

BIODEGRADABILITY OF PLA AND PET BASED PLASTICS IN SOIL

by

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ABSTRACT

BIODEGRADABILITY OF PLA AND PET BASED PLASTICS IN SOIL

Plastics are widely used because of their advantages. In order to deal with the plastic accumulation problem, the term biodegradation and developing technology bioplastic seems to be promising for this problem. This study investigates the change in biodegradation potentials for biodegradable polylactide acid (PLA) and conventional polyethylene terephthalate (PET) polymer. These two types of polymers have similar uses in industry. Soil burial experiment was conducted by following the Standard Test Method ASTM D5988 – 12 to observe evaluation and compare the biodegradability of two plastic types. Plastic samples were buried in the soil, which is the source of matrix and inoculum. The test method consists of determining the aerobic biodegradability of the selected plastic materials. For this purpose, the carbon dioxide generated by the microorganisms was measured and biodegradability was revealed as a function of time. PLA based and PET based plastic samples showed 1.0775 % and 0.3683 % biodegradation respectively at the end of the experiment which last 190 days. In order to be sure about the environmental conditions, positive reference material was used in the test. It also proves that the soil is fertile enough for a biodegradation test. Positive reference material showed 100% biodegradation and demonstrated that test is conducted in the required conditions. The amount of carbon which was used by the microorganism indicated the biodegradation tendency of the buried polymer. This study revealed that not only conventional plastics but also selected bioplastic were very resistant to the environmental conditions and microbial attack.

ÖZET

PLA VE PET ESASLI PLASTİK BİYOBOZUNURLUĞU

Plastikler, sağladıkları avantajlar nedeniyle yaygın olarak kullanılmaktadırlar. Plastik birikim problemiyle başa çıkmak için, biyobozunma ve biyoplastik terimi umut verici görünmektedir. Bu çalışma, benzer kullanım alanları olan iki tür polimerin biyolojik olarak parçalanma özelliklerini incelemektedir. Bu iki polimer biyolojik olarak parçalandığı bilinen polilaktit asit (PLA) ve yaygın olarak kullanılan polietilen tereftalat (PET)'dir. Bu çalışmada biyolojik bozulmayı gözlemlemek için ASTM D5988 – 12 Standart Test Yöntemi takip edilerek toprağa gömme deneyi gerçekleştirilmiştir. Seçilen plastik numuneler, mikroorganizma bakımından zengin toprağa gömülmüştür. Test yöntemi, seçilen plastik malzemelerin aerobik biyobozunurluğunun belirlenmesinden oluşur. Bu amaçla, mikroorganizmalar tarafından ortama salınan karbondioksit ölçülür ve zamanın bir fonksiyonu olarak biyobozunurluk ortaya çıkar. PLA bazlı numuneler, 190 günlük toprak gömme testinin sonunda % 1.0775 biyobozunurluk gösterirken, PET bazlı numuneleri % 0.3683 biyobozunurluk göstermiştir. Deneyin çevresel koşullarından emin olmak ve biyolojik bozunma testinde toprağın yeterince verimli olduğunu kanıtlamak için testte pozitif referans materyali kullanılmıştır. Pozitif referans materyali, deney sonunda % 100 biyobozunma göstererek deneyin gereken koşullarda yapıldığını göstermiştir. Mikroorganizma tarafından kullanılan karbon miktarı, gömülü polimerin biyolojik bozunma eğilimini gösterir. Bu çalışma, sadece geleneksel plastiklerin değil, aynı zamanda seçilen biyoplastiğin de çevresel koşullara ve mikrobiyal saldırılara karşı çok dirençli olduğunu ortaya koymuştur. Bu çalışma ile plastiklerin doğal ortama gelişigüzel atılmaması gerektiği sonucuna ulaşılmıştır.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation
O ₂	Oxygen
CO ₂	Carbon Dioxide
H ₂ O	Dihydrogen Monoxide
C ₃ H ₄ O ₂	Polylactic Acid
C ₁₀ H ₈ O ₄	Polyethylene Terephthalate
C	Carbon
N	Nitrogen
(NH ₄) ₂ HPO ₄	Diammonium Phosphate
Ba(OH) ₂	Barium Hydroxide
BaCO ₃	Barium Carbonate
HCl	Hydrochloric Acid
BaCl ₂	Barium
W	Weight
Abbreviation	Explanation
ASTM	American Society for Testing and Materials
PLA	Polylactic Acid
PET	Polyethylene Terephthalate
NPK	Nitrogen – Phosphorus - Potassium Compound Fertilizer
CMR	Cumulative Measurement Respirometric
GMR	Gravimetric Measurement Respirometric
CL	Confidence Limit

1. INTRODUCTION

Plastic is a material consisting of inorganic and organic raw materials such as carbon, oxygen, hydrogen, nitrogen, chloride and silicon (Shah et al., 2008). They can be produced by the conversion of natural products and raw material obtained from petroleum, coal or natural gas. Plastics can be much lighter, stronger, corrosion resistant, durable and better insulator than wood, metal, glass, leather, paper and rubber. With these properties plastic material serves for the same purpose in an economical way, which makes it most widely preferred material (Gervet, 2007). In the last 50 years, plastics production increased from 15 million to 311 million tons from 1964 to 2014 (Barnes et al., 2009).

The packaging industry is the largest market where plastics are used due to the alteration of the packaging products from reusable form to single use form (Geyer et al., 2017). Therefore, the percentage of plastics concentration has increased gradually in solid waste (Meng et al., 2015). The plastic quality has improved in over time. However, stability and durability have progressed so that plastics are very resistant to environmental conditions (Mueller, 2006). Conventional polymers, such as polyethylene and polypropylene, are known to last in the environment after disposal or entering the environment as litter (Barnes et al., 2009). It can be stated that commonly used plastic types can stay in the environment for a long time.

The advantages of plastic material have turned into extreme plastic accumulation both in the environment and in the disposal areas. Therefore, studies and researches continue to suggest solutions to these increasing problems. The most promising term among these studies appears to be biodegradation. Biodegradation is a possible way that may occur after the plastic material has entered the environment. Biodegradable indicates a material that its decomposition is capable to undergo into carbon dioxide, methane, water, inorganic compounds, or biomass (Song et al., 2009). This process may offer a solution to the unwanted plastic waste accumulation since the polymer is used as a substrate by the microorganisms. Biodegradation is a process whereby bacteria, fungi, yeasts and their enzymes can utilize plastic (Gautam et al., 2013). Aerobic biodegradability occurs according to this basic formula (Pagga, 1997).



Recent decades, there have been a great number of developments in the bioplastic field, especially for food contact materials and particularly in the packaging field. Conventional plastics are known to be dangerous for human health. Bioplastics are offering a solution to plastic waste. The term bioplastic is used for the definition of plastic bio-based or biodegradable (Andreeßen et al., 2018).

Plastics can be produced from natural origins (plants, animals or microorganisms). For instance, polylactic acid (PLA) is a widely used plastic type, which belongs to these types of plastics (Rujnić-Sokele et al., 2017). PLA is chosen as a test specimen in this study for the encouraging feature. There are plastics which are made from petrochemical sources and so they do not have the biodegradation ability. This group consists of classical or traditional plastics (Rujnić-Sokele et al., 2017). Polyethylene terephthalate (PET) based samples are used in this study which represents to achieve the opportunity to compare the biodegradation abilities.

The aim of this study is to obtain biodegradability of a plastic sample after entering the environment. This study will provide information for industries to develop more environmentally friendly products and municipalities to build more sustainable waste management models. In order to obtain a comparative result, two types of plastics are chosen and soil burial test was conducted by following standard method ASTM D5988 – 12. The test method consists of determination biodegradability under aerobic conditions. For this purpose, carbon dioxide production is measured, expressed as a fraction of calculated carbon content and reported with respect of time. The results are evaluated to achieve more precise information toward the fate of PLA and PET based plastics after entering the environment. Most biodegradation studies conducted with plastic samples recommends to inoculum the soil with proper microorganism before the experiment. Powdering the sample, irritating the surface of the sample, etc. are also recommended steps that provides higher biodegradation percentages. Since this study investigates the natural occurring process of a sample entered the environment, biodegradation speeding steps have been avoided.

2. LITERATURE REVIEW

Plastics are assumed to be the most widely used polymers in our daily life. The annual production of petroleum based plastics exceeded 300 million tons in 2015 (Mekonnen et al., 2013). But, plastics for single use or short-term use, especially the packaging materials, are dominated in the market. Approximately 30% of plastic is used in packaging applications like food, pharmaceuticals, cosmetics, detergents and chemicals (Shah et al., 2008). The excessive utilization of plastics causes an increase in plastic waste that is disposed in landfills, incinerated or recycled as waste. There is also a big amount that escaped into the environment. Such as roadsides, parks, beaches, ocean and natural areas are inundated with plastic debris pollution (Gómez et al., 2013).

Over time the quality of plastics have been improved. So the stability and durability have progressed so that plastics are very resistant to ambient conditions (Mueller, 2006). It is obvious that this will cause a plastic accumulation in the nature with time. Therefore, the pollution of the natural environment with plastic waste is a growing concern. That also forces industries to investigate and develop new technologies. The term biodegradation and bioplastics are drawing promising attention for this issue.

2.1. Plastics and Classification

Plastics are (mostly) synthetic (human-made) materials, made from polymers, which are long molecules built around chains of carbon atoms, typically with hydrogen, oxygen, sulfur, and nitrogen filling in the spaces.








Plastics can be classified into four types for their origins and biodegradability. These four types are conventional plastic, bio-based plastic, biodegradable plastic and biodegradable bio-based plastic (Table 2.1).

Table 2.1. Classification of plastics (Gómez et al., 2013).

Class	Source	Biodegradable	Example
I	Petroleum / natural gas	No	Polyethylene, polypropylene.
II	Petroleum / natural gas	Yes	Polylactic acid from petroleum.
III	Biomass (Corn, sugar cane, etc)	No	Polyethylene derived from corn ethanol.
IV	Biomass (Corn, sugar cane, etc)	Yes	Polyhydroxyalkanoates based resin, Polylactic acid derived from starch.

Group I plastics are made from petrochemical sources. They are not capable to biodegrade. This group consists of plastics, which are known as classical or traditional plastics, like polyethene, polypropylene, polystyrene, polyvinyl chloride, etc (Rujnić-Sokele et al., 2017). Table 2.1 describes some commonly used plastics and their applications (Alshehrei et al., 2017).

Table 2.2. Plastics and applications (Alshehrei et al., 2017).

Plastic Recycling Symbol	Plastic Name	Where to Find This Plastic in Your Home	This Plastic is Valued For
	Polyethylene Terephthalate	water and soda bottles	clarity strength impermeability to gas and moisture
	High Density Polyethylene	milk jugs, grocery bags and toiletry bottles	stiffness strength resistance to moisture permeability to gas
	Polyvinyl Chloride	water pipes, blister packaging for non-food items	strength ease of blending with other materials versatility
	Low – density Polyethylene	food bags, squeezable bottles, cling films, disposable cups	flexibility ease of processing ease of sealing barrier to moisture
	Polypropylene	microwaveable containers, yogurt cups, disposable plates/cups	strength resistance to heat, chemicals, oils and moisture
	Polystyrene	disposable plates, cups, cutlery, containers and packing peanuts	clarity versatility molding ease
	Other (often Polycarbonate or ABS)	beverage bottles, CD's, lenses for glasses, riot shields	properties depending upon the mixture of polymers may contain BPA

The second group plastics are made from petrochemical sources. This type of plastic is biodegradable. But, this group has great potential to contribute to greenhouse gas emission. In addition to the greenhouse gas emitted while production, they release carbon dioxide when they biodegrade (Gómez et al., 2013). They are mainly used in combination with both biomass and fossil-derived resin to decrease manufactory prices, increase the bio-based content and improve material performance (Song et al., 2009). Petrochemical based biodegradable polymers such as aliphatic polyesters (e.g. polyglycolic acid, polybutylene succinate and polycaprolactone), aromatic co-polyesters (e.g. polybutylene succinate terephthalate) and polyvinyl alcohol are known to be produced from monomers that are derived from petrochemical refining. This source material has certain degrees of inherent biodegradability (Song et al., 2009).

Group III plastics are produced from biomass. They do not have any biodegradation property. These plastics are mainly made from bioethanol biofuel like polyethene. The bioethanol is used for ethylene and bio-polyethene production. These groups consist of bio-polyvinyl chloride, bio-

polyethene terephthalate or bio-polypropylene plastics (Kawada et al., 2003; Lütke-Eversloh et al., 2001; Rujnić-Sokele et al., 2017).

Plastics in the fourth group are produced from natural origins (plants, animals or microorganisms) and have biodegradation property. The examples in this group are polysaccharides (e.g. starch, cellulose, lignin and chitin), proteins (e.g. gelatine, casein, wheat gluten, silk and wool) and lipids (e.g. plant oils and animal fats). Natural rubber and certain polyesters both produced by microorganism / plant (e.g. polyhydroxyalkanoates and poly-3-hydroxy-butyrates) or synthesized from bio-derived monomers (e.g. polylactic acid) (Gómez et al., 2013; Rujnić-Sokele et al., 2017; Tokiwa et al., 2009). It is important for plastics to be bio-based or biodegradable except for this classification. The term bioplastic is used for bio-based or biodegradable plastics. Bioplastics are offering solution to the plastic accumulation problem (Andreeßen et al., 2018).

Bio-based plastics are produced from biological and renewable resources (Gómez et al., 2013; Tokiwa et al., 2009). The term biodegradable used for polymers or plastics to indicate a material which has the capacity to separate into carbon dioxide, methane, water, inorganic compounds, or biomass (Song et al., 2009). All bio-based plastics are not biodegradable. (Jariyasakoolroj et al., 2018) On the other hand, not all biodegradable plastics are bio-based (Rujnić-Sokele et al., 2017).

Biodegradable plastic can be defined as a plastic in which all the organic carbon can be converted into biomass, water, carbon dioxide, and/or methane via the action of naturally occurring microorganisms such as bacteria and fungi (ASTM D883). Biodegradable plastics have been developed from the viewpoint of biodegradability, which possibly provides a solution to the plastic waste problem (Iwata, 2015).

In recent decades, there have been a great number of developments of bioplastics particularly in packaging field. According to current estimates, the global production of bioplastics is expected to grow at an annual rate of up to 30% in the coming decade to reach 3.5 million tons in 2020 (Shen et al., 2010). This is because of the rise in environmental awareness and the implementation of coercive environmental regulations (Jariyasakoolroj et al., 2018). On the other hand, plastic waste is becoming a major environmental problem and creates a great concern with the increase in the use of plastics. In addition to the environmental awareness, the depletion of fossil resources has established a market for bio-based plastics, whereas most plastics are produced from petrochemical sources (Anderson et al., 2016). Plastics are generally known for their resistance of biodegradation. In other words, plastics are known as they cannot be separated or degraded into basic primary

compounds such as air, water or soil, although they have passed through a certain period of time in contact with microorganisms (Bura, 2019).

Global environmental awareness increase has developed new generation materials, products and processes. Research from the 1990s onwards has led to the introduction of many new bio-based products (Mohanty et al., 2005). New generation food packages are being produced from natural biodegradable plastics such as starch, polylactic acid, and poly-hydroxy alkanates (Jariyasakoolroj et al., 2018). Polycaprolactone, polyhydroxyalkanoates and polylactic acid have biodegradable capacities. These polymers are produced in diverse shaped products like bottles and sheets (Guzman et al., 2011; Mohanty et al., 2005).

PLA is produced from lactic acid. PLA is rigid (Riaz et al., 2018) and strength. This property makes it highly preferable in the packaging industry. PLA addition gives biodegradable, antimicrobial and antioxidant properties (Jamshidian et al., 2010). PLA are synthesized by microorganisms (a fermentation process) that use glucose obtained from corn, sugar beet, sugar cane, potatoes, and other biomasses as the carbon source. This microbial synthesis is being known as biorefinery method (Gupta et al., 2007; Iwata, 2015). There are also industrial methods to produce PLA. To reduce the costs and environmental footprint, engineered yeast is being used to convert sugar to lactic acid (Guzman et al., 2011). The chemical structure of PLA is shown in Figure 2.1.

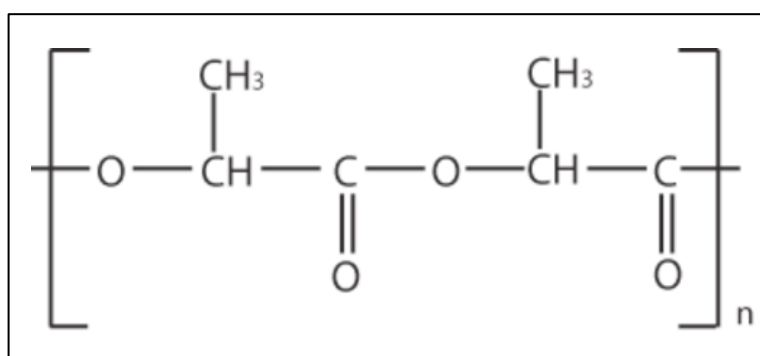


Figure 2.1. Chemical structure of PLA.

PLA is a water insoluble polymer when its molecular weight is sufficiently high. As the water penetrates the bulk of the polymer, it subjects to degrade. The long polymer chains convert into short polymer chains with low molecular weight, which are water soluble monomers (Middleton et al., 2000). The water-soluble monomer is able to liberate from the matrix. The degradation process begins since the oligomers are close to the surface. The amount of carboxylic chain ends increases,

which auto-catalyses the ester hydrolysis, during the degradation process. Water-soluble oligomers contributed to the autocatalytic effect (Gupta et al., 2007; Guzman et al., 2011; Middleton et al., 2000).

PLA polymer properties (Jariyasakoolroj et al., 2018) and performance (Jariyasakoolroj et al., 2018; Riaz et al., 2018) are similar to PET. PET chemical structure is shown in Figure 2.2.

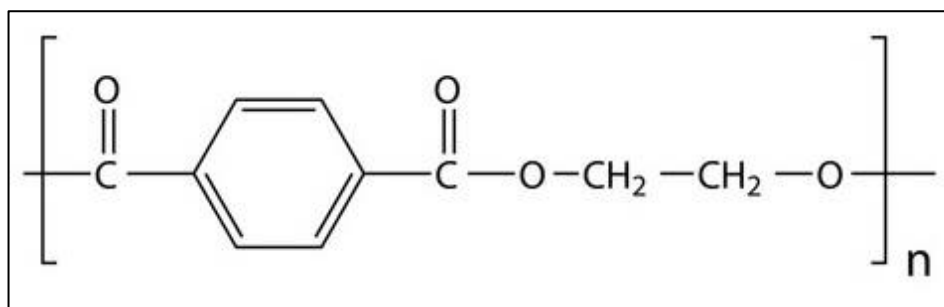


Figure 2.2. Chemical structure of PET.

PET is commercially used for food packaging applications. PET also provides great material property. It is also very resistant to atmospheric and biological agents (Eubeler et al., 2010; Fotopoulou et al., 2017; Mueller, 2006). PET is produced partially based on biomass-derived precursors as bulk. Generally, PET is used in packaging applications especially bottles and containers. The bio-based PET is produced from ethanol, which is synthesized from cornstarch or sugar cane. This ethanol is then converted in mono-ethylene glycol and combined with fossil based terephthalic acid by conventionally applied transesterification in order to yield partially bio-based PET (Andreeßen et al., 2018).

2.2. Biodegradation

Chemical, physicochemical (photo-degradation, thermal degradation, and mechanical degradation), or biological processes cause some changes on the physical or chemical properties of a plastic material, which is termed as degradation (Fotopoulou et al., 2017). It is proven that the term biodegradation is a consequence of microbial activity grown on the surface and/or inside the plastic (Mohee et al., 2008). So, the biodegradation can be defined as a process whereby bacteria, fungi, yeasts and their enzymes can utilize plastic as a food source so that its original form disappears (Gautam et al., 2013). On this basis, it is possible to say that biodegradation consists of three steps: (1) Biodegradation, is the mechanical, chemical and physical modifications of the polymer caused by microorganisms (Emadian et al., 2017). (2) Bio fragmentation is the process

where high molecular weight polymers are fragmented into a mixture of oligomers and/or monomers. It is an essential step for the sequent process called assimilation (Guzman et al., 2011). (3) Assimilation, is the integration of atoms, from fragments of polymeric materials, inside a microbial cell. This is the mechanism that provides the necessary source of energy, electrons and elements (i.e. carbon, nitrogen, oxygen, phosphorus, sulfur and so forth) that is consequently used for cell structure formation (Lucas et al., 2008).

The microorganisms present in the soil are playing role in biodegradation process differ from each other and have their own optimal growth conditions. Since the polymers are potential substrates for heterotrophic microorganisms, the biodegradation of plastics precedes under different soil conditions (Shah, 2007). The physical and chemical structures of the polymers, type of organisms present in the environment are the basic properties that affect biodegradation. Besides molecular structure, biodegradation depends on crystallinity and complexity of polymer formula, etc. In fact, the specific functional groups are selected by enzymes and can be processed. Moreover, the environment, in which the polymers are placed or disposed of, plays a key factor on their biodegradation. The pH, temperature, moisture and the oxygen content are among the most significant environmental factors that must be considered in the biodegradation of polymers (Fotopoulou et al., 2017; Massardier-Nageotte et al., 2006) (Figure 2.3).

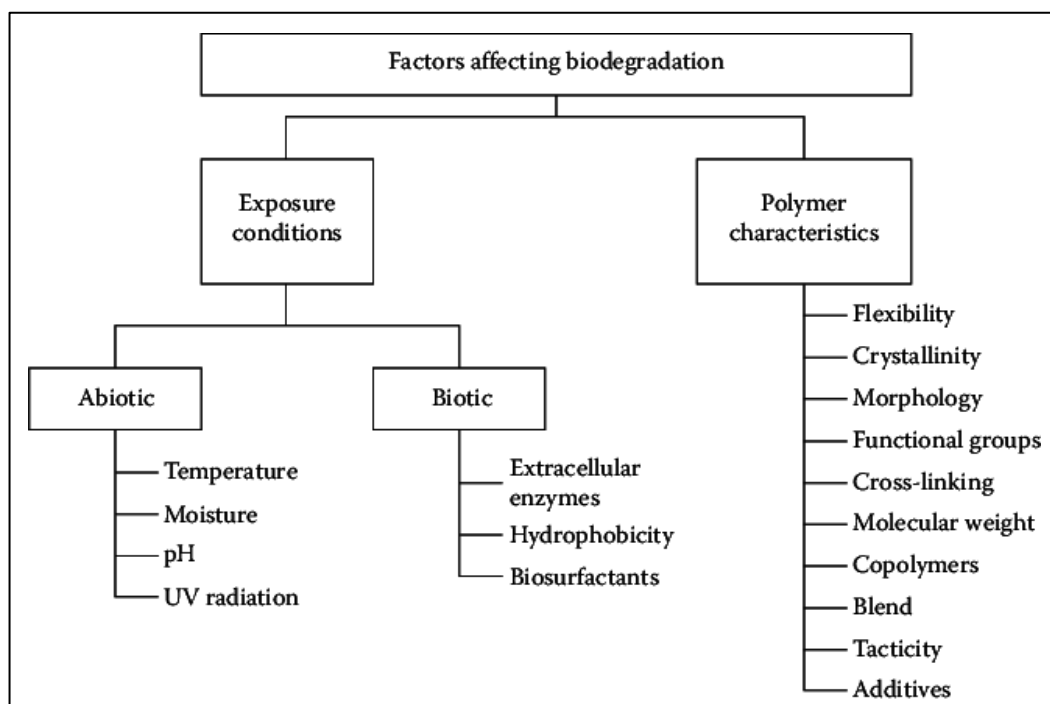


Figure 2.3. Biodegradation affecting factors (Kijchavengkul et al., 2008).

There are many methods and procedures to measure and/or calculate biodegradation. Visual observations, weight loss measurements, changes in mechanical properties and molar mass, radiolabeling, clear-zone formation, enzymatic degradation, controlled composting test, oxygen consumption and carbon dioxide evolution are some of these methods (Shah, 2007). Among many ways, carbon dioxide evolution is measured according to standard method ASTM D5988. This test is conducted under aerobic conditions. Microorganisms use oxygen to oxidize carbon and generate carbon dioxide as metabolic product under this condition. Consequently, the formation of carbon dioxide is measured as the indicator in order to measure biodegradation of the polymer (Hoffmann et al., 1997; Shah, 2007). To obtain the biodegradation accurately, background respiration is also identified in this test.

Plastics are known as the most versatile materials used worldwide, which are synthetic chemicals and the extensive use causes a huge plastic waste accumulation in the environment. Therefore, bioplastics seem to be an alternative due to their biodegradability. Consequently, studies were conducted to investigate the biodegradability of bioplastics under different environmental conditions such as soil, compost marine and aquatic environments. Some researchers reported successful biodegradation results where some did not observe any biological degradation at all. Due to the microbial diversity, most studies were conducted in soil and compost environment (Anstey et al., 2014).

A study on the plastic biodegradability was conducted by Adhikari et al. (2016) to analyze the biodegradation potential of three kinds of bioplastics: Poly (butylene succinate) and Poly (butylene succinate)-starch and PLA, and a non-biodegradable petrochemical plastic. Plastic samples were powdered and buried in an agricultural field. The surface structures of these samples were observed using a scanning electron microscope after 2 years test period. Observations showed that the surfaces of bioplastic samples drastically changed, where the non-biodegradable plastic samples remained unchanged. Even though the degradation rates differed among the bioplastics, confirmed the decay and biodegradability of bioplastics in the soil environment.

Further studies conducted under controlled environmental conditions in order to investigate the biodegradability of PLA. Whereas some studies stated the rate of PLA degradation in the soil is relatively slow (Ahn et al., 2011; Ohkita et al., 2006; Shogren et al., 2003). In order to shorten time and ensure biodegradation of PLA in soil Apinya et al. (2015) conducted a study also in soil, which was inoculated with a selected type of microorganism. The biodegradability was evaluated by measuring carbon dioxide produced from each reactor containing mixtures of PLA films and

enriched soil. The study stated that the PLA biodegradation in the soil which was inoculated with the selected microorganism higher than that in un-inoculated soil.

In a study stated that PLA is relatively expensive and suggests blending natural biomaterials to reduce the PLA composites price (Wu, 2012). Sisal was claimed to be an abundant natural fibers resource that can improve the mechanical properties. Therefore, sisal fibers composites and PLA samples were used for a 6-10 weeks soil burial test. The result showed that the degree of biodegradation increased with increasing sisal fibers composite content.

Another supporting investigation was conducted with PLA, PLA/NPK (NPK: fertilizer), and bioplastic coated fertilizers composite samples. Thermal properties of bioplastic coated fertilizers composites were investigated by thermogravimetric analysis, differential scanning calorimetry, and morphological and degradation properties were analyzed by scanning electron microscopy and soil burial test was conducted to observe the biodegradability for time periods varying between 2 to 8 weeks. The result showed that the biodegradation rates differ with the natural additives (Harmaen et al., 2015). Further study was conducted by Lv et al. (2018) in order to investigate the biodegradation of starch/PLA composites. The study obtained that starch has accelerated the biodegradation of PLA based materials.

The most commonly used method to calculate the percentage of biodegradation of polymers is measuring the carbon dioxide generated during aerobic biodegradation. The ASTM method works with this principle. According to ASTM method, carbon dioxide generated from each reactor was trapped with an absorbing solution and is then titrated in order to report biodegradation.

A study was conducted to accelerate the biodegradation of PLA samples upon reducing the molecular weight of the samples by ultraviolet irradiation. PLA sheets were buried in soil amended with various microbial sources. The aerobic biodegradation of the buried PLA samples carried out according to the standard test methods of ASTM D5338 – 11 and ASTM D5988 – 12. Carbon dioxide generated during the test, external morphology of PLA samples, the pH and the number of bacteria in the soil mixtures are determined in order to reveal the biodegradation (Apinya et al., 2015).

Another investigation examined the influence of the chain extender and montmorillonite clay on the biodegradation of PLA blends. Carbon dioxide generated from each flask was trapped by a potassium hydroxide solution and biodegradation was calculated according to the standard method

ASTM D 5988 – 12. The surfaces of films were observed by scanning electron microscopy and FTIR spectroscopy before and after biodegradation (Freitas et al., 2017).

Another study that confirms the PLA degradation in natural environment was conducted in South Finland (Gallet et al., 2001). PLA polymers were buried for two years test period. The change in polylactide matrix was observed via comparing the original un-aged samples with samples recovered from soil after 8, 12, 20 and 24 months soil burial test.

It is stated that biodegradation is a temperature depended process. In order to investigate the biodegradation under different temperature, Shi et al. (2010) conducted an anaerobic test. Three different PLA samples were used under two different temperatures, 35 and 50 °C. It was found that PLA's anaerobic biodegradation significantly decreased with temperature.

Further investigations also proof the temperature effect on biodegradation rate of PLA. For instance Karamanlioglu et al. (2013), conducted a test with PLA pieces at range temperatures of 25°, 37°, 45°, 50° and 55°C in soil and compost environment. This comparative study obtained the results via measuring loss of molecular weight and tensile strength. However, it stated that there was not any change observed in molecular weight or tensile strength in low temperature. The study also stated that non-biodegradability of PLA under low temperature suggests accumulation problem of PLA in the environment and can cause future pollution issue.

Various environmental factors that affect the biodegradation process were investigated in further studies. These studies research differences in soil and climatic conditions (Boyandin et al., 2013). Polyhydroxyalkanoate samples were buried in two close locations, which differed in microbial communities. As the test was conducted in Vietnam, high temperature and humid environmental conditions were expected during the 10-12 months of soil burial period. Air, soil temperatures and also humidity values were monitored. The result shows that polyhydroxyalkanoate degradation in tropical soils is influenced by polymers chemical composition, specimen shape, and microbial genera.

Studies in literature showed that not only the environmental conditions but also the morphological specification of the test material could affect the biodegradation process. Further studies were conducted to enrich the existing knowledge about the long-term biodegradation in soil environment by using PLA films of various thicknesses and of PLA fibers. Biodegradation was studied in full-scale field experiments by simulated soil burial experiments under controlled

laboratory conditions. After 11 months burial, samples were analyzed using visual inspection, mechanical testing, differential scanning calorimetry method and fourier transform infrared spectroscopy analysis. This investigation showed that thickness and also form of material is playing an important role in the biodegradation process (Rudnik et al., 2011).

Besides aerobic conditions, biodegradable plastics were also tested under anaerobic conditions. The investigation stated that the environmental degradability of plastics is a complex process that is influenced by the nature of the plastics and the conditions to which they are exposed (Mohee et al., 2008).

In addition, further biodegradation studies have been carried out with PLA in real and simulated composting conditions in order to compare the obtained results (Kale et al., 2007). The study asserted that the current standard methodologies, ASTM and ISO provide a traditional way of testing. Further investigations with PLA were conducted by using compost as a microbial community for the biodegradation (Mihai et al., 2014; Tabasi et al., 2015). Composting is a process in which the organic matter is converted to carbon dioxide and a soil-like material (humus) by microbial activity (Kale et al., 2007). As defined by the American Society for Testing and Materials (ASTM), compostable plastic, is a plastic that undergoes by biological processes during composting to yield carbon dioxide, water, inorganic compounds, and biomass (ASTM D6400 – 04). Therefore, it is possible to say that a compostable plastic is biodegradable, but a biodegradable plastic could not always be compostable (Kale et al., 2007).

Ahn et al. (2011) studied the biodegradability of three types of bioplastic pots in a compost environment. The experiment was conducted under laboratory conditions and biodegradation was observed by measuring carbon dioxide production. It is reported that the reactors which were containing mixtures of pot fragments and compost inoculum were kept at 58°C for 60 days. The biodegradability of pots was reported as follows: first pot type (containing 5% poultry feather, 80% PLA, 15% starch), and the second pot type (containing 50% poultry feather, 25% urea, 25% glycerol), 53% and 39%, respectively. It is observed that more than 85% of the total biodegradation of these bioplastics occurred within 38 days. On the other hand, this investigation reported only 13% degradation on the third pot type that was composed of 100% PLA.

PLA bottles were tested for biodegradability by Kale et al. (2007). Different from the study conducted by Ahn et al. (2011), study results showed that PLA bottles have a high tendency to biodegrade in compost environment. Biodegradation was reported as 84.2% and 77.8% for

cumulative measurement respirometric (CMR) and gravimetric measurement respirometric (GMR) systems respectively. It is reported that biodegradation observed for PLA bottles in both simulated and real environmental conditions explored in the study matches well with theoretical degradation and biodegradation mechanisms.

Sarasa et al. (2009) investigated the biodegradation degree of PLA with and without corn in its composition via subjecting the samples to aerobic degradation at a constant temperature of $58 \pm 2^\circ\text{C}$ for 90 days. The average biodegradation degree of PLA samples, with and without corn, was reported as 63.6% and 79.7% respectively.

Biodegradable and conventional plastic has nearly similar uses in industry; there are many differences in polymer type and behavior. Therefore, two polymer types have been investigated in many studies Janczak et al. (2018) conducted an experiment in order to examine biodegradation of PLA and PET in the soil in which selected plant species were cultivated. Additional studies conducted on degraded PET bottles collected from the bottom of the Aegean Sea showed that PET surfaces remained unchanged for almost 15 years (Ioakeimidis et al., 2016).

3. MATERIALS AND METHODS

Laboratory scale soil burial test was set up to understand the biodegradation rate of the selected plastic materials. For this, the ASTM D5988 – 12 Standard Method was followed. This test method involves the determination of the degree of aerobic biodegradability of plastic materials in contact with soil and formulation additives under laboratory conditions.

Soil and test specimens are prepared before the experiment, as described in the initial preparation section. In this study, the soil is the natural environment, where the microorganisms present, expected to biodegrade the selected and buried plastic samples. After the prepared soil and test specimens are loaded to the reactors, they demonstrate real environmental conditions to understand the behavior of plastic in the environment.

Samples are buried and the soil is incubated in the sealed reactor during the test. Generated carbon dioxide is trapped via designated absorbing solution in the biodegradation process. Before the trapping capacity of the solution exceeds, the reactors are opened and carbon dioxide generated during the incubation period is measured by titrating the solution. This calculated data enables to observe the biodegradation rate in time.

The test was conducted for 190 days as the method suggests. End of the test, soil and test specimens are analyzed and characterized in order to reveal changes besides the biodegradation.

3.1. Initial Analysis

210 mm desiccators were used as a reactor. This test requires two types of blanks for an accurate result. One blank set is used without the addition of any test material to determine the background activity of soil itself. The other blank set is used as a technical control. The technical control contains only the absorbing solution without any soil or test material. This set will allow measuring the carbon dioxide introduced to the system by the air.

The degradation of the positive reference material is used as an indication that biodiversity persists and fragmentation continues. However, it is not a measure of the corresponding biodegradation (Briassoulis et al., 2018). GE Healthcare – Whatman branded Chromatography sheets were used as positive reference material. These sheets are known to be produced from pure

cellulose. So, they were used in the experiment to observe the viability of the soil microbial community (Figure 3.1).



Figure 3.1. Chromatography sheets.

A real environment simulation was established in the test. For this purpose, natural and fertile soil was collected from the surface layers of a nearby forest. The soil has been taken from multiple and diverse locations to maximize biodiversity and to be sure that the soil have not been exposed to pollutants which cause significant perturbations of the microbial population. The soil has been sieved to less than 2 mm particle size. Plant materials, stones and other inert materials have been removed (Figure 3.2). It has been stored in a sealed container at 5°C, for 3 weeks. The soil has been analyzed for pH, moisture (total solids—dry solids) and ash (total solids—volatile solids) contents. At this stage of the study, it is important that the pH between 6.0 and 8.0 as stated in the methodology followed.



Figure 3.2. Sieved soil.

PLA glasses and PET bottles were fragmented for this study. These two different and widely used plastic products related to compare the biodegradation abilities. PLA based plastic sample was provided from Bionatic GmbH & Co. KG. This brand is known with producing sustainable bioplastics and offers environmentally friendly packaging for food and food service. 500 mL water bottle used for PET specimen which was used widely in Turkey. PET is the other buried test specimen in this study. This experiment will show the biodegradability and compare the biodegradation of these two plastic types.

The standard method for this study indicates that it is important the test specimens have sufficient carbon content to yield enough carbon dioxide. This method evinces that approximately 200 mg to 1000 mg carbon is proper for 500 g soil to supply a good environment for suitable the degradation process. Plastic samples were loaded to the reactors so that each reactor contained 600 mg of carbon content. Where (1 mole PLA ($C_3H_4O_2$), contains 36 g carbon and weight 72 g, it was appropriate to bury 1.2 g PLA sample. 1 mole PET ($C_{10}H_8O_4$) that contains 120 g carbon and weights 192 g burying 0.96 g PET sample loaded the system with 600 mg carbon.

The method followed for the study suggests using the fragmented test specimens. So, the samples were cut into the same sized pieces as possible as shown in Figure 3.3.



Figure 3.3. Plastic fragments.

3.2. ASTM Procedure

ASTM method was used in the experiment. The method is summarized below:

500 g of soil was placed in the bottom of the reactors. Soil was amended with nitrogen to give a Carbon:Nitrogen (C:N) ratio between 10:1 and 20:1 (by weight) by adding the proper volume of ammonium phosphate solution. Distilled water was added to bring the moisture content of soil between 80 to 100 %. The reactors weight was recorded. plastic samples and positive reference materials were added to the soil.

100 mL of 0.025 N barium hydroxide solution in a 150 mL beaker and 50 mL of distilled water in a 100 mL beaker placed on the perforated plate inside the reactors. The reactors were sealed and placed it in the dark cabinet. Barium hydroxide solutions were titrated with hydrochloric acid and the consumed amount is then used to asses biodegradability of the buried plastic samples.

3.3. Reactor Set Up and Operation

The test is performed in triplicate for each plastic samples and soil blanks. For this purpose, three reactors are loaded with PLA based plastic samples and three other reactors are installed with PET based plastic samples. Two parallel reactors are loaded for technical control and positive reference material. Experimental setup is summarized in the Table 3.1.

Table 3.1. Experimental setup.

Number of reactors	Content	Scope	Reactor Name
3	Soil and PLA based plastic samples	To observe the biodegradability of PLA	PLA 1, 2, 3
3	Soil and PET based plastic samples	To compare the biodegradability of PLA and PET	PET 1, 2, 3
3	Soil only blank	To find out the background activity of soil itself.	Soil only blank
2	Absorbing solution without soil (Technical control blank)	To account carbon dioxide introduction into the system via air	Technical Control
2	Positive reference material	To observe the viability of the soil microbial community	Cellulose

500 g soil is loaded to each reactor. 600 mg carbon loaded to each reactor via plastic samples. The soil is enhanced with nitrogen by adding the proper volume of ammonium phosphate solution. Same amount of nitrogen is added to the soil only blank reactor and positive reference material reactors. Table 3.2 shows the exact weights of specimens loaded to each reactor.

Table 3.2. Amount of the test specimens loaded to the reactors.

Reactors	Weight, g
PLA 1	1.2040
PLA 2	1.2087
PLA 3	1.2060
PET 1	0.9643
PET 2	0.9620
PET 3	0.9629
Cellulose 1	1.3507
Cellulose 2	1.3505

Deionized water is added to bring the moisture content up to 80 % to 100 % for each soil pile designated in the reactors. Reactors are weighed in order to keep the moisture content in the requested rang by adding deionized water in every carbon dioxide analysis (Table 3.3).

Table 3.3. Weight of reactors.

Reactors	Reactor + Lid, g	Reactor, g
SOIL 1	4387	3228
SOIL 2	4474	3344
SOIL 3	3519	2441
PLA 1	4352	3072
PLA 2	4408	3231
PLA 3	4534	3255
PET 1	4353	3230
PET 2	4401	3226
PET 3	3717	2519
Cellulose 1	4460	3112
Cellulose 2	4280	3106
Technical Control	-	-

Plastic samples and positive reference materials are placed in the soil to maximize the surface and contacted with soil in order to provide a good biodegradation environment. They are buried as shown in Figure 3.4.



Figure 3.4. Soil burial.

In this experiment, the parameter indicating the biodegradation is the amount of carbon dioxide released into the closed system. For this purpose, 0.025 N barium hydroxide solution (in a 150 mL beaker) is placed in each reactor, as carbon dioxide absorbing solution. The carbon dioxide produced reacts with barium hydroxide and precipitated as barium carbonate. Because of the static incubation, the barium carbonate builds upon the surface of the liquid (Figure 3.5). This thin surface is broken up periodically by shaking the reactor gently during the experiment. This ensures the absorption of the evolved carbon dioxide.

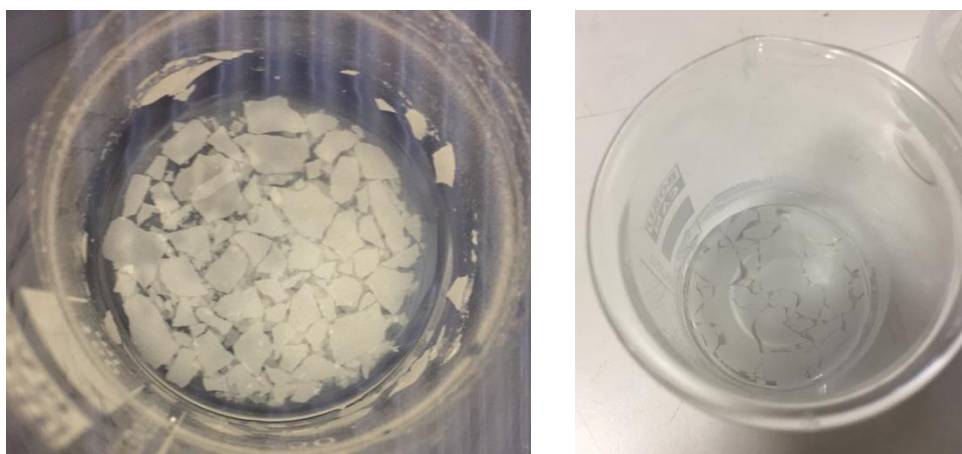


Figure 3.5. Barium carbonate layer formed on the surface of absorbent.

Besides the carbon dioxide absorbing solution, 50 mL deionized water in a 100 mL beaker is placed on the perforated plate to provide humidity into the reactor. The schematic view of the prepared reactors is summarized in Figure 3.6. The amount of carbon dioxide produced is determined by titrating the remaining barium hydroxide with 0.05 N hydrochloric acid to a phenolphthalein endpoint. The barium hydroxide traps are removed and titrated before their capacity is exceeded. The test procedure recommended the titration frequency as, every 3 to 4 days

for the first 2 to 3 weeks and thereafter every 1 to 3 weeks. It is also reminded that the period of time will vary with soil and test materials used in the test.

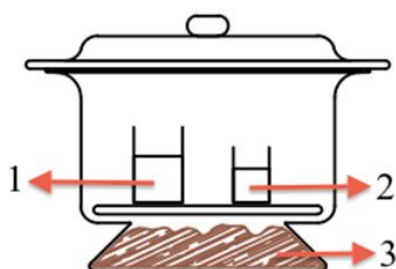


Figure 3.6. Schematic representations of reactors [(1) Barium hydroxide solution (carbon dioxide absorption solution) (2) Water (3) Soil (mixed with plastic sample / Soil only or empty for blank)].

The procedure followed in this test, recommends allowing the reactors remain open a minimum of 15 min and a maximum of 1 hour. All reactors were remained open for around 35 minutes during the experiment. The air in the reactors is refreshed before replacing 100 mL of fresh barium hydroxide and resealing the reactors. All reactors were weighed to follow moisture loss from the soil and deionized water is added back periodically to the soil to maintain the initial weight of the reactors. Finally, all reactors are sealed and placed again to the dark cabinet until to the next titration day (Figure 3.7).



Figure 3.7. Sealed reactors placed in a dark cabinet.

3.4. Calculation of Biodegradation

Measurement of the carbon dioxide evolved by the microorganism as a function of time is issued to assess the degree of biodegradation. This section describes the calculation method used to determine the biodegradability of plastics.

Carbon (C) content loaded into a reactor is liberated as carbon dioxide, with the presence of oxygen (O₂), into the closed and sealed reactor via biodegradation process. Theoretical quantity of carbon dioxide (CO₂) evolution is:



The reactors are loaded with PLA and PET based plastic fragments that corresponds 0.6 g carbon to the system. Thus,



If all carbon loaded to the system by the test specimen completely biodegraded 2200 mg carbon dioxide would be generated in each reactor. This is the theoretical carbon dioxide amount that will further be used in calculations.

Generated carbon dioxide is trapped by the barium hydroxide (Ba(OH)₂) placed into the reactor. Barium carbonate (BaCO₃) and water (H₂O) is formed by the following equation:



Following chemical equations take place at titration:



Titration was performed with hydrochloric acid. It is not possible to do the calculation before the end of the 190 days experiment. We can estimate the biodegradation rate by evaluating the

amounts of hydrochloric acid used for titration during the experiment. This evaluation is introduced in the Results section (See hydrochloric acid consumed).

Normality of hydrochloric acid and amount of carbon dioxide evolved is expressed as follows:

$$m \text{ moles of CO}_2 = (0.05N \times \text{mL HCl}) / 2 \quad (3.5)$$

According to the titration equation, when one mole of carbon dioxide is evolved into the medium (here reactor), it is titrated with 0.5 mole hydrochloric acid. According to this, the amount of carbon dioxide evolved into a reactor between titration days, can be calculated by the following equation. The evaluation of this data is introduced in the Results section (mg CO₂ evolved):

mg of carbon dioxide produced:

$$\text{mg of CO}_2 = (0.05N \times \text{mL HCl} \times 44) / 2 \quad (3.6)$$

Then the amount of carbon dioxide generated by the biodegradation of the plastics loaded into the reactors can be calculated by correcting the results with data from technical control and soil blank reactors. The evaluation of this data is introduced in the Results section (mg carbon dioxide generated).

Biodegradation is determined as, percent of carbon converted to carbon dioxide. It is obtained by measuring the monitored average carbon dioxide yield from the plastic samples and dividing it by the theoretical amount.

Finally, the percentage of carbon dioxide generated is calculated as:

$$\% \text{ of CO}_2 \text{ generated} = (\text{mg CO}_2 \text{ generated}) / (\text{mg CO}_2 \text{ theoretical}) \quad (3.7)$$

4. RESULTS AND DISCUSSION

Standard ASTM D5988 – 12 test method was followed to determine, compare and find out the aerobic biodegradation behavior of PLA and PET based plastic samples. Biodegradation is briefly determined as the percent of carbon conversion to carbon dioxide in a time-period. In other words, biodegradation is measuring the percentage or amount in mass of carbon converted to carbon dioxide in a process. It relates directly to the biodegradation of the carbon based polymer (Ashter, 2016).

Plastic samples are buried and expected to biodegrade according to the method. This process takes place in closed reactors in order to not give any access to air and concomitant carbon dioxide. It is important to open the reactors and titrate barium hydroxide before its capacity exceeded. Titration procedure held at periodic intervals allows the data to calculate the amount of carbon dioxide formed during the incubation time.

The incubation period depends on soil and type of the buried plastic sample. The time-period between titration procedures decreases slowly as the carbon content of soil or of buried plastic sample reduces. The methodology followed in this study recommended starting the test by titrating the absorbing solutions in every 3 to 4 days for the first 2 to 3 weeks. Temperature is crucial for the biodegradation process. The temperature is monitored daily during the 190 days test period and summarized in Figure 4.1.

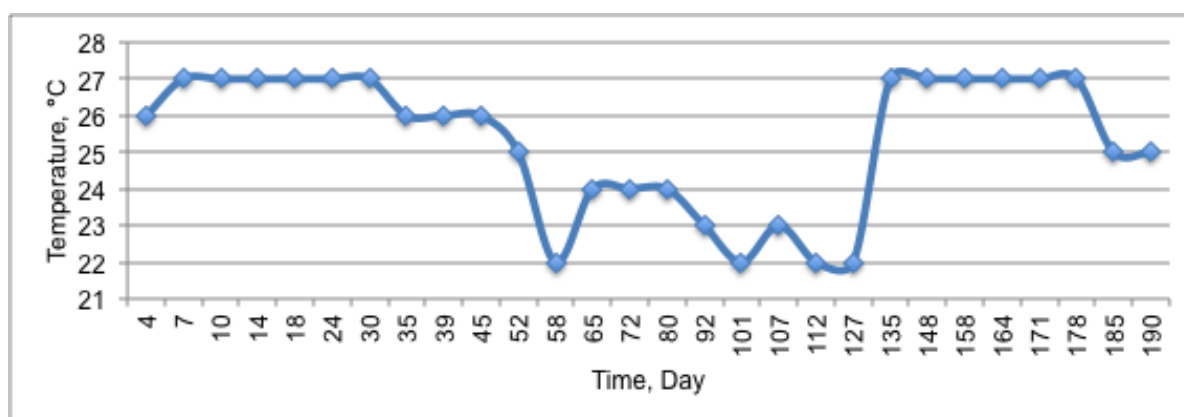


Figure 4.1. Temperature monitoring of reactors.

Carbon dioxide evolves in the reactors and forms barium carbonate via reacting with barium hydroxide according to the methodology. In order to determine the carbon dioxide evolved in the

reactors, the remaining barium hydroxide is titrated with hydrochloric acid. The remaining barium hydroxide presents the amount of absorbing solution which is not reacted with the evolved carbon dioxide. This procedure has been followed 28 times during the 190 days incubation period. The hydrochloric acid consumed are summarized in Tables 4.1, 4.2 and 4.3 for each reactor.

4.1. Hydrochloric Acid Consumed

The purpose of this study is to examine biodegradability of the buried plastic samples, but biodegradability of a buried sample can be calculated after the experiment is completed. The method followed here is based on titration, so the consumed amount of titrant, here hydrochloric acid, is able to give the opportunity to make predictions about the reactors before the study is completed. The consumed amount of hydrochloric acid gives the evolved carbon dioxide value for the reactor. The amount of solution consumed during titration was noted and discussed in this section for each reactor.

In this study, two parallel reactors are used as technical control. These reactors contain only the absorbing solution. Soil or plastic sample is not placed in these reactors. Technical control reactors indicated the carbon dioxide amount present in every reactor's headspace and the carbon dioxide interrupting during the titration procedure. The carbon dioxide amount calculated by technical control reactors shows the amount that all reactor contains. Additionally, technical control reactors do not give any result that corresponds to biodegradation or microbial activity.

Technical control reactors have the highest and nearly the same hydrochloric acid consumption during each titration period. These values varied between 49.75 and 49.90 mL (Table 4.1). This value indicates that the amount of barium hydroxide remained after the incubation period and which is titrated with hydrochloric acid afterwards is also high. While the titration is carried out with the remaining amount of absorbing solution, it is obvious that the evolved carbon dioxide amount mentioned with the two sources listed is very few.

The soil only blanks are the reactors that contain only soil. Carbon dioxide evolved in these reactors originates from two sources. First, the carbon dioxide that is present in headspace and suspend during titration procedure, as technical control reactors. Second the carbon dioxide produced from soil. Carbon dioxide produced from microbial activity that is present in soil is revealed by correcting the evolved carbon dioxide in these reactors with technical control reactors.

Table 4.1. Hydrochloric acid consumed for titrating samples from technical control and soil only blank reactors.

Time (Day)	Tech. Cont. R. 1, mL	Tech. Cont. R. 2, mL	Soil R. 1, mL	Soil R. 2, mL	Soil R. 3, mL
4	49.85	49.90	40.00	40.20	40.25
7	49.85	49.90	40.50	40.80	40.95
10	49.80	49.80	40.90	40.95	41.00
14	49.80	49.85	41.10	41.30	41.35
18	49.90	49.90	41.55	41.75	41.80
24	49.90	49.90	41.70	42.00	42.15
30	49.80	49.95	42.50	42.55	42.60
35	49.85	49.85	42.20	42.90	43.00
39	49.90	49.90	43.20	43.40	43.45
45	49.80	49.80	43.55	43.60	43.65
52	49.90	49.90	43.75	43.80	43.85
58	49.80	49.85	43.80	43.85	43.90
65	49.90	49.90	44.45	44.65	44.70
72	49.85	49.95	44.85	44.90	44.95
80	49.65	49.95	44.40	45.10	45.20
92	49.80	49.90	45.10	45.15	45.20
101	49.90	49.90	45.50	45.70	45.75
107	49.90	49.90	45.55	45.60	45.65
112	49.75	49.80	45.85	46.05	46.10
127	49.80	49.90	46.10	46.15	46.20
135	49.75	49.90	45.75	46.45	46.55
148	49.70	49.85	46.30	46.35	46.40
158	49.75	49.90	46.50	46.55	46.60
164	49.80	49.90	46.35	46.65	46.80
171	49.70	49.80	46.50	46.70	46.75
178	49.80	49.90	46.65	46.70	46.75
185	49.90	49.90	46.70	46.90	46.95
190	49.85	49.90	46.50	47.20	47.30

Three reactors were run with PLA based plastic samples. These reactors contain soil and PLA based plastic samples, where these specimens are considered as organic matter source for microorganisms. Due to the additional organic matter, a higher carbon dioxide evolution is considered in these reactors. Consumed hydrochloric acid values varied between 40.1 mL to 46.95 mL for PLA containing reactors. Hydrochloric acid consumption is lower for reactors loaded with PLA based samples compared to technical control reactors.

There are three carbon dioxide related mechanisms in these reactors: (1) the carbon dioxide present in headspace and the amount interrupts during titration procedure, (2) the microbial activity

that naturally takes place in soil, (3) the carbon dioxide generated by the biodegradation of the buried PLA based plastic samples. As the carbon dioxide evolution in a reactor increases, the amount of hydrochloric acid used to titration remaining barium hydroxide reduces (Table 4.2).

Table 4.2. Hydrochloric acid consumed for titrating samples from PLA loaded reactors.

Time (Day)	PLA 1, mL	PLA 2, mL	PLA 3, mL
4	40.05	40.25	40.00
7	40.65	40.85	40.60
10	40.80	41.05	40.70
14	41.20	41.25	41.15
18	41.60	41.80	41.55
24	41.75	41.90	41.75
30	42.10	42.15	42.05
35	41.75	41.95	41.70
39	42.05	42.30	41.95
45	42.30	42.35	42.25
52	41.75	41.95	41.70
58	41.30	41.55	41.20
65	41.35	41.40	41.30
72	42.75	42.90	42.75
80	43.30	43.55	43.20
92	43.65	43.70	43.60
101	44.15	44.35	44.10
107	44.30	44.55	44.20
112	45.30	45.35	45.25
127	45.75	45.95	45.70
135	46.05	46.25	46.00
148	46.25	46.30	46.20
158	46.45	46.65	46.40
164	46.50	46.75	46.40
171	46.60	46.65	46.55
178	46.60	46.80	46.55
185	46.75	46.90	46.75
190	46.95	47.00	46.90

The other three reactors were operated with PET based plastic samples. Because of this additional organic matter, a higher carbon dioxide evolution is considered and observed in these reactors compared to soil only blank reactors. The hydrochloric acid values varied between 39.20 mL to 47 mL for PET based samples reactors (Table 4.3).

Table 4.3. Hydrochloric acid consumed for titrating samples from PET loaded reactors.

Time (Day)	PET 1, mL	PET 2, mL	PET 3, mL
4	39.35	39.15	39.10
7	39.90	39.70	39.65
10	40.20	40.00	39.85
14	40.50	40.45	40.35
18	41.15	40.95	40.90
24	41.55	41.40	41.40
30	42.20	42.15	42.10
35	42.50	42.35	42.25
39	43.30	43.05	42.95
45	43.45	43.40	43.25
52	44.00	43.35	43.30
58	44.50	43.20	43.10
65	44.45	44.40	44.35
72	44.85	44.70	44.70
80	45.00	44.75	44.65
92	45.15	45.10	45.05
101	45.70	45.50	45.45
107	45.75	45.50	45.40
112	46.05	46.00	45.95
127	46.25	46.05	46.00
135	46.40	46.20	46.15
148	46.35	46.30	46.25
158	46.70	46.50	46.45
164	46.80	46.55	46.45
171	46.70	46.65	46.60
178	46.85	46.65	46.60
185	46.95	46.80	46.80
190	47.05	47.00	46.95

It is possible to see from Tables 4.1, 4.2 and 4.3, the highest hydrochloric acid is consumed for technical control reactors. This was an expected value since no biological activity can take place in these reactors. To compare with technical control reactors, soil only blank reactors had a lower hydrochloric acid consumption. This decrease can be interpreted as the activity of the naturally occurring microorganisms present in soil. On the other hand, the lowest hydrochloric acid consumption was observed for reactors that contain PLA based plastic samples. This also means that the highest carbon dioxide is evolved in these reactors.

So, increase in carbon dioxide sources is decreasing the remaining amount of absorbent solution. Therefore, it is possible to indicate that hydrochloric acid consumption was lower in blank reactors.

4.2. Carbon Dioxide Evolved

The carbon dioxide amount evolved in the reactors was obtained by examining the consumed amount of hydrochloric acid and is explained here. Correcting the evolved carbon dioxide amounts with technical control reactors gives the produced carbon dioxide amounts for both soil only blank and plastic sample containing reactors.

The amount of carbon dioxide evolved in a reactor is calculated via titration. 100 mL 0.025 N barium hydroxide is placed in every reactor as absorbent. The amount remaining is then assessed via titrating it with 0.05 N hydrochloric acid. All tables and figures are presented by taking the average of the mean of reactors that were run in parallel.

The methodology stated that the incubation should be continued until there is no carbon dioxide evolution noted between consecutive measurements taken four weeks apart, from the reactors. The test was conducted for 190 days to be on the safe side. Where there is not any carbon dioxide evolution noted after day 158 for PLA and day 171 for PET based plastic samples.

The carbon dioxide evolved in technical control reactor was titrated, calculated and is shown in Figure 4.3. Data in Figure 4.3 is the average of two reactors. The carbon dioxide evolved in technical control reactors varies between 0.11 mg and 0.28 mg (Figure 4.3). The evolved amount of carbon dioxide did not decrease or increase with time. The reason is that the technical control reactor does not give any result that is corresponded to biodegradation or any microbial activity. The small amount of carbon dioxide is formed via the air (also the carbon dioxide) present in the headspace of the reactors.

The amount of carbon dioxide evolved in reactors was calculated from the amount of base remaining in the absorbing solution. As 0.025 N barium hydroxide was used as the absorbent, the amount of remaining was assessed by titrating with 0.05 N hydrochloric acid. Thus, 50 mL hydrochloric acid would be needed to titrate 100 mL barium hydroxide.

Since 1 mol of carbon dioxide is evolved for every mol of barium hydroxide reacted to barium chloride and 2 mol of hydrochloric acid are needed for the titration of the remaining barium hydroxide, and given that the molecular weight of carbon dioxide is 44 g, the weight of carbon dioxide evolved (mg) was calculated as follow for the technical control reactors:

$$0.05 \times (50 - \text{mL HCl titrated}) \times 44 / 2 = \text{mg CO}_2 \text{ evolved} \quad (4.1)$$

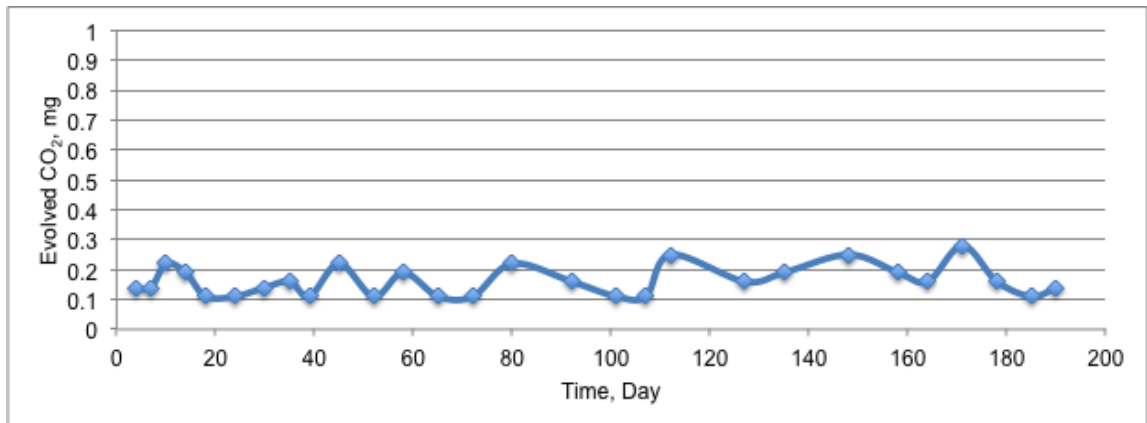


Figure 4.2. Carbon dioxide evolved in technical control reactors.

Table 4.4. Carbon dioxide evolved in technical control reactors.

Time (Day)	mg CO₂ evolved Tech. Cont.	Time (Day)	mg CO₂ evolved Tech. Cont.
4	0.14	80	0.22
7	0.14	92	0.16
10	0.22	101	0.11
14	0.19	107	0.11
18	0.11	112	0.25
24	0.11	127	0.16
30	0.14	135	0.19
35	0.16	148	0.25
39	0.11	158	0.19
45	0.22	164	0.16
52	0.11	171	0.28
58	0.19	178	0.16
65	0.11	185	0.11
72	0.11	190	0.14

The carbon dioxide which was not related with any microbial activity which was calculated by technical control reactors, was then corrected with soil only blank reactors. So the amount of carbon dioxide which was just produced from the background activity of the microorganisms that naturally present in the soil was calculated (Figure 4.4).

Soil placed in reactors was different from the real environment while the reactors were closed tightly. Therefore, it is not possible to mention any further carbon entrance to the soil. Consequently, the carbon dioxide produced from the microbial activity decreased gradually, while the carbon source of the soil decreased. Carbon dioxide produced decreased from 10.7 mg to 3.16 mg during the test, where the total amount of produced carbon dioxide was 168.72 mg (Table 4.4, Table 4.5).

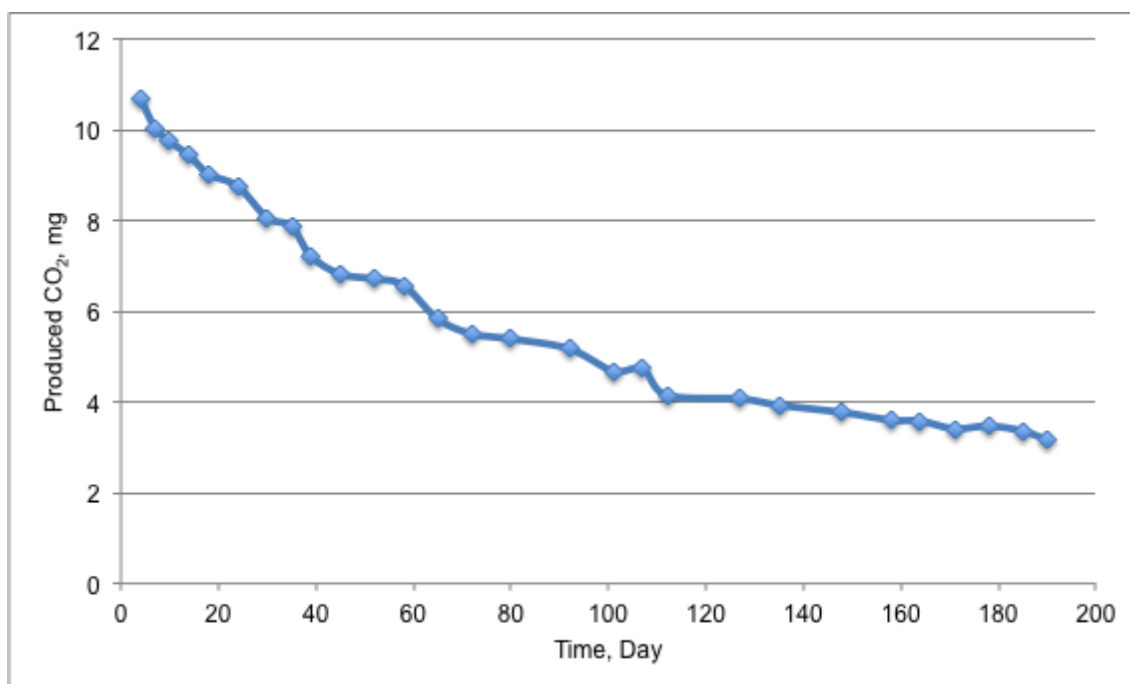


Figure 4.3. Carbon dioxide produced from soil.

Table 4.5. Carbon dioxide produced from soil.

Time (Day)	mg CO ₂ produced from Soil	Time (Day)	mg CO ₂ produced from Soil
4	10.70	80	5.39
7	10.04	92	5.17
10	9.74	101	4.68
14	9.43	107	4.73
18	9.02	112	4.15
24	8.75	127	4.07
30	8.06	135	3.93
35	7.87	148	3.77
39	7.21	158	3.60
45	6.82	164	3.58
52	6.71	171	3.41
58	6.57	178	3.47
65	5.83	185	3.36
72	5.50	190	3.16
		TOTAL	168.72

In order to examine carbon dioxide generated from biodegradation of PLA based plastic samples, two-stage correction is needed. All hydrochloric acid consumptions are corrected with technical control reactors.

After this, it is possible to obtain the carbon dioxide amount produced both from soil background activity and biodegradation process. As carbon dioxide produced from soil background

activity is computable with soil blanks, carbon dioxide generated with biodegradation is also computable. After two steps of correction, the achieved result refers the carbon dioxide amount that is generated during the biodegradation process.

Carbon dioxide generated from biodegradation of PLA based plastic samples was calculated as described and shown in Figure 4.4 The highest carbon dioxide generation was observed between day 24 and 135. The sum of the generated carbon dioxide amount between these days was 22.94 mg, which corresponded 96.75 % of the total amount of carbon dioxide generated during the test. Low carbon dioxide generation was observed at the beginning of the test and this can be explained with the adaptation behaviour of the microorganisms and is called as lag phase.

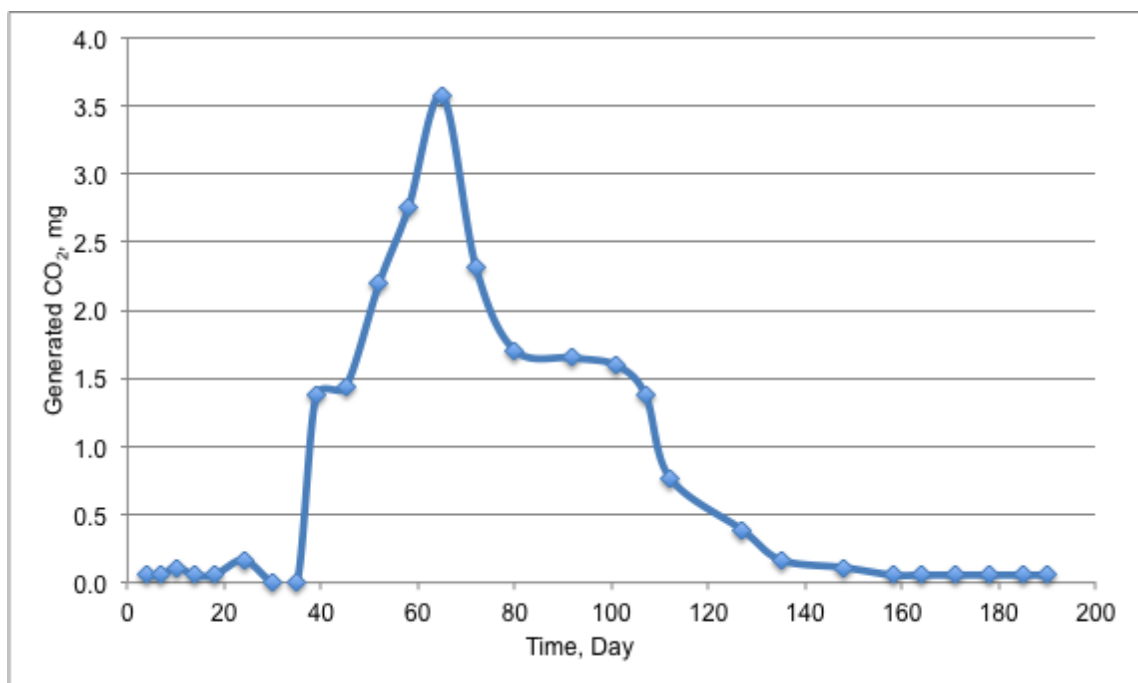


Figure 4.4. Carbon dioxide generated from PLA based plastic samples.

A peak value of carbon dioxide generation was observed on day 65, this can mean that the highest carbon dioxide was generated between day 58 and day 65. 15.08 mg carbon dioxide generation was computed on day 65, which is 15.08 % of the total generated amount (Figure 4.5, Table 4.6).

Table 4.6. Carbon dioxide generated from PLA based plastic samples.

Time (Day)	mg CO₂ generated from PLA	Time (Day)	mg CO₂ generated from PLA
4	0.055	80	1.705
7	0.055	92	1.650
10	0.110	101	1.595
14	0.055	107	1.375
18	0.055	112	0.770
24	0.165	127	0.385
30	0.495	135	0.165
35	0.990	148	0.110
39	1.375	158	0.055
45	1.430	164	0.055
52	2.200	171	0.055
58	2.750	178	0.055
65	3.575	185	0.055
72	2.310	190	0.055
		TOTAL	21.725

Carbon dioxide generated from the biodegradation of PET based plastic samples was calculated with the same way of PLA based samples and shown in Figure 4.5. The highest carbon dioxide generation was observed with in the first 80 days. The total amount of generated carbon dioxide on these days was 7.77 mg, which corresponded 95.93 % of the total amount generated during the test. Carbon dioxide generation was very low after day 80 (Table 4.7).

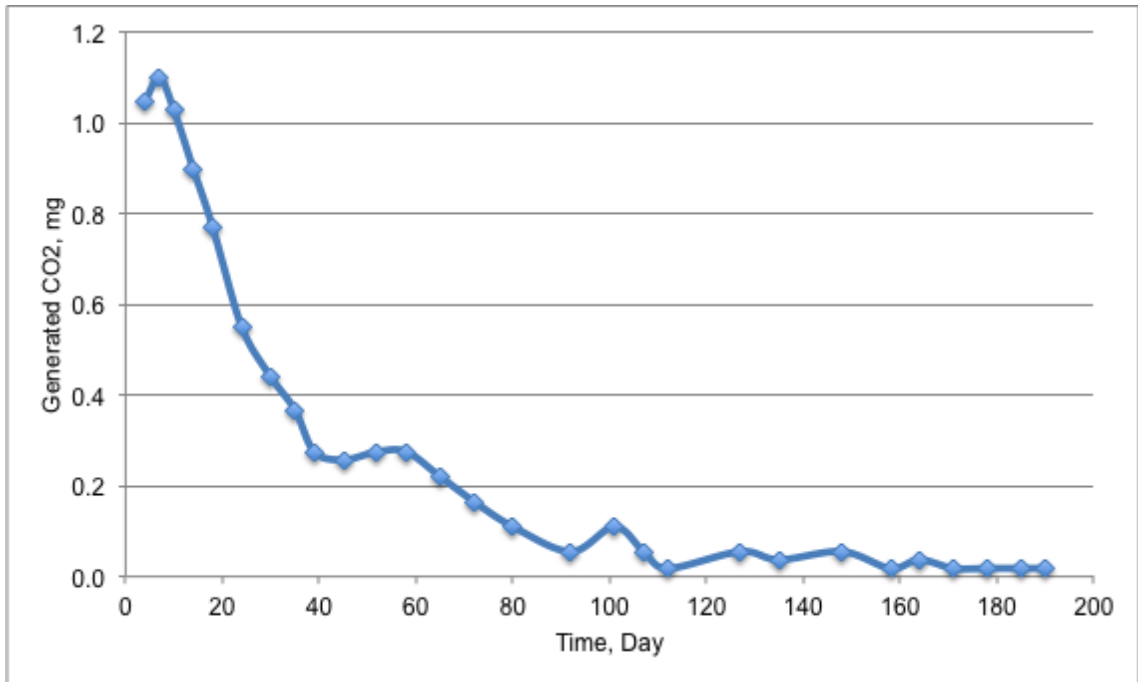


Figure 4.5. Carbon dioxide generated from PET based plastic samples.

Figure 4.5 shows that a rapid decrease in carbon dioxide generation was observed from the first day. The generated amount of carbon dioxide was smaller for PET based plastic samples compared to PLA (Table 4.7).

Table 4.7. Carbon dioxide generated from PET based plastic samples.

Time (Day)	mg CO ₂ generated from PET	Time (Day)	mg CO ₂ generated from PET
4	1.045	80	0.110
7	1.100	92	0.055
10	1.027	101	0.110
14	0.898	107	0.055
18	0.770	112	0.018
24	0.550	127	0.055
30	0.440	135	0.037
35	0.367	148	0.055
39	0.275	158	0.018
45	0.257	164	0.037
52	0.275	171	0.018
58	0.275	178	0.018
65	0.220	185	0.018
72	0.165	190	0.018
		TOTAL	8.286

4.3. Cumulative Carbon Dioxide Generated

To examine the biodegradability of a buried sample, cumulative carbon dioxide generation was calculated by adding values obtained at each titration day. Recording hydrochloric acid consumptions gave the opportunity to calculate cumulative amount of carbon dioxide generated during the whole test. Cumulative carbon dioxide is the main feature used to calculate biodegradability of a plastic samples in soil. Cumulative carbon dioxide amount generated in a reactor is used to assess the biodegradability in each reactor.

Higher amount of carbon dioxide was generated from the biodegradation of PLA based plastic samples at the end of 190 days test period (Table 4.8, Figure 4.6). The cumulative carbon dioxide generated from PLA based samples was 23.705 mg. Meanwhile the amount of cumulative carbon dioxide was 8.287 mg at the end of the test from the PET based samples (Figure 4.7).

Table 4.8. Cumulative carbon dioxide generated from PLA and PET based plastic samples.

Time (Day)	Cumulative CO₂ generated, mg PLA	Cumulative CO₂ generated, mg PET
4	0.055	1.045
7	0.110	2.145
10	0.220	3.172
14	0.275	4.070
18	0.330	4.840
24	0.495	5.390
30	0.990	5.830
35	1.980	6.197
39	3.355	6.472
45	4.785	6.728
52	6.985	7.003
58	9.735	7.278
65	13.310	7.498
72	15.620	7.663
80	17.325	7.773
92	18.975	7.828
101	20.570	7.938
107	21.945	7.993
112	22.715	8.012
127	23.100	8.067
135	23.265	8.103
148	23.375	8.158
158	23.430	8.177
164	23.485	8.213
171	23.540	8.232
178	23.595	8.250
185	23.650	8.268
190	23.705	8.287

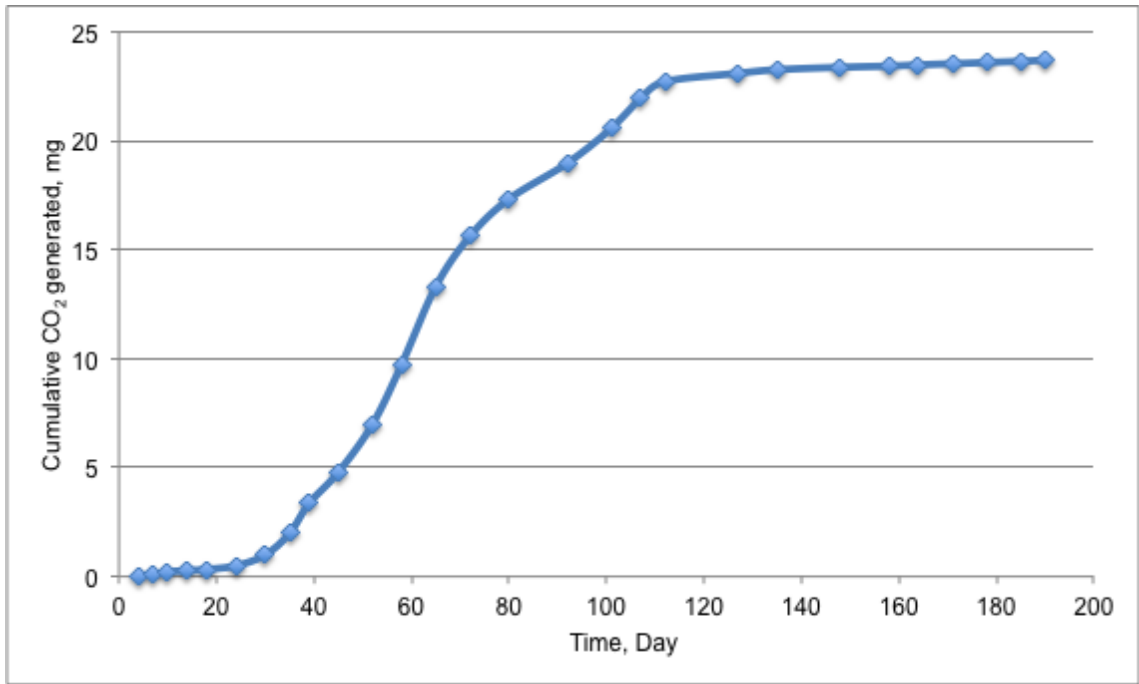


Figure 4.6. Cumulative carbon dioxide generated from PLA based plastic samples.

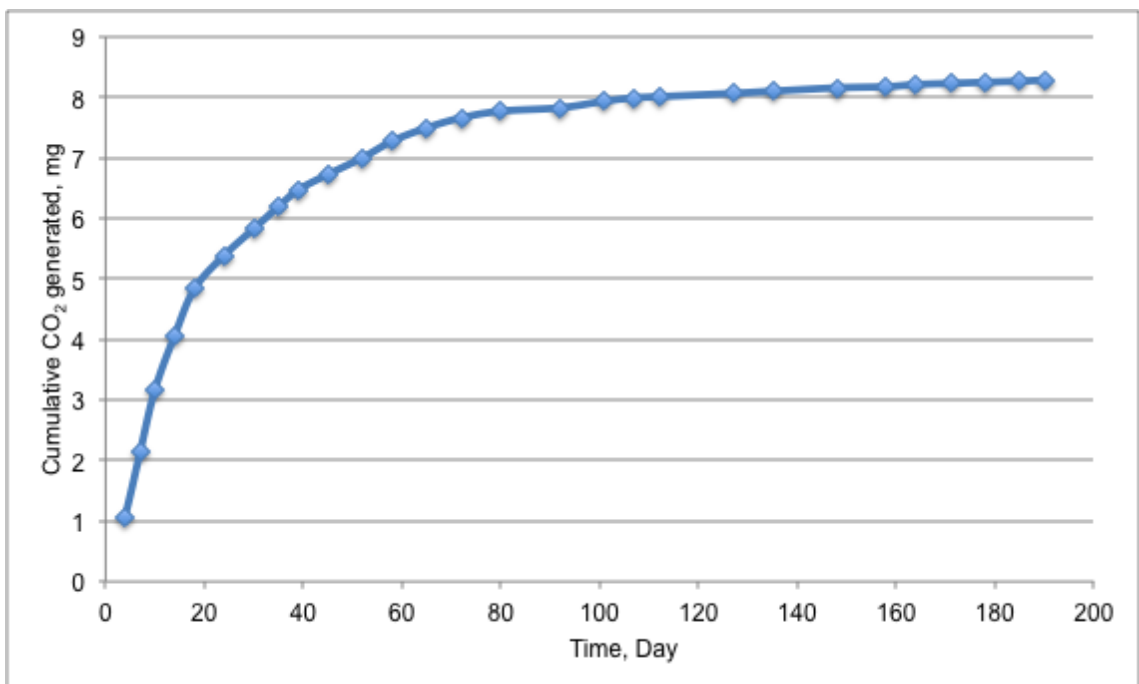


Figure 4.7. Cumulative carbon dioxide generated from PET based plastic samples.

4.4 Biodegradation

If PLA or PET based plastic samples could have been completely biodegraded (100 %), 2200 mg carbon dioxide would have been generated at the end of the experiment (the utilized part of polymers that is used in cellular life process by microorganisms is neglected). The term biodegradation is defined as the percentage of carbon source converted into carbon dioxide. Finally, the per cent of converted carbon was calculated and so the biodegradation curves of the buried plastic samples are plotted by following the standard method ASTM D 5988 – 12.

$$\% \text{ of CO}_2 \text{ generated} = (\text{mg CO}_2 \text{ generated}) / (\text{mg CO}_2 \text{ theoretical}) \quad (4.2)$$

So,

$$(23.705 \text{ mg CO}_2 \text{ generated}) / (2200 \text{ mg CO}_2 \text{ theoretical}) = 1.077 \%$$

biodegradation is reported for PLA based plastic samples and,

$$(8.287 \text{ mg CO}_2 \text{ generated}) / (2200 \text{ mg CO}_2 \text{ theoretical}) = 0.376 \%$$

biodegradation is reported for PET based plastic samples.

Table 4.9. % Biodegradation of PLA and PET based plastic samples.

Time (Day)	% Biodegr. PLA	% Biodegr. PET
4	0.002	0.047
7	0.005	0.097
10	0.010	0.144
14	0.012	0.185
18	0.015	0.220
24	0.022	0.245
30	0.045	0.265
35	0.090	0.281
39	0.152	0.294
45	0.217	0.305
52	0.317	0.318
58	0.442	0.330
65	0.605	0.340
72	0.710	0.348
80	0.787	0.353
92	0.862	0.355
101	0.935	0.360
107	0.997	0.363
112	1.032	0.364
127	1.050	0.366
135	1.057	0.368
148	1.062	0.370
158	1.065	0.371
164	1.067	0.373
171	1.070	0.374
178	1.072	0.375
185	1.075	0.375
190	1.077	0.376

As can be seen from Figure 4.8 and Table 4.9 biodegradation of PLA based plastic samples increased with time. At the end of 190 days of incubation, the biodegradability of PLA based plastic samples was 1.077 % and the amount of cumulative carbon dioxide generated was 23.71 mg. The first titration procedure was held after 4 days of incubation period. After the first 4 days, the amount of generated carbon dioxide was 0.06 mg and this amount corresponds to 0.003 % biodegradation of PLA samples.

A lag phase was observed at the beginning of the test (Figure 4.9). The lag phase took place from the first day until day 24, where the biodegradability could solely reach to 0.023%. Biodegradation took place between day 24 and 112, and it reached from 0.023 % to 1.033 %.

Finally, between day 112 and 190 where biodegradation reached a plateau, and it is possible to say that utilization of the substrate was mostly completed. There the biodegradation varied between 1.033 % and 1.078 % and the cumulative carbon dioxide generated increased from 22.72 mg to 23.71 mg.

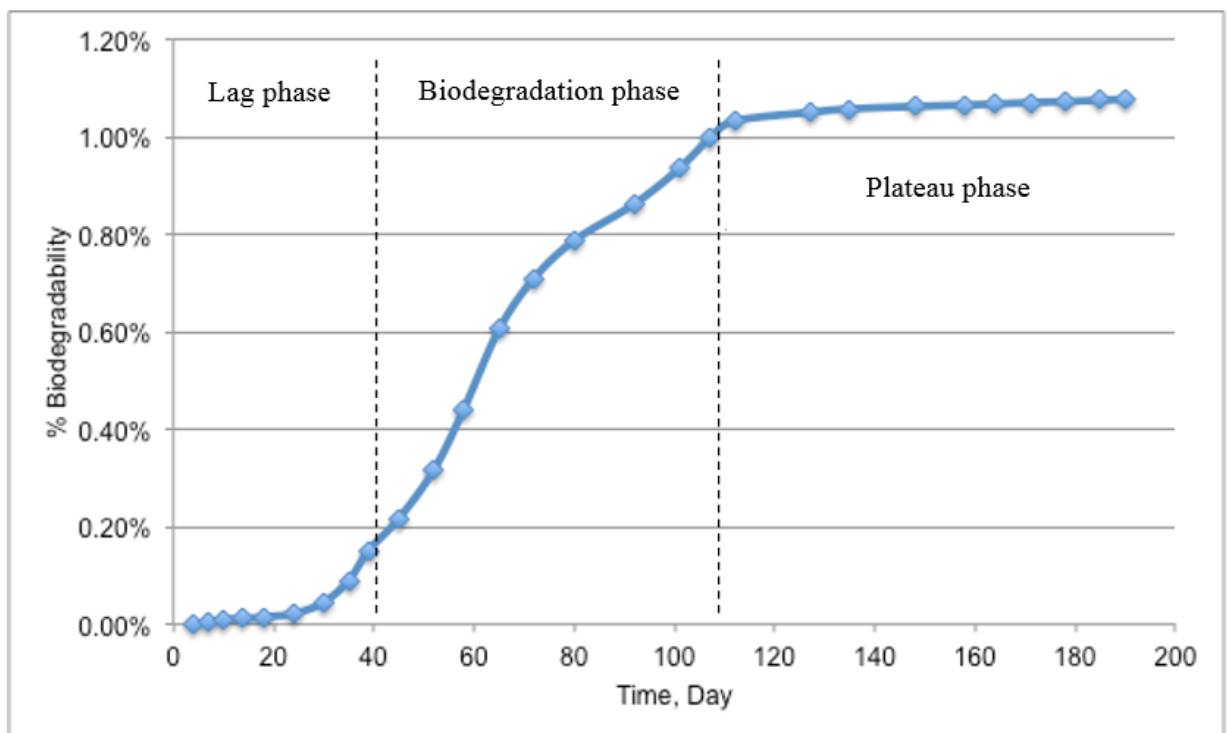


Figure 4.8. Biodegradability of PLA based plastic sample.

PLA is known to be a water-insoluble polymer when its molecular weight is sufficiently high. The polymer subjects to degrade as a result of hydrolysis on the ester group when water penetrates into the bulk of the polymer. Long polymer chains were converted into short polymer chains with

low molecular weight, which are water-soluble monomers. The reduction in molecular weight gives the polymer a suitable form which is able to biodegrade (Gupta et al., 2007; Guzman et al., 2011; Middleton et al., 2000). The lag and biodegradation phases, seen in Figure 4.8, can be explained with this process. Lag phase before biodegradation of PLA/PBAT blends was also observed in the study conducted by Freitas et al. (2017).

The biodegradability of PET based plastic samples reached to 0.376 %. This corresponded to 8.287 mg cumulative carbon dioxide generated. Unlike to PLA based plastic samples, there was no lag phase during the biodegradation of PET based plastic samples (Figure 4.9). Also, there was a rapid rise for the first 68 days, where the biodegradation varied from 0.048 % to 0.331 % and the cumulative carbon dioxide generated increased from 1.05 mg to 7.28 mg. Corresponding to 0.341 % of biodegradation. A plateau was reached after 65 days of incubation. The study which was conducted by Chiellini et al. (2003) with synthetic polymers, did not also observe any lag phase during the biodegradation test.

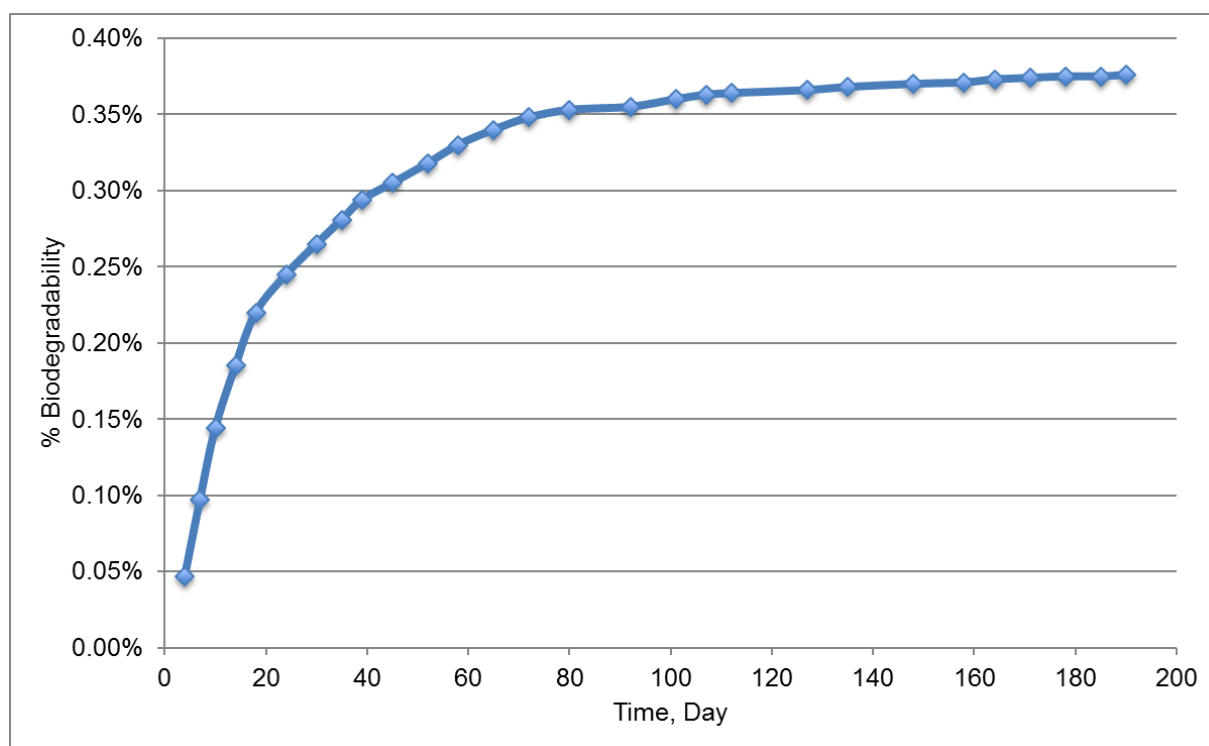


Figure 4.9. Biodegradability of PET based plastic sample.

This study aimed to compare the biodegradation tendencies of two different type plastic samples. PLA and PET based plastic samples were buried for this purpose. So by assessing carbon which was converted to carbon dioxide, biodegradation of samples were calculated easily. Figure 4.10 shows the biodegradability of two buried plastic types together.

A higher amount of carbon dioxide was generated from PLA based plastic samples at the end of the test. This result indicates that the PLA based plastic samples had higher biodegradation tendency compared to the PET based plastic samples. On the other hand, biodegradability of PET based plastics did not show any lag phase, unlike to PLA based plastic sample. Moreover, PET based plastic samples showed a lower biodegradation tendency.

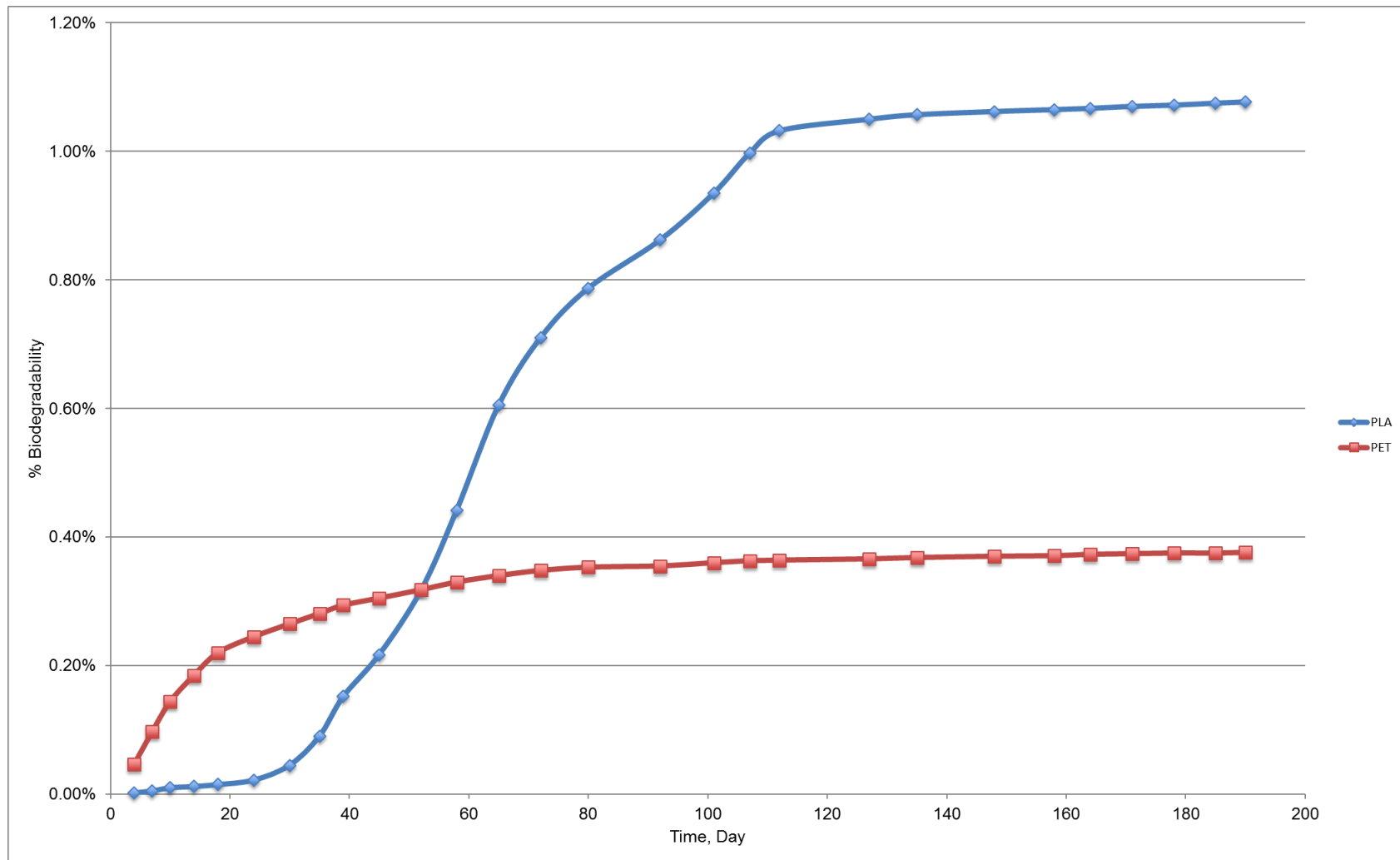


Figure 4.10. Biodegradability of PLA and PET based plastic samples.

A higher amount of carbon dioxide was generated from the biodegradation of PET based plastic samples for the first 24 days. This case can be explained with the lag phase that occurred before PLA started to biodegrade. The cumulative carbon dioxide generated increased from 1.05 mg to 5.39 mg and the % biodegradation increased from 0.047 % to 0.245 % for PET based plastic samples where cumulative carbon dioxide generated reached from 0.06 mg to 0.49 mg and % biodegradation reached from 0.002 % to 0.022 % for PLA based plastic samples (Figure 4.10). After the first 30 days, generated carbon dioxide was higher for PLA based plastic samples. The biodegradability of PLA based plastic samples reached to a plateau after 112 days of incubation, though PET based samples reached to plateau after 65 days.

The amount of cumulative carbon dioxide generated from PLA based samples reached to 23.71 mg and 1.077 % of biodegradation was observed at the end of the experiment. Within this time the biodegradation of PET based plastic samples was 0.376 % and the cumulative carbon dioxide generated was 8.287 mg (Figure 4.8, Figure 4.9).

Adhikari et al (2015) stated that the biodegradation rate of bioplastic in soil was closely related to the main components in the bioplastics. With the conducted test, he reported that the PLA based plastic samples did not biodegrade in soil after the test ended. Where bioplastic blends showed 1 to 7 % biodegradability. Ahn et al (2005) also stated the biodegradability of bioplastic composed of 100% PLA as very low ($13 \pm 3\%$) even the test was conducted under high temperature (58°C) conditions.

The 95% confidence limits (CL) for the buried samples was calculated as 1.0775 ± 0.991 and 0.3683 ± 0.924 for PLA and PET based plastic samples respectively. This presents low biodegradability interval for both samples. On the other hand, the 95% CL of the difference between means crosses zero for PET based specimen where the difference could be zero, and that means there could be no difference at all.

4.5. Other Parameters

4.5.1. Positive Reference Material Results

In this study, Cellulose (Whatman cellulose chromatography paper) was selected as the standard positive reference material. This paper is a bio-based and biodegradable material and was used as a positive reference in most international standards to test biodegradation (Briassoulis, 2018). As seen in Figure 4.11 positive reference material was completely biodegraded (100%). This result indicates that the soil used in this study was fertile enough and the condition of this test was suitable for any biodegradation study.



Figure 4.11. Positive reference material, before and after the biodegradation test.

4.5.2. Weight Change (%)

The PLA and PET fragments were weighed with an analytical balance with an accuracy of 0.001 g before and after the biodegradation process. After 190 days of incubation period the remaining fragments were washed with distilled water to remove adherent soil and dried in an oven. The fragments were stored in a desiccator for 48 hours until they reached a constant weight before weighing. The weight losses are consistent with biodegradation rates of PLA and PET based plastic samples.

Table 4.10. Weight differences of PLA and PET based plastic samples before and after the biodegradation test.

Reactor	Weight before test, g	Weight after test, g	% change in weight
PLA 1	1.2040	1.2040	0.0000
PLA 2	1.2087	1.2085	0.0165
PLA 3	1.2060	1.2060	0.0000
PET 1	0.9643	0.9642	0.0104
PET 2	0.9620	0.9620	0.0000
PET 3	0.9629	0.9629	0.0000
Pos. Ref. 1	1.3507	0.0000	100
Pos. Ref. 2	1.3505	0.0000	100

The weight of PLA based plastic samples have slightly decreased, confirming the low decay of these bioplastics. On the other hand, PET did show a very few change after 190 days of biodegradation (Table 4.10).

4.5.3. pH

The pH of the soil before and after the biodegradation test is given in Table 4.11. Initial pH of the soil was 6.69. The method followed in this study requires that the pH remains between 6.0 and 8.0 before installing the reactors.

Table 4.11. pH values before and after biodegradation test.

REACTOR	pH before test	pH after test
PLA 1	6.69	6.66
PLA 2	6.69	6.67
PLA 3	6.69	6.68
PET 1	6.69	6.70
PET 2	6.69	6.69
PET 3	6.69	6.69
Pos. Ref. 1	6.69	7.70
Pos. Ref. 2	6.69	7.71

Slight decrease in soil pH was observed after biodegradation of PLA based plastic samples. This may be occurred due to the low biodegradation, which consists of lactic acid like as it is stated in the study conducted by (Janczak, 2018). The presence of PET had no effect on the pH value of the soil. This may be resulted from the very low biodegradation of PET based plastic samples. On the other hand, positive reference material showed an increase in pH after the study. It is possible to observe that with biodegradation of additional carbon source, the pH of soil increases.

4.5.4. Observation of the Bioplastic Surface by SEM

The plastic samples buried in soil for 190 days were also examined under Scanning Electron Microscope (SEM), and changes in the plastic surfaces were analyzed. SEM uses an electron source to image and characterise the samples. It generates a magnified image of the sample by bombarding it with an electron beam and detecting the electrons that are emitted from the surface as a result of the interaction of the incident electrons with the sample under study (Suri et al, 2019).

The images were enlarged 1000 to 5000 times. The surfaces of PLA based plastic samples were slightly changed, confirming the low decay of these bioplastics. White spots that can be clearly noted on the surface of the PLA based sample-after the experiment (Figure 4.13) prove disintegration while the surface of the material is rather smooth before the experiment (Figure 4.12). On the other hand, PET did not change after 190 days of biodegradation, thus the non-biodegradable petrochemical plastic seemed to be very resistant to biodegradation in the soil environment (Figure 4.14, Figure 4.15).

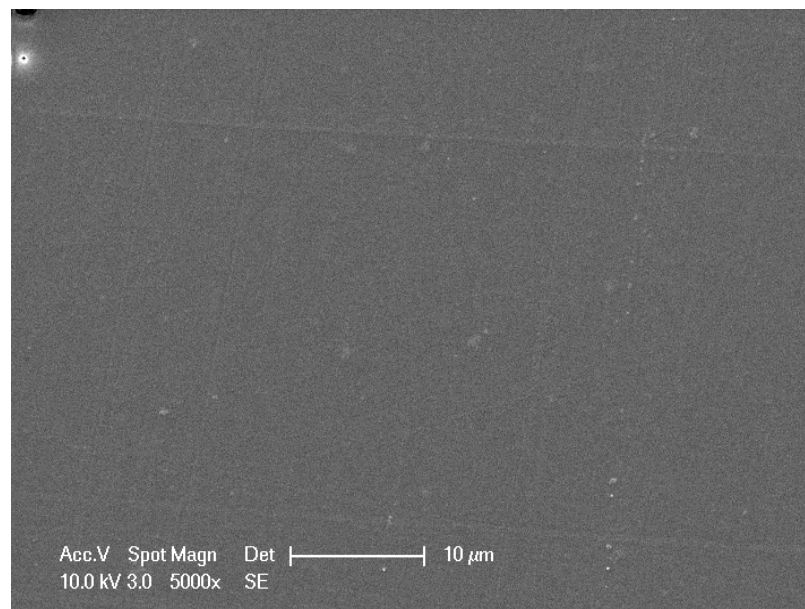


Figure 4.12. SEM analysis of surface of PLA based plastic samples before biodegradation

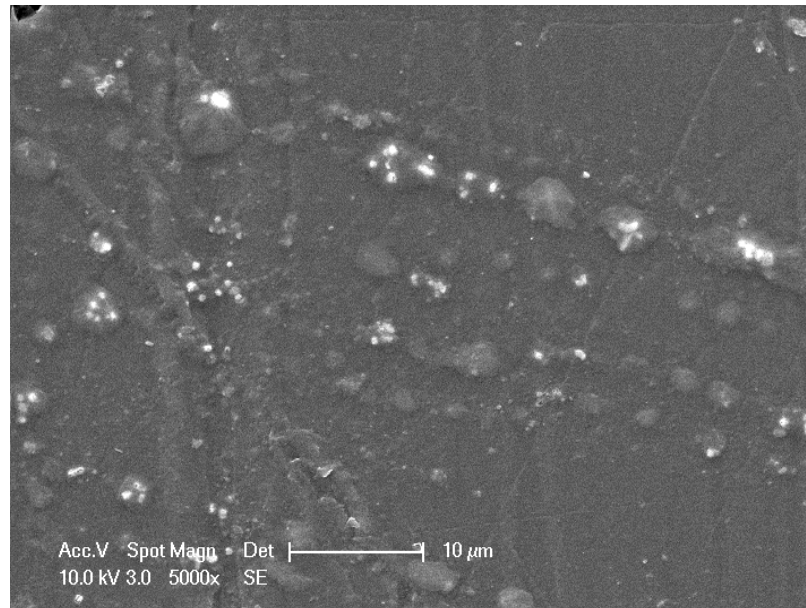


Figure 4.13. SEM analysis of surface of PLA based plastic samples after biodegradation

Adhikari (2016) stated after 2 years of biodegradation test and observation by SEM, that the structures of buried bioplastics were biodegradable and decayed in the soil environment, but the degradation rates differed between bioplastics. PBS (butylene succinate) and PBS-starch were easily degraded, but PLA needed much longer time for a significant degradation.

The biodegradability of the plastics in soil environment differed considerably, depending on the main polymer component. The biodegradation rate of PLA was faster than PET based plastic samples after 190 days. Biodegradation of PLA based plastic required both structural change and microbial attack (Sakai et al., 2001). Therefore, for biodegradation of PLA based plastics, microbial attack at a high temperature (such as by thermophilic bacteria under a composting process) might be needed. For the treatment of waste bioplastics in the soil, the biodegradability of each bioplastic should be considered.

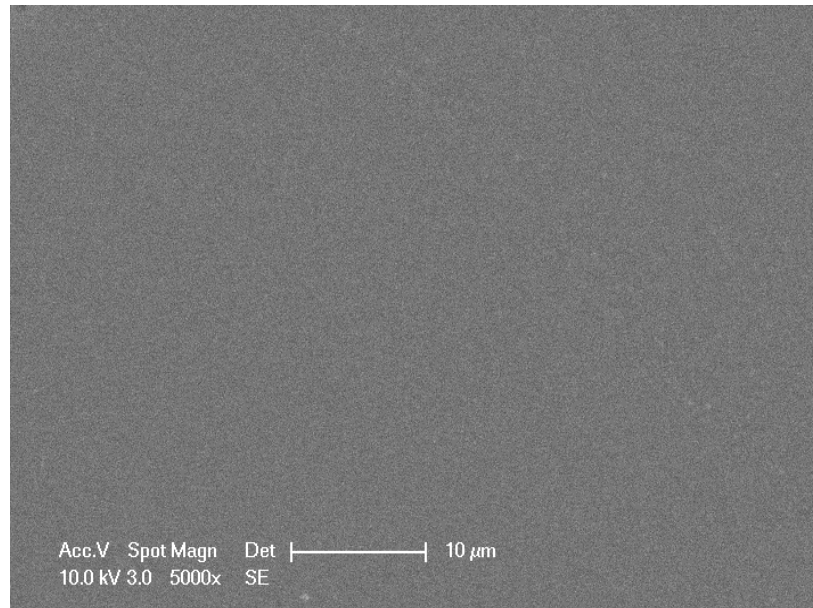


Figure 4.14. SEM analysis of surface of PET based plastic samples before biodegradation

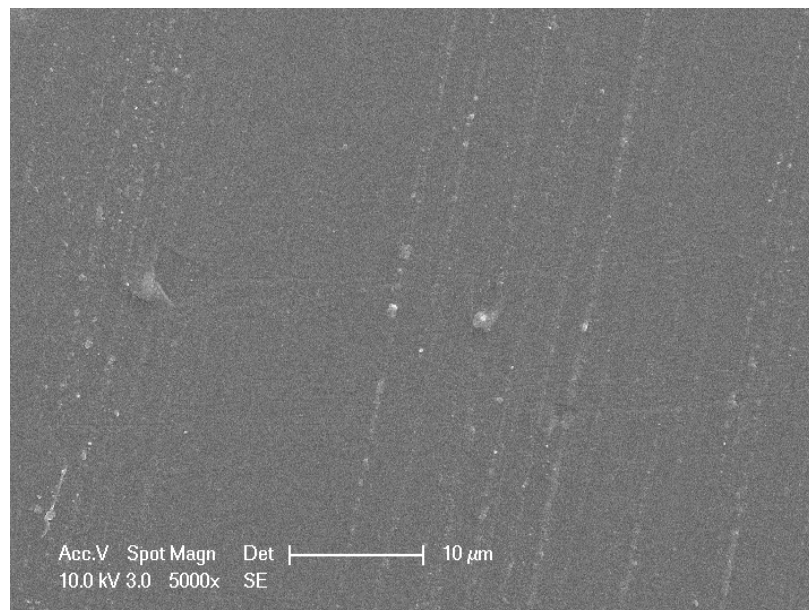


Figure 4.15. SEM analysis of surface of PET based plastic samples after biodegradation

5. CONCLUSION

Plastics are known to protect their structures for a very long time in nature. It is mentioned that plastic can stay in the environment for more than 1.000 years. Plastic wastes are also considered as a problem in the waste management system. Independent from the management system, plastics have the bad reputation of having high volume.

Durability and high volumes are the main problems for plastic. Besides this, plastics cannot be prevented from being released into the natural environment. Plastic protects its form for a long time in the soil environment.

The term biodegradation and bioplastics are offering solution to the plastic problem. Biodegradation is a process whereby microorganisms takes place and degrades the polymer via using the carbon source as a substrate. Bioplastics are the type of plastics that are stated to be whether biodegradable or bio-based.

A soil burial test was conducted to monitor the behavior of plastic in the soil environment to give a solution to this problem. PLA and PET based plastics were specially selected for the test according to their nearly similar use in packaging application. These selected plastic specimens were buried and reported by following the standard method ASTM D5988 – 12.

Based upon the 190 days experiment, the following results were obtained:

- PLA and PET based plastic samples biodegraded 1.0775% and 0.3683% respectively after 190 days burial.
- The same test was also conducted to positive reference material as control material. 100% biodegradation was obtained from the positive reference material containing reactors. This part of the test proves that the environmental conditions where the test was conducted was suitable for a biodegradation process. To add the soil used as inoculum in this study was fertile enough to give the opportunity to the buried samples to biodegrade.

- Despite this condition PLA and PET based samples remained nearly unchanged. That contributes to an accumulation problem even for PLA-based plastic products. Where PLA is used in this study as promising polymer.

- Most biodegradation studies conducted with plastic samples recommends to inoculum the soil with proper microorganism before the experiment. Powdering the sample, irritating the surface of the sample, etc. are also recommended steps that provides higher biodegradation percentages. Since this study investigates the natural occurring process of a sample entered the environment, biodegradation speeding steps have been avoided.

- PLA based samples showed a slightly higher biodegradation potential. This result shows that PLA based plastic products can be more preferable compared to PET based plastics.

- This study reports that both PLA and PET based plastic products do not offer an effective solution to the plastic accumulation problem due to their low biodegradation. Today's technology is not enough to deal with the problem where it does not have a fully or at least high biodegradation potential.

- With this test is possible to state that, after the industrial processes held to provide a solid and stable product to the end user, the plastic reaches to a form that is non-biodegradable.

- According to the visual observation, these products presented a very resistant behavior to natural occurring activities like microbial attacks. PLA-based plastic is literally stated as biodegradable. Studies conducted with PLA blends also reported high biodegradations. On the other hand, like the study conducted here, there are other studies that state that PLA based plastic as end product has a low biodegradation potential.

- pH is playing a very important role in the soil biochemistry. It is possible that PLA and PET based plastic products do not have any effect on disturbing this balance. Despite this result, plastic release to the environment is very dangerous.

6. RECOMMENDATIONS

Standard Method ASTM D5988 – 12 was followed in this study; the test was conducted for 6 months (190 days) time-period. Where low biodegradation is observed by choosing processed end products, further investigations may held on raw material or on powdered and irritated plastic samples to meet higher biodegradation. On the other hand, processed end products are reported as non-biodegradable with this study, which establishes that plastic accumulation may last until the plastic wastes are properly disposed or more environmentally products are produced.

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