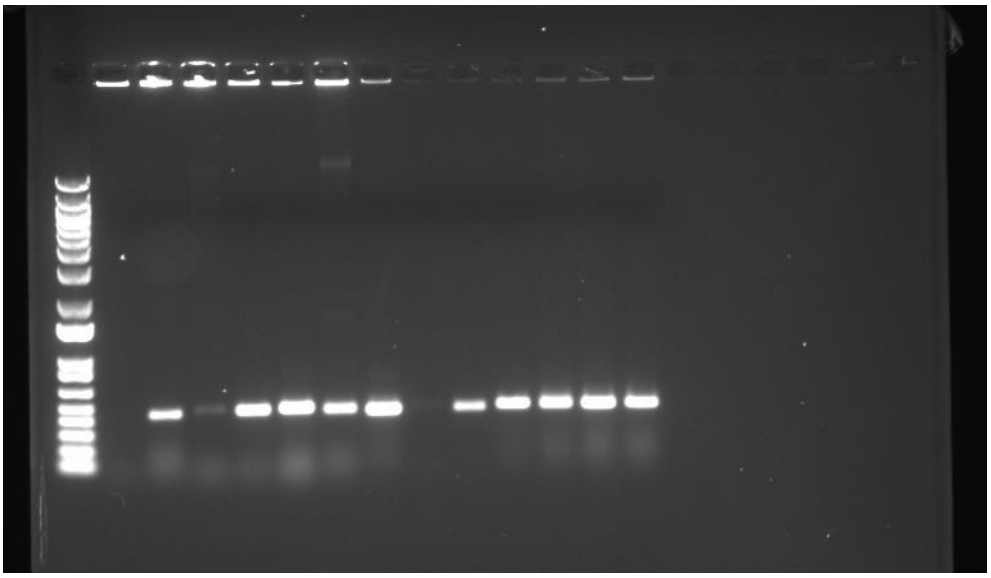

Figure A. 6. Image of gel after gel electrophoresis used for 16S rRNA DNA sequencing.

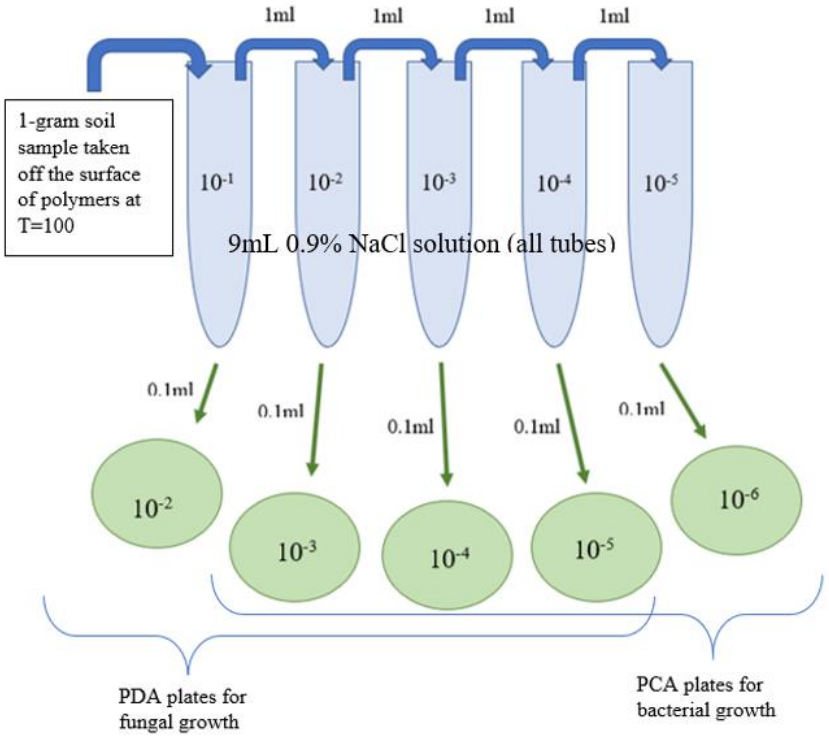

Figure A. 5. Dilution process used for plating and culturing of bacteria and fungi.

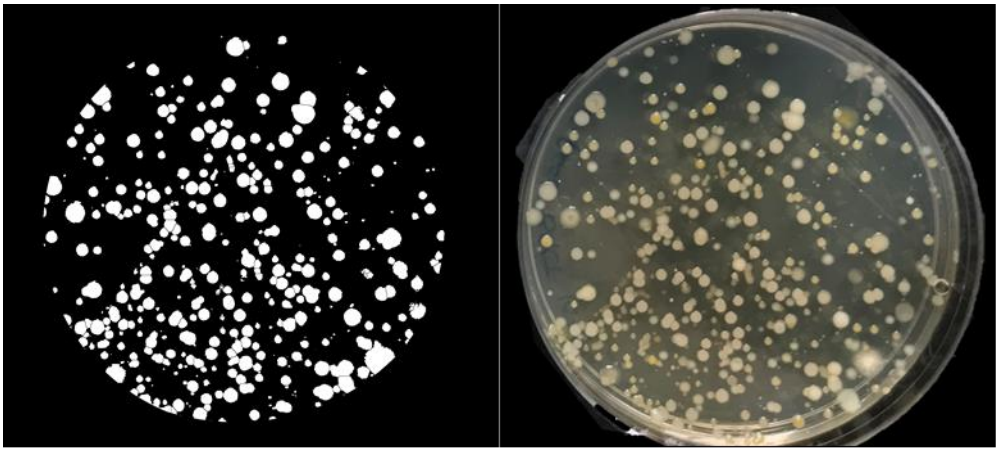

Figure A. 7. ImageJ processed image of colonies formed on a plate [33].

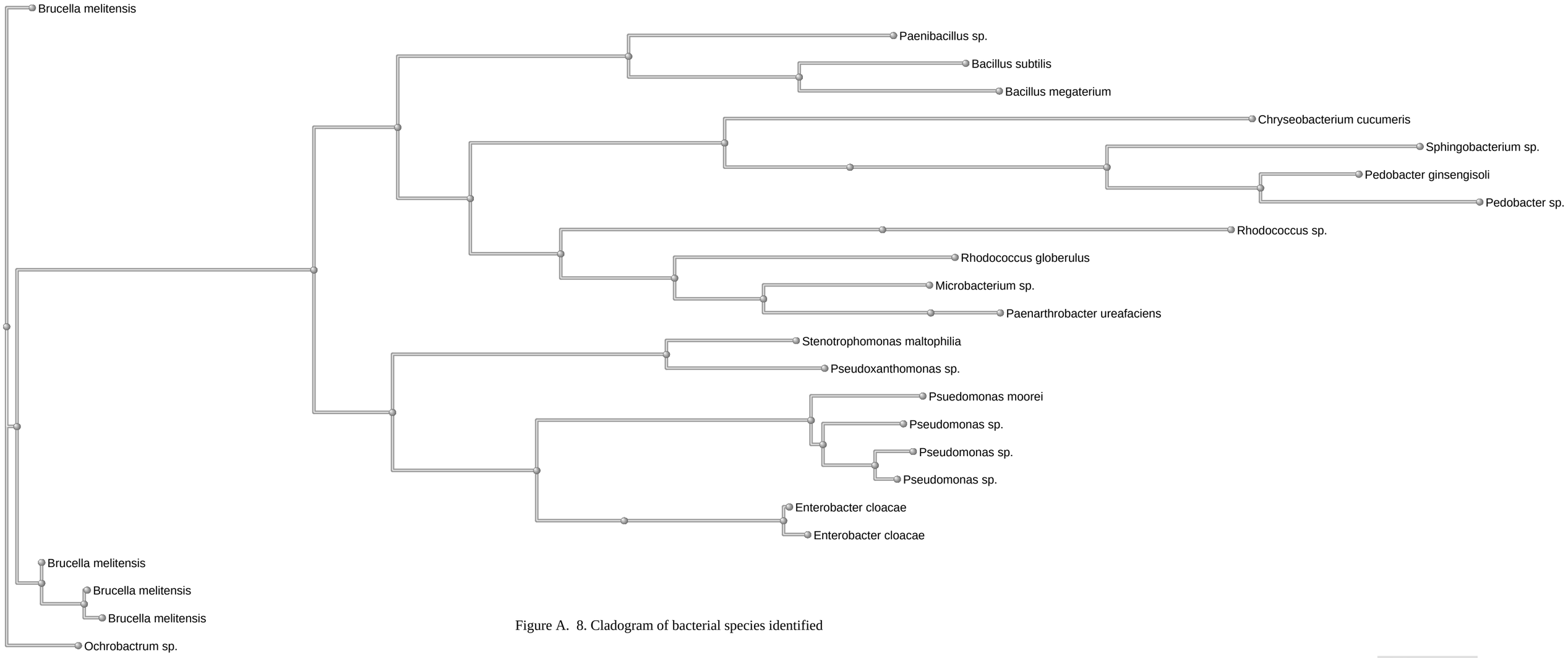

Figure A. 8. Cladogram of bacterial species identified

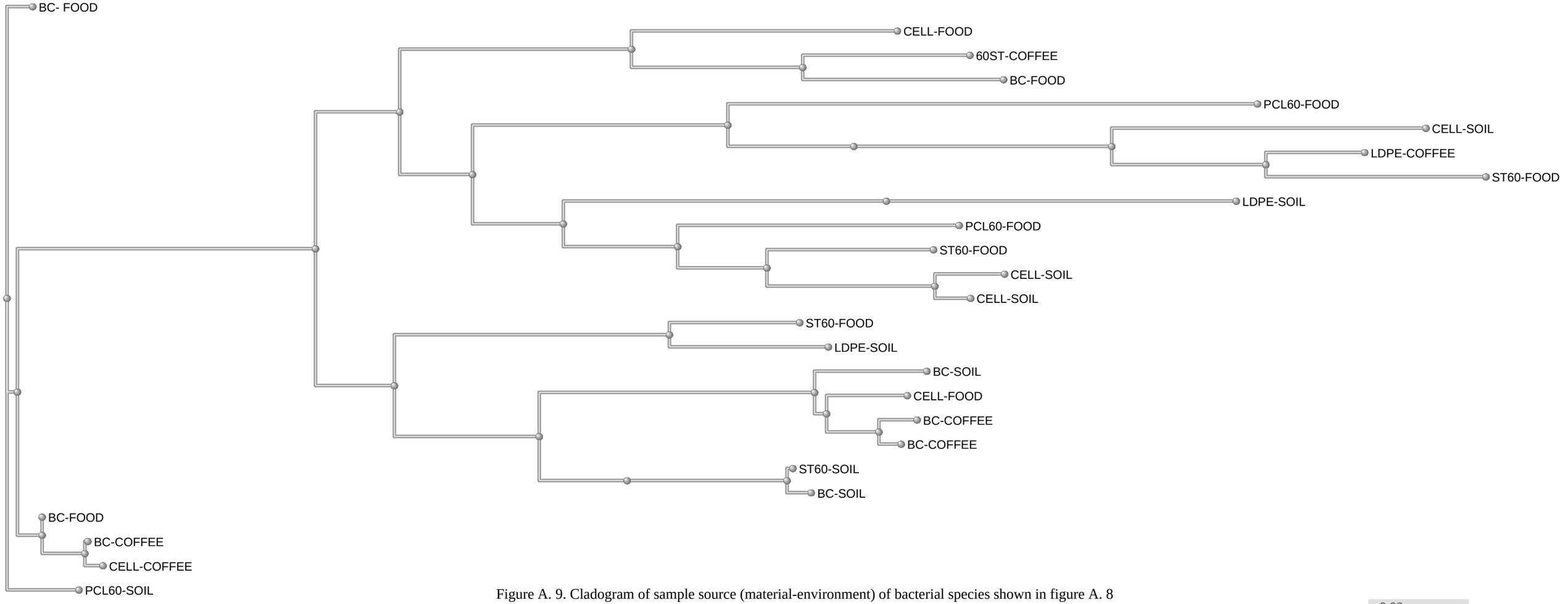

Figure A. 9. Cladogram of sample source (material-environment) of bacterial species shown in figure A. 8