Development of Polypropylene-Polylactide Blends and their Degradation by Bacterial Isolates

Thesis

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In

Biotechnology

By

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September 2017

Dedicated

To my beloved parents

Mr. Pawan Kumar Jain and

Mrs. Manju Jain

For their endless love, support and encouragement

DECLARATION

I hereby declare that the work which is being presented in this thesis "**Development of Polypropylene-Polylactide blends and their degradation by bacterial isolates**" submitted by me for the award of the degree of **Doctor of Philosophy** in the Department of Biotechnology, Thapar Institute of Engineering & Technology, Patiala, is true and original record of my own independent and original research work carried out under the supervision of Dr. M. Sudhakara Reddy, Professor, Department of Biotechnology, Thapar Institute of Engineering & Technology, Patiala and Dr. Haripada Bhunia, Professor, Department of Chemical Engineering, Thapar Institute of Engineering & Technology, Patiala, India. The matter embodied in this thesis has not been submitted in part or full to any other university or institute for the award of any degree in India or Abroad.

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CERTIFICATE

Certified that the thesis **"Development of Polypropylene-Polylactide blends and their degradation by bacterial isolates"** submitted by Ms. Kimi Jain, in fulfillment of the requirement for the award of the degree of **Doctor of Philosophy** in the Department of Biotechnology, Thapar Institute of Engineering & Technology, Patiala, is a record of candidate's own independent and original research work carried out by her under our supervision and guidance. The matter embodied in this thesis has not been submitted in part or full to any other University or Institute for the award of any degree.

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ABSTRACT

Polypropylene (PP) is widely used polymer in packaging applications as it is flexible, economical, resistant to water and water-borne organisms and possesses excellent barrier properties. But it is non-biodegradable in nature and causes environmental pollution as well as also harms the wildlife. Therefore, it is essential to find some ways to biodegrade the polymer. The biodegradation of polypropylene (PP) can be enhanced by using two approaches; one, by blending polypropylene with some biodegradable polymer and secondly, by isolating efficient microorganisms having capability to degrade polypropylene. In our study, both the approaches have been used simultaneously to make polypropylene degradable. Firstly, polypropylene was blended with poly-L-lactide (PLLA) in varying ratios and with/without compatibilizer maleic anhydride grafted polypropylene (MAPP) to develop biodegradable polymers which were optimized on the basis of mechanical properties. Secondly, effect of blend composition and compatibilizer content on the physicochemical properties of blends was investigated via X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Scanning electron microscopy (SEM) and Thermogravimetric analysis (TGA). Thirdly, degradation of selected blends was studied using abiotic and biotic factors.

PP/PLLA blends with ratio of 80:20 (without compatibilizer, PP80) and 80:20:6 ratio (with compatibilizer, PP80C6) showed optimum mechanical properties. The results of physicochemical properties revealed that PP80C6 possessed more mechanical strength, better thermal stability, improved interfacial adhesion as compared to PP80 due to interactions formed between PP and PLLA by the addition of MAPP. Therefore, two blends namely PP80 and PP80C6 were selected for further studies. The biodegradation of selected blends was studied by using bacteria isolated from compost. Rationally, sixteen bacteria (P1-P16) were isolated by using enrichment technique. P3, P6, P8, P10 and P13 were selected on the basis of their degradation efficiency and identified as *Bacillus* sp. The bacterial isolate P8 i.e., *Bacillus thuringenesis* showed the maximum potential to degrade blends in synthetic media as well as in soil (microcosm) under laboratory conditions. Field study was conducted using consortium made of all the five bacterial isolates. It showed decrease in mechanical strength and thermal stability of blends after degradation for six months. The blends degraded by bacterial isolates in soil according to ASTM D 5338 showed percentage biodegradation of 9-12% and the highest

degradation was caused by P8. The results suggest that bacterial isolates have potential to degrade PP/PLLA blends in an efficient manner and P8 showed the largest potential. So, the bacterial isolate P8 can be further studied for enzyme production and genes responsible for it.

LIST OF CONTENTS

Chapters	Page no.
Abstract	i
List of Contents	iii
List of Figures	ix
List of Tables	xiii
List of Abbreviations	xv
1. INTRODUCTION	**1-8**
1.1 Plastics	1
1.2 Degradation of plastics	3
1.3 Microbial/Biotic degradation of plastics	3
1.4 Polymer blends	7
1.5 Gaps in study	8
1.6 Objectives	8
2. LITERATURE REVIEW	**9-44**
2.1 Brief preface on plastics	9
2.1.1 Types of plastics	10
2.1.2 History of plastics	11
2.2 Scenario of global production and demand of plastic materials	13
2.2.1 Global production of plastics (1950-2015)	13
2.2.2 Global production of plastics (2006-2015)	14
2.2.3 Global demand of plastics (in 2015)	15
2.3 Biodegradable polymers	16
2.3.1 Natural polymers	18
2.3.1.1 Starch	18
2.3.1.2 Cellulose	21

2.3.2 Synthetic biodegradable polymers 23
2.3.2.1 Polylactic acid 23
2.3.2.2 Polycaprolactone 27
2.3.2.3 Polyglycolic acid 29
2.3.3 Microbial polymers/ Polyhydroxyalkanoates (PHA) 29
2.4 Non-degradable synthetic polymers 33
2.4.1 Polyethylene 34
2.4.2 Polypropylene 39
3. MATERIALS AND METHODS 45-77
3.1 Development of degradable polypropylene blends 45
3.1.1 Materials 46
3.1.2 Development of PP-PLLA blends 47
3.1.3 Mechanical properties 48
3.2 Effect of blend composition and compatibilizer content on the physicochemical properties of blends 49
3.2.1 X-ray diffraction (XRD) 50
3.2.2 Fourier Transform Infrared Spectroscopy (FTIR) 50
3.2.3 Scanning electron microscopy (SEM) 50
3.2.4 Thermogravimetric analysis (TGA) 51
3.3 Abiotic and biotic degradation studies 52
3.3.1 Isolation and identification of polypropylene degrading bacteria 53
3.3.1.1 Compost 53
3.3.1.2 Characterization of compost 53
(a) Determination of pH and Electrical conductivity 53
(b) Organic Carbon 54
(c) Determination of Total Phosphorus 55
(d) Determination of Available Phosphorus 56
(e) Determination of Total Nitrogen 57

3.3.1.3 Isolation of polypropylene degrading bacteria 58
3.3.1.4 Morphological and biochemical characterization of bacteria 58
(a) Gram staining 58
(b) Capsule staining 58
(c) Oxidase test 59
(d) Catalase test 59
(e) Nitrate reduction test 59
(f) Starch hydrolysis test 60
(g) Simmons citrate agar test 60
(h) Carbohydrate utilization test by isolated bacteria 60
(i) Antibiotic susceptibility test for bacteria 60
3.3.1.5 Molecular characterization 61
(a) Isolation of genomic DNA from polypropylene degrading bacteria 61
(b) Agarose gel electrophoresis of DNA 61
(c) DNA quantification 61
(d) Amplification of 16S rRNA gene 62
(e) Purification of PCR products 62
(f) Ligation of purified 16S rRNA gene in pTZ57R/T vector 62
(g) Genetic transformation by $CaCl_2$- induced method 63
(h) Screening for recombinant plasmids 63
(i) Isolation of plasmid DNA from recombinant bacteria 64
(j) Size screening for recombinant plasmids 64
(k) Purification of plasmid DNA 64
(l) Sequencing 65
(m) DNA sequence analysis 65
3.3.2 Biodegradation of polymers by bacterial isolates 66
3.3.2.1 Methods of analysis 66
(a) Growth of microorganisms 66

(b) Protein estimation 66
(c) FTIR 67
(d) SEM 67
(e) TGA 67
3.3.3 Microcosm studies 68
3.3.3.1 Sample collection 68
3.3.3.2 Procedure 68
3.3.3.3 Bacterial growth in soil (cfu/gm soil) 69
3.3.3.4 FTIR 69
3.3.3.5 TGA 70
3.3.4 Biodegradation study of polymers in open field 70
3.3.5 Aerobic biodegradation of polymers 71
3.3.5.1 Materials 71
3.3.5.2 Apparatus 72
3.3.5.3 Procedure 74
3.3.5.4 Calculations 75
3.3.6 Abiotic degradation of polymers 76
3.3.6.1 Heat treatment 76
3.3.6.2 UV- irradiation 76
3.3.6.3 Chemical disinfection of films 76
3.3.6.4 FTIR 76
3.3.6.5 Biotic treatment 76
3.4 Statistical analysis 77
4. RESULTS AND DISCUSSION 78-134
4.1 Development of degradable polypropylene blends 78
4.1.1 Mechanical properties 79
Conclusions and salient features 84
4.2 Effect of blend composition and compatibilizer content on the 85

physicochemical properties of blends
4.2.1 X-ray diffraction (XRD) 85
4.2.2 Fourier Transform Infrared Spectroscopy (FTIR) 87
4.2.3 Morphology (SEM) 88
4.2.4 Thermogravimetric analysis (TGA) 90
Conclusions and salient features 92
4.3 Abiotic and biotic degradation studies 93
4.3.1 Isolation and identification of polypropylene degrading bacteria 94
4.3.1.1 Morphological and biochemical characterization of bacteria 95
(a) Carbohydrate utilization 96
(b) Antibiotic susceptibility 96
4.3.1.2 Molecular characterization of isolates 97
4.3.2 Biodegradation of polymers using bacterial isolates in liquid media 101
4.3.2.1 Methods of analysis 101
(a) Growth of bacteria 101
(b) Protein estimation 103
(c) FTIR 104
(d) SEM 105
(e) TGA 107
4.3.3 Microcosm studies 109
4.3.3.1 Cfu/gm count 110
4.3.3.2 FTIR 112
4.3.3.3 TGA 114
4.3.4 Aerobic biodegradation of polymers 116
4.3.5 Biodegradation study of polymers in field 121
4.3.5.1 Mechanical properties of the blends 121
4.3.5.2 TGA analysis 124
4.3.6 Abiotic degradation of polymers 126

4.3.6.1 FTIR of blends 126
4.3.6.2. Growth kinetics 128
4.3.6.3. Protein estimation 130
Conclusions and salient features 132
Summary **135-138**
References **139-158**
Appendix I **159-164**
Appendix II **165-174**
Appendix III

LIST OF FIGURES

Figure No.	Title	Page No.
Figure 1.1	Polymer biodegradation scheme	4
Figure 2.1	The different types and categories of plastics	9
Figure 2.2	Global production of Plastics from 1950 to 2015	13
Figure 2.3	Global production of Plastics from 2006 to 2015	14
Figure 2.4	Global Demand of Plastics Materials in 2015 by Types	15
Figure 2.5	The classification of biodegradable polymers	17
Figure 2.6	The chemical structures of (a) amylose and (b) amylopectin	19
Figure 2.7	The chemical structure of cellulose	21
Figure 2.8	The chemical structure of Polylactic acid	23
Figure 2.9	The current scheme of PLA production	25
Figure 2.10	The degradation of Cargill Dow's bioplastic in compost facility within 45 days	26
Figure 2.11	The chemical structure of Polycaprolactone	27
Figure 2.12	The chemical structure of Polyglycolic acid	29
Figure 2.13	The chemical structures of various polyhydroxyalkanoates; (a) PHB, (b) PHV, (c) PHBV	31
Figure 2.14	Schematic route of the PP-MAPP-PLLA blend	44
Figure 3.1	Schematic diagram for development of degradable polypropylene blends	45
Figure 3.2	Schematic diagram for studying effect of blend composition and compatibilizer content on the physico-chemical properties of blends	49

Figure 3.3	Schematic diagram for abiotic and biotic degradation studies	52
Figure 3.4	The set-up of microcosm experiment in dark room	68
Figure 3.5	The glass vessels containing soil and samples	73
Figure 3.6	Biodegradability testing apparatus for determining percentage biodegradation	73
Figure 4.1	Changes in tensile strength and elongation at break by varying PP composition in blend	81
Figure 4.2	Changes in tensile strength and elongation at break by varying MAPP composition in PP80 blend	83
Figure 4.3	XRD patterns of PLLA100, PP100, PP80, and PP80C6	86
Figure 4.4	FTIR spectra of PP100, PP80, PP80C6 and PLLA100	88
Figure 4.5	Scanning electron micrographs of (A) PP100, (B) PP80, (C) PP80C6, and (D) PLLA100 at 1000x magnification	89
Figure 4.6	Thermogravimetric curves of virgin polymers and blends	91
Figure 4.7	16S rDNA amplification of bacterial isolates, Lane M: 1 Kb marker	97
Figure 4.8	Neighbour-joining tree from 16S rRNA sequences showing the relationship of present study isolates and other closely related *Bacillus* species retrieved from the GenBank (accession number). Bootstrap values (1000 replicates) are shown on the branches	100
Figure 4.9	Growth curve of (a) isolate P3 (b) isolate P6 (c) isolate P8 (d) isolate P10 (e) isolate P13 in synthetic medium amended with polymer PP80	102
Figure 4.10	FTIR spectra of (a) PP80 before degradation; (b) PP80 after degradation; (c) PP80C6 before degradation and (d) PP80C6 after degradation by the isolate P3	105

Figure 4.11	Scanning electron micrographs of polymers 'PP80' (a) before degradation and b) after degradation by isolate P3 at 500X	106
Figure 4.12	Scanning electron micrograph of biofilm formed on the surface of polymer PP80 after degradation by bacteria P3 at 5000X	106
Figure 4.13	Effect of degradation on thermal stability of blend with isolate P8 (TG curves)	107
Figure 4.14	Effect of degradation on the thermal stability of blends with isolate P8 (DTG curves)	109
Figure 4.15	cfu/gm count of bacteria in microcosm at different intervals (a) bacterial isolate 'P3', (b) bacterial isolate 'P6', (c) bacterial isolate 'P8', (d) bacterial isolate 'P10', (e) bacterial isolate 'P13' and (f) consortium	111
Figure 4.16	FTIR spectra of polymer PP80 treated with different bacterial isolates as well as consortium	113
Figure 4.17	FTIR spectra of polymer PP80C6 treated with different bacterial isolates as well as consortium	114
Figure 4.18	Effect of degradation on thermal stability of blend PP80C6 with different isolates as well as consortium	115
Figure 4.19	Cumulative amount of carbon dioxide produced (grams) by the polymer blends and cellulose after incubation with bacterial isolates for 45 days	118
Figure 4.20	Cumulative percent biodegradation of PP/PLLA blends by bacterial isolates after 45 days of incubation	120
Figure 4.21	The blends PP80 and PP80C6 after ageing with consortium in field study	121
Figure 4.22	Effect of biodegradation on mechanical properties of blends (a) Tensile strength, (b) Elongation at break and (c) Tensile modulus.	123

Bars are mean ± SD (n = 3); *P < 0.05 and **P < 0.01

Figure 4.23 TG curves of blends before and after degradation with consortium in field study 124

Figure 4.24 FTIR spectra of untreated, UV-, Thermal-, Aquaregia- and Fenton's reagent treated blend PP80 degraded by consortium 127

Figure 4.25 FTIR spectra of untreated, UV-, Thermal-, Aquaregia- and Fenton's reagent treated blend PP80C6 degraded by consortium 127

Figure 4.26 Growth curve of (a) unpretreated polymers (b) thermally treated (c) UV-treated (d) Aquaregia reagent treated (e) Fenton's reagent treated polymers in synthetic medium degraded by consortium 129

LIST OF TABLES

Table No.	Title	Page no.
Table 3.1	Trade names, sources and characteristics of polymers and compatibilizer used	46
Table 3.2	Composition of PP-PLLA Blends	48
Table 4.1	Mechanical properties of blends	79
Table 4.2	Degree of crystallinity and crystallite size of polymers from XRD	87
Table 4.3	Physicochemical characteristics of compost	94
Table 4.4	Biochemical characteristics of bacterial isolates of present study	95
Table 4.5	Fermentation of carbon substrates and antibiotic profiling of bacterial isolates of the present study	96-97
Table 4.6	Bacterial isolates and their closest relative species inferred from 16S rRNA gene sequences of EzTaxon-e database	98
Table 4.7	The homologies among the sequences as calculated by Clustalw (multiple sequence alignment)	99
Table 4.8	Protein content (mg/ml) estimated in biofilm + media of different polymers treated with bacterial isolates	104
Table 4.9	TG analysis of polymers before and after degradation with isolate P8 in synthetic media	108
Table 4.10	Physiochemical characteristics of soil	110
Table 4.11	TG analysis of polymers before and after aging with bacteria in microcosm studies	115
Table 4.12	Theoretical carbon content (%) and theoretical amount of CO_2 (g) evolved from cellulose and polymer blend samples	117
Table 4.13	CO_2 evolved (in grams) by the bacterial isolates and Cumulative	118

percentage biodegradation at the end of test

Table 4.14 Mechanical properties of the blends before and after ageing with consortium in field study 122

Table 4.15 TG analysis of polymers before and after degradation with consortium in field study 125

Table 4.16 Carbonyl index (CO*i*) of blends PP80 and PP80C6 before and after pretreatment 128

Table 4.17 Protein content (mg/ml) estimated in biofilm + media of untreated, UV-, Thermal-, Aquaregia- and Fenton's reagent treated polymers degraded by consortium 131

List of Abbreviations

%	percent
°C	Degree centigrade
ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
bp	Base pair
C	Carbon
cm	Centimeter
Da	Dalton
DNA	Deoxyribonucleic acid
DNS	Dinitrosalicylic acid
dNTP	2'-deoxynucleoside-5'-triphosphate
DSC	Differential Scanning Calorimetry
DTA	Differential Thermal Analysis
DTG	Differential Thermogravimetry
E_b	Elongation at Break
EDTA	Ethylenediamine-tetra acetic acid
FTIR	Fourier Transform Infrared
g	Gram
HDPE	High Density Polyethylene
HPLC	High Performance Liquid Chromatography
hr	Hour
IPTG	Isopropyl-β-thiogalactoside
kb	Kilo base
KOH	Potassium hydroxide
LDPE	Low Density Polyethylene
LLDPE	Linear Low Density Polyethylene

LB	Luria-Bertani
MA	Maleic Anhydride
MAPP	Maleic Anhydride *grafted* Polypropylene
MFI	Melt Flow Index
MPa	Mega Pascal
MW	Molecular Weight
m	Metre(s)
M	Molar
mg	Milligram
min	Minutes
ml	Millilitre
mm	Millimetre
mM	Millimolar
N	Nitrogen
NA	Nutrient agar
NaCl	Sodium chloride
NaOH	Sodium hydroxide
ng	Nanogram
PCR	Polymerase chain reaction
PCL	Poly(ε-caprolactone)
PE	Polyethylene
PET	Polyethyleneterephthalate
PHA	Polyhydroxyalkanoates
PHB/ P(3HB)	Poly(3-hydroxybutyrate)
PHBV	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
phr	Parts Per Hundred of Resin
PLA	Poly(lactic acid)
PLLA	Poly(L-lactide)

PP	Polypropylene
ppm	Parts per million
PS	Polystyrene
PU	Polyurethane
PVC	Polyvinylchloride
RFLP	Restriction fragment length polymorphism
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SE	Standard error
SEM	Scanning Electron Microscopy
TG	Thermogravimetry
TGA	Thermogravimetric Analysis
T_i	Initiation temperature
T_f	Final degradation temperature
T_m	Melting point
TOC	Total Organic Carbon
U.V.	Ultraviolet
v/v	Volume by volume
w/v	Weight by volume
w/w	Weight by weight
WAXD	Wide-angle X-ray Difraction
X-gal	5-Bromo-4-chloro-3-indolyl-β-D-galactoside
XRD	X-ray Diffraction
µg	Microgram
µl	Microlitre
µM	Micromolar

Chapter 1
Introduction

1.1 Plastics

Plastics can be defined as the synthetic long chain polymeric molecules (Zheng et al., 2005) which are extensively used in clothing, shelter, food, transportation, construction, medical and recreation industries because of their strength, light-weight and durability (Konduri et al., 2010). About 140 million tonnes of plastics are produced globally every year (Shimao, 2001). These have almost substituted paper and cellulose based products meant for packaging purposes due to better physico-chemical properties. The most extensively used plastics in packaging are polyethylene (PE) [low density polyethylene (LDPE), linear low density polyethylene (LLDPE), medium density polyethylene (MDPE), and high density polyethylene (HDPE)], polypropylene (PP), polystyrene (PS), polyurethane (PUR), polyethylene terephthalate (PET), polybutylene terephthalate (PBT), polyvinyl chloride (PVC), nylons. Their uses are described as follows. Polyethylene (PE) is the most commonly used polymer in food packaging films, plastic bags, toys, milk and water bottles, motor oil bottles etc. The second most extensively used polymer is polypropylene (PP) for bottle caps, medicine bottles, drinking straws, disposable syringes, car seats, bumpers, car batteries, carpet backing etc. Similarly, polystyrene (PS) is used for disposable cups, laboratory ware, packaging materials and certain electronic uses. Polyurethane (PU) is used in making tyres, gaskets, bumpers, sponges, life jackets, in refrigerator insulation etc; Polyvinyl chloride (PVC) is used for automobile seat covers, raincoats, bottles, shower curtains, electricity pipes, shoe soles, garden hoses etc. Polyethylene terephthalate (PET) is utilized in processed meat packages, carbonated soft drink bottles, textile fibres, pillow and sleeping bag filling, peanut butter jars etc. Nylon or polyamides are utilized in small bearings, windshield wipers, speedometer gears, football helmets, inks, racehorse shoes, water hose nozzles, cellophane, rainwear, clothing parachute fabrics etc. Polycarbonate (PC) is utilized in street lighting, making nozzles on paper making machinery, safety visors, baby bottles; PC are also utilized in sky-lights as well as in roofs of greenhouses, varandahs and sunrooms. Polytetrafluoroethylene (PTFE) is widely utilized in a variety of industrial and household applications such as specialized chemical plant; as coating on non-stick saucepans and frying pans; electronics and bearings etc. (Shah et al., 2008).

Polyolefins (i.e. PE and PP) have become the materials of choice for packaging applications due to their toughness, flexibility and outstanding barrier properties. They are resistant to water and water-borne organisms and are used in distribution of perishable foodstuffs. In agriculture, they are used as mulching films to grow soft fruits and vegetables (Scott and Wiles, 2001). But, the problem is that synthetic polymers are made from petrochemicals due to which they are non-biodegradable in nature. It leads to generation of environmental pollution and also harms wildlife after dispersed in nature. And, the use of long-term polymers for short-term applications like packaging, catering, surgery etc. is undesirable (Averous and Pollet, 2012). Over 40 million tonnes of plastics generated every year are discarded into environment (Yang et al., 2007). The plastics are non-degradable due to lack of water solubility and the high molecular weight by which it cannot transport directly to the microbial cells (Sudhakar et al., 2008).

The careless disposal of plastic waste causes environmental pollution and it has become serious problem all over the world. The main strategies available to manage the waste are landfill sites, recycling and incineration. Most of the plastic waste ends up in landfill but they are recalcitrant in nature. So, the landfill capacity is getting diminished and new sites become increasingly difficult to find (Orhan and Buyukgungor, 2000). It is estimated that approximately 20-30% of the volume of landfill sites is covered by plastics only and they remain there for several years (Ishigaki et al., 2004). Recycling of plastics is also economically unfeasible as they are often soiled by foodstuffs and other biological substances (Gross and Kalra, 2002). Furthermore, incineration of plastic waste generates toxic emissions like dioxins. In composting plants too, plastic waste does not get completely eliminated so, the fragments of polymer contaminate the compost and require screening or other processes for their elimination (Ojeda et al., 2009). Therefore, the use of biodegradable polymers or the biodegradation of persisting polymers can be the solution to this problem.

In the previous years, studies related to the replacement of synthetic plastics by biodegradable materials either completely or partially has increased which can be helpful in the solution of the solid waste management problems to some extent. Presently, it is required that disposable items like packing bags; coffee and tea cups; milk, water, and soft drinks cartons; and agricultural mulch films, should be biodegradable and get degraded into safe by-products under normal composting conditions (Basu et al., 2002). Previously, some biodegradable polymers have been introduced into the market. Though, none of them is capably

biodegradable in landfills. Therefore, none of the products have achieved extensive use. Thus, there is a need to find efficient microorganisms and their products to resolve this worldwide issue (Kathiresan, 2003).

1.2 Degradation of plastics

Degradation of plastics is the process that leads to physical or chemical change in the polymer properties due to environmental factors like heat, light and moisture; chemical state or biological activity. The polymer degradation can be categorized into three types according to the factors involved in degradation of the polymers – (i) photodegradation, (ii) thermo-oxidative degradation and (iii) biodegradation (Sangale et al., 2012).

Photodegradation occurs due to absorption of high-energy radiation in the ultraviolet region of the spectrum. During the process of photodegradation, the polymer chains are broken at various places, and the plastic waste gets destabilized through embrittlement. Ultimately it becomes fragile and gets fragmented. Moreover, the erosion by wind and rain causes the breakdown of embrittled plastic into friable powder (Kyrikou and Briassoulis, 2007).

Thermo-oxidation occurs due to combined action of heat and oxygen. In this method, free radicals and some reactive chemical species are generated which break down the polymer chains by the creation of branching and crosslinking of polymer molecules. It ultimately causes reduction in the molecular weight, and crystallinity of polymers which consequently, causes deterioration in the mechanical properties. The thermo-oxidation depends on several factors such as the manufacturing process, degree of aging, and the content and nature of antioxidant additives (Real and Correia, 2012).

1.3 Microbial/ Biotic degradation of plastics

Biodegradation is considered as the best approach for degrading polymers as it is a cheap and eco-friendly process in which the microorganisms utilize the polymers as substrate for their growth (Bhardwaj et al., 2013). Previously, biodegradation was defined as the decomposition of materials solely by the action of microorganisms but now, it is understood that both abiotic and biotic factors act synergistically to degrade the organic material and some studies show that abiotic degradation precedes biotic degradation. The biodegradation of polymers is a complex process and can be divided into various steps as shown in Figure 1.1. The first step

is biodeterioration in which the biodegradable materials are fragmented into tiny fractions by the combined action of microorganisms, other decomposer organisms and abiotic factors. The next step is depolymerisation in which the polymeric molecules are broken down to produce low molecular weight compounds like oligomers, dimers and monomers by the enzymes or free radicals secreted by microorganisms. This leads to reduction in molecular weight of polymeric molecules so that they can go across the plasmic membrane. After getting transported into the cytoplasm, the tiny molecules get integrated with metabolic pathways. This step is known as assimilation and it causes production of energy, new biomass, various primary and secondary metabolites, and storage vesicles. Subsequently, mineralisation occurs in which CO_2, H_2O, CH_4, N_2, and different salts are released into the environment (Lucas et al., 2008).

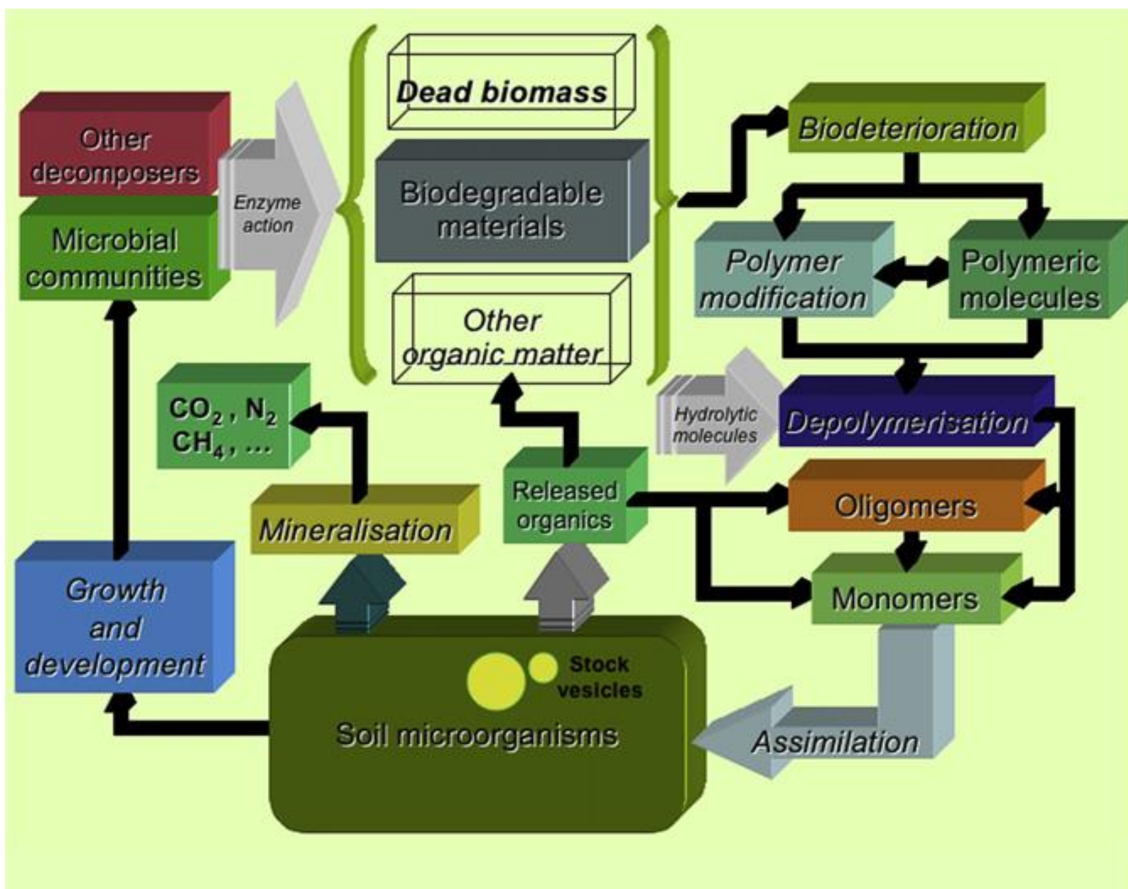


Figure 1.1 Polymer biodegradation scheme (Lucas et al., 2008)

The degradation varies from one microorganism to another as they possess different characteristics. It depends on the type of enzymes produced by microorganisms (i.e. extracellular or intracellular), characteristics of the organisms, kind of polymer and kind of

treatment required. The degradation of polymers is indicated by discoloration, phase separation, erosion, cracking and delamination. The old bonds are broken, new functional groups are synthesised and transformation occurs due to chemicals (Bhardwaj et al., 2013). The growth and activity of microorganisms is affected by various parameters like temperature, pH, humidity, and salinity; the supply of nutrients and the presence or absence of oxygen. So, such conditions should be controlled during the biodegradability testing of polymers (Muller, 2005).

There are several microorganisms reported in the literature which can degrade synthetic plastics for example, *Brevibacillus borstelensis, Rhodococcus ruber* and *Penicillium simplicissimum* YK are reported to degrade polyethylene (PE). *Comamonas acidovorans* TB-35, *Curvularia senegalensis, Pseudomonas chlororaphis, Fusarium solani, Aureobasidium pullulans, Cladosporium* sp., can degrade polyurethane (PU). Polyvinyl chloride (PVC) is degraded by *Pseudomonas putida* AJ, *P. fluorescens* B-22, *Ochrobactrum* TD, *Aspergillus niger* van Tieghem F-1119 etc. *Aureobasidium pullulans* can degrade plasticized Polyvinyl chloride and *Thermomonospora fusca* can degrade BTA-copolyester (Shah et al., 2008).

Similarly, various microorganisms have also been reported to degrade natural plastics for example; *Alcaligenes faecalis*, *P. lemoignei, Schlegelella thermodepolymerans* can degrade poly-3-hydroxybutyrate (PHB); *Clostridium botulinum*, *C. acetobutylicum*, *F. solani* can degrade polycaprolactone (PCL); *F. moniliforme*, *Amycolatopsis* sp., *Penicillium roquefort*, *Bacillus brevis*, *Rhizopus delemer* can degrade polylactic acid (PLA); *Schlegelella thermodepolymerans, P. indica* K2 are reported to degrade poly(3-hydroxybutyrate-co-3-mercaptopropionate); *Streptomyces* sp. SNG9 can degrade the poly(3-hydroxybutyrate)poly(3-hydroxybutyrate-co-3-hydroxyvalerate); *Ralstonia pikettii* T1, *Acidovorax* sp. TP4 can degrade poly(3-hydroxybutyrate-co-3-hydroxypropionate) etc. The blends of starch-polyethylene are reported to be degraded by *Penicillium funiculosm*, *Aspergillus niger*, *Phanerochaete chrysosporium;* and the blends of starch/polyester is reported to be degraded by *Streptomyces*, and *Phanerochaete chyrsosporium* (Shah et al., 2008).

Biodegradation of polymers can be more efficient if degrading microorganisms form the biofilm on surface of polymers (Hadad et al., 2005). The microorganisms attach to the surface of polymer to form the biofilm and secrete extracellular polymeric substances (EPS). Initially, the cells are attached reversibly as they are held by physical forces and are removed

by a mild wash but later on, the adhesion becomes irreversible as it is mediated by cation bridging, hydrogen bonding, and receptor ligand interaction. EPS helps in adhesion, protection and immobilization of microorganisms and also facilitate spatial arrangement of dissimilar species (Muthukumar et al., 2014). The major amount of biofilm, upto 97% is bound water (Welch et al., 2012). The metabolic activity of microorganisms is higher in biofilm as compared to suspended bacteria due to greater carbon availability in the biofilm (Gilan et al., 2004). It is a common method of living adopted by microbes which can attach to any kind of surface-water interface and it facilitates the biodegradation. Biofilms also increase the survival potential of microorganisms in harsh conditions by promoting the vital interactions among the microbes and surface. It also promotes the use of surface as substrate. Although there are some harmful effects of biofilm in natural, industrial and clinical environments but they are useful in degradation of synthetic polymers. Since, the surface of synthetic polymer is hydrophobic therefore; bacterial surface should also be hydrophobic to form a stable biofilm. There is a correlation between bacterial hydrophobicity and carbon starvation (Gilan et al., 2013a; Gilan et al., 2013b).

Regarding biodegradability of polymers, there is always a dispute between polymer scientists and microbiologists that for polymer scientists, the degradation can be defined as the deterioration in mechanical or physical properties while microbiologists take interest in complete transformation of materials into CO_2 and biomass (Koutny et al., 2006). The amount of CO_2 evolved or oxygen consumed during the transformation of polymer is considered as the best way to determine total biodegradability. The organic carbon present in polymer gets converted to inorganic carbon mainly CO_2 when the sample is exposed to controlled environmental conditions like soil, activated sludge, compost etc. (Siotto et al., 2012). There are some standard testing procedures found for biodegradability of plastics which are issued by American Society for Testing and Materials International (ASTM, http://www.astm.org), International Organization for Standardization (ISO, www.iso.org), European Committee for Standardization (CEN, http://www.cenorm.be), German Institute for Standardization (DIN, http://www2.din.de) etc. ISO is internationally recognized standardization body while ASTM and CEN are taken as regional standardization bodies (Krzan et al., 2006).

There are various techniques to determine the polymer degradation like scanning electron microscopy (SEM), Fourier Transform Infrared spectroscopy (FTIR), High Performance

Liquid Chromatography (HPLC), Thin Layer Chromatography (TLC) and Gas Chromatography-Mass Spectrometry (GC-MS). SEM is utilized to observe the level of scission as well as the attachment of microorganisms on the polymer surface before and after biodegradation. FTIR is an important tool to quantify the carbonyl groups produced after degradation. Besides this, the reduction of native bonds indicates that the polymer has fragmented into smaller chains (Rajandas et al., 2012). TLC, HPLC and GC-MS are used to characterize the products from polymer degradation. The physical changes occurred in polymer after degradation are determined by weight loss, change in tensile strength and percentage of elongation (Sangale et al., 2012).

1.4 Polymer blends

The polymer blends are produced by mixing of two or more polymers via regular processing techniques. Nowadays, it is a well-known process for acquiring appropriate materials having specific end uses; for example, the production of partially biodegradable polymers. These polymers are produced by blending inert synthetic polymers (PE, PP) with biodegradable polymers (starch, cellulose, PLA etc.). The substitution of commercial plastics (PE, PP, PVC, nylon etc.) by the completely biodegradable polymers is a useful solution for solid waste management problems but the cost of manufacturing such polymers is very high and they show inferior properties too. So, there is a trend to produce partially biodegradable polymer by blending as a compromise between cost and performance. Presently, it is mandatory that the disposable items like packing bags; coffee and tea cups; milk, water and soft drink cartons; agricultural mulch films etc. should be biodegradable and get converted into safe by-products when dispersed in nature after use (Singh et al., 2010; Singh et al., 2011; Singh et al., 2012).

Polymer blends are generally categorized into three groups which are described as follows:

1. **Miscible/homogeneous polymer blends**: It is a single-phase structure. It possesses only single glass transition temperature.
2. **Immiscible polymer blend (heterogenous polymer blend)**: It is the most populous group that exhibits immiscibility. There are two glass transition temperature observed.
3. **Compatible polymer blends**: It is an immiscible blend which exhibits macroscopically uniform physical properties. This uniformity in properties is due to strong interactions among the component polymers.

1.5 Gaps in study

- Little research has been carried out on blending polylactide with non-biodegradable polymers. Since none of the PLLA blends are commercially available presently; therefore, blending PLLA with other polymers needs to be explored. The research outcome will help as a step in developing the low-cost and eco-friendly material.
- Previously most of the efforts were made to synthesize biodegradable polymers, but less attention has been given to the identification of environmental requirements and testing of biodegradable polymers.
- The degradability of plastics in environment is a complex process which is strongly affected by the kind of plastics; and biotic and abiotic conditions to which plastics are exposed. But, only a few studies on biodegradability have been conducted under controlled conditions.

1.6 Objectives

The overall objective of the research is to develop degradable polypropylene films which will maintain its functional properties during its lifespan and at the same time be able to break down quickly & effectively after utilization. The specific objectives are:

- Development of degradable polypropylene blends having optimum performance properties based on polypropylene (PP) and polylactide with and without compatibilizer.
- Investigating the effect of blend composition and compatibilizer content on the physico-chemical properties of blends.
- Abiotic and biotic degradation study of blended films and their characterization.

The blends of polypropylene (PP) and polylactide (PLA) have been studied earlier too but for different purposes. Our purpose was to introduce degradability in packaging films which are difficult to segregate and recycle. Moreover, the blends of PP and PLA are not commercially available. So, blending PLA with PP would be a new combination to explore and it may be better than the blends of PP with other polymers such as starch, cellulose etc. due to better mechanical properties of PLA. The blends of PP/PLA developed would be degradable so they can be expected to start with environmental and aerobic conditions and then likely move to anaerobic conditions, which is what typically happens in S landfills.

Chapter 2

Literature Review

2.1 Brief preface on plastics

The word plastic is derived from the Greek word *plastikos*, meaning 'able to be molded into different shapes' (Joel, 1995). They are made from organic and inorganic raw materials for example carbon, hydrogen, oxygen, nitrogen, silicon, and chloride. These materials are generally derived from oil, coal and natural gas (Seymour, 1989).

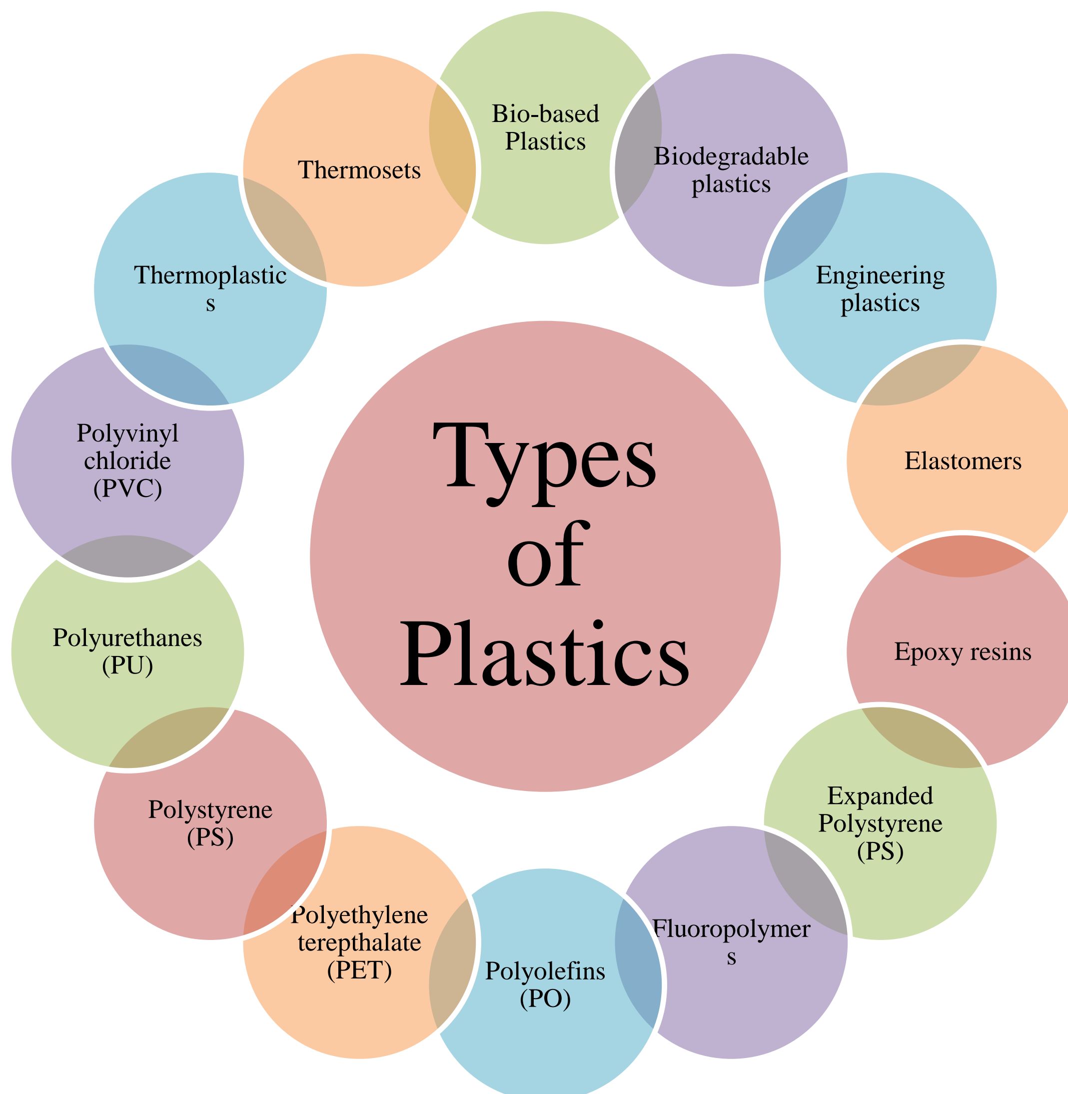


Figure 2.1 The different types and categories of plastics

(Source: http://www.plasticseurope.org/what-is-plastic/types-of-plastics-11148.aspx)

2.1.1 Types of plastics

Plastics are of many types as shown in Figure 2.1 but they are broadly classified into two major groups on the basis of chemical structure: (i) Thermoplastics and (ii) Thermosets. Thermoplastics are the kind of plastics which can be softened and hardened repetitively by heating and cooling. It means that they can be moulded into any shape by heating and cooling for several times without affecting their mechanical properties. These polymers have a simple linear structure where the atoms and molecules are joined end-to-end into a series of long carbon chains and each chain is independent of the other. The backbone of thermoplastics is completely made of carbon atoms, due to which they are resistant to degradation as well as hydrolytic cleavage of chemical bonds. Therefore, thermoplastics are regarded as non-degradable plastics. The thermoplastics are manufactured by 'addition polymerisation' reaction in which the double bonds present in olefins are broken to produce new carbon-carbon bonds for example, polyethylene and polypropylene (Zheng et al., 2005; Singh and Sharma, 2008). Examples of Thermoplastics are:

- Polyethylene - PE
- Polypropylene - PP
- Polystyrene - PS
- Polyethylene terephthalate - PET
- Polycarbonate – PC
- Acrylonitrile butadiene styrene - ABS
- Poly(methyl methacrylate) – PMMA
- Poly(vinyl chloride) - PVC
- Expanded Polystyrene - EPS

On the other hand, thermoset plastics are those which become permanently hard and rigid after cooling so that they cannot be re-molten and reformed. It means that the transformation from liquid to solid state is irreversible. Thermoset plastics possess extremely cross-linked structure where the small molecules are joined together by chemical bonds to form complex inter-connected network structures. The main chain is built of hetero-atoms, making these polymers susceptible to degradation by the hydrolytic cleavage of chemical bonds like ester or amide bonds. The thermoset plastics are manufactured by 'step-growth polymerization' in which the elimination of water molecule takes place between a carboxylic acid and an alcohol

or amine for example, polyester or polyamide (Singh and Sharma, 2008; Zheng et al., 2005). Examples of Thermosets are:

- Phenol-formaldehyde (PF)
- Polyurethane (PUR)
- Epoxide (EP)
- Polytetrafluoroethylene - PTFE
- Unsaturated polyester resins (UP)

2.1.2 History of plastics

Parkesine (nitrocellulose) was the first man-made plastic which was produced by Alexander Parkes. He unveiled it at the 1862 Great International Exhibition in London. It was an organic substance obtained from cellulose which could be molded upon heating and could retain its shape on cooling (Bellis, 2017).

Celluloid was invented by John Wesley Hyatt in 1868 as a substitute for the ivory in the manufacture of billiard balls. It is made from cellulose and alcoholized camphor. In the beginning, he tried using a natural substance called collodion, after spilling a bottle of it and found that the material dried into a tough and flexible film. But the material was not very strong so it could not be used as a billiard ball. Afterwards, camphor (a derivative of laurel tree) was added resulting in new celluloid which could be molded into a durable shape with heat and pressure. Besides billiard balls, celluloid also became famous in photography and movie film industries (Bellis, 2017).

After cellulose nitrate, the next product was formaldehyde that advanced the technology of plastic. Around 1897, efforts were made to produce white chalkboards which led to casein plastics (i.e., milk protein mixed with formaldehyde). Galalith and Erinoid (tradenames) are the two early examples of casein plastics. In 1899, Arthur Smith got a British Patent for phenol-formaldehyde resins which could be used in electrical insulation as an ebonite substitute. However, in 1907, a Belgian chemist Leo Hendrik Baekeland invented the first fully synthetic resin named ‘Bakelite’ to become commercially successful by improving the phenol-formaldehyde reaction techniques (Bellis, 2017).

The development of commodity plastics began in early twentieth century which is described below (Andrady and Neal, 2009):

Polyvinyl chloride (PVC) was first synthesized in 1872 by Eugen Baumann but its commercial production started in the late 1920s in the USA (Andrady and Neal, 2009).

Polystyrene (PS) was first created by the German company BASF (I G Farben) in the 1930s but it was introduced into the USA in 1937. Expanded polystyrene (used in building insulation, packaging purposes, cups and trays) was invented by the Dow Chemical Company in 1954 (Andrady and Neal, 2009).

In March 1933, Polyethylene (PE) was discovered by two research chemists, Reginald Gibson and Eric Fawcett, at Imperial Chemical Industries (ICI) Winington Laboratory in the UK and it was first manufactured as low-density resin (LDPE) in 1935 (Andrady and Neal, 2009).

Polyethylene terephthalate (PET) was discovered in 1941 by the chemists Whinfield and Dickson, employees of the Calico Printers' Association of Manchester. Ultimately, it was licensed to DuPont for use in the USA and to ICI for use in the rest of the world. It is one of the few polymers which are suitable for bottles due to transparency, lightweight, gloss and resistance to CO_2 permeation resulting in complete replacement of glass in Europe (Andrady and Neal, 2009).

Polypropylene (PP) was discovered by Giulio Natta in 1954, and its commercial production began in 1957 (Andrady and Neal, 2009).

2.2 Scenario of global production and demand of plastic materials

2.2.1 Global production of plastics (1950-2015)

There is a continuous growth in the global production of plastics for more than 50 years. It has risen from 1.5 million tonnes in 1950 to 322 million tonnes in 2015. This statistics of production volume of plastics has been shown in Figure 2.2. In 2015, the global production of plastics grew by 3.4% as compared to 2014. From 1950 to 2015, the compound annual growth rate (CAGR) of plastics was about 8.6% (PlasticsEurope, 2015). Due to comparatively low cost of plastic materials, the demand for plastics is growing continuously but there are not adequate methods for disposal of such materials. Millions of tons of plastic materials end up in either landfills or oceans each year.

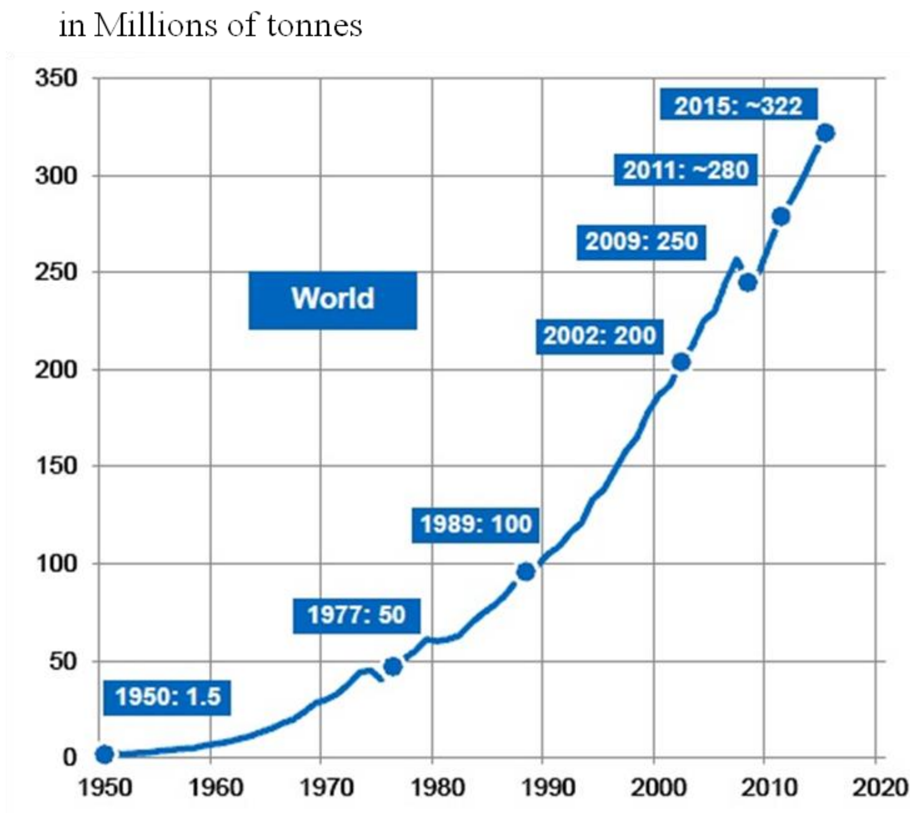


Figure 2.2 Global production of Plastics from 1950 to 2015 (PlasticsEurope, 2015)

(includes Thermoplastics, Polyurethanes, Thermosets, Adhesives, Elastomers, Coatings and Sealants and PP-Fibers but does not include PET-, PA- and Polyacryl - Fibers

Source: PlasticsEuropeMarket Research Group (PEMRG) / ConsulticMarketing & IndustrieberatungGmbH)

2.2.2 Global production of plastics (2006-2015)

Figure 2.3 shows the global production of plastic materials from 2006 to 2015 (PlasticsEurope, 2015). As observed, the Plastic production is shifting towards Asia. This region (Asia) produced more than 49% of the global plastic in 2015, with China alone producing nearly 28% of the world's plastic. The other leading regions are Europe and NAFTA, each account for around 18 to 19% of the global plastic production. Middle East and Africa produces 7% of the global plastic. Latin America (4%), Japan (4%) and CIS (3%) comprise the smallest share of global plastic production.

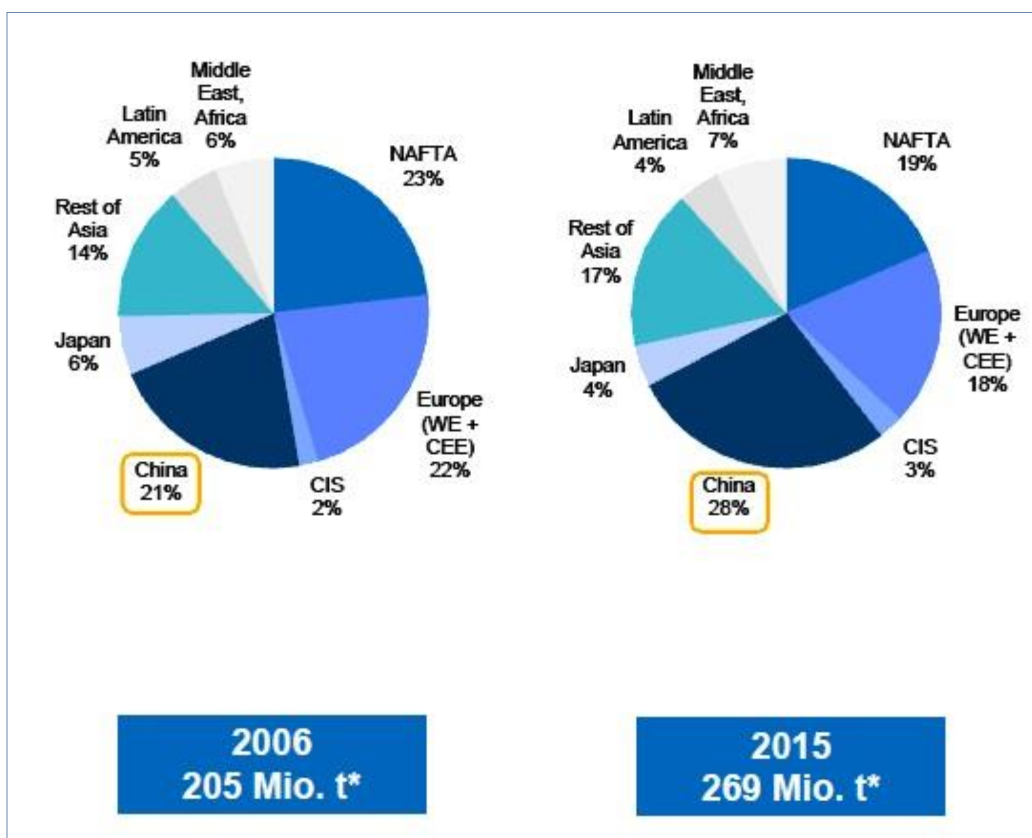


Figure 2.3 Global production of Plastics from 2006 to 2015 (PlasticsEurope, 2015)

(*does not include Thermosets, Elastomers, Adhesives, Coatings and Sealants and PP-Fibers nor included PET-, PA- and Polyacryl-Fibers, 2014 = ~53 million tonnes

Source: PlasticsEuropeMarket Research Group (PEMRG) / ConsulticMarketing & IndustrieberatungGmbH)

2.2.3 Global demand of plastics (in 2015)

Figure 2.4 shows the percentage distribution of plastic materials (2015) in terms of demand (PlasticsEurope, 2015). It was observed that around 55% of the plastics material demand is employed by polyolefins *i.e.,* LDPE, LLDPE, HDPE and PP. Polyethylene (LDPE, LLDPE, HDPE) accounts for approximately 32% and Polypropylene accounts for approximately 23%. Polyvinylchloride (PVC) is the second largest resin after polyolefins which accounts for 16%. Apart this, it was found that the standard plastics (polyolefins, PVC, PS, PET and EPS) account for about 85% of the total demand. Standard plastics are the basic materials. Rest all other plastics like ABS, ASA, SAN, PA, PC etc. are 'Engineering plastics' which are a small but valuable part of the market (approximately 10% of the global plastic demand).

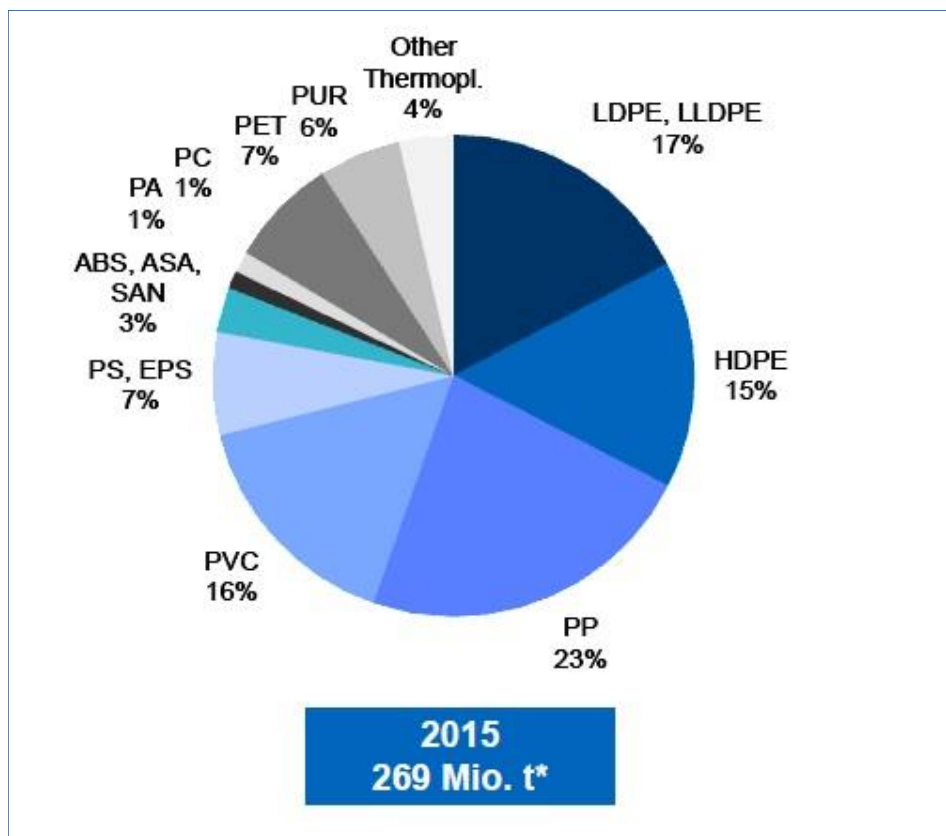


Figure 2.4 Global Demand of Plastics Materials in 2015 by Types (PlasticsEurope, 2015)

(*does not include Thermosets, Elastomers, Adhesives, Coatings and Sealants and PP-Fibers nor included PET-, PA- and Polyacryl-Fibers = ~53 million tonnes

Source: PlasticsEuropeMarket Research Group (PEMRG) / ConsulticMarketing & IndustrieberatungGmbH)

2.3 Biodegradable polymers

Biodegradable polymers are described as the polymers which are capable of undergoing disintegration into carbon dioxide (CO_2), water, methane (CH_4), inorganic compounds and biomass by the enzymatic action of microbes. Biodegradable polymers are compostable too i.e., they can undergo decomposition in a compost site. Initially decomposition may involve abiotic or biotic processes to break the polymer into low-molecular weight compounds. But, the resultant fragments should be fully utilized by microorganisms to avoid any environmental and health consequences (Narayan, 2006a; Narayan, 2006b). Biodegradable polymers can be divided into two types on the basis of their origin: bio-based (renewable resources) and petrochemical-based (non-renewable resources). The bio-based polymers are mainly biodegradable in nature and are formed from natural origins such as plants, animals or microorganisms. It includes (i) polysaccharides (e.g. starch, cellulose, chitin and lignin), (ii) proteins (e.g. casein, wheat gluten, silk, gelatine and wool) and (iii) lipids (e.g. animal fats and plant oils). Some polyesters either produced by microorganisms/plants (e.g. polyhydroxyalkanoates and poly-3-hydroxybutyrate) or manufactured from bio-derived monomers (e.g. polylactic acid) also come under this category. Petrochemical-based biodegradable polymers are synthesized from monomers obtained from petrochemical refining; for example aliphatic polyesters (e.g. polycaprolactone, polybutylene succinate, polyglycolic acid), aromatic copolyesters (e.g. polybutylene succinate terephthalate) as well as poly(vinylalcohol). These polymers possess certain degree of inherent biodegradability (Song et al., 2009).

In general, biodegradable polymers are classified into following three types (Mitrus et al., 2009):

1. Natural polymers: The polymers which are extracted either directly or from natural materials such as plants are called natural polymers. Examples of natural polymers include polysaccharides (like starch, cellulose etc.) and proteins (like casein, wheat gluten etc.).
2. Synthetic polymers: These are the polymers which are produced from renewable resources by chemical synthesis. For example, polylactate produced by polymerization of lactic acid monomers. Lactic acid itself is produced by the fermentation of carbohydrate feedstock.
3. Microbial polymers: These polymers are produced by microbes or genetically transformed bacteria. For example, polyhydroxyalkanoates (PHA) which mainly includes polyhydroxybutyrate (PHB) and copolymers of hydroxybutyrate (HB) and

hydroxyvalerate (HV). This categorization of biodegradable polymers is shown in Figure 2.5.

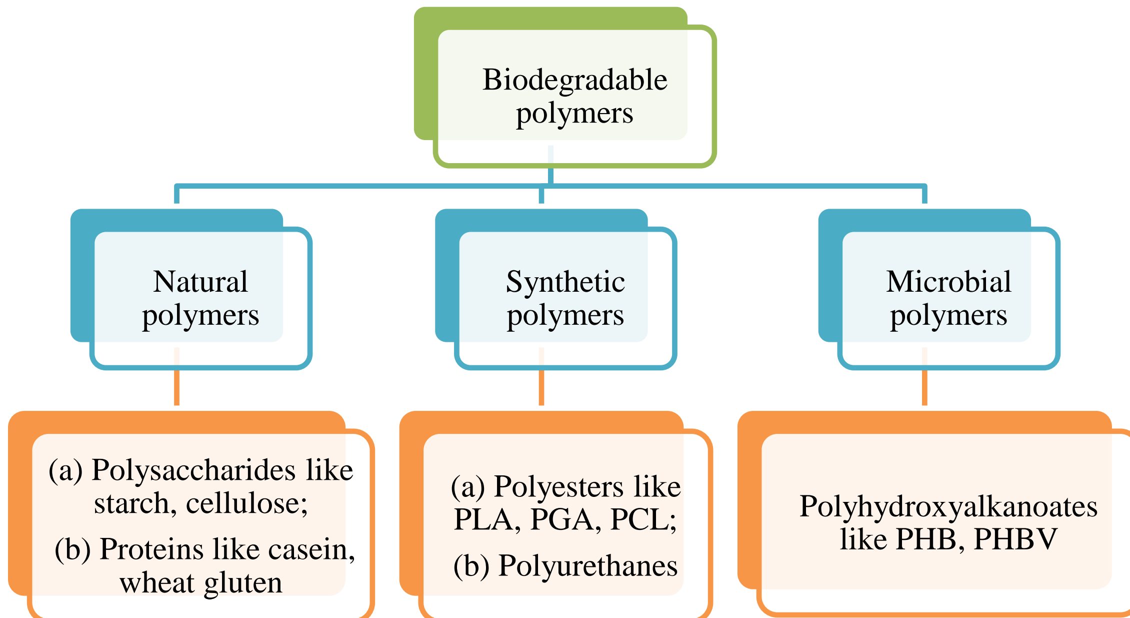


Figure 2.5 The classification of biodegradable polymers

Biodegradable polymers are extensively used in many medical applications like drug delivery, tissue engineering, regenerative medicine, gene therapy, temporary implantable devices, coatings on implants, etc. The basic criteria for choosing a polymer to be used as degradable biomaterial are: (i) the mechanical properties and degradation rate; which should match the needs of an application so that adequate strength remains till the surrounding tissue has been cured, (ii) biocompatibility, (iii) non-toxic degradation products, (iv) shelf life or stability, (v) processability and cost. For drug delivery applications, the time of release governs the type of polymer, shape and size of device. For example, the lactide and glycolide polymers are the clinically approved polymers that can be used in any application (Doppalapudi et al., 2014; Nair and Laurencin, 2007).

Biodegradable plastics are widely used in agriculture section too. The main motive for using biodegradable plastics in agriculture is the rising utilization of plastics in agriculture which allowed the farmers to enhance their crop production. Some of the plastics employed in agriculture are recyclable such as silage films, greenhouse films, and fertiliser sacks, pipes and other plastic products while others are difficult to recycle such as thin mulching films,

thin low tunnel and direct cover films. Such films are very thin and are often contaminated by soil and foreign substances. So, the attractive alternative for non-recyclable plastic waste is biodegradation. Consequently, the utilization of biodegradable plastics is increasing in the agricultural applications. Such applications include chiefly the mulching films as well as plant pots, nets for agriculture and forestry (including animal nets), guide strings or nets for climbing plants, compost bags etc. (Briassoulis and Dejean, 2010a; Briassoulis et al., 2010b).

Though biodegradable polymers can be used in different applications such as packaging, medical and agricultural areas but the commercialization of biodegradable polymers is often hampered due to competition with commodity plastics which are cheap as well as familiar to the customer. Also, the infrastructure needs to be developed for disposal of biodegradable polymers in bioactive environments which will require the capital investments (Gross and Kalra, 2002). Moreover, the biodegradable polymers available today possess inferior physical properties such as strength and dimensional stability and their processing is technically difficult (Luckachan and Pillai, 2011). The general production, biodegradation, biocompatibility and applications of some of the most promising biodegradable polymers used today are described here.

2.3.1 Natural polymers

The polymers which are produced under natural conditions within the cells of the organisms are known as natural polymers. These are produced during the growth cycle of organisms by complex metabolic processes. Starch and cellulose are most interesting one for materials applications. Though, there are other complex hydrocarbon polymers too which are formed by bacteria and fungi; for example, xanthene, pullulan, curdlan, and hyduromic acid (Mitrus et al., 2009).

2.3.1.1 Starch

Starch is considered as the most promising one among all natural biopolymers as it is inexpensive, easily available, biodegradable, and one of the most abundant polysaccharide in nature. It is the main type of stored carbohydrate in plants for example corn, rice, wheat, potatoes etc. (Tang and Alavi, 2011; Pal et al., 2006). Starch is made up of two homopolymers of D-glucose: (i) amylose and (ii) amylopectin. Amylose is a linear polymer consisting of α-D(1→4) linked glucose molecules while amylopectin is highly branched

structure whose backbone is same as of amylose but consist of many α(1→6) linked branching points. Amylopectin contains short side chains of around 30 glucose molecules that are attached after each 20-30 glucose molecules along the chain. The chemical structures of amylose and amylopectin are displayed in Figure 2.6. The ratio of amylose and amylopectin in starch granules depends on the origin of starch. It ranges from 10-20% amylose and 80-90% amylopectin. There are many hydroxyl groups found on the starch chains; (i) two secondary hydroxyl groups are found at C-2 and C-3 of each glucose moiety and (ii) one hydroxyl group is found at C-6 when it is not linked. So, it is evident that starch is hydrophilic in nature. It can be oxidized and reduced and can participate in the formation of hydrogen bonds, ethers and esters. Amylose forms a helical structure with a hydrophobic core as hydrogen groups are exposed in the interior of the helix. Amylopectin provides the bulk to the starch granules and contains the crystalline portion of the granules. The branches of the amylopectin form double helices which are further arranged in crystalline structures. The crystalline regions are supposed to be hard and brittle (Lu et al., 2009; Glenn et al., 2014).

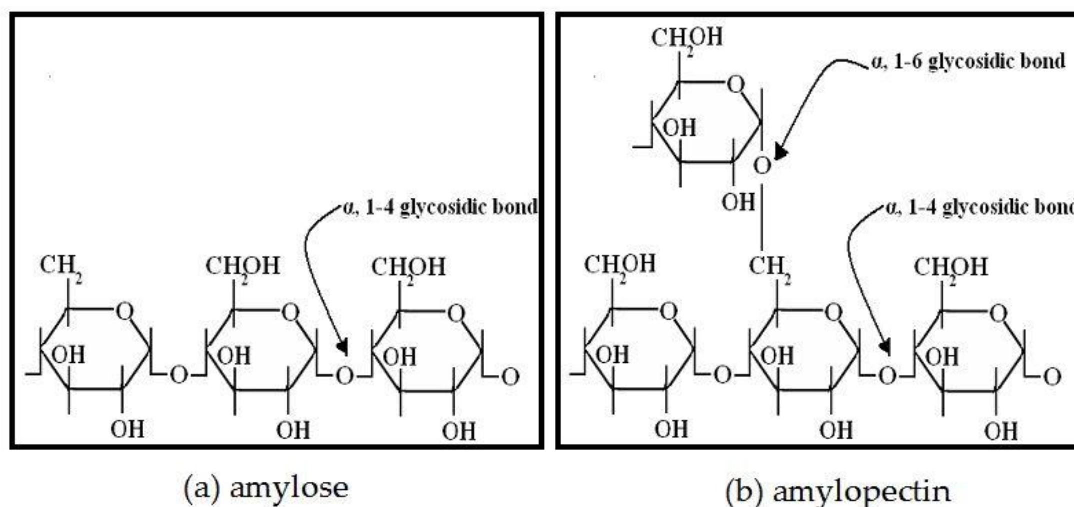


Figure 2.6 The chemical structures of (a) amylose and (b) amylopectin

(Source: http://usmle.biochemistryformedics.com/action-of-alpha-amylase-on-starch/)

The major problems of using starch is their limited processability, poor dimensional stability and also the poor mechanical properties due to which native starch cannot be used directly to produce the blown thin films for packing applications. But it can be used as filler to form degradable blends. The addition of starch to undegradable/non-degradable plastics helps in promoting the products' biodegradability (Ray and Bousmina, 2005; Lu et al., 2009).

Native starch is not a true thermoplastic material but when it is subjected to high temperature in the presence of plasticizers such as water, sorbitol, glycol etc., it readily melts and fluidizes thus enabling its use as extruded or injected materials, similar to conventional synthetic plastics. This process of transformation is termed as gelatinization which leads to the formation of thermoplastic starch (TPS). In this process, the semi-crystalline starch granules get transformed into homogenous materials due to disruption of hydrogen bonds present between the macromolecules under shear and pressure. When plasticizer is added to the starch and blended thoroughly, the new hydrogen bonds are formed between plasticizer and starch synchronously with the destruction of hydrogen bonds present between starch molecules; thus the starch is plasticized. Plasticizers help in increasing the flexibility and processability of starch due to the ability of reducing internal hydrogen bonds between polymer chains; thus increasing molecular space. Various polyols are used as plasticizers such as glycerol, sorbitol, glycol, sugars and ethanolamine, but the main plasticizer used is glycerol. The physical properties of TPS depend on the proportion of plasticizer as well as the chemical nature of plasticizer (Huneault and Li, 2006; Prachayawarakorn et al., 2010).

But, TPS cannot be used in many applications due to poor mechanical properties and sensitivity to humidity. They are mainly used in soluble compostable foams, shape molded parts, expanded trays etc. The TPS products have been commercialized by BIOTEC (Emmerich, Germany) as Bioplast TPS® for foamed trays and boxes application (Tang and Alavi, 2011). The limitations of TPS can be overcome by two approaches, (i) the use of natural fibers to reinforce TPS; for example, Curvelo et al., (2001) reported that reinforcement of glycerol plasticized corn starch with Eucalyptus pulp helped in increasing the tensile strength and modulus. Similarly, corn starch plasticized by urea and formamide was reinforced by using winceyette fibers (Ma et al., 2005) (ii) blending TPS with synthetic polymers (Pierre et al., 1997) but the two components (TPS and synthetic fibers) tend to separate from each other due to incompatibility between their chemical structures (Prachayawarakorn et al., 2010). Some starch-based blends have been commercialized also like Mater-Bi© from Novamont (Italy). These blends are produced by blending starch with non-biodegradable polymers such as polyolefins or with biodegradable polyesters like PLA, PCL etc. (Averous, 2004).

2.3.1.2 Cellulose

Cellulose occurs in plant-based materials such as wood, cotton, hemp etc. and it is the most abundant organic matter on the earth. The content of cellulose in cotton and wood is 90% and 50%, respectively (Huang et al., 2014). It is readily extracted from plant cell walls. It is also synthesized by bacteria, tunicates and algae. It is biodegradable, quite cheap and also edible (Brito et al., 2012). The physical and morphological structure of cellulose in higher plants is found to be complex and heterogeneous. Moreover, cellulose molecules are closely associated with other polysaccharides and lignin in plant cell walls, leading to even more complex morphologies. Because of infusibility and insolubility of cellulose, it is usually converted into its derivatives to make it more easily processed such as cellulose acetate, methyl cellulose etc. But, little attention has been given to develop biodegradable plastics from these materials due to high cost, slow degradation rate, and processes that generate pollutants (Tajeddin et al., 2010; Siro and Plackett, 2010).

Cellulose is a linear polysaccharide, consisting of large number of glucose units with β-1,4-glycosidic linkages. The chemical structure of cellulose is shown in Figure 2.7. The cellulose chains are aggregated into microfibrils via extensive inter- and intra-molecular hydrogen bonding and van der Waals interactions (Endler and Persson, 2011). The microfibril composed of about 24 to 36 hydrogen-bonded chains of β-1,4-glucose. These microfibrils are non-soluble because the glucan chains are aggregated laterally to produce crystalline structure of parallel chains (Somerville et al., 2004). In crystalline cellulose, the consecutive sugars along the chains are rotated by 180 degrees, meaning that the cellobiose (disaccharide) is the repeating unit. Cellulose contains both well-ordered crystalline regions as well as disordered amorphous regions. The complete depolymerisation of cellulose yields only one product *i.e.,* glucose (Horn et al., 2012).

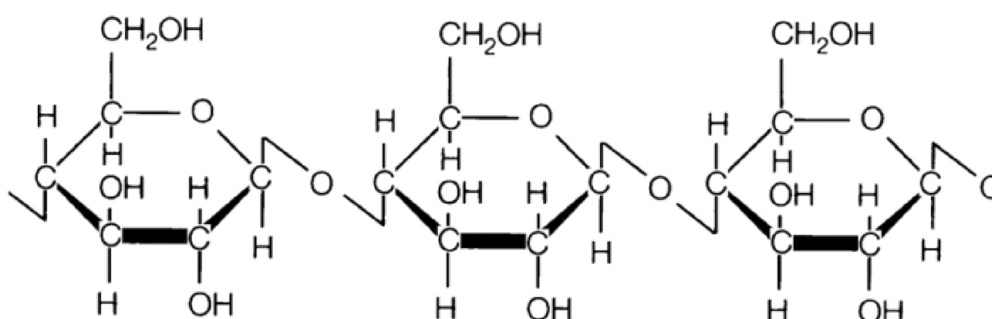


Figure 2.7 The chemical structure of cellulose

Cellulose is mainly hydrolyzed by microorganisms such as bacteria and fungi, which produce either free cellulolytic enzymes (under aerobic conditions) or extracellular enzyme complexes *i.e.,* cellulosomes (under anaerobic conditions). Cellulolytic microorganisms mainly include bacteria from aerobic order *Actinomycetales* (phylum *Actinobacteria*) and anaerobic order *Clostridiales* (phylum *Firmicutes*). The use of cellulose by cellulolytic microorganisms or enzymes for production of biofuels has gained much attention by the biotechnologist (Song et al., 2013; Keshwani and Cheng, 2009; Demain et al., 2005; Lynd et al., 2002). Song et al. (2013) studied degradation of cellulose by a mesophilic strain *Caulobacter* sp. FMC1 under aerobic as well as anaerobic conditions. It was observed that the main fermentative product formed under anaerobic conditions was ethanol.

The degradation of cellulose (cellulolysis) is basically controlled by the enzymes of cellulase system. It comprises of synergistic action of three classes of extracellular enzymes: Endo-1,4-β-glucanases, Exo-1,4-β-glucanases, and β-glucosidases (cellobiase or β-D-glucoside glucohydrolase). Endoglucanases randomly cleave the β-1,4-glycosidic bonds (*i.e.,* internal bonds) along the cellulose chain. Exoglucanases attack the non-reducing end of glucose chain and is responsible for splitting of elementary fibrils from the crystalline cellulose. β-1,4-glucosidase enzyme converts cellobiose (the major product of endo- and exo-glucanases) to glucose. All these enzymes act synergistically to produce glucose from complete hydrolysis of cellulose. The cleavage of glycosidic bonds takes place via hydrolysis *i.e.,* by the addition of water molecule (Gupta et al., 2012; Horn et al., 2012). The cellulase system can be extracted from microbial system found in the gut of organisms which thrive on the cellulosic biomass as their major feed; for example, insects such as termites (*Isopteran*), bookworm (*Lepidoptera*) etc. (Dillon and Dillon, 2004). There are many bacterial and fungal species reported to have cellulosic activities. For example, *Chaetomium, Funsarium Myrothecium, Trichoderma, Penicillium, Aspergillus* etc. are some of the fungal species which can hydrolyze cellulosic biomass. The cellulolytic bacteria include *Trichonympha, Clostridium, Bacteroides succinogenes, Actinomycetes, Butyrivibrio fibrisolvens, Ruminococcus albus,* and *Methanobrevibacter ruminantium* (Milala et al., 2005; Schwarz, 2001). The cellulase enzyme is widely used in many industrial processes like biofuels such as bioethanol, agricultural and plant waste management, triphasic biomethanation, chiral separation and ligand binding studies (Gupta et al., 2012).

2.3.2 Synthetic biodegradable polymers

Fully biodegradable synthetic polymers mainly consist of polymers like polylactic acid (PLA), polyglycolic acid (PGA), polycaprolactone (PCL) etc. These polymers are regarded as biodegradable, biocompatible and highly safe due to which they are widely used in biomedical applications particularly in the areas of controlled drug delivery system and tissue engineering. Due to degradable nature of polymeric implants, there is no requirement of surgical intervention to remove the implant at the end of the functional life (Gliding and Reed, 1979). In tissue engineering applications, synthetic polymers are mainly used in scaffolds which provide suitable mechanical support and show favourable surface properties like adhesion, proliferation and differentiation of cells (Asghari et al., 2016). The synthesis, applications and degradation of some of the important synthetic polymers is discussed here.

2.3.2.1 Polylactic acid

Polylactic acid (PLA) is linear aliphatic polyester produced from lactic acid (2-hydroxy propionic acid, $C_3H_6O_3$). Lactic acid itself is obtained by either fermentation of carbohydrate crops (like corn, sugar beets, tapioca roots, wheat, barley and sugarcane) or chemical synthesis (Datta and Henry, 2006). The fermentation process is more preferable than synthetic route as synthetic route is unable to produce only the desirable L-isomer, as well as its high manufacturing costs whereas fermentation process produces the L-isomer in high purity (99.5%). In general, PLA is produced from pure L-isomer. Lactic acid has two enantiomers- L(+) and D(-) which are produced by bacterial fermentation of carbohydrates. The bacteria used in fermentation process belong to *Lactobacillus* genus like *Lactobacillus delbrueckii, L. bulgaricus, L. amylophilus,* and *L. leichmanii* (Jamshidian et al., 2010). The chemical structure of PLA is shown in Figure 2.8.

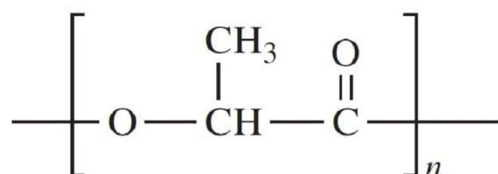


Figure 2.8 The chemical structure of Polylactic acid

PLA is principally produced via three different processes: condensation polymerization of lactic acid (LA), condensation reaction in an azeotropic solution, and ring opening

polymerization of an intermediate called lactide. The 1st method (polycondensation) involves the esterification of monomers in presence of suitable solvents and water (byproduct) is removed azeotropically under reduced pressure (vacuum) and high temperature. Tin (II) chloride is the most commonly used catalyst in this method and it can be recovered at the end of the reaction. This method is the least expensive route but it cannot produce solvent-free high molecular weight PLA having superior mechanical properties. The 2nd method involves the condensation reaction of lactic acid in an azeotropic solution. This process yields high molecular weight PLA without the utilization of chain extenders or adjuvants. The 3rd method (ring opening polymerization process) is the most frequently used procedure to produce high molecular weight PLA. It involves three steps: (i) condensation of lactic acid monomers to give low-molecular weight PLA, (ii) depolymerisation of the PLA into the lactide (cyclic dimmer of lactic acid), and (iii) the ring opening polymerization of lactide unit in presence of metal catalysts resulting in PLA with a high molecular weight (Hamad et al., 2015).

The production of high molecular weight PLA is industrially accomplished by polymerization through lactide formation. Lactide is a cyclic dimer of lactic acid produced by removing water in mild conditions and without the use of solvent. There are three types of products of L-lactic acid and D-lactic acid: L-lactide, D-lactide and meso (L,D) lactide (Jamshidian et al., 2010). On this basis, PLA also exists in three stereoforms: PLLA, PDLA and PDLLA. Out of three, PLLA and PDLA are semicrystalline polymers that show high tensile strength and low elongation whereas PDLLA is more amorphous polymer and show random distribution of both the isomers (Tokiwa and Calabia, 2006). The ROP of L-lactide unit can be carried out in melt or solution by stannous octoate ($SnOct_2$) as the initiator, which avoids racemization at high temperature and trans-esterification. PLA is less toxic and accepted by the US Food and Drug Administration (Puaux et al., 2007). The process of PLA production by ROP using $SnOct_2$ as an initiator is shown in Figure 2.9.

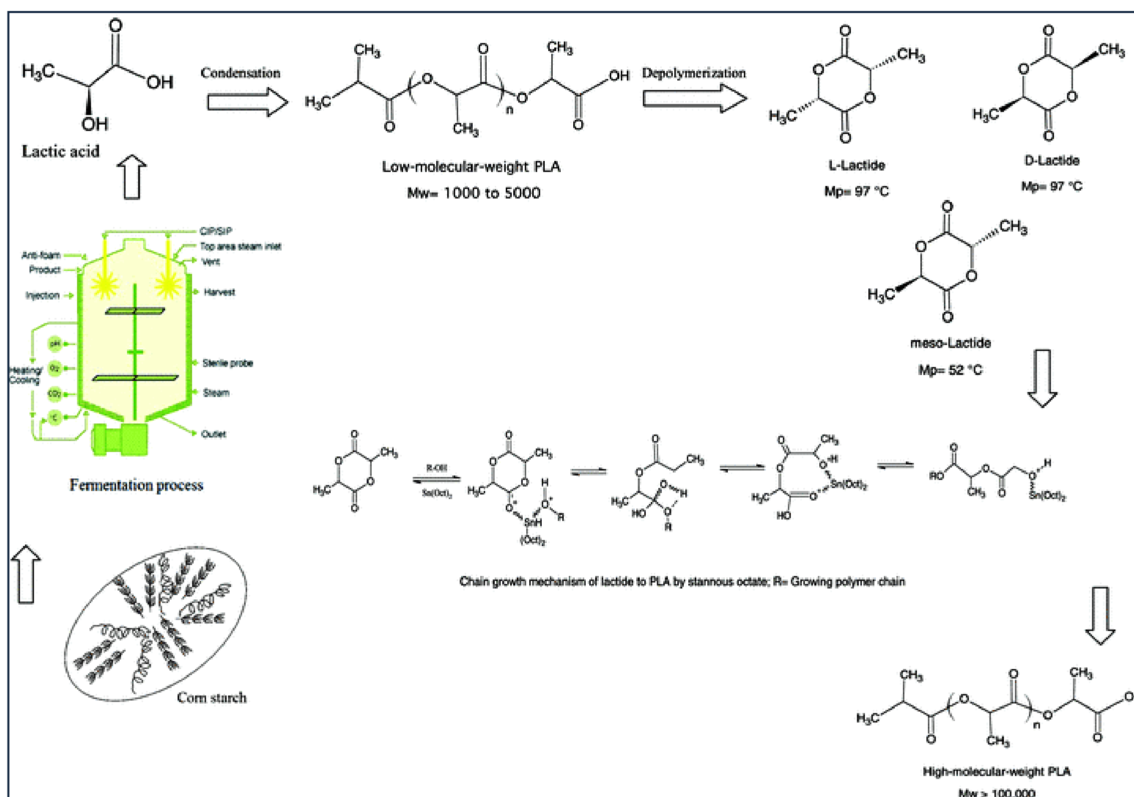


Figure 2.9 The current scheme of PLA production (Jamshidian et al., 2010)

The synthesis of PLA from lactic acid was initiated in 1932 by Carothers. They created low-molecular weight PLA via heating lactic acid under vacuum (Holten, 1971; Lunt, 1998). Latter, Dupont developed the high-molecular weight PLA and the procedure was patented in 1954 (Hamad et al., 2015; Lowe, 1954). In 1972, Ethicon produced the copolymer of PLA and PGA for the application of bioabsorbable surgical suture. At present, NatureWorks® LLC is the leading company in polylactic acid technology. It developed a solvent-free, low-cost continuous method for the synthesis of PLA from corn starch (Dechy-Cabaret et al., 2004). The process begins from the fermentation of corn starch to yield L-lactic acid; L-lactic acid condenses to produce lactic acid oligomers, and subsequently catalytic depolymerization takes place under reduced pressure to yield pure lactic acid containing minimum 95% of L-LA. The resultant L-LA is polymerized by $Sn(Oct)_2$ to produce high molecular weight PLA (Raquez et al., 2013). There are other major companies too which produce PLA for different applications such as PURAC Biomaterials (The Netherlands), Mitsui Toatsu (Japan), Shimadzu (Japan), LACTRON (Kanebo Goshen, Japan), SOLANYL (Rodenburg Biopolymers, Holland), GALACTIC (Belgium), Reliance Life Sciences etc. (Nampoothiri et al., 2010). According to resources, it is found that plastics made from Cargill Dow's corn-

resin pellets can break down in an industrial compost facility within 45 days as shown in Figure 2.10 (Katsnelson, 2005).

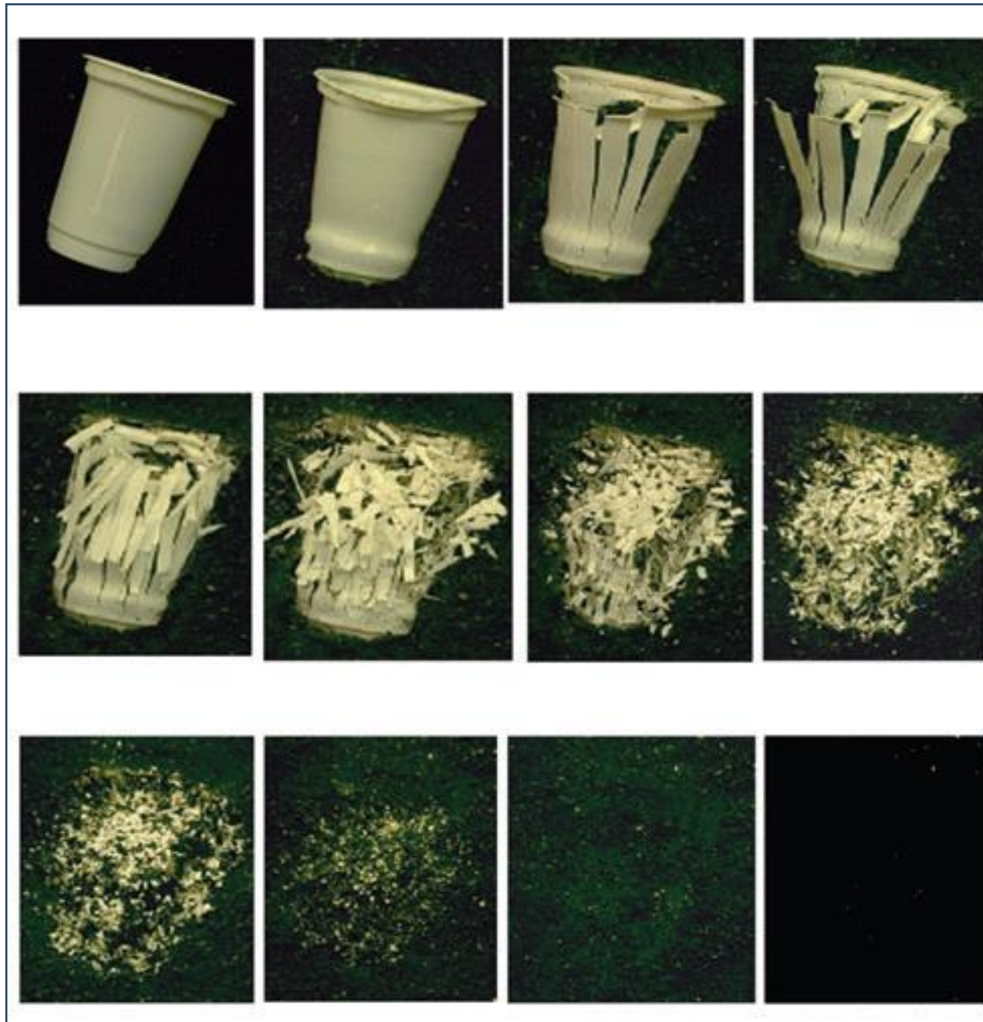


Figure 2.10 The degradation of Cargill Dow's bioplastic in compost facility within 45 days (Katsnelson, 2005)

Polylactic acid has vast range of applications. Currently, it is the most widely used material by the food packaging industry in disposable cutlery, plates, cups, lids etc. However, it cannot be used in flexible films due to poor ductility as well as poor thermal and barrier properties (Arrieta et al., 2014). PLA is extensively used in medical applications too due to its unique characteristics like biodegradability, biocompatibility, eco-friendliness and thermoplastic processability. Moreover, PLA works very well and offers outstanding properties at low price. It is used for preparing various devices like degradable sutures, nanoparticles, drug releasing microparticles etc. The use of biodegradable polymers over the non-degradable polymers in medical applications has an advantage that it eliminates the need

to remove implants. They remain temporarily in the body and disappear on degradation (Lasprilla et al., 2012). The physical properties of PLA (like transparency or mechanical properties) are comparable to polystyrene and poly(ethylene terepthalate) but due to high cost (compared to PP, PE, PS etc.), brittleness, low viscosity, medium gas barrier properties, high moisture sensitivity; its use becomes restricted for various applications. So, the efforts are being made to improve the properties of PLA by blending. Some investigations are found on blends of PLA with other polymers like poly(ε-caprolactone), poly(hydroxyl butyrate), poly(ethylene glycol), poly(hexamethylene succinate) etc. But the blends produced were immiscible and resulted in poor mechanical properties (Hamad et al., 2011a; Omura et al., 2006; Ren et al., 2006).

2.3.2.2 Polycaprolactone

Polycaprolactone (PCL) is synthetic linear polyester which is derived from crude oil. It is manufactured by ring opening polymerization of ε-caprolactone (Khatiwala et al., 2008). The molecular structure of PCL is given in Figure 2.11. It is a hydrophobic, semi-crystalline and biodegradable polymer, with a low melting point of 60°C and glass transition temperature of -60°C. It is resistant to water, oil, solvent and chlorine. The number average molecular weight of PCL ranges from 3000 - 90,000 g/mol. With increase in molecular weight, the crystallinity of PCL tends to decrease. It is fully biodegradable under composting conditions and mainly used in the biomedical field. It acts as a stiffening material for shoes and orthopaedic splints as well as for completely biodegradable compostable bags, fibers and sutures. It is also used in thermoplastic polyurethanes, adhesives, resins etc. (Mohamed and Yusoh, 2016; Kunioka et al., 2007). PCL is also used in tissue engineering (Sarasam et al., 2006).

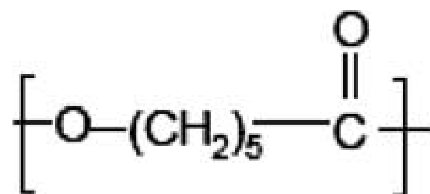


Figure 2.11 The chemical structure of Polycaprolactone (Ray and Bousmina, 2005)

PCL is readily biodegradable in diverse environments like marine water, soil, sewage sludge, and compost ecosystems; hence it is widely used in drug delivery systems. The biodegradation of PCL occurs through either enzymes or simple hydrolysis or both. But, the

hydrolytic degradation is very slow due to hydrophobicity and crystallinity of PCL. There are several parameters which influence the biodegradation of PCL like molecular weight, crystallinity, thickness of films, and degradation parameters. The microorganisms secrete extracellular depolymerises which degrade the polymer and utilize it as a source of carbon (Khatiwala et al., 2008; Liu et al., 2000).

The enzymatic degradation of polycaprolactone has been studied mainly in the presence lipase enzymes which help in accelerating the biodegradation of PCL (Liu et al., 2000) for example *Rhizopus delemer* lipase (Fukuzaki et al., 1990), *Rhizopus arrhizus* lipase (Mochizuki et al., 1995), and *Pseudomonas* lipase (Gan et al., 1997; Gan et al., 1999).

Some studies have been performed on biodegradation of PCL (Khatiwala et al., 2008). Chen et al. (2000) observed that the enzyme lipase could accelerate the degradation of polycaprolactone microparticles and the degradation rate of PCL is not greatly influenced by the surface area of PCL. Murphy et al. (1996) revealed that the depolymerise enzyme produced by *Fusarium moniliforme* was cutinase. Oda et al. (1995) isolated five fungal strains having capability to degrade two polymers: PHB and PCL. One of the fungal strains was identified as *Paecilomyces lilacinus.* The degradation of polycaprolactone has also been studied by the bacteria *Alcaligenes faecalis* (Oda et al., 1997; Khatiwala et al., 2008). Abdel-Motaal et al. (2014) found that *Pseudomonas japonica*-Y7-09 (yeast) produced the extracellular cutinase enzyme which degraded PCL by 93.33% in 15 days. The mechanism of biodegradation of PCL has also been studied in detail by some researchers. It is believed that PCL depolymerases preferentially attack the amorphous areas of polymer and degradation occurs due to endo- and exo- cleavage (Khatiwala et al., 2008).

The various physico-chemical and mechanical properties of polycaprolactone can be altered by either co-polymerization or by blending with other polymers efficiently. The co-polymerization helps in altering the chemical property of PCL which further affect many properties like crystallinity and solubility which results in a modified polymer having desired attributes for drug delivery. On the other hand, blending helps in changing the physical properties and biodegradation along with mechanical properties leading to polymers preferable for tissue engineering. PCL has been found to compatible with natural polymers (starch, hydroxyl apatite, chitosan) as well as synthetic polymers such as polyurethane (PU), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyethylene oxide (PEO), polylactic

acid and polylactic co-glycolic acid (PLGA). These modifications are useful in formulations for drug delivery (Azimi et al., 2014).

2.3.2.3 Polyglycolic acid

It is the simplest linear and aliphatic polyester (Figure 2.12) which is produced by polycondensation reaction between glycol and aliphatic dicarboxylic acids. The constituents are derived from renewable resources such as glycol obtained from glycerol and organic acids are obtained via fermentation. PGA is a soft and biodegradable material; possess good touch and high melting point (approximately 200°C). It has excellent material properties like aromatic PET. Commercial production of PGA is carried out by Dupont either in the form of aliphatic-aromatic copolymer under (Biomax®) or as aramid fibers (Kevlar®) (Flieger et al., 2003).

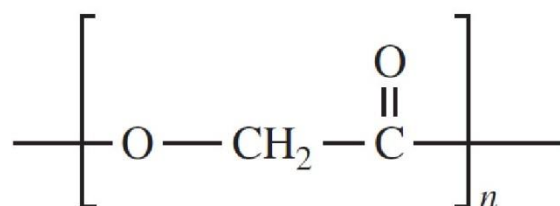


Figure 2.12 The chemical structure of Polyglycolic acid

Both PGA as well as its copolymer poly(glycolic acid-co-lactic acid) are widely used in medical applications for degradable and absorbable sutures. They can easily degrade in the aqueous surroundings like body fluids via hydrolysis of the ester backbone. Furthermore, the breakdown products of PGA get metabolized to CO_2 and water (Mitrus et al., 2009; Fields et al., 1974).

2.3.3 Microbial polymers/ Polyhydroxyalkanoates (PHA)

Polyhydroxyalkanoates (PHA) are biodegradable biopolyesters which are completely synthesized by microorganisms like bacteria and fungi and some plants too. There are many bacteria which can synthesize PHA like those found in activated sludge or high seas or extreme environments. More than 30% of soil-inhabiting bacteria are capable of synthesizing PHA (Chen, 2010). Some examples of PHA-producing bacteria are *Alcaligenes latus*, *Pseudomonas oleovorans*, *Azotobacter vinelandii*, recombinant *Alcaligenes eutrophus* and recombinant *Escherichia coli* (Salehizadeh and Loosdrecht, 2004). Microorganisms like

Ralstonia eutropha and recombinant *Escherichia coli* are capable of accumulating PHA as much as 90% (w/w) of their dry cell mass in a nutrient-limited media *i.e.,* media deprived of essential nutrients like nitrogen or phosphorus or oxygen but carbon should be present in excess. The most general limitation is observed with nitrogen (*Azotobacter spp.*) but the most efficient limitation is oxygen. PHA is insoluble in water due to which it accumulates as carbon or energy source within the intracellular granules (Chen, 2010; Verlinden et al., 2007).

Poly(3-hydroxybutyrate) (PHB) was the first PHA to be discovered in the bacterium *Bacillus megaterium* by a French Scientist, Maurice Lemoigne in the year 1926. PHB is the most common and well studied polymer of the polyhydroxyalkanoates family. He reported that this bacterium could accumulate PHB intracellularly and it is a homopolymer made up of 3-hydroxybutyric (3HB) acid molecules. The molecules are joined together by ester bonds formed between 3-hydroxyl group of one monomer and carboxylic group of other monomer. Since then, many other bacteria have been identified to accumulate PHB in their cells both aerobically and anaerobically. But PHB possess poor physical properties. It is too stiff and brittle to be used in most commodity products. Later on, PHA containing other monomers apart from 3-hydroxybutyric acid (3HB) were discovered in activated sludge such as 3-hydroxyvalerate (3HV). Afterwards in the mid-1980s, Imperial Chemical Industries (ICI) produced a copolymer *i.e.* poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) by the incorporation of propionate to the fermentation broth. The incorporation of just a few percent of 3HV units into the polymer helped in improving the flexibility and also reduced brittleness. Since then, many companies such as ICI and later on, Zeneca and Monsanto, have started production of PHBV on an industrial scale (Poirier, 1999; Zinn et al., 2001; Chee et al., 2010; Keshavarz and Roy, 2010). So far, the most commercial production of PHAs is from Tianan Biologic Material Co. in Ningbo, China. Their capacity of production has increased to 900 million tons per year. The commercial PHA produced by Tianan contains about 5% valerate, although some developmental grades contain upto 15% valerate. Valerate improves the flexibility of the polymer. The other companies to produce PHAs are Metabolix (Cambridge, MA) (Glenn et al., 2014). The chemical structures of some members of PHAs are given in Figure 2.13.

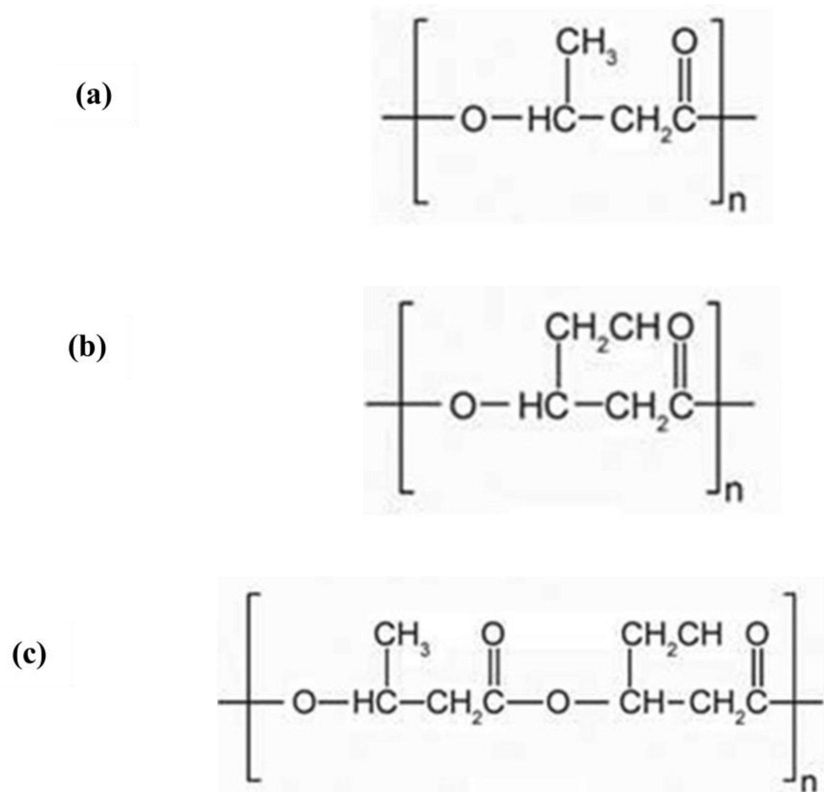


Figure 2.13 The chemical structures of various polyhydroxyalkanoates; (a) PHB, (b) PHV, (c) PHBV (Bugnicourt et al., 2014)

Till now, more than 150 kinds of monomers have been discovered forming PHAs like 3-hydroxybutyrate (3HB), 3-hydroxyvalerate (3HV), 3-hydroxyhexanoate (3HHx), 3-hydroxyoctanoate (3HO), 3-hydroxydecanoate (3HD) and 3-hydroxydodecanoate (3HDD) as well as 4-hydroxybutyrate (4HB). These monomers are used to form homopolymers, block copolymers or random polymers. In this way, materials with diverse properties can be made (Wang et al., 2014). PHAs are mainly categorized into two types: (i) short-chain-length PHAs (scl) consisting of monomer units with only 3-5 carbon atoms. These polymers are produced by bacteria like *Alcaligenes latus* and *Cupriavidus necator*; (ii) medium-chain-length PHAs (mcl) that consist of monomer units with 6-14 carbon atoms. These are accumulated by bacteria like *Pseudomonas putida* and *Aeromonas caviae.* PHA copolymers also exist with scl-copolymers for example, P(3HB-co-4HB) and P(3HB-co-3HV); mcl-copolymers for example, P(3HHx-xo-3HO); and scl-mcl-copolymers like P(3HB-co-3HHx) (Leong et al., 2014).

PHAs are thermoplastic and/or elastomeric, biocompatible, non-toxic, water-insoluble, inert, indefinitely stable in air, enantiomerically pure, optically active *i.e.* possess only the R-

configuration, piezoelectric *i.e.,* helps in wound healing and also stimulates the bone growth. They show better resistance to UV degradation than polypropylene (PP) but are less solvent resistant. The most important characteristic is that they are completely biodegradable. Due to these properties, PHAs are widely used in biomedical applications like orthopaedy (screws, bone graft substitutes, scaffolds for cartilage engineering), cardiovascular system devices (scaffolds for regeneration of arterial tissues, vascular grafts, cardiovascular stents, heart valves etc.), wound management (sutures, dressings, dusting powders), urological stents, controlled drug-delivery (tablets, micro-carriers, implants) and others. Like PVC and PET, PHAs also exhibit good barrier properties so they are used in areas of packaging also such as shampoo bottles, cosmetic containers, milk cartons and films, cover for cardboard and papers, pens, combs, bullets, moisture barrier in nappies and sanitary towels etc. It would help in solving the problems of environmental pollution caused by non-degradable synthetic polymers (Leong et al., 2014; Laycock et al., 2014, Bugnicourt et al., 2014; Keshavarz and Roy, 2010).

There are some drawbacks of using these polymers like (i) the cost of producing PHAs is very high as compared to conventional petroleum-based plastics; (ii) the processing of PHA is more difficult than conventional petroleum-based plastics due to their slow crystallization process (iii) their mechanical and thermal characteristics are not consistent as compared to petrochemical plastics (iv) they are further required to be developed for wider range of applications of PHA and for large-scale production (v) the quality and uniformity of PHA need to be optimized. So, further investigations are required to reduce the production cost and to increase their industrial sustainability and commercialization (Wang et al., 2014; Leong et al., 2014, Bugnicourt et al., 2014).

The two polyesters namely PLA and PHA have their own advantages and disadvantages. Typically, polylactic acid (PLA) is cheaper whereas PHA is more expensive than PLA. Therefore, the application research of PLA is ahead of PHA’s (Chen, 2009).

2.4 Non-degradable synthetic polymers

Since 1940s, the synthetic polymers have become technologically significant and they are widely used in packaging industry. Packaging industry has been mainly revolutionized by the oil-based polymers like polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET) and polyvinyl chloride (PVC). There are major advances in the manufacturing, synthesis and processing of these materials but still this industry faces two major problems: (i) non-renewable, oil-based chemicals are used for manufacturing of commodity polymers, (ii) the eventual fate of the waste materials (Amass et al., 1998). Generally, the packaging materials account for about 25% of all the plastics. They have substituted the paper as well as cellulose-based products in packaging due to superior physical and chemical properties. Also, the cost of producing these materials is lesser and the energy efficiency is more than paper packaging. Currently, the synthetic polymers are widely utilized in food processing industry for packaging of milk, fruits and vegetable products. Packaging materials are usually non-toxic but after disposal, causes significant impact on environment as litter and also contribute to landfill costs. So, the approach of waste minimisation and waste management is required (Jayasekara et al., 2005). Synthetic polymers find their uses in the biomedical field too. For example, medical supplies like syringes, vials, catheters, blood transfusion bags, dialysers for blood purification, etc. Synthetic polymers are highly durable, hydrophobic, and resistant to weathering, photo-degradation, and biological attack (Alariqi et al., 2006).

The common mechanism of biodegradation of polyolefins involves assimilation from the "ends" of substrate molecules. As industrial polyolefins possess high molecular weight so, few ends are available on or near the surface of polymers (Wiles and Scott, 2006). Polyolefins or saturated polymers are produced from the cheap petrochemical feed stocks via efficient catalytic polymerisation process. It includes polyethylene and polypropylene which are the most extensively used linear hydrocarbon polymers and are expressed as C_nH_{2n}. They are used in various applications which include food packaging, lab equipments, textiles, and automotive components (Arutchelvi et al., 2008). Several studies have been done on the biodegradation of PE, PP and PS in natural environments which suggested that some soil microorganisms like bacteria or fungi have metabolic capability to digest these polymers as source of carbon and energy required for their growth (Muenmee et al., 2015). The

manufacturing, biodegradation, biocompatibility and applications of non-degradable polymers (mainly PE and PP) are discussed here.

2.4.1 Polyethylene

Polyethylene is the most widespread synthetic polymer worldwide. It is a linear hydrocarbon polymer which is made up of long chains of ethylene (C_2H_4) monomers. The general formula of PE is C_nH_{2n}, where 'n' defines the no. of carbon atoms. It is widely used in large amount for the production of bottles, carry bags, garbage containers, disposable articles, water pipes and milk jugs. Among the synthetic plastic waste generated, PE constitutes about 64%. Similarly, in the marine water only, it constitutes about 60-80% of total marine waste. The whole PE waste along with other plastic waste goes into the marine water via rivers, canals and municipal drainages. Consequently, the beaches are reported to be the reservoirs for polyethylene waste. A small portion of PE waste is recycled; rest enters into the landfills where it takes hundreds of years for degradation (Sangale et al., 2012). PE is of different types: (i) Low Density Polyethylene (LDPE), (ii) Linear Low Density Polyethylene (LLDPE), (iii) High Density Polyethylene (HDPE) and (iv) Cross Linked Polyethylene (XLPE).

PE, mainly as thin films, is extensively used in packaging applications due to its outstanding mechanical properties, less cost, barrier properties against water borne pathogens etc. But, the feature of recalcitrance towards microorganisms, which made PE a popular option for packaging purposes, has nowadays turn into a matter of much criticism. Most of the PE films after use, ends-up in landfill sites, where it remains as such for thousands of years due to non-degradability. PE is resistant towards biological attack due to various factors like hydrophobicity, high molecular weight and lack of functional groups identifiable by microorganisms. To enhance the biodegradation of polyethylene, carbonyl groups can be directly incorporated in the main chain or pro-oxidants such as UV activators can be introduced at the time of processing (Roy et al., 2008).

The degradation of polyethylene can be categorized into two types based on the factors responsible for degradation *i.e.,* abiotic and biotic. Abiotic degradation occurs due to environmental factors like UV irradiation, temperature etc. whereas biotic degradation occurs due to the action of microorganisms which modify and consume the polymer resulting in changes in its properties. Although the damage caused to polyethylene is classified into two

types, but in nature both factors act cooperatively (Restrepo-Florez et al., 2014; Hakkarainen and Albertsson, 2004). There are number of research studies published on biodegradation of polyethylene, which mention that the process of PE degradation under normal conditions is very slow (Arutchelvi et al., 2008).

The biodegradation of polyethylene is a complex phenomenon and is not fully understood. However, to elucidate the mechanism of PE degradation, two approaches are found in the literature. The first approach includes the use of pure strains to degrade PE. It is a convenient way to examine the metabolic pathways or elucidate the effect of different environmental conditions. But it has a disadvantage that it ignores the possibility of degradation due to cooperative process between the different species. To overcome these limitations, there is a second approach found, in which the complex environments or microbial communities are used for degradation. For example, the use of soil, compost or marine water comes under the second approach. Over the past 50 years, many microorganisms have been identified for their ability to colonize polyethylene or degrade it or both. The list includes bacteria as well as fungi which is as follows (Restrepo-Florez et al., 2014).

The bacterial strains reported to degrade polyethylene are *Acinetobacter Baumannii, Arthrobacter viscosus, Bacillus amyloliquefaciens, Bacillus thuringiensis, Micrococcus luteus, Micrococcus lylae, Paenibacillus macerans*, *Pseudomonas fluorescens, Rahnella aquatilis, Staphylococcus cohnii, Staphylococcus xylosus* (Nowak et al., 2011), *Arthrobacter* spp. (Satlewal et al., 2008; Balasubramanian et al., 2010), *Arthrobacter paraffineus* (Albertsson et al., 1995; Albertsson et al., 1998), *Bacillus brevis* (Watanabe et al., 2009), *Bacillus cereus* (Roy et al., 2008; Satlewal et al., 2008; Sudhakar et al., 2008; Nowak et al., 2011), *Bacillus circulans* (Watanabe et al., 2009), *Bacillus halodenitrificans* (Roy et al., 2008), *Bacillus mycoides* (Seneviratne et al., 2006; Nowak et al., 2011), *Bacillus pumilus* (Roy et al., 2008; Satlewal et al., 2008; Nowak et al., 2011), *Bacillus sphericus* (Kawai et al., 2004; Sudhakar et al., 2008), *Brevibacillus borstelensis* (Hadad et al., 2005), *Delftia acidovorans*, *Flavobacterium spp, Ralstonia spp, Rhodococcus erythropolis, Stenotrophomonas spp* (Koutny et al., 2009), *Microbacterium paraoxydans* (Rajandas et al., 2012), *Nocardia asteroides* (Bonhomme et al., 2003; Koutny et al., 2006b), *Pseudomonas spp* (Balasubramanian et al., 2010; Yoon et al., 2012; Tribedi and Sil, 2013), *Pseudomonas aeruginosa* (Koutny et al., 2009; Rajandas et al., 2012), *Rhodococcus ruber* (Gilan et al., 2004; Sivan et al., 2006; Santo et al., 2013), *Rhodococcus rhodochrous* (Bonhomme et al.,

2003; Koutny et al., 2006b; Fontanella et al., 2010), *Staphylococcus epidermidis* (Chatterjee et al., 2010), *Streptomyces badius*, *Streptomyces setonii*, *Streptomyces viridosporus* (Pometto et al., 1992).

The different fungal strains reported to biodegrade polyethylene are *Acremonium kiliense* (Karlsson et al., 1988), *Aspergillus niger* (Raghavan and Torma, 1992; Volke-Sepulveda et al., 2002; Manzur et al., 2004), *Aspergillus versicolor* (Karlsson et al., 1988; Pramila and Ramesh, 2011b), *Aspergillus flavus* (Koutny et al., 2006b; Pramila and Ramesh, 2011a), *Chaetomium spp* (Sowmya et al., 2012), *Cladosporium cladosporioides* (Bonhomme et al., 2003; Koutny et al., 2006b), *Fusarium redolens* (Albertsson, 1980; Karlsson et al., 1988; Albertsson and Karlsson, 1990), *Glioclodium virens* (Manzur et al., 2004), *Mortierella alpina* (Koutny et al., 2006b), *Mucor circinelloides* (Pramila and Ramesh, 2011a), *Penicillum simplicissimum* (Yamada-Onodera et al., 2001), *Penicillum pinophilum* (Volke-Sepulveda et al., 2002; Manzur et al., 2004), *Penicillum frequentans* (Seneviratne et al., 2006), *Phanerochaete chrysosporium* (Manzur et al., 1997; Orhan and Büyükgüngör, 2000; Manzur et al., 2004), *Verticillium lecanii* (Karlsson et al., 1988).

Some of the studies are described here. Albertsson and Karlsson, (1990) studied the biodegradation of UV irradiated (for 26 days) and non-irradiated PE films in soil. It was observed that less than 0.5% carbon by weight (as CO_2) was released by the UV-irradiated sample after 10 years of soil burial whereas less than 0.2% CO_2 was evolved from non-irradiated samples.

Yamada-Onodera et al., (2001) isolated a fungus *Penicillium simplicissimum,* YK from soil which was able to utilize polyethylene as the sole carbon source. The strain was further used for degradation of UV irradiated (for 500 hours) and non-irradiated polyethylene in synthetic media for 3 months. The degradation was monitored by high-temperature gel permeation chromatography to study the molecular weight distribution of polyethylene and FTIR analysis for detection of functional groups in PE. It was observed that irradiated PE undergoes more degradation than the non-irradiated samples due to generation of carbonyl groups in PE after irradiation and there was a minimal decrease in molecular weight too.

Volke-Sepulveda et al., (2002) studied the biodegradation of thermo-oxidised LDPE with *Aspergillus niger* and *Penicillium pinophilum* strains. The samples were preheated at 80°C for 15 days and then incubated with fungi, in presence/absence of ethanol, at 30°C for 31

months in liquid media. The degradation was monitored by GC analysis (for determination of CO_2 and O_2), FTIR, XRD, DSC and SEM techniques. *P. pinophilum* showed higher degradation efficiency than *A. niger*. The mineralization rate obtained were 0.64% and 0.37% for *P. pinophilum* whereas 0.50% and 0.57% for *A. niger* in case of samples with and without ethanol, respectively. Moreover, there was decrease in oxidation (without ethanol), increase in oxidation (with ethanol), increase in double bond index, decrease in crystallinity and increase in small crystal content as well as the mean crystallite size indicating that ethanol favours the biodegradation of thermo-oxidised LDPE.

Gilan et al., (2004) isolated a bacteria *Rhodococcus ruber* (C208) from soil through two-step enrichment procedure that could utilize the PE films as sole carbon source. The strain was further tested for biodegradation of UV irradiated (for 60 h) PE films in minimal media. It was observed that bacteria were able to make biofilm on the polymer surface and reduced the weight of PE up to 8% (gravimetrically) in 30 days. The addition of mineral oil to media further enhanced the biodegradation of polyethylene by approximately 50% after 4 weeks.

Hadad et al., (2005) isolated a thermophillic bacterial strain *Brevibaccillus borstelensis* 707 from soil that could utilize LDPE as the only source of carbon. The strain was further used for degrading photo-oxidized PE films. The films were UV irradiated for 60 h and then subjected to biodegradation in synthetic media for 30 days at 50°C. It was found that the gravimetric and molecular weight of LDPE reduced by 11 and 30%, respectively. FTIR analysis showed reduction in carbonyl groups after degradation with bacteria. Moreover, the strain was also capable of degrading LDPE in presence of another carbon source i.e., mannitol.

Sivan et al., (2006) further examined the biofilm growth kinetics of *Rhodococcus ruber* (C208) on the surface of LDPE. It was observed that the polymer lost about 7.5% of its weight in 8 weeks and the degradation rate was 0.86% per week in basal media. Moreover, the strain was able to produce dense biofilm on the polymer surface and the ratio of population of biofilm with those of planktonic cells was 60:1 after 10 days of incubation. The determination of extracellular polymeric substances showed that the amount of polysaccharides was 2.5 folds higher than the proteins.

Roy et al., (2008) studied the biodegradation of PE containing pro-oxidant (cobalt stearate) using consortium having three bacteria namely, *Bacillus cereus, Bacillus pumilus* and

Bacillus halodenitrificans. The films were UV-irradiated (λ_{max} at 313 nm) and subsequently incubated with the bacteria. The degradation was monitored by FTIR, mechanical properties, GC-MS, DSC, TGA, SEM, melt flow index, weight loss, and cfu count. It was observed that there was decrease in carbonyl index (by FTIR analysis), formation of low molecular weight compounds (by GC-MS studies), increase in initial decomposition temperature (TGA), formation of biofilm on the polymer surface (by SEM analysis), weight loss of polymer by 8.4% and increase in the bacterial count (by cfu count).

Satlewal et al., (2008) studied the relative biodegradation of HDPE and LDPE polymers by the indigenously developed consortium. The consortium was made up of bacterial strains isolated from soil and identified as *Bacillus cereus, Bacillus pumilus* and *Arthrobacter* sp. The biodegradation studies of HDPE/LDPE using consortium were carried out in minimal broth for 2 weeks and monitored by FTIR as well as thermogravimetric-differential thermogravimetry-differential thermal analysis. From TG analysis, the weight loss of pure HDPE and LDPE samples was observed as 2.5% and 4.5%, respectively at 400°C while the weight loss of HDPE and LDPE, after treatment with consortium increased to 22.4% and 21.7%, respectively.

Fontanella et al., (2010) studied the biodegradation of HDPE, LDPE and LLDPE films containing pro-oxidants (Mn^{2+}, Fe^{3+}, Co^{2+}) with a bacterial strain of *Rhodococcus rhodochrous* in mineral medium. The films were UV treated ($\lambda \geq 300$ nm) for 10-40 h and then thermally treated at 60°C for 192-1224 h. The abiotically treated samples were subjected to biodegradation with *Rhodococcus rhodochrous* in mineral media for 180 days. The degradation was monitored by FTIR, SEC, NMR, SEM, ATP and ADP assays. It was concluded that HDPE film show less oxidation than the other two types of PE; whatever may be the pro-oxidant. The biodegradation of PE depended more on the nature of pro-oxidant and lesser on the type of matrix. Cobalt at high concentration was toxic for the strain and inhibited growth of bacterial cells.

Yoon et al., (2012) isolated a mesophilic bacterium *Pseudomonas* sp. E4 from a beach soil that could utilize low-molecular-weight polyethylene (LMWPE) as the only carbon source. The biodegradation of LMWPE was carried out in compost inoculated with the isolated strain at 37°C for 80 days. The results showed that 4.9-28.6% of the carbon in the test sample was mineralized into CO_2 and the biodegradability decreased with increase in molecular weight of LMWPE. Moreover, alkane hydroxylase gene (*alk*B) from the isolated strain was amplified

and expressed in *E. coli* BL21 to test whether the recombinant bacterial cells could mineralize LMWPE. It was observed that recombinant cells were as active as of *Pseudomonas* sp., and mineralized 19.3% of the carbon into CO_2 indicating that the *alk*B gene played a central role in degradation of LMWPE.

Santo et al., (2013) studied the role of extracellular laccase, a copper binding enzyme, in the degradation of PE. The enzyme was excreted by the actinomycete *Rhodococcus ruber* C208, previously isolated by Gilan et al., (2004). From the results of RT-PCR, it was found that laccase mRNA levels increased up to 13 fold after the addition of copper and thus, increased the biodegradation of LDPE by 75% as compared to non-amended samples indicating that copper had great effect on induction and activity of laccase enzyme. The average molecular weight of polymer was decreased by 20% and average molecular number was decreased by 15% after incubation with laccase enzyme collected from copper-induced cells. The study demonstrated that enzyme laccase has a vital role in biodegradation of PE.

Sowmya et al., (2015) isolated few fungal strains from soil capable of using PE as the solitary source of carbon which were identified as *Penicillium simplicissimum, Alternaria alternata, Curvularia lunata,* and *Fusarium* sp. The degradation of surface sterilized polyethylene by pure cultures and consortium was conducted in mineral salt medium for 3 months and it was assesssed by weight loss, SEM and FTIR analysis. The weight loss shown by *P. simplicissimum, C. lunata, A. alternata, Fusarium* sp. and consortium were 7.7%, 1.2%, 0.8%, 0.7% and 27%, respectively indicating that the microbial consortium degraded the polyethylene better than the individual microorganisms. The polyethylene degrading enzymes were also screened and they were identified as laccase and manganese peroxidase.

2.4.2 Polypropylene

Polypropylene is the second-most utilized polymer after PE. It possesses one methyl group extra as compared to PE on every other carbon, due to which it exists in three stereoisomeric types - atactic, isotactic, and syndiotactic. PP was first manufactured by Zieglar and Natta using propylene as the monomer. Mettalocene catalysts are also be utilized for its production (Arutchelvi et al., 2008). It is light in weight, resistant to chemicals, acids, solvents etc., have flexibility. According to a report by Plastemart.com, 2015; the consumption of PP worldwide in 2007 was 45 million tons which is expected to rise to 85 million tons by 2017. The manufacturing capacities in China and India alone will be expanded by 7.5 million tons till

2017. The figures show that the enormous plastic waste will get accumulated in the environment after their utilization and disposal (Arkatkar et al., 2010). PP is a very popular polymer for synthesis of medical disposables due to less cost, good transparency, high mechanical strength, and chemical resistance over other polymers. But, it is badly affected by γ-radiations resulting in oxidative degradation when sterilised in air. The addition of small amount of ethylene in PP makes it stable to γ-radiations (Alariqi et al., 2006).

Polypropylene is widely used for packaging purposes too but the biodegradation of PP has received less attention. The first report on biodegradation of PP is found by Cacciari et al., (1993). The consortium named as 'community 3S' was used to degrade isotactic polypropylene in a mineral medium supplemented with sodium lactate and glucose for 5 months. The consortium consisted of three isolates, *Pseudomonas chlororaphis*, *P. stutzeri* and *Vibrio* sp. It was found that the concentration of dichloromethane crude extracts increased with incubation time whereas the weight of sample (PP) decreased. Through spectral analyses, the extract was found to be a combination of hydrocarbons ($C_{10}H_{22}$ to $C_{31}H_{64}$).

However, majority of the studies deal with the biodegradation of pretreated polypropylene. Arkatkar et al., (2009a) pretreated the polypropylene films at high temperature (at 80°C for 10 days) and found that thermally pretreated polypropylene films undergo better degradation than unpretreated films when subjected to biodegradation with mixed soil culture in minimal medium for twelve months. During this time, the weight loss of 10.7% and 0.4% was found in thermally treated PP and untreated PP, respectively. Moreover, the tensile strength of pretreated PP and untreated PP was reduced by 51.8% and 28.3%, while the crystallinity increased (determined using DSC) by 28% and 33%; and the isotacticity also increased (determined through FTIR) by 3% and 9%, respectively. After 1 year of degradation experiment, one culture was also isolated from the thermally pretreated sample which was identified as *Bacillus flexus*. Arkatkar et al., (2010) pretreated the polypropylene films using short UV (at 225 nm for 6 days), thermally (at 100°C for 8 days), Aquaregia reagent (for 3 days) and Fenton's reagent (for 7 days). Afterwards, unpretreated and pretreated films were subjected to biodegradation in minimal media for 12 months with four bacterial species, namely *Pseudomonas azotoformans, Pseudomonas stutzeri, Bacillus subtilis* and *Bacillus flexus.* The maximum weight loss of about 2.5% after one year was found in case of short UV treated PP treated with *Bacillus flexus*. Moreover, *P. azotoformans* and *B. subtilis* were able

to produce higher amount of carbohydrate and protein than the other two organisms. The surface energy of all the polymers increased after 12 months showing that the surface became hydrophilic. Fontanella et al., (2013) reported the biodegradation of PP films containing pro-oxidants based on Mn/Fe, Mn and Co. The polypropylene films were abiotically pretreated by photooxidation (for 20-45 hours) and then thermooxidation (at 60°C for 336-2712 h). Afterwards, the films were biodegraded with a bacterial strain *Rhodococcus rhodochorus* in mineral media for 180 days. The metabolic activity of bacterial cells was determined by estimating ATP content and ADP/ATP ratio while the analysis was carried out by FTIR, SEC and NMR (Nuclear magnetic resonance) spectroscopy. It was concluded that Co derivatives were toxic for the strain while Mn and Mn/Fe gave positive results. Moreover, the biodegradability of oxidised polypropylene films was observed to be less efficient than oxidised polyethylene films.

Previously, polypropylene has been blended with other polymers too such as starch, cellulose, poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV), poly-3-hydroxybutyrate (PHB) etc. so as to improve the biodegradability of PP. Contat-Rodrigo et al., (2001) reported the biodegradation study of PP blended with additive ‘Bioefect’ which is made up of starch and other compounds that enhance the biodegradation. The polymer blends of PP/starch were subjected to outdoor soil burial experiment for 21 months. By the means of thermogravimetric analysis (TGA), it was observed that additive in the blend was getting more affected by the degradation process in soil than the polymeric (PP) matrix. Ramis et al. (2004) also studied the biodegradation of PP/starch blends by soil burial method for 1 year. TGA was employed to determine the thermal stability of blends before and after degradation in soil. It was concluded that biodegradation mainly occurs in starch of the additive and there was no effect on polypropylene matrix. Morancho et al., (2006) studied the biodegradation of photo-oxidised samples of PP/starch blends in soil. Photo-oxidation was carried out for a period of 98.7 hours followed by biodegradation in soil for a period of one year. The crystallinity and thermal stability of polymers were assessed using DSC and TGA, respectively. Due to photo-oxidation, the crystallinity of samples decreased indicating for the generation of free radicals that favour chain-elongation reactions whereas due to degradation in soil, the crystallinity increased showing the breakdown of polymer chains in the amorphous region of starch. Moreover, the thermal stability of starch in the blends was increased after burial in soil showing that biodegradation affected the starch units but did not affect the PP whereas the photo-oxidation process reduced the overall stability of the blend.

Kaczmarek et al., (2005) studied the degradation PP/cellulose blends. The samples were pre-irradiated (at 254 nm wavelength for 10 hours) and subsequently composted in garden soil for 6 months under laboratory conditions. The analysis of degradation was carried out using FTIR analysis, SEM technique and mechanical properties. The efficiency of photo-oxidation and biodegradation was higher in blends as compared to pure components but the incorporation of cellulose to PP caused the significant reduction in mechanical strength as compared to polypropylene alone. Sadi et al., (2013) studied the biodegradation of photo-degraded samples of PP/PHB blends in compost. The samples were exposed to UV light (at 340 nm) for 4 weeks and then studied for biodegradation in compost by Bartha respirometric tests. The analysis of degradation was carried out using SEM, SEC, and FTIR technique. It was observed that prior photo-degradation enhanced the biodegradation of PP/PHB blends due to formation of hydroxyl and carbonyl groups as well as reduction in molecular weight. Masood et al., (2014) studied biodegradation of PP/PHBV blends. The degradation of blends was carried out in mineral salt media (MSM) as well as in garden soil. In MSM, the degradation was carried out in flasks incubated at 30°C under shaking conditions (at 150 rpm) for four weeks using a consortium made up of *Bacillus cereus* FC11, *Bacillus cereus* FB11, and *Bacillus cereus* S10. On the other hand, the degradation of PP/PHBV blends in fields was carried out by soil burial test from April to July. The analysis of degradation was carried out using SEM, FTIR and TGA techniques. It was found that pits, grooves, agglomerates and holes formed on the polymer surface (using SEM technique); carbonyl peak index increased (by FTIR technique), and thermal stability of blends increased (by TGA technique) after the soil burial test. This shows that the degradation of polypropylene was enhanced by the incorporation of PHBV into it.

There are some studies reported on PP/PLLA blends describing their various properties but less attention has been given to biodegradability studies of PP/PLLA blends. Reddy et al., (2008) developed the blends of PLA/PP to create fibres and characterized them through mechanical properties, SEM, XRD and DSC techniques. The blends showed partial compatibility between PLA and PP; and their mechanical properties were inferior to the pure polymers. But blends showed better resistance to hydrolysis and biodegradation as well as better dyeabilty than pure PLA. Nishida et al., (2009) prepared the blends of PLLA and PP with and without catalyst MgO; and characterized them through SEC, NMR, FTIR, SEM and TGA techniques. From TGA analysis, it was found that the addition of MgO in the blend selectively accelerated the depolymerization of PLLA component in the blend leading to

generation of L,L-lactide as a main volatile product. Hamad et al., (2011b) blended PLA with PP in different ratios and studied their rheological and mechanical properties. The rheological results of blends revealed that the true viscosity was between that of pure polymers while the flow activation energy was less than that of the pure polymers. The mechanical tests showed that there is incompatibility between the two polymers. Choudhary et al., (2011) blended PLA with PP at various ratios with and without compatibilizers i.e., maleic anhydride grafted PP (MAPP) and glycidyl methacrylate. The blends were characterized by mechanical tests, DSC, TGA, FTIR and SEM techniques. The results revealed that blend of PLA/PP in ratio of 90:10 had optimum mechanical properties which led to improved melt processability of PLA. The study depicted that PP forms an immiscible blend with PLLA due to high polarity difference between them. The interaction between these two polymers can be improved by the addition of suitable compatibilizer like MAPP. It is an effective compatibilizer which mediates the polarity at the interface of two polymers. The PP part of MAPP is compatible with PP while the anhydride part reacts with PLLA; thus a favourable interface is formed as shown in Figure 2.14.

In our study, PP was blended with PLLA [Poly(L-lactide)] to develop partial biodegradable polymers. Both the polymers have same processing temperatures which make it suitable for blend preparation. The plan of present work was to investigate the biodegradation of the PP/PLLA blends with and without compatibilizer (MAPP), and its individual constituents in the presence of bacterial isolates, using standard test methods such as ASTM D5338. To our knowledge a very few studies have been made on the biodegradability of such blends. Biodegradation studies were carried out in synthetic media as well as in soil. To monitor and describe polymers' degradation; FTIR, SEM and TGA techniques were employed.

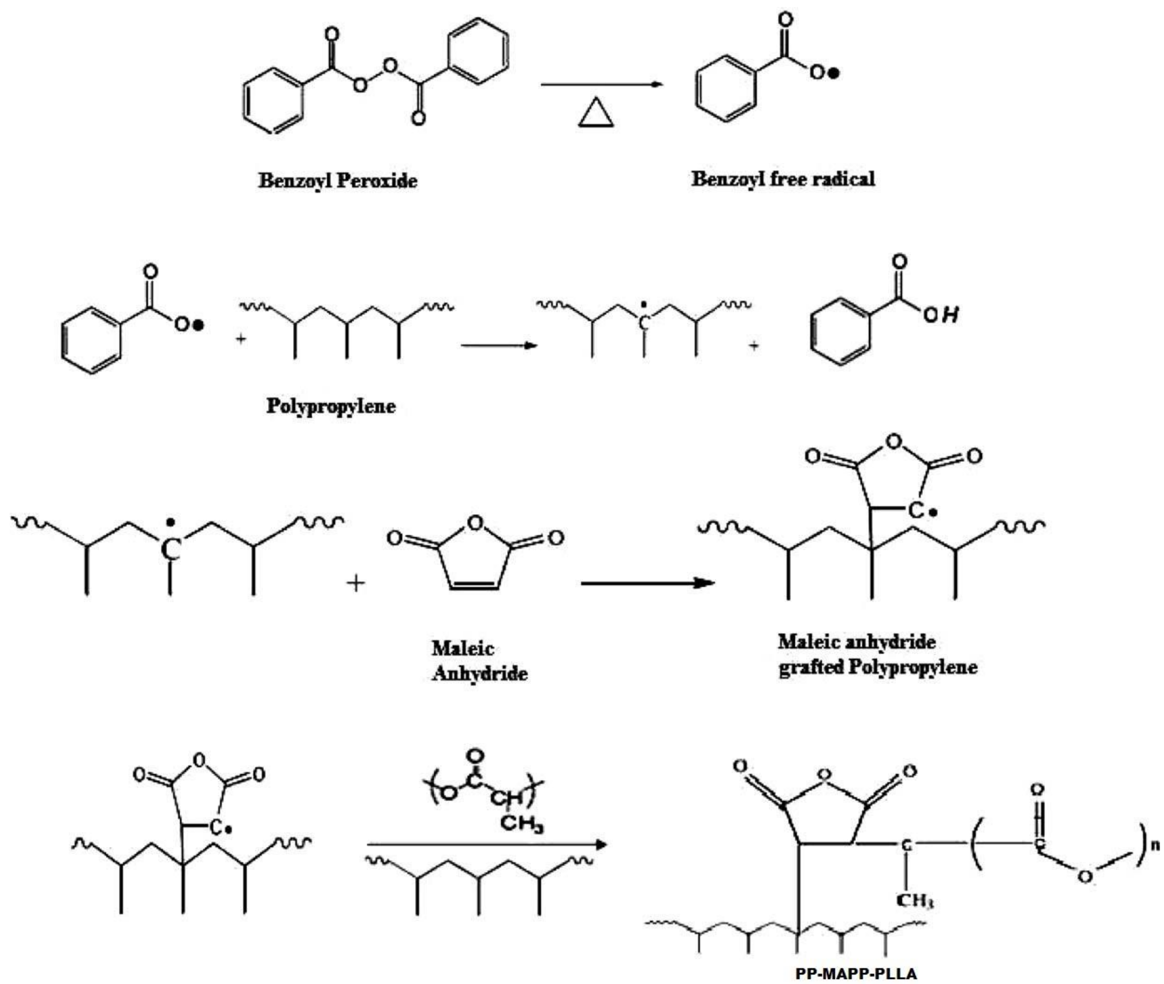


Figure 2.14 Schematic route of the PP-MAPP-PLLA blend (Choudhary et al., 2011)

Chapter 3

Materials and Methods

3.1 Development of degradable polypropylene blends

The overall scheme for development of degradable polypropylene blends is given in Figure 3.1. Firstly, the commercial grade polymers i.e., Polypropylene (PP), Poly-L-Lactide (PLLA) and maleic anhydride grafted polypropylene (MAPP) were procured to produce degradable blends. Afterwards, the polymers (PP and PLLA) were mixed in desired ratios with and without compatibilizer (MAPP) to prepare the blends by using melt-blending technique. Thereafter, all the blends were tested for their mechanical properties to select two blends for degradation studies.

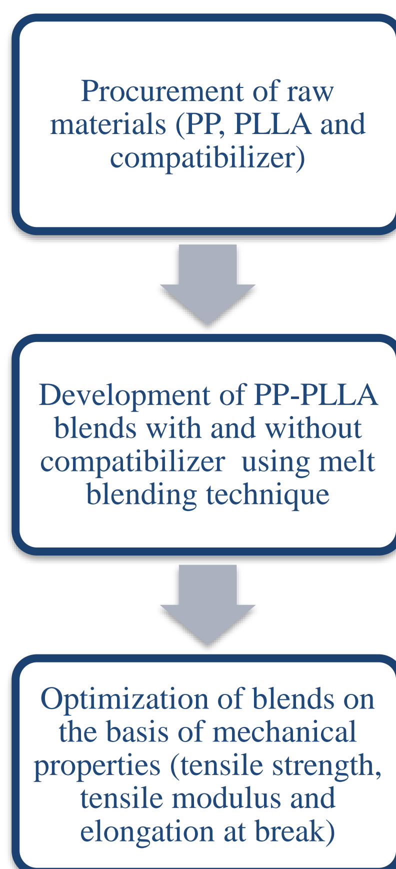


Figure 3.1 Schematic diagram for development of degradable polypropylene blends

3.1.1 Materials

The different types of polymers used in this study were – polypropylene (PP), poly-L-lactide (PLLA) and maleic-anhydride grafted polypropylene (MAPP, compatibilizer). They were of commercial grade; whose sources and properties are mentioned in Table 3.1.

Table 3.1 Trade names, sources and characteristics of polymers and compatibilizer used

Polymers	**Trade name**	**Source**	**Density (g/cc)**	**MFI (g/10 min)**	**T_m (°C)**
PP	Halene-P, F103	Haldia Petrochemicals Ltd., Kolkata, India (www.haldiapetrochemicals.com)	0.90	3.3	152
PLLA	Biomer L9000	Biomer Forst-Kasten-Str Kailling, Germany (http://www.biomer.de)	1.25	3.0	190
MAPP (MA content = 0.5-0.8%)	Optim P406	Pluss Polymers Pvt., Ltd., Gurgaon, India (http://www.pluss.co.in/)	0.91	40	163

Polypropylene (PP) (Trade name Halene-P F103, Density 0.90 g/cc, MFI 3.3 g/10 minutes, melting point i.e., T_m 152°C) was supplied by Haldia Petrochemicals Limited, India. The company produces this polymer by the latest generation Spheripol II Technology. The polymer produced by them is mainly appropriate for Monolayer or Coextruded Biaxially Oriented Polypropylene (BOPP) films. They possess outstanding gloss, clarity, mechanical strength, high impact and puncture resistance and barrier to moisture, fats, aroma, and oils. The material produced from F103 is also appropriate for Food Contact application.

The second polymer, Poly-L-lactide (PLLA) (Trade name Biomer L9000, Density 1.25 g/cc, MFI 3.0 g/10 minutes, melting point i.e., T_m 190°C) was procured from Biomer Forst-Kasten-Str Kailling, Germany. It is commercially produced by polymerization of naturally occurring L-lactate. It is thermoplastic polyester of 2-hydroxy lactate (lactic acid). The formula of the subunit is: -[O-CH(CH_3)- CO]-. PLLA is transparent and its surfaces are suitable for optical purposes. It is waterproof and can be processed as classic thermoplastics. Chemically they are alpha hydroxyl esters. These are prone to hydrolysis (degradation).

Hydrolysis occurs fast in humid conditions at temperatures above the glass transition temperature i.e., 50 - 60°C (c.f. biodegradation in composts). However hydrolysis also occurs at room temperature, although at very low rates.

The compatibilizer, maleic-anhydride grafted polypropylene (MAPP) (Trade name Optim P-406, Density 0.91 g/cc, MFI 40 g/10 minutes, melting point i.e., T_m 163°C) was obtained from Pluss Polymers Pvt., Ltd, Gurgaon, India. It contains 0.5 - 0.8% of maleic anhydride. It is a grafted polypropylene with anhydride and acid functionality and is white to light yellow colored free flowing granules/ pellets. It acts as wetting and dispersing aid for colors and pigments. In case of extruded and moulded products, it facilitates printability, paintability and receptivity to adhesives due to polar functionality. It offers enhanced interfacial adhesion too between the filler and polymer matrix.

3.1.2 Development of PP-PLLA blends

The blends of PP-PLLA in different ratios, with and without compatibilizer (MAPP), were developed by melt blending technique. The equipment used for preparing PP-PLLA blends was Brabender Plasticorder (Model PLE-651) fitted with cam type counter rotors. Prior to melt blending, the ingredients i.e., PP, PLLA and compatibilizer were mixed manually in the desired compositions as shown in Table 3.2. The melt mixing was conducted at 180°C with a rotor speed of 60 rpm for 4 min. The fill factor was 0.8. Then, the molten mass was passed through a two-roll mill (Schwabentan, Berlin, Germany) with a nip gap of 2 mm while the rolls were maintained at room temperature.

Afterwards, the blends were molded into thin films in a compression moulding hydraulic press (George E. Moore Press, UK) at 180°C and 3 MPa for 2 min to obtain films of 50 ± 10 µm thickness. While molding, Teflon sheets were placed in between the mold plates so as to prevent the molten mass sticking to it. The overall dimensional stability of the samples was maintained by cooling the moldings under pressure by circulating cold water through the platens. Neat PP (PP100) and neat PLLA (PLLA100) films were also prepared in a similar fashion as reference stocks.

The different ratios of polymer blends of PP/PLLA were 95/5, 90/10, 85/15, 80/20, 70/30, 60/40 and 50/50 (by weight, Table 3.2). To assess the effects of MAPP on the physico-

chemical properties of blends, the contents of the compatibilizer was varied as 2, 4, 6 and 8 phr (parts per hundred of resin) in 80/20 ratio of PP/PLLA.

Table 3.2 Composition of PP-PLLA Blends

Sr. No.	Sample	PP (wt %)	PLLA (wt %)	MAPP (phr)
1	PP100	100	0	0
2	PP95	95	5	0
3	PP90	90	10	0
4	PP85	85	15	0
5	PP80	80	20	0
6	PP70	70	30	0
7	PP60	60	40	0
8	PP50	50	50	0
9	PLLA100	0	100	0
10	PP80C2	80	20	2
11	PP80C4	80	20	4
12	PP80C6	80	20	6
13	PP80C8	80	20	8

3.1.3 Mechanical properties

Mechanical or physical properties of polymers such as tensile strength at yield, elongation at break, and elastic modulus were determined according to ASTM D 882-91 standard on a Zwick universal testing machine (model Zwick Roell Z010, Germany) with a clamp separation of 100 mm. The crosshead speed was kept at 50 mm/min, and the test was conducted at room temperature. Three specimens were tested for each composition, and the average values have been reported.

3.2 Effect of blend composition and compatibilizer content on the physico-chemical properties of blends

The blends selected on the basis of mechanical properties were further characterized by various techniques to determine the effect of blend composition as well as compatibilizer content on the physical and chemical properties of blends. The overall scheme is given in Figure 3.2. The physical properties of blends were determined by X-ray diifraction as well as Thermogravimetric analysis; chemical properties were assessed by Fourier Transform Infrared Spectroscopy; and surface properties were assessed by Scanning electron microscopy.

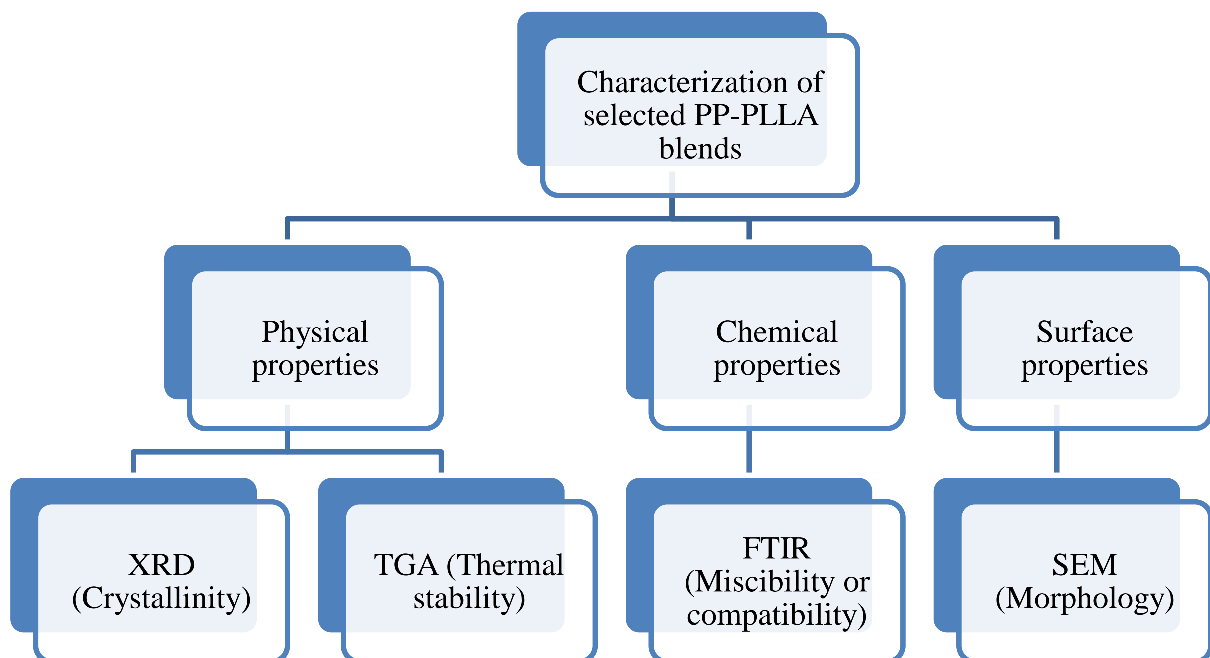


Figure 3.2 Schematic diagram for studying effect of blend composition and compatibilizer content on the physico-chemical properties of blends

3.2.1 X-ray diffraction (XRD)

XRD is a standard method to examine the crystalline structures. It also provides the important information about polymers such as degree of crystallinity. XRD spectra for film samples were obtained at room temperature using a Philips X'Pert diffractometer (Almelo, The Netherlands) with monochromatic CuKα radiation of wavelength (λ) = 1.5418 Å operating at 45 kV and current 40 mA. The scanning speed and diffraction angles (2θ) were kept at 5°/min and 5-60°, respectively. The percentage of crystallinity (χ) and crystallite size (*P*) were also measured using the XRD spectra according to Equations (1) and (2), respectively as follows (Maji et al., 2010):

$$Xc = \frac{Ic}{Ic+Ia} * 100 \text{.......Equation (1)}$$

where *Ic* is integrated intensity of crystalline phase and *Ia* is integrated intensity corresponding to amorphous phase.

$$P = \frac{k\lambda}{\beta \cos\theta} \text{..........Equation (2), also known as } \textit{Scherer's equation}$$

where λ is the wavelength of the X-ray radiation (1.54 A°), β is half width (in radian) of the crystalline peak and k is the Scherer constant having value of 0.9.

3.2.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR technique is used to measure changes occurring in the spectral intensities of the polymer that correlates to the formation and disappearance of functional groups within the polymer films. The interactions among the PP, PLLA and compatibilizer were also studied. Film samples were scanned using FTIR spectrophotometer (Cary 600 Series, Agilent Technologies, USA) in attenuated total reflectance (ATR) mode from 400 to 4000 cm^{-1}. A total of five scans per sample were accumulated at a resolution of 4 cm^{-1}. The spectra were analyzed using Agilent Resolution Pro software.

3.2.3 Scanning electron microscopy (SEM)

The morphology of polymer surface was examined by using Scanning electron microscope (JSM-6510LV, JEOL, Japan). The specimens were coated with gold (50 µm thin film) in an

automatic sputter coater (JFC1600) to avoid charging of samples under the electron beam. The instrument was operated at 10 kV. The surface view of the polymer films were taken at a magnification of 500x to 5000x.

3.2.4 Thermogravimetric analysis (TGA)

The thermal stability of the polymers was assessed by thermogravimetric analyzer (Q-500, TA Instruments, USA). This method or analysis is used to measure the changes occurred in weight of a material at definite temperatures and time intervals. The weight reduction rate of a polymer under definite conditions is inversely proportional to the size of polymer.

In the present study, samples weighing 10±2 mg were heated from 30°C to 600°C at a heating rate of 10°C/min under dry nitrogen and weight loss of the sample was measured. The flow rate of nitrogen was maintained at 50 ml/min. The initiation degradation temperature (T_i) and final degradation temperature (T_f) were also recorded using the TGA data. T_i corresponds to 1% weight loss in the polymer sample and T_f corresponds to 1% residue left after which no considerable loss is achievable.

3.3 Abiotic and biotic degradation studies

The selected PP-PLLA blends were further subjected to degradation studies by abiotic as well as biotic factors and the overall scheme is shown in Figure 3.3. For abiotic degradation, the blends were subjected to thermal, UV and chemical pretreatments followed by biodegradation with consortium of bacterial isolates. For biodegradation of blends, initially the PP degrading bacteria were isolated from compost and then, the bacterial isolates were tested for their efficiency to degrade blends in synthetic media as well as in soil.

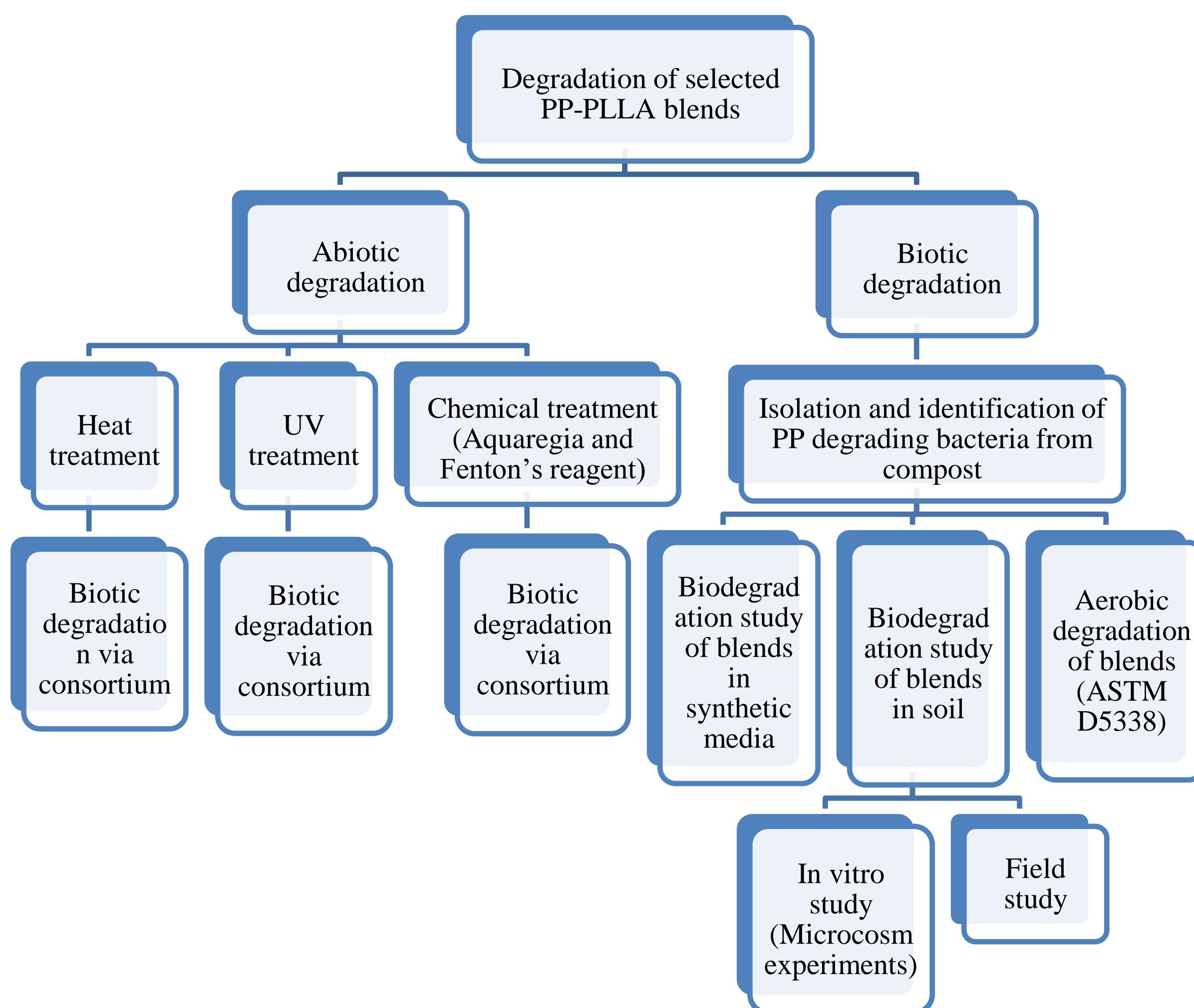


Figure 3.3 Schematic diagram for abiotic and biotic degradation studies

3.3.1 Isolation and identification of polypropylene degrading bacteria

3.3.1.1 Compost

Compost was collected from municipal solid waste, Okhla, New Delhi. They were crushed and sieved by 2 mm size mesh to remove larger inert particles. The samples were pooled together to make homogeneous sample and were characterized for their physico chemical properties as described below:

3.3.1.2 Characterization of compost

(a) Determination of pH and Electrical conductivity

pH of compost is measured to determine whether the soil or compost is acidic or alkaline. It can be defined as the negative logarithm of concentration of hydrogen ions.

Electrical conductivity of soil or compost is measured to determine the amount of available nutrients present in sample. It can be defined as the ability of soil or compost to conduct electric current through it. Its units are milliSeimens per centimetre (mS/cm).

To determine the pH and electric conductivity of compost sample, the compost and water were taken in a ratio of 1: 2.5 (w/v).

Procedure:

20 g of air-dried compost was taken in a beaker and 50 ml of distilled water was poured into it. It was mixed properly for 2-3 minutes using a glass rod. Then it was kept under shaking conditions (at 120 rpm) for 3 hrs. Let it stand for 30 min. In the meantime, the pH instrument was calibrated using two buffer solutions - one acidic (pH 4.0) and other alkaline (pH 9.0). pH electrode was cleaned properly by rinsing it with distilled water and then, wiped with tissue paper. Afterwards, pH of the compost was determined by submerging the pH electrode in above prepared supernatant solution of compost. The measurement was recorded when the pH reading was stable.

The compost sample was mixed with water and stirred as described previously. KCl solution (0.01 M; EC = 1.413 mS m^{-1}) was used to calibrate the meter. The electrode was dipped in the supernatant solution and recorded the EC.

(b) Organic Carbon

The amount of organic carbon (%) present in compost was calculated as per the protocol of Walkley and Black, (1934).

Procedure:

1.0 g of dried compost was weighed and put in a 150 ml conical flask. To the compost, 10 ml of 1N potassium dichromate, $K_2Cr_2O_7$ (Appendix I) was added and mixed the contents properly by swirling the flask. Afterwards, 20 ml of concentrated sulphuric acid, H_2SO_4 was added from the sides of the flask and in the meantime, flask was swirled to mix it properly. Let it stand for about 30 min and then, 70 ml of water was poured into it. Again, the flasks were shaken thoroughly and let the compost particles to settle overnight. Next day, the supernatant was discarded and color intensity was read using red filter at 660 nm. The blank was also prepared in same way except the compost was not added. To make a standard curve, different amount of anhydrous sucrose crystals (0, 10, 20, 30, 40 and 50 mg) were taken in separate 100 ml volumetric flasks and the same protocol was repeated as mentioned above.

Calculations: To calculate the percentage of carbon (%) present in sucrose, the amount of sucrose (0, 10, 20, 30, 40 and 50 mg) was multiplied by factor of 0.4207. This can be explained as follows:

Since, 10 mg of sucrose molecules = 4.207 mg of carbon

And let, 1.0 g of compost has 4.207 mg of carbon

Therefore, $100\ mg\ of\ compost = \frac{4.207*100}{1000} = 0.4207\ mg\ carbon$

So, the percentage of organic carbon in a sample can be calculated as follows:

$$Organic\ carbon\ (\%) = Colorimetric\ reading * 0.0042$$

(c) Determination of Total Phosphorus

Total phosphorus present in compost was calculated according to the protocol given by Kitson and Mellon, 1944. In this method, vanadomolybdate reagent is used which consists of ammonium molybdate as well as ammonium (meta) vandate. Under acidic conditions, vanadomolybdate reagent reacts with phosphorus to produce a yellow colored compound i.e., vanadomolybdophosphoric acid as well as a heteropoly acid is also formed.

$HNO_3/HClO_4$ digestion:

The digestion of sample with $HNO_3/HCLO_4$ was carried out to release the mineral elements from compost. One gram of air dried compost sample was taken in digestion tube and 10 ml conc. HNO_3 was added. The contents were digested on electric heater at 145°C for 1 hr in acid proof digestion chamber having fume exhaust system. It was allowed to cool. Further, 10 ml conc. HNO_3 and 5 ml $HClO_4$ was added and heated at about 100°C for the first one hour and then raised the temperature to about 200°C. The digestion was continued till the contents became colorless and only white fumes appeared. Reduced the acid contents till white matter left in the digestion tube. Afterwards, removed from the heating mantle and cooled. 50% dil. HCl was added and filtered through whatman filter paper no. 42. Then, 2 or 3 washings with 50% dil. HCl was given and final volume made was 50 ml with diluted 50% HCl. This was used to determine total phosphorus.

Procedure:

10 ml of acid digest of compost was put in 50 ml volumetric flask. Added 10 ml of vanadomolybdate reagent (Appendix I) and diluted to 50 ml. Mixed well and let it stand for 10 minutes. A yellow color was developed whose intensity was proportional to phosphate concentration. Read the conc. of Phosphorus using spectrophotometer at 420 nm. A standard graph was made by taking different amount of stock Phosphorus solution (Appendix I) such as 0, 1, 2, 3, 4 and 5 ml separately in six different volumetric flasks and the color was developed as mentioned above.

Calculations:

$$\mathrm{P}\left(\frac{\mathrm{mg}}{\mathrm{kg}}\right)=\frac{\left[\text{volume make up after digestion (ml)} * 50 * \mathrm{P}\left(\frac{\mathrm{mg}}{\mathrm{L}}\right)\right]}{[\text{weight of sample (g)} * \text{volume of digest used to develop color (ml)}]}$$

where volume make up after digestion = 50 ml, weight of sample = 1 g, volume of digest taken = 10 ml and P (mg/L) is spectrophotometric reading

(d) Determination of Available Phosphorus

Available phosphorus present in compost was estimated according to the protocol given by Olsen et al. (1954).

Procedure:

The compost sample (2.5 g) was placed in 100 ml flask containing 50 ml of extracting solution (Appendix I) and kept on shaker for 30 minutes. The contents were filtered through whatman filter paper No. 42. Then, 10 ml of filtrate was transferred to 50 ml volumetric flask. 1 ml of 2.5 M H_2SO_4 was added into it to lower the pH to 8.5 followed by addition of 15.5 ml distilled water. 8 ml of freshly prepared ascorbic acid solution (Appendix I) was added and final volume made was 50 ml with distilled water. The samples were kept for 10 minutes and colour intensity was measured at 882 nm. The blank was also prepared in the same manner as described above using 10 ml of extracting solution in place of compost extract.

A standard graph was made by taking different amount of working P solution (KH_2PO_4, Appendix I) i.e., 0, 2, 5, 10, 15 and 20 ml of 1 mg/L in six different 50 ml volumetric flasks. Then, 10 ml of extracting solution, 1 ml of 2.5 M H_2SO_4 and 8 ml of ascorbic acid were put in each flask; and final volume made was 50 ml with distilled water. The concentration of Phosphorus in these solutions was 0.04, 0.1, 0.2, 0.3 and 0.4 ($\mu g\ ml^{-1}$ or $mg\ L^{-1}$) respectively.

Calculations:

$$Available\ Phosphorus\ in\ sample\ (\frac{mg}{kg}) = P\ in\ extract\ \left(\frac{mg}{L}\right) * 20$$

Here, 20 is the compost-to-solution ratio.

(e) Determination of Total Nitrogen

To assess the total nitrogen present in compost sample, Kjeldahl method was used as per protocol of Piper, 1960.

Procedure:

1. 5 g compost sample was mixed thoroughly with 30 ml of sulphuric salicylic acid (Appendix I) and followed by 5 g of sodium thiosulphate. Heating was carried out for 5 minutes followed by cooling and then 10 g of digestion mixture (Appendix I) was added. The contents were mixed well in a kjeldahl flask. The sample was taken in duplicates. Blank was also set in duplicates; which was having all the contents as of sample except the soil.
2. The flasks were kept in a digestion chamber for two hours at 100°C.
3. The color of contents was changed from dark brown to greenish white; after that the contents were cooled to room temperature and 300 ml distilled water was added.
4. 20 ml of the digested sample, 15-20 ml of 0.02 N NaOH and glass beads were added to the distillation flasks through the open end of the condenser attachment and stoppered. Water flow was maintained through the condenser.
5. The distillate was collected through a receiver tube in a beaker containing 15 ml boric acid (Appendix I) and 2 drops of mixed indicator (Appendix I) was added till the end point color changes from pink to green.
6. The distillate was titrated against 0.02 N H_2SO_4 until the end point color was changed from green to pink.

Calculations:

$$\text{Total N (\%)} = \frac{[(\mathrm{T} - \mathrm{B}) * \text{normality of } \mathrm{H_2SO_4} * 1.4 * 300]}{\text{Weight of sample}}$$

T is the titer value for sample, B is for blank

3.3.1.3 Isolation of polypropylene degrading bacteria

One gram of compost was placed in Erlenmeyer flask containing 10 ml of autoclaved distilled water. It was mixed on vortex for 2 minutes and left undisturbed for 30 minutes. Five ml of the compost suspension was inoculated in autoclaved synthetic media (100 ml) supplemented with 0.5% polypropylene (Haldia Petrochemicals Ltd., Kolkata) as the only source of carbon. Polypropylene was added after autoclaving the media. The composition of synthetic media is given in Appendix I. The flasks were incubated at 37°C and 120 rpm in incubator shaker (New Brunswick Scientific, USA). When microbial growth was found to be sufficient as observed spectrophotometrically at OD_{600}, five ml of this culture was transferred to fresh sterile 100 ml synthetic medium having 0.5% of PP. Similarly, second transfer was done under the same conditions. After sufficient time of incubation, 100 µl of this culture was spread plated on agar plates containing 0.5% PP in synthetic media. The plates were kept in incubator for 14 days at 37°C; then, single colonies were picked and streaked on nutrient agar (Appendix I) media.

3.3.1.4 Morphological and biochemical characterization of bacteria

(a) Gram staining

Gram staining of bacteria was carried out via Gram staining kit (HiMedia Laboratories, Mumbai, India). A thin smear of actively growing fresh bacterial cultures in nutrient broth (Appendix I) was made on clean microscopic slide and heat fixed. Flooded the smear with Gram's crystal violet and let it stay as such for 1 minute. The slides were washed gently with distilled water for few seconds. Then, the slides were covered with Gram's iodine for 1 minute and again washed with distilled water. Afterwards, the smear was decolorized using Gram's decolourizer for 5-10 seconds till no more colour flows from the slides. Further, the smear was flooded with counterstain (0.5% safranin), let it stay as such for 1 minute and rinsed with distilled water. Then the slides were air dried and observed microscopically under oil-immersion (100X). The bacteria which stained blue/purple were described as Gram positive bacteria while those which stained pink/red were referred as Gram negative bacteria.

(b) Capsule staining

The capsule stain is a differential stain, which selectively stains external capsules surrounding bacterial cells. Capsules are highly ordered polymers of sugars and proteins that surround

some bacterial cells, and can be easily dislodged by heat or water. The primary stain applied is crystal violet, which stains both the bacterial cell and the surrounding capsule. A 20% copper sulfate solution is then applied, which serves a dual function as both decolorizer and counterstain. It removes and replaces the crystal violet in the capsule only. At the end of the staining procedure, the capsule appears as a faint blue or white halo around a purple cell observed under microscope.

(c) Oxidase test

Oxidase discs (HiMedia Laboratories, Mumbai India) were used to identify the bacteria which can produce oxidase enzyme. The enzyme oxidises N,N-dimethyl-p-phenylenediamine (a colorless dye) to form indophenol blue (a colored compound). The oxidase discs are already coated with N,N-dimethyl-p-phenylenediamine oxalate, ascorbic acid and a-naphthol. Bacterial isolates were grown on nutrient agar plates, kept at 37°C for 24 hours and a well isolated colony was touched with toothpick and spreaded on the oxidase disc. A positive reaction turned the discs to blue within 10 seconds. Either no change in color or change after 10 seconds was regarded as negative reaction.

(d) Catalase test

The test is used to identify those bacteria that can produce catalase enzyme. The enzyme breakdown the molecules of hydrogen peroxide (H_2O_2) to produce O_2 and water. A small amount of bacterial colony grown in nutrient broth was placed on a clean microscope slide using a toothpick. Then, few drops of hydrogen peroxide, H_2O_2 (3%) were placed on the smear. Rapid evolution of O_2 bubble was considered as the positive result. Either no bubble or only a few scattered bubbles indicated negative result.

(e) Nitrate reduction test

Nitrate reagent discs were used for detection of nitrate reduction by microorganisms. Bacterial isolates were grown on nitrate agar (Appendix I) plates and incubated at 37°C for 24 hours. The nitrate reagent disc was placed on bacterial colony and one or two drops of rehydrating fluid was added on the disc. A positive reaction was indicated by red or pink color formation on the discs.

(f) Starch hydrolysis test

A single colony of bacteria was streaked on starch agar plates (Appendix I) and kept in incubator for 24 hours at 37°C. After the growth of cultures, surface of agar was flooded with iodine. Starch hydrolysis was seen as a colorless zone surrounding the colonies. Iodine turns blue-black in the presence of starch so, a blue or purple zone indicated that starch is not hydrolyzed.

(g) Simmons citrate agar test

Simmons citrate agar (HiMedia Laboratories, Mumbai India) was used to detect citrate utilization by bacteria as the sole carbon source. Slants were made from Simmon's citrate agar in test tubes and bacteria were grown in it at 37°C for 24 hours. Bromothymol blue was added as pH indicator. A positive reaction was indicated by a change in color of media from green to blue.

(h) Carbohydrate utilization test by isolated bacteria

HiCarbohydrate^{TM} kit (HiMedia Laboratories, Mumbai, India) was used for carbohydrate utilization test. The kit contained twelve wells corresponding to 12 different sugars. These 12 different sugars were Lactose, Maltose, Xylose, Dextrose, Fructose, Sucrose, Galactose, Raffinose, Melibiose, Trehalose, L-Arabinose and Mannose. A single bacterial colony was inoculated into nutrient broth and incubated at 37°C until the turbidity of cultures increased to $\geq$ 0.5 at 600 nm. 50 µl of this inoculum was inoculated in each well aseptically and incubated at 37°C for 24-48 hours. If color changed from red to yellow due to acid production then test was positive otherwise negative.

(i) Antibiotic susceptibility test for bacteria

Ready precoated octo discs (HiMedia, India) were used to check the sensitivity of bacterial isolates. The 8 antibiotics were Penicillin G (P), Vancomycin (VA), Cephalothin (CEP), Clindamycin (CD), Co-trimoxazole (COT), Erythromycin (E), Gentamicin (GEN) and Ofloxacin (OF). The bacterial cells were grown in nutrient broth till the turbidity became 1.0 at 600 nm. 100 µl of grown cultures was spread on nutrient agar plates and antibiotic discs were placed on it. The plates were incubated at 37°C for 24 hours and inhibition zone was noted.

3.3.1.5 Molecular characterization

(a) Isolation of genomic DNA from polypropylene degrading bacteria

The genomic DNA of polypropylene degrading bacteria was extracted from freshly grown cultures according to the method described in Sambrook and Russel (2000). A single colony of each isolate was picked from agar plate using toothpick and transferred in 20 ml of nutrient broth (Appendix I) taken in 250 ml Erlenmeyer flasks. The flasks were incubated for 16-20 h at 37°C and 120 rpm. The bacterial cells were harvested from the above 1.5-2.0 ml stationary phase culture by centrifugation (sterile microfuge tubes) at 8000 rpm for 5 minutes. The supernatant was decanted and pellet of each bacterial cell was resuspended in 800 µl saline-EDTA buffer. 50 µl of freshly prepared lysozyme solution was added and mixed properly by inversion for 10-15 times. The microfuge tubes were incubated for 20 minutes at 37°C. Afterwards, 200 µl SDS (10%) was added into it, mixed well and again incubated in water bath at 60°C for 15 min. Equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) solution was added into the cell suspension to remove proteins and cell debris; and centrifuged at 12,000 rpm for 10 min. The upper aqueous phase was taken in another sterile microfuge tube and equal volume of isopropanol was added. Centrifuged at 10000 rpm for 10 minutes and decanted the supernatant. The DNA pellets were washed with 300 µl of ethyl alcohol (70%) and centrifuged at 8000 rpm for 5 minutes. Finally, 30 µl of milliQ water was added to dissolve the pellets and stored at 4°C for further use.

(b) Agarose gel electrophoresis of DNA

0.7% (w/v) agarose gel was made in a conical flask by dissolving 0.35 g of agarose powder in 50 ml of 0.5X TBE buffer, pH 8.0 (Appendix I) and poured in tray. Before pouring, ethidium bromide (0.5 µg/ml) was added. DNA samples were loaded in wells using 6X loading dye (Appendix I) and electrophoresed at 3 volts/cm for 45-60 minutes. Then gel was visualized with an UV transilluminator.

(c) DNA quantification

The concentration of DNA was measured by spectrophotometric measurement at 260 nm (E_{260}). For double stranded DNA, OD of 1.0 at 260 nm in 1 cm cuvette is equal to the DNA concentration of 50 µg/ml. The quality of DNA was estimated via ratio $E_{260/280}$ and $E_{230/260}$.

$E_{260/280}$ ratio of 1.8 and $E_{230/260}$ ratio of 0.3-0.9 is accepted as pure for DNA. Ratio ($E_{260/280}$) lower than 1.8 indicates for protein or phenol contamination and greater than 2.0 indicate the presence of RNA.

(d) Amplification of 16S rRNA gene

The amplification of 16S rRNA gene from genomic DNA of bacteria was done by using polymerase chain reaction (PCR). PCR was performed in Veriti 96 well Thermocycler (Applied Biosystems, USA) using primers: 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-ACGGGCGGTGTGTTC-3'). The reaction volume of 25 µl consisted of autoclaved milliQ water 15.7 µl; 10X PCR buffer (Fermentas, USA) 2.5 µl; $MgCl_2$ (50 mM; Fermentas, USA) 1.5 µl; dNTPs mix (200 µM) 2.0 µl; each primer (10 µM) 1.0 µl; Taq DNA Polymerase (5 U/µl; Fermentas, USA) 0.3 µl; and DNA sample 1.0 µl. PCR protocol used was: preheating at 94ºC 5 min; 35 cycles of 94ºC 1 min, 55ºC 30s, 72ºC 30s; and a final extension at 72ºC 5 min.

(e) Purification of PCR products

PCR products obtained were purified using "HiPurA^{TM} Quick Gel Purification Kit" (HiMedia Laboratories, India). PCR products were loaded on 1% agarose gel using 6X loading dye and electrophoresed at 3 volts/cm for 45-60 minutes. The DNA band of 1.5 kb was excised from the gel, dissolved in Gel Bind Buffer and applied to HiElute Miniprep Spin Column. Finally purified PCR products were eluted with 30 µl of elution buffer and were further cloned into T-vector.

(f) Ligation of purified 16S rRNA gene in pTZ57R/T vector

The purified 16S rRNA amplicons were ligated in pTZ57R/T vector using "Thermo Scientific InsTAclone PCR Cloning Kit" as per the manufacturer's protocol. The reaction mixture of 10 µl was prepared as follows and incubated overnight at 4°C.

Autoclaved milliQ water	5 µl
5X Ligation Buffer	1 µl
Vector pTZ57R/T, 55 ng/µl	1 µl
DNA sample	2 µl
T4 DNA ligase, 5 U/µl	1 µl

(g) Genetic transformation by $CaCl_2$- induced method

The ligated products were transformed into *E. coli* DH5α cells. A single colony of *E. coli* DH5α was transferred into 25 ml of Luria broth, LB (Appendix I) taken in a conical flask and incubated in shaker for 16-20 h at 37°C and 120 rpm. 200 µl of above saturated culture was aseptically transferred into 50 ml of LB taken in another flask. The culture was again incubated in shaker for 2-3 h at 37°C and 120 rpm. The growth was monitored by taking OD_{590} at every one hour (OD_{590} should be approx. 0.5). The above culture was taken in an autoclaved, ice-cold 50 ml polypropylene tube. The culture was cooled to 0°C by storing the tube in ice for 10 minutes and cells were harvested by centrifugation at 5000 rpm for 10 minutes at 4°C. Decanted the supernatant and 10 ml of ice-chilled $CaCl_2$ (0.1 M) was added to the pellet. The tube was stored on ice for 10-15 minutes. The cells were harvested by centrifugation at 5000 rpm for 10 minutes at 4°C. Afterwards, 1 ml of ice-chilled $CaCl_2$ (0.1 M) was added to it and stored in ice for 16 hrs to make competent cells. To 100 µl of this suspension taken in sterile and prechilled microfuge tubes, 5µl of plasmid DNA sample was added; the contents were mixed gently and stored in ice for 30 minutes for binding of plasmid DNA. Then, microfuge tubes were incubated in water bath which has been preheated to 42°C for exactly 2 minutes without shaking. Rapidly transferred the tubes in ice bath and chilled the cells for 1-2 minutes. Subsequently, one ml of LB broth was added in each tube and incubated at 37°C for 45-60 minutes so that bacteria can recover and express the antibiotic resistance marker encoded by the plasmid. 100 µl of transformed cells were spreaded on Luria agar-Ampicillin-X gal-IPTG plates (Appendix I) and incubated overnight at 37°C for appearance of colonies.

(h) Screening for recombinant plasmids

Transformed cells were spreaded on LA-amp-X gal-IPTG plates for screening of colonies containing a recombinant plasmid. If the insert was present in multiple cloning site (mcs) of pTZ57R/T vector's lacZα gene, then a non-functional β-galactosidase was produced which resulted in positive transformants (white colonies). Blue colored colonies were formed when insert was absent in mcs and a functional β-galactosidase is formed. White colonies were selected and grown in 2 ml of LB-ampicillin for isolation of plasmid and simultaneously respective clones were patched on LA-ampicillin plates.

(i) Isolation of plasmid DNA from recombinant bacteria

The plasmid DNA from recombinant bacteria was isolated by the alkaline lysis method. The recombinant colonies were grown in 2ml of LB medium containing ampicillin. 1.5 ml of the above-saturated culture was transferred to microfuge tube and cells were harvested by centrifugation at 8000 rpm for 5 minutes. The supernatant was discarded. The bacterial pellet was resuspended in 200 µl of ice-cold Solution I (Appendix I) by vigorous vortexing to ensure complete dispersion in the solution and kept for 3 minutes at room temperature. Further 400 µl of freshly made Solution II (Appendix I) was added; mixed the contents by gentle inversion five to ten times and kept on ice for 3 minutes. Then 300 µl of Solution III (Appendix I) was added, mixed by inversion so that Solution III can diffuse throughout the viscous bacterial lysate and stored in ice for 15 minutes. Finally 400 µl of chilled phenol: chloroform: isoamyl alcohol (25:24:1) solution was added for protein denaturation and centrifuged at 12000 rpm for 10 minutes. Transferred the upper aqueous phase in another tube and added equal volume of chilled isopropanol, mixed well and let it stay as such at room temperature for 10 minutes. The tubes were centrifuged at 10000 rpm for 10 minutes. The supernatant was decanted. The pellet was washed with 300 µl of ethyl alcohol (70%) and centrifuged at 8000 rpm for 5 minutes. Finally the pellets were air-dried; dissolved in 30 µl of milliQ water/TE buffer (pH 8.0) and stored at 4°C for further use.

(j) Size screening for recombinant plasmids

The recombinant plasmids containing 1.5 kb 16S rDNA inserts were identified by PCR technique using M13 forward and M13 reverse primer (Appendix I). The PCR products were observed by agarose gel (1.0% w/v) electrophoresis.

(k) Purification of plasmid DNA

The plasmid DNA was purified from overnight grown cultures of transformed colonies in LB-ampicillin using “QIAprep Spin Miniprep Kit” as per the manufacturer’s protocols. 5 ml of culture was used to isolate purified plasmid DNA in QIAprep spin column. After washing with buffer, DNA was eluted out using 30 µl of EB buffer.

(l) Sequencing

The 16S rRNA inserts in the plasmids were sequenced for both strands by Sanger DNA sequencing technique using M13 forward and M13 reverse primers. The DNA sequencing was performed by chain termination method in an automated DNA sequencer (Delhi University, South Campus, New Delhi).

(m) DNA sequence analysis

The sequences obtained were compared with those available in EzTaxon-e database (http://eztaxon-e.ezbiocloud.net) for similar sequences (Kim et al., 2012). The sequences of closely related bacteria were aligned using ClustalW program (Thompson et al., 1994). The phylogenetic tree (Neighbour-Joining method) was constructed by means of MEGA 6.0 software (Tamura et al., 2013) and the bootstrap values were inferred from 1000 replicates.

3.3.2 Biodegradation of polymers by bacterial isolates

The isolated bacteria were tested for their capability to use polypropylene blends as the sole carbon source. The conical flasks having 100 ml of synthetic media were amended with polymer films and bacterial culture. Polymer films were added after autoclaving of media. The composition of synthetic media is given in Appendix I. Apart from these; minimum amount of glucose (0.1%) was added for initial growth of bacteria so that they could utilize blend as sole source of carbon after its consumption. Before transferring to media, the polymer films were cut into 3 cm x 3 cm size, washed with 70% ethanol for 30 minutes and dried in laminar air flow.

The bacterial isolates were grown overnight in NB at 37°C under shaking conditions (120 rpm). 1 ml of each culture was centrifuged (Biofuge pico benchtop centrifuge, Heraeus) at 11000 rpm for 1 minute and then supernatant was discarded. Afterwards, the pellet was resuspended in 1 ml of synthetic media. Samples were centrifuged and resuspended a second time to ensure removal of the entire residual NB. This ensures that polymer was the only source of carbon. Then cells corresponding to OD_{600} 0.2 were added to each flask containing 100 ml media. Degradation was carried out for 15 days at 37°C temperature and at 120 rpm. All the samples were set in triplicates. Flasks having bacterial culture but no polymers were also set as control.

3.3.2.1 Methods of analysis

(a) Growth of microorganisms

The growth of bacteria was monitored everyday by taking OD at 600 nm in UV-Visible spectrophotometer for 15 days. At the end of 15 days, protein estimation was done and other testing was performed to confirm the biofilm forming ability of bacteria.

(b) Protein estimation

The population density of bacteria within biofilm was estimated by protein content. The polymer samples taken out from the media were rinsed with distilled water to remove loosely attached bacterial cells and subjected to mild sonication in 1.0 ml of saline (0.85%) for a total of 4 min at 1 min intervals. This saline solution was used for protein estimation. The protein

content present in growth media as well as in the biofilm was estimated by Bradford's method. 100 µl of saline as well as media was taken in test tubes to which 5.0 ml of Bradford reagent was added; kept for 5 min and then OD was taken at 595 nm.

(c) FTIR

The changes occurred in polymer surface after degradation was monitored by FTIR technique in ATR mode (Cary 600 series, Agilent Technologies, USA). FTIR was carried out within the wavenumber range of 400-4000 cm^{-1} with a resolution of 4 cm^{-1}. Prior to FTIR, the biofilm on the polymer surface was removed by mild sonication.

(d) SEM

SEM is a powerful tool to elucidate the fine structure of living systems and it has been used for biofilms. It can demonstrate even a single bacterium as well as the relation of biofilm to the polymer surface. After degradation period of 15 days, polymer samples were monitored for biofilm formation by SEM images. SEM micrographs were taken after gold sputtering at 5 kV under different magnifications. Prior to coating, the polymers were washed with autoclaved distilled water and dried.

(e) TGA

After degradation, the thermal stability of blends was also assessed using thermogravimetric analysis. Samples weighing 10 ± 2 mg were heated from 30°C to 600°C at a heating rate of 10°C/min under dry nitrogen and the weight loss of the sample was recorded. The flow rate of nitrogen was maintained at 50 ml/min. The initiation degradation temperature (T_i) and final degradation temperature (T_f) were also recorded using the TGA data.

3.3.3 Microcosm studies

3.3.3.1 Sample collection

Soil was collected from garden area of CORE (The Centre of Relevance and Excellence), Thapar Institute of Engineering & Technology, Patiala. It was dried, sieved (size < 2 mm) and ground. The crushed soil was analyzed for physiochemical properties like pH, electrical conductivity, TOC, Kjeldahl nitrogen, total phosphorus, available phosphorus (procedures are described above in section 3.2.2). Soil was loamy sand in texture, having pH 8.1; electric conductivity 0.14 mS/cm; organic carbon 0.3%; organic matter 0.5%; total Phosphorus 211 mg/kg; available Phosphorus 3.2 mg/kg and total nitrogen 0.03%. The soil was autoclaved for 60 min at 121°C and bacterial isolates were inoculated into it.

3.3.3.2 Procedure

The microcosm studies were carried out to study degradation potential of bacterial isolates in the natural environment. 45 microcosms consisting of 150 gram of soil each were prepared in glass jars that were 4 (width) by 12 (height) cm as shown in Figure 3.4.

Figure 3.4 The set-up of microcosm experiment in dark room

The cultures grown in NB were centrifuged at 11,000 rpm for 1 min and the pellet was rinsed twice with sterilized distilled water to remove any traces of media from the cells. The cells were inoculated at a concentration of 1.0 x 10^7 cells per gram of soil. A consortium was also prepared and inoculated into the soil. For preparation of consortium, the isolates were grown overnight in 20 ml of nutrient broth at 37°C and 120 rpm. Afterwards, the volume of cultures with an absorbance reading of 0.2 at 600 nm was centrifuged separately at 11000 rpm for 1

minute. Then supernatant was thrown and pellet was washed twice with autoclaved distilled water to remove any traces of media from it. The consortium (having $O.D_{600\ nm} = 1.0$) was prepared by mixing pellet of all the cultures and this was used for biodegradation experiments.

The sterile polymer films of size 5 cm x 2 cm (single strip in each microcosm) were put in jars containing soil amended with bacterial cultures. Three replicates of each type of bacteria were set in this way. The moisture content of the soil was adjusted to 40% using sterile distilled water. The amount of water lost during evaporation was measured by weighing the microcosms every month, and was replenished by using the sterile distilled water. Moreover, microcosms were maintained at temperature matching to the natural ones and in the dark to avoid any photochemical degradation. This regimen was followed for 6 months. After 6 months, polymer films were recovered, and the degree of degradation was determined by FTIR and TGA. The sample of soil from each microcosm was taken out after every month and used for analysis.

3.3.3.3 Bacterial growth in soil (cfu/gm soil)

The growth of bacteria in microcosm was measured by recording the colony forming units (CFU) of bacteria via serial dilution plating on nutrient agar. Soil sample (1.0 g) was suspended in 10 ml of autoclaved distilled water. Afterwards, the suspension was diluted tenfold up to 10^{-4} and 10^{-5} concentration. Then, 0.1 ml suspension of 10^{-4} and 10^{-5} dilution was spread on nutrient agar plates and incubated at 37°C in a BOD incubator. After 24 h visible pinhead colonies were counted.

3.3.3.4 FTIR

The structural changes occurred in polymer surface after degradation was observed by ATR-FTIR technique. It was carried out in FTIR spectrophotometer (Cary 600 Series, Agilent Technologies, USA) in the wave number range of 400-4000 cm^{-1}. A total of five scans per sample were accumulated at a resolution of 4 cm^{-1}. The spectra were analyzed using Agilent Resolution Pro software.

3.3.3.5 TGA

After degradation, the thermal stability of blends was assessed by thermogravimetric analysis. Samples weighing 10 ± 2 mg were heated from 30°C to 600°C at a heating rate of 10°C/min under dry nitrogen and the weight loss of the sample was recorded. The flow rate of nitrogen was maintained at 50 ml/min. The initiation degradation temperature (T_i) and final degradation temperature (T_f) were also recorded using the TGA data.

3.3.4 Biodegradation study of polymers in open field

The polymers were tested for biodegradation in open field too. Two pits were dug in garden area of CORE, Thapar Institute of Engineering & Technology, Patiala. The consortium was prepared as described above and mixed with soil taken out from one of the dig. The polymer strips of PP80 and PP80C6 (size 15 cm x 2.5 cm) were kept in duplicates in dig and again covered with soil for 6 months during the months of October, 2015 through March, 2016. One dig was kept as control in which no bacteria were mixed with soil. After the incubation of 6 months, the polymers were tested for their mechanical properties and thermal stability (TGA).

3.3.5 Aerobic biodegradation of polymers

The biodegradability of organic substances can be defined as the extent of the changes occurring in physical and chemical properties of the organic materials as well as their molecular structure through degradation by bacteria. The estimation of biodegradability of plastics under aerobic or anaerobic conditions is essential for their use in the future (Mohee and Unmar, 2007).

The degradation of polymer substrate in the environment causes its breakdown into organic waste. In this regard, composting can be used as an alternative tool to incineration or disposal in landfills. During composting, the organic material gets converted to humic substances that can be used in the agricultural applications as high quality fertilizer. Therefore, composting is regarded as environmental friendly process since it saves energy, minimizes the waste stream into landfills, and permits the valuable nutrients and organic substances to be used in agriculture. In the method of composting, a degradation curve is plotted by determining the amount of CO_2 evolved with time and it involves three phases: lag phase, growth phase and stationary phase (Leejarkpai et al., 2011).

3.3.5.1 Materials

Soil was collected from garden area of CORE, Thapar Institute of Engineering & Technology, Patiala. It was dried, sieved (size < 2 mm) and ground. The crushed soil was analyzed for physiochemical properties like pH, electrical conductivity, TOC, Kjeldahl nitrogen, total phosphorus, available phosphorus (procedures are described above in section 3.2.2).

Barium hydroxide, $Ba(OH)_2.8H_2O$ having molecular weight of 315.46 was purchased from HiMedia Laboratories Pvt. Ltd., India. Approximately, 0.024 N $Ba(OH)_2$ solution was made by dissolving 4.0 g of barium hydroxide in one litre of distilled water and then standardized against HCl to verify the normality of solution. The solution thus prepared was filtered through filter paper and then stored in a sealed container to avoid absorption of carbon dioxide from the air.

Hydrochloric acid, HCl with 35% concentration was procured from HiMedia Laboratories Pvt. Ltd., India. 0.05 N HCl was prepared and then standardized with sodium carbonate (Na_2CO_3) prior to use.

Microcrystalline cellulose powder of analytical grade (positive reference) was obtained from Sigma-Aldrich, India.

3.3.5.2 Apparatus

The biodegradability testing apparatus (Figure 3.6) was used to measure CO_2 evolved by the bacterial isolates on utilizing the polymers as their carbon source. The apparatus had different components which are described as follows:

1. Composting vessels/ bioreactors: There were 12 composting vessels (Figure 3.5) placed in a temperature controlled chamber. The vessels were made up of glass and had a total volume of 1 L. There were two ports on each vessel: (i) inlet port at the bottom for incoming air, and (ii) outlet port at the top for release of CO_2 gas.

2. Temperature controlled chamber: This chamber was meant for maintaining the temperature of composting vessels at 58 ± 2°C. The vessels were placed in 3 level partitions i.e., 4 vessels on each stage of the chamber.

3. Supply system for inlet air: It was meant for supplying CO_2 free air to every composting vessel at constant aeration rate. A cylinder was used to supply the CO_2 free air to a common inlet of rotameters. There were 12 rotameters attached to the chamber having flow rate capacity of 0-100 ml/min.

4. CO_2-trapping apparatus: The CO_2 released from each vessel was absorbed in barium hydroxide solution contained in conical flasks. There were 3 conical flasks of 500 ml volume attached in series to each vessel and they were fitted with gas sparging system. The inlet pipe to the flasks was long enough so that the solution can absorb the CO_2 gas and the outlet pipe was short so as to allow the gas from only headspace of the flasks to carry forward into next flask.

Figure 3.5 The glass vessels containing soil and samples

Figure 3.6 Biodegradability testing apparatus for determining percentage biodegradation

3.3.5.3 Procedure

The aerobic biodegradation of polymer blends, PP80 and PP80C6, was assessed as per ASTM D5338-98. It is a standard method meant for determining the aerobic biodegradation of polymers under controlled composting conditions. The test was little modified- (i) instead of compost; soil was used for degradation studies, and (ii) the soil was amended with cultures to examine the degradation efficiency of isolates. The vessels were packed with three types of mixtures, each was set in triplicates:

1. Blank: consisting of soil and polymers but no bacteria were added.
2. Positive control: consisting of soil and 500 mg of cellulose. The test is considered valid only if positive control undergoes more than 70% degradation after 45 days.
3. Test samples: containing soil, polymer sample and bacterial cultures (Figure 3.5). 400 g of soil was taken in each vessel; bacterial cells were inoculated at the concentration of 1 x 10^7 cfu per gram of soil and strips of polymer (size 2 x 2 cm) were added into them (Figure 3.5). The 5 strips of polymer (each having weight of about 100 mg; so total of 500 mg per vessel) were added into each vessel.

The test was initiated by supplying CO_2-free air to samples at a flow rate of 60 ± 10 ml/min and the vessels were incubated in dark for a time period of 45 days under aerobic conditions. The moisture content was maintained by addition of water at regular intervals. The temperature was maintained at 58 ± 2°C (Figure 3.6). The vessels were shaken weekly to make sure that air and moisture are getting distributed uniformly throughout the samples. The biodegradability was measured in terms of amount of carbon dioxide evolved and percentage biodegradation was also calculated by the end of experiment. The concentration of carbon dioxide in outgoing air was determined by absorbing it in barium hydroxide solution (0.024 N) where carbon dioxide reacts with barium hydroxide solution to form the insoluble precipitates of barium carbonate ($BaCO_3$). The residual amount of $Ba(OH)_2$ solution was measured by titrating 30 ml of $Ba(OH)_2$ solution with HCl solution (0.05 N). Phenolphthalein was used as an indicator in titration method. The measurements were taken daily for 45 days.

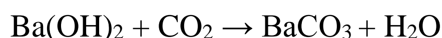
3.3.5.4 Calculations

Initially, the total carbon content of test samples was determined by CHNS analyzer (Thermo Finnigan, Italy) and from carbon content, the theoretical amount of CO_2 evolved was calculated as follows:

$$Theoretical\ amount\ of\ CO_2 = \frac{44 * C_{sample}}{12} \quad \text{.......... (Eq. 3.1)}$$

where C_{sample} is the theoretical carbon content of the polymer determined by CHNS analyzer; 44 and 12 are molecular weight of CO_2 and carbon respectively

In this method, the exhaust air from vessels was made to pass through absorber bottles i.e. [$Ba(OH)_2$]; where CO_2 reacts with $Ba(OH)_2$ to form insoluble precipitates of Barium carbonate ($BaCO_3$) as follows:



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

Then, remaining amount of barium hydroxide in solution is determined by titration with HCl with phenolphthalein as an indicator.

So, the number of mmoles of CO_2 produced is:

$$mmoles\ of\ CO_2 = mmoles\ of\ Ba(OH)_2\ at\ start - \frac{(mmoles\ of\ HCl)}{2} \quad \text{............ (Eq. 3.2)}$$

So, the amount of CO_2 produced by each vessel was calculated and the mean of three replicates was taken. Afterwards, the net amount of carbon dioxide released was determined by subtracting the amount of CO_2 evolved from blank from that of test substance and then the percentage of biodegradation were calculated by the following equation:

$$(\%)\ \text{Biodegradation} = \frac{(CO_2)_T - (CO_2)_B}{CO_2(Th)} * 100 \quad \text{............. (Eq. 3.3)}$$

where $(CO_2)_T$ = amount of CO_2 released by the test sample, $(CO_2)_B$ = amount of CO_2 evolved by the blank and $CO_2(Th)$ is the theoretical CO_2 released from the polymer sample.

3.3.6 Abiotic degradation of polymers

The polymer films were subjected to various abiotic factors like UV treatment, thermal treatment, Aquaregia and Fenton's reagent treatment. The abiotic factors cause oxidation of the polymer which leads to either formation of new bonds or breaking of old bonds.

3.3.6.1 Heat treatment

The films of PP80 and PP80C6 were kept in hot air oven preheated at 100°C with both sides exposed to air for a maximum of 10 days. The samples were placed in duplicates.

3.3.6.2 UV- irradiation

The films of PP80 and PP80C6 were irradiated in a chamber under a long-wave lamp (365 nm) for 8 days with exposure on both sides. The samples were taken in duplicates.

3.3.6.3 Chemical disinfection of films

The chemical treatment was given by placing films of PP80 and PP80C6 in a covered beaker containing fresh solution of Aquaregia reagent (Appendix I) and Fenton's reagent (Appendix I) separately for a period of 7 days.

3.3.6.4 FTIR

After abiotic treatment FTIR analysis was carried out to monitor changes occurred in surface of polymers.

3.3.6.5 Biotic treatment

Followed by abiotic treatment, polymers were biotically treated with consortium in synthetic media as of the method described above in section 3.3.3. The unpretreated films of blends were also subjected to similar biodegradation studies. The biotic treatment was conducted for 15 days at 37°C and 120 rpm. The growth of cells was measured daily by taking O.D at 600 nm in UV-visible spectrophotometer. To quantify bacterial growth at the end of 15 days, protein content was measured in the media and biofilm.

3.4 Statistical analysis

The measurements were recorded in triplicate and their mean and standard deviation were calculated. Data was analyzed by means of analysis of variance (ANOVA) and the means were compared by Tukey's honestly significant different test. All the analysis was done using GraphPad prism 5.0 software.

Chapter 4

Results and Discussions

4.1 Development of degradable polypropylene blends

Blending of polymers is an efficient method to obtain new materials with "tailor-made" properties such as functional physical properties and biodegradability. But, most of the polymer pairs are immiscible due to low entropy of mixing which results in the poor mechanical properties and formation of coarse phase morphologies having sharp interfaces between phases. To compatibilize the immiscible polymer blends and to improve their mechanical properties, copolymers are frequently used as interfacial modifiers. Copolymers are made up of monomers or segments that are miscible with both the phases and thus act as effective compatibilizers such as diblock or triblock copolymers, multiblock or graft copolymers. A compatibilized blend may not be miscible but it possesses fine phase morphology and better affinity between the phases (Chen et al., 2007).

Polypropylene also shows the poor interactions with other polymers due to lack of chemical functionalities. It cannot produce miscible blends or even adhere to the other polymers. Therefore, adhesion of PP with many materials can be improved by the addition of polar groups such as maleic anhydride modified PP (Arcana et al., 2007).

The objective of our study was to increase the biodegradability of PP by blending it with biodegradable polyester i.e., PLLA. As both the polymers are immiscible with each other, therefore, maleic anhydride grafted polypropylene (MAPP) was used to compatibilize the blends. The polymer blends of PP with PLLA were prepared by melt blending technique. The following compositions of PP: PLLA (in weight percentage) were prepared- 95: 5, 90: 10, 85: 15, 80: 20, 70: 30, 60: 40 and 50: 50. Pure PP and pure PLLA films were also prepared by the same technique. Subsequently, 2, 4, 6 and 8 phr of MAPP was added to the PP-PLLA blend with a ratio of 80: 20 to prepare the PP-PLLA-MAPP compatibilized blends. The films thus formed were tested for their mechanical properties.

4.1.1 Mechanical properties

The mechanical properties of the virgin PP and its blends (such as tensile strength, elongation at break, and tensile modulus) are depicted in Table 4.1.

Table 4.1 Mechanical properties of blends

S. No.	Blends code	Tensile strength at yield (MPa)	Elongation at break (%)	Tensile modulus (MPa)
1	PP100	45.4 ± 2.3 bc	3.3 ± 0.2 b	941 ± 47.1 cd
2	PP95	42.9 ± 2.1 c	2.6 ± 0.1 c	934 ± 46.7 cd
3	PP90	40.0 ± 2.0 c	3.8 ± 0.2 a	1210 ± 40.5 a
4	PP85	32.4 ± 1.6 d	2.1 ± 0.1 d	788 ± 39.4 ef
5	PP80	31.3 ± 1.6 d	3.2 ± 0.2 b	1010 ± 50.5 bc
6	PP70	19.8 ± 0.9 e	1.5 ± 0.1 ef	1100 ± 55.0 ab
7	PP60	18.1 ± 0.9 e	1.3 ± 0.1 f	732 ± 36.6 f
8	PP50	12.5 ± 0.6 f	0.7 ± 0.0 g	694 ± 34.7 f
9	PP80C2	42.5 ± 2.1 c	1.8 ± 0.1 de	707 ± 35.4 f
10	PP80C4	50.7 ± 2.5 ab	2.5 ± 0.1 c	824 ± 41.2 def
11	PP80C6	52.1 ± 2.6 a	2.0 ± 0.1 d	1080 ± 54.0 ab
12	PP80C8	42.9 ± 2.2 c	1.5 ± 0.1 ef	870 ± 43.5 de

Values sharing same letters within the columns are not significant at $P < 0.05$. Values are mean ± SD (n = 3)

Neat PP (i.e., PP100) exhibited tensile strength of 45.4 MPa and tensile modulus of 941 MPa, which decreased on the addition of PLLA (Table 4.1). This is mainly due to the brittle nature of PLLA. Moreover, there is polarity difference between the two polymers (Choudhary et al., 2011). PLLA is a polar polymer whereas PP is non-polar polymer. Therefore, it is difficult to mix these two polymers to produce the blends having regular particle size of PLLA phase in PP phase. Higher the amount of PLLA, lesser was the tensile strength. Thus, the PP-PLLA blend with ratio of 50:50 had the lowest strength amongst all the blends. According to Reddy et al. (2008) an ideal compatible polymer blend possesses higher strength than or in between that of the component polymers. But in the case of PP-PLLA, the blends had lower strength than neat polypropylene which evidence for the incompatibility between the two polymers. The addition of 20% PLLA to PP caused the reduction in tensile strength from 45.4 MPa to 31.3 MPa i.e., approximately 69% of pure PP. The further increase in proportion of PLLA such as up to 30%, the tensile strength decreased drastically to 19.83 MPa, which is only 46% of neat PP. Thus, PP/PLLA blend with 80:20 ratios (PP80) was chosen for further studies. The results of tensile strength along with elongation at break (E_b) are displayed graphically in Figure 4.1. PP80 had almost similar value of E_b as of PP100, but further increasing the amount of PLLA led to continuous decrease in E_b. Furthermore, the results of tensile modulus also showed decreasing trend with increase in amount of PLLA, but it was quite higher in case of blends PP80 and PP70 (Table 4.1). The high value of tensile modulus signifies that the substance is rigid hence; more stress is required to generate certain amount of strain and it resists deformation or stretch (Ibrahim et al., 2009). Therefore, PP80 (80% PP + 20% PLLA) was selected as optimum composition in view of the better retention of mechanical properties and comparatively low proportion of expensive PLLA. Kaczmarek et al. (2005) reported that even 5% addition of cellulose in PP caused reduction in tensile strength and elongation at break by 43% and 55%, respectively. Reddy et al. (2008) reported that the tensile strength as well as the elongation at break of PLA/PP blends was inferior to the pure fibres. The incorporation of only 20% of PLA into polypropylene caused the decrease in elongation at break by around 45% as compared with pure PP and the incorporation of 20% of polypropylene into PLA caused the decrease in elongation by 28%. Hamad et al. (2011b) reported that both the stress at break and strain at break of the blend PP/PLA decreased up to the addition of 50% PLA and increased on addition of more than 50% PLA. In contrast, Young’s modulus of the blends showed intermediate values between that of PP and PLA.

Besides, the results of ANOVA (Table 4.1) revealed that the values of mechanical properties for PP80 are quite dissimilar as compared with other blends and the data is quite significant at $P < 0.05$.

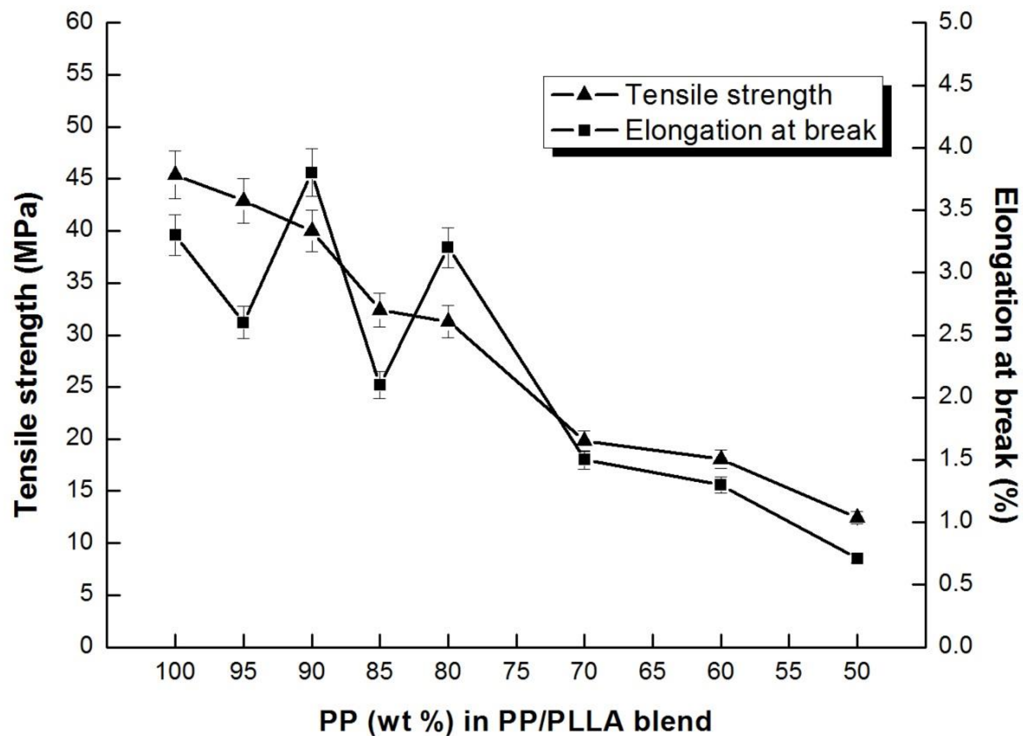


Figure 4.1 Changes in tensile strength and elongation at break by varying PP composition in blend

The effect of incorporation of compatibilizer on the mechanical strength of polymer blends was also studied and the results of tensile strength as well as elongation at break of PP/PLLA blend with a ratio of 80/20 containing varying amount of MAPP are graphically shown in Figure 4.2. The different amount of compatibilizer (MAPP) added was 2, 4, 6 and 8 phr (parts per hundred of resin). It was evident that the tensile strength increased to 42.5 MPa, 50.7 MPa, 52.1 MPa and 42.9 MPa in the blends PP80C2, PP80C4, PP80C6 and PP80C8, respectively as compared to 31.3 MPa in PP80 blend. There was increase in tensile strength up to the addition of 6 phr of compatibilizer, afterwards it decreased. This happened for the reason that compatibilizer with the excessive mass cannot disperse regularly on the interface between PP and PLLA which leads to difficulty in mixing as well as compatibility of the two

polymers. As a result, the mechanical strength of polymer blends decreases on raising the compatibilizer proportion above the critical content (Hoang and Giang, 2004). Moreover, the tensile modulus of blend PP80 was 1010 MPa which increased to 1080 MPa on addition of 6 phr of compatibilizer. The results of tensile strength and tensile modulus of PP80C6 are higher than that of pure polypropylene too. Thus, addition of compatibilizer enhanced the tensile strength and tensile modulus of blends but decreased the value of E_b. This increment in tensile properties was attributed to fine bonding occurred between PP and PLLA molecules by the addition of compatibilizer. So, 6 phr of compatibilizer in the blend was considered as optimum amount due to retention of better tensile properties. There are various reports found on compatibilization effect of the blends. Basu et al. (2002) developed the blends of polypropylene with amylose (AM) as well as with dodecanoyl ester of amylose (DODAM) in different ratios. It was found that the mechanical strength of PP/AM blends decreased with the increase in AM proportion. But, the blends containing both AM and DODAM showed enhancement in mechanical properties due to improved homogeneity of the blends by DODAM. Sadi et al. (2012) reported that the mechanical strength of PP/PHB blends without compatibilizer had intermediary values between that of pure PP (27 MPa and 71.5%) and pure PHB (29 MPa and 2.0%). But on addition of compatibilizer, the mechanical strength increased significantly as compared to PP/PHB blend. This means effective compatibilization has taken place. Similarly, Choudhary et al. (2011) found that the tensile strength of PP/PLA blends improved significantly on incorporation of compatibilizer (maleic anhydride grafted polypropylene). The tensile strength of PP/PLA blend was 37.12 MPa which increased to 46.32 MPa by incorporation of 3 wt % compatibilizer, i.e. approximately by 19.8% and it was almost comparable with pure PLA. On the other hand, the tensile modulus of blend increased by only 6% after the addition of compatibilizer.

Moreover, the results of ANOVA (Table 4.1) revealed that the values of PP80C6 are different as compared to the other blends and the data is quite significant at $P < 0.05$. Hence, PP80 (without compatibilizer) and PP80C6 (with 6 phr compatibilizer) were chosen for further studies and characterized and compared with the neat polymers.

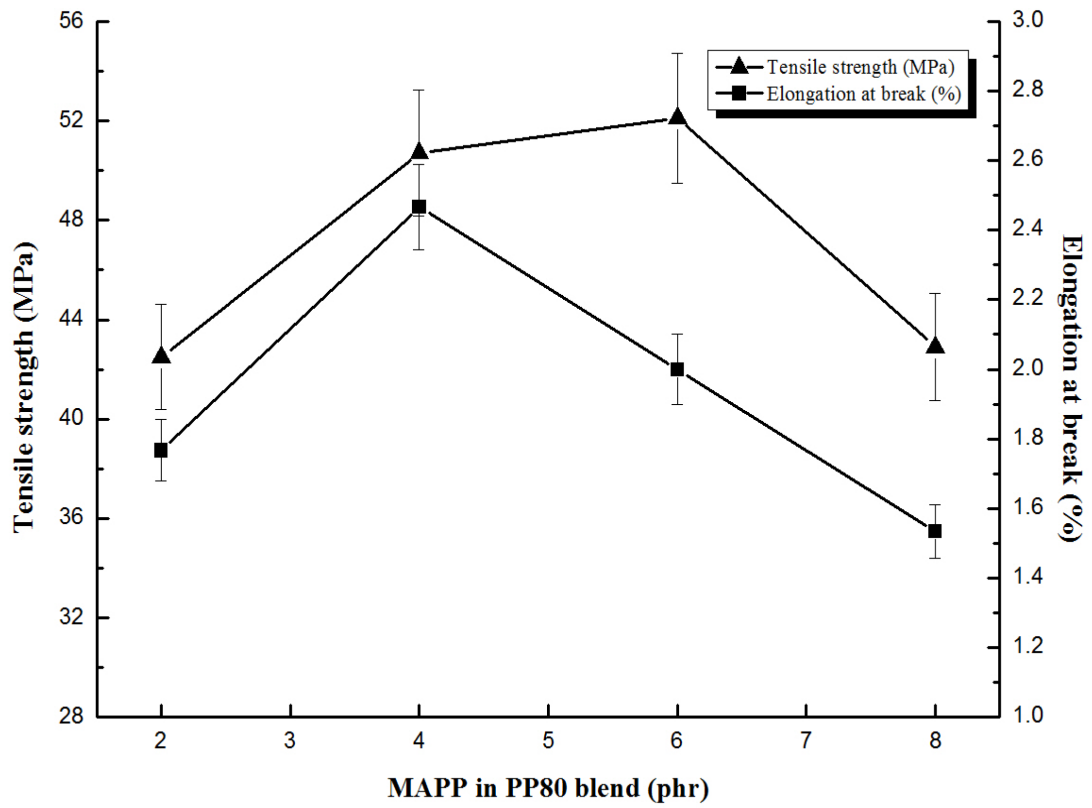


Figure 4.2 Changes in tensile strength and elongation at break by varying MAPP composition in PP80 blend

Conclusions and salient features

Experimental findings revealed that the polymer blends of PP and PLLA with and without compatibilizer (MAPP) were successfully prepared using the melt-blending technique in Brabender Plasticorder PLE-651. Moreover, the mechanical strength of PP-PLLA blends depended on the ratio of component polymers as well as on the compatibilizer content.

The results of determination of mechanical properties showed that the tensile strength, elongation at break and tensile modulus of PP reduced with the incorporation of PLLA. However, the addition of compatibilizer (MAPP) improved the mechanical strength of the polymer blends. This is due to the fact that the compatibilizer MAPP acts as adhesive between PP and PLLA. The chain of PP in MAPP get mixed and adhered with PP whereas the anhydride part of MAPP reacts with PLLA, thus forming a favourable interface. Among the different compositions of polymer blends prepared without compatibilizer, the blend with 80/20 ratio (PP80) had the optimum amount of mechanical strength and relatively low amount of expensive PLLA. The further increase in amount of PLLA showed remarkably reduction in mechanical strength of blends. Thus, PP80 was selected for all the following biodegradation research. The incorporation of compatibilizer (MAPP) into the PP/PLLA blend of 80/20 ratio caused the increase in mechanical strength as compared to PP80. This increase in tensile strength, elongation at break and tensile modulus of blends were observed upto the addition of 6 phr of compatibilizer (PP80C6). Thereafter, the mechanical strength decreased by raising the compatibilizer content above 6 phr due to the critical point. Thus, the compatibilized blend PP80C6 gave the highest values for mechanical strength and was chosen for further studies.

4.2 Effect of blend composition and compatibilizer content on the physico-chemical properties of blends

The production of valuable products from polymer blends requires the compatibility between the component polymers. Moreover, compatibility depends on the physical and chemical properties of the component polymers and on the blending parameters such as temperature, pressure, shear, humidity etc. Presently, there are many techniques to assess the degree of compatibility between the polymers which are generally divided into mechanical, thermal, spectroscopic, morphological, and scattering techniques (Biresaw et al., 2008). In the present work, the effect of compatibility on physico-chemical properties of the blends was investigated by X-ray diffraction, Fourier transform infrared spectroscopy, Scanning electron microscopy and Thermogravimetric analysis. This work demonstrates the reactive compatibilization of polypropylene with PLLA in order to produce new materials with improved physical and chemical properties and lower cost so that they can be used for a variety of applications.

4.2.1 X-ray diffraction (XRD)

XRD was used to examine the various crystalline properties of pure polymers as well as the polymer blends such as percentage crystallinity, crystal symmetry and size of crystallites. The diffractograms of PP100, PP80, PP80C6 and PLLA100 are shown in Figure 4.3. The XRD spectra of PP100 showed prominent peaks at $2\theta = 14.1°$, 16.8°, 18.6°, and 21.8° representing the (110), (040), (030) and (041) planes, respectively. This confirms for the α-monoclinic form of polypropylene (Mathakari et al., 2008). On the other hand, PLLA100 showed two major peaks one at about 16.7° and other at about 19° corresponding to (200)/(110) and (203) planes (Teng et al., 2015). When PLLA was added into PP to form the polymer blends i.e., PP80 and PP80C6, the XRD spectra showed no shift in the peaks as compared to pure PP indicating for the minimum or no changes in crystal lattice dimensions of blends as compared with neat polypropylene. This means that polymer blends possibly comprise a crystal lattice which is more similar to polypropylene than PLLA.

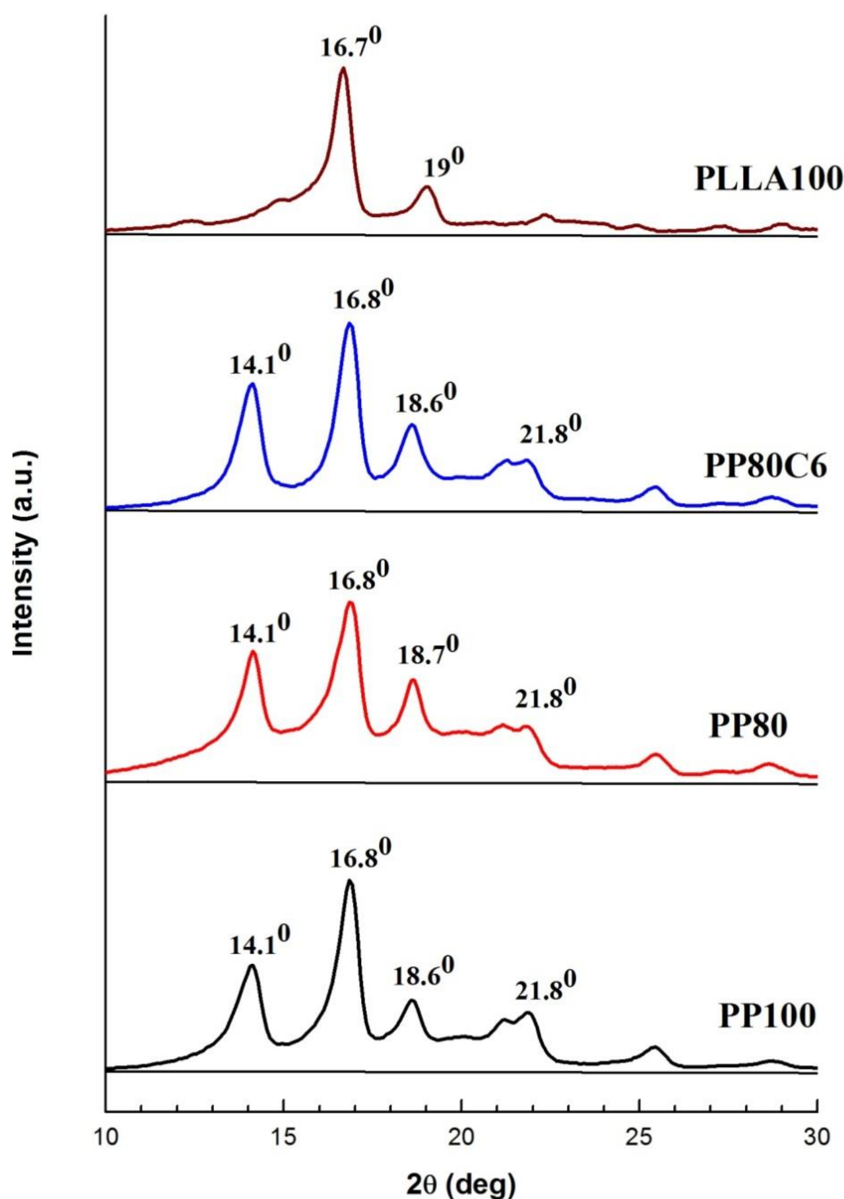


Figure 4.3 XRD patterns of PLLA100, PP100, PP80, and PP80C6

The relative crystallinity and size of crystallite of pure polymers and polymer blends are depicted in Table 4.2. The addition of PLLA into PP caused the increase in crystallinity of the polymers and hence, PP80 had a percentage crystallinity of 86.7%. However, compatibilized blend i.e., PP80C6 showed almost the same degree of crystallinity (76.3%) as of PP100 (79.7%). Moreover, PP100 and PP80C6 have the same crystallite size (337 Å), whereas the crystallite size of PP80 (289 Å) is intermediate of PP100 and PLLA100. This investigation shows that MAPP improved the compatibility between the component polymers.

Table 4.2 Degree of crystallinity and crystallite size of polymers from XRD

Blend name	Degree of crystallinity (%)	Crystallite size (A°)
PP100	79.7	337
PP80	86.7	289
PP80C6	76.3	337
PLLA100	62.6	270

4.2.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is an effective tool to determine the specific interactions in a blend. The FTIR spectra of PP100, PP80, PP80C6 and PLLA100 are depicted in Figure 4.4. The IR spectrum of PP100 showed transmittance peaks at 2915, 1450, and 1367 cm^{-1} that corresponded to C-H stretching, CH_3 bending, and C-H bending, respectively. On the other hand, PLLA100 showed transmittance peaks at 1746, 1178, and 1084 cm^{-1}, which represented C=O stretching, C-O-C symmetric stretching, and asymmetric CH_3 bonds, respectively (Ploypetchara et al., 2014). The spectrum of blend PP80 showed transmittance peaks around 2915, 1746, 1450, 1367, 1191, and 1084 cm^{-1} representing the native bonds present in both the component polymers i.e., PP and PLLA. It indicates that there are no chemical interactions between PP and PLLA resulting in the formation of immiscible and uncompatibilized blend. This explains the decrease in mechanical strength of PP80 as compared to PP100. However, the addition of compatibilizer (MAPP) into the blend resulted in emergence of a new peak at 1757 cm^{-1} that was referred to carbonyl of ester linkage stretching. Thus there was a clear shift in the characteristic peak of C=O group from 1746 to 1757 cm^{-1} which indicates for the occurrence of chemical interactions between polymers and MAPP. This can be attributed to formation of ester bond between PLLA and anhydride part of MAPP. Both PLLA and MAPP are polar molecules having functional groups in their molecules. Therefore, the compatibilizer MAPP was able to enhance the compatibility between the two component polymers i.e., PP and PLLA. These results support the results of XRD and tensile testing too.

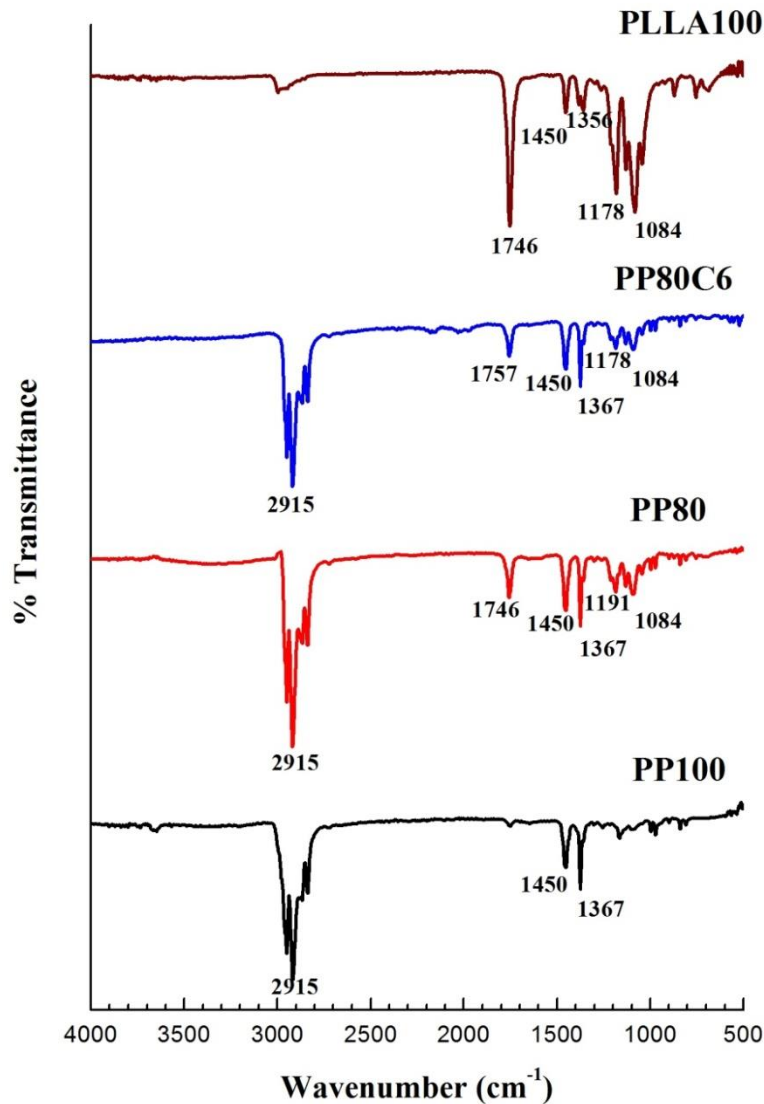


Figure 4.4 FTIR spectra of PP100, PP80, PP80C6 and PLLA100

4.2.3 Morphology (SEM)

The morphology of blends is important in determining the structure as well as property relationship. It provides information such as particle size, the distribution of components in binary blend, the crystalline phase, the consequence of interfacial addition on particle size, dispersal or agglomeration of particles, as well as distribution of fillers in the blend (Bijarimi et al., 2012). The mechanical properties are also influenced by the morphology of polymers; therefore, SEM analysis was carried out to better understand the correlation with morphology and structure of blends. The scanning electron micrographs of PP100, PP80, PP80C6, and PLLA100 are shown in Figure 4.5.

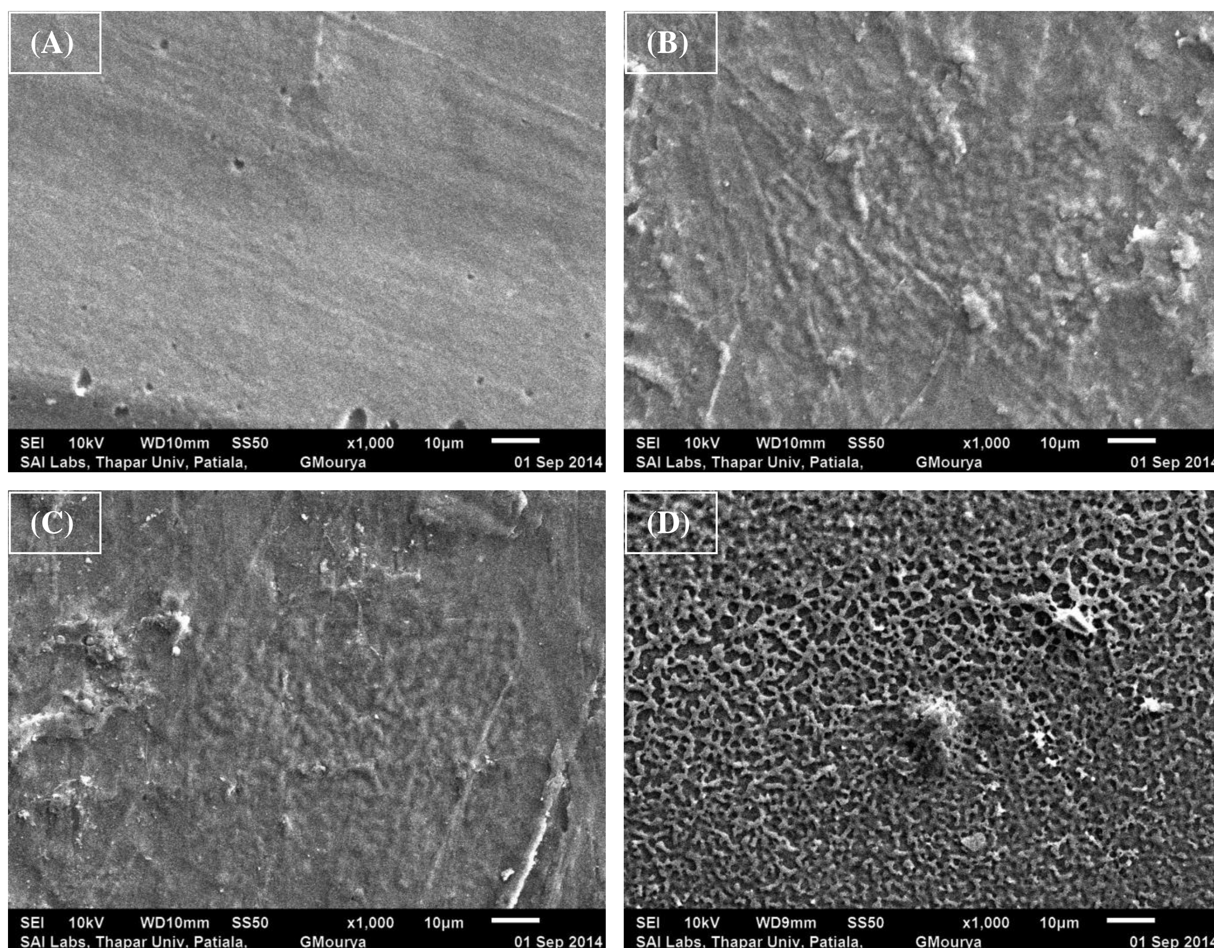


Figure 4.5 Scanning electron micrographs of (A) PP100, (B) PP80, (C) PP80C6, and (D) PLLA100 at 1000x magnification

The micrographs revealed that the surface of PP100 was smooth (Figure 4.5A) whereas the surface of PLLA100 (Figure 4.5B) was rough that contains some cracks and pits due to the brittle nature of PLLA. The micrographs of the blend PP80 showed dispersion of PLLA in PP matrix (Figure 4.5C), but phase separation and boundary layer between the two components could be seen evidently. It depicts poor interfacial adhesion and immiscibility between the two components. This was the actual cause of lower mechanical strength of PP80 too as compared to PP100. However, the scanning electron micrograph of compatibilized blend i.e., PP80C6 (Figure 4.5D) showed better adhesion between PP and PLLA. As MAPP improves the miscibility between PP and PLLA, therefore PLLA gets uniformly dispersed in the PP matrix resulting in increase in the tensile strength too. Therefore, it may be concluded that MAPP was successful in improving the interfacial adhesion between PP and PLLA due to the chemical bond formed between PLLA and MAPP. Similar morphological features have been reported previously in the literature for blends of polypropylene with other biodegradable

polymers. Kaczmarek et al. (2005) reported that PP exhibited a smooth surface while cellulose showed entangled fibres of various lengths. On blending these two polymers, a surface with high inhomogeneity is produced and the roughness of surface increased. Choudhary et al. (2011) reported that SEM micrographs of PLA/PP blends revealed two phase morphology whereas the addition of compatibilizer (maleic anhydride grafted PP) led to improved miscibility between PP and PLA. Sadi et al. (2012) reported that blend of PP-PHB without compatibilizer showed poor adhesion between the two phases owing to disparity in the chemical structures. But addition of compatibilizer led to improved adhesion between the matrix and dispersed phase. Different types of compatibilizers were used and the best efficiency was possessed by poly(ethylene-*co*-methylacrylate-*co*-glycidylmethacrylate).

4.2.4 Thermogravimetric analysis (TGA)

TGA is an important tool to evaluate the thermal stability of polymeric materials. Higher the decomposition temperature, more is the stability of polymer or blend. In this method, the weight loss of substance after decomposition is examined as a function of temperature or time. The blending of polymers can have positive (stabilizing) effect or negative (destabilizing) effect on thermal stability of blends which can be grouped into three categories: (i) If thermal stability of a polymer blend is more than the stable constituent, then there is clear-cut enhancement in thermal stability because of blending. It confirms for the presence of strong interactions between the components of a blend, (ii) If the thermal stability of a polymer blend is less than the least stable constituent, then it indicates for the decline in thermal stability because of blending. It reveals that there are no fruitful interactions between the components of a blend and it forms phase-separated, immiscible blend (iii) If the thermal stability of a polymer blend is in-between those of the constituents then it can be improvement, decline or no change in thermal stability because of blending. For such TGA data, careful analysis is required to understand the TGA results. Hence, it can be said that the thermal stability of polymer blends is assessed comparative to that of its constituents (Biresaw et al., 2008).

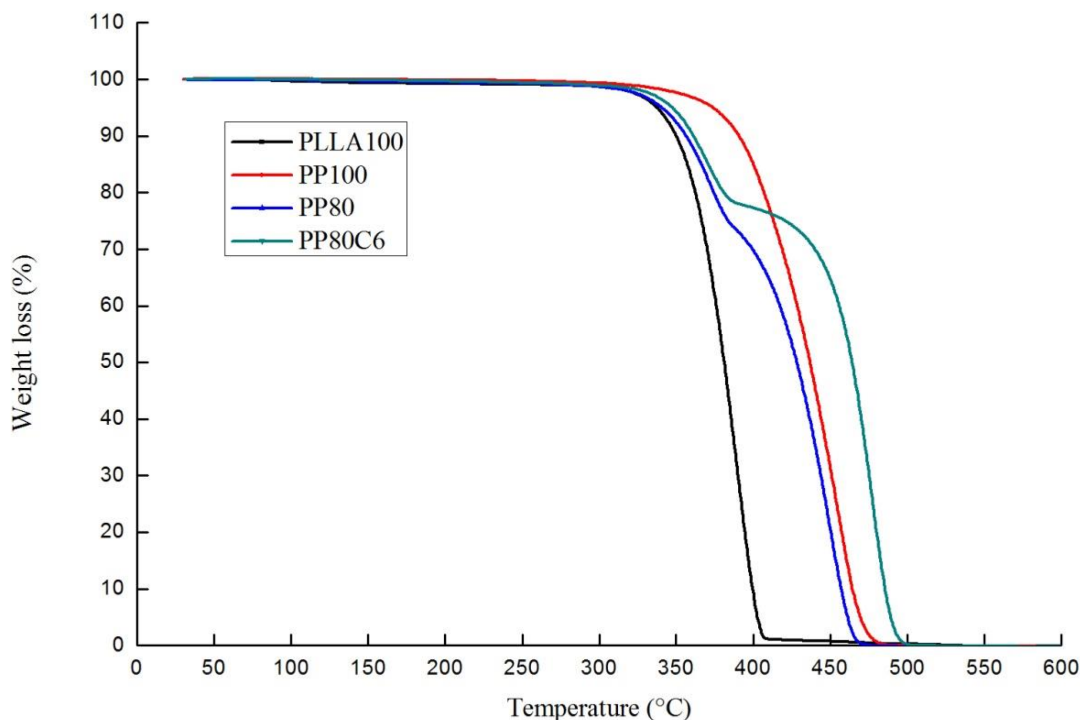


Figure 4.6 Thermogravimetric curves of virgin polymers and blends

The TG curves of PP100, PP80, PP80C6 and PLLA100 are shown in Figure 4.6. The thermograms of polymers PP100 and PLLA100 showed single stage degradation ranging from 319-478°C and 275-417°C, respectively. It implies that polypropylene is thermally more stable than poly-L-lactide. But, the thermograms of blend PP80 showed two stages of degradation. The first stage ranging from 282°C to 390°C was due to thermal decompositon of PLLA and the second stage (390°C to 470°C) was attributed to the decomposition of PP matrix. The compatibilized blend (PP80C6) also showed the two stages of degradation similar to blend PP80. The first stage ranged from 295°C to 390°C and second stage ranged from 390°C to 495°C corresponding to degradation of PLLA and PP, respectively. These results indicate for the partial compatibility between the blend components. However, it is found that degradation started earlier in PP80 (282°C) than PP80C6 (295°C) which implies that the thermal stability of PP80C6 is more than PP80. Therefore, it can be concluded that the physical and chemical bonding formed between MAPP and polymers resulted in the enhancement of thermal stability. Masood et al. (2014) reported that the mass loss of pure polypropylene occurred in one step whereas blend of PP-PHBV showed two step degradation. Choudhary et al. (2011) reported that thermal stability of PP is more than PLA.

The incorporation of 10% PP to PLA caused reduction in thermal stability of overall material which was enhanced by the incorporation of compatibilizer into the blend.

Conclusions and salient features

The effect of addition of MAPP as compatibilizer to the blends was investigated through different techniques such as FTIR, XRD, SEM and TGA. From FTIR analysis of PP80C6, it was confirmed that a chemical bond is formed between MAPP and PLLA which led to shift in the characteristic transmittance peak of C=O group. XRD analysis revealed that the crystallinity increases on addition of PLLA into PP but compatibilized blend possess the same degree of crystallinity as well as same size of crystallite as of pure polypropylene. The morphological findings showed that the PLLA was uniformly dispersed in PP matrix after addition of compatibilizer (PP80C6) in it resulting in improved interfacial adhesion as compared to the uncompatibilized blend (PP80). This was also reflected in improved mechanical properties of the blend PP80C6 as compared with PP80. Thermogravimetric analysis of pure polymers (PP100 and PLLA100) showed single stage degradation whereas blends (PP80 and PP80C6) showed two stage degradation which indicates for the partial compatibility between PP and PLLA. Moreover, the thermal stability of compatibilized blend (PP80C6) was higher than uncompatibilized blend (PP80). So, it can be concluded that the incorporation of compatibilizer in the blends resulted in improved interactions between the two polymers and led to increase in mechanical strength too as compared to the blend without compatibilizer. Hence, the results suggested that MAPP was efficient in reducing the interfacial tension between the two immiscible polymers (PP and PLLA).

4.3 Abiotic and biotic degradation studies

Degradation is a desired feature in polymers designed for single use purposes such as packaging and mulching, where gathering of post-use plastic waste create realistic difficulties. It can be categorized into two groups according to the factors responsible for degradation: abiotic and biotic. Abiotic degradation takes place due to environmental factors such as temperature, UV irradiation etc. while biotic degradation/biodegradation occurs due to the action of microorganisms like bacteria or fungi which modify and consume the polymer leading to changes in its properties. In nature, both the methods of degradation occur co-operatively (Restrepo-Flórez et al., 2014). During the abiotic oxidation, low molecular weight products are formed which are further degraded and mineralized by microorganisms to form new oxidized products. The biodegradation is a cheap and widely accepted method. It is of two kinds: aerobic and anaerobic. The degradation which occurs in the presence of oxygen is known as aerobic degradation and it produces CO_2 and water as by-products. On the other hand, the degradation that occurs in the absence of oxygen is known as anaerobic degradation and it produces CO_2, methane and water as by-products. In fact, the mineralisation of long chain polymers to CO_2 and water is a complex process which requires different microorganisms: (i) which lead to breakdown of polymers into its monomers, (ii) which consumes the monomers produced and release simple compounds as by-products, (iii) which utilizes the simple compounds (Sangale et al., 2012). Biodegradation of polymers depend on several factors such as molecular weight; branched or unbranched; biological factors and physico-chemical factors. The most significant factor is the molecular weight. With increase in molecular mass, tendency towards biodegradation decreases. The limit of molecular mass for linear paraffins has been reported to a maximum of 620 (n-tetratetracontane, $C_{44}H_{90}$). In low molecular weight paraffins, the microbial growth increases linearly on solid hydrocarbons whereas exponential growth is found on liquid hydrocarbons. The degradation of long chain n-alkanes start with the oxidation of terminal methyl groups which end up at the formation of fatty acids. The fatty acids enter the β-oxidation pathway by which acetyl-CoA is formed after the cleavage of two carbons (Roy et al., 2014).

During the process of biodegradation, biomass is also formed along with low molecular weight compounds and it increases with the growth of microbes. Thus, biodegradation of polymers can be determined by different methods such as measuring the chemical

characteristics of the new products produced, physical deterioration, and the examination of bacterial growth on the polymer surface (Jayasekara et al., 2005).

4.3.1 Isolation and identification of polypropylene degrading bacteria

The present study was carried out to isolate those microorganisms in compost having the capability to use PP as carbon source, leading to biodegradation of the polymer. The isolated bacteria were identified through biochemical characteristics as well as 16S rRNA sequence analysis. Compost was obtained from Municipal Council, Okhla, New Delhi. The sample was cleaned manually to remove large inert materials and sieved on a screen of less than 2 mm. The physiochemical characteristics of the compost are depicted in Table 4.3.

Table 4.3 Physicochemical characteristics of compost

S. No.	Parameter	Concentration
1.	pH	7.2
2.	Total solids (at 100-105°C)	81%
3.	Volatile solids (at 550°C)	18%
4.	Organic Carbon	4.3%
5.	Organic matter	7.4%
6.	Available Phosphorus (Olsen Phosphorus)	19 mg/kg
7.	Total Phosphorus	1051 mg/kg
8.	Total Nitrogen	0.7%

The ability of compost microflora to degrade polypropylene was assessed by isolating bacteria living in presence of polypropylene. The use of no carbon source in synthetic media except the polypropylene itself ensured that the microorganisms were degrading only PP. Sixteen bacterial isolates were isolated from composted sample capable of consuming polypropylene as a solitary carbon source. The isolates were named serially from P1 to P16. The individual colonies of bacteria were grown on nutrient agar medium and maintained at 4°C. Afterwards, the isolated bacterial cultures were spread on agar plates containing 1 ml/l of hexadecane and incubated at 58°C for 1 week to observe the clear zone formed. On the

basis of clear zone formation, five bacterial isolates (P3, P6, P8, P10 and P13) were selected for further studies.

4.3.1.1 Morphological and biochemical characterization of bacteria

The selected bacteria were studied for their morphological and biochemical features which are tabulated in Table 4.4. All the bacterial isolates were found to be Gram positive and rod shaped. For capsule staining, all the isolates except P3 showed negative reaction. P8 and P13 were oxidase positive while P3, P6 and P10 were catalase positive. All the isolates except P3 were able to reduce nitrate. Starch hydrolysis was observed by P3, P6, and P8. Simmon's citrate agar test was positive only for P6 while rest other isolates showed negative reaction. The characteristics of isolates were compared to that of bacteria found in literature as shown in Table 4.4.

Table 4.4 Biochemical characteristics of bacterial isolates of present study

Characteristics	**Bacteria**							
	P3	**P6**	**P8**	**P10**	**P13**	***B. licheniformis*[a]**	***B. cereus*[b]**	***B. thuringenesis*[c]**
Gram staining	+	+	+	+	+	+	+	+
Morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Capsule staining	+	-	-	-	-			
Oxidase test	-	-	+	-	+	Variable	-	+
Catalase test	+	+	-	+	-	+	+	+
Nitrate reduction test	-	+	+	+	+	+	+	+
Starch hydrolysis test	+	+	+	-	-	+	+	+
Simmon's citrate agar test	-	+	-	-	-	+	+	-

[a]Ghani et al. (2013), [b]Liang et al. (2015), [c]Wu et al. (2015)

+ Positive reaction, - Negative reaction

(a) Carbohydrate utilization

The ability to ferment various carbohydrates by these bacterial isolates was determined. Majority of the isolates were capable of fermenting different carbon substrates (Table 4.5). P3 was able to ferment eight carbon sources, P6 fermented seven carbon sources, P8 and P10 fermented five carbon sources while P13 fermented only two carbon sources.

(b) Antibiotic susceptibility

Bacterial isolates were checked for their sensitivity to different antibiotics too (Table 4.5). Total of eight different antibiotics were used. P8 and P10 were found to be resistant to some antibiotics whereas rest all other isolates (P3, P6 and P13) were sensitive to all the antibiotics used in the present study. P8 was resistant to Penicillin G and Co-trimoxazole whereas P10 isolate was resistant to Co-trimoxazole and Gentamicin.

Table 4.5 Fermentation of carbon substrates and antibiotic profiling of bacterial isolates of the present study

Bacterial isolate	Carbohydrates utilized[#]	Antibiotic sensitivity*
P3	Maltose, Fructose, Dextrose, Galactose, Raffinose, Trehalose, Melibiose, Sucrose	Penicillin G, Vancomycin, Cephalothin, Clindamycin, Co-Trimoxazole, Erythromycin, Gentamicin, Ofloxacin
P6	Maltose, Fructose, Dextrose, Trehalose, Sucrose , L-arabinose, Mannose	Penicillin G, Vancomycin, Cephalothin, Clindamycin, Co-Trimoxazole, Erythromycin, Gentamicin, Ofloxacin
P8	Maltose, Fructose, Dextrose, Trehalose, Sucrose	Vancomycin, Cephalothin, Clindamycin, Erythromycin, Gentamicin, Ofloxacin
P10	Maltose, Dextrose, Trehalose, Sucrose , Mannose	Penicillin G, Vancomycin, Cephalothin, Clindamycin, Erythromycin, Ofloxacin

P13	Dextrose, Trehalose	Penicillin G, Vancomycin, Cephalothin, Clindamycin, Co-Trimoxazole, Erythromycin, Gentamicin, Ofloxacin

[#]Total of 12-carbohydrate fermentation tests was performed with isolated bacterial species using HiCarbo Kit (Himedia Lab., Bombay, India).

*Total 8 antibiotics were used with isolated bacteria using octodiscs (Hi-Media Lab., Mumbai, India).

4.3.1.2 Molecular characterization of isolates

The selected five bacterial isolates were also characterized by amplification of 16S rRNA gene using universal primer, and amplicon of about 1.5 kb was observed in all the isolates (Figure 4.7). 16S rRNA amplified products were purified and ligated into pTZ57R/T vector. Ligated products were transformed into *E. coli DH5α* cells and the DNA sequences were generated using Applied Biosystems automatic sequencer. The primers used for sequencing reactions were M13-F and M13-R.

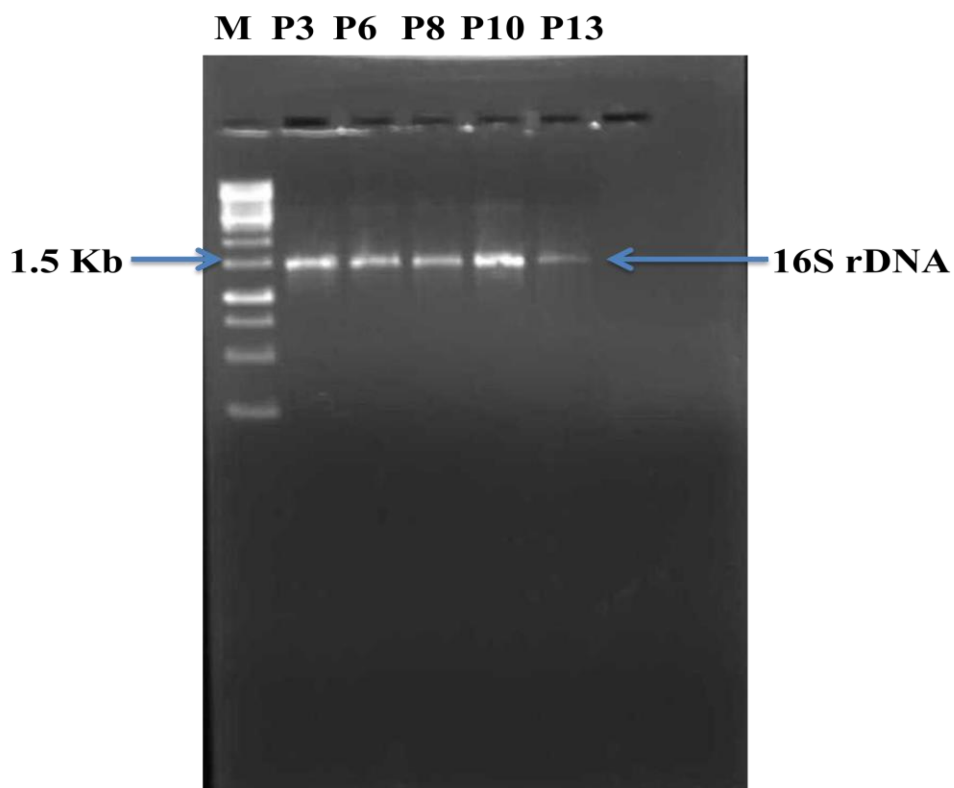


Figure 4.7 16S rDNA amplification of bacterial isolates, Lane M: 1 Kb marker (Fermentas)

The sequences generated were compared for the similarity in the EzTaxon-e database as shown in Table 4.6. The results showed that isolate P3 is 99.8% similar with *Bacillus cereus,* P6 is 99.8% similar with *Bacillus licheniformis*, P8 is 99.6% similar with *Bacillus thuringenesis,* P10 is 99.6% similar with *B. thuringenesis* and P13 is 99.7% similar with *B. cereus*. It has been reported that sequence identities of over 98% represent same species while the sequences sharing an identity below 98% are generally regarded as part of the same genus (Sadowsky et al., 1996).

Table 4.6 Bacterial isolates and their closest relative species inferred from 16S rRNA gene sequences of EzTaxon-e database

Bacterial isolate	**Nearest match**	**Phylum**	**Query coverage (%)**	**Percentage similarity (%)**	**NCBI Accession no.**
P3	*Bacillus cereus*	Firmicutes	94.2	99.78	KT267059
P6	*Bacillus licheniformis*	Firmicutes	100.0	99.80	KT267060
P8	*Bacillus thuringenesis*	Firmicutes	94.3	99.57	KT267061
P10	*Bacillus thuringenesis*	Firmicutes	94.2	99.57	KT267062
P13	*Bacillus cereus*	Firmicutes	94.3	99.72	KT267063

The related sequences showing similarity in database were retrieved from GenBank and aligned using ClustalW program (multiple sequence alignment) to check the similarities among the isolates (Table 4.7). The homologies among the sequences varied from 93 to 99% among isolates. Minimum of 93% similarity was found in P6 with P13 and maximum 99% similarity was found in P3 with P10; P8 with P10; and P10 with P13 (Table 4.7).

Table 4.7 The homologies among the sequences as calculated by Clustalw (multiple sequence alignment):

SeqA	Name	Length	SeqB	Name	Length	Score
1	P3	1408	2	P6	1513	94.03
1	P3	1408	3	P8	1410	99.64
1	P3	1408	4	P10	1408	99.72
1	P3	1408	5	P13	1412	99.64
2	P6	1513	3	P8	1410	93.9
2	P6	1513	4	P10	1408	93.96
2	P6	1513	5	P13	1412	93.63
3	P8	1410	4	P10	1408	99.72
3	P8	1410	5	P13	1412	99.5
4	P10	1408	5	P13	1412	99.72

The phylogenetic tree was constructed using neighbour-joining method of MEGA 6.0 package. For analysis, 1000 bootstrap replicates were performed to assess the statistical support for the tree. Gaps were treated as missing data. *E. coli* was used as an outgroup taxa. Phylogenetic tree separated the sequences into different clusters. Isolates P3, P8, P10 and P13 were grouped into one cluster mainly consists of *Bacillus cereus* and *B. thuringenesis*. However isolate P6 formed a separate cluster with *B. licheniformis* sequences (Figure 4.8). The 16S rRNA gene sequences of the present study bacteria have been deposited in NCBI database under the accession numbers KT267059, KT267060, KT267061, KT267062 and KT267063 for isolates P3, P6, P8, P10 and P13 isolates, respectively (Appendix II).

These bacteria are well-known for their degradation capability. *B. cereus* is a ubiquitous soil bacterium which produces a variety of hydrolytic enzymes like proteases by which it can degrade feather, wool, hair, horn keratin, fibrous protein (Laba et al., 2015). *B. licheniformis* secrete various hydrolytic enzymes that enable it to grow on different substrates. It is generally regarded as safe (GRAS) bacteria and used at large scale to produce exoenzymes

(Parrado et al., 2014). *B. thuringiensis* is a widely used biopesticide and produces insecticidal crystal proteins (ICPs). Besides this, it has the capability to remove various chemical wastes like diesel fuel, dimethyl phthalate, pentachlorophenol, alachlor, and organophosphorus pesticides (Chen et al., 2015).

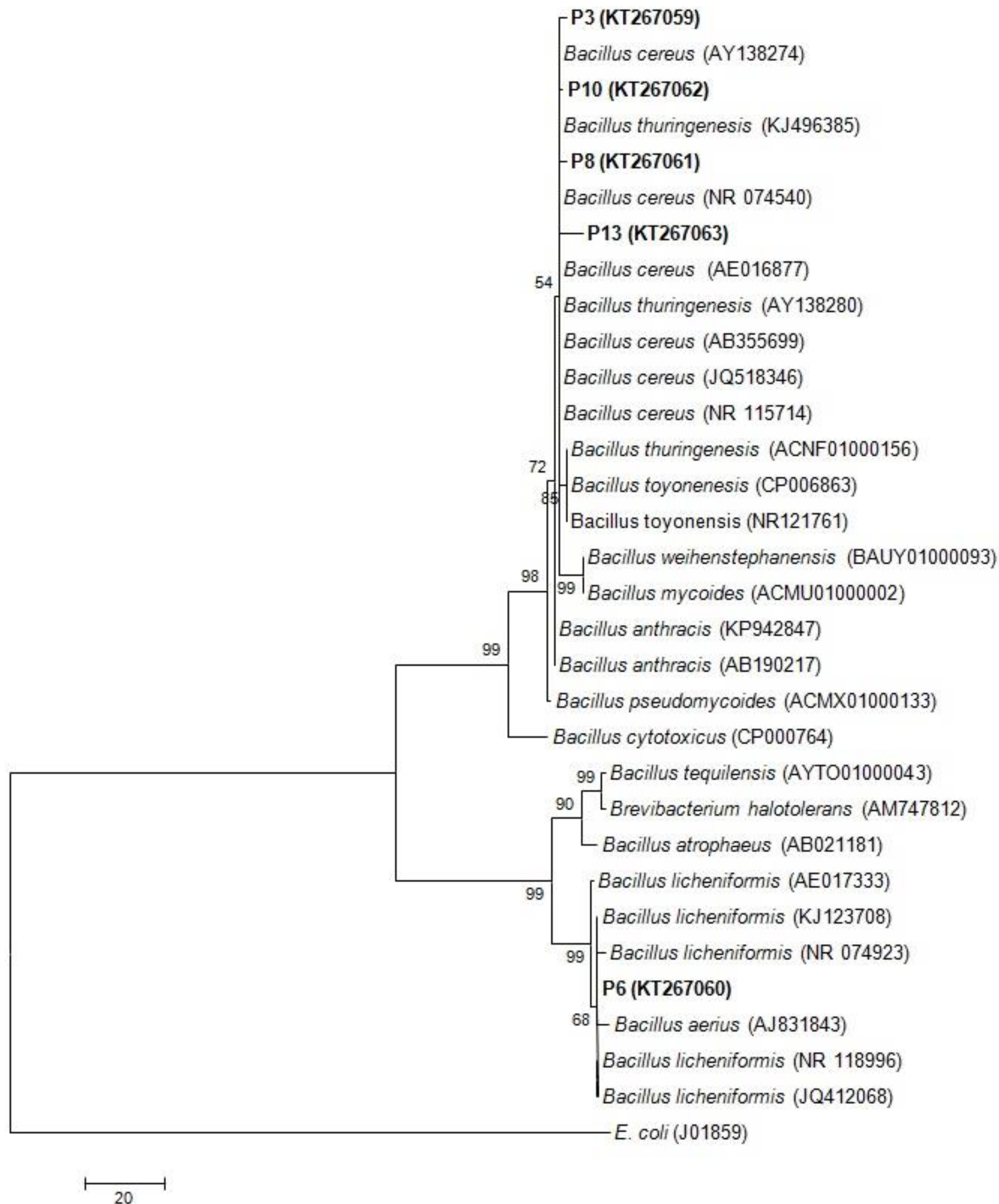


Figure 4.8 Neighbour-joining tree from 16S rRNA sequences showing the relationship of present study isolates and other closely related *Bacillus* species retrieved from the GenBank (accession number). Bootstrap values (1000 replicates) are shown on the branches

4.3.2 Biodegradation of polymers using bacterial isolates in liquid media

The biodegradation tests of polymers with five identified bacteria within 15 days of incubation were carried out in synthetic media. The analysis of biodegradation was carried out using growth curve of bacteria, protein estimation, FTIR, SEM and TGA analysis.

4.3.2.1 Methods of analysis

(a) Growth of bacteria

The degradation ability was monitored by determining the growth of bacteria in minimal medium supplemented with polymer films and the profile for these bacteria are shown in Figure 4.9. The bacterial isolates showed a diauxic growth in presence of polymers which is due to the fact that bacteria first utilized the easily oxidisable carbon source i.e. glucose followed by utilization of polymers for their carbon and energy source (Figure 4.9). But, only one lag phase was found in control samples indicating that the isolates were growing only in the presence of glucose. This proves for the utilization of polymers by the isolates as their carbon source. The maximum growth was observed in case of isolates P8 and P10 (Figure 4.9c and 4.9d) whereas the least in P6 (Figure 4.9b). Besides it, the turbidity (optical density) was observed more in control samples as compared to polymer amended medium. This was ascribed to formation of biofilm on the polymer surface which led to decrease in turbidity of bacteria in samples as compared to control. The formation of biofilm can be an indication that isolates have the potential to degrade blends. Sivan et al. (2006) reported that polyethylene films exposed to *Rhodococcus ruber* (C208) in synthetic media showed adherence of bacterial cells to polyethylene film and moreover, the density of bacterial cells in biofilm was 1.5 times more than the planktonic cells.

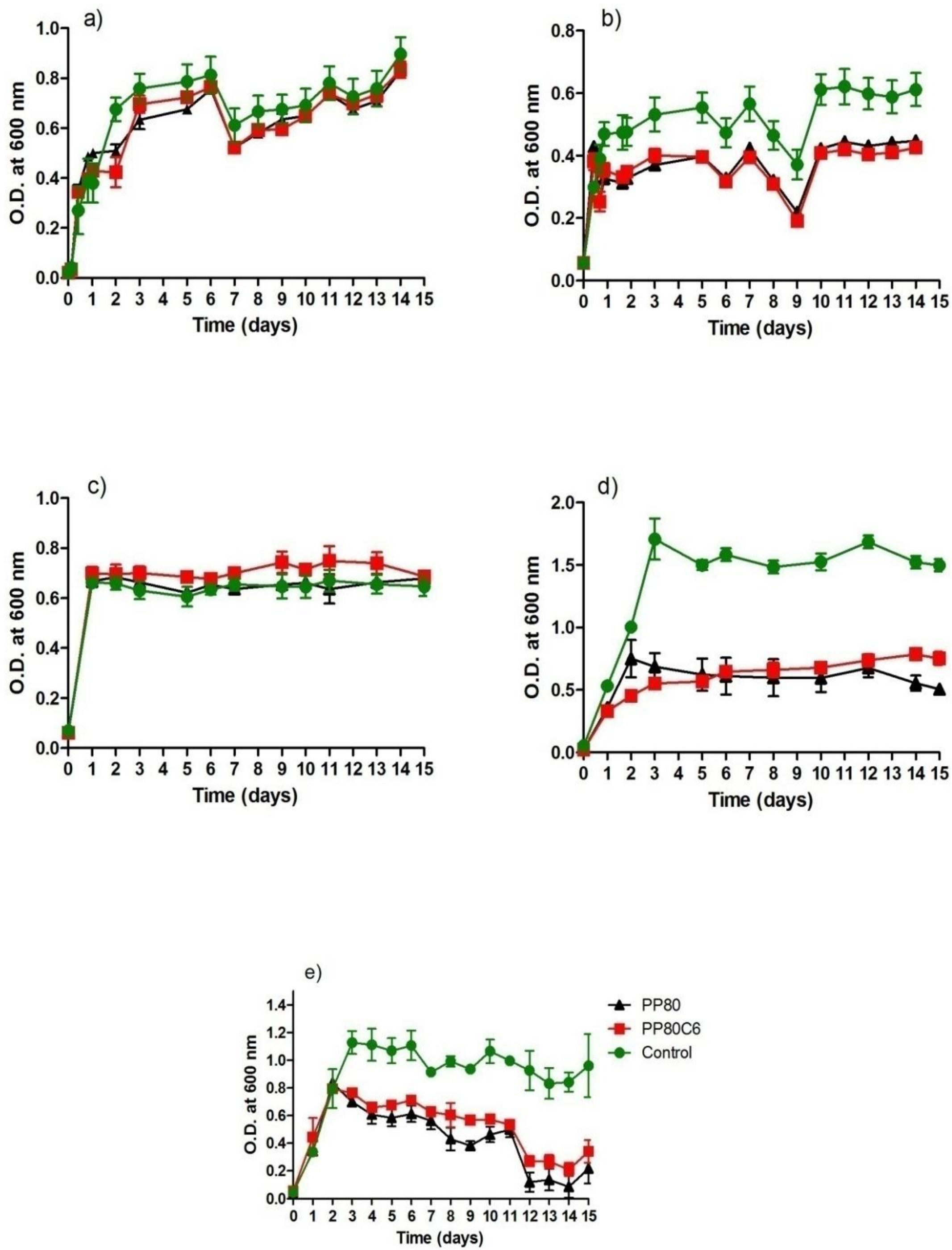


Figure 4.9 Growth curve of (a) isolate P3 (b) isolate P6 (c) isolate P8 (d) isolate P10 (e) isolate P13 in synthetic medium amended with polymer PP80

(b) Protein estimation

The protein content in control and polymer supplemented medium was estimated to observe the biomass density by bacterial isolates. It was observed that protein content in control was lower than their respective samples grown in presence of polymer (Table 4.8). For example, protein content in control was 0.117 mg/ml for isolate P3 whereas it was 0.166 mg/ml for PP80. The same trend was observed in all the isolates. This proves that the bacterial isolates were able to consume the blends as their carbon source and formed biofilm on the surface. In case of control, the maximum protein content was found to be 0.171 mg/ml secreted by P6 whereas in case of polymer amended samples, the maximum protein content was observed as 0.276 mg/ml for PP80 and 0.478 mg/ml for PP80C6 by the isolate P8. These results indicate that P8 is able to degrade the blends better than other isolates (Table 4.8). Moreover it was also found that protein content was higher in case of polymer PP80C6 than PP80 which shows that PP80C6 undergo easy degradation than PP80. The results of ANOVA (Table 4.8) indicated that all the five bacterial isolates produce different content of protein on different blends and the data is statistically significant ($P < 0.05$). Earlier reports are also found on estimation of protein content in the biofilm. Gilan et al. (2004) determined the protein concentration in the biofilm formed on the surface of UV irradiated PE by *Rhodococcus ruber*. The protein content increased rapidly during 2-3 days of incubation and then decreased sharply due to consumption of mineral oil in early stages of incubation. Hadad et al. (2005) estimated the concentration of extractable protein in the biofilm formed on the UV-irradiated polyethylene surface by *Brevibacillus borstelensis.* It was observed that the amount of extractable protein increased during the 1st day, afterwards remained constant for four days and subsequently declined till the 20th day. However, the addition of Tween 85 and mineral oil in the medium improved the colonization. Sivan et al. (2006) also reported the estmation of protein content in the biofilm formed on polyethylene surface by *R. ruber.* It increased till the 10th day of incubation and then it decreased.

Table 4.8 Protein content (mg/ml) estimated in biofilm + media of different polymers treated with bacterial isolates

Isolates	Protein content (mg/ml)		
	Control	**PP80**	**PP80C6**
B. cereus (P3)	0.117 ± 0.02bA	0.166 ± 0.00bA	0.204 ± 0.04abA
B. licheniformis (P6)	0.171 ± 0.02aB	0.235 ± 0.02abA	0.179 ± 0.01bB
B. thuringenesis (P8)	0.157 ± 0.01aB	0.276 ± 0.01aAB	0.478 ± 0.04aA
B. thuringenesis (P10)	0.119 ± 0.01bB	0.210 ± 0.02abAB	0.294 ± 0.04abA
B. cereus (P13)	0.015 ± 0.00cA	0.034 ± 0.00cA	0.023 ± 0.00cA

Values sharing a common lower case letter within the columns and common upper case letters within the rows are not significant at $P < 0.05$. Values are mean ± SD (n = 3)

(c) FTIR

The oxidation of polymers is an initial step and the attack by microbes is secondary step during the biodegradation process. Figure 4.10 shows the FTIR spectra of polymer PP80 before degradation (a), polymer PP80 after degradation in synthetic media by the isolate P3 (b), polymer PP80C6 before degradation (c), and polymer PP80C6 after degradation in minimal media with the isolate P3. Before degradation, the FTIR spectra of polymer PP80 showed peaks at 2915, 1746, 1450, 1367, 1191 and 1084 cm^{-1} which represents C-H stretching, C=O stretching, CH_3 bending, CH_2 wagging, C-O-C symmetric stretching and asymmetric CH_3 bonds, respectively. These peaks are also found in spectra of individual polymers i.e. PP and PLLA; which proves for the immiscibility between the two polymers (Figure 4.10a). After degradation, the peak at 1746 cm^{-1} was reduced drastically. This peak is associated with C=O stretching of PLLA counterpart. The reduction in intensity of this peak shows that the isolate attacked at this functional group and hence formed biofilm on the surface (Figure 4.10b). The FTIR spectra of PP80C6 before degradation (Figure 4.10c) showed peaks at 2915, 1757, 1450, 1367, 1178 and 1084 cm^{-1}. There is a new peak formed at 1757 cm^{-1} that is associated with C=O bond of ester stretch. This shows that bonding has taken place between PP and PLLA due to the addition of compatibilizer (MAPP) in the blend. The spectra of PP80C6 after degradation (Figure 4.10d) showed the reduction in intensity of

peak at 1757 cm^{-1} and formation of new peak at 2123 cm^{-1} (C≡C, alkyne bond). It means that bacteria attacked on easily available polar group (C=O ester bond) found in PP80C6 and led to decrease in the intensity of carbonyl absorption band. The formation of new alkyne bonds revealed that dehydrogenation has taken place in the presence of bacteria. So we can say that both the blends can undergo degradation if given suitable conditions.

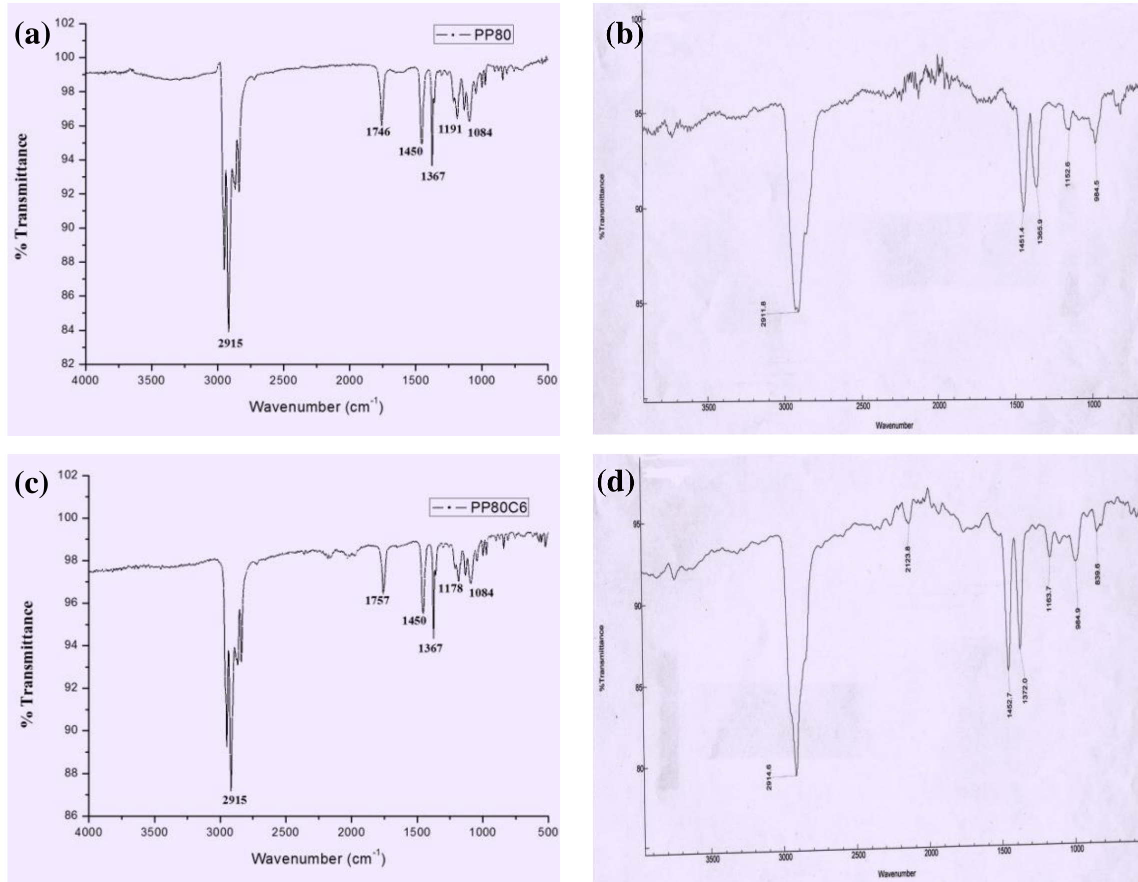


Figure 4.10 FTIR spectra of (a) PP80 before degradation; (b) PP80 after degradation; (c) PP80C6 before degradation and (d) PP80C6 after degradation by the isolate P3

(d) SEM

The formation of biofilm and surface morphology of blends after degradation was monitored by SEM technique. Figure 4.11 demonstrates the SEM images of polymer 'PP80' before and after degradation by the isolate P3. PP80 is a blend of PP and PLLA in a ratio of 80: 20. The two polymers are not miscible with each other and form a heterogeneous blend due to which the surface observed was rough as shown in Figure 4.11a. After degradation with isolate P3, some irregularities were found to appear on the polymer surface (Figure 4.11b) which shows

that surface erosion has taken place. Shah et al. (2008) reported the formation of cracks, pits, grooves, agglomerates on the polymer surface after degradation in soil due to microbial enzymatic activity.

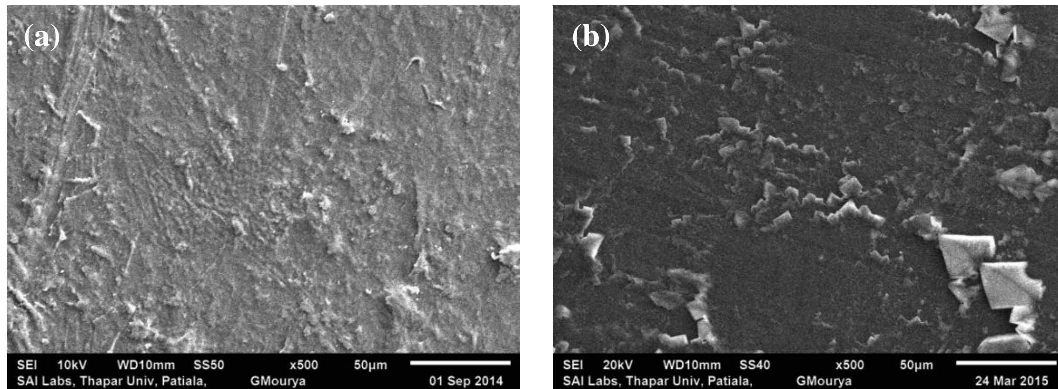


Figure 4.11 Scanning electron micrographs of polymer 'PP80' (a) before degradation and b) after degradation by isolate P3 at 500X

The biofilm formed by isolate P3 was also analyzed under scanning electron microscope and the rod shaped bacteria were seen to colonize the polymer surface (Figure 4.12). It shows that bacteria were able to adhere and colonize the polymer surface within 15 days. Sudhakar et al. (2008) reported that the biofilm formation is requisite for the consumption of polymers for microbial growth. Santhoskumar, (2014) reported the formation of biofilm and generation of pores on the surface of LDPE- additive blend after biodegradation in municipal solid compost for 90 days.

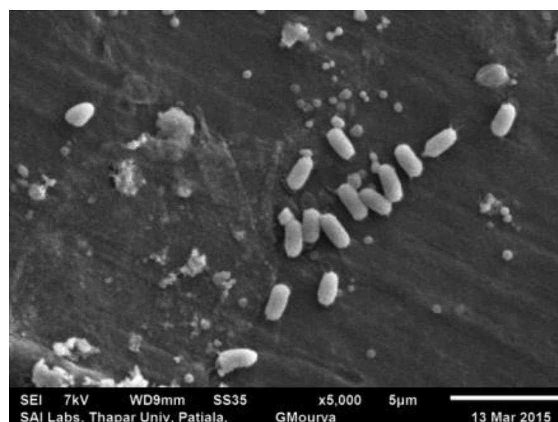


Figure 4.12 Scanning electron micrograph of biofilm formed on the surface of polymer PP80 after degradation by bacteria P3 at 5000X

(e) TGA

TGA technique is used for determining the thermal stability of a substance. It measures the weight loss of sample with respect to change in temperature. TGA allows the accurate control of conditions for example, variable temperature range and precise heating rate. Moreover, it needs little quantity of sample for testing (George et al., 1996). Figure 4.13 and Figure 4.14 shows the thermogravimetric (TG) curves and differential thermogravimetric (DTG) curves of blends, respectively before and after incubation with isolate P8 for 15 days in synthetic media supplemented with polymer. The TG curves of blends 'PP80 and PP80C6' (Figure 4.13) showed two-stage degradation indicating for the immiscibility between the two polymers; the first stage represents decomposition of PLLA and second stage corresponds to decomposition of PP. After degradation, there was decrease in thermal stability of both the blends (Figure 4.13). Moreover, both the stages in TG curves of blends are showing shift towards lower temperature. The first step of thermal degradation indicated that the bacteria were able to consume PLLA content of blends and the degradation of second step was due to decomposition of PP chains. It indicates that the thermal stability of both the components (PLLA and PP) has decreased after degradation.

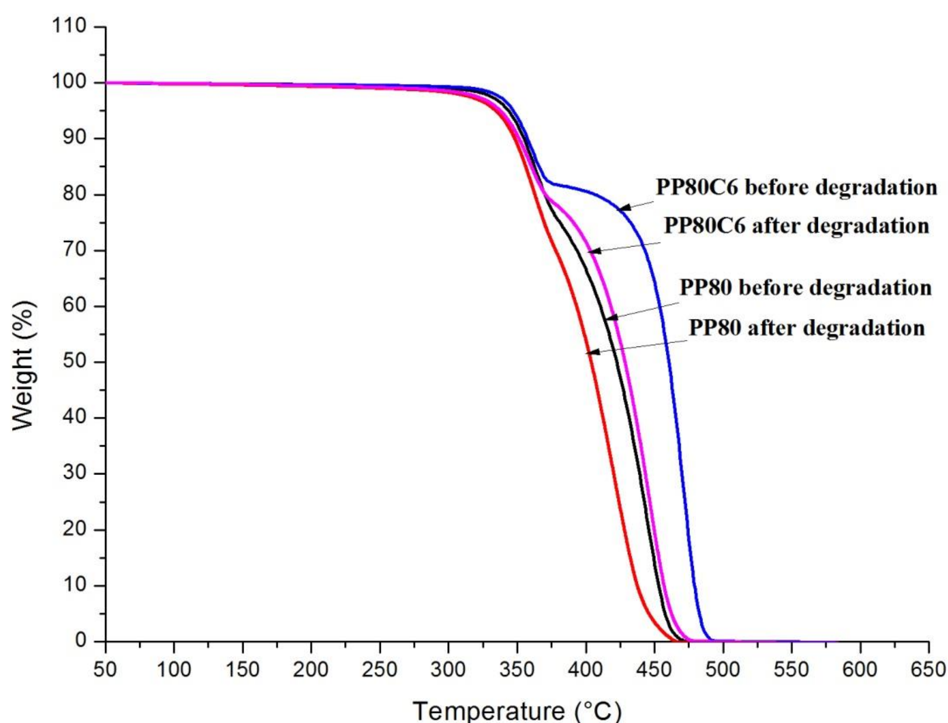


Figure 4.13 Effect of degradation on thermal stability of blend with isolate P8 (TG curves)

Before degradation, the thermal decomposition of PP80 started at around 318°C (T_i) and completed at 487°C (T_f). On the other hand, biodegraded sample of PP80 showed decrease in thermal decompositon temperatures i.e., 198°C (T_i) and 474°C (T_f). Similarly, T_i of PP80C6 decreased from 235°C to 203°C and T_f decreased from 475°C to 471°C (Table 4.9). This means that the thermal stability of blends has decreased to a good extent within 15 days by bacterial isolate P8. Morancho et al. (2006) reported that the blend of PP-starch showed two-stage degradation; the first corresponding to starch and the second corresponding to PP. After degradation via soil burial test for one year, only the first stage in TG curve was shifted towards higher temperature indicating that starch stabilized during the biodegradation process but there was no change in second stage corresponding to PP.

Table 4.9 TG analysis of polymers before and after degradation with isolate P8 in synthetic media

Polymer	**T_i (°C)**	**T_f (°C)**
PP80 before degradation	318.2	487.9
PP80 after degradation	198.1	474.9
PP80C6 before degradation	235.1	475.3
PP80C6 after degradation	203.8	471.7

The DTG curves (derivative thermogravimetric curves) also verify this degradation profile. DTG curves are the indication of the temperature at which the maximum weight loss of sample is triggered. As seen in Figure 4.14, the DTG curves of the blends possess two peaks corresponding to PLLA and PP, respectively. The maximum rate of mass loss in case of polymer PP80 before degradation was achieved at 363°C and 445°C whereas in case of PP80C6, it was observed at 360°C and 470°C. But after degradation, both the blends showed DTG peaks at significant lower rates as compared to pure samples. This proves for the occurrence of biodegradation.

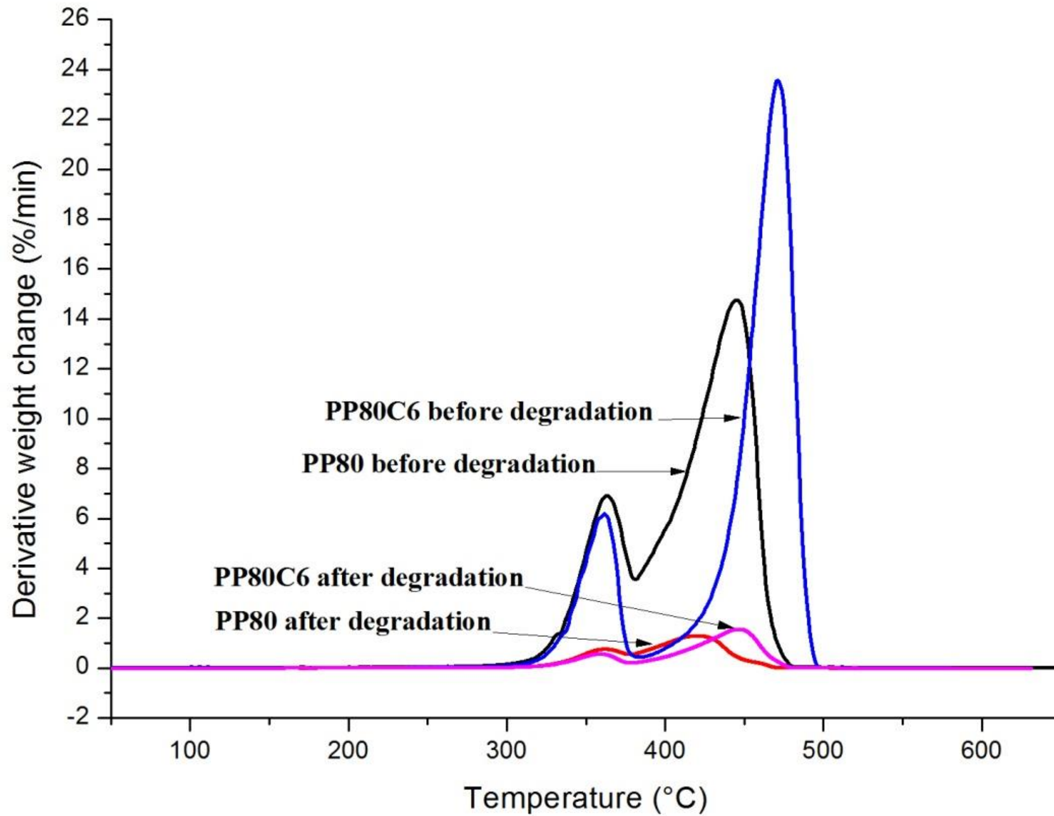


Figure 4.14 Effect of degradation on the thermal stability of blends with isolate P8 (DTG curves)

4.3.3 Microcosm studies

Lab-scale microcosms are generally used to study the biodegradation efficiencies of microorganisms and the results obtained can be used for developing corrective pilot test specifications. The microcosm studies also act as standard test methods which can be modified according to diverse environmental conditions (Farahat and El-Gendy, 2008). In fact, laboratory microcosms are representative of ecosystems by which a part of the natural environment such as soil or water can be confined and studied (Grenni et al., 2012). Moreover, it is very important to characterize the biodegradation process occurring in soil so that the destiny of contaminants in polluted soils can be determined (Arias et al., 2008).

In our study, *in-situ* microcosm experiments were set up to monitor efficiency of bacterial isolates to degrade PP-PLLA blends in soil taken from garden area of CORE, Thapar Institute

of Engineering & Technology, Patiala. The physiochemical features of soil are given in Table 4.10.

Table 4.10 Physiochemical characteristics of soil

S. No.	Parameter	Concentration
1.	pH	8.1
2.	Electrical conductivity	0.14 mS cm^{-1}
3.	Organic Carbon	0.3%
4.	Organic matter	0.5%
5.	Available Phosphorus (Olsen Phosphorus)	3.2 mg/kg
6.	Total Phosphorus	211 mg/kg
7.	Total Nitrogen	0.03%

4.3.3.1 Cfu/gm count

The growth of all the five bacterial isolates as well as of consortium (in soil) in presence or absence of polymer blends is given in Figure 4.15. The growth was monitored at a regular interval of two months and the average of three replicates was calculated. It was observed that growth of all the bacteria in soil increased with time. The maximum increase in growth or cfu/g was found in case of consortium. The CFU/g increased from 14 x 10^5 (in 2nd month) to 1067 x 10^5 (in 6th month) in case of polymer 'PP80'. In case of polymer 'PP80C6', the increase in CFU/g was 19 x 10^5 (in 2nd month) to 3033 x 10^5 (in 6th month) whereas the control microcosm having no polymer in it showed the increase from 35 x 10^5 (in 2nd month) to 3467 x 10^5 (in 6th month). It is observed that the growth of bacteria was higher in control cultures than the test sample. Similar trend was observed in all the isolates. The lower growth in test samples was attributed to the formation of bacterial biofilm on the blend surface which shows that the bacteria were proficient in utilizing the blend surface as their carbon source. The development of biofilm on surface of polymer is prerequisite to biodegradation (Gilan et al., 2004; Hadad et al., 2005). The second highest growth was found in culture P3 followed by P10, P8 and P13 whereas the least growth was found in isolate P6. But all the cultures

showed increase in growth with time which shows that all the bacterial isolates were capable to utilize polymer as the carbon source.

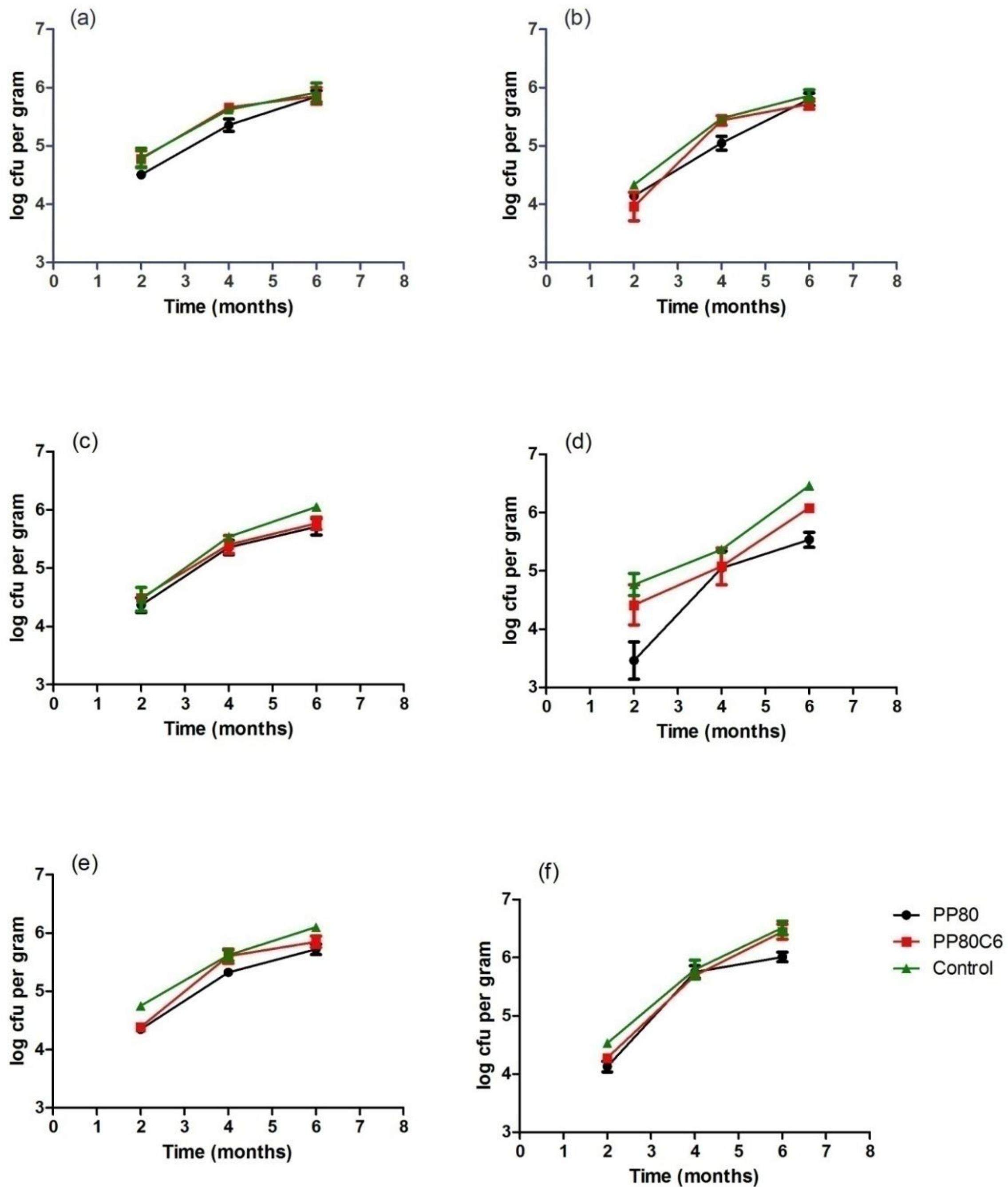


Figure 4.15 cfu/gm count of bacteria in microcosm at different intervals (a) bacterial isolate ‘P3’, (b) bacterial isolate ‘P6’, (c) bacterial isolate ‘P8’, (d) bacterial isolate ‘P10’, (e) bacterial isolate ‘P13’ and (f) consortium

4.3.3.2 FTIR

FTIR helps in identifying the functional groups present in a compound. During the degradation process, new functional groups are formed and old bonds are broken which can be identified by FTIR technique. The main region in FTIR spectra is 1700-1800 cm^{-1} which indicates the presence of oxidized groups (Arkatkar et al., 2009a; Arkatkar et al., 2010). In our study two polymer blends were used for degradation study- PP80 and PP80C6. PP80 is a blend of PP and PLLA in a ratio of 80:20. The two polymers are immiscible with each other and form a heterogenous blend. The main peaks found in FTIR spectra of PP80 (Figure 4.16) were 2915 cm^{-1}, 1746 cm^{-1}, 1450 cm^{-1}, 1367 cm^{-1}, 1191 cm^{-1}, and 1084 cm^{-1} which are associated with C-H stretching, C=O stretching, C-H bending, CH_3 bending, CH_2 wagging, C-O-C symmetric stretching, and asymmetric CH_3 bonds, respectively. The peaks at 2915 cm^{-1}, 1450 cm^{-1} and 1367 cm^{-1} are found in spectra of pure PP and peaks at 1746 cm^{-1}, 1191 cm^{-1}, and 1084 cm^{-1} are found in spectra of pure PLLA; indicating for the absence of chemical interactions between the two polymers. After degradation in soil for six months, the spectra of polymer blend PP80 showed some changes. All the isolates showed the decrease in intensity of peak at 1746 cm^{-1} corresponding to C=O stretching of PLLA counterpart. This shows that bacteria were capable to utilize blend as the carbon source by attacking on carbonyl bonds. Several reports show that there is a reduction in carbonyl group on exposure to microorganisms (Chiellini et al., 2003; Gilan et al., 2004; Hadad et al., 2005; Arkatkar et al., 2009b; Arkatkar et al., 2010). The decrease in peak related to carbonyl bond can be due to chain scission resulting in the reduction in molar mass of polymer.

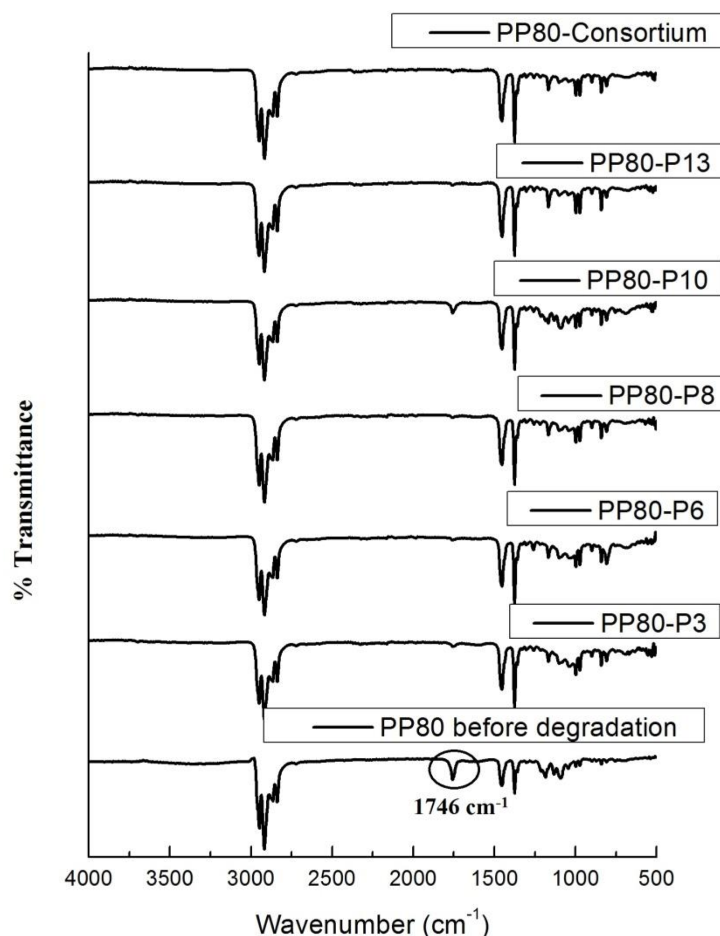


Figure 4.16 FTIR spectra of polymer PP80 treated with different bacterial isolates as well as consortium

Similarly, the spectra of PP80C6 (Figure 4.17) shows major peaks at 2915 cm^{-1}, 1757 cm^{-1}, 1450 cm^{-1}, 1367 cm^{-1}, 1178 cm^{-1} and 1084 cm^{-1}. There is emergence of new peak at 1757 cm^{-1} representing the carbonyl bond of ester stretching. This shows that addition of compatibilizer (MAPP) helped in improving miscibility between two polymers. But after degradation by various isolates, this peak was found to be reduced in intensity. This also proves for the degradation of polymer blend PP80C6 and their utilization by bacteria as carbon source.

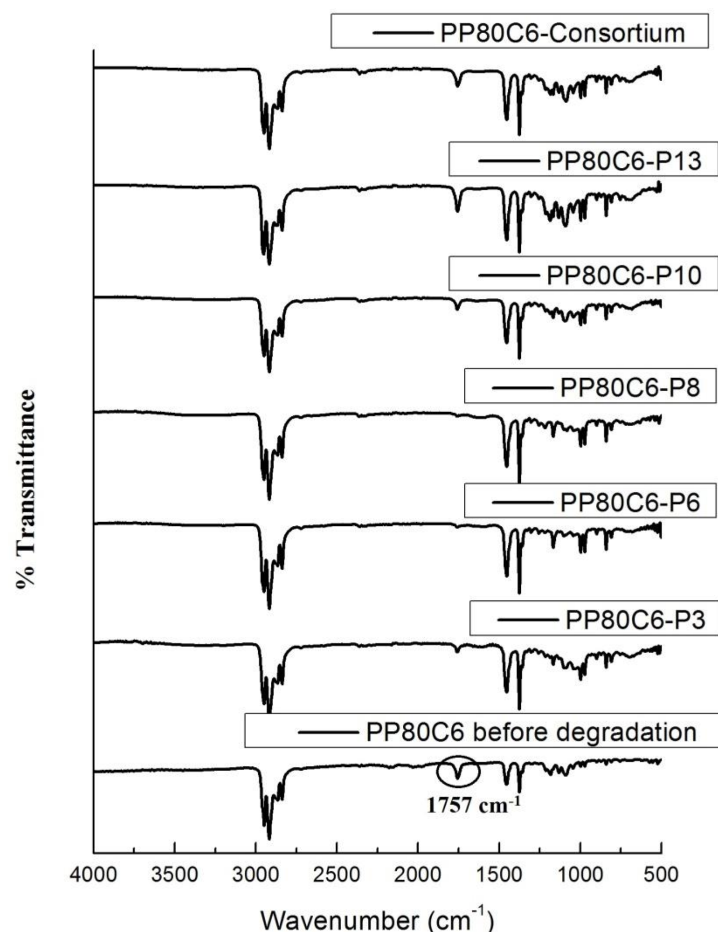


Figure 4.17 FTIR spectra of polymer PP80C6 treated with different bacterial isolates as well as consortium

4.3.3.3 TGA

TGA analysis was employed to determine thermal stability of blends after ageing with bacteria in soil as shown in Figure 4.18. The values of T_i and T_f temperatures are shown in Table 4.11. It was observed that the values of both T_i and T_f decreased after degradation with each of the isolate but the values of T_f are very close to each other. Therefore, based on the decrease in values of T_i, the efficiency of cultures to degrade polymers is P8 > P3 > Consortium > P13 > P6 > P10 which implies that the largest decrease in thermal stability was caused by P8 and the least by P10. Martelli et al. (2009) also reported that both onset and maximum degradation temperatures of LDPE/PHB blend decreased after soil burial test for 180 days. But, Masood et al. (2014) reported the increase in thermal stability of PP/PHBV blend after soil burial due to conversion into brittle and crystalline material.

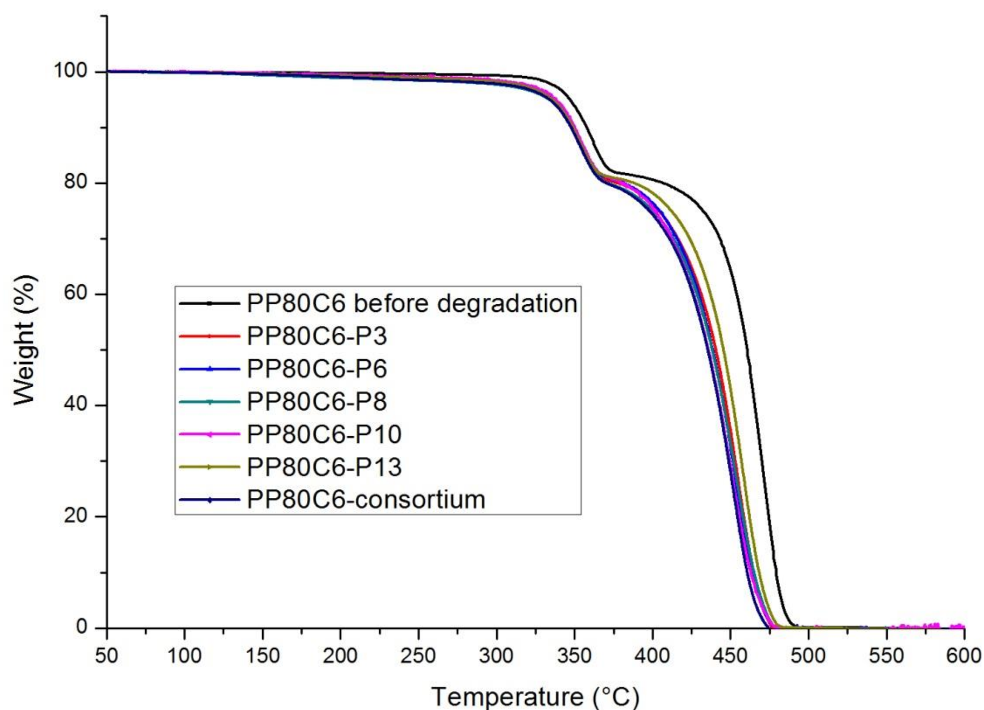


Figure 4.18 Effect of degradation on thermal stability of blend PP80C6 with different isolates and consortium

Table 4.11 TG analysis of polymers before and after aging with bacteria in microcosm studies

Samples	**T_i (°C)**	**T_f (°C)**
PP80C6 before degradation	318.2	487.9
PP80C6- P3	198.1	474.9
PP80C6-P6	235.1	475.3
PP80C6-P8	186.7	476.1
PP80C6-P10	259.8	475.7
PP80C6-P13	231.5	478.9
PP80C6-consortium	203.8	471.7

4.3.4 Aerobic biodegradation of polymers

The aerobic biodegradation occurs in the presence of oxygen in which the microorganisms attack on polymer molecules and the carbon present in polymer gets converted into carbon dioxide (CO_2), water and biomass. So, the extent of biodegradation can be determined by calculating the amount of CO_2 released from the polymer sample. But it does not involve the amount of carbon that gets transformed to biomass which in turn, is not mineralised to produce CO_2 during the test.

The biodegradability of blends PP80 and PP80C6 via bacterial isolates was studied under aerobic conditions according to ASTM D5338-98 (modified). The test was conducted using soil taken from garden area of CORE, Thapar Institute of Engineering & Technology, Patiala. It was loamy sand in texture, having pH 8.1; electric conductivity 0.14 mS/cm; organic carbon 0.3%; organic matter 0.5%; total Phosphorus 211 mg/kg; available Phosphorus 3.2 mg/kg and total nitrogen 0.03%. The theoretical amount of carbon (%) present in the polymers was determined using CHNS analyzer whose values are depicted in Table 4.12. From the values of theoretical carbon (%), the theoretical amount of CO_2 evolved was calculated as follows:

$$CO_2 \text{ (theoretical) for cellulose} = \frac{0.222*44}{12} = 0.815$$

$$\text{Similarly, } CO_2 \text{ (theoretical) for PP80 sample} = \frac{0.395*44}{12} = 1.445$$

$$CO_2 \text{ (theoretical) for PP80C6 sample} = \frac{0.405*44}{12} = 1.49$$

Here, 44 and 12 are the molecular weights of CO_2 and carbon, respectively.

Since, we have taken 500 mg of each sample in vessels; therefore, theoretical amount of carbon dioxide was also calculated for 0.5 g sample (Table 4.12).

Table 4.12 Theoretical carbon content (%) and theoretical amount of CO_2 (g) evolved from cellulose and polymer blend samples

Sample	C-content by CHNS analyzer (%)	Weight of carbon in 0.5 g of polymer (g)	Theoretical CO_2 evolution (g) from 0.5 g of sample
Cellulose	44.4	0.222	0.815
PP80	78.5	0.395	1.45
PP80C6	81.4	0.405	1.49

The results of cumulative carbon dioxide produced (in grams) by different cultures utilizing polymer as the sole carbon source are presented in Figure 4.19 as well as the values are depicted in Table 4.13. It can be deduced that cellulose started to degrade within 5 days of incubation and other polymers also showed beginning of CO_2 evolution within 5 days of incubation but to a much lesser extent. The maximum amount of CO_2 was released by the cellulose, as expected (0.591 g). Among the test samples, the blend PP80C6 degraded by isolate P3 (i.e., PP80C6-P3) produced the highest amount (0.184 g) followed by PP80-P8 (0.176 g), PP80-P3 (0.173 g), PP80-P10 (0.168 g), PP80-P13 (0.163 g), PP80-P6 (0.151 g), PP80C6-P6 (0.140 g) and PP80C6-P8 (0.135 g). The results of ANOVA (Table 4.13) show that values for carbon dioxide evolved (g) are different from one another and data is quite significant at $P < 0.05$.

The percent biodegradation of polymer blends degraded by different cultures is shown in Figure 4.20 and the values are depicted in Table 4.13. The percent biodegradation was calculated according to the Eq. 3.3. The calculations for percent biodegradation can be understood by illustrating an example.

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

Therefore, Molarity of $Ba(OH)_2$ = $\frac{0.024}{2}$ = 0.012 M

Volume of $Ba(OH)_2$ taken for titration was = 30 ml

Table 4.13 CO_2 evolved (in grams) by the bacterial isolates and Cumulative percentage biodegradation at the end of test

Samples	Cumulative CO_2 produced (g)	Percent biodegradation (%)
Cellulose (Positive reference)	0.591 ± 0.030 a	72.40 ± 3.62 a
PP80-P3	0.173 ± 0.009 bc	11.94 ± 0.59 b
PP80-P6	0.151 ± 0.008 bcd	10.39 ± 0.52 b
PP80-P8	0.176 ± 0.009 b	12.09 ± 0.61 b
PP80-P10	0.168 ± 0.008 bcd	11.57 ± 0.58 b
PP80-P13	0.163 ± 0.008 bcd	11.24 ± 0.56 b
PP80C6-P3	0.184 ± 0.009 b	12.27 ± 0.61 b
PP80C6-P6	0.140 ±0.007 cd	9.34 ± 0.47 b
PP80C6-P8	0.135 ± 0.007 d	9.01 ± 0.45 b

Values sharing common letter within the column are not significant at $P < 0.05$. Values are mean ± SD (n=3).

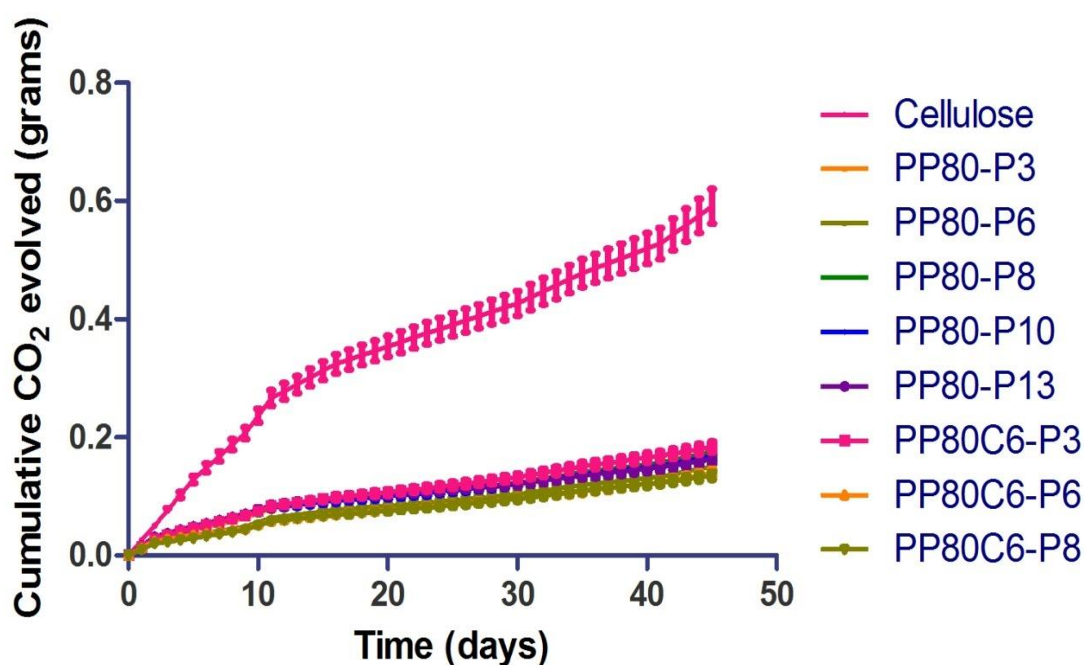


Figure 4.19 Cumulative amount of carbon dioxide produced (grams) by the polymer blends and cellulose after incubation with bacterial isolates for 45 days

So, mmoles of $Ba(OH)_2$ taken = 30 x 0.012 = 0.36 mmoles

Taking an example of PP80 degraded by P3 on 2nd day:

Volume of HCl used = 2.2 ml

Normality of HCl was = 0.05

So, Molarity of HCl = 0.05

Hence, mmoles of HCl used = 2.2 x 0.05 = 0.1079 mmoles

Now,

mmoles of CO_2 = mmoles of $Ba(OH)_2$ at start – mmoles of HCl/2

= 0.36 – 0.1079/2

= 0.3060 mmoles

As we know that,

1 mol of CO_2 = 44 g/L

So, 1 mmol of CO_2 = 44 x 10^{-3} = 0.044 g/L

Hence, grams of CO_2 produced = 0.3060 x 0.044 = 0.013466 g

In this way, the readings were taken at regular intervals for 45 days and the cumulative amount of CO_2 produced for PP80-P3 was 0.173 g.

Finally, percentage biodegradation was calculated:

$$\% \text{ biodegradation} = \frac{0.173-0.0008*100}{1.45} = 11.9\%$$

The ultimate biodegradation degree observed was 72.40% for cellulose after 45 days of incubation. Among the test samples, the percent biodegradation for PP80 incubated in soil amended with bacterial isolates namely P3, P6, P8, P10 and P13 was 11.94%, 10.39%,

12.09%, 11.57% and 11.24%, respectively. The percent biodegradation observed for PP80C6 degraded by the isolates P3, P6 and P8 was 12.27%, 9.34% and 9.01%, respectively. So, the blend which degraded the most was PP80-P8 (12.09%) and PP80C6-P3 (12.27%) while the least degradation was observed in the blends degraded by P6 which indicates that 'P8' possess the highest degrading potential whereas P6 has the least. As the values of percentage biodegradation range from 9 to 12%, it indicates that the bacterial isolates have the potential to degrade PP-PLLA blends in an efficient manner. Madhu et al. (2014) reported the percentage biodegradation of 4.57% and 4.43% for the blends HDPE/PLA/MAH and HDPE/PLA, respectively under controlled composting conditions for 45 days according to same standard ASTM D5338. Mandal et al. (2016) reported that acrylic acid grafted polypropylene undergoes maximum biodegradation of 6.85% for 90.5% grafting. Mandal et al. (2017b) reported that the acrylic acid grafted PP undergoes biodegradation of 5.5% under the same conditions for 34.55% grafting. But higher values were reported for PP films containing pro-oxidants such as calcium stearate (7.65%) and cobalt stearate (8.34%); suggesting that the polypropylene films containing pro-oxidants were more degraded (Mandal et al., 2017a).

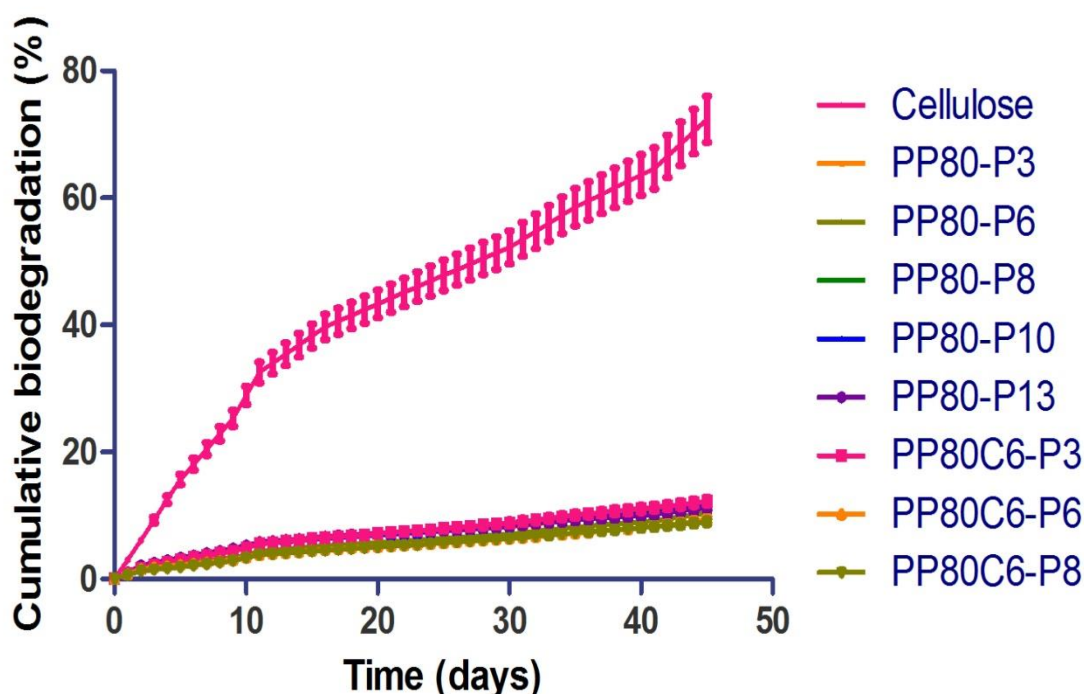


Figure 4.20 Cumulative percent biodegradation of PP/PLLA blends by bacterial isolates after 45 days of incubation

4.3.5 Biodegradation study of polymers in field

The objective of this test was to study the biodegradation of new developed blends under open field conditions. After the soil burial for 6 months, the changes occurred in physical appearance of the films were monitored as well as changes occurred in mechanical and thermal properties. It was visually observed that the films of PP80C6 degraded faster than PP80. The films of PP80C6 disintegrated and the cracks were formed after the incubation period as depicted in Figure 4.21 while the films of PP80 started breaking down.

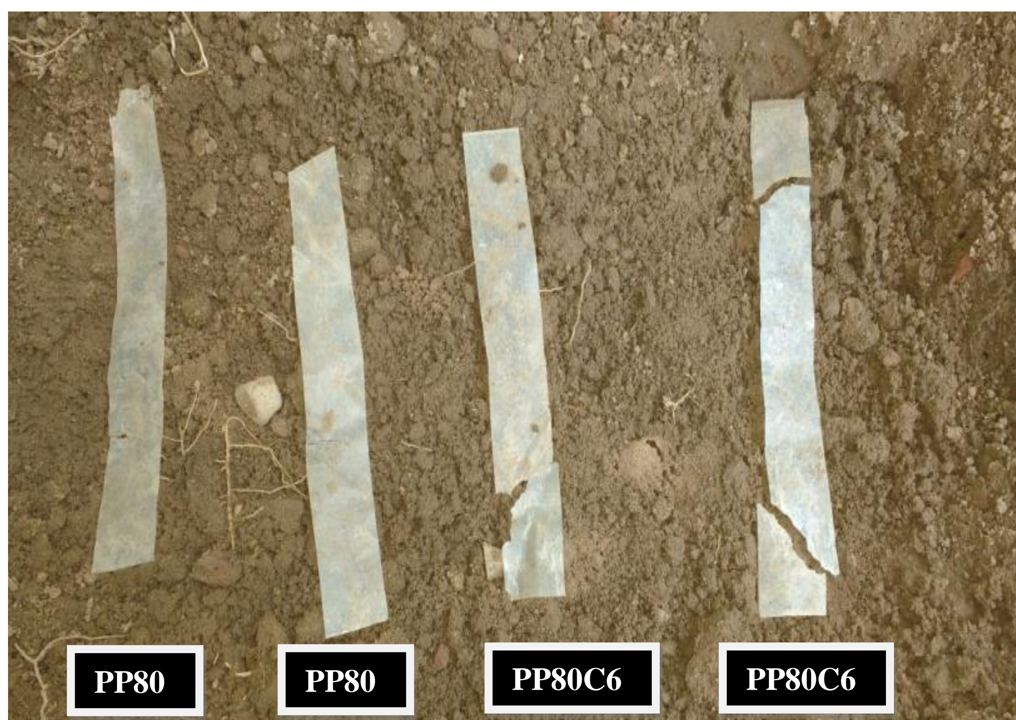


Figure 4.21 The blends PP80 and PP80C6 (in duplicates) after ageing with consortium in field study

4.3.5.1 Mechanical properties of the blends

The loss in mechanical/tensile strength is one of the most important practical criterions to determine the degradation of films. The mechanical properties of polymer blends buried in soil mixed with the bacterial cells of consortium in fields are shown in Table 4.14. The average values of duplicate samples were taken. After six months, the films of both the blends became brittle and there was decrease in mechanical strength. The tensile strength of PP80 decreased from 31.3 MPa to 13.75 MPa, i.e., by about 56% and that of PP80C6 decreased from 52.1 MPa to 28.8 MPa, i.e., by about 45% (Table 4.14 and Figure 4.22).

Similarly, % elongation (E_b) values also dropped from 3.2% to 1.05% for PP80 (by about 67%) and 2.0% to 0.52% for PP80C6 (by about 74%). But, there was increase in tensile modulus of both the blends indicating for the brittleness of polymers. The results of tensile strength and E_b proved that mechanical strength of polymers has decreased to a large extent and disintegration also took place. So, we can say that microorganisms have capability to degrade blends.

Table 4.14 Mechanical properties of the blends before and after ageing with consortium in field study

Samples	Tensile strength (MPa)	Elongation at break (%)	Tensile modulus (MPa)
Blend PP80			
Before degradation	31.3 ± 1.83 a	3.2 ± 0.68 a	1010 ± 56.57 a
After degradation	13.75 ± 0.07 b	1.05 ± 0.07 b	1965 ± 91.92 b
Blend PP80C6			
Before degradation	52.1 ± 5.79 a	2.0 ± 0.99 a	1080 ± 98.99 a
After degradation	28.8 ± 1.27 b	0.52 ± 0.04 b	2130 ± 198.0 b

Values sharing common letter within the columns are not significant at $P < 0.05$. Values are mean ± SD (n = 2)

The ANOVA analysis of mechanical strength (Figure 4.22 and Table 4.14) shows that there is large difference in the mechanical strength of blends before degradation vs. after degradation and data is quite significant at $P < 0.05$ (*) and $P < 0.01$ (**). Earlier reports are also found on decrease in mechanical strength of polymers after degradation. Konduri et al. (2010) reported that there was reduction in tensile strength, percentage of elongation and breaking load of abiotically treated and untreated HDPE films exposed to fungal strains. The tensile strength of UV irradiated HDPE films exposed to different fungal strains such as *Aspergillus oryzae*, *Aspergillus niger* and *Aspergillus flavus* was reduced by 63%, 46% and 32%, respectively; elongation at break was reduced by 72%, 52% and 40%, respectively; and the breaking load was reduced by 56%, 39%, and 27%, respectively. On the other hand, untreated HDPE samples exposed to same fungal strains showed reduction in tensile strength by 5.5%, 3.7% and 2.8%, respectively; elongation at break by 15.0%, 9.4% and 6.6%, respectively; and the breaking load by 6.2%, 3.3% and 1.7%. Sudhakar et al. (2008) also

reported the decrease in tensile strength of untreated and thermally pretreated LDPE and HDPE samples as well as unpretreated LDPE-starch blend samples after exposure to marine micro-organisms i.e., *Bacillus sphericus* and *Bacillus cereus* for 1 year. The tensile strength of HDPE, thermally pretreated LDPE and unpretreated LDPE-starch blend with *B. sphericus* was decreased by 14.8%, 27% and 30.5%, respectively.

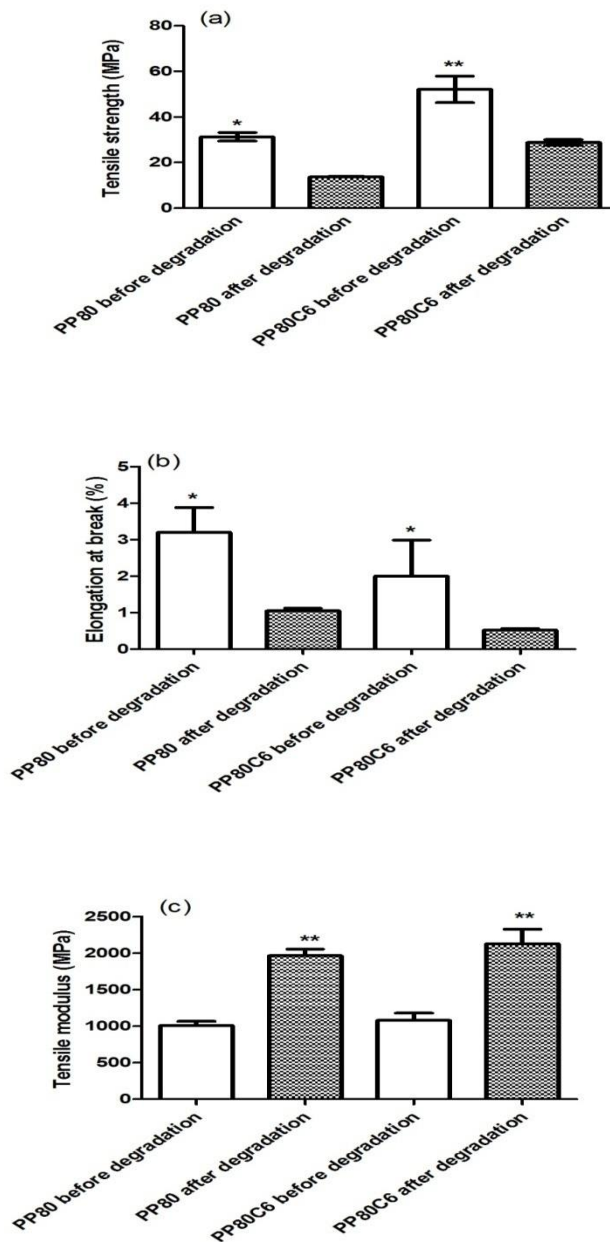


Figure 4.22 Effect of biodegradation on mechanical properties of blends (a) Tensile strength, (b) Elongation at break and (c) Tensile modulus. Bars are mean ± SD (n = 3); *P < 0.05 and **P < 0.01.

4.3.5.2 TGA analysis

Figure 4.23 shows the thermal behavior of blends before and after degradation with consortium in open field conditions. As observed, thermal stability of both the blends decreased after degradation with consortium. The decrease in T_i of PP80 was from 300.5°C to 283.7°C and that of PP80C6 was from 318.2°C to 240.8°C (Table 4.15). Moreover, it can be seen that the thermal stability of both the stages corresponding to PLLA and PP; was decreased after degradation indicating that biodegradation led to reduction in overall thermal stability of the blends. Furthermore, the decrease in values of T_i was more in PP80C6 (24.3% of the initial value) as compared with PP80 (5.6% of the initial value) which indicates that PP80C6 is more degradable than PP80. So, we can say that blends have undergone sufficient degradation after treatment with soil amended with consortium.

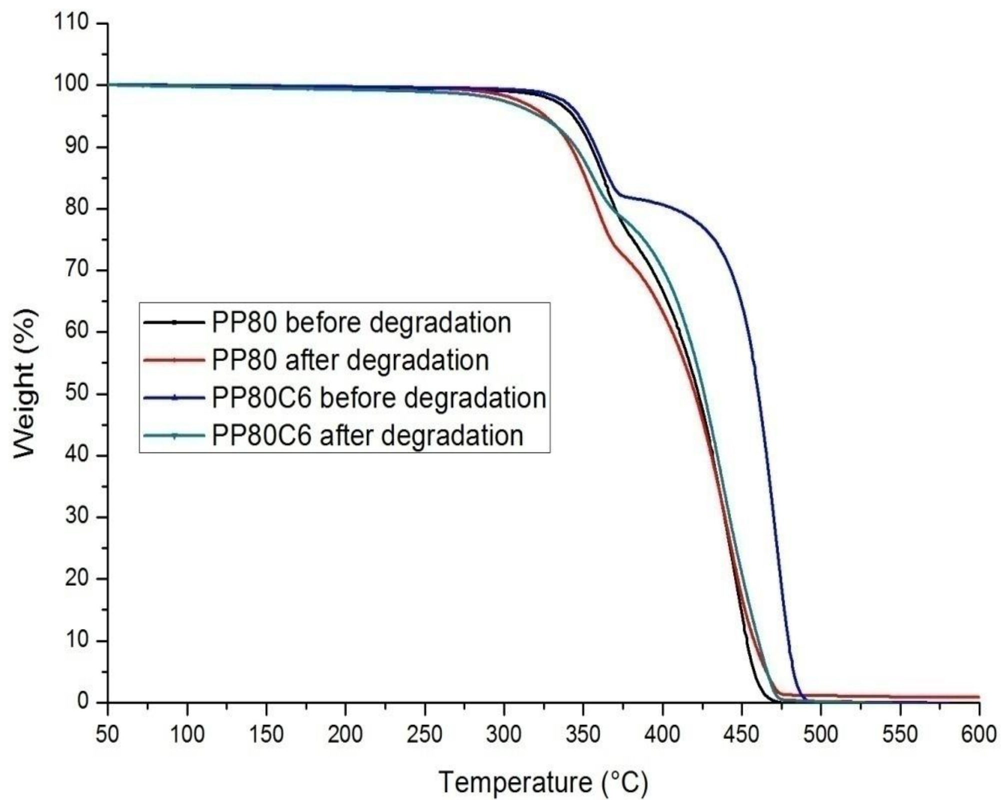


Figure 4.23 TG curves of blends before and after degradation with consortium in field study

Table 4.15: TG analysis of polymers before and after degradation with consortium in field study

Polymer	**T_i (°C)**	**T_f (°C)**
PP80 before degradation	300.5	466
PP80 after degradation	224.9	462.7
PP80C6 before degradation	318.2	487.9
PP80C6 after degradation	253.3	463.8

4.3.6 Abiotic degradation of polymers

The blends were subjected to different types of pretreatments: UV-, thermal and chemical (Aquaregia and Fenton's reagent) pretreatments. Though chemical pretreatment given to blends is very extreme which may begin the degradation of pure PLLA but the blends of PP/PLLA showed no visual changes in surface after pretreatment which may be due to the fact that PLLA is dispersed in PP matrix. However, there may be some micropores formed on the surface of blends due to degradation of PLLA which could not be seen by naked eyes.

During abiotic pretreatment, the oxidation occurs due to generation of radicals that further propagate to form peroxides. PP possess three carbon atoms where radicals can form i.e., primary (methyl group), secondary (in the chain, not attached to methyl group) and tertiary position (in the chain to which methyl group is attached). There are three paths reported in the literature that lead to the formation of peracid. The first path is due to the oxidation at the primary carbon which leads to the formation of methyl radical. The second path is due to abstraction of intra-molecular hydrogen of tertiary alkoxy radical that lead to formation of primary alkyl radical. The third path is the formation of peracid due to the oxidation of oxidized products. Initially, the peroxides formed decompose at a slower rate because of the restricted mobility of the polymer, but the formation of primary oxidation products lead to the formation of peracids. The decomposition of peracids further produces ketones and esters which can be analyzed through FTIR analysis (Arkatkar et al., 2010; Arkatkar et al., 2009a).

4.3.6.1 FTIR of blends

The FTIR spectra of unpretreated and pretreated polymer blends of PP80 and PP80C6 are shown in Figure 4.24 and Figure 4.25, respectively. The spectra of blend PP80C6 (Figure 4.25) showed that peak found at 1757 cm^{-1} was reduced in intensity after treatment with each of the abiotic stress. But after the addition of UV treatment, an additional peak is formed at 2213 cm^{-1} in case of polymer PP80C6. This indicates that UV treatment was the most effective in oxidizing the polymer.

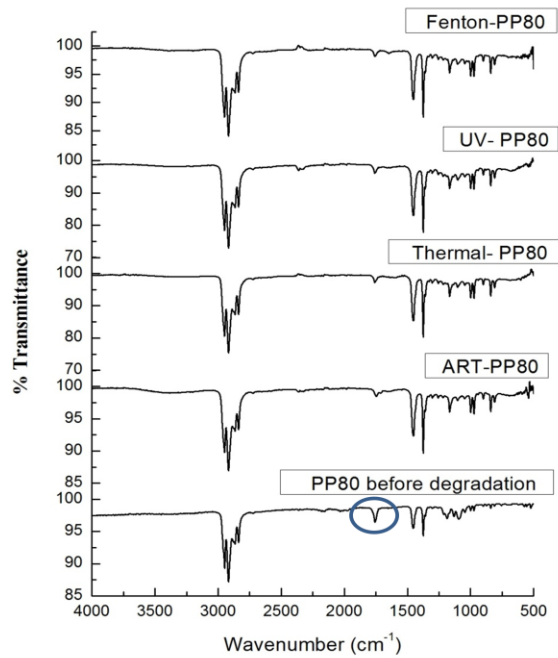


Figure 4.24 FTIR spectra of untreated, UV-, Thermal-, Aquaregia- and Fenton's reagent treated blend PP80 degraded by consortium

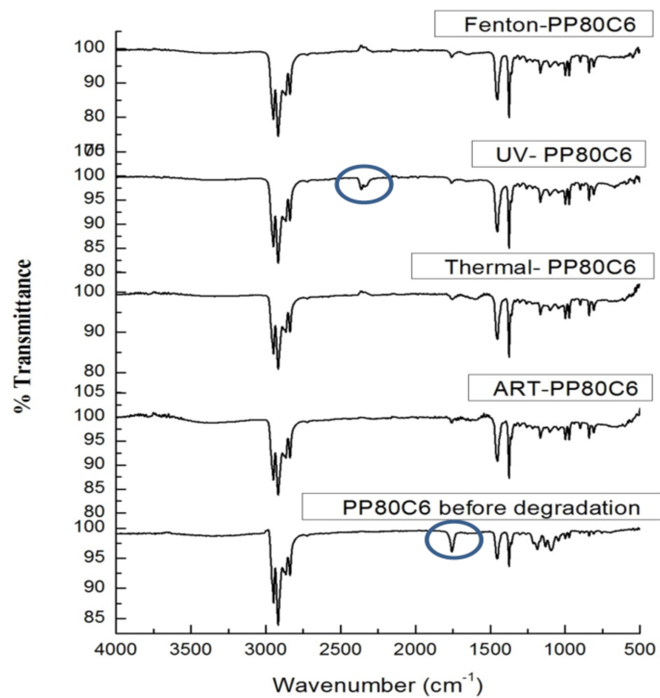


Figure 4.25 FTIR spectra of untreated, UV-, Thermal-, Aquaregia- and Fenton's reagent treated blend PP80C6 degraded by consortium

The oxidation occurred in the test samples can be measured by calculating carbonyl index (CO*i*) from FTIR data. The carbonyl index (CO*i*) expresses the ratio of optical density of the absorption band between 1700 and 1780 cm^{-1} (carbonyl peak) and the optical density of the absorption band at 1463 cm^{-1} ($-CH_2-$ scissoring peak) (Corti et al., 2010). The results of carbonyl index obtained from FTIR spectra of blends PP80 and PP80C6 before and after various types of pretreatment are shown in Table 4.16. There was an increase in the CO*i* of both the blends after abiotic pretreatments; showing that the oxidation has occurred in both the blends after all the pretreatments. There are several reports found on polyethylene which mention the increase in carbonyl index during abiotic treatment followed by decrease during the biotic treatment (Gilan et al., 2004; Hadad et al., 2005; Sudhakar et al., 2008). The oxidation of polymers leads to increase in hydrophillicity and further enhance the degradation by microbes. The maximum increase was found in the case of UV-pretreated PP80 and Fenton's reagent treated PP80C6. The increase in CO*i* of UV-pretreated blends PP80 and PP80C6 is attributed to initiation of chain scission leading to generation of shorter and more readily crystallisable fragments.

Table 4.16: Carbonyl index (CO*i*) of blends PP80 and PP80C6 before and after pretreatment

Samples	Carbonyl index	Samples	Carbonyl index
PP80 before degradation	1.008	PP80C6 before degradation	1.001
PP80-Thermal	1.105	PP80C6-Thermal	1.081
PP80-UV	1.118	PP80C6-UV	1.086
PP80-Aquaregia	1.048	PP80C6-Aquaregia	1.065
PP80-Fenton's	1.064	PP80C6-Fenton's	1.106

4.3.6.2. Growth kinetics

The unpretreated and pretreated polymers were subjected to biodegradation *via* consortium in synthetic media for 15 days at 37°C and 120 rpm. The growth of bacteria was monitored every day by measuring O.D. at 600 nm to determine whether the consortium was able to utilize blends as their carbon source as shown in Figure 4.26.

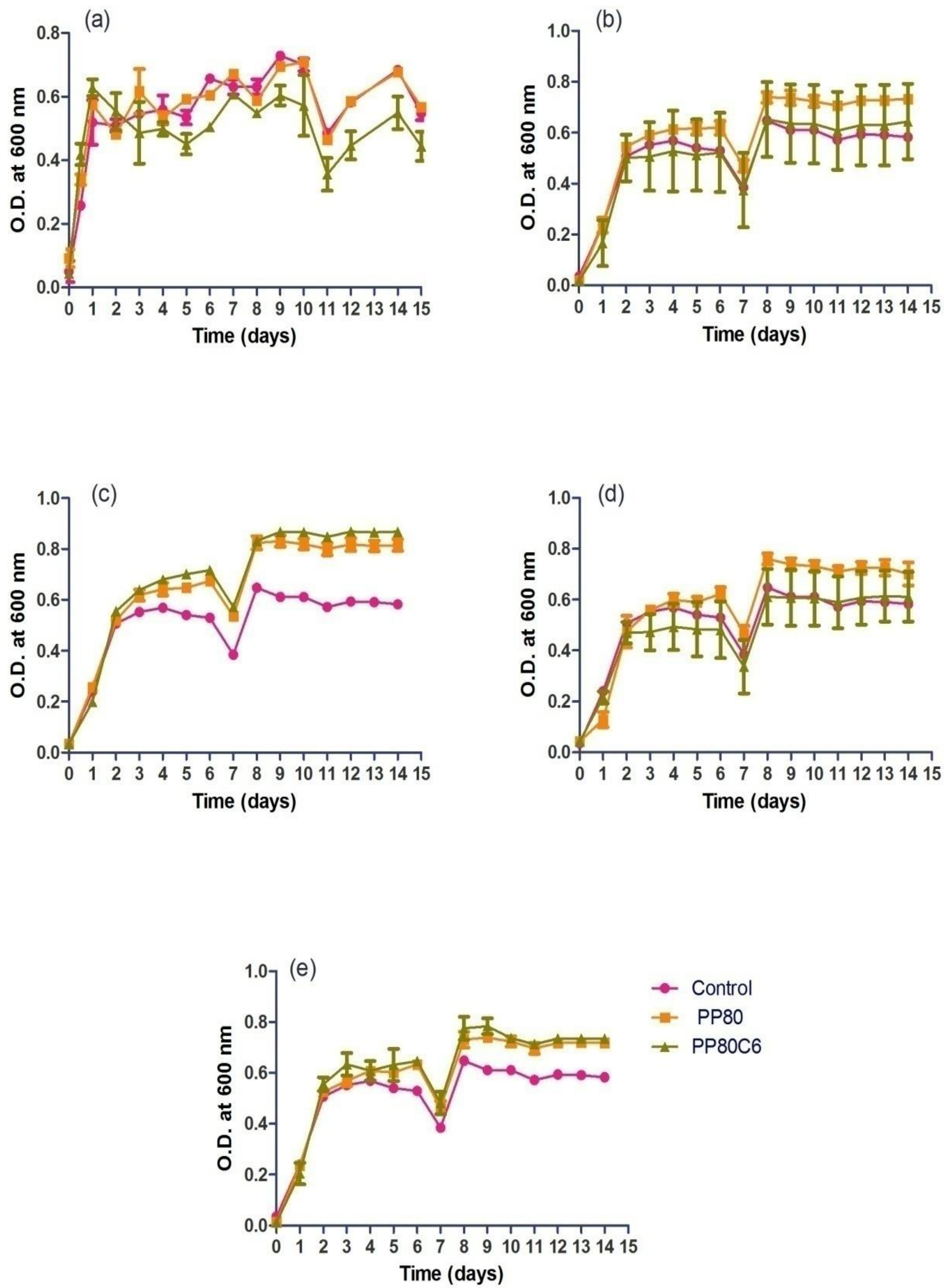


Figure 4.26 Growth curve of (a) unpretreated polymers (b) thermally treated (c) UV-treated (d) Aquaregia reagent treated (e) Fenton's reagent treated polymers in synthetic medium degraded by consortium

The growth in control sample was higher than the test samples in case of unpretreated samples (Figure 4.26a) which indicate that the consortium was able to form biofilm on the polymer surface while the growth in control sample was less than the test samples in case of pretreated samples (Figure 4.26b, 4.26c, 4.26d and 4.26e) which may be due to the fact that consortium was not able to form biofilm on the polymer surface in much efficient manner after the pretreatments. But, earlier reports show that pretreatments help in enhancing the growth of microbes. Sudhakar et al. (2008) reported that thermally pretreated LDPE and HDPE samples had more CFU count than the corresponding unpretreated samples. Arkatkar et al. (2010) reported that CFU/ml count increased in all the samples of unpretreated and pretreated PP films (UV, thermal, Aquaregia and Fenton's reagent) exposed to four different bacteria for one year. But, the highest increase was observed in thermally pretreated films exposed to *B. subtilis.* Moreover, the samples without PP showed less growth than the polymer amended samples.

4.3.6.3. Protein estimation

The protein content in control and polymer supplemented medium was determined to observe the biomass density by bacterial isolates. The protein content was lower in control sample (without polymer) as compared to the test samples i.e., presence of polymer (Table 4.17) which proves for the utilization of polymers as the carbon source. The maximum protein content was found in case of unpretreated samples which means that consortium is working more efficiently without pretreatment. This was also reflected in growth profile of consortium in synthetic media. Among the pretreated samples, the maximum protein content was observed in case of UV-treated samples and least in case of Fenton's reagent-treated samples indicating for the higher growth of bacteria and thus better formation of biofilm in UV-treated samples as compared to other pretreatments. This proves that UV pretreatment was more effective in oxidation of polymers than the other pretreatments and it is also reflected by FTIR spectra of polymers. The values of protein content in unpretreated samples of blends PP80 and PP80C6 were 0.341 mg/ml and 0.409 mg/ml, respectively. Similarly, the values of protein content in UV-treated samples of PP80 and PP80C6 was 0.187 mg/ml and 0.364 mg/ml, respectively. Moreover, it was also observed that protein content of unpretreated samples degraded by consortium shows the higher values than that of polymers degraded by pure cultures (Table 4.8). For example, the maximum protein content was found to be 0.276 mg/ml for PP80 and 0.478 mg/ml for PP80C6 by the isolate P8. But the values of UV-treated

samples are lesser than that of isolate P8. This shows that consortium had high degradation potential at par to isolate P8 even without pretreatments. Moreover, the results of ANOVA (Table 4.17) show that data for unpretreated polymers is significantly different from pretreated polymers and data of PP80 is different from PP80C6 ($P < 0.05$). Some reports are found on estimation of protein content in the biofilm. Arkatkar et al. (2010) reported the estimation of protein content in the growth media and in biofilm formed on the surface of unpretreated as well as pretreated PP (UV, thermal, Aquaregia and Fenton's reagent). The maximum content of protein was observed in UV pretreated PP films. Moreover, *P. azotoformans* and *B. subtilis* produced higher protein content than *P. stutzeri* and *B. flexus*. Masood et al. (2014) also reported the estimation of proteins released by a consortium during degradation of PHBV/PP blend and it was significantly higher.

Table 4.17 Protein content (mg/ml) estimated in biofilm + media of untreated, UV-, Thermal-, Aquaregia- and Fenton's reagent treated polymers degraded by consortium

Treatment		Protein conc. (mg/ml)	
		PP80	**PP80C6**
Control (without polymer)	0.128 ± 0.045		
Unpretreated polymers		0.341 ± 0.002 aA	0.409 ± 0.083 aB
UV treatment		0.187 ± 0.029 bA	0.364 ± 0.251 aB
Thermal treatment		0.169 ± 0.014 bA	0.141 ± 0.009 bA
Aquaregia reagent		0.141 ± 0.012 bA	0.127 ± 0.003 bA
Fenton's reagent		0.12 ± 0.049 bA	0.092 ± 0.002 bA

Values sharing common lower case letter within the columns and common upper case letters within the rows are not significant at $P < 0.05$. Values are mean ± SD ($n = 2$)

The aim of the present work was to explore the compost sample for the presence of polypropylene degrading bacteria. Polypropylene degrading bacteria were successfully isolated from municipal solid waste compost. The bacterial isolates which were capable of growing in the presence of hexadecane on synthetic agar media were screened for biodegradation studies. Based on this, five bacterial isolates were selected and characterized on the basis of biochemical properties and 16S rDNA sequence analysis. The selected bacterial isolates belonged to phylum Firmicutes and genus *Bacillus*. Sequence alignment of closely related species showed about 99% similarity. The five selected isolates were *Bacillus cereus* P3*, Bacillus licheniformis* P6*, Bacillus thuringenesis* P8*, Bacillus thuringenesis* P10 and *Bacillus cereus* P13 and their sequences have been deposited in NCBI database under the accession no. from KT267059 to KT267063.

The biodegradation of polypropylene in nature is a very slow process and the formation of biofilm on its surface is a pre-requisite for its utilization as the carbon and energy source by the microorganisms. The addition of PLLA into PP can enhance the formation of biofilm formation due to its biodegradable nature. But as we know, PP/PLLA is an expensive combination and cannot be used extensively. Therefore, further research can be carried out to investigate the potential of less expensive biodegradable components for their ability to promote biodegradation of synthetic plastics. Here, the biodegradation studies of PP-PLLA blends by bacterial isolates were carried out in different conditions for example, in synthetic media for 15 days, in soil under laboratory conditions for six months (microcosm experiments), in fields using the same soil for six months, aerobic biodegradation with same soil for 45 days (ASTM D5338) and by giving different kind of abiotic pretreatments followed by biotic treatment with consortium.

Biodegradation of polymer blends PP80 and PP80C6, in the presence of suitable medium containing no other source of carbon and energy than the polymer films was measured by growth kinetics. In addition, the biomass density of bacteria at the end of test was determined by protein content (mg/ml). The oxidation occurred in the polymer surface was monitored by FTIR technique and the surface changes were visualized by SEM technique. Moreover, the thermal stability of polymers before and after degradation was also determined by TGA

technique. The results showed that the bacterial isolate P8 i.e., *Bacillus thuringenesis* had the highest degradation potential among all the isolates.

Microcosm experiments were set to study biodegradation of PP-PLLA blends via bacterial isolates in laboratory conditions. The soil was previously sterilized and treated with pure culture of isolated bacteria so that the degradation efficiency of bacterial isolates can be monitored. All soil microcosms were kept in dark at room temperature for six months. The biodegradation was estimated in terms of growth kinetics via cfu/g count and the changes occurred in chemical properties as well as thermal stability of polymers was determined by FTIR and TGA techniques, respectively. The maximum growth was observed in case of consortium whereas TGA analysis showed that the largest decrease in thermal stability of polymers was caused by isolate P8.

Field study was conducted to monitor the degradation of PP80 and PP80C6 via consortium in the natural conditions. The biodegradation was estimated on the basis of mechanical properties and TGA analysis was carried out to determine any changes in thermal stability. Firstly, the blends were visually found to be disintegrated in the soil after treatment for six months. Moreover, the mechanical strength and thermal stability of the blends decreased after incubation with consortium cells in soil which confirms for the biodegradation efficiency of bacterial isolates.

The biodegradation behavior of blends PP80 and PP80C6 was also studied in soil under controlled laboratory conditions according to ASTM D5338. According to this method, if a polymer undergoes 70% degradation within 45 days, it is said to be biodegradable. In our study, the positive reference i.e., cellulose showed about 72% degradation in 45 days while the blends degraded by different bacterial isolates showed percentage biodegradation from 9-12%. The highest degradation potential was shown by isolate P8. The blends are not completely biodegradable but the values reported are better than the earlier reports on biodegradation of polyolefins. Thus, we can say that blends are partially biodegradable.

Abiotic degradation studies were also conducted to test whether the pretreatments affect the rate of biodegradation of polymers. The different pretreatments given were UV-, thermal-, Aquaregia- and Fenton's reagent treatment. FTIR was conducted on polymers after abiotic pretreatment to determine the change in level of oxidation. The carbonyl index of both the blends increased after each of the abiotic pretreatment showing that oxidation has occurred in

all the samples. The maximum increase in CO*i* was observed with UV- pretreatment in PP80 while in PP80C6 the maximum increase was found in Fenton's treated polymer followed by UV-treated polymer. Hence, we can say that UV pretreatment is best in oxidizing the polymer among the given pretreatments. Moreover, unpretreated and pretreated samples of blends were subjected to biodegradation via consortium in synthetic media for 15 days. The growth profile was checked and protein estimation was done at the end of 15 days. It was observed that biofilm formation occurs better in unpretreated samples as compared with pretreated samples. However, among the pretreated sample, UV-treated sample showed the maximum biomass density of bacterial cells in terms of protein content (mg/ml).

The experimental data showed here reveals that the isolated bacteria have the potential to degrade polymer blends PP80 and PP80C6. It can be concluded that polymer blends can be biodegraded by the action of variety of microorganisms found in compost. However, the presence of PLLA and the acceleration of biodegradation by bacterial isolates do not turn the overall material biodegradable but make it more reactive to biotic reactions. Hence, we can say that the blends prepared were partial biodegradable but they can be used for long term use applications like packaging and mulching.

Summary

The objective of the present work was to develop the PP-PLLA blends as an alternative to packaging materials; which will be biodegradable as well as possess strong mechanical strength. The blends developed are non-toxic as prepared from non-toxic components i.e., Polypropylene (PP), Poly-L-Lactide (PLLA) and maleic-anhydride grafted Polypropylene (MAPP). The blends were prepared by melt-blending technique, followed by compression molding to produce thin films of thickness about 50 ± 10 µm. Moreover, the experimental results showed that the polymer blend of PP/PLLA with a ratio of 80: 20 is mechanically strong (before degradation). But, the blends of PP-PLLA are immiscible in nature due to polarity difference. Therefore, MAPP was used to compatibilize the blends. The addition of MAPP enhanced the tensile properties of PP-PLLA blends and the blend containing 6 phr (parts per hundred of resin) of compatibilizer (MAPP) showed the best mechanical strength. Hence, based on the mechanical properties, two blends PP80 and PP80C6 were selected for further studies.

The selected films i.e., PP80 and PP80C6 along with pure PP and pure PLLA were characterized for physical (XRD), chemical (FTIR-ATR), morphological (SEM) and thermal (TGA) properties. The addition of PLLA into PP (PP80) caused the increase in crystallinity as compared with neat PP while the blend PP80C6 showed almost same degree of crystallinity as of PP100. The FTIR spectra indicated the existence of chemical interactions in the blend PP80C6 which caused the shift of characteristic peak of carbonyl group to longer wavelength. Phase separation and boundary layer between the two components was clearly revealed in blend PP80 by the observation of its morphology but the addition of MAPP improved the interfacial adhesion between two polymers leading to formation of miscible and compatible blend. Thermal stability of blend PP80C6 was more than PP80 showing that compatibility has improved the thermal properties. The neat polymers showed one-stage degradation while blends showed two stage degradation corresponding to decomposition of two polymers separately. From the characterization, it can be concluded that PP and PLLA are immiscible in nature due to polarity difference and form a heterogeneous blend but compatibility was improved by the addition of MAPP.

After the synthesis and optimization of polymer blends, the blends were studied for the degradation by abiotic and biotic factors. Initially, the bacteria were isolated from municipal

solid waste compost using enrichment technique having capability to use PP as the sole carbon source. There was no other source of carbon except PP in synthetic media. Sixteen different bacteria were isolated from compost and they were further screened for their degradation ability in agar media containing hexadecane. On the basis of that, five bacterial isolates were selected (P3, P6, P8, P10 and P13) and they were identified as *Bacillus cereus* P3*, Bacillus licheniformis* P6*, Bacillus thuringenesis* P8*, Bacillus thuringenesis* P10 and *Bacillus cereus* P13 on the basis of 16S rDNA gene sequencing. The five isolates were also characterized for their morphological and biochemical features such as Gram staining, capsule staining, oxidase test, catalase test, nitrate reduction test, carbohydrate utilization test and antibiotic susceptibility test. The results showed that there are marked differences among the five isolates due to which degradation also varies from one isolate to another. It depends on the type of enzymes produced by bacteria (i.e. extracellular or intracellular), characteristics of the organisms, kind of polymer and kind of treatment required.

Afterwards, the selected five bacterial isolates were tested for their efficiency to degrade the polymer blends PP80 and PP80C6 in different media such as synthetic media, soil etc. In synthetic media, blends were treated with pure cultures for 15 days at 37°C under shaking (120 rpm). The results showed that all the isolates were able to colonize the polymers and P8 had the maximum potential to degrade it. Moreover, the blend PP80C6 undergoes degradation more easily than PP80. FTIR analyses showed the reduction in intensity of carbonyl bonds in both the blends and formation of new alkynes bond in PP80C6; SEM micrographs revealed the formation of biofilm as well as the occurrence of cracks and pits on polymer surface after degradation; and the thermal stability of blends decreased after incubation.

The degradation of blends via pure cultures and consortium was carried out in soil too; one under laboratory conditions (microcosm) and the other in open fields (field study). In microcosm studies, cfu/g count of all the microcosms increased with time but the maximum increase was observed in consortium. FTIR analysis showed the reduction in carbonyl bonds and thermal stability of blends was also decreased. The field study was conducted on blends PP80 and PP80C6 via consortium only. There was decrease in mechanical strength of polymers after the six months of incubation period and also the thermal stability was decreased proving that bacterial isolates have the capability to degrade the polymer blends.

The aerobic biodegradation of the polymer blends PP80 and PP80C6 was carried out in soil according to ASTM D5338 standard for 45 days. The results of percentage degradation were as cellulose (72.40%), PP80-P3 (11.9%), PP80-P6 (10.4%), PP80-P8 (12.1%), PP80-P10 (11.6%), PP80-P13 (11.2%), PP80C6-P3 (12.2%), PP80C6-P6 (9.3%) and PP80C6-P8 (9.0%). the isolate P8 showed the maximum potential and P6 showed the least potential to degrade polymer blends. Obviously, the blends have been degraded to a good extent by bacterial isolates.

The abiotic degradation of polymers was also carried out via UV-, thermal, aquaregia and Fenton's reagent pretreatments. The pretreatments caused the increase in oxidation level of blends as evident from FTIR analysis and UV treatment was found to be the most effective. The pretreated as well as unpretreated polymers were further subjected to biodegradation via consortium in synthetic media for 15 days. The bacterial growth and protein content was more in case of untreated samples than abiotically treated samples.

The evident differences among the peak positions and peak intensities of FTIR spectra, the variation in internal morphologies obtained through SEM analysis, difference in thermal stability determined via TGA analysis, and decrease in mechanical strength of polymer blends PP80 and PP80C6 before and after degradation prove that PP-PLLA films are biodegradable. Moreover, it was found that PP80C6 was more degradable than PP80. This can be attributed to the degree of crystallinity of polymers. The amorphous regions of polymers undergo more degradation than crystalline regions. It means that more is the crystallinity of polymers (i.e., ordered arrangement of molecules), lesser is the degradation. As shown in Table 4.2, PP80C6 is less crystalline as compared to PP80; therefore, it undergoes more degradation. Finally, it can be recommended that PP-PLLA film with 80:20 ratio and PP-PLLA-MAPP film of 80:20:6 ratio would be commendable from material preparation point of view but from biodegradation point of view, the biodegradation in soil, i.e. solid state process will be more preferable than liquid state (synthetic medium) degradation process.

Future work

During the biodegradation process in the open fields, the polymers breakdown and release various kind of constituents to the soil. It is important to determine that whether the constituents have any ecotoxic effects on crops or animals etc.

There is vast variety of microorganisms present in different sources such as soil, compost, water etc. These sources need to be explored to find efficient polypropylene degrading microbes which can be multiplied at large scale to commercialize the polypropylene degradation.

Few studies have been conducted on elucidation of polymer degradation mechanisms and fate of the polymers inside the microorganisms. Until now, it has been suggested that polymers may undergo degradation by the means of tricarboxylic acid cycle (TCA cycle) but still it needs to be proved that this is happening.

The enzymes responsible for oxidising and breaking the polypropylene chains should be isolated and identified so that the degradation mechanism of polypropylene can be elucidated. The genes responsible for production of those enzymes can be characterized and used to enhance the polypropylene degradation capacity using other easily available microorganisms.

From the morphological studies of polymers after degradation, it can be concluded that the amorphous regions undergo degradation more easily than crystalline regions. But, it still needs to be find out that the highly organized crystalline regions can undergo microbial degradation and at what rate.

Since, the biodegradation in real environment is a complex process therefore; the role of ecological interactions between the different microorganisms in the process of degradation of polypropylene should be established.

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Appendix I

(A) Reagents for TOC

1. **1 N Potassium dichromate ($K_2Cr_2O_7$)**

   Dissolved 49.04 g of Potassium dichromate in distilled water and volume was made up to 1L.

2. **0.5 N ferrous ammonium sulphate**

   Dissolved 198 g ferrous ammonium sulphate in distilled water and volume was made up to 1L.

(B) Reagents for Total P

1. **Vanadomolybdate solution**

   i. **Solution A** – 25 g ammonium molybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$] was dissolved in 300 ml water in a 500 ml beaker

   ii. **Solution B** – 1.25 g of ammonium(meta)vandate (NH_4VO_3) was dissolved in 300 ml boiling water. It was cooled and 250 ml of conc. HNO_3 was added to it. It was cooled again.

   Solution A was added to Solution B and volume was made up to 1L in a volumetric flask.

2. **Phosphorus stock standard solution (50 mg/l P)**

   0.2195 g of KH_2PO_4 was dissolved in distilled water, mixed thoroughly. To it, 25 ml of 7N H_2SO_4 was added and volume was made up to 1L. 4-5 drops of toluene was added to prevent microbial activity.

(C) Reagents for Available P

1. **0.5 M $NaHCO_3$ extracting solution**

   42.0 g of sodium bicarbonate was added in distilled water and the volume was made up to 1L. The pH was adjusted to 8.5 with 1M or 1N NaOH.

2. **Reagent A (Molybdate - tartarate solution)**

   i. Dissolved 12 g of ammonium molybdate in about 250 ml distilled water.

   ii. Dissolved 0.291 g of antimony potassium tartarate in 100 ml of distilled water.

   Added the above two solutions to 1L of 2.5M H_2SO_4. Mixed thoroughly and made the volume up to 2L with distilled water.

3. **Reagent B (freshly prepared)**

   1.058 g of ascorbic acid was dissolved in 200 ml of reagent A.

4. **Sulphuric acid (2.5 M)**

   140 ml of concentrated H_2SO_4 diluted to 1L.

5. **Stock standard P solution (50 mg/L)**

   0.2917 g of KH_2PO_4 was dissolved in 1L of distilled water. Added 3-4 drops of toluene to diminish microbial activity (KH_2PO_4 should be dried at 100°C for 1 hour and cooled in a desiccator before weighing).

6. **Working standard P solution (1 mg/L)**

   Diluted 20 ml of 50 mg/L P solution (stock) to 1L. Mixed it thoroughly.

(D) Reagents for Total Nitrogen

1. **Sulphuric salicylic acid**

   Mixed 5.0 g of salicylic acid with 150 ml of concentrated sulphuric acid (H_2SO_4).

2. **Boric acid (4%)**

   Dissolved 4.0 g of boric acid in 100 ml of distilled water.

3. **Mixed indicator**

   Dissolved 0.066 g of methyl red and 0.099 g of bromocresol in 100 ml of ethyl alcohol.

4. **Sodium Hydroxide (NaOH, 50%)**

   Dissolved 50 g of NaOH in 100 ml of distilled water.

5. **Digestion mixture**

   Mixed 5 g of HgO, 2.5 g of $CuSO_4$ and 50 g of K_2SO_4. The ratio is 2:1:20.

6. **0.02 N H_2SO_4**

   Add 0.28 ml of conc. H_2SO_4 in 500 ml of distilled water.

(E) Nutrient broth

Ingredient	**Quantity (g/l)**
Peptone	5.0
Beef extract	1.5
Yeast extract	1.5
Sodium chloride	5.0

Boiled to dissolve the medium completely, sterilized by autoclaving at 15 lbs pressure (121°C) for 15 min, pH 7.4 ± 0.2

(F) Nutrient agar

Ingredient	Quantity (g/l)
Peptone	5.0
Beef extract	1.5
Yeast extract	1.5
Sodium chloride	5.0
Agar	15.0

Boiled to dissolve the medium completely, sterilized by autoclaving at 15 lbs pressure (121°C) for 15 min, pH 7.4 ± 0.2

(G) Synthetic media (Hadad et al., 2005)

Ingredient	Quantity (g/l)
NH_4NO_3	1.0 g/l
$MgSO_4.7H_2O$	0.2 g/l
K_2HPO_4	1.0 g/l
$CaCl_2.2H_2O$	0.1 g/l
KCl	0.15 g/l
Yeast extract	0.1 g/l
$FeSO_4.6H_2O$	1.0 mg/l
$ZnSO_4.7H_2O$	1.0 mg/l
$MnSO_4$	1.0 mg/l

(H) Nitrate agar

Ingredient	Quantity (g/l)
Agar	12.0
Beef extract	3.0
Peptic digest of animal tissue	5.0
Potassium nitrate	1.0

Boiled to dissolve the medium completely, sterilized by autoclaving at 15 lbs pressure (121°C) for 15 min

(I) Starch Agar

Ingredient	Quantity (g/l)
Peptic digest of animal tissue	5.0
Meat extract	3.0
Starch (soluble)	2.0
Agar	15.0

Final pH was adjusted to 7.2± 0.1

(J) TBE Buffer (5x)

Tris base	54 g/l
Boric acid	27.5 g/l
0.5 M EDTA, pH 8.0	20 ml/l

(K) Agarose Gel Loading Dye (6X)

Bromophenol blue	0.25%
Xylene cyanol FF	0.25%
Glycerol in water	30.0%

(L) Primers

M13 forward primer	5''-GTAAAACGACGGCCAGT-3'
M13 reverse primer	5'-CAGGAAACAGCTATGAC-3'
16S rDNA forward primer	5'-AGAGTTTGATCCTGGCTCAG-3'
16S rDNA reverse primer	5'-ACGGGCGGTGTGTTC-3'

(M) Luria-Bertani (LB) Medium

Ingredient	Quantity (g/l)
NaCl	10.0
Beef extract	5.0
Tryptone	10.0

pH adjusted to 8.0 with 1N NaOH, sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 min.

(N) LB/amp+ agar plates

Prepared LB broth as above, added agar (15 g/l), autoclaved, and cooled to 50°C, added ampicillin 50 μg/ml, Poured in plates and stored at 4°C.

(O) X-Gal (2 ml)

100 mg of 5-bromo-4-chloro-3-indolyl-β-D-galactoside was dissolved in 2 ml of N,N-dimethylformamide (DMF), covered with aluminum foil and stored at -20°C.

(P) IPTG stock solution (0.1M)

To 1.2 g IPTG, added water to 50 ml final volume, filtered with 0.22 µm membrane and stored at 4°C

(Q) LB plates with Ampicillin/X-Gal/IPTG

Prepared LB plates with ampicillin as above; 100 μl of 100 mM IPTG and 20 μl of 50 mg/ml X-Gal spread over the surface of LB ampicillin plate and allowed to absorb for 30 minutes at 37°C prior to use.

(R) Plasmid Extraction Solution I (10X)

Glucose	50 mM
Na_2EDTA	10 mM
Tris-HCl	25 mM (pH 8.0)

(S) Plasmid Extraction Solution II (freshly prepared)

NaOH	0.2 M
SDS	1 %

(T) Plasmid Extraction Solution III

3.0 M with respect to potassium and 5.0 M with respect to acetate

(For 100 ml solution III: 60 ml of 5.0 M potassium acetate, 11.5 ml of glacial acetic acid and water to a final volume of 100 ml, pH~5.0)

(U) Aquaregia reagent

Nitric acid and HCl were dissolved in ratio of 1:3 (v/v).

Precaution: While preparing aquaregia reagent, always add Nitric acid to HCl, never vice-versa.

Disposal: Pour excess and waste aquaregia into a large quantity of ice (500g of ice per 100 ml of aguaregia). Neutralize the mixture with an aqueous basic solution such as 1M or 10% (NaOH) or saturated sodium bicarbonate ($NaHCO_3$) in water until pH is neutral. The neutralized solution may then be poured down drain.

(V) Fenton's reagent

FeSO4 (II) in water, addition of H_2O_2 dropwise till pH becomes 5.5.

Appendix II

16S rRNA Sequences of Bacterial Isolates

Bacillus cereus strain P3 16S ribosomal RNA gene, partial sequence

GenBank: KT267059.1

FASTA Graphics

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  JOURNAL   Unpublished
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Bacillus licheniformis strain P6 16S ribosomal RNA gene, partial sequence

GenBank: KT267060.1

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GenBank: KT267061.1

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  TITLE     Isolation and characterization of bacteria capable of polypropylene
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  JOURNAL   Unpublished
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Bacillus thuringiensis strain P10 16S ribosomal RNA gene, partial sequence

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Bacillus cereus strain P13 16S ribosomal RNA gene, partial sequence

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REFERENCE   2  (bases 1 to 1412)
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Appendix III

Research Papers published in SCI Journals

Kimi Jain, Gaurav Madhu, Haripada Bhunia*, Pramod K. Bajpai, Golok B. Nando and Mondem S. Reddy

Physico-mechanical characterization and biodegradability behavior of polypropylene/poly(L-lactide) polymer blends

Abstract: Partially biodegradable polymer films from the blends of polypropylene (PP) and poly(L-lactide) (PLLA) were prepared in an internal mixer by melt blending technique, with and without compatibilizer, maleic anhydride grafted polypropylene (MAPP), followed by compression molding. With regard to tensile properties, 80/20 (PP/PLLA) and 80/20/6 (PP/PLLA/MAPP) were found as the optimum blends with best combination of the ingredients. Therefore, the blend samples, namely, PP80 (80% PP+20% PLLA) and PP80C6 (80% PP+20% PLLA+6 phr MAPP) were selected as 'optimized' blends and further characterized for their physical, chemical, morphological, and thermal properties. X-ray diffraction studies showed that neat PP and PP80C6 had the same crystallite size indicating compatibility between PP and PLLA due to MAPP. Fourier transform infrared spectroscopy and scanning electron microsopy investigations revealed that the two polymers were completely immiscible in absence of the compatibilizer. Bacterial biodegradation of the samples was performed by exposure to *Pseudomonas stutzeri* for 60 days and measured in terms of weight loss, optical density, and thermal stability of the samples before and after degradation. The results showed that 80/20 (PP/PLLA) blends undergo considerable degradation. Reduction in thermal stability of the film samples was also observed through thermogravimetric analysis, which was useful in accelerating their biodegradation.

Keywords: biodegradation; morphology; packaging films; physico-mechanical; PP/PLLA.

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1 Introduction

Polypropylene (PP) is an outstanding polymer material and used mainly in flexible packaging applications such as food packaging, trash bags, etc. due to its low cost and excellent physical, mechanical, and thermal properties [1, 2]. But, after their usage period, it is carelessly discarded in open dumps, landfills, or as simple litter. And, owing to its large molecular weight, hydrophobicity, and non-biodegradable nature, it is hard to be metabolized due to which it accumulates in land and water bodies [3]. A rapid development of researches on blending of non-biodegradable polymers with the biodegradable ones to enhance biodegradation has been witnessed during the last few decades [4, 5]. However, most of these studies have been done using starch or cellulose for introducing biodegradability in polyolefin, which results in degradation of starch/cellulose, only leaving behind the polyolefin part. Moreover, blends with starch/cellulose do not show good mechanical properties [6]. In fact, the performance of polymer blends depends on the composition of ingredients, their properties, and their morphology [7].

Blends of PP with poly(L-lactide) (PLLA) have been studied earlier to improve dyeability of PLLA [8], melt processability of PLLA [9], and rheological and mechanical properties [3]. PP and PLLA form incompatible blends, and a compatibilizer is needed to improve compatibility between them. Maleic anhydride grafted polypropylene (MAPP) is a well-known compatibilizer used for PP-based blends and composites. It follows the free-radical mechanism – the anhydride part of MAPP has free radical site at α-carbon of carbonyl group, which forms ester linkages with PLLA, and the PP part of MAPP shows compatibility with PP matrix [9]. In this way, it acts as a bridge and helps in improving the interfacial adhesion between PP and PLLA.

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A number of efforts have been done to study the degradation behavior of PP and increase the same through various means [10–15]. It can be achieved if somehow PP is degraded to low-molecular-weight compounds so that microbes can easily attack the polymer surface [16]. Addition of PLLA into PP will make its surface hydrophilic to some extent, which helps in accelerating degradation process by the microorganisms. PLLA is a well-known biodegradable polymer and has high strength, modulus, and biocompatibility. It is widely used for various applications like biomedical devices, tissue engineering, etc. It has potential to resolve the current problems concerning global warming on account of its carbon neutrality. However, its brittleness and low heat distortion temperature limit the application of neat PLLA [17–19]. Some of the previous studies also reported that *Pseudomonas stutzeri* bacterium shows potential to form biofilm, i.e., attachment of microorganisms to polymer surface, on the surface of PP [20, 21].

The purpose of our study was to develop partially biodegradable polypropylene films which should retain their mechanical and other requisite properties for flexible packaging applications during their lifespan and then undergo effective biodegradation after their useful life. In the present study, PLLA was blended with PP, with/without compatibilizer, in different ratios, and their mechanical, physical, and morphological characterizations were performed through various techniques. Biodegradation of the films prepared from PP/PLLA blends was also analyzed using bacteria *P. stutzeri* in laboratory conditions.

2 Materials and methods

2.1 Materials

The polymers used in this study, PP, PLLA, and the compatibilizer (MAPP) were of commercial grade whose sources and properties are mentioned in Table 1. *Pseudomonas stutzeri* (MTCC number 2643) was procured from Microbial Type Culture and Gene Bank, Institute of Microbial Technology (IMTECH, Chandigarh, India). It is a Gram-negative, motile, and rod-shaped bacterium having an optimum growth at 35°C. Nutrient broth (NB, in liquid media) and nutrient agar (in solid media) were obtained from HiMedia Laboratories Ltd. (Mumbai, India). For degradation studies, synthetic medium containing (g/l of distilled water): 1.0 g NH_4NO_3, 0.7 g $MgSO_4 \cdot 7H_2O$, 1.0 g K_2HPO_4, 1.0 g KH_2PO_4, 0.005 g NaCl, 2 mg/l each of $FeSO_4 \cdot 6H_2O$ and $ZnSO_4 \cdot 7H_2O$, and 1 mg/l of $MnSO_4 \cdot H_2O$ was used. All these chemicals were obtained from HiMedia Laboratories Ltd. (Mumbai, India).

2.2 Blend preparation

Blends of PP/PLLA, with and without compatibilizer MAPP, were prepared in a Brabender Plasticorder PLE-651 mixer by keeping rotor speed of 60 rpm. The fill factor was 0.8, and mixing was carried out at 180°C for 4 min. The extrudate thus obtained was sheeted out through a two-roll mill set at room temperature with a nip gap of 2 mm, and the films were casted in a compression molding hydraulic press (George E. Moore Press, UK) electrically heated at 180°C for 2 min at a pressure of 3 MPa. While molding, Teflon sheets were used to prevent the molten mass from sticking to the mold. To maintain the overall dimensional stability of the samples, moldings were allowed to cool under pressure by circulating cold water through the platens. Neat PP and neat PLLA films were also prepared in similar fashion. The composition of prepared films is described in Table 2.

3 Testing methodologies

3.1 Mechanical properties

Tensile strength at yield, elongation at break, and elastic modulus were measured according to American Society for Testing and Materials (ASTM) D 882-91 standard on a universal testing machine (Zwick Roell Z010, Germany) with a clamp separation of 100 mm. The crosshead speed was kept at 50 mm/min, and the test was conducted at room temperature. Three specimens were tested for each composition, and the average values were taken.

Table 1 Names, sources, and characteristics of polymers and compatibilizer used.

Material	Trade name	Source/Supplier	Density (g/ml)	MFI (g/10 min)	T_m (°C)
PP	Halene-P, F103	Haldia Petrochemicals Ltd., Kolkata, India (www.haldiapetrochemicals.com)	0.90	3.3	152
PLLA	Biomer L9000	Biomer Forst-Kasten-Str Kailling, Germany (http://www.biomer.de)	1.25	3.0	190
MAPP	Optim P406	Pluss Polymers Pvt., Ltd., Gurgaon, India (http://www.pluss.co.in/)	0.91	40	163

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Table 2 Composition of PP/PLLA blends.

S. no.	Sample name	Composition		
		PP (wt%)	PLLA (wt%)	MAPP (phr)
1.	PP100	100	0	0
2.	PP95	95	5	0
3.	PP90	90	10	0
4.	PP85	85	15	0
5.	PP80	80	20	0
6.	PP70	70	30	0
7.	PP60	60	40	0
8.	PP50	50	50	0
9.	PLLA100	0	100	0
10.	PP80C2	80	20	2
11.	PP80C4	80	20	4
12.	PP80C6	80	20	6
13.	PP80C8	80	20	8

3.2 X-ray diffraction

X-ray diffraction (XRD) spectra were obtained at room temperature using a Philips X'Pert diffractometer (Almelo, The Netherlands) with monochromatic CuKα radiation (λ=1.5418 Å) operated at 45 kV and current 40 mA. The scanning speed and diffraction angle (2θ) were kept at 5°/min and 5–60°, respectively. From XRD analysis, percentage of crystallinity (χ) and crystallite size (P) was measured according to Equations (1) and (2), as follows [22]:

$$\chi_c = \frac{I_c}{I_c + I_a} \times 100 \quad (1)$$

where I_c and I_a are integrated intensities corresponding to crystalline and amorphous phases, respectively.

$$P = \frac{k\lambda}{\beta \cos\theta} \quad (2)$$

where β is half width (in radian) of the crystalline peak, λ is the wave length of the X-ray radiation (1.54 Å), and k is the Scherer constant having a value of 0.9.

3.3 Fourier transform infrared spectroscopy

The interactions between PP, PLLA, and MAPP were studied by Fourier transform infrared spectroscopy (FTIR) analysis in attenuated total reflectance mode. It was carried out using FTIR spectrophotometer (Cary 600 Series, Agilent Technologies, USA) within the wave number range of 400–4000 cm^{-1}. A total of five scans were accumulated at a resolution of 4 cm^{-1}. The spectra were analyzed using Agilent Resolution Pro software.

3.4 Morphology

Scanning electron microscope (JSM-6510LV, JEOL, Japan) was used to examine the surface morphology of polymer films. The specimens were coated with gold in an automatic sputter coater (JFC1600) to avoid charging effect and to enhance emission of secondary electrons. The instrument was operated at 10 kV.

3.5 Biodegradability studies

Biodegradability studies were performed using pure culture in synthetic media. The culture (*P. stutzeri*) was in freeze-dried form in a sealed depressurized ampoule, which was broken carefully with sterile file in laminar air flow (Thermodyne Pvt. Ltd., India), and 300 μl of sterilized NB was added for suspension preparation. NB was sterilized by autoclaving at 121°C and 15 psi pressure for 15 min. The prepared suspension was added to 250 ml Erlenmeyer flasks containing 100 ml NB and incubated for 24 h at 35°C and 120 rpm in a shaker incubator (New Brunswick Scientific, USA). This grown culture was used as inoculum in biodegradation studies. Synthetic media (100 ml) was placed in 250 ml conical flasks, and the film samples (2×2 cm) were added in duplicates and inoculated with 5 ml of freshly grown culture. Prior to degradation, polymer films were weighed, disinfected in 70% ethanol, and dried under laminar air flow. The flasks were incubated in the shaker incubator at 35°C and 120 rpm for 60 days. Polymers (PP100, PP80, PP80C6, and PLLA100) were added as sole source of carbon and energy in synthetic media. Biodegradation was monitored in terms of weight loss, optical density (OD) of bacteria, and thermogravimetric (TG) analysis. OD at 600 nm was taken in UV-visible spectrophotometer (Lambda 35, Perkin Elmer, USA) after every 5 days to verify the formation of biofilm on polymer surfaces. Weight loss was measured after 60 days of incubation in the media. Prior to weight measurement, biofilm was removed by washing the polymer film samples with 2% sodium dodecyl sulfate for 4 h and subsequently with distilled water. The washed samples were dried overnight in an oven at 60°C and then weighed.

The TG and derivative TG (DTG) analysis of the polymer films before and after aging was determined by using TG analysis (Q-500, TA Instruments, USA) under a nitrogen flow of 50 ml/min. Samples weighing 10±2 mg were heated from room temperature to 600°C at a heating rate of 20°C/min.

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4 Results and discussion

4.1 Mechanical properties

The mechanical properties (tensile strength, elongation at break, and tensile modulus) of the PP/PLLA blends are shown in Table 3. As observed, neat PP had tensile strength of 45.4 MPa and tensile modulus of 941 MPa, which decreased on addition of PLLA due to its brittle nature. The increase in amount of PLLA caused the decrease in tensile strength, and the blend of 50:50 had the lowest strength amongst all the blends. In an ideal or highly compatible polymer blend, the blends are expected to have strength higher than or at least in between that of the pure polymers [8]. In the case of PP/PLLA, the blends had lower strength than neat polypropylene. With the addition of 20% PLLA, tensile strength reduced to 31.3 MPa. But on further increasing the proportion of PLLA, i.e., up to 30%, the tensile strength decreased drastically to 19.83 MPa, which is only 46% of neat PP. The elongation at break (E_b) showed zig-zag trend. The blend of 80:20 showed almost similar value of E_b as of PP, but further addition of PLLA led to continuous decrease in E_b. This trend of tensile strength and elongation at break in graphical form is shown in Figure 1. The tensile modulus showed decrease in its value with increase in content of PLLA, but it was quite higher in the case of PP80 and PP70. Therefore, PP80 (80% PP+20% PLLA) was chosen as optimum considering the better retention of mechanical properties as well as the relatively low proportion of expensive PLLA.

The effect of adding compatibilizer from 2 phr upto 8 phr was investigated for the blend containing 80% PP. The addition of compatibilizer increased the tensile strength and tensile modulus than pure PP but decreased the value of E_b. This increase in tensile strength and modulus can be attributed to fine bonding and formed interaction between PP and PLLA molecules with the addition of compatibilizer. The changes in tensile strength and elongation at break by varying MAPP composition in PP80 blend is shown in Figure 2. So 6 phr of compatibilizer in the blend was considered as optimum amount by which the blend showed better tensile properties.

Hence, PP80 (without compatibilizer) and PP80C6 (with 6 phr compatibilizer) were chosen for further studies and characterized and compared with the neat polymers.

Table 3 Mechanical properties of blends.

S. no.	Blend name	Tensile strength at yield (MPa)	Elongation at break (%)	Elastic modulus (MPa)
1.	PP100	45.4±2.3	3.3±0.2	941±47.1
2.	PP95	42.9±2.1	2.6±0.1	934±46.7
3.	PP90	40.0±2.0	3.8±0.2	1210±40.5
4.	PP85	32.4±1.6	2.1±0.1	788±39.4
5.	PP80	31.3±1.6	3.2±0.2	1010±50.5
6.	PP70	19.8±0.9	1.5±0.1	1100±55.0
7.	PP60	18.1±0.9	1.3±0.1	732±36.6
8.	PP50	12.5±0.6	0.7±0.0	694±34.7
9.	PP80C2	42.5±2.1	1.8±0.1	707±35.4
10.	PP80C4	50.7±2.5	2.5±0.1	824±41.2
11.	PP80C6	52.1±2.6	2.0±0.1	1080±54.0
12.	PP80C8	42.9±2.2	1.5±0.1	870±43.5

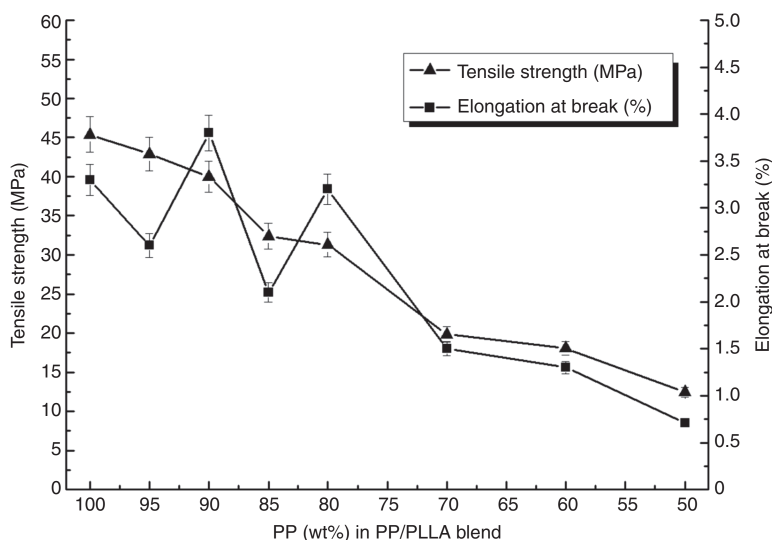

Figure 1 Changes in tensile strength and elongation at break by varying PP composition in blend.

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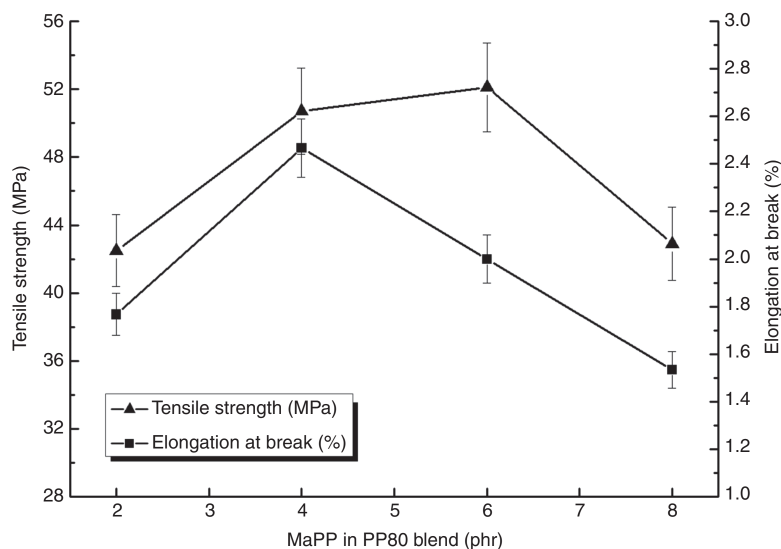

Figure 2 Changes in tensile strength and elongation at break by varying MAPP composition in PP80 blend.

4.2 X-ray diffraction

The XRD patterns of the selected film samples are shown in Figure 3. It was observed that PP100 showed prominent peaks at 2θ=14.1°, 16.8°, 18.6°, and 21.8°, which corresponds to the α-monoclinic form of polypropylene, and PLLA100 showed major peaks at 2θ=16.7° and 19°. This has been reported in literature also [2, 8]. After addition of PLLA into PP as in the case of PP80 and PP80C6, the spectra matched very closely to neat PP. This indicates no or minimum change in crystal lattice dimensions of blends as compared with neat polypropylene.

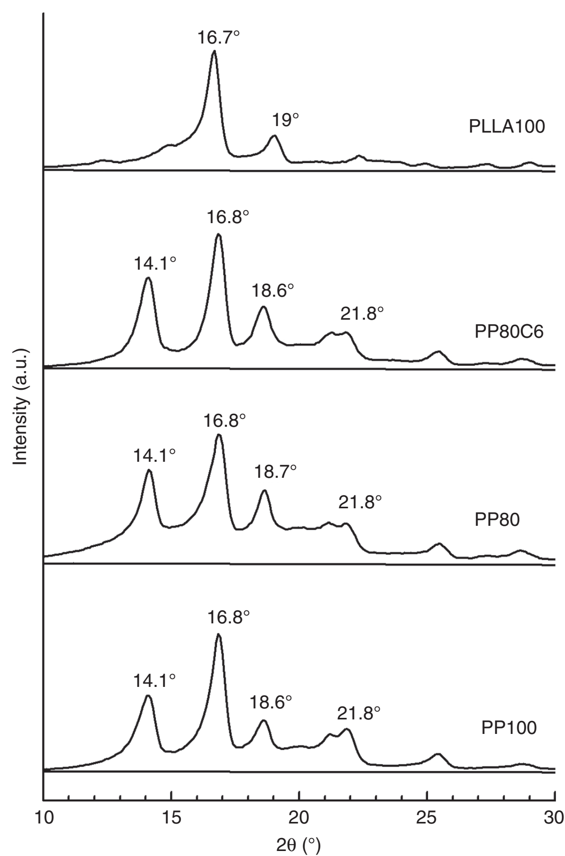

Figure 3 XRD patterns of PLLA100, PP100, PP80, and PP80C6.

The percentage of crystallinity of different polymers and crystallite size has been shown in Table 4. The addition of PLLA into PP causes increase in percentage of crystallinity from 79.7% to 86.7%, whereas compatibilized blend possesses almost the same crystallinity as of pure PP. Pure PP and PP80C6 have the same crystallite size (337 Å), whereas the crystallite size of PP80 (289 Å) is intermediate of pure PP and PLLA. This investigation showed that MAPP improved compatibility between the polymers.

4.3 FTIR studies

FTIR was carried out to study the occurrence of any chemical reaction between PP and PLLA. Figure 4 depicts the FTIR spectra of neat PP, neat PLLA, PP/PLLA, and PP/PLLA/MAPP blends. PLLA100 showed peaks at 1746, 1178,

Table 4 Degree of crystallinity and crystallite size of polymers from XRD.

Blend name	Degree of crystallinity (%)	Crystallite size (Å)
PP100	79.7	337
PP80	86.7	289
PP80C6	76.3	337
PLLA100	62.6	270

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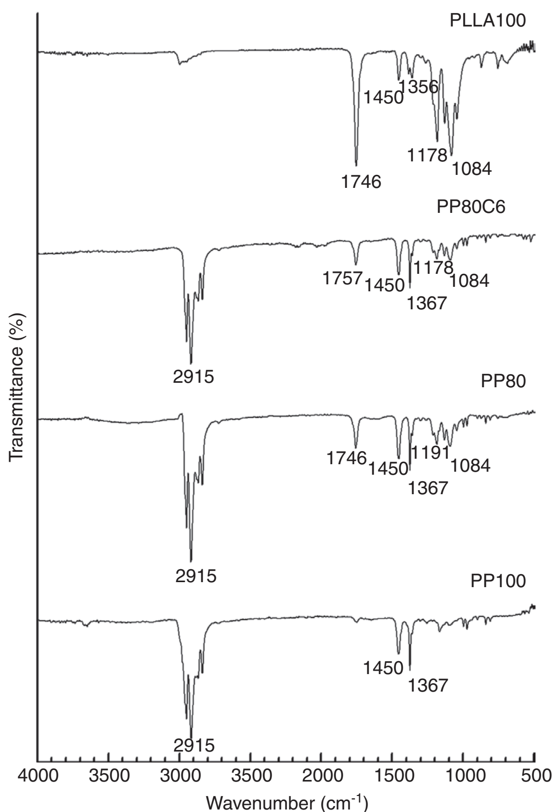

Figure 4 FTIR spectra of PLLA100, PP100, PP80, and PP80C6.

and 1084 cm^{-1}, which were associated with C=O stretching, C-O-C symmetric stretching, and asymmetric CH_3 bonds, and these peaks were absent in pure PP. PP100 showed C-H stretching, C-H bending, CH_3 bending, and CH_2 wagging corresponding to 2915, 1450, and 1367 cm^{-1}, respectively. The spectrum of PP80 showed transmittance peaks around 2915, 1746, 1450, 1367, 1191, and 1084 cm^{-1}, corresponding to both PP and PLLA indicating the absence of chemical interactions between the two components. Thus, there was decrease in mechanical properties of PP80 as compared with PP100. But addition of MAPP into the blend resulted in emergence of a new peak at 1757 cm^{-1} representing the carbonyl of ester linkage stretching. This indicates the interaction of MAPP with the PP/PLLA blend, which supports the XRD and tensile testing results. FTIR studies also showed a little chemical interaction between the two components after adding MAPP, which were not observed in the blend not containing the compatibilizer.

4.4 Morphological analysis

Mechanical properties of blends largely depend on morphology; therefore, scanning electron microsopy (SEM) analysis was carried out to better understand the correlation with morphology and structure of blends. The surface images of PP100, PP80, PP80C6, and PLLA100 are shown in Figure 5A, B, C, and D, respectively. It was

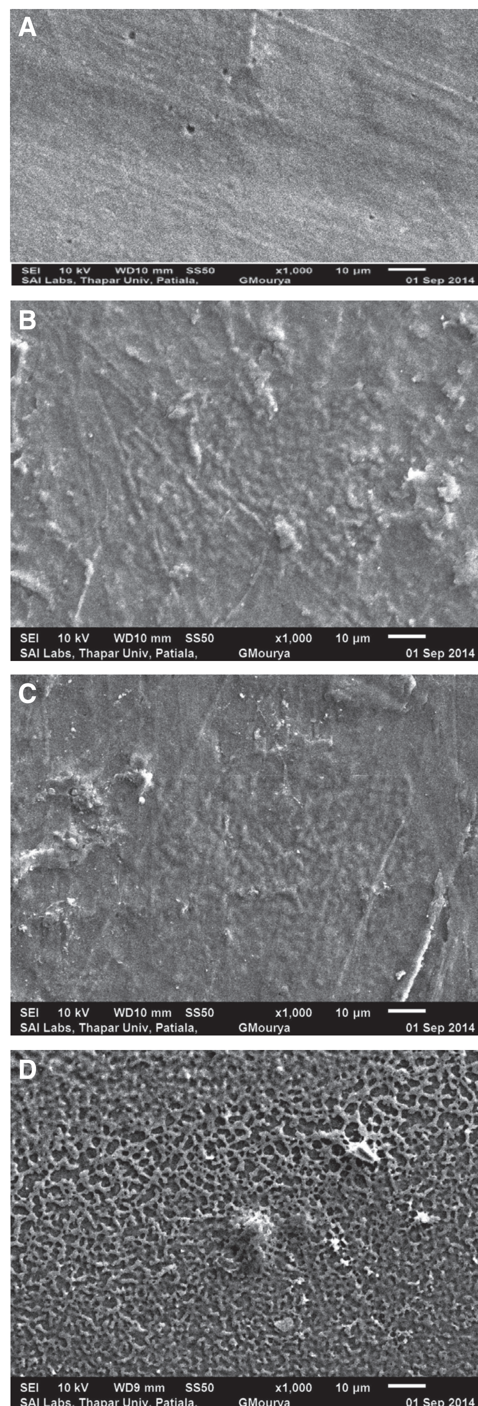

Figure 5 (A) Scanning electron micrograph of PP100 at 1000× magnification. (B) SEM of PP80 at 1000× magnification. (C) SEM of PP80C6 at 1000× magnification. (D) Scanning electron micrograph of PLLA100 at 1000× magnification.

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noticed that the surface of PP100 is smooth and that of PLLA100 is rough that contains some cracks and pits due to its brittle nature. The surface morphology of the blend PP80 showed dispersion of PLLA in PP matrix, but phase separation and boundary layer between the two components could be seen clearly. It depicts low interfacial adhesion and immiscibility between the two components. It was due to this that the addition of PLLA caused reduction in tensile strength of PP. However, addition of MAPP introduced compatibility between PP and PLLA in their blend, and as shown in the micrograph of PP80C6, PLLA was almost uniformly dispersed in the PP matrix, which resulted in increase in the tensile strength of PP80C6 sample, as described earlier. Therefore, it may be concluded that MAPP has improved the interfacial adhesion between PP and PLLA due to reaction between PLLA functional group and the anhydride group present in MAPP [9].

4.5 Biodegradability studies

4.5.1 Weight loss

The weight loss in polymer samples after 60 days of incubation was found to be 1.21%, 1.67%, 2.41%, and 3.01% for PP100, PP80, PP80C6, and PLLA100, respectively. PLLA100 showed the maximum weight loss. As the surface of PLLA is hydrophilic, more microorganisms attached to its surface and caused effective degradation, while in the case of PP, microorganisms took some time to form a biofilm on the surface; therefore, least degradation was observed in PP. This observation indicates that the bacterial strain *P. stutzeri* has noticeable potential to degrade the blends when kept in media.

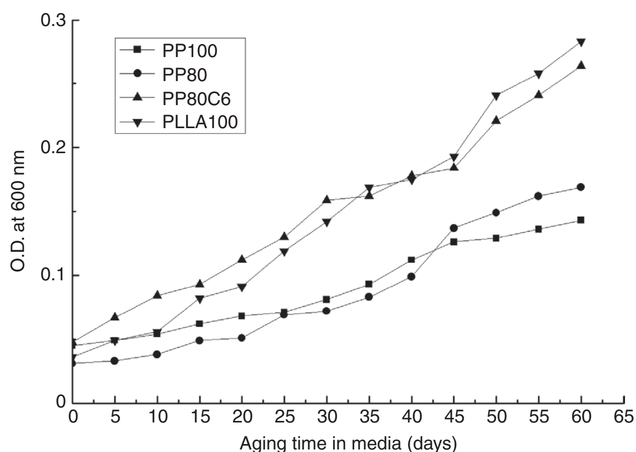

Figure 6 OD of bacteria with time.

4.5.2 Optical density

The survival of bacteria in the presence of polymers was monitored by determining the growth in synthetic media. The OD of the synthetic media at 600 nm was observed every 5 days incubation period and is shown in Figure 6. The maximum growth was found in the case of PLLA, indicating that the microorganisms grow faster in media with PLLA as carbon source. In the case of PP, although OD increased, the extent was very much less as compared with PLLA. The OD for PP80C6 was found to be large enough, almost closer to PLLA. These data comply with weight loss data and show that PP/PLLA blends show

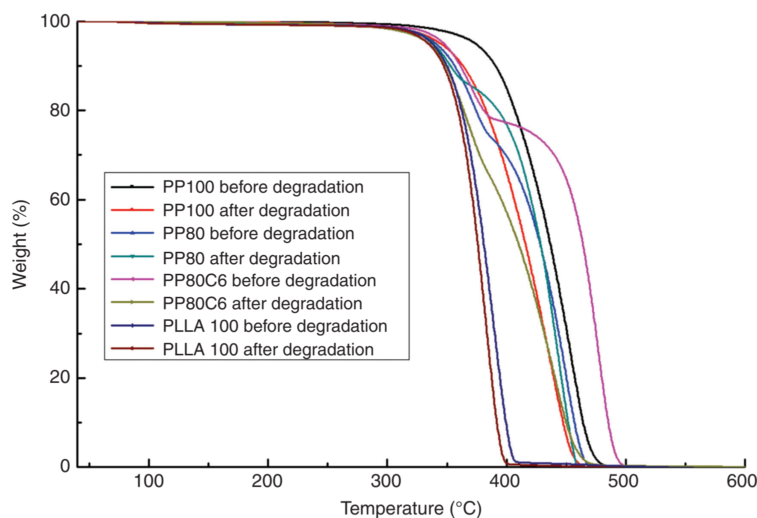

Figure 7 TG curves of polymer before and after aging.

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more effective degradation than PP alone, and bacteria were able to utilize polymer samples as sole source of carbon which resulted in their partial degradation.

4.5.3 Thermogravimetric analysis (TGA)

The TG and DTG curves of polymers before and after aging are illustrated in Figures 7 and 8, respectively. As observed, TG curves of both PP100 and PLLA100 showed one-stage degradation, but PP/PLLA blends showed two-stage degradation. Two distinct peaks at 320°C and 390°C were identified in the TG curves of blends (PP80 and PP80C6) corresponding to the thermal degradation of PLLA and PP, respectively, which indicated partial compatibility between the blend components. Furthermore, it was found that the thermal stability of PP80C6 was more than PP80, which means that compatibility has been improved by adding MAPP into the blend. The DTG curves showed two maxima for blends whereas single maxima for PP100 and PLLA100, indicating that there is phase separation between the two components. After aging, the maxima shifted toward lower temperature due to decrease in thermal stability of the samples.

The initiation temperature (T_i), final temperature (T_f) and percent weight loss are shown in Table 5. The initial degradation temperature (T_i) corresponds to 1% weight loss of the polymer sample, and the final degradation temperature (T_f) corresponds to 1% residual left after which no appreciable loss is possible [23]. The weight loss in virgin PP100 started at 319°C (T_i) and reached maximum at 478°C (T_f). This polymer sample, after exposure to bacterial aging for 60 days, shows 9°C decrease both in T_i and T_f, which indicates that it was not degraded much. But in the case of PP 80 sample, decrease in T_i was 22°C and that in T_f was 8°C. Similarly, 18°C decrease in T_i and 27°C decrease in T_f was noted in PP80C6, while in the case of PLLA100, 16°C decrease in T_i and 18°C decrease in T_f was observed. These data reveal that thermal stability of polymers reduced after aging in media, indicating considerable degradation of polymers. Maximum reduction in thermal stability was found in PP80C6 film sample.

Table 5 TG analysis of polymers before and after aging.

Blend name	T_i (°C)		T_f (°C)	
	Before degradation	After degradation	Before degradation	After degradation
PP100	319	310	478	469
PP80	282	260	466	458
PP80C6	295	277	495	468
PLLA100	275	259	417	399

5 Conclusions

Blends of PP/PLLA, with and without compatibilizer, were prepared in an internal mixer, and those blends were given the shape of films by compression molding technique. The ratio of PP/PLLA was optimized (separately for those with compatibilizer and without compatibilizer) on the basis of their mechanical properties. PP80 (80% PP and 20% PLLA) and PP80C6 (80% PP, 20% PLLA, and 6 phr MAPP) were found to be the best possible compositions, and, therefore, evaluated further for their physical, chemical,

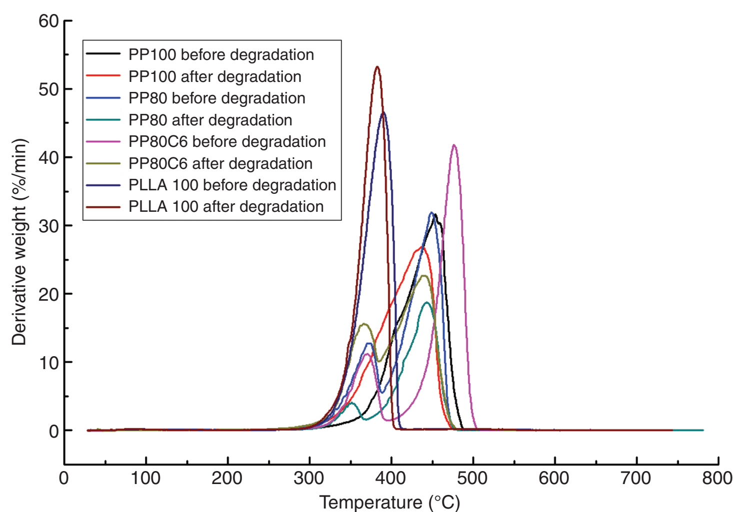

Figure 8 Derivative thermogravimetric curves of polymer before and after aging.

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and biodegradation behavior. The physical and chemical properties showed that PP and PLLA form immiscible blend due to poor interfacial adhesion and polarity difference between them. This was also supported by mechanical strength data which showed decrease in mechanical strength of blends with increasing amount of PLLA. By adding 6 phr compatibilizer into the blend, improvements in mechanical strength, compatibility, and thermal stability were observed. The same indication was given by the XRD results. Biodegradation studies with *P. stutzeri* shows that PP/PLLA blends undergo more biodegradation than neat PP, and PP80C6 showed maximum degradation. Hence, these partially biodegradable blends of PP and PLLA could be used for some flexible packaging applications replacing neat PP, thereby reducing plastic pollution to a certain extent.

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Kinetics of Biodegradation of Polypropylene/ Polylactide Blends

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ABSTRACT

Thin films of polypropylene (PP) and poly(L-lactide) (PLLA) blends were prepared in ratios PP/ PLLA = 100/0, 90/10, and 80/20 (with/without compatibilizer) and investigated for their biodegradation by Pseudomonas stutzeri bacteria under aerobic conditions. The biodegradation of the samples was estimated in terms of weight loss and the bacterial biomass colonizing the polymers. The blended films were also characterized for their thermal and morphological properties, before and after degradation. High growth of the bacterium was noticed for PLLA in the synthetic media as compared with PP/PLLA blends. After 60 days of incubation period, the weight loss observed was 3.01% for PLLA (neat), 2.41% for 80:20 PP/PLLA blend, 1.21% for 90:10 PP/PLLA blend and only 0.25% for PP (neat) films respectively. Thermogravimetric analysis (TGA) showed that the degradation of PP/PLLA blends is more than the virgin PP films. Surface morphology (SEM) studies indicated degradation of the blends incubated with Pseudomonas stutzeri in the form of pits and cracks developed on their surfaces.

KEYWORDS: *Polypropylene, Polylactide, Blends, Biodegradation kinetics, Pseudomonas stutzeri.*

INTRODUCTION

Polypropylene (PP) is a widely used in a variety of applications, like packaging segment which accounts for over 35% of the global consumption of the total plastics. PP is normally tough and flexible, especially when copolymerized with ethylene, which allows polypropylene to be used as an engineering plastic. It is rugged and unusually resistant to many chemical solvents, bases and acids and hence remains inert to degradation. The global consumption of PP in 2010 was 50 million tons and moreover, per capita consumption of plastic

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in India is 5 kg[1]. These figures reveal an idea about the huge plastic waste which deposits in the environment after their use and careless disposal, and therefore creating serious and critical problem all over the world[1-3]. In last two decades, a steady increase in the investigations concerning the total or partial substitution of synthetic plastics by biodegradable materials have proven to be useful, in solving the problem of solid waste management of plastics[4-9]. Poly(L-lactide) (PLLA), derived from renewable resources, has been attracting the attention of many researchers because of its biomass origin, recyclability (based on its biodegradation), hydrolysis, and thermal depolymerization functions[10-14].

In environment, the dumped plastic waste is subjected to deterioration or degradation by solar radiation, ultraviolet (UV) rays and heat, which mainly affect their surface properties, but this process degrades the plastic extremely slowly[15-16]. 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[17]. 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[18]. The oxidation of the polymer surface occur when treated by UV rays, thermal radiation and/or chemical radiation and forming carbonyl, carboxyl and ester functional groups, which ultimately decreases the hydrophobicity of the surface and therefore, support the formation of microbial biofilm on the surface[19-21]. Thus, pre-treatment helps the biodegradation of the polymer to be more effectual. It has also been seen that there is a synergistic interaction between photo-oxidation and biodegradation of polyethylene[22]. An increase in UV exposure time enhances the biodegradation of polythene. However, an extensive literature on the biodegradation of low density polyethylene (LDPE) is available[2,23], but the data and literature on biodegradation of PP is very limited. A fungal species, *Aspergillus niger* and microbial community including *Pseudomonas* and *Vibrio* species have been reported to biodegrade PP[1,24]. A mixture of hydrocarbons (between $C_{10}H_{22}$ and $C_{31}H_{64}$), in the form of methylene chloride, amounting to approx. 40%, was observed on isotactic PP when exposed to bacterial consortia for 175 days[24]. It is also evident that fungus is also able to utilize PP as its sole carbon source and 30-60% growth of *A. niger* was observed on gamma irradiated films after 6 weeks[25]. When thermally pretreated PP was exposed to mixed soil consortia under *in vitro* conditions, the polymer lost approx. 10% of weight[18].

In this research article, biodegradation of PP and PP/PLLA blends under aerobic conditions with the bacterial species *Pseudomonas stutzeri* is studied extensively along with the changes in their thermal and morphological properties before and after degradation.

EXPERIMENTAL

Materials

Polymers

The trade name, company name, and the key properties of the polymers used in the study are summarized in Table 1 below.

TABLE 1. Names and properties of polymers and compatibilizer used

Sr. No.	Material	Trade name	Company name	Properties
1	Polypropylene (PP)	Halene P, F103	Haldia Petrochemicals Limited, Kolkata, India (www.haldiapetrochemicals.com)	MFI = 3.3 g/10 min Density = 0.90 gm/cc T_m = 152°C
2	Poly(L-lactic acid) (PLLA)	Biomer L 9000	Biomer Forst-Kasten-Str Kailling, Germany (http://www.biomer.de)	MFI = 3.0 g/10 min, M_w = 20 kDa, T_m = 190°C
3	Maleic anhydride grafted polypropylene (MAPP)	Optim P406	Pluss Polymers Pvt., Ltd., Gurgaon, India (http://www.pluss.co.in/)	MFI = 40 g/10 min, Density = 0.91 g/ml, T_m = 163°C MA content = 0.5-0.8%

Bacterium

Pseudomonas stutzeri (MTCC number 2643) was procured from Microbial Type Culture and Gene Bank, Institute of Microbial Technology (IMTECH, Chandigarh, India). This bacterium was mesophilic, gram-negative, motile and rod-shaped with an optimum growth at 35°C.

Chemicals

All the chemicals used to prepare the media (viz. nutrient broth, nutrient agar etc.) were obtained from Himedia Laboratories Ltd. (Mumbai, India).

METHODS

Media for cultivation

Nutrient broth (NB); per liter containing 5 g peptic digest of animal tissue, 5 g sodium chloride, 1.5 g beef extract and 1.5 g yeast extract and Nutrient agar (NA); per liter containing 5 g peptic digest of animal tissue, 5 g sodium chloride, 1.5 g beef extract, 1.5 g yeast extract and 15 g agar was used to maintain the bacterial culture (Figure 1).

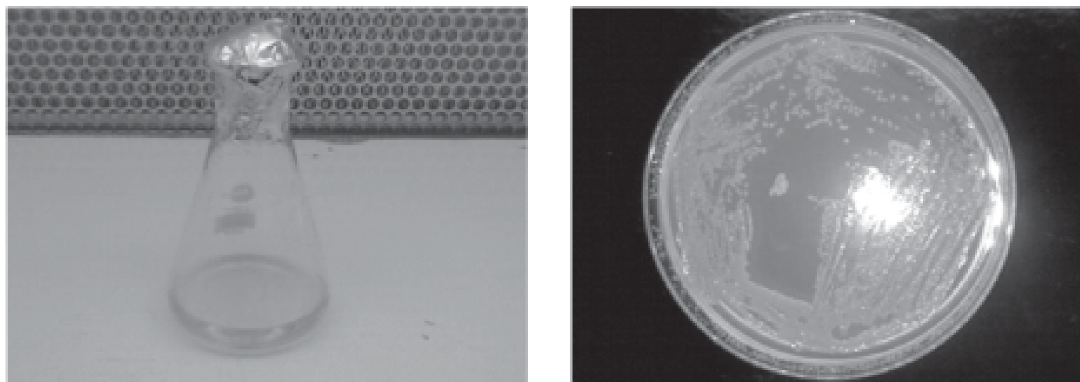

Fig. 1. Bacteria cultivated in (a) Nutrient broth and (b) Nutrient agar

Media for inoculation and degradation

As the bacterial strain should be capable of utilizing the polymer as the sole source of carbon and energy, therefore, they were grown on minimal medium containing (g/l of distilled water): 1 g NH_4NO_3, 0.7 g $MgSO_4.7H_2O$, 1 g K_2HPO_4, 1 g KH_2PO_4, 0.005 g NaCl, 2 mg/l each of $FeSO_4.6H_2O$ and $ZnSO_4.7H_2O$ and 1 mg/l of $MnSO_4.H_2O$. The set-up used for degradation experiments is shown in Figure 2.

Fig. 2. Shaker incubator used for degradation experiments

Polymer blends preparation

Blends of PP and PLLA with/without compatibilizer in varying ratios were prepared in a Haake Rheocord instrument (Haake Rheocord, Burladingen, Germany), fitted with cam type counter rotors with a rotor speed of 60 rpm at 190°C for 4 min. using a fill factor of 0.8. The blends in semi-solid state were then passed through a two-roll mill (Schwabenthan, Berlin, Germany) at room temperature with a nip gap of 2 mm. Finally, the films (of 30-60 µm thickness) were casted in a compression molding hydraulic press (George E. Moore, Birmingham, England) electrically heated at 190°C for 2 min at a pressure of 5 MPa. The mold was then cooled to ambient temperature by circulating water through the platens, while maintaining the pressure to maintain the dimensional stability of the samples. While molding, Teflon sheets were used to prevent the molten mass sticking to the mold. The notation and composition of the various blends is described in Table 2.

TABLE 2. Notation and composition of various blends of PP/PLLA

Sr. No.	Blend Notation	Composition (phr)		
		PP	PLLA	MAPP
1	PP100	100	0	0
2	PP90	90	10	0
3	PP90C4	90	10	4
4	PP80C6	80	20	6
5	PLLA100	0	100	0

Revival of culture

The bacterium was provided in freeze-dried form in a sealed depressurized ampoule. The ampoule was marked near the middle of the cotton wool with a sharp

file. The surface around the mark was disinfected with ethanol. A thick cotton wool was wrapped around the ampoule and it was then broken. The pointed top of the ampoule was removed gently (this step had to be performed very carefully as hasty opening might release bacterial spores into the atmosphere).

Approximately 200 ml of nutrient broth was sterilized by autoclaving at 15 psi and 121°C for 15 minutes. Sterilized nutrient broth medium (0.3 to 0.4 ml) was added in ampoule to make a cell suspension, which was then taken into two Erlenmeyer flasks (of 250 ml capacity) each containing 100 ml nutrient broth. These flasks were incubated for 24 hours at 35°C and 120 rpm in a shaker incubator (New Brunswick Scientific, USA).

Estimation of aerobic biodegradation

Aerobic biodegradability of the polymer blends by *Pseudomonas stutzeri* was determined as per ASTM D5247 standard. The polymer films were cut into small strips (2 × 2 cm), weighed, disinfected and dried in laminar air flow. Afterwards, the films were taken in Erlenmeyer flasks containing 100 ml minimal medium inoculated with the culture and incubated in the shaker incubator at 35°C and 120 rpm under aerobic conditions for 60 days.

Growth curve in nutrient broth

Two Erlenmeyer flasks containing 100 ml nutrient broth were taken. One flask was inoculated with culture of *Pseudomonas stutzeri* and the other one was taken as blank (not inoculated). Both flasks were kept in shaker incubator at 35°C and 120 rpm. Optical density (OD) was measured in regular intervals of 1 hour at 620 nm in UV-Vis Spectrophotometer (Perkin Elmer, USA). The growth pattern of the *Pseudomonas stutzeri* under controlled conditions was determined.

Growth curve in synthetic media (with glucose as carbon source)

Similar to the above, two Erlenmeyer flasks containing 100 ml minimal media with 3% glucose as a sole source of carbon were taken. Overnight grown culture of *Pseudomonas stutzeri* was inoculated in one of the flasks and the other one was taken as blank (not inoculated). Both flasks were kept in shaker incubator at 35°C and at 120 rpm for the growth of bacteria. The growth curve was obtained by measuring OD at regular intervals of 1 hour representing the behavior of culture in different carbon source i.e., glucose.

Growth kinetics in synthetic media (with polymers as carbon source)

Polymer strips were immersed in 100 ml of minimal media and 3 ml of inoculum (24 hour old culture in nutrient broth) in 250 ml Erlenmeyer flasks. The growth of bacteria in presence of polymer films was monitored by taking OD at 620 nm and in terms of colony forming units (CFU) count. The flasks were consistently monitored after equal interval of 5 days to verify the formation of biofilm on polymer surfaces. 2 ml of media was taken from the flasks and OD was measured at 620 nm to ensure the culture growth and utilization of polymers as its carbon source. CFU count was taken by serial dilution and spread plating technique.

Weight loss

One of the important aspects of polymer's degradation is determining the weight loss as it is a result of reduction in its integrity. After 60 days of retaining period (degradation time), the bacterial biofilm was washed off from the film surface 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.

Protein estimation of microbial biofilm

The population density of the biofilm on the polymer surfaces was estimated by determining the concentration of extractable protein. Colonized polymer samples were washed and centrifuged at 10,000 rpm at 4°C for 15 min; the supernatant was kept aside and the pellet was subjected to the same procedure repeatedly. The two supernatants thus obtained, were mixed and the protein concentration was determined by Bradford method, which is a dye binding method using bovine serum albumin (BSA) as a standard.

Thermogravimetric analysis (TGA)

TGA determines the amount and rate of weight loss of the sample as a function of temperature and thus, is a useful tool to characterize thermal degradation and

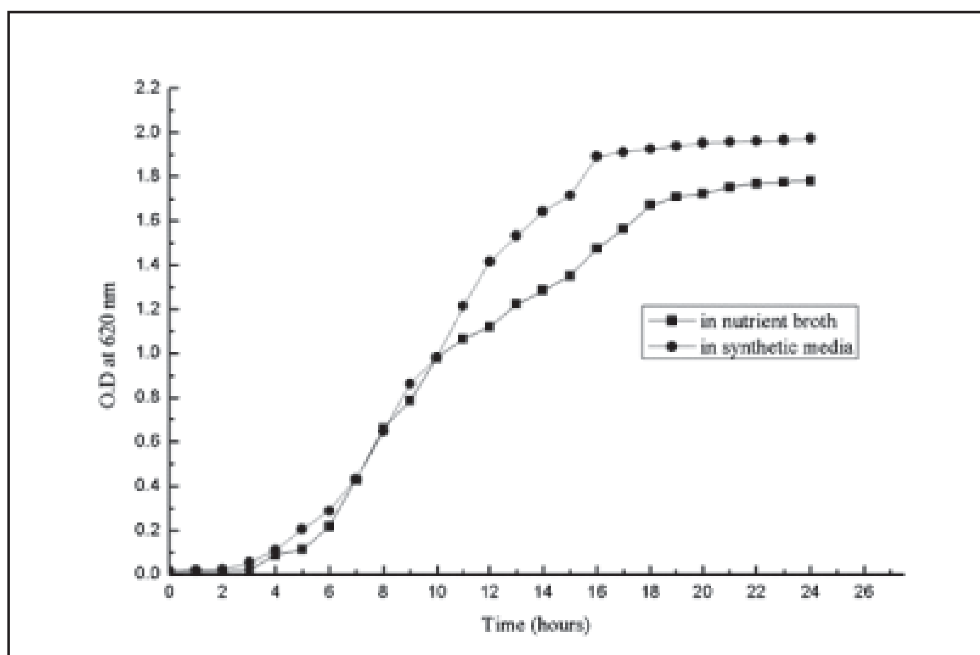

Fig. 3. Growth kinetics of *Pseudomonas stutzeri* in nutrient broth and synthetic media (with glucose as carbon source)

thermal stability of polymer materials. It was done using TGA apparatus (Q500, TA Instruments, USA). The sample weight was 5 mg and the temperature range was 35-750°C with a heating rate of 20°C per minute under inert atmosphere (nitrogen flow). The flow rate of nitrogen was kept at 50 ml/min.

Scanning electron microscopy (SEM)

Scanning electron micrographs of the films were taken with a scanning electron microscope (JSM 6510LV, JEOL, Japan). 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 and the accelerating voltage was kept at 10 kV.

RESULTS AND DISCUSSION

Growth kinetics

The purpose of studying growth curve of *Pseudomonas stutzeri* in nutrient broth was to determine the time period at which exponential phase starts as the culture during mid-exponential phase was taken as inoculum for the purpose. The growth pattern of *Pseudomonas stutzeri* in nutrient broth and in synthetic medium with glucose as carbon source is shown in Figure 3.

The lag phase continued for 5-6 hours, log (or exponential) phase occurred for 6-17 hours and then stationary phase started with little or no growth, which is due to lack of nutrients. In case of synthetic medium, the growth curve followed almost the same pattern as that of nutrient broth.

Figure 4 represents the growth behavior in synthetic media with polymer(s) as the carbon source. The ability of a microorganism to utilize any substrate depends on its growth and its adherence to form a biofilm. Readings were

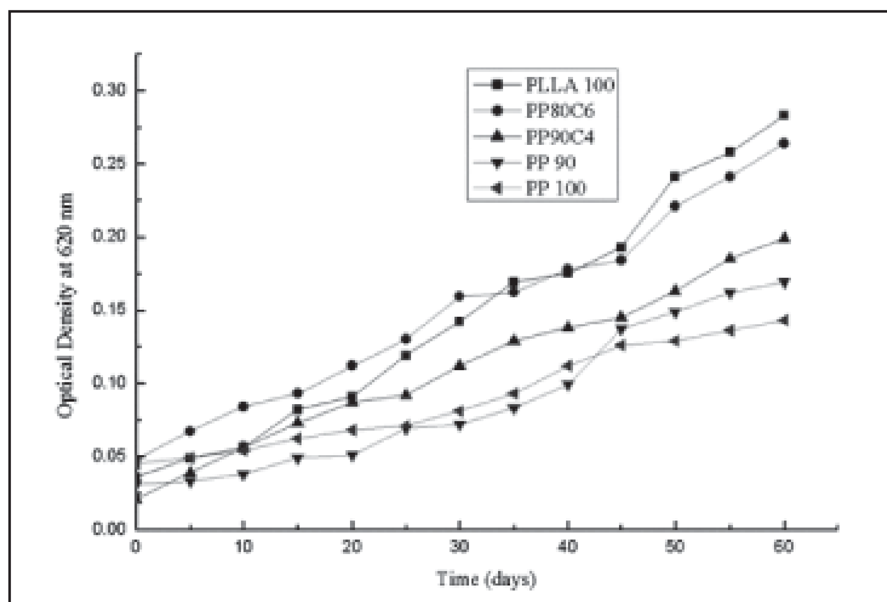

Fig. 4. Growth kinetics of Pseudomonas stutzeri in synthetic media (with polymers as carbon source)

taken at 5 days interval to determine the microbial activity. It can be easily evaluated from Figure 4 that the growth of *Pseudomonas stutzeri* is higher in case of PLLA as compared to PP and PP/PLLA blends.

As the surface of PLLA is hydrophilic, therefore more microorganisms attached to its surface and caused rapid degradation while in case of PP microorganisms took longer period (of some days) to first make the surface hydrophilic initially by the release of certain enzymes (particularly esterase), and then attached to the surface and used it as their carbon source. The PP/PLLA blends follow the similar growth pattern as that of PLLA.

Growth in terms of colony forming units (CFU)

The survival of this strain was monitored by determining the growth in the form of colony forming units (CFU) observed at every 5 days incubation period. The graph for the observations is plotted in Figure 5.

The maximum growth (CFU count) was found in case of PLLA (in the range of 10^5 colonies/ ml), which comply with the maximum OD observed, indicating that the microorganisms grow faster in media with PLLA as carbon source, which in turn lead to its degradation. In case of PP, though CFU/ml is increasing, but the extent is very much less as compared to PLLA and PP/PLLA blends. CFU count for PP80C6 was found to be large enough, almost closer to PLLA. It indicated that the bacterial strain *Pseudomonas stutzeri* has noticeable potential to degrade the polymers when kept in media.

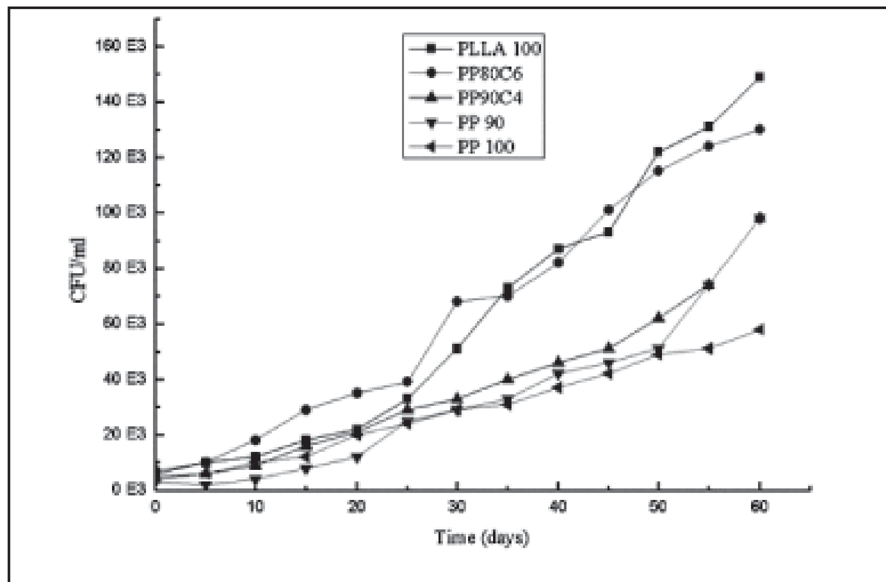

Fig. 5. CFU (or total viable count) during degradation of different polymer samples Weight loss

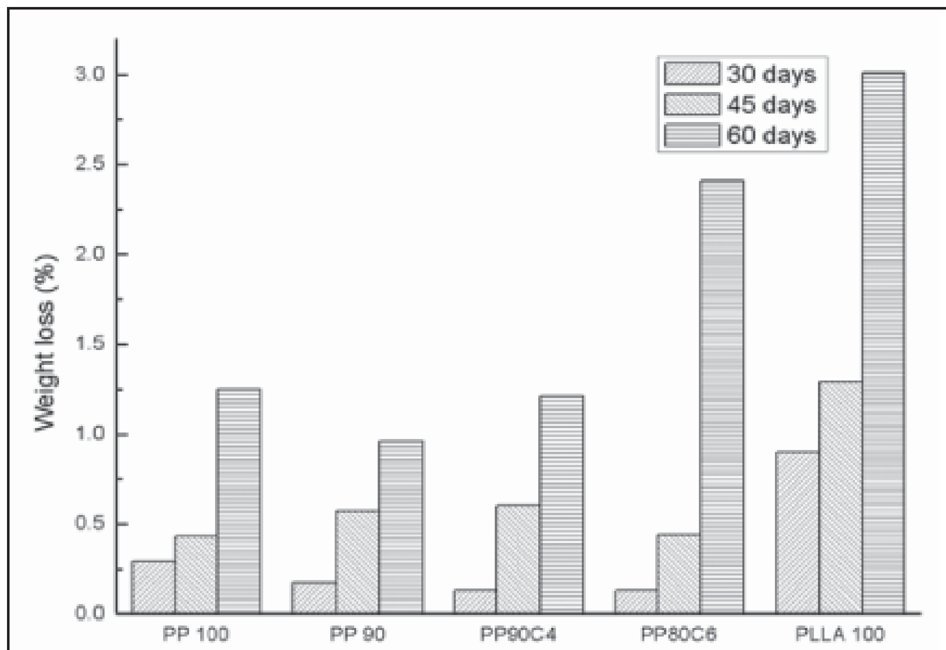

Fig. 6. Percentage weight loss comparisons of different polymer samples Protein estimation of microbial biofilm

Weight loss

The observations of reduction in weights of the different samples, calculated after each incubation period of the samples, i.e. at 30, 45 and 60 days interval, are given in Figure 6. At the end of 60 days, the weight loss by 3.01% for PLLA, 2.41% for PP80C6, 1.25% for PP90C4, 1.21% for PP90 and 0.25% for PP100 samples respectively. PLLA showed higher weight loss than others. Second largest weight loss was observed in PP80C6. The above data shows that PP/PLLA blends show more effective degradation than PP alone and bacteria were able to utilize polymer samples as sole source of carbon which resulted in their partial degradation.

Protein estimation of microbial bio films

Growth of microorganisms (i.e. biofilm) on the surface of the polymer was monitored by quantifying the total proteins extracted from the biofilm. The OD of a known sample i.e. bovine serum albumin (BSA) was taken as standard.

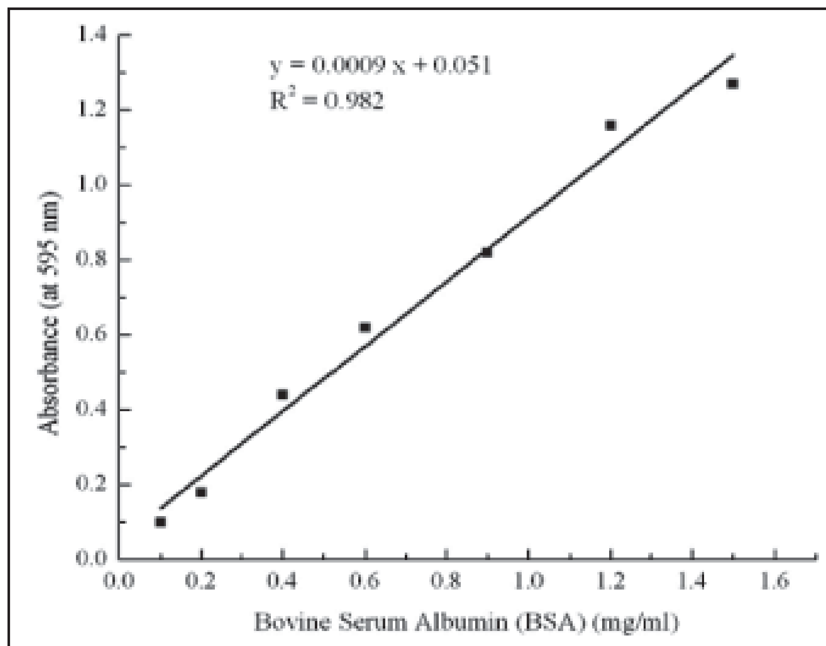

Fig. 7. Standard curve of BSA

The standard curve of bovine serum albumin BSA was obtained using known concentrations of BSA and shown in Figure 7.

The plot for the protein estimation is given in Figure 8. It represents the changes in protein concentration of the biofilm which was formed on the polymer surfaces. There observed an increase in protein concentration consistently which reflects an increase in the biomass. The figure also depicts the comparison between the concentrations of proteins extracted from different polymer samples.

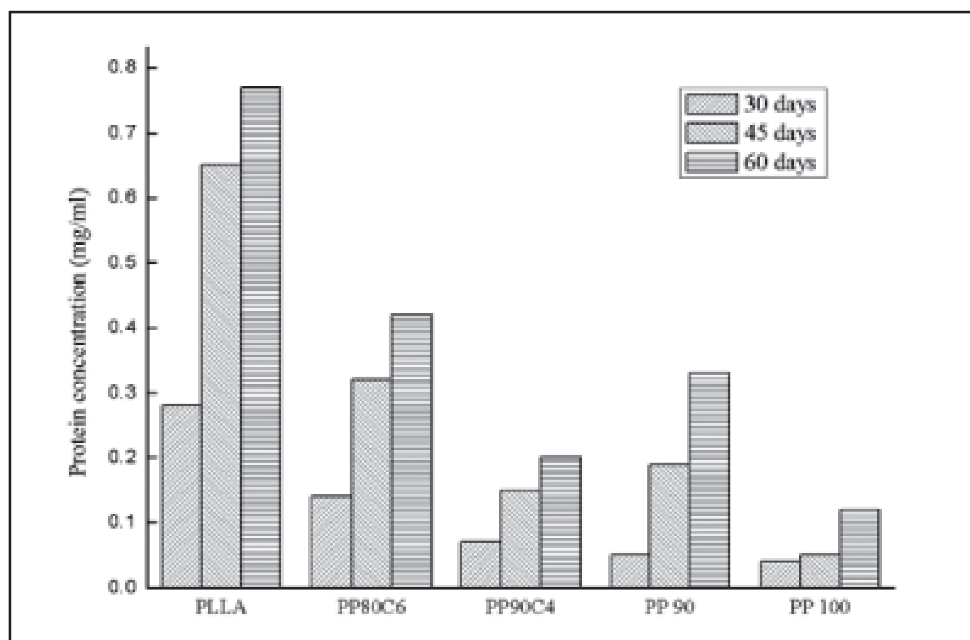

Fig. 8. Protein content (of microbial biofilm) comparisons on the surfaces of the blends

TABLE 3. TG analysis of pure polymers and the blends

Sr. No.	Blend Name	T_i (°C)		T_f (°C)		Weight loss (%)
		before degradation	after degradation	before degradation	after degradation	
1	PP100	319	310	478	469	100
2	PP90	306	280	491	449	100
3	PP90C4	301	282	490	458	100
4	PP80C6	295	277	495	468	100
5	PLLA100	275	259	417	399	100

Thermal properties

Thermogravimetric (TG) curves of virgin (pure) polymers and the blends are illustrated in Figure 9 and the results including the thermal degradation initiation temperature (T_i) and final temperature (T_f) are summarized in Table 3. T_i corresponds to the value of the temperature after just 1% weight loss in the polymer sample, and T corresponds to that after 99% weight loss of the sample during the TGA test.

It was observed that the thermal degradation of both PP100 and PLLA100 was one-stage degradation, but that of PP/PLLA blends was two-stage degradation. The two distinct stages (values) correspond to the thermal degradation temperatures of PLLA and PP respectively.

Virgin PP (i.e. PP 100) showed the value of T_i as 319°C and that of T_f as 478°C. This polymer

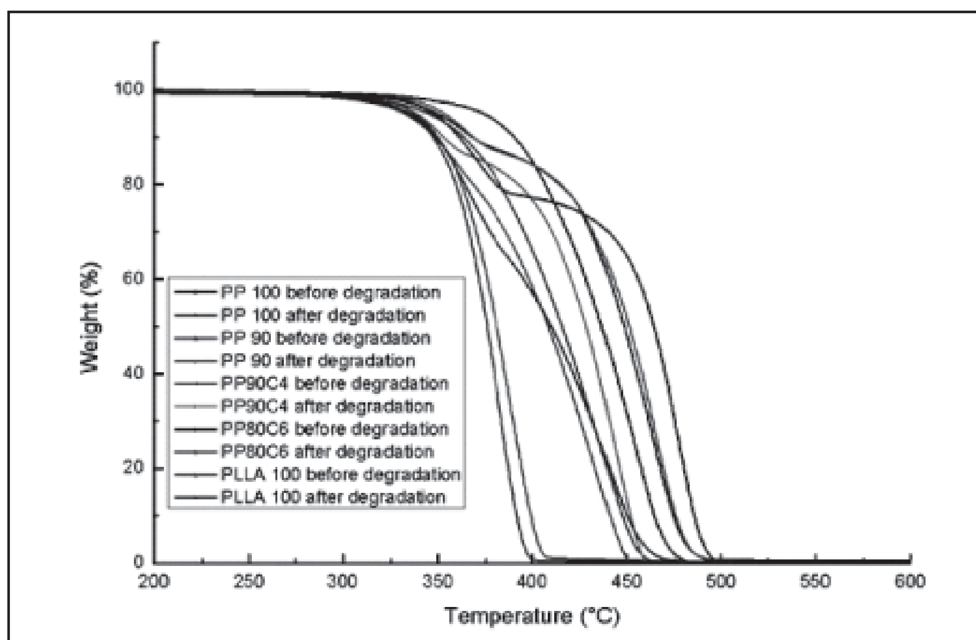

Fig. 9. Thermogravimetric (TG) curves of the polymer blends before and after degradation

sample, after exposing to bacterial degradation for 60 days exhibits only 9°C decrease both in T_i and T_f, indicating minor degradation. But in case of PP 90 sample, decrease in T_i was 26°C and that in T_f was 42°C. Similarly, 19°C decrease in T_i and 32°C decrease in T_f was noted in PP90C4; while in case of PP80C6, 18°C decrease in T_i and 27°C decrease in T_f was noted. PLLA100 showed 16°C decrease in T_i and 18°C decrease in T_f after degradation. This data revealed that the thermal stability of the polymer blends was reduced, which was a clear indication of the polymer backbone chain cleavage by the microorganisms helping in their degradation.

Morphological properties

The surface changes and bio-deterioration of polymer films were evaluated from scanning electron micrographs (SEM) of the films, which are shown in Figure 10.

The structural changes in the form of pits and erosions indicate the damages on the surface of different polymer samples incubated with *Pseudomonas stutzeri.* Surface of PLLA was observed to be rough with well-defined numerous shallow pits. As the surface of PLLA is hydrophilic, microorganisms easily adhere to the surface and after the removal of microorganisms; the surface became physically eroded. The microbial attack on PP/PLLA blends caused rearrangement of the polymer chains and changes in the PP amorphous phase and interphases of the different polymers which lead to the surface erosion. There were several irregular cracks and pits of variable sizes on the surfaces of PP90C4

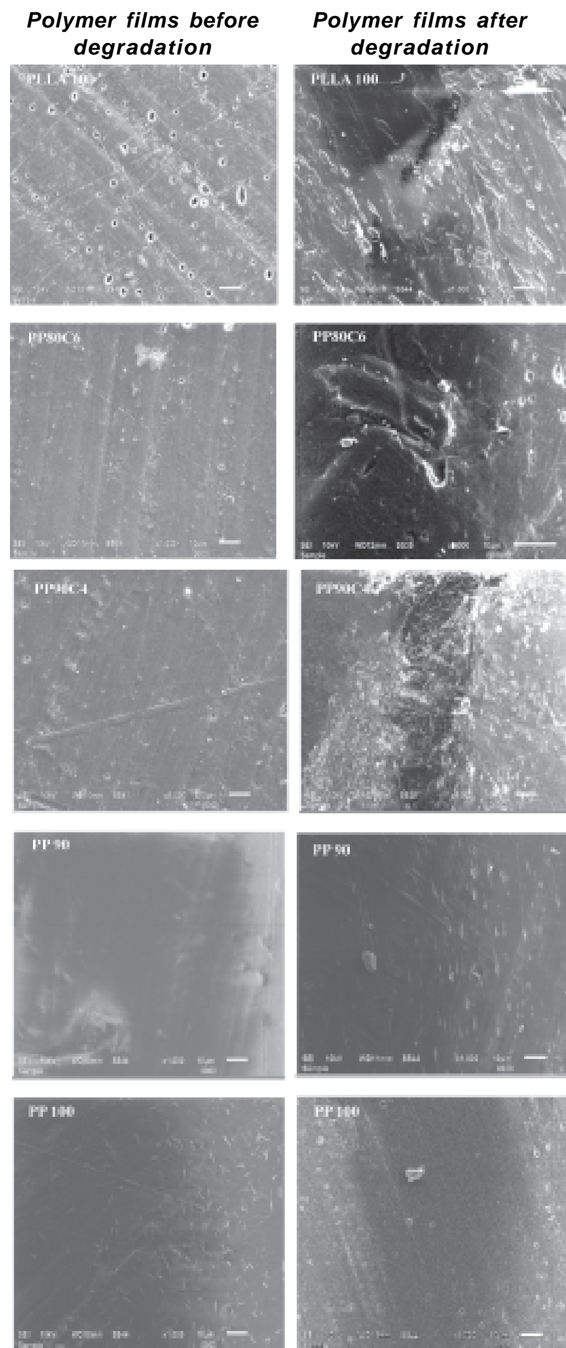

Fig. 10. SEM Micrographs of different polymer samples before and after degradation at 1000 X

and PP80C6, while the surface of PP90 sample was smooth with very few deformities. In conclusion, PP is resistant to decomposition by microorganisms, although the appearance of cracks occurred, caused by contraction of the surface layers as one of the consequences of 'chemi-crystallization'. The surface of PP100 sample, after degradation, was observed rough as compared to the sample before degradation but no marked holes and pits were noticed.

CONCLUSIONS

This study revealed that the PP/PLLA blends with the used additives can be biodegraded to significant levels, in the microbial strain used under aerobic conditions. Culture enrichment methods were found effective for enhancing the capabilities of a bacterium in utilizing the polymer(s) as the sole carbon and energy source. Maximum biodegradation was observed in case of PLLA followed by PP80C6 and then PP90C4. Although biodegradation of PP was very minute, but the culture was able to survive in media containing PP and thus, the polymer showed a little degradation. It is clear from the study that the bacterium *Pseudomonas stutzeri* is able to degraded carbon backbone of the polymer. Blending PLLA with PP increased its surface hydrophillicity, which is prerequisite for biodegradation. Hence, the degradation was more facile in PP/PLLA blends as compared to neat PP. Biodegradation by *Pseudomonas stutzeri* is also supported by the SEM micrographs, which showed drastic surface changes in the polymer samples.

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