Biodegradability studies of HDPE/PLLA blends under controlled composting environment

Dissertation

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Submitted

by

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Declaration

I, the undersigned, hereby declare that the research work presented in the M. Tech. dissertation entitled "**Biodegradability studies of HDPE/PLLA blends under controlled composting environment**" has been carried out by me under the supervision and guidance of Prof. P.K. Bajpai and Dr. Haripada Bhunia, Department of Chemical Engineering, Thapar University, Patiala.

Further, I declare that no part of this Dissertation has been submitted for a degree or any other qualification of any other university or examining body in India/elsewhere

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CERTIFICATE

This is to certify that dissertation entitled, "**Biodegradability studies of HDPE/PLLA blends under controlled composting environment**" submitted by Ms. Jasmeet Kaur in partial fulfillment of the requirements for the award of M. Tech. in Biotechnology at Thapar University, Patiala is an authentic work carried out by her under our supervision and guidance.

To the best of our knowledge, the matter embodied in this dissertation has not been submitted to any other University/ Institute for award of any Degree or Diploma.

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Jasmeet Kaur

Abstract

Degradation of plastics, especially those used in flexible packaging and similar other applications is necessary to prevent environmental pollution by littering of these plastics after their use. To make polyethylene degradable, blending with biodegradable polymers such as poly(L-lactic acid) (PLLA) is required, which facilitates the enzymatic degradation of the blends by the microbes present in compost and/or soil. In the present research, biodegradability of eight different HDPE/PLLA blend samples i.e. with compatibilizer (X1, X2, X3 and X4) and without compatibilizer (B1, B2, B3, and B4) were studied as per the guidelines of ASTM D 5338-98 (modified) standard. One gram approx by weight of each sample was taken for carrying out the experiment. The conditions required for the degradation in composting environment were optimized such as temperature, humidity, and flow rate of CO_2-free air etc. The properties of the municipal compost used were also analyzed; viz. N_2 content and C/N ratio which will facilitate the growth of the microbes responsible for the polymer blend degradation. The biodegradability testing apparatus, to carry out the experiments, was designed and developed according to the guidelines of the mentioned standard. The CO_2 evolved was measured as a function of time and the percentage of biodegradability was obtained by determining the percentage of carbon in the test substance that is converted to CO_2 during the duration of the test. It was found that after 45 days of test period, the percent biodegradability of blend X4 was maximum i.e. 3.5% and minimum i.e. 1.84% for negative (HDPE) as compared to positive control (MCE) i.e. 16.5%. After degradation period, weight loss and protein estimation of X4 was found to be maximum i.e. 2.54% and 0.773 gm/ml respectively. In conclusion, the blend may be considered as 'safe' for disposal through solid-waste composting plants.

Keywords: Biodegradation, ASTM D 5338, Blend.

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Abbreviations

AcC	Acetyl Cellulose
ASTM	American Society of Testing and Materials
CPCB	Central Pollution Control Board
C & D	Construction and Demolition
CRU	Constitutional Repeating Units
FTIR	Fourier-Transformed Infrared Spectroscopy
GC-MS	Gas Chromatography Mass Spectroscopy
HDPE	High-Density Polyethylene
IS	Indian Standards
ISO	Indian Standard Organisation
LDPE	Low-Density Polyethylene
LLDPE	Linear Low-Density Polyethylene
MCE	Microcrystalline Cellulose
NA	Nutrient Agar
NY11	Nylon 11
PBS	Poly(Butylenes Succinate)
PBSA	Poly (Butylenes-*co*-Adipate)
PCL	Poly Ɛ-caprolactone
PDLA	Poly D-lactic acid
PE	Polyethylene
PET	Polyethylene Terephthalate
Phr	Parts per hundred of resin
PLA	Poly-lactic Acid
PLLA	Poly L-lactic Acid
PUR	Polyurethane
SEM	Scanning Electron Microscopy
SLA	State Level Advisory

Chapter: 1
INTRODUCTION

Polymer is a substance which is composed of molecules characterized by repetition of one or more monomer or constitutional repeating units (CRU) linked together by covalent bonds formed by polymerization **[1]**. Polymers are broadly classified in two categories: natural polymers (e.g. proteins, carbohydrates, natural rubber) and synthetic polymers (e.g. plastics, fibres, elastomers). Plastic is the most commonly exploited synthetic polymer. The word 'plastic' is derived from a Greek word 'plastikos' which means able to be molded into different shapes **[2]**. Plastic is solid, non-metallic and high molecular weight material composed of repeated units of one or more monomers. They are synthesized chemically from petrochemical feedstock **[3]**. Plastics are inert, durable, lightweight, strong, flexible, malleable, resistant to various degradative forces, easy processibility and low cost production widens the horizons of its applications **[4]**. Plastics are of two different types: thermoplastics and thermoset plastics. A brief comparison between the two types is given in Figure 1.1

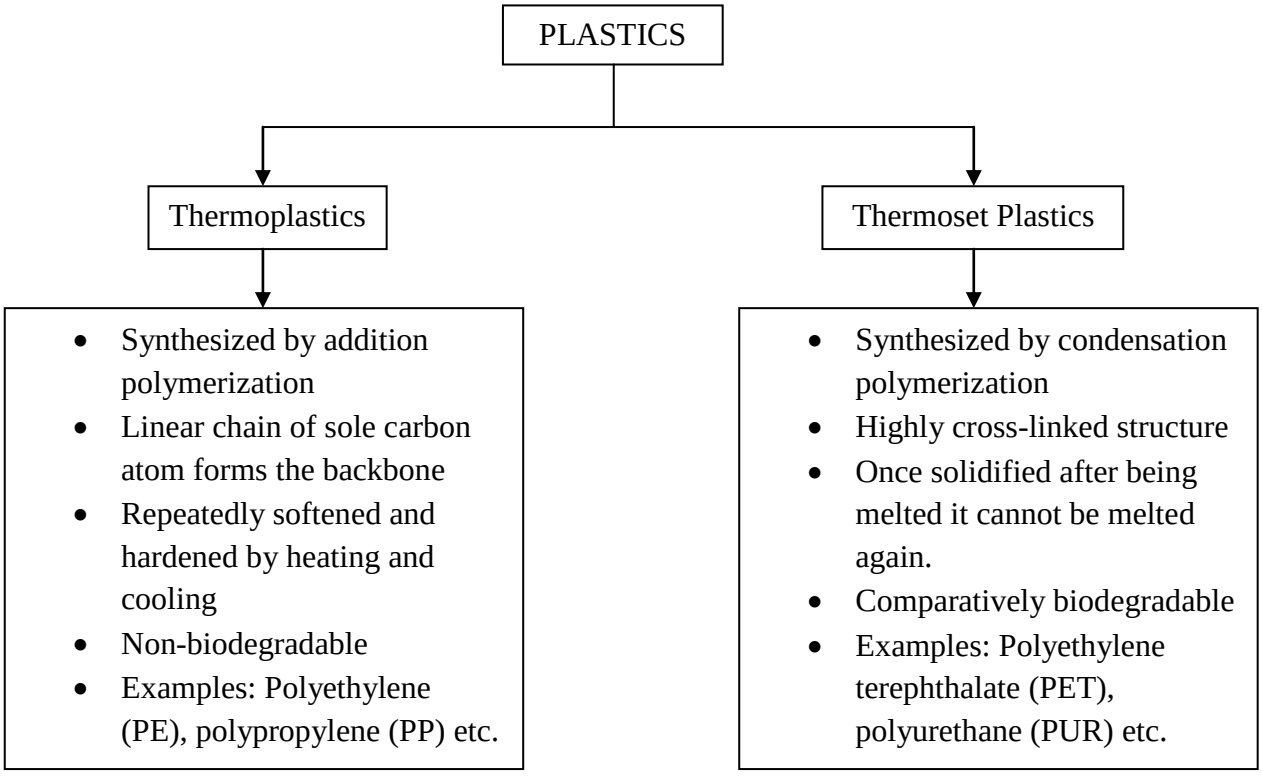

Figure 1.1: Comparison between thermoplastics and thermoset plastics **[5]**.

Thermoplastics like polyethylene (PE), polypropylene (PP), polystyrene (PS) etc are widely used in packaging, fabrication of bottles and films, transportation, automobiles, industrial machinery, construction, electrical and electronics, adhesives and other applications mentioned in Table 1.1.

Table 1.1: Application of plastics **[5]**

Plastics	**Uses/Applications**
LDPE, LLDPE, PVC	Films and packaging
PET, HDPE, PVC	Bottles, tubes, pipes and insulation molding
PS, PP, PVC	Tanks, jugs and containers
LDPE, LLDPE	Bags
PUR	Coating, insulation, paints and packing

1.1 Global Plastic Consumption

Due to diverse field of application for plastics, their production is increasing day by day. Among all the synthetic plastics, PE is the commodity leader among various synthetic polymers with a current global production of ca. 140 million tons per year **[6]**. PE accounts for 64% of the total synthetic plastics which mainly include bottles, carry bags, disposable articles, garbage containers, margarine tubs, milk jugs, and water pipes etc **[7]**. Annually about 500 billion to 1 trillion of polythene bags are routinely used all over the globe. Due to increase in demand, plastics production has reached to 180 million tons per year. The amount of plastic consumption in major area of the world is given in the Table.1.2.

Table 1.2: Worldwide scenario of plastic consumption **[8]**

Main World Areas	**Plastic Consumption (in Million tons)**
Europe	40,000
America	56,000
Africa	3,000
India	4,000
China	19,000
Japan	11,000
Rest	17,000
Total	150,000

Overproduction of plastic commodities has led to generation of huge amount of waste which cannot be recycled or reused as a result gets accumulated in the environment. Non-biodegradable plastics such as PE, PP etc are accumulating in the environment at the rate of 25 million tons per year **[9]**.

70% of the consumed plastic is disposed off in the environment **[10]**. There are different sectors which contribute to plastic waste such as municipal waste, commercial waste, residential waste, industrial waste, construction and demolition (C&D) waste, E-waste (electronic) etc **[11]**. Packaging applications add maximum to the post consumer plastic waste followed by other applications which is depicted in Figure 1.2. The continuous disposal of plastic waste is leading to various types of pollution which is of great environmental concern.

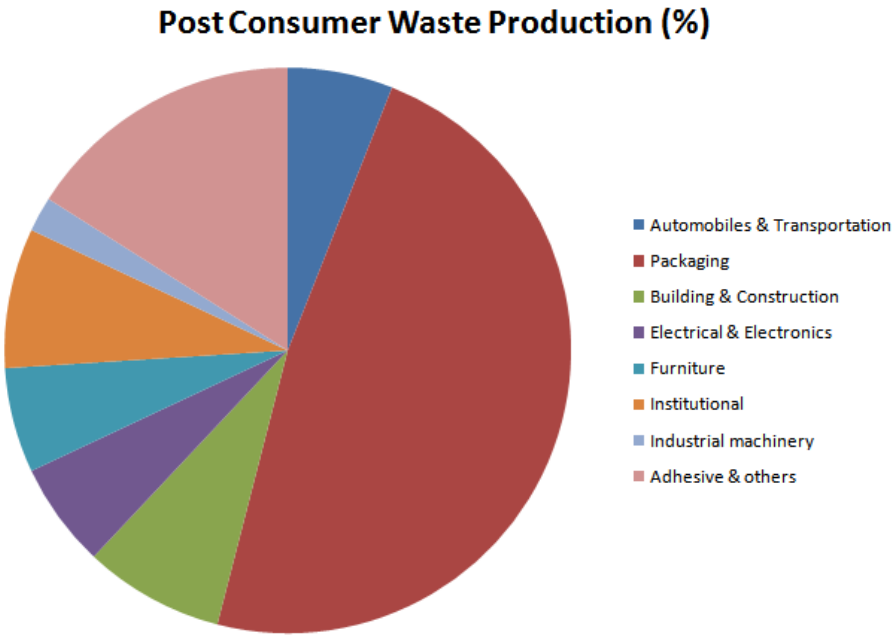

Figure 1.2: Global scenario of post consumer plastic waste **[8]**

1.2 Plastic Pollution

Plastic is considered as one of the most toxic pollutant as it takes up to 500-1000 years to degrade in natural environment. Plastic pollution is the result of accumulation of plastics in the environment which adversely affects the environment and its inhabitants. Pollution caused by plastics contributes to various types such as marine pollution, land/soil pollution, air pollution and aesthetic pollution. Marine or coastal area is the ultimate dumping site for almost all type of wastes. Plastic waste shares about 60-80% by mass of the total marine waste **[7]**. Plastic waste accumulates at an increasing rate not only on the shoreline or coast but also on sea floor as shown in figure 1.3.

Figure 1.3: Plastic waste found on and under sea surface **[12]**

According to National Oceanographic and Atmospheric Administration, 100,000 marine mammals die due to ingestion of plastic debris. The sea inhabitants and other animals ingest the polymeric material accidently which is responsible for intestinal blockage in small fishes or suffocation in other animals such as dolphins, turtle etc **[13]**. It also enters the food chain and remains there for very long time which is detrimental for the living system **[14]**. Around 267 species including seabirds, mammals etc in the marine environment are affected by plastic pollution **[15]**. 50 to 80 percent of sea turtles found dead are known to have ingested plastic marine debris **[12]**. Research into the stomach contents of dead Fulmars from the Netherlands, between 1982 and 2001, found that 96 percent of the birds had plastic fragments in their stomachs with an average of 23 plastic pieces per bird **[16]** as shown in Figure 1.4.

Figure 1.4: Plastic debris found in the corpse of fish and bird **[12]**

Soil infertility and toxicity are two major issues associated in context with land or soil pollution. The plastic dumping in landfills results in reduced fertility of soil due to the barrier properties of plastics. The natural degradation process of polymer consequently releases toxic products like lead,

cadmium pigments (used as additives) etc which get leached into the soil and thereby increase the soil toxicity. Air pollution is caused both during manufacturing and destruction of plastic material. During polymerization and manufacturing process various types of emissions are released. Incineration or open burning of the plastics waste releases toxic pollutants like carbon monoxide, furans, amides, benzenes, furan, dioxin etc **[10]**. Most of these pollutants are carcinogenic which can adversely affect human health.

1.3 Remedies for Plastic Pollution

There are different strategies which can be adopted to reduce littering and accumulation of plastic waste in the environment which are as follows:

- Recycling
- Reusing
- Energy recovery
- Prevention

Recycling is an acceptable remedy worldwide but it's not applicable for all types of plastic material. The collection and cleansing of plastic waste can be costly **[17]**. The properties of the recycled plastics are not as good as virgin plastics so their useful life is reduced. Moreover, the disposal of recycled plastics is more detrimental for environment. Reusing is also not as acceptable as recycling. Energy recovery from plastic waste is a costly method so it's not practiced widely.

In India, Central Pollution Control Board (CPCB) has given some norms for recycling and reducing plastic waste which are as follows **[10]**:

- Plastic carry bags used for various purposes should not be less than 40µ thickness.
- Carry bag should be made of compostable plastics as per IS/ISO: 17088: 2008.
- Every plastic carry bag should bear "recycled" label as per IS: 14534: 1998.
- Carry bags should not be provided to the customer free of cost.
- Every State Government should have a State Level Advisory (SLA) to monitor the implementation of rules.

So, prevention is the best option for tackling the plastic waste. Replacing the conventional plastics with biodegradable plastics is the best method for plastic pollution.

1.4 Research Objectives

The overall objective of the research is the assessment of biodegradation of non-biodegradable polymers which are hugely employed for packaging applications. Non-biodegradable polymers such as HDPE is highly resistant to degradation, there is need to make them prone to degradation which can be achieved by blending with biodegradable polymers such as PLLA. The specific objective(s) are:

- Biodegradability assessment of different blends of HDPE/PLLA that can be utilized for packaging applications.
- Optimizing the conditions which can enhance the rate of biodegradation.

1.5 Thesis Overview

This thesis consists of five chapters. Chapter 1 contains a brief introduction to the research topic. Chapter 2 contains a more detailed literature review on assessment of biodegradation. In chapter 3, materials and methods briefly discussed about the instrumentation and methodology involved. In chapter 4, results obtained are shown, analyzed and discussed. The final chapter (chapter 5) includes the conclusions that can be drawn from the results obtained as well as recommendations for future work.

2.1 Bioplastics

Depletion of petroleum resources and accumulation of plastics in the environment has led to the development of biodegradable polymers. Bioplastic is the type of plastic derived from renewable biomass sources such as vegetable oil, cornstarch, and microorganisms. Bioplastics are widely classified on the basis of their source or production method into three categories as shown in Figure 2.1 **[18, 19]**:

- Renewable resource-based bioplastics: They are made from sole natural sources such as plants, animals and microorganisms. For example starch, proteins, chitosan, PLA, PHB, PHA etc.
- Petroleum-based bioplastics: They are synthesized from petroleum resource yet are biodegradable. For example polyƐ-caprolactone (PCL), poly(butylenes adipate-*co*-terephthalate) (PBAT) etc.
- Bioplastics from mixed sources: They are produced as a result of combination of bio-based and petroleum monomers. For example poly (trimethylene tereph-thalate) (PTT), bio-thermosets etc.

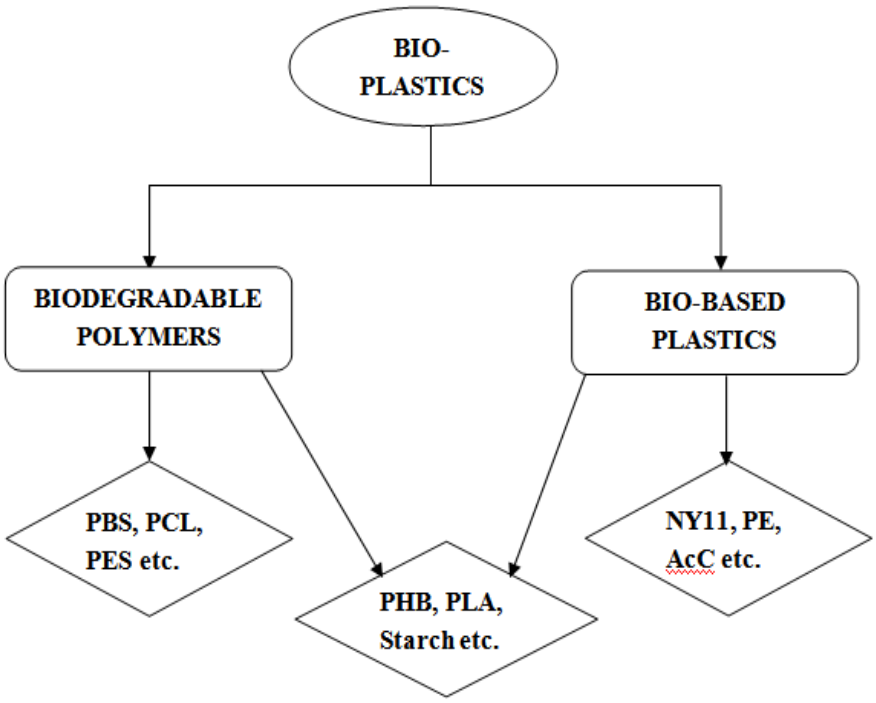

Figure 2.1: Types of bioplastics **[19]**

2.2 Biodegradable polymers

Biodegradable polymer is a promising eco-friendly solution to the problem of plastic pollution. They are the polymeric materials that undergo decomposition to form carbon dioxide, water, biomass and methane under the influence of various degradative forces **[20]**. These polymers can be synthesized from both renewable and petroleum feedstocks. Although biodegradable polymers have a lot of advantages, there are also some disadvantages associated such as performance, cost and processing. The tensile properties of most of the biodegradable polymers are good but their brittleness, low heat distortion temperature, vapor permeability, poor resistance to processing operation limits their applications **[21]**. Cost is an important factor associated with the production of biodegradable polymers. The usage of biodegradable polymers is encouraged all around the world due to efficient waste management systems. They can be recycled, reused and at the end of their useful life they can be disposed off in the environment which will not affect the environment as they get decomposed to natural products **[22]**. Polyglycolic acid (PGA), polylactic acid (PLA), polyhydroxybutyrate (PHB), polyhydroxyvalerate (PHBV), polycaprolactone (PCL), polyvinyl alcohol (PVOH) etc are some of the biodegradable polymers widely used for various packaging, industrial and biomedical applications. Some of the applications are tabulated in Table 2.1.

Table 2.1: Applications of biodegradable polymers **[23]**

Biodegradable Polymer	Uses and applications
PGA	For specialized biomedical applications like controlled drug releases, implantable composites, bone fixation parts etc.
PHB	Bottles, bags, wrapping films, control drug release carriers etc.
PCL	Mulch and agricultural films, long term items, slow or extended release of drugs etc.
PVOH	Packaging and bagging applications
PHBV	Film and paper coatings, sustained drug release and other biomedical applications

2.2.1 Polylactic acid

Polylactic Acid (PLA) is biocompatible and biodegradable polyester which is produced from renewable feedstocks **[18]**. Lactic acid is the monomer which is produced by microbial fermentation and it is further polymerized to form polylactic acid by ring opening polymerization (ROP) or direct polycondensation of lactic acid **[24]**. Since the monomer i.e. lactic acid contains

chiral centre, PLA exhibits stereoisomerism. PLA exists in different forms namely poly(L-lactide) (L-PLA), poly(D-lactide) (D-PLA) and poly(DL-lactide) (DL-PLA). L-isomer of PLA is naturally occurring while other isomers are synthesized chemically. The presence of $–CH_3$ side group makes PLA a hydrophobic polymer **[25]**. Stereo-regularity of PLA makes it highly crystalline in nature. Highly crystalline PLA contains less D-isomer content i.e. (<2%) and fully amorphous PLA contains >20% of D-isomer content. Stereochemistry of PLA significantly affects its physicochemical and mechanical properties. The density of solid amorphous PLA is ~ 1.25 gm/cm^3 whereas for purely crystalline PLA, it ranges from 1.37-1.49 g/cm^3. PLA exhibits a melting temperature (T_m) and glass transition temperature (T_g) of 63.8˚C and 170˚C -180˚C respectively. The mechanical properties of PLA are good with tensile strength of 32.2 MPa but its elongation at break i.e. 30.7% contributes to brittleness. PLA is a brittle polymer, therefore for improving the elasticity it is blended with other polymers and additives. Brittleness and poor thermal stability are some of the disadvantages of PLA. PLLA is hard, transparent and semi-crystalline polyester as compared to PDLA which is amorphous in nature. PLLA exhibits stronger tensile strength than PDLA with elongation at break of 85%-105% and tensile strength of 45-70 MPa. So, PLLA is more widely used for blending with various non-biodegradable polymers such as HDPE, PP, LDPE etc. The manufacturing of PLA was started by Carothers in 1932 **[19]**. PLA is produced semi-commercially by several companies which are mentioned in Table 2.2.

Table 2.2: World's leading manufacturers of PLA **[18, 26]**

Country	Name of the Company
USA	Natureworks LLC
	DuPont Chemicals
Japan	Mitssui Chemicals
	Mistubishi
	Shimadzu
	Toyota
	Dainippon Ink Chemicals
	Toyobo
	Teijin
China	Hiusan Biosciences
	Jiangsu Jiulding
Belgium	Futerro
Germany	Biomer
Netherlands	Purac
	Synbra
	Tate & Lyle

2.3 Degradation of Plastics

Polymer degradation involves change in properties such as tensile strength, color, thickness of the polymer under the influence of various environmental conditions that can be physical, chemical or biological which results in chemical transformation of polymer **[27]**. Polymer degradation is of various types which are as follows **[28]**:

- Photo-oxidative degradation
- Thermal degradation
- Ozone-induced degradation
- Mechano-chemical degradation
- Catalytic degradation
- Biodegradation

Photo-oxidative degradation involves decomposition of the polymeric material due to the action of light. UV and visible lights are used for initiation of degradation in most of the synthetic polymers. Sunlight which is a near UV-radiation (290-400 nm) determines the service life of polymer for outdoor applications, as it has negative influence on polymer **[29]**. The light mainly attacks the ether part of the polymer and generates functional groups like ester, formate, propyl, aldehyde etc which makes it more susceptible for degradation **[30]**. UV radiation possess sufficient amount of energy for cleaving C-C bond **[31]**. Different polymers exhibit maximum degradation at different wavelength of radiation such as 300 nm for PE, 370 nm for PP etc. Upon irradiation with light of appropriate wavelength, different types of changes are observed in polymer such as yellowing (visible effect), loss of mechanical properties and change in molecular weight and molecular weight distribution **[32-34]**. Degradation can be enhanced at an optimum temperature and moisture content. Photo-degradation methods can be of two types: natural method and artificial/laboratory method. The rate of degradation is slow initially but its propagation is fast. The method is eco-friendly if high energy radiation is not used. The method is acceptable, but is costlier than conventional ones.

Thermal degradation refers to molecular deterioration of the polymer due to elevation in temperature. It is a bulk phenomenon unlike photo-oxidative degradation which is a surface phenomenon **[35]**. Thermal degradation of polymers brings about random and chain degradation, which can start anywhere in the polymeric chain. The chemical reaction involved in thermal degradation leads to physical and optical changes in properties of the polymer. Polyolefins degrades thermally at a temperature of 350˚C-450˚C, which produces organic compounds such as phenol, quinine, naphthalene etc **[36]**. Thermal degradation is influenced by various factors such as

heating rate, pressure, reaction medium, reactor geometry and viscosity of polymer **[37]**. There are three different methods of thermal degradation namely batch reactor method, thermogravimetric analysis and pyrolysis GC/MS method. The rate of degradation is fast but the method is not acceptable.

Ozone-induced degradation refers to the polymer degradation in the presence of ozone. Although ozone is present in atmosphere in very small concentrations, it significantly accelerates the aging of polymeric materials **[38]**. The degradation of polymers result in formation of ether, vinyl and hydroxyl functional groups which results in impairment of structural and mechanical properties of the polymer **[39]**. Ozone primarily attacks C=C bonds, aromatic rings and saturated hydrocarbon linkages **[40]**.

Mechano-chemical degradation is the degradation of polymer under the influence of mechanical stress and ultrasonic irradiations **[41]**. Mechanical stress is often aided by chemical reaction. When excessive mechanical stress is applied, the molecular chain breaks to form a pair of free radicals, which undergo subsequent chemical reactions resulting in reduction of polymer's average molecular weight **[42]**. Agitation, extrusion and grinding are some of the mechano-chemical methods for polymer degradation.

Catalytic degradation refers to the catalytic transformation of polymer into hydrocarbons. Polyolefins such as PE, PP, PS are thermally and catalytically degraded to produce gases and oils. It involves addition of catalyst to polymer which not only reduces the temperature for decomposition but also improves the quality of products after pyrolysis. Zeolite is used as a solid acid catalyst for this purpose **[43]**. Batch autoclave method is often used for this type of polymer degradation.

The types of degradation mentioned above are not applicable for the huge amount of plastic waste which is generated around the globe. Therefore, biodegradation is the appropriate method for polymer degradation. Even though the rate of degradation is moderate but the method is eco-friendly, cheap and much acceptable.

2.4 Biodegradation of Plastics

2.4.1 Biodegradation definitions

There are different definitions for biodegradation and biodegradable plastics given by different standards which are as follows **[44, 45]**:

- According to DIN FNK 103.2 (German working group), biodegradation is defined as a process, caused by biological activity, which leads under change of the chemical structure to naturally occurring metabolic products.
- As per ASTM sub-committee D20-96, biodegradable plastic is a degradable plastic in which the degradation results from the action of naturally occurring microorganisms such as bacteria, fungi and algae.
- Japanese Biodegradable Plastics Society defines biodegradable polymers as the polymeric materials which are changed into lower molecular weight compounds where at least one step in the degradation process is through metabolism in the presence of naturally occurring organisms.
- According to ISO 472, biodegradable polymer is defined as a plastic designed to undergo a significant change in its chemical structure under specific environmental conditions resulting in a loss of some properties that may vary as measured by standard test methods appropriate to the plastic and the application in a period of time that determines its classification. The change in the chemical structure results from the action of naturally occurring microorganisms.
- CEN defines biodegradable plastics as a degradable material in which the degradation results from the action of microorganisms and ultimately the material is converted to water, carbon dioxide and/or methane and a new cell biomass. Further biodegradation is defined as degradation caused by biological activity, especially by enzymatic action, leading to a significant change in the chemical structure of a material **[44]**.

2.4.2 Types of biodegradation

Biodegradation is a natural process of transformation of complex polymeric material into simpler molecules by micro-biota present in the environment under specific conditions like moisture, temperature, pH etc. The biodegradation can occur in two ways either aerobically i.e. in compost or anaerobically i.e. in landfills. Aerobic biodegradation leads to the formation of water, carbon dioxide and biomass as per the following equation **[46]**:

$$C_{polymer} + O_2 \longrightarrow CO_2 + H_2O + C_{biomass} \quad (1)$$

Whereas in anaerobic biodegradation an additional compound methane is also formed as per the mentioned equation:

$$C_{polymer} \longrightarrow CO_2 + H_2O + CH_4 + C_{biomass} \quad (2)$$

2.4.3 Mechanism of biodegradation

The mechanism of biodegradation is very complex (Figure 2.2). Due to the hydrophobic nature and large size of polymeric materials, the microbes are not able to uptake them directly into the cells where majority of metabolic or biochemical reactions takes place. Therefore, the biodegradation occurs in two major steps, first is the secretion of extracellular enzymes which act on long polymeric chains and break down them into oligomers, dimmers and monomers which are easily transported across the cell membrane and consequently utilized in appropriate metabolic pathway. The second step is mineralization or bioassimiliation which involves the conversion of polymeric material into gases, water and residual biomass. The oligomeric units are decomposed to produce water, carbon dioxide, methane and biomass. Biodegradation is often referred as surface erosion as the large size of extracellular enzyme does not allow their penetration inside the polymer **[47]**.

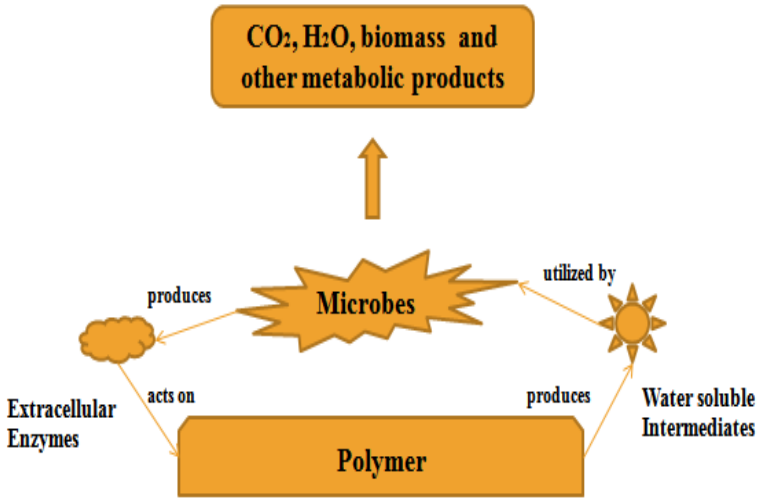

Figure 2.2: Mechanism of biodegradation **[47]**

2.4.4 Factors influencing biodegradation

There are various factors which affect biodegradation of polymer **[19, 25]**:

- Presence of suitable microbial population in the compost or soil.
- Physical properties of polymer such as surface area, hydrophobicity, melting temperature, glass transition temperature, modulus of elasticity etc.
- Chemical properties of the polymers such as chemical structure, molecular weight, molecular weight distribution, chemical bonding, crystal structure, crystallinity etc.
- Method of synthesis of polymer
- Environmental factors such as presence or absence of oxygen, moisture, temperature, light etc.

- Pre-treatment of the polymer material such as thermal treatment, addition of pro-oxidants or other additives etc **[4, 48, 49]**.

2.5 Biodegradation of Natural Plastics

Natural plastics are produced by biotic sources such as plant, microbes etc. A variety of bacteria produce polyhydroxyalkanoates (PHA) which is utilized as energy storage material. Similarly polyhydroxybutyrate (PHB) is produced by various microbes **[50]**. These natural plastics are easily metabolized by extracellular hydrolases secreted by microbes. The enzyme cleaves the polymeric chain to produce the monomer which easily diffuses in the cell and where they are metabolized by β-oxidation and tricarboxylic acid cycle (TCA) to produce CO_2, H_2O and biomass, methane is also produced if conditions are anaerobic.

2.6 Biodegradation of Synthetic Plastics

There is variety of synthetic polymers such as PE, PP, PS, PCL, PVC, PET etc. which are resistant to degradative force due to their huge molecular weight and hydrophobic nature. So as to facilitate the biodegradation of non-biodegradable polymers such PE, PS, PP etc. they are blended with biodegradable polymers such as PLA, starch or additives like pro-oxidants, which make polyethylene susceptible to biodegradation **[51]**. Biodegradation of polyethylene occurs by two different mechanisms: Hydro-biodegradation and oxo-biodegradation **[52]**. The addition of starch or pro-oxidants reduces the hydrophobicity and facilitates fragmentation of polymeric chain. Various species of bacteria and fungi such as *Streptomyces*, *Bacillus*, *Aspergillus*, *Pseudomonas*, *Penicillium* etc extensively take part in degradation of polyethylene **[53-58]**.

2.7 Biodegradability Testing

There are different types of biodegradability testing methods which can be adopted for biodegradability assessment and the result and conclusion varies depending on the condition of the test, viz. field test, simulation test, and laboratory test.

Field Test: This test refers to the testing which is performed in real environmental conditions. Field tests include burying plastic in soil, placing the plastic in river or lake or it can be a full-scale composting process. The problems with field test are that the conditions in real environment cannot be controlled, the rate of degradation cannot be monitored and the residues or intermediates cannot be quantified after degradation. At the end of test period, degradation can be analyzed by visible physical changes and weight loss. Since mere physical disintegration cannot be regarded as biodegradation, therefore the field test alone cannot be considered reliable for biodegradability assessment **[4]**.

Simulation Test: The degradation of the polymer takes place in compost, soil, or sea water etc which is carried out under controlled conditions in laboratory. The conditions are very close to real environment but the parameters such as pH, temperature etc. are controlled and adjusted as per the requirement. The intermediates and residues obtained after degradation can be analyzed; the rate of degradation can be determined by quantifying the amount of CO_2 evolved or O_2 consumed. Controlled composting test **[59]**, landfill simulating test and aqueous aquarium test **[60]** are some of the tests which are carried for simulation studies.

Laboratory Test: This test provide the most reproducible biodegradation results. The media is defined, microbial population is selective and all the other parameters are optimized so the rate of degradation in this test is much higher than the test performed in natural environment. Absolute degradation can be determined and exact conclusion can be drawn from the experiments. The advantages of adopting this method include study of biodegradation mechanism and kinetic studies of biodegradation. Various analytical methods can be used for biodegradability assessment which are as follows:

- Visual observations by SEM or atomic force microscopy AFM **[61]**
- Change in mechanical properties and weight loss **[62]**
- CO_2 evolution or O_2 consumption by titration, GC/MS etc **[59]**
- Determination of biogas by ASTM D 5511, ASTM D 5210, ISO/DIS 15985 **[63]**
- Clear zone formation for checking the ability of microbes in de-polymerizing the polymeric substrate **[64]**

3.1 Materials

3.1.1 Blended Films: The blended films which are used as test samples were obtained from Department of Chemical Engineering (ChED), Thapar University Patiala. The samples are composed of HDPE, PLLA and maleic anhydride grafted polyethylene (M-*g*-P). The compositions of the blended films along with their codes are mentioned in the table 3.1.

Table 3.1: Composition of HDPE/PLA blended films

Sr. No.	Sample Code	Composition		
		HDPE (wt%)	**PLA (wt%)**	**M-*g*-P (phr)**
1	B1	95	5	0
2	B2	90	10	0
3	B3	85	15	0
4	B4	80	20	0
5	X1	80	20	2
6	X2	80	20	4
7	X3	80	20	6
8	X4	80	20	8

3.1.2 Chemical reagents

- 0.024N Barium hydroxide solution which was prepared by dissolving 4.0 g $Ba(OH)_2$ per litre of double distilled water and was further standardized.
- 0.05N HCl solution was prepared by dissolving 1.55 ml of concentrated HCl in 1 litre of double distilled water.
- Microcrystalline cellulose of size <20µ used as positive control
- HDPE used as negative control
- Phenolphthalein indicator

3.1.3 Compost soil: The mature compost (municipal solid waste) was obtained from Delhi Jal Board composting plant, Municipal Council, Okhla, New Delhi, India. The compost inoculums were stabilized at the laboratory in order to obtain a low CO_2 production. The compost was screened through 10 mm mesh sieve to remove large inert substances (pieces of glass, stone, wood, etc.). The mature compost had the following basic properties or characteristics: pH 7.7; C/N ratio 20.6; dry solids 52%; moisture 74%; total dry solids 73.7%; total volatile solids 20.4%.

3.1.4 Biodegradability testing apparatus: The laboratory-scale Biodegradability Testing Apparatus was designed as per the guidelines of ASTM D 5338-98 at the Department of Chemical Engineering, Thapar University, Patiala and fabricated by M/s. Andel Equipment Ltd., Mohali,

India. The apparatus comprises three different components. The first component is the carbon dioxide-free air supply i.e. cylinder and the rotamers for controlling the amount of CO_2 free air to be supplied. The second component is a temperature controlling chamber i.e. incubator in which sample along with compost is present in different bioreactors. The third component is carbon dioxide trapping assembly in which there are three conical flasks in series containing CO_2 scrubbing solution i.e. barium hydroxide for each sample.

3.2 Methodology

3.2.1 Standard testing method : ASTM D5338

ASTM D 5338 determines the rate and extent of aerobic biodegradation for plastic materials under controlled-composting conditions. The test materials are exposed to compost inoculums obtained from municipal solid waste. The aerobic composting takes place in an environment where temperature, aeration and humidity are closely controlled and monitored. This test method is designed so as to yield reproducible results. The rate of biodegradation is determined by the conversion of carbon present in the sample to carbon dioxide. This test method is equivalent to IS0 14852. The percentage of biodegradability is obtained by determining the percentage of carbon in the test substance that is converted to CO_2 during the duration of the test. It does not include the amount of carbon converted from the test substance which is utilized for the metabolism of cell biomass during the course of test **[65]**.

3.2.2 Evaluation of compost viability

Compost viability is a significant factor which will directly affect the degree of biodegradation. Therefore, to check whether the microbial population in the compost are enough to facilitate biodegradation, it is necessary to carry out isolation of the microbes by microbiological experiments. One gram of compost was mixed in 9 ml of distilled water to made stock sample. Serial dilutions were prepared from the stock samples such as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, etc. These serially diluted samples were inoculated on NA (Nutrient agar) plates by spreading. The NA plates were incubated at 37ºC for 24-48 hours. Then, the colony formation on the plates was checked.

3.2.3 Biodegradability test

Evolution of carbon dioxide or methane from carbon source indicates bio-assimilation or mineralization which is quantified by the amount of carbon dioxide evolved in aerobic conditions and by the amount of methane evolved in anaerobic conditions.12 composting vessels were taken and labelled according to sample names/codes. One gram of film sample and mature compost of

250 grams were weighed for each composting vessel. Most aerobic standard tests apply continuous aeration, so a continuous stream of pressurized CO_2-free air is set for supplying to the composting vessels with a fixed aeration rate of 60 ml/min which ensures atleast 6% oxygen level in the exhaust air. The composting vessels were incubated in dark inside the biodegradability testing apparatus for a period of 45 days. Initially, the incubation temperature is kept 35˚C(±2˚C) which is referred as mesophillic start-up phase for 1 day, then the temperature is raised to 58˚C(±2˚C) for 4 days and it is considered as sanitizing period. The temperature is then reduced to 50˚C(±2˚C) till day 28 for maintaining optimum composting conditions, temperature is further reduced to 35˚C(±2˚C) for the remaining test period i.e. till day 45. The exit stream of air can be directly analyzed continuously using a carbon dioxide monitor (usually infrared detectors) or titrimetrically after sorption in dilute alkali. CO_2 concentrations in the outgoing air is measured daily after the first week for the remainder of the test by titration of $Ba(OH)_2$ scrubbing solution present in the CO_2-trapping apparatus against hydrochloric acid (HCl).

3.2.4 Weight Loss

Weight loss is a significant and convenient way for determining the biodegradation of polymer which is directly proportional to the reduction in polymer's integrity. After 45 days of test period (degradation time), the microbial biofilms were washed with 2% (v/v) aqueous sodium dodecyl sulphate solution for 4 hours followed by distilled water. The washed polymer samples were placed on a filter paper and dried overnight at 60°C before calculating the weight loss **[66]**.

3.2.5 Estimation of the protein content of biofilms

The total protein content of the biofilm biomass was determined using alkaline hydrolysis method. Polymer films were sampled from bioreactors containing soil compost, washed with water to remove compost debris and boiled for 20 min in 4.0 ml of 0.5N sodium hydroxide (NaOH). The suspension was centrifuged at 10,000 rpm at 4°C for 15 min. The supernatant was kept aside and pellet was subjected to the same procedure repeatedly. The two supernatants were mixed and the protein concentration was determined spectrophotometrically at 280 nm and 260 nm **[67]**. The ratio of absorbance at 280 nm: 260 nm was calculated to determine corresponding factor (F) and the protein concentration was calculated using the following formula:

$$\text{Protein concentration (mg/ml)} = F \times \text{Absorbance at 280 nm} \times 1/d$$

where d is the cuvette width in cm.

4.1 Compost Viability

The experiment was carried out for evaluation of compost viability and it was found that the compost contains sufficient amount of microbes which are beneficial for the biodegradation of the plastic material as shown in Figure 4.1. Since biodegradation is primarily mediated by microbes, more the number of microbes, greater will be the degree of biodegradation.

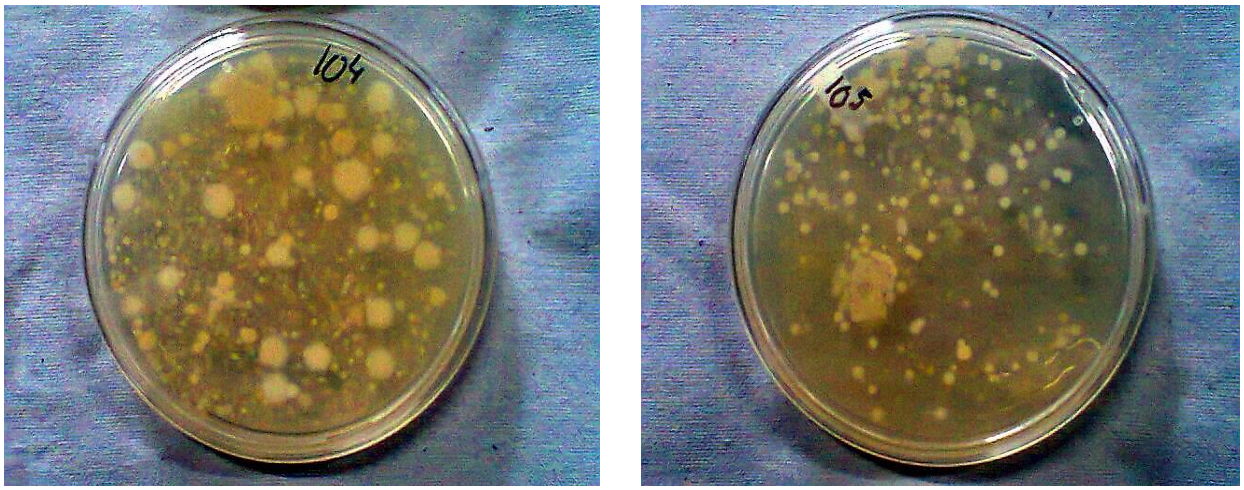

Figure 4.1: Microorganism population isolated from the compost used for biodegradation

4.2 Theoretical Carbon Dioxide Evolved from the Test Sample by Elemental Analysis

Molecular formula of HDPE: $(CH_2{=}CH_2)_n$

Molecular weight of HDPE used = 28000 grams/mole

Molecular weight of monomer i.e. ethylene = 28

$$n = \frac{28000}{28} = 1000 \text{ units}$$

As 1 mole (unit) of HDPE contains 24 grams of carbon, therefore, 1000 units contain 24000 grams of carbon

Hence, 1 mole = 24000 grams of carbon

For 1 gram of HDPE, $\text{number of moles} = \frac{1}{28000} = 0.0000357$

Amount of carbon in 1 gram sample of HDPE = $0.0000357 * 24000 = 0.857$ g.

Similarly,

Molecular formula of PLLA: $(C_3H_4O_2)_n$

Molecular weight of PLLA used = 9000 grams/mole

Molecular weight of monomer i.e. lactic acid = 72

$$n = \frac{9000}{72} = 125\ units$$

1 mole (unit) of PLLA contains 36 grams of carbon,

Therefore, 125 units will contain =125×36 =4500 grams of carbon

Hence, 1 mole = 4500 grams of carbon

For 1 gram sample, $number\ of\ moles = \frac{1}{9000} = 0.00011$

Amount of carbon in 1 gram sample of PLLA = 0.00011 ×4500 = 0.5 g.

According to the above calculations, the amount of carbon present in each HDPE/PLLA blended films (1 gram approx. by weight) was calculated and the theoretical CO_2 was also calculated for each sample as per the following formula:

$$Theoretical\ carbon\ dioxide\ (ThCO_2) = \frac{Amount\ of\ carbon\ present \times 44}{12} \quad (3)$$

The values of carbon content in the samples and the theoretical amounts of CO_2 evolved from the respective samples are shown in Table 4.1:

Table 4.1: Carbon content and theoretical carbon dioxide of various samples

Sr. No.	Sample Name	Carbon content (in grams) in 1 gm of sample (film)	Theoretical CO_2 evolved (in grams)
1.	B1	0.83815	3.070
2.	B2	0.81930	3.004
3.	B3	0.80045	2.935
4.	B4	0.78160	2.866
5.	X1	0.79876	2.930
6.	X2	0.81573	2.991
7.	X3	0.83270	3.050
8.	X4	0.84967	3.104
9.	Negative	0.85700	3.142
10.	Positive	0.40000	1.467

4.3 Determination of Experimental Carbon Dioxide Evolved from the Test Sample

The amount of CO_2 produced in the composting vessel was trapped in CO_2 scrubbing solution i.e. barium hydroxide and reacted to form barium carbonate ($BaCO_3$) as shown below:

$$Ba(OH)_2 + CO_2 \rightarrow BaCO_3 + H_2O \quad (4)$$

The amount of CO_2 is determined by the difference in volume of titrant, between the test substance and blank, trapped in $Ba(OH)_2$ by performing titration with 0.05 N HCl. The $BaCO_3$ so formed is insoluble and precipitates. The amount of $Ba(OH)_2$ remaining in solution is determined by end-point titration with HCl using phenolphthalein as an indicator according to the following equation:

$$Ba(OH)_2 + 2\ HCl \rightarrow BaCl_2 + 2\ H_2O \quad (5)$$

Formulae used (ASTM D 5338)

Carbon dioxide evolution: The number of mmoles of CO_2 produced is calculated by using the following formula:

$$mmoles\ of\ CO_2 = mmoles\ of\ Ba(OH)_2 - \frac{mmoles\ of\ HCl}{2} \quad (6)$$

Further, the amount of carbon dioxide evolved can be determined by using the following formula:

$$Amount\ of\ CO_2\ evolved\ (in\ grams) = mmoles\ of\ CO_2\ obtained \times 0.044 \quad (7)$$

Percentage biodegradation: The percentage biodegradation was calculated by dividing the average net gaseous-carbon production of the test compound by the original average amount of carbon in the test compound and multiplying by 100:

$$\%biodegradation = \frac{Mean\ CO2\ (sample) - Mean\ CO2\ (blank)}{Thoeretical\ carbon\ dioxide\ in\ sample} \times 100 \quad (8)$$

Calculation for carbon dioxide evolution

Barium hydroxide: 0.024N $Ba(OH)_2$ scrubbing solution is used to capture carbon dioxide

Normality of $Ba(OH)_2$ = 0.024N

Molarity of 0.024N $Ba(OH)_2$ = 0.024×2 = 0.048 moles = 48 mmoles of $Ba(OH)_2$

If 1000 ml of 0.024N $Ba(OH)_2$ contains 48 mmoles of $Ba(OH)_2$

Then 1 ml of 0.024N $Ba(OH)_2$ will contain = 48/1000 = 0.048 mmoles of $Ba(OH)_2$

Since 10 ml of $Ba(OH)_2$ is used for titration,

so 10 ml of 0.024N $Ba(OH)_2$ will contain = 0.048 ×10 = 0.48 mmoles of $Ba(OH)_2$

Hydrochloric acid (HCl): 0.05N HCl is used for titration of $Ba(OH)_2$

Normality of HCl =0.05N

Molarity of 0.05N HCl= 0.05 × 1= 0.05moles= 50 mmoles of HCl

If 1000 ml of 0.05N HCl contains 50 mmoles of $Ba(OH)_2$

Then 1 ml of 0.05N HCl will contain = 50/1000 = 0.05 mmoles of $Ba(OH)_2$

For example: Volume of titrant i.e. HCl for sample B1 flask 3 is 14.8 ml

If 14.8 ml of 0.05N HCl is used for titrating 10 ml of 0.024N $Ba(OH)_2$

Then, mmoles of HCl required to titrate 0.48 mmoles of $Ba(OH)_2$ = 14.8×0.05 = 0.74 mmoles of HCl

For calculating mmoles of carbon dioxide produced

$$mmoles\ of\ CO_2 = mmoles\ of\ Ba(OH)_2 - \frac{mmoles\ of\ HCl}{2}$$

$$mmoles\ of\ CO_2 = 0.48 - \frac{0.74}{2}$$

$$= 0.48 - 0.37 = 0.11 \text{ mmoles of } CO_2$$

1 mole of carbon dioxide = 44 g/l

1000 mmoles of carbon dioxide = 44 g/l

1 mmole of carbon dioxide = 0.044 g/l

So, amount of CO_2 in grams = 0.11×0.044 = 0.00484 grams of CO_2

Similar calculations were done for all the samples. The amount of CO_2 evolved and hence the extent of biodegradation of the samples was calculated. Table 4.2.1 and 4.2.2 tabulate the titration results for each sample and the amount of CO_2 produced and percentage biodegradation is shown in tables (Table 4.3 and 4.4) and also depicted in figures (Figure 4.2, 4.3, 4.4 and 4.5).

The biodegradability of different samples i.e. with compatibilizer (X1, X2, X3 and X4) and without compatibilizer (B1, B2, B3 and B4) was evaluated under controlled aerobic composting conditions in the laboratory. Biodegradation is generally measured as the degree of mineralization or percent biodegradation. The biodegradable components of the blended film samples eventually get mineralized to form CO_2 and H_2O due to microbial activity at the end of test period of 45 days. The

amount of theoretical CO_2 for each sample is shown in Table 4.1. As shown in Figure 4.2, the amount of CO_2 evolved from blank (compost only) is almost negligible i.e. 0.0093 g which is deducted from each sample to estimate the net CO_2 evolved from each sample. The positive control (MCE) evolves maximum amount of CO_2 i.e. 0.08152 g with corresponding percent biodegradation of 16.5% as compared to negative control (100% HDPE) with 0.0158 g of CO_2 evolution and 1.84% biodegradation. Among all the samples, X4 (HDPE/PLLA/M-g-P: 80/20/8) exhibits maximum biodegradation i.e. 3.5% with corresponding CO_2 evolution of 0.04504 g followed by X3 (HDPE/PLLA/M-g-P: 80/20/6), X2 (HDPE/PLLA/M-g-P: 80/20/4), X1 HDPE/PLLA/M-g-P: 80/20/2), B4 (HDPE/PLLA: 80/20), B3 (HDPE/PLLA: 85/15), B2 (HDPE/PLLA: 90/10), B1(HDPE/PLLA: 95/5) and negative control (100% HDPE) with 0.04147 g, 0.04037 g, 0.0396 g, 0.0346 g, 0.03263 g, 0.03158 g, 0.02973 g and 0.0158 g of carbon dioxide respectively. The amount of carbon dioxide continuously increases as the ratio of PLLA in the blend increases which is evident from amount of CO_2 evolved from B1, B2, B3 and B4. The CO_2 evolution further increases when M-*g*-P is added to the blends i.e. in case of X1, X2, X3 and X4. Similar increasing trend for percent biodegradation was also observed as shown in Figure 4.4 and 4.5. The addition of M-*g*-P reduces the phase separation between HDPE and PLLA and introduces some structural modifications viz. branching, which enables the microbes to utilize HDPE as carbon source. Blending of non-biodegradable polymers with biodegradable polymers and other additive made them more prone to degradation.

Table 4.2 (a): Titration data of blank, positive, negative, B1, B2 and B3.

Time (in Days)	Volume of titrant (in ml)																	
	Blank			Negative			Positive			B1			B2			B3		
1	14.6	15.1	16.2	13.4	14.9	16.5	9	10.5	11.2	13.1	14.4	14.8	12.9	13.9	14.2	12.2	13	13.6
2	14.4	15	16.4	13.3	14.8	16.5	8.6	9.8	11	12.9	14.3	14.8	12.8	13.9	14.1	12.2	12.8	13.6
3	14.2	15	16.2	13.1	14.7	16.3	9.4	10.5	10.9	12.8	14.1	14.8	12.7	13.8	14.2	12.1	12.9	13.6
4	14.1	15	16.2	13.1	14.5	16.2	9.1	10.1	10.8	12.7	13.8	14.8	12.5	13.7	14	12.1	12.7	13.5
5	14.1	14.9	16.1	13	14.3	16.2	8.8	9.8	10.6	12.8	13.7	14.8	12.6	13.7	13.9	11.9	12.6	13.4
6	14	14.9	16	12.8	14.2	16.2	8.5	9.6	10.5	12.5	13.5	14.8	12.4	13.5	13.8	11.7	12.4	13.1
7	13.8	14.9	16.1	12.7	14.1	16.1	8.4	9.4	10.3	12.2	13.3	14.8	12.1	13.6	13.9	11.8	12.3	13
8	13.7	14.7	16	12.6	14.1	16.1	8.2	9.1	10.2	12.1	13.2	14.8	12.2	13.5	13.7	11.6	12.1	12.9
9	13.6	14.8	16	12.5	14	16.1	8	8.9	10.1	11.9	13	14.7	12	13.3	13.6	11.5	11.9	12.6
10	13.6	14.7	16	12.4	13.9	16	7.8	8.6	10	12	12.9	14.5	12	13.2	13.4	11.5	12	12.5
11	13.5	14.7	16	12.4	13.7	15.9	7.7	8.4	9.8	11.9	12.7	14.2	11.8	13	13.1	11.3	11.7	11.9
12	13.5	14.6	16	12.3	13.7	15.9	7.4	8.2	9.7	11.8	12.8	14	11.7	12.8	13	11.3	11.6	11.9
13	13.5	14.6	16	12.4	13.7	15.9	7.4	8.1	9.5	11.6	12.6	13.7	11.5	12.5	12.8	11.2	11.5	11.9
14	13.4	14.6	16	12.4	13.6	15.9	7	8	9.4	11.6	12.5	13.4	11.5	12.3	12.5	11.1	11.5	11.8
15	13.4	14.5	16	12.4	13.5	15.8	6.7	7.8	9.3	11.3	12.2	13.2	11.2	12.2	12.5	11	11.3	11.8
16	13.3	14.5	16	12.4	13.4	15.8	6.4	7.6	9.3	11.2	12.1	13	11	12.1	12.5	10.8	11.1	11.7
17	13.3	14.5	16	12.2	13.3	15.8	6.2	7.4	9.2	11	12	12.8	11	12	12.4	10.5	11	11.7
18	13.3	14.5	16	12.1	13.3	15.8	5.9	7.2	9.1	10.9	12	12.8	10.9	11.8	12.3	10.4	10.9	11.5
19	13.2	14.5	16	12.1	13.2	15.8	5.7	7.1	9	10.8	11.9	12.5	10.5	11.6	12.2	10.3	10.8	11.5
20	13.2	14.4	16	12.1	13.1	15.8	5.5	7	8.8	10.7	11.6	12.3	10.1	11.2	12	10	10.5	11.5
21	13.2	14.4	16	12	13.1	15.7	5.2	6.8	8.8	10.5	11	12.1	10	10.2	12	9.8	10.4	11.3
22	13.2	14.3	16	12	13	15.7	5.1	6.6	8.6	10.3	11	11.8	9.9	11	11.6	9.8	10.2	11
23	13.1	14.3	16	11.9	13	15.7	4.9	6.4	8.5	10	10.8	11.5	9.4	10.7	11.2	9.2	10	10.8
24	13.1	14.2	16	11.9	12.9	15.7	4.6	6.3	8.5	9.9	10.3	11.2	9.1	10.3	11	9	9.7	10.8
25	13.1	14.2	16	11.9	12.8	15.7	4.3	6.1	8.3	9	10	10.9	9	9.9	10.7	8.8	9.3	10.5
26	13	14.2	16	11.8	12.8	15.7	4.1	6	8.2	9.3	9.8	10.5	8.6	9.7	10.4	8.5	9.3	10
27	13.1	14.2	16	11.8	12.8	15.7	3.8	5.8	8.2	8.5	9.4	10.2	8.2	9.3	10	8.1	9	9.5
28	13	14.2	16	11.8	12.7	15.7	3.5	5.7	8.1	8.4	9.2	10	8.1	9.1	9.9	8	8.9	9.5
29	13	14.2	16	11.7	12.8	15.7	3.1	5.4	8	8.3	9.1	10	8	9	9.9	7.8	9	9.4
30	13	14.2	16	11.7	12.7	15.7	2.6	5.1	7.8	8.1	8.6	9.8	7.6	8.6	9.6	7.4	8.4	9.2
31	12.9	14.2	16	11.6	12.7	15.7	2.1	4.8	7.6	7.9	8.4	9.5	7.3	8.3	9.4	7.2	8.1	9
32	12.9	14.2	16	11.6	12.6	15.7	1.7	4.5	7.5	7.8	8.2	9.3	7.1	8.2	9.3	7	8	9.1
33	12.9	14.2	16	11.6	12.6	15.7	1.1	4.2	7.3	7.8	8.4	9.1	7.2	8.2	9.1	7	8	9
34	12.8	14.2	16	11.5	12.6	15.7	0.5	3.9	7.1	7.7	8.2	9	7	8.1	8.8	7	8	8.7
35	12.8	14.2	16	11.5	12.6	15.7	0	3.3	6.7	7.7	7.9	8.9	7	8	8.8	6.9	7.9	8.6
36	12.8	14.2	16	11.5	12.6	15.7	0	2.8	6.5	7.6	7.6	8.5	6.8	7.9	8.5	6.7	7.6	8.6
37	12.8	14.2	16	11.4	12.6	15.7	0	2.3	6.2	7.6	7.4	8.3	6.5	7.7	8.4	6.3	7.5	8.4
38	12.8	14.2	16	11.4	12.6	15.7	0	2	5.8	7.6	7.1	8.1	6.3	7.4	8.1	6.1	7.1	8
39	12.8	14.2	16	11.4	12.5	15.7	0	1.4	5.5	7.4	6.9	8	6	7.1	7.9	5.8	6.6	7.9
40	12.8	14.2	16	11.4	12.5	15.7	0	1	5.2	7.2	6.4	7.8	5.7	6.4	7.8	5.6	6.2	8
41	12.8	14.2	16	11.4	12.5	15.7	0	0.4	4.8	7.2	6.8	7.7	5.6	6.7	7.7	5.5	6.4	7.6
42	12.8	14.2	16	11.4	12.5	15.7	0	0	4.3	7	6.7	7.7	5.6	6.7	7.6	5.5	6.4	7.5
43	12.8	14.2	16	11.4	12.5	15.7	0	0	4.1	6.9	6.5	7.7	5.5	6.5	7.6	5.5	6.4	7.5
44	12.8	14.2	16	11.4	12.4	15.7	0	0	3.8	6.5	6.5	7.7	5.4	6.4	7.6	5.4	6.4	7.5
45	12.8	14.2	16	11.4	12.5	15.7	0	0	3.4	6.2	6.5	7.6	5.4	6.4	7.4	5.3	6.2	7.4

Table 4.2 (b): Titration data of B4, X1, X2, X3 and X4

Time (days)	Volume of titrant (in ml)														
	B4			X1			X2			X3			X4		
1	12	12.5	13.3	12.2	13.1	13.8	12	12.8	13.5	12	12.5	13.5	11.8	12.4	13.3
2	11.9	12.3	13.3	12.1	13.1	13.6	11.9	12.6	13.5	11.8	12.3	13.4	11.6	12.2	13.2
3	11.8	12.4	13.2	11.9	13	13.5	11.7	12.3	13.4	11.7	12.2	13.4	11.5	12.1	13.2
4	11.7	12.3	13	11.8	12.9	13.5	11.8	12.1	13.3	11.7	12	13.2	11.7	11.9	13
5	11.5	12.1	13.1	11.6	12.8	13.2	11.5	12	13.1	11.6	11.8	13.2	11.4	12	12.8
6	11.2	12	12.9	11.3	12.6	13.1	11.6	11.9	13.1	11.4	11.6	13	11.2	11.7	12.6
7	11	11.8	12.8	11.4	12.7	13.1	11.4	11.8	13	11.3	11.7	13.1	11.3	11.5	12.7
8	11.4	11.9	12.6	11.2	12.5	12.9	11.2	11.6	12.9	11.1	11.5	12.8	11.1	11.4	12.6
9	11.4	11.7	12.5	11.2	12.5	12.7	11	11.5	12.7	11	11.2	12.4	11	11.1	12.3
10	11.4	11.8	12.4	11.2	12.3	12.6	11	11.4	12.5	10.8	11.1	12.4	10.7	10.9	12
11	11.3	11.6	11.8	11.1	12.2	12.4	10.9	11.4	12.4	10.7	11	12.1	10.5	10.7	11.9
12	11.2	11.6	11.8	11	11.8	12.1	10.5	11.6	12.1	10.5	11	12	10.4	10.5	11.6
13	11	11.4	11.8	10.8	11.3	11.7	10.4	11.2	11.9	10.3	10.9	11.8	10.3	10.4	11.4
14	11	11.3	11.7	10.7	11.8	11.9	10.4	11.1	11.7	10.3	10.8	11.6	10.2	10.3	11.4
15	10.9	11.1	11.6	10.6	11.4	11.8	10.5	11	11.4	10.2	10.8	11.4	10.2	10.6	11
16	10.8	11	11.5	10.5	11.3	11.8	10.3	10.9	11.4	10.1	10.5	11.2	10	10.4	10.8
17	10.6	11	11.4	10.5	11.2	11.8	10.2	10.7	11.4	10	10.5	11	9.9	10.3	10.8
18	10.4	10.8	11.2	10.6	11.1	11.7	10.2	10.6	11.3	10	1.4	10.9	9.8	10.2	10.6
19	10.2	10.6	11.2	10.5	11.1	11.7	10.3	10.5	11.4	9.9	10.2	10.9	9.8	10.2	10.5
20	9.9	10.3	11	10.2	11	11.5	10	10.4	11.1	9.7	10	10.5	9.6	9.9	10.4
21	9.7	10.2	10.8	10	10.6	11	9.5	10.1	10.8	9.4	9.8	10.3	9.2	9.3	9.8
22	9.5	10	10.7	9.9	10.2	10.8	8.9	9.7	10.4	9	9.5	10	8.8	8.9	9.5
23	9.2	9.9	10.4	9.7	10.1	10.8	8.6	9.7	10	8.5	9.2	9.8	8.3	8.6	9.4
24	8.9	9.5	10	9.4	9.9	10.5	8.3	9.3	9.8	8.1	8.8	9.3	8	8.2	9.1
25	8.5	9	9.8	9.1	9.4	10.3	8.2	9.1	9.8	8	8.5	9.2	7.7	8	9
26	8.3	8.7	9.5	8.6	9.1	9.8	8.2	8.9	9.4	8	8.3	8.8	7.4	7.9	8.7
27	8.1	8.9	9.4	8.6	9	9.5	8.1	8.9	9.4	8	8.2	8.9	7.2	7.8	8.6
28	7.8	8.7	9.2	8.5	8.9	9.4	7.8	8.6	9.2	7.7	8.2	8.9	7	7.6	8.5
29	7.7	8.7	9.1	8.3	8.8	9.4	7.6	8.4	9	7.6	8.1	8.8	6.8	7.5	8.5
30	7.3	8.4	9	7.8	8.5	9.3	7.6	8.5	9	7.5	8	8.5	6.7	7.3	8.4
31	7.1	8	8.8	7.5	8.1	8.8	7.3	8	8.8	7.3	8	8.5	6.7	7.2	8.3
32	7	7.9	8.8	7.3	7.9	8.7	7.1	7.8	8.6	7	7.6	8.4	6.5	7	8
33	7	7.7	8.8	7.2	7.9	8.8	7.1	7.9	8.5	7	7.5	8.3	6.4	6.8	8
34	6.9	7.4	8.4	7.2	7.6	8.7	7.1	7.7	8.5	6.8	7.5	8.2	6.1	6.4	7.7
35	6.6	7.1	8.3	7.2	7.6	8.8	7.1	7.5	8.5	6.6	7.2	8	6	6.2	7.7
36	6.7	7	8.3	7.2	7.5	8.8	7.1	7.5	8.3	6.5	7.1	8.4	5.9	6	7.4
37	6.3	7	8.1	7	7.3	8.8	6.9	7.2	8.3	6.5	7	8.4	5.8	6	7.1
38	6	6.7	8	6	7.3	8.7	6.5	7	8.2	6.3	6.8	8.2	5.6	5.8	6.9
39	5.8	6.5	7.8	6.5	7.1	8.7	6.3	6.9	8.2	6.1	6.6	8.1	5.5	5.7	6.8
40	5.6	6.1	7.8	6.1	7	8.6	6	6.8	8.4	6	6.4	8.1	5.3	5.6	6.7
41	5.5	6.2	7.5	6	7	8.5	6	6.8	8.3	5.8	6.3	8.1	5.1	5.5	6.7
42	5.4	6.1	7.5	6.1	7.1	8.5	6	6.9	8.3	5.8	6.3	8	4.8	5.4	6.6
43	5.4	6	7.4	6.1	7	8.5	6	6.7	8.2	5.6	6.3	8	4.5	5.3	6.5
44	5.3	6	7.4	6	6.9	8.5	6	6.8	8.2	5.5	6.3	8	4.3	5.1	6.3
45	5.3	6	7.2	5.8	6.8	8.3	5.7	6.6	8	5.4	6.2	7.8	4	5	6.3

Table 4.3: Carbon dioxide evolved (in grams) from different samples

Time (in days)	Carbon dioxide evolved (in grams)										
	Blank	**Negative**	**Positive**	**B1**	**B2**	**B3**	**B4**	**X1**	**X2**	**X3**	**X4**
1	0.0001	0.00102	0.01562	0.00153	0.0016	0.002	0.0024	0.00179	0.00205	0.00184	0.00187
2	0.0002	0.00225	0.02701	0.0031	0.0032	0.003	0.0044	0.00356	0.00418	0.00275	0.00394
3	0.0005	0.00434	0.03904	0.0043	0.0053	0.005	0.0074	0.00638	0.00729	0.00516	0.00682
4	0.0009	0.00523	0.04197	0.0058	0.0072	0.0074	0.0094	0.00942	0.01032	0.00938	0.01104
5	0.0011	0.00606	0.05383	0.0068	0.0088	0.009	0.0131	0.01247	0.01265	0.01274	0.01251
6	0.0013	0.00621	0.05604	0.00738	0.0111	0.0123	0.0154	0.01492	0.01529	0.01617	0.01493
7	0.0018	0.00702	0.05868	0.00968	0.01368	0.0149	0.0181	0.01701	0.01851	0.01882	0.01757
8	0.002	0.00729	0.06032	0.01036	0.01578	0.01813	0.0201	0.01916	0.02273	0.02351	0.02084
9	0.00209	0.00819	0.06353	0.01225	0.01698	0.0199	0.02131	0.02083	0.02398	0.02406	0.02217
10	0.00198	0.00868	0.06353	0.01314	0.0172	0.02001	0.02222	0.02145	0.02409	0.02442	0.02475
11	0.00187	0.00885	0.06386	0.01391	0.01808	0.02133	0.02332	0.02266	0.02464	0.0253	0.02552
12	0.00187	0.00887	0.06485	0.01435	0.01852	0.02144	0.02343	0.02288	0.02497	0.02563	0.0264
13	0.00154	0.00923	0.06628	0.01556	0.01962	0.02199	0.0242	0.02332	0.02519	0.02618	0.02695
14	0.00176	0.00908	0.06672	0.01578	0.01995	0.02199	0.02387	0.02453	0.02574	0.02651	0.02761
15	0.00176	0.00917	0.06815	0.01666	0.02039	0.02232	0.02464	0.02528	0.02651	0.02706	0.02805
16	0.00198	0.00911	0.06815	0.01688	0.0205	0.02265	0.02475	0.02552	0.02684	0.02739	0.02827
17	0.00165	0.00956	0.06881	0.01776	0.02105	0.02342	0.02541	0.02618	0.02717	0.02772	0.02838
18	0.00176	0.00961	0.06903	0.01776	0.02138	0.02375	0.02508	0.0264	0.0275	0.02838	0.02904
19	0.00253	0.00962	0.06848	0.01754	0.02138	0.0232	0.02563	0.02651	0.02783	0.02871	0.02926
20	0.00283	0.01009	0.06851	0.0179	0.02218	0.02356	0.02621	0.02662	0.02805	0.02893	0.0297
21	0.00297	0.01046	0.0687	0.01886	0.02215	0.02397	0.02662	0.02705	0.02794	0.02926	0.02981
22	0.0033	0.01076	0.06925	0.01908	0.02259	0.02419	0.02684	0.02739	0.02871	0.03014	0.03047
23	0.00341	0.01117	0.06969	0.01985	0.0238	0.02518	0.0275	0.0286	0.02992	0.03091	0.03223
24	0.00363	0.01153	0.07079	0.02062	0.02435	0.02551	0.02849	0.02937	0.03146	0.03209	0.03344
25	0.00374	0.01199	0.07178	0.02216	0.02534	0.02639	0.02959	0.0297	0.03223	0.03311	0.03443
26	0.00418	0.01207	0.07123	0.02205	0.02589	0.02683	0.03003	0.03058	0.03322	0.03454	0.03553
27	0.00429	0.01218	0.07167	0.02359	0.0271	0.02804	0.03003	0.03167	0.03354	0.03519	0.03619
28	0.0044	0.01251	0.07189	0.02403	0.02743	0.02815	0.03069	0.03211	0.03421	0.03575	0.03696
29	0.00484	0.01269	0.07189	0.02381	0.02721	0.02793	0.03047	0.03355	0.03432	0.03575	0.0374
30	0.00495	0.0129	0.07277	0.02469	0.02831	0.02924	0.03124	0.03377	0.0352	0.03608	0.03795
31	0.00506	0.01323	0.07343	0.02535	0.02908	0.0298	0.03201	0.03421	0.03586	0.03641	0.03828
32	0.00517	0.01334	0.0742	0.02579	0.02941	0.02991	0.03212	0.0352	0.03575	0.03696	0.03872
33	0.00528	0.01356	0.07442	0.02568	0.02941	0.02991	0.03223	0.03652	0.03685	0.03718	0.03894
34	0.0055	0.01356	0.07475	0.0259	0.02985	0.03002	0.03289	0.03707	0.03751	0.03806	0.03971
35	0.00627	0.01386	0.07475	0.02557	0.02919	0.02936	0.03289	0.03707	0.03751	0.03828	0.04004
36	0.00638	0.01387	0.07585	0.02634	0.02974	0.03002	0.03278	0.03751	0.03773	0.03861	0.04114
37	0.00671	0.01414	0.07607	0.02645	0.03007	0.03046	0.03311	0.0374	0.03795	0.03938	0.04147
38	0.00704	0.01417	0.07662	0.02667	0.03062	0.03123	0.03355	0.03751	0.03817	0.03916	0.04213
39	0.00748	0.01434	0.07695	0.02678	0.03106	0.03178	0.03377	0.03795	0.03872	0.03927	0.04257
40	0.0077	0.01447	0.07728	0.027	0.03101	0.03211	0.03388	0.0385	0.03949	0.03993	0.0423
41	0.00781	0.01511	0.07816	0.02711	0.03103	0.03233	0.03443	0.03883	0.03982	0.04048	0.04356
42	0.00792	0.01519	0.07871	0.02871	0.03103	0.03233	0.03454	0.03949	0.04026	0.04081	0.044
43	0.00814	0.01544	0.08014	0.02909	0.03104	0.03211	0.03454	0.03971	0.04015	0.04114	0.04433
44	0.0086	0.01577	0.08119	0.02913	0.03108	0.03263	0.0347	0.03949	0.04004	0.04125	0.04492
45	0.0093	0.01582	0.08152	0.02973	0.03158	0.03263	0.0346	0.0396	0.04037	0.04147	0.04504

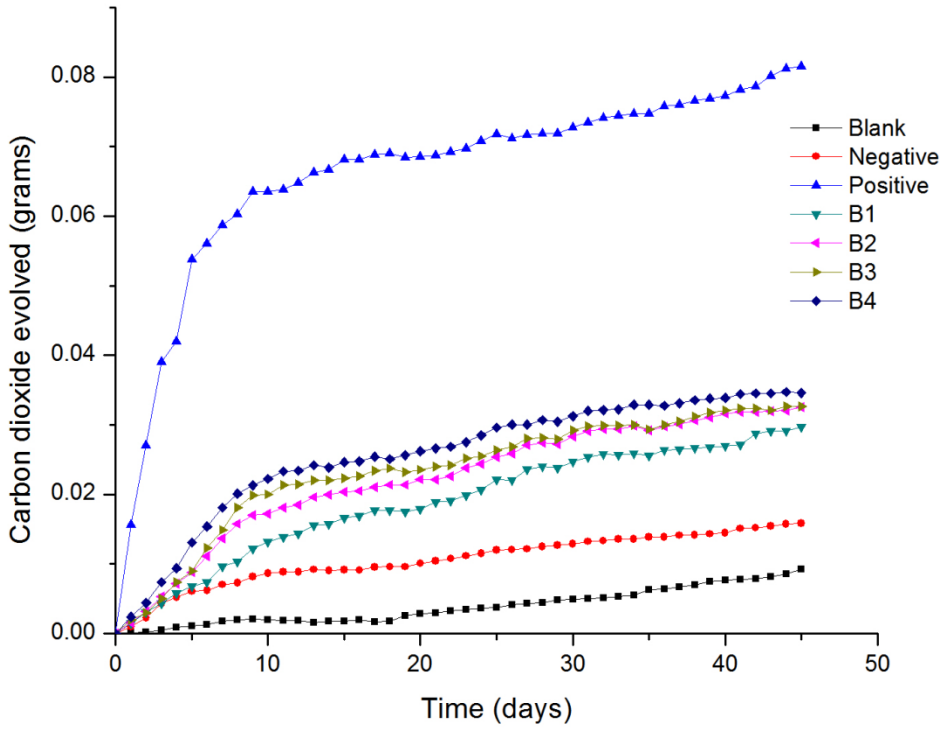

Figure 4.2: Carbon dioxide evolution from blank, negative, positive, B1, B2, B3 and B4

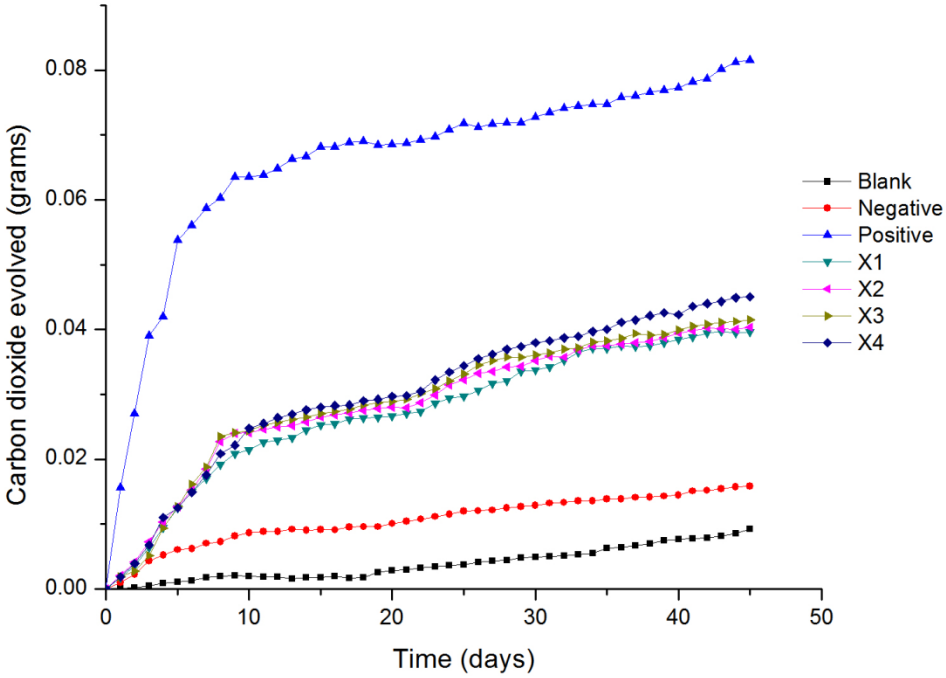

Figure 4.3: Carbon dioxide evolution from blank, negative, positive, X1, X2, X3 and X4

Table 4.4: Percentage biodegradation of different samples

Time (in days)	Percentage biodegradation									
	Positive	**Negative**	**B1**	**B2**	**B3**	**B4**	**X1**	**X2**	**X3**	**X4**
1	3.527	0.119	0.170	0.183	0.237	0.294	0.199	0.226	0.196	0.196
2	6.093	0.262	0.346	0.366	0.349	0.537	0.395	0.463	0.282	0.416
3	8.759	0.506	0.453	0.585	0.562	0.882	0.673	0.771	0.499	0.684
4	9.334	0.61	0.584	0.768	0.812	1.087	0.953	1.044	0.91	1.087
5	11.984	0.707	0.68	0.939	0.986	1.535	1.285	1.281	1.265	1.213
6	12.44	0.724	0.725	1.196	1.374	1.803	1.542	1.555	1.629	1.451
7	12.927	0.819	0.94	1.45	1.636	2.085	1.678	1.827	1.827	1.644
8	13.254	0.85	0.997	1.681	2.015	2.317	1.897	2.296	2.342	1.981
9	13.963	0.955	1.212	1.817	2.224	2.459	2.084	2.427	2.387	2.117
10	13.988	1.012	1.331	1.857	2.252	2.589	2.189	2.467	2.457	2.446
11	14.088	1.032	1.436	1.978	2.431	2.744	2.368	2.562	2.589	2.563
12	14.313	1.035	1.488	2.032	2.446	2.758	2.396	2.602	2.628	2.666
13	14.713	1.077	1.672	2.206	2.554	2.899	2.533	2.71	2.774	2.809
14	14.763	1.059	1.672	2.22	2.527	2.828	2.63	2.723	2.760	2.835
15	15.088	1.07	1.777	2.273	2.568	2.927	2.724	2.818	2.826	2.887
16	15.038	1.063	1.777	2.26	2.582	2.913	2.699	2.809	2.813	2.861
17	15.263	1.115	1.922	2.367	2.719	3.039	2.86	2.926	2.932	2.951
18	15.288	1.121	1.908	2.394	2.747	2.983	2.864	2.939	2.985	3.003
19	14.988	1.122	1.790	2.3	2.582	2.955	2.685	2.791	2.84	2.848
20	14.927	1.177	1.798	2.361	2.589	2.991	2.624	2.744	2.794	2.829
21	14.938	1.22	1.895	2.341	2.623	3.025	2.642	2.696	2.8	2.809
22	14.988	1.255	1.882	2.354	2.609	3.011	2.602	2.710	2.826	2.809
23	15.063	1.303	1.961	2.489	2.719	3.082	2.726	2.831	2.892	2.99
24	15.263	1.345	2.027	2.528	2.733	3.081	2.768	2.966	2.981	3.180
25	15.463	1.399	2.197	2.636	2.829	3.171	2.781	3.034	3.077	3.307
26	15.238	1.408	2.132	2.649	2.829	3.197	2.781	3.047	3.143	3.307
27	15.313	1.421	2.302	2.784	2.967	3.249	2.89	3.059	3.195	3.293
28	15.338	1.459	2.342	2.81	2.967	3.314	2.918	3.115	3.236	3.363
29	15.238	1.48	2.263	2.733	2.884	3.262	2.988	3.020	3.13	3.279
30	15.413	1.505	2.355	2.851	3.034	3.301	2.988	3.101	3.143	3.363
31	15.538	1.543	2.42	2.831	3.09	3.314	3.015	3.145	3.157	3.448
32	15.688	1.556	2.46	2.858	3.09	3.340	3.012	3.115	3.196	3.448
33	15.713	1.582	2.433	2.845	3.077	3.340	3.05	3.122	3.196	3.448
34	15.738	1.582	2.433	2.872	3.063	3.387	3.063	3.149	3.249	3.504
35	15.563	1.617	2.302	2.797	2.884	3.236	3.071	3.061	3.091	3.405
36	15.788	1.618	2.381	2.851	2.953	3.34	3.068	3.061	3.104	3.377
37	15.763	1.649	2.355	2.851	2.967	3.301	3.002	3.071	3.117	3.377
38	15.813	1.653	2.342	2.878	3.022	3.301	2.933	2.953	3.011	3.391
39	15.788	1.673	2.302	2.778	3.035	3.249	2.878	2.912	2.919	3.363
40	15.813	1.688	2.302	2.818	3.049	3.165	2.891	2.953	2.945	3.349
41	15.988	1.763	2.309	2.831	3.063	3.288	2.905	2.966	2.985	3.405
42	16.088	1.772	2.410	2.818	3.049	3.314	3.088	2.993	2.998	3.405
43	16.363	1.801	2.449	2.804	2.994	3.301	3.056	2.926	2.985	3.377
44	16.497	1.84	2.449	2.865	3.002	3.262	3.088	2.799	2.888	3.339
45	16.413	1.845	2.437	2.841	2.914	3.111	3.097	2.668	2.746	3.236

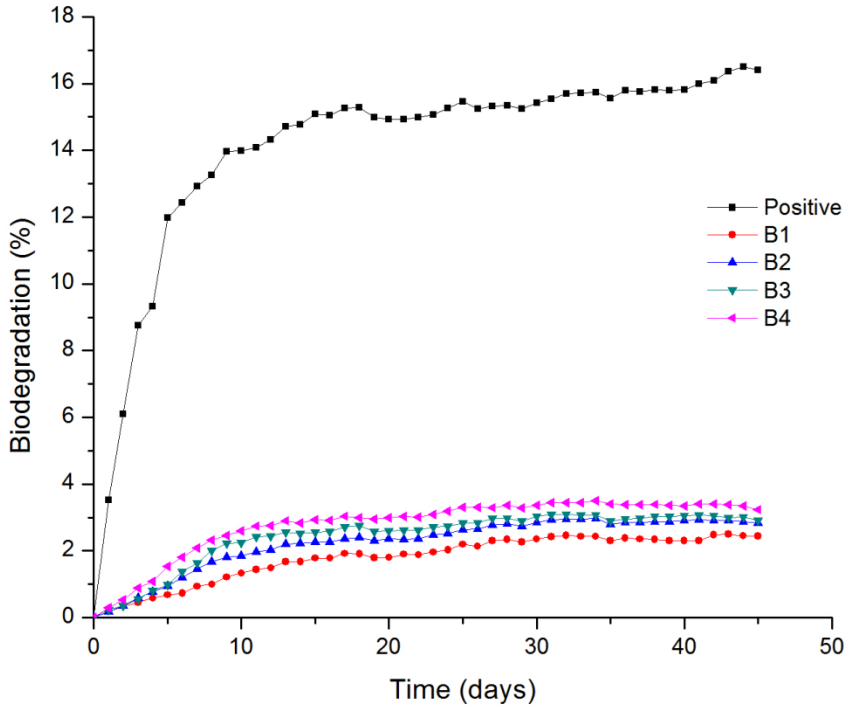

Figure 4.4: Percentage biodegradation of positive, B1, B2, B3 and B4

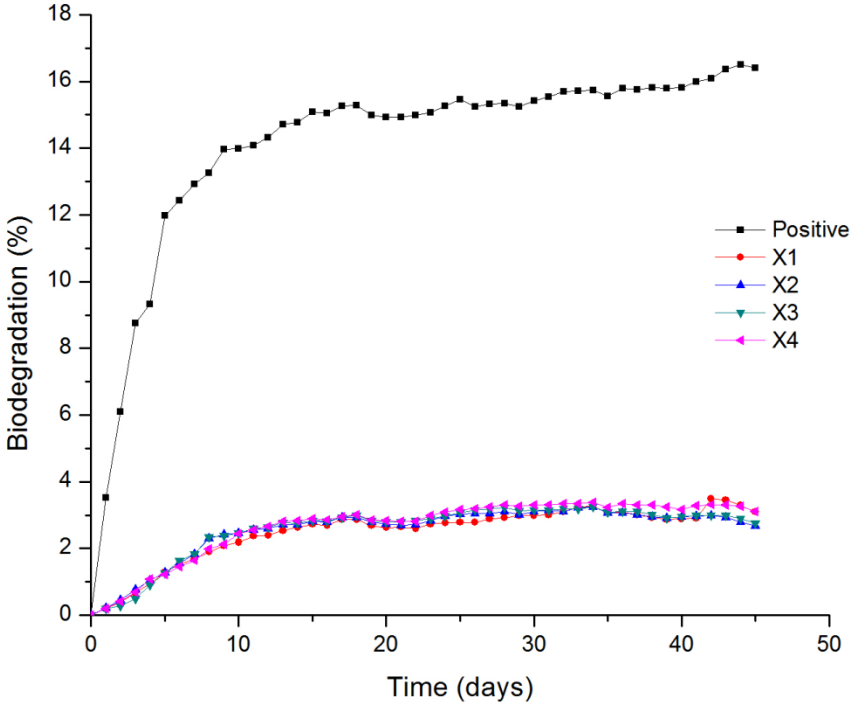

Figure 4.5: Percentage biodegradation of positive, X1, X2, X3 and X4

4.4 Weight Loss

After 45 days of degradation period, weight loss in all the samples was calculated and it was observed that the weight of the samples was reduced. The results of weight loss estimation are shown in the Figure 4.6. The maximum weight loss was found in sample X4, i.e., 2.54%; whereas it was minimum in negative control sample (100% HDPE), i.e. 0.112% only. All the other samples had intermediate values of weight loss. The figure shows that the blended samples undergo more effective degradation as compared to pure HDPE (negative) which mean that microbes utilizes the blended sample as carbon source.

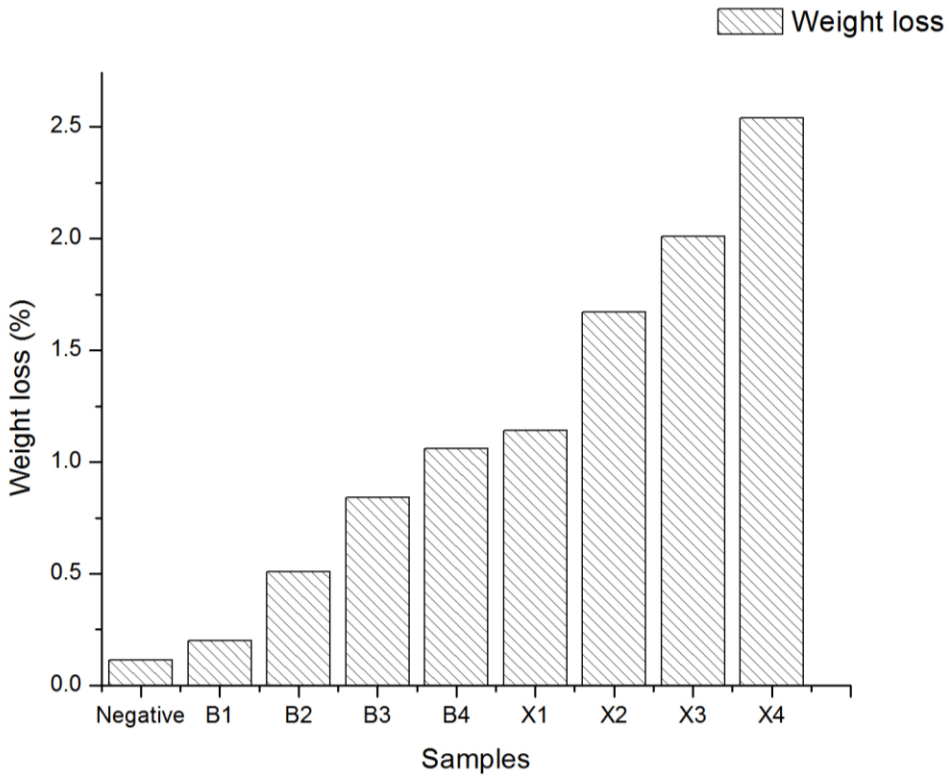

Figure 4.6: Weight loss percentages of different samples

4.5 Protein Estimation

Protein quantification is a significant measure for estimating the microbial growth on the surface of polymer during the degradation period. Protein content on the polymer surface is directly

proportional to the microbial population. The presence and concentration of protein on the surface of polymer confirms that the microbes utilize it as carbon source. The protein extracted from different samples is shown in terms of protein concentration in mg per ml of sodium hydroxide which is depicted in bar diagram in Figure 4.7.

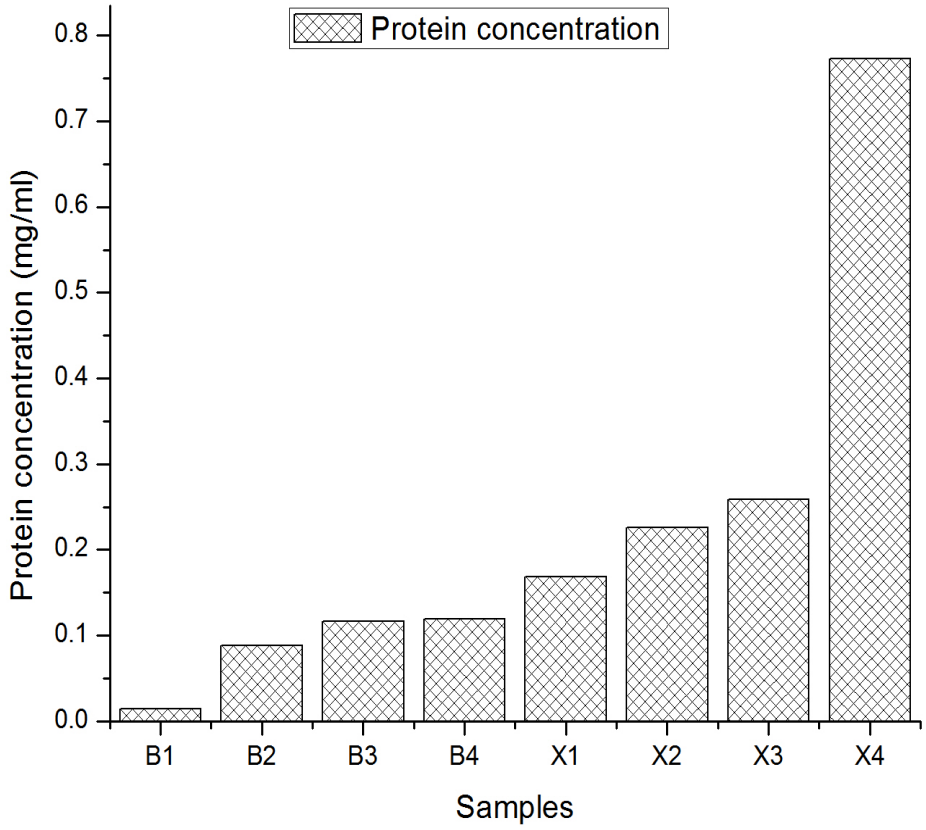

Figure 4.7: Protein content extracted from the surface of different blended samples

As expected, it was observed that X4 sample contains maximum (i.e. 0.773 mg/ml) amount of protein content on its surface which might be due to the greater density of microbial population on the film surfaces utilizing it as carbon source. The protein content on other sample films is comparatively lesser than X4 and minimum for B1. Since, HDPE is made of sole carbon backbone which is very difficult to break and B1 is mainly composed of HDPE (i.e. 95% by weight) and microbes are not able to properly utilize HDPE as carbon source. As the ratio of HDPE in blended samples decreases, the amount of microbes growing on the film surface increases.

Chapter: 5
CONCLUSIONS

5.1 Conclusions

The experiment carried out for 45 days showed that the blends incubated in the composting environment under defined experimental conditions have degraded which is evident from the amount of carbon dioxide evolved and increases in percent biodegradation with time. Hence, it can be concluded that the microbes in the composting environment grow at a good rate and utilize the polymer blend material as their energy (carbon) source and thereby degraded the test sample(s). Eight different blends of HDPE/PLLA, with and without compatibilizer, were tested as per ASTM D 5338-98 (modified) standard. In the HDPE matrix, the PLLA was blended maximum upto 20 wt% and in 80/20 (HDPE/PLLA) blend, the amount of compatibilizer, M-*g*-P was varied from 2-8 phr. After a degradation period of 45 days, the sample X4 showed maximum biodegradation of 3.5%, which could be due to the highest content of the biodegradable polymer PLLA and compatibilizer in it. Further, weight loss and protein content analysis confirms the effective biodegradation of samples under controlled composting environment. X4 sample is the most biodegradable blend among all the blends. Therefore, sample X4 could be an appropriate blend for flexible packaging applications. As evident from the results of compost viability and percentage biodegradation, the compost could be considered as a reliable candidate for biodegradation of plastics. The partial biodegradability of the blend was verified from the ASTM method.

5.2 Recommendations

This research work can be extended for the latest techniques of making the packaging polymers (bio)degradable, viz. pre-treatment through acid etching, thermal degradation, irradiation etc. which makes the polymer films more susceptible to microbial attack. Secondly, pro-oxidants, e.g. metallic strearates (cobalt stearate, manganese stearate and iron stearate) can also be added in the polymers, which are required in trace amounts to make the polymers degradable by photobiodegradation. Compost with more microbial density can also increase the rate of biodegradation so vermicompost is a very good alternative on which biodegradability assessment can be evaluated. Life cycle analysis of the sample blends can also be determined for estimation of useful life of the sample for various applications.

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PUBLICATIONS

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