ENHANCED DEGRADATION OF POLYLACTIC ACID (PLA) -BASED COMPOSTABLE PLASTICS

By

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ENHANCED DEGRADATION OF POLYLACTIC ACID

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Abstract: Plastics are utilized extensively because of their array of functional properties, but plastic pollution has become a global environmental problem. These concerns have spurred the development of numerous compostable plastics. However, many commercial composters don't want to handle them because their rate of degradation is slower than other typical compost substrates. As a result, most compostable plastics still end up in landfills, where they do not disintegrate.

The overall goal of the project was to develop some pretreatment process to increase the rate of disintegration of compostable plastic. The pretreatment could be applied to compostable plastics at a local level before being taken to a regional commercial compost facility; and hopefully the pretreated plastics would then be a welcome addition to the commercial compost facility. This study was focused on Polylactic acid (PLA) based compostable plastic, because it is the most commonly used. Both biotic and abiotic factors were evaluated to determine their effect on the rate of degradation of compostable plastic. Abiotic pretreatments included soaking the plastic in basic solution, acidic solution, heating the plastic in 75^{o} water, and steam pretreatment. Biotic pretreatments included the use of a spent mushroom compost waste, commercially available compost starter, and compost material from previous batches as inoculum. ISO 20200:2004 standard methodology was used. Experiments were conducted at two different sizes: 6 L vessels and 250 ml flasks.

Use of inoculum and mature compost increased the rate of mycelial growth in the synthetic waste and use of various pretreatments increased the hydrolysis in PLA coupons, making it easier for microbes to degrade, thereby increasing the rate of disintegration. The PLA coupons disintegrated completely in all the treatments and the control, but at different rates. The best performing treatment was a combination of autoclaved plastic and compost starter, which took about 19 days to complete, while the control took about 46 days. Overall, all the treatments were significantly better than control, except mushroom spent waste.

Colorimetric analysis was performed on periodically sampled coupons. A general trend was observed in all the samples, irrespective of the treatment. SEM imaging revealed different surface changes that occurred during the compost process.

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Keywords: PLA, compostable, degradation, spent mushroom, pretreatment, inoculum, colorimetry, SEM

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CHAPTER 1

INTRODUCTION

1.1 Introduction

It is difficult to imagine a world without plastics, which have become an integral part of our lives. The versatile nature of plastic, ease of manufacture and low cost have made it one of the most sought-after materials for construction, packaging, textile, sports, electronics and even aerospace. But plastics come with a price; it is not biodegradable, difficult to recycle, and the low cost makes consumers careless with its use and it is usually discarded after a single use. The discarded plastic ends up in landfills or the ocean where it is a threat to the environment, plants, animals and even to humans. In developing countries, to save space and time it is improperly burned, which releases toxic gases into the atmosphere polluting our air.

Biodegradable and compostable plastics have been developed to reduce the use of conventional plastics and their impact on nature. These plastics, however, come with their own set of problems. The terms bioplastics and compostable have become synonymous with biodegradable and consumers often do not know the difference between them. Consumers are less mindful when they throw away these plastics, thinking they will degrade in a landfill or the ocean. Compostable plastics do not usually degrade naturally. They require specific temperature, pH and high

microbial activity to be maintained to completely breakdown. These conditions are seldom found in nature, landfills, or marine environments, resulting in these plastics being just as bad as conventional plastic. Without proper segregation and disposal, compostable plastics fail to achieve their purpose of being environmentally friendly.

Polylactic acid (PLA) is a commonly used compostable plastic that has met the standard for being 100% compostable. It is marketed and sold as an environmentally friendly alternative to conventional plastic, but PLA does not degrade in natural soil or marine environments; it must be disposed of in a commercial compost facility. Even under composting conditions, the breakdown of PLA is slow compared to other substrates present in a composting pile. When properly disposed in a commercial composting facility, PLA tends to not disintegrate completely and is left behind in the compost as small chunks. This makes the compost undesirable, and for those reasons commercial composting facilities do not want to handle compostable plastics. Consumers are also misled with "green" packaging, and usually tend to throw away PLA with regular trash thinking it will degrade naturally in landfills.

Compostable plastics provide a great opportunity to reduce our plastic waste and reduce the load on landfills and oceans and preserve our natural environment. However, they must be disposed of properly in order to be beneficial. Composting is a safe and environmentally friendly method to reduce plastic waste, as it breaks down compostable plastics into non-toxic compounds and converts the complex organic matter into a commercially useful product. The challenge here is to increase the rate of breakdown of compostable plastics, which will make it easier for commercial facilities to handle these plastics. Some form of pretreatment or using a substrate rich in microbes/enzymes that will help break down PLA faster is needed to make it more desirable for commercial facilities to accept these plastics and dispose of them safely.

1.2 Objectives

In order for compostable plastics to serve as an environmentally friendly alternative to conventional plastics, they need to break down faster during composting. The overall goal of this project is to find some pretreatment strategies for compostable plastics that could aid in their rate of disintegration so that more compostable plastics will be processed in commercial compost facilities. The specific objectives are:

1. To determine whether the use of pretreatments on PLA coupons such as heat, acid, and base will reduce the time taken for complete disintegration under composting conditions.
2. To evaluate the effectiveness of using commercial compost inoculants in breaking down PLA
3. To evaluate the characteristic changes that occur in PLA during composting

1.3 Hypothesis

Our hypothesis is that some form of pretreatment for compostable plastics could increase their rate of degradation, making them a welcome component in commercial composting facilities.

CHAPTER 2

LITERATURE REVIEW

2.1 The Plastic Problem

Plastic use has increased tremendously since it became popular in the 1950s due to its wide range of applications, functional properties, ease of manufacturing, and longevity. Although the term "plastic' is a broad term for synthetic and semi-synthetic material, it usually refers to Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Polyvinylchloride (PVC), Polyethylene terephthalate (PET), and polyurethanes (PUR) (Geyer et al, 2017). None of these plastics biodegrade and remain intact for hundreds of years. From food packaging and electronics to aerospace, plastics have become an integral part of our everyday life. It is estimated that around 3900 million metric tons of plastics were produced between 2002 and 2015, out of which approximately 30% are still in use (Tsakona et al, 2021).

Packaging accounted for about 36% of all plastic usage and 50% of the plastic waste generated, with food packaging contributing to more than a third of all the packaging (Tsakona et al,. 2021 and Ncube et al,. 2021). Out of all the plastic discarded, approximately 79% has ended up in landfills, marine environments, and other terrestrial environments. This has resulted in large garbage patches in the oceans like the great pacific garbage patch. Plastics are lethal to marine life since they are a choking hazard, toxic, block sunlight, and entangle with living organisms reducing their mobility. These plastics also fragment into microplastics and enter the food chain and even make their way into our diet.

Only about 9% of the plastic is collected for recycling and less than 5% is successfully recycled (Ncube et al, 2021). Additives added to plastics like dyes and plasticizers, contaminated food packaging, and segregation of different types of plastic, all pose a difficulty to recycling. Recycling also results in plastics losing their physical properties like strength, making them less desirable compared to virgin material. Around 12% of plastics are incinerated, with or without the generation of electricity. When burned improperly, plastics release many hazardous gases, ash, and persistent organic pollutants. Open burning results in the loss of energy and resources that could otherwise have been recovered by recycling or generating electricity. The huge demand for plastic and the subsequent consequences associated with it led to the development and marketing of bioplastics or bio-based plastics as an alternative to synthetic plastics.

2.2 Bioplastics

The global production capacity of biobased plastics is only about 4 million metric tons, a small fraction compared to 340 million metric tons of synthetic plastic (Geyer et al, 2017). The last decade has seen rapid growth in the production of biobased plastics due to growing awareness of non-biodegradable, fossil-based plastics. Although biobased plastics like celluloid have existed since the late 1800s (Stevens, 2002), they became popular in the late 1980s as an alternate to

fossil-based plastics. They find applications in packaging, automobile, and the agricultural industry. Being biodegradable is certainly an advantage that bioplastics have over conventional plastics. They are also known to generate less greenhouse gas emissions and use fewer fossil fuels for production and have mechanical properties like conventional plastic.

While this may seem like bioplastics solve most of the problems associated with conventional plastics, this is not always the case. It is important to first understand the term bioplastic since the word can be misleading. Bioplastic refers to materials that are either bio-based i.e. plant-based or biodegradable fossil-based plastics. Plant-based bioplastics can further be divided into biodegradable, compostable, and non-biodegradable. Some of the most commonly available bioplastics are plant-based: Polyhydroxlbutyrate (PHA-PHB), Thermoplastic starch (TPL), Polylactic acid (PLA), and polybutylene succinate (PBS), and fossil fuel-based: Polycaprolactone (PCL) (Greene et al, 2014). Consumers mistake the term bioplastics with the term biodegradable and are less concerned while throwing it away. This results in them ending up in landfills and oceans similar to conventional plastics (Adamcova, 2019). While some bioplastics like PHA-PHB and TPL degrade in natural soil and marine environment, others like PLA only degrade under industrial composting conditions. When these plastics end up in landfills and the marine environment they do not degrade, can fragment into microplastics, and even release the toxic additives into the environment during the breakdown process. A plastic bag made of bioplastic poses as much threat as a conventional plastic bag to marine life.

PET bottle recycling is relatively easy, but it is impossible to distinguish a PET and PLA bottle visually. When these plastics end up in a recycling facility, they create a problem since the two materials have different melting points (Alaerts et al, 2018). Most life cycle analyses of bioplastic have focused on CO_2 emissions and a complete life cycle analysis needs to be further explored. While composting is a good solution to most bioplastics, plastic manufacturing companies are taking advantage of the standard for terming plastic as “compostable”. If 90% of the plastic

degrades in 12 weeks at 60°C under composting conditions, the plastic can be designated as compostable. However, in most industrial composting facilities organic waste is composted for a period of 4 weeks. This results in the incomplete disintegration of compostable plastics which can contaminate the final compost material. For these reasons most composting facilities do not handle compostable plastics.

2.3 Polylactic Acid

Polylactic acid (PLA) is a polyester made from a low molecular weight biomolecule, namely lactic acid (Stevens, 2002). It can be processed by already existing methods like extrusion, injection molding, thermoforming, and fiber spinning. It can also be made flexible or rigid and clear or opaque depending on the requirement. It is made by first fermenting starch, usually from corn, into L-lactic acid. Lactic acid can also be produced from petrochemicals, but the method has a high negative environmental impact compared to the fermentation route (Lunt, 1998). It also produces a racemic mixture of L- and D-Lactic acid. The percentage of D-isomer determines the crystallinity of the polymer (Fukushima et al, 2012). PLA can be produced as an amorphous, semi-crystalline, and highly crystalline polymer, which results in a glass transition temperature between 60 and 65°C (Lunt, 1998).

Lactic acid can then be polymerized into PLA by three different processes: Condensation polymerization, Azeotropic dehydrative condensation polymerization, and ring-opening polymerization. Direct condensation polymerization is the simplest method of producing PLA. However, only low and medium molecular weight polymers can be made using this method because not all the water and solvent can be removed. Azeotropic dehydrative condensation removes water by azeotropic distillation and therefore high molecular weight PLA can be produced. Finally, ring-opening polymerization is one of the best methods to produce PLA, developed by Cargill Inc. in 1992 (Drumwright et al, 2000).

Ring-opening polymerization is a solvent-free and continuous process and can therefore produce large quantities of commercial PLA. Figure 2.1 shows the reaction chemistry of PLA production via prepolymer and lactide. The process is both low cost and has environmental benefits since it does not make use of expensive and environmentally unfriendly solvents. This is possible because lactide and high molecular weight PLA are produced in the melt using a tin catalyst instead of using a solvent. The result is a commercially viable process for the production of compostable polymer made from natural resources.

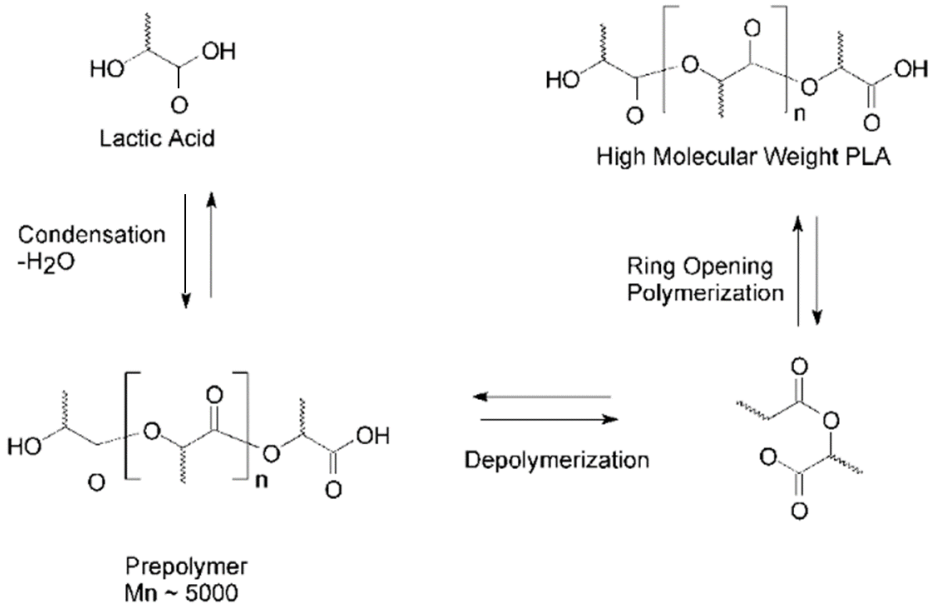


Figure 2.1: Schematic of PLA production via ring-opening polymerization. Adapted from Drumwright et al, 2000.

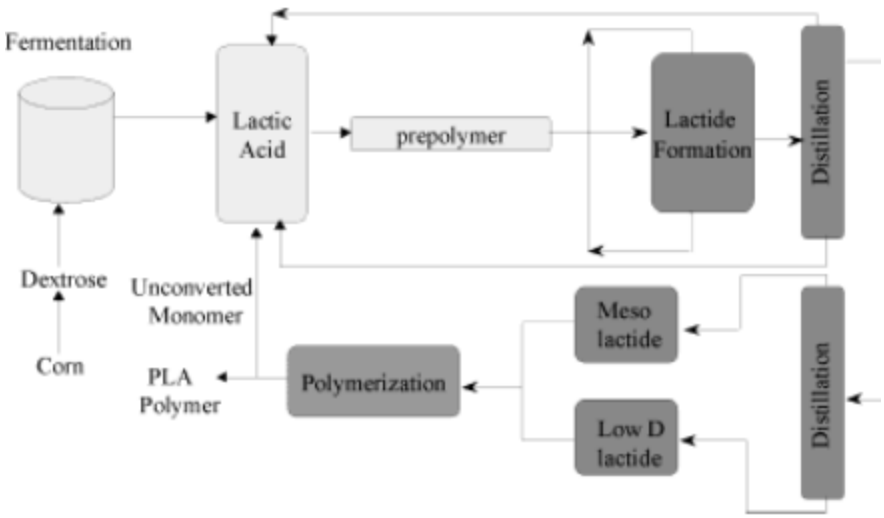


Figure 2.2: Process Flow diagram of PLA production via ring-opening polymerization. Adapted from Drumwright et al, 2000.

Higher molecular weight PLA has higher transition temperature and better mechanical properties. It can have a tensile strength of 50-70 MPa, which is comparable to conventional plastics. Based on the application the type of PLA (amorphous/crystalline, high molecular weight/low molecular weight) can be selected. High crystalline PLA has a higher melting point, is very stiff, and has low permeability and high chemical resistance making it suitable for cutlery, textile fabric, and films. Amorphous and semicrystalline are more susceptible to biodegradation making them suitable for biomedical devices.

PLA has been branded as 100% compostable (Nature works Safety data sheet for product 2003D), but it is, however, not a biodegradable polymer, meaning it does not degrade in natural soil and marine environment. The temperature, pH and bacterial activity in soil is not ideal to decompose PLA. PLA breakdown is a two-stage process. In the first step the abiotic factors, namely temperature, and moisture hydrolyze PLA. PLA is a linear polymer with a carboxylic acid backbone that is susceptible to hydrolysis. With the increase in temperature, the rate of hydrolysis increases. As the temperature nears 60°C, the glass transition temperature, PLA becomes soft and

flexible in the presence of moisture, enhancing the degradation process (Farah et al., 2016). During the hydrolysis process, the molecular weight of PLA reduces rapidly, depending on the initial crystallinity (Gorrasi et al, 2012). This is where natural soil and marine environment fail to degrade PLA, because the soil temperature is usually around 25°C, and the moisture in the soil is low, compared to composting conditions, which is not enough to hydrolyze PLA (Siakeng, et al., 2020). PLA is also insoluble in water making it practically undegradable in marine environment. The second step is microbial degradation. Hydrolysis can reduce the molecular weight of PLA to as low as 10,000 Da, at this point microorganisms can break down PLA. Microorganisms break down PLA into carbon dioxide, water, and biomass. Under composting conditions, the temperature reaches around 55 to 60°C, has a moisture content of around 55% to 60%, and is rich with microorganisms. These conditions make it ideal for breaking down polylactic acid into non-toxic products.

2.4 Hydrolysis of Polylactic Acid

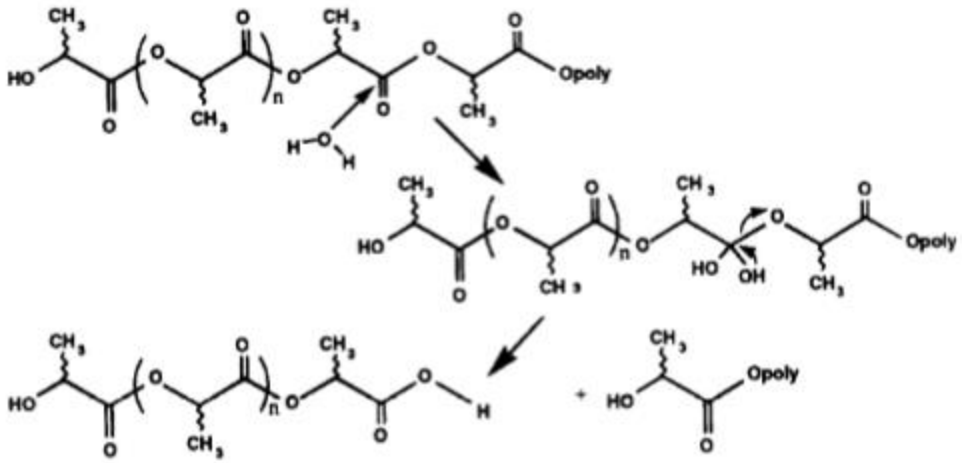


Figure 2.3: Hydrolytic chain scission of PLA. Adapted from Lunt, 1997.

The hydrolysis of PLA has been studied extensively for its medical application (Siepmann et al., 2000). PLA is used for slow release of drug delivery, and in bone regeneration applications (Stevens et al, 2002). PLA is biocompatible and hydrolyses inside the body and the rate of

hydrolysis can also be modified, making it suitable for medical applications like delivering heparin, cancer drug, and as a scaffold for bone repair.

Degradation of PLA starts either through hydrolytic or enzymatic chain scission of the ester bonds to low molecular weight oligomers and monomers as shown in Figure 2.3. Temperature, pH, and moisture content accelerate the rate of hydrolysis, while thermal stabilizing additives tend to reduce the rate of hydrolysis. The chain cleavage proceeds preferentially in the amorphous regions. This leads to an increase in crystallinity. The carboxylic end groups act as a catalyst for the hydrolytic degradation of PLA, making it an autocatalyzed process (Elsawy, 2017). For these reasons, it has been proposed that hydrolysis of PLA follows third-order kinetics since the rate of hydrolysis is dependent on polymer bonds, water, and hydrolysis products (Gironi et al., 2012). The reaction mechanism is as follows:

$$(PLA)_n + LA + H_2O \rightarrow 2LA + (PLA)_{n-1}$$

The hydrolytic degradation of PLA proceeds on the surface or heterogenous erosion and also in the bulk or homogeneous erosion, with homogenous reaction being more predominant for PLA. Heterogenous erosion degradation occurs only on the outermost polymer layers since the polymer degradation is much faster than water intrusion and vice-versa in the case of homogeneous erosion. PLA erodes through both pathways, but homogenous erosion is more predominant for PLA.

The degradation of semicrystalline PLA matrices can be accounted for by two stages. Firstly, random hydrolytic scission of ester bonds proceeds with the diffusion of water into the amorphous regions. The degree of crystallinity increases as the degradation proceeds. In the next step, hydrolytic attack occurs from the edge towards the center of the crystalline domains with the degradation of the major portions of the amorphous area. The lactic acid monomers generated in

the process tend to dissolve in water. In the bulk of the plastic, the lactic acid helps in the self-catalyzed and self-maintaining process of PLA breakdown.

2.5 Composting Process

Composting is a complex process used to break down waste, like vegetable peel, farmyard waste, and other complex organic matter into simple molecules which can then be used by plants as nutrients. It is a solid-state fermentation process where organic matter is broken down into simpler compounds by either microorganism: aerobic and anaerobic, or by earthworm (vermicomposting). Composting is used to transform organic waste into organic manure, thus recycling nitrogen, phosphorous, potassium, and carbon in an economical way (Gao et al., 2009). Composting is usually carried out in thermophilic conditions i.e at elevated temperatures (usually between 54 and 65°C), which eliminates pathogenic microorganisms and therefore total hygienization (Nasreen et al., 2012, Awasthi et al., 2016). Incinerating and landfilling organic matter produces huge quantities of greenhouse gases, and loss of energy, nutrients, and agricultural land. Composting is an environmentally beneficial method of recycling and reducing organic waste. Composting results in humification, which can then be used on agricultural land, thereby reducing the requirement for chemical fertilizers. The quality of organic waste tends to have a better quality than inorganic fertilizers (Onwosi et.al, 2017).

Composting is an oxygen-demanding process in which organic matter is hydrolyzed into humus (Pepe et al., 2013). The physiological activity of different microbes is responsible for the nutrition content and subsequent effect on agricultural productivity. Bacteria such as Stenotrophomonas, Pseudomonas, Achromobacter, etc., can enhance the nitrogen content in the compost and also have the ability to suppress the growth of pathogens by producing antimicrobial compounds and by exuding heat. Composting itself is a three-stage process (Bhatia et al, 2013). The first stage is the mesophilic stage, where mesophilic organisms break down the readily available, soluble

organic matter and produce metabolites which in turn increase the temperature of the compost. The rise in temperature paves the way for the growth of thermophilic microorganisms. These microorganisms have the ability to break down more complex nutrients, namely, polysaccharides, proteins, and fats, enabled by the high temperature (Maheshwari et al., 2000). During the thermophilic stage pathogens and any seeds present in the composting pile are destroyed due to high temperature. In the final stage, after the breakdown of complex organic matter, the compost cools down leading to the growth of the mesophilic bacteria and the compost matures. The final stage is especially important because it stabilizes the compost, making it suitable for plant growth (Bhatia et al, 2013).

Composting is influenced by the following factors: temperature, pH, C/N ratio, oxygen supply, and moisture. Turning frequency affects the distribution of microorganisms, and nutrients and increases oxygenation (Bhave, 2019). While it may seem like more mixing is better, it is, however, counterintuitive to over mix the compost. Overmixing results in bacteria not having enough time to adjust to the nutrients and the environment and time to grow. Not enough mixing will result in compaction of the compost, resulting in oxygen depletion and reduced porosity (Awasthi et al., 2014). Mixing is also essential to maintain the right moisture content. Moisture is lost from the surface and tends to settle at the bottom, leading to a moisture gradient within the compost. Mixing will ensure an even distribution of moisture. It is therefore vital to optimize the mixing regime to ensure an even distribution of nutrients for the good growth of bacteria.

Temperature plays a very important role in composting. Composting is naturally an exothermic process, which depends on the initial temperature, the biodegradability of the substrates, and the presence of a diverse culture of microbes. However, the efficiency of composting decreases with a temperature rise. Very high temperatures can kill both mesophilic and thermophilic bacteria which are responsible for the breakdown of the compost substrates. It has been determined that the optimum temperature for composting is between 54 and 60 °C (Vuorinen et al,.1997).

Although some bacteria and fungi can survive temperatures above 60 °C, the majority of the microbes cannot, resulting in their death. If sufficient temperature is not reached by the initial activity of the mesophilic microbes, then the substrates will not degrade completely. It can also result in the growth of pathogens which can subsequently harm plants and animals which consume these plants.

During the composting process, microbes break down organic compounds into simpler compounds. They do so by consuming and excreting nutrients, namely nitrogen, potassium, phosphorous, and carbon. They break down complex forms of these nutrients making them easily available for plants. Among all the nutrients present in the compositing substrates, carbon and nitrogen play the most important role in the growth of microorganisms. Carbon is the source of energy. Microbes break down complex carbon molecules into simpler organic molecules, carbon dioxide, and water. Nitrogen is used for cell growth. Every protein molecule contains nitrogen, and since proteins are the building blocks of life, nitrogen plays an important role in the growth of microbes. These factors in turn affect the overall stability and maturation of the compost (Guo et al., 2012). For these reasons, the C/N ratio is measured in the compost pile. Excess carbon results in low microbial growth and the composting process will not proceed, while a high nitrogen content results in nitrogen being converted to ammonia gas (Lazcano et al., 2008). Based on this it has been estimated that a C/N ratio between 20:1 and 40:1 is most suitable for composting process.

2.6 Degradation of PLA

The degradation of PLA is affected by both biotic and abiotic factors (Nampoothiri., 2010). Studies have been conducted on the disintegration of PLA in soil and composting conditions at different temperatures. While some studies have stated that PLA does degrade in natural soil and marine environments (Stevens, 2002), others have shown that there is very little degradation in

PLA even after being buried in soil for a year at 25°C and 30°C (Karamanlioglu, 2013). Coupons buried at temperatures above 45 °C have been reported to turn opaque in soil and compost, while at 25 °C PLA remained transparent. Loss in tensile strength of PLA coupons was also observed only at temperatures above 45 °C. Under composting conditions, PLA has been reported to degrade completely after 45 to 60 days at 50 to 60 °C (Karamanlioglu, 2013). Some studies have also shown more than 90% reduction in mass after 28 days under composting conditions (Arrieta et al., 2013). Tensile strength of coupons recovered from soil and compost at 45 °C have been reported to be significantly different. At 45 °C weight loss of more than 20% was observed in coupons buried in compost just after 21 days, while it took about 8 weeks in soil (Al et al., 2019). Fungal activity on the surface of PLA coupons has also been reported in samples buried in compost above 45 °C. Temperature and higher microbial activity (found in compost) is therefore necessary for quick degradation of PLA coupons.

2.7 Design of Reactor

Solid particles behave differently compared to fluids. While fluids are mixed using molecular diffusion and by inducing turbulent flow, solids tend to segregate. Segregation can be induced by vibration and during flow in a cylindrical drum. Segregation usually occurs due to dissimilarity in shape, size, and density, with larger and lighter particles moving to the top and smaller and denser particles settling at the bottom. Segregation can be both useful and undesirable. In the case of ores, segregation has a positive application. But in cases like composting, pharmaceutical field, and polymers, it can have a negative impact (Yu et al., 2015). In the case of composting, it is important that all the nutrients are evenly mixed for the best growth of microorganisms. Improper distribution of nutrients can lead to slower growth and a longer time for a complete breakdown of compostable waste. For effective mixing, traditionally baffles, set at the outer periphery of the drum, have been used. However, Shi et al. (2007) have shown that axially located baffles have a better quality of mixing. In their study, Jiang et al. (2010), studied the effect of "+","-","*"

shaped baffles versus drum mixers without baffles. The particles that were being mixed were 1.5 mm and 3 mm in diameter and the volume ratio of the two particles was 1:1. The effect of the length of the baffles was also studied.

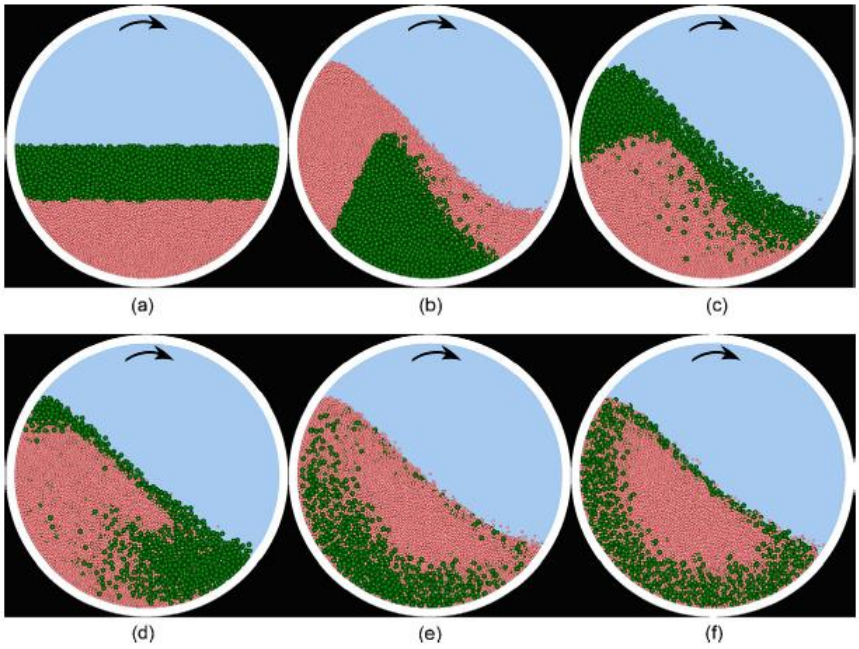


Figure 2.4: Snapshots of mixing process of binary particles in the drum without any internal baffle, with the larger (smaller) particles drawn in dark (light) color: (a) t=0s, (b) t=2s, (c) t=4s, (d) t=8s, (e) t = 16 s and (f) t = 100 s. Adapted from Jiang et al, 2011

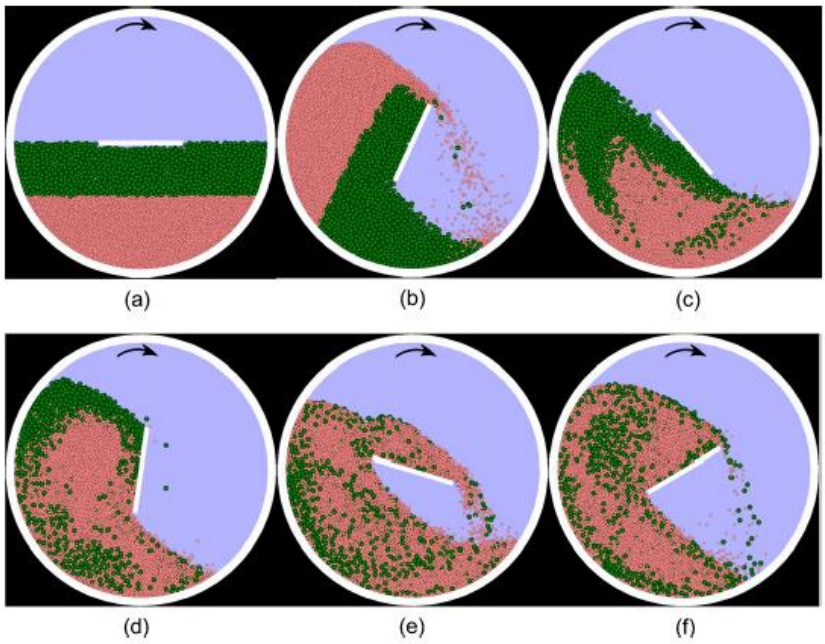


Figure 2.5: Snapshots of mixing process of binary particles in the drum with "-" baffle, with the larger (smaller) particles drawn in dark (light) color: (a) t=0s, (b) t=2s, (c) t=4s, (d) t=8s, (e) t = 16 s and (f) t = 100 s. Adapted from Jiang et al, 2011

In the first simulation, binary particles are mixed without any baffles as shown in Figure 2.4. Initially, the particles are incomplete segregation and eventually arrive at a stage of mixing after 100s. Due to the absence of baffle and difference in particle size, segregation of particles can be seen and the particles are still not homogenously mixed. To improve particle mixing, a "-" shaped baffle has been fitted as shown in Figure 2.5, such that the baffle rotates with the drum. In this case, however, it can be seen that at the end of the 100s the particles have reached a state of homogeneity. Mixing in both baffled and unbaffled drums is affected by mechanisms of particle segregation, but segregation has been avoided in the case of a baffled drum. Size segregation phenomenon occurs in rapid free surface flow, and the "-" baffle greatly reduces the free surface flow and ultimately enhances overall interparticle mixing. Based on the study it was determined that a baffle of any shape and length is much better than a drum with no baffle. To quantitatively describe the mixing degree of binary mixtures in a rotating drum, the Lacey index is used. In

practical terms, the Lacey mixing index is the ratio of ‘mixing achieved’ to ‘mixing possible’. A Lacey mixing index of zero would represent complete segregation and a value of unit would represent a completely random mixture. Practical values of this mixing index are found to lie in the range of 0.75 to 1.0. In the case of the unbaffled rotating drum, the lacey index was found to be around 0.5, a very poor value. In the case of the “-“ shaped baffle, the length of the baffle was found not to significantly affect the value of the Lacey index. However, the maximum value was obtained for an L/D ratio of 1/3, where L is the length of the baffle and D is the diameter of the drum.

Apart from the presence of the baffle, the speed of rotation also affects effective mixing. If the speed of rotation is too slow, then the solids tend to slip and slide at the bottom of the drum, resulting in no mixing (Timoshenko et al., 1951). If the speed is too fast, centrifugal forces come into effect the solids stick to the inner surface of the drum, also resulting in improper mixing. To have effective mixing rolling and cascading of particles in a rotating drum is necessary. The Froude number specifies the tendency of the particles to roll and cascade during mixing in a rotating drum. The Froude number is given below:

$$Fr = \frac{\omega^2 R}{g}$$

Where ω is the angular velocity, R is the radius of the drum and g is the acceleration due to gravity. A Froud number between 0.001 and 0.1 is recommended for effective mixing.

2.8 Colorimeter

A colorimeter is an instrument used to quantify the color of a substance. It works on the principle of Beer-Lambert’s law, where the amount of light absorbed by a substance is measured (Kumar et al., 2018). Sample is placed above the detector and a light of visible range (400-800 nm) is passed

through the sample. Based on the absorption, the L*, a* and b* values are obtained. The International Commission on Illumination expresses color as three values: L* for lightness, which has a value from 0 to 100, a* for red (+ value) to green (- value) and b* for blue to yellow (Mazur et al., 2022). Colorimeter is extensively used in the food industry to test fruits, vegetables and meat. During the composting process PLA undergoes color changes from transparent to opaque and then yellow.

2.9 Scanning Electron Microscope

In a scanning electron microscope accelerated electron beam is used to scan the surface topography and morphology. SEM is capable of achieving a detailed visual image of a particle with high quality and special resolution of 1 nm and magnification of up to 300,000 x. (Akhtar, 2018) A typical SEM has a very powerful electron gun which emits an electron beam. This beam is then focused on the sample using a lens. When the beam hits the surface of the sample, signals in the form of electrons and X-rays are generated. These signals are picked up by the detector which then produces the surface image of the sample.

SEM is a very useful tool and has a number of applications, especially in the nanotechnology field. In the polymer field it is important to assess the morphology of cross-section fractures since they are closely related to the mechanical properties of polymer (Mazur et al., 2022). SEM images of PLA after composting has revealed cavities and pinholes and growth of biofilm on the surface of the sample (Stloukal et al., 2014, Brdl et al., 2021) SEM is also used extensively to study the shape and structures of microorganisms in situations where the magnification of optical microscopes is not sufficient (Golding et al, 2016).

CHAPTER 3

METHODOLOGY

The ISO 20200:2004, titled “Plastics- Determination of the degree of disintegration of plastic materials under simulated composting conditions in a laboratory-scale test” is the standard that was followed throughout the study, with some modifications detailed in this report (ISO 20200:2004, 2004).

3.1 Preparation of Wet Synthetic Waste

To simulate good compost substrates, sawdust, rabbit feed, mature compost, corn starch, sugar, corn seed oil, and urea made up the “wet synthetic waste”. These substrates provide all the necessary nutrients required for microbial growth. Sawdust is used as a bulking agent and was sifted through 2000 micron mesh to remove large objects like wood chips. The rest of the ingredients provided the required nutrients for a well-balanced composting mixture. Well aerated peat humus compost was used as inoculum. The inoculum was sifted on a screen mesh of 4000 microns to remove any inert objects like glass, stones, or pieces of metal. Wet synthetic waste was prepared by manually mixing the different components listed in Table 3.1.

Table 3.1: List of material in wet synthetic waste expressed as dry percentage weight.

Material	Percentage Dry Weight (%)	Source
Sawdust	40	Gooch sawmill LLC
Rabbit feed	30	Small world® Rabbit feed
Mature compost	10	Timberline® Peat hummus
Corn starch	10	Agro® Corn starch
Sugar	5	Great value® granulated sugar
Corn seed oil	4	Great value® cornseed oil
Urea	1	Scotts® Turf Builder
Compost starter	NA	Jobe Organics

All the materials listed in Table 3.1 except sawdust were bought at the local Walmart®. Chlorine-free tap water was added to bring the moisture content to 55%. The above mixture served as the control for all the trials in the experiment. Sawdust was replaced with mushroom spent waste as one of the treatments.

Compost starter was added as a treatment to increase the concentration of microbes in the wet synthetic waste. Table 3.2 is a list of bacterial and fungi in Jobe's Organic compost starter.

Table 3.2: List of bacteria and fungi in Jobe's Organics Compost starter.

Bacteria	Fungi
Arthrobacter globiformis	*Glomus intraradices*
Azotobacter chroococcum	*Glomus aggregatum*
Azospirillum lipoferum	*Glomus etunicatum*
Pseudomonas fluorescens	*Glomus mosseae*

3.2 Measurement of Moisture Content

The moisture content of each of the ingredients in the synthetic waste was determined. Each ingredient was carefully sampled from its container and weighed. It was then placed in an air convection oven set at 105°C (Govett et al., 2010). Weight was monitored until a constant weight was observed. Dry weight was then calculated using the formula below:

$$Dry\ \% = \frac{(W_i - W_b) - (W_f - W_b)}{(W_i - W_b)} \times 100$$

$$MC = 1 - Dry\%$$

Where,

W_i is the initial weight of the sample

W_b is the weight of the weighing boat

W_f is the final weight of the sample

MC is the moisture content expressed as a percentage

The moisture content of each of the ingredients in the synthetic waste is shown in Table 3.3 The final moisture content of the wet synthetic waste was also measured using the same procedure.

Table 3.3: Moisture content of each of the components in the wet synthetic waste.

	Component	Moisture Content, Trial 1 (%)	Moisture Content, Trial 2 (%)	Moisture Content, Trial 3 (%)	Moisture Content, Trial 4 (%)	Moisture Content, Trial 5 (%)	Moisture Content, Trial 6 (%)
1	Sawdust	20	20	30	20	20	30
2	Rabbit Feed	4	4	4	4	4	4
3	Mature Compost	12	5	0	0	0	0
4	Corn Starch	17	17	17	17	17	17
5	Sugar	0	0	0	0	0	0
6	Urea	0	0	0	0	0	0
7	Mushroom Spent Waste	0	0	0	0	0	0

3.3 Experimental Setup

The tests were performed on two different scales. The majority of the trials were performed on a small scale in 250 mL conical flasks as shown in Figure 3.1 a. A large batch (~2500 kg) of wet synthetic waste was prepared as per the standard. Out of this, 75 g was carefully weighed and placed in each flask, and each small scale trial consisted of 30 flasks (10 treatments x 3 replicates of each). PLA sheet was cut into 20x 20 mm size pieces. 10 pieces, weighing approximately 1.2 g total, was accurately weighed and buried in the wet synthetic waste. The flask was then closed with a rubber stopper with air vents to maintain aerobic conditions.

Large-scale composting was performed in 6 L Polypropylene cylindrical bins as shown in Figure 3.1 b. A 6.5 cm width by 20 cm length rectangular piece was cut from a PVC sheet to make the baffle. This was then mounted on a PVC pipe which acted as the shaft. Holes were drilled at the bottom of the bin and the cap to fit the shaft. PVC shaft along with the baffle was glued to the

bottom of the bin. Two holes were drilled on the side of the reactor for ventilation. One kg of wet synthetic waste was individually prepared for each of the composting bins numbered 1 to 12. PLA was cut into 25 x 25 x 0.032 mm pieces. 26 pieces weighing approximately 5 g in total, were accurately weighed and buried in 1 kg of wet synthetic waste. The total weight of the reactor was then weighed.

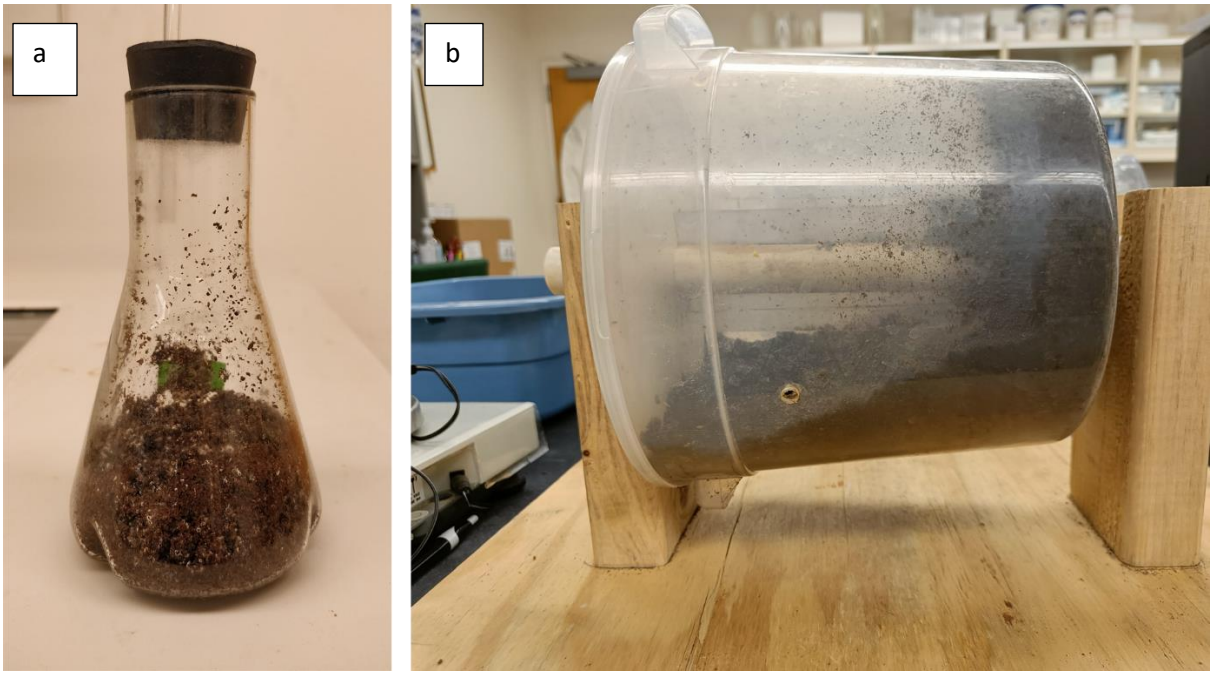


Figure 3.1: Experimental setup in a) Small scale 250 ml flasks, b) Large scale 6 L bins

3.4 Plastic Preparation and Pretreatment

3.4.1 Preparing PLA Coupons

PLA clear containers with lids were obtained from EcoProducts (16 oz container EP-RC16), and small coupons were cut from the lids for use in composting experiments. The thickness of the lid was measured to be 0.032 mm using a vernier calipers. Squares measuring 25 mm x 25 mm, 20 mm x 20 mm and 15 mm x 15 mm were carefully marked on the surface and cut to form coupons

which were used in large bins, small flasks and small flask treatments with smaller sized coupons respectively.

3.4.2 Hot Water

Plastic pieces were cut to the desired dimensions. The water bath was set at 70°C or 75°C and a beaker containing 200 mL of water was placed in the water bath. Once the desired temperature was reached, plastic pieces were carefully placed in the water to prevent them from sticking together. It was allowed to cook in the water bath for 2 h or 4 h, depending on the treatment.

3.4.3 Basic Solution

20% NaOH solution was prepared by dissolving 10 g of NaOH pellets in 50 mL of distilled water. This was then carefully added to 200 mL water to increase the pH to 10. Plastic pieces were then soaked in the solution for 2 h.

For treatments involving heating, 200 mL of 10 pH solution was prepared using the above procedure. It was then placed in a water bath set at 75 °C. Plastic pieces were then added to the solution and allowed to cook for 2 h.

3.4.4 Acidic Solution

10 mL of 97% HCl was diluted to 20% solution by dissolving it in 90 mL of water. This solution was then used to bring down the pH of 200 mL of water to 4 pH. Plastic pieces were then placed in the solution for 2 h.

For treatments involving heating, 200 mL of 4 pH solution was prepared using the above procedure. It was then placed in a water bath set at 75°C. Plastic pieces were then carefully dropped into the beaker and allowed to cool for 2h.

3.4.5 Food Steamer

Water was poured into the food steamer bowl and the steamer was set to the rice option (Temperature approximately 100°C). Plastic pieces were placed in the plastic steamer tray and placed above the water bath after steam was observed. Plastic pieces were removed after 1 hr. In trial 6, treatment 11, plastic pieces were removed after 5 minutes.

3.4.6 Autoclave

Plastic pieces were placed on an autoclave safe tray and placed inside the autoclave. The autoclave was set to the gravity option (34 minute cycle, 121°C) and plastic pieces were removed after the completion of the cycle.

3.5 Treatments

3.5.1 Trial 1

Trial 1 was conducted in small flasks, with 75 g of wet synthetic waste. Synthetic waste was prepared as per the formula recommended by the ISO standard which included sawdust, rabbit feed, mature compost, corn starch, sugar, cornseed oil and urea. The control consisted of untreated PLA coupons embedded in wet synthetic waste. The effect of temperature and time on effectively breaking down the plastic was studied by pretreating the plastic at two different temperatures, 70° and 75° C for 2 and 4 hours. Two different types of compost starters were used to increase the rate of mycelial growth, and PLA coupons of smaller sizes were used to test the effect of size on the rate of disintegration. Three replicates of each treatment were conducted. All treatments in trial 1 are listed in Table 3.4

Table 3.4: Treatments in Trial 1.

Treatment

Control
Sample treated with water at 70 °C for 2 hours
Sample treated with water at 70 °C for 4 hours
Sample treated with water at 75 °C for 2 hours
Sample treated with sodium hydroxide solution, pH 10, for 2 hours
Sample treated with hydrochloric acid, pH 4, for 2 hours
5 g of compost accelerator added to the synthetic waste
5 g of compost starter added to the synthetic waste
5 g of mature compost from the previous batch added to the synthetic waste
Size of the PLA coupons changed to 15 mm x 15 mm

3.5.2 Trial 2

Trial 2 also consisted of 10 treatments in the small flasks. Since both the compost starter and several pretreatments worked well in trial 1, some of the new treatments consisted of combinations of the compost starter and the pretreatments. A new treatment included the use of spent mushroom waste to replace some of the sawdust (40% dry mass). The mushroom waste was generated during the growth of oyster mushroom. After the mushrooms were harvested, the left over material was air dried and stored. All treatments evaluated in trial 2 are shown in Table 3.5.

Table 3.5: Treatments in Trial 2.

Treatment
Control
Mushroom spent waste

Mushroom spent waste and 5 g of compost starter added to the synthetic waste
Mushroom spent waste and Sample treated with water at 75°C for 2 hours
5 g of compost starter added to synthetic waste and sample treated with base, pH 10, and heated at 75°C for 2 hours
5 g compost starter added to synthetic waste and sample treated with acid, pH 4 and heated 75°C for 2 hours
5 g compost starter added to synthetic waste and sample heated in water at 75°C for 2 hours
5 g of mature compost from the previous batch added to the synthetic waste
5 g of mature compost from the previous batch and 5 g of compost starter were added to the synthetic waste
5 g of mature compost from the previous batch was added to synthetic waste and the size of the PLA coupons changed to 15 mm x 15 mm

3.5.3 Trial 3

Trial 3 was performed in large composting bins to test the effect of scale on the time taken to compost PLA coupons. The best-performing treatments from the small flasks were selected and evaluated in the larger bins. All treatments were performed in triplicate. All treatments in trial 3 are listed in Table 3.6

Table 3.6: Treatments in Trial 3.

Treatment
Control
Compost starter added to synthetic waste and sample treated with base, pH 10
Compost starter added to synthetic waste and sample heated in water at 75°C for 2 hours
Compost starter added to the synthetic waste

3.5.4 Trial 4

Trial 4 consisted of 10 treatments (with 3 replicates of each) in the small flasks. From the previous trials, heat had a very good effect on the hydrolysis; hence a food steamer, which had a temperature of about 100 °C, and an autoclave, which had a temperature of about 121°C were both evaluated in an attempt to hydrolyze the plastic to the maximum extent. Combinations of various treatments were also evaluated. The concentration of the mature compost was also increased to check the effect of more mature compost on the breakdown of PLA. All treatments evaluated in trial 4 are shown in Table 3.7.

Table 3.7: Treatments in Trial 4.

Treatment
Control
5 g of compost starter added to the synthetic waste
5 g of mature compost from the previous batch
5 g of compost starter added to synthetic waste and sample treated in a food steamer
5 g of compost starter added to synthetic waste and sample treated in autoclave
50% mature compost and 50% synthetic waste
5 g of mature compost from the previous batch was added to synthetic waste, size of the PLA coupons was changed to 15 mm x 15 mm and heated in water at 75°C
50% mature compost from a previous batch and 50% synthetic waste
Mushroom spent waste and 5 g of compost starter added to the synthetic waste
5g of the previous mushroom spent compost

3.5.5 Trial 5

Trial 5 consisted of 4 treatments (and 3 replicates of each) in the large scale bins. Since the autoclave treatments worked well in the previous trial, the goal of this one was to evaluate the autoclave treatments in the larger scale process. Table 3.8 shows the treatments in trial 5.

Table 3.8: Treatments in Trial 5.

Treatment
Control
Sample treated in autoclave
Compost starter added to synthetic waste and sample treated in autoclave
Compost starter was added to the synthetic waste and the sample was treated with hot water at 75°C for 1 hour

3.5.6 Trial 6

The best performing pretreatments were repeated in trial 6. These included autoclave, food steamer and hot water. Compost starter and mature compost was added in treatments to make a good comparison. In treatment 11, PLA coupons were heated only for 5 minutes to see if same results could be obtained with lesser time.

Table 3.9: Treatments in Trial 6.

Treatment
Control
5g of compost starter added to the synthetic waste
5 g of previous mature compost added to the synthetic waste
Sample treated for 1 hr in the food steamer
5 g of compost starter added to synthetic waste and sample treated for 1 hr in a food steamer
5 g of previous mature compost added to synthetic waste and sample treated for 1 hr in a food steamer
Sample treated in autoclave
5 g of compost starter added to synthetic waste and Sample treated in autoclave
5 g of mature compost added to synthetic waste and Sample treated in autoclave
5 g of compost starter added to synthetic waste and sample treated for 1 hr in hot water at 75°C
5 g of compost starter added to synthetic waste and sample treated for 5 min in a food steamer

3.6 Composting Procedure

For the large scale tests, bins were placed in an air convection oven and the temperature was set at 58 °C. The airflow rate was adjusted to prevent excessive loss of moisture. A tray of water was

placed inside the air convection oven to maintain humidity (Ghorpade et al., 1999). Each reactor was removed periodically (according to standard), and the weight was recorded. Water was added to restore the weight to the initial mass. The synthetic waste was then mixed thoroughly and placed back in the air convection oven. Table 3.10 gives a detailed description of the specific day the operations were performed.

Table 3.10: Schedule of watering and mixing as prescribed by ISO standard.

Time from start (days)	Operation
0	Recorded initial mass of the reactor
1,2,3,4,7,9,11,14	Reactor was weighed and water added to restore initial mass. Compost material mixed.
8,10,16,18,21,23,25,28	Reactor was weighed and water added to restore initial mass. Compost material was not mixed.
30-45	Reactor was weighed and water added to restore 80% of initial mass. Compost material was mixed.
45-60	Reactor was weighed and water added to restore 80% of initial mass. Compost material was not mixed.
60 onward	Reactor was weighed and water added to restore 80% of initial mass. Compost material was mixed.

The mixing and watering regime listed in Table 3.10 was carefully followed. In the large-scale experiment, mixing was performed by rotating the drum for 50 rotations at approximately 2 rotations per second to satisfy the Froude number requirement. In the smaller scale experiments, flasks were placed into a floor incubator set at 58°C for composting. For mixing, the compost was mixed carefully with a glass stirring rod, without mechanically damaging the plastic pieces. For both small- and large-scale experiments, loss in weight was monitored and the appropriate amount of water was added before placing it back in the air convection oven or incubator.

3.7 End of Composting

End of composting was reached when pieces of plastic were no longer visible. Once the plastic had completely disintegrated, the lid of the reactor was removed, and the compost was dried in the air circulation oven at 58 °C. Once completely dry, lumps were carefully broken up and the

compost was sieved using standard sieves, to confirm complete disintegration. It was first sieved through a 4000 micron sieve and subsequently through a 2000 micron sieve. In all our trials none of the plastic pieces remained on the sieve.

3.8 Calculation of Degree of Disintegration

If any plastic material was recovered from sieving it was to be considered non-composted material. The material which passed through the sieves is considered disintegrated. The degree of disintegration D is calculated as a percentage of the original weight of the test material using the equation:

$$D = \frac{m_i - m_f}{m_i} \times 100$$

Where,

m_i is the initial dry mass of the test material

m_f is the dry mass of the residual test material recovered by sieving

In all our treatments and control all plastics broke down completely. Hence the degree of disintegration in all the cases was 100%.

3.9 pH

The initial and final pH of the compost was measured. Two g of the wet synthetic waste was accurately weighed and placed in a beaker, and 10 mL of distilled water was added and stirred well. The pH of the slurry was measured using a pH meter and recorded.

3.10 C/N Ratio

Samples were submitted to The Soil, Water and Forage Analytical Laboratory (SWFAL) at Oklahoma State University for carbon and nitrogen analysis. C/N ratio was measured using a Leco carbon/nitrogen analyzer. The sample is first burned in a furnace at 950 °C. The gases are collected and passed through a secondary burner at 850°C which oxidizes the gases completely. Moisture is then removed using a filter. The gases are passed through the infrared detector to determine the CO2 concentration. The gases are then passed through a hot copper tube to convert NOx to N2. A thermal conductivity cell is then used to determine the nitrogen content. A list of treatments and their C/N ratio is shown in Table 3.11.

Table 3.11: C/N ratio of different treatments.

Treatment	C/N ratio
Wet synthetic waste	29:1
Wet synthetic waste with compost starter	20:1
Mushroom spent waste	14:1
Mushroom spent waste with compost starter	12:1

3.11 Determination of Dry Mass and Volatile Solids

The dry mass of individual components (sawdust, rabbit feed, mature compost, etc) was determined by weighing a small sample and drying it in an air convection oven at 105°C. It was then removed, weighed, and placed back in the air convection oven and allowed to dry until constant mass was reached. The same procedure was repeated for wet synthetic waste before and after the composting process. Once the wet synthetic waste was dried, volatile solids were determined by calcination. The dry sample was accurately weighed and placed in a muffle furnace and the temperature was set to 550°C for 6 to 8 hours. The sample was removed and

placed in a desiccator until it cooled down. It was then weighed, and the mass was noted. The decrease in volatile solids was then determined using the below formula:

$$R = \frac{\{m_i \times (DM)_i \times (VS)_i\} - \{m_f \times (DM)_f \times (VS)_f\}}{\{m_i \times (DM)_i \times (VS)_i\}}$$

Where,

m_i and m_f are initial and final mass of the synthetic waste, respectivel

$(DM)_i$ and $(DM)_f$ are initial and final dry mass of the synthetic waste, expressed as a percentage

$(VS)_i$ and $(VS)_f$ are initial and final volatile solids content of synthetic waste, expressed as a percentage

3.12 Statistical Analysis

Analysis of variance (one-way ANOVA) and Tukey's Honestly Significant Difference test was performed on Microsoft Excel, to evaluate the statistical significance of any change in time taken for complete disintegration of PLA coupons by different treatments in comparison to the control. The significance threshold was set at α=0.05.

For the Tukey Honestly Significant Difference Q value was obtained from the Q table at α=0.05. Critical value was then calculated from the Q value using the below formula

$$C.V. = Q * \sqrt{\frac{MSE}{n}}$$

Where,

C.V is critical value

MSE is mean square error

and

n is number of terms in a group

The absolute difference in means was then calculated for each pair of groups. If the absolute difference in mean was greater than critical value for any pair of treatments, then they were classified as significantly different from each other.

3.13 Colorimetric Analysis

Color properties of the plastic during composting were measured using a MINOLTA colorimeter by measuring the CIELab color coordinates L (lightness), a* (red to green), and b* (yellow to blue). The measurements were performed using the petri dish option since most of the samples had fragmented, where the sample is placed in a petri dish. The instrument was first zero calibrated and then white calibration was performed. An untreated PLA coupon was used as a test and the samples extracted on different days were then analyzed.

3.14 Scanning Electron Microscopy (SEM)

During the composting process, PLA coupons undergo surface erosion. To study the changes SEM was be used to visually see the changes. Sampled plastic was first trimmed and fixed on an adhesive tape on an aluminum holder. It was then placed in Blazers MED 010 to coat the surface with 1 nm of Gold-Palladium. It was then placed in the ThermoFisher Scientific FEI Quanta 600F SEM and set at an accelerated voltage of 20 kV. Images were captured at different magnifications from 37 x to 7000 x magnification.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Breakdown of PLA: General Trends

Throughout the different composting trials and subsequent treatments that were conducted, there were some general trends observed in the degrading PLA coupons. The breakdown of PLA initially starts with a color change. The clear plastic, due to heat and moisture, starts turning opaque. This is a sign of breakdown of PLA into its monomers by cleaving. Initially, the amorphous PLA chains are more susceptible to hydrolysis, hence the color changes from transparent to white. With the passage of time and further hydrolysis, PLA becomes completely white. However, at this stage, the plastic still maintains its structural integrity. Continued exposure to moisture and heat results in PLA losing its mechanical properties and becoming brittle. After this stage, if there is microbial growth in the compost media, small white spots can be observed on the surface of the polylactic acid. Finally, there is a color change to dark brown towards the end of the process and some of the compost material also adheres to the surface of the plastic. At this stage, there is a complete loss of mechanical properties, the PLA becomes soft, and dissolves into the compost. Figure 4.1 shows the general trend observed in disintegrating PLA coupon.

Figure 4.1: PLA sampled on days 7, 10, 14, 21 and 28. The four different treatments included 1) Control, 2) Autoclaved Plastic composted in control, 3) Autoclaved plastic with compost starter, 4) Heat treated plastic with compost starter

4.2 Breakdown of Wet Synthetic Waste

During the composting process, several changes occur in the compost as well. The changes in odor, color, and pH will be discussed.

4.2.1 Odor

Initially, the wet synthetic waste has a mixture of odors, mainly the smell of the sawdust and rabbit feed. Immediately after a day or two of composting, the odor changes to a sour smell due to the spoilage of wet synthetic waste and drop in pH. This trend is observed irrespective of the

presence of compost starter or mature compost from previous batches. There are no further changes in the odor until there is mycelial growth in the compost. At this point, the odor changes from sour to the smell of ammonia, a very pungent odor. This lasts for 2 to 3 days. The pungent oror is more prominent in compost with compost starter and mature compost from the previous batch and less so in control synthetic waste treatments. The pungent odor lasts for about 5 days after mycelial growth in treatments with compost starter. Once the pungent odor is gone, the compost has an earthy odor, similar to soil. There are no further changes in odor after this point.

4.2.2 Changes in Appearance

The wet synthetic waste has a light brown appearance when prepared, due to the presence of a large quantity of sawdust. There are no changes in the physical appearance until mycelial growth is observed. Mycelial growth appears as white spots within the compost. These quickly multiply and release large amounts of ammonia. The color also changes quickly from light brown to dark brown. Mycelial growth is observed in the compost for 10 to 15 days. At the end of the process, the compost turns completely black. Figure 4.2 shows the changes in the appearance of the compost. There is also a considerable loss in mass at this stage, but it was not possible to quantify this loss in mass. It was possible to observe this loss in mass qualitatively since the compost could no longer hold the same amount of water as it initially did. When the compost was replenished with water, waterlogging was observed.

Start of Compost process

Mycelial Growth Stage

Final Result

Figure 4.2: Different stages of the composting process and their visual appearance.

4.2.3 Changes in pH

Changes in the pH of the compost with the plastic pieces could not be performed because pH measurement would result in the loss of compost material, since the material has to be taken out and discarded. Only the initial and the final pH of compost with plastic pieces were recorded. A separate compost mixture was prepared as per the standard and 5 g of compost starter was added, to study the changes in the pH of a typical compost process. This is shown in Figure 4.3. Initially, the pH of the wet synthetic waste is almost neutral around 6. In the first week, there is a drop in pH and the synthetic waste starts turning acidic. After mycelial growth is observed, the pH quickly changes to basic due to evolution of ammonia. Once this composting is completed the pH drops back again to a neutral pH of around 7.5.

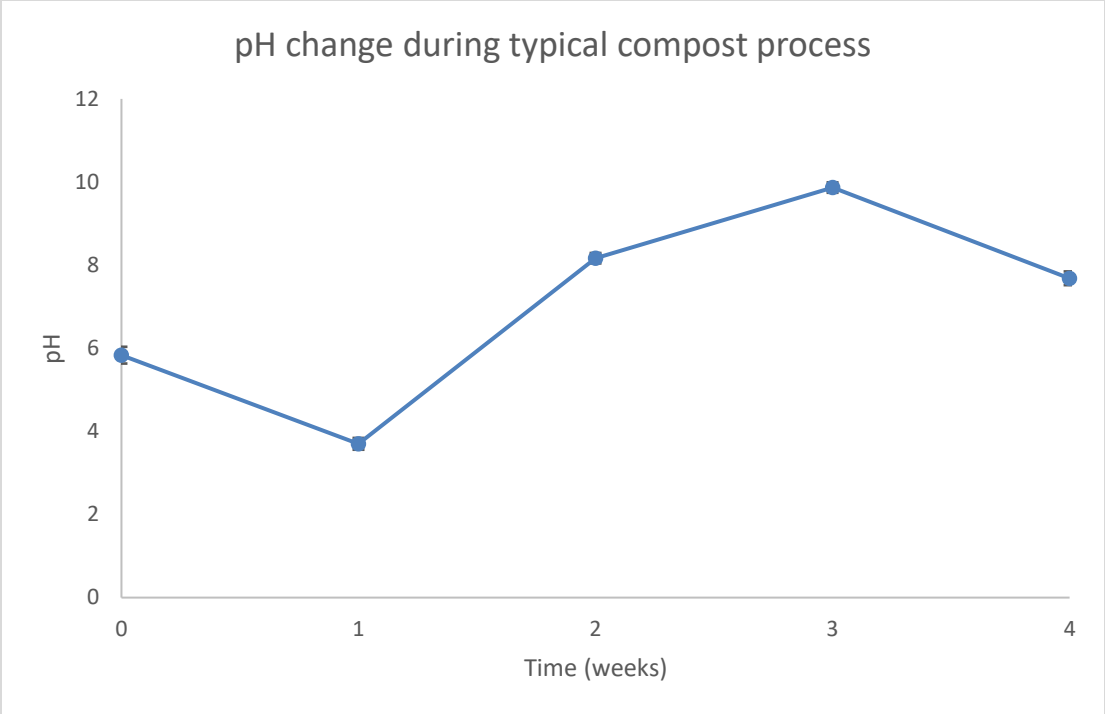


Figure 4.3: pH change over time in wet synthetic waste.

4.3 PLA Disintegration in Trial 1

The first trial with PLA coupons consisted of nine different treatments and a control with three replicates of each. The treatments included pretreating PLA coupons in hot water, base and acid, use of two different types of commercial inoculants, recycled synthetic waste and reduced PLA coupon size. Individual treatments were performed to check the effectiveness of each treatment in reducing the time taken to disintegrate polylactic acid compared to the control. Figure 4.4 shows all the treatments and the average time for complete disintegration of PLA. Hot water treatment of PLA coupons was done to hydrolyze PLA, which in turn reduces the time taken for complete disintegration of PLA by facilitating the growth of microorganisms on the surface. The time taken for PLA to break down after pretreatment with hot water was shorter than the control. Similarly, acidic and basic treatments were evaluated to check their effect on hydrolysis. Overall, all the treatments performed better than the control. The best-performing treatments were the use

of commercial inoculants, treatments 7 and 8, which took about 42 and 45 days respectively. The use of inoculants reduced the time taken for the growth of mycelia, making the entire process faster. The control was the longest time to break down PLA, which was about 60 days.

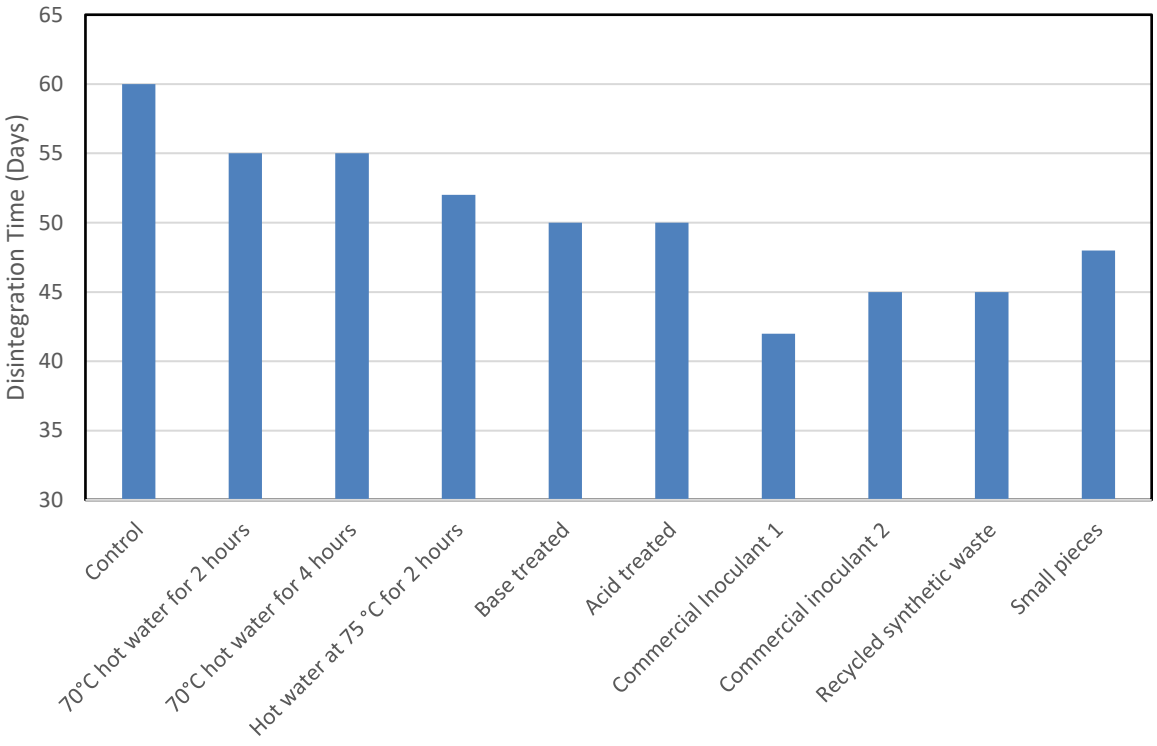


Figure 4.4: Total time taken for complete disintegration of PLA in trial 1 with nine different treatments.

4.4 PLA Disintegration in Trial 2

The results obtained in trial 2 are shown in **Error! Reference source not found.** and graphically represented in Figure 4.5 . Mushroom spent waste was substituted for saw dust in treatments 1, 2, and 3. Plastic pieces were treated with hot water in treatment 2 and commercial inoculant was used in treatment 3. Based on the best-performing treatments from trial 1, the treatments in trial 2 also included some combinations of the previous treatments. Plastic was pretreated with hot water, hot basic solution and hot acidic solution with compost starter to hasten the growth of

mycelia in treatments 5, 6, and 7. An additional treatment was the use of mature compost from trial 1 as a compost starter. A combination of mature compost with commercial inoculant and mature compost with small pieces was also evaluated. The idea was that the mature compost will have bacteria that can specifically break down PLA. This was a success, and the mature compost treatment was a good performing treatment, which was significantly better than the control. All the treatments, except the treatments with spent mushroom waste, were significantly better than the control ($P<0.05$). In one of the treatments, PLA coupons were heated in basic solution at 75°C with a pH of 10 for 2 hours before placing it in wet synthetic waste with a compost starter. Heating the plastic in basic solution was probably responsible for a high degree of hydrolysis, and with the compost starter providing the necessary microbial culture for quick composting, the combination degraded the plastic in just 25 days, compared to the control which in this trial took about 43 days. It was also the best performing treatment and was significantly better than all other treatments except plastic heated in an acidic solution of pH 4 for 2 hours with the use of commercial inoculant, which took about 31 days. The treatments with spent mushroom waste did not perform better than the control. There was also no mycelial growth observed in the spent mushroom waste, which is probably the reason why the performance was not better than control. However, the plastic pieces did fragment into tiny pieces which passed through the 2000 micron mesh, and as per the standard, it can be termed composted. However, in all the other treatments, including control, the plastic pieces completely disintegrated into the compost and there were no visible pieces remaining.

From trial 1 it was evident that the biological disintegration of plastics began after the growth of mycelia. In **Error! Reference source not found.** time taken for mycelial growth has been reported. In treatments with either compost starter or previous compost material, disintegration was much faster compared to the control. which did not have additional microbial culture. Mycelia appeared as white spots in the wet synthetic waste. At this stage biological degradation

of the plastic started. After this stage the plastic coupons turned soft from brittle and disintegration was more rapid. This transformation was not observed in treatments with mushroom spent waste. This shows microbial activity is very important for the quick disintegration of PLA coupons.

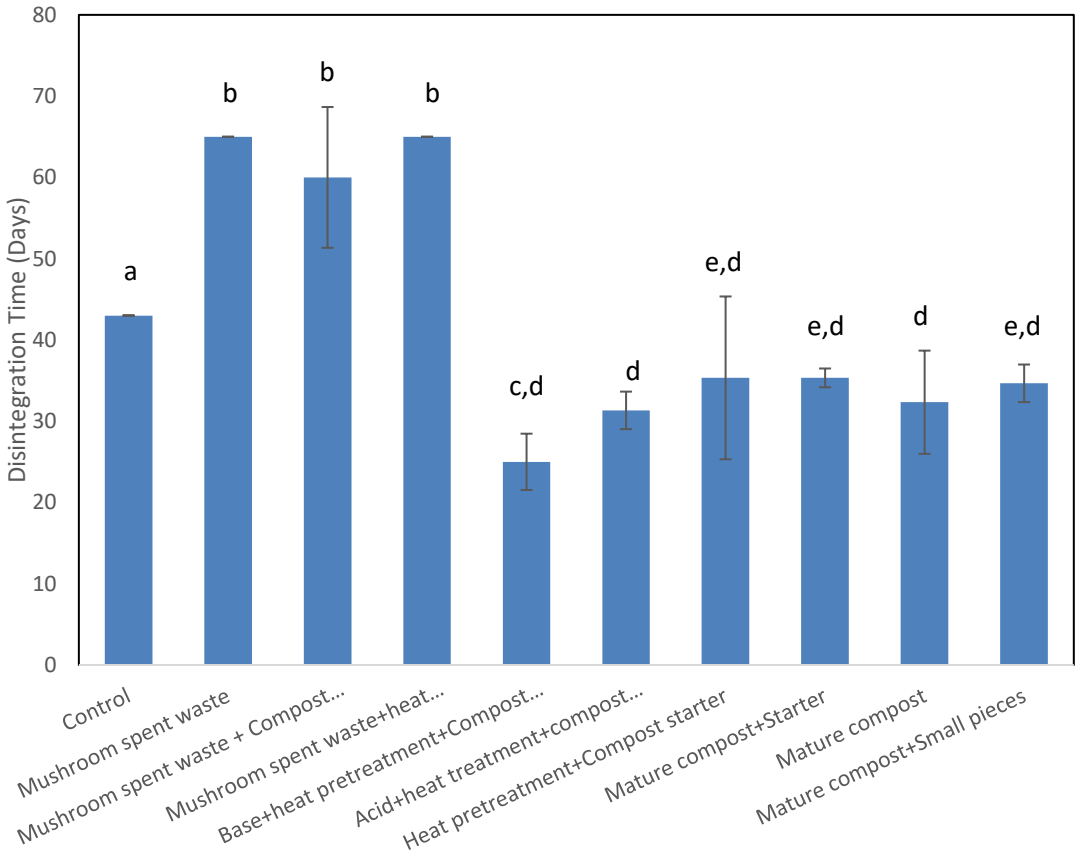


Figure 4.5: Total time taken for complete disintegration of PLA in trial 2. Treatments with the same letter are not significantly different (α=0.05). Error bars represent standard deviation.

Table 4.1: Time taken for mycelial growth and complete disintegration of PLA coupons during trial 2.

Treatment	Flask No.	Mycelial Growth (Days)	Complete Disintegration (Days)	Average Days for Complete Disintegration	Standard Deviation
Control	1A	34	43	43	0
	1B	34	43		
	1C	34	43		
Mushroom Spent Waste	2A	NA	65	65	0
	2B	NA	65		
	2C	NA	65		
Mushroom Spent Waste + Compost Starter	3A	NA	65	60	8.7
	3B	NA	65		
	3C	34	50		
Mushroom Spent Waste + Heat Treated Sample	4A	NA	65	65	0
	4B	NA	65		
	4C	NA	65		
Base + Heat Pretreatment + Compost Starter	5A	15	29	25	3.5
	5B	10	23		
	5C	10	23		
Acid + Heat Treatment + Compost Starter	6A	15	34	31	2.3
	6B	10	30		
	6C	12	30		
Heat Pretreatment + Compost Starter	7A	30	45	35	10
	7B	18	36		
	7C	10	25		
Mature Compost + Starter	8A	10	34	35	1.2
	8B	10	36		
	8C	10	36		
Mature Compost	9A	10	25	32	6.4
	9B	10	36		
	9C	10	36		
Mature Compost + Small Pieces	10A	10	32	35	2.3
	10B	10	36		
	10C	10	36		

4.5 PLA Disintegration in Trial 3

Trial 3 was performed in the large size compost bins to check the effectiveness of the treatments from the previous batches when scaled up. The treatments included control, pretreating plastic with base, and hot water. Commercial inoculant was added to all the treatments except the control. The results are shown in Figure 4.6 and **Error! Reference source not found.**. Again, all the treatments were significantly better compared to the control, but not better compared to each other at a significance level α of 0.05. The test proved that scale-up is possible and that the tests perform well even at a larger scale. It is also important to note that in one of the triplicates of the control, there was no mycelial growth. Again, after about 60 days, all the plastic pieces had broken down enough to pass through the standard 2000 micron sieve. The best performing treatment was plastic heated in hot water at 75°C with the addition of commercial inoculant, which took an average of about 28 days for complete disintegration.

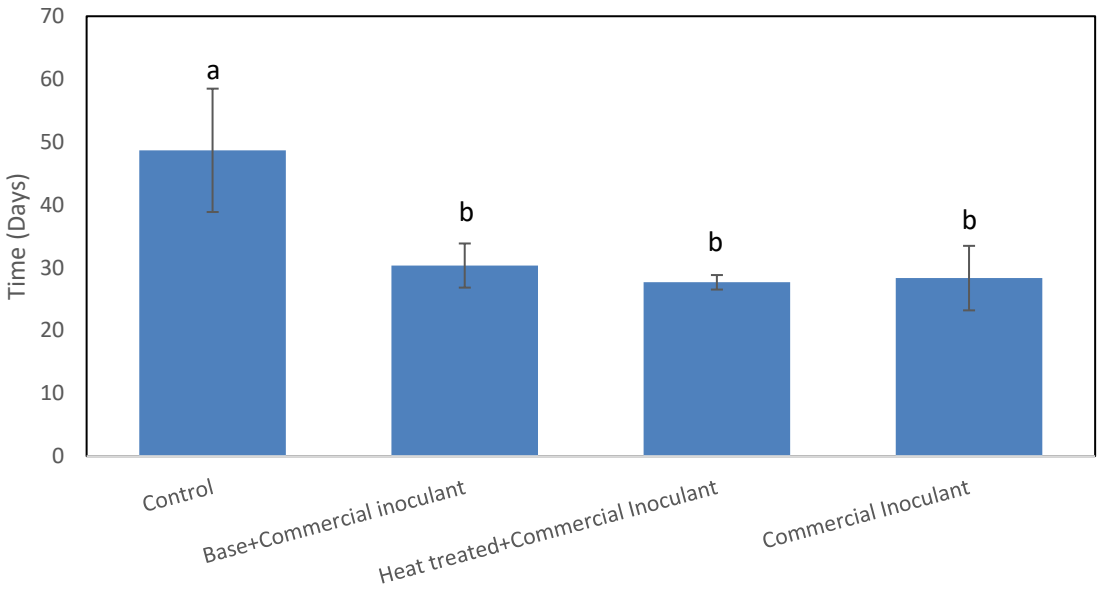


Figure 4.6: Time taken for complete disintegration of PLA in trial 3. Treatments with same the letter are not significantly different (α=0.05). Error bars represent standard deviation.

Table 4.2: Time taken for mycelial growth and complete disintegration of PLA coupons during trial 3 in 6 L compost bins.

Treatment	Bin No.	Mycelial Growth (Days)	Complete Disintegration (days)	Average Days for Complete Disintegration	Standard Deviation
Control	1	29	43	49	9.81
	2	NA	60		
	3	30	43		
Base+Commercial Inoculant	4	17	34	30	3.51
	5	16	30		
	6	12	27		
Heat Treated+Commercial Inoculant	7	10	27	28	1.15
	8	12	29		
	9	12	27		
Commercial Inoculant	10	10	27	28	5.13
	11	25	34		
	12	8	24		

4.5 PLA Disintegration in Trial 4

Trial 4 consisted of a normal control, as well as controls with compost starter and mature compost, which served as controls for tests with pretreated plastic. To test the effect of increased microbial activity, 50% previous compost was mixed with 50% fresh wet synthetic waste on a dry basis in treatment number 8, and plastic pieces were buried in it. For trial 6, compost was prepared in lab following the procedure for preparation of wet synthetic waste, compost starter added and allowed to mature. 50% dry weight of this mature compost was mixed with 50% dry weight fresh synthetic waste and PLA coupons were buried in the mixture. This was done to compare treatment 6 and 8 to see if there was specific PLA degrading microbes present in treatment 8 which could degrade PLA faster, which would be absent in treatment 6. In order to test the effects of increased temperature of preheating, the PLA coupons were heated in a food steamer (~100°C) and an autoclave (121°C), which served as treatments 4 and 5. Among all the treatments, the best performing treatment was the autoclaved plastic with compost starter in the synthetic media, which took about 19 days for complete disintegration. Food steamer treated PLA also on average took about the same amount of time. The other results are shown in Figure 4.7.

It was observed that although mycelial growth occurred very quickly in treatments 6 and 8 (which were mature compost), it did not adhere to the plastic immediately. They were also not significantly better than using just compost starter, even though the microbial concentration was, presumably, much higher. Treatments 6 and 8 were also not significantly different from each other. It took much longer for microbial growth to occur on the surface of the plastic compared to the pretreated plastics. This proves the importance of pretreating the plastic before composting. Overall, the treatments 6 and 8 took about 24 days to complete.

In the case of treatments with mushroom spent waste, microbial growth was observed in the treatment with compost starter. However, after microbial growth, there was a very strong pungent

ammoniacal smell. The quality of the compost was also different from the general trend observed. It was very sticky, had a bad odor, and was difficult to mix. On further analysis, it was understood that the C/N ratio of the mushroom spent compost was less than the recommended 20:1 at 14:1 without commercial inoculant and 12:1 with commercial inoculant and was likely the reason for the observations of poor performance.

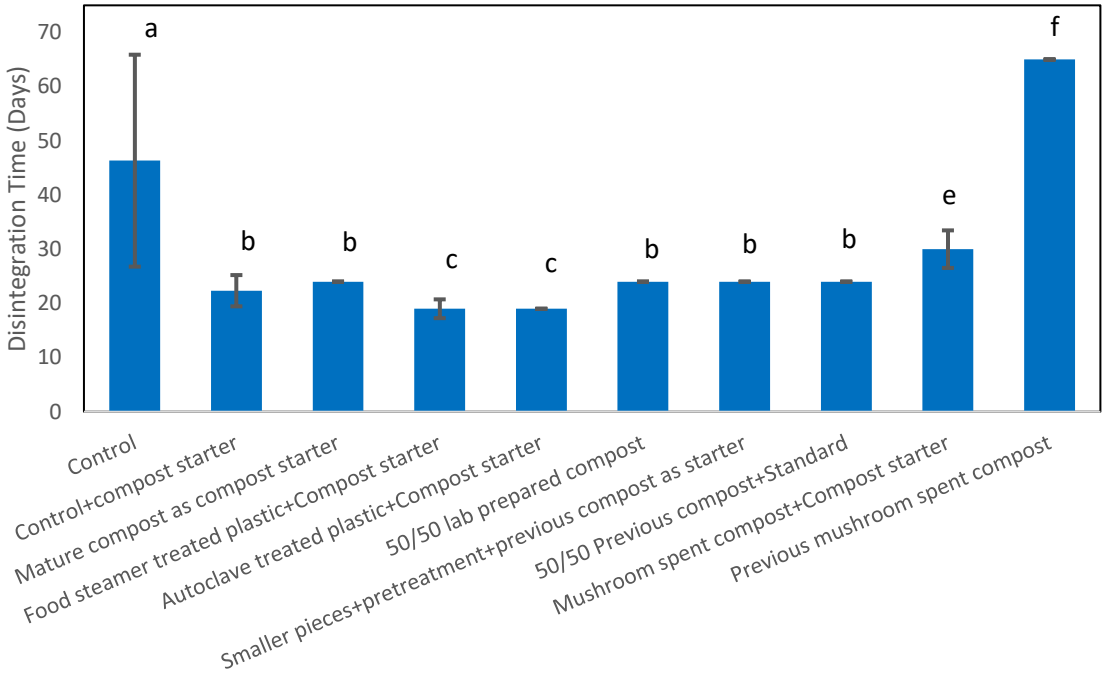


Figure 4.7: Time taken for complete disintegration of PLA in trial 4. Treatments with the same letter are not significantly different(α=0.05). Error bars represent standard deviation.

Table 4.3: Time taken for mycelial growth and complete disintegration of PLA coupons during trial 4.

Treatment	Flask No.	Mycelial Growth (Days)	Complete Disintegration (Days)	Average Days for Complete Disintegration	Standard Deviation
Control	1A	NA	65	46	19.55
	1B	34	48		
	1C	8	26		
Control + Compost Starter	2A	12	24	22	2.89
	2B	8	24		
	2C	12	19		
Mature Compost as Compost Starter	3A	8	24	24	0.00
	3B	12	24		
	3C	12	24		
Food Steamer Treated Plastic+Compost Starter	4A	8	17	19	1.73
	4B	10	20		
	4C	12	20		
Autoclave Treated Plastic+Compost Starter	5A	10	19	19	0.00
	5B	10	19		
	5C	10	19		
50/50 Lab Prepared Compost+Standard	6A	10	24	24	0.00
	6B	10	24		
	6C	10	24		
Smaller Pieces+ Heat Pretreatment+ Previous Compost as Starter	7A	10	24	24	0.00
	7B	12	24		
	7C	14	24		
50/50 Previous Compost+Standard	8A	8	24	24	0.00
	8B	8	24		
	8C	8	24		
Mushroom Spent Compost+Compost Starter	9A	24	28	30	3.46
	9B	24	34		
	9C	17	28		
Previous Mushroom Spent Compost	10A	NA	65	65	0.00
	10B	NA	65		
	10C	NA	65		

4.6 PLA Disintegration in Trial 5

Trial 5 was performed in the large 6 L bins. Treatments included control and autoclave treated plastic, autoclave treatment with compost starter and heat treatment at 75°C with compost starter. Treatment with autoclaved plastic was significantly better than control ($P<0.05$). This proves that autoclaved plastic breaks down much more easily than untreated plastic at the same microbial activity as control. The autoclaved plastic took about 19 days to disintegrate which was the same result observed in the previous trial. There was no significant difference between heat treated plastic and autoclaved plastic and it also took about 19 days for complete disintegration of PLA coupons. The results of trial 5 are reported in Figure 4.8 and Table 4.4. These results suggest that both heat treatment and added microbial inoculum are important for fast disintegration of PLA.

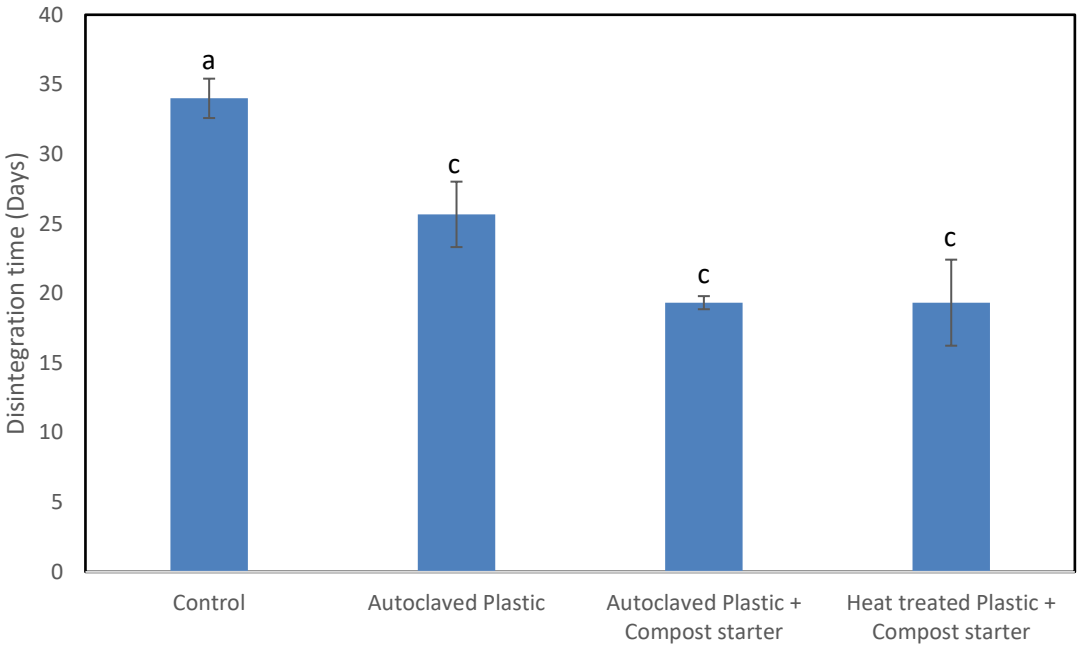


Figure 4.8: Time taken for complete disintegration of PLA in trial 5. Treatments with the same letter are not significantly different(α=0.05). Error bars represent standard deviation.

Table 4.4: Time taken for mycelial growth and complete disintegration of PLA coupons during trial 5.

Treatment	Bin No	Mycelial Growth (Days)	Complete Disintegration (days)	Average Days for Complete Disintegration	Standard Deviation
Control	1	21	35	34	1.41
	2	23	32		
	3	21	35		
Autoclaved Treated Plastic	4	21	29	26	2.35
	5	20	24		
	6	19	24		
Autoclaved plastic + Compost Starter	7	9	20	19	0.47
	8	9	19		
	9	9	19		
Heat Treated Plastic	10	9	15	19	3.09
	11	9	21		
	12	9	22		

4.7 PLA Disintegration in Trial 6

Trial 6 was performed in small flasks to compare the best performing pretreatments with autoclave, hot water treatment and food steamer in combination with compost starter and mature compost. Results are shown in Figure 4.9 and **Error! Reference source not found.**. The best performing treatments were hot water treated and autoclaved plastic in combination with compost starter, which took about 17 and 18 days respectively. Overall, all the treatments, except food steamer treated plastic which took about 45 days for complete disintegration, performed better than the control. The reason was that the time taken for mycelial growth in treatments 4 was more than control, even though there was no difference in the wet synthetic waste. There was no significant difference between autoclaved plastic (treatment 7) and the control which took about 35 and 36 days respectively for complete disintegration. This observation is different from the previous trial, where there was a significant difference between the same two treatments. Food steamer treated PLA with compost starter and mature compost took about 23 and 22 days, respectively, for complete disintegration and were not significantly different from each other.

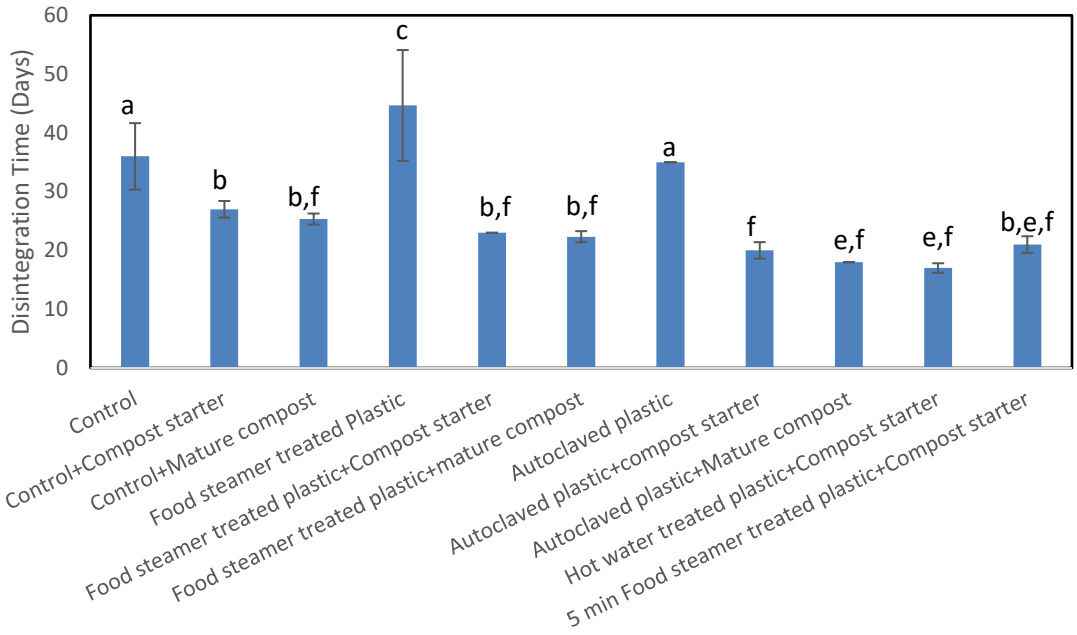


Figure 4.9: Time taken for complete disintegration of PLA in trial 6. Treatments with same the letter are not significantly different (α=0.05). Error bars represent standard deviation.

Table 4.5: Time taken for mycelial growth and complete disintegration of PLA coupons during trial 6.

Treatment	Flask No.	Mycelial Growth (Days)	Complete Disintegration (Days)	Average Days for Complete Disintegration	Standard Deviation
Control	1A	28	40	36	5.66
	1B	28	40		
	1C	23	28		
Control + Compost Starter	2A	15	25	27	1.41
	2B	20	28		
	2C	18	28		
Control + Mature Compost	3A	18	26	25	0.94
	3B	18	26		
	3C	8	24		
Food Steamer Treated Plastic	4A	32	38	45	9.43
	4B	30	38		
	4C	45	58		
Food Steamer Treated Plastic+Compost Starter	5A	15	23	23	0
	5B	14	23		
	5C	15	23		
Food Steamer Treated Plastic+Mature Compost	6A	15	21	22	0.94
	6B	15	23		
	6C	15	23		
Autoclave Treated Plastic	7A	28	35	35	0
	7B	28	35		
	7C	28	35		
Autoclave Treated Plastic+Compost Starter	8A	14	21	20	1.41
	8B	14	21		
	8C	12	18		
Autoclave Treated Plastic+Mature Compost	9A	8	18	18	0
	9B	8	18		
	9C	8	18		
Hot Water Treated Plastic+Compost Starter	10A	8	17	17	0.82
	10B	8	16		
	10C	8	18		
5 min Food Steamer Treated Plastic+Compost Starter	11A	14	23	21	1.41
	11B	8	20		
	11C	8	20		

4.8 Color Properties

To quantify the color changes in the plastic, samples were analyzed using the Minolta Spectrophotometer CM-3500d to measure the CIE (The international commission on Illumination) L*, a*, and b* parameters. These represent:

L*- Black (0) to white (100)

a*- Red (+a*) to green (-a*)

b*- Yellow (+b*) to blue (-b*)

Error! Reference source not found., Figure 4.15, Figure 4.18,

Figure 4.21,

Figure 4.21 and

Figure 4.24 show the changes in the L* values of plastic pieces of all treatments, sampled on different days of trials 2, 3, and 4 respectively. The L* value of clear plastic was very low. However, with the progress of composting, the L* value increases with the increase in opacity of the plastic as the plastic starts turning white. It reaches a maximum value before the growth of mycelia. After the growth of mycelia, the plastic starts turning darker again. This can be seen by a decrease in the L* value and it continues to drop until the end of the composting process. This trend can be seen irrespective of the treatment.

Changes in the a* values are graphically represented in Figure 4.13, Figure 4.16,

Figure 4.19,

Figure 4.22 and

Figure 4.25. Initially, the a* has a negative value, indicating greenness. With the progress of composting and at the stage where the plastic initially starts turning yellow, the a* value continues to drop. Eventually, when the plastic starts turning brown, the value shifts to positive indicating an increase in the redness.

The b* values are plotted in

Figure 4.14, Figure 4.17,

Figure 4.20,

Figure 4.23 and

Figure 4.26. Visually b* values have very high significance since the plastic undergoes yellowing. Initially the plastic is blue, but with continued composting the plastic turns yellow and the value of b* changes from negative to positive. Visual changes in the PLA over time are shown in Figure 4.10 and Figure 4.11.

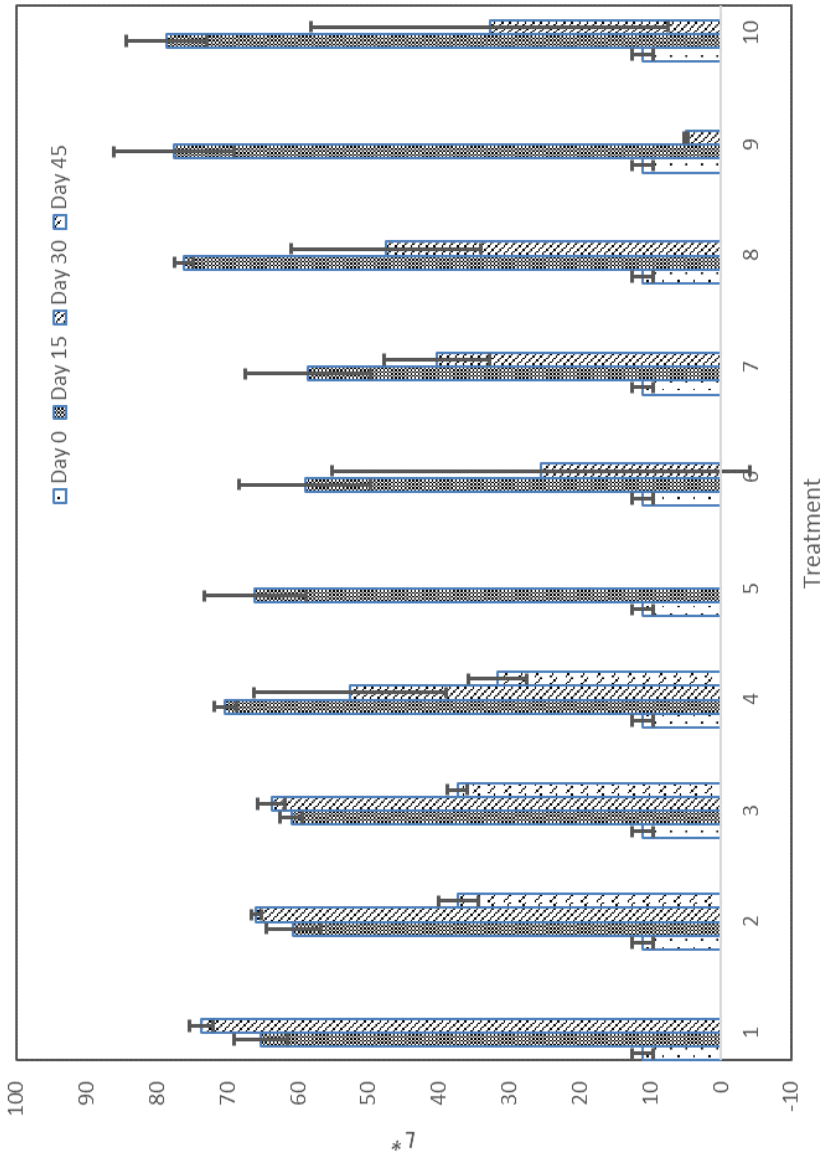
When PLA is pretreated in hot water and autoclave, it changes color from transparent to white. Figure 4.27 we can see that there is a significant difference in the *L** values of untreated, hot

water treated and autoclaved plastic. This shows that autoclaved plastic has probably undergone more hydrolysis compared to hot water treated plastic.

ANOVA was performed on the *b** values of trial 4, day 14 as shown in figure 4.28. There was significant difference between the *b** values of different treatments. However, the significant differences were not an indication of stage of degradation and treatments with higher value were not the best performing treatment. For example treatment 6 had a higher *b** value than treatment 5 (autoclaved plastic with compost starter), but towards the end, treatment 5 was the better performing treatment in terms of days for complete disintegration.

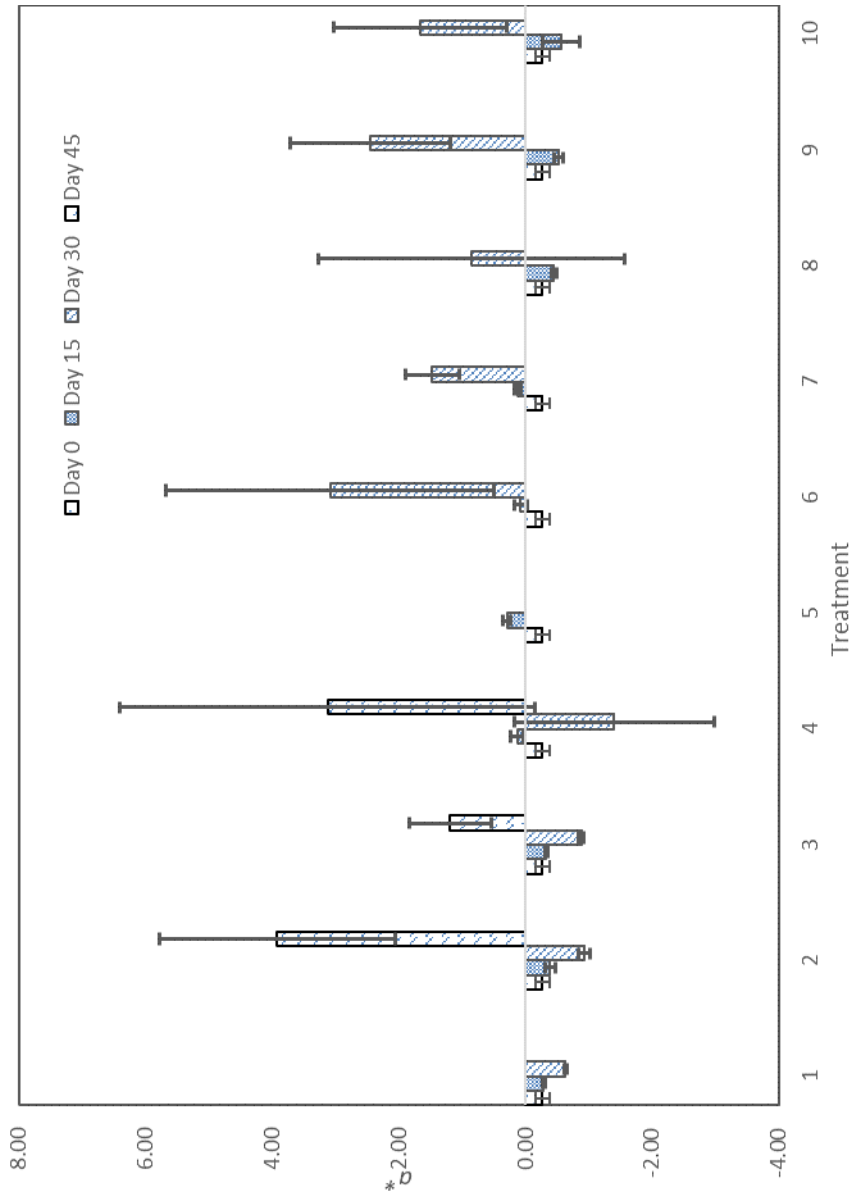
Figure 4.10: Color changes in plastic sampled on day 7,14 and 21 during trial 4 treatment 2.

Figure 4.11: Color changes in plastic sampled on day 7, 14, 21, 28 and at the end of the process during trial 4 treatment 10



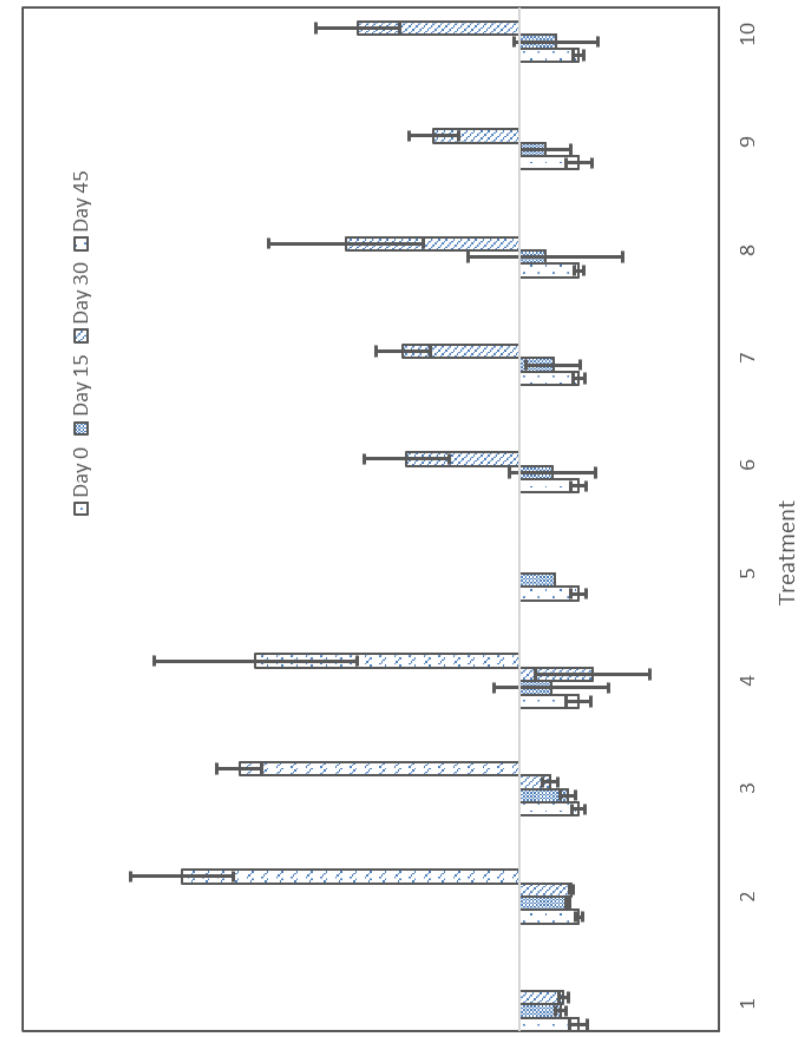
- - .	Treatment
1	Control
2	Mushroom Spent Waste
3	Mushroom Spent Waste + Compost Starter
4	Mushroom Spent Waste + Heat Treated Sample
5	Base + Heat Pretreatment + Compost Starter
6	Acid + Heat Treatment + Compost Starter
7	Heat Pretreatment + Compost Starter
8	Mature Compost + Starter
9	Mature Compost
10	Mature Compost + Small Pieces

Figure 4.12: L* values of plastic pieces sampled from all the treatments in trial 2 on days 0 (Pretrial) 15, 30 and 45. Error bars represent standard deviation.



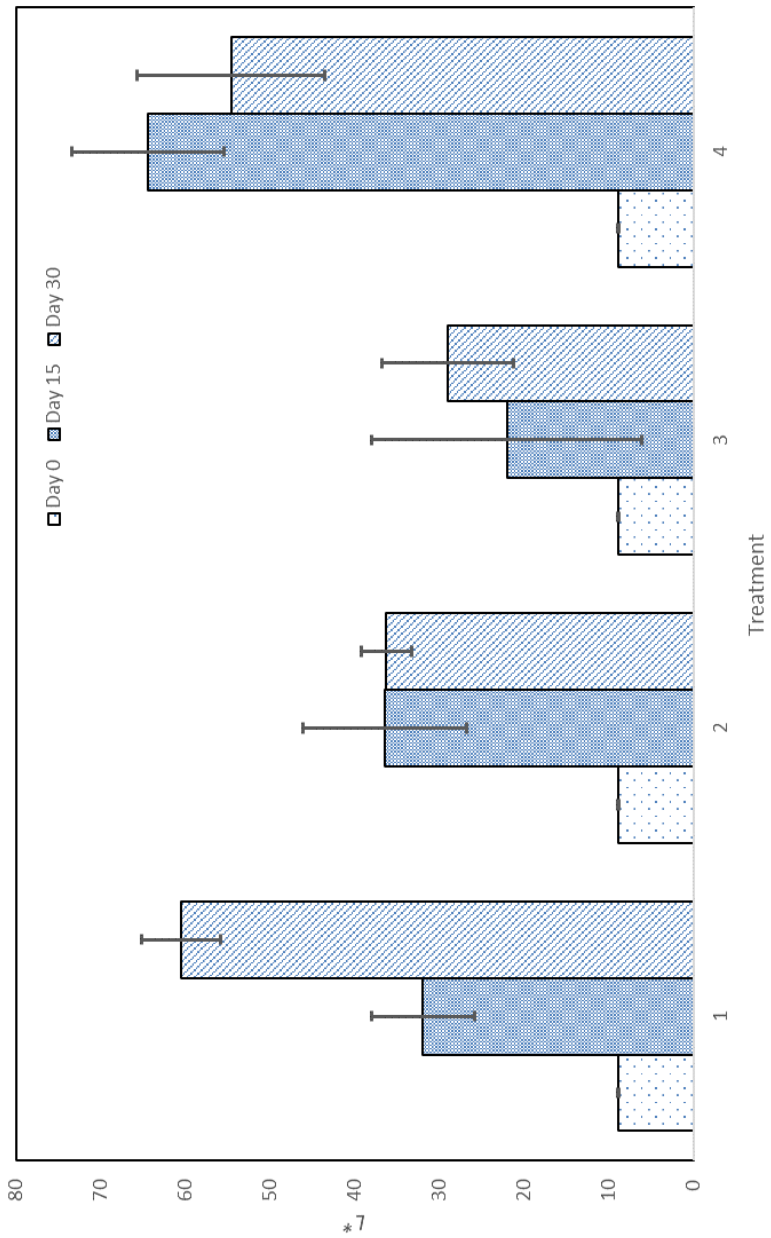
	Treatment
1	Control
2	Mushroom Spent Waste
3	Mushroom Spent Waste + Compost Starter
4	Mushroom Spent Waste + Heat Treated Sample
5	Base + Heat Pretreatment + Compost Starter
6	Acid + Heat Treatment + Compost Starter
7	Heat Pretreatment + Compost Starter
8	Mature Compost + Starter
9	Mature Compost
10	Mature Compost + Small Pieces

Figure 4.13: a* values of plastic pieces sampled from all the treatments in trial 2 on days 0 (pretrial), 15, 30 and 45. Error bars represent standard deviation.



	Treatment
1	Control
2	Mushroom Spent Waste
3	Mushroom Spent Waste + Compost Starter
4	Mushroom Spent Waste + Heat Treated Sample
5	Base + Heat Pretreatment + Compost Starter
6	Acid + Heat Treatment + Compost Starter
7	Heat Pretreatment + Compost Starter
8	Mature Compost + Starter
9	Mature Compost
10	Mature Compost + Small Pieces

Figure 4.14: b* values of plastic pieces sampled from all the treatments in trial 2 on days 0 (pretrial), 15, 30 and 45. Error bars represent standard deviation.



	Treatment
1	Control
2	Base+Commercial Inoculant
3	Heat Treated+Commercial Inoculant
4	Commercial Inoculant

Figure 4.15: L* values of plastic pieces sampled from all the treatments in trial 3 on days 0 (pretrial), 15, and 30. Error bars represent standard deviation.

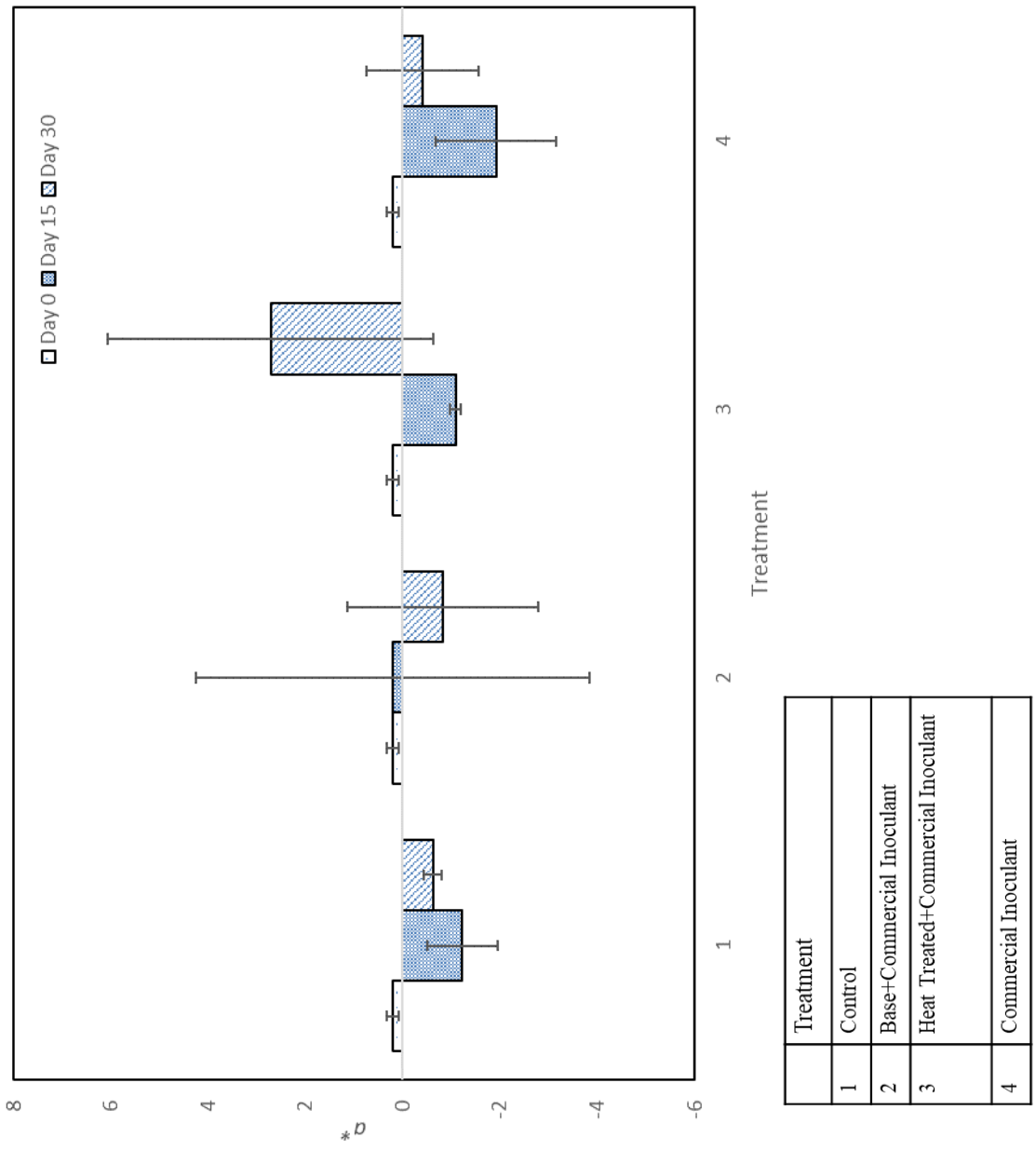


Figure 4.16: a* values of plastic pieces sampled from all the treatments in trial 3 on days 0 (pretrial), 15, and 30. Error bars represent standard deviation.

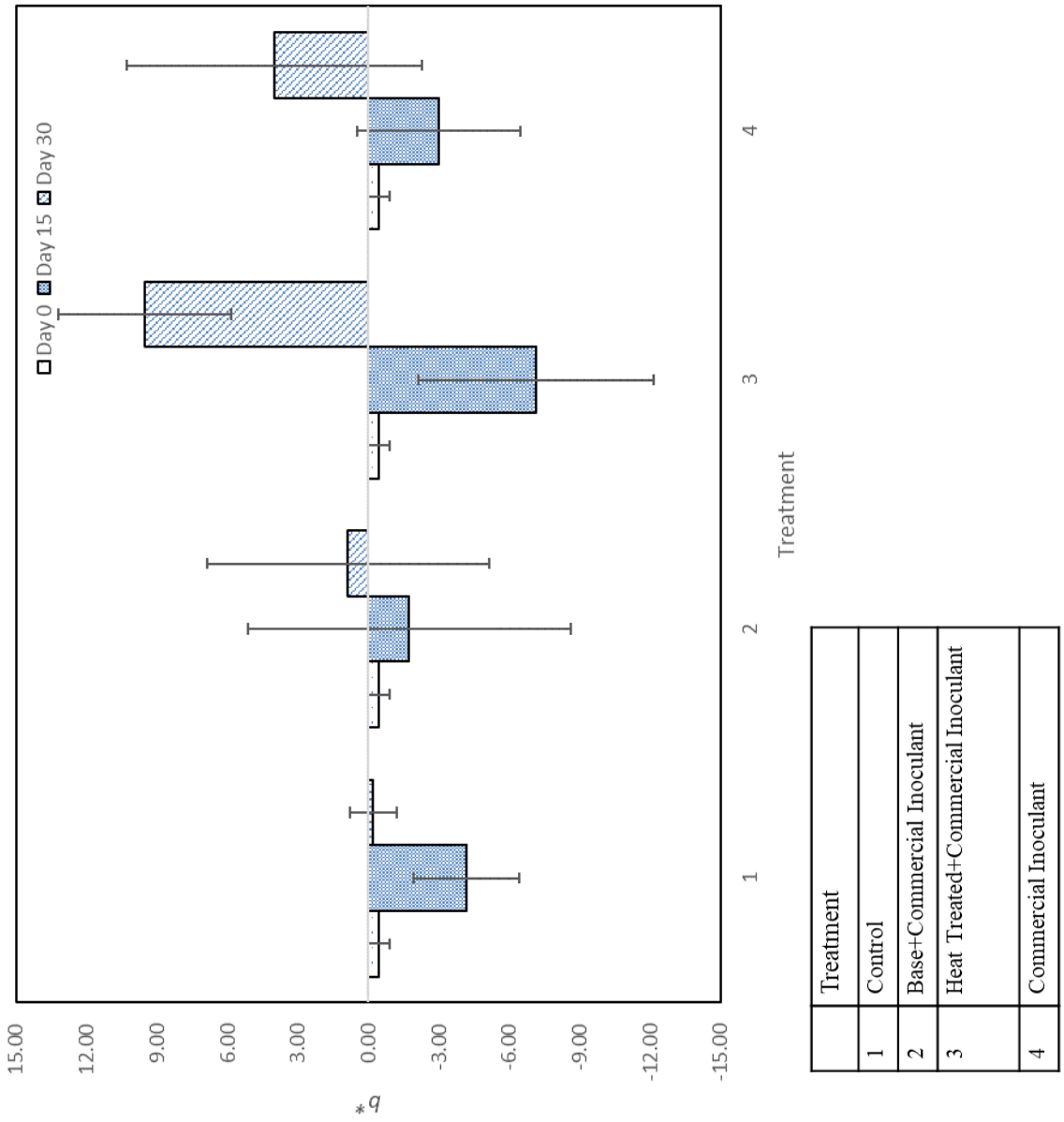
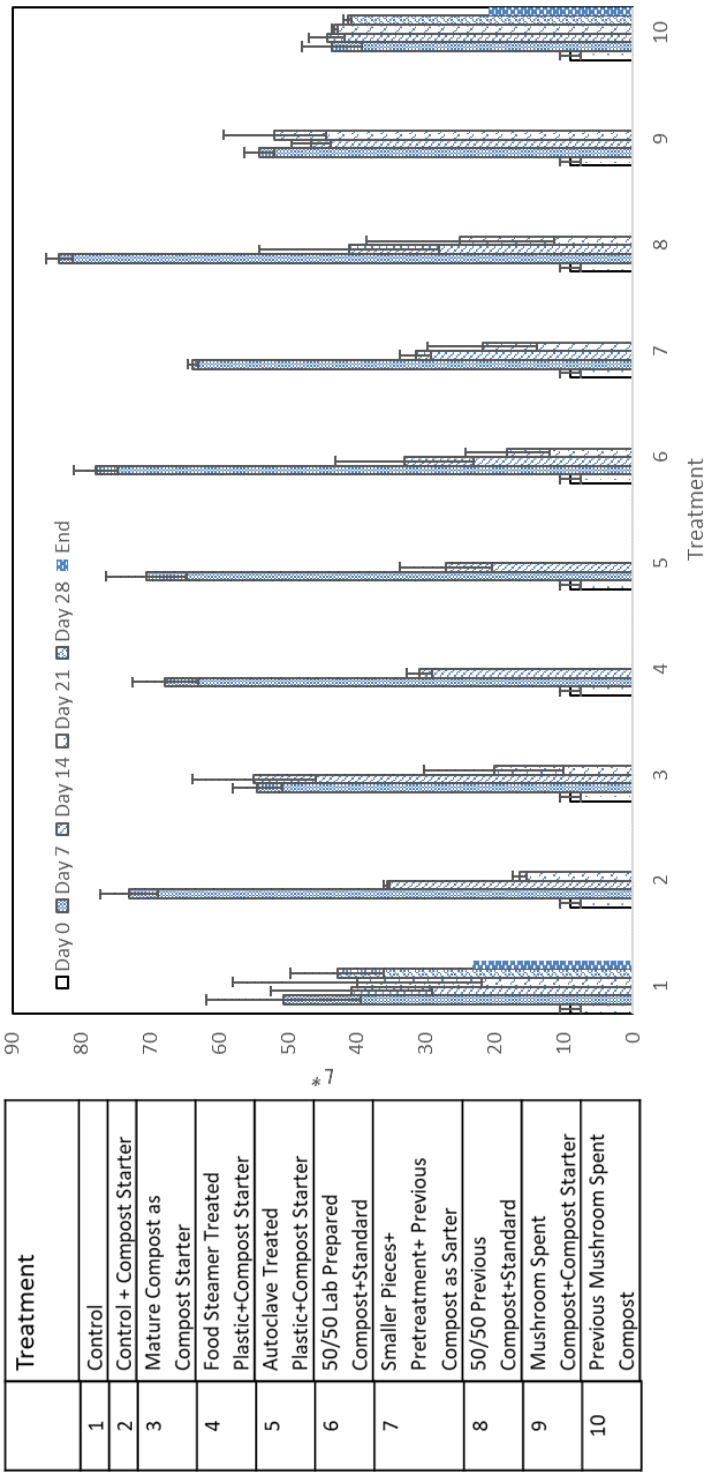
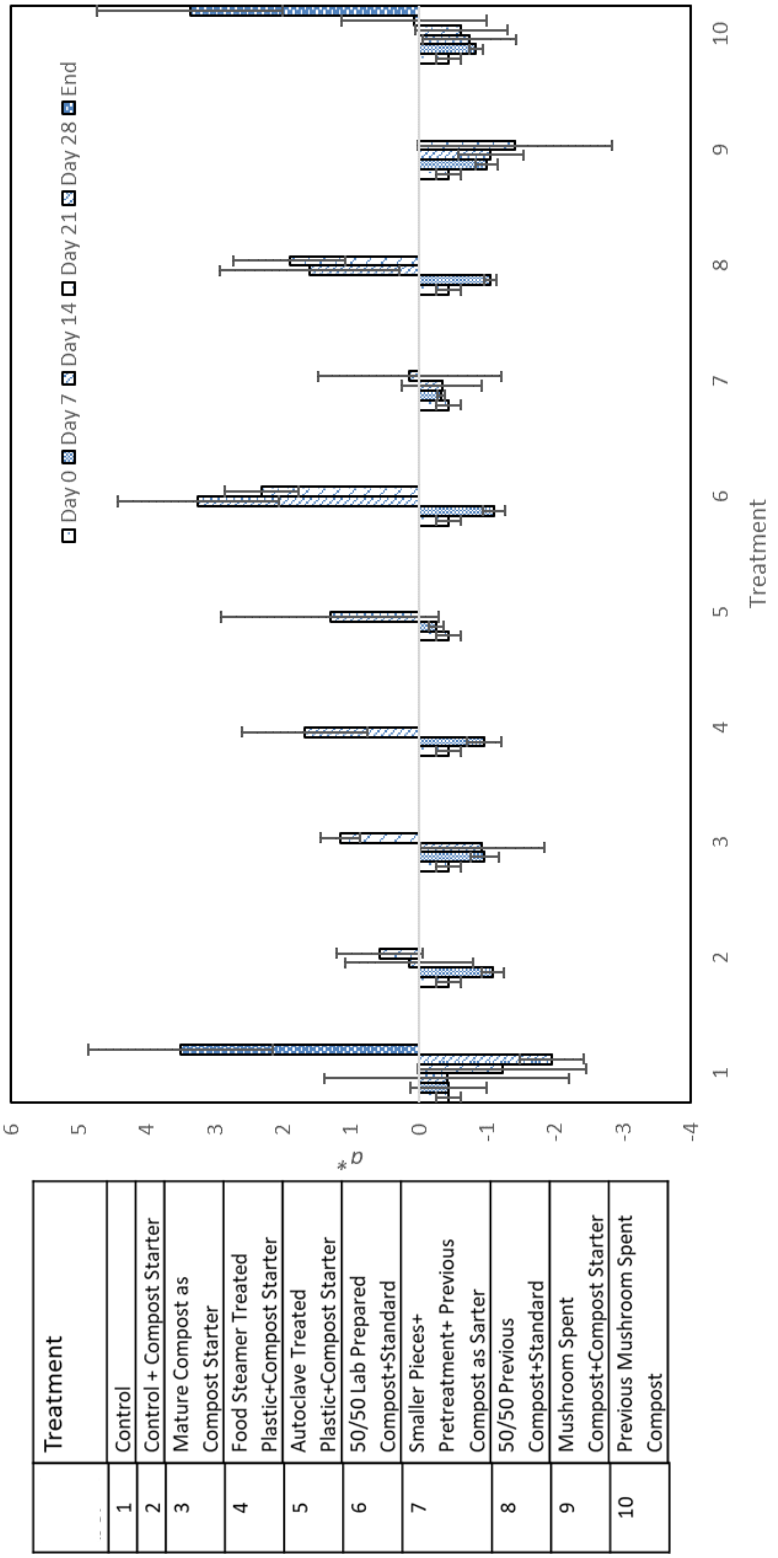


Figure 4.17: b* values of plastic pieces sampled from all the treatments in trial 3 on days 0 (pretrial), 15, and 30.



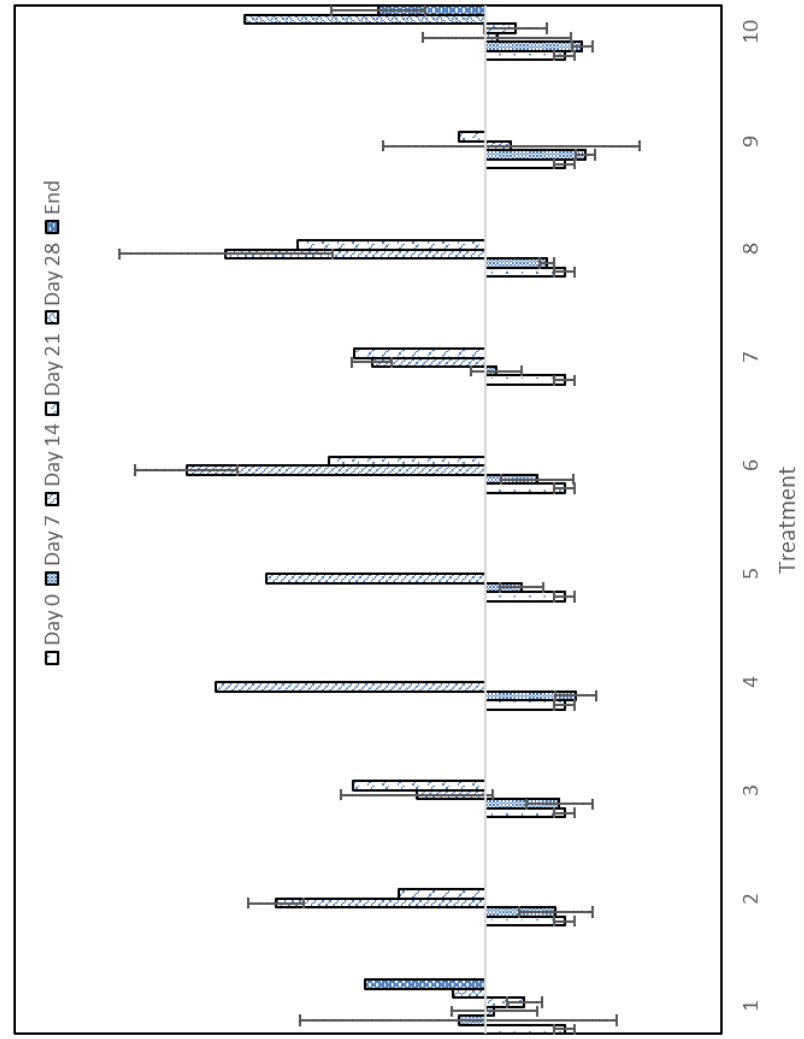
	Treatment
1	Control
2	Control + Compost Starter
3	Mature Compost as Compost Starter
4	Food Steamer Treated Plastic+Compost Starter
5	Autoclave Treated Plastic+Compost Starter
6	50/50 Lab Prepared Compost+Standard
7	Smaller Pieces+ Pretreatment+ Previous Compost as Sarter
8	50/50 Previous Compost+Standard
9	Mushroom Spent Compost+Compost Starter
10	Previous Mushroom Spent Compost

Figure 4.18: L* values of plastic pieces sampled from all the treatments in trial 4 on days 0 (pretrial), 7, 14, 21 and 28. Error bars represent standard deviation.



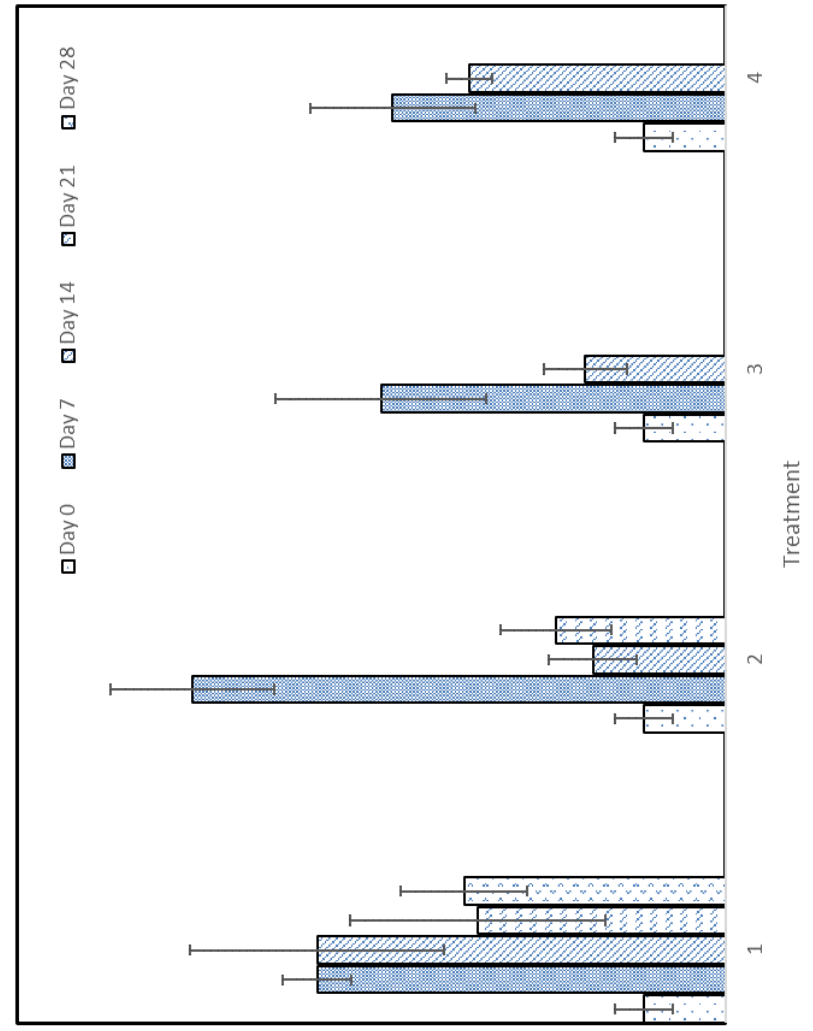
	Treatment
1	Control
2	Control + Compost Starter
3	Mature Compost as Compost Starter
4	Food Steamer Treated Plastic+Compost Starter
5	Autoclave Treated Plastic+Compost Starter
6	50/50 Lab Prepared Compost+Standard
7	Smaller Pieces+ Pretreatment+ Previous Compost as Sarter
8	50/50 Previous Compost+Standard
9	Mushroom Spent Compost+Compost Starter
10	Previous Mushroom Spent Compost

Figure 4.19: a* values of plastic pieces sampled from all the treatments in trial 4 on days 0 (pretrial), 7, 14, 21 and 28. Error bars represent standard deviation.



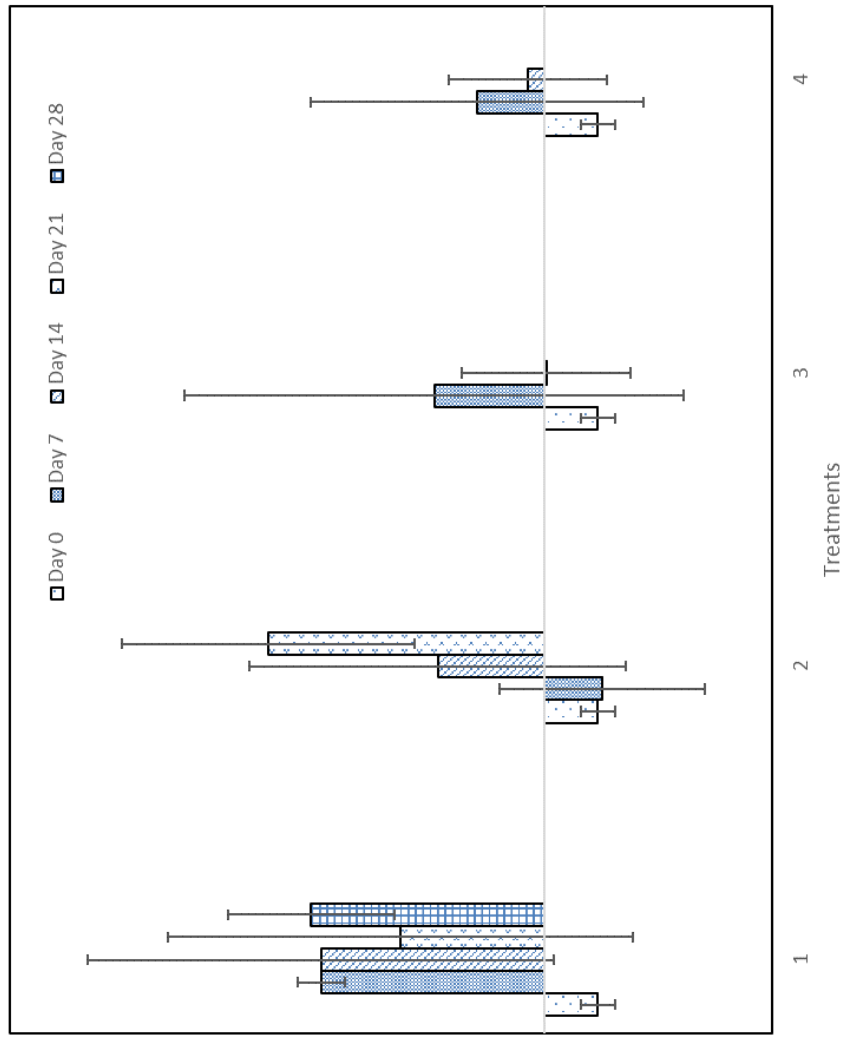
	Treatment
1	Control
2	Control + Compost Starter
3	Mature Compost as Compost Starter
4	Food Steamer Treated Plastic+Compost Starter
5	Autoclave Treated Plastic+Compost Starter
6	50/50 Lab Prepared Compost+Standard
7	Smaller Pieces+ Pretreatment+ Previous Compost as Sarter
8	50/50 Previous Compost+Standard
9	Mushroom Spent Compost+Compost Starter
10	Previous Mushroom Spent Compost

Figure 4.20: b* values of plastic pieces sampled from all the treatments in trial 4 on days 0 (pretrial), 7, 14, 21 and 28. Error bars represent standard deviation.



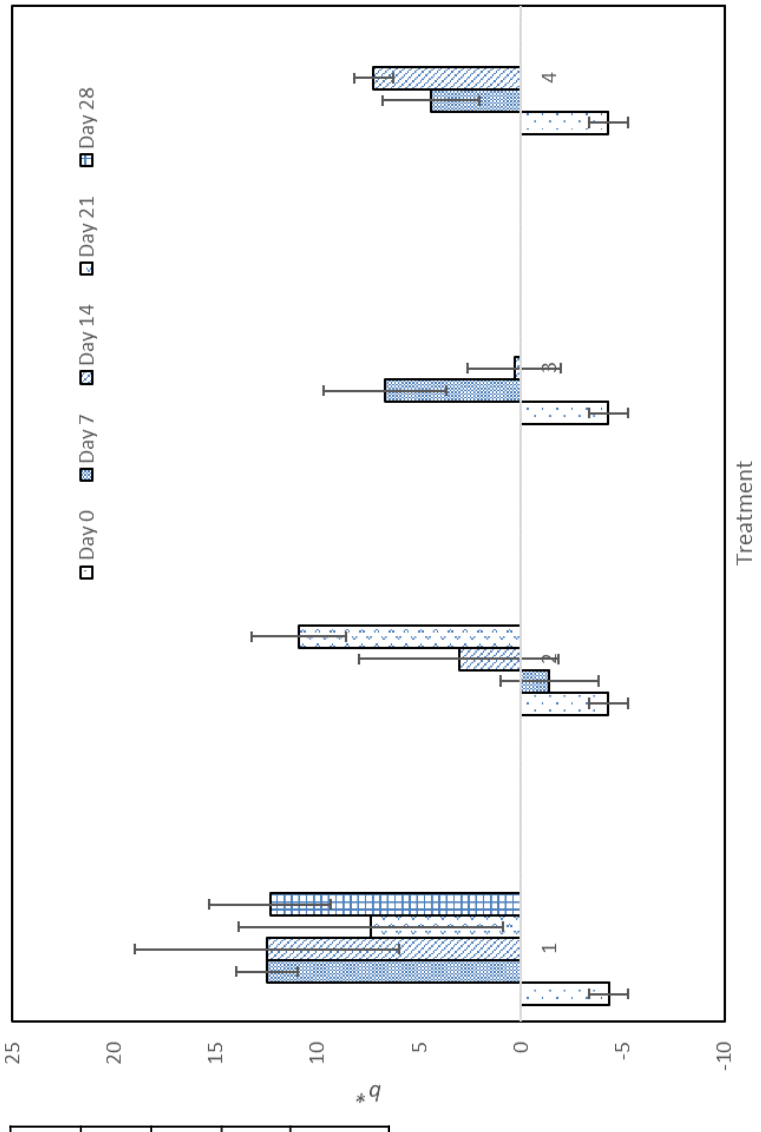
	Treatment
1	Control
2	Autoclave treated
3	Autoclave Treated+Compost Starter
4	Hot Water Treated+Compost Starter

Figure 4.21: L* values of plastic pieces sampled from all the treatments in trial 5 on days 0 (pretrial), 7, 14, 21 and 28. Error bars represent standard deviation.



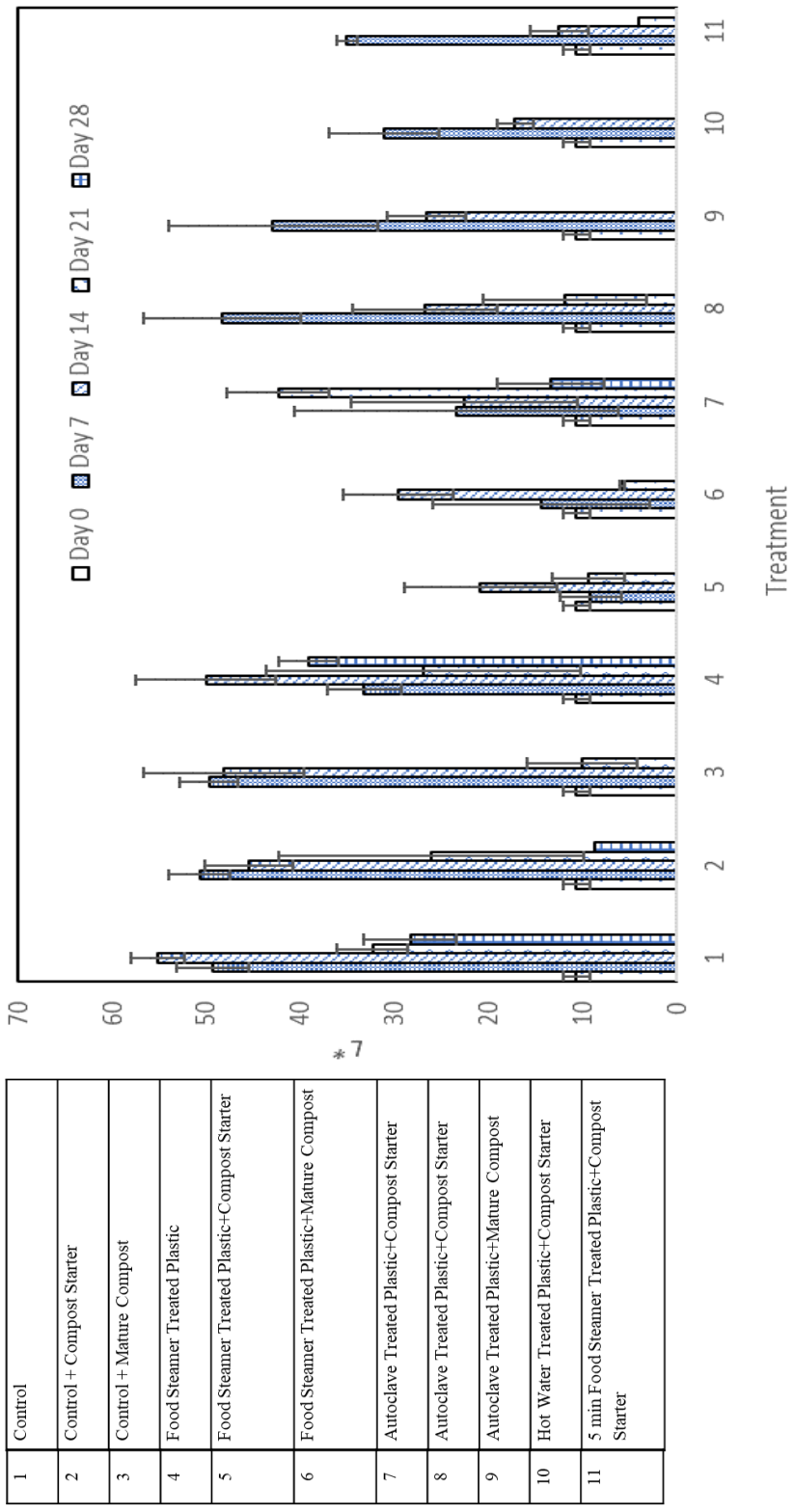
	Treatment
1	Control
2	Autoclave treated
3	Autoclave Treated+Compost Starter
4	Hot Water Treated+Compost Starter

Figure 4.22: a* values of plastic pieces sampled from all the treatments in trial 5 on days 0 (pretrial), 7, 14, 21 and 28. Error bars represent standard deviation.



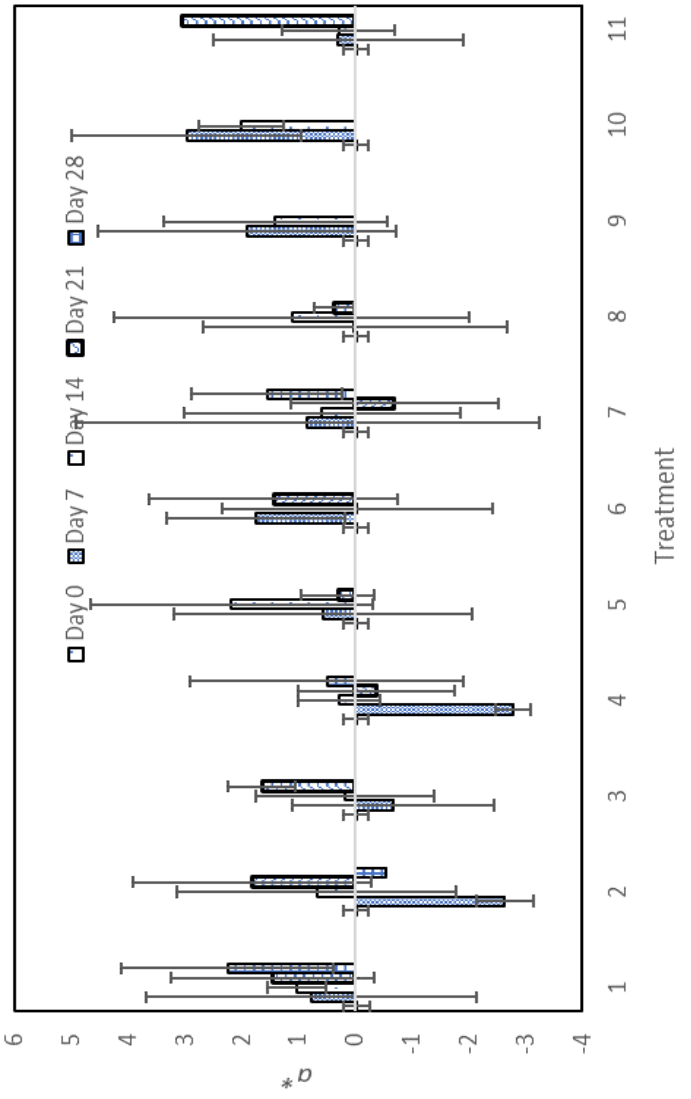
	Treatment
1	Control
2	Autoclave treated
3	Autoclave Treated+Compost Starter
4	Hot Water Treated+Compost Starter

Figure 4.23: b* values of plastic pieces sampled from all the treatments in trial 5 on days 0 (pretrial), 7, 14, 21 and 28. Error bars represent standard deviation.



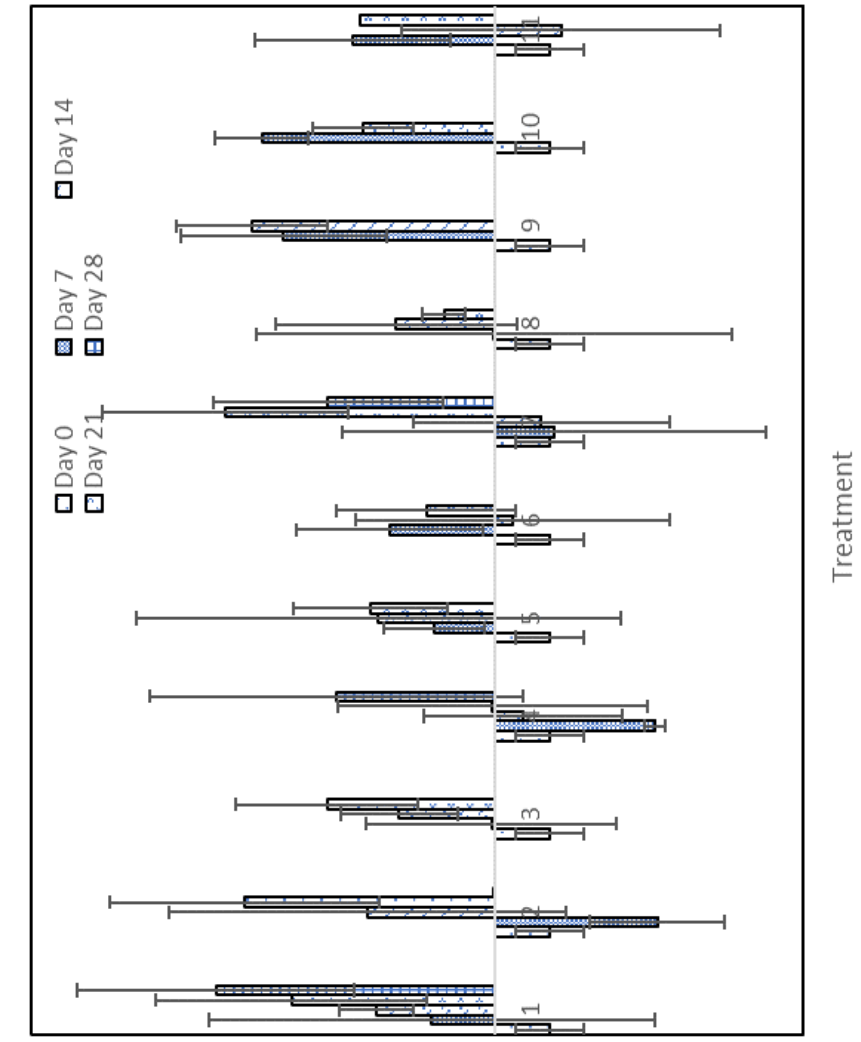
1	Control
2	Control + Compost Starter
3	Control + Mature Compost
4	Food Steamer Treated Plastic
5	Food Steamer Treated Plastic+Compost Starter
6	Food Steamer Treated Plastic+Mature Compost
7	Autoclave Treated Plastic+Compost Starter
8	Autoclave Treated Plastic+Compost Starter
9	Autoclave Treated Plastic+Mature Compost
10	Hot Water Treated Plastic+Compost Starter
11	5 min Food Steamer Treated Plastic+Compost Starter

Figure 4.24: L* values of plastic pieces sampled from all the treatments in trial 6 on days 0 (pretrial), 7, 14, 21 and 28. Error bars represent standard deviation.



1	Control
2	Control + Compost Starter
3	Control + Mature Compost
4	Food Steamer Treated Plastic
5	Food Steamer Treated Plastic+Compost Starter
6	Food Steamer Treated Plastic+Mature Compost
7	Autoclave Treated Plastic+Compost Starter
8	Autoclave Treated Plastic+Compost Starter
9	Autoclave Treated Plastic+Mature Compost
10	Hot Water Treated Plastic+Compost Starter
11	5 min Food Steamer Treated Plastic+Compost Starter

Figure 4.25: a* values of plastic pieces sampled from all the treatments in trial 6 on days 0 (pretrial), 7, 14, 21 and 28. Error bars represent standard deviation.



1	Control
2	Control + Compost Starter
3	Control + Mature Compost
4	Food Steamer Treated Plastic
5	Food Steamer Treated Plastic+Compost Starter
6	Food Steamer Treated Plastic+Mature Compost
7	Autoclave Treated Plastic+Compost Starter
8	Autoclave Treated Plastic+Compost Starter
9	Autoclave Treated Plastic+Mature Compost
10	Hot Water Treated Plastic+Compost Starter
11	5 min Food Steamer Treated Plastic+Compost Starter

Figure 4.26: b* values of plastic pieces sampled from all the treatments in trial 6 on days 0 (pretrial), 7, 14, 21 and 28. Error bars represent standard deviation.

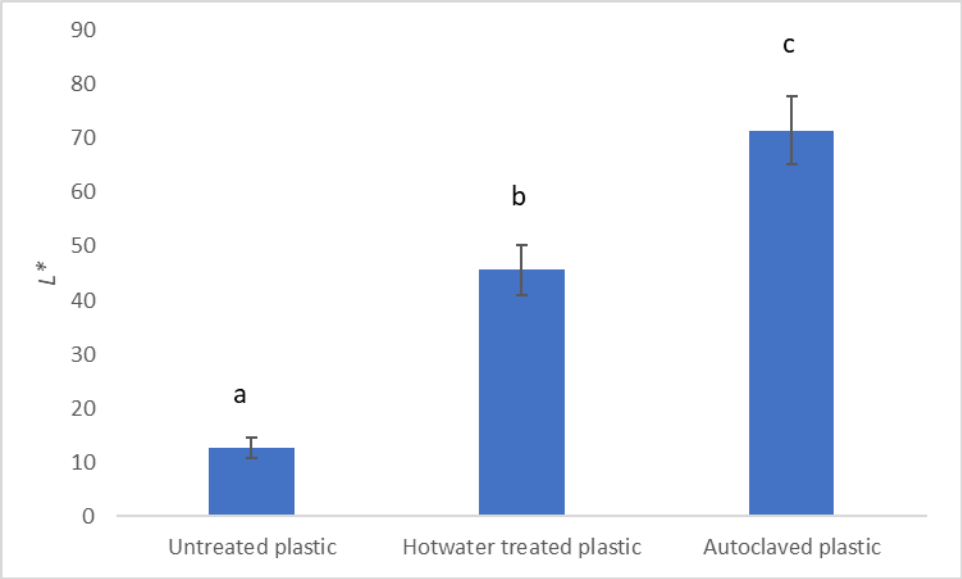
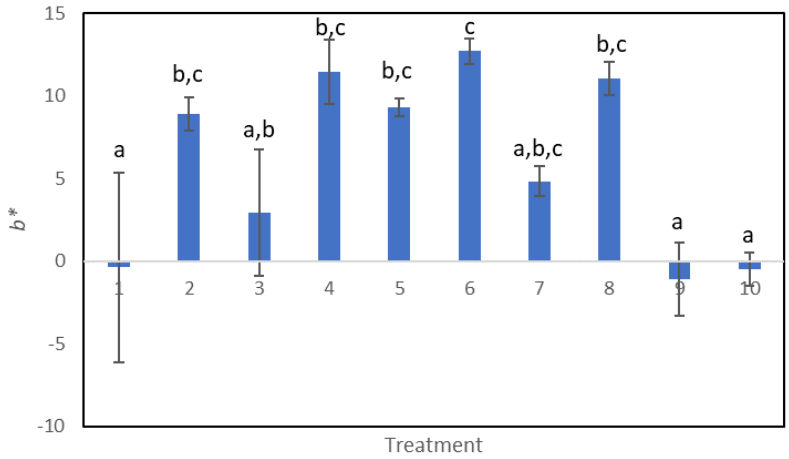


Figure 4.27: L* values of heat treated and untreated plastic on day 0 (pretrial). Treatments with the same letter are not significantly different(α=0.05). Error bars represent standard deviation.



	Treatment
1	Control
2	Control + Compost Starter
3	Mature Compost as Compost Starter
4	Food Steamer Treated Plastic+Compost Starter
5	Autoclave Treated Plastic+Compost Starter
6	50/50 Lab Prepared Compost+Standard
7	Smaller Pieces+ Pretreatment+ Previous Compost as Sarter
8	50/50 Previous Compost+Standard
9	Mushroom Spent Compost+Compost Starter
10	Previous Mushroom Spent Compost

Figure 4.28: b* values of different treatments sampled on day 14, trial 4. Treatments with the same letter are not significantly different(α=0.05). Error bars represent standard deviation.

4.9 Scanning Electron Microscopy

The scanning electron microscope uses a focused electron beam to visualize the surface topography of a sample, and in this case was used to view the surface of the PLA coupons during composting. Figure 4.29 a shows a clear piece of plastic captured at 500x magnification. It can be seen that there are no blemishes, and the plastic has a smooth surface. Comparatively, in the case of the autoclaved plastic, Figure 4.29 b, we can see that the plastic has undergone some changes resulting in crater like dents on the surface of the plastic. The food steamer treated plastic (Figure 4.29c) also appears to have a smooth surface, but the heat-treated plastic (Figure 4.29d) has some blemishes on the surface. After 14 days of composting, cracks start appearing on the surface of the plastic. This can be seen in the untreated plastic after 14 days (Figure 4.29e). However, in the case of the heat-treated plastic there are holes on the surface. Continued degradation increases the cracks on the surface and cracks also appear on the surface of untreated plastic, as seen in Figure 4.30 a) and b). These holes combined with the cracks seem to accelerate the rate of degradation in treated plastic.

In Figure 4.30 c), d) e) and f) we can see the growth of mycelia at different magnifications. Initially at day14 there is less amount of mycelial growth, and there is not much compost adhering to the surface. At day 21, the growth has increased exponentially, and it looks like a biofilm. There is also a lot of compost material that has adhered to the surface. These images are in line with the theory that the degradation of PLA is a two-stage process. The first stage where the plastic undergoes hydrolysis and there is a reduction of molecular weight, and the second stage is when the molecular weight reaches below 10000 Da and microbial activity disintegrates the plastic completely.

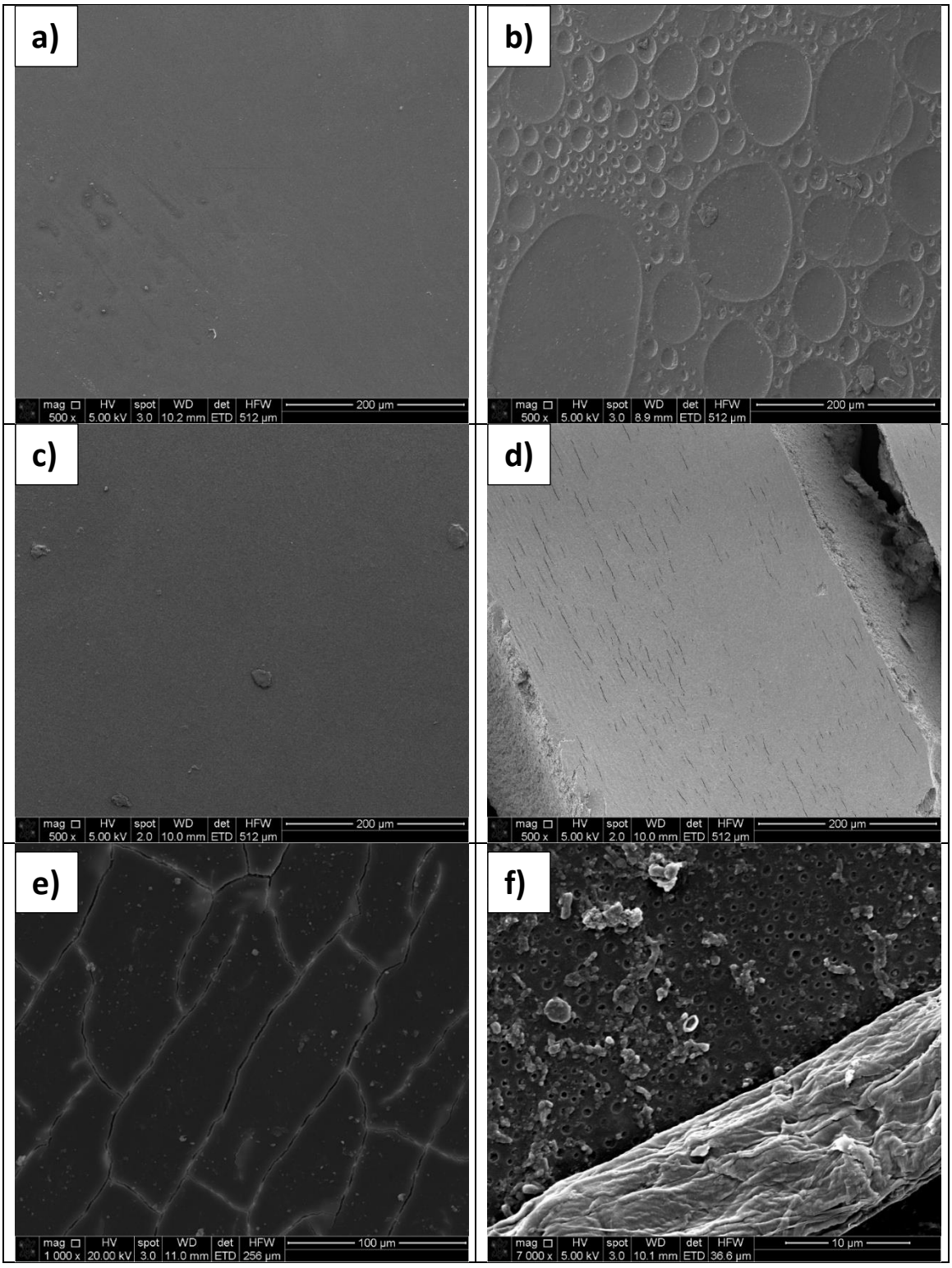


Figure 4.29: SEM images; a) Untreated plastic on day 0 at 500 x magnification b) Autoclaved plastic at 500 x magnification, c) Food steamer plastic at 500 x magnification, d) Hot water treated plastic at 500 x magnification, e)Untreated plastic sampled after 14 days 1000 x magnification, f) Heat-treated plastic sampled after 14 days 7000 x magnification

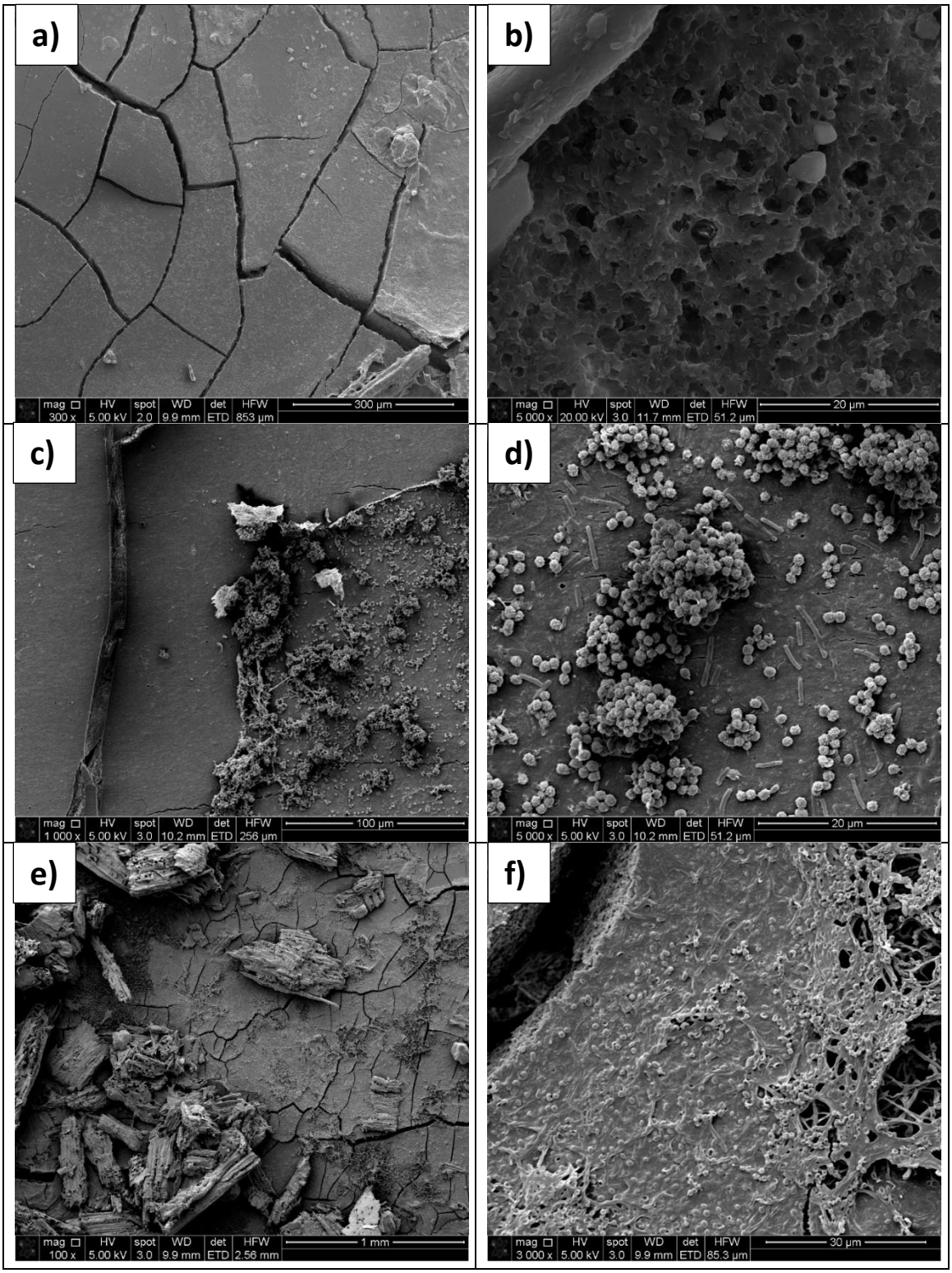


Figure 4.30: SEM images; a) Untreated plastic sampled after 21 days 500 x magnification, b) Heat-treated plastic sampled after 21 days at 5000 x magnification, c) Microbial growth after 14 days at 1000 x magnification d) Microbial growth after 14 days at 5000 x magnification, e) Microbial growth sampled after 21 days 100x magnification f) Microbial growth sampled after 21 days at 3000 x magnification.

4.10 Decrease in Volatile Solids

During the composting process the wet synthetic waste loses organic matter in the form of carbon dioxide. It also loses nitrogen as ammonia. This loss in mass can be quantified by measuring the volatile solids content before and after composting. It therefore is a good indicator that organic matter has undergone decomposition (Namkoong et al., 2013). However, it has been reported that measurement of decrease in volatile solids is not very specific nor sensitive (Finstein et al., 1986). As per the ISO standard, the decrease in volatile solids should be at least 30% in a successful composting process. Table 4.6 to Table 4.9 report the decrease in volatile solids in each of trials 1-4. It can be seen that based on changes in volatile solids, most of the treatments resulted in successful composting processes, with most of them being well above the 30% threshold.

Table 4.6: Average reduction in volatile solids in each treatment of trial 1.

	Treatment	**Average Decrease in Volatile Solids (%)**
1	Control	36.83 ± 5.23
2	Sample treated with water at 70 °C for 2 hours	33.96 ± 3.25
3	Sample treated with water at 70 °C for 4 hours	30.16 ± 1.88
4	Sample treated with water at 75 °C for 2 hours	24.03 ± 3.56
5	Sample treated with sodium hydroxide solution, pH 10, for 2 hours	34.08 ± 6.25
6	Sample treated with hydrochloric acid, pH 4, for 2 hours	25.27 ± 5.82
7	5 g of compost accelerator added to the synthetic waste	42.04 ± 0.76
8	5 g of compost starter added to the synthetic waste	32.37 ± 1.23
9	5 g of mature compost from the previous batch added to the synthetic waste	43.91 ± 2.68
10	Size of the PLA coupons changed to 15 mm x 15 mm x thickness	46.24 ± 2.59

Table 4.7: Average reduction in volatile solids in each treatment of trial 2.

	Treatment	**Average Decrease in Volatile Solids (%)**
1	Control	35.15 ± 1.79
2	Mushroom spent waste	35.12 ± 23.36
3	Mushroom Spent waste and 5 g of compost starter added to the synthetic waste	15.36 ± 2.17
4	Mushroom spent waste and Sample treated with water at 75°C for 2 hours	54.48 ± 5.00
5	5 g of compost starter added to synthetic waste and Sample treated with base, pH 10, and heated at 75°C for 2 hours	44.18 ± 2.74
6	5 g Compost starter added to synthetic waste and Sample treated with acid, pH 4 and heated 75°C for 2 hours	47.92 ± 2.95
7	5 g Compost starter added to synthetic waste and Sample heated in water at 75°C for 2 hours	42.47 ± 1.02
8	5 g of mature compost from the previous batch added to the synthetic waste	45.07 ± 2.66
9	5 g of mature compost from the previous batch and 5 g of Compost starter were added to the synthetic waste	49.72 ± 6.95
10	5 g of mature compost from the previous batch was added to synthetic waste and size of the PLA coupons changed to 15 mm x 15 mm x 0.32 mm	54.32 ± 6.85

Table 4.8: Average reduction in volatile solids in each treatment of trial 3.

	Treatment	**Average Decrease in Volatile Solids (%)**
1	Control	50.27 ± 3.44
2	Compost starter added to synthetic waste and sample treated with base, pH 10	49.17 ± 1.70
3	Compost starter added to synthetic waste and sample heated in water at 75°C for 2 hours	51.26 ± 4.74
4	Compost starter added to the synthetic waste	46.32 ± 5.727

Table 4.9: Average reduction in volatile solids in each treatment of trial 4.

	Treatment	**Average Decrease in Volatile Solids (%)**
1	Control	40.09 ± 0.56
2	5 g of compost starter added to synthetic waste	38.21 ± 7.14
3	5 g of mature compost from the previous batch	34.83 ± 3.19
4	5 g of compost starter added to synthetic waste and sample treated in a food steamer	55.70 ± 1.52
5	5 g of compost starter added to synthetic waste and sample treated in autoclave	42.28 ± 0.67
6	50% mature compost and 50% synthetic waste	44.82 ± 1.30
7	5 g of mature compost from the previous batch was added to synthetic waste, Size of the PLA coupons was changed to 5 mm x 15 mm x 0.32 mm and heated in water at 75°C	34.97 ± 0.24
8	50% mature compost from a previous batch and 50% synthetic waste	46.60 ± 0.76
9	Mushroom Spent waste and 5 g of compost starter added to the synthetic waste	57.35 ± 1.51
10	5g of the Previous mushroom spent compost	47.04 ± 2.33

CHAPTER 5

CONCLUSIONS

In order to reduce the time taken to biodegrade PLA, different treatments were applied to both the compost, in the form of microbial inoculants, and the plastic itself, in the form of preheating. Plastics were sampled periodically and were analyzed using a colorimeter and SEM. The overall conclusions are as follows:

1. The use of commercial inoculants reduced the time taken for mycelial growth. This in turn significantly reduced the overall time taken for the breakdown of PLA from about 46 to 22 days. The control, which had no commercial inoculant, took significantly longer to degrade PLA compared to treatments containing the commercial inoculant.
2. Use of mature compost had a similar effect on the compost as use of commercial inoculant. Disintegration times with mature compost were significantly lower than the control samples. Mycelial growth in treatments with mature compost was more consistent (about 10 days), compared to compost starter which took different amounts of time.
3. Pretreating the plastic with temperature in the presence of moisture (food steamer, autoclave, hot water etc.,) significantly improved the rate of disintegration. Higher temperatures performed better for lesser treatment time. Average disintegration times were 19 days for autoclave pretreatment, 19 days for food steamer treatment, and 28 days for hot water treatment, compared to 46 days for the control samples.

4. It is also important to note that the pretreatments and microbial inoculants worked even better together. The fastest rate of degradation was achieved with a combination of autoclaved PLA coupons and compost starter.
5. Results support the theory of a 2-stage breakdown process for PLA. It was observed that with pretreatment without microbial growth, there was little to no degradation. The best case for disintegration included physical pretreatment followed by rigorous microbial growth.
6. The use of mushroom spent waste did not perform very well. Upon further investigation it was observed that this was likely due to a very low carbon to nitrogen ratio (12:1). This is much lower than the recommended range between 20:1 and 40:1. This resulted in very little microbial growth, and when microbial growth was observed the final material was not similar to other treatments.
7. Colorimetric analysis showed a similar trend in the way plastic coupons disintegrated, irrespective of the treatment. The color changes occurred faster in faster treatments in slower in slower treatments. Colorimetric analysis shows that the plastic can break down very quickly, provided the right conditions of temperature, pH, microbial activity and hydrolysis are met.
8. Visual appearance of different pretreated PLA and sampled PLA was observed under the SEM. PLA treated in the autoclave had crater like depression on the surface. Hot water treated and food steamer treated plastic did not initially look any different from an untreated piece of plastic. They did develop pores and cracks over the course of composting, untreated plastic piece only seemed to develop cracks. It also revealed the growth of micro-organisms on the surface of the plastic.

While literature (Cosate et al., 2016) has shown that mechanical recycling is the most environmentally friendly option for handling plastic waste, recycling cannot always be

performed. Plastics contaminated with food waste are difficult or impossible to recycle. Composting is therefore a very good option for disposal of contaminated plastics.

5.1 Suggestions for Future Work

1. Evaluate processing options for scaling up the pretreatment process to make it desirable for commercial composters to handle compostable plastics.
2. Measure molecular weight of the plastic pieces before and after different pretreatment processes in order to determine their effectiveness.
3. Measure tensile strength of sampled plastic before pretreatment, after pretreatment and sampled on different days
4. Identify the fungi and bacteria growing on the surface of the plastic and in the compost.
5. Conduct further evaluation of spent mushroom waste with better C/N ratio.

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APPENDICES

APPENDIX A

Trial 2 ANOVA and Tukey Significance test

Anova: Single Factor

SUMMARY

Group number	*Groups*	*Count*	*Sum*	*Average*	*Variance*
1	Control	3	129	43	0
2	Mushroom spent waste	3	195	65	0
3	Mushroom spent waste+Compost starter	3	180	60	75
4	Mushroom spent waste+heat treated sample	3	195	65	0
5	Base+heat+Starter	3	75	25	12
6	Acid+heat+Starter	3	94	31	5.3
7	Heat+Compost starter	3	106	35	100.3
8	Mature compost+starter	3	106	35	1.33
9	Mature compost	3	97	32	40.33
10	Mature compost+small pieces	3	104	34	5.33

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6050.966667	9	672.3296	28.0527	1.95E-09	2.392814
Within Groups	479.3333333	20	23.96667			
Total	6530.3	29				

Comparison	Difference of Averages	Significant (If difference>Critical value)
G1 vs G2	22	Yes
G1 vs G3	17	Yes
G1 vs G4	22	Yes
G1 vs G5	18	Yes
G1 vs G6	11.67	Yes
G1 vs G7	8.33	Yes
G1 vs G8	8.33	Yes
G1 vs G9	10.67	Yes
G1 vs G10	8.33	Yes
G2 vs G3	5	No
G2 vs G4	0	No
G2 vs G5	40	Yes
G2 vs G6	33.67	Yes
G2 vs G7	30.33	Yes
G2 vs G8	30.33	Yes
G2 vs G9	32.67	Yes
G2 vs G10	30.33	Yes
G3 vs G4	5	No
G3 vs G5	35	Yes
G3 vs G6	28.67	Yes
G3 vs G7	25.33	Yes
G3 vs G8	25.33	Yes
G3 vs G9	27.67	Yes
G3 vs G10	25.33	Yes
G4 vs G5	40	Yes
G4 vs G6	33.67	Yes
G4 vs G7	30.33	Yes
G4 vs G8	30.34	Yes
G4 vs G9	32.67	Yes
G4 vs G10	30.33	Yes
G5 vs G6	6.33	No
G5 vs G7	9.66	Yes
G5 vs G8	9.66	Yes
G5 vs G9	7.33	No
G5 vs G10	9.67	Yes
G6 vs G7	4	No
G6 vs G8	4	No
G6 vs G9	1	No
G6 vs G10	3.333333	No

Comparison	Difference	Significant
G7 vs G8	0	No
G7 vs G9	3	No
G7 vs G10	0.666666667	No
G8 vs G9	3	No
G8 vs G10	0.666666667	No
G9 vs G10	3	No

Trial 3 ANOVA and Tukey Significance test

Anova:
Single Factor

SUMMARY

Group Number	*Groups*	*Count*	*Sum*	*Average*	*Variance*
1	Control	3	146	48	96.33333
2	Base+Commercial inoculant	3	91	30	12.33333
3	Heat treated+Commercial Inoculant	3	83	27	1.333333
4	Commercial Inoculant	3	85	28.	26.33333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	901.5833333	3	300.5278	8.817441	0.006454	4.066181
Within Groups	272.6666667	8	34.08333			
Total	1174.25	11				
$Q_{0.05(4,8)}$	4.53	CV	15.26893			

Comparison	Difference	Significant
G1 vs G2	18.33333333	Yes
G1 vs G3	21	Yes
G1 vs G4	20.33333333	Yes
G2 Vs G3	2.666666667	No
G2 Vs G4	2	No
G3 Vs G4	0.666666667	No

Trial 4 ANOVA and
Tukey Significance test

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Control	3	139	46	382
Control+compost starter	3	67	22.	8
Mature compost as compost starter	3	72	24	0
Food steamer treated plastic+Compost starter	3	57	19	3
Autoclave treated plastic+Compost starter	3	57	19	0
50/50 lab prepared compost	3	72	24	0
Smaller pieces+pretreatment+previous compost as starter	3	72	24	0
50/50 Previous compost+Standard	3	72	24	0
Mushroom spent compost+Compost starter	3	90	30	12
Previous mushroom spent compost	3	195	65	0

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5808.033	9	645.337	15.90806	2.77E-07	2.392814
Within Groups	811.3333	20	40.56667			

Total	6619.367	29	
$Q_{0.05(10,20)}$	5.01	Critical value	2.35979

Comparison	Difference	Significant
G1 vs G2	24	yes
G1 vs G3	22.333333	yes
G1 vs G4	27.333333	yes
G1 vs G5	27.333333	yes
G1 vs G6	22.333333	yes
G1 vs G7	22.333333	yes
G1 vs G8	22.333333	yes
G1 vs G9	16.333333	yes
G1 vs G10	18.666667	yes
G2 vs G3	1.6666667	No
G2 vs G4	3.3333333	yes
G2 vs G5	3.3333333	yes
G2 vs G6	1.6666667	No
G2 vs G7	1.6666667	No
G2 vs G8	1.6666667	No
G2 vs G9	7.6666667	yes
G2 vs G10	42.666667	yes
G3 vs G4	5	yes
G3 vs G5	5	yes
G3 vs G6	0	No
G3 vs G7	0	No
G3 vs G8	0	No
G3 vs G9	6	yes
G3 vs G10	41	yes
G4 vs G5	0	No
G4 vs G6	5	yes
G4 vs G7	5	yes
G4 vs G8	5	yes
G4 vs G9	11	yes

Comparison	Difference	Significance
G4 vs G10	46	yes
G5 vs G6	5	yes
G5 vs G7	5	yes
G5 vs G8	5	yes
G5 vs G9	11	yes
G5 vs G10	46	yes
G6 vs G7	0	No
G6 vs G8	0	No
G6 vs G9	6	yes
G6 vs G10	41	yes
G7 vs G8	0	No
G7 vs G9	6	yes
G7 vs G10	41	yes
G8 vs G9	6	yes
G8 vs G10	41	yes
G9 vs G10	6	yes

Trial 5 ANOVA and Tukey
Significance test

Anova: Single Factor

SUMMARY

Group Number	*Groups*	*Count*	*Sum*	*Average*	*Variance*
1	Control	3	102	34	3
2	Autoclaved	3	77	25	8.333333
3	Autoclaved Plastic + Compost starter	3	58	19	0.333333
4	Heat treated Plastic + Compost starter	3	58	19	14.33333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	434.9166667	3	144.9722	22.30342	0.000306	4.066181
Within Groups	52	8	6.5			
Total	486.9166667	11				
$Q_{0.05(4,8)}$	4.53	CV	6.667979			

Comparison	Difference	Significant
G1 vs G2	8.33	Yes
G1 vs G3	14.67	Yes
G1 vs G4	14.67	Yes
G2 Vs G3	6.33	No
G2 Vs G4	6.33	No
G3 Vs G4	0	No

Trial 6 ANOVA and Tukey Significance test

Anova: Single Factor

SUMMARY

Group Number	*Groups*	*Count*	*Sum*	*Average*	*Variance*
1	Control	3	108	36	48
2	Control+Compost starter	3	81	27	3
3	Control+Mature compost	3	76	25	1.333333
4	Food steamer treated Plastic	3	134	45	133.3333
5	Food steamer treated plastic+Compost starter	3	69	23	0
6	Food steamer treated plastic+mature compost	3	67	22	1.333333
7	Autoclaved plastic	3	105	35	0
8	Autoclaved plastic+compost starter	3	60	20	3
9	utoclaved plastic+Mature compost	3	54	18	0
10	Hot water treated plastic+Compost starter	3	51	17	1
11	5 min Food steamer treated plastic+Compost starter	3	63	21	3

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2274.969697	10	227.496969	12.89931	4.84E-07	2.29669
Within Groups	388	22	17.6363636			
Total	2662.969697	32				

Comparison	**Difference**	**Significance**
G1 vs G2	9.00	Yes
G1 vs G3	10.67	Yes
G1 vs G4	8.67	Yes
G1 vs G5	13.00	Yes
G1 vs G6	13.67	Yes
G1 vs G7	1.00	No
G1 vs G8	16.00	Yes
G1 vs G9	18.00	Yes
G1 vs G10	19.00	Yes
G1 vs G11	15.00	Yes
G2 vs G3	1.67	No
G2 vs G4	17.67	Yes
G2 vs G5	4.00	No
G2 vs G6	4.67	No
G2 vs G7	8.00	Yes
G2 vs G8	7.00	Yes
G2 vs G9	9.00	Yes
G2 vs G10	10.00	Yes
G2 vs G11	6.00	No
G3 vs G4	19.33	Yes
G3 vs G5	2.33	No
G3 vs G6	3.00	No
G3 vs G7	9.67	Yes
G3 vs G8	5.33	No
G3 vs G9	7.33	Yes
G3 vs G10	8.33	Yes
G3 vs G11	4.33	No
G4 vs G5	21.67	Yes

Comparison	Difference	Significance
G4 vs G6	22.33	Yes
G4 vs G7	9.67	Yes
G4 vs G8	24.67	Yes
G4 vs G9	26.67	Yes
G4 vs G10	27.67	Yes
G4 vs G11	23.67	Yes
G5 vs G6	0.67	No
G5 vs G7	12.00	Yes
G5 vs G8	3	No
G5 vs G9	5	No
G5 vs G10	6	No
G5 vs G11	2	No
G6 vs G7	12.67	Yes
G6 vs G8	2.33	No
G6 vs G9	4.33	No
G6 vs G10	5.33	No
G6 vs G11	1.33	No
G7 vs G8	15	Yes
G7 vs G9	17	Yes
G7 vs G10	18	Yes
G7 vs G11	14	Yes
G8 vs G9	2	No
G8 vs G10	3	No
G8 vs G11	1	No
G9 vs G10	1	No
G9 vs G11	3	No
G10 vs G11	4	No

APPENDIX B

Plastic Weights

Trial 1

Flask No.	Plastic Weight
1A	1.141
1B	1.143
1C	1.157
2A	1.222
2B	1.206
2C	1.208
3A	1.206
3B	1.221
3C	1.225
4A	1.220
4B	1.190
4C	1.195
5A	1.198
5B	1.206
5C	1.207
6A	1.216
6B	1.220
6C	1.203
7A	1.197
7B	1.198
7C	1.225
8A	1.189
8B	1.200
8C	1.184
9A	1.204
9B	1.200
9C	1.198
10A	0.81
10B	0.789
10C	0.776

Trial 2

Flask No.	Plastic Weight
1A	1.225
1B	1.225
1C	1.239
2A	1.217
2B	1.236
2C	1.24
3A	1.238
3B	1.249
3C	1.241
4A	1.231
4B	1.206
4C	1.263
5A	1.229
5B	1.244
5C	1.231
6A	1.215
6B	1.225
6C	1.25
7A	1.203
7B	1.241
7C	1.241
8A	1.241
8B	1.234
8C	1.253
9A	1.225
9B	1.225
9C	1.235
10A	1.034
10B	1.013
10C	1.013

Trial 3

Bin No.	Plastic Weight
1	5.74
2	5.737
3	5.861
4	5.973
5	5.846
6	5.833
7	5.807
8	5.79
9	5.559
10	5.555
11	5.981
12	5.99

Trial 4

Flask No.	Plastic Weight
1A	1.215
1B	1.238
1C	1.216
2A	1.230
2B	1.244
2C	1.232
3A	1.25
3B	1.241
3C	1.231
4A	1.218
4B	1.25
4C	1.232
5A	1.242
5B	1.235
5C	1.220
6A	1.220
6B	1.208
6C	1.205
7A	1.033
7B	1.068
7C	1.08
8A	1.220
8B	1.226
8C	1.228
9A	1.225
9B	1.241
9C	1.253
10A	1.224
10B	1.225
10C	1.238

Trial 5

Bin No.	Plastic weight
1	5.09
2	5.023
3	5.02
4	5.065
5	5.058
6	5.058
7	5.052
8	5.071
9	5.096
10	5.062
11	5.046
12	5.088

Trial 6

Flask No.	Weight of plastic
1A	1.51
1B	1.489
1C	1.449
2A	1.503
2B	1.462
2C	1.498
3A	1.495
3B	1.461
3C	1.432
4A	1.529
4B	1.545
4C	1.513
5A	1.469
5B	1.509
5C	1.44
6A	1.482
6B	1.515
6C	1.438
7A	1.431
7B	1.459
7C	1.5
8A	1.511
8B	1.447
8C	1.467
9A	1.502
9B	1.512
9C	1.5
10A	1.414
10B	1.431
10C	1.415
11A	1.415
11B	1.512
11C	1.492

APPENDIX C

Composition of Wet Synthetic waste

Trial 1							
Sawdust	Rabbit Feed	Mature Compost	Corn Starch	Sugar	Oil	Urea	Water
600	375	136	144	60	48	12	1295

Flask No.	Empty Bottle Weight	Cap Weight	Wet Synthetic Waste
1A	132	29.2	75
1B	136.1	28.6	75.2
1C	133.1	28.5	75.5
2A	129.7	28.6	75
2B	140.8	28.5	75
2C	132.5	28	75.5
3A	126.1	29.3	76
3B	133.4	28.5	75.2
3C	127.1	28.3	75.8
4A	137.7	28.3	75.3
4B	135.9	29	75.9
4C	129.3	30	75.1
5A	127.6	28.8	75
5B	133	28	76
5C	132	28	75
6A	137	29	75.3
6B	123.2	21.8	75
6C	136	28	75.2
7A	106.5	22.5	75
7B	128	28	75.8
7C	122	21	75.7
8A	124	27	75.1
8B	131	28	75.3
8C	134	22.6	75
9A	134	28.2	75.8
9B	127.5	29.5	75.4
9C	124.9	22.4	75.2
10A	105	22	75
10B	136	29.5	75
10C	106	22	74.9

Trial 2							
Sawdust	Rabbitfeed	Mature compost	Corn starch	Sugar	Oil	Urea	Water
375	234	85	90	38	30	7.6	810
Mushroom Spent waste	Rabbitfeed	Mature compost	Corn starch	Sugar	Oil	Urea	Water
140	109	40	42	18	14	3.5	414

Flask No.	Empty bottle weight	Cap weight	Weight added (Sawdust Based Synthetic Waste)
1A	132	29.2	75
1B	136.1	28.6	75.1
1C	133.1	28.5	75.5
5A	127.6	28.8	75.3
5B	133	28	75.2
5C	132	28	75.4
6A	137	29	75.3
6B	123.2	21.8	75.5
6C	136	28	75.4
7A	106.5	22.5	75.1
7B	128	28	75.3
7C	122	21	75
8A	124	27	75.1
8B	131	28	75
8C	134	22.6	75
9A	134	28.2	75.3
9B	127.5	29.5	75.2
9C	124.9	22.4	75.6
10A	105	22	75.3
10B	136	29.5	75.2
10C	106	22	75

Flask No.	Empty bottle weight	Cap weight	Weight added (Mushroom Spent Waste Based Synthetic Waste)
2A	129.7	28.6	75
2B	140.8	28.5	75
2C	132.5	28	75.3
3A	126.1	29.3	75
3B	133.4	28.5	75.1
3C	127.1	28.3	75.1
4A	137.7	28.3	75
4B	135.9	29	75.2
4C	129.3	30	75

Trial 3										
Bin No.	Bin Weight	Cap Weight	Sawdust	Rabbitfeed	Mature Compost	Corn Starch	Sugar	Oil	Urea	Water
1	436	59	257.3	141.3	45	54.5	23	18	4.5	456
2	438	60	257.8	142	45.1	54.8	22.8	18.5	4.6	454
3	412.7	59	257.6	141.9	45.3	55	22.6	18.8	4.7	454
4	421.8	61	257.2	141.6	45.2	55.1	22.8	19	4.6	455
5	435.3	59.1	257.1	142.1	45.1	54.2	22.7	18.5	4.5	456
6	425.5	59.1	257.5	142	45.2	55.1	22.9	18.6	4.6	454
7	422	59.5	257.3	141	45	55.1	22.8	18.4	4.5	456
8	421.7	59.7	257.5	141.5	45.1	55	23	18.3	4.5	455
9	434.1	60.2	257.8	141.7	45.2	54.6	22.7	18.9	4.6	455
10	434.7	59	257.4	141.6	45.1	55.1	22.6	18	4.5	456
11	435.9	59.8	257.6	141.3	45.2	56.1	22.9	18.8	4.5	454
12	438.5	59.4	257.5	141.5	45	54.1	22.6	18.5	4.6	456

Trial 4							
Sawdust	Rabbitfeed	Mature Compost	Corn Starch	Sugar	Oil	Urea	Water
375	234	85	90	38	30	7.6	810
Mushroom Spent waste	Rabbitfeed	Mature compost	Corn starch	Sugar	Oil	Urea	Water
140	109	40	42	18	14	3.5	414

Flask No.	Empty Bottle Weight	Cap Weight	Weight added (sawdust based synthetic waste)
1A	132	29.2	75.2
1B	136.1	28.6	75.3
1C	133.1	28.5	75
2A	129.7	28.6	75.1
2B	140.8	28.5	75.2
2C	132.5	28	75.6
3A	126.1	29.3	75.8
3B	133.4	28.5	75.9
3C	127.1	28.3	75.5
4A	137.7	28.3	75
4B	135.9	29	75.4
4C	129.3	30	75
5A	127.6	28.8	75.2
5B	133	28	75.6
5C	132	28	75.5
6A	137	29	75
6B	123.2	21.8	75.4
6C	136	28	75.1
7A	106.5	22.5	75
7B	128	28	75
7C	122	21	75.2
8A	124	27	75.3
8B	131	28	75.4
8C	134	22.6	75.3

Flask No.	Empty Bottle Weight	Cap Weight	Weight added (sawdust based synthetic waste)
9A	134	28.2	75.3
9B	127.5	29.5	75.2
9C	124.9	22.4	75.5
10A	105	22	75.3
10B	136	29.5	75.4
10C	106	22	75.3

Trial 5										
Bin No.	Bin weight	Cap Weight	Sawdust	Rabbitfeed	Mature compost	Corn starch	Sugar	Oil	Urea	Water
1	436	59	225.6	142	45	54.8	22.5	18.2	4.5	487
2	438	60	225.8	141.8	45.2	55	22.8	19	4.6	486
3	412.7	59	225.9	141	46	55.6	22.9	18.6	4.6	485
4	421.8	61	226	142	45.5	55.5	22.5	18.8	4.5	485
5	435.3	59.1	226	141.6	45.6	55.4	23	18.5	4.6	485
6	425.5	59.1	225.8	142	45.8	55.3	23.1	19	4.7	484
7	422	59.5	225.5	142.1	45.9	54.9	22.9	18.6	4.5	486
8	421.7	59.7	225.7	142.2	45.8	54.8	22.7	18	4.6	486
9	434.1	60.2	226	141.9	45.6	54.6	22.7	18.5	4.5	486
10	434.7	59	225.8	142	45.9	55	22.8	18.3	4.5	486
11	435.9	59.8	226.7	141.8	45.8	56	22.9	18.2	4.7	484
12	438.5	59.4	225.9	141.6	45.6	55.1	23	18	4.5	486

Trial 6							
Sawdust	Rabbitfeed	Mature compost	Corn starch	Sugar	Oil	Urea	Water
686	375	136	144	60	48	12	1210

Flask No.	Empty Bottle Weight	Cap Weight	Wet Synthetic Waste
1A	132.4	27.9	76
1B	136	28.6	75.1
1C	132.8	28.2	75
2A	129.6	28.5	75.6
2B	129	29.3	75.6
2C	132.4	28.8	75.8
3A	125.6	27.5	75.4
3B	133.3	27.7	75.3
3C	132.4	28.6	75.2
4A	137.6	28.6	75.5
4B	135	28.7	75.8
4C	129	29.2	75.9
5A	129	27.7	75.7
5B	133.6	29.3	76
5C	132	28.7	75
6A	137	28.5	75.8
6B	123.1	22	75
6C	137	30.1	75.4
7A	106	21.6	75.3
7B	128.4	28.4	75.8
7C	134.5	28.8	75.7
8A	123.5	28.1	75.5
8B	131.5	27.7	75.4
8C	132.6	22.3	75
9A	134.6	28.6	75.6
9B	127.5	28.3	75.4
9C	135.9	27.7	75.5
10A	105.7	21.7	75.5
10B	124.8	22.5	75
10C	106.8	22.5	75.3
11A	163.5	19.8	75.2
11B	150.2	20.3	75.6
11C	160	19.5	75.4

VITA

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