BIODEGRADATION OF MODIFIED POLYPROPYLENE FILMS

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Master of Technology

in

Environmental Sciences and Technology

By

Pallavi Nayak

M. Tech. 2nd year

(Roll No. 601001022)

UNDER THE GUIDANCE OF

Dr. Anita Rajor
Assistant Professor
Department of Biotech & Env Sciences
Thapar University, Patiala

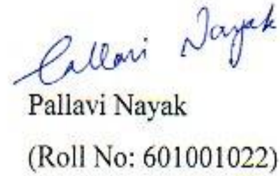
Dr. Haripada Bhunia
Associate Professor
Department of Chemical Engineering
Thapar University, Patiala

Department of Biotechnology and Environmental Sciences
Thapar University
Patiala-147004, Punjab

July 2012

CERTIFICATE

This is to certify that the thesis entitled **"Biodegradation of modified Polypropylene films"**, is an authentic record of my own work carried out as requirements for the award of degree of Master of Technology in Environmental Science & Technology from Thapar University, Patiala, under the guidance of Dr. Anita Rajor (Assistant Professor, DBTES) and Dr. Haripada Bhunia (Associate Professor, ChED) during January to June 2012.

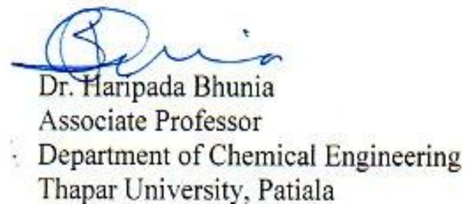


Date: 21/06/2012

Pallavi Nayak
(Roll No: 601001022)

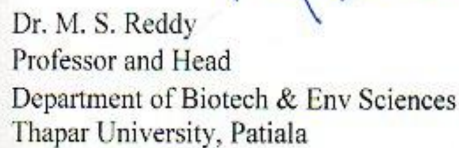
It is certified that the above statement made by the student is correct to the best of our knowledge and belief.

Dr. Anita Rajor
Assistant Professor
Department of Biotech & Env Sciences
Thapar University, Patiala



Dr. Haripada Bhunia
Associate Professor
Department of Chemical Engineering
Thapar University, Patiala

Countersigned by



Dr. M. S. Reddy
Professor and Head
Department of Biotech & Env Sciences
Thapar University, Patiala

Dr. S. K. Mohapatra
Dean, Academic Affairs
Thapar University, Patiala

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Place: Thapar University, Patiala

Pallavi Nayak
(Roll No: 601001027)

ABSTRACT

The ability of microbes to attack / degrade certain polymers, viz. polypropylene (PP) and its blends with poly-L-lactic acid (PLLA) with/without compatibilizer, maleic anhydride was investigated in pure shake-flask culture conditions. The mesophilic bacterium *Bacillus flexus* (MTCC number 2909) and fungus *Aspergillus niger* (MTCC number 478) utilized PP and its blends with PLLA as the sole carbon and energy source(s) and degraded them. Incubation of the mentioned polymers and polymer blends with *Bacillus flexus* and *Aspergillus niger* for 60 days and 30 days respectively at 30°C and 120 rpm, under aerobic conditions, reduced their weights. As the growth of microbes proportionally increased in synthetic media so it was predicted that the microbes were solely dependent on the polymer samples for its carbon source. Firstly, the biodegradation of all the polymer samples was observed in terms of their respective weight loss, which is an established indicator of the degradation. It was observed that PLLA degraded maximum by *Bacillus flexus* i.e. 8.93% (after 60 days) followed by M-*g*-PP 90/4, which degraded by 1.08%. Pure polymer sample, PP 100 degraded by 0.78%. The above data showed that blend of PP and PLLA undergo more degradation than PP alone. The biodegradation of the films were attributed to microbial attack, with major changes occurring at the surface of the polymer which was confirmed by scanning electron microscopy (SEM). PLLA degrade maximum by *Aspergillus niger* i.e. by 0.91% (after 30 days) followed by PP 90, which degraded by 0.85% and no weight loss was noticed in PP 100.

TABLE OF CONTENTS

Item Description	Page No.
Certificate	ii
Acknowledgements	iii
Abstract	iv
Table of Contents	v
List of Figures	viii
List of Tables	ix
List of Abbreviations	x
Chapter 1: INTRODUCTION	1
Chapter 2: LITERATURE REVIEW	8
Chapter 3: MATERIALS & METHODS	16
3.1 Materials	16
3.1.1 Polypropylene and its Blends	16
3.1.2 Bacterial strain	17
3.1.3 Media for cultivation	17
3.1.4 Media for inoculation & degradation (bacteria)	17
3.1.5 Fungal Strain	17
3.1.6 Media for cultivation of fungus	17
3.1.7 Media for Degradation studies (fungus)	18

3.2 Methodolgy 18

3.2.1 Aerobic biodegradation by *Bacillus flexus* 18

3.2.1.1 Pretreatment of polymers 18

3.2.1.2 Culture conditions 19

3.2.1.3 Culture Enrichment 19

3.2.1.4 Degradation Studies 20

3.2.2 Aerobic Biodegradation using *Aspergillus niger* 20

3.2.2.1 Pretreatment of polymers 20

3.2.2.2 Culture Conditions 21

3.2.2.3 Culture Enrichment 21

3.2.2.4 Degradation Studies 21

3.3 Analytical/ Testing procedures 22

3.3.1.1 Growth pattern in Nutrient Broth 22

3.3.1.2 Viable cell count by Spectrophotometer 22

3.3.1.3 Colony forming unit measurement 22

3.3.1.4 Determination of bacterial biomass 23

3.3.1.5 Residual weight measurement of polymer film 23

3.3.1.6 Morphological evaluation using scanning electron microscopy (SEM) 23

3.3.2.1 Growth kinetics of fungal strain 23

3.3.2.2 Determination of fungal biomass 24

3.3.2.3 Residual weight measurement of polymer film 24

Chapter 4: RESULTS & DISCUSSIONS 25

4.1.1 Aerobic biodegradation by *Bacillus flexus* 25

4.1.1.1 Growth pattern of *Bacillus flexus* 25

4.1.1.2 Viable cell count by spectrophotometer 27

4.1.1.3 Growth of bacteria in terms of Colony forming unit/ml 28

4.1.1.4 Determination of biomass 29

4.1.1.5 Residual weight measurement 30

4.1.1.6 Morphological evaluation using scanning electron microscopy (SEM) 31

4.1. 2 Aerobic Biodegradation using *Aspergillus niger* 33

4.1.2.1 Growth pattern of *Aspergillus niger* in CY media containing Sucrose as C-source 33

4.1.2.2 Growth pattern of *Aspergillus niger* in CY media containing Polymers as C-source 35

4.1.2.3 Residual weight measurement of polymer inoculated with *Aspergillus niger* 36

Chapter 5: CONCLUSIONS & FUTURE PROSPECTS 38

5.1 Conclusions 38

5.2 Future prospects 38

REFRENCES 39

LIST OF FIGURES

Figure No.	Title	Page No.
1.1	The inter-relationship between biodegradable plastics and bio-based plastics	5
2.1	Schematic diagram of polymer degradation under aerobic and anaerobic conditions	9
3.1	Flasks containing polymer films kept for degradation	20
3.2	Pretreatment of polymer film	21
4.1	Growth curve of *Bacillus flexus* in nutrient broth	26
4.2	Comparison of O.D over 60 days	27
4.3	Comparison of CFU over 60 days	28
4.4	Comparison of increase in Biomass over 60 days	29
4.5	%Weight loss over 60 days	31
4.6	Scanning electron microscope image of polymer films before and after 60 days of degradation	32-33
4.7	Growth curve of *Aspergillus niger* in CY broth	34
4.8	Biomass after 30 days	35
4.9	Average % weight loss over 30 days	37

LIST OF TABLES

Table No.	Title	Page No.
3.1	Blends Compositions with/without Compatibilizer	16
4.1	Growth kinetics of Bacillus flexus in nutrient broth	26
4.2	Comparison of O.D. over 60 days	27
4.3	Comparison of CFU over 60 days	28
4.4	Comparison of increase in Biomass over 60 days	29
4.5	Weight loss studies over 60 days	30
4.6	Growth kinetics of *Aspergillus niger* in CY broth	34
4.7	Biomass after 30 days	35
4.8	Weight loss studies over 30 days	36

LIST OF ABBREVIATIONS

AM	Amylose
ASTM	American Society for Testing and Materials
BOPP	Bio-oriented polypropylene
CFU	Colony forming units
DODAM	Dodecanoyl ester of amylose
FTIR	Fourier Transform Infra-Red Spectroscopy
HDPE	High Density Polyethylene
LDPE/LDP	Low Density Polyethylene
LLDPE	Linear Low Density Polyethylene
MA/ MAH	Maleic anhydride
M-*g*-PP	Maleic anhydride grafted Polypropylene
MTCC	Microbial Type Culture Collection
NB	Nutrient Broth
NY11	Nylon 11
OD	Optical Density
PBS	Poly (butylene succinate)
PBSA	Poly (butylene succinate-co-adipate)
PCL	Polycaprolactone
PE	Polyethylene
PET	Polyethylene teraphthalate
PHB	Poly (hydroxybutyrate)
PHB/V	Poly (-hydroxybutyrate-*co*-hydroxyvalerate)
PLA	Polylactic Acid
PLLA	Poly L-Lactic Acid
PP	Polypropylene

PP-TT	Thermally pretreated polypropylene
PP-UT	Un-pretreated polypropylene
PS	Polystyrene
PVC	Polyvinyl chloride
SEM	Scanning Electron Microscopy
TPS	Thermoplastic starch
US EPA	United States Environmental Protection Agency
UV	Ultra-Voilet rays
UV-Vis	Ultra-Voilet Visible Spectroscopy

Chapter 1

INTRODUCTION

Polymers are synthetic and natural macromolecules composed of smaller units called monomers that are bonded together (Kyrikou and Briassoulis, 2007). More than half a century ago synthetic polymers started to substitute natural materials in almost every area and nowadays plastics have become an indispensable part of our life (Shah *et al*., 2008).

The polymers we use today are made from inorganic and organic raw materials, such as carbon, silicon, hydrogen, nitrogen, oxygen and chloride. The basic materials used for making plastics are extracted from oil, coal and natural gas. Due to the relatively low cost of production involved, and the ease of which they can be manufactured, plastics are used in a wide variety of products. They are versatile and durable, and therefore have already replaced many traditional construction materials, such as leather, wood, stone, bone, metal, ceramics and glass.

Examples of natural polymers include proteins, polysaccharides, and nucleic acids (Chandra and Rustgi, 1998). Synthetic polymers have been developed for durability and resistance to all forms of degradation (Chiellini *et al*., 2003). These characteristics and others, such as rigidity, permeability and transparency can be controlled by changing the polymer synthesis, molecular weight and/or by the use of specific additives. The resulting plastics' versatility allows them to be used in a very wide range of applications, including packaging and agriculture.

Production of synthetic polymers is expected to increase to about 200 million tons per year (Shah Ali, 2008; Shah *et al*., 2009). A 25-fold increase in the production of plastic resin products between 1960 and 2000, and a recovery rate of less than 5%, has resulted in an unparalleled rate of plastic materials entering the environment (McDermid and McMullen, 2004; Moore, 2008).

The world consumes 100 million tones of plastic materials – 36.8 million tons in Europe, 5 million tons in the UK – and growing at 3 to 4 percent each year. The largest single sector, 37.3 percent, is in packaging. There are about 50 different groups of plastics with hundreds of different varieties.

The amount of plastic wastes generated annually in the UK was estimated at 3 million tons in 2001. Although all types of plastics could be recycled, only 7 percent actually were. The rest

were buried in landfills (80 percent) or incinerated (8 percent). Most plastics are non-biodegradable, which means they take a long time to break down naturally.

Statistics published by the United States Environmental Protection Agency in 2003 indicated that, before recycling, approximately 236 million tons of municipal solid waste (MSW) was generated in the United States in that year, of which 11.8% was composed of plastics (American Chemistry Council, 2007). Only a small fraction of this plastic waste (mostly soft drink and other bottles) was recovered. Thus the remaining quantity of plastic waste was required to be disposed. Most of this plastic waste has been accumulating in landfills. Therefore, in order to save capacity for plastic waste disposal, there is a growing interest both in the development of newer, readily biodegradable plastics and in the biodegradation of conventional plastic waste (US EPA, 2005).

In India according to future flow analysis the total virgin plastics consumption is expected to reach 20,000 KT by the year of 2030 and over 18,800 KT of waste can be generated. Polyolefins like Polypropylene (PP), low density polyethylene (LDPE) and high density polyethylene (HDPE) account for about 60% of the total plastics consumption in India. Dumping of plastic in the environment at such a large amount is causing already serious problems to the flora and fauna. The conventional method like incineration is a source of secondary hazardous product.

Traditional applications of synthetic polymers are mostly based on their inertness to environmental degradations (hydrolysis, oxidation, biodegradation, and so on). A number of synthetic polymers are now widely applied for the raw material of textile, film, and various containers. The quantity of plastics waste, therefore, has become so large that their waste spoils our environment (Ikada, 1999).

In recent times, the excessive consumption of synthetic plastics derived from petroleum has had an adverse impact on the environment because the majority of these synthetic plastics do not degrade in the environment, and incineration of plastics generates CO_2 and dioxin (Maeda *et al.*, 2005). These molecules increase the warming of the Earth and environmental pollution.

Plastics are resistant against microbial attack, since during their short time of presence in nature evolution could not design new enzyme structures capable of degrading synthetic polymers. The most important externalities created by inefficient plastic disposal include the aesthetic damage created by landfills containing plastic waste, the impact to marine life of plastic residuals,

hazardous emission of plastic incineration, and the economic inefficiencies created by the difficulty of plastic reutilization from recycling (Andrady, 2003; Elias, 2003).

Amongst several compounds in waste landfill sites, plastics are estimated to make up approximately 20–30% of the volume of municipal solid waste landfill sites (Ishigaki *et al.*, 2004). Because plastics are recalcitrant to microbial degradation, they would remain in landfill sites semi-permanently.

Plastic waste comprises 60% to 80% of marine debris litter accumulated in ocean shores. Effects on marine life of plastic waste include the entanglement and ingestions of harmful plastics by marine vertebrates and the bioaccumulation of toxicants causing reduced reproduction capacities in certain species (Andrady, 2003).

Gases released from decomposition processes from plastics present in MSW include cyclic chlorinated hydrocarbons believed to be toxic (Andrady, 2003). Specifically, the release of hydrogen chloride and dioxins from PVC incineration can be very toxic to animal species. The corrosive fumes released into the atmosphere from plastic combustion can also increase acidity levels of the environment. A potential effect from such emissions is the generation of acid rain.

Total management of polymer wastes requires complementary combinations of recycling, incineration for energy, and biodegradation. Polymers prepared from renewable and sustainable resources can be designed, synthesized, and engineered by environmentally compatible routes and can be disposed after use by biodegradation (composting, etc.).

Changes in polymer properties due to chemical, physical or biological reactions resulting in bond scissions and subsequent chemical transformations are categorized as polymer degradation (Pospisil *et al.*, 1998). Depending upon the nature of the causing agents, polymer degradations have been classified as photo-oxidative degradation, thermal degradation, ozone-induced degradation, mechanochemical degradation, catalytic degradation and biodegradation (Grassie and Scott, 1985).

Biodegradation has been defined in various ways by different investigators. It is defined as change in surface properties or loss of mechanical strength (Lemm *et al.*, 1981), assimilation by microorganisms (Potts *et al.*, 1973), and degradation by enzymes (Swift, 1992), backbone chain breakage and subsequent reduction in the average molecular weight of the polymers (Ratner *et*

al.,1988; Hergenrother *et al*.,1992). Degradation can occur by any of the above mechanisms alone or in combination with one another. According to the standard ASTM D5247, biodegradation is defined as "process which is capable of decomposition of materials into carbon dioxide, methane, water, inorganic compounds, or biomass in which the predominant mechanism is the enzymatic action of microorganisms, that can be measured by standard tests, in a specified period of time, reflecting available disposal conditions."

The degradation of most synthetic plastics in nature is a very slow process that involves environmental factors and action of wild microorganisms. The primary mechanism for biodegradation of polymers (plastics) is the oxidation or hydrolysis by enzymes to create functional groups that improve its hydrophylicity. Consequently, the main chains of polymer are degraded resulting in polymer of low molecular weight and feeble mechanical properties, thus, making it more accessible for further microbial assimilation (Shah *et al*., 2009).

To overcome these problems some aliphatic polymer types have been developed as biodegradable plastics (BPs), which have been designed to be easily degraded by microorganisms and to be absorbed by the natural environment or by waste landfills, are gaining public endorsement as a possible alternative to petroleum-derived plastic. They can be derived from renewable feedstock, thereby reducing greenhouse gas emissions. Biodegradable plastics have several excellent properties and may provide solutions to global environmental problems. First, biodegradable plastics are degraded by microorganisms in the natural environment (Ishigaki *et al*., 2000; Suyama *et al*., 1998). Second, they can be composted, and burn with a lower calorific value than that of synthetic plastic materials (Maeda *et al*., 2005).

Biodegradable plastics offer a lot of advantages such as increased soil fertility, low accumulation of bulky plastic materials in environment (which invariably will minimize injuries to wild animals), and reduction in the cost of waste management. Furthermore, biodegradable plastics can be recycled to useful metabolites (monomers and oligomers) by microorganisms and enzymes. A second strategy involves degradation of some petroleum-derived plastics by biological processes.

Bio-plastics consist of either biodegradable plastics (*i.e.,* plastics produced from fossil materials) or bio-based plastics (*i.e.,* plastics synthesized from biomass or renewable resources).

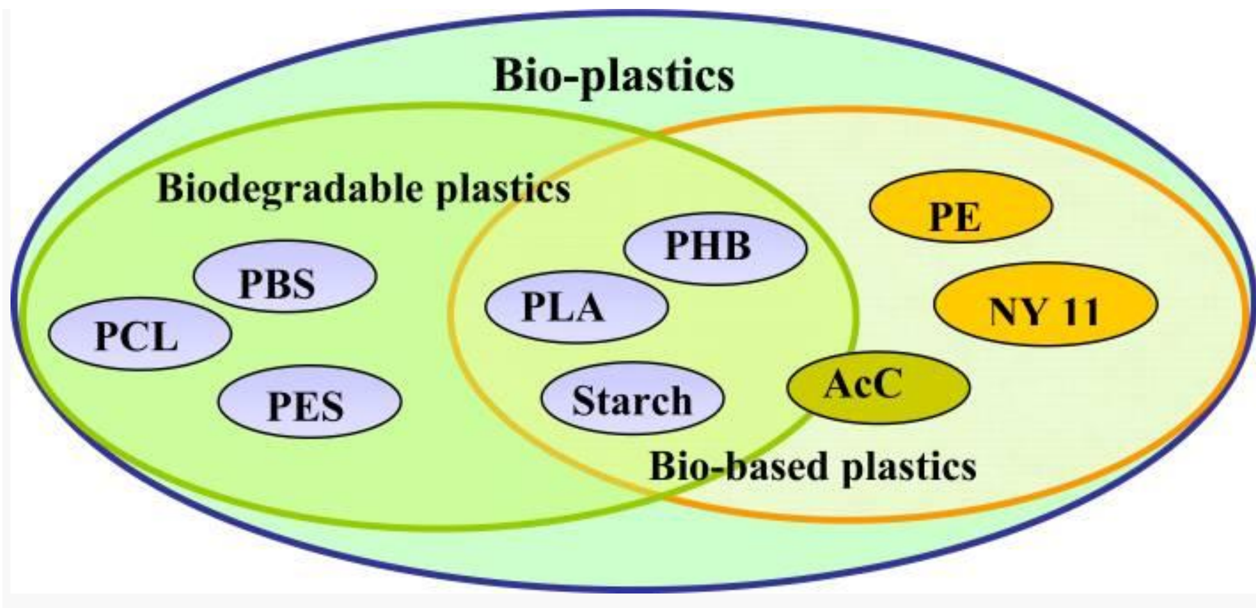


Figure-1.1: The inter-relationship between biodegradable plastics and bio-based plastics (Tokiwa *et al.,* 2009).

Polycaprolactone (PCL), and poly (butylene succinate) (PBS) are petroleum based, but they can be degraded by microorganisms. On the other hand, poly (hydroxybutyrate) (PHB), poly (lactide) (PLA) and starch blends are produced from biomass or renewable resources, and are thus biodegradable. This has clearly been depicted in Figure 1. Despite the fact that polyethylene (PE) and Nylon 11 (NY11) can be produced from biomass or renewable resources, they are non-biodegradable (Tokiwa *et al.,* 2009).

The production of biodegradable plastics that get decomposed completely in nature have received remarkable attention globally as they are totally ecofriendly and helpful in waste landfill management. Addition of natural polymers to thermoplastics having long-term potential is one of the approaches to enhance biodegradability (Dave *et al*., 1997). To prepare biodegradable plastic involves adding special additives to the synthetic polyolefins, which make it susceptible to microbial degradation, and these additives also disconnect the continuity of C–C chain of polyolefin. Few additives, having hydrophilic groups, make plastic hydrophilic and susceptible for photo- and chemical degradation. Modification of backbone through copolymerization and through anchoring monosaccharide with polyolefin or blending with nutrients and biodegradable fillers makes the plastic degradable (Galgali *et al*., 2002; Ratajska & Boryniec, 1999; Zuchowska *et al*., 1999; De Graaf & Janssen, 2000).

The degradation of a polymer may be achieved by two major paths, namely, (1) the design of a polymer from monomers that are vulnerable to microorganisms and (2) the incorporation of biodegradable additives or groups in the polymer. This, in turn, can be done by two methods. The first one involves the copolymerization of biodegradable monomers with a non-degradable monomer, and the second method involves the blending of a biodegradable additive/polymer with a non-degradable polymer (Singh *et al.*, 2011).

A wide variety of organic materials are easily degraded under aerobic conditions. In aerobic metabolism, O_2 is the terminal electron acceptor. When biodegradation follows this pattern, microbial populations quickly adapt and achieve high densities. As a result, the rate of biodegradation soon becomes limited by the rate of supply of oxygen or some of the nutrients, not the inherent microbial capacity to degrade the polymer or other contaminant. Some organic compounds also degrade under anaerobic conditions. Under anaerobic conditions, in the absence of oxygen, nitrate (NO_3^-), sulphate (SO_4^{2-}), ferric iron (Fe^{3+}), manganese (Mn^{3+}, Mn^{4+}), and bicarbonate (HCO_3^-) can serve as final electron acceptor, in presence of appropriate enzyme system in the microorganism. Usually the rate of anaerobic degradation is limited by inherent reaction rate of the active microorganism, showing slow adaptation spanning months or years, and metabolic activity results in the formation of incompletely oxidized simple organic substances, like organic acids, and by-products viz. methane or hydrogen gas. Hence aerobic treatment is considered to be the safer alternative over anaerobic treatment. Microorganisms acclimatize easily and early in aerobic conditions and release of undesirable/toxic gasses does not occur. Also complete oxidation of organic substances could be achieved by aerobic degradation.

Mixing of two or more polymers to produce blends by common processing steps is today a well-established approach for obtaining suitable materials for specific end-uses. Specific applications of biodegradable blends have drawn marked attention in offering an attractive route to further improve environmental waste management (Maiti and Jana, 2005). In the last few years, studies concerning the total or partial substitution of synthetic plastics by biodegradable materials have been increasing steadily and have proven to be very useful in the solution of the solid waste management problem of plastics, at least to some extent (Singh *et al*., 2011).

The aim of this work was to investigate the aerobic biodegradation of composites of Poly (L-lactic acid) PLA and Polypropylene (PP) with and without the coupling agent Maleic anhydride (MA), and its individual constituents in pure shake-flask culture studies, using standard test methods designed for biodegradable plastics.

Chapter 2

LITERATURE REVIEW

The drastic rise in the use of non-biodegradable plastic materials during the past 3 decades has not been accompanied by corresponding development procedures for the disposal or degradation of these polymers (Vijaya and Reddy, 2008). Because of their high durability, they accumulate in the environment at a rate of 25 million tons per year (Orhan and Buyukgungor, 2000).

The global consumption of polypropylene (PP) in 2010 may touch 50 million tons with an average annual growth of about 8%. The per capita consumption of plastic in India is 5 kg (Plastic Industry Statistic Report by CIPET, 2008).

In Western Europe alone it is estimated that 7.4% of municipal solid wastes are plastics, which are classified as 65% Polyethylene/polypropylene (PP/PE), 15% Polystyrene (PS), 10% PVC, 5% Polyethylene terephthalate (PET) & remaining others (Premraj & Doble, 2005).

These figures give us an idea about the huge plastic waste which will be deposited in the environment after their use and disposal. In environment this dumped plastic waste is subjected to solar radiation, UV rays and heat, which affect their surface as well as to some extent their bulk properties. But this deterioration or degradation process is very slow (Suits & Hsuan, 2003).

In addition, the bacteria, fungi and other microorganisms present in the environment also attack the polymer in order to utilize it as a carbon source. The primary step for initiation of this biodegradation is the attachment of the microorganism to the polymer surface, thus forming a biofilm (Hadad et al., 2005).

Biodegradation (i.e. biotic degradation) is a chemical degradation of materials (i.e. polymers) provoked by the action of microorganisms such as bacteria, fungi and algae. Biodegradation is expected to be the major mechanism of loss for most chemicals released into the environment. This process refers to the degradation and assimilation of polymers by living microorganisms to produce degradation products.

Natural polymers (i.e., proteins, polysaccharides, nucleic acids) are degraded in biological systems by oxidation and hydrolysis (Kyrikou & Briassoulis, 2007). Biodegradable materials

degrade into biomass, carbon dioxide and methane. In the case of synthetic polymers, microbial utilization of its carbon backbone as a carbon source is required (Jayasekara *et al*., 2005).

The biodegradation process can be divided into (1) aerobic and (2) anaerobic degradation. If oxygen is present, aerobic biodegradation occurs and carbon dioxide is produced. If there is no oxygen, an anaerobic degradation occurs and methane is produced instead of carbon dioxide (Leja and Lewandowicz, 2009) (Figure 2.1).

Aerobic biodegradation:

$$\text{Polymer} + O_2 \longrightarrow CO_2 + H_2O + \text{biomass} + \text{residue(s)} \quad (1)$$

Anaerobic biodegradation:

$$\text{Polymer} \longrightarrow CO_2 + CH_4 + H_2O + \text{biomass} + \text{residue(s)} \quad (2)$$

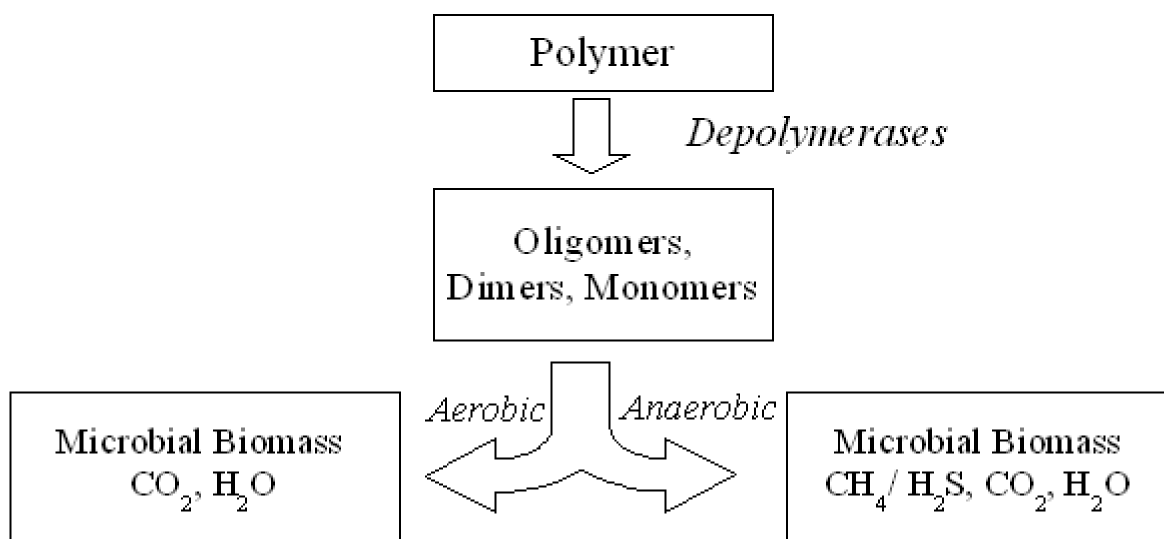


Fig 2.1 Schematic diagram of polymer degradation under aerobic and anaerobic conditions (Leja and Lewandowicz, 2010)

In the case of PP, since it is highly hydrophobic with high molecular weight, lacking active functional group, with continuous chain of repetitive methylene units, it shows resistance to biodegradation (Arkatkar *et al*., 2009).

Fungal species (*Aspergillus niger*) and microbial communities including *Pseudomonas* and *Vibrio* species have been reported to biodegrade PP (Cacciari *et al*.,1993). Isotactic polypropylene exposed to bacterial consortia for 175 days had 40% methylene chloride

extractable compounds, and this extract was identified to be a mixture of hydrocarbons (between $C_{10}H_{22}$ and $C_{31}H_{64}$) (Cacciari *et al*., 1993). 30 – 60% growth of *A. niger* was observed on gamma irradiated PP films at the end of 6 weeks, which indicated that the fungus was able to utilize this polymer as its sole carbon source (Alariqi *et al*., 2006). Nearly 10% of weight loss was observed in thermally pretreated PP when subjected to mixed soil consortia under in vitro conditions (Arkatkar *et al*., 2009).

The polymer films which are not pre-treated and the ones which are pre-treated with the reagents Aquaregia and Fenton; and thermal and short UV were subjected to biodegradation in vitro in minimal medium with four soil cultures, namely *Pseudomonas azotoformans*, *Pseudomonas stutzeri*, *Bacillus subtilis* and *Bacillus flexus* separately for 12 months. All the organisms made use of the polymer as their carbon source. The carbonyl indices decreased in one year in the case of pretreated polymer and increased in the case of untreated polymer, indicating only abiotic oxidation in the absence of pretreatment. Increase in surface energy indicated that the polymer became more hydrophilic when compared to the original. *P. stutzeri* had marginal effect on the polymer. Highest weight loss (2.5%) was observed in the case of short UV treated polymer exposed to *B. flexus* after one year (Arkatkar *et al.*, 2010).

Arkatkar *et al.* in 2008 conducted in vitro biodegradation studies on un-pretreated (PP-UT) and thermally pretreated polypropylene (PP-TT) films in minimal medium with mixed soil culture for 12 months. In this period 10.7 and 0.4% weight loss was observed with PP-TT and PP-UT, respectively. The tensile strength decreased by 51.8 and 28.3%, the crystallinity increased by 28 and 33% and isotacticity increased by 3 and 9%, respectively, over the same time period. The ester carbonyl index in PP-TT increased up to 9 months and later decreased indicating abiotic followed by biotic process. No such changes were observed with PP-UT. Methyl group index decreased in both the cases indicating oxidation at the primary carbon. Increase in surface energy indicated that the polymer became hydrophilic. Surface changes were observed by SEM and AFM. A single culture was isolated at the end of 12 months and it was identified as *Bacillus flexus*. The morphology of the organism was rods in a chain and it was present in the form of an endospore.

Sharma and Sharma (2004) assessed tensile strength, elongation, percentage extension, CFU, BOD & turbidity of plastics to study the extent of degradation of LDP & PP using *Pseudomonas*

stutzeri under laboratorytest conditions. Throughout the investigation both the plastic types were found to undergo qualitative and quantitative changes by bacteria but PP was found to be more biodegradable as compared to LDP.

Longo *et al*. in 2011 analyzed the degradability/biodegradability of polypropylene films (PP) and Bio-oriented polypropylene (BOPP). The major reduction was observed in the PP, since its crystallinity is a consequence of polymerization instead of chain orientation processes, as in BOPP by thermal analysis, chemical analysis via FTIR spectrometry demonstrated that buried PP and BOPP films experienced structural alterations. Cracks and erosion of the polymer surface were detected in both PP and BOPP, indicating degrading processes by microorganisms. The major effects were observed on samples buried for 11 months at a 2 m depth in the sanitary landfill.

The possibility of replacing commercial polymers such as polyethylene, polypropylene, polyvinyl chloride, nylons and polyesters etc. by totally biodegradable polymers for clean environment is remote. Attainment of high performance of the former by the latter is not yet possible. Secondly, biodegradable polymers are meant for single use i.e. these are to be thrown to the garbage after only one time use. These polymers, therefore, should be cheap. To overcome this limitation, partially biodegradable polymers have been developed as a compromise between cost and performance (Singh *et al*., 2011).

The use of biodegradable and renewable materials to replace conventional petroleum plastics for biodegradable and renewable materials for disposable applications is becoming popular (Motoyama *et al.,* 2007). In this regard, poly (l-lactic acid) (PLLA), a synthetic biopolymer derived from agricultural feedstock, is a promising polymer because of its biomass-origin and recyclability based on its biodegradation, hydrolysis, and depolymerization functions (Omura *et al.*, 2006). But, the cost of PLA is still higher than that of petroleum plastics for disposable applications due to its complicated processing procedures (Acioli-Moura & Sun, 2008).

As PLA is absorbed in animals and humans, the use of this polymer in medicine has been extensively developed. The degradation of the polymer in animals and humans is thought to proceed via non-enzymatic hydrolysis. Several enzymes can degrade the polymer: proteinase K, pronase and bromelain; however, few have been characterized with regards to microbial degradation of the polymer. Only a few PLA-degrading microorganisms have been identified

and are not thought to be widespread within the environment. Pranamuda *et al.* (1997) analyzed 45 soil samples collected from various places around Tsukuba City, Japan, but only one soil sample contained PLAdegrading microorganisms. These organisms were identified by their ability to form a clear zone in an agar plate containing PLA powder. This ratio is much lower than that observed for PCL-degrading microorganisms. A PLA-degrading actinomycete, an *Amycolatopsis* sp. Strain isolated from the sample, reduced 100 mg of PLA film by ~60% after 14 days in liquid culture at 30°C. In addition to this strain, a thermophilic bacterium, *Bacillus brevis*, with PLA-degrading properties has been isolated from soil. This strain degraded PCL molecules in a random manner and decreased 50 mg of PLA film by ~20% after 20 days in liquid culture at 60°C. PLA-degrading enzymes of these strains have not been examined. It might be said that PLA is too persistent or recalcitrant for microbes to attack. On the other hand, in a solid waste disposal site of Japan, PLA-degrading bacteria were detected together with bacteria capable of degrading other polymers, such as PHB and PCL. PLA is degraded readily in compost: 14C-labeled PLA was mineralized in compost to CO2 (about 90% degradation after 90 days), although involvement of microorganisms in the depolymerization of PLA is unclear (Shimao, 2001).

Blending PLLA with other polymers can substantially modify the mechanical and thermal properties, degradation rate, and permeability. PLLA/poly (ε-caprolactone) (PCL) blends have been extensively studied. Various compatibilizers such as P (LA-*co*-CL) copolymer were used to improve the miscibility between PLA and PCL. PLLA was also blended with other non biodegradable polymers, including polyethylene, poly (ethylene oxide), poly (ethylene glycol), poly (vinyl acetate), poly (4-vinylphenol), and polyacrylates. Varying degrees of property modifications of PLLA were achieved by blending with these polymers. Many of these blends are immiscible or only partially miscible and may need compatibilizers to increase their compatibility (Singh *et al*., 2011).

Gilmore *et al.* (1993) have carried out degradation study of six types of plastics and plastic blends in municipal wastewater. Samples consist of 6% starch in PP, 12% starch in linear LDPE, 30% Polycaprolactone in LDPE, and poly(-hydroxybutyrate-*co*-hydroxyvalerate) (PHB/V), a microbially produced polyester in activated sludge of 5 months and found no sign of degradation of blended samples except PHB/V, which has showed a considerable loss of mass and a

significant loss of tensile strength in municipal wastewater. Biodegradable polymers can be synthesized by the modification of natural polymers by blending and fermentation.

Composites were prepared by two methods, (i) graft copolymerization (GFC) of isotactic polypropylene (PP) with maliec anhydride, (MAH) followed by esterification with coir fiber and (ii) by direct reactive mixing (DFC) of polypropylene (PP) and ethylene–propylene (EP) copolymers with MAH and peroxide with coir fiber. These composites, after molding in films were examined for susceptibility to biological attack by measuring the percentage weight loss in compost up to 6 months, periodically, and fungal colonization on surface of the samples, when kept as sole carbon source for the growth of *Aspergillus niger* in culture medium up to 40 days. Significant changes were observed depending on the preparation methods during photodegradation and bio-disintegration of composites. DFCs samples were disintegrated faster than GFCs during the composting whereas, in culture, GFCs were covered highly in well uniform way by fungi. It was observed that photo-oxidative ageing directly enhanced the biodegradability of composites as the increase in fungal growth rate and decrease in weight during composting were found. It was concluded that extent of compatibilization had a profound effect on photo-oxidation and bio-disintegration of composite material; consequently ester bonds were main units during fungal consumption (Kumar *et al.*, 2006).

Yang *et al*., 2004 tested biodegradation of plastics in compost made with animal fodder. Specimens in film shape as well as in powder shape were subjected to the biodegradation tests to investigate dependence of the test results on the shape of the specimens. Polypropylene (PP) was chosen as a non-degradable plastic. Poly (L-lactic acid) (PLLA) and poly (butylene succinate) (PBS) were selected as slowly degrading plastics while polycaprolactone (PCL) and poly (butylene succinate-co-adipate) (PBSA) were chosen as easily degradable plastics. Biodegradation results of PLLA and PBS depended on their shape all through the biodegradation test. In contrast, the shape of PCL and PBSA exerted influences on their biodegradability only at the early stage of the biodegradation, while at the late stage; the biodegradation proceeded almost independently of their shape.

Singh *et al.* (2012) studied, the degradability of linear low-density polyethylene (LLDPE) and poly (L-lactic acid) (PLLA) blend films under controlled composting conditions according to modified ASTM D5338 (2003). Differential scanning calorimetry, X-ray diffraction, and Fourier

transform infrared spectroscopy were used to determine the thermal and morphological properties of the plastic films. LLDPE 80 (80 wt % LLDPE and 20 wt % PLLA) degraded faster than grafted low-density polyethylene–maleic anhydride (M-g-L) 80/4 (80 wt % LLDPE, 20 wt % PLLA, and 4 phr compatibilizer) and pure LLDPE (LLDPE 100). The tensile strength of LLDPE 100, LLDPE 80 and M-*g*-L 80/4 decreased by 20%, 54%, and 35% respectively. The films, as a result of degradation, exhibited a decrease in their mass. This investigation revealed that introduction of PLLA into LLDPE led to rapid degradation on composting and (LLDPE and PLLA) blend films are more susceptible to biodegradation compared to compatibilizer blend films.

Reddy *et al.* (2008) produced polyblend fibers from five ratios of polylactic acid/polypropylene (PLA/PP) in an effort to improve the resistance to hydrolysis and biodegradation, and to improve the dyeability of PLA. When made into polyblend fibers, the two polymers, PLA and PP, show partial compatibility and the mechanical properties of the blends are inferior compared to the pure PLA or PP fibers. However, PLA in the blends had substantially better resistance to biodegradation and hydrolysis, and dyeability with disperse dyes, resulting in a polyblend fiber with much better resistance to hydrolysis and similar dyeability to PLA.

Goncalves et al (2009) prepared films of poly (hydroxybutyrate-co-hydroxyvalerate) (PHBV) and poly(propylene) (PP), PP/PHBV (4:1) blends by melt-pressing and investigation was done with respect to their microbial degradation in soil after 120 days. Biodegradation of the films was evaluated by Fourier transform infrared spectroscopy, scanning electron microscopy, differential scanning calorimetry, and X-ray diffraction. The biodegradation and/or bio-erosion of the PP/PHBV blend was attributed to microbiological attack, with major changes occurring at the interphases of the homopolymers. The PHBV film was more strongly biodegraded in soil, decomposing completely in 30 days, while PP film presented changes in amorphous and interface phase, which affected the morphology.

Iovino *et al*. (2008) investigated the aerobic biodegradation of a composite under controlled composting conditions using standard test methods. Composite was formed by poly (lactic acid) (PLA), with and without the addition of maleic anhydride (MA), acting as coupling agent, thermoplastic starch (TPS) and short natural fiber (coir). At the end of the incubation period, TPS appeared to be the most bio-susceptible material being totally biodegraded and the matrix

showed a higher level of biodegradation than PLA. SEM micrographs showed the formation of patterns and cracks on the surface of the materials aged in the compost evidencing a profound loss of structure. Moreover, an extended biofilm (evident also with optical microscopy observation) was detected on the biodegraded materials, thus indicating the growth of a large number of bacteria and fungi on their surfaces.

Basu *et al.* (2002) blended polypropylene (PP) with amylose (AM) and/or dodecanoyl ester of amylose (DODAM) in an effort to make it biodegradable. The content of AM/DODAM was varied from 0 to 40% in the blends. The biodegradability, mechanical properties, melt flow indexes (MFIs), and morphologies of the blends were studied. Biodegradability increased with increases in AM/DODAM content. Maximum biodegradation was observed in blends containing 5% DODAM substitution at all blend compositions.

Chapter 3

MATERIALS AND METHODS

3.1 Materials

The following materials were used for carrying out the research work –

- Polypropylene Blends (described in table 3.1)
- Bacterial Strain
- Media for Cultivation
- Media for Degradation Studies (Bacteria)
- Fungal Strain
- Media for Cultivation of Fungus
- Media for Degradation Studies (Fungus)

The specifications and other details of all the above mentioned materials are given in following sections.

3.1.1 Polypropylene and its Blends

Sheets of Polypropylene (PP) and poly-L-lactic acid (PLLA) were taken. In addition, the sheets of Polypropylene and poly-L-lactic acid blends were used, in which compatibilizer maleic anhydride was added in different ratios (Table 3.1).

Blend Codes	PP (% weight)	PLLA (% weight)	M-g-PP (% weight)
PP 100	100	0	0
PP 90	90	10	0
PLLA 100	0	100	0
M-g-PP- 80/6	80	20	6
M-g-PP- 90/4	90	10	4

Table 3.1 Blends Compositions with/without Compatibilizer

3.1.2 Bacterial Strain

Bacillus flexus (MTCC Number 2909) was procured from Microbial Type Culture & Gene Bank, Institute of Microbial Technology (IMTECH), Chandigarh. This is an aerobic, mesophilic bacterium having optimum growth temperature of 30°C.

3.1.3 Media for cultivation

Nutrient broth (NB) (g/l of distilled water: 5 g peptic digest of animal tissue, 5 g NaCl, 1.5 g beef extract & 1.5 g yeast extract) & nutrient agar (NA) (g/l of distilled water: 5 g peptic digest of animal tissue, 5g NaCl, 1.5g beef extract, 1.5g yeast extract and 15g agar) obtained from HI-MEDIA Laboratories Ltd., India, were used to maintain the bacterial culture. In case of nutrient broth, Liquid cultures (100ml) were incubated in 250ml flasks on shaking incubator (New Brunswick Scientific, USA, Excella E-24) at 120 rpm, 30°C. In case of nutrient agar, medium was first poured in a Petri-plates then the bacterium was cultured by spread plate method by taking 1ml inoculum.

3.1.4 Media for Inoculation and Degradation Studies (Bacteria)

Bacterial strain used was grown on minimal medium containing (g/l of distilled water): 0.7g KH_2PO_4, 0.7g K_2HPO_4, 0.7g $MgSO_4.7H_2O$, 1g NH_4NO_3 and micronutrients (mg/l of distilled water): 5mg NaCl, 2mg $FeSO_4$, 2mg $ZnSO_4$, 1mg $MnSO_4$. All chemicals were obtained from HIMEDIA Laboratories Ltd., India.

3.1.5 Fungal Strain

Aspergillus niger (MTCC number 478) was procured from Microbial Type Culture & Gene Bank, Institute of Microbial Technology (IMTECH), Chandigarh. This is an aerobic, mesophilic fungus having optimum growth temperature of 30°C and incubation time of 7 days.

3.1.6 Media for cultivation of fungus

Czapek yeast extract broth (CYB) (g/l of distilled water): 1g K_2HPO_4, 5g yeast extract, 30g Sucrose and Czapek concentrate (10ml/l) [g/100ml containing: 30g $NaNO_3$, 5g KCl, 5g $MgSO_4.7H_2O$, 0.1g $FeSO_4.7H_2O$] & Czapek yeast extract agar (CYA) (g/l of distilled water): 1g K_2HPO_4, 5g yeast extract, 30g Sucrose, 15g agar and Czapek concentrate (10ml/l) [g/100ml

containing: 30g $NaNO_3$, 5g KCl, 5g $MgSO_4.7H_2O$, 0.1g $FeSO_4.7H_2O$] obtained from HIMEDIA Laboratories Ltd., India, were used to maintain the bacterial culture. In case of CYB, Liquid cultures (100ml) were incubated in 250ml flasks on shaking incubator (New Brunswick Scientific, USA, Excella E-24) at 120 rpm, 30^oC. In case of CYA, medium was first poured in a Petri-plates then the fungus was cultured by point culture method.

3.1.7 Media for Degradation Studies (Fungus)

Fungal strain used was grown on minimal salt medium containing (g/l of distilled water): 0.7g KH_2PO_4, 0.7g $MgSO_4$, 1g NH_4NO_3 and micronutrients (mg/l of distilled water): 5mg NaCl, 2mg $FeSO_4$, 1mg $MnSO_4$. All chemicals were obtained from HIMEDIA Laboratories Ltd., India.

3.2 Methodology

Aerobic Biodegradation

The aerobic biodegradability of degradable polymers by specific microorganisms is determined as per the guidelines of ASTM D5247 standard. For biodegradation studies specific microorganisms used were *Bacillus flexus* and *Aspergillus niger* and the polymers used were different blends of PP and PLLA with/without compatibilizer maleic anhydride. Pre-weighed films of the polymers were taken in 250 ml Erlenmeyer flasks containing 150 ml minimal medium inoculated with the culture. The flasks were incubated in shaker incubator at 120 rpm, 30^{0}C under aerobic conditions for 30, 45, 60 days for bacteria and 30 days for fungus as inoculum. ASTM is currently developing standard practices for exposing degradable plastics to ‘real systems’ environments and reporting the resulting data. The specific microorganisms test method does not represent any real world waste management infrastructure but provides a standard test method to quantify biodegradability using well-defined microbial cultures commonly present in the environment.

3.2.1 Aerobic biodegradation by *Bacillus flexus*

3.2.1.1 Pretreatment of polymers

The polymer films were cut into small pieces (3cm x 3cm) and then, the weighed films were disinfected for 30 min. in 70% ethanol. Ethanol was used to remove any organic matter present

on the surface. Afterwards, the films were added to flasks, each containing 150 ml of the minimal media.

3.2.1.2 Culture conditions

The bacterium was provided in freeze-dried form in a sealed depressurized ampoule. The ampoule was marked near the middle of cotton wool with the help of sharp file. The surface was disinfected with ethanol. Thick cotton wool was wrapped around the marked area of ampoule and it was carefully broken. Pointed top of the ampoule was removed gently and carefully to avoid release of dried culture into air of laboratory.

50ml nutrient broth was sterilized by autoclaving at 121°C and 15 psi pressure for 15 min. 300 µl of sterilized nutrient broth medium was added to the ampoule for suspension preparation. The prepared suspension was added to 250 ml Erlenmeyer flasks each containing 100 ml nutrient broth. The flasks were incubated at 30°C for 24 hrs in the shaking incubator (Excella-24, New Brunswick Scientific, USA) at 120 rpm. The suspension was also streaked on NA plates and kept in incubator at 30°C for 24 hrs.

3.2.1.3 Culture Enrichment

Enrichment of *Bacillus flexus* was done by using nutrient broth as a medium. 1 ml of culture was inoculated in 250 ml Erlenmeyer flask containing 100 ml NB. The flasks were incubated at 30°C for 24 hrs in the shaking incubator at 120 rpm.

3.2.1.4 Degradation Studies

Fig 3.1 Flasks containing polymer films kept for degradation

Pre-weighed disinfected films (3cm x 3cm) were aseptically transferred to 150 ml sterilized culture media. The set ups were kept in shaking incubator for 24 hrs prior to inoculation to ensure asepsis. 3 ml culture from mid-exponential phase was then inoculated into the set up and were incubated in shaking incubator at 120 rpm for 15, 30, 45 & 60 days at 30°C. Each set-up contained 3 polymer films. Blanks, each containing 2 disinfected polymers films (3cm x 3cm) and minimal media were also kept at the same incubation temperature and condition for 30 & 60 days.

3.2.2 Aerobic Biodegradation using *Aspergillus niger*

3.2.2.1 Pretreatment of polymers

The polymer films were cut into small pieces (2cm x 2cm) and weighed then films were disinfected (30 mins in 70% ethanol). Ethanol was used to remove any organic matter present on the surface. Then films were added to flasks, each containing 150ml of minimal salt media.

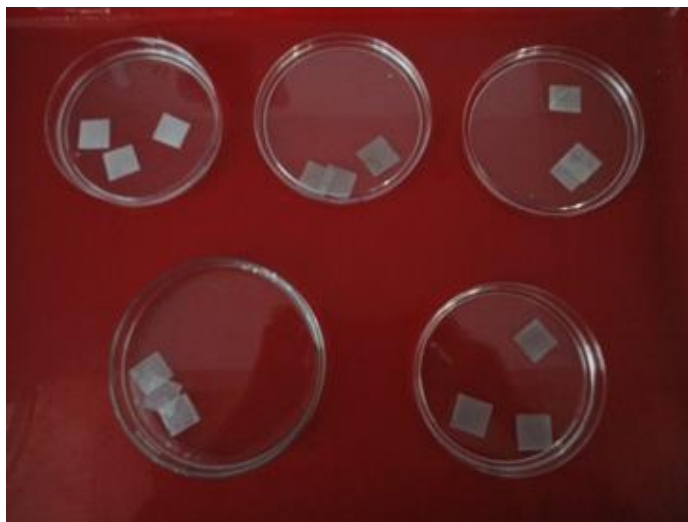


Fig 3.2 Pretreatment of polymer film

3.2.2.2 Culture Conditions

The fungus was provided in freeze-dried form in a sealed depressurized ampoule. The ampoule was marked near the middle of cotton wool with the help of sharp file. The surface was disinfected with ethanol. Thick cotton wool was wrapped around the marked area of ampoule and it was carefully broken. Pointed top of the ampoule was removed gently and carefully to avoid release of dried culture into air of laboratory.

50 ml CYB was sterilized by autoclaving at 121°C and 15 psi pressure for 15 min. 300 µl of CYB sterilized medium was added to the ampoule for suspension preparation. The prepared suspension was transferred on to CYA petri-plates by point culture method and was kept in incubator at 30°C for 7 days.

3.2.2.3 Culture Enrichment

Enrichment of *Aspergillus niger* was done by using CYA as a medium. 3 mm culture was taken with the help of a cork boarer and was sub-cultured onto CYA plates. The petri-plates were kept at 30°C for 7 days in the incubator.

3.2.2.4 Degradation Studies

Pre-weighed disinfected films (2cm x 2cm) were aseptically transferred to 150 ml sterilized minimal salt media. The set ups were kept in shaking incubator for 24 hrs prior to inoculation to ensure asepsis. 9 mm of 7 days old heavily sporulated culture was then inoculated in the set up

and was incubated in shaking incubator at 120 rpm for 30 days at 30°C. Each set-up contained 3 polymer films. Blanks, each containing 2 disinfected polymers films (2cm x 2cm) and minimal salt media were also kept at the same incubation temperature and condition for 30 days.

3.3 Analytical/Testing Procedure

3.3.1 *Bacillus flexus*

3.3.1.1 Growth kinetics in NB

The flasks containing *Bacillus flexus* in NB media were kept in shaking incubator at 30°C & 120 rpm. OD was taken at 620 nm in UV-Visible Spectrophotometer (Lambda 35 model, Perkin Elmer, USA) at the time of inoculation i.e. at 0 hr & at regular interval of 1 hr up to 26 hrs. The growth pattern of the bacterium *Bacillus flexus* i.e. lag, log, stationary & death phases under controlled condition were estimated by measuring OD at regular intervals. OD represents the logarithm of the number of microorganisms.

3.3.1.2 Measurement of O.D by Spectrophotometer

The bacterial growth was monitored by estimation of bacterial biomass i.e. by measuring absorbance of sample at 600 nm in UV-Visible spectrophotometer. Aliquot was drawn from the flasks for quantification of bacteria i.e. at time of inoculation (at 0 hr) and at regular intervals of 7 days.

3.3.1.3 Colony forming unit (CFU) measurement

CFU measurement method was applied to monitor growth of bacteria in minimal media using only polymer film as carbon source. 1 ml sample from each set up was collected at the time of inoculation & at regular interval of 7 days. These samples were serially diluted with distilled water to make 10^{-2} to 10^{-5} dilutions. 100 µl of culture from 10^{-4} & 10^{-5} dilutions were then transferred to NA plates and spreading was performed using spreader. These plates were kept in incubation at 30°C for 24 hrs and then isolated bacterial colonies were carefully counted and multiplied with the dilution factor to get representative bacterial count in 1 ml culture of each set up.

3.3.1.4 Determination of bacterial biomass

Samples taken from each set up were centrifuged at 10,000 rpm, 4^oC for 15 min; the supernatant was discarded and dry weight of the eppendorf was measured after drying it overnight at 60^oC. Comparing initial and final dry weight of the eppendorf, biomass was estimated. The procedure was repeated at regular interval of 7 days.

3.3.1.5 Residual weight measurement of polymer film

By measuring weight loss of polymer film we can directly observe the effect of bio-degradation. Microorganism when grown on surface of polymer films leads to increase in weight of the polymer due to biofilm production, whereas loss of polymer strength leads to weight loss. Weight loss is proportional to the surface area as bio-degradation is initiated, usually, at the surface of polymer sheet.

For proper weight measurement of polymers films, the films were kept in 2% (v/v) sodium dodecyl sulphate solution for 4 hrs & then repeatedly washed with distilled water. These washed films were kept on a filter paper & were dried overnight at 60^oC prior to weighing.

3.3.1.6 Morphological evaluation using scanning electron microscopy (SEM)

The films were washed in 70 % ethanol solution to remove cell mass from the residual film to the maximum possible and then dried at 60^0C for 24 hrs. These films were used to analyze the surface change and bio-deterioration. Scanning electron micrographs (SEM) of the films were taken with a scanning electron microscope (JEOL, Model JSM 6510 LV). The accelerating voltage was kept at 10 kV. The specimens were coated with 50 µm of thick gold film in an automatic sputter coater (Polaron) to avoid charging under the electron beam prior to SEM studies.

3.3.2 *Aspergillus niger*

3.3.2.1 Growth kinetics of fungal strain

Mycelia obtained from 10 ml sample was placed on pre-weighed filter paper (Whattman #1) and washed twice with distilled water. The filter paper was then dried in an oven at 90^oC for 24 hrs

before weighing. The growth pattern of fungus was determined by measuring weight of dry mycelia in every 24 hrs for 7 days.

3.3.2.2 Determination of fungal biomass

Fungal biomass was determined by filtering the whole content of the flask containing fungal culture through filter paper (Whattman #1) and washed twice with distilled water. Biomass on the filter paper was dried at $90^{o}C$ for 24 hrs to constant weight. The filter paper was weighed to get the dry biomass subtracting the weight of filter paper.

3.3.2.3 Residual weight measurement of polymer film

By measuring weight loss of polymer film we can directly observe the effect of bio-degradation. Microorganism when grown on surface of polymer films leads to increase in weight of the polymer due to biofilm production, whereas loss of polymer strength leads to weight loss. Weight loss is proportional to the surface area as bio-degradation is initiated usually at the surface of polymer sheet.

For proper weight measurement of polymers films, the films were kept in 2% (v/v) sodium dodecyl sulphate solution for 4 hrs & then repeatedly washed with distilled water. These washed films were kept on a filter paper & were dried overnight at $60^{o}C$ prior to weighing.

Chapter 4

RESULTS AND DISCUSSIONS

4.1 Aerobic Treatment

In current study, biodegradation tests were carried out with *Bacillus flexus* (bacteria) and *Aspergillus niger* (fungus) on polypropylene and its blends with PLLA and M-g-PP by keeping them in minimal media for 60 days and 30 days respectively (as per the guidelines of ASTM D5247 standard). During this period the ability of *Bacillus flexus* and *Aspergillus niger* to degrade various blends of PP and PLLA was evaluated.

4.1.1 Aerobic biodegradation by *Bacillus flexus*

4.1.1.1 Growth pattern of *Bacillus flexus*

Growth pattern of *Bacillus flexus* was studied to determine lag, log, stationary and death phases of the bacterium. 1 ml of inoculum was inoculated in 100 ml of nutrient broth in 250 ml Erlenmeyer flask and kept in shaking incubator at 120 rpm and 30°C. Blank (without the inoculums) was also kept under same conditions. Absorbance readings in terms of optical density (OD) were taken in regular intervals at 620nm.

Time (hours)	OD (620nm)
0	0.002
1	0.004
2	0.009
3	0.095
4	0.237
5	0.465
6	0.535
7	0.583
8	0.654
9	0.743
10	0.843
11	0.876
12	0.909
13	0.94

14	0.989
15	1.123
16	1.154
17	1.178
18	1.204
19	1.238
20	1.304
21	1.306
22	1.306
23	1.314
24	1.32
25	1.328
26	1.354

Table 4.1 Growth kinetics of *Bacillus flexus* in nutrient broth

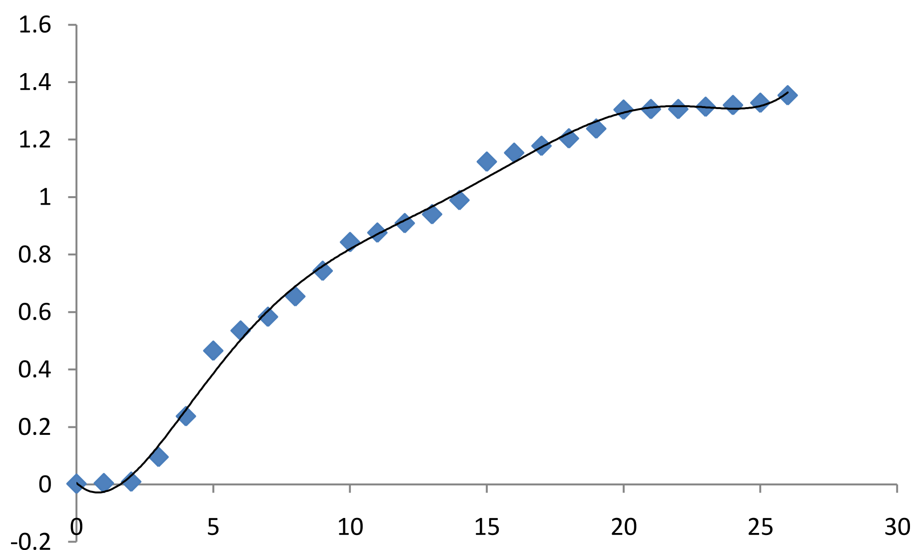


Fig. 4.1 Growth curve of *Bacillus flexus* in nutrient broth

From Table 4.1 and Fig. 4.1, we can conclude that the lag phase continued for 3 hrs followed by the exponential phase, for 3-22 hours. Stationary phase was observed with little or no growth due to lack of nutrients. In this study, culture present in mid-exponential phase was taken for degradation purpose.

4.1.1.2 Measurement of O.D by spectrophotometer

With the increasing microbial growth in the minimal media containing Polymer blends as the sole carbon source, readings were taken in an interval of 7 days at 600nm to determine the growth of bacteria.

Polymer sample code	O.D.								
	0 day	**7 day**	**14 day**	**21 day**	**28 day**	**35 day**	**42 day**	**49 day**	**56 day**
PP100	0.077	0.089	0.15	0.181	0.192	0.194	0.197	0.198	0.199
PP90	0.045	0.083	0.133	0.136	0.138	0.151	0.15	0.166	0.166
PLLA	0.053	0.131	0.203	0.209	0.209	0.211	0.212	0.239	0.268
M-g-PP- 90/4	0.041	0.096	0.186	0.188	0.193	0.206	0.208	0.228	0.233
M-g-PP- 80/6	0.041	0.093	0.196	0.196	0.204	0.21	0.235	0.24	0.24

Table 4.2 Comparison of O.D over 60 days

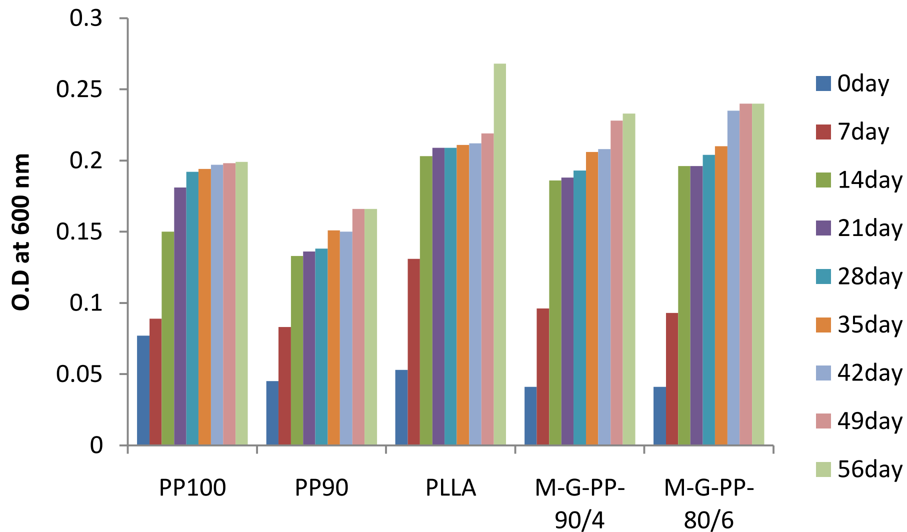


Fig. 4.2 Comparison of O.D over 60 days

From Table 4.2 and Fig. 4.2, it is noticed that after first 7 days, O.D., which represents the bacterial biomass, increased minutely in flask containing PP-100 sample as carbon source and thereafter, increased gradually upto 56 days. Gradual increase in O.D. was observed in all the

flasks containing 5 different blends of polymer. Maximum increase in O.D. from 0.053 to 0.268 was observed in flasks containing PLLA sample as carbon source.

4.1.1.3 Growth of bacteria in terms of Colony forming unit/ml

The growth of bacteria in presence of polymer films was quantified after every 7 days for a period of 60 days by serial dilution and spread plating technique and results were interpreted in terms of CFU/ml. Results showed a similar pattern to that of optical density (O.D.) data.

Polymer sample code	CFU/ml								
	0 day	**7 day**	**14 day**	**21 day**	**28 day**	**35 day**	**42 day**	**49 day**	**56 day**
PP100	$3x10^5$	$7x10^5$	$52x10^5$	$73x10^5$	$81x10^5$	$84x10^5$	$86x10^5$	$93x10^5$	$97x10^5$
PP90	$1x10^5$	$8x10^5$	$43x10^5$	$63x10^5$	$65x10^5$	$73x10^5$	$76x10^5$	$79x10^5$	$83x10^5$
PLLA	$2x10^5$	$14x10^5$	$65x10^5$	$91x10^5$	$95x10^5$	$95x10^5$	$98x10^5$	$116x10^5$	$114x10^5$
M-g-PP-90/4	$1x10^5$	$11x10^5$	$59x10^5$	$82x10^5$	$85x10^5$	$89x10^5$	$94x10^5$	$95x10^5$	$96x10^5$
M-g-PP-80/6	$2x10^5$	$8x10^5$	$62x10^5$	$86x10^5$	$94x10^5$	$96x10^5$	$98x10^5$	$101x10^5$	$103x10^5$

Table 4.3 Comparison of CFU over 60 days

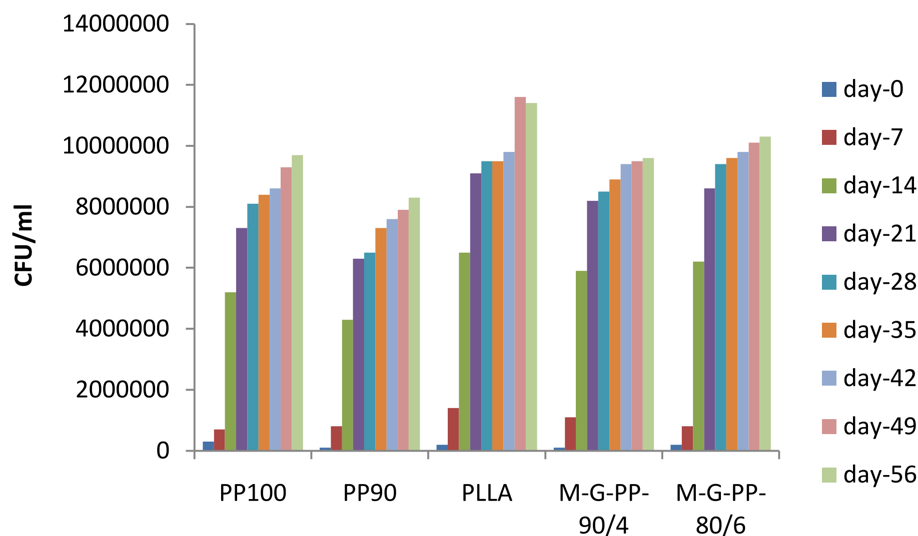


Fig 4.3 Comparison of CFU over 60 days

From Fig 4.3, it was observed that CFU/ml increased gradually in all the 5 set-ups. At first, increase in CFU/ml was very slow till 7 days and there after it increased substantially in all the 5

set-ups containing different blends of polymer(s) as carbon source. This result showed similar pattern of increasing O.D.; after 56 days, maximum CFU/ml count was found in flask containing PLLA as carbon source, where the CFU/ml increased from $2x10^5$ initially to $114x10^5$on 56th day. Set up containing M-*g*-PP 80/6 as carbon source showed an increase in CFU/ml from $2x10^5$ initially to $103x10^5$on 56th day, which was the 2nd largest increase. The results indicated that the bacteria were able to utilize all the polymer blends as C-source but was able to hydrolyze PLLA and M-*g*-PP 80/6 more easily.

4.1.1.4 Determination of biomass

The biomass can be correlated with the growth of the bacterium. Biomass was evaluated at regular interval of 7 days for 60 days.

Polymer codes	Biomass Concentration (in g/ml)								
	0 day	7 day	14 day	21 day	28 day	35 day	42 day	49 day	56 day
PP100	0	0.0001	0.0003	0.001	0.002	0.002	0.002	0.003	0.003
PP90	0	0.0002	0.0006	0.001	0.002	0.002	0.002	0.002	0.002
PLLA	0	0.0002	0.0006	0.002	0.003	0.004	0.004	0.004	0.004
M-g-PP-90/4	0	0.0004	0.0005	0.002	0.002	0.002	0.002	0.002	0.002
M-g-PP-80/6	0	0.0003	0.0005	0.001	0.002	0.002	0.002	0.003	0.003

Table 4.4 Comparison of increase in Biomass over 60 days

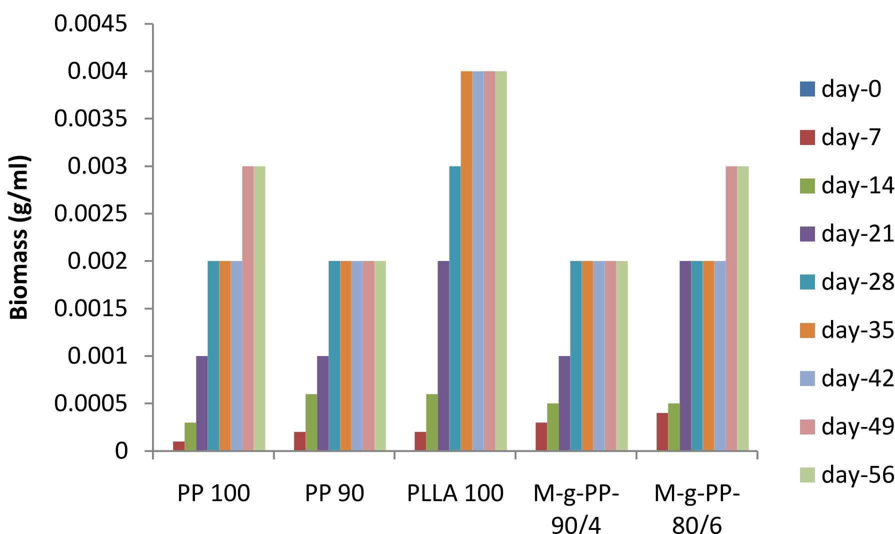


Fig 4.4 Comparison of increase in Biomass over 60 days

From Table 4.4 and Fig 4.4, it can be concluded that in the beginning, the increase of biomass was very slow (upto 14 days) in all the 5 set ups. But from 21st day onwards, drastic increase in biomass was seen in the set-up containing PLLA and M-*g*-PP 80/6 as C-source. In set-ups containing PP-90 and M-*g*-PP as C-source, the biomass remained constant from 28th day to 56th day and no further increase in biomass was observed. Set up containing PLLA as C-source showed the maximum increase in biomass from 0.0006g/ml in 14th day to 0.004g in 35th day and it remained constant thereafter till 56th day. Growth in biomass in set-ups containing PP-100 and M-*g*-PP 80/6 was observed to be more gradual, where increase in biomass was even seen on 49th day also.

4.1.1.5 Residual weight measurement

The weight loss of polymer films during incubation in minimal media in presence of *Bacillus flexus* could be taken as an indicator of biodegradation of plastics.

Polymer codes	**Initial weight (mg)**	**Final dry weight (mg) 30days**	**% weight loss 30days**	**Initial weight (mg)**	**Final weight (mg) 45 days**	**% weight loss 45 days**	**Initial weight (mg)**	**Final weight (mg) 60 days**	**% weight loss 60 days**
PP100	230	229	0.43	154.2	153	0.78	114.9	114	0.78
PP90	129.8	129	0.62	168.1	167	0.65	197.8	196.4	0.70
PLLA	274.6	262.6	4.37	254	242	4.72	229.5	209	8.93
M-g-PP-90/4	243.7	243	0.29	115.1	114	0.95	202.6	200.4	1.08
M-g-PP-80/6	243.7	243	0.29	196	195	0.51	62.5	61.9	0.96

Table 4.5 Weight loss studies over 60 days

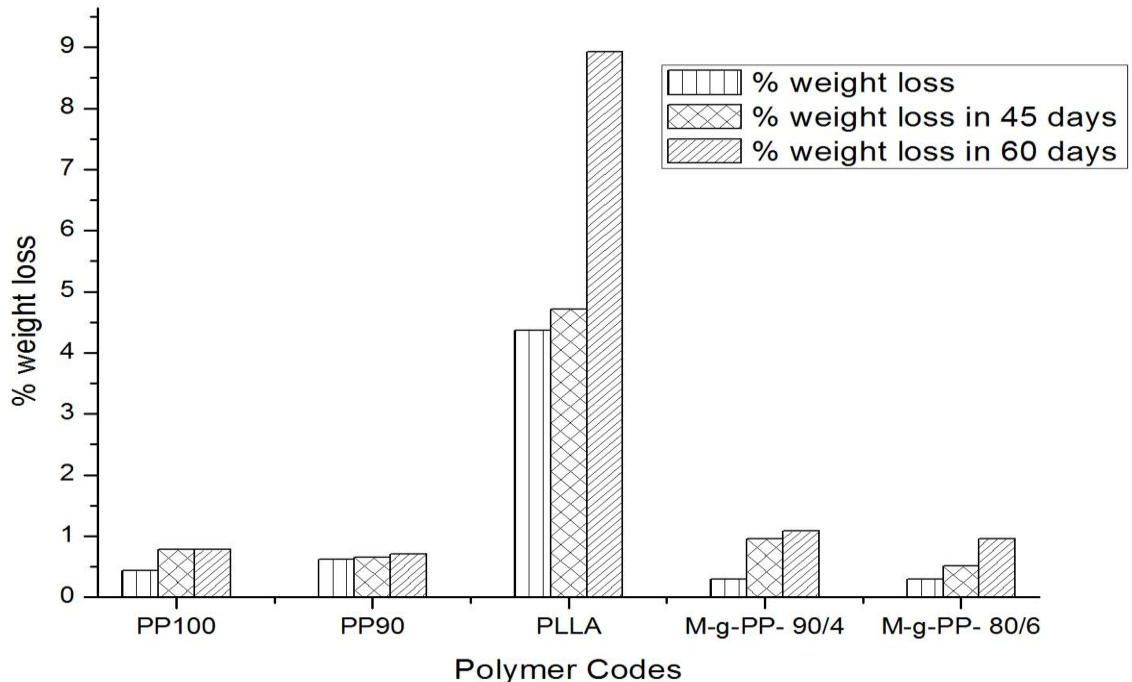


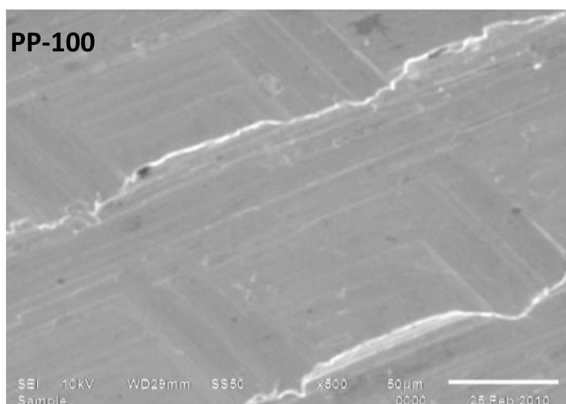
Fig 4.5 %Weight loss over 60 days

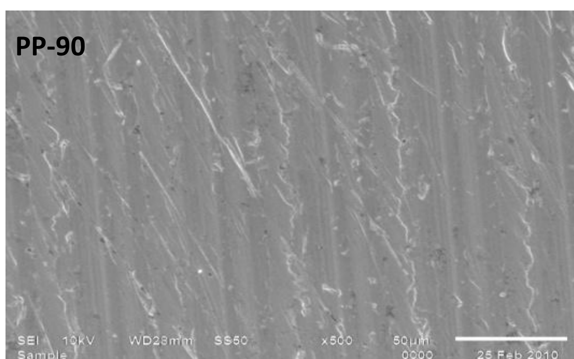
Weight loss in polymer films was calculated to find out the ability of the bacteria *Bacillus flexus* to degrade different polymer blends. From Table 4.5 and Fig 4.5, it is clearly indicated that weight loss occurred in all polymer blends over a period of 30, 45 and 60 days. However the maximum weight loss of 8.93% was observed in the set-up containing PLLA as C-source over a period of 60 days. And the least weight loss was observed in set up containing PP-90 as C-source, where the weight loss was found out to be 0.7% only after 60 days. So, it is proved that the bacterium is capable of degrading the polymer samples.

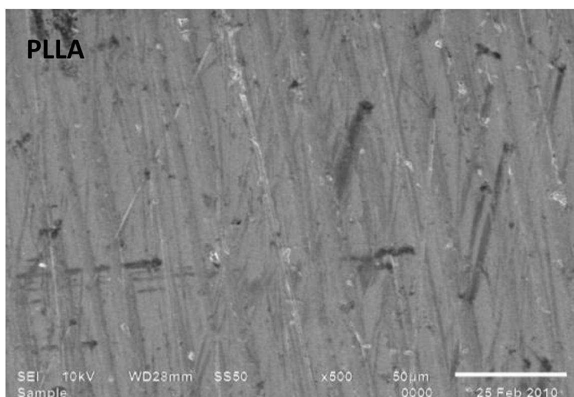
4.1.1.6 Morphological evaluation using scanning electron microscopy (SEM)

For evaluation of the surface changes and bio-deterioration in polymer films scanning electron micrographs of the films after 60 days of degradation studies with *Bacillus flexus* were taken with a scanning electron microscope (JEOL, Model JSM 6510 LV).

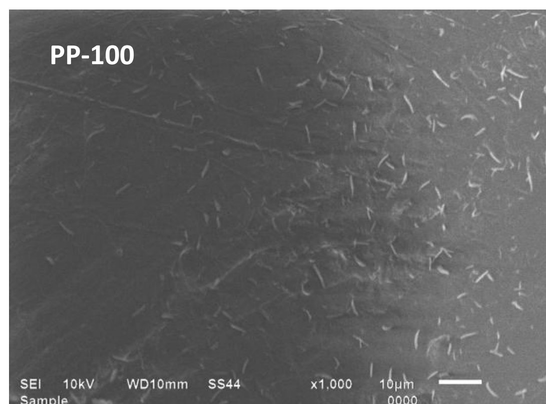
Polymer films before degradation

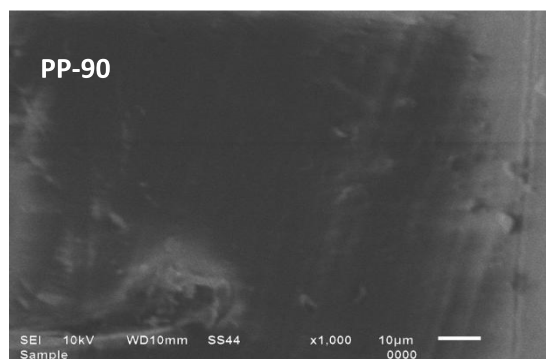


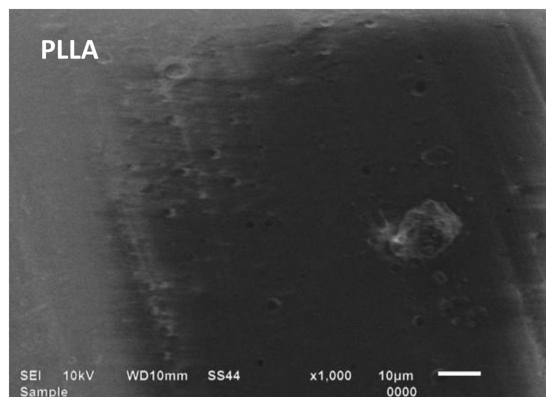




Polymer films after degradation







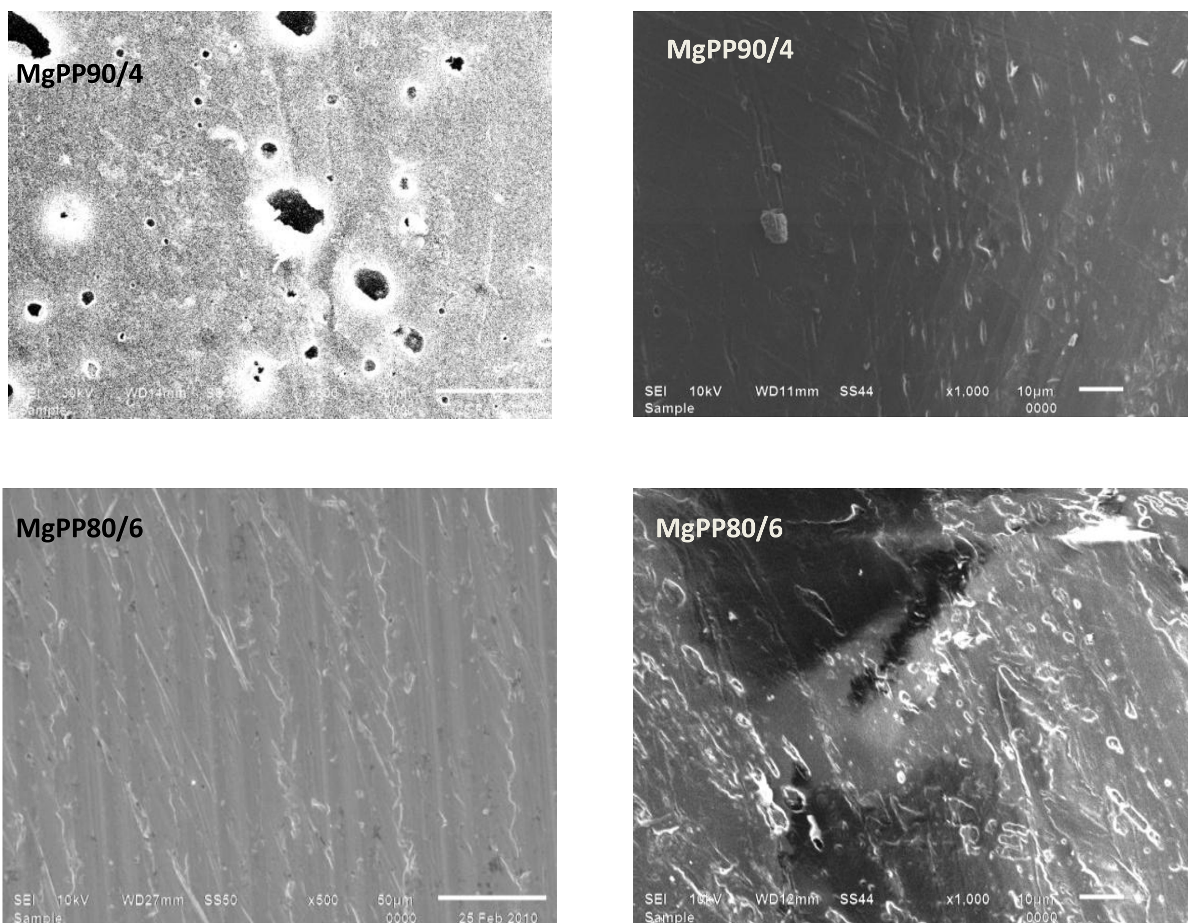


Fig 4.6 Scanning electron microscope image of polymer films before and after 60 days of degradation

After analyzing Fig. 4.6, it is concluded that the surface of the PP 100 and PP 90 was smooth but change in surface can be observed. Surface of PLLA was observed to be fairly smooth with well-defined shallow pits. There were numerous irregular cracks and erosion pits of variable sizes on the surface M-g-PP 90/4 and M-g-PP 80/6 and M-g-PP 80/6 showed a very rough appearance unlike that of the control.

4.1.2 Aerobic Biodegradation using *Aspergillus niger*

4.1.2.1 Growth pattern of *Aspergillus niger* in CYB media containing sucrose as C-source

Growth pattern of *Aspergillus niger* was studied in terms of biomass to determine lag, log, stationary and death phases of the fungus. 5 mm disc of 7 days old fully grown fungus was inoculated in 50 ml of CY broth in 250 ml Erlenmeyer flask and kept in shaking incubator at 120

rpm and 30°C. Seven nos. of such flasks were kept in shaking incubator. One flask was taken out after every 24 hrs from 0th day to 7th day and biomass was determined.

Day	Initial dry weight (g)	Final dry weight (g)	Weight of disc (5mm)	Biomass Concentration (g/ml)
0	1	1.061	0.041	0.02
1	1.059	1.158	0.041	0.058
2	1.058	1.197	0.041	0.098
3	1.034	1.214	0.041	0.139
4	1.073	1.323	0.041	0.209
5	1.03	1.359	0.041	0.288
6	1.076	1.514	0.041	0.397
7	1.06	1.579	0.041	0.478

Table 4.6 Growth kinetics of *Aspergillus niger* in CY broth

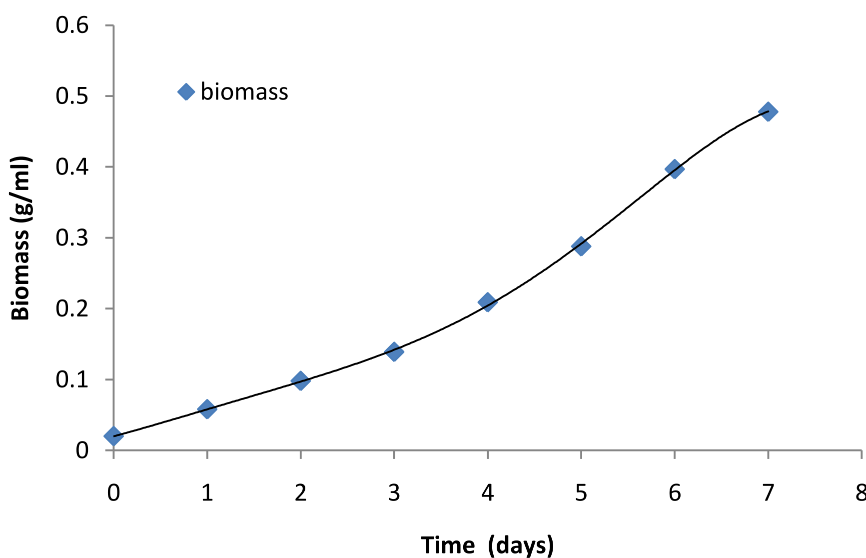


Fig. 4.7 Growth curve of *Aspergillus niger* in CY broth

In this study, culture present in mid-exponential phase was taken for degradation purpose. From Table 4.6 and Fig. 4.6, we conclude that exponential phase continued for 1-7 days and lag phase passed before 24 hrs.

4.1.2.2 Growth pattern of *Aspergillus niger* in CY media containing Polymers as C-source

Direct determination of biomass was done after 30 days to study the growth of fungus and to study its ability to hydrolyze different polymer blends.

Polymer Codes	Initial dry weight (g)	Final dry weight (g)	Biomass Concentration (g/ml)
PP 100	1.087	1.134	0.047
PP 90	1.058	1.135	0.077
PLLA	1.048	1.14	0.092
M-g-PP 90/4	1.083	1.148	0.065
M-g-PP 80/6	1.054	1.12	0.066

Table 4.8 Biomass after 30 days

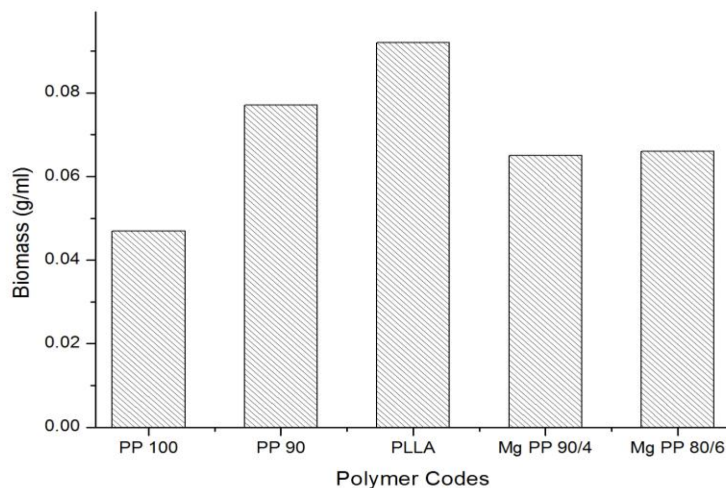


Fig 4.8 Biomass after 30 days

From Table 4.7 and Fig 4.7, it was noticed that the growth in biomass occurred in all the 5 flasks after incubation for 30 days. Maximum biomass of 0.092g/ml was obtained in flask containing PLLA sample as sole C-source and the least biomass of 0.047g/ml was found in flask containing

PP-100 sample as C-source. From this, we can conclude that *Aspergillus niger* is able to utilize PLLA and other the polymer blends as C-source.

4.1.2.3 Residual weight measurement of polymer inoculated with *Aspergillus niger*

The weight loss of polymer films during incubation in minimal salt media in presence of *Aspergillus niger* could be taken as an indicator of biodegradation of plastics.

Polymer sample code	Initial dry weight (mg)	Final dry weight (mg)	% weight loss after 30 days	Average % weight loss (of triplicates)
PP 100	168.9	168.9	0	0
	157.2	157.2	0	
	152.1	152.1	0	
PP 90	149.3	148	0.87	0.85
	161.4	160	0.867	
	123	122	0.81	
PLLA	141.2	140	0.85	0.91
	107	106	0.93	
	136.4	135.1	0.95	
M-g-PP 90/4	128	127	0.78	0.26
	128	128	0	
	128	128	0	
M-g-PP 80/6	112	112	0	0.149
	112	111.5	0.446	
	112	112	0	

Table 4.9 Weight loss studies over 30 days

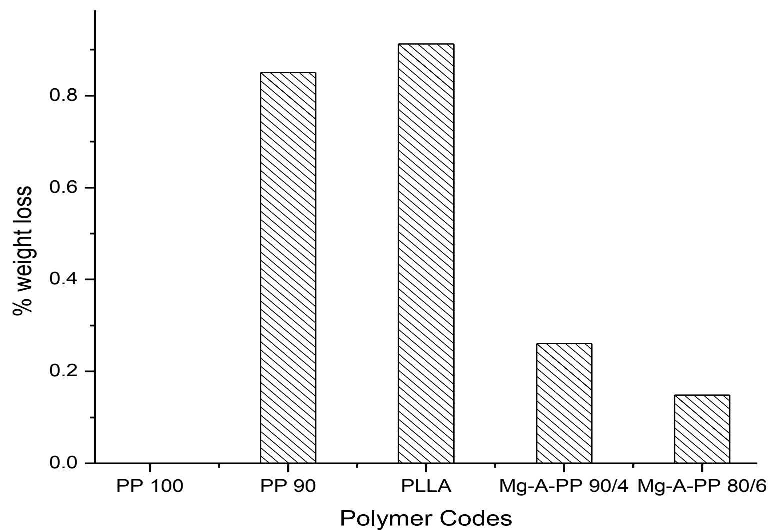


Fig 4.9 Average % weight loss over 30 days

Weight loss in polymer films was calculated to determine the ability of the fungus to degrade different polymer blends. From Table 4.8 and Fig 4.8, it is clearly indicated that weight loss occurred in all polymer blends over a period of 30 days, except in PP-100 sample. However, the maximum weight loss of 0.91% and 0.85% respectively was observed in the set-up containing PLLA sample and PP-90 over the period of 30 days, but no weight loss was noticed in the flask containing PP-100 sample.

Chapter 5

CONCLUSIONS & FUTURE PROSPECTS

5.1 Conclusions

One of the key advantages of a pure culture biodegradation assay is that we can identify the part of the biodegradation which is due to chemical degradation. This study reveals that the selected polymer and its blends with appropriate additives can be biodegraded provided the right microbial strain is used under appropriate conditions. Culture enrichment methods were found effective for enhancing the capabilities of a bacterium in utilizing the polypropylene (PP) and its blends with poly-L-lactic acid (PLLA) as the sole carbon and energy source. Maximum biodegradation by the bacterium *Bacillus flexus* in terms of weight loss was observed in case of PLLA followed by M-*g*-PP 90/4 and M-*g*-PP 80/6. The fact that M-*g*-PP 80/6 is biodegraded by the mentioned bacterium is supported by the SEM results, which showed most drastic surface change in the polymer sample. In case *Aspergillus niger*, biodegradation in terms of weight loss, was found out to be maximum in PLLA followed by PP 90. Although biodegradation of PP was very minute, *Bacillus flexus* was able to survive in media containing PP and thus, the polymer showed a little degradation. Biodegradation leads to decrease in molecular weight. It is clear from the study that the bacteria *Bacillus flexus* and fungus *Aspergillus niger* are able to degrade the CH_2 backbone of the polymer under consideration and its blends with PLLA with/without traces of compatibilizer.

5.2 Future prospects

1. Modify the polymer for microbial utility by (i) increasing the percentage of natural/biodegradable polymers; (ii) using pro-oxidants as additive; and (iii) pretreatment (e.g. photodegradation / UV radiation) of the polymer(s).
2. Modify the microbes to utilize the polymer by (i) varying and optimizing the medium composition and thus enhancing the utilization of polymer; and (ii) using genetic engineering, thus enabling the microorganism to utilize the polymer more.
3. Over-expressing the gene to increase the enzyme production, which is responsible for biodegradation, and purify the same to re-utilize for the purpose.

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