Soil Degradation of a Plastic Blend of Opuntia Ficus-indica juice, animal protein and beeswax

A Senior Project

presented to

the Faculty of California Polytechnic State University, San Luis Obispo

In Partial Fulfillment

of the Requirements for the Degree

Bachelor of Science in Materials Engineering

By

Isabel Gamez and Daniel Kava

Advisor: Dr. Trevor Harding

Sponsor: Dr. Sandra Pascoe Ortiz

June 2022

ABSTRACT

Soil Degradation of a Plastic Blend of Opuntia Ficus-indica juice, animal protein, and beeswax

Isabel Gamez and Daniel Kava

Since the introduction of single use plastics in the early 1900s, their usage has increased exponentially. Unfortunately, due to this exponential increase there are negative environmental implications of their production, which requires further consideration. Currently, there are various waste disposal practices for SUPs, including landfilling, incineration, and recycling. Recent data revealed that only 14 % of plastic waste was recycled, with 40% of plastic waste being landfilled, while the rest of the plastic waste was incinerated or released to the environment at 14 % and 32 %, respectively. A potential solution to the plastic waste issue is developing a polymer that can easily biodegrade in the environment. Using this idea, Dr. Sandra Pascoe-Ortiz has produced a cactus-based biopolymer with six different formulations. Each of these contains various amounts of nopal juice, protein, beeswax, and glycerol. This project will investigate how these cactus-based biopolymers will degrade in soil by testing the samples' physical properties and structure throughout the degradation process. The degradation testing will consist of 28 days in a temperature and humidity-controlled environment. The biopolymer's mechanical and thermal properties will be monitored using thermogravimetric analysis, differential scanning calorimetry, and a scanning electron microscope. Finally, to track the changes in chemical structure throughout degradation, Fourier-transform infrared spectroscopy was utilized.

ACKNOWLEDGMENTS

We would like to thank Dr. Trevor Harding of the Cal Poly Materials Engineering Department for his continual support of this project.

We would also like to thank Dr. Pascoe Ortiz for allowing us to study her material, and for being a great resource.

Lastly, we would like to thank Mr. Eric Beaton of the Cal Poly Materials Engineering Department for maintaining the labs and assisting when equipment was down.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. Introduction

Since the introduction of single-use plastics (SUPs) in the early 1900's, their usage has increased exponentially. Since the 1950's, the global production of plastics (irrespective of purpose or design) has increased at a rate of 9 % per year, with total plastic production reaching approximately 360 million metric tons in 2018, as shown in Figure 1. SUPs are estimated to account for half of this amount. Over the next two decades, it is predicted by the MacArthur Foundation that global plastic production will double.[1] Given the environmental implications of SUPs and the impacts of their production, further consideration on this matter is needed.

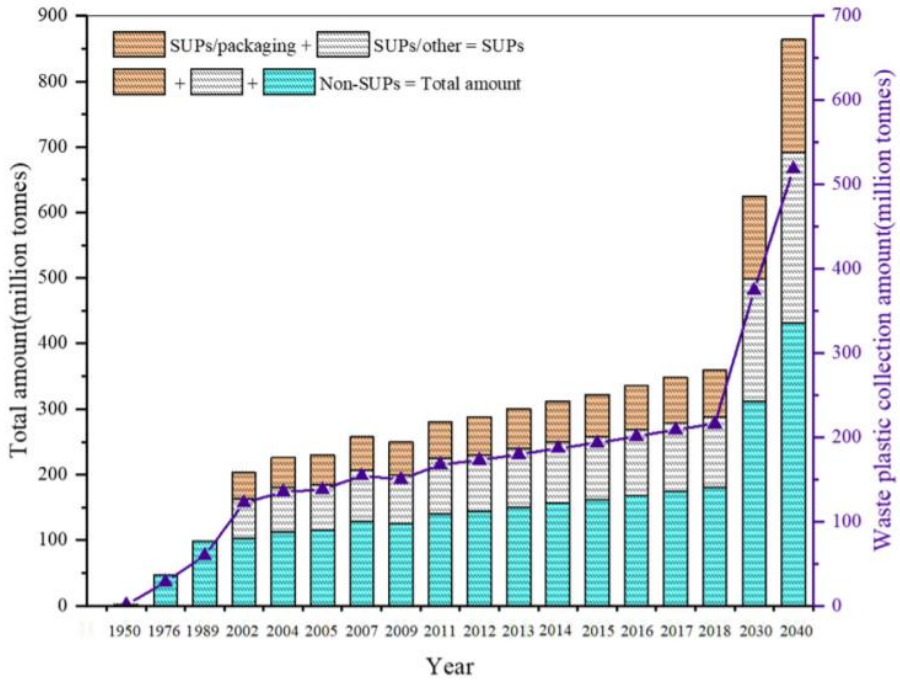

Figure 1. Global plastic and SUP production growth trends[1]

1.1 Consumer Plastics

Consumer plastics serve a multitude of uses and designs. Referring to global plastic production and specifically SUPs, approximately 40 % of SUP production is dedicated towards SUP packaging.[1] Figure 2 details the distribution of SUP production worldwide, split into different regions. This figure also breaks down sector distributions for the purposes of SUPs.

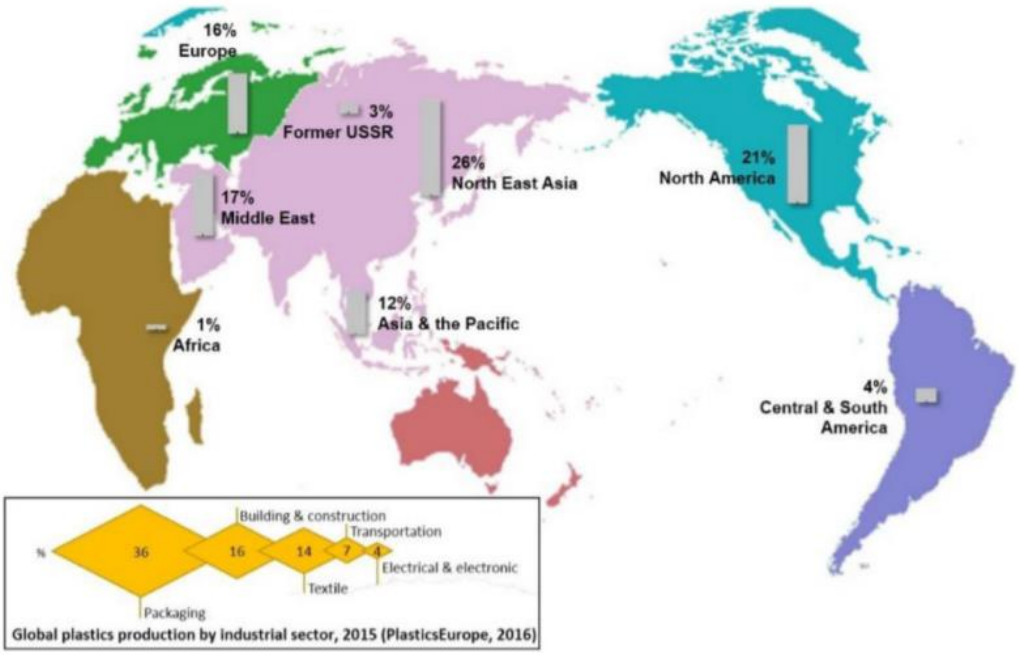

Figure 2. Regional production distribution of SUPs, 2014[1]

According to this figure from 2014 and 2015, Northeast Asia produces the highest proportion of SUPs at 26 %, followed by North America at 21 %, the Middle East at 17 %, Europe at 16 %, Asia and the Pacific at 12 %, and marginal proportions of SUP production from Central/South America, regions formerly under USSR control, and Africa. In 2015, packaging made up 36 % of global SUP production, followed by building/construction at 16 %, textile production at 14 %, 7 % for transportation, and electrical use/electronics production at 4 %.[1]

There are currently over 300 types of plastic produced, with approximately 60 of those being the most popular to use. The most well-known of these plastics, which are intended for both general use and engineering design, include polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), polyurethane (PU) and phenolic resin.[2] PP and PE are the most commonly used among these plastics for single-use products, including water bottles and plastic wrap for individually packaged food items. Especially in developing countries, SUPs are also being used for greenhouse materials and mulching film.[1]

Increasing the plasticity of these polymers to fit the needs of a plastic's design involves the use of different chemicals, including phthalates or phthalic acid esters (PAEs) for tableware, plastic bags, and food containers. Dimethyl phthalate (DMP), diethyl phthalate (DEP), benzyl butyl phthalate (BBP), and diethylhexyl phthalate (DEHP) have been reported as chemicals used for SUP water bottles, particularly in Saudi Arabia. Table 1 expands on the most commonly used chemicals for SUPs, including the purposes of said SUPs.

Table 1: Chemical usage in selected SUP products[1]

Plastic product (polymer)	Hazardous chemical content mg/kg	Standard Limit (mg/kg)	Detection protocol/method
Tableware (PP, PS,PET,PE)	(i) acetic acid (11658), (ii) n-hexane (274), (iii) DEHP (5.22–227.92), (iv) DBP (5.03–19.51)	(i) 30, (ii) 60, (iii) 5, (iv) 5	GB 18006.1–2009
(1)Plastic bags, (2)PVC bags,(3)PVC films, (4)plastic cups, (5)plastic bowls, (6)plastic bottles	(1)ΣDEHP, DEP, BBP, DBP, DnOP, DMP(6568) (2)ΣDEHP, DEP, BBP, DBP, DnOP, DMP(3.205) (3)ΣDEHP, DEP, BBP, DBP, DnOP, DMP(3.059) (4)ΣDEHP, DEP, BBP, DBP, DnOP, DMP(2.926) (5)ΣDEHP, DEP, BBP, DBP, DnOP, DMP(5.667) (6)ΣDEHP, DEP, BBP, DBP, DnOP, DMP(4.354)	5	Gas chromatograph with a DB-5 capillary column
Sanitary pad& Diapers (release amount)	Sanitary pad: DBP (0.052–7.82), DEHP (0.0055–0.197), VOC (nd-5.471 × 10^{-3}); Diapers: DBP (0.0134–1,0.6097), DEHP (0.0126–0.0628), VOC (nd- 0.397 × 10^{-3})	0.0001	Liquid Chromatography/Mass Spectrometry/Mass Spectrometry for PAEs; Gas chromatograph for VOC
Medical extension lines, drip chambers and bags.	DiDP, DINCH, DiNP>1000, DEHP<85	20 for DiDP, DINCH, DiNP; 2 for DEHP	Gas chromatography and mas spectrometry
Fast food container (PP, PS, GPPS, PE film)	DEHP(65.6)	5	GB/T 21928–2008
Bag (PE,HDPE, PE film)	DEP (nd-10.3), DIBP (nd −6.4), DBP (nd −7.2), DEHP (nd −34.3)	5	GB/T 21928–2008
Food packaging (PVC)	DEHP(nd-21,455), DBP(nd −86)	5	GB/T 21928–2008
Food Packaging (PET, HDPE, PE,PP,PS, PVDC, PES)	DBP (22. 89–267.6), DEHP (nd-899.8)	5	High Performance Liquid Chromatographic method (HPLC)

NOTE: not detected (nd).

There is a concern among the general population regarding the use of chemicals as plasticizers, fillers, flame retardants, etc. for single use plastics. Many of the chemicals used in plastics are known to cause health issues in people. Particularly, some of these chemicals can disrupt the human endocrine system. For example, phthalates used in plastics, when humans are exposed to them, can cause reduced sperm quality and increased sperm DNA damage in males. Additives from SUPs in landfills can also leech into the ground, contaminating groundwater and rendering it non-potable.[3]

The general process used to make plastics involves extracting oil, natural gas, and (sometimes) coal, refining them into monomers, polymerizing the monomers into hydrocarbons, and malt blending the hydrocarbons plus other raw materials into plastic.[4] There are multiple concerns surrounding this general method to manufacturing plastic. Chiefly, the processes of extracting oil (fracking and offshore drilling, as examples) are destructive to the environment while the furnaces used to heat oil give off greenhouse gases like methane and carbon dioxide. Additionally, the additives used in plastics (such as DEHP, n-hexane, and phthalates) are known to be toxic when exposed to humans, as explored earlier.

1.2 Waste Disposal / Life Cycle

There are various disposal practices for SUPs which are in use currently. Chiefly among these practices of disposal include landfilling, incineration, and recycling. Additionally, thermal cracking and carbonization are also practices used to dispose of single use plastics, however they are not as popular.[1] Recent data revealed that only 14 % of plastic waste was recycled, with 40 % of plastic waste being landfilled, while the rest of the plastic waste was incinerated or released to

the environment at 14 % and 32 %, respectively. This data was reported by the World Economic Forum in 2016 based on worldwide plastic waste disposal practices.[1]

Landfilling of plastics simply involves throwing out plastics into standard garbage containers where it is then shipped off to plots of land specifically used to store trash. The obvious concerns with landfilling of plastic include the release of greenhouse gases to the environment from methods of transportation, while toxic chemicals may seep from these plastics and contaminate the land it is stored on, destroying that part of the environment. Additionally, longitudinal data across 2015 to 2018 from the USEPA and the US Census Bureau reports that a greater portion of plastic in the US is being landfilled in 2018, as compared to 2015, as shown in Figure 3.[5]

Incineration of plastics refers to the practice of burning plastics into ash and using the emitted gases as a form of energy. Additionally, incineration is used as a practice of reducing the physical amount of plastic waste so as not to over-fill landfills. It is estimated that incineration of plastics achieves a waste volume reduction between 90 and 99 percent.[1] However, incineration is not a perfect method of plastic disposal. The primary purpose of incineration is material destruction, not material recovery, as is often assumed with incineration. These "Waste-to-Energy" facilities are primarily working to destroy plastic material, as opposed to generating energy as they are often advertised as. A large concern surrounding incineration is the release of toxic ash and smoke, which pollutes the surrounding air. When waste is burned, approximately 10-15 % of the original waste volume remains in the form of toxic ash containing heavy metals. This byproduct must then be transported to a hazardous waste facility.[5]

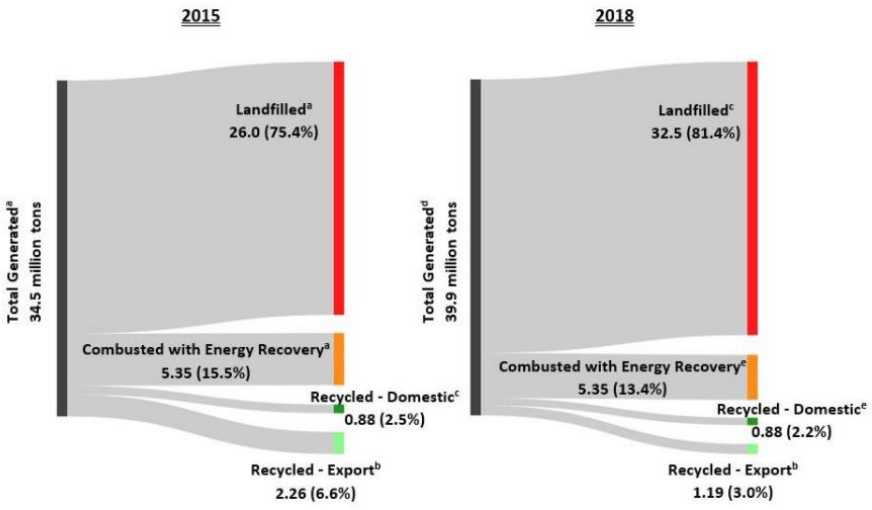

Figure 3. Longitudinal disposal practice data, US plastic waste (2015 vs 2018)[5]

The purpose of recycling plastic waste is to reuse that plastic and convert it into new products, such as water bottles, disposable cutlery, and food containers. This is a practice that is encouraged worldwide due to its inclusion as a part of the circular economy concept. The difficulties related to plastic recycling relate to SUPs and the likeliness that sorting equipment

used to process solid waste streams will clog from these SUPs. This increases the difficulty for municipal waste processing centers to effectively sort solid waste streams into proper groupings of waste.[1] Additionally, product manufacturers of plastic products have an unwillingness to use more recycled plastic for multiple reasons. First, new plastic is preferred for product manufacturing because it is cheaper, has a higher material quality, and has a more stable supply chain. Second, the use of recycled plastic poses contamination risks and increased delivery difficulty. Many trucks and drivers must be involved in the processing of plastic waste for recycling, which includes waste collection, moving equipment to sort said waste, and cleaning facilities to convert the waste plastic into usable material.[5]

Noted attempts to resolve the issues surrounding SUPs usually include legislative interventions from local and national governments. In Austin, Texas, a Single Use Bag Ordinance was enacted in 2013 to ban the use of single-use plastic bags in businesses.[6] San Jose, CA enacted their own ban on single-use plastic bags in 2012.[5] Currently, consumer fees for SUP bags are enforced in 30 countries worldwide, while 27 countries worldwide currently enact taxes on SUP bag production.[1] In California, most cities and towns have bans on plastic bags and/or plastic straws. India is currently committed to phase out their use of SUPs by 2022.[1] While legislative efforts to end the use of SUPs are important and in multiple cases have reduced the amount of plastic litter and waste, these efforts will not be enough to combat the adverse environmental and public health effects of single-use plastics. Even as the use of SUPs decreases, the simple fact that SUPs will, to an extent, still be used means the health of humanity and the health of the environment will continue to suffer.

2. Biopolymers

2.1 What are Biopolymers

A biopolymer is a polymer produced from natural sources that can either be chemically synthesized from a biological material or biosynthesized by organisms[10]. These biopolymers are important in the development of biodegradable polymers that can be used for consumer applications. Because they are often synthesized from starch, sugar, and natural fibers of organic components, they will degrade in the presence of bacteria in soil, compost, and marine sediment. Therefore, placing these polymers in waste disposal can significantly reduce the CO_2 emission by using a different waste disposal method. Polymers can be classified in four ways in combination with their renewability and degradability. First, biopolymers such as chitosan, polylactic acid, and cellulose both come from renewable resources and are biodegradable[10]. Conversely, conventional polymers are petroleum-based and not biodegradable. There are also two classes of polymers that fall between the two already mentioned. These polymers can be bio-based but not biodegradable or petroleum-based and biodegradable. Polymers such as bio-PE, PP, and PET are renewable and not biodegradable. On the other hand, polycaprolactone (PCL) and polybutylene succinate (PBS) are synthetic and biodegradable[11]. The figure below shows a schematic comparing these classes of polymers.

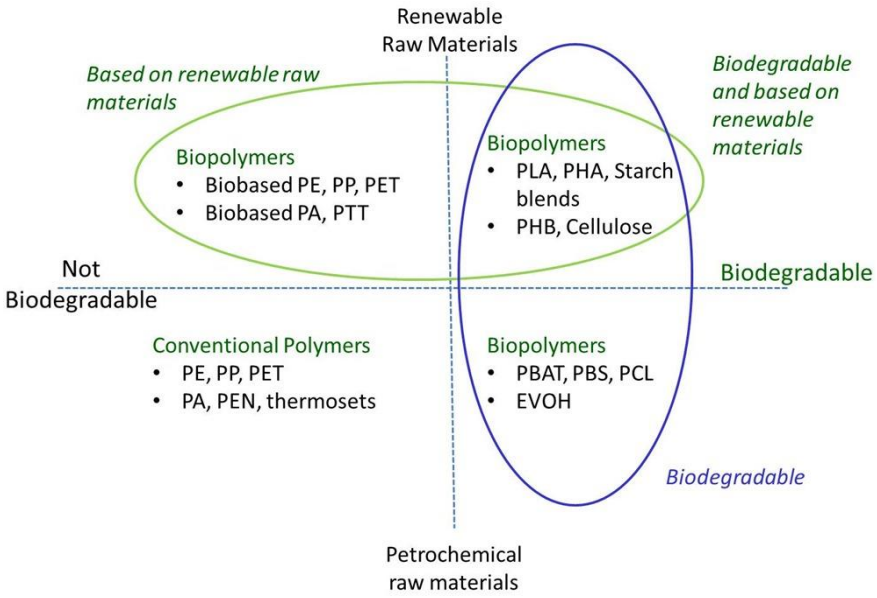

Figure 4. Matrix of key characteristics that make a biopolymer[11]

Research on biopolymers has been a field of interest for scientists for many years. The first intentionally designed biopolymer was in the 1980s, they blended a synthetic polymer such as PE with starch to make "biodegradable trash bags". This was unsuccessful because landfills are anaerobic, but the microorganisms require oxygen to reach the starch[10]. If the starch were successfully degraded there would still be leftover polyethylene in the landfill, which does not solve the problem of disposing plastics.

Biodegradation in the engineering field is defined as when a polymer provides uncompromised functionality until the end of its useful life and is followed by accelerated biotic degradation. Degradation can be any chemical or physical change to a polymer by environmental factors such as UV, heat, chemical, mechanical, moisture, and biological/marine[10]. This type of degradation can occur in vivo (within a living organism), in vitro (lab-based), or in enviro. For this report, we will be looking deeper into in enviro degradation of biopolymers.

2.2 Drawbacks of Current Biopolymers

Unfortunately, there are many drawbacks of current biopolymers that make it unfavorable for companies to make the switch. One of the biggest disadvantages is that they are not cost-competitive to the petroleum-based polymers already in use. Biopolymers are typically two to three times more expensive than commodity plastics like PE and PET[14]. The cause of this comes from low yields and smaller manufacturing plants. Another large concern with biopolymers is that because most are made from crops like corn, their production could harm food supplies[10]. However, there have been some innovations in using food waste that could help alleviate this issue. Furthermore, because crop-based biopolymers require fertile land, fertilizers and water are important in keeping these crops alive which can cause more harm to the environment. Most

biopolymers also have a shorter lifetime than oil-based polymers due to their weaker mechanical properties, water permeability, and brittleness[14]. This makes them less practical for commercial applications.

The terms compostable and biodegradable sound great to many consumers, however many products that are marketed with these terms require a specific disposal procedure and industrial level composting[14]. These processes tend to be expensive and not common enough to prevent the biopolymer product from being incinerated or going to a landfill[14]. This problem is common when the local authorities lack this type of facility.

3. Cactus-Based Biopolymer

3.1 What is a cactus-based biopolymer

To continue, the subject of biopolymers, it is important to also consider the natural resources used in the making of biopolymers. The use of cacti as a source to make biopolymers is sensible, given that cacti themselves require little water to survive and can survive in harsh conditions. This means that cacti can be grown in a large variety of places across the world.[8] Specifically for this report, the *nopal* species of cacti is used due to its abundance in Mexico and the American Southwest. A picture of a nopal, more commonly known as the "prickly" pear cactus is shown in Figure 5.[9]

Figure 5. Matured cactus pad from the nopal species[9]

The juices present in cacti (in this case, the nopal species of cacti) contain considerable amounts of sugars and gums, which themselves favor the formation of the biopolymer being tested.[8] With the resources and assistance of Dr. Pascoe Ortiz, this biopolymer is made using a combination of

nopal juice (which is produced by blending cacti leaves), animal protein, natural wax, and the inclusion of glycerin as a plasticizer.

As an end-of-life option for biopolymers, soil degradation is an effective method of sustainably disposing of a biopolymer, with the benefit of nullifying the need for collection and cleaning products for these biologically produced plastics. Additional benefits of degrading biopolymers in soil include active substances, such as herbicides or fertilizers, being released into the soil, which strengthens its overall quality.[12] However, these benefits are also dependent on the biopolymer decomposing at a quick rate. Should an excessive amount of time be needed for a biopolymer to degrade, substances will form that decrease the quality of the soil and may in fact damage it. For example, if a dehydrated biopolymer gel is exposed to degradation in a high-hydration soil, these gels will undergo swelling due to water absorption, which can reduce a soil's strength close to 10x its original strength.[13] For this report, the effects of soil degradation for both the soil and the cactus-based biopolymer will be investigated across multiple compositions and degradation times.

Cactus-based biopolymers have certain advantages over other types of biopolymers which are important to mention. As stated previously, the inclusion of cactus juice as a naturally found ingredient in biopolymers is sensible due to the ability of cacti to survive in a large variety of environments. This is partially due to the low amount of water required for a cactus to grow and fully develop, which means the biopolymer made with cactus juice will not require as much water as other biopolymers. Additionally, cacti are extremely abundant in various regions of the Americas, which gives merit to their use as an ingredient in the production of biopolymers.

3.2 Nopal Juice

Nopal juice comes from the *nopal* species of cacti and has a large abundance in the Southwest Americas[18]. Nopal juice was used as the base ingredient for the biopolymer product. Prickly pear cacti are commonly made into a popular beverage as an antioxidant for many health-conscious consumers in Mexico[18]. The antioxidant properties come from the high content of carbohydrates and phenolics. D-galactose, glucuronic acid, L-rhamnose, and D-glucose are all saccharides found in nopal juice and have been shown to have some plasticizer properties[17]. This can be useful in the formation of a film; however, added components will still be required for improved film formation.

3.3 Animal Protein

Collagen is an animal protein that can be found in mammals, which makes it easier to harvest. Collagen fiber is fiber in the extracellular matrix of connective tissues and characterized by elongation and made up of collagen glycoprotein[19]. Typically, it will be arranged in branching bundles with indefinite length. This fiber is strong, insoluble, and occurs in the skin, tendon, ligaments, bone, and cartilage[19]. In tendons the collagen fibers are arranged in parallel which strengthens the overall structure[17]. Collagen has amino acids in each chain that are arranged in a regular pattern, which contributes to its ability to strengthen the structure of tissues. Because

polymers are made of repeating monomer units, the amino acid sequence will be ideal in strengthening the structure of the biopolymer.

3.4 Natural Wax

Candelilla wax is a natural wax that can be derived from the Mexican shrub *Euphorbia Antisyphilitica* and is usually a deep yellow with a mild pleasant odor[20]. This type of natural wax could be a substitute for beeswax in this biopolymer. Waxes are a simple lipid of a long-chain alcohol and fatty acid. Wax is found as a coating for leaves and stems with the purpose of preventing the plant from losing excess amounts of water[21]. This implies that the addition of natural wax to the cactus-based biopolymer will increase the hydrophobicity and decrease the water vapor permeability. These properties are important for food packaging applications to protect the food and prevent anything from reacting with hydroxyl groups in water[17].

3.5 Glycerin

Glycerin (also referred to as glycerol) is a type of plasticizer which can be used in polymers to reduce the stiffness of the plastic and introduce more elasticity into the material's behavior. Plasticizers themselves are, according to Issue 3 of the European Polymer Journal from 2011, "an important class of low molecular weight non-volatile compounds that are widely used in polymer industries as additives. The primary role of such substances is to improve the flexibility and processability of polymers by lowering the second order transition temperature, [known as] the glass transition temperature (T_g)."[15] In the case of glycerin ($C_3H_8O_3$), it is a colorless, odorless hygroscopic liquid that is miscible and boils at 290 °C. Glycerin can be sourced from plants such as soybeans and palms, or from tallow in animals. Additionally, glycerin can be synthetically manufactured from propene.[16] Glycerin, like most plasticizers, has a low molecular size which can fit in the intermolecular spaces between polymer chains. This, therefore, results in two effects. First, the 3-D molecular organization of the polymer will be altered, which reduces the energy needed to induce molecular motion and the formation of hydrogen bonds, which themselves will increase a polymer's stiffness. Second, the secondary forces present in polymers will be reduced, further lowering its stiffness. These two effects lead to an increase in the free volume of a polymer, as well as an increase in molecular mobility.[15]

4. Research Question

Given the above sections, including motivations for this project plus information surrounding single-use plastics and cactus-based biopolymers, this report will attempt to answer the following questions about cactus-based biopolymers and their degradation in soil:

How is the degradation rate of six different compositions of cactus-based biopolymer (made of Nopal juice, animal protein, natural wax, and glycerin) affected during forest-type soil degradation over 56 days under aerobic conditions? Additional questions to consider include:

- How does the amount of Nopal juice (60% to 70 wt%) affect the thermo-mechanical properties and soil degradation of this biopolymer?
- How does the amount of animal protein (10% and 20 wt%) affect the thermo-mechanical properties and soil degradation of the biopolymer?
- How does the amount of natural wax (0 to 20 wt%) affect the thermo-mechanical properties and soil degradation of this biopolymer?
- How does the amount of glycerin (0% to 20 wt%) affect the thermo-mechanical properties and soil degradation of this biopolymer?

5. Methodology

5.1 Safety

All experiments were performed safely by referring to the safety protocol, SOPs, and general lab safety rules. General PPE for all experiments is safety goggles, closed-toe shoes, long pants, no loose jewelry, and wearing gloves when handling samples to prevent contamination. Lab spaces remained at a reduced capacity due to COVID regulations and the importance of cleanliness was enforced.

5.2 Sample Preparation

Six compositions of cactus biopolymers were delivered in 20 cm x 20 cm sheets then cut into 10 cm x 1.5 cm strips for testing. Table 2 lists the formulations for each of the six compositions. Each sample was cut using a razor blade to attempt to avoid damaging the microstructure of the samples. Seven samples were cut from each composition making a total of 42 samples. One sample from each composition is intended to be used for varying amounts of soil degradation; the samples were placed in six different bins which represent a certain length of degradation, these were chosen to be 0, 1, 3, 5, 7, 14, and 28 days. The samples which had zero days of degradation were never placed in soil and will be used as reference samples. At each day mark, a sample of each composition was removed from the soil and placed in a plastic bag and kept in a fridge.

Table 2: Formulations for Each Composition

Composition 1	**Composition 2**	**Composition 3**	**Composition 4**	**Composition 5**	**Composition 6**
60 % juice nopal	70 % juice nopal	60 % juice nopal	65 % juice nopal	65 % juice nopal	70 % juice nopal
20 % protein	10 % protein	20 % protein	20 % protein	20 % protein	10 % protein
10 % wax	10 % wax	20 % wax	8 % wax	0 % wax	0 % wax
10 % glycerol	10 % glycerol	0 % glycerol	7 % glycerol	15 % glycerol	20 % glycerol

5.3 Soil burial degradation testing

A soil burial degradation test was used to measure the degradation of the biopolymers. Six plastic bins were filled with standard organic potting soil and polypropylene sheets were used to form six separate areas. This was to keep each sample separated, organized within the bin, and to help ensure all of the sample is retrieved; a key was drawn on the lid to ensure samples are not mixed up. Each of the bins will contain one sample from each composition. The six bins will be representative of the length of time samples were in the soil, so they will be labelled as day 1, 3, 5, 7, 14, and 28. To ensure a somewhat controlled environment, the test was conducted indoors with temperature and humidity monitored using a wireless sensor. When the samples were removed from the bin, they were stored in a fridge to help stop the degradation process and allow time for testing each sample. Figure 6 shows how each sample was laid out in the soil degradation bins.

Figure 6. Soil degradation testing bins

5.4 Scanning Electron Microscopy

To obtain high-resolution images of the biopolymers, a Scanning Electron Microscope was employed to achieve this. Under normal circumstances, a biopolymer would have to be coated in an electrically conductive material (i.e. gold) as the SEM normally can only image samples if said sample is itself electrically conductive or coated in an electrically conductive material in a high-vacuum setting. However, these images were taken using the SEM in a low-vacuum mode which circumvents the requirement for electrical conductivity. Various parameters, including voltage, probe current, brightness, contrast, etc. were adjusted to achieve photos with optimal

brightness, contrast, and resolution with minimal-to-zero charging on the edges of the samples.

5.5 Thermo-mechanical testing

5.5.1 Tensile Testing

To test the mechanical properties of the biopolymer, a tensile test was performed using an INSTRON Mini 550 and ASTM D882. Measurements of the length, width, and thickness were taken for each sample before testing. These values are later used for determination of mechanical property data. The sample was held vertically using metal clamps with sandpaper as an aid to prevent slipping. Before each test, the elongation was zeroed and the instrument was balanced. Before the actual application of the elongation, a pre-test load of 1 newton was applied to each sample. Each test performed was at a strain rate of 75 mm/min for all compositions. NOTE: This data was inconclusive due to the extremely low mechanical strength of the biopolymer after only one day in soil. Therefore, a qualitative approach was taken by documenting observations and how each sample's mechanical properties have changed throughout the 28 days of testing.

5.5.2 Differential scanning calorimetry (DSC)

Differential scanning calorimetry tests were run on samples which could be recovered from the soil. Each sample was cut using a sharp blade such that it could fit into a small aluminum pan and had a recorded mass between 5-10 mg. Each pan was covered with a lid and clamped closed for testing. The DSC was programmed to run a heat/cool/heat cycle, beginning at room temperature (~25℃), heating up to 200℃, cooling down to 20℃, and heating back up to room temperature. The ramp rate for each dynamic heating/cooling cycle was 10℃ per minute.

5.5.3 Thermogravimetric analysis (TGA)

Thermo-Gravimetric Analysis tests were run on samples which could be found within the soil. The samples were cut into small pieces such that they fit within a small alumina pan used for the TGA. The TGA was set up to begin the test at room temperature (25℃) and heat up to 450℃ at a ramp rate of 20℃ per minute. The nitrogen flow used to cool the sample and machine was set to 100ml per minute to keep each sample test time efficient.

5.6 Characterization

5.6.1 Fourier-transform infrared spectroscopy (FTIR)

A scan range of 400 - 4000 cm^{-1} was utilized for infrared spectroscopy. The number of scans was set to 32 while the sensitivity of the FTIR was set to 4 cm^{-1}. Before testing the first sample, a background scan was necessary to calibrate the FTIR. The sample surface and bottom tip of the positioning arm were wiped down with a Kimwipe and isopropyl alcohol before and after every scan to prevent contamination. Each sample was then placed underneath the positioning arm and scanned for approximately thirty seconds. After scanning, a graph of the sample spectra was produced from the software.

6. Results and Discussion

6.1 Soil Degradation

Throughout the degradation of the biopolymer compositions (6 compositions each across 6 days of removal) as they were buried in soil, each sample was photographed upon removal. The photographs of these samples were organized both into progression of degradation for each composition across the entire degradation period, and into each day with each composition present. Of all compositions, composition 3 exhibited the lowest rate of biodegradation given it was the only composition to be found for every day of removal from soil.

Figure 7. Composition 3 biodegradation across all periods of time

Although this is only one picture which happens to show all samples of composition 3 being retrieved from the soil in which it was buried, there are characteristics of this biodegradation which are consistent with all compositions. As the samples experienced more time buried in the soil, they absorbed increasing amounts of water and, because of said water absorption, soil stuck to the samples in increasing amounts. Additionally, samples retrieved at later dates (in general) broke down into more numerous pieces of small size or would break down upon removal from the soil despite every effort being undertaken to prevent breakage from human handling. Examining the degradation-over-time of compositions 1 and 2 leads reveals another important observation about these samples.

Figure 8. Composition 1 biodegradation across all periods of time

Figure 9. Composition 2 biodegradation across all periods of time

Aside from the characteristic color differences between the above three compositions (due to the differences in ingredient amounts utilized), compositions 1 and 2 both do not show all 6 of the original samples buried in soil. These two compositions, along with the other five, exhibited sufficiently fast degradation rates such that they fully degraded into the soil before day 28, the final day of sample removal-from-soil. In fact, multiple compositions fully degraded by day 7, a full two weeks before they would have been removed for the final time. The primary takeaway is that compositions of the cactus-based biopolymers degrade at different rates in soil due to variations in the amounts of each ingredient used.

6.2 Scanning Electron Microscopy

Images were taken of samples before and after 1 day of degradation at a range of magnification. The best results were seen at 200x magnification. Figure 10 shows composition 2 and 3 before

any soil degradation testing. In the left image there is a darker matrix which is most likely the surface of the material, there are also some white spheres which are believed to be protein crystals or nucleation sites. Composition 2 contains 10% glycerol, according to literature the addition of glycerol is causing this initiation of the crystals. This is confirmed in composition 3, which has 0% glycerol, because these prominent spheres are not present. Instead, there is a bumpier surface, which is most likely protein that has not had time to crystallize without the addition of glycerol.

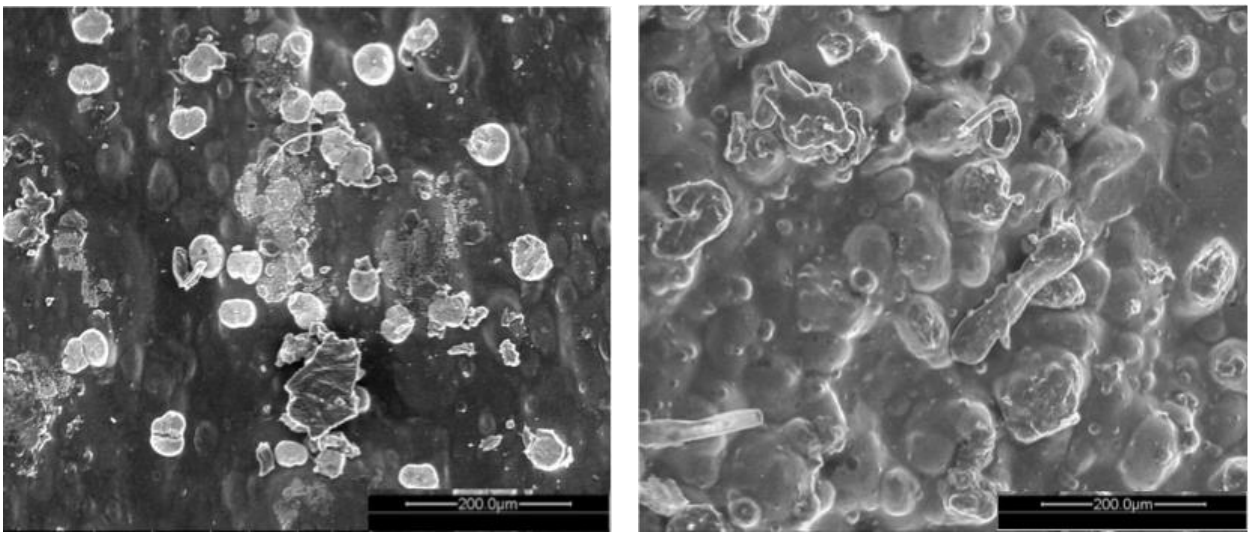

Figure 10. SEM 200x of composition 2 (left) and composition 3 (right) before degradation

Figure 11 shows the same samples from the previous figure, but after one day in the soil. The left image shows the spheres becoming more prominent on the surface and larger, while the gray background material begins to degrade. The protein crystals stay prominent because the enzymes causing the degradation will only eat the amorphous regions of the material, and not the crystallized animal proteins.

In composition 3, on the right, you can also see the degradation of the surface material in the background and the protein crystals just beginning to come through on the surface as sphere-like shapes. This occurs because the enzymes are degrading the amorphous regions around the crystals that were previously under the surface of the material.

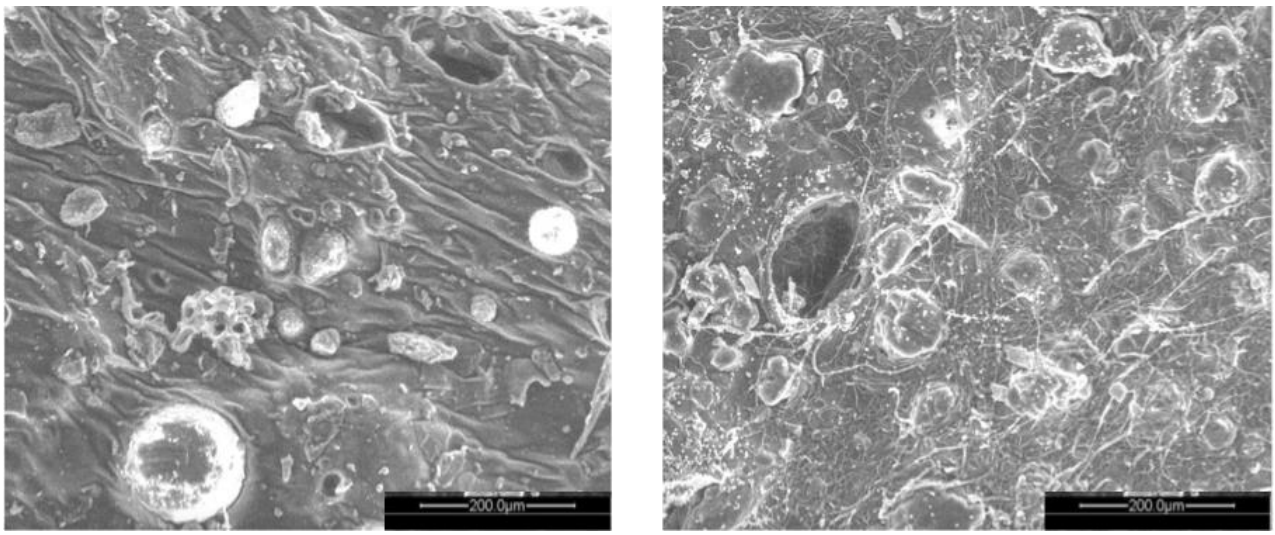

Figure 11. SEM 200x of composition 2 (left) and composition 3 (right), 1 day of degradation

6.3 Infrared Spectroscopy

6.3.1 Differences between compositions

Referring to Table 2, each composition is formulated using differing amounts of nopal juice, protein, wax, and glycerol. To determine structural differences between the formulations, the FTIR spectra of the samples before degradation were stacked for easy comparison.

The first broad peak which appears at approximately 3290 cm^{-1} across all compositions is an OH functional group which undergoes stretching during degradation. The next two peaks in series, which start at approximately 2915 cm^{-1}, are expected to be a C-H bond stretching within the alkane functional group. Ultimately, the peak which proved to be the most useful for analyzing the degradation of these compositions over time was the peak at 1630 cm^{-1}. This peak most likely shows the presence of peptide bonds, which is expected given that each composition contains some amount of animal protein. The intensity of the "peptide" peak is directly related to the amount of protein present within the composition. For example, composition 6, which only has 10 percent protein present, shows a less-intense peak as compared to a sample with higher protein, such as composition 4 (which has 20 percent protein present in its makeup). It is important to note that unlike typical biopolymers like PLA, there are no esters in this structure because the ester bonds usually come from processing and these samples were not processed. Examining the degradation of each composition over time indicates certain trends related to the amount of glycerol and animal protein present (see Figure 13 and Figure 14). Compositions 2 and 3 will be shown here to illustrate this. Over time, the peak for the peptide bond experiences a general decrease in intensity, which points to some sort of enzymatic degradation which is breaking the bonds. Additionally, the OH peak around 3290 cm^{-1} increases in intensity over the course of the degradation period (except for the very last day before complete degradation), which may show hydrolysis of the samples over time.

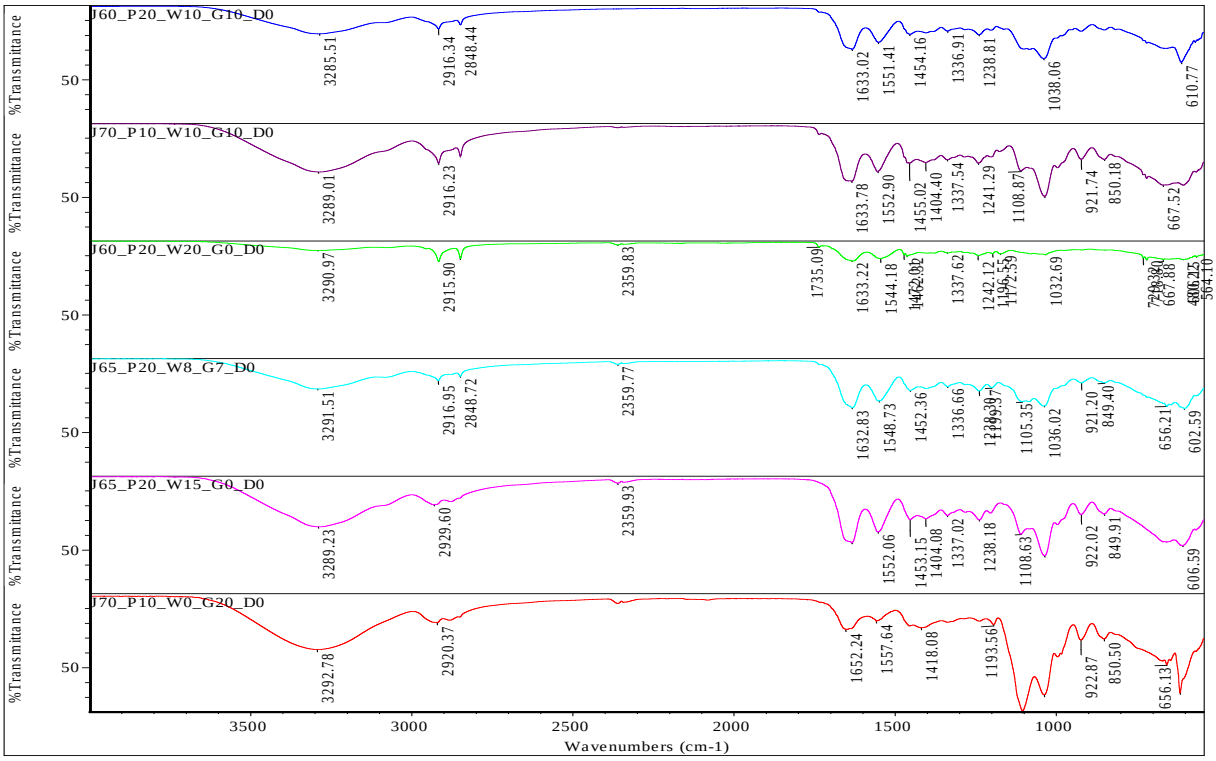

Figure 12. FTIR spectra for each composition before degradation

Figure 13. FTIR spectra overlay for composition 2 over time

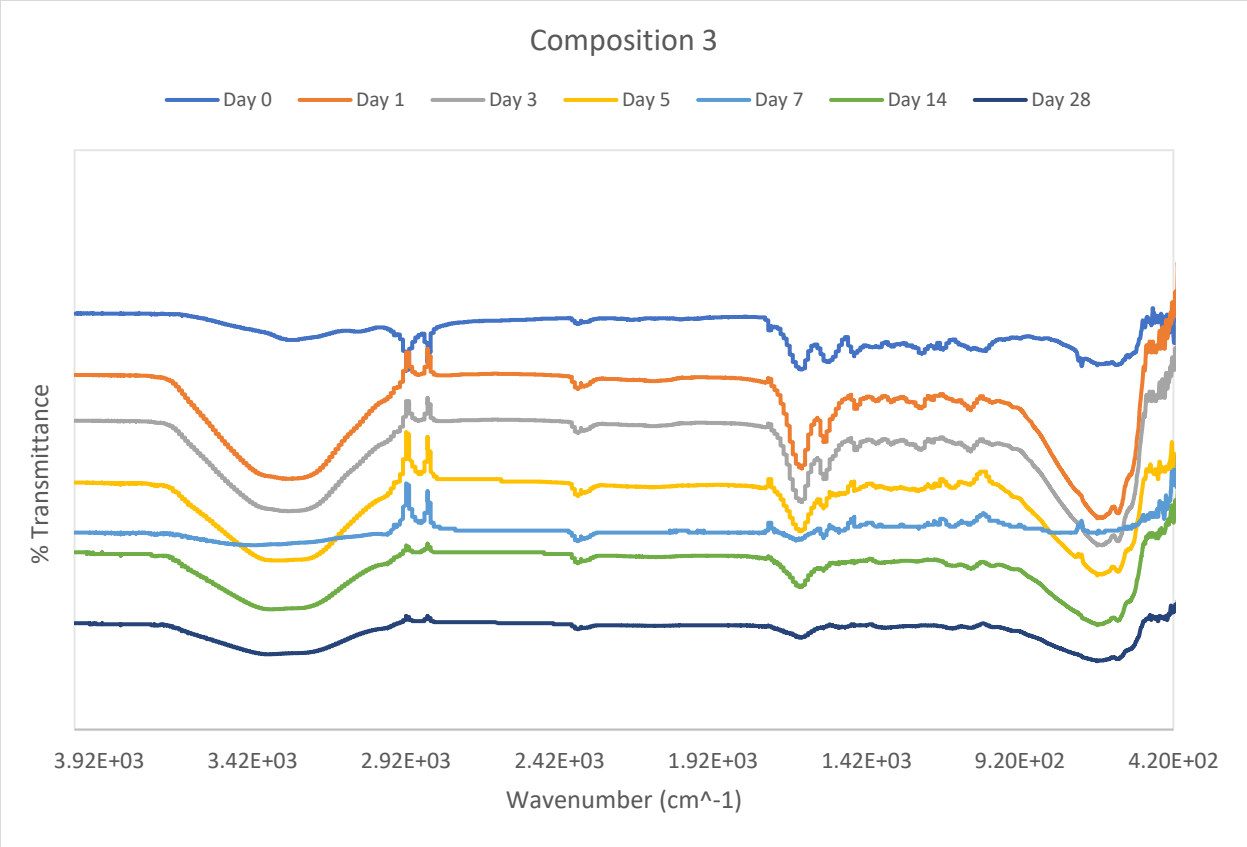

Figure 14. FTIR spectra overlay for composition 3 over time

6.4 Thermo-Mechanical Properties

6.4.1 TGA

Thermogravimetric analysis of these cactus-based biopolymers reveals variations in degradation onset temperature resulting from the amounts of the ingredients used in each composition. The range of temperatures on Figure 15 covers just above 180℃ to just under 310℃.

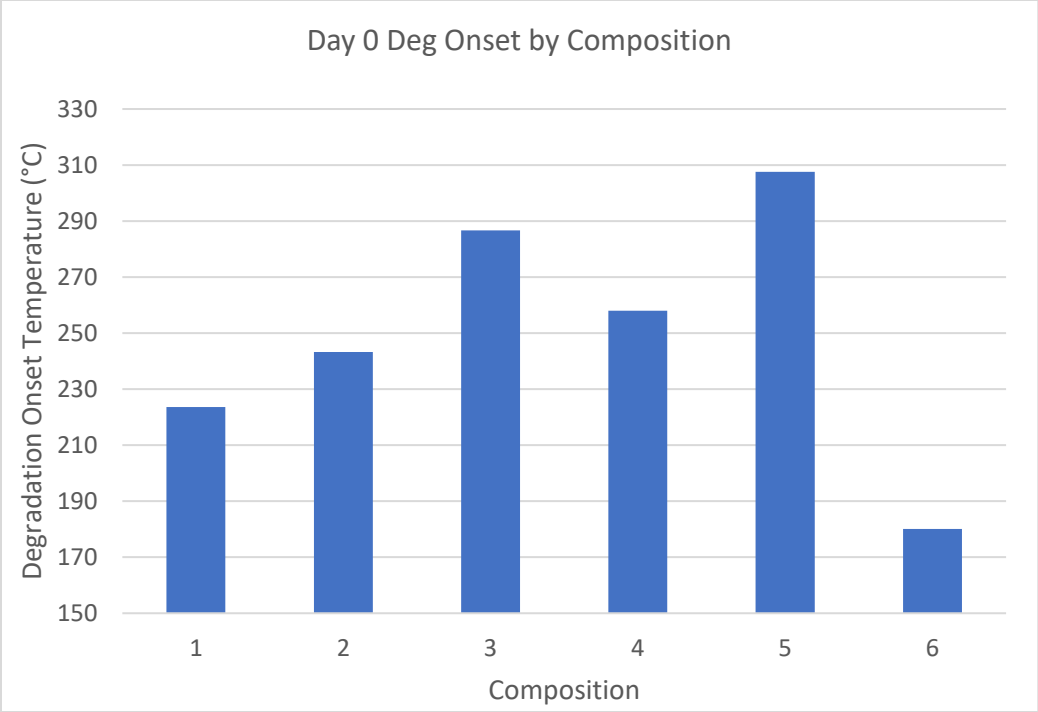

Figure 15. TGA degradation onset temperatures, Day 0

Compositions 2 and 3 exhibit degradation onset temperatures near 300℃, while composition 6 has a degradation onset temperature of ~180℃. What seems to be happening is that samples with higher amounts of glycerol have lower degradation onset temperatures, while samples with little or no glycerol present have higher degradation onset temperatures, close to or exceeding 300℃. Figure 16 details the degradation onset temperatures over time for every composition.

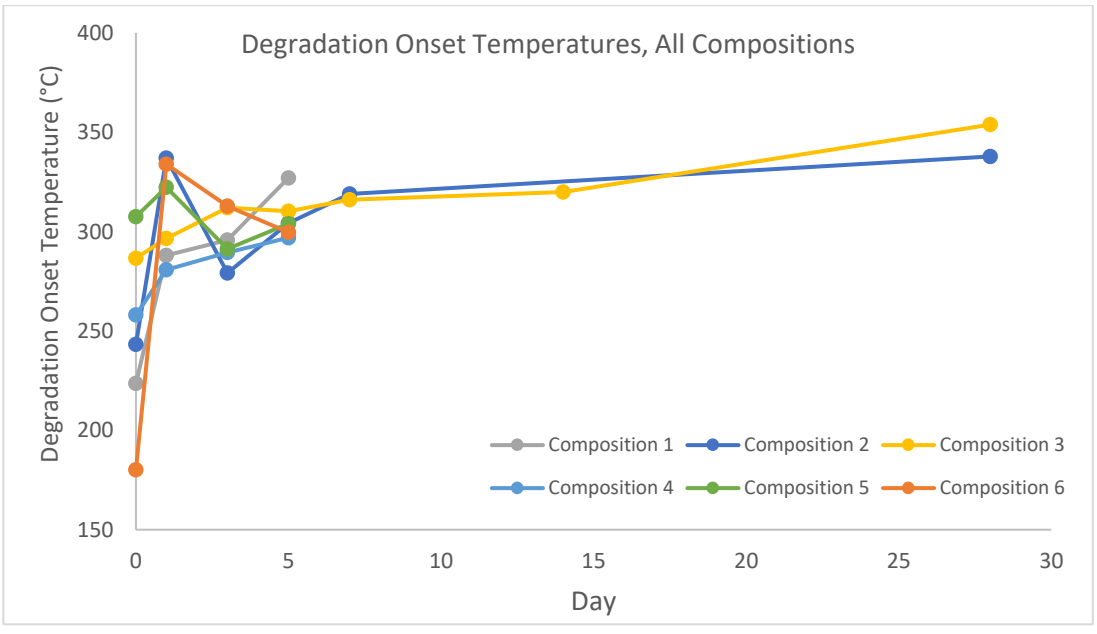

Figure 16. TGA degradation onset temperatures, all compositions

Across all compositions, Figure 16 indicates that the degradation onset temperature experiences an increase (in varying amounts) from Day 0 to Day 1. However, this trend is not 100 percent definitive given compositions 2, 5, and 6 experience some form of degradation onset temperature decrease at differing timepoints across the entire period of soil-based degradation.

6.4.2 DSC

The results of the DSC testing show multiple endothermic peaks, which may be related to two different phases in the system. Unfortunately, due to the design of experiment it has become very difficult to identify which component of the formulation is causing the different phases, Due to this fact, glycerol is the focus of the analysis by comparing composition 3 and 6. According to the TGA results glycerol is degrading first, and will not crystallize, so it is assumed that a composition with high glycerol and low protein will have much less intense peaks on the endothermic region of the heating cycle. This is proven in Figure 17, which shows the DSC heating curve for composition 6 before degradation. Figure 18 then shows the DSC heating curve of composition 3, which has no glycerol added, and much more intense peaks are seen in the endothermic region. This is due to the absence of the plasticizer, making the material much more brittle and crystalline. The larger areas under these peaks also imply a higher degree of crystallinity in the sample before any degradation begins. This also corresponds with the qualitative observations of the mechanical strength of the sample.

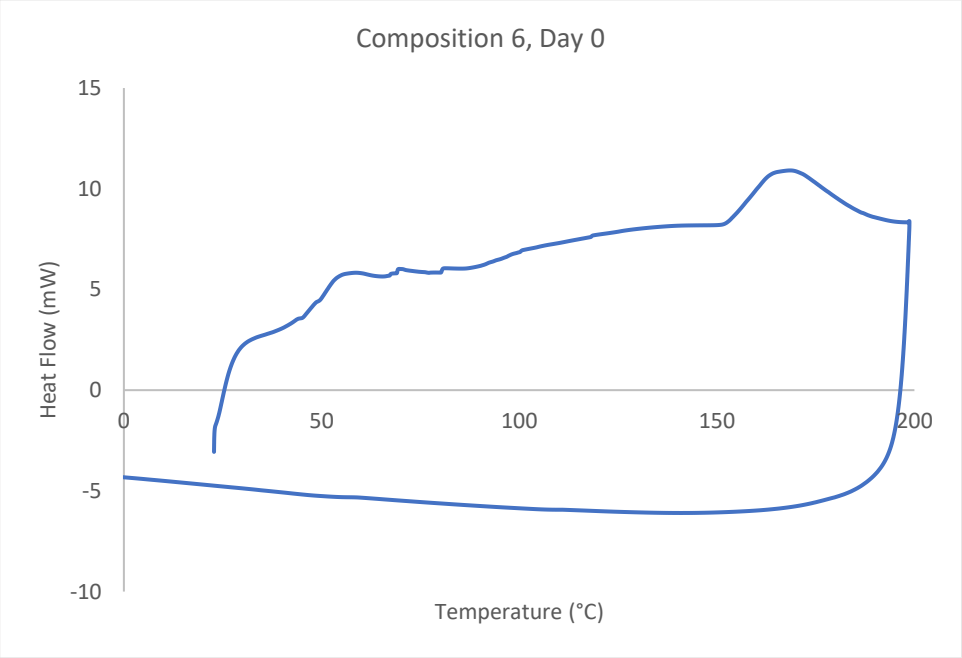

Figure 17. DSC heating cycle, composition 6, day 0

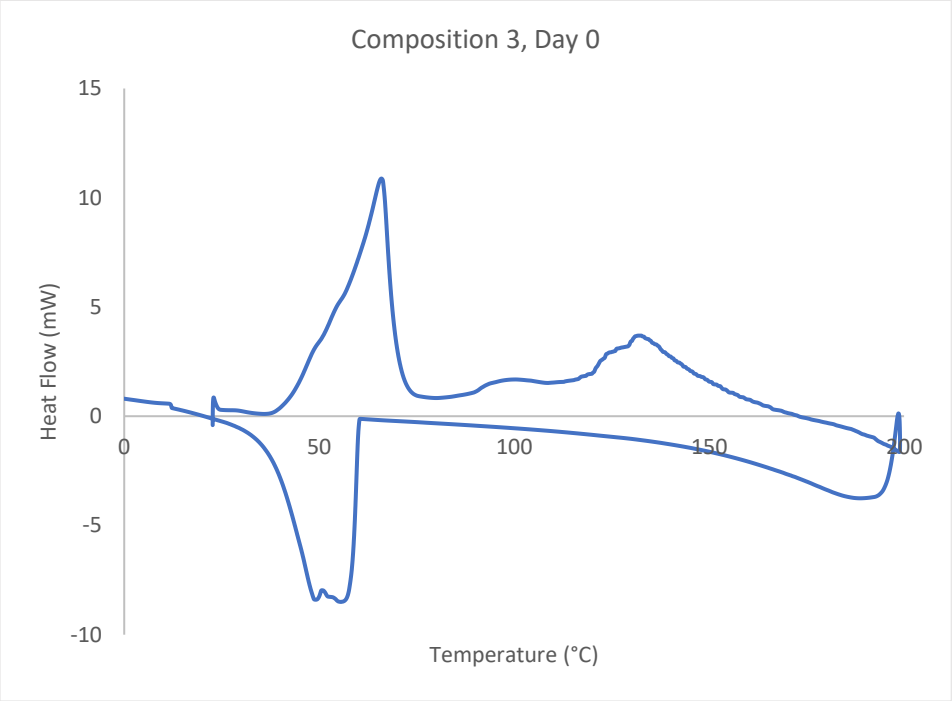

Figure 18. DSC heating cycle, composition 3, day 0

6.5 Gravimetric analysis

Although gravimetric analyses were initially determined to be important to characterizing these biopolymers throughout their degradation processes, multiple factors contributed to this team's decision to exclude any data related to gravimetry. The initial plan was to measure the mass of each sample pre-soil burial and re-measure them post-removal from the soil. Upon inspection of the samples across various time points in the degradation, soil was physically sticking to the samples and could not be removed without causing tears or permanently damaging the samples. This is possibly a result of the amount of water absorbed by each sample as it was buried in the soil. As such, any data that could be collected would be completely unrepresentative of the actual mass changes resulting from water absorption and/or biological degradation. Additionally, samples removed from time periods after day 5 exhibited degradation to the point that pieces of the original sample were completely indistinguishable from the soil or had completely disintegrated into the soil. Therefore, it was not possible to reliably collect every single part of the original sample in a condition suitable for gravimetric analysis. Any data which would be legitimately representative of the mass and gravimetric changes occurring to these samples during burial would be better reported by TGA.

6.6 Mechanical Testing

As stated in the methodology section of this report under Tensile testing, it was reported that the data collected was determined to be inconclusive due to "the extremely low mechanical strength of the biopolymer after only one day in soil." As for the reasons behind this limited mechanical

strength, it is assumed that the intention behind the design of these cactus-based biopolymers was to induce fast degradation the moment these samples were buried in soil. As such, the bacteria which started to “eat away” at these samples were extremely effective in weakening the samples’ mechanical strength to the point that even the most minimal of elongation strains would induce tears and/or permanent plastic deformation. It must also be noted that a large number of the mechanical failures which were experienced by these samples in the pretest load application of 1 newton. Additionally, a number of these pretest failures occurred at the ends of each sample, near the clamps. This may suggest these samples are sensitive to even slight variations in clamp grip strength and would therefore affect the results of these tensile tests.

7. Conclusions

Over the course of the degradation of the samples in garden soil and subsequent characterization of said samples, there were multiple conclusions which also point to areas of future work. During the 28 days of soil burial testing, these samples experienced rapid degradation. Within one week, four of the six different compositions experienced complete enzymatic breakdown and could not be recovered in any capacity past day 7, suggesting their decomposition into the soil. The only two compositions to survive past one week, compositions 2 and 3, were fully degraded by day 28. Additionally, the inclusion of glycerol as a part of the formulation of each sample induced significant effects on the thermo-mechanical stability of the biopolymer samples. Glycerol acted not only as an inducer for post-day 1 protein crystallization but decreased the degradation onset temperature of the samples and induced enzymatic breakdown of all amorphous regions present in these biopolymers. Examining each sample under SEM indicated various degrees of physical degradation on the surface of the sample, depending on how long said sample was buried in soil. Additionally, tensile testing of the samples was impossible after only one day buried in soil. Sufficient enzymatic breakdown of the samples occurred within that time such that many samples would tear in the pre-test loading phase (which only applies 1 newton of tensile strain) of the tensile tests which would have occurred.

Given the above conclusions reached from the burial tests and post-test characterization, there are three areas of future work this group recommends are undertaken to better understand the mechanisms of degradation which are happening to these biopolymer samples. First, each individual component should be characterized to understand the influence such a component would have on the degradation the biopolymer. Second, improvements to experimental design would include utilizing samples which change the amount of each component present in a consistent interval to better understand the trends which may be identified after material characterization testing. Last, additional DSC testing should be undertaken to understand the changing thermo-mechanical properties more fully throughout the duration of the degradation.

8. References

1. A. K. Awasthi, F. Wei, Q. Tan, and J. Li, “Single-use plastics: Production, usage, disposal, and adverse impacts,” *Science of The Total Environment*, vol. 752, p. 141772, Jan. 2021.
2. “Plastic: It's not all the same,” *Plastic Oceans International*, 30-Oct-2021. [Online]. Available: https://plasticoceans.org/7-types-of-plastic/. [Accessed: 05-Dec-2021].
3. H. K. Karapanagioti and H. Takada, “Conclusions of ‘Hazardous Chemicals associated with plastics in environment,’” *The Handbook of Environmental Chemistry*, pp. 297–305, Jul. 2018.
4. J. Payal, “How Is Plastic Made? A Simple Step-By-Step Explanation,” *How is Plastic Made? - British Plastic Federation*. [Online]. Available: https://www.bpf.co.uk/plastipedia/how-is-plastic-made.aspx. [Accessed: 05-Dec-2021].
5. J. Dell, “Six times more plastic waste is burned in U.S. than is recycled,” *Plastic Pollution Coalition*, 30-Apr-2019. [Online]. Available: https://www.plasticpollutioncoalition.org/blog/2019/4/29/six-times-more-plastic-waste-is-burned-in-us-than-is-recycled. [Accessed: 05-Dec-2021].
6. “Single-use carryout bags,” *Single-Use Carryout Bags | AustinTexas.gov*. [Online]. Available: http://austintexas.gov/bags. [Accessed: 06-Dec-2021].
7. Palanisamy, K., Jeyaseelan, A., Murugesan, K., & Palanisamy, S. B. (2019). Biopolymer Technologies for Environmental Applications. *Nanoscience and Biotechnology for Environmental Applications*, 55–83. https://doi.org/10.1007/978-3-319-97922-9_3
8. A. Peters, “This new biodegradable plastic is made from Cactus,” *Fast Company*, 20-Jun-2019. [Online]. Available: https://www.fastcompany.com/90365199/this-new-biodegradable-plastic-is-made-from-cactus. [Accessed: 06-Dec-2021].
9. “Nopal,” *Wikipedia*, 28-Nov-2021. [Online]. Available: https://en.wikipedia.org/wiki/Nopal. [Accessed: 06-Dec-2021].
10. Harding, T. (2021). Biopolymers and Bionanocomposites Lectures from MATE 420
11. Gotro, J., says, clive C., & Cooper, clive. (2012, June 28). *Do you know the three key aspects of bioplastics?* Polymer Innovation Blog. Retrieved December 6, 2021, from https://polymerinnovationblog.com/do-you-know-the-three-key-aspects-of-bioplastics/.
12. H.-J. Endres and A. Siebert-Raths, “End-of-Life Options for Biopolymers,” in *Engineering biopolymers: Markets, manufacturing, properties, and applications*, Cincinnati, OH: Hanser Publishers, 2011, pp. 225–243.
13. I. Chang, J. Im, and G.-C. Cho, “Introduction of microbial biopolymers in soil treatment for future environmentally-friendly and sustainable geotechnical engineering,” *Sustainability*, vol. 8, no. 3, p. 251, 2016.
14. *Advantages of bioplastics vs. disadvantages: Memo for Product Designers*. QualityInspection.org. (2020, March 26). Retrieved December 6, 2021, from https://qualityinspection.org/advantages-of-bioplastics-vs-disadvantages/.
15. M. G. Vieira, M. A. da Silva, L. O. dos Santos, and M. M. Beppu, “Natural-based plasticizers and biopolymer films: A Review,” *European Polymer Journal*, vol. 47, no. 3, pp. 254–263, Dec. 2010.
16. “Glycerol,” *Wikipedia*, 24-Nov-2021. [Online]. Available: https://en.wikipedia.org/wiki/Glycerol. [Accessed: 06-Dec-2021].
17. M. Glozer, M. Bolling, A. Wolfson, “Effects of UV exposure on the thermo-mechanical properties of cactus based biopolymers,” June. 2021.

18. Ware, M. (2019, October 9). *Nopal cactus: Benefits, recipes, and Nutrition*. Medical News Today. Retrieved December 6, 2021, from https://www.medicalnewstoday.com/articles/320293#benefits.
19. *Collagen fiber*. Biology Articles, Tutorials & Dictionary Online. (2021, September 6). Retrieved December 6, 2021, from https://www.biologyonline.com/dictionary/collagen-fiber.
20. Essentials, P. by R., & Essentials, R. (2019, September 21). *Understanding different types of waxes: Raw essentials*. Raw Essentials | Candles & Soap Making Supplies. Retrieved December 6, 2021, from https://www.rawessentials.com.sg/2019/09/21/understanding-which-type-of-waxes/.
21. Libretexts. (2020, August 11). *Wax*. Chemistry LibreTexts. Retrieved December 6, 2021, from https://chem.libretexts.org/Bookshelves/Biological_Chemistry/Supplemental_Modules_(Biological_Chemistry)/Lipids/Non-glyceride_Lipids/Wax#:~:text=A%20wax%20is%20a%20simple,losing%20excessive%20amounts%20of%20water.
22. *IR Spectrum Table & Chart*. IR Spectrum Table. (n.d.). Retrieved June 6, 2022, from https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/analytical-chemistry/photometry-and-reflectometry/ir-spectrum-table
23. Kijchavengkul, T., Auras, R., Rubino, M., Alvarado, E., Camacho Montero, J. R., & Rosales, J. M. (2010). Atmospheric and soil degradation of aliphatic–aromatic polyester films. *Polymer Degradation and Stability*, *95*(2), 99–107. https://doi.org/10.1016/j.polymdegradstab.2009.11.048
24. Oberlintner, A., Bajić, M., Kalčíková, G., Likozar, B., & Novak, U. (2021). Biodegradability study of active chitosan biopolymer films enriched with Quercus polyphenol extract in different soil types. *Environmental Technology & Innovation*, *21*, 101318. https://doi.org/10.1016/j.eti.2020.101318
25. Mohanan, N., Montazer, Z., Sharma, P. K., & Levin, D. B. (2020). Microbial and enzymatic degradation of synthetic plastics. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.580709
26. Van de Velde, K., & Kiekens, P. (2002). Biopolymers: Overview of several properties and consequences on their applications. *Polymer Testing*, *21*(4), 433–442. https://doi.org/10.1016/s0142-9418(01)00107-6
27. Müller, C. M., Pires, A. T., & Yamashita, F. (2012). Characterization of thermoplastic starch/poly(lactic acid) blends obtained by extrusion and thermopressing. *Journal of the Brazilian Chemical Society*. https://doi.org/10.1590/s0103-50532012000300008

Appendix A

Figure 19. Composition 1 visual degradation, starting at day 1

Figure 20. Composition 4 visual degradation, starting at day 1

Figure 21. Composition 5 visual degradation, starting at day 1

Figure 22. Composition 6 visual degradation, starting at day 1

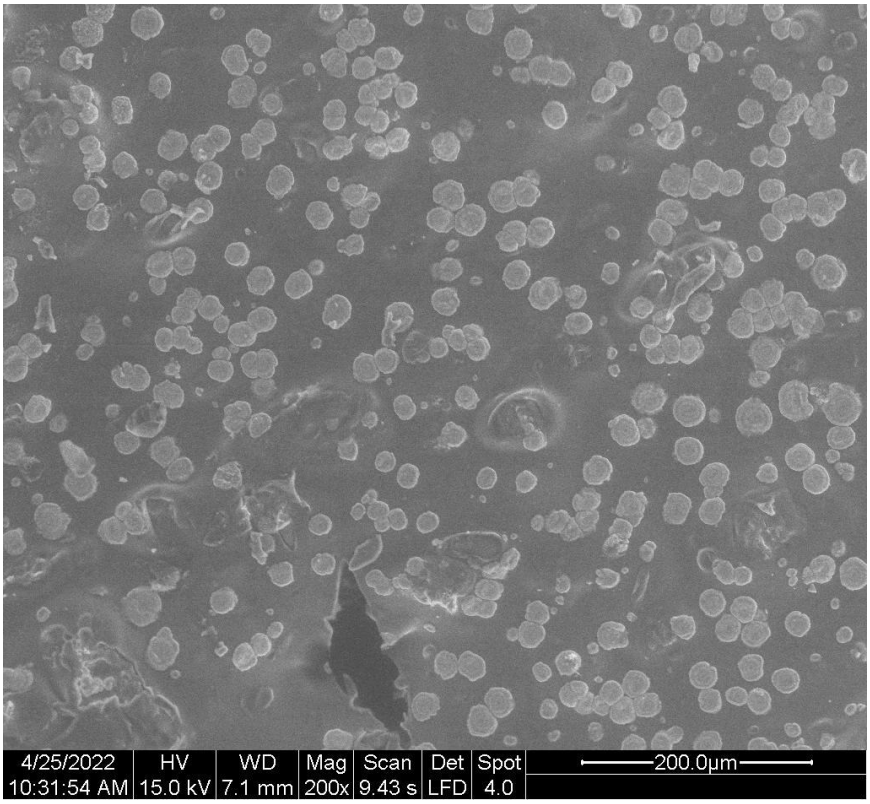

Figure 23. SEM 200x magnification, composition 1, before degradation, day 0

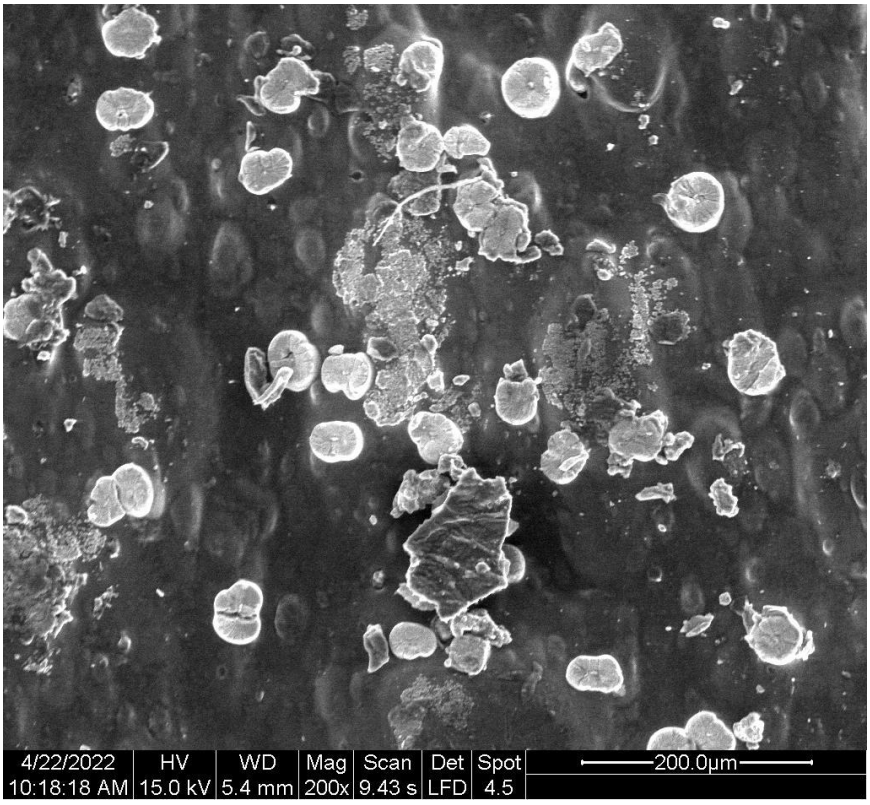

Figure 24. SEM 200x magnification, composition 2, before degradation, day 0

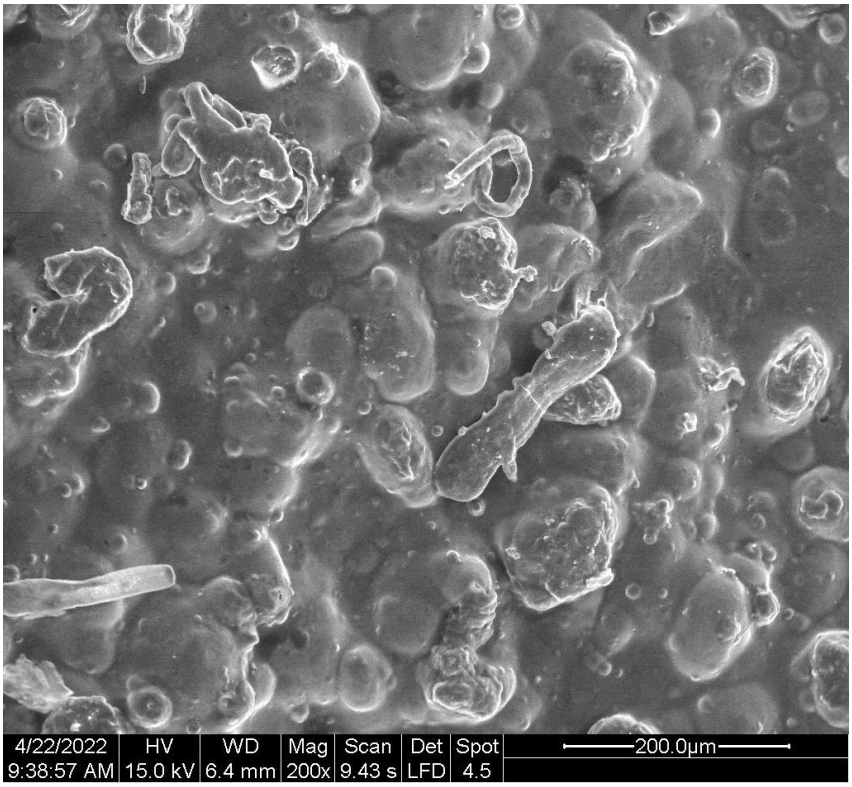

Figure 25. SEM 200x magnification, composition 3, before degradation, day 0

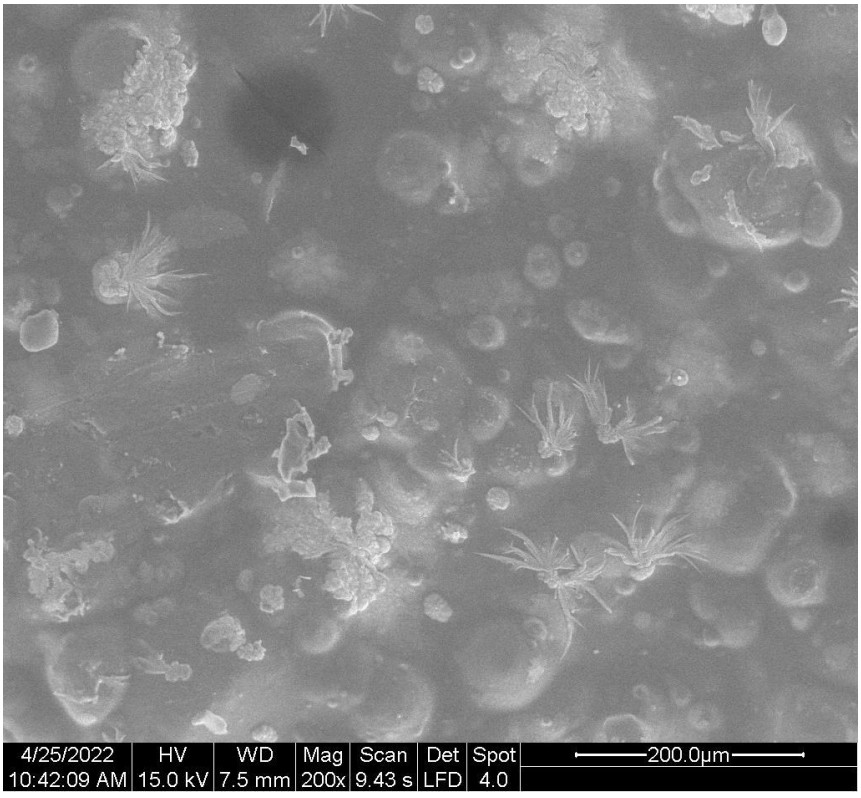

Figure 26. SEM 200x magnification, composition 4, before degradation, day 0

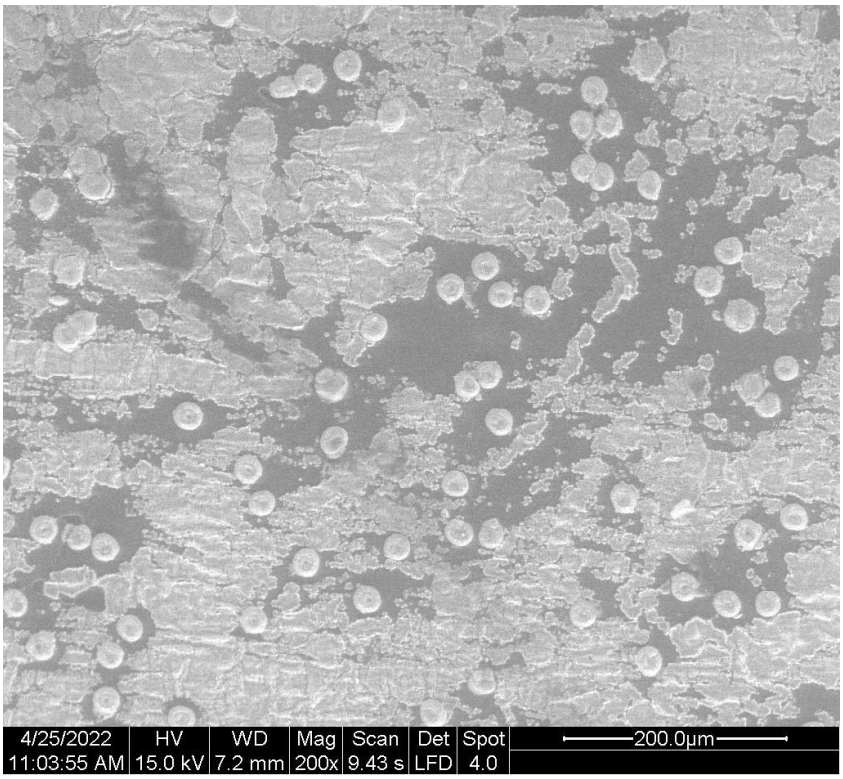

Figure 27. SEM 200x magnification, composition 5, before degradation, day 0

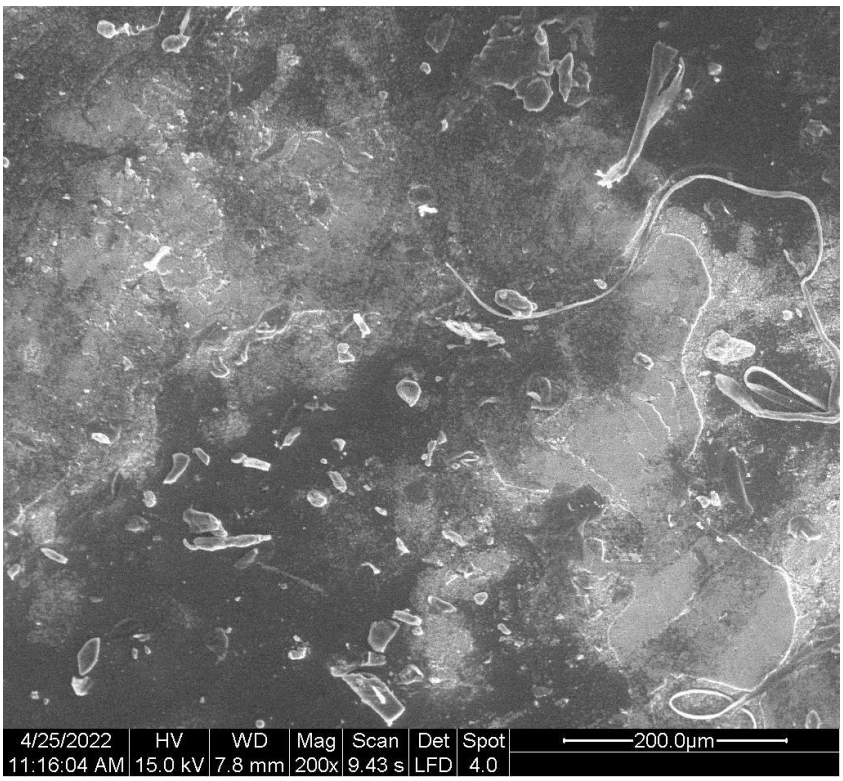

Figure 28. SEM 200x magnification, composition 6, before degradation, day 0

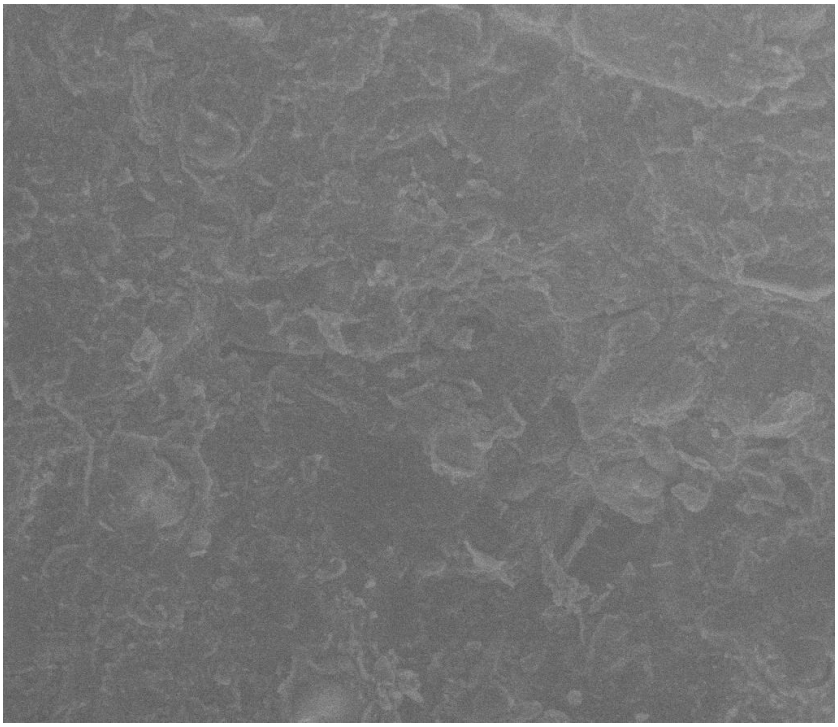

Figure 29. SEM 200x magnification, composition 1, day 1

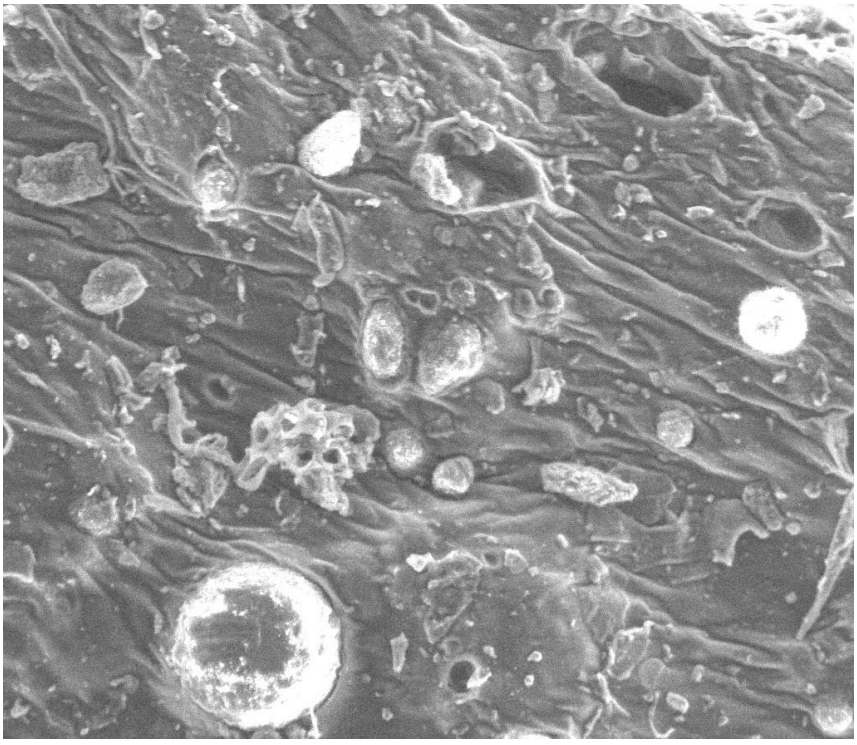

Figure 30. SEM 200x magnification, composition 2, day 1

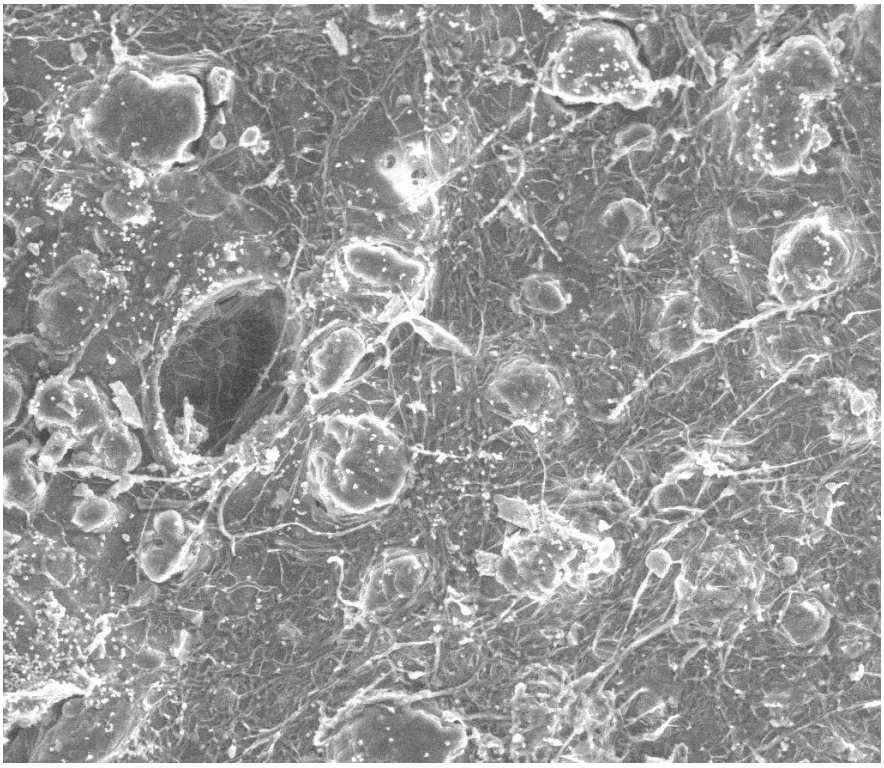

Figure 31. SEM 200x magnification, composition 3, day 1

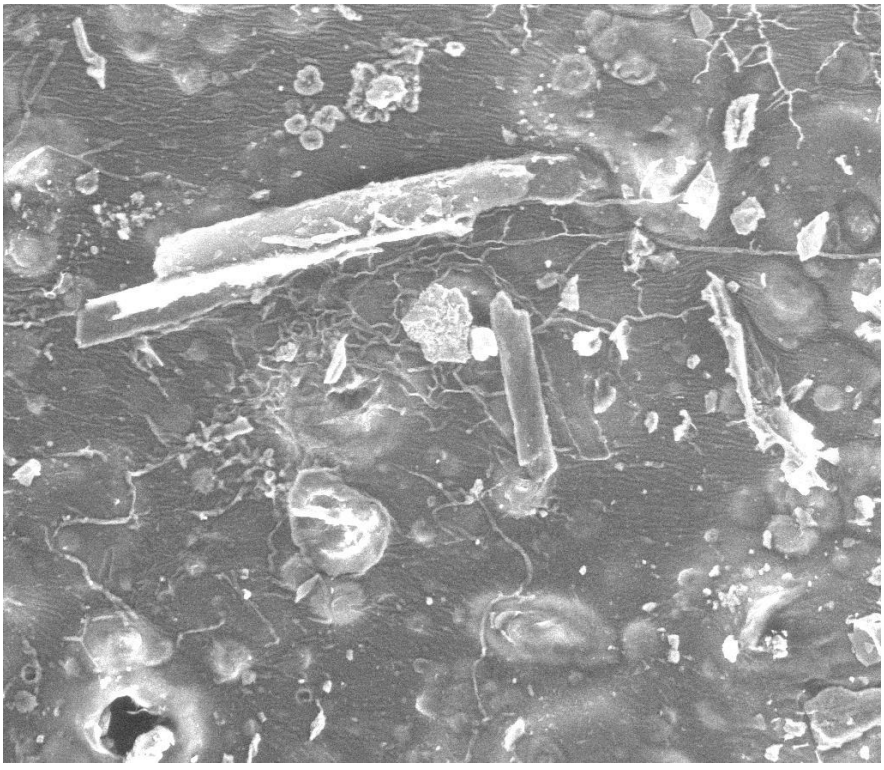

Figure 32. SEM 200x magnification, composition 4, day 1

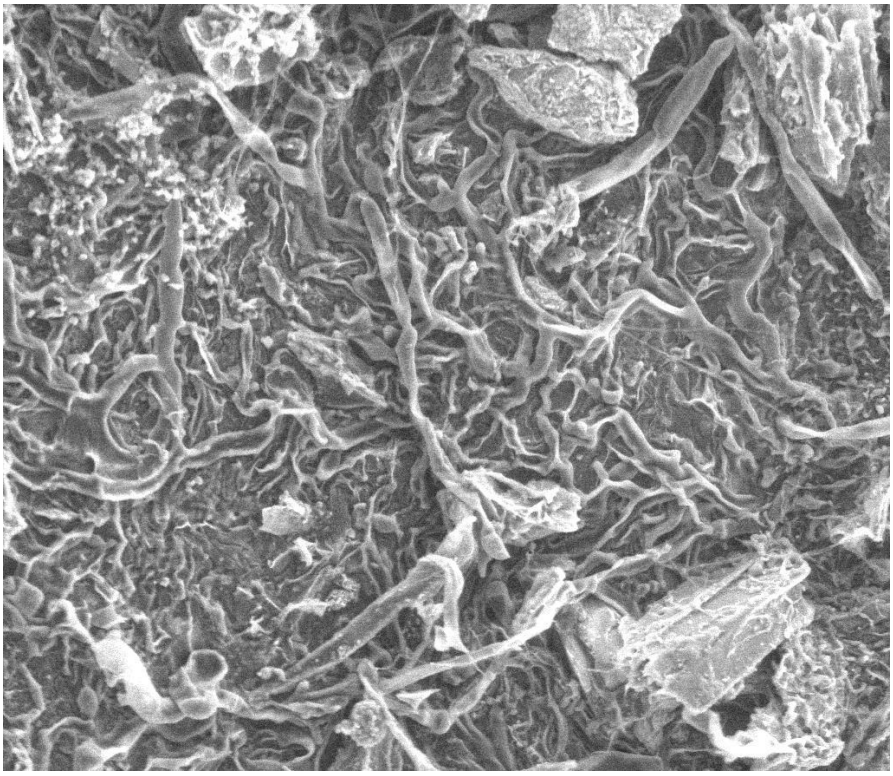

Figure 33. SEM 200x magnification, composition 5, day 1

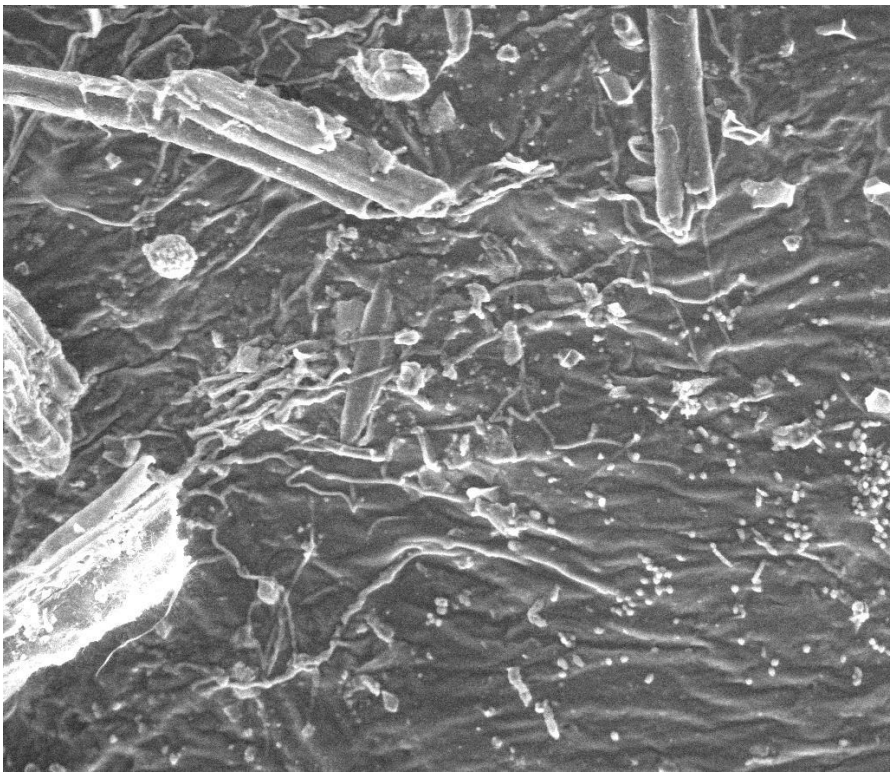

Figure 34. SEM 200x magnification, composition 6, day 1

Appendix B

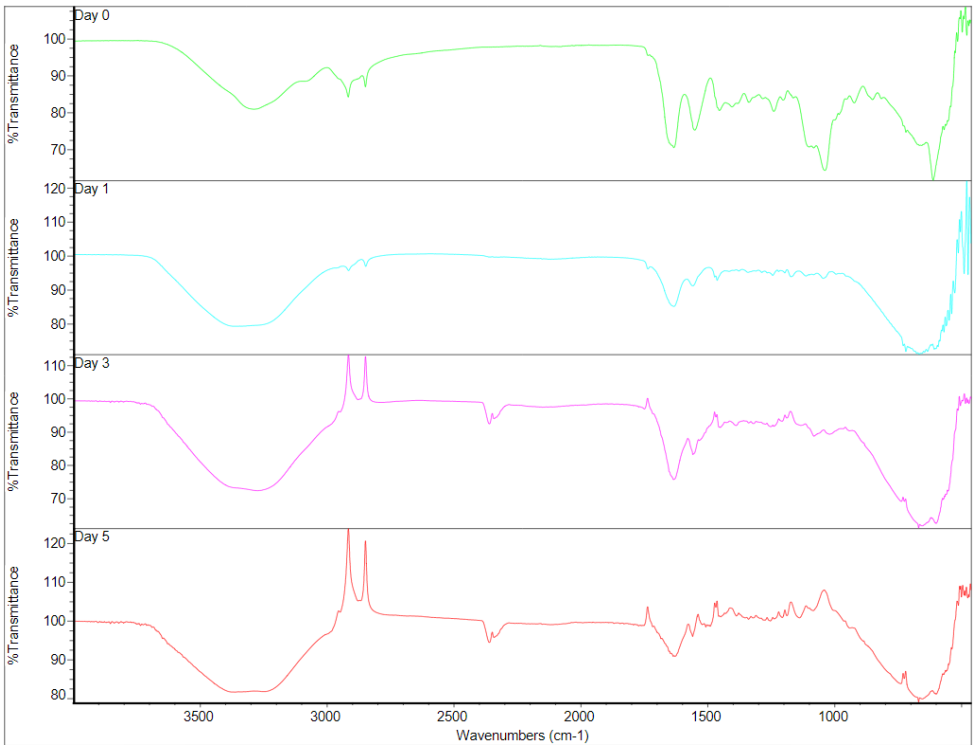

Figure 35. FTIR Overlays, Composition 1

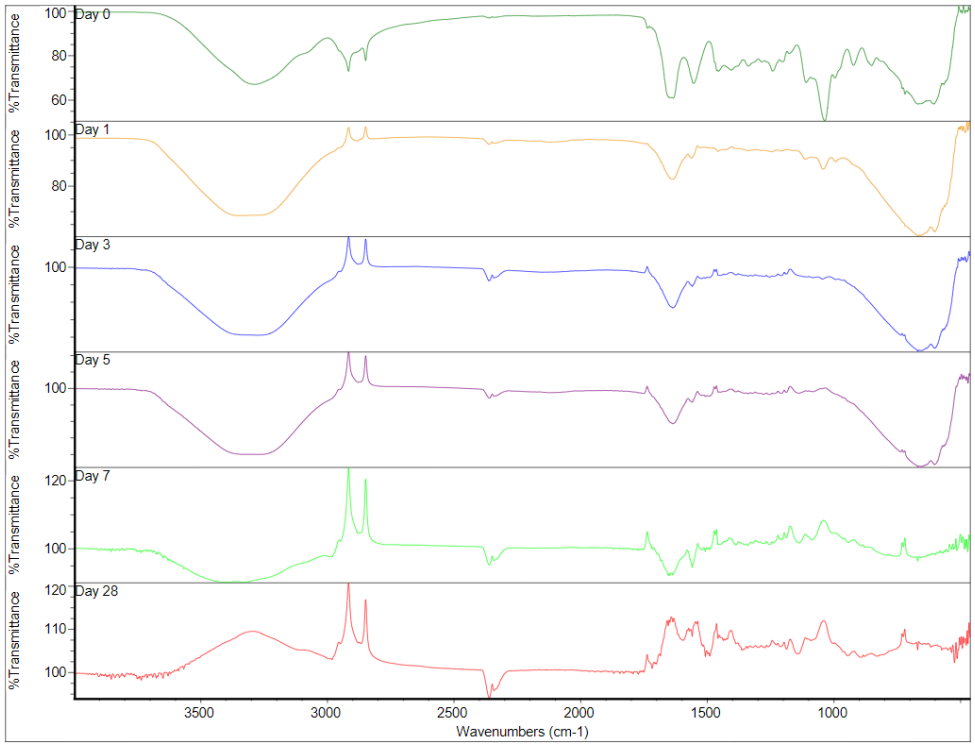

Figure 36. FTIR Overlays, Composition 2

Figure 37. FTIR Overlays, Composition 3

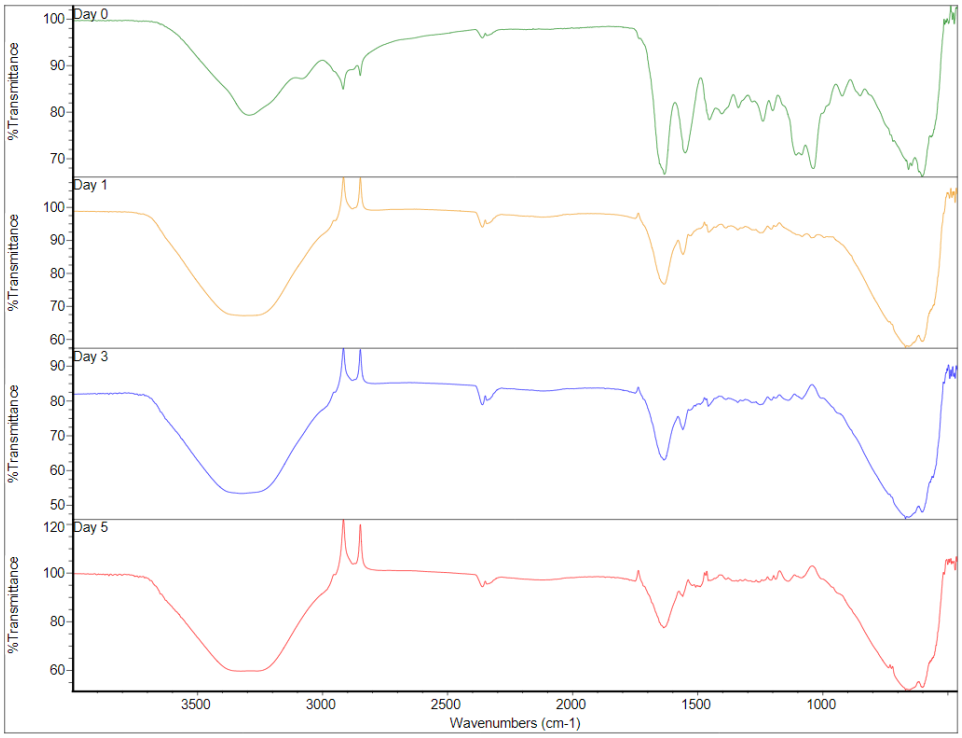

Figure 38FTIR Overlays, Composition 4

Figure 39. FTIR Overlays, Composition 5

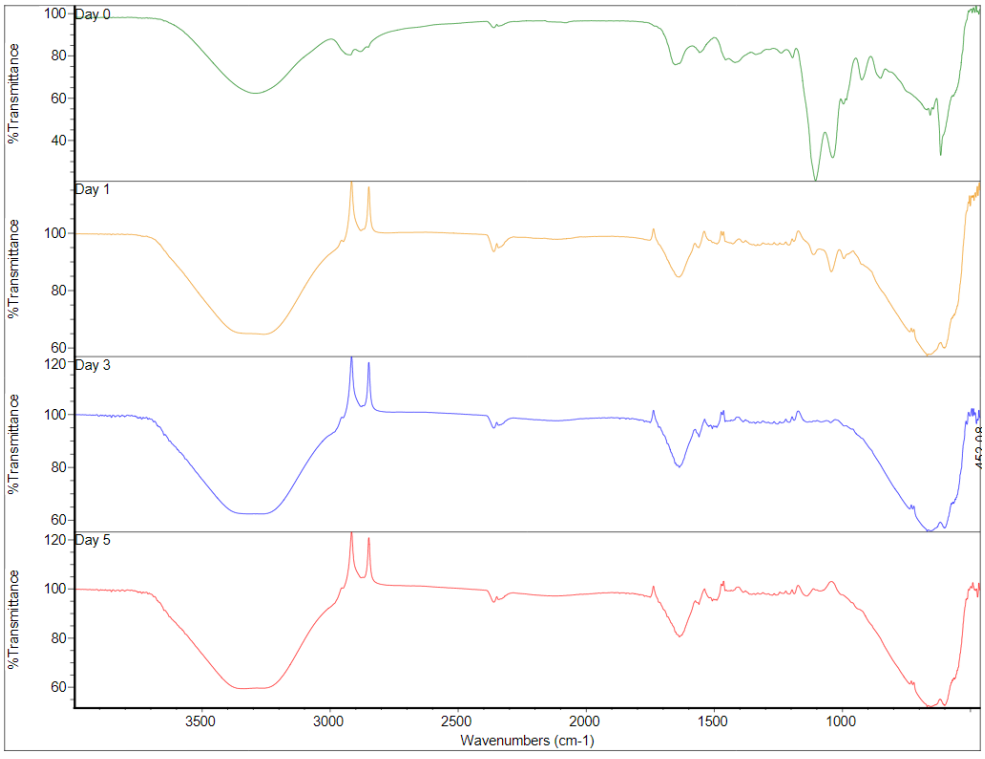

Figure 40. FTIR Overlays, Composition 6

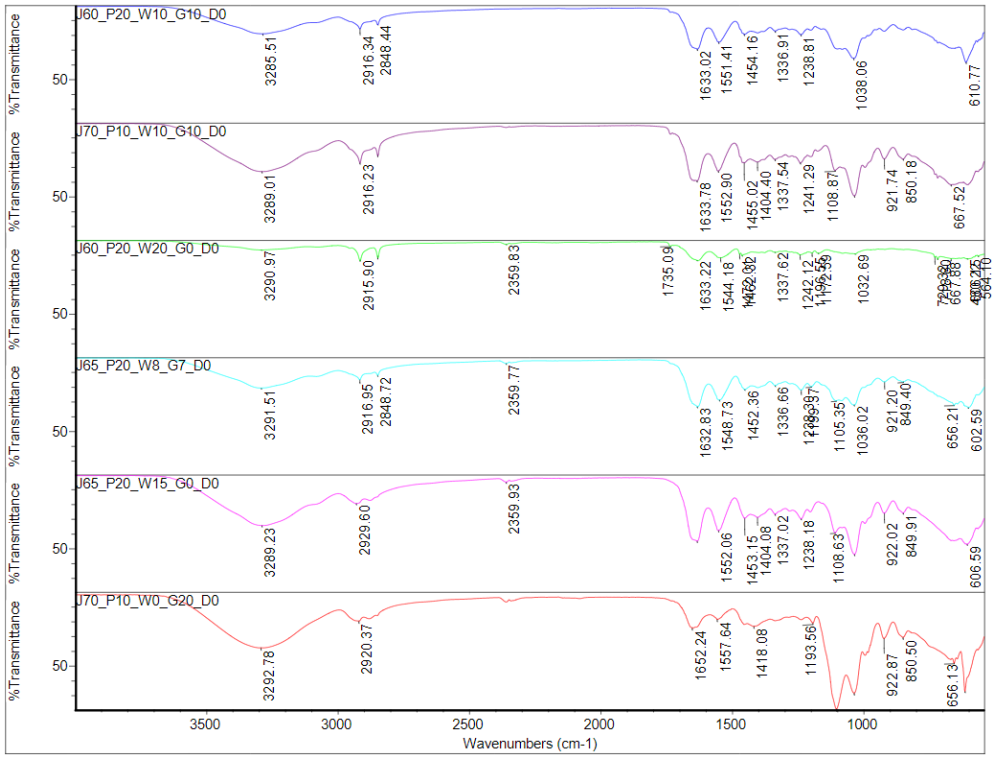

Figure 41. FTIR Overlays, Day 0

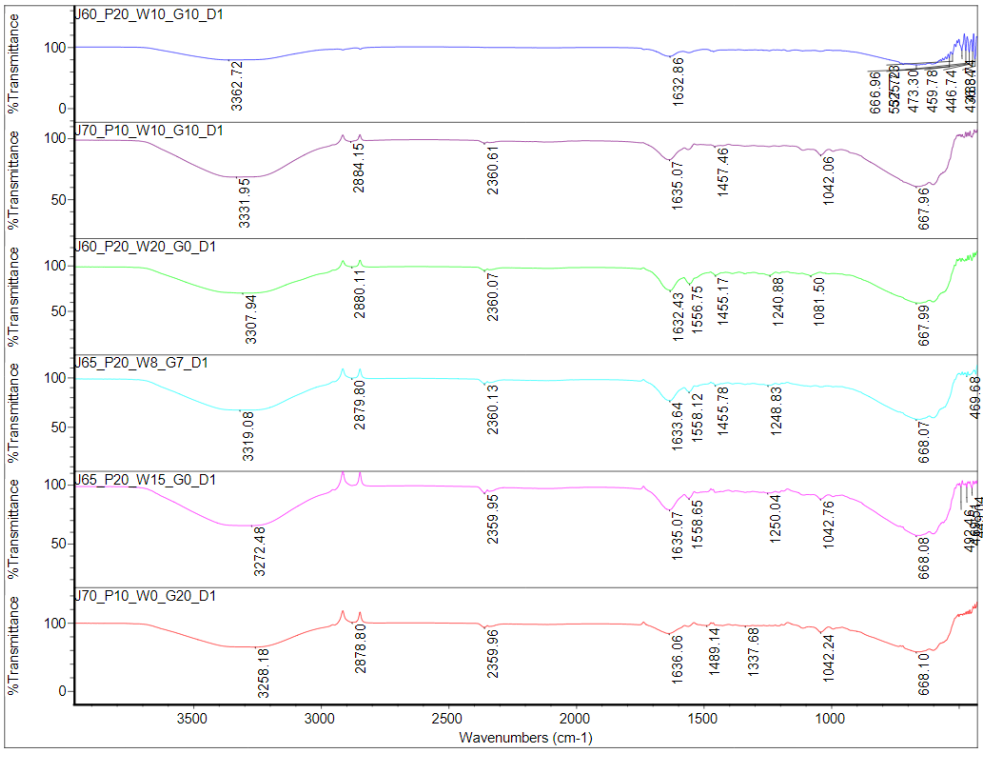

Figure 42. FTIR Overlays, Day 1

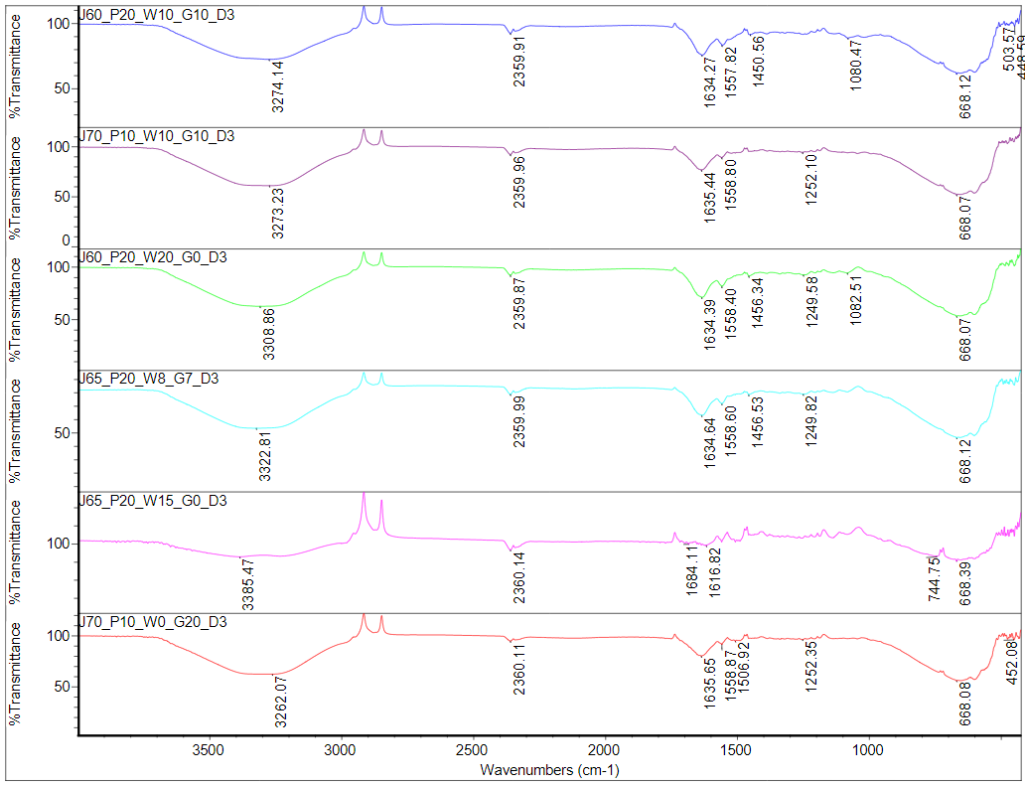

Figure 43. FTIR Overlays, Day 3

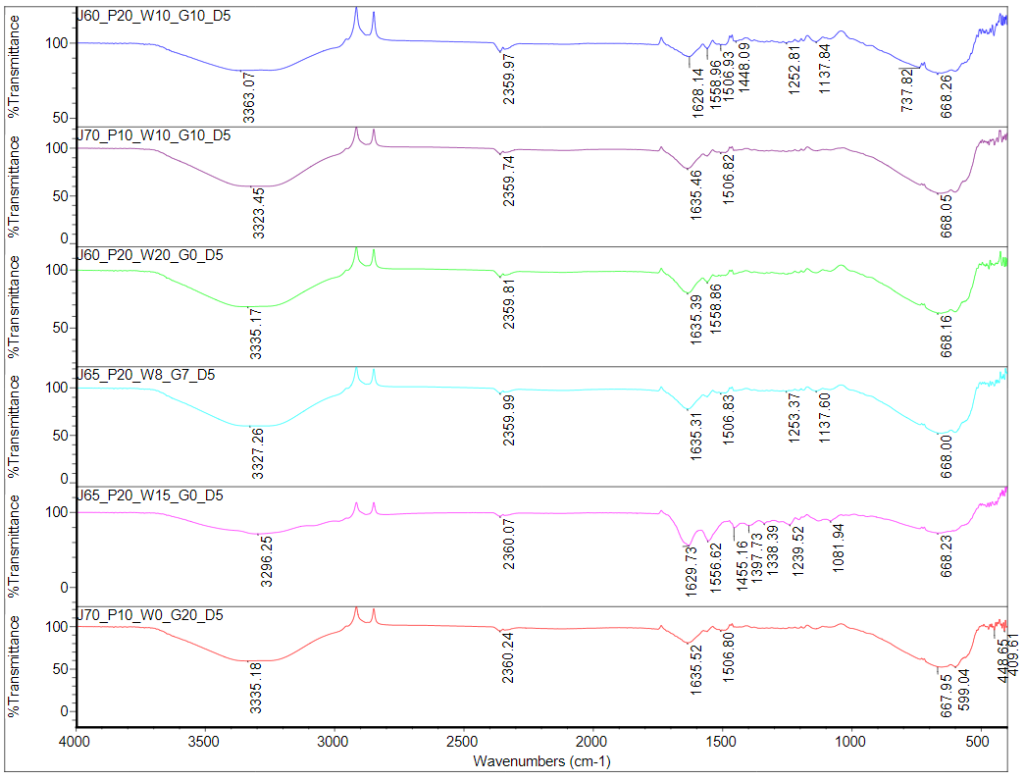

Figure 44. FTIR Overlays, Day 5

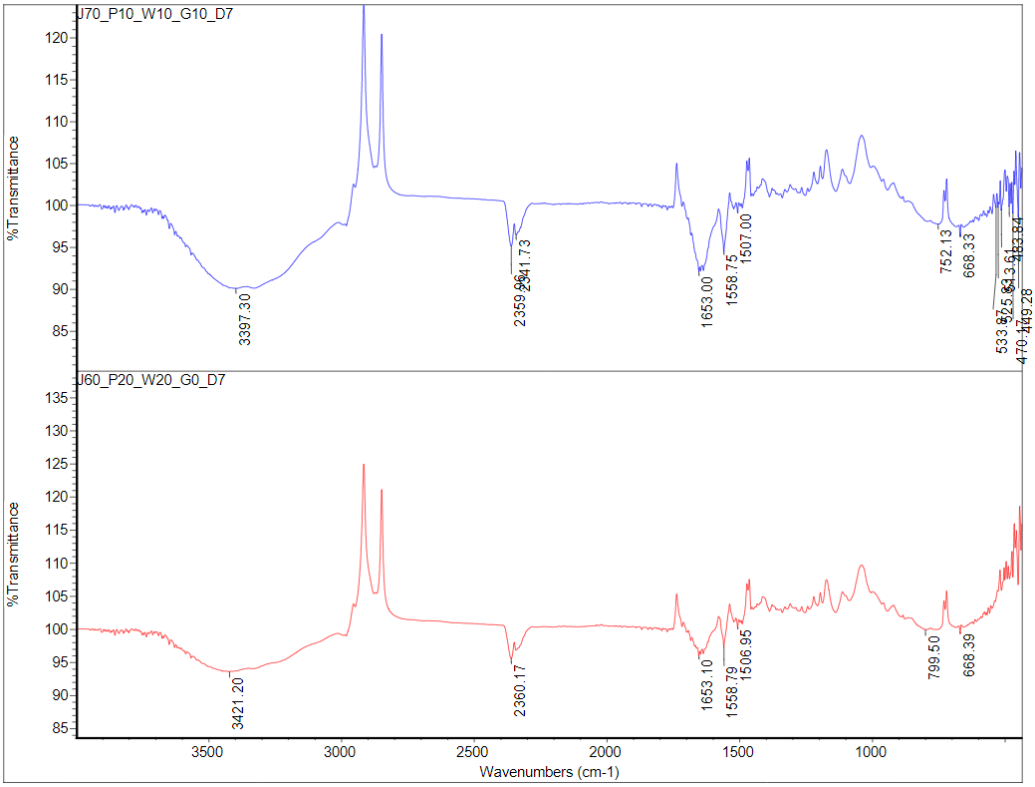

Figure 45. FTIR Overlays, Day 7

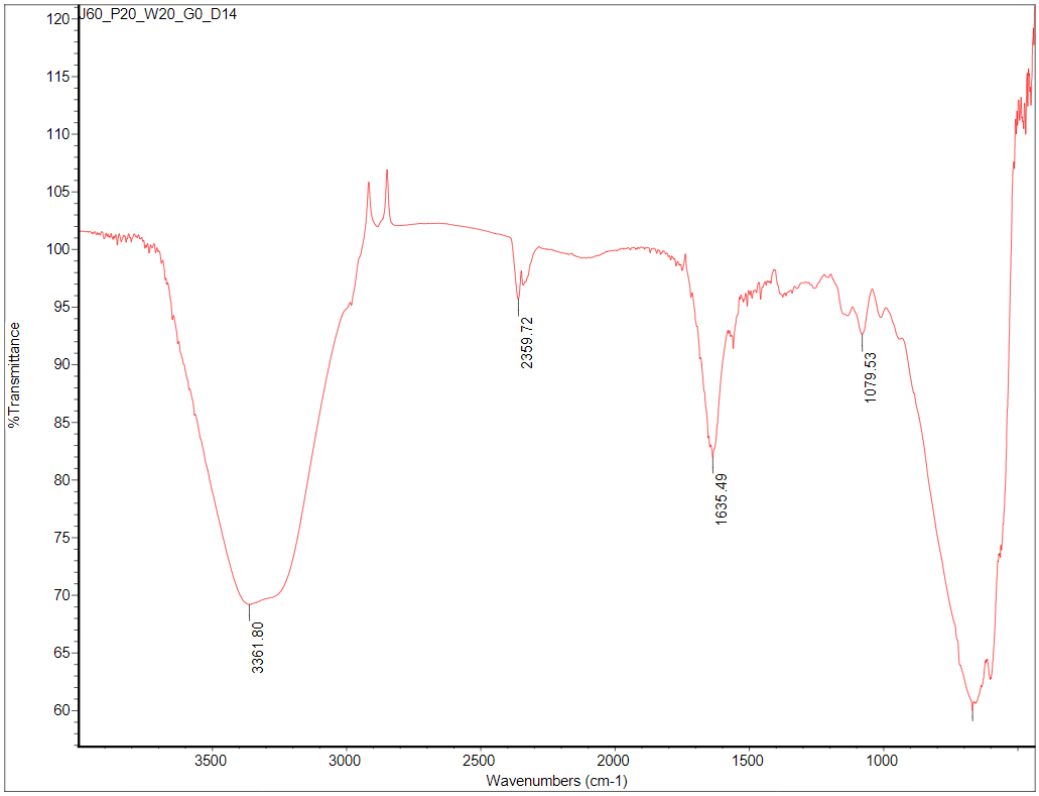

Figure 46. FTIR Overlay, Day 14

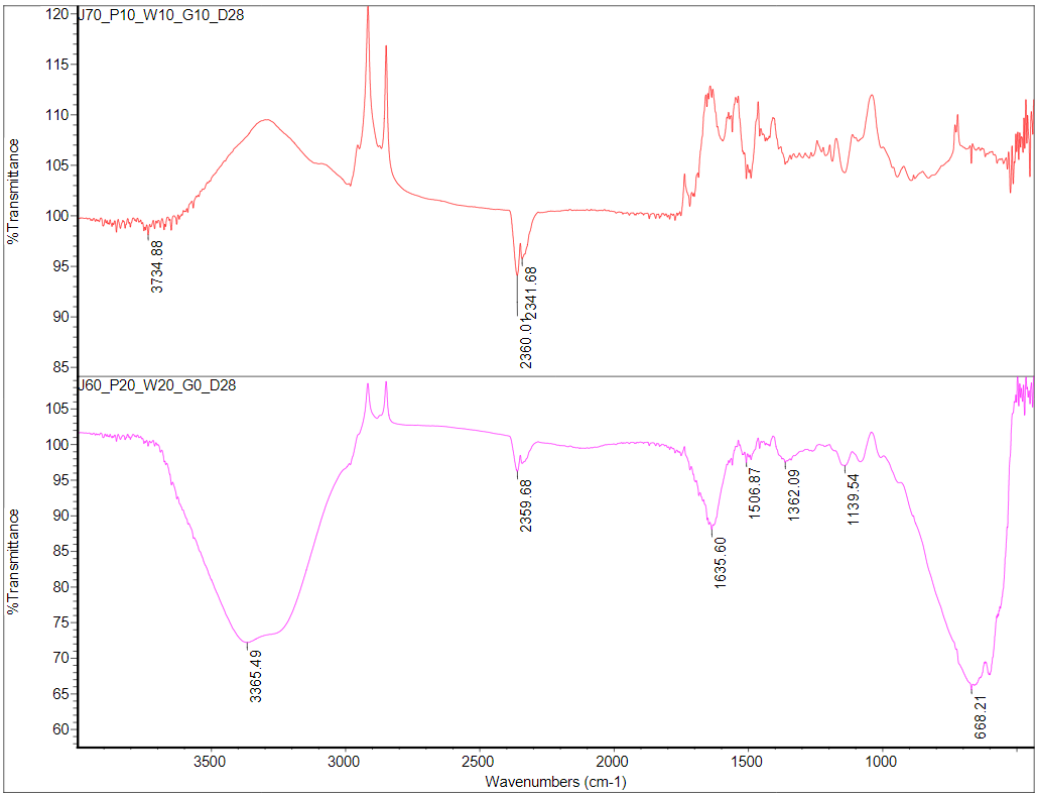

Figure 47. FTIR Overlays, Day 28

Appendix C

Table 3: Thermogravimetric analysis raw data for each composition during degradation

Composition 1		
Day	Degradation Onset (°C)	Water Content (%)
0	223.66	0%
1	288.01	53.293%
3	295.73	13.568%
5	327.06	60.216%
7	N/A	N/A
14	N/A	N/A
28	N/A	N/A

Composition 2		
Day	Degradation Onset (°C)	Water Content (%)
0	243.29	0%
1	337.03	17%
3	279.23	60.67%
5	304.26	30.00%
7	318.93	15.51%
14	N/A	N/A
28	337.82	0%

Composition 3		
Day	Degradation Onset (°C)	Water Content (%)
0	286.67	0.1
1	296.64	28.780%
3	312.09	18.020%
5	310.27	33.278%
7	316.06	43.167%
14	319.97	0%
28	353.84	0%

Composition 4		
Day	Degradation Onset (°C)	Water Content (%)
0	258.03	0%
1	280.72	35.926%%
3	289.48	71.88%
5	296.94	66.93%
7	N/A	N/A
14	N/A	N/A
28	N/A	N/A

Composition 5		
Day	Degradation Onset (°C)	Water Content (%)
0	307.56	0%
1	322.31	60.160%
3	291.32	50.991%
5	303.94	50.454%
7	N/A	N/A
14	N/A	N/A
28	N/A	N/A

Composition 6		
Day	Degradation Onset (°C)	Water Content (%)
0	180.09	8%
1	333.96	64%
3	312.82	10.43%
5	299.7	47.07%
7	N/A	N/A
14	N/A	N/A
28	N/A	N/A

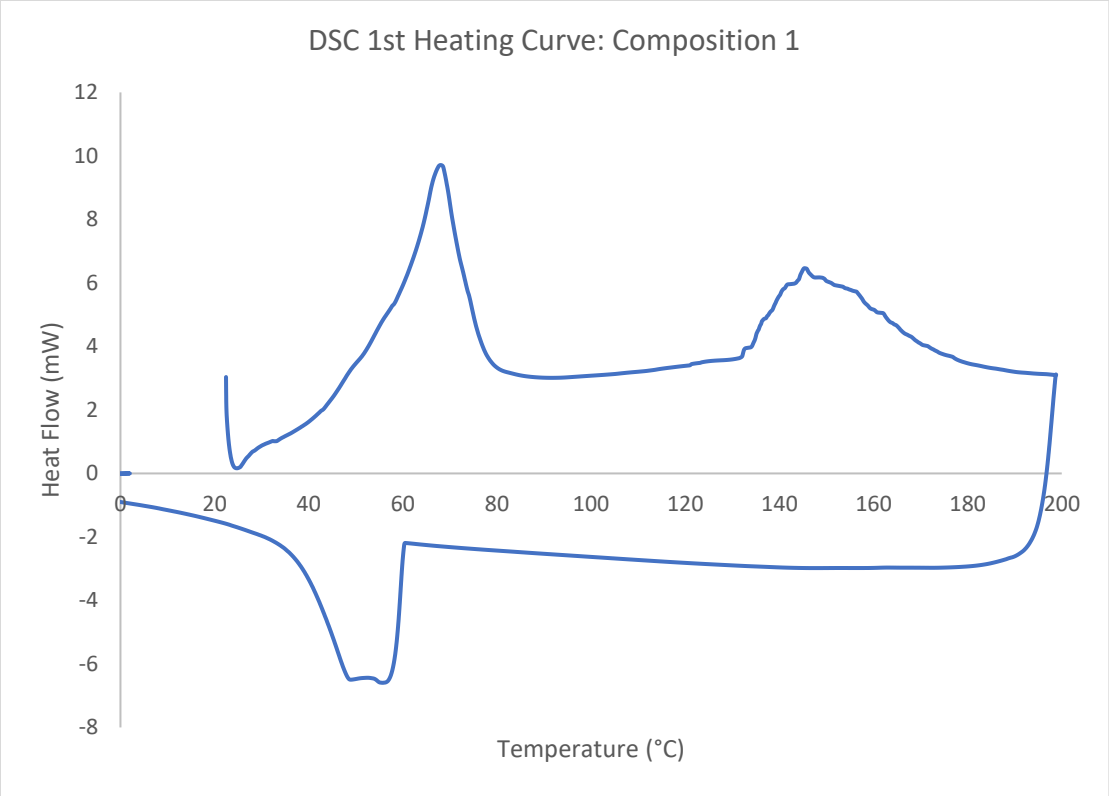

Figure 48. DSC first heating curve, Composition 1, Day 0

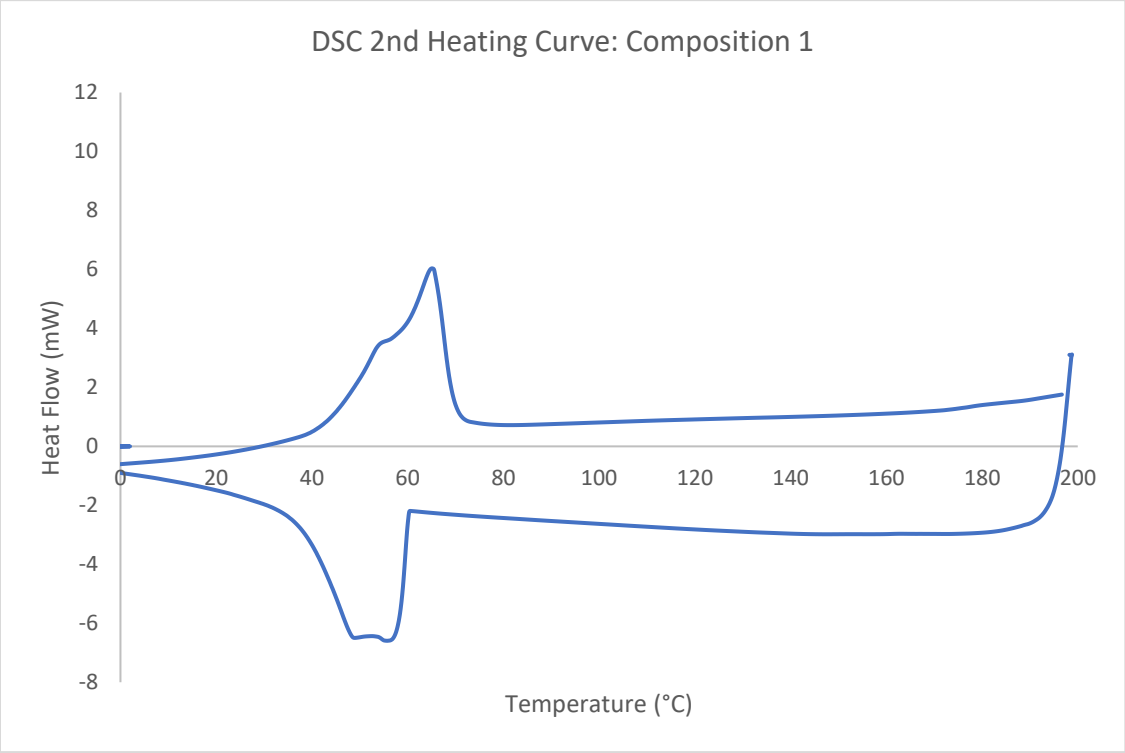

Figure 49. DSC 2nd heating curve, Composition 1, Day 0

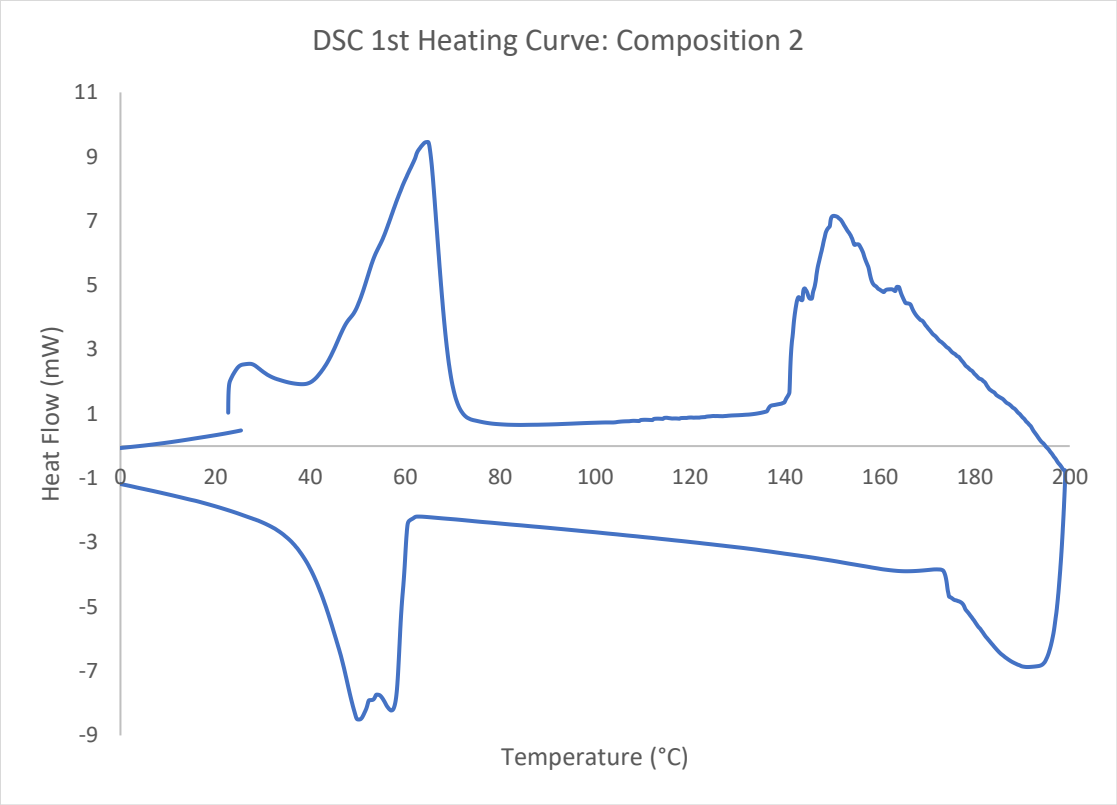

Figure 50. DSC first heating curve, Composition 2, Day 0

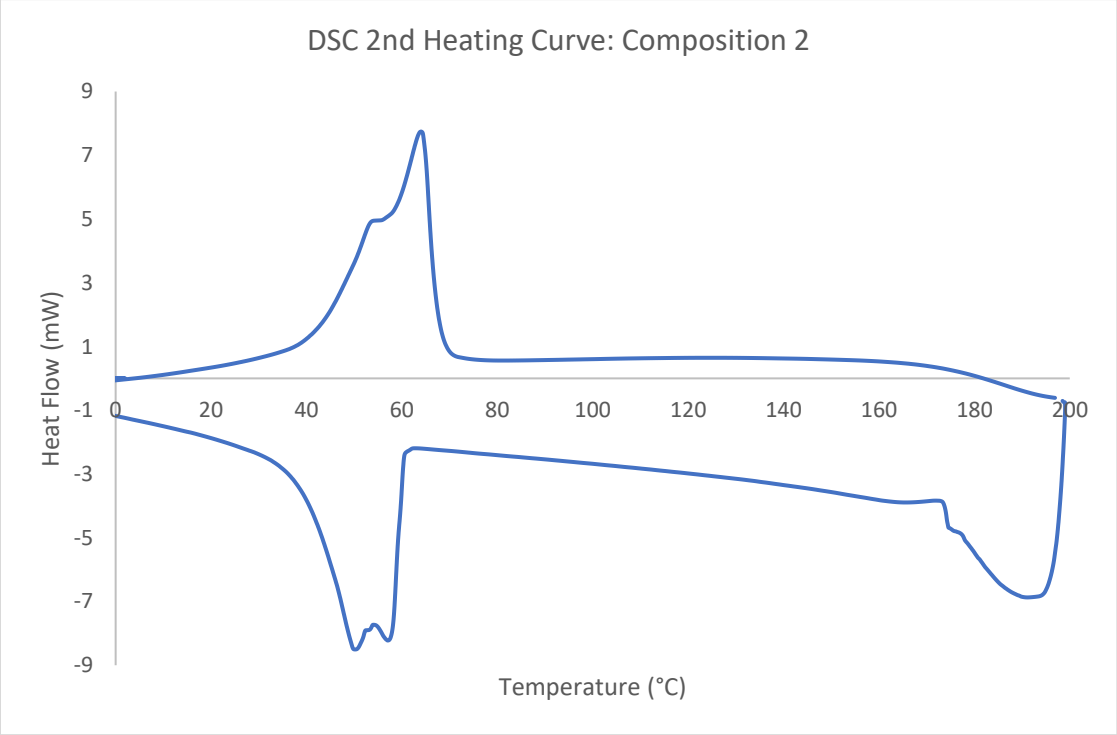

Figure 51. DSC 2nd heating curve, Composition 2, Day 0

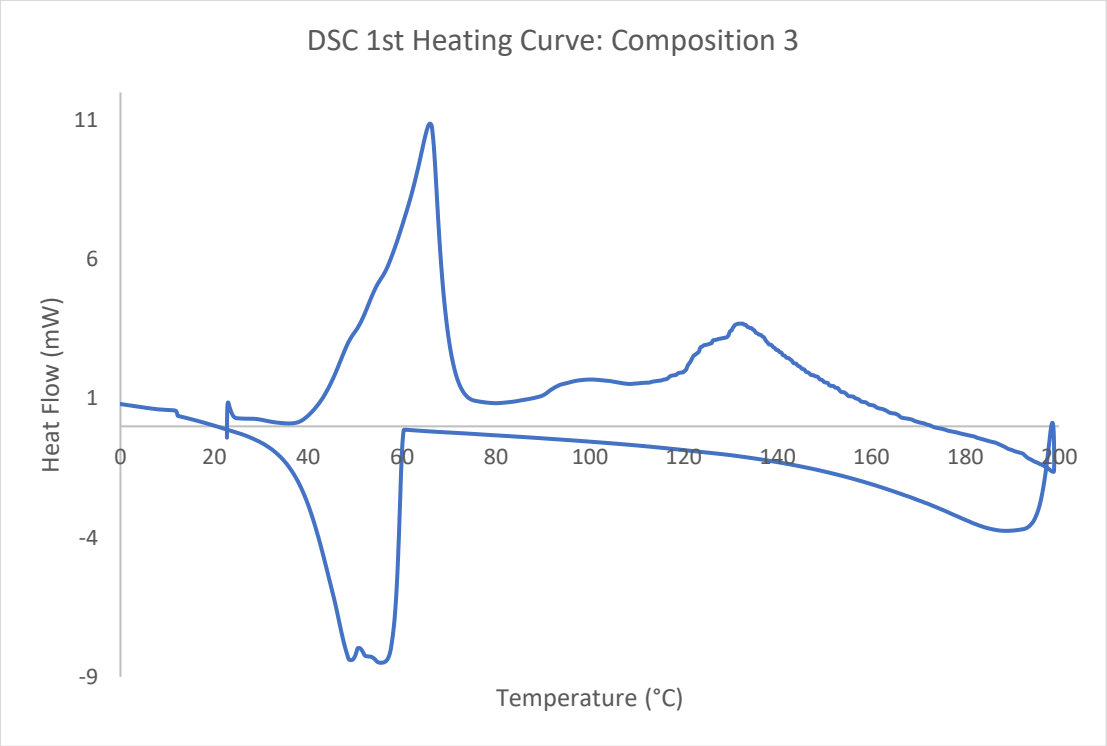

Figure 52. DSC first heating curve, Composition 3, Day 0

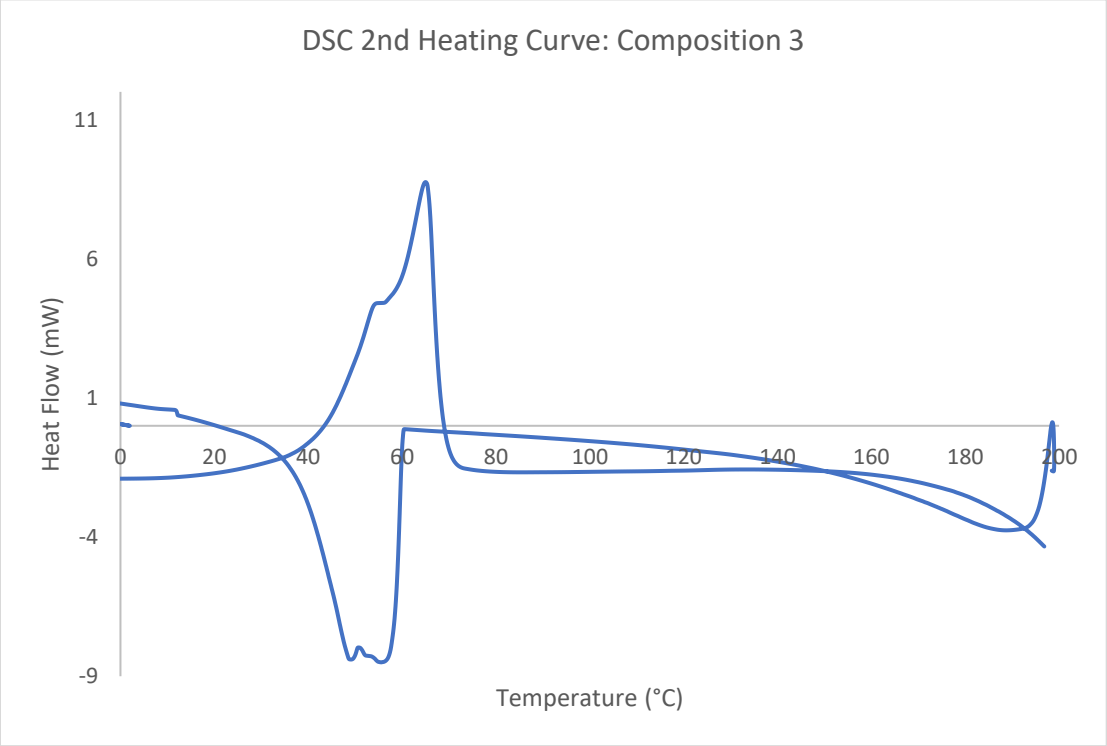

Figure 53. DSC 2nd heating curve, Composition 3, Day 0

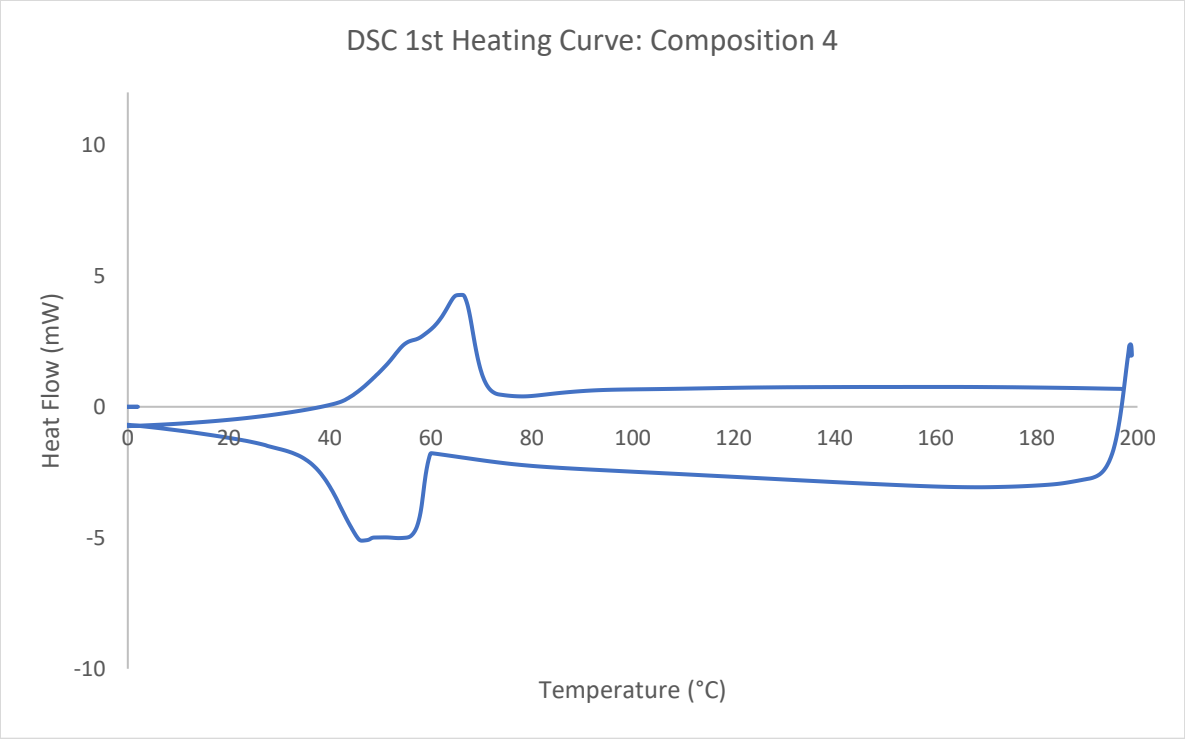

Figure 54. DSC first heating curve, Composition 4, Day 0

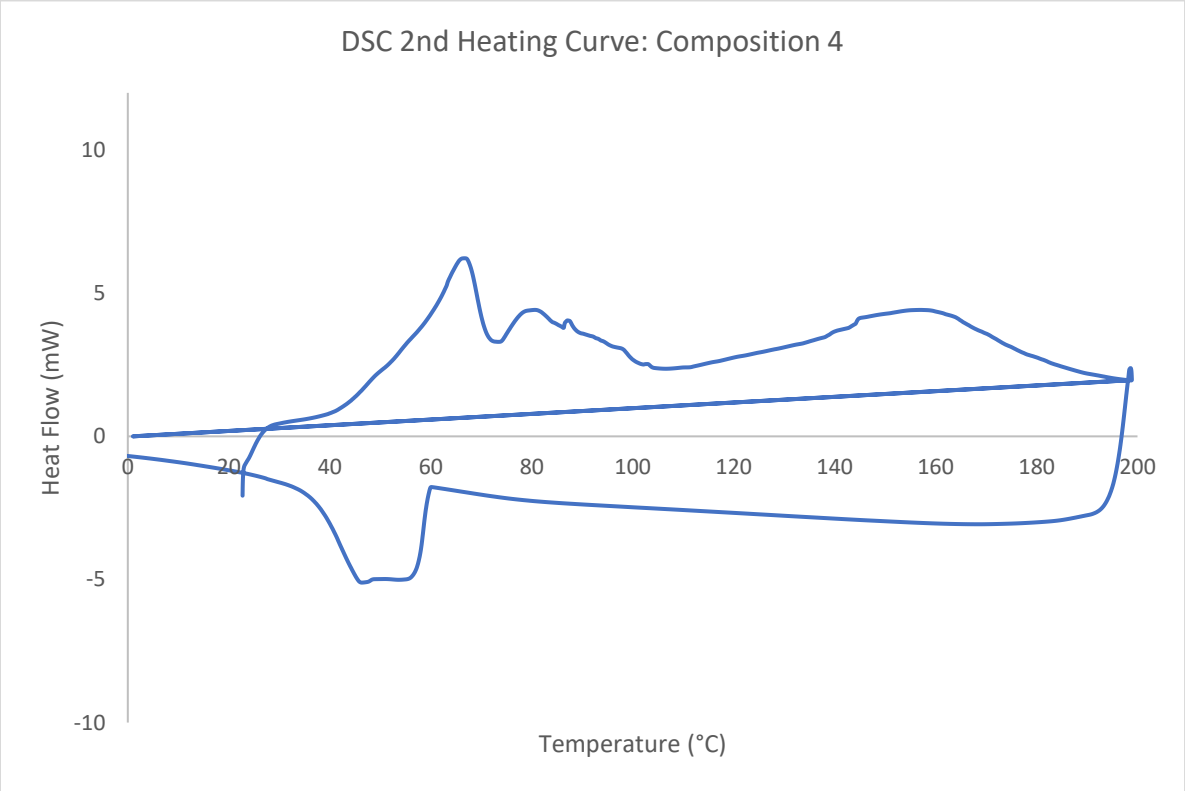

Figure 55. DSC 2nd heating curve, Composition 4, Day 0

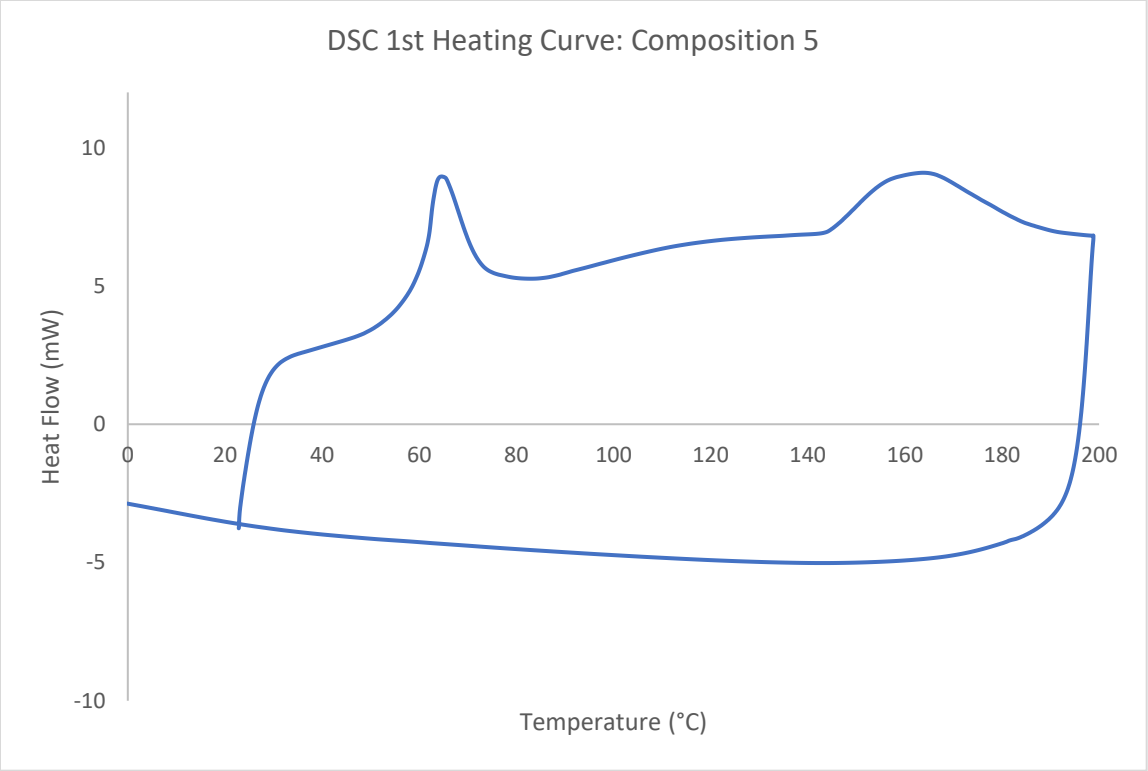

Figure 56. DSC first heating curve, Composition 5, Day 0

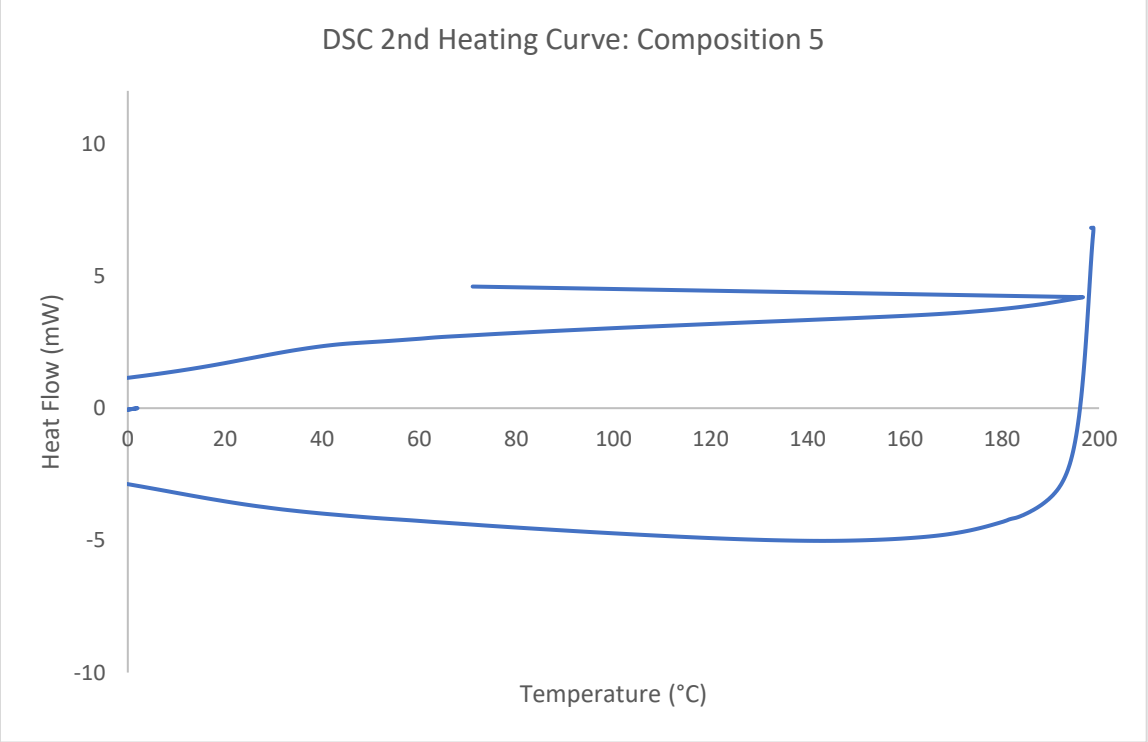

Figure 57. DSC 2nd heating curve, Composition 5, Day 0

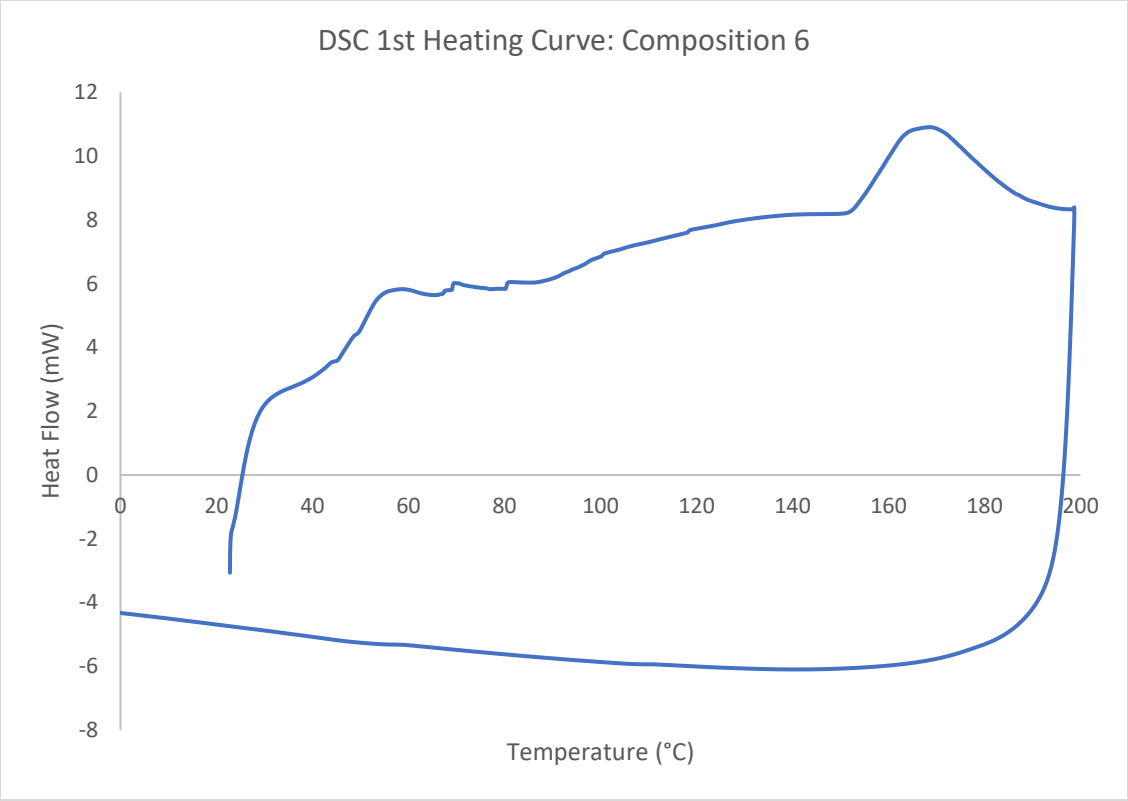

Figure 58. DSC first heating curve, Composition 6, Day 0

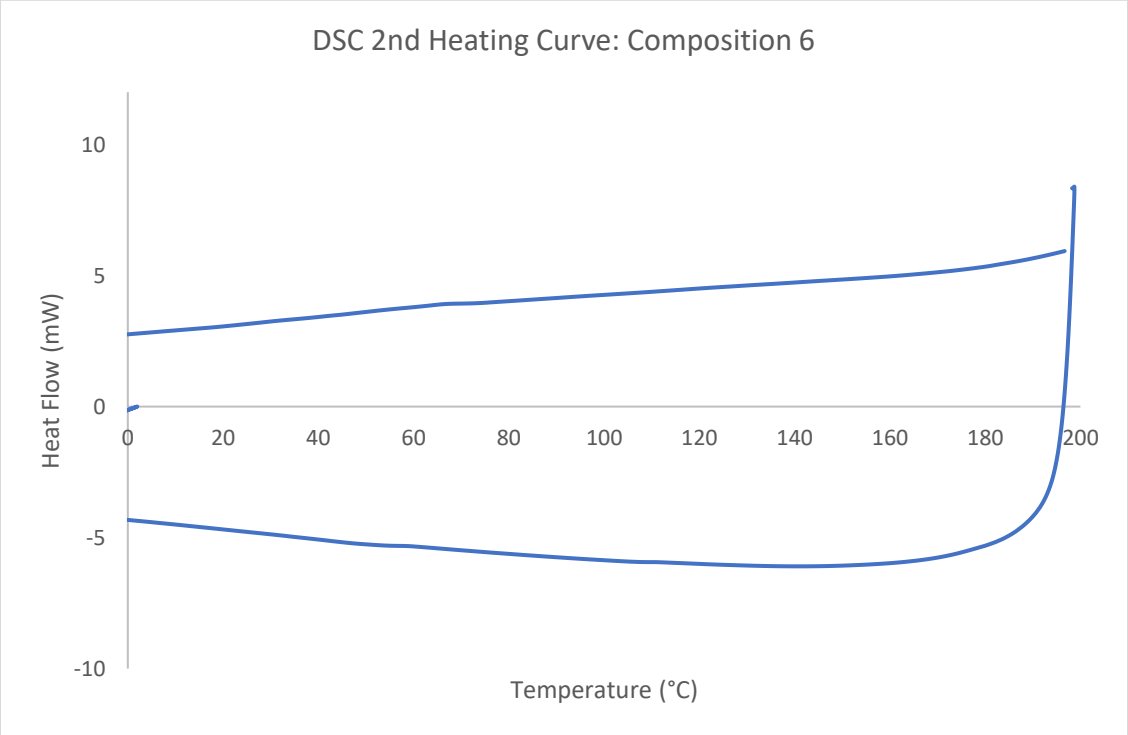

Figure 59. DSC 2nd heating curve, Composition 6, Day 0