

Studies on Biodegradation of Bioplastics under Composting Conditions and its Ecotoxicology Impact on Crop Sustainability

*Thesis submitted in partial fulfillment of the requirements for the
degree of*

DOCTOR OF PHILOSOPHY

Submitted by

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*Dedicated to My
Beloved Parents for
their Endless
Sacrifices, Love,
Support and
Encouragement*









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CERTIFICATE

This is to certify that the research work in the thesis entitled “**Studies on Biodegradation of Bioplastics under Composting Conditions and its Ecotoxicology Impact on Crop Sustainability**”, is carried out by me at the Chemical Engineering Department, Indian Institute of Technology Guwahati, under the supervision of **Professor Vimal Katiyar** and **Professor Ajay Kalamdhad**. The results documented in this thesis are achieved by me and has not been submitted to any other University or Institute for the award of any degree or diploma.

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CERTIFICATE

This is to certify that the thesis entitled “**Studies on Biodegradation of Bioplastics under Composting Conditions and its Ecotoxicology Impact on Crop Sustainability**”, being submitted by **Naba Kumar Kalita** for the award of Ph.D. Degree has been carried out by him at the Chemical Engineering Department, Indian Institute of Technology Guwahati, under our guidance and supervision. The work documented in this thesis has not been submitted to any other University or Institute for the award of any degree or diploma.

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“giramasma Ekamaksharam”







ABSTRACT

The use of plastic products increases problems associated with waste disposal and management. Growing concerns related to environmental degradation has directed research towards the development of plastics that could undergo biodegradation. Biodegradable materials that are commonly present in the market are poly (lactic acid) PLA, polycaprolactone PCL, and poly(butylene adipate-co-terephthalate)], PBAT. Other examples include cellulose, protein, chitosan, gum arabic etc. PLA, a linear aliphatic polyester has significant potential to replace the conventional petroleum based plastics to overcome such disposal problem. Most of these plastics are specifically targeted towards packaging applications that may help to facilitate scrap collection for their composting. Collection of these scraps and commercial composting facilities are increasing day-by-day across the globe with increasing use of those biodegradable plastics. Nowadays, many researches have also targeted towards specific microbial strains, which can degrade conventional plastics, and have succeeded up to a certain extent as microorganisms play a key role in the biodegradation of polymers in the real environment conditions. Plastic materials originating or produced from bio-sources like corn, starch, sugar etc., unlike conventional plastics originated from fossil-based sources, are known as bio-based plastics, and also called “plant-derived products”. Hence, plastics that biodegrade within a certain time period are said to be biodegradable plastics. Main motivation behind the current work is to understand and to observe the extent of biodegradation of biopolymer based biocomposites and blends under composting and natural soil conditions for development of a suitable biopolymer based composite with high biodegradability. Biodegradable polymers like PLA and PCL are having excellent polymeric properties comparable with their conventional counterparts. Still there are several drawbacks and questions related to biodegradability and its end-of-life surrounding environmental conditions and parameters.

These led to the development of various biodegradable polymers using natural biofillers (modified chitosan, modified gum, functionalized cellulose nanocrystals (CNC), cellulose microcrystalline (MCC) and polymeric blends) for enhancing certain plastic properties like permeability, mechanical strength etc. This work also focused on biodegradation through biofilm formation. In this work, ASTM D5338-15 was followed for laboratory setup and standard protocol with a little modification for accessing percentage biodegradation. Indigenously built composting reactors were designed and built for the composting process with accordance to ASTM International D5338-15. PLA and PCL can be synthesized by polymerization of precursor lactic acid or its derivative lactide and monomer caprolactone respectively. Mixing optimized concentrations of biofillers (modified chitosan, modified gum, functionalized cellulose nanocrystals (CNC), cellulose microcrystalline (MCC)) and algae biomass with PLA and PCL enhanced plastic properties with biodegradability. Hence, its percentage biodegradation and biodegradability tested under different environmental conditions. Biodegradation kinetics and percentage biodegradation of these blends and biocomposites revealed modified chitosan of having higher rate of biodegradation than other tested samples. This was due to presence of nitrogen as amines in the chitosan, which lead to higher microbial assimilation of the biocomposite. For supporting this thought algae biomass with high nitrogen content was used as biofiller in PLA based biocomposites. It was found that it has increased the biodegradation rate of the bioplastic due to formation of high microbial colony growth and early attraction of microbes in the film surface during the degradation period than neat PLA making it more susceptible towards microbial assimilation at a much faster rate than rest of the filler used under composting environment. Thus, algal biomass can be used in PLA as well as in PCL based biocomposite for enhancing the biodegradation rate of the biopolymer under composting condition. PLA/PCL/MCC: 80/20/3 also showed higher biodegradation rate than neat PLA and neat

PCL under composting environment. Under natural soil condition, it was found that both commercial PCL and our lab synthesized PCL got biodegraded under 180 days of time. It was also found that addition of certain biofillers slows the rate of biodegradation in PLA based biocomposites under composting condition prepared for improving properties like mechanical strength and permeability. Another study demonstrated the plant growth study of potato (*Solanum tuberosum* L) on used (biopolymer biodegradation biomass) compost as fertilizer or growth substrate under controlled greenhouse condition. Molecular weight of the samples with biopolymer and without biopolymer was found to be 400 Da and 430 Da signifying zero uptake of any oligomers from the biopolymers. Despite of high pH of 7.8, the growth was good, assuming null effect of high pH on plant growth. Biodegradation of the polymers during the composting did not increase the conductivity of the tested growth medium which showed good growth in the potato plant with good salt uptake. This study revealed current understanding to identify the knowledge gaps and future research priorities related to biopolymers as well as biopolymers “nature-to-nature” sustainability. Future detailed analysis can reveal more details related to reuse of compost biomass after biopolymer biodegradation in more sustainable approach. Therefore, this doctoral thesis mainly focuses on the biodegradation study of PLA and PCL based biocomposites and blends under composting as well as in natural soil condition. Characterization of the test samples after biodegradation for better understanding and underlying a biodegradation mechanism with an assessment of biodegradation and summarizing up all the results for obtaining best material characterization is the main goal to enhance biodegradability of the test samples, which has been summarized in eleven different chapters with detailed explanation in subsequent sections.

Keywords: *biopolymers; composting; biodegradation; biofillers; biofilm;*







Table of Contents

Acknowledgement	a
Abstract.....	i
Chapter 1	1
<i>Introduction and Literature Review</i>	1
1.1 INTRODUCTION.....	2
1.2 SCOPE AND STRUCTURE OF THESIS.....	9
1.3.1 Biodegradation and composting of polymers and its characterization	16
1.3.1.1 Visual observations.....	23
1.3.1.2. Changes in mechanical properties and molar mass	23
1.3.1.3 Weight loss measurements: Determination of residual polymer.....	24
1.3.1.4 Clear zone formation	25
1.3.1.5 Isolation and selection of aerobic microorganisms for PLA biodegradation investigation.....	25
1.3.1.6 The Microbial communities and other factors which acts as biological agent in biodegradation of polyester	26
1.3.2 Isolation and identification of various microorganisms and enzymes associated with biopolymer biodegradation	26
1.3.2.1 Actinomycetes	29
1.3.2.2 Fungus	29
1.3.2.3 Bacteria.....	30
1.3.4 Enzymes:.....	31
1.3.4.1 PLA-degrading enzymes	31
1.3.5 Biodegradation kinetics.....	33
1.3.6 Biodegradable polymers.....	35
Poly(lactic acid) PLA	36
Polycaprolactone (PCL)	39
Chitosan	40
Cellulose based derivatives ((Cellulose nanocrystals (CNC) and microcrystalline cellulose (CMC))	41
Gum Arabica	43
Motivation.....	44

Chapter 2	47
<i>Materials and Methods</i>	47
2.1 MATERIALS	49
2.2 METHODS	49
2.2.1 Development of the composting setup	49
2.2.2 Testing material preparation	50
2.2.3. Compost preparation	52
2.2.4 Biodegradation test:	53
2.2.5 Biodegradation mechanism and kinetics	56
2.2.6 Molecular weight analysis.....	58
2.2.7 Microbial analysis	59
2.2.7.1 Microbial counts	59
2.2.7.2 16sRNA analysis	59
2.2.7.3 Analysis of microbial biofilm formation.....	60
2.2.8 Differential Scanning Calorimeter (DSC).....	61
2.2.9 POM analysis	62
2.2.10 FTIR and UV-Visible spectroscopy analysis.....	63
2.2.11 Contact angle measurements.....	63
2.2.12 Morphological analysis	63
2.2.13 Nuclear Magnetic Resonance (NMR).....	64
2.3 Biodegradation under soil condition	64
2.3.1 Experiment procedure	64
2.4 Reutilization of the used compost biomass as fertilizer for plant growth.....	66
2.4.1 Growth characterization	66
2.5 Abiotic degradation	67
2.5.1. Mathematical model Description	67
2.5.2 Mass loss test and visual observation.....	69
2.5.3. Density and pH measurement	69
Chapter 3	71
<i>Development of composting setup and protocol</i>	71
Abstract	71
3.1 INTRODUCTION.....	72
3.2. Results and discussion.....	74

3.3. Conclusion.....	79
Chapter 4	82
<i>Biodegradation behavior and kinetics of PLA based films (Neat PLA; PLA/chitosan; PLA/Gum Arabic; PLA/CNC; under controlled aerobic composting conditions.....</i>	82
Abstract.....	83
4.1 INTRODUCTION.....	84
4.2 RESULTS AND DISCUSSION	88
4.2.1 Elemental analysis.....	88
4.2.2 Biodegradation test.....	88
4.2.3 Biodegradation mechanism and kinetics.....	92
4.2.4 Microbiological analysis and isolation of the degrading strain	97
4.2.5 Morphological Studies	99
4.2.6 Molecular weight analysis.....	100
4.2.8 Differential Scanning Calorimeter (DSC).....	105
4.2.9 Contact angle analysis.....	109
4.2.10. Fourier transformed infrared spectroscopy (FTIR) and UV-visible spectroscopy	117
4.2.11 Degradation mechanism and morphological analysis.....	121
4.3 CONCLUSIONS	126
Chapter 5	129
<i>Biodegradation behaviour of PLA/LLDPE/MCC biobased poly-blends under controlled aerobic composting conditions by analysis of evolved carbon-dioxide and its characterization.....</i>	129
Abstract.....	130
5.1 INTRODUCTION.....	131
5.2 RESULTS AND DISCUSSIONS.....	133
5.2.1 Biodegradation test	133
5.2.2 Molecular weight analysis	139
5.2.3 Polarizing optical microscopy (POM)	141
5.2.4 Differential Scanning Calorimetry (DSC)	144
5.2.5 Morphological analysis	147
5.2.6 Water contact angle analysis	149
5.3 CONCLUSION	151

Chapter 6	154
<i>Biodegradation behaviour of PLA/PCL/MCC based blends under controlled aerobic composting conditions by analysis of evolved carbon-dioxide and its characterization</i>	<i>154</i>
Abstract.....	155
6.1 INTRODUCTION.....	156
6.2 RESULTS AND DISCUSSIONS.....	159
6.2.1 Biodegradation test	159
6.2.2 Microbial analysis and biofilm formation study	162
6.2.3 Molecular weight analysis	166
6.2.4 Spherulite growth analysis	169
6.2.5 Wettability analysis	173
6.2.6 FT-ATR analysis.....	175
6.2.7 DSC analysis	180
6.2.8 Morphological analysis.....	186
6.3 CONCLUSION	188
Chapter 7	191
<i>Potential of algal biomass in accelerating the biodegradation of PLA based composites under composting condition and comparison of lab synthesized PCL with PLA</i>	<i>191</i>
7.1 INTRODUCTION.....	193
7.2 RESULTS AND DISCUSSION	196
7.2.1 Algal biomass characterization	196
7.2.2 Case Study 1.....	198
7.2.2.1 Abiotic degradation	198
7.2.3 Case study 2	207
7.2.3.1 Biodegradation under composting condition	207
7.3 CONCLUSIONS	226
Chapter 8	229
<i>Studies on various biopolymeric blends and composites under outdoor natural soil condition</i>	<i>229</i>
Abstract.....	230
8.1 INTRODUCTION.....	231
8.2 RESULTS AND DISCUSSION	233
8.2.1 Outdoor temperature, humidity profiling and manual observation.....	233

8.2.2 Molecular weight analysis.....	238
8.2.3 Weight loss analysis and size(thickness) analysis of the test samples.....	247
8.2.4 Infrared spectroscopy analysis	251
8.2.5 Contact angle analysis.....	259
8.2.3 Conclusion.....	262
Chapter 9	265
<i>Comparison of all the test materials under different environment</i>	<i>265</i>
Abstract.....	266
9.1 INTRODUCTION.....	267
9.2 RESULTS AND DISCUSSIONS	269
9.2.1 Comparative biodegradation under controlled environment	269
9.2.2 Degradation of NPLA under different types of compost	273
9.2.3 Comparative biodegradation under natural outdoor soil (uncontrolled) environment	274
9.3 Conclusions	275
Chapter 10	279
<i>Study of plant growth using the compost used in biodegradation and inducing biopolymers in both soil as well as natural soil condition.....</i>	<i>279</i>
Abstract.....	280
10.1 Introduction	281
10.2 Results and Discussions	284
10.2.1 Germination rate of the samples under different medium	284
10.2.2 Molecular weight analysis of the test samples	285
10.2.3 Electrical conductivity and pH of soil	286
10.2.4 Potato stem length and thickness measurement	287
10.2.5 Salt uptake capacity of the test samples measured using inductively coupled plasma mass spectrometry (ICP-MS).	289
10.3 CONCLUSION	292
Chapter 11	294
Conclusion and Future Prospects.....	294
11.1 Conclusions	294
11.2 Future Prospects	298
11.2.1 SCOPE FOR FUTURE WORKS.....	299
11.3. RESEARCH OUTPUTS.....	300





List of Tables

Table no.	Table caption	Page no.
Chapter 1		
Table 1.3.1:	Selected biodegradation tests in composting conditions reported in the literature about the samples, compost and the main methods for assessing biodegradation.	19-22
Table 1.3.2:	Showing different strains isolated from different sources that acts upon PLA based films and were responsible for biodegradation of PLA with the enzymes they release during the biodegradation process.	28
Chapter 2		
Table 2.1.:	Composition of the polyblend composites.	52
Table 2.2.	Composition of the different polyblend composites	53
Chapter 4		
Table 4.1:	Kinetic model parameters and coefficient of determination (R^2)	93
Table 4.2:	Number Average Molecular weight (M_n) of the test samples before and after biodegradation. Here (d) represents days and (da) represents Dalton	102
Table 4.3:	Microbial colony count during the biodegradation process	104
Table 4.4:	Crystallinity (χ %) of the test samples before and after biodegradation.	106
Table 4.5:	Contact angle images at different temperature and using two different solvents.	115-116
Table 4.6:	Transmission of the test samples during the biodegradation period.	121
Chapter 5		
Table 5.1:	Composition of the polyblend composites	133
Table 5.2:	Kinetic model parameters and coefficient of determination (R^2)	139

Table 5.3:	Number average molecular weight analysis with retention times for samples) before biodegradation and after biodegradation	140
Table 5.4:	Percentage crystallization of the test samples before and after biodegradation	145
Table 5.5:	Water contact angle of samples before and after biodegradation	150

Chapter 6

Table 6.1:	Composition of the different polyblend composites	159
Table 6.2:	Colony count of the test samples before and after biodegradation.	163
Table 6.3:	Absorbance of the test samples before and after biodegradation	163
Table 6.4.:	Molecular weight analysis of the test samples before and after biodegradation	167
Table 6.5:	Rate constant values of the samples before and after biodegradation.	168
Table 6.6:	Water contact angle of the samples before and after biodegradation under composting conditions, measured initially, on the 30 th day and the 60 th day (note: It was difficult to measure the contact angle of the samples after 60 th day as they became very disoriented).	174
Table 6.7:	Percentage crystallinity of the samples before and after biodegradation.	185

Chapter 7

Table 7.1:	Parameters used in the model to fit the experimental data of the abiotic degradation.	200
Table 7.2:	Percentage crystallization of samples before and after abiotic degradation at two different temperature.	206
Table 7.3:	Molecular weight analysis of the test samples before and after biodegradation.	210

Chapter 8

Table 8.1 (a-b):	Molecular weight of the test samples before and after biodegradation under natural soil condition	242-243
Table 8.2:	Chain scission rate constants with regression coefficient for the test samples	245
Table 8.3:	Thickness measurement of test samples before and after degradation under soil condition	251
Table 8.4:	Water contact angle of samples before and after biodegradation under soil condition	260

Chapter 10

Table 10.1:	Germination % of the test samples grown under various soil conditions	284
Table 10.2:	Electrical conductivity of soil measured initially and after 120 days of growth period	287
Table 10.3:	pH measurement of the soil before and after 120 days of plant growth	287
Table 10.4:	Stem length (mm) and stem thickness (mm) measurement of the crop measured for 30 days	289
Table 10.5:	Salt and mineral concentration in soil medium used for potato cultivation	291
Table 10.6:	Salt and mineral concentration in test samples or whole potato.	291

List of figures

Figure No.	Figure caption	Page No.
Chapter 1		
Figure 1.1:	Thesis content orientation	13
Figure 1.2:	Basic mechanism of biopolymer biodegradation	17
Figure: 1.3:	Flowchart of biodegradation mechanism of biodegradable polymers (a) and proposed carbon degradation during aerobic composting (b).	33
Figure 1.4:	Different Source of biodegradable polymers and their classification adopted from various sources	35
Figure 1.5:	Poly(lactic acid) structure	36
Figure 1.6:	Different optical forms of PLA	37
Figure 1.7:	Synthesis method for High molecular weight PLA	38
Figure 1.8:	PCL an aliphatic biodegradable linear polyester derived from fossil fuels	39
Figure 1.9:	Chitosan grafted PLA structure	41
Figure 1.10:	Cellulose nanocrystals (CNC) grafted PLA structure	42
Figure 1.11:	Cellulose microcrystalline structure (MCC) structure	42
Figure 1.12:	Gum Arabica-PLA structure	44
Chapter 2		
Figure 2.1:	Representing graphical abstract of the composting process with the characterization	48
Figure 2.2:	Graphical abstract representing the biodegradation method and characterization under natural soil condition	65
Chapter 3		
Figure 3.1:	Image showing different reactors connected to the Gas Chromatography system	77
Figure 3.2:	Specification of the bioplastic composting reactors or composting vessels (CV)	78

Figure 3.3:	Biodegradation (C-CO ₂ evolution) of 99.99 % cellulose tested under controlled composting condition for validation of the experimental setup.	79
--------------------	--	-----------

Chapter 4

Figure 4.1	Aerobic biodegradation process in accordance to ASTM D5338-15. Flowchart design used for biodegradation	89
Figure 4.2:	Carbon-dioxide evolution from compost plus test samples (the bar is standard error) during the composting period.	90
Figure 4.3:	Percentage biodegradation of the test samples under AC condition (the bar is standard error).	90
Figure 4.4:	Proposed biodegradation mechanism of biodegradable polymers and carbon degradation during AC. C_r , C_m and C_s are three different kinetic forms of SC, which are, rapidly, moderately and slowly hydrolysable respectively. Intermediates are mainly amorphous region attacked by microbes at first, leaving behind the crystalline polymer region.	94
Figure 4.5:	Kinetic of percentage CO ₂ evolutions during biodegradation: (a) NPLA (b) PLA/Chitosan (c) PLA/CNC and (d) PLA/Gum (the bar is standard error).	95
Figure 4.6	Electrophoresis documenting microbial community	98
Figure 4.7:	Based on sequence alignment and Phylogenetic analysis the 16S sample has been matched to <i>Bacillus flexus</i> .	98
Figure 4.8:	FESEM images of different test samples before and after biodegradation under composting condition.	100
Figure 4.9:	Samples were randomly taken out and weight at different times of composting duration. Samples are small pieces and cameras were zoomed before taking the pictures for better visibility.	101

Figure 4.10:	Molecular weight analysis of the test samples and their predicted data according to the first order reaction.	103
Figure 4.11:	Identification of rod shaped bacteria is acting and is gram negative as the pink colour is retained after staining during the composting process.	104
Figure 4.12 (a-d):	DSC thermograms of the test samples before and after biodegradation.	107-108
Figure 4.13 (a-d):	Contact angle of the test samples before and after biodegradation at 27 °C with standard errors.	110-112
Figure 4.14 (a-d):	Contact angle of the test samples before and after biodegradation at 58 °C with standard errors.	112-114
Figure 4.15 (a-d):	IR footprint of the test samples before and after biodegradation	119-121
Figure 4.16 (a):	Degradation mechanism of NPLA test sample under composting condition	122
Figure 4.16 (b):	Degradation mechanism of PLA/Chitosan test sample under composting condition.	123
Figure 4.16 (c):	Degradation mechanism of PLA/CNC test sample under composting condition.	124
Figure 4.16 (d):	Degradation mechanism of PLA/Gum test sample under composting condition.	125

Chapter 5

Figure 5.1:	Laboratory composting setup used for biodegradation study and the samples that were tested under the system.	134
Figure 5.2. (a-e):	Kinetics of percentage biodegradation of (a) NPLA (b) PLA/LLDPE (c) PLA/LLDPE/ 1 % MCC, (d) PLA/LLDPE/ 3 % MCC and (e) PLA/LLDPE/ 5 % MCC.	135-137
Figure 5.3 (a-b):	Number average molecular weight analysis with retention times for samples (a) before biodegradation and (b) after biodegradation.	140

Figure 5.4:	Polarizing optical micrographs of the samples done at 120 °C before biodegradation and 100 °C after biodegradation.	142
Figure 5.5 (a-b):	Spherulite diameter growth (a) before biodegradation and (b) after biodegradation.	143
Figure 5.6:	Spherulite density shown before biodegradation (initial density) after biodegradation (final density) for all the test samples.	144
Figure 5.7 (a):	DSC thermograms before biodegradation	145
Figure 5.7 (b):	DSC thermograms after biodegradation	146
Figure 5.8 (a-j):	FESEM images of the test samples before and after biodegradation (90 th day).	148
Figure 5.9 (a-d):	TEM image of the samples (a) PLA/LLDPE/ 1 % MCC before biodegradation (b) PLA/LLDPE/ 5 % MCC before biodegradation (c) PLA/LLDPE/ 1 % MCC after biodegradation (d) PLA/LLDPE/ 5 % MCC after biodegradation.	149
Figure 5.10 (a-j):	Water contact angle analysis of samples namely (a) NPLA, (b) PLA/LLDPE/ 5 % MCC, (c) PLA/LLDPE (d) PLA/LLDPE 3 % MCC (e) PLA/LLDPE/ 1 % MCC before biodegradation respectively and water contact angle analysis of samples namely (f) NPLA, (g) PLA/LLDPE/ 5 % MCC, (h) PLA/LLDPE, (i) PLA/LLDPE / 3 % MCC (j) PLA/LLDPE/ 1 % MCC after biodegradation.	151

Chapter 6

Figure 6.1 (a-b):	Percentage biodegradation of all the test samples under composting condition.	161
Figure 6.2:	Test samples before and after biodegradation.	162
Figure 6.3:	Growth absorbance curve of the isolated microbial strains observed at 600	164
Figure 6.4:	Growth of the isolated microbes on three different media plates: (a) Eosin methylene blue agar (b)	165

	Potato dextrose agar (c) Plate count agar (d) magnified representation of the plate count agar showing only bacterial growth.	
Figure 6.5:	Molecular weight analysis of the test samples and their predicted data according to the first order reaction kinetics.	168
Figure 6.6:	Spherulite growth size of the samples before and after biodegradation	170
Figure 6.7:	Spherulite density of samples before and after biodegradation	171
Figure 6.8:	Micrographs showing spherulite formation before and after biodegradation, captured at times 10 seconds (initial), 10 minutes and 15 minutes.	172
Figure 6.9:	Micrographs showing spherulite formation before and after biodegradation captured at times 10 seconds (initial), 10 minutes and 15 minutes.	173
Figure 6.10:	Representative image highlighting the surface of the polymer during the initial stage and after the biodegradation process of 30 days, under composting conditions.	175
Figure 6.11 (a-h):	FTIR representation of the samples before and after biodegradation	176-180
Figure 6.12(a-h):	DSC thermograms of the samples before and after biodegradation (a) nPLA (b) nPCL (c) PLA/PCL: 80/20 (d) PLA/PCL/MCC: 80/20/1 (e) PLA/PCL/MCC: 80/20/3. (f) PLA/PCL: 90/10 (g) PLA/PCL/MCC: 90/10/1 (h) PLA/PCL/MCC: 90/10/3.	181-184
Figure 6.13:	FESEM images representing the test samples before biodegradation and after biodegradation under composting conditions.	187

Chapter 7

Figure 7.1:	FTIR spectra of the whole algal biomass.	197
--------------------	--	------------

Figure 7.2:	TGA thermogram of the algal biomass representing $T_{50\%}$.	197
Figure 7.3:	SEM image representation the morphology of the algal biomass.	198
Figure 7.4:	Manual observation of the test samples during the abiotic degradation time interval (initial to 30 days)	199
Figure 7.5:	$\overline{M}_n$ (change with time) is the number average molecular weight (M_n) normalized by its initial value. Straight line represents the predicted value and the discrete points represents the experimental data.	200
Figure 7.6:	pH variation of the buffer solution with time during the abiotic degradation process.	202
Figure 7.7:	Mass loss (%) of the test samples observed during the abiotic degradation process.	202
Figure 7.8 (a-d):	DSC thermograms of the test samples at different temperature on the 30 th day of the abiotic degradation process.	204-205
Figure 7.9:	Morphology of the test samples before and after abiotic degradation observed using POM.	207
Figure 7.10:	Flowchart representing different stages of the biodegradation of a biopolymer (here PCL is used to understand the different stages.	211
Figure 7.11(a-f):	DSC thermograms of the test samples before and after biodegradation under composting condition.	213-215
Figure 7.12(a-f):	FTIR analysis of the test samples before and after biodegradation representing peak changes during the biodegradation process	216-219
Figure 7.13 (a-d):	¹ H NMR spectra of two representative test samples before and after biodegradation under composting condition	221-222
Figure 7.14:	Morphological changes observed using SEM during the biodegradation process.	223

Figure 7.15:	Surface morphology of PCL samples during the biodegradation process which represents formation of Acetyl-CoA and other intermediates on the surface.	224
Figure 7.16:	Microbial attachment comparison on the PLA/algae 5% ES and neat PLA ES films on the 15 th day of the composting process. This shows how algae as filler increasing the synergy of the degradation environment.	225

Chapter 8

Figure 8.1:	Graph representing outdoor temperature profile during the degradation period	234
Figure 8.2:	Graph representing temperature profile of soil under 20 cm deep.	234
Figure 8.3:	Graph representing the humidity (%) of the degradation environment	235
Figure 8.4(a-c):	Manual observation of test samples before and after biodegradation	236-238
Figure 8.5 (a-c):	Molecular weight analysis of the test samples and their predicted data according to the first order reaction.	246-247
Figure 8.6:	Residual weight fraction of the test samples before and after biodegradation under soil condition	249
Figure 8.7:	Thickness changes in the test samples during the degradation period	250
Figure 8.8 (a-l):	IR spectroscopy analysis of samples before and after biodegradation under soil condition	253-258
Figure 8.9 (a-b):	Water contact angle of test samples under natural soil condition (a) initial contact angles (b) contact angle after 180 th day of degradation	261

Chapter 9

Figure 9.1:	Biodegradation of various PLA based biocomposites	270
Figure 9.2:	Biodegradation of PLA based biobased polyblends done under thermophilic composting condition	271

Figure 9.3:	Biodegradation of PLA/PCL based polyblends under thermophilic composting condition	272
Figure 9.4:	Graph representing the best compost used for the biodegradation of PLA under thermophilic composting condition	273
Figure 9.5:	Biodegradation of test samples done under natural soil environment	275

Chapter 10

Figure 10.1:	Showing the plant growth on different media throughout the growth period	285
Figure 10.2:	Representative image of the potato samples cultivated after 120 th day	285
Figure 10.3(a):	Stem growth was measured using scale and callipers as shown (representative image)	288
Figure 10.3(b):	Representative image during stem length measurement	288



Abbreviations

ATR	Attenuated total reflectance
CDCl₃	Deuterated chloroform
CO₂	Carbon dioxide
CNC	Cellulose Nanocrystals
CV	Composting Vessel
DSC	Differential Scanning Calorimetry
FESEM	Field Emission Scanning Electron Microscopy
FTIR	Fourier Transmission Infrared Spectroscopy
GC	Gas Chromatography
GPC	Gel Permeation Chromatography
GRG	Generalized Reduced Gradient
ISO	International Standards Organization
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LLDPE	Linear Low Density Polyethylene
MWD	Molecular Weight Distribution
NMR	Nuclear Magnetic Resonance
NPLA	Neat Poly(lactic acid)
PBAT	Poly(butylene adipate-co-terephthalate)
PCL	Polycaprolactone
PLA	Poly(lactic acid)
PDI	Polydispersity Index
PPB	Parts Per Billion
POM	Polarized Optical Microscope
TGA	Thermogravimetric Analysis
TEM	Transmission Electron Microscopy
UV	Ultraviolet

Notations

C_c	Carbon Mineralization Percentage
C_{C0}	Solid Carbon Fraction
C_{FC}	Remaining Solid Carbon
M_n	Number Average Molecular Weight
M_w	Weight Average Molecular Weight
T_{cc}	Crystallization Temperature
T_m	Melting Temperature
ΔH_m	Enthalpy of Melting
ΔH_{cc}	Enthalpy of Crystallization
Da	Dalton
T_g	Glass Transition Temperature
T_m	Melting Temperature
k	Rate Constant
R^2	Regression Coefficient
X_c	Percentage Crystallinity







Introduction and Literature Review

This chapter mainly focuses on biodegradation of biodegradable polymers like poly(lactic acid) PLA and polycaprolactone (PCL) under composting and natural soil conditions. Various standard protocols are followed for assessing the biodegradation of biopolymers set by international agencies such as ASTM International and International standard organization (ISO), etc. for terming a polymer as biodegradable. Standards like ASTM International D5338 and D6400 has been discussed thoroughly. Biodegradation of biopolymers under aerobic composting releases CO₂ and water. This CO₂ is measured cumulatively either by titration or by any other respirometric methods, which is evaluated in terms of percentage biodegradation of a material. This chapter also focuses on material characterization of the test samples to understand the extent of biodegradation during the degradation time interval. Assessing microbial flora and biofilm formation on the surface, which leads to biodegradation under the surrounding environment of the test material, are discussed with various analytical techniques. It discusses the role of biodegradation kinetics in order to understand the characteristics and behavior of the test material during biodegradation, which helps in designing and development of material characterization and for enhancing and tuning biodegradability of a biopolymer under prescribed environmental condition.

Publications

- Naba Kumar Kalita, Melakuu Tesfaye, Purabi Bhagabati and Vimal Katiyar, 2017, book chapter titled as ‘Trends of end-of-life Options: Recycling, Reusing and Composting of Waste Food Packaging’ in book ‘Bio-based Plastics for Food Packaging Applications’. (Smithers Rapra, ISBN: 9781910242582).

1.1 Introduction

Utilization of non-degradable plastic materials leads to environmental problems due to improper waste disposal. Issues like environmental sustainability has promoted tremendous research towards the development of biopolymers, which could undergo biodegradation (Cadar et al. 2012; Valapa et al. 2015). Some of the representative biodegradable polymers which are commonly present in the shelf are poly(lactic acid) (PLA), polyvinyl alcohol (PVA), polyhydroxybutyrate (PHB), poly(butylene adipate-co-terephthalate) (PBAT) (Qi et al. 2017). However, biopolymers including cellulose, chitosan, gums etc. (Lucas et al. 2008) can also be used for the development of biopolymer based biocomposite products. Among all, PLA, has significant and durable properties to replace fossil-based plastics (Garlotta 2002; Shah et al. 2008; Dhar et al. 2015; Valapa et al. 2016). Most of these plastics are specifically targeted towards packaging applications since they undergo degradation during composting (Ge et al. 2016). It is observed that biopolymers such as cellulose (Dhar et al. 2016), chitosan (Pal and Katiyar 2016a), gums (Tripathi and Katiyar 2017), when dispersed in PLA, can enhance certain required properties for food packaging. Studies show that cellulose nanocrystal (CNC), one of the most abundantly available biopolymer, has significantly improved thermo-mechanical properties of PLA based composites (Bitinis et al. 2013). Most biopolymers are hydrophilic in nature, but there are certain grafting techniques that can be applied to change its surface properties from hydrophilic to hydrophobic. In-line with above, recently, chitosan and gum arabic have been applied as reinforcing phase for improvement of gas barrier properties of PLA films for application in food packaging (Pal and Katiyar 2016a; Tripathi and Katiyar 2017). However, these improved modified composite films must meet certain criteria regarding their biodegradability, like 90% organic carbon present in the composite film should be converted to CO₂, water etc., under either compost or soil, following certain standards. In

this regard, ASTM Internationals (ASTM D 5338-15) has detailed protocol about biodegradation (ASTM International 2015). However, this standard is inherent to different composites and substrates, though the protocols for testing remain similar. Composting has the potential to transform biodegradable waste materials into fertile soil, which may enhance the growth of agro-economy.

Several studies suggested that PLA undergo biodegradation under composting condition within a short period (Castro-Aguirre et al. 2017). The rate of PLA biodegradation may vary, depending upon various factors such as temperature and moisture content (Kale et al. 2007b; Pradhan et al. 2010a). Biodegradation of PLA follows two steps, which includes (1) hydrolytic degradation and (2) biodegradation (Pradhan et al. 2010a; Zenkiewicz et al. 2012; Sikorska et al. 2015). During hydrolytic degradation of PLA, degradation occurs by water molecules through random chain scission, reducing the molar mass that leads to low molecular chain formation as low as <20000 Da. Low molecular weight polymer chains are required for microbial digestion. Whereas, these intermediates are assimilated by microbes for energy and decompose the short polymer chains into various compounds like CO₂ and water (Torres et al. 1999; Gu 2003). Though no such microbes are identified which is said to degrade high molecular weight PLA directly (Sikorska et al. 2015), but various microorganisms and enzymes have been identified as PLA degrader (Lucas et al. 2008; Leja and Lewandowicz 2010; Sikorska et al. 2015; Castro-Aguirre et al. 2017). In the current investigation, authors have prepared and investigated PLA films and biocomposites using melt extrusion process with the intention to use these films for thermoformed or vacuum formed products. The leeway of research for improvements in PLA based food packaging application is very broad (Nieddu et al. 2009; Petinakis et al. 2010; Luo et al. 2012; Weng et al. 2013; Arrieta et al. 2014). There are reports that showed that PLA is

biodegradable under the composting condition and takes more time to degrade under soil conditions (Kale et al. 2007a; Weng et al. 2013; Kucharczyk et al. 2016).

Hence, CO₂ evolution measurement is an utmost tool to measure biodegradation as it can help to monitor kinetic parameters during biodegradation. Various analytical techniques are used to measure the CO₂ evolution for assessing percentage biodegradation of PLA and its derivative materials under different environments which includes direct measurement respirometry (DMR), non-dispersive infrared (NDIR), cumulative measurement respirometry (CMR) titration etc. are used to monitor the evolved CO₂ (Kijchavengkul et al. 2006, 2008; Kale et al. 2007b; Petinakis et al. 2010; Pradhan et al. 2010a; Gómez and Michel 2013; Castro-Aguirre et al. 2017). Results observed from these literature state that reproducibility is one of the main issues for this kind of evaluations. Gas chromatography is another online technique to monitor CO₂ evolved can be measured for assessing biodegradation. Kinetic studies exhibited that carbon mineralization is a first-order reaction (Komilis 2006; Leejarkpai et al. 2011). Microbial identification and DNA sequencing also revealed various PLA biodegrading microbes responsible for biodegradation at both mesophilic and thermophilic temperature (Fontanella et al. 2010; Walczak et al. 2015) as this highlights the microbes responsible for biodegradation at elevated temperatures. As a consequence of the development of various online CO₂ evolution test, we also developed a test according to the ASTM D5338-15 with few modifications for measuring the CO₂ evolution using a gas chromatography system having 16 port valve connected with the composting reactors.

PLA is known to have shown good biodegrading behavior under controlled composting condition for a certain time, as well as in industrial and real composting environment. Various parameters like high temperature, moisture content and pH plays a vital role which accompany PLA biodegradation under composting condition (Kalita et al., 2020).

Degradation of PLA based products has been widely studied under different environmental conditions like hydrolytic degradation and UV-radiations (De Jong et al., 2001; Tsuji et al., 2006; R. Valapa et al., 2016). Biodegradation of PLA is a sequential process followed by two steps namely, chemical hydrolysis or hydrolytic degradation which occurs during the composting process, followed by biodegradation which follows a certain biopolymer degradation mechanism (Kalita et al., 2019; Kucharczyk et al., 2016; Leejarkpai et al., 2011; R. Valapa et al., 2016). In the first step PLA degradation occurs via random chain scission i.e. hydrolytic degradation, which reduces molar mass of the polymeric chains and leads to intermediate formation of low molecular weight chains. These intermediates are further assimilated by the microbes in the second step for energy and production of various compounds like CO₂ and H₂O (Han and Pan, 2009; Kalita et al., 2019; Zumstein et al., 2019). Authors have identified and studied various microorganisms and enzymes which degrades PLA based biocomposites as well as other products (Castro-Aguirre et al., 2017; Kitadokoro et al., 2012; Qi et al., 2017; Tsuji et al., 2006). Authors have prepared and investigated PLA films as well as PLA composites because these offers wide scope of research for further improvement of PLA based packaging systems (Kale et al., 2007a; Kalita et al., 2019; Pantani and Sorrentino, 2013; Pradhan et al., 2010a, 2010c; Torres et al., 2015). Series of literature investigated PLA based biocomposite packaging systems under different environments, like in soil, composting and industrial composting conditions (Gómez and Michel, 2013; Leejarkpai et al., 2011; Musioł et al., 2016; Weng et al., 2013b).

Various studies used different analytical techniques that have been employed for evaluation of biodegradation during composting of biopolymers using either direct or an indirect approach. These techniques like visual observations, weight loss measurements, changes in mechanical properties, and changes in molecular weight, can provide insights into the

degradation process of a polymer, and demonstrate biodegradation (Jayasekara et al., 2001; Rudeekit et al., 2008).

Biodegradability is steadily gaining importance in the plastic industry due to ever increasing environmental concerns related to non-biodegradable products and their ecological impacts. Conventional polymers like linear low-density polyethylene (LLDPE), polyethylene (PE), polypropylene (PP) are very cheap and are therefore used extensively in packaging industries as well as in the manufacture of daily household goods. The cost of biodegradable plastics is high and so, its use is limited to developed countries largely. Hence, it is required to find alternatives to use bioplastics in order to reduce the overuse of conventional plastics. Blending is one such option of reducing the cost of bioplastics. Blending of conventional polymers with bioplastics may help in the improvement of the properties of these bio-based blends. Poly(lactic acid) (PLA), a potential biodegradable polymer, is comparable to its conventional counterparts due to its thermoplastic nature and can be used in these blends (Dhar et al., 2016; Gupta et al., 2018). Recent research studies suggest that blending of bioplastics like PLA with conventional plastics like LLDPE is a growing trend, although their biodegradability remains a key issue (Bhasney et al., 2018; Siracusa et al., 2008; Trongsatitkul and Chaiwong, 2017). Biodegradable polymers are either partially or completely degradable under certain conditions, but their degradation properties might change due to blending with conventional polymers. Various literatures report that the polymers PLA, PCL and cellulose are both biodegradable and compostable, having various applications ranging from packaging to commodity usage (Narancic et al., 2018; Pekařová et al., 2018). Although PLA and PCL have been extensively studied, their use is still not popular because these polymers have some disadvantages like high barrier properties, high rigidity as well as low glass transition temperature (T_g), which makes it difficult to use them in specific applications (A. D. Campos et al., 2011; Dulnik et al., 2016;

Garlotta, 2002; Vieira et al., 2010). Literatures suggest that the undesirable properties of a polymer can be tuned by blending it with another polymer (A. D. Campos et al., 2011; Narancic et al., 2018). PCL, a petroleum-derived biodegradable polymer, has enormous potential to replace its conventional counterparts. Thus, blending of PLA with PCL along with other biofillers might alter their properties and affect their end-of-life characteristics under various environmental conditions. Most of the countries in North-America, Europe and Japan have legislation for composting of bioplastics under different environments (Siracusa et al., 2008).

Under controlled composting condition analytical tools and measurement techniques are of utmost importance (Zumstein et al., 2019).

Even though techniques like visual observations, weight loss measurements, changes in mechanical properties, and changes in molecular weight, can provide insights into the degradation process of a polymer, they do not necessarily demonstrate biodegradation [Shah et al., 2008]. Therefore, respirometric methods, in which the consumption of oxygen and/or the evolution of carbon dioxide (CO_2) is measured, have become the preferred technique for such assessment (Zumstein et al., 2019). Gas chromatography is also an utmost tool for measuring the evolved CO_2 if appropriate measuring and process parameters are determined during biodegradation. It is however not very clear how to develop a proper PLA degrading setup where every process parameter can be calculated during biodegradation for a more precise biodegradation study. Modified chitosan, modified gum Arabica and functionalized cellulose nanocrystals (CNC) has been used extensively by various authors (Dhar et al., 2016a; Pal and Katiyar, 2016a; Tripathi and Katiyar, 2016) in PLA matrix. These fillers tend to improve certain properties of PLA based packaging films. These improved properties makes the composites more superior than their nPLA counterparts. Modified chitosan and modified gum Arabica when mixed with PLA

matrix improves permeability of the packaging material. Whereas, functionalized CNC improves the mechanical as well as thermal stability of the PLA based green composites. Although, its biodegradability needs to be understood for tuning the properties and material characterization for making it more biodegradable by pertaining its improved properties. Use of bioplastics is still in commercial debate table due to its biodegradability in natural soil or unmanaged environmental condition. Although, industrial composting is highly suitable for developed countries due to their high waste segregation ratio then countries like India. So setting up industrial composting setup exclusively for bioplastic end-life management is not economically feasible.

Hence, in order to solve this problem more research is needed in order to grow plants using the biomass of the after biodegradation compost. Limited research is available regarding toxicological impact on the plant growth using PLA or PCL. Fewer have studied the impact of compost used for biodegradation study of biopolymers and reusing that compost as fertilizer or as soil for plant growth (Adamcová et al., 2019; Tuominen et al., 2002). In this study, authors studied complete biodegradation of all tested lactic acid polymers and their biodegradation was confirmed. Bio tests clearly showed that poly(esterurethane) where lactic acid prepolymers were chain linked with 1,4-butane diisocyanate did not exhibit any ecotoxicological effect, and neither did poly(ester-amide) or poly-(lactide). However, they discussed about the importance of composting process, in order to understand the degradation products of polymers in compost and its effect in plant growth (Tuominen et al., 2002). Authors here carried out experiments suggesting materials should also be tested in real conditions that differ from those in a laboratory, as these samples are intended for clients who can compost them in actual conditions. This study opens new doors for materials.

However, search and research for bioplastics that can biodegrade under natural environmental condition is also of paramount necessity. Although use of fillers like algal biomass extract and modified chitosan has proven to be good accelerator for biodegradation of the PLA based composites, its degradation in unmanaged soil condition can have much more positive social impact in the development of the developing countries of the globe thereby, reducing plastic pollution. Biodegradation of biopolymers is an essential end-of-life parameter for a bioplastic for entering the market of biodegradable polymers. Bioplastics such as PLA and PCL are one of the most extensively studied and used biopolymers for various applications. The addition of biofillers like modified chitosan, modified gum, functionalized CNC, MCC, algal biomass, and wood-dust are known to have improved certain properties like permeability, thermal stability and mechanical strength of PLA and PCL based composites when added in optimized quantity. However, their biodegradability remains a concern under different environmental conditions

1.2 Scope and Structure of Thesis

In the preceding section, brief overview of biodegradation of PLA and PCL based biocomposites, blends have been presented, and designing of a material by enhancing biodegradability under composting condition based upon the results obtained. Traditionally composting of biodegradable polymers is done by using compost and adding inoculum in it. Moreover, biodegradation of neat PLA takes almost 90 to 180 days to biodegrade under composting condition. Reducing the biodegradation time of PLA based composites is a challenging task.

Biofillers like modified chitosan, modified gum Arabica and functionalized cellulose nanocrystals have been used to enhance certain physical as well as chemical properties of the PLA based green biocomposites for improving various properties according to their

uses. However, these techniques might improve or deter biodegradation. Despite significant research on PLA and PCL based blends and composites its biodegradation study with proper carbon footprint measurement is very limited. Despite use of certain fillers like modified chitosan and algal biomass which accelerated the biodegradation of PLA based composites under composting condition but its biodegradability under soil condition remains an issue which needs to be addressed.

Moreover, reuse of the after biodegradation biomass as fertilizer for plant growth is the most challenging task in order to bring the biodegradable plastics into the nature-to-nature approach category.

As discussed previously, most of the literatures lack proper characterization of the test samples after biodegradation and enhancement and acceleration of biodegradation of PLA based samples is very limited. Therefore, this PhD Research work focuses on addressing the issues related to biodegradation of biopolymers under composting as well as soil condition. It also focuses on enhancing biodegradation of PLA and PCL based biocomposites under composting and soil condition. The noteworthy results and other research activities are compiled in the thesis as eight chapters from (Chapter 3-chapter 10).

The organization of the thesis and the content of each (excluding the present one) are briefly discussed accordingly and a graphical abstract of the same has been presented in **figure 1.1**

Chapter 2, provides the detailed methodology and characterization procedures that were followed in order to study biodegradation under composting as well as soil condition. Analytical procedures for microbial biofilm formation and other methodologies related to microbes are discussed. The analytical instrumentation techniques and mathematical models that were followed and were carried out in this study have been discussed in detail.

Chapter 3. deals with the design and fabrication of the composting setup according to ASTM D5338. Development of the lab-scale composting setup with proper protocol so that aerobic biodegradation can be studied under composting condition with precision.

Chapter 4. describes the biodegradation study of PLA based biocomposites under composting condition. This chapter deals with PLA/modified chitosan, PLA/functionalized CNC, PLA/Gum Arabica and neat PLA films. Their biodegradation rate and kinetics were studied using a biodegradation model for understanding the biodegradation parameters. Test samples were also characterized using various analytical techniques for assessing biodegradation.

Chapter 5, details the biodegradation study of PLA/Linear low-density polyethylene (LLDPE)/microcrystalline cellulose (MCC) under paper mill sludge compost. The detailed biodegradation study was done to understand the extent of biodegradation of PLA in non-degradable polymeric blends. Addition of MCC also affected the biodegradation rate of the polymeric blend.

Chapter 6, describe the biodegradation of PLA/PCL/MCC based blends of various proportions under water hyacinth compost. The detailed biodegradation study showed that addition of higher proportion of PCL and MCC in the PLA based composites increased the biodegradation rate. This study provides insights into biodegradation and material characterization of PLA/PCL based blends under composting condition.

Chapter 7, this chapter showed the potential of algal biomass in increasing the biodegradation rate of PLA based composites under composting condition.

Chapter 8, details about different PLA, PCL biocomposites and PLA/PCL based blends under natural soil condition. This chapter provides insight about the extent of

biodegradation of different filler based PLA and PCL based composites and blends under natural soil environment.

Chapter 9, summarized and compared biodegradation of all the test samples and different compost used under composting condition in previous chapters

Chapter 10, details about reuse of the after biodegradation compost biomass for seasonal crop growth

Chapter 11, summarized the conclusion of all the chapters and highlights the importance of technical and fundamental issues highlighted in each chapter. Based on the general conclusions from the results some future research work has been presented.



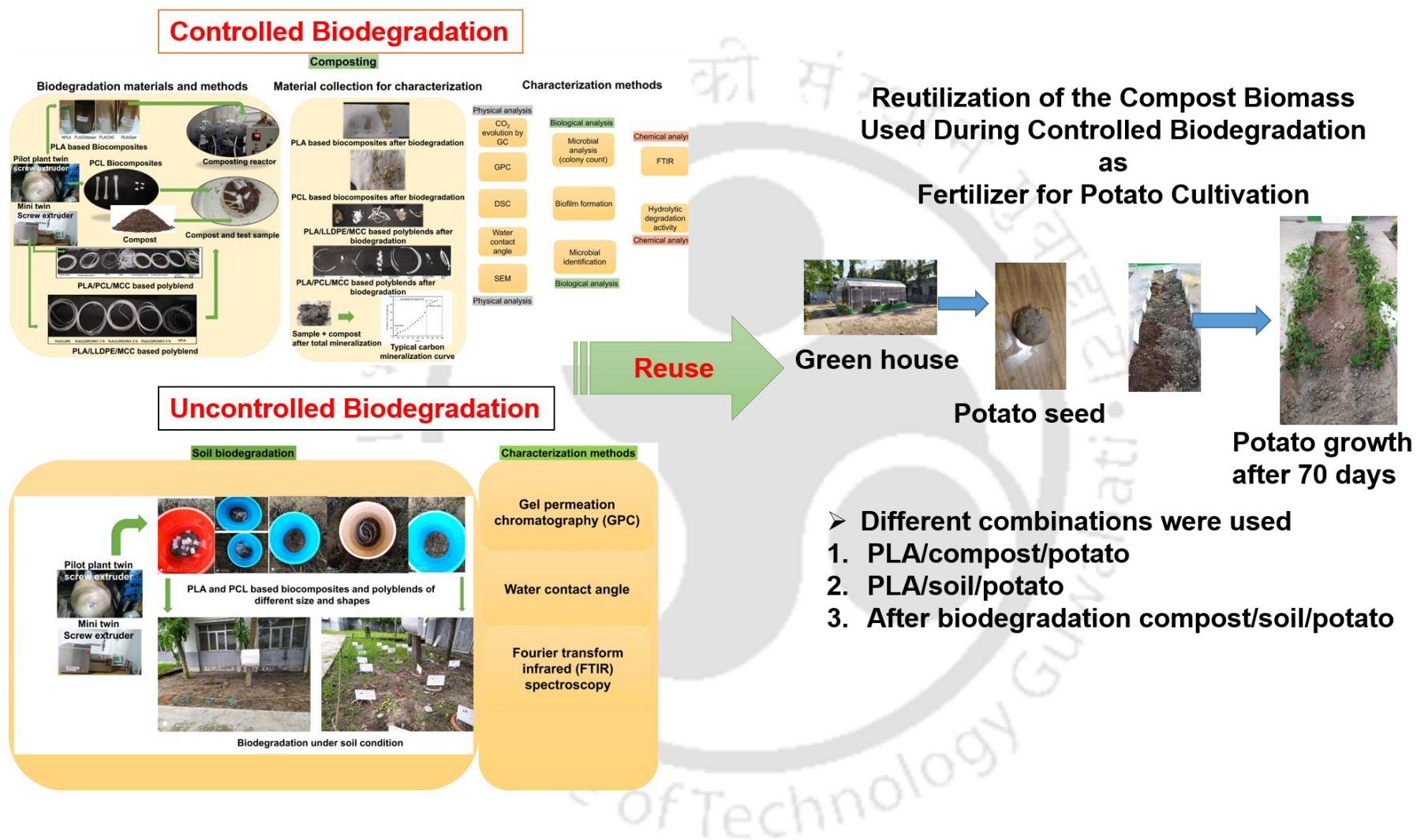


Figure 1. 1: Thesis content orientation

1.3 Literature Review

Conventional plastics cause severe pollution and is tremendously affecting the day-to-day activity of various species in both land and water. Many of the present one time use plastics takes years of time to degrade, which blocks drainage and clogs seas and rivers creating a mess for the organism living there. This plastic in landfill also increases toxicity of the land and water table of the nearby landfill area. Therefore, biodegradable polymers having similar properties as conventional plastics are promoted as their counter alternatives. These biopolymers get degraded under various environment (Dhar et al., 2016b; Gupta et al., 2018; Kalita et al., 2019; Pradhan et al., 2010a). However, their degradation in managed and unmanaged environments is not well understood. Understanding degradation mechanism and secretion of plastics as non-biodegradable compostable and biodegradable is very tedious job in countries like India. Hence, development of plastics which can be thrown to the soil and which degrades within certain time period without managing for its degradation is the utmost necessity. This kind of strategy will be more effective in developing biopolymer in order to replace its conventional counterpart. Although non-biodegradable polymers can be recycled, but after recycle, various properties of the plastics decreases limiting its application (Tesfaye et al., 2017; Yoshida et al., 2016). Whereas, biodegradable plastics offers end-of-life management like composting which tremendously benefits the environment and non-biodegradable plastics lacks this kind of end-of-life management system(Kale et al., 2007b; Ramani and Charles, 1999; Yoshida et al., 2016; Zumstein et al., 2019) . Biodegradable polymers are materials that can be decomposed into CO₂, CH₄, H₂O, inorganic compounds or biomass (ASTM International, 2015a; Narancic et al., 2018). Among the commonly used biopolymers Poly(lactic acid) PLA and polycaprolactone (PCL) can be biodegraded within 180 days of time under composting condition (A. D. Campos et al., 2011; Kale et al., 2007b; Narancic et al., 2018). PLA one

of the most commercially produced synthetic biopolymer which accounts for 25% of the global biopolymer production. PLA is biodegradable in various environmental conditions and can be biodegraded both biotically or abiotically (Kucharczyk et al., 2016).

Literatures suggest degradation of PLA at 58 °C under composting condition. Various microorganisms has been isolated from compost and has been shown to biodegrade PLA at different thermophilic temperature (Al Hosni et al., 2019a; Walczak et al., 2015). Biodegradation also depends upon various shapes and sizes of the plastics and processing types. Extruded sheets of PLA have a different biodegradation rate than extruded PLA strip of same grade (Kale et al., 2007b; Pantani and Sorrentino, 2013). Therefore, processing condition and type can influence biodegradation of PLA and PLA based composites. PCL another biopolymer derived from petrochemicals, is a thermoplastic. PCL is biodegradable and is known to be compatible with various other biopolymers and has been demonstrated in various applications (Bhagabati et al., 2019; Narancic et al., 2018). Like PLA, PCL is also biodegradable under various natural environments like soil, water and compost (A. De Campos et al., 2011; Narancic et al., 2018; Siparsky et al., 1998). These polymers are susceptible to biodegradation due to breakage of ester bonds and the enzymes like lipase and protease pathway helps in microbial assimilation (Al Hosni et al., 2019a). Various literatures suggest use of algal biomass in large quantity for bio-refinery and other pharmaceutical purposes. Its use is immense from biotechnology to chemical industries (Bi and He, 2013; Tibbetts et al., 2015).

Application of agro wastes as fillers and as blends for preparing biocomposites of PLA and PCL (Dhar et al., 2016b; Dulnik et al., 2016) has generated immense scientific interests due to its pragmatic environmental effects and high-end applications with tremendous tunability. These include (a) biodegradability (b) reduction in carbon footprint (c) for reducing cost of the biopolymer-based products etc. Use of algal biomass as filler in

polymer preparation can prove to be a profitable as well as probable strategy for increasing biodegradability. Study used residual microalgal biomass in Poly(butylene adipate-co-terephthalate) for preparation of green biocomposites and found good mechanical properties (Torres et al., 2015).

Unlike recycling of bioplastics through processing, nature carries out recycling through composting. Composting leads to biodegradation of several organic wastes like food, manure, leaves, grass, paper, wood etc. turning them into numerous valuable organic fertilizers. Composting of biodegradable plastics is a biological process, which is generally conducted under controlled and aerobic conditions. This process breaks down organic matter into simpler substances under the action of various microorganisms, such as bacteria and fungi. The effectiveness of this process depends upon the environmental conditions and constituents of the composting system i.e. temperature, oxygen, organic matter, moisture, the size and activity of microbial populations and material disturbance. In natural environment, composting occurs on a continuous basis and organic matter is metabolized by microorganisms. This is further consumed by invertebrates that in turn, act as nutrients for the soil and support plant growth (ASTM International, 2015a; A. D. Campos et al., 2011; Jayasekara et al., 2005; Pekařová et al., 2018; Yagi et al., 2014).

1.3.1 Biodegradation and composting of polymers and its characterization

Deterioration or a loss in physical integrity of a material is often mistaken for biodegradation. Biodegradation, however, is a natural and complex process of decomposition facilitated by biochemical mechanisms and successive mineralization of the polymer material (**Figure 1.2**). Biodegradable plastics are seen by many as a promising solution to the increasing carbon footprint because they are environmental-friendly.

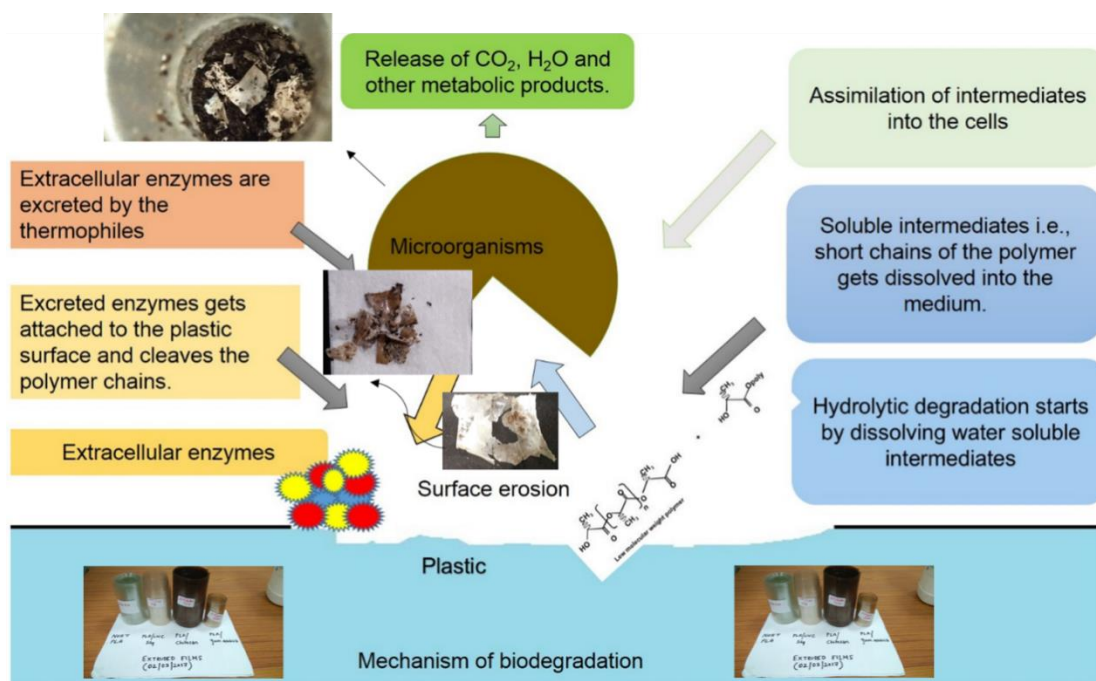


Figure 1. 2: Basic mechanism of biopolymer biodegradation

They can be derived from renewable feedstocks, thereby reducing greenhouse gas emissions. For instance, polyhydroxyalkanoates (PHA) and lactic acid (raw materials for PLA) can be produced by fermentative biotechnological processes using agricultural products and microorganisms (Cadar et al., 2012). Interest in biodegradable plastic packaging arises primarily from the use of renewable raw materials (crops instead of crude oil) and end-of-life waste management by composting or anaerobic digestion to reduce landfilling (Song et al., 2009; Yagi et al., 2014). Plastic materials originating or produced from bio-sources like corn, starch, sugar etc. unlike conventional fossil-based sources, are known as biobased plastics, also called “plant-derived products” (Leja and Lewandowicz, 2010). Hence, plastic that biodegrades within a certain period is called biodegradable plastic. According to the ASTM standard, compostable plastics undergo bio-degradation via biological processes and in this case, composting yields CO₂, H₂O, different inorganic compounds. Thus, compostable materials leaves no mark of any visible and distinguishable, or toxic residue and convert 90 % carbon content of the material into CO₂ (ASTM International, 2015a).

Testing Methods:

There is a concern regarding the testing conditions for monitoring the biodegradation process. Tests can be further subdivided into laboratory tests, simulation tests and field test.

For simulation test includes:

- Laboratory Reactors: Water, Soil, Compost
- Very complex environment
- Controlled and defined environment

As an alternative for field test, various simulation tests in the laboratory have been used for measuring the biodegradability of plastics. Here parameters are controlled in order to have a complete and reliable understanding as well as observation regarding the biodegradation process and its mechanism using different substrates. This simulation tests or laboratory tests are reproducible. Hence, its utility for scientific data and understanding is very vital for biodegradation of plastics research. Various organizations like ASTM and ISO etc. have framed their conditions as standards which might be followed for proper understanding of the biodegradation process (Castro-Aguirre et al., 2017; Leja and Lewandowicz, 2010; Qi et al., 2017).

Different analytical techniques are used for biodegradation observation and analysis:

Mostly analytical techniques used observe or monitor the degradation process depends solely on the aim of the investigation and the environment used. Some analytical methods and polymers used will be discussed in more detail in **table 1.1**.

Table 1. 1: Selected biodegradation tests in composting conditions reported in the literature about the samples, compost and the main methods for assessing biodegradation.

Sample Materials	Form	Thickness (mm)	% Biodegradation	Time (days)	Key Conclusions	Ref.
PLA PLA/thymol 6%/1% silver nanoparticles PLA/thymol 8%/1% silver nanoparticles	Film	-	D	14	Visual inspection as well as weight loss (%), Observed for 14 days under compost at 58 °C, Disintegrability of the polymer films were observed as a mode of assessing biodegradation.	Ramos et al., 2016
	Film	-	D	14		
	Film	-	D	14		
PLA 4042 D PLA/CAB 80/20 PLA/CAB 50/50 PLA/CAB/PEG 80/20/20	Film	0.04-0.06	CD	30	Visual inspection, Complete disintegrability of the polymer films were observed.	Kunthadong et al., 2015
	Film	0.04-0.06	CD	>90		
	Film	0.04-0.06	CD	>90		
	Film	0.04-0.06	CD	90		
PLA 4032 D PLA-PEG PLA-PHB-PEG	Film	0.2	100	28	Weight loss and molecular weight loss as the mode of assessing biodegradation. Though high molecular weight PLA was used, biodegradation was observed.	Arrieta et al., 2014
	Film	0.2	100	28		
	Film	0.2	100	28		

Sample Materials	Form	Thickness (mm)	% Biodegradation	Time (days)	Key Conclusions	Ref.
PBAT (Manure compost)	Film	0.04	67	45	CO ₂ evolution (DMR-NDIR) Different types of compost were used, for assessing biodegradation. Observed for 45 days.	Kijchavengkul et al., 2010
PBAT (Yard compost)	Film	0.04	34	45		
PBAT (Food waste compost)	Film	0.04	45	45		
PLA 7000 D	Sheet	3	60	80	CO ₂ evolution (DMR-NDIR) Observed for 80 days. % Biodegradation was different for every PLA based blends.	Petinakis et al., 2010
PLA60/Starch40	Sheet	3	>80	80		
PLA90/Starch10	Sheet	3	>60	80		
PLA90/Wood-flour10	Sheet	3	50	80		
Microcrystalline cellulose	Powder		>70	45	CO ₂ evolution (CMR Titration). % Biodegradation in PLA mixed with wheat straw took 60 days to reach higher than 60%. Whereas, NPLA took 80 days to reach the same % biodegradation.	Pradhan et al., 2010a
Industrial recycled cellulose	Particle	size < 2.8 mm	>70	45		
PLA (Biomer L 9000)	Particle	size < 2.8 mm	>60	80		
Wheat straw	Particle	size < 2.8 mm	>70	45		
Soy straw	Particle	size < 2.8 mm	>60	45		
PLA-Wheat straw (50:50)	Particle	size < 2.8 mm	>60	60		
PLA-Soy straw (50:50)	Particle	size < 2.8 mm	>60	60		

Sample Materials	Form	Thick-ness (mm) & compatibility	% Biodegradation	Time (days)	Key Conclusions	Ref.
PLA PET Corn starch	bottle		64	63	CO ₂ evolution DMR-NDIR 64 % biodegradation was observed for PLA bottles. Biodegradation was observed under compost pile.	Kijchave-ngkul et al., 2006
	bottle		3	63		
	powder		72	63		
PLA (96% L-lactide)	bottle		CD	<30	Visual inspection Observed for complete disintegrability under compost pile at 65 °C. Observed for less than 60 days.	Kale et al., 2007
PLA tray (94% L-lactide)	tray		CD	<30		
PLA container (94% L-lactide)	Container		CD	<30		
PLA/PCL(80/20) PLA/PBS (80/20) PLA/PHB (80/20)	Blend	compatible	90	75	CO ₂ evolution industrial composting. Surprisingly, they found PLA/PCL(80/20) as home compostable that too in 260 days of time.	Narancic et al., 2018
	Blend	inconclusive	90	75		
	Blend	compatible	90	75		
PLA	Strips/pellets			S300	Weight percentage Observed at 27 °C, 37 °C and 58 °C. PCL degraded in all condition at different temperature.	Al Hosni et al., 2019
PCL	Strips/pellets			C91/S300		
PHB	Strips/pellets			S300		
PLA	foil		70	100	CO ₂ evolution industrial composting. Surprisingly, they concluded PHB of being better in terms in biodegradability. Although for blends their compatability was an issue and found to incompatible since structural defects were observed. Which leads to disintegration and faster degradation.	Sednickova et al., 2018
PLA/triacetine	Composite foil	compatible	80	100		
PLA/PHB triacetine	Blend foil	inconclusive	90	100		

Sample Materials	Form	Thickness (mm)	% Biodegradation	Time (days)	Key Conclusions	Ref.
PLA (2002 D)	Sheet	1	55	90	CO ₂ evolution (CMR Titration). Biodegradation was observed for 90 days. Biodegradation of PLA based blends have different rate of biodegradation within a certain time period.	Lovino et al., 2008
TPS	Sheet	1	87	90		
PLA/TPS 75/25	Sheet	1	61	90		
PLA/TPS/Coir 52/17/30	Sheet	1	59	90		
PLA/TPS/MA 75/25/1	Sheet	1	57	90		
PLA/TPS/Coir/MA 52/17/30/1	Sheet	1	54	90		
PCL	Particle size	<10 mesh	52	45	CO ₂ evolution (CMR Titration). They found that 8% biodegradation in the LDPE samples in 45 days. Compost used had 43 C/N ratio, and 52% dry solids They used PCL to study biodegradation for 45 days.	Calil et al., 2006
CA	Particle size	<10 mesh	22	45		
LDPE	Particle size	<10 mesh	8	45		
Cellulose	Particle size	<10 mesh	70	45		
PCL/CA 60/40	Particle size	<10 mesh	56	45		
PCL/CA 40/60	Particle size	<10 mesh	65	45		

Notes: Cells without values indicate that the authors did not report or calculate these values, films are samples with thickness 0.254 mm, sheets are samples with thickness > 0.254 mm. Mn: number average molecular weight, Mw: weight average molecular weight, CD: complete disintegration, NS: not specified, PLA: poly(lactic acid), CAB: cellulose acetate butyrate, PEG: poly(ethylene glycol), PHB: poly(hydroxybutyrate), ATBC: acetyl-tri-n-butyl citrate, LA: lactic acid, EG: ethylene glycol, SA: succinic acid, MA: malonic acid, PHBV: poly(hydroxybutyrate-co-hydroxyvalerate), PBAT: poly(butylene adipate-co-terephthalate), PCL: poly(caprolactone), DDGS: distillers dried grains with solubles, PP: poly(propylene), EPI: environmental product Inc. containing 3% of totally degradable plastic additive, Mater-Bi: starch/hydrophilic-biodegradable resin blend, TPDAS: thermoplastic dialdehyde starch, TPS: thermoplastic starch, CA: cellulose acetate, LDPE: low-density polyethylene, PET: poly(ethylene terephthalate), Direct Measurement Respirometry (DMR), non-dispersive infrared (NDIR), Cumulative measurement respirometry (CMR), D-Disintegration

1.3.1.1 Visual observations

The evaluation of visible changes in plastics can be performed in almost all tests. Effects used to describe degradation include roughening of the surface, formation of holes or cracks, defragmentation, change in color or formation of biofilms on the surface. These changes do not prove the presence of a biodegradation process in terms of metabolism, but the parameter of visual changes can be used as a first indication of any microbial attack. To obtain information about the degradation mechanism, observations that are more sophisticated can be made using either scanning electron microscopy (SEM) or atomic force microscopy (AFM) (Kalita et al., 2019; Rudeekit et al., 2008; Tsuji and Ikada, 1999).

1.3.1.2. Changes in mechanical properties and molar mass

Visual observations alone along with changes in material properties cannot be used to prove biodegradability with regard to metabolism of the polymer material. Changes in mechanical properties are often used only when minor changes in the mass of the test specimens are observed, like in hydrolytic degradation, (Castro-Aguirre et al., 2017), which is an initial step to fulfill biodegradation or to attain biodegradation of a polymeric material. Properties such as tensile strength are very sensitive to changes in the molar mass of polymers, which is also often taken as an indicator of degradation (Jayasekara et al., 2005). Therefore, this type of measurement is often used for materials where an abiotic process is responsible for the first degradation step, e.g., chemical hydrolysis for poly (lactic acid) or oxidation for modified polyethylene (Abrusci et al., 2011; Husorova et al., 2014; Kucharczyk et al., 2016). (Le Marec et al., 2014; Tayal and Khan, 2000), used molar mass loss or decrease in molecular weight of the polymers as extensive technique for monitoring biodegradation of polymers using gel permeation chromatography (GPC) technique. (Rudeekit et al., 2008). Rudeekit, Y. et al., 2008 also used molar mass loss of the sample material as a tool to monitor biodegradation. Most recently (Chinaglia et al., 2018; Sikorska et al., 2015) used

GPC elugrams for analyzing the molecular weight loss of PLA and PLA/a-PHB based samples in industrial composting conditions for showcasing biodegradation through molar mass loss, and suggested that PLA has a bimodal molar mass distribution after incubation under industrial composting conditions. They observed this phenomenon after 7 days of degradation of the investigated material in the container system and after 70 days of incubation in the composting pile. The formation of the distribution is most probably caused by the thickening of the sample, which led to heterogeneous degradation of the materials caused by the absorption of water during their incubation.

This is consistent with the general hydrolysis pattern of thick PLA samples, as diffused water degrades the polymer molecules from the interior of the material, thus generating a significant amount of carboxylic acid groups, which are difficult to diffuse out as opposed to those in the surface phase. A higher concentration of acidic groups results in a higher degradation rate of the interior part than the exterior part. Bimodal distribution may thus be formed (Musioł et al., 2016; Sikorska et al., 2015)

1.3.1.3 Weight loss measurements: Determination of residual polymer

The mass loss of test specimens such as films or test bars is widely applied in degradation tests (especially in field- and simulation tests), although again no direct proof of biodegradation is obtained. Problems can arise with proper cleaning of the specimen, or if the material disintegrates excessively. For finely distributed polymer samples (e.g., powders) the decrease in residual polymer can be determined by an adequate separation or extraction technique (polymer separated from biomass or polymer extracted from soil or compost). By combining a structural analysis of the residual material and the low molecular-weight intermediates, detailed information regarding the degradation process can be obtained, especially if a defined synthetic test medium is used (Li et al., 2015).

1.3.1.4 Clear zone formation

A very simple semi-quantitative method is so-called clear-zone test. This is an agar-plate test in which the polymer is dispersed as very fine particles within the synthetic medium agar; this results in the agar having an opaque appearance. After inoculation with microorganisms, the formation of a clear halo around the colony, indicates that the organisms are at least able to depolymerize the polymer, which is the first step of biodegradation. This method is usually applied to screen organisms that can degrade a certain polymer (Abou-Zeid et al., 2004; Suyama and Tokiwa, 1998), but it can also be used to obtain semi-quantitative results by analyzing the growth of the clear zones (Castro-Aguirre et al., 2017).

1.3.1.5 Isolation and selection of aerobic microorganisms for PLA biodegradation investigation.

Identification and isolation of microorganisms in different environments like compost, soil, or wastewater, which are generally used as substrate for monitoring biodegradation activity of biodegradable polymers, is of utmost necessity for understanding the biodegradation mechanism that occurs during the biodegradation process. Various literature suggest that different family of microbes like fungi, bacteria or actinomycetes are responsible for biodegradation at different temperature along with other physical conditions of the substrate like pH, moisture content in case of compost, etc. Overall, microbial growth depends upon the set conditions or standard protocols like ASTM standards under which the biodegradation procedure is followed. It is observed from noted mechanism of biodegradation of polymers that microbes form biofilm on the polymer surface which further enhances the complete biodegradation of the polymers by disintegrating them into oligomers, monomers as well as other major metabolites which get dissolved with the substrate used (Arkatkar et al., 2010; Walczak et al., 2015).

The principle of enriching the desirable target organisms from the microcosm that coexist in a habitat was first developed by Winogradsky and Beijerinck (Walczak et al., 2015). For successful isolation of a given organism into pure culture, the organism generally must (Suyama and Tokiwa, 1998)

Apart from being completely biodegradable, PLA also does not pollute the environment after biodegradation. With the development of modern molecular biological techniques (e.g., PCR, high throughput sequencing technology) [Sangwan and Wu 2008] and analytical techniques (e.g., ¹H NMR, ESI-MS, IR, DSC, X-ray, SEM, FTIR-ATR, GPC) (Jarerat et al., 2006; Kim et al., 2006; Lee, 2002; Suyama and Tokiwa, 1998). Some studies have detected the biochemical processes and microbial community composition in the biodegradation of PLA.

1.3.1.6 The Microbial communities and other factors which acts as biological agent in biodegradation of polyester

The biological environment, i.e. the biological surroundings in which polymers are present, includes the biological agents responsible for the deterioration of polymeric substances.

Biological agents such as bacteria, fungi and their enzymes consume a substance as a food source so that its original form disappears. Under appropriate conditions of moisture, temperature and oxygen availability, biodegradation is a relatively rapid process.

1.3.2 Isolation and identification of various microorganisms and enzymes associated with biopolymer biodegradation

Two types of microorganisms are of particular interest in the biodegradation of natural and synthetic polymers; these are bacteria and fungi. Bacteria include the general bacteria and actinomycetes. Many species or types of microorganisms are found broadly in nature (Zumstein et al., 2019). Therefore, researches on isolation of PLA-degrading microorganisms have been raised in recent years. Currently, multiple types of microbes

(**Table 1.2**) that are able to degrade PLA have been isolated from soil or water. However, they mostly are actinomycetes, and only a fraction of them belongs to bacteria and fungi. The biological environment, i.e. the biological surroundings in which polymers are present, includes the biological agents responsible for the deterioration of polymeric substances.

Biological agents such as bacteria, fungi and their enzymes consume a substance as a food source so that its original form disappears. Under appropriate conditions of moisture, temperature and oxygen availability, biodegradation is a relatively rapid process (Al Hosni et al., 2019b; Kalita et al., 2019; Zumstein et al., 2019).

Table 1. 1: Showing different strains isolated from different sources that acts upon PLA based films and were responsible for biodegradation of PLA with the enzymes they release during the biodegradation process.

Strains	Sample source	Type of enzymes	Reference
Bacteria			
<i>Bacillus brevis</i>	Soil		(Tomita et al., 1999)
<i>Bacillus stearothermophilus</i>	Soil		
<i>Bacillus smithii</i> strain PL21	Garbage	Esterase	(Sakai et al., 2001)
<i>Bacillus licheniformis</i>	Compost		(Arena et al., 2011)
<i>Paenibacillus amylolyticus</i> strain TB-13	Soil	Lipase	(Akutsu-Shigeno et al., 2003)
<i>Alcaligenes</i> sp.		Lipase	(Hoshino and Isono, 2002)
<i>Geobacillus thermocatenulatus</i>	Soil		(Tomita et al., 2004)
<i>Pseudomonas</i> sp. strain DS04-T	Activated sludge	Lipase	(Wang et al., 2011)
<i>Pseudomonas tamsuii</i> TKU015 PLA	Soil	Lipase	(Liang et al., 2016)
<i>Elizabethkingia meningoseptica</i> LK3 (accession number KT715773)	Compost		(M. Walczak et al. 2015)
<i>A. aurescens</i> LK9 (accession number KT715774)	Compost		M. Walczak et al. 2015)
Fungus			
<i>Cryptococcus</i> sp. strain S-2	Wastewater	Cutinase	(Masaki et al., 2005)
<i>Thermomyces lanuginosus</i> (KC762239)	Compost		[Karamanlioglu et al., 2013 and Karamamlioglu 2014]
<i>Aspergillus fumigatus</i> (KC762248)			
Actinomycete			
<i>Amycolatopsis</i> strain HT-32, No.3118, KT-s-9	Soil	Protease	(Kawai, 2010)
<i>Pseudonocardia</i> sp. RM423	Compost		[Apinya et al. 2015]
<i>T. flexuosa</i> FTPLA	Compost		[Husárová et al. 2014]
<i>Thermopolyspora</i> sp.	Compost		
<i>Thermobifida fusca</i> DY-3	Compost		

1.3.2.1 Actinomycetes

Actinomycetes are filamentous bacteria, mainly found in the soil, that are well-known for being antibiotic producers. Some actinomycetes efficiently degrade polyesters, including PLA, PCL, PHB and poly (ethylene succinate). The first reported PLA degrader was *Amycolatopsis* HT-32, which was successfully isolated from soil by applying the plate count and clear-zone methods (Castro-Aguirre et al., 2017; Pranamuda et al., 1997). In this study, the strain and 100mg film were added to fluid medium and about 60 mg film was degraded after 14 days of culture. They analyzed 25 strains of *Amycolatopsis* after that, of which 15 strains were able to degrade PLA pellets. The study shows that PLA degraders are distributed generally in this genus. The fermentation process for a poly (L-lactide) (PLA)-degrading enzyme production by a newly isolate of thermophilic PLA degrading *Actinomadura* sp. T16-1 was investigated. The strain produced 33.9 U/mL of enzyme activity after cultivation at 50 °C under shaking.

The optimal concentration of PLA film and gelatin obtained by response surface methodology (RSM) for the highest production of PLA-degrading enzyme was 0.035% (w/v) and 0.238% (w/v), respectively. Under these conditions, the model predicted 40.4 U/mL of PLA-degrading activity and the verification of the optimization showed 44.6 U/mL of PLA-degrading enzymatic activity in the flasks experiment. The maximum PLAdegrading activity reached 150 U/mL within 72 h cultivation in the 3-L airlift fermenter (Sukkhum et al., 2009).

Soil burial experiments show that PLA materials will take a long time for degradation to start, and the degradation rate is quite slow (Rudeekit et al., 2008; Weng et al., 2013b).

1.3.2.2 Fungus

Fungal degradation of PLA has very few studies in the reported literature (**Table 1.2**). The study on fungal degradation of PLA was made in 1996 by (Torres et al., 1996). They found that only two of 14 filamentous fungal strains (*Fusarium moniliforme* and *Penicillium*

roqueforti) were able to assimilate DL-lactic acid in liquid cultures. Moreover, fungal degradation of PLLA had also been studied by using *Tritirachium album* (ATCC 22563) in a liquid culture (Jarerat and Tokiwa, 2001). It shows that most of the PLA film is degraded after 14 days of cultivation by the addition of gelatin. However, many studies mainly focused on the degradation of PLA by the pure cultures of fungi. Some researchers have explored the fungal degradation of PLA in soil and compost condition (Pranamuda et al., 1997). It shows that temperature is a key parameter governing the fungal degradation of PLA. In addition, the biodegradation of PLA and some plasticized PLA with *Trichoderma viride* fungus, have been assessed in liquid medium and controlled laboratory conditions (Lipsa et al., 2016).

There is little research involving the quorum sensing of microorganisms in degradation of PLA. Hence, the future studies on biodegradation of PLA should also focus on the microbial quorum sensing. In addition, knowledge of the behavior of bacterial communities also is crucial for understanding the organization of the microbial community involved in the disruption of materials (Gu, 2003)

1.3.2.3 Bacteria

Compared to actinomycetes, the bacterial degradation of PLA was reported rarely in the literature. As summarized by Jeon et al., 2013; and Penkhrue et al., 2015; the PLA-degrading bacteria include *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, etc. Some studies show that PLA-degrading bacteria are not distributed widely in the natural environment. The first reported PLA-degrading bacteria was, *Bacillus brevis*, which was isolated from 144 soil samples using an enrichment culture medium (Tomita et al., 1999). After that, some PLA-degrading bacteria were successfully isolated using an enrichment culture medium as shown in (**Table 1.2**). The exploration based on bacterial degradation of PLA will provide vast useful information for the studies on PLA degrading enzymes. According

to reported classification, the PLA degrading bacteria mainly came from firmicutes. Moreover, most of them belong to thermophilic bacteria.

1.3.4 Enzymes:

Enzymes are, first, catalysts. Structurally, enzymes are complex and highly specialized proteins, which are produced by the cell, in order to catalyze specific types of chemical functions. Enzymes are generally classified into six groups, depending on the reaction they catalyse: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases (Bhardwaj et al., 2013; Webb et al., 2013). Polyester cleaving enzymes belong to the group of hydrolases, which catalyses the hydrolytic cleavage of the C-O and C-N- bonds. Hydrolases include lipases, esterases, PHB-depolymyerases and serine endopeptidases (Webb, 1992). A common feature for the serine hydrolases is the presence of a specific sequence Gly-X-Ser-X-Gly. It has been well demonstrated that serine from this sequence Gly-X-Ser-X-Gly- in the active center, attacks the ester bond nucleophilically (Castro-Aguirre et al., 2017; Lim et al., 2005; Qi et al., 2017).

The biodegradation of polymers by microorganisms is catalyzed by extracellular, degradative enzymes that produce water soluble, low molecular weight products from the macromolecular substrates. These products are water soluble and can diffuse into the surrounding aqueous environment and are taken up by the cells of the microorganisms and used as nutrients. The extracellular degradative enzymes were produced by microorganisms degrading natural and synthetic polyesters (Bhardwaj et al., 2013; Lim et al., 2005; Qi et al., 2017).

1.3.4.1 PLA-degrading enzymes

PLA-degrading enzymes from microorganisms play a vital role in biodegradation of PLA. (Williams, 1981) first reported the enzymatic hydrolysis of PLLA by proteinase K from *Tritirachium album*. And after that, PLA-degrading activity of 56 commercially available

proteases was tested by (Oda et al., 2000). It shows that acid and neutral proteases have little or no activity but some alkaline proteases form appreciable numbers of lactic acid from PLA. However, the commercial proteases can't be applied to PLA plastics of real environment due to limited conditions. Therefore, purification and characterization of some extracellular PLA-degrading enzymes from microorganisms have been addressed by the modern molecular biological techniques, e.g., sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE), UV-vis spectrophotometry, 16S rDNA (Jarerat et al., 2006; Sukkhum et al., 2009) The recent researches of PLA depolymerases show that protease-type have preference toward PLLA (type I) and lipase/ cutinase-type have preference to PDLA (type II) (Kitadokoro et al., 2012). In addition, the maximum activity of these enzymes depends on the optimum pH, temperature and PLA property (chain stereochemistry and material crystallinity). Serine proteases as the main member of PLA-degrading proteases (such as proteinase K, α -chymotrypsin, subtilisin, trypsin and elastase) show vast potential for the future exploration of PLA degradation (Lim et al., 2005). (Leejarkpai et al., 2011)

New advances of catalytic mechanism should come from the isotope tracing method and fluorescent mark. In addition to find the new depolymerase of PLA, the future studies on PLA-degrading enzymes may be directed to explore catalytic mechanism and enzyme engineering technology.

1.3.5 Biodegradation kinetics

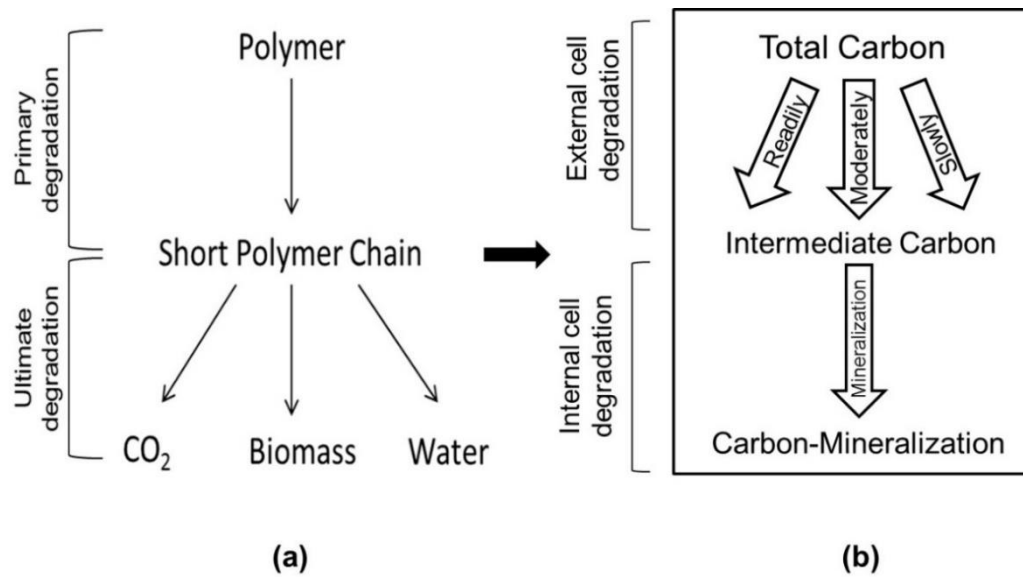


Figure 1. 3: Flowchart of biodegradation mechanism of biodegradable polymers (a) and proposed carbon degradation during aerobic composting (b).

$$\frac{dC_r}{dt} = -k_{hr} \cdot C_r \dots \dots \dots 1.1$$

$$\frac{dC_m}{dt} = -k_{hm} \cdot C_m \dots \dots \dots 1.2$$

$$\frac{dC_s}{dt} = -k_{hs} \cdot C_s \dots \dots \dots 1.3$$

$$\frac{dC_{aq}}{dt} = (k_{hr} \cdot C_r + k_{hm} \cdot C_m + k_{hs} \cdot C_s) - k_{aq} \cdot C_{aq} \dots \dots \dots 1.4$$

$$\frac{dC_T}{dt} = k_{aq} \cdot C_{aq} \dots \dots \dots 1.5$$

The proposed mechanism is illustrated in (**Figure 1.3**). The changes of three types of dry solid carbon into intermediate (water-soluble carbon) are illustrated in Eqs. (1)–(3). The change of water-soluble C is illustrated in Eq. (4). The mineralization of water-soluble carbon into carbon dioxide is illustrated in Eq. (5). where, C_T is percentage of cumulative C-CO₂ production; C_r , C_m , C_s are percentages of readily, moderately and slowly

hydrolysable solid carbon fractions, respectively; C_{aq} is percentage of intermediate solid carbon; k_{hr} , k_{hm} , k_{hs} are readily, moderately and slowly hydrolysis

Rate constants (day^{-1}); k_{aq} is the mineralization rate constant of water-soluble carbon into carbon dioxide (day^{-1}); $t = t - c$; c is the lag time before C-CO₂ (CT) product evolves (day); t is time (day).

$$C_{T_t} = \left(\begin{aligned} & C_{aq0} \cdot (1 - e^{-k_{aq} \cdot (t-c)}) \\ & + \left[C_{r0} \cdot \left(1 - \frac{k_{aq}}{k_{aq}-k_{hr}} \cdot e^{-k_{hr} \cdot (t-c)} + \frac{k_{hr}}{k_{aq}-k_{hr}} \cdot e^{-k_{aq} \cdot (t-c)} \right) \right] \\ & + [C_{m0} \cdot (1 - \frac{k_{aq}}{k_{aq}-k_{hm}} \cdot e^{-k_{hm} \cdot (t-c)} + \frac{k_{hm}}{k_{aq}-k_{hm}} \cdot e^{-k_{aq} \cdot (t-c)})] \\ & + [C_{s0} \cdot (1 - \frac{k_{aq}}{k_{aq}-k_{hs}} \cdot e^{-k_{hs} \cdot (t-c)} + \frac{k_{hs}}{k_{aq}-k_{hs}} \cdot e^{-k_{aq} \cdot (t-c)})] \end{aligned} \right) \dots\dots\dots 1.6$$

for $t > c$ or 0 for $t \leq c$ where, C_{T_t} is percentage of cumulative C-CO₂ production at time t ; C_{r_t} , C_{m_t} , C_{s_t} are percentages of readily, moderately and slowly hydrolysable solid carbon fractions at time t , respectively; C_{aq_t} is percentage of intermediate solid carbon at time t ; C_{r0} , C_{m0} , C_{s0} are percentages of initial readily, moderately and slowly hydrolysable solid carbon fraction, respectively; C_{aq0} is the percentage of initial intermediate solid carbon fraction (Cadar et al., 2012; Leejarkpai et al., 2011; Stloukal et al., 2015).

1.3.6 Biodegradable polymers

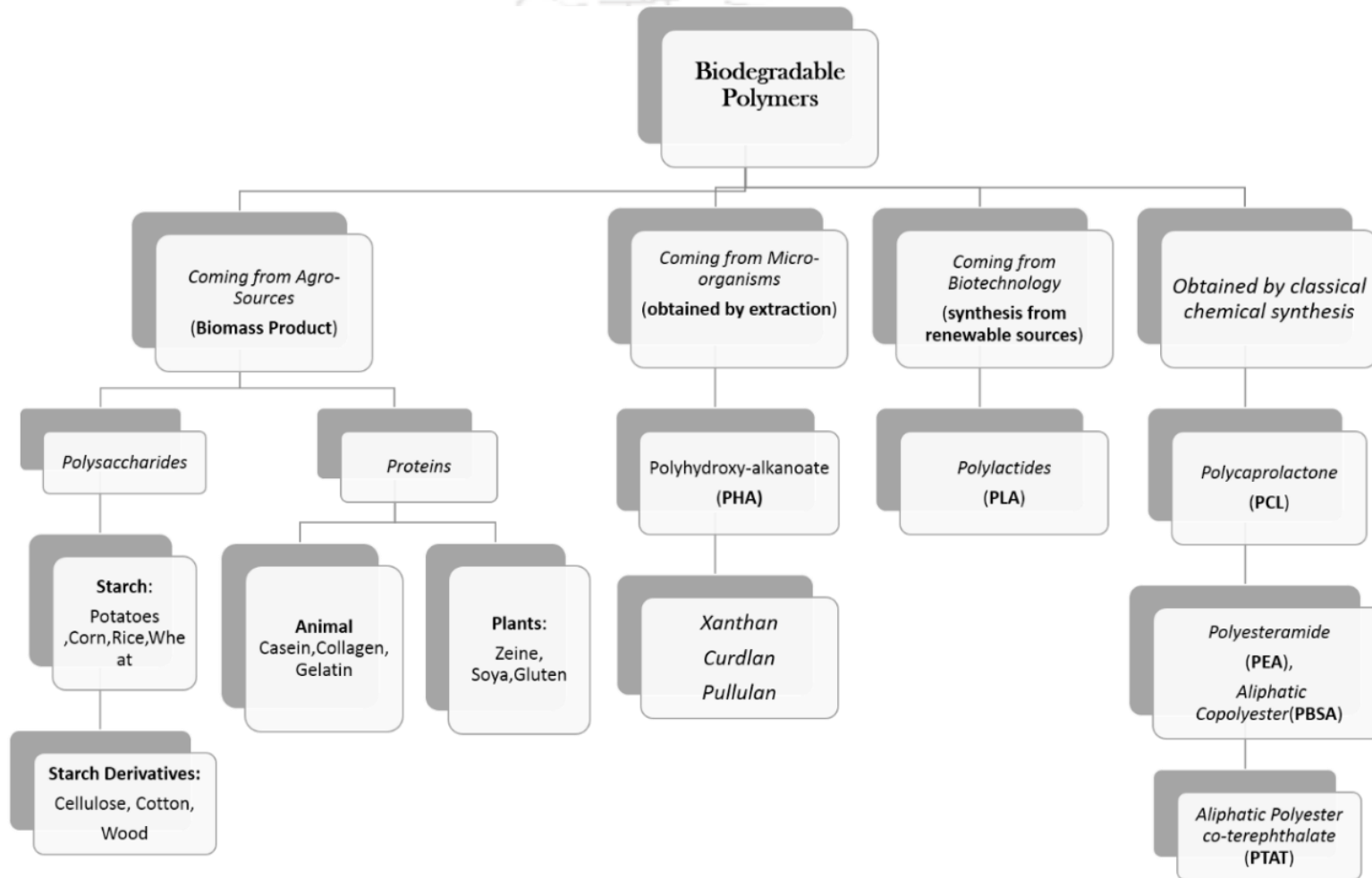


Figure 1. 4: Different Source of biodegradable polymers and their classification adopted from various sources.

Biodegradable polymers are those polymers which are obtained from various natural resources as carbon source (**Figure 1.4**). They can degrade and assimilate in various environments like soil or compost and doesn't produce any toxic substances during the degradation process. Various microbes like fungus, bacteria and actinomycetes are generally responsible for the degradation of the biopolymers (Zumstein et al., 2019).

Poly(lactic acid) PLA

PLA is an aliphatic polyester commonly made from hydroxy acids (**Figure 1.5**), which include polyglycolic acid or polymandelic acid, and are biodegradable and compostable in a certain time frame. It is a thermoplastic polymer having high-strength, high-modulus that can be made from renewable resources for yielding articles for use in industrial packaging field or biocompatible/bioabsorbable medical devices. Main advantage of this polymer is that it is easily processed on standard plastics equipment to yield molded parts, film, or fibers. Similar to polystyrene, high-molecular-weight PLA is a stiff thermoplastic polymer which is color-less and glossy (Garlotta, 2002; Gupta et al., 2018; Tesfaye et al., 2017; Tsuji and Ikada, 1999). PLA can be made into forms having different optical purity from +D Lactic acid to –L Lactic acid (**Figure 1.6**)

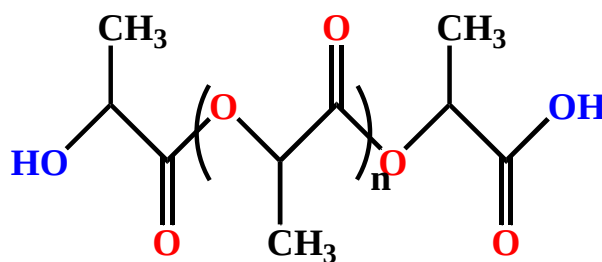


Figure 1. 5: Poly(lactic acid) structure

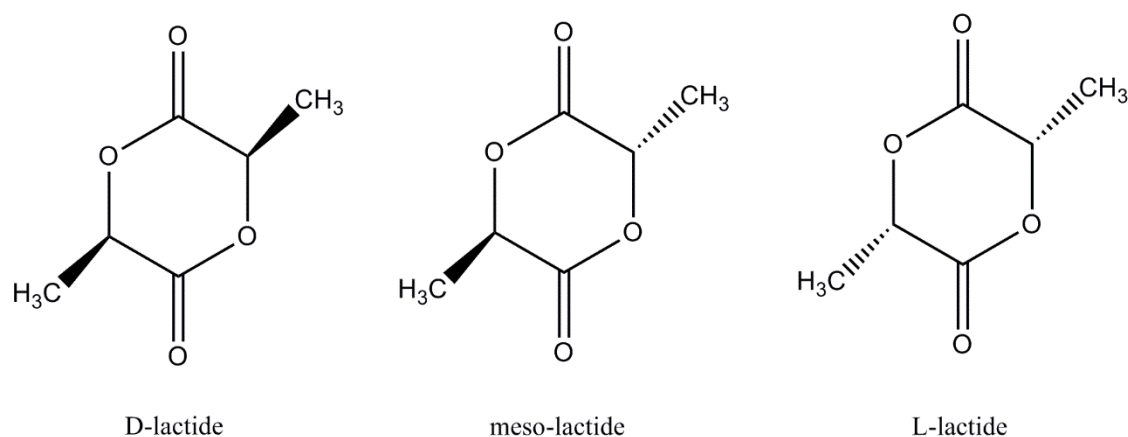


Figure 1. 6: Different optical forms of PLA

Synthesis of PLA

The basic building block for PLA is lactic acid. Lactic acid is manufactured by carbohydrate fermentation or chemical synthesis, though fermentation is the dominant process which ensures proper formation of lactic acid. Synthesis of lactic acid conversion to high molecular weight PLA can follow two different synthetic routes of polymerization, as depicted in **figure. 1.7**. Here, Lactic acid is condensation polymerized to yield a low-molecular-weight, brittle, glassy polymer, which, for the most part, is unusable for any applications unless external coupling agents are used to increase its molecular weight. In **figure1.7** known routes to high-molecular weight PLA: azeotropic dehydrative condensation, condensation/coupling, or ring-opening polymerization of lactide, are depicted. Condensation polymerization is the most inexpensive route, but its main constraint is the production of high molecular weight PLA in a solvent free system, and therefore the use of coupling agents or esterification-promoting adjuvants is required, adding cost and complexity. Self-condensation of lactic acid produces low-molecular-weight product with hydroxyl and carboxyl end-groups having an equimolar concentration of the functional groups respectively. To increase the molecular weight, chain-coupling agents must be added, which will preferentially react with either the hydroxyl or carboxyl

group, leading to different kinetic reaction rates of coupling (Garlotta, 2002; Katiyar and Nanavati, 2011)

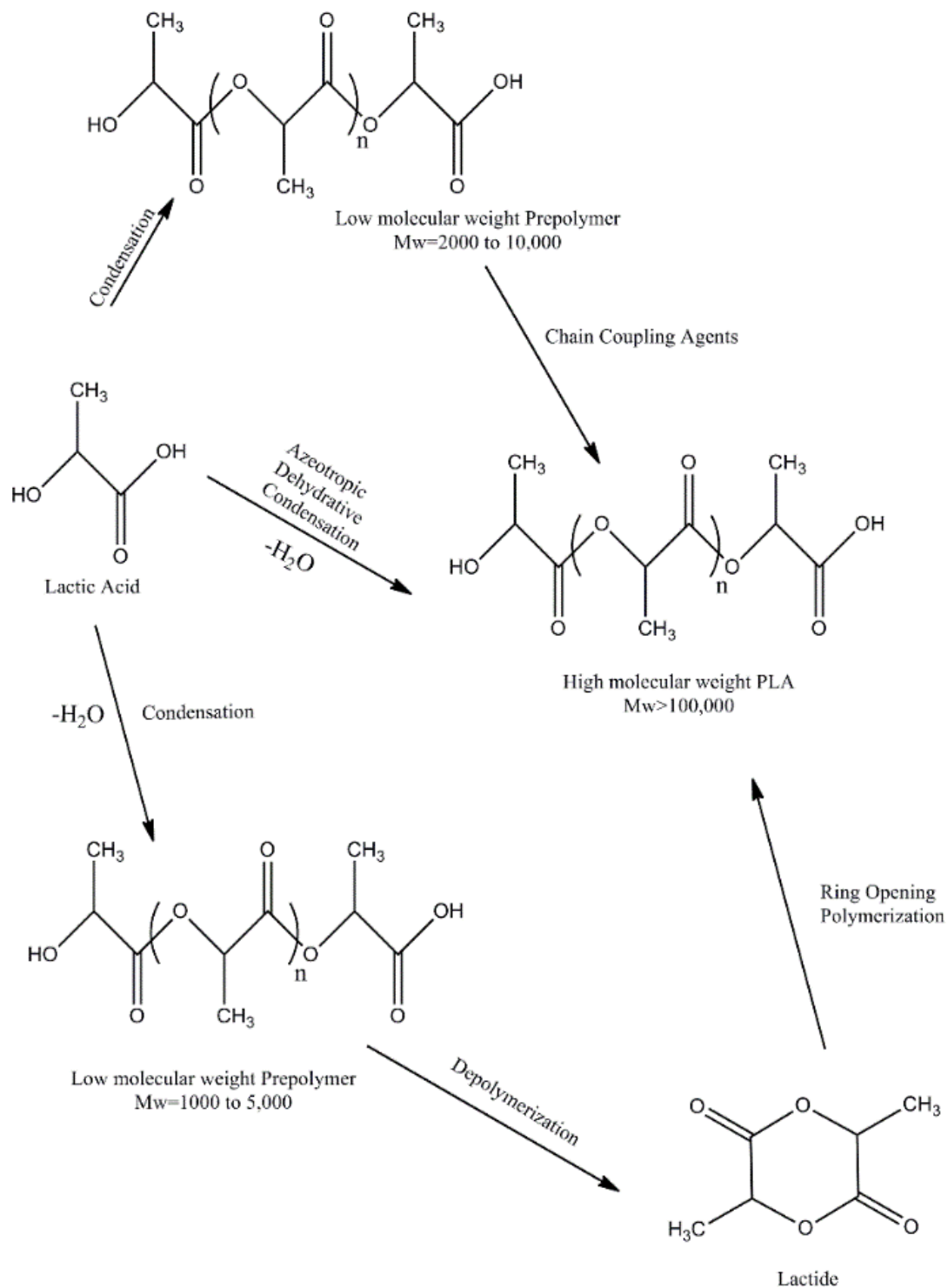


Figure 1. 7: Synthesis method for High molecular weight PLA

Polycaprolactone (PCL)

PCL a petroleum derived biodegradable polymer, has enormous potential to replace its conventional counterparts. Poly(ϵ - caprolactone) PCL (**Figure 1.8**) is a polyester that has emerged as an effective alternate of various non-biodegradable polymers and characterized by its versatility in property via tuning its molecular weight, degree of crystallinity, compatible blending with multiple polymers etc., creating another roadway to scientists and researchers for its utilization in the industrial scale. Synthesis of PCL can be carried out by following two chemical pathways: condensation polymerization of 6-hydroxycaproic (6- hydroxyhexanoic) acid and ring-opening polymerization (ROP) of ϵ - caprolactone (Bhagabati et al., 2019). The advantages of easy and convenient polymerization with tuneable high molecular weight and narrow polydispersity without the formation of by-products make the ROP a preferred method to follow the synthesis of PCL. ROP of ϵ -caprolactone are done through selection of four different categories of catalyst that follows anionic, cationic, monomer activated and co-ordination insertion reaction mechanism. While, use of alkali metal based catalyst like phenyl lithium at 170 °C for a few hours led to production of PCL of moderate molecular weight, cyclopentadienyl sodium catalyst was reported to have produced high molecular weight PCL ($M_n = 130,000$ Da) with narrow PDI. Alkaline earth metal based catalyst like alkyl or aryl group containing magnesium based complexes were capable of forming high molecular weight PCL ($M_n = 43,100$ Da) with narrow PDI ~ 1.1 . While metals like aluminium based alkyl or aryl complexes were also used for the synthesis of PCL, but the control over reaction was difficult, which led to molecular weight of the PCL not reaching up to the expectation (Kim et al., 2005; Labet and Thielemans, 2009). Moreover is PCL is compostable under natural as well as controlled composting condition (Narancic et al., 2018).

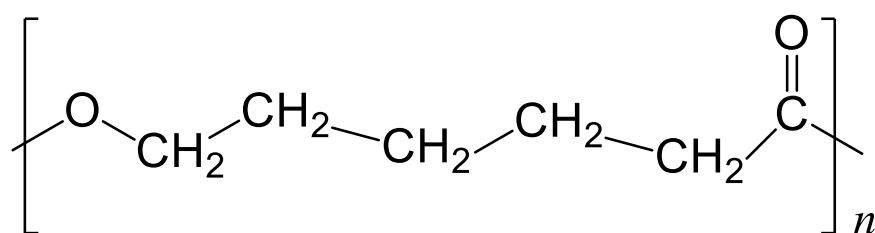


Figure 1. 8: PCL an aliphatic biodegradable linear polyester derived from fossil fuels

Chitosan

Chitosan is also one of the best studied and highly promising biopolymer, which has ability to change the current scenario of consumption of conventional synthetic polymers in various applications from food packaging to other medicinal uses. It is a partly acetylated (1-4)-2-amino-2-deoxy -D glucan with linear backbone of glucosamine (Furuike et al., 2015; Pal and Katiyar, 2016a). Biodegradable, non-toxic and flexible chitosan is extracted by alkaline hydrolysis of chitin, which is the most available and used natural biopolymer after cellulose (Zhang et al., 2007). Generally, chitin is extracted from various exoskeleton of marine as well as land crustacean creatures, the cell walls of fungi and insects. Chitosan, a cheap and natural resource, exhibits various unmatched properties such as film forming behaviour, biocompatibility and biocompostibility due to the nitrogen attached at C-2 position. This makes it prominent for various applications such as biotechnology, agriculture, biomedicine, wastewater treatment, and environmental protection. The limitations of chitosan are its hydrophilic nature but recently various researches have been done to convert hydrophilic chitosan into hydrophobic chitosan. Chitosan can be utilized in food packaging application, if its hydrophilic nature converts into hydrophobic as shown in the **figure 1.9** below (Pal and Katiyar, 2016a). Chitosan as a filler is used in various biopolymers to make films for packaging applications and its compostability will make it a unique biopolymer for preparation of compostable polymers.

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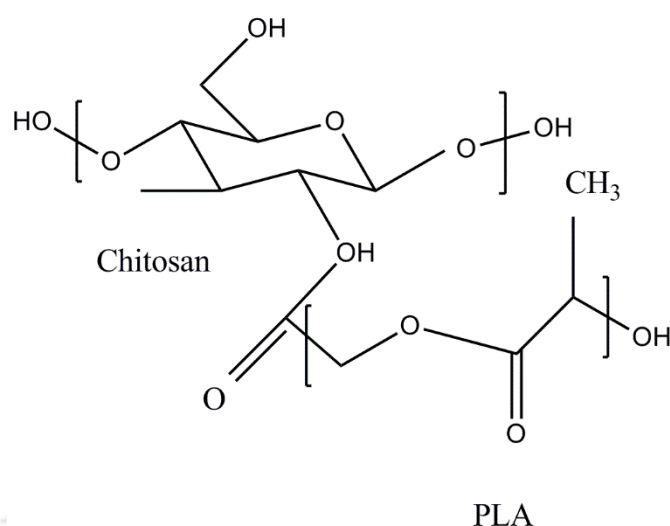


Figure 1. 9: Chitosan grafted PLA structure

Cellulose based derivatives ((Cellulose nanocrystals (CNC) and microcrystalline cellulose (MCC))

Cellulose is the most used and available renewable biopolymer, either used in pure form or as a derivative in packaging as well as pharmaceutical industry. Recently great deal of research and developments have been undertaken in cellulose-based nanotechnology through various processes, and have gained great attention in the fields of packaging and cosmetics as well as other applications (Dhar et al., 2015). CNCs are promising bionanoparticles of the twenty-first century with unique material properties: a high aspect ratio, controlled morphology, high surface reactivity, specific surface area, specific strength, interesting optical properties, biodegradability, non-toxicity and biobased origin. Through novel surface modification approaches and the fabrication process, CNCs have been widely used as a reinforcing agent in polymer nanocomposites (Borkotoky et al., 2018; Miao and Hamad, 2016). CNCs usually consist of glucose repeating units (10–100 units), each unit having six hydroxyl groups, which provide enhanced functionality and reactivity of the CNC surface toward chemical moieties (**Figure 1.10**) (Dhar et al., 2015). Synthesis of such biopolymer-supported noble metal

nanoparticles has shown improved dispersibility, reduced coagulation/agglomeration-related problems and drastically enhanced stability_(Dhar et al., 2016a). CNCs, if used in compostable plastics would create a green compostable plastic for use in packaging industry which would contribute to a green environment with reduction in the use of conventional plastic material (Kalita et al., 2019).

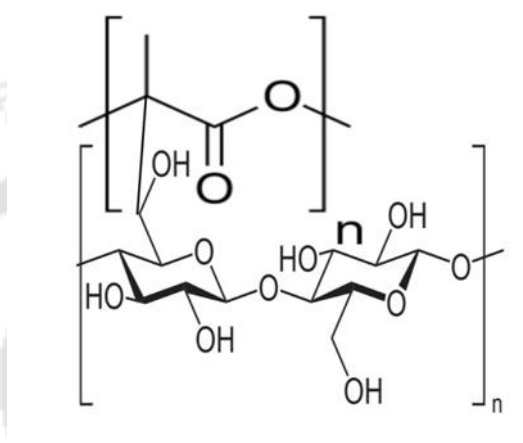


Figure 1. 10: Cellulose nanocrystals (CNC) grafted PLA structure

Considering its widespread usage in various fields, such as food, pharmaceutical, medical, cosmetic and polymer composites industries, microcrystalline cellulose (MCC) (Figure 1.11) is becoming impellent due to increasing demand of alternatives to non-renewable and scarce fossil materials.

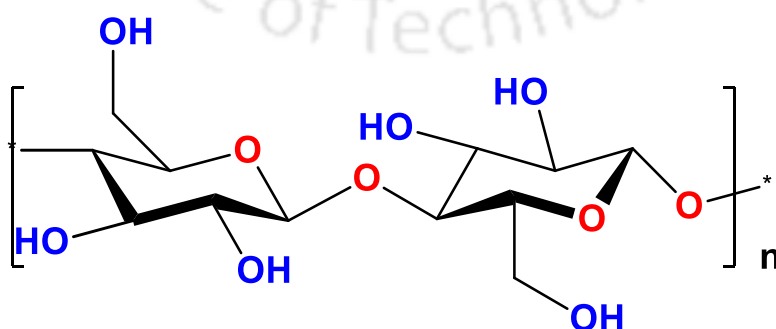


Figure 1. 11: Cellulose microcrystalline structure (MCC) structure

Although it still suffers from some drawbacks, MCC has recently gained more interest owing to its renewability, non-toxicity, economic value, biodegradability, high mechanical properties, high surface area and biocompatibility. New sources, new isolation processes, and new treatments are currently under development to satisfy the increasing demand of producing new types of MCC-based materials on an industrial scale (Murphy and Collins, 2018; Thoorens et al., 2014; Trache et al., 2016).

Gum Arabica

Polysaccharide based gums are natural biopolymers, which are mostly available in nature. These gums have industrial applications in various fields due to their nontoxicity, renewability, and biodegradability. Gum Arabic is a very complex polysaccharide which is obtained as an exudate from Acacia trees such as *Acacia senegal* and *Acacia seyal*. Polydisperse Gum Arabic is composed of approximately 90% AG fraction (which has very little associated protein), around 10% AGP fraction (which contains 10% of protein component), and about 1% GP fraction (which contains 20–50% of protein component) (Tripathi and Katiyar, 2016; Zhang et al., 2018). Gum Arabica is a non-toxic and hydrophilic biopolymer. It has good emulsion property and has been used in various bioactive compounds for encapsulation processes. It has been used as a filler in PLA based films with some modifications in its structural properties. This modification (**Figure 1.12**) decreased the gas-transport through the films, and its OTR and WVTR decreased rapidly, signifying its used a food packaging film (Tripathi and Katiyar, 2017).

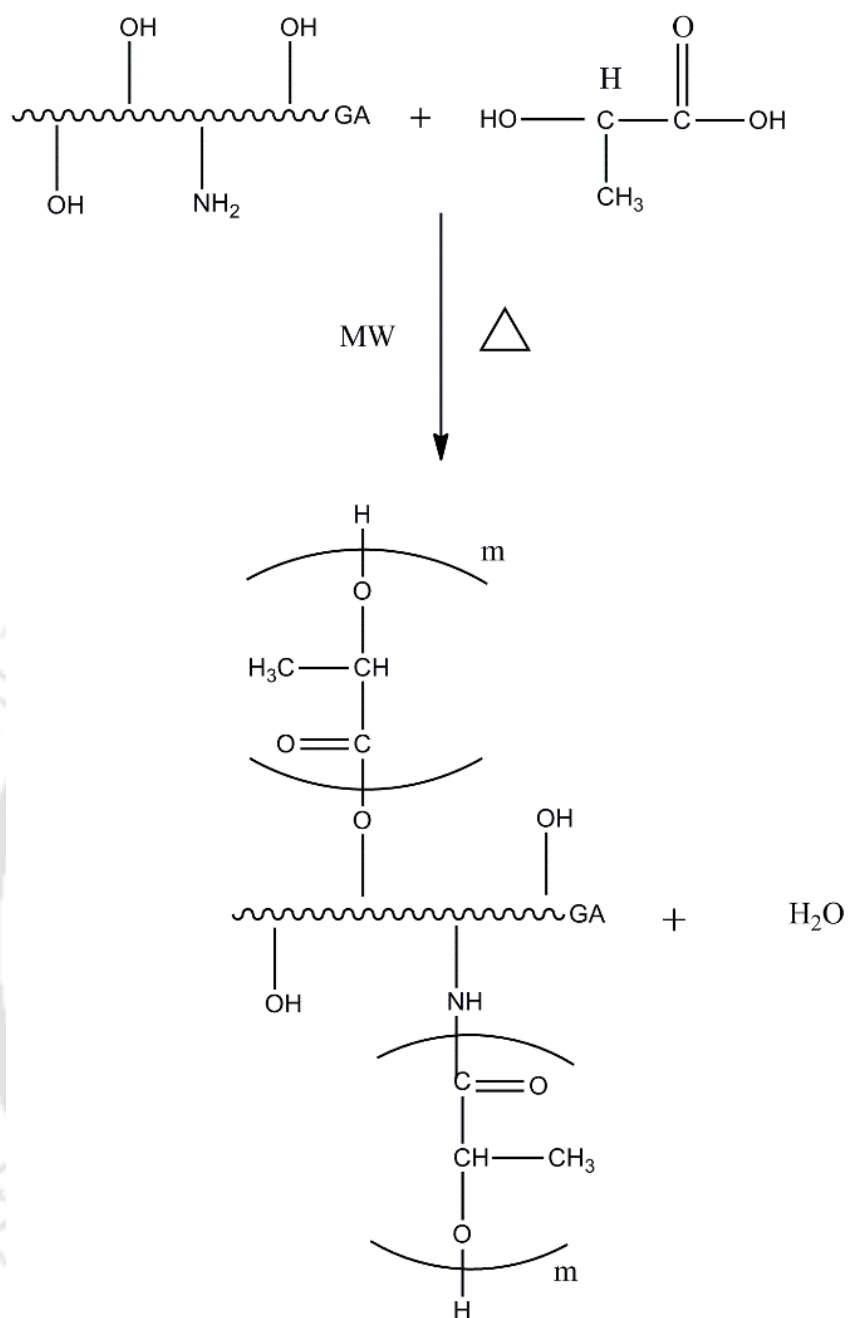


Figure 1. 12: Gum Arabica-PLA structure.

Motivation

A recent research on study of PLA biodegradation shows the use of soil bioaugmentation with a specific PLA-degrading actinomycete for accelerating PLA biodegradation in the natural soil microcosm.

From above viewpoint the biodegradation of PLA and PCL based blends can be tried and conducted in a simulated system without addition of any microbial strains, and utilizing the

strains already present in the compost. For better acceleration of the biodegradation process, polymers can be tuned using as either blends or biocomposites. Nevertheless, many studies were conducted to establish the simulated system of PLA and PCL based blends under different environments both managed and unmanaged. However, their mechanism and microbial consortium information are limited in the prior art. Uses of different types of compost might also affect the biodegradation rate of these blends and composites under simulated conditions. Therefore, these findings might give a thorough understanding of the PLA and PCL based biodegradation under different process conditions. Accordingly, a high efficient system of PLA biodegradation based on the specific microbial community, or compost as substrate may be established in the near future.

Analysis of biochemical processes might also show that biopolymer biodegradation is specifically related to the presence of the microorganisms and environmental conditions. The modern molecular biological techniques might offer a better understanding of the organization of the microbial community involved in the attack on biopolymeric materials. Breakthroughs in this field are still very difficult to achieve before the following critical problems are solved (1) The construction of a more robust system for PLA and PCL based biodegradation studies will bring new information, this may include polymer modification , microbial identification etc,. (2) Reutilization of the after biodegradation compost biomass as fertilizer for plant growth (3) Degradation of these biopolymers under uncontrolled and natural outdoor soil condition in a short span of time as it does in controlled composting condition.



Materials and Methods

This chapter provides detail information about the materials, chemicals, and equipment used to prepare and analyse biodegradation of the biocomposites and blends (Figure 2.1). This chapter also details about the modelling of experimental data for performing the kinetics study. Fabrication and development of the indigenously built composting setup following ASTM D5338-15 is discussed in details. Material changes its behavior during biodegradation, and its analysis during biodegradation is detailed with instrumentation. The chapter will also discuss about the various biodegradation kinetics study required for rate estimation of biodegradation under composting condition. Use of fillers in PLA and PCL processing for biocomposite and blend preparation is discussed in details. Detailed analysis and preparation of compost with various analytical parameters is also presented in this chapter.

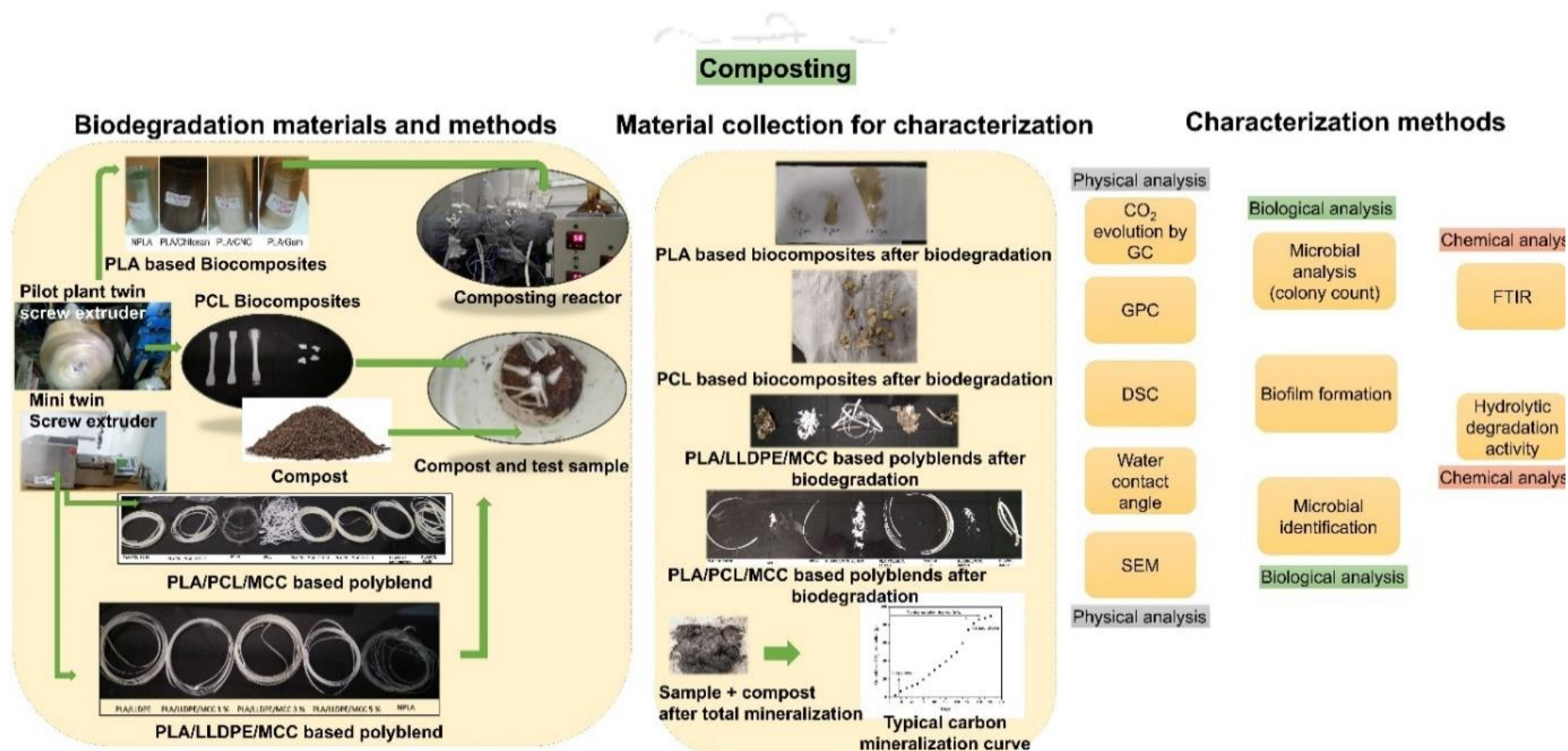


Figure 2. 1: Representing graphical abstract of the composting process with the characterization methods used

2.1 Materials

PLA granules were purchased from Nature Works® 2003D, having an number average molecular weight (M_n) of 140 kDa when analysed using polystyrene standard, glass transition temperature 57 °C and melting point 180 °C. Three-month-old mature compost was used for the process of composting. Modified chitosan and gum arabic master batches were used by authors prepared in the laboratory and its detailed characterization were mentioned elsewhere (Pal and Katiyar, 2016a; Tripathi and Katiyar, 2017), H_2SO_4 hydrolyzed CNC (Dhar et al., 2016a) were sourced as materials for preparation of the extruded films.

Poly(lactic acid) polymer 4032D (number average molecular weight (M_n) = 140 kDa) was obtained from Nature Works®. LLDPE were purchased from Haldia Petrochemicals limited, India and MCC purchased from Sigma Aldrich India. PCL polymer Capa 6500, having a M_n of 95 kDa was obtained from Perstorp UK Limited. MCC was obtained from Sigma Aldrich, India. Algal biomass extraction was used as fillers in PLA based biocomposites. Lab-made PCL having M_n of 40 kDa was sourced as material for PCL based biocomposites. These materials were extruded in the form of films, extruded dumbbells and extruded foams.

2.2 Methods

2.2.1 Development of the composting setup

The composting setup consists of reactors or composting vessels made up of Borosil glass, having capacity of 2L volume, connected to a gas chromatography (GC) system, through a 16-port gas analysing valves. H_2SO_4 was used to trap the moisture placed in cylindrical glass vessel in between the composting vessels and the gas valve is connected to the GC. Air compressor was used to give aerobic condition inside the composting vessels (CV). Rotameter purchased locally was used to control airflow. Gas Chromatography system

(Thermo Scientific, model: Trace 1310) was used for measuring the evolved CO₂ gas. Helium, hydrogen and Zero Air with 99.9% purity was used as mobile phase for GC. The setup was developed accordingly with ASTM D5338-15 (Kalita et al., 2019; Pradhan et al., 2010a).

2.2.2 Testing material preparation

The PLA based biocomposite films were fabricated in a pilot scale twin screw extruder cum-sheet casting unit (Boolani Engineering Corporation, India) (with length/diameter (L/D) ratio =48) at ~200rpm, which has five temperature zones maintained at T₁=180°C, T₂=185°C, T₃=190°C, T₄=195°C and T₅=200 °C respectively (with slit die width ~20 cm) and residence time of ~3 minutes. The extrusion process and parameters were kept fixed for all the bioplastic materials taken, Neat PLA (NPLA), PLA/Chitosan 5% (PLA/Chitosan), PLA/CNC-SO₄ (1%) (PLA/CNC) and PLA/Gum Arabic 5% (PLA/gum), respectively. The testing films were having a thickness of 0.45 mm average. NPLA, PLA/Chitosan, PLA/CNC and PLA/gum were used to code the test samples later in results and discussions.

PLA based biocomposite strips were fabricated in a twin screw extruder (Thermofisher Scientific). Processing temperature was maintained at 180 °C with ~2 minutes residence time. All the parameters were kept same for the fabrication of different polyblends of PLA, namely, neat PLA (NPLA), PLA/LLDPE, PLA/LLDPE/ 1 % MCC, PLA/LLDPE/3% MCC and PLA/LLDPE/5% MCC (Bhasney et al., 2018). The thickness of the entire sample strips were maintained as 0.45 mm on an average and their composition is presented in **table 2.1**.

Table 2. 1: Composition of the polyblend composites.

Sample designation	PLA (g)	LLDPE (g)	MCC (g)	MCC (wt % of polymer)
NPLA	10	0	0	0
LLDPE	0	10	0	0
PLA/LLDPE	8	2	0	0
PLA/LLDPE/1% MCC	7.92	1.98	0.1	1
PLA/LLDPE/3% MCC	7.76	1.94	0.3	3
PLA/LLDPE/5% MCC	7.6	1.9	0.5	5

Twin screw extruder (D76227, Haake Thermo Fisher, Germany) with L/D (length/diameter) ratio 16:1, was used for fabrication of the samples. Granules of PLA and PCL and MCC powder were used for melt blending. Strips of nPLA, PLA/PCL blend and polyblends of PLA, PCL and MCC were processed at 190 °C for the biodegradation study using varied composition of test materials, whereas nPCL was extruded at 100 °C. The screw speed was adjusted to 100 min⁻¹ and a recycling time of 2 min was maintained. Average thickness of all the testing materials was kept as ~0.815 mm. The composition of the different polyblend test samples is presented in **table 2.2**.

Table 2. 2: Composition of the different polyblend composites.

Sample Designation	PLA (g)	PCL (g)	MCC (g)
nPLA	10	0	0
nPCL	0	10	0
PLA/PCL : 80/20	8	2	0
PLA/PCL : 90/10	9	1	0
PLA/PCL/MCC : 80/20/1	7.92	1.98	0.1
PLA/PCL/MCC : 80/20/3	7.76	1.94	0.3
PLA/PCL/MCC : 90/10/1	9	0.9	0.1
PLA/PCL/MCC : 90/10/3	8.40	1.3	0.3

PLA granules were extruded using a twin-screw extruder. The PLA based biocomposite films were fabricated in a pilot scale twin screw extruder cum-sheet casting unit (Boolani Engineering Corporation, India). The extrusion process and parameters were kept fixed for all the testing neat PLA films and PLA/5% algae extract films. Extrusion foam process was used in processing of neat PLA and PLA/5% algae extract foams of 400 µm width

2.2.3. Compost preparation

For compost preparation, vegetable waste, dry leaves were collected in the month of october 2016 from different hostels of IIT Guwahati institute campus, India. Cow dung and saw dust was collected from nearby villages and dairy farms from the nearby Amingaon village, Guwahati, India. Prior to composting, the maximum particle size in the mixed waste was restricted to 1 cm in order to provide better aeration and moisture control. Preparation of the compost was made in line with our earlier investigation as mentioned elsewhere (Kalamdhad and Kazmi, 2009; Varma and Kalamdhad, 2014). Physio-chemical

characteristics of the compost are listed below: pH-7.74, Moisture content (MC)-50.15 %, volatile solids (VS)-65.43%, Oxygen uptake ratio (OUR)-4.12 mg g⁻¹ VS day⁻¹, C/N ratio- 20.15 and Total Organic Carbon (TOC)- 40.65%.

Paper mill sludge waste was collected in the month of October 2017 from Jagiroad Paper Mill (Morigaon, Assam) and dry leaves were collected from the campus of Indian Institute of Technology (IIT) Guwahati (Assam, India). Cow dung and saw dust were obtained from Amingaon village (Guwahati, Assam, India). The compost was prepared as described in an earlier investigation (Varma and Kalamdhad, 2014).

Preparation of the compost was done as discussed in our earlier investigation (Varma and Kalamdhad, 2014). Physio-chemical characteristics of the compost were modified according to the characteristics mentioned in the ASTM International D5338-15 standard protocol. The composting setup consisted of composting vessels (CV) of 3 L volume which were designed indigenously and were made of glass, and connected to a gas chromatography (GC) system through a 16-port gas analyzing valve for measuring CO₂ released from CV's (Kalita et al., 2019; Pradhan et al., 2010a).

2.2.4 Biodegradation test:

Biodegradation test was done for samples namely, NPLA, PLA/Chitosan, PLA/CNC and PLA/Gum under simulated aerobic composting conditions. The aerobic biodegradation process was in accordance to ASTM D5338-15 as shown in Fig. 1. Set of three composting vessel (CV) were used for every test material. Each CV was filled with 50g of test material (width 12 cm and thickness of 200 micron) mixed with 700 g of compost. The contents were thoroughly mixed and incubated at 58 °C (±5 °C) in the dark. A set of CV with only compost material was kept as blank, and another set of CV with cellulose (Whatmann No. 1 filter papers) were kept as positive control and incubated at 58 °C (±5 °C) in the dark as per ASTM test protocol. All CV's were supplied with humidified compressed air and all

exiting gases were continuously purged through the GC (subtracting 0.05% of CO₂ from the GC, which is the amount of CO₂ present in normal air, since, compressed air was used in the CV's) within a time interval for assessing the CO₂ accumulated inside the CV. At an interval of 7 days, distilled water is added to replenish lost moisture (Pradhan et al., 2010b). Temperature, pH and aeration were closely monitored and controlled during the experiment.

Neat PLA, PLA/Chitosan 5%, PLA/CNC-SO₄ (1%) and PLA/Gum Arabic 5% under simulated aerobic composting conditions were used as test samples for biodegradability test. The biodegradation process were in accordance to ASTM D5338-15 with little protocol changes. Three CV were used for each test sample, and each CV was filled with 700 g of compost and 50 g of test material films. The contents were analysed for moisture and pH tests, and incubated at 58 °C (±5 °C). A set of CV with only compost was kept as blank, and other another set of CV with cellulose (Whatmann No. 1 filter papers) were kept as positive control and incubated at 58 °C (±5 °C) as per ASTM test protocol. At an interval of 7 days, distilled water was mixed for maintaining the moisture in the CV (Pradhan et al., 2010b). Samples were analysed only after the 40th day of the biodegradation process. This number of days is actually needed by cellulose to attain desired percentage biodegradation in accordance with the standard (ASTM Standard D 5338, 2003) to say whether the test can be further conducted or not. Various parameters used for monitoring the chemical properties of the inoculums in both the tests were determined according to the standards mentioned in ASTM D5338-15 respectively for the test system. Samples were taken out from the CV's after 40th day and the samples were analyzed using different characterization tools within a time interval of 10 days till the 80th day instead of measuring the CO₂ evolution as mentioned in the ASTM D5338 standard. This little modification of taking out

the samples within a time interval would provide insights about the biodegradation effect on the test samples.

In this test, all the prepared blends were subjected to biodegradation under simulated aerobic composting conditions in accordance to ASTM D5338-15 standard. 50g of test material strips was buried under 500g of compost in the CV's and the experiments were done in triplicate. Thorough mixing of the compost and sample was done, followed by incubation for a time period of 140 days at a temperature of $58\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$ and relative humidity (RH) of 60%. Routine observations were made to ensure a constant oxygenated environment of more than 15% to maintain a proper aerobic condition during the composting process at a constant temperature. At an interval of every 7 days, any moisture loss was replenished with distilled water and the compost mixes were returned to the CV (Pradhan et al., 2010b). Chemical properties of the test were monitored according to ASTM D5338-15 (ASTM Standard D 5338, 2003).

Cumulative evolution of CO_2 and net cumulative CO_2 production was determined according to ASTM D5338-15 standard:

$$\% \text{Biodegradation} = \frac{(\text{CO}_2)T - (\text{CO}_2)B}{\text{ThCO}_2} \times 100 \quad (\text{Eq. 1})$$

Where, $(\text{CO}_2)T$ was the cumulative amount of CO_2 evolved in each composting flask containing the polymer sample, unit- g/flask;

$(\text{CO}_2)B$ was the mean cumulative amount of CO_2 evolved in blank flask, unit- g/flask;

ThCO_2 was the theoretical amount of CO_2 evolved from the test materials assuming that all the carbon of the test material was transformed into CO_2 .

The samples (**Table 2.1 and 2.2**) were composted under simulated aerobic conditions for analyzing biodegradation. The study was done according to the ASTM International D5338-15 standard. Triplicate sets of CVs were taken and filled with 500 g of compost and 50 g of test material in each vessel. Thorough mixing of the compost and sample was done

and incubation was done at 58 ± 5 °C temperature and 60% relative humidity. Routine observations were made to ensure constant oxygen supply of more than 6% to maintain proper aerobic conditions during simulated composting at a constant temperature. Any moisture loss was replenished with distilled water (Pradhan et al., 2010b). All the parameters of the test method were determined accordingly (ASTM International, 2015a). Cumulative evolution of CO₂ and net cumulative CO₂ production was determined according to the ASTM D5338-15 standard protocol as follows:

$$\% \text{Biodegradation} = \frac{(CO_2)T - (CO_2)B}{ThCO_2} \times 100 \quad (1)$$

Where, (CO₂)T, (CO₂)B and ThCO₂ were carbon dioxide produced from sample plus compost, carbon dioxide produced from blank and amount of carbon dioxide produced by 12g of carbon respectively (Pradhan et al., 2010a).

2.2.5 Biodegradation mechanism and kinetics

Biodegradation of polymers is a complex process, comprising of a series of intricate steps. The biodegradation mechanism is followed by three kinetically different forms of solid carbon namely: rapidly, moderately and slowly hydrolysable, at different rates, until finally a common water soluble intermediate is reached, which mineralizes readily to release carbon-dioxide, thus achieving biodegradation. The rate equations, depicting the change of each component involved in the entire mechanism, are as follows:

$$\frac{dC_r}{dt} = -k_{hr} \cdot C_r \quad (2)$$

$$\frac{dC_m}{dt} = -k_{hm} \cdot C_m \quad (3)$$

$$\frac{dC_s}{dt} = -k_{hs} \cdot C_s \quad (4)$$

$$\frac{dC_{aq}}{dt} = (k_{hr} \cdot C_r + k_{hm} \cdot C_m + k_{hs} \cdot C_s) - (k_{aq} \cdot C_{aq}) \quad (5)$$

$$\frac{dC_T}{dt} = k_{aq} \cdot C_{aq} \quad (6)$$

Here, C_r , C_m & C_s are the percentages of rapidly, moderately and slowly hydrolysable solid carbon in the initial polymer chain, respectively. C_{aq} is the percentage of intermediate water soluble carbon, and C_T depicts the percentage of mineralization, that is, cumulative C-CO₂ production of the polymer sample. Now, k_{hr} , k_{hm} & k_{hs} are the hydrolysis rate constants of rapidly, moderately and slowly hydrolysable carbon fractions, respectively; k_{aq} is the ultimate mineralization rate constant, describing the formation of carbon dioxide from the water soluble intermediate.

Analytical solutions for biodegradation kinetics

The above stated rate equations are differential equations (eq. 2-6) of the first order, and hence their solutions can be derived using an integrating factor. While solving, a lag phase, c , is taken for each substance undergoing biodegradation, so as to account for the initial days of the process, where first-order biodegradation kinetics is not followed. The analytical solutions were modified in order to satisfy our experimental observations during the lag phase which are more suitable to the solutions given in prior art (Leejarkpai et al., 2011; Stloukal et al., 2015), where they have taken lag phase to be a period of negligible carbon mineralization.

$$C_{r_t} = C_{ro} \cdot e^{-k_{hr}(t-c)} \quad ; \text{for } t > c, \quad (7)$$

$$C_{m_t} = C_{mo} \cdot e^{-k_{hm}(t-c)} \quad ; \text{for } t > c, \quad (8)$$

$$C_{s_t} = C_{so} \cdot e^{-k_{hs}(t-c)} \quad ; \text{for } t > c, \quad (9)$$

$$\begin{aligned} C_{aq_t} = & C_{aqo} \cdot e^{-k_{aq}(t-c)} + C_{ro} \cdot k_{hr} \cdot \frac{(e^{-k_{hr}(t-c)} - e^{-k_{aq}(t-c)})}{k_{aq} - k_{hr}} \\ & + C_{mo} \cdot k_{hm} \cdot \frac{(e^{-k_{hm}(t-c)} - e^{-k_{aq}(t-c)})}{k_{aq} - k_{hm}} + C_{so} \cdot k_{hs} \cdot \frac{(e^{-k_{hs}(t-c)} - e^{-k_{aq}(t-c)})}{k_{aq} - k_{hs}} \end{aligned} \quad (10)$$

for $t > c$,

$$C_{T_t} = \left(\begin{array}{l} C_{aq0} \cdot (1 - e^{-k_{aq} \cdot (t-c)}) \\ + \left[C_{ro} \cdot \left(1 - \frac{k_{aq}}{k_{aq}-k_{hr}} \cdot e^{-k_{hr} \cdot (t-c)} + \frac{k_{hr}}{k_{aq}-k_{hr}} \cdot e^{-k_{aq} \cdot (t-c)} \right) \right] \\ + [C_{mo} \cdot (1 - \frac{k_{aq}}{k_{aq}-k_{hm}} \cdot e^{-k_{hm} \cdot (t-c)} + \frac{k_{hm}}{k_{aq}-k_{hm}} \cdot e^{-k_{aq} \cdot (t-c)})] \\ + [C_{so} \cdot (1 - \frac{k_{aq}}{k_{aq}-k_{hs}} \cdot e^{-k_{hs} \cdot (t-c)} + \frac{k_{hs}}{k_{aq}-k_{hs}} \cdot e^{-k_{aq} \cdot (t-c)})] \end{array} \right) \quad (11)$$

for $t > c$, or $(C_c/c) \cdot t$ for $t \leq c$.

Here, C_{ro} , C_{mo} & C_{so} refer to the initial percentages of rapidly, moderately and slowly hydrolysable carbon fractions, respectively, while C_{r_t} , C_{m_t} & C_{s_t} , respectively, are the percentages of the same fractions at time t . C_{aq0} is the initially present percentage of the intermediate water soluble carbon, and C_{aq_t} denotes the same at time t . C_{T_t} , gives the cumulative percentage of C-CO₂ production at time t . C_c denotes the C-CO₂ %. The slow hydrolysis rate constant is assumed to be zero. All the kinetic parameters are constrained to be positive.

Mathematically, the constraints are,

$$C_{C0} = C_{ro} + C_{mo} + C_{so} + C_{aq0}$$

$$C_{FC} = C_{r,final} + C_{m,final} + C_{s,final} + C_{aq,final}$$

$$0 \leq k_{hm} < k_{hr} < k_{aq}, k_{hs}=0$$

$$C_{T_t} = (C_c/c) \cdot t, \text{ for } t \leq c$$

2.2.6 Molecular weight analysis

Molecular weight analysis was conducted using a Shimadzu, Japan, gel permeation chromatography (GPC). The number average molecular weight (M_n) was determined by using a GPC instrument with refractive index detector (RID-10A) standardized using polystyrene for calibration. Before analysis the solution was filtered with 0.25 μ m syringe filters. The process of molecular weight analysis was followed accordingly, (Rudeekit et

al., 2008). Molecular weight analysis of the samples were done before and after burying the samples under composting condition.

The evolution of molecular weight during abiotic hydrolysis was modelled. The analytical solution of the model is given by Eq. 1.

$$M_{n,t} = M_{n,0} \cdot e^{-ut} \dots\dots\dots(12)$$

Where, $M_{n,t}$ and $M_{n,0}$ (Dalton) are final and initial number average MW at time t , and $t = 0$, respectively. Parameter u (day^{-1}) is the rate constant.

2.2.7 Microbial analysis

2.2.7.1 Microbial counts

Microbial counts in compost soil was determined by growth colonies on nutrient agar (NA) and plate count agar (PCA) plates. The pH medium of the media was adjusted to 6.5 prior to autoclaving. 1 g compost, were dissolved in 9 ml sterile water using a vortex mixer. Each of the sample were diluted to 10^{-3} by adding 1 ml of the soil suspensions to 9 ml sterile water. Prepared plate samples were incubated at 55°C for 48 h. After incubation, each plate (NA and PCA), was measured by a colony counter. Then, gram staining was done to identify either it is gram negative or positive, observed using an Optical Polarisation Microscope (Nikon H600L, Japan).

2.2.7.2 16sRNA analysis

Microbial counts of compost from the CV's was determined by counting the growth colonies on nutrient agar (NA) plates. Three samples of both the media plates were prepared for measuring microbial count. Compost samples of 1 g, were dissolved in 9 ml sterile water using a vortex mixer. They were then diluted to 10^{-6} by adding 1 ml of the compost suspensions to 9 ml sterile water. From the diluted solution samples, 100 μl were spread onto NA plates, and were incubated at 55°C for 72 hours. Colonies were isolated to purity. Genomic DNA was isolated using the InstaGeneTM Matrix DNA isolation kit. In the first PCR round, 27F and 1492R (AGAGTTTGATCATGGCTCAG and

TACGGCTACCTTGTTACGACTT, respectively) were used to amplify nearly the full length 16S rRNA gene. Target gene fragment was amplified using TeledynePrime Thermal Cycler. DNA fragments are amplified using 1 µl of template DNA in 20 µl of total PCR reaction mixture using 27F/1492Rprimers (50 pmol) and 35 amplification cycles with following program:

1. Denaturation: 94°C for 50 sec,
2. Annealing: 52°C for 55 sec,
3. Chain Elongation: 72°C for 65 sec.

2.2.7.3 Analysis of microbial biofilm formation

For microbial analysis, three different kinds of media were used, namely eosin methylene blue agar (EMBA), potato dextrose agar (PDA) and plate count agar (PCA). 1 g of compost from the CVs were taken on the 3rd day and 100th day of the biodegradation experiment, and mixed with sterile distilled water and vortexed. The mixture was then diluted to 10⁻³ strength and the sample solutions were spread on the media plates (EMBA, PDA and PCA) and incubated. PDA and PCA was incubated at 55 °C for 72 hours, whereas, EMBA was incubated at 37 °C. After incubation, each plate was analyzed for colony formation. Colony formation unit was calculated and expressed in cfu/ml.

For biofilm analysis, a new assay was performed. All the test samples before and after biodegradation were dipped in chloroform and their absorbance was measured at 600 nm. Colonies were again incubated at 55 °C for 48 hours and purified. Microbial growth was measured at the end of 48 hours at 600 nm using a UV-visible spectrophotometer (Perkin-Elmer, Germany). Test samples after biodegradation were supposed to show similar absorbance as the isolated microbes from the CVs, since microbes get attached to the polymer surface during biodegradation.

2.2.8 Differential Scanning Calorimeter (DSC)

First heating and second heating cycles were analysed to understand the impact of biodegradation on the test samples. Indium standards were used for precalibration of the instrument. (~5–6 mg) pieces of film samples were used for analysis. The analysis was programmed for two thermal scans i.e. the first cycle was from 30 to 200 °C at 10 °C min⁻¹ followed by two minutes isothermal condition at 200 °C and then cooling from 200 to 30 °C with a cooling rate of 10 °C min⁻¹. The second cycle was same as first cycle. Crystallinity of all the test samples before and after biodegradation for 80 days was evaluated from the DSC second heating cycles. Melting enthalpy of 93.1 J/g for 100% crystalline PLA was taken to determine percentage crystallinity (Kontou and Anthoulis, 2007). The equation used for percentage crystallinity is given below,

$$\chi(\%) = \left[\frac{\Delta H_m - \Delta H_{cc}}{\Delta H_0} \right] \times 100 \dots \dots \dots (13)$$

Where, ΔH_m is the melt enthalpy and ΔH_{cc} is the cold crystallization enthalpy, ΔH_0 is enthalpy of melting for a 100% crystalline PLA samples.

The DSC instrument (Netzsch, Germany) were pre-calibrated using indium standards. ~5-6 mg of the test samples were used for this analysis. Second heating cycle was programmed to measure the crystallinity percentage as mentioned elsewhere (Kalita et al., 2019). Melting enthalpy of 100% crystalline PLA was taken as 93.1 J/g for the determination of percentage crystallinity as follows:

$$X_c(\%) = \left[\frac{\Delta H_m - \Delta H_{cc}}{\Delta H_0} \right] \times 100 \dots \dots \dots (14)$$

Where, ΔH_m was the melt enthalpy, ΔH_{cc} was the cold crystallization enthalpy, and ΔH_0 was the enthalpy of melting of 100% crystalline PLA samples (Pantani and Sorrentino, 2013).

The samples were analyzed by running two thermal scans in a differential scanning calorimeter (Netzsch, Germany) to study about the crystallinity of the test samples both

before and after biodegradation during the second heating cycle (Kalita et al., 2019). For nPCL, the the scan was programmed from -80 °C to 120 °C. The following equation was to calculate the percentage crystallinity of the test samples:

$$X(\%) = \left[\frac{\Delta H_m - \Delta H_{cc}}{\Delta H_0 \left(1 - \frac{\% \text{ filler}}{100}\right)} \right] \times 100 \quad (15)$$

Where, ΔH_m was the melting enthalpy, ΔH_{cc} was the cold crystallization enthalpy, and $\Delta H_0 = 93.5 \text{ J/g}$ was the enthalpy of melting of 100% crystalline PLA samples (Pantani and Sorrentino, 2013).

2.2.9 POM analysis

POM was used to analyze the spherulite density and spherulite size before and after biodegradation. It was performed using Nikon h600L (Nikon, Japan) mounted on a Linkam TST350 hot stage (Linkam Scientific Instruments). At first, the samples were mounted on a glass slide cover and heated to 200 °C at the rate of 50 °C/minute. In the next cycle, the same sample was cooled to 100 °C at the rate of 50 °C/min and kept for 30 min in isothermal conditions. At this isothermal juncture, optical micrograms having the data of spherulite growth and size were obtained using the transmission mode (Pal and Katiyar, 2016a). The analysis was done at 120 °C for samples before biodegradation and at 100 °C after biodegradation, since the recrystallization temperature of the samples after biodegradation decreased to almost or little less than 100 °C.

POM was used to calculate the spherulite density and spherulite size of the test samples both before and after biodegradation. Analysis was done at 120 °C before biodegradation and at 100 °C after biodegradation since the recrystallization temperature of the samples decreased to almost or little less than 100 °C after biodegradation. POM was done using Nikon H600L microscope (Nikon, Japan) mounted on a Linkam TST350 hot stage (Linkam Scientific Instruments). At first, the samples were mounted on a glass slide cover and heated to 200 °C at the rate of 50 °C/min. The samples were then cooled to 100 °C at 50 °C/min

rate. Next, the samples were kept for 30 min in isothermal conditions and their spherulite growth and size were measured (Pal and Katiyar, 2016a).

2.2.10 FTIR and UV-Visible spectroscopy analysis

Fourier transform infrared (FTIR) spectroscopy (Perkin Elmer, Frontier) in attenuated total reflection (ATR) mode, was performed on the degraded test samples and measured in the range of 4000-500 cm^{-1} with resolution of 4 cm^{-1} for 128 scans. The transparency of the films (dimensions: 20mm x 50mm) was also measured using UV-Vis spectrophotometer (Perkin Elmer) scanned in the range of 600 – 200 cm^{-1} . Both IR and UV-Visible spectroscopy was performed on test samples before and after biodegradation.

2.2.11 Contact angle measurements

The test samples before and after biodegradation were placed on glass slides and 4 μL drop of distilled water and diiodo-methane was deposited on the specimens at two different temperatures 27 and 58 ± 2 °C. The drop rate was 0.16 mL/min and thus the water contact angle was measured using a Kruss, DSA-25 Expert model. The procedure was repeated in triplicate and the mean deviation was calculated. The films had been rinsed with distilled water, dried cut into 20-30 mm pieces for measurement (Stloukal et al., 2015)

The water contact angle of the test samples was measured using a Kruss, DSA-25 Expert model, Germany). Analysis was done accordingly (Kalita et al., 2019) with water as solvent.

The test samples were rinsed with distilled water before and after biodegradation

2.2.12 Morphological analysis

Field emission scanning electron microscopy (FESEM) (ZEISS, USA) was used to study the morphology of the test materials. This study was used to understand the action of biodegradation on the blends. Similarly, transmission electron microscopic (TEM) (JEOL JEM 2100) images were captured at an accelerating voltage of 200 kV, to study the samples before and after biodegradation.

2.2.13 Nuclear Magnetic Resonance (NMR)

NMR was carried out in order to have a clear idea about its formations after biodegradation under composting condition. The ^1H NMR spectra of the compounds were recorded using Bruker 600 MHz NMR spectrometer using trimethylsilane (TMS) as an internal standard. The samples were prepared by dissolving 10 mg specimens in 1 mL deuterated chloroform (CDCl_3). The prepared solutions were then filtered using syringe filter (pore size $0.25\ \mu\text{m}$) and were transferred into the NMR tubes before analysis. A spectral range of 0–10 ppm were considered at room temperature for analysis of ^1H NMR.

2.3 Biodegradation under soil condition

2.3.1 Experiment procedure

For conducting the biodegradation experiments under soil condition samples were placed 20 cm deep inside soil (**Figure 2.2**). Every month samples are dugged out from the soil for characterization. As shown in **figure 2.2**, samples were characterized for Gel permeation chromatography (GPC), water contact angle and FTIR, observation of spherulite formation at room temperature.



Figure 2. 2: Graphical abstract representing the biodegradation method and characterization under natural soil condition

2.4 Reutilization of the used compost biomass as fertilizer for plant growth

All the compost after biodegradation study was used as fertilizer for plant growth inside a green house. Green house (Bhanu Biotech, India) built equipped with temperature control, humidity control, light control, and moisture control. Proper drainage system for water circulation within the growth area is available. The green house is fully automated with the required parameters and is a hydroponic system having polycarbonate walls for protecting it from UV rays. *Solanum tuberosum* common name potato was grown for the growth study. 20 °C and 70 % RH was maintained with 12 hours light cycle. For analysis six different samples were prepared namely PLA/compost/potato; PLA/soil/potato; after biodegradation compost/soil/potato; compost/soil/potato/PLA/LLDPE. Compost/potato and soil/potato are used as control.

2.4.1 Growth characterization

Electrical conductivity of soil, pH, growth index etc was measured on 7-day basis. Electrical conductivity and pH was measured using (Water Analyser 371, Systronics, India). For conductivity 40 ml of media was mixed with 150 ml of distilled water and then the conductivity of the solution was measured accordingly (Kapanen et al., 2002). For pH measurement 1g of the soil sample from each test sample type and then added in 10 ml of water. Test samples were measured on every 7 day time period.

For growth analysis stem thickness and stem length was measured for 30 days in order to analyze the *RshG* %

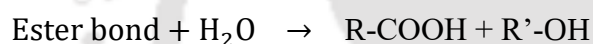
$$\text{Relative shoot growth (RshG)\%} = \frac{\text{Mean shoot length in test samples}}{\text{Mean shoot length in control}} \times 100 \dots\dots\dots 16$$

2.5 Abiotic degradation

For abiotic degradation, 1M NaOH buffer solution was used which was standardized to pH 7.8. Extruded films and foams were cut into 5×5 cm pieces and dipped in 50 ml of the buffer solution at 58 °C for 1 month. Parameters like pH and density were measured after every 7 days. Film samples were tested after every 7 days for 1 month.

2.5.1. Mathematical model Description

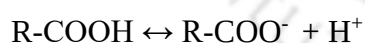
A general model on hydrolytic degradation and erosion of biodegradable polymers proposed by Jingzhe Pan and coworkers [18,19] is utilized here to predict the polymer degradation. On hydrolytic reaction water molecules react with the ester group and cleaves it into hydroxyl and carboxylic group thus the rate of polymer chain scission is governed by the equation



$$\frac{dR_s}{dt} = k_1 C_e + k'_2 C_e C_{H^+} \dots\dots\dots (17)$$

In which R_s , C_e , C_{H^+} represent the mole concentration of chain scission, concentration of ester bonds, concentration of H^+

The acid dissociation of carboxylic is expressed as



Since the reaction is reversible the K_a equilibrium constant for dissociation of carboxylic acid is given by

$$K_a = \frac{C_{H^+} C_{\text{COO}^-}}{C_{\text{COOH}}} \dots\dots\dots (18)$$

$$\text{Since for charge balance} \quad C_{H^+} = C_{\text{COO}^-} \quad \dots\dots\dots (19)$$

thus substituting 18 & 19 into 17 give

$$\frac{dR_s}{dt} = k_1 C_e + k_2 C_e [\text{COOH}]^{1/2} \dots\dots\dots (20)$$

$$k_2 = k_2' [k_a]^{1/2}$$

Where, the empirical relation between production of short chain represented by R_{ol} and chain scission is given by Han(et 2010) is used where C_{e0} is the initial ester bond concentration, α and β are empirical parameters

$$\frac{R_{ol}}{C_{e0}} = \alpha \left(\frac{R_s}{C_{e0}} \right)^\beta \dots\dots\dots (21)$$

Since each chain scission produces COOH group thus

$$C_{\text{COOH}} = C_{\text{chain0}} + R_s \dots\dots\dots (22)$$

Where C_{chain0} is the initial number of polymer chain per unit volume .

Similarly the relation between short chain and ester bonds is expressed by successively substituting eq(4) into it we get

$$C_e = C_{e0} - R_{ol} = C_{e0} \left[1 - \alpha \left(\frac{R_s}{C_{e0}} \right)^\beta \right] \dots\dots\dots (23)$$

Thus substituting eq (7) into eq 4 we get

$$\frac{dR_s}{dt} = \left(1 - \alpha \bar{R}_s^\beta \right) \left[k_1 + \bar{k}_2 \left(N_{dp0}^{-1} + \bar{R}_s \right)^{\frac{1}{2}} \right] \dots\dots\dots (24)$$

Where $\bar{R}_s$, N_{dp0} , $\bar{k}_2$, is R_s/C_{e0} , $C_{e0}/\text{Chain0}$ and $C_{e0}^{0.5*k_2}$ respectively.

And $\bar{M}_n$ was calculated as following.

$$\bar{M}_n = \frac{M_n}{M_{n0}} = \frac{1 - \alpha \bar{R}_s^\beta}{1 + N_{dp0} \left(\bar{R}_s - \left(\frac{\alpha}{m} \right) \bar{R}_s^\beta \right)}$$

The first order differential equation eq(4) was solved using numerical approach thus, comparative study of model vs experimental data was done .

For solving the hydrolytic degradation model Microsoft excel 2016, Origin 9 and Turbo C++ were used for data analysis.

2.5.2 Mass loss test and visual observation

The abiotic degradation was carried out at 58 ± 2 °C. In order to determine the mass loss of the samples, following relationship was used to study the residual weight fraction as a function of degradation time.

$$\Phi = \frac{W_t}{W_0} \times 100 \dots \dots \dots (25)$$

Here, Φ = residual weight fraction, W_t and W_0 were the dry weight of the samples after certain period.

Visual observation of all the test samples was done using a 16 Mega pixel camera.

2.5.3. Density and pH measurement

Density of the degraded samples were measured before and after abiotic degradation. pH of the abiotic solution was recorded in order to understand the change in pH of the solution, when it comes in contact with polymer. Density change and pH variation during the abiotic degradation process were plotted with respect to degradation time.



Development of Composting Setup and Protocol

This chapter deals with development and design of the indigenously built composting setup. This setup was built following the protocol of ASTM International D5338. Although no proper definition of the composting reactor is provided in the ASTM International D5338 protocol, here the composting reactor was designed in order to have proper aeration and which can be connected to either titration flasks or multiple port Gas Chromatography system.

Abstract

The biodegradation test system was built according to ASTM International D5338-15. The system has been developed for studying biodegradation of natural as well as conventional polymeric materials under controlled composting condition. The system is indigenously built and is connected with a Gas Chromatography system through 16-port gas valve. The system has been automated for data collection. Temperature control and moisture control were optimized according to the type of biopolymer used in the system. The system was able to maintain optimum pH for the compost. 100 percent cellulose based whatmann filter paper was used for validity of the test system. C-CO₂ conversion took almost 50 days while maintaining the optimal condition parameters.

3.1 Introduction

Testing Methods:

There is a concern regarding the testing conditions for monitoring the biodegradation process. Tests can be further subdivided into laboratory tests, simulation tests and field test.

For simulation test includes:

- Laboratory Reactors: Water, Soil, Compost
- Very complex environment
- Controlled and defined environment

As an alternative for field test, various simulation tests in the laboratory have been used for measuring the biodegradability of plastics. Here parameters are controlled in order to have a complete and reliable understanding as well as observation regarding the biodegradation process and its mechanism using different substrates. This simulation tests or laboratory tests are reproducible. Hence, its utility for scientific data and understanding is very vital for biodegradation of plastics research. Various organizations like ASTM and ISO etc. have framed their conditions as standards, which might be followed for proper understanding of the biodegradation process. Some of the primary ASTM test methods related to compostability of plastics is listed below.

ASTM D5338: This is the standard protocol for testing how samples will biodegrade in a composting facility. Though the standard does not confirm the experiment as successful or unsuccessful, it provides details about performing the experiment [ASTM standards]. Quantification of biodegradability is of utmost importance to say if a product is biodegradable or not. Quantitative methods help in establishing disposal policies for various packaging plastic materials. For addressing issues related to biodegradation, various test methods have been developed in recent times based on the product type and

application. Biodegradation occurs in two different steps, namely aerobic biodegradation and anaerobic biodegradation. The mass balance equation for biodegradation that occurs in aerobic environment is given in Equation 1, which is characterized by total carbon conversion.

$$C_T = CO_2 + C_R + C_B \dots\dots\dots(1)$$

Where, C_T is total carbon content of the polymeric material, C_R is the residue left by the polymeric material or any other by-product formed during the degradation; whereas, CO_2 is the gaseous product that is measured out during the degradation process, and C_B is the biomass produced by the microbes through growth and reproduction during the degradation process.

ASTM D6400: A set of three tests that includes ASTM D5338, and must meet the standard of pass/fail criterion for judging the compostability of the plastics either in industrial or pilot plant based composting facility. This criterion signifies that the product that has passed through this process can be termed/claimed to be compostable. Scope of this standard state that it covers plastics and various products made from plastics that are designed to be composted in both municipal and industrial composting facilities. Moreover, the properties of the composted product should not diminish the compost utility [ASTM standards].

Analysis of biochemical processes might also show that PLA biodegradation is specifically related to the presence of microorganisms and environmental conditions. The modern molecular biological techniques might offer a better understanding of the organization of the microbial community involved in the attack on PLA materials. Breakthroughs in this field are still very difficult to achieve before the following critical problems are solved that includes (1) Analysis of the different enzymatic families involved in PLA biodegradation. (2) Exploration the efficient degradation methods based on PLA-biodegrading microorganisms, which are crucial for accelerating its degradation process. (3) The

simulated system based on aerobic biodegradation of PLA is currently limited due to limitations in information on process parameters. The construction of a more robust system for PLA biodegradation studies will bring new information.

Even though techniques like visual observations, weight loss measurements, changes in mechanical properties, and changes in molecular weight, can provide insights into the degradation process of a polymer, they do not necessarily demonstrate biodegradation [Shah et al., 2008]. Therefore, respirometric methods, in which the consumption of oxygen and/or the evolution of carbon dioxide (CO₂) is measured, have become the preferred technique for such assessment. Gas chromatography is also an utmost tool for measuring the evolved CO₂ if appropriate measuring and process parameters are determined during biodegradation. It is however not very clear how to develop a proper PLA degrading setup where every process parameter can be calculated during biodegradation for a more precise biodegradation study.

3.2. Results and discussion

Several respirometric biodegradation test systems were built by different researchers following standards like ASTM D 5338 and ISO 14855 (Dagnon et al., 2014; Dřimal et al., 2007; Jayasekara et al., 2001; Way et al., 2010).

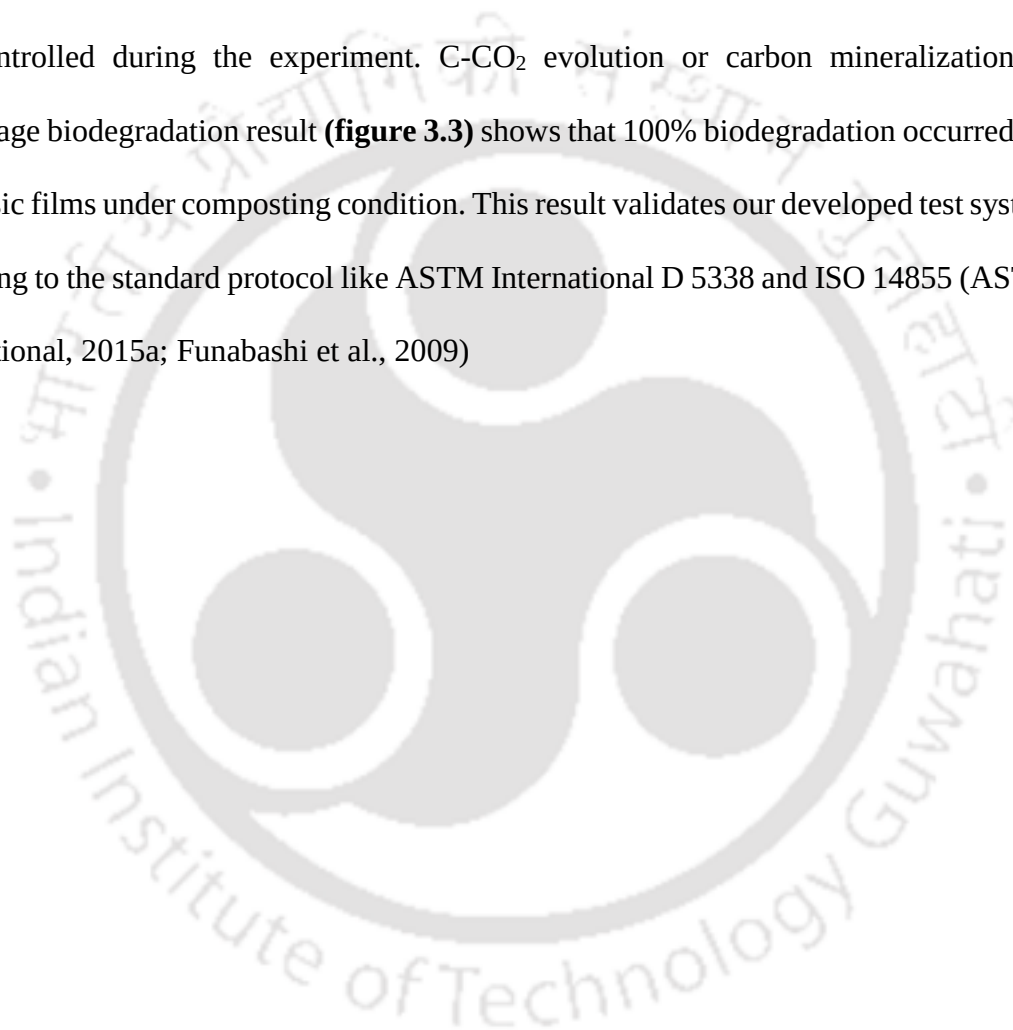
Our in-built test method determines the degree and rate of aerobic biodegradation of plastic materials on exposure to a controlled-composting environment under laboratory conditions, at thermophilic temperatures. This test method is designed to yield reproducible and repeatable test results under controlled conditions those resembling composting conditions, where thermophilic temperatures are achieved. The test substances are exposed to an inoculum that is derived from compost collected from municipal solid waste. The aerobic composting takes place in an environment where temperature, aeration, and humidity are closely monitored and controlled.

This test method is designed to yield a percentage of conversion of carbon in the sample to carbon dioxide. The rate of biodegradation is monitored as well. This test method is designed to be applicable to all plastic materials, which are intended to be composted in facilities that achieve thermophilic temperatures. Biodegradation of a plastic within a composting unit is an important phenomenon because it may affect the decomposition of other materials enclosed by the plastic and the resulting quality and appearance of the composted material. Hence, biodegradation of plastics will also allow the safe disposal of these plastics through large, professionally managed composting plants and well-run residential units, where thermophilic temperatures are achieved. This procedure has been developed to permit the determination of the rate and degree of aerobic biodegradability of plastic products when placed in a controlled composting process.

There are wide variations in the construction and operation of composting facilities and because regulatory requirements for composting systems vary, this procedure is not intended to simulate the environment of any particular composting system. However, it is expected to resemble the environment of a composting process operated under optimum conditions where thermophilic temperatures are achieved. More specifically, the procedure is intended to create a standard laboratory environment that will permit a rapid and reproducible determination of the aerobic biodegradability under controlled composting conditions.

The aerobic biodegradation process was in accordance to ASTM D5338-15 as shown in **figure 3.1**. Set of three composting vessel (CV) were used for every test material. Each CV (**Figure 3.2**) was filled with 50g of test material (width 12 cm and thickness of 200 micron) mixed with 700 g of compost. The contents were thoroughly mixed and incubated at 58 °C (± 5 °C) in the dark. A set of CV with only compost material was kept as blank, and another set of CV with cellulose (Whatmann No. 1 filter papers) were kept and incubated at 58 °C

(± 5 °C) in the dark as per ASTM test protocol. All CV's were supplied with humidified compressed air and all exiting gases were continuously purged through the GC (subtracting 0.05% of CO₂ from the GC, which is the amount of CO₂ present in normal air, since, compressed air was used in the CV's) within a time interval for assessing the CO₂ accumulated inside the CV. At an interval of 7 days, distilled water is added to replenish lost moisture (Pradhan et al., 2010b). Temperature, pH and aeration were closely monitored and controlled during the experiment. C-CO₂ evolution or carbon mineralization or percentage biodegradation result (**figure 3.3**) shows that 100% biodegradation occurred for cellulosic films under composting condition. This result validates our developed test system according to the standard protocol like ASTM International D 5338 and ISO 14855 (ASTM International, 2015a; Funabashi et al., 2009)



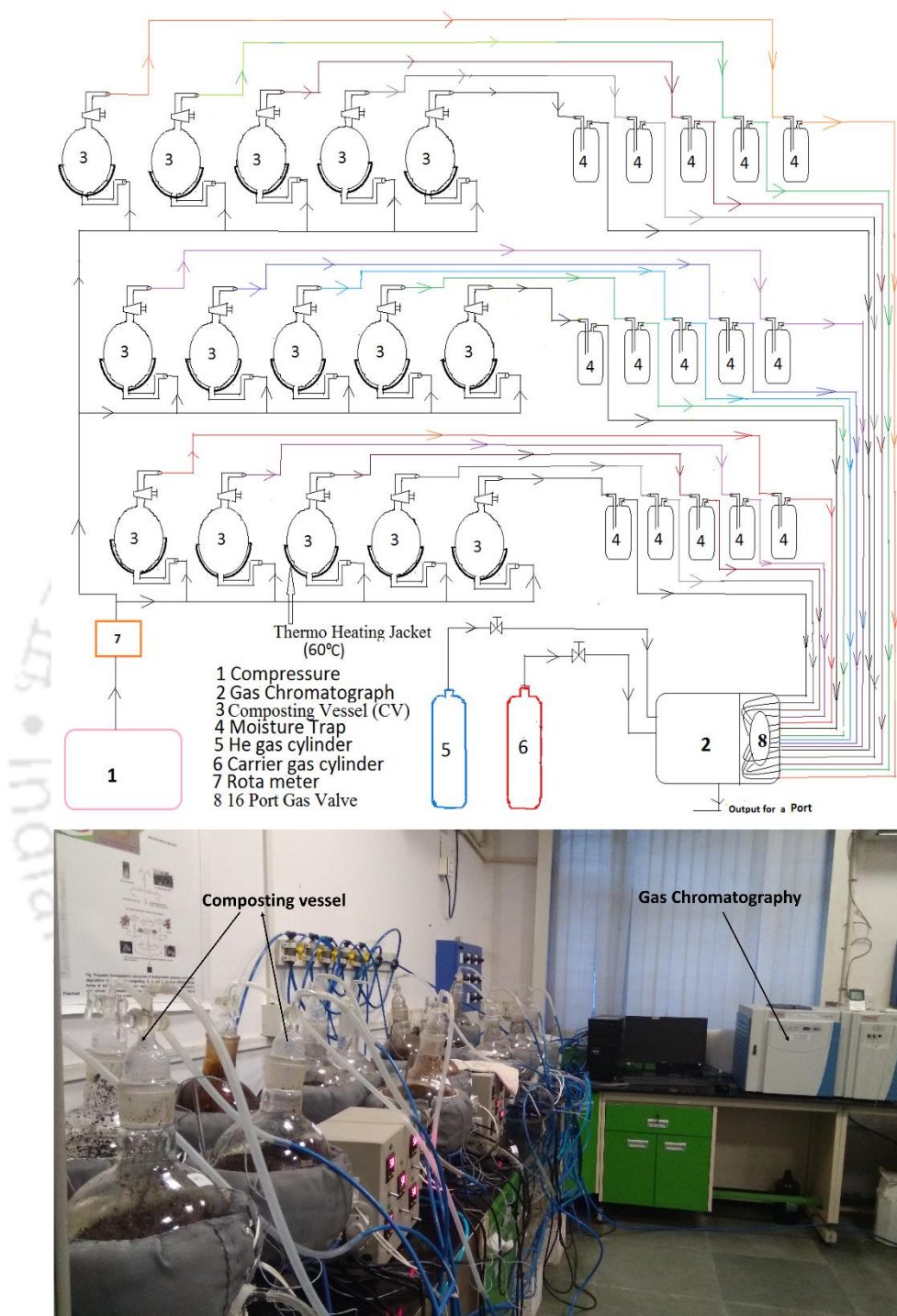


Figure 3. 1: Image showing different reactors connected to the Gas Chromatography system

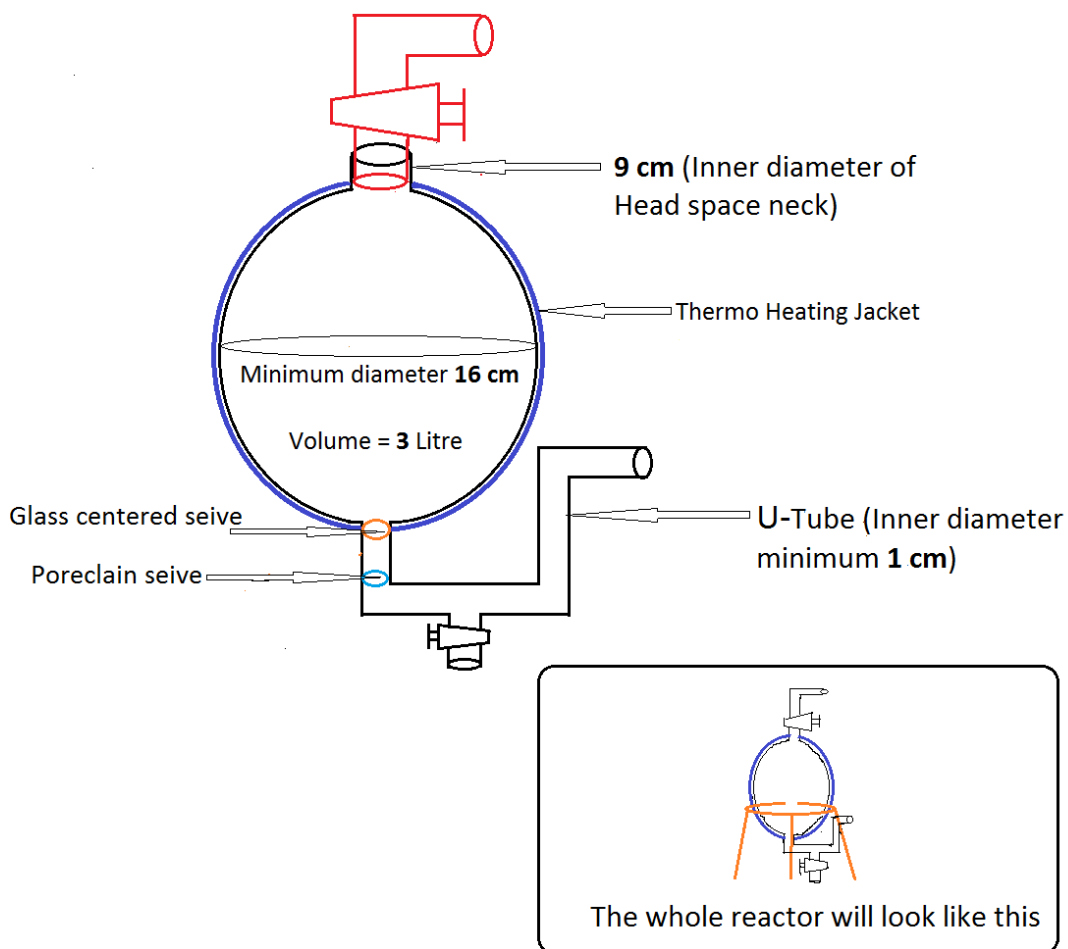


Figure 3. 2: Specification of the bioplastic composting reactors or composting vessels (CV).

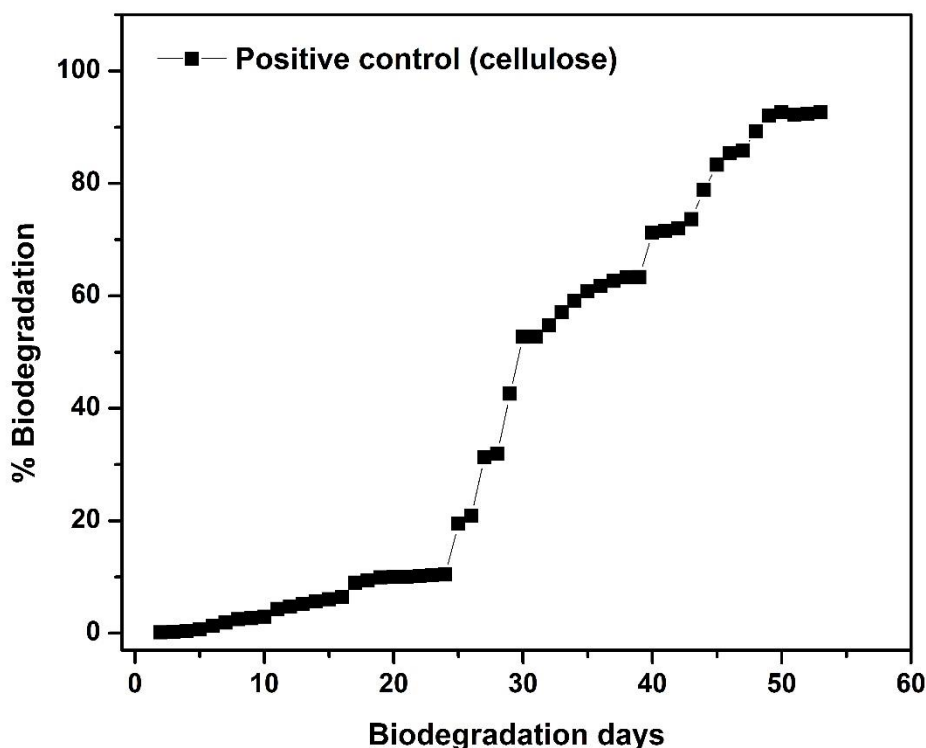


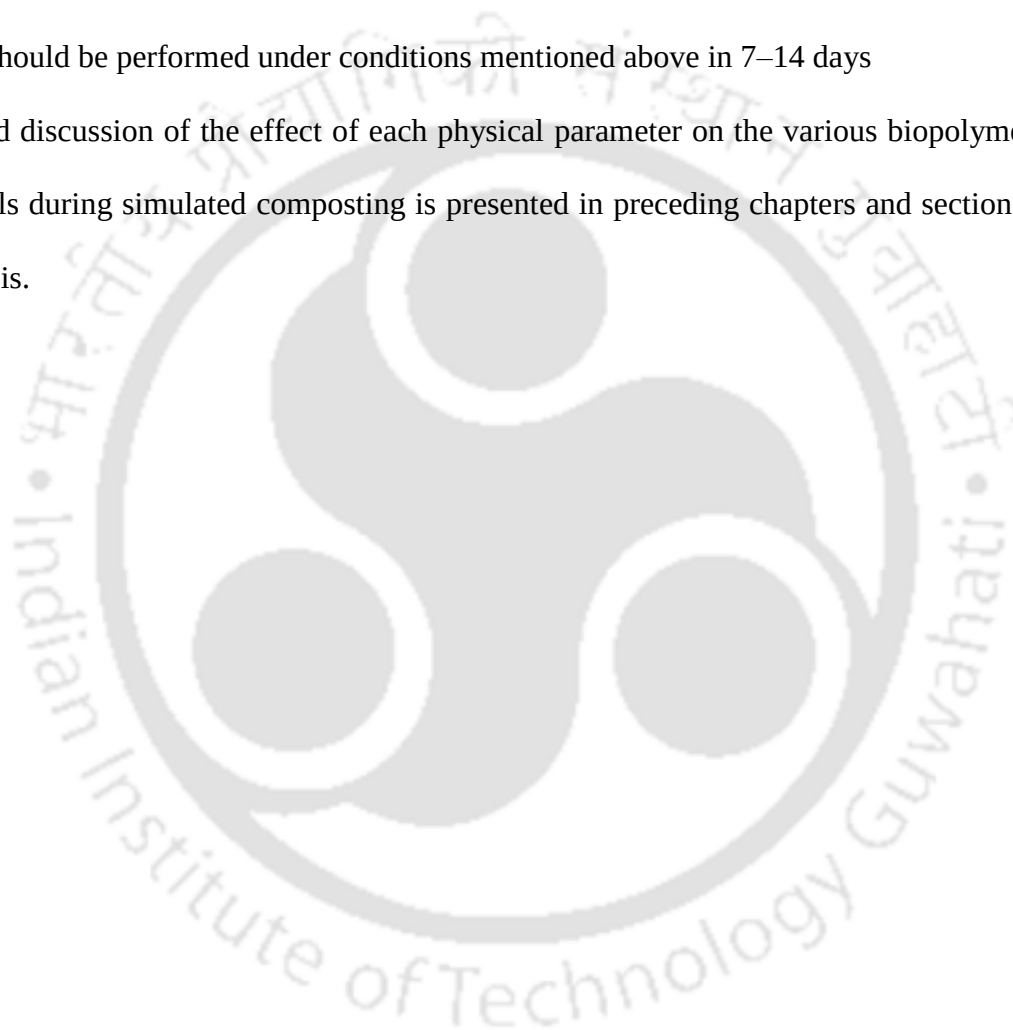
Figure 3. 3: Biodegradation (C-CO₂ evolution) of 99.99 % cellulose tested under controlled composting condition for validation of the experimental setup.

3.3. Conclusion

Well-established biodegradation tests use biogenously evolved carbon dioxide (CO₂) as an analytical parameter to determine the ultimate biodegradability of substances. So, design considerations through process optimization remains an important parameter in order to attain precise biodegradability under laboratory condition. The developed setup succeeded in initial trial of the biodegradation process. An in-house built biodegradation system performed the biodegradation tests in which temperature, RH, airflow rate, CO₂ concentration, and time were monitored and measured. Temperature and pH were stable at $58 \pm 5^\circ\text{C}$ and 7-7.5, respectively. The flow rate of air passing in each composting vessel (CV) was adjusted throughout the testing period in order to maintain more than 12 % oxygen level inside the reactor in order to have better microbial growth and assimilation in other

words microbial respiration. Moisture content was measured periodically in a control bioreactor to determine the amount of water required for adjustment. The frequency of analyses of CO₂ content in the gaseous phase was approximately 24 hours always with respect to the actual “endogenous” respiration of compost itself and to anticipated sample degradability. For preserving aerobic test conditions throughout frequent control is perhaps necessary with unknown samples. In case O₂ content decreases to this limit, aeration of test flasks should be performed under conditions mentioned above in 7–14 days

Detailed discussion of the effect of each physical parameter on the various biopolymeric materials during simulated composting is presented in preceding chapters and sections in the thesis.





Biodegradation behavior and kinetics of PLA based films (Neat PLA; PLA/chitosan; PLA/Gum Arabic; PLA/CNC; under controlled aerobic composting conditions

Modified chitosan, modified gum Arabica, and functionalized cellulose nanocrystals (CNC) has been used extensively by various authors in PLA matrix. These fillers tend to improve certain properties of PLA based packaging films. These improved properties make the composites more superior than their nPLA counterparts do. Modified chitosan and modified gum Arabica when mixed with PLA matrix improves permeability of the packaging material. Whereas, functionalised CNC improves the mechanical as well as thermal stability of the PLA based green composites. Although, its biodegradability needs to be understood for tuning the properties and material characterization for making it more biodegradable by pertaining its improved properties.

Publications:

- Naba Kumar Kalita, Nagar, M. K., Mudenur, C., Kalamdhad, A., & Katiyar, V. (2019). Biodegradation of modified Poly (lactic acid) based biocomposite films under thermophilic composting conditions, *Polymer Testing*, 76, 522-536. (IF: 3.275)
- Naba Kumar Kalita, Anubhav Sharma, Siddharth Mohan Bhasney, Ajay Kalamdhad and Vimal Katiyar Demonstrating an Ideal Compostable Plastic using Biodegradability Kinetics of Poly(lactic acid) based Green Biocomposite Films under Aerobic Composting (AC) Conditions. *Journal of environmental management*, (Under review).

Abstract

This chapter demonstrates the kinetics of aerobic biodegradation of melt extruded poly(lactic acid) (PLA) based biocomposite films, by online monitoring of CO₂ using gas chromatography technique in accordance with ASTM International D5338-15. Biodegradation study of PLA and its biocomposites were carried out in presence of compost microbes, without addition of any external inoculum. First order kinetics model is modified by incorporating linear lag phase for each test sample and results are discussed after regression analysis of the experimental data. Bacterial identification by 16s-RNA gene sequencing suggests *Bacillus flexus* is one of the microbes, which is responsible for biodegradation of exposed films at thermophilic temperature. Neat PLA (NPLA) and PLA/Chitosan composite films found to produce high amounts of C-CO₂ (carbon-to-carbon dioxide) evolution. The C-CO₂ rate is highest for PLA/chitosan sample at 1.13 day⁻¹. The experimental data of all the test samples showed a good fit ($R^2 \sim 99.99$) with the kinetic model. Various analytical techniques like molecular weight analysis, differential scanning calorimetry (DSC), contact angle analysis, and microbial colony count were used for analysis of biodegradation. Colony formation was analyzed and was found to be 5.12×10^6 cfu/mL in PLA/Chitosan sample. Contact angle measurement was employed for understanding biodegradation over a period of 70 days for the bio composites. Biodegradation study was also confirmed through decrease in transparency and major peak shift in infrared (IR) spectroscopy. DSC studies showed increase in crystallinity for all the degraded test samples, which confirms biodegradation. The results confirmed modified PLA films as biodegradable and confirmed suitability of the test system for studying biopolymer biodegradation.

4.1 Introduction

The use of non-degradable plastic materials leads to environmental problems due to improper waste disposal. Issues like environmental sustainability has promoted tremendous research towards the development of biopolymers, which could undergo biodegradation (Cadaru et al., 2012; Valapa et al., 2015). Some of the representative biodegradable polymers which are commonly present in the shelf are poly(lactic acid) [PLA], polyvinyl alcohol [PVA], polyhydroxybutyrate [PHB], poly(butylene adipate-co-terephthalate) [PBAT] (Qi et al., 2017). However, biopolymers including cellulose, chitosan, gums etc. (Lucas et al., 2008) can also be used for the development of biopolymer based biocomposite products. Among all, PLA, has significant and durable properties to replace fossil-based plastics (Dhar et al., 2015; Garlotta, 2002; Shah et al., 2008; R. Valapa et al., 2016). Most of these plastics are specifically targeted towards packaging applications since they undergo degradation during composting (Ge et al., 2016). It is observed that biopolymers such as cellulose (Dhar et al., 2016b), chitosan (Pal and Katiyar, 2016a), gums (Tripathi and Katiyar, 2017), when dispersed in PLA, can enhance certain required properties for food packaging. Studies show that cellulose nanocrystal (CNC), one of the most abundantly available biopolymer, has significantly improved thermo-mechanical properties of PLA based composites (Bitinis et al., 2013). Most biopolymers are hydrophilic in nature, but there are certain grafting techniques that can be applied to change its surface properties from hydrophilic to hydrophobic. In-line with above, recently, chitosan and gum arabic have been applied as reinforcing phase for improvement of gas barrier properties of PLA films for application in food packaging (Pal and Katiyar, 2016b; Tripathi and Katiyar, 2017). However, these improved modified composite films must meet certain criteria regarding their biodegradability, like 90% organic carbon present in the composite film should be converted to CO₂, water etc., under either compost or soil, following certain

standards. In this regard, ASTM Internationals (ASTM D 5338-15) has detailed protocol about biodegradation (ASTM International, 2015a). However, this standard is inherent to different composites and substrates, though the protocols for testing remain similar. Composting has the potential to transform biodegradable waste materials into fertile soil, which may enhance the growth of agro-economy.

Several studies suggested that PLA undergo biodegradation under composting condition within a short period (Castro-Aguirre et al., 2017). The rate of PLA biodegradation may vary, depending upon various factors such as temperature and moisture content (Kale et al., 2007b; Pradhan et al., 2010b). Biodegradation of PLA follows two steps, which includes (1) hydrolytic degradation and (2) biodegradation (Pradhan et al., 2010a; Sikorska et al., 2015; Zenkiewicz et al., 2012). During hydrolytic degradation of PLA, degradation occurs by water molecules through random chain scission, reducing the molar mass that leads to low molecular chain formation as low as <20000 Da. Low molecular weight polymer chains are required for microbial digestion. Whereas, these intermediates are assimilated by microbes for energy and decompose the short polymer chains into various compounds like CO₂ and water (Gu, 2003; Torres et al., 1999). Though no such microbes are identified which is said to degrade high molecular weight PLA directly (Sikorska et al., 2015), but various microorganisms and enzymes have been identified as PLA degrader (Castro-Aguirre et al., 2017; Leja and Lewandowicz, 2010; Lucas et al., 2008; Sikorska et al., 2015). In the current investigation, authors have prepared and investigated PLA films and biocomposites using melt extrusion process with the intention to use these films for thermoformed or vacuum formed products. The leeway of research for improvements in PLA based food packaging application is very broad (Arrieta et al., 2014; Luo et al., 2012; Nieddu et al., 2009; Petinakis et al., 2010; Weng et al., 2013b). There are reports that showed that PLA is biodegradable under the composting condition and takes more time to

degrade under soil conditions (Kale et al., 2007a; Kucharczyk et al., 2016; Weng et al., 2013b).

Hence, CO₂ evolution measurement is an utmost tool to measure biodegradation as it can help to monitor kinetic parameters during biodegradation. Various analytical techniques are used to measure the CO₂ evolution for assessing percentage biodegradation of PLA and its derivative materials under different environments which includes direct measurement respirometry (DMR), non-dispersive infrared (NDIR), cumulative measurement respirometry (CMR) titration etc. are used to monitor the evolved CO₂ (Castro-Aguirre et al., 2017; Gómez and Michel, 2013; Kale et al., 2007c; Kijchavengkul et al., 2006, 2008; Petinakis et al., 2010; Pradhan et al., 2010b). Results observed from these literature state that reproducibility is one of the main issues for this kind of evaluations. Gas chromatography is another online technique to monitor CO₂ evolved can be measured for assessing biodegradation. Kinetic studies exhibited that carbon mineralization is a first-order reaction (Komilis, 2006; Leejarkpai et al., 2011). Microbial identification and DNA sequencing also revealed various PLA biodegrading microbes responsible for biodegradation at both mesophilic and thermophilic temperature (Fontanella et al., 2010; Walczak et al., 2015) as this highlights the microbes responsible for biodegradation at elevated temperatures.

Therefore, this chapter focuses on the biodegradation behavior and to highlight the extent of biodegradation of a new class of PLA based green biocomposites under the same composting setup that has been described in Chapter 3. ASTM D 6003-96 and ASTM D 5512 was used to validate our process as well as results. Melt extruded composite films of NPLA, PLA/Chitosan 5%, PLA/CNC-SO₄ (1%) and PLA/Gum Arabic 5% are used for this study. This study helped in checking the test protocol, validation of the system and understanding the compostability of the green biocomposites with the compost. Kinetic

study corresponding to PLA based biocomposite biodegradation, were employed and experimental data were analysed to understand various phases and forms of carbon produced during biodegradation. In such a case, information obtained from these analytical parameters leads to a better and more reliable prediction of biodegradability for the PLA based composites films. Assessment of microbial flora involved during thermophilic state of biodegradation and by analyzing the biocomposite films, before and after biodegradation for surface morphology.

Various studies used different analytical techniques that have been employed for evaluation of biodegradation during composting of biopolymers using either direct or an indirect approach. These techniques like visual observations, weight loss measurements, changes in mechanical properties, and changes in molecular weight, can provide insights into the degradation process of a polymer, and demonstrate biodegradation (Shah et al., 2008).

Various authors studied characterization techniques used for confirmation of biodegradation and for understanding the behavior of PLA films under composting condition (ASTM Standard D 5338, 2003; Rudeekit et al., 2008; Stloukal et al., 2015) based upon molecular weight loss, crystallinity percentage, contact angle analysis, etc.

Citing this, objective of the present chapter was to summarize the results for evaluating the biodegradation of modified PLA based composites under thermophilic phase of the composting process. This study focuses on the biodegradability and the changes that occurred during biodegradation of plastic samples using various analytical techniques. Melt extruded composites of neat PLA (NPLA), PLA/chitosan 5 % (modified) (PLA/Chitosan), PLA/CNC-SO₄ (1 %) (PLA/CNC) and PLA/Gum Arabic 5 % (modified) (PLA/Gum) were used for this study due to their excellent barrier and thermomechanical properties as reported by the authors elsewhere [9-11]. The outcome of the study defined the compostability of modified PLA films with the compost.

4.2 Results and Discussion

4.2.1 Elemental analysis

Elemental analysis of the samples was done using an elemental analyzer- Eurovector EA 3000. C% of NPLA, PLA/Chitosan, PLA/CNC and PLA/Gum Arabic was found to be ~50.26%, ~50.06%, ~50.05% and ~49.99%, respectively.

4.2.2 Biodegradation test

AC tests were conducted under laboratory conditions at 58 °C (± 5) °C and 60% relative humidity (**Figure 4.1**). The standard cellulose films degraded more than 90% within 37 days which confirms the liability of our composting setup according to ASTM D5338-15. CO₂ evolution test results for samples namely Neat PLA, PLA/Chitosan 5%, PLA/CNC-SO₄ (1%) and PLA/Gum Arabic 5% CRs are presented in **figure 4.2**. From **figure 4.2**, it can be observed that ~435g of CO₂ evolved in 113 days from the PLA/Chitosan sample plus the inoculum which is followed by NPLA, PLA/CNC-SO₄ (1%) and PLA/Gum Arabic 5% in ascending number of days. Different amount of CO₂ evolution from the samples plus inoculum represents the samples viability or compatibility to degrade with that particular compost in a particular duration. Factors, which might be responsible are microbial consortium, biodegradability of the test sample with the compost and moisture content in different composting reactors (CR's). This can be further understood from the evolution of CO₂ from respective samples representing the percentage biodegradation as shown in **figure 4.3**.

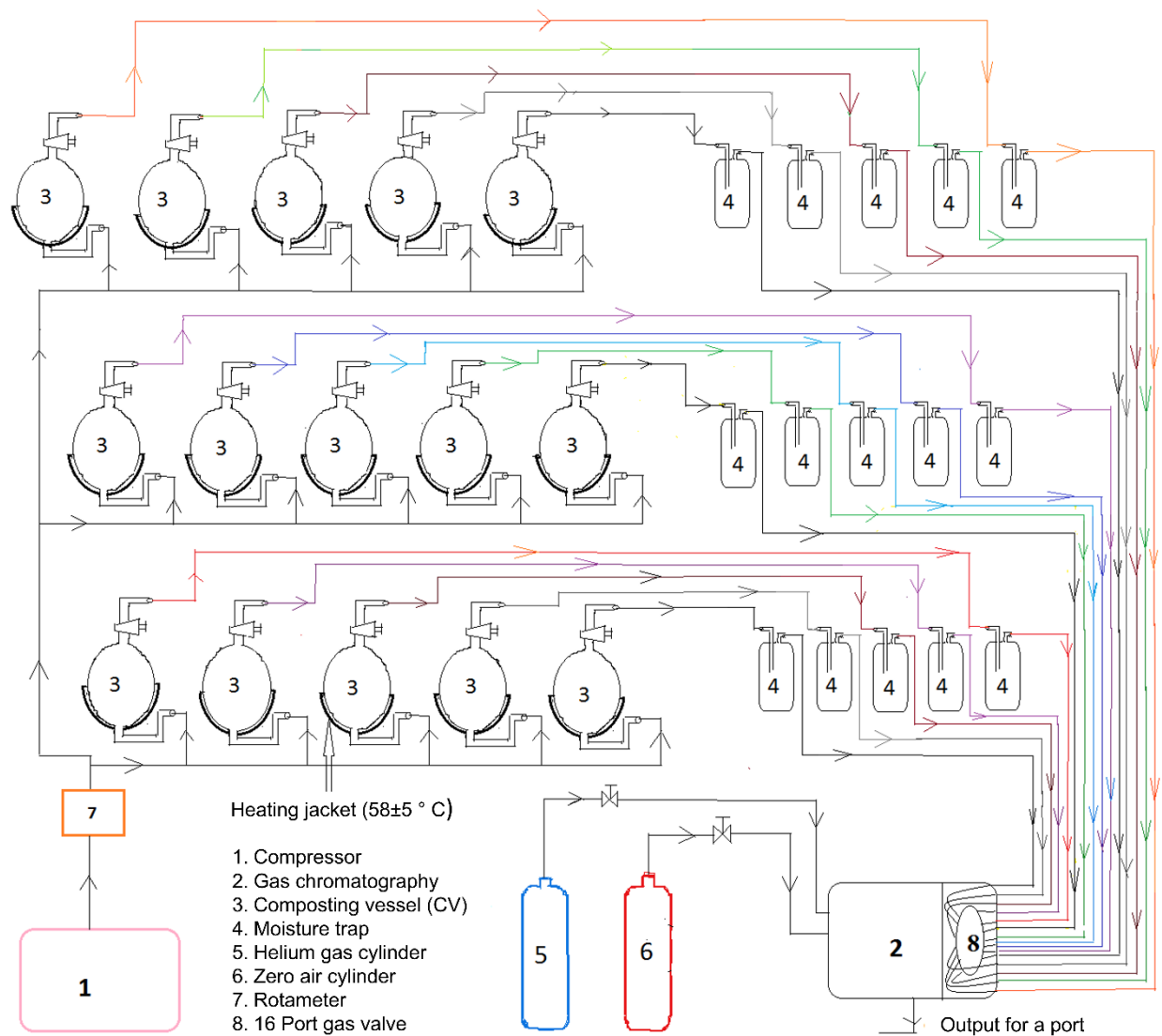


Figure 4. 1: Aerobic biodegradation process in accordance to ASTM D5338-15.
Flowchart design used for biodegradation.

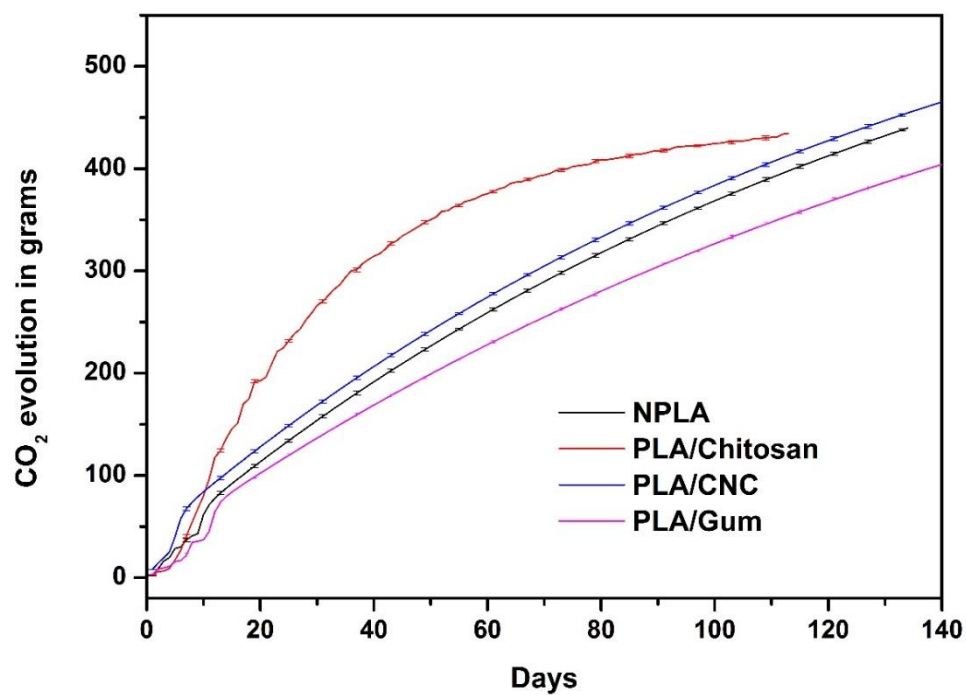


Figure 4. 2: Carbon-dioxide evolution from compost plus test samples (the bar is standard error) during the composting period.

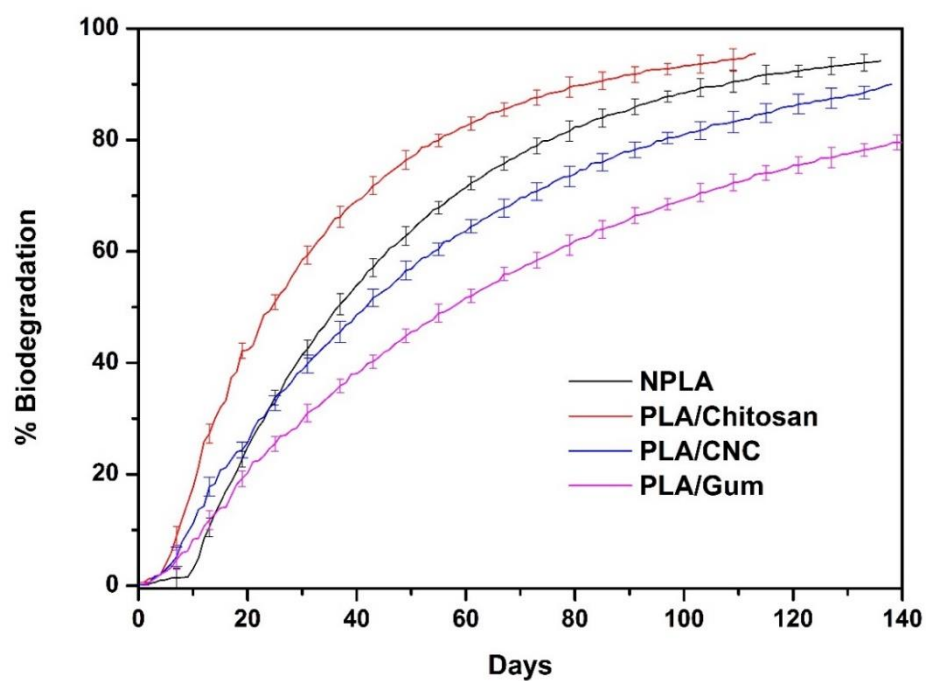


Figure 4. 3: Percentage biodegradation of the test samples under AC condition (the bar is standard error).

The trend for percentage biodegradation as follows PLA/Chitosan 5% >NPLA> PLA/CNC-(SO₄) (1%) > PLA/Gum Arabic 5% Further in case of NPLA, 94.20 % biodegradation was observed in 136 days, whereas, 88.30 % biodegradation was observed for PLA/CNC in 138 days and 80.50% biodegradation was observed for PLA/Gum. After hydrolytic degradation, microorganisms present in compost metabolize the PLA oligomers and other intermediates, and converts them to carbon dioxide, water and hummus. This step occurs at the surface of the material through formation of biofilm (Fukushima et al., 2009; Kale et al., 2007a). It has been observed that 85% of the composite fraction has been degraded within 70 days, against 95 days in case of NPLA. Almost similar observation was reported elsewhere, however, the lag phase has not been reported which provide valuable information in comparing the two systems (Kale et al., 2007a; Rudeekit et al., 2008). It further establishes our results for compostability of PLA in the used compost. The degradability of the PLA/Chitosan samples significantly enhanced in comparison to neat PLA. Whereas, in case of PLA/CNC-SO₄ (1percentage) and PLA/Gum Arabic 5% biodegradation rate is comparatively slow. This might be due to several reasons like compost suitability, molecular structure of the PLA-CNC material and mechanical strength of the material, and all these factors were found to be higher than the neat PLA before biodegradation. Authors have also observed a slower disintegration rate for the composites PLA/CNC/NR (natural rubber) blend under composting condition (Bitinis et al., 2013).. They attributed this behaviour to a higher resistance to water uptake and diffusion through the composites compared to neat PLA. Parameters which might have affected the rate are percentage of crystallinity and the hydrophilicity of the surface of the biocomposites (Picard et al., 2011). Whereas, in case of PLA-Gum Arabica barrier properties increased significantly for the processed films (Tripathi and Katiyar, 2016), like oxygen solubility as well as water solubility. Good barrier properties of the films might prove to be another

reason behind slow biodegradation of PLA/Gum biocomposite, as low water solubility and oxygen solubility will hinder the hydrolysis process as well as growth of aerobic microorganisms on the film surface. In another work (Lee, 2002), the improved gas barrier properties of the nanocomposites is found to have similar detrimental effect upon its biodegradability. In this study, the PLA biocomposites had certain improved properties such as mechanical and gas barrier properties as compared with NPLA as supported by reported literatures. From our observation it is clear that material properties like high mechanical strength or gas barrier characteristics plays a crucial role in determining its rate of biodegradation.

4.2.3 Biodegradation mechanism and kinetics

It begins with the hydrolysis where PLA chain scission by hydrolytic attack H_2O on ester group of PLA primary backbone. As the entire chain is not identical in its affinity towards hydrolysis, the primary chain is assumed composed of three different kinetic forms of SC, which are, rapidly, moderately and slowly hydrolysable. This difference could be on the account of their position with respect to the chain ends, percentage crystallinity etc. Once the initial hydrolysis commences, the short polymer chains formed are susceptible to microbial assimilation, leading to formation of biofilm on its surface. This facilitates enzymatic secretion on the polymer, which promotes biodegradation. Proposed biodegradation mechanism shown in **figure 4.4** is followed by all three forms of SC, at different rates, until finally a common water-soluble intermediate form of carbon is reached, which mineralizes readily to release carbon dioxide, thus achieving biodegradation. To further upgrade the existing model developed by previous authors, during the lag phase, for the sake of uniformity, the mineralization is assumed to occur linearly, before the onset of first order mechanism. Unique duration and percentage

conversion is considered for the lag phase of each sample, as determined experimentally. Biodegradation rate of all the test materials are given in **table 4.1** and the trend are shown in **figure 4.5 (a)-(d)**.

The obtained results show a faster rate of biodegradation for PLA/Chitosan 5% biocomposite films compared with the extruded neat PLA film. This is in agreement with various literatures where authors found to have increased biodegradability of PLA based biocomposites upon addition of biopolymers as reinforcements (Pradhan et al., 2010b, 2010a; Qi et al., 2017; Stloukal et al., 2015), but are varying with our results with other test samples like PLA/CNC-SO₄ (1%) and PLA/Gum Arabic 5%.

Table 4. 1: Kinetic model parameters and coefficient of determination (R²)

Material	NPLA	PLA/Chitosan	PLA/CNC	PLA/Gum
k_{aq} (day⁻¹)	0.99	1.13	0.99	1.00
k_{hr} (day⁻¹)	0.02	0.04	0.01	0.01
k_{hm} (day⁻¹)	0.02	0.02	0.01	0.01
k_{hs} (day⁻¹)	0	0	0	0
C_{aq0} (%)	0.98	0.94	2.00	3.31
C_{r0} (%)	46.90	46.59	45.96	44.36
C_{m0} (%)	50.13	50.56	48.03	45.73
C_{s0} (%)	1.97	1.89	3.98	6.57
Lag Phase (days)	8	3	3	4
R² (%)	99.99	99.99	99.99	99.96
RMSE	0.17	0.17	0.27	0.16

Model data analysis revealed, CO₂ evolution percentage was within the range of 90% - 70% in 113 days for all the test samples. This signifies that the prepared green biocomposites are suitable for industrial composting due to the rate of their biodegradation.

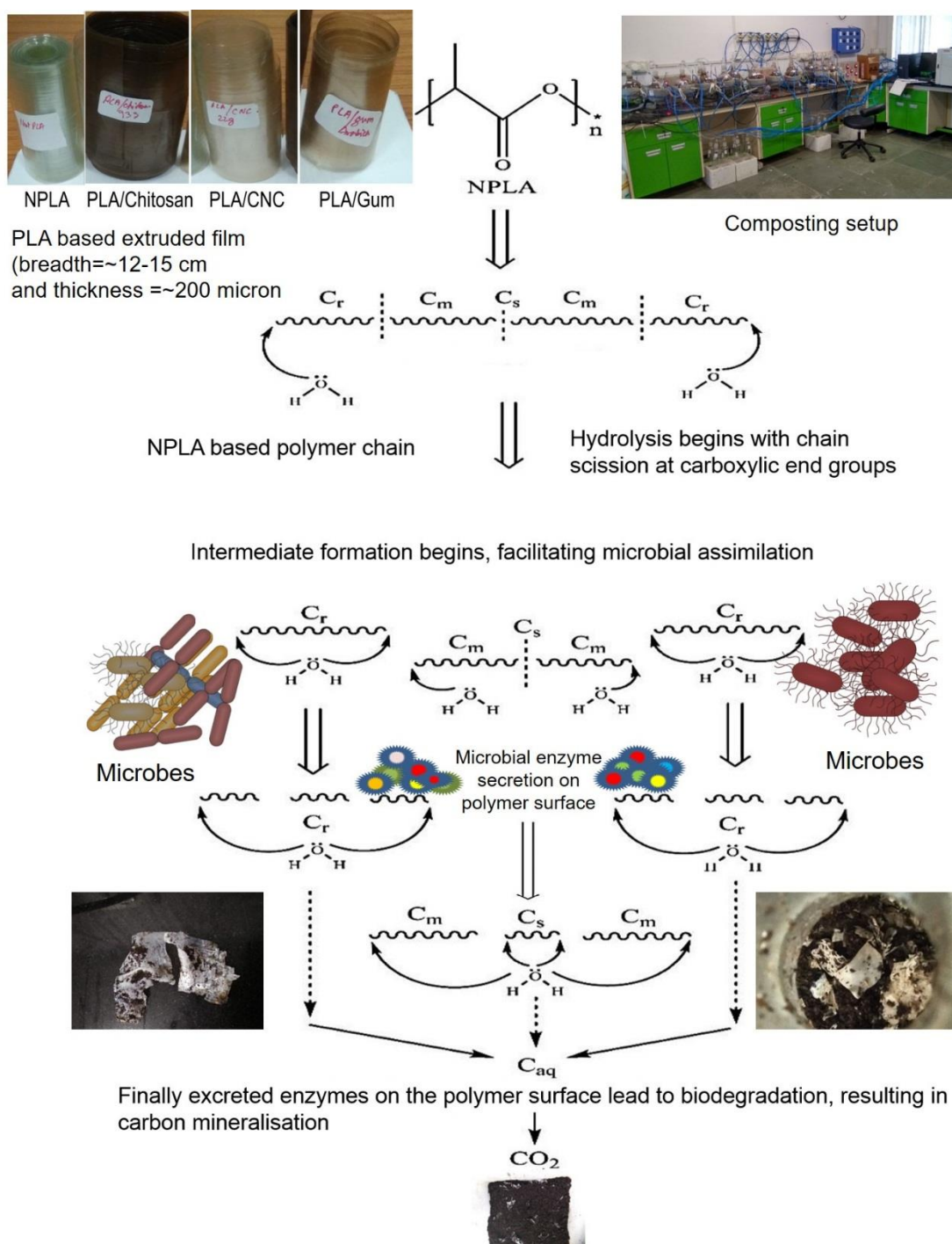


Figure 4. 4: Proposed biodegradation mechanism of biodegradable polymers and carbon degradation during AC. C_r , C_m and C_s are three different kinetic forms of SC, which are, rapidly, moderately and slowly hydrolysable respectively. Intermediates are mainly amorphous region attacked by microbes at first, leaving behind the crystalline polymer region.

Ready biodegradation in PLA/chitosan occurs because of the activity of higher intermediate carbon mineralization 1.13 day^{-1} leading to faster rate of biodegradation. It can be

mentioned that faster rate of rapidly hydrolysable carbon 0.04 day^{-1} resulted in faster biodegradation of the sample compared to NPLA or other test samples. The moderately hydrolysable carbon hydrolysis rate is 0.02 day^{-1} , compared to 0.01 day^{-1} for PLA/CNC.

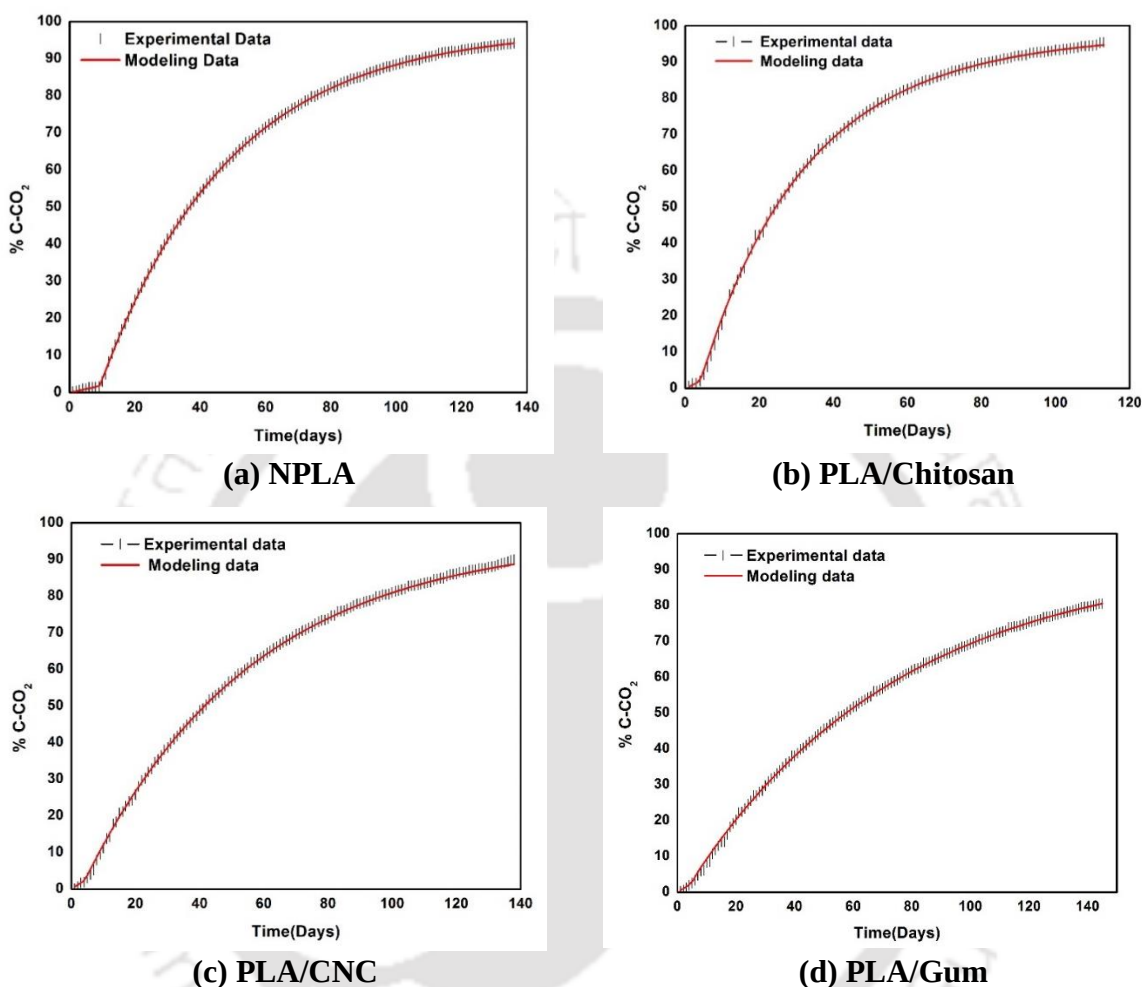


Figure 4. 5: Kinetic of percentage CO₂ evolutions during biodegradation: (a) NPLA (b) PLA/Chitosan (c) PLA/CNC and (d) PLA/Gum (the bar is standard error).

Intermediate rate constant was almost similar to NPLA, which can be signified from its biodegradation rate. However, PLA/gum shows the hydrolysable rate constant for rapidly and moderately hydrolysable carbon and is very low compared to other samples studied i.e., 0.01 day^{-1} and 0.01 day^{-1} respectively. Whereas, it's intermediate carbon mineralization is found quite high 1.00 day^{-1} as compared with NPLA and PLA/CNC. This may be due to the complex structure of Gum Arabica formed with the PLA matrix making hydrolysis difficult but once intermediates are formed carbon mineralization became faster. We have

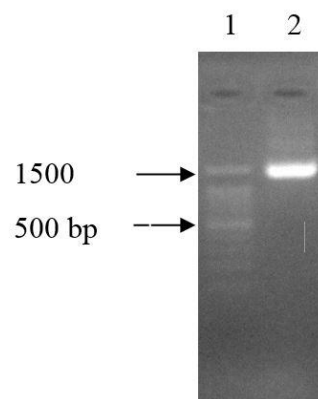
incorporated the effect of lag phase to C-CO₂ evolution, since our experiments showed some amount of CO₂ being released during the lag phase, which was close to zero in some studies (Komilis, 2006; Leejarkpai et al., 2011; Stloukal et al., 2015). The kinetic model adopted in our calculation of relevant parameters was initially developed by (Komilis and Ham, 2003)) (Komilis and Ham, 2003). Further, with the incorporation of the concept of lag phase and non-predetermined calculation of the mineralization rate constant were taken to be 10⁶ day⁻¹ by Komilis, (2006) (Komilis, 2006). The kinetic model was further modified (Leejarkpai et al., 2011). However, in the assumptions put forth by the author, the lag phase is taken to be a period in the initial days of the experiment, where the carbon mineralization percentage is non-existent. In contrast, as observed experimentally during the composting process, results are found different. Moreover, due to the differences in the characteristic of each polymer blend used in the biodegradation process, every material has its own lag phase and a unique conversion percentage in the given period. Hence, to further upgrade the existing model developed by authors (Komilis, 2006; Leejarkpai et al., 2011) during the lag phase, for the sake of uniformity, the mineralization is assumed to occur linearly, before the onset of first order mechanism. As per experimental data, a unique duration and percentage conversion is considered for the lag phase of each sample, as determined experimentally. The stationary phase or the plateau commenced after that, indicating reduction in depolymerization rate after the growth phase.

It is generally assumed that biocomposites derived by dispersion of bio-fillers in bioplastics enhance its biodegradation capacity. Nevertheless, it is observed from experiments that it is not a necessary occurrence. As showed by Komilis, (2006) various carbon forms follow different biodegradation pathways, and most importantly, at different rates. These discrepancies in the molecular structures of the composites are what have led to different kinetic parameters observed in our experimental analysis. Gum Arabic, is a

complex polysaccharide (natural plant origin) used with Neat PLA, has shown the lowest rate of biodegradation, and highest quantity of slowly HCF, which is in accordance with the classification put forth by author (Komilis, 2006), pertaining to the biodegradation rates of various carbon forms. According to our modelled data PLA/CNC showing a higher quantity of readily HCF and a lower quantity of slowly HCF, as compared with PLA/Gum Arabic. Again, this supports Komilis, (2006) (Komilis, 2006) classification, which claims a higher biodegradation rate of cellulose compared to proteins. The next filler involved in our set of experiments is chitosan, which is obtained by deacetylation of Chitin. Chitosan a bioactive polysaccharide / a marine polysaccharide derived from Chitins has shown an immense increase in biodegradation capacity when infused with PLA. This has been validated experimentally by the highest mineralization rate constant of PLA/Chitosan, even surpassing NPLA.

4.2.4 Microbiological analysis and isolation of the degrading strain

Microbes were isolated at 58 °C, which were further purified for colony isolation and genomic DNA analysis. From the microbial analysis, it was confirmed that the differences in biodegradation rate between different PLA based biocomposites in this study could be explained by participating microorganism's compatibility with the biocomposites. This study also reveals that different biodegradable fillers has different biodegradation rate. Through bacterial identification (**Figure 4.6 & 4.7**), we were able to identify one of the bacterial strain, which was acting during the thermal biodegradation process. The strain is identified by 16S RNA sequencing as highly similar to *Bacillus flexus* (*Bacillus flexus* strain NK1 16S ribosomal



Lane 1- 100 BP LADDER; **Lane 2-**16S sample

Figure 4. 6: Electrophoresis documenting microbial community.

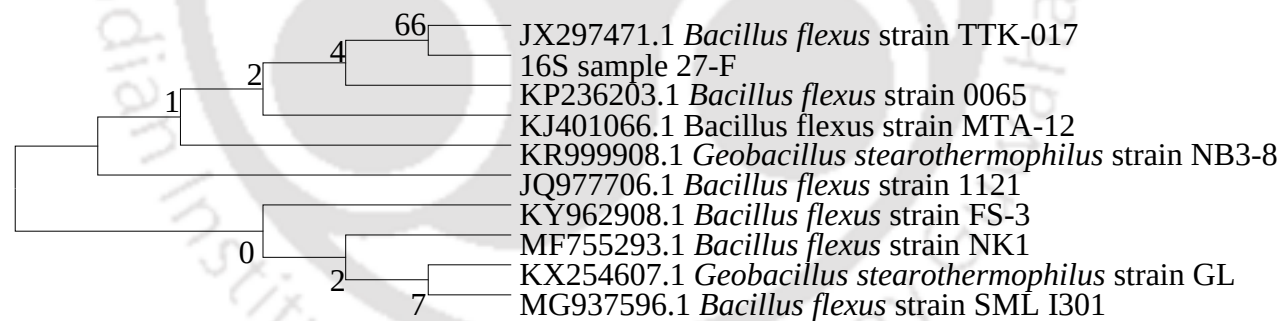


Figure 4. 7: Based on sequence alignment and Phylogenetic analysis the 16S sample has been matched to *Bacillus flexus*.

RNA gene, partial sequence, accession number MF755293.1, 97% identity) belonging to the phylum of bacteria firmicutes.

From this study it is clear that *Bacillus flexus* can degrade high molecular weight PLA based biocomposites. This strain can survive at extreme conditions and forms endospores. From **figure 4.6 & 4.7** it is clear that specific strain was only detected using the universal bacteria detecting primer. To summarize, *Bacillus flexus* strain was never been reported for PLA based biocomposites biodegradation.

4.2.5 Morphological Studies

The biodegradability and model results were corroborated by FESEM, as shown in **figure 4.8**. The FESEM images after composting shows degradation on the PLA based green biocomposites surface. This morphological change was observed on the 40th and 80th day of the composting process. Microbial assimilation of the amorphous regions as well as hydrolytic degradation led to the intermediate's formation during the biodegradation process. This resulted in large holes and cracks formation, which can be seen on the polymer surface and cross-section over the time of biodegradation, which attributes the action of microbes or biofilm formation on the surface of the polymers and diffusion of the cells to the core of polymer, leading to surface erosion, and leaving cavities on PLA bulk. Moisture inside the CR diffuses into the amorphous domains, which initiates the hydrolytic degradation for further microbial assimilation of the polymeric intermediates. With progress in the hydrolytic degradation process the fillers tends to move away from the matrix towards the compost resulting in holes, cavities and cracks which was observed on the 40th as well as 80th day. This disorientation of the films during biodegradation also suggest that short chains are assimilated by microbes first and then attacks the long crystalline chains, though chain scission mechanism. These morphological changes also

prove that these new class of PLA based composites are biodegradable under controlled composting environment.

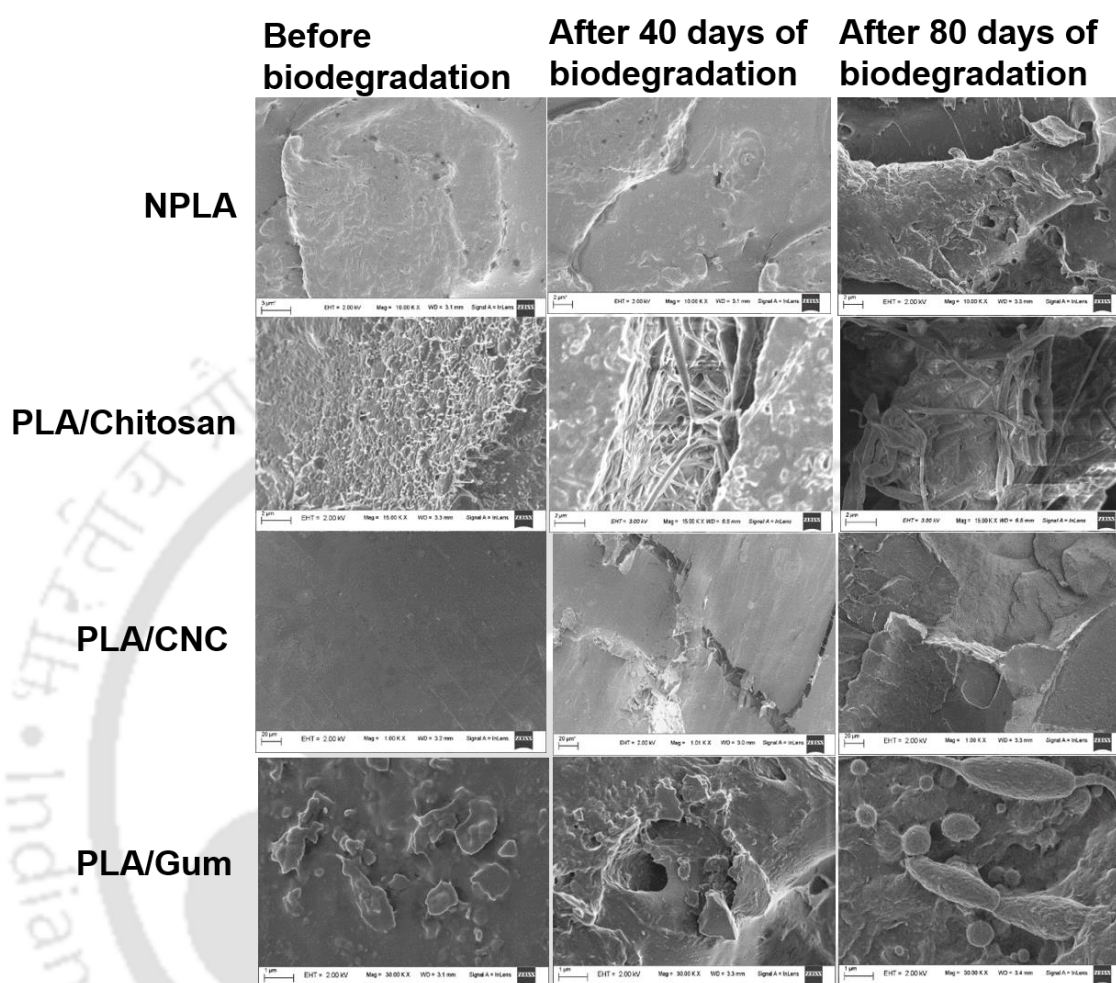


Figure 4. 8: FESEM images of different test samples before and after biodegradation under composting condition.

4.2.6 Molecular weight analysis

From the molecular weight analysis data shown in **table 4.2**, it can be observed that GPC chromatograms showed two to three peaks (both higher and lower molecular weight area), on the 50th day in some samples and from the 60th day for the left of the test samples. This indicates biodegradation was taking place through chain scission of the high molecular weight chains and converting them into intermediates of low molecular weight chains. This process increases the degradation of the test materials under composting condition. It was

observed that number average molecular weight (M_n) of NPLA decreased from 109 kDa to 49 kDa, with formation of low molecular weight chains of $M_n \sim 331$ and ~ 82 Da was observed on the 80th day of the biodegradation process. Similar behaviors were recorded for all the test samples, which confirm degradation of the test material under the composting condition. Though, in case of PLA/Gum on the 70th day of the biodegradation process only high molecular weight area of the sample were recorded but with decreased M_n as reported in **table 4.2**. The chain cleaved in all the PLA based test material also indicates that ester bonds converted to hydroxyl groups during biodegradation leading to loss in molecular weight of the samples (Rudeekit et al., 2008). Reduction of molecular weight also leads to embrittlement of the PLA based test samples making the samples opaque as shown in **figure 4.9**.



Figure 4. 9: Samples were randomly taken out and weight at different times of composting duration. Samples are small pieces and cameras were zoomed before taking the pictures for better visibility.

which also increases the crystallinity percentage of the samples during the biodegradation process. When the molecular weight decreases after hydrolytic degradation of modified PLA chains microorganisms present in compost can metabolize the macromolecules, converting them to CO₂, hummus and water (De Jong et al., 2001; Leja and Lewandowicz, 2010).

Table 4. 2: Number Average Molecular weight (Mn) of the test samples before and after biodegradation. Here (d) represents days and (da) represents Dalton.

Samples	(Mn) Initial (kda)	(Mn) 40d (kda)	(Mn) 50d (kda)		(Mn) 60d (kda)		(Mn) 70d (kda)		(Mn) 80d (kda)	
			High Mn & Low Mn	(area %)	High Mn & Low Mn	(area %)	High Mn & Low Mn	(area %)	High Mn & Low Mn	(area %)
NPLA	109	102	72		55	98.38	51	100	49.	92.60
					0.09	1.61			0.33	2.18
									0.08	5.20
PLA/CNC	63	49	44		33	67.90	31	88.85	24	91.16
					0.50	29.50	0.36	11.15	0.36	3.42
					0.09	0.72			0.08	5.14
PLA/Chitosan	90	51	43		46	95.54	26	100	28	95.57
					0.32	2.5			0.31	4.11
					0.09	1.8				
PLA/Gum	95	64	18	72.34	17	92.93	11	100	17	47.80
			2	27.65	0.33	5.55			0.86	41.09
					0.09	1.51			0.07	11.06

Moreover, decrease in number average molecular weight was in line with various authors who investigated degradation of various blends and composites of PLA under different environments (Rudeekit et al., 2008). The data obtained were evaluated by applying the Eq. 1 for first-order kinetics describing the random scission of ester bonds and are shown in **figure 4.10**. The coefficients of determination (R^2) of about 94.3% for PLA/Chitosan,

88.4% for PLA/CNC, 79.89% for PLA/Gum and 72.5% for NPLA were found. This study also indicate the lack of adequacy of the model for some of the test samples.

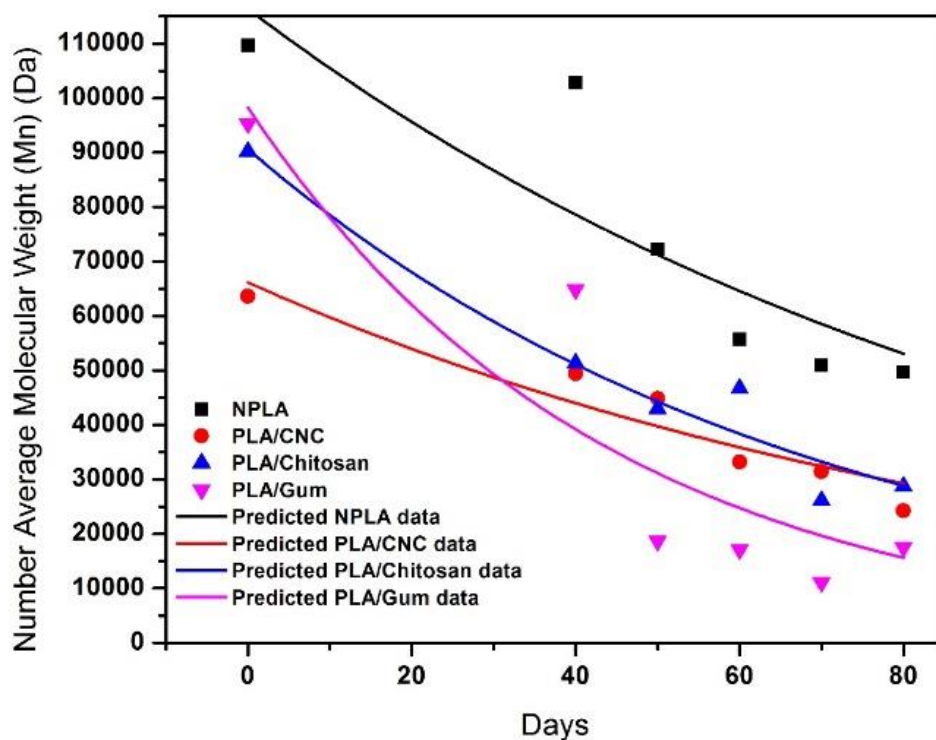


Figure 4. 10: Molecular weight analysis of the test samples and their predicted data according to the first order reaction.

4.2.7. Microbial count and colony formation

For microbial analysis samples were incubated at 60 °C. Sample plates were observed for colony formation (cfu/mL) for all the test samples studied and are presented in **table 4.3**. It was found that colony formation was much higher in PLA/Chitosan compared to other samples. So, microbial colony formation indicates that higher the colony formation inside a reactor, higher its biodegradation rate, which can be further correlated with R^2 values of molecular weight data (Husorova et al., 2014a). These results might comply with the type of compost used, that might have provided a better microbial synergism, which needs a thorough investigation for further proof of concept. The plates were analyzed by gram

staining and found that all of them contain gram-negative bacteria as they retained the pink colour after the staining process and was confirmed by observing in an optical microscope as shown in **Figure 4.11**.

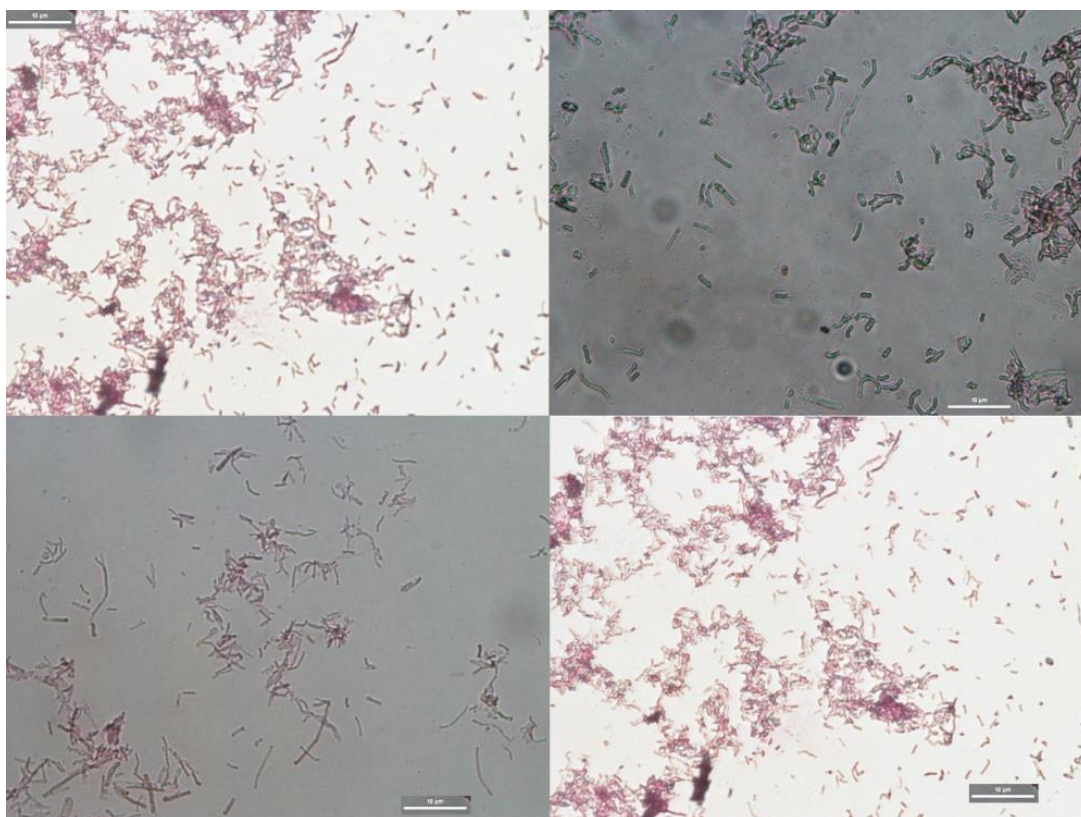


Figure 4. 11: Identification of rod shaped bacteria is acting and is gram negative as the pink colour is retained after staining during the composting process.

Table 4. 3: Microbial colony count during the biodegradation process.

Sample	Microbial count
NPLA	3.56×10^6 cfu/ml
PLA/CNC	2.43×10^6 cfu/ml
PLA/Chitosan	5.12×10^6 cfu/ml
PLA/Gum	2.20×10^6 cfu/ml

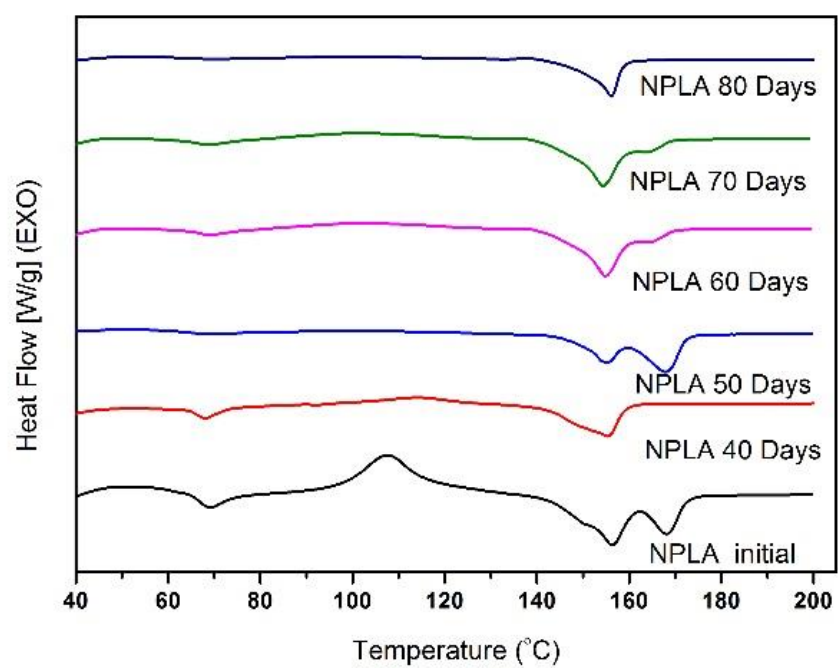
4.2.8 Differential Scanning Calorimeter (DSC)

Comparison of the thermographs of the second heating of all the test samples which underwent biodegradation under composting condition to those of the samples before biodegradation is shown in **figure 4.12(a-d)**. During the biodegradation period of 80 days all the test samples revealed relevant difference between the test samples before biodegradation and after biodegradation. With increasing biodegradation time, the differences become clearer after 40 days. The NPLA samples during the second heating, loses the capacity to crystallize and melt (**Figure 4.12(a)**). With increase in biodegradation time, percentage crystallinity (χ) also increased which signifies that amorphous portions of the test samples were biodegraded leaving behind only the crystalline portions, which were reported in **table 4.4**. Literatures showed that it might be possible that the absence of melting and cold crystallization can be related to the mixing upon the heating of PLA with the enzymes during the biodegradation period (Pantani and Sorrentino, 2013). So our temperature of 60 °C/58 °C (± 5 °C) maintained during biodegradation has a vital role to play. Similar behavior was also observed in case of PLA/Chitosan based samples during biodegradation (**figure 4.12(b)**). It was found that from the 60th day, PLA/Chitosan samples lost its capacity to crystallize, which might be due to sample losing its capacity to cold crystallize. It suggests that PLA/Chitosan samples crystallinity increased with decrease in the amorphous portion. Whereas, in test samples PLA/CNC and PLA/Gum, effect of filler reinforcement on the PLA matrix can be observed **figure 4.12(c-d)**, effecting crystallinity percentage during biodegradation as well as its melting and recrystallization capacity. From the thermographs it can be observed that as compared to NPLA and PLA/Chitosan samples, PLA/CNC and PLA/Gum had less effect in increase in crystallinity percentage, as these two test samples showed less tendency to lose melting and recrystallization behavior during biodegradation. It was also observed that

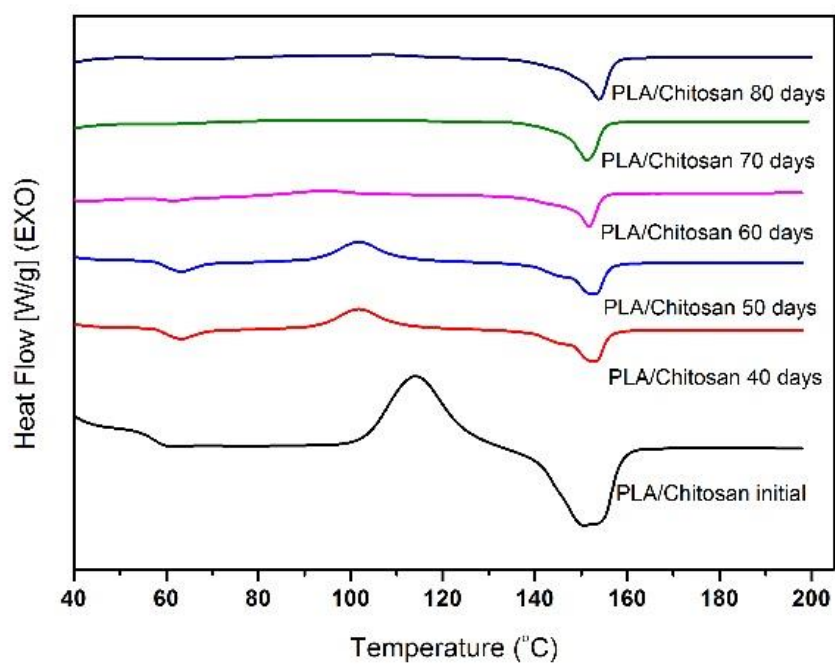
NPLA and PLA/Chitosan test samples showed increase in crystallinity percentage (χ %) with time. Whereas, PLA/CNC and PLA/Gum showed decrease in (χ %) from 70th to the 80th day as shown in **table 4.4**. This behavior was observed due to the chain scission and decrease in molecular weight of both the samples. So cleavage of more crystalline chains into semi-crystalline or amorphous nature might have taken place facilitating the growth of microbes, making them more accessible to the polymers during the later stage of the biodegradation process (Ramos et al., 2016). These results might encourage further research for crystalline portion biodegrading microcosm for PLA, under composting condition.

Table 4. 4: Crystallinity (χ %) of the test samples before and after biodegradation.

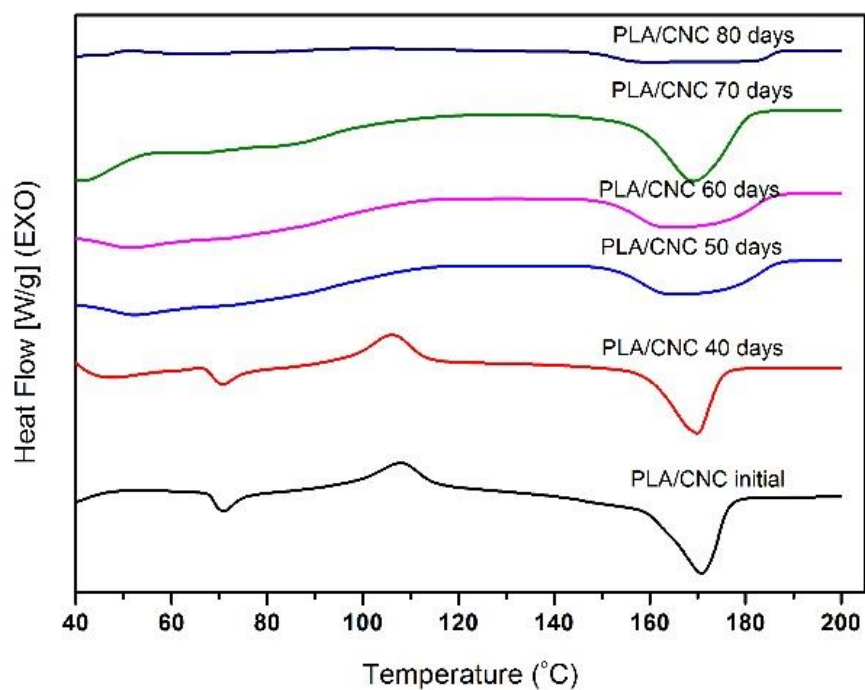
Samples	Before biodegradation (χ %)	40 days (χ %)	50 days (χ %)	60 days (χ %)	70 days (χ %)	80 days (χ %)
NPLA	25.00	35.00	40.00	42.12	51.00	71.57
PLA/Chitosan	23.57	24.56	36.89	37.97	39.95	48.38
PLA/CNC	33.37	43.23	53.61	73.23	49.99	38.70
PLA/Gum	32.39	51.23	56.32	79.66	62.36	42.60



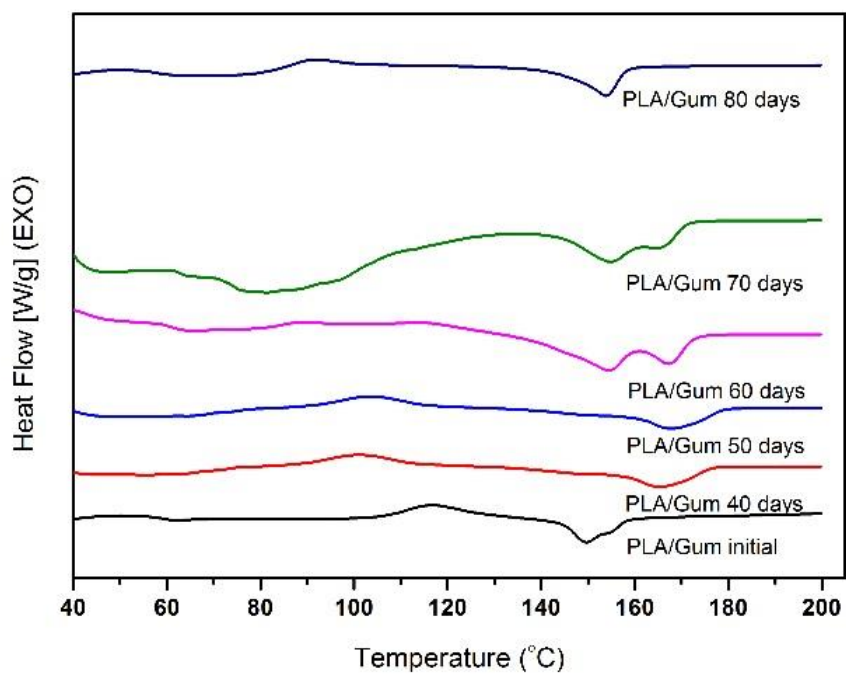
(a) NPLA



(b) PLA/Chitosan



(c) PLA/CNC



(d) PLA/Gum

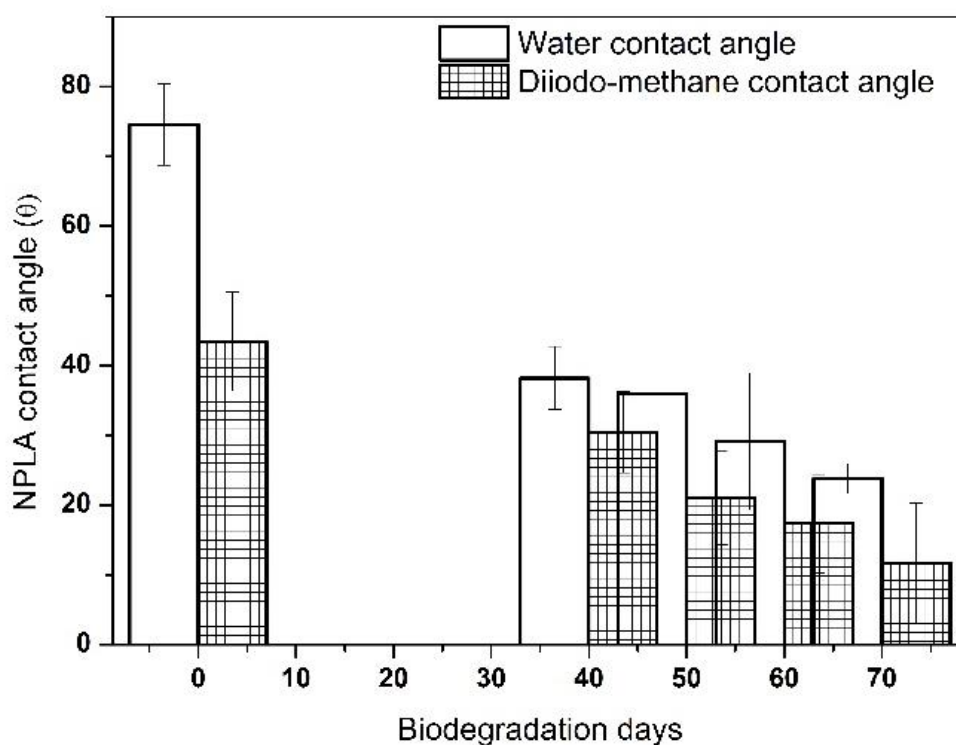
Figure 4. 12(a-d): DSC thermograms of the test samples before and after biodegradation.

4.2.9 Contact angle analysis

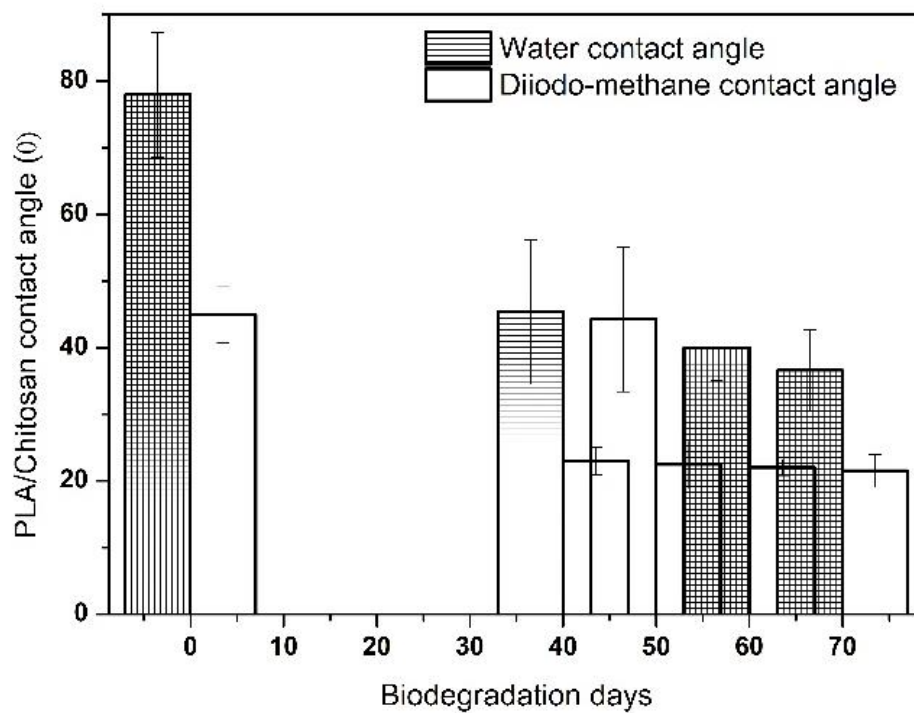
For contact angle analysis the samples were analyzed at two different temperatures 27 °C and 58±2 °C or composting temperature (**Figure 4.13(a-d)**). This was done to have insights on how the wettability of the material is changing with composting temperature and the test materials' molecular activity during the biodegradation process. Tests were performed with two different solvents namely, water (polar solvent) and diiodo-methane (non-polar solvent) and readings were recorded at 5 seconds to 300 seconds and average contact angle was analyzed for results. This process also helps in identifying the stability of the solvent drop on the sample surface. Higher the standard deviation of the contact angles more the drop is unstable during that time period of analysis. It can be observed that both water contact angles decreased significantly for all the test samples at 27 °C. For diiodo-methane solvent, standard deviation was low for all the test samples which signifies polar surface group formation on the surface of the test samples after biodegradation. Here, diiodo-methane contact angles decreased with increase in number of biodegradation days. This decreasing trend was observed for both water contact angle and diiodo-methane contact angle at 58 ± 2 °C for PLA/Gum samples as shown in **table 4.5**. These decrease in contact angles suggests that samples surfaces were becoming hydrophilic in nature as shown in **figure 4.14(a-d)**

Though, many studies documented that crystallinity percentage increases in biodegraded samples due to loss of amorphous phase at a faster rate. But this explanation won't serve the results obtained regarding our decreased contact angles in most of the cases, as it is seen that normally contact angles increases with increase in crystallinity percentage for polymeric materials. Ester bond hydrolysis typically creates new end groups which is carboxyl and hydroxyl group that may improve the hydrophilicity of PLA microsphere (Stloukal et al., 2015). It was observed that solvent drops were more stable at 27 °C, as less

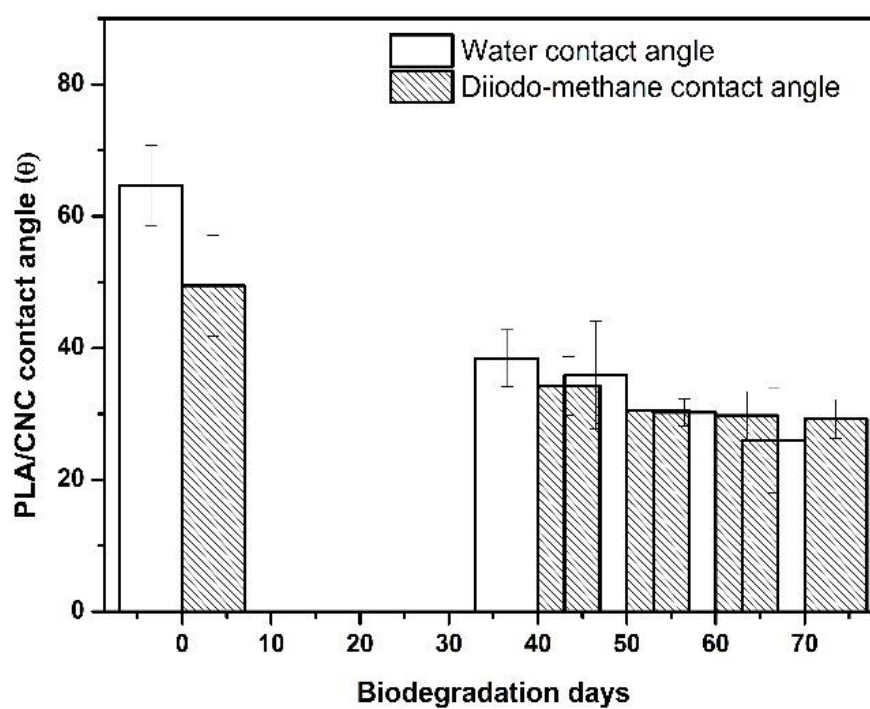
standard deviation among the angles were measured for any particular test sample, for both the solvents. Whereas, higher standard deviation values were obtained for samples analyzed at $(58 \pm 2)^\circ\text{C}$ (**Figure 4.14 (a-d)**). This might be due to the decrease in T_g of all the test samples, which increased chain mobility of the polymeric test samples at $(58 \pm 2)^\circ\text{C}$. Chain mobility further increased hydrophilicity and increased the solvent solubility within that time period. This temperature condition might have increased biodegradation rate of our test samples. During analysis temperature played a crucial role for understanding the biodegradation behavior of the studied test samples. This higher standard deviation in contact angles might also attributed to holes and cracks that have occurred on the biodegradable samples, which can be observed from our FESEM studies. This process of contact angle measuring gives the insight of the degraded surfaces which leads to its hydrophilicity or hydrophobicity.



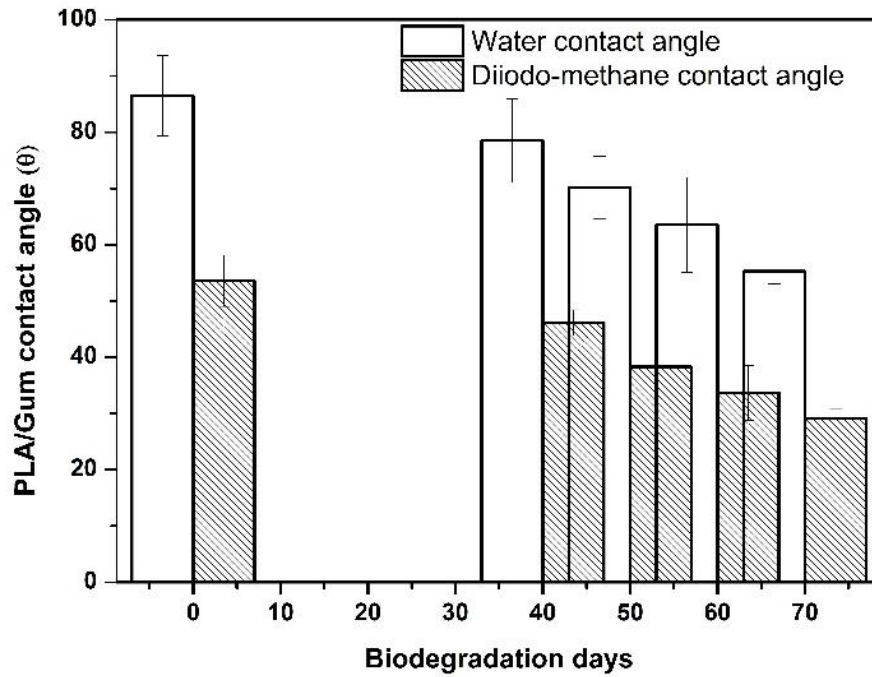
(a) NPLA



(b) PLA/Chitosan

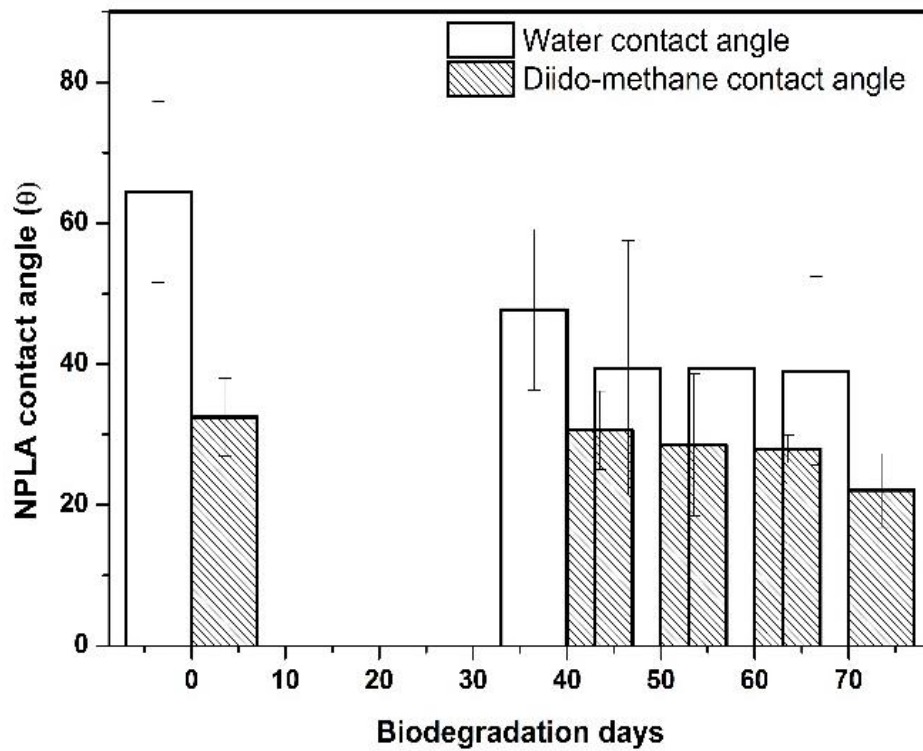


(c) PLA/CNC

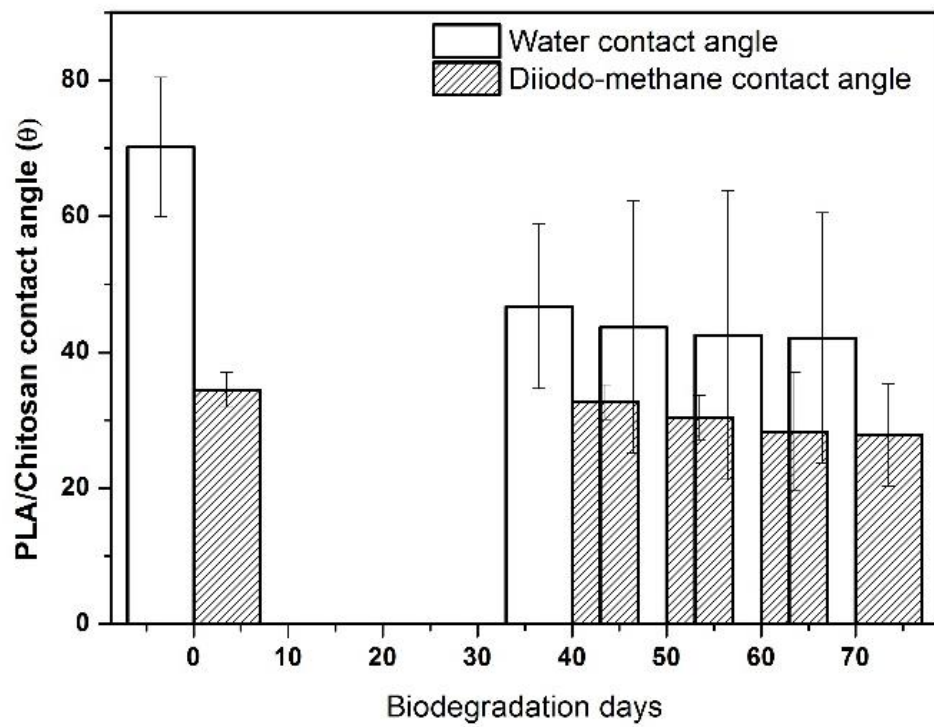


(d) PLA/Gum

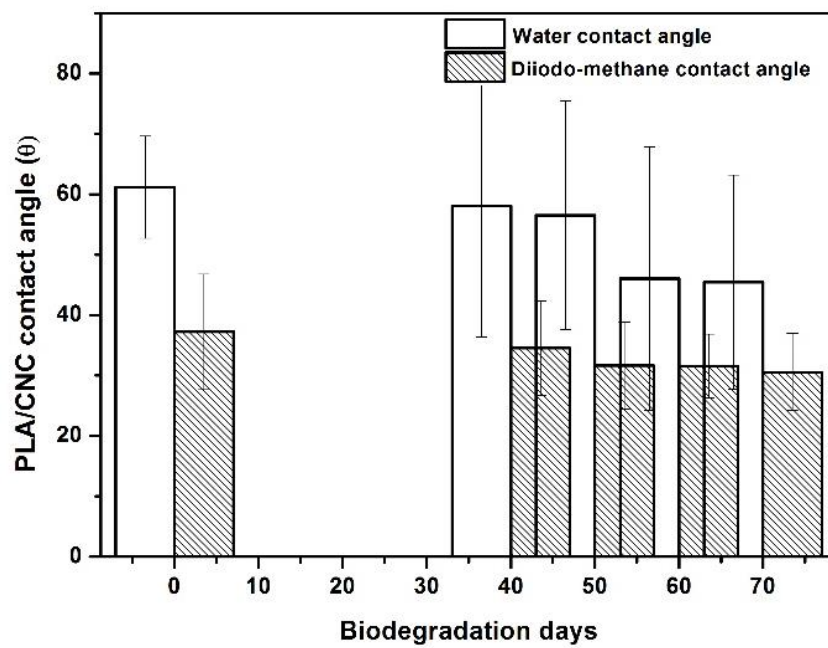
Figure 4. 13 (a-d): Contact angle of the test samples before and after biodegradation at 27 °C with standard errors.



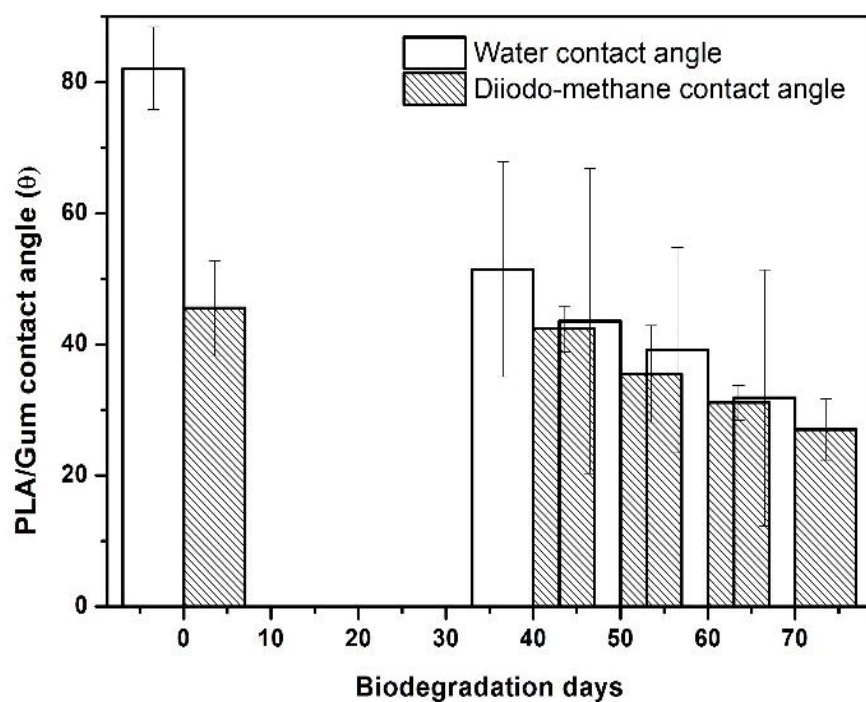
(a) NPLA



(b) PLA/Chitosan



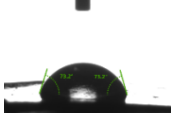
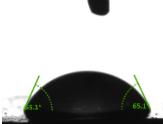
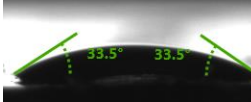
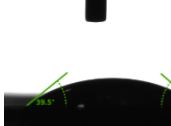
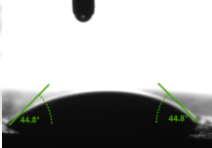
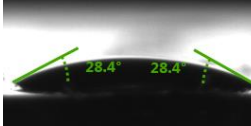
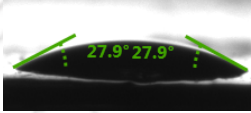
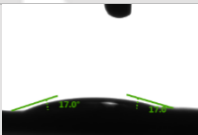
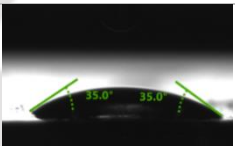
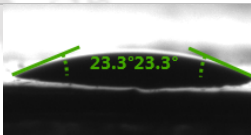
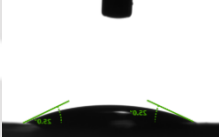
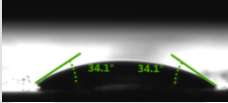
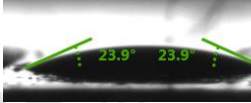
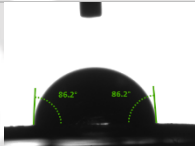
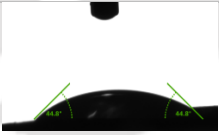
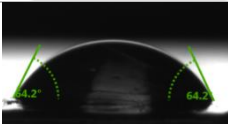
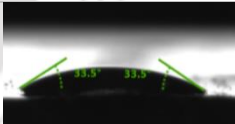
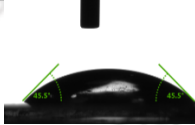
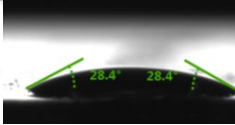
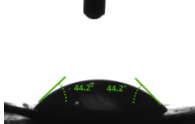
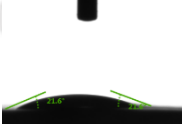
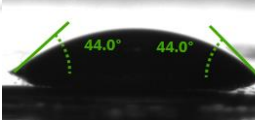
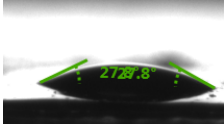
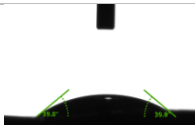
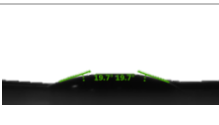
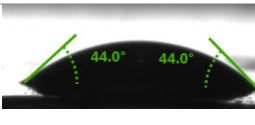
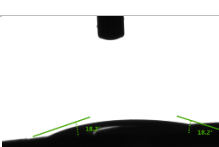
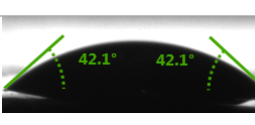
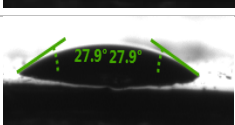
(c) PLA/CNC

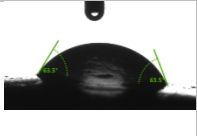
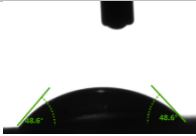
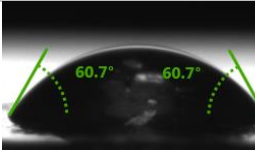
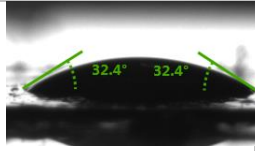
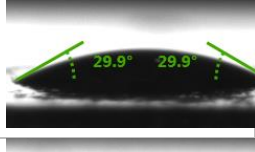
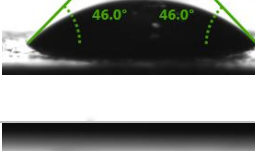
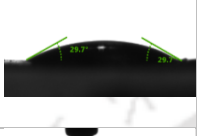
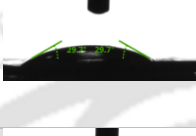
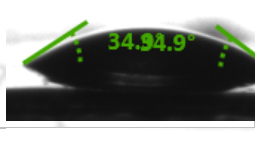
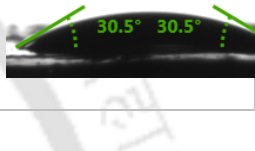
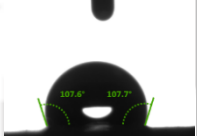
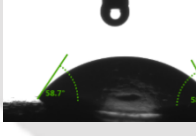
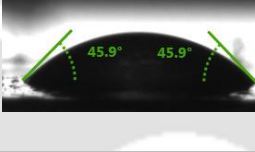
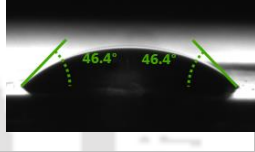
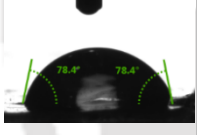
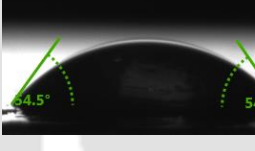
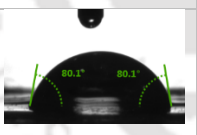
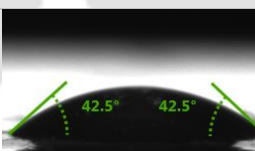
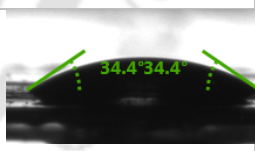
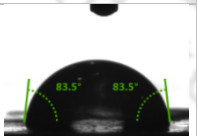
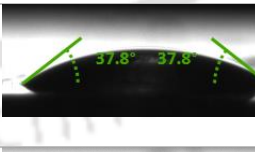
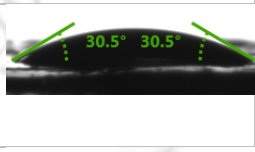
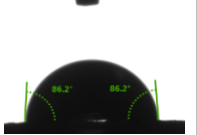


(d) PLA/Gum

Figure 4. 14 (a-d): Contact angle of the test samples before and after biodegradation at 58 °C with standard errors.

Table 4. 5: Contact angle images at different temperature and using two different solvents.

Samples	Analysis at 27 °C		Analysis at 60 °C	
	Water Contact Angle	Diiodo-methane Contact Angle	Water Contact Angle	Diiodo-methane Contact Angle
NPLA initial				
NPLA 40d				
NPLA 50d				
NPLA 60d				
NPLA 70d				
PLA/Chitosan initial				
PLA/Chitosan 40d				
PLA/Chitosan 50d				
PLA/Chitosan 60d				
PLA/Chitosan 70d				

PLA/CNC initial				
PLA/CNC 40d				
PLA/CNC 50d				
PLA/CNC 60d				
PLA/CNC 70d				
PLA/Gum initial				
PLA/Gum 40d				
PLA/Gum 50d				
PLA/Gum 60d				
PLA/Gum 70d				

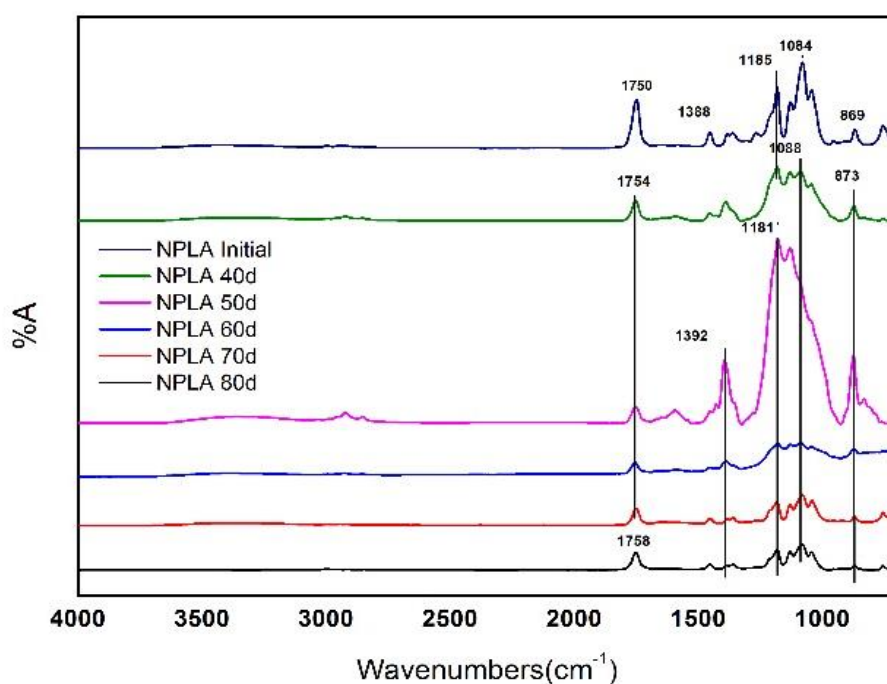
4.2.10. Fourier transformed infrared spectroscopy (FTIR) and UV-visible spectroscopy

FTIR samples were analyzed for 80th day and are shown in **figure 4.15 (a)-(d)**. It was found that there were shifting of peaks from 1750 cm⁻¹ to 1758 cm⁻¹ which corresponds to the carbonyl (-C=O-) stretching. This shift in peaks confirms degradation. This shifting of peaks was in accordance with many authors (Weng et al., 2013a, 2013b), who found same shifting of peaks during biodegradation of PLA at different environments. 1388 cm⁻¹ is assigned to CH₃ deformation vibration and -C-O-stretching vibration, are assigned to peaks at 1185 cm⁻¹, which got shifted during biodegradation on 50th day with increased peak intensity but was consistent till the 80th day with slight decrease in peak intensity. This shifting of peaks mainly confirms the degradation of the NPLA test samples. For PLA/Gum samples there was increase in peak shifting at 1750 to 1758 cm⁻¹ with decrease in peak intensity upto 80th day which is assigned to corresponding absorption band of carbonyl (-C=O-) stretching vibrations. Peaks at 1451 cm⁻¹ shifted to 1456 on the 40th day with very less peak intensity, represents the (-CH₃) bending vibration. This shifting represents degradation of the sample due to change in chemical structure of the samples during biodegradation. This peak range was representation of the PLA/Gum bionanocomposite (Tripathi and Katiyar, 2016). Peaks at 1185 cm⁻¹ represents the C-N stretch representing aliphatic amine group, which shifted towards 1180 cm⁻¹ representing biodegradation was able to affect the structure of the aliphatic amine groups on the 80th day of degradation. Peak shifting from 868-872 cm⁻¹ with smaller intensity, represents biodegradation of aromatic functional groups. This kind of peak shifting was also observed in PLA/CNC samples, like 1750-1754 cm⁻¹ representing C=O stretch which signifies presence and degradation of ester bonds to carboxylic groups during the biodegradation process. Small intensity peaks shifting from 1083 cm⁻¹ to 1075 cm⁻¹ represents the C-O stretching

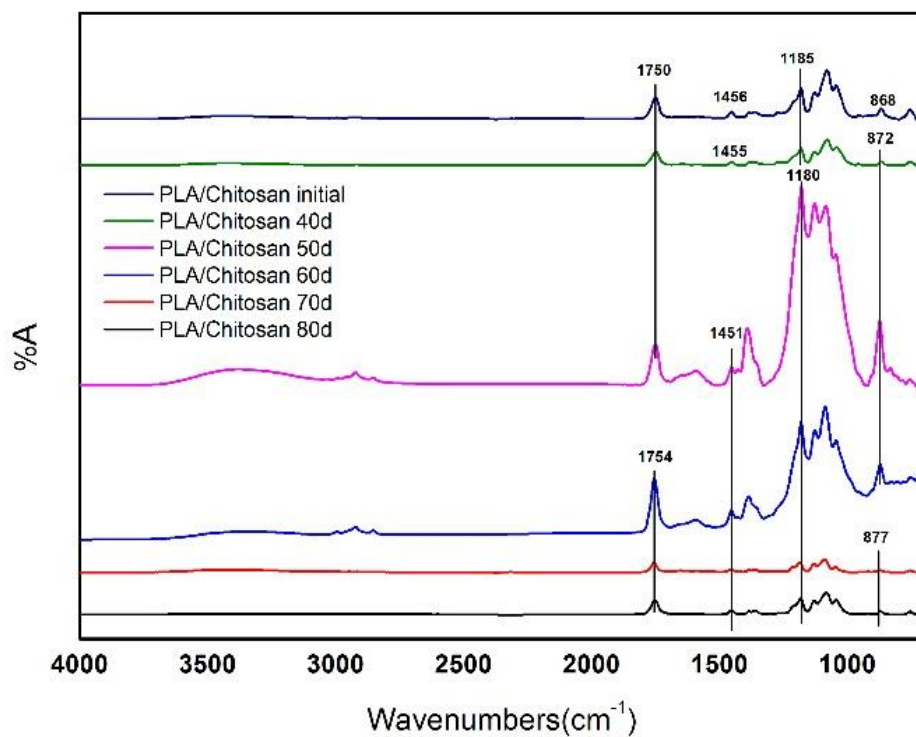
vibrations of alcohols and carboxylic acids functional groups, which confirms degradation of PLA/CNC samples and effect of hydrolytic degradation forming intermediates which are having less intensity peaks. FTIR graphs for PLA/Chitosan samples show same peak shifting as our other studied samples. It was observed that medium intensity peaks at 1185 cm^{-1} shifted towards 1180 cm^{-1} with no significant change in peak intensity represents the C-N stretching vibrations which corresponds to the aliphatic amines functional groups. This peak confirms degradation had an effect on the amine groups present in the sample. Thus, for PLA based samples it is clear that aerobic biodegradation caused higher molecular samples to degrade into low molecular weight samples, causing peak shifting. This kind of phenomenon was also observed by authors but in different environment for different kind of biopolymers and their blends (Karamanlioglu et al., 2017; Pantani and Sorrentino, 2013; Paul et al., 2005; Sedlarik et al., 2008).

The transparency measurements for NPLA, PLA/CNC, PLA/Chitosan and PLA/Gum composite samples before as well as after biodegradation were performed using UV-vis spectrophotometer. The transmittance (%) values obtained in the wave length region of 575 nm is presented in **table 4.6**. From transmittance graphs, it is observed that when NPLA is subjected to visible light, it showed around ~79% transmittance but after it was subjected to biodegradation its transmittance gradually decreased to ~3%. This might be due to the increase in crystallinity percentage, formation of biofilms or might be due to microbial extracts that may have affected colour as well as properties like increase in brittleness. This similar behavior has been observed with other test samples, but with some variations. From the graphs as well from the **table 4.6**, it has been observed that samples other than NPLA showed sudden increase in transparency on the 50th day and further decreasing consecutively. Occurrence of this behavior might be due to the filler and PLA matrix interaction. It was observed visually that colour of the samples changed too from opaque

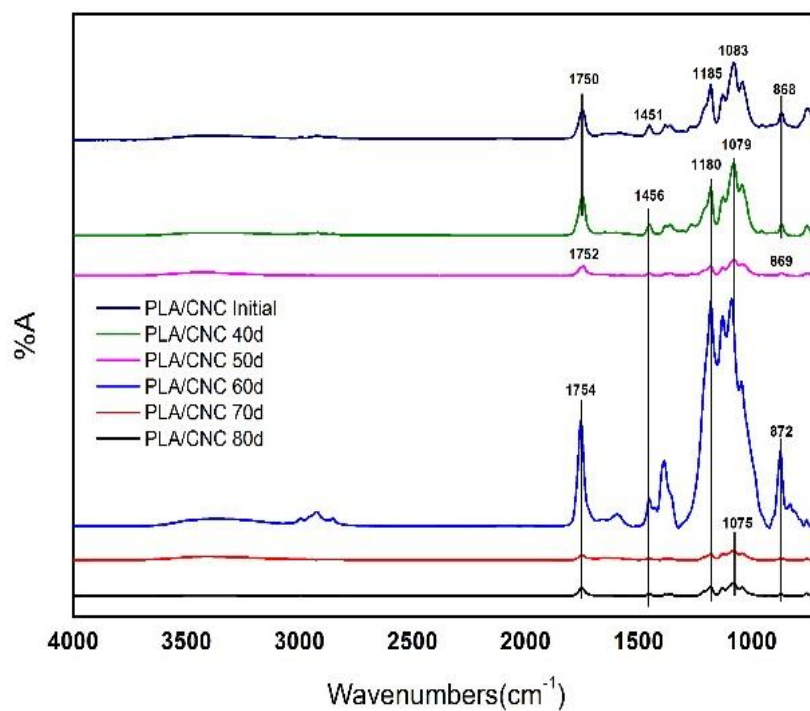
to transparent and again to opaque because of the degradation of fillers at a faster rate than NPLA matrix in the particular time period. This results were consistent with all the test samples except NPLA. Other reason might be due to the washing of the degraded samples before analysis.



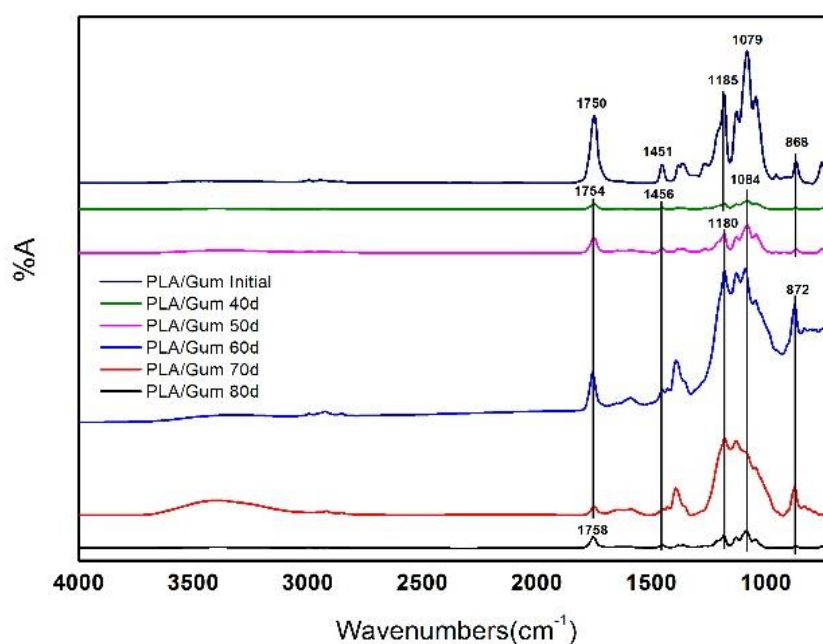
(a) NPLA



(b) PLA/Chitosan



(c) PLA/CNC



(d) PLA/Gum

Figure 4. 15 (a-d): IR footprint of the test samples before and after biodegradation

Table 4. 6: Transmission of the test samples during the biodegradation period.

Sample	Initial (T%)	40 days (T%)	50 days (T%)	60 days (T%)	70 days (T%)
NPLA	~79	~35	~32	~9	~3
PLA/Chitosan	~50	~12	~17	~2	~2
PLA/Gum	~70	~35	~50	~9.6	~2
PLA/CNC	~58	~65	~38	~25	~13

4.2.11 Degradation mechanism and morphological analysis

As shown in **figure 4.16 (a)** it can be seen that H₂O attacks on C(C=O) forming carboxyl groups and hydroxyl groups. The hydrolytic degradation process paves the way for the degradation of PLA by formation of intermediates of lower molecular weight which can be assimilated by the microbes further for biodegradation forming CO₂, water and energy (Torres et al., 1999). Modified PLA biocomposites used in our study namely PLA/Chitosan, PLA/CNC and PLA/Gum will also follow the same path or first step of

biodegradation of PLA. In case of PLA/Chitosan following possible mechanism was proposed: H_2O attacks on $C(C=O)$ forming carboxyl groups and hydroxyl groups. H^+ attack on $N(NH-C)$ and make N^+H_2 due to high E_N , leading to bond breakdown making separate molecule PLA and chitosan as shown in **figure 4.16 (b)**. In case of PLA/Gum and PLA/CNC also follows the same path as depicted in **figure 4.16 (c-d)**. From the depicted mechanism it was clear that once intermediates are formed further biodegradation is possible for the modified PLA biocomposites.

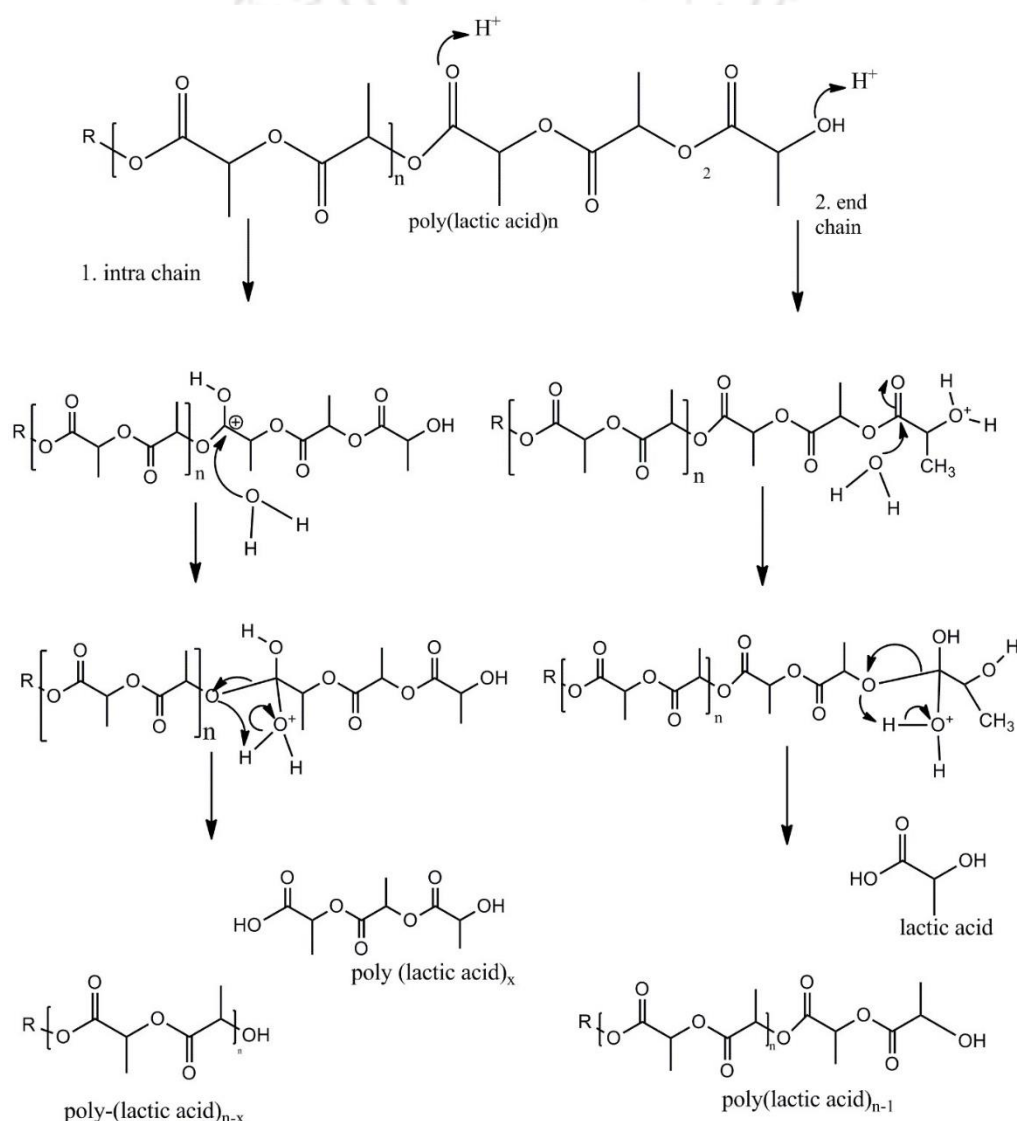


Figure 4.16(a): Degradation mechanism of NPLA test sample under composting condition.

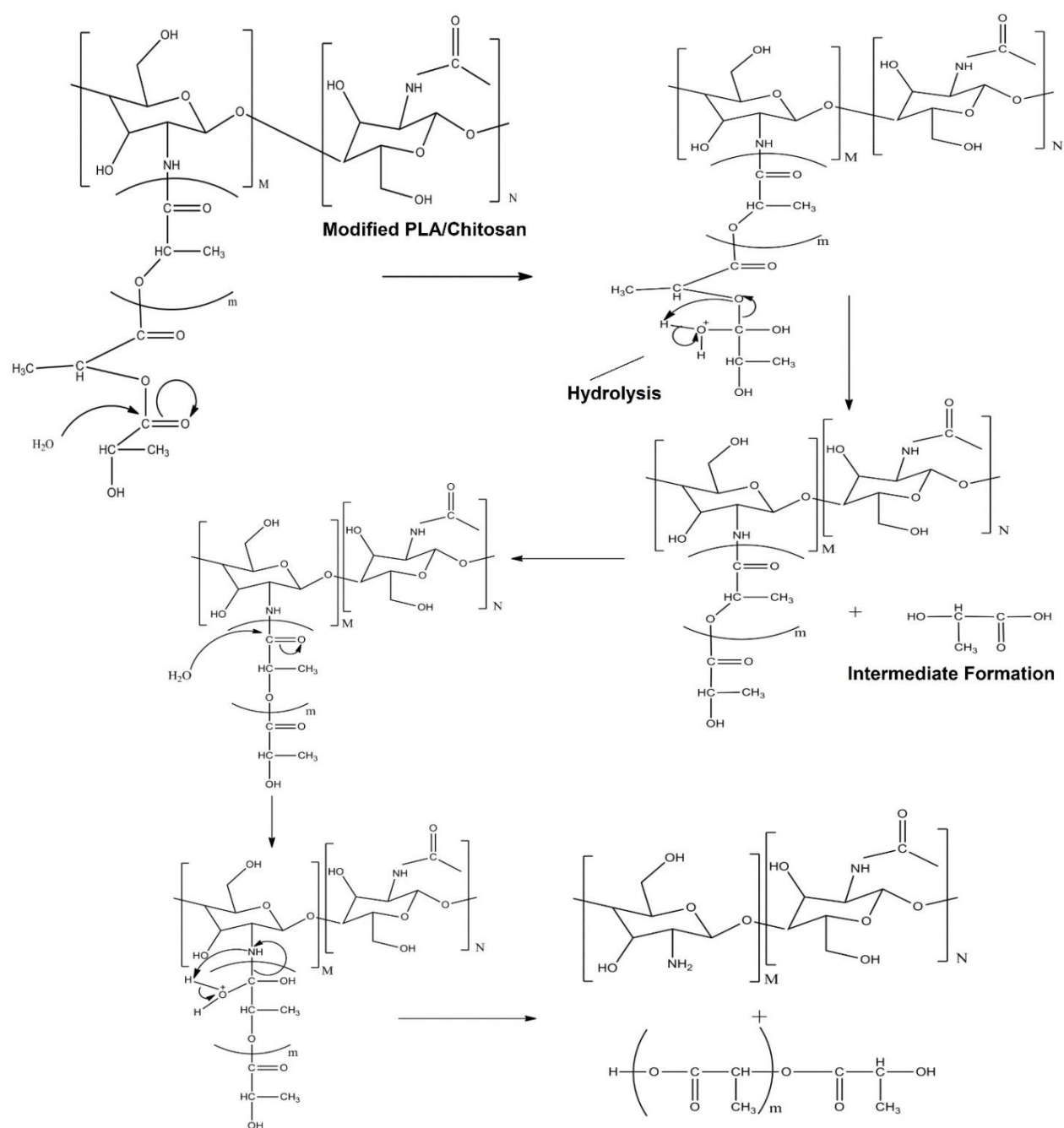


Figure 4.16 (b): Degradation mechanism of PLA/Chitosan test sample under composting condition.

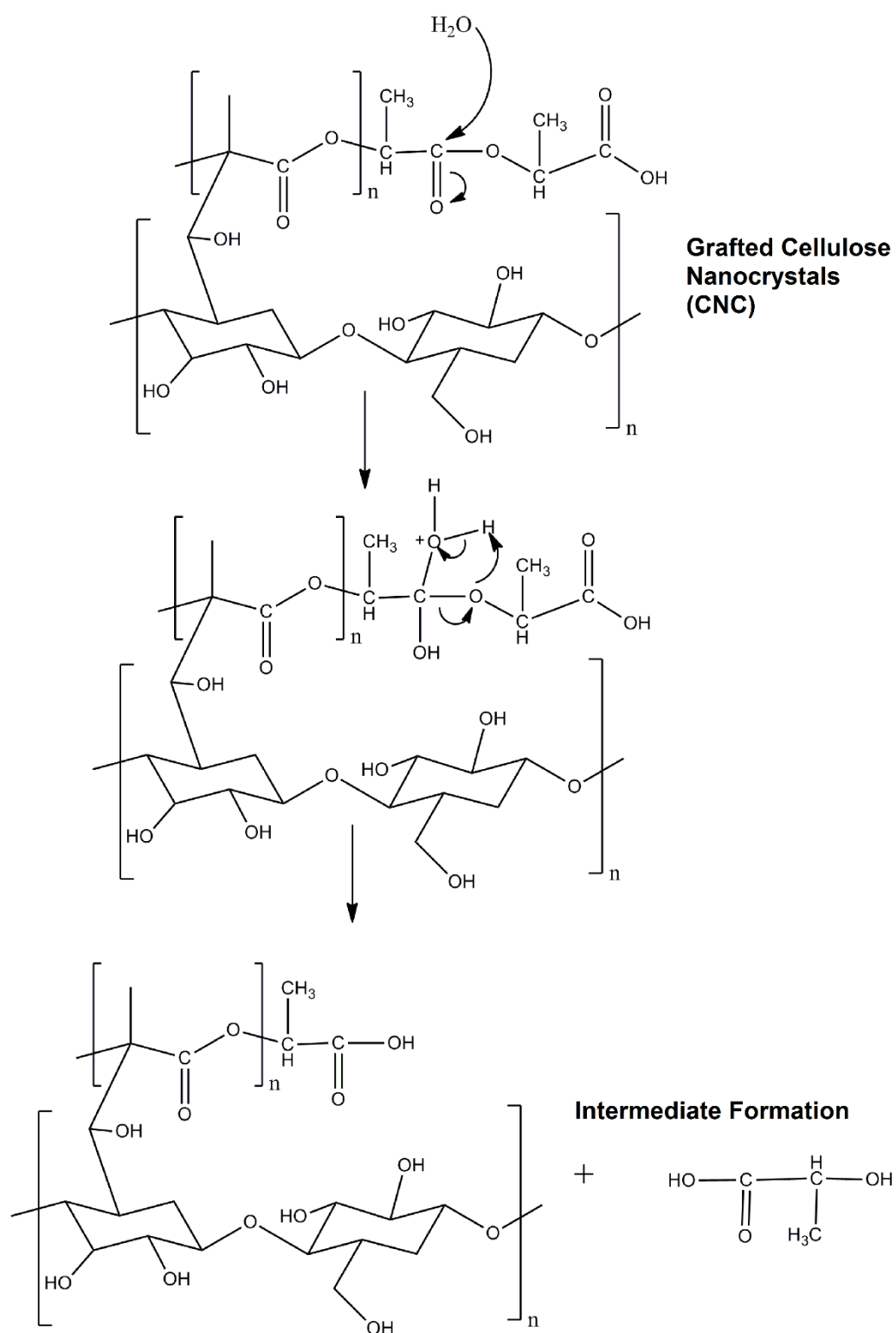


Figure 4.16 (c): Degradation mechanism of PLA/CNC test sample under composting condition.

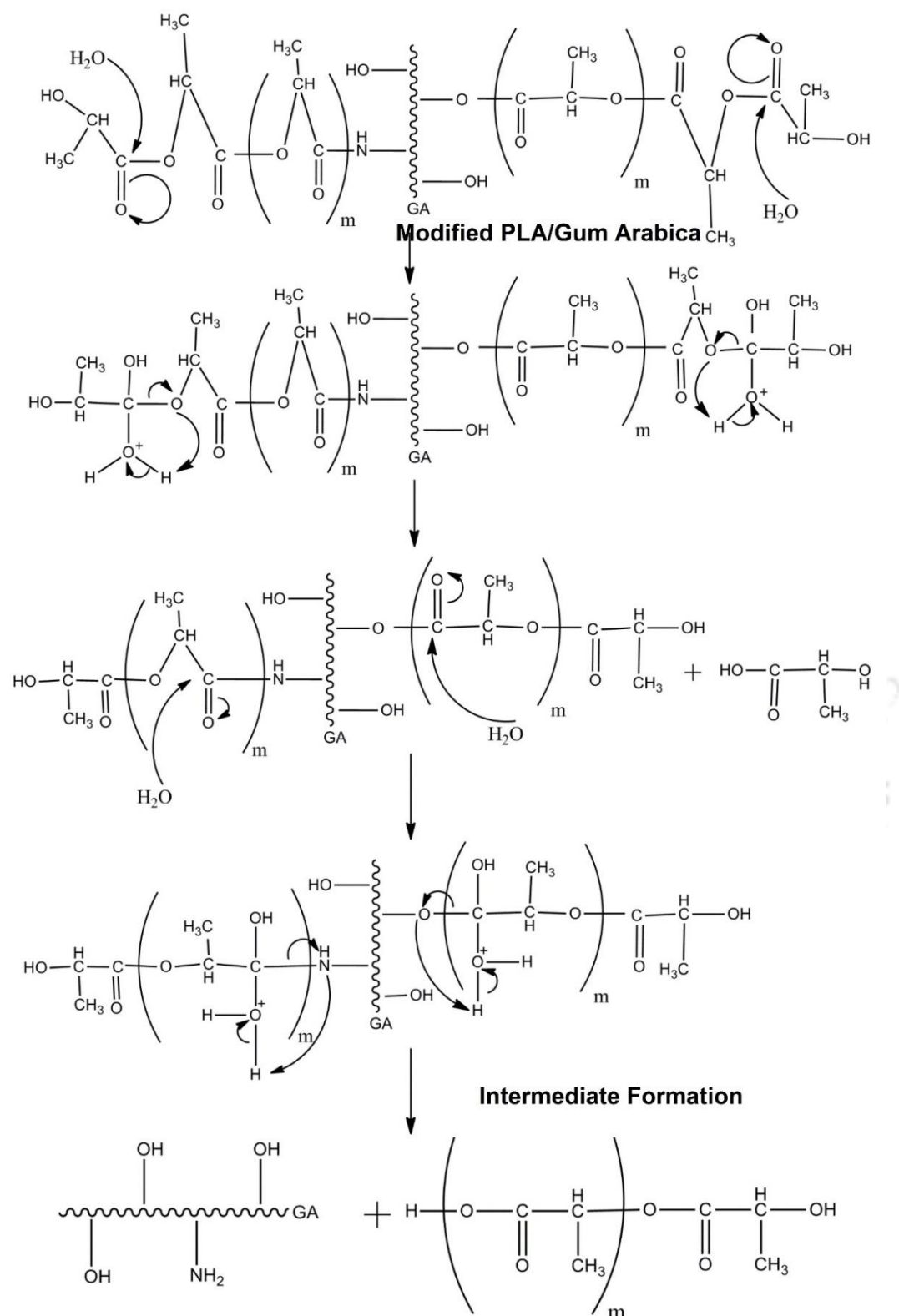


Figure 4.16 (d): Degradation mechanism of PLA/Gum test sample under composting condition.

4.3 CONCLUSIONS

The present study successfully demonstrates the biodegradability of PLA/Chitosan, NPLA, PLA/CNC and PLA/Gum, whose percentage biodegradation were observed to be 97%, 94.2%, 88.3% and 80.5%, respectively in almost 120-145 days. The first order reaction kinetics which is in streak with lag-phase, showed the best fitting with experimental datasets. Various kinetic parameters including initial solid carbon fraction (readily, moderately, and slowly hydrolysable carbon fraction), mineralization rate, hydrolysis rate constant (ready, moderate, and slow hydrolysis rate), and lag time have been investigated. Initial solid carbon in NPLA, PLA/Chitosan, PLA/CNC and PLA/Gum consists of readily, moderately hydrolysable carbon fractions and intermediate solid carbon. Whereas, high amount of slowly hydrolysable carbon was present in PLA/CNC and PLA/Gum, which signifies their slow rate of biodegradation as compared with NPLA, and PLA/Chitosan as predicted from the biodegradation model. This study also enhances the possibility of using some modified biopolymers in the biocomposites for sustainable packaging application. Microbial analysis and its 16S-RNA data revealed *Bacillus flexus* was responsible for PLA based biocomposite biodegradation, which was not been reported elsewhere. So, its not necessary to add inoculum before biodegradation if proper and high quality compost is used and might give insights about strains degrading PLA based composites at thermophilic temperature. This investigation further opened the window for single strain degradation of PLA based biocomposites under composting condition with microbes naturally occurring in compost. Morphological analysis revealed cracks and holes caused due to microbial assimilation and release of bio-fillers from the PLA matrix. This kind of sustainable strategy can benefit to satisfy the 90% carbon constituent degradation/biodegradation requirements for the modified biopolymers in a compostable PLA based composites within 180 days.

PLA films containing reinforcements like modified chitosan (PLA/Chitosan), functionalized CNC (PLA/CNC) and modified gum Arabic (PLA/Gum) prepared by melt extrusion, and their biodegradation behaviour under composting conditions were studied and compared with NPLA. The first-order kinetic model was applied to data from GPC analysis, showed PLA/chitosan samples were having good 94.3 % fit, as compared with the other test samples, which confirms hydrolysis or chain scission reaction and had a significant effect on the biodegradation process for microbial assimilation of low molecular weight chains. This possible hydrolysis mechanism of the modified PLA biocomposites was shown for having a better insight of the first sequential step of biodegradation. Microbial colony count shows that PLA/Chitosan sample showed higher 5.12×10^6 cfu/mL of colony formation value as compared to other test samples. This attribute of higher colony formation in PLA/Chitosan sample can be correlated to highest decrease in number average molecular weight of the sample and were never reported elsewhere. The employed contact angle analysis method confirmed the increase in hydrophilicity of the test samples which signifies biodegradation. Peak shifting occurred during FTIR analysis for each test sample and were due to formation of hydroxyl and carboxyl group formation on the film samples. Medium intensity peaks at 1185 cm^{-1} shifted towards 1180 cm^{-1} representing the C-N stretching vibrations corresponding the aliphatic amines functional groups present in PLA/Chitosan samples. This confirms effect of biodegradation on the amine groups. Transparency also decreased from 79 % to mere 3% for NPLA, 50% to 2% for PLA/Chitosan, 70% to 2% for PLA/Gum and 58% to 13% in PLA/CNC samples. TGA analysis further states that NPLA, PLA/Chitosan and PLA/gum samples thermal stability was altered due to degradation under composting condition. Crystallinity percent of NPLA and PLA/Chitosan increased from 25% to 71% and 23% to 48% respectively. Crystallinity percentage increased due to assimilation of the amorphous polymeric chains by the

microbes, which resulted in relative increase of crystallinity of the samples at different time intervals of the biodegradation process. This biodegradation study confirmed that the method implemented for biodegradation is suitable for modified PLA based biocomposites. This study mainly signifies the importance of material characterization and design of experiments for neat PLA and modified PLA films.



Biodegradation behaviour of PLA/LLDPE/MCC biobased poly-blends under controlled aerobic composting conditions by analysis of evolved carbon-dioxide and its characterization

Linear Low density Polyethylene (LLDPE) is one of the most commonly used non-biodegradable plastics as packaging material due to superior elasticity and plasticity. However, it takes hundreds of years to degrade which in turn chocks our drains and oceans. Therefore, its plastics properties can be blending with PLA by melt mixing it in PLA matrix for property improvement as well as pertain some amount of biodegradability. This can help in disintegrate LLDPE much quicker, which takes more time to degrade and chocks drains and ocean floors. Microcrystalline cellulose (MCC) another biodegradable material can incorporated in the matrix for further acceleration of the disintegration process of the polyblend. For this, its biodegradability needs to be understand in order to tune material properties.

Publications:

- Naba Kumar Kalita, Siddharth Mohan Bhasney, Ajay Kalamdhad and Vimal Katiyar, (2020), Biodegradable Kinetics and Behavior of Bio-based Polyblends under Simulated Aerobic Composting Conditions, Journal of Environmental Management, 261, 110211. (IF: 5.647)

Abstract

This study demonstrates the biodegradation of melt extruded PLA based blends. Neat PLA (NPLA), and polyblend composites of PLA/LLDPE having different concentration of MCC samples were analyzed to understand biodegradation behaviour of the blends under composting condition. Biodegradation kinetics revealed that higher content of MCC and PLA accelerates the biodegradation process of the polymeric blends. Gel Permeation Chromatography (GPC) results revealed molecular weight loss of the biodegraded samples due to chain scission and number average molecular weight of the polyblends decreased from 104 kDa to 32 kDa. Moreover, an increase in spherulite growth size and the decrease in spherulite density of the biodegraded samples confirms a decrease in amorphous portion due to microbial assimilation leaving behind the crystalline portion. It was proved by analyzing using Differential Scanning Calorimetry (DSC) results, which showed an increase in percentage crystallinity of the test samples and a decrease in melting peaks of PLA in the polyblends after degradation. This suggests that biodegradation was taking place for all the polyblends. Surface morphological analysis revealed that the samples have undergone surface erosion prior to bulk degradation, until the 90th day of the biodegradation process and PLA forming fibril-like structure after degradation. Based on the current investigation it can be concluded that these biobased blends can undergo upto 50-80 % biodegradation. This study is helpful in designing and preparation of biobased commercial blends.

5.1 Introduction

Biodegradability is gaining importance in plastic industry due to increasing environmental concerns. Cost of biodegradable plastics is high and its use is limited to developed countries. Poly Lactic Acid (PLA), a potential biodegradable thermoplastic, is comparable with its conventional counterparts (Dhar et al., 2016b; Gupta et al., 2018). Conventional polymers like Linear Low Density Polyethylene (LLDPE), Polyethylene (PE), Polypropylene (PP) are very cheap and extensively used for packaging as well as daily household goods (Siracusa et al., 2008). Due to high cost of the bioplastics, one needs to find alternatives in order to reduce the overuse of conventional plastics. Blending is one such option for reducing cost of bioplastics. Blending conventional polymers with bioplastics may help in improve property of the bioplastic based blends. Recent research studies suggest that blending bioplastics with conventional plastics like LDPE and LLDPE etc., are a growing trend. Although their biodegradability, remains a key issue (Bhasney et al., 2018; Trongsatitkul and Chaiwong, 2017).

Biodegradable polymers are totally or partially biodegradable under certain conditions, but their biodegradable properties might change during blending with conventional polymers. Biodegradation of plastics according to ASTM International (ASTM D-5338-15) standard, is a process in which 90 percent of the carbon content of the individual plastic material is converted into CO₂, water and heat. Composting is the process through which biodegradation of biopolymer/blends of conventional and biopolymers can be studied under certain controlled conditions. Though composting is an end-use technology, basically it is used for producing organic manure. Biodegradation of biopolymers has been demonstrated via composting under controlled condition by following international standards such as ASTM D-6400, International Organization for Standardization (ISO 14855-2). Organizations like ASTM International and ISO has maintained certain basic

protocols for terming plastics as biodegradable, which is followed by researchers as well as industries. These standards allow conducting biodegradation experiments under various environments and controlling various composting parameters. Parameters such as moisture, heat, humidity, volatile solids, carbon content of the substrate are necessary to control during the composting process (Astm, 2014; Kalita et al., 2019).

Biodegradation of Poly(lactic acid) PLA follows two sequential steps: hydrolysis followed by biodegradation. During hydrolytic degradation of PLA, degradation occurs by water molecules through random chain scission, reducing molar mass which subsequently leads to low molecular weight chain formation (as low as <20000 Da). It is noteworthy to be mentioned that low molecular weight to middle molecular weight polymer chains are required for microbial action, whereas, these intermediates are assimilated by microbes for energy and decompose the short polymer chains into various compounds like CO₂ and water (Pradhan et al., 2010a; R. babu Valapa et al., 2016). Studies have been carried out on various biocomposites, based on PLA during the composting process, using various analytical techniques (Rudeekit et al., 2008; Weng et al., 2010). Large number of analytical techniques are used to measure the CO₂ evolution for assessing percentage biodegradation of PLA and its derivatives materials under different environments which includes direct measurement respirometry (DMR), non-dispersive infrared (NDIR), cumulative measurement respirometry (CMR) titration etc. (Kale et al., 2007c; Leejarkpai et al., 2011; Stloukal et al., 2015). Among them CO₂ evolution measurement is a very distinct technique for measuring biodegradability. In one of our previous work, we used Gas Chromatography as a tool for measuring the evolved CO₂ for predicting biodegradation. This technique is very precise in measuring aerobic biodegradation of test materials for various PLA based biocomposites.

Our present study demonstrated a new class of melt extruded PLA/LLDPE based blends whose biodegradation activity has never been reported under thermophilic phase of the composting process and comparing its activity with NPLA. The outcome of the test revealed compostability of the blends with the compost. In this study melt extruded films of Neat PLA (NPLA), PLA/LLDPE, PLA/LLDPE/ 1 % MCC, PLA/LLDPE/ 3 % MCC and PLA/LLDPE/ 5% MCC were used.

5.2 Results and Discussions

All the test samples used in this study is presented in **table 5.1**.

Table 5. 1: Composition of the polyblend composites

Sample designation	PLA (g)	LLDPE (g)	MCC (g)
NPLA	10	0	0
PLA/LLDPE	8	2	0
PLA/LLDPE/1% MCC	7.92	1.98	0.10
PLA/LLDPE/3% MCC	7.76	1.94	0.30
PLA/LLDPE/5% MCC	7.60	1.90	0.50

5.2.1 Biodegradation test

Elemental analysis showed almost 50 % organic carbon for all the test samples and 40% organic carbon content (OC) for the compost. Aerobic composting tests were maintained at 58 ± 2 °C and 60% RH condition. The standard cellulose films degraded more than 90% within 37 days which confirms the liability of our composting setup according to ASTM D5338-15. **Figure 5.1** representing before and after biodegradation samples. It can be seen that after biodegradation, colour of the test samples changed from opaque white to brown in colour. This is mainly due to diffusion of soil, enzymes and moisture in the samples.

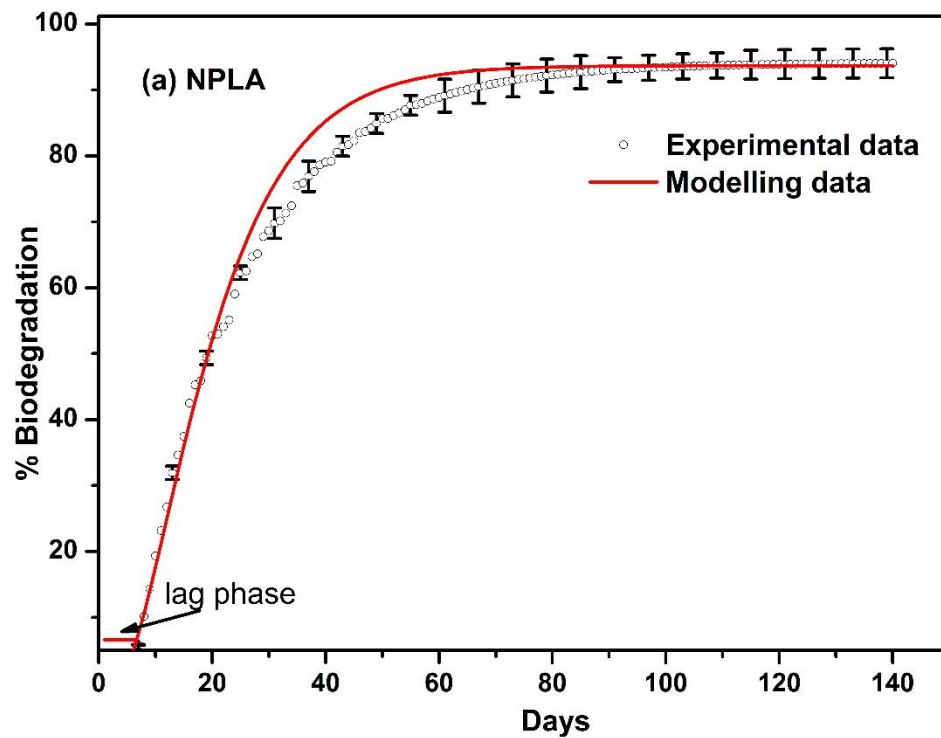
Percentage biodegradation measured using CO₂ evolution test results for samples namely PLA/LLDPE/ 5 % MCC, NPLA, PLA/LLDPE/ 3 % MCC, PLA/LLDPE/ 1 % MCC and PLA/LLDPE are presented in figure 5.2 (a-e). From the % biodegradation test results it can be seen that NPLA got more than 90% biodegraded under paper industry sludge compost. PLA/LLDPE/ 5 % MCC showed almost 80% degradation in 140 days, which is higher than PLA/LLDPE/ 3 % MCC which was having degradation of 75 % in 140 days. PLA/LLDPE showed only 50% degradation in 140 days of time, whereas, MCC, PLA/LLDPE/ 1 % MCC showed almost 70% biodegradation.



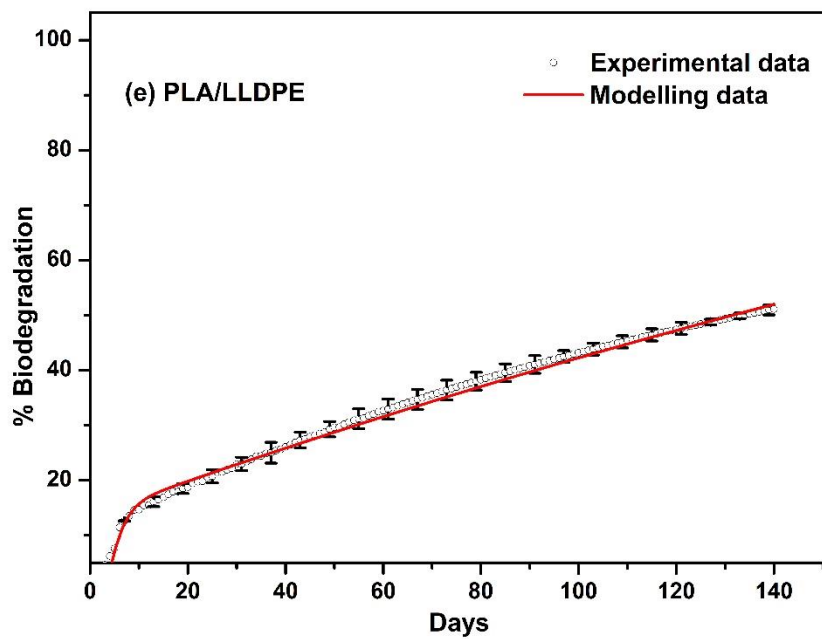
Figure 5. 1: Laboratory composting setup used for biodegradation study and the samples that were tested under the system.

This clearly insists that increase in MCC % in the test samples accelerated biodegradation, whereas, less MCC amount in all the polyblends indicated less degradation. Here, it's the degradation of PLA and MCC which induced biodegradation and not LLPDE, since LLDPE showed almost negligible amount of carbon mineralization. From biodegradation percentage results it can be observed that under 140 days the samples showed following trend for biodegradation: NPLA > PLA/LLDPE/5 % MCC > PLA/LLDPE/ 3 % MCC >

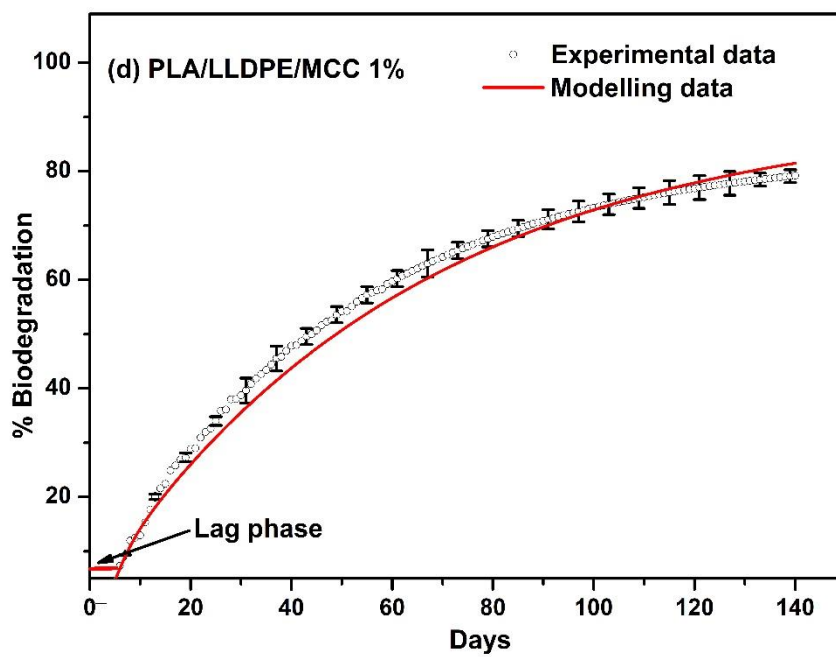
PLA/LLDPE/ 1 % MCC > PLA/LLDPE. From this results it is clear that biobased PLA based blends can attain almost 50-60% biodegradability if mixed with conventional polymers which would reduce carbon footprint in the environment.



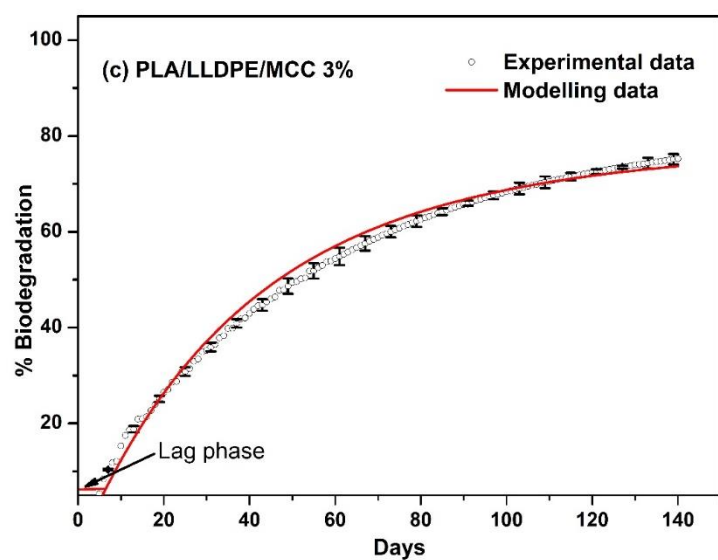
(a)



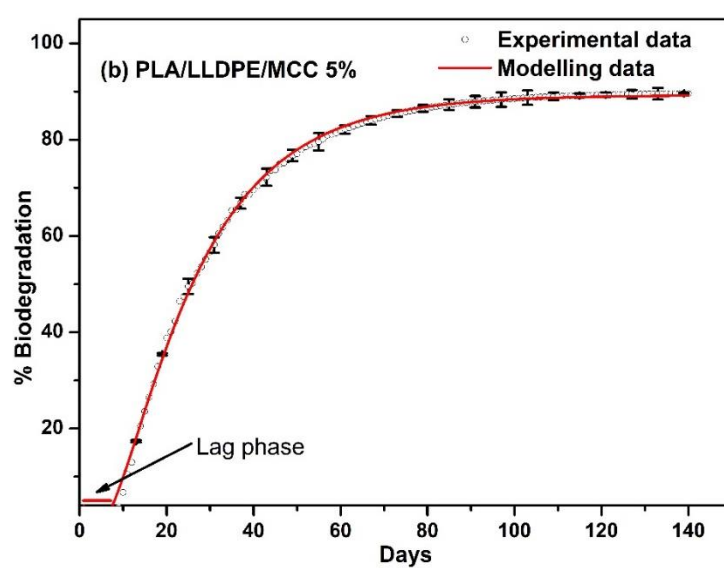
(b)



(c)



(d)



(e)

Figure 5. 2(a-e): Kinetics of percentage biodegradation of (a) NPLA (b) PLA/LLDPE (c) PLA/LLDPE/ 1 % MCC, (d) PLA/LLDPE/ 3 % MCC and (e) PLA/LLDPE/ 5 % MCC.

As the entire chain is not identical in its affinity towards hydrolysis, the primary chain is assumed to be composed of three different kinetic forms of solid carbon, which are rapidly, moderately and slowly hydrolysable, according to the biodegradation kinetics governing equation. Results showed a faster rate of biodegradation for NPLA biocomposite films when compared with the other test samples from data analysis of CO₂ evolution or biodegradation percentage. From model data analysis the percentages of CO₂ evolution for all the samples tested, ranged from 90% to ~70% in 140 days, signifying that the materials NPLA, PLA/LLDPE/ 5 % MCC, PLA/LLDPE/ 3 % MCC are suitable for industrial composting due to the rate of their decomposition. Ready biodegradation in NPLA and PLA/LLDPE/ 5 % MCC may be due to the initial amount of Cr_0 leading to faster rate of biodegradation, as observed from modelled data and shown in **table 5.2**. Amount of Cr_0 for test samples PLA/LLDPE/ 1 % MCC and PLA/LLDPE was very low, due to which ready biodegradation was also low, although the ultimate mineralization rate was almost same for all the test samples as shown in **table 5.2**. Higher amount of Cm_0 & Cs_0 hinders biodegradation which is obvious from the data obtained from the modelled data and shown in **table 5.2**. Lower rate of biodegradation can also be attributed to the lower rate constant value k_r for test samples PLA/LLDPE/ 1 % MCC and PLA/LLDPE. From the modelled data analysis, it can be observed that with addition of MCC component, mineralization rate increased. So addition of MCC in biobased samples can increase the rate of biodegradation and biodegradability of the test samples which is a cost effective approach in making biobased polymeric blends.

Table 5. 2: Kinetic model parameters and coefficient of determination (R^2).

Sample	PLA/LLDPE/ 5 % MCC	NPLA	PLA/LLDPE/ 3 % MCC	PLA/LLDPE/ 1 % MCC	PLA/LLDPE
k_{aq}	1	1	0.99	1	0.99
k_r	0.04	0.05	0.02	0.02	0.007
k_m	0.04	0.04	0.02	0.02	0.006
k_s	0	0	0	0	0
Cr_0	40.17	42.79	36.78	35.72	31.52
Cm_0	44.49	48.38	38.42	37.06	31.97
Cs_0	10.18	5.88	16.43	18.01	24.04
Caq_0	5.14	2.94	8.35	9.19	12.45
Lag Phase	8	5	4	4	4
R^2	99.87	99.9	99.81	99.87	99.92

5.2.2 Molecular weight analysis

To prepare the samples for molecular weight analysis the test samples were at first dipped in chloroform overnight. The results are basically the molecular weight of PLA that dissolved in chloroform mixed with the LLDPE. From the molecular weight analysis data, it is clear that PLA matrix got degraded under the composting condition which is due to its biodegradability. It was observed that blending PLA with conventional polymer doesn't affect its biodegradability. Since, there was significant molecular weight loss during the degradation period which is evident from the **figures 5.3 (a-b)** where peaks become broad and less intense with decrease in retention time for the samples, suggesting degradation due to chain scission mechanism (Musioł et al., 2016). Molecular weight loss also suggests feasibility of the blend since it can reduce carbon footprint as well as biopolymer cost.

Molecular weight of the samples before and after degradation is shown in **table 5.3**. It is important to mention that the molecular weight analysis was targeted to be done in case of PLA but not the LLDPE. Hence, chloroform being non-solvent of LLDPE at room temperature will remain undissolved in the form of microparticles and can be filtered out easily.

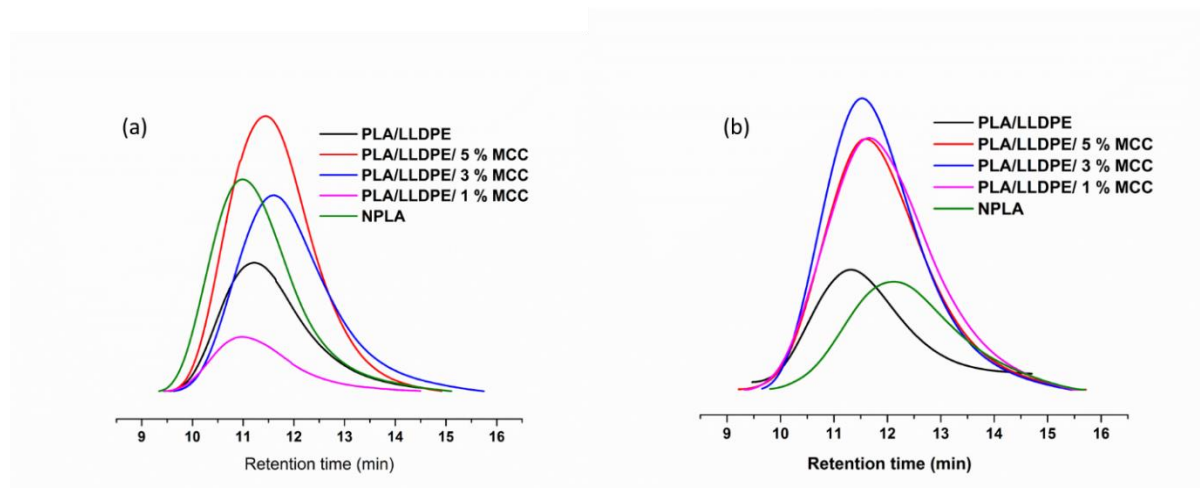


Figure 5. 3 (a-b): Number average molecular weight analysis with retention times for samples (a) before biodegradation and (b) after biodegradation.

Table 5. 3: Number average molecular weight analysis with retention times for samples) before biodegradation and after biodegradation

MOLECULAR WEIGHT (KDA)		
SAMPLES	Before biodegradation	After Biodegradation
NPLA	104	32
PLA/LLDPE/ 5 % MCC	75	21
PLA/LLDPE	74	45
PLA/LLDPE/ 3 % MCC	87	38
PLA/LLDPE/ 1 % MCC	73	41

5.2.3 Polarizing optical microscopy (POM)

POM was done to understand the spherulite density and spherulite size of the samples before and after biodegradation. There was a significant increase in spherulite size in case of NPLA after biodegradation than in NPLA samples before biodegradation as shown in **figure 5.4**. Spherulite size observed during 30-minute study had increased from $\sim 30\mu\text{m}$ in initial sample to $\sim 150\mu\text{m}$ in degraded as shown in **figure 5.5 (a-b)**. This increase in spherulite size in degraded samples may be attributed to the space between the spherulites, which contained amorphous material and also attached compost within the polymer that were unable to crystallize. After biodegradation of the amorphous portion, crystalline region got more space for chain folding, chain mobility and spherulite formation of bigger diameter than the initial samples. Formation of large spherulites after biodegradation can also be attributed to longer chains of higher molecular weight PLA left behind as crystalline portion, due to microbial assimilation of medium and shorter chains. Moreover, crystallization temperature plays a crucial role in spherulite growth and density change. The spherulite growth rate also decreases due to decrease in nucleation density leading to larger spherulite size making it difficult for chain motion and therefore alignment and ordering (Owen, 2013). This phenomenon can be understood well from the DSC data where T_c is decreasing for all the biodegraded samples, leading to decrease in spherulite density as shown in **figure 5.6**. This analysis helps in understanding that microbes were readily degrading the amorphous region leaving behind crystalline region which leads to increase in crystal size and decrease in crystal density. Here, lamella formation occurs due to joining of the molecular chains to the nuclei, which changes the dimensions of the lamella causing increase in spherulite diameter. For all the test samples similar results were obtained.

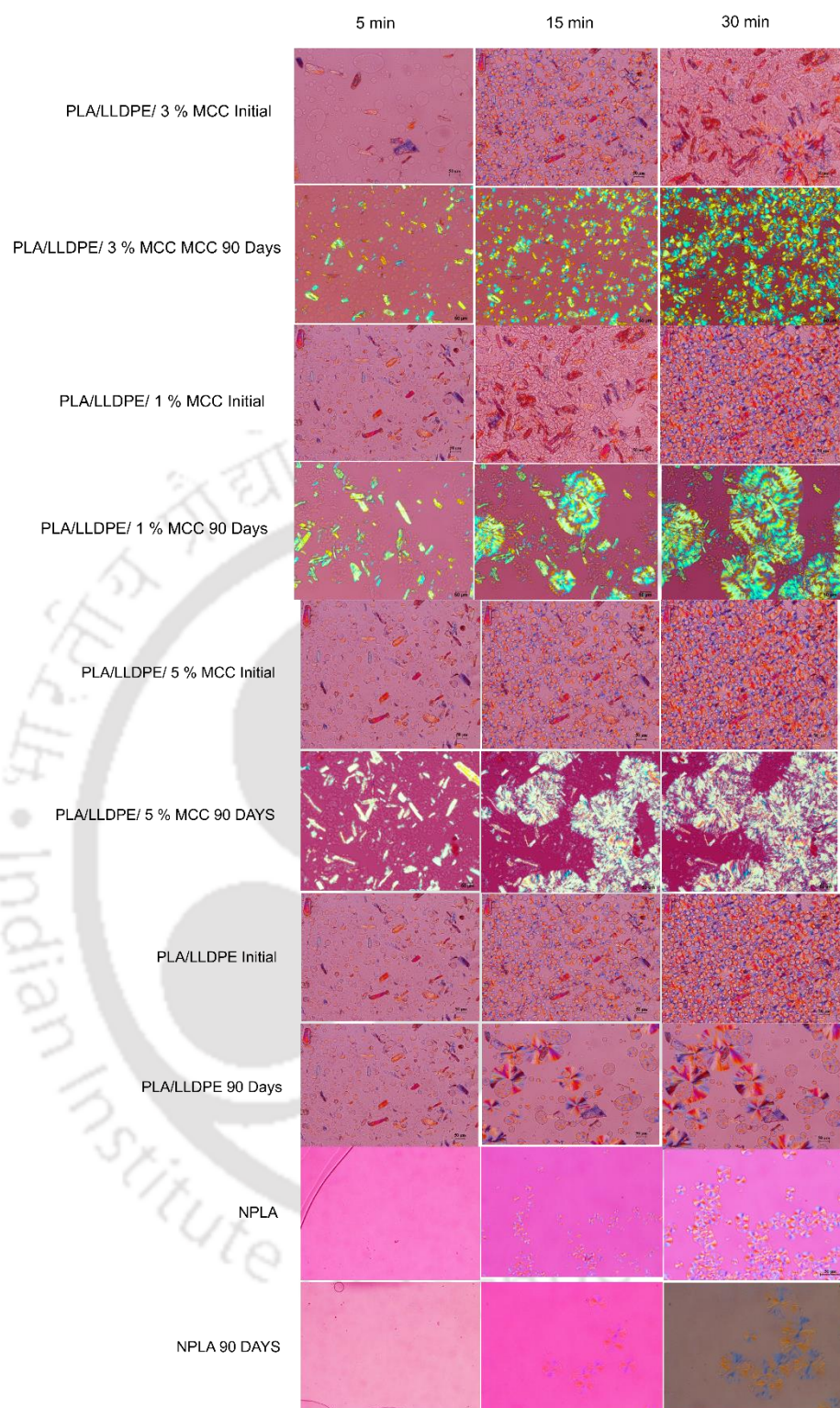


Figure 5. 4: Polarizing optical micrographs of the samples done at 120 °C before biodegradation and 100 °C after biodegradation.

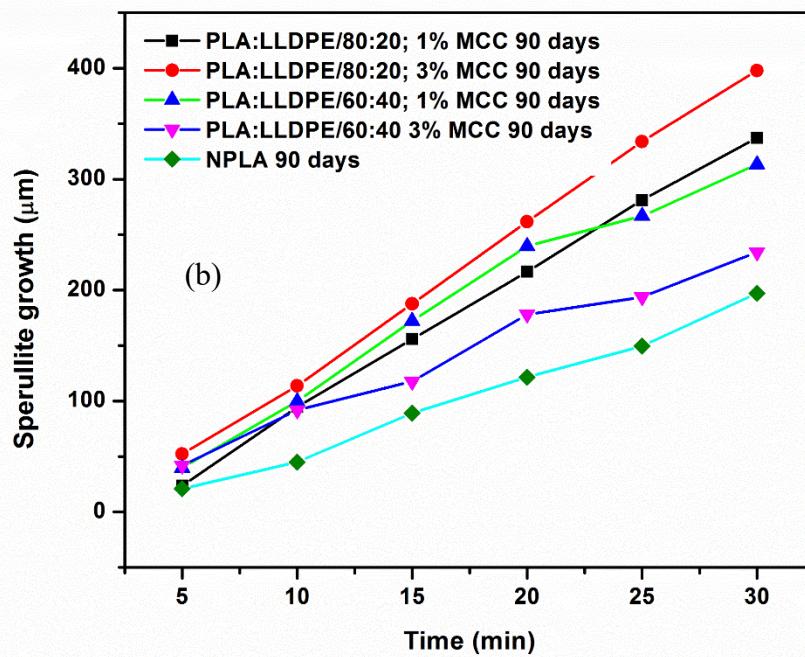
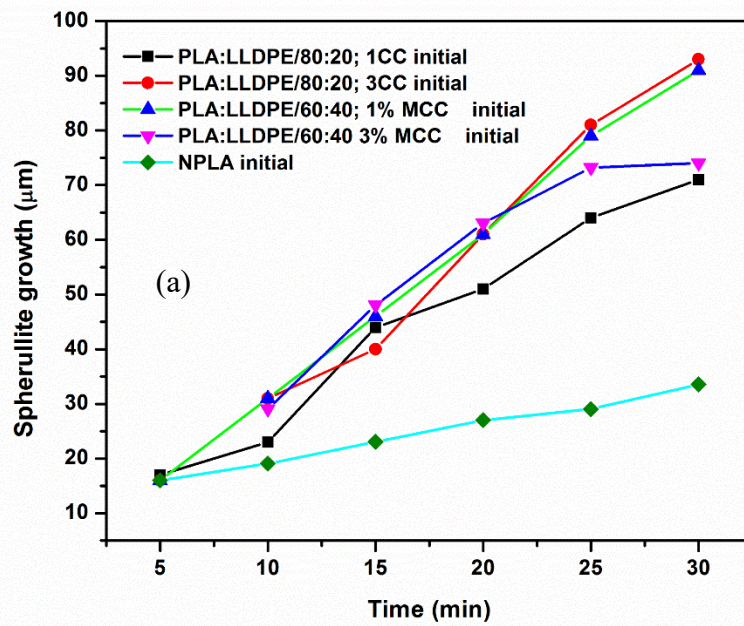


Figure 5. 5 (a-b): Spherulite diameter growth (a) before biodegradation and (b) after biodegradation.

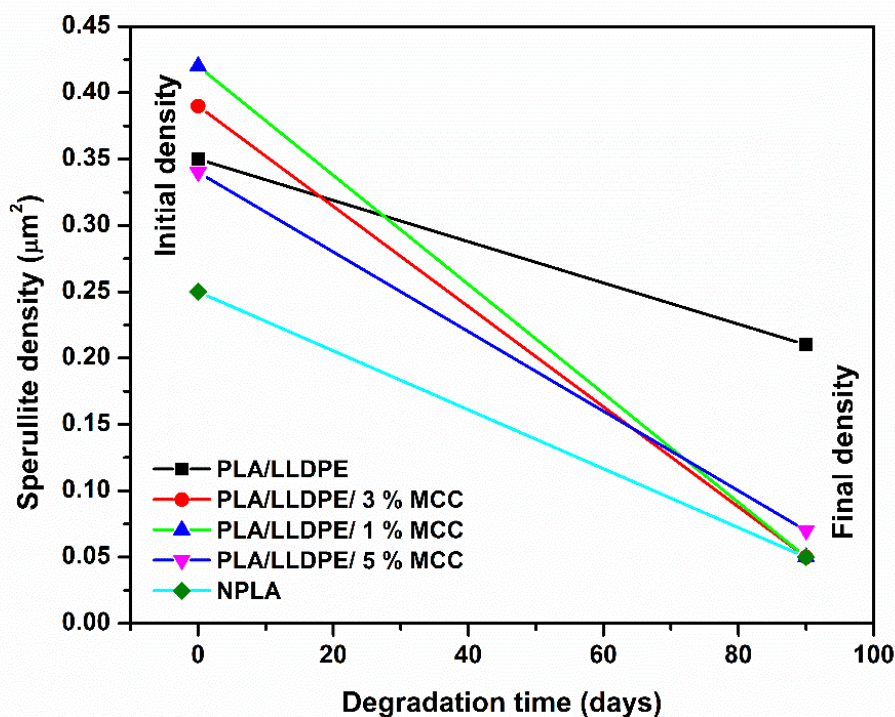


Figure 5. 6: Spherulite density shown before biodegradation (initial density) after biodegradation (final density) for all the test samples.

5.2.4 Differential Scanning Calorimetry (DSC)

Thermograms of samples before and after biodegradation is shown in **figure 5.7. (a-b)**. All the test samples were studied for 90 days which revealed significant difference after biodegradation. The NPLA and PLA component in the polyblends loses the capacity to crystallize and melt (**Figure 5.7. (a)**) during the second heating cycle. With increase in biodegradation time, percentage crystallinity ($X\%$) also increased as shown in **table 5.4**. This happens because amorphous portions of the test samples were biodegraded leaving behind only the crystalline portion (Pantani and Sorrentino, 2013) as shown previously from our POM results.

Table 5. 4: Percentage crystallization of the test samples before and after biodegradation

	Before Biodegradation (X%)	After Biodegradation (X%)
NPLA	~7	~27
PLA/LLDPE/ 3 % MCC	~16	~31
PLA/LLDPE/ 5 % MCC	~14	~38
PLA/LLDPE/ 1 %	~23	~40
PLA/LLDPE	~20	~30

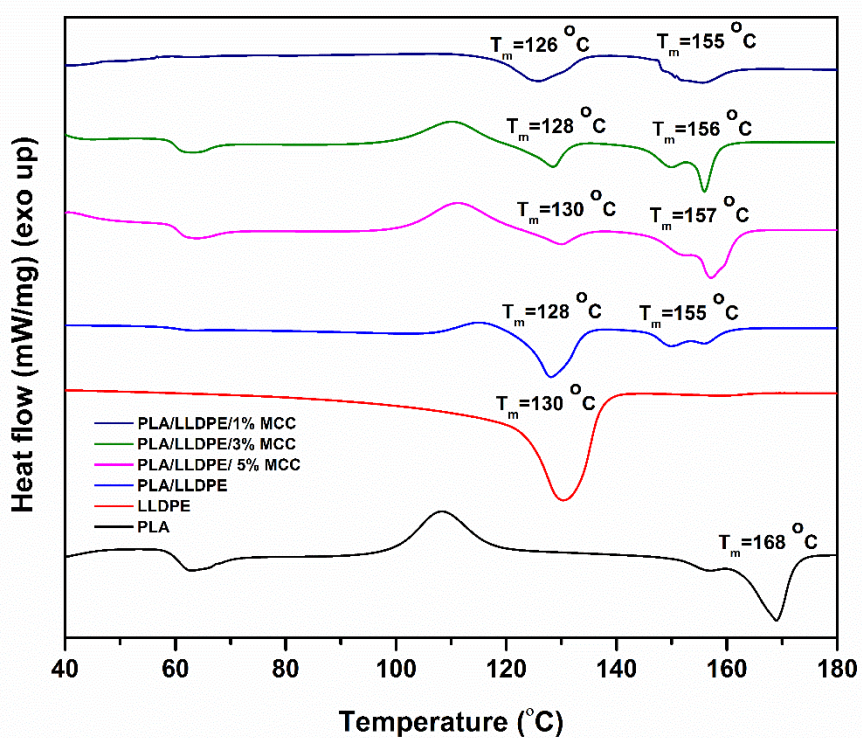


Figure 5.7. (a): DSC thermograms before biodegradation

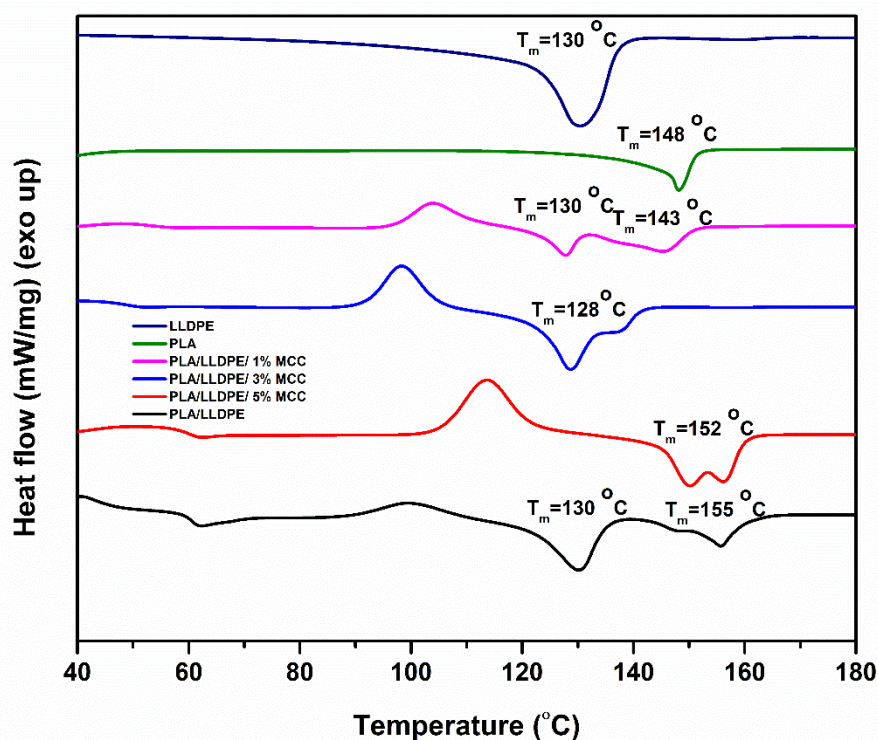


Figure 5.7. (b): DSC thermograms after biodegradation.

Samples having higher percentage of PLA and MCC tends to degrade faster as seen from our kinetic results, which can be further proved from our percentage crystallization values as shown in **table 5.4**. From the DSC thermograms it can be seen that melting point of the samples decreased gradually which suggests biodegradation. From the DSC thermograms it can be noticed that the melting peak of LLDPE in the PLA/LLDPE/ 5 % MCC, PLA/LLDPE/ 3 % MCC, PLA/LLDPE/ 1 % MCC and PLA/LLDPE samples didn't undergo significant decrease in melting temperature as biodegradation progressed. This indicates LLDPE doesn't degrade during biodegradation. With decrease in molecular weight, the melting temperature also got decreased significantly which suggest biodegradation of the test samples (Stloukal et al., 2015). Similarly, for all the test samples recrystallization temperature decreased significantly during the course of biodegradation.

5.2.5 Morphological analysis

Surface morphology of the test samples are shown in **figure 5.8.1(a-j)**. FESEM was done in order to understand the surface morphology of all the test samples before and after biodegradation. This morphological changes were observed on the 90th day of the composting process. Large holes and cracks formation were observed on the polymer surface over the time of biodegradation which can be attributed to the action of microbes or biofilm formation on the surface of the polymers and diffusion of the cells inside the polymer. This can be attributed to surface erosion, on the PLA based biocomposites characterized by the presence of cavities (Arrieta et al., 2014; Leejarkpai et al., 2011). Water (moisture inside the CV) diffuses into the amorphous domains which initiates the hydrolytic degradation. With progress in the hydrolytic degradation process the fillers tends to move away from the matrix towards the compost resulting in holes, cavities and cracks which was observed on the 90th day. In the **figure 5.8.1(a-j)** surface morphology reveals how PLA is getting degraded where LLPDE is attached in cavities after the PLA forming fibril like structure in the sample. This suggests biodegradation through surface erosion scheme. Similar behavior was also observed for all the test samples except neat PLA samples which indicates formation of fibrils under degradation process. From TEM analysis of couple of the samples (**figure 5.8.2 (a-d)**) namely, PLA/LLDPE/ 1 % MCC and PLA/LLDPE/ 1 % MCC it can be seen that after biodegradation, PLA gets degraded rapidly forming voids as shown in **figure 5.8.2 (c)**, leaving behind LLDPE which were embedded under the PLA matrix (Bhasney et al., 2018) before biodegradation. From TEM analysis it's clear that after the biodegradation of the biobased blend LLDPE remains with the remaining PLA matrix and also suggests that this kind of biobased blends are compostable under certain environmental conditions.

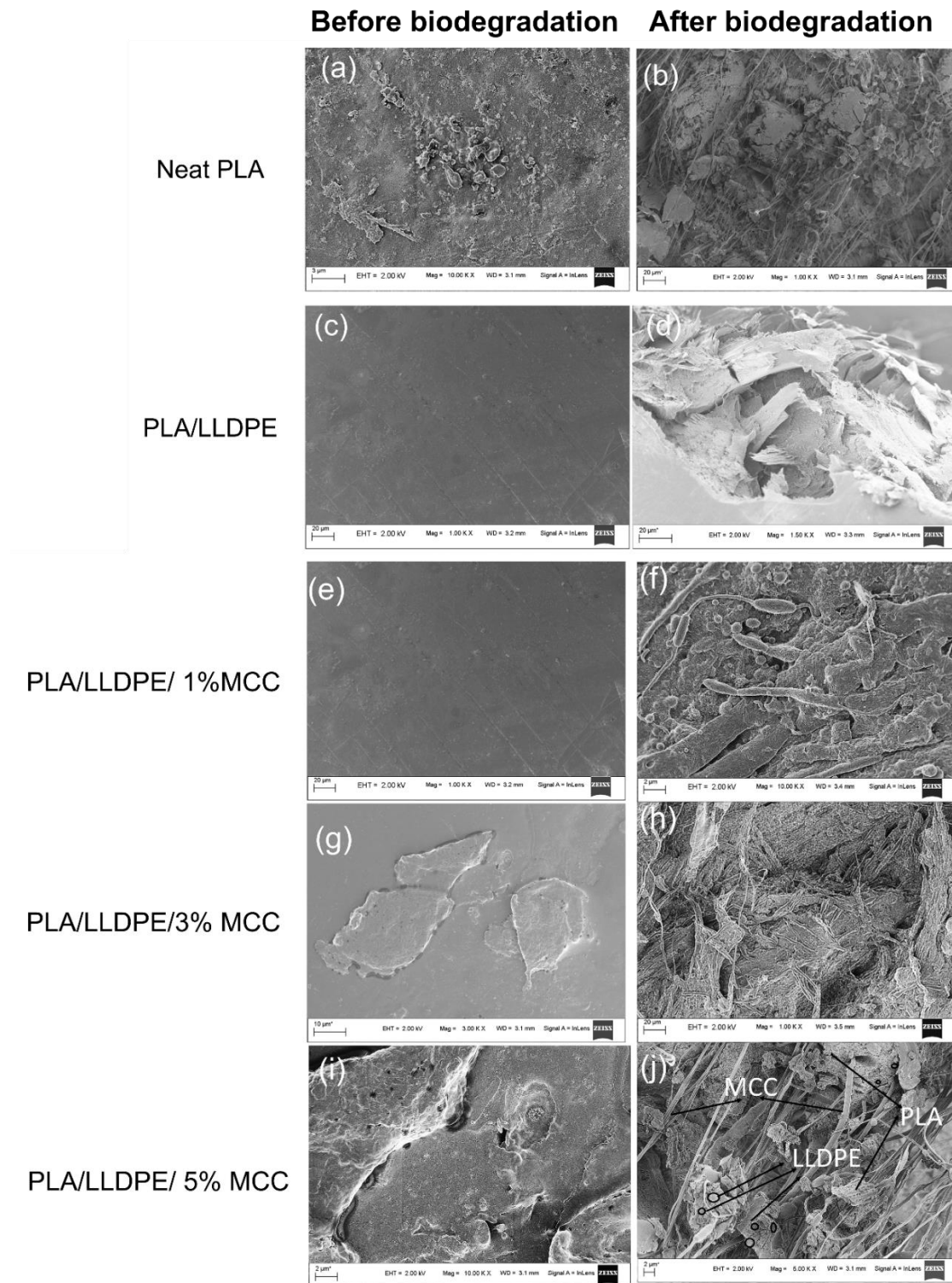


Figure 5.8.1(a-j): FESEM images of the test samples before and after biodegradation (90th day).

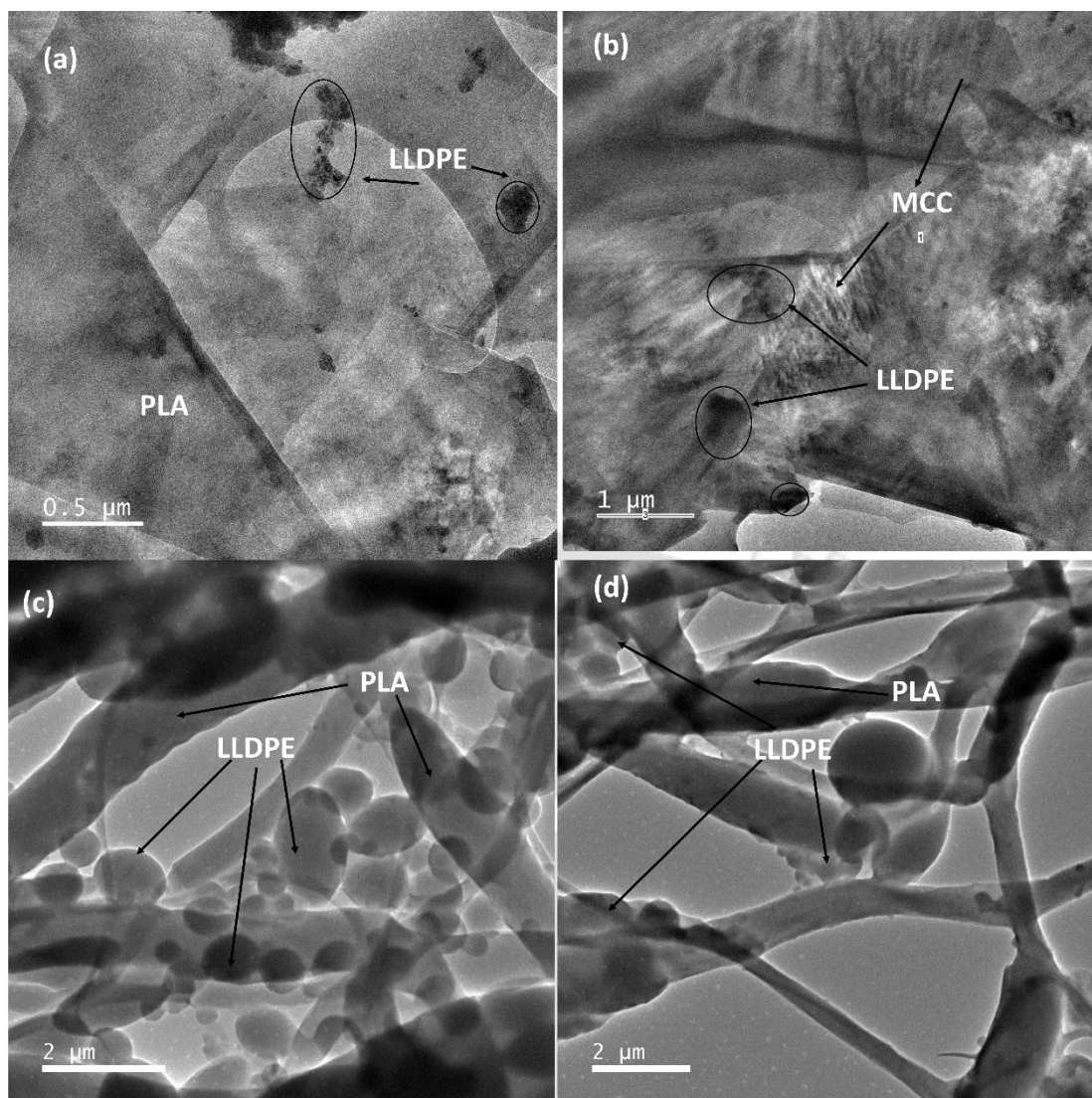


Figure 5.8.2 (a-d): TEM image of the samples (a) PLA/LLDPE/ 1 % MCC before biodegradation (b) PLA/LLDPE/ 5 % MCC before biodegradation (c) PLA/LLDPE/ 1 % MCC after biodegradation (d) PLA/LLDPE/ 5 % MCC after biodegradation.

5.2.6 Water contact angle analysis

The blend matrix before and after biodegradation was observed for wettability changes occurring on the surface of the polyblends which underwent biodegradation. It was found that all the test samples hydrophilicity decreased for all the test samples with increase in degradation time. This behavior is attributed to formation of carboxylic and hydroxyl group on the blend surface as well as the presence of cavities on the PLA matrix leading to

hydrophilicity and higher chain mobility which leads to microbial assimilation of the surface. Water contact angle of NPLA decreased from 78 ± 5 to 50 ± 2 . Similar decrease in contact angle was observed for all the test samples as depicted in **figure 5.9 (a-j)** and shown in **table 5.5**.

Table 5. 5: Water contact angle of samples before and after biodegradation.

Sample	Before biodegradation Water contact angle (°)	After biodegradation Water contact angle (°)
NPLA	78 ± 5	50 ± 2
PLA/LLDPE/ 5 % MCC,	66 ± 4	54 ± 7
PLA/LLDPE/ 3 % MCC	69 ± 3	55 ± 5
PLA/LLDPE/ 1 % MCC	70 ± 2	39 ± 4
PLA/LLDPE	81 ± 3	69 ± 5

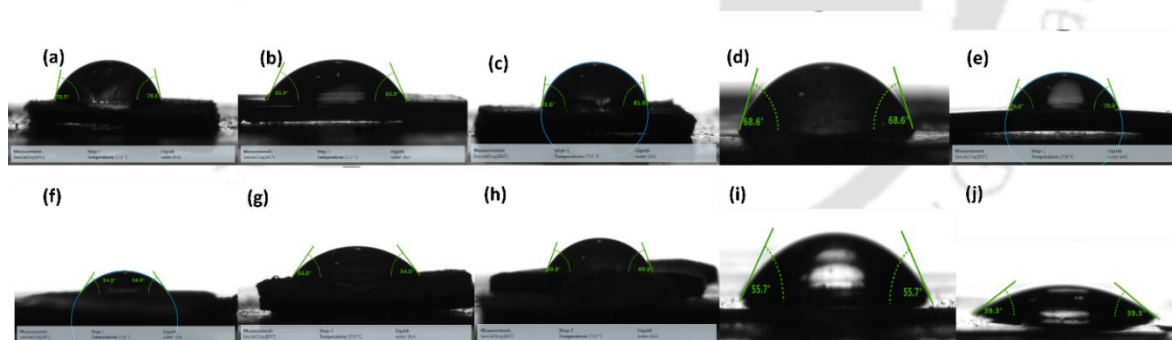


Figure 5.9(a-j): Water contact angle analysis of samples namely (a) NPLA, (b) PLA/LLDPE/ 5 % MCC, (c) PLA/LLDPE (d) PLA/LLDPE 3 % MCC (e) PLA/LLDPE/ 1 % MCC before biodegradation respectively and water contact angle analysis of samples namely (f) NPLA, (g) PLA/LLDPE/ 5 % MCC, (h) PLA/LLDPE, (i) PLA/LLDPE / 3 % MCC (j) PLA/LLDPE/ 1 % MCC after biodegradation.

5.3 Conclusion

Extruded PLA/LLDPE/ 5 % MCC, PLA/LLDPE/ 3 % MCC, PLA/LLDPE/ 1 % MCC, PLA/LLDPE and NPLA blends were analysed for biodegradation study. Biodegradation percentage results showed that with higher MCC and PLA component in the test samples biodegradation was higher as compared to lower percentage of PLA and MCC in the blended test samples. Kinetic data revealed that higher amount of Cr_0 hinders biodegradation and lower rate of biodegradation can be attributed to the lower rate constant value k_r for test samples PLA/LLDPE/ 1 % MCC and PLA/LLDPE. From modelled data, it was observed that with addition of MCC mineralization rate increased for test samples, which signifies that addition of MCC accelerates the rate of biodegradation in the prepared blends. Decrease in molecular weight analysis proved that chain scission mechanism occurred leading to formation of intermediates, which further attributes to microbial assimilation. Microbes attacks the amorphous region leaving behind the crystalline portion which was proved by using POM studies which showed increase in spherulite size and spherulite density due to spaces formed after erosion of the amorphous region from the blend surface. Thus, cavities formed were observed using FESEM which revealed PLA forming fibril like structures due to degradation under compost. From TEM analysis its clear that after the biodegradation of the biobased blend LLDPE remains with the remaining PLA matrix and also suggests that this kind of biobased blends are compostable under certain environmental conditions. These disorientations due to biodegradation leads to decreasing melting peaks in the blends and decreasing recrystallization temperature which was observed from the DSC thermograms. DSC thermograms further revealed the increase in percentage crystallinity of the test samples due to microbial assimilation of the amorphous portion leaving behind the crystalline portion. Water contact angle study suggested breakage of the polar carbonyl group and formation of hydroxyl and carboxyl

group thus increasing hydrophilicity of the sample surface and facilitating biodegradation. Results obtained signifies that PLA based biobased blends prepared are almost 50-80% biodegradable and it may reduce cost and carbon footprint. This tests will also help in material characterization and preparation of the test samples.





Biodegradation behaviour of PLA/PCL/MCC based blends under controlled aerobic composting conditions by analysis of evolved carbon-dioxide and its characterization

Poly(lactic acid) PLA is a linear aliphatic biodegradable polyester, compostable under industrial composting condition in 180 days of time. PLA is highly brittle and have low elasticity with high melting point of 180 °C and high molecular weight PLA is not biodegradable under natural environment in short span of time (180 days). Whereas, PCL another linear polyester with low melting point (60 °C) and high impact properties is used extensively for various biomedical applications and in production of polyurethanes. PCL is also biodegradable under natural environment condition. Both these polymer can be blended in desired quantities in order to achieve better properties and to achieve higher biodegradability. Microcrystalline cellulose (MCC) a thermally stable and highly biodegradable material can be used as filler for increasing its biodegradability.

Publications:

- Naba Kumar Kalita, Siddharth Mohan Bhasney, Chethana Mudenur, Ajay Kalamdhad and Vimal Katiyar, (2020) End-of-life evaluation and biodegradation of Poly (lactic acid)(PLA)/Polycaprolactone (PCL)/Microcrystalline cellulose (MCC) polyblends under composting conditions, Chemosphere, 247, 125875. (IF: 5.778)

Abstract

The present study evaluates biodegradation of the polyblends of poly(lactic acid) (PLA), polycaprolactone (PCL) and microcrystalline cellulose (MCC) in different compositions and comparison of the properties of those blends with that of neat PLA (nPLA) and neat PCL (nPCL). The samples were melt extruded and blended into 0.816 mm strips to evaluate the environmental fate of the polyblends under simulated composting conditions following ASTM International D5338-15 protocol. It was found that the blends having a higher concentration of PCL and MCC in the PLA matrix showed higher carbon mineralization percentage compared to the blends having low PCL and MCC components. Molecular weight analysis of the samples showed a decrease in their weight due to chain scission mechanism leading to the formation of intermediates. Analytical techniques used to study biofilm formation revealed the formation of microbial biofilms on the blended biopolymeric surfaces. It was further confirmed by Fourier Transform Attenuated total reflectance (FT-ATR) analysis, which showed the formation of amide bonds (N-H bending) at 1596 cm^{-1} after the 30th day of the study, revealing enzyme attachment on the biopolymeric surfaces of the test samples. Field emission scanning electron microscopy (FESEM) showed the formation of fibril-like structures by PLA, and the formation of rough patches on the PCL surface re-confirmed biodegradation of the samples. Water contact angle and differential scanning calorimetric (DSC) analysis also established the biodegradation of the test samples. Percentage crystallinity of the test samples calculated using DSC showed a rapid rise but decreased gradually after the 60th day of the study. This work fuels the interest in the material characterization of PLA/PCL/MCC based polyblends and tuning the biodegradability of the studied samples according to the demands.

6.1 Introduction

Magnanimous use of conventional plastics has made the world population completely dependable on its commodity products as well as its high performance utility objects. Though plastics has made life easier in many ways, its overuse has left the environment rattled by polluting land, sea, and rivers. Recent developments and research have been mainly focused on the development of biodegradable and compostable polymers to counter these issues affecting our environment. These biopolymers are derived from natural sources and have properties almost similar to conventional polymers (Dhar et al., 2016b; Kalita et al., 2019).

Poly(lactic acid) (PLA), polycaprolactone (PCL), cellulose in various forms like microcrystalline cellulose (MCC), starch, chitosan, etc., are some of the most commonly studied and used biopolymers that have immense potential to replace conventional plastics. Various literature report that the polymers PLA, PCL and cellulose are both biodegradable and compostable, having various applications ranging from packaging to commodity usage (Bhasney et al., 2018; Castro-Aguirre et al., 2018; Selke et al., 2015; Vieira et al., 2010). Although PLA and PCL have been extensively studied, their use is still not popular because these polymers have some disadvantages like high barrier properties, high rigidity as well as low glass transition temperature (T_g), which makes it difficult to use them in specific applications (Dhar et al., 2015; Vieira et al., 2010). Literatures suggest that the undesirable properties of a polymer can be tuned by blending it with another polymer (Bhasney et al., 2019). PCL, a petroleum-derived biodegradable polymer, has enormous potential to replace its conventional counterparts. Similarly, PLA is one of the world's most commercially viable bioplastic. Thus, blending of PLA with PCL along with other biofillers might alter thier properties and affect their end-of-life characteristics under various environmental conditions. Various literature show that industrial composting of bioplastics is one of the

most desirable end-of-life management system for the biopolymers (Pradhan et al., 2010a; Sikorska et al., 2015). Most of the countries in North-America, Europe and Japan have legislation for composting of bioplastics under different environments. Certifying agencies like ASTM International have set rules and various protocols for designating a polymer as biodegradable. According to ASTM International D5338 standard, a polymer can be labeled as compostable only if 90% of its carbon content gets converted into CO₂. This standard further clarifies about the protocols that have to be followed while performing a biodegradation test of the biopolymers under simulated composting conditions (ASTM International, 2015a). Various studies have reported biodegradation of PLA and PCL based biocomposites under simulated composting environments (Pradhan et al., 2010a; Vieira et al., 2011). It was found that PLA based biocomposites having rice husk as fillers showed better biodegradation rate than neat PLA (nPLA) films. Previous researchers have shown that PLA/PCL in a 80/20 ratio had better mechanical properties than nPLA, but its relative biodegradability was only 75% in 110 days (Narancic et al., 2018) compared to that of nPLA which was found to be in the range of 80-90% within 100 days under simulated composting conditions (Kijchavengkul et al., 2009; Leejarkpai et al., 2011; Pradhan et al., 2010b; Stloukal et al., 2015). Moreover, many reports show slow degradation of PLA based composites and films under normal soil conditions, which generates concerns related to its end-of-life management in landfill and soil (A. De Campos et al., 2011; Qi et al., 2017; Weng et al., 2013b). Therefore, composting of bioplastics becomes a necessary end-of-life management tool to be used effectively for environmental management.

In the present study, PLA, PCL and MCC were polyblended in various concentrations to understand their material behaviour under simulated composting conditions. The different samples studied were as follows: Neat PLA (nPLA), neat PCL (nPCL), PLA/PCL : 80/20, PLA/PCL/MCC : 80/20/3, PLA/PCL/MCC : 80/20/1, PLA/PCL : 90/10, PLA/PCL/MCC :

90/10/3, and PLA/PCL/MCC : 90/10/1. These materials were subjected to degradation under simulated composting conditions as per ASTM D 5338 standard. The consequences of biodegradation were examined using various analytical techniques. This study paved the way for material characterization of various blends of PLA/PCL with MCC for numerous commodity applications through biodegradation study. Biodegradability was analyzed by carbon dioxide (CO₂) evolution test (percentage carbon mineralization), molecular weight analysis, manual observation of the films, water contact angle (WCA) analysis, spherulite growth, Fourier transform attenuated total reflection (FT-ATR) spectroscopy, differential scanning calorimetry (DSC) and field emission scanning electron microscopy (FESEM). Furthermore, the assessment of biofilm formation on the film surface revealed the precision of the analytical methods used for the first time to determine biofilm formation under the composting conditions for PLA/PCL based blends. The results helped in better predictability of the biodegradation of PLA/PCL/MCC polyblends under composting conditions.

6.2 Results and Discussions

All the test samples used in this study is presented in **table 6.1**.

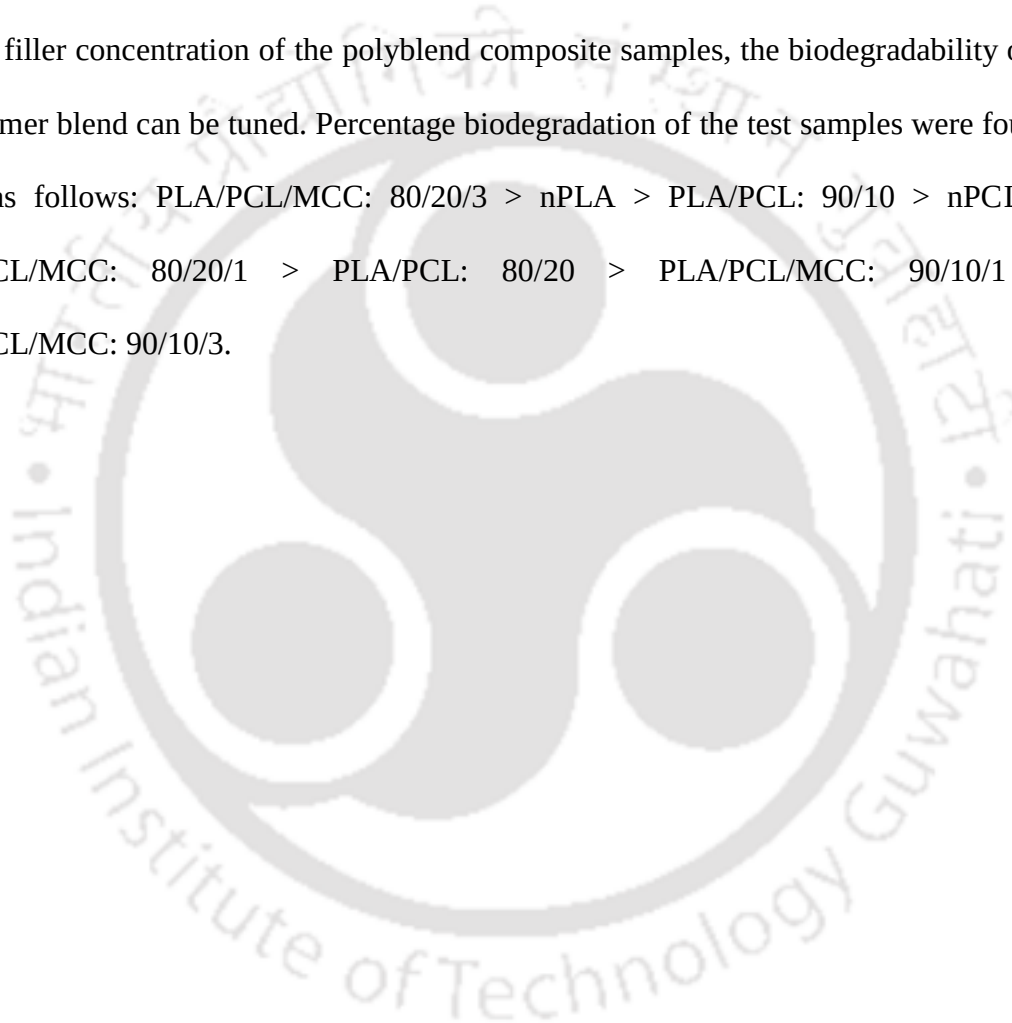
Table 6. 1: Composition of the different polyblend composites.

Sample Designation	PLA (g)	PCL (g)	MCC (g)
nPLA	10	0	0
nPCL	0	10	0
PLA/PCL : 80/20	8	2	0
PLA/PCL : 90/10	9	1	0
PLA/PCL/MCC : 80/20/1	7.92	1.98	0.1
PLA/PCL/MCC : 80/20/3	7.76	1.94	0.3
PLA/PCL/MCC : 90/10/1	9	0.9	0.1
PLA/PCL/MCC : 90/10/3	8.73	1.3	0.3

6.2.1 Biodegradation test

Elemental analysis showed that the test samples contained almost 50% organic carbon (OC) whereas the OC content of the compost was 40%. More than 90% of the standard cellulose films degraded within 37 days, which confirmed that our composting setup was prepared correctly in accordance to the ASTM D5338-15 standard. Percentage biodegradation of the polyblend composite samples was measured by the estimation of CO₂ and are presented in **figure 6.1(a-b)**. Manual observation of all the samples recorded using a 13 Mega Pixel Camera is presented in **figure 6.2**. Percentage carbon mineralization test results showed that 90% of nPLA biodegraded under the water hyacinth compost. It was also found that the addition of MCC to the PLA/PCL: 80/20 sample increased its percentage biodegradation (**Figure 6.1**). MCC being a biofiller increased the biodegradation percentage of the blends and around 80% biodegradation was observed for the

PLA/PCL/MCC: 80/20/3% and PLA/PCL/MCC: 80/20/1% test samples. But, contradictory results were obtained when MCC was added to PLA/PCL: 90/10 blend samples. Here, the addition of MCC decreased the percentage biodegradation as compared to PLA/PCL: 80/20 blends. These results signify that the blend composition and the filler concentration plays a crucial role in biodegradation behavior of the composite test materials. Or, it could be concluded that by introducing variations in the blend composition and the filler concentration of the polyblend composite samples, the biodegradability of a biopolymer blend can be tuned. Percentage biodegradation of the test samples were found to be as follows: PLA/PCL/MCC: 80/20/3 > nPLA > PLA/PCL: 90/10 > nPCL > PLA/PCL/MCC: 80/20/1 > PLA/PCL: 80/20 > PLA/PCL/MCC: 90/10/1 > PLA/PCL/MCC: 90/10/3.



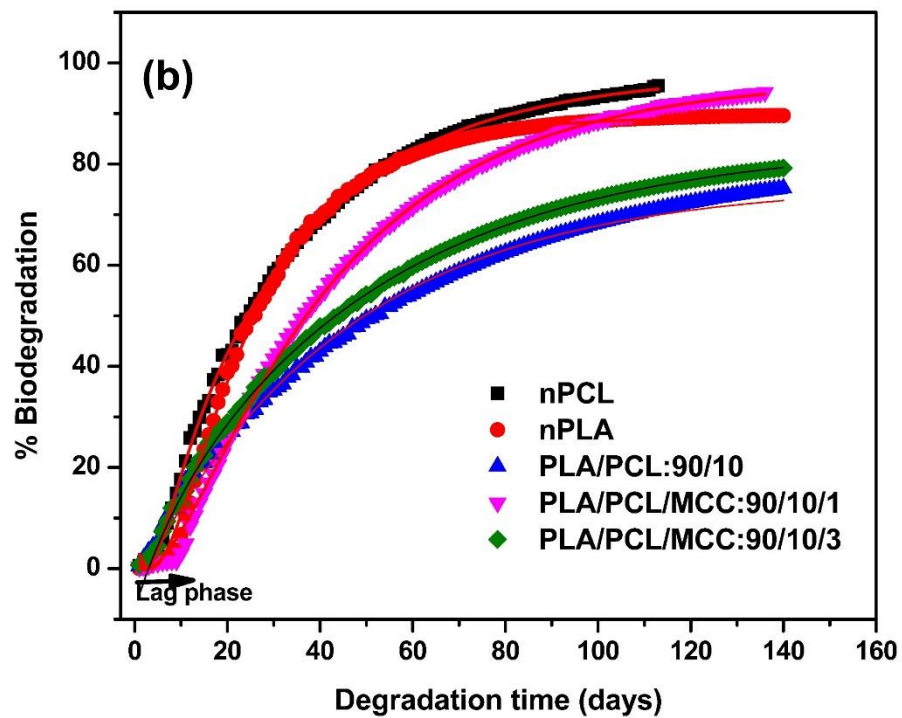
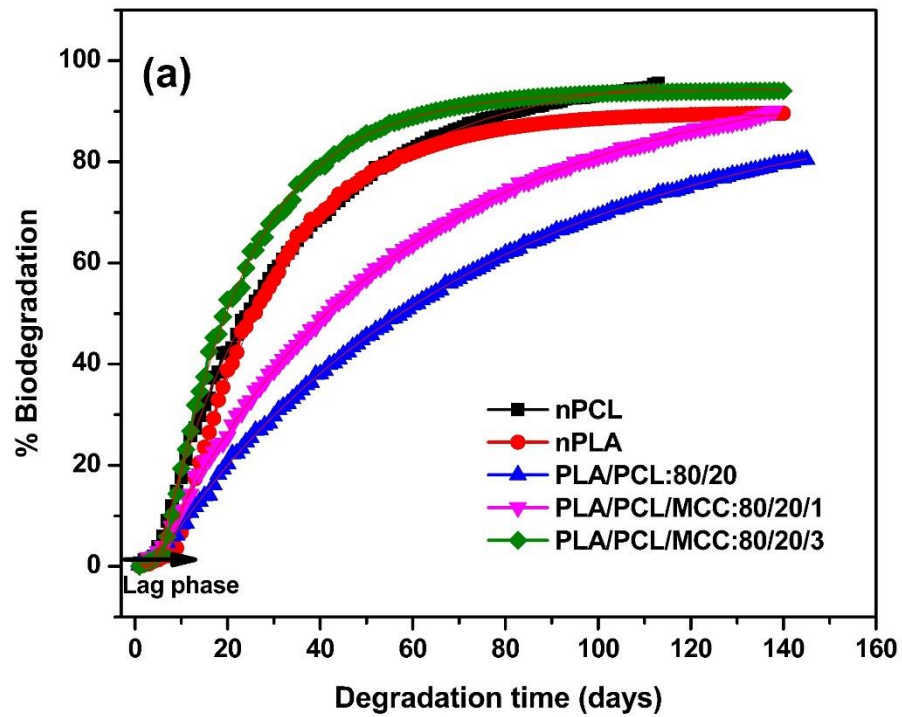


Figure 6. 1 (a-b). Percentage biodegradation of all the test samples under composting condition.

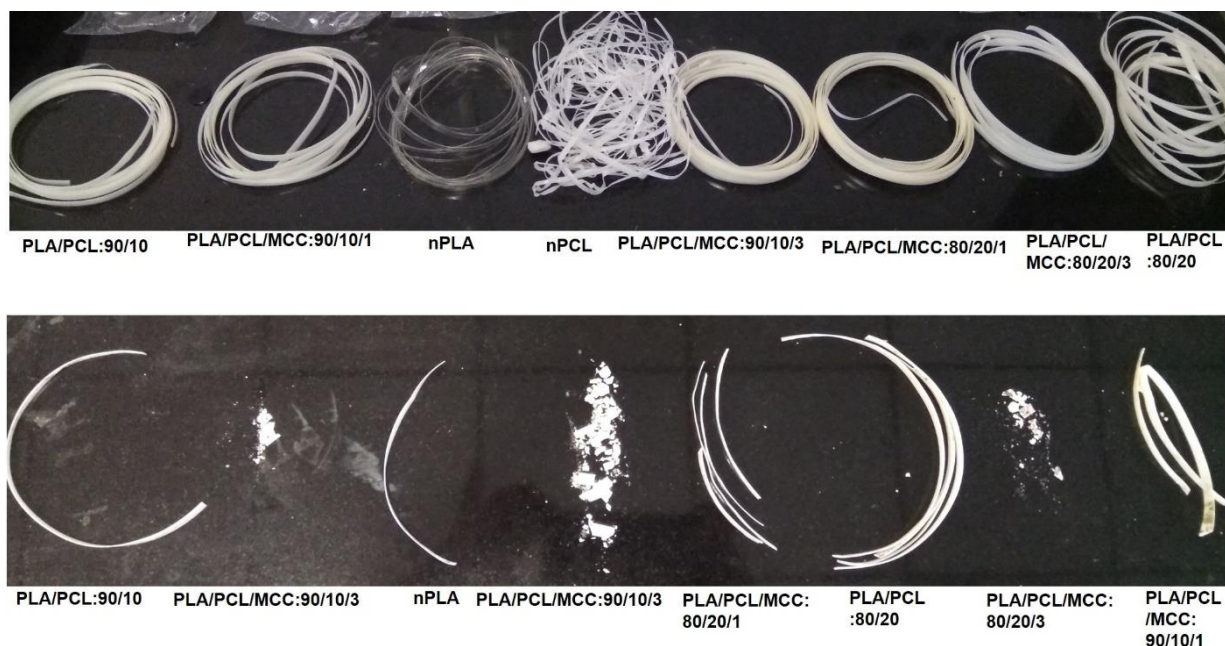


Figure 6. 2: Test samples before and after biodegradation.

6.2.2 Microbial analysis and biofilm formation study

For the microbial analysis, soil samples were examined during the biodegradation process by microbial colony count method (**Table 6.2.**). From the colony formation results, it was clear that microbial growth affected the percentage biodegradation of a sample. Gram staining confirmed the presence of gram-negative bacteria in the degradation process. The isolated cultures were then cultivated in nutrient agar media to check their absorbance activity (**Figure 6.3.**). For the biofilm analysis, absorbance of the test samples both before and after biodegradation was measured at 600 nm from 8 to 48 hours of incubation (**Table 6.3.**). It was found that after biodegradation, the absorbance of the samples varied between 0.5-0.6. This indicated the presence of biofilm on the surface of the biodegraded samples due to surface as well as bulk degradation in the test samples.

Table 6. 2: Colony count of the test samples before and after biodegradation.

Sample designation	Colony count (cfu/ml) before biodegradation	Colony count (cfu/ml) after biodegradation
nPLA	1.4×10^6	2.4×10^7
nPCL	1.5×10^6	3.6×10^7
PLA/PCL:90/10	1.5×10^6	4.0×10^6
PLA/PCL;80/20	1.5×10^6	4.5×10^6
PLA/PCL/MCC:80/20/1	1.3×10^6	9.0×10^7
PLA/PCL/MCC:80/20/3	1.5×10^6	10.5×10^7
PLA/PCL/MCC:90/10/1	1.6×10^6	8.0×10^7
PLA/PCL/MCC:90/10/3	1.7×10^6	8.73×10^7

Table 6. 3: Absorbance of the test samples before and after biodegradation.

Sample designation	Absorbance (λ_{600})	
	Before biodegradation	After Biodegradation
nPCL	0.01	0.64
PLA/PCL:90/10	0.14	0.58
PLA/PCL:80/20	0.03	0.52
PLA/PCL/MCC:90/10/1	0.03	0.50
PLA/PCL/MCC:90/10/3	0.04	0.55
PLA/PCL/MCC:80/20/1	0.07	0.60
PLA/PCL/MCC:80/20/3	0.05	0.65
nPLA	0.02	0.63

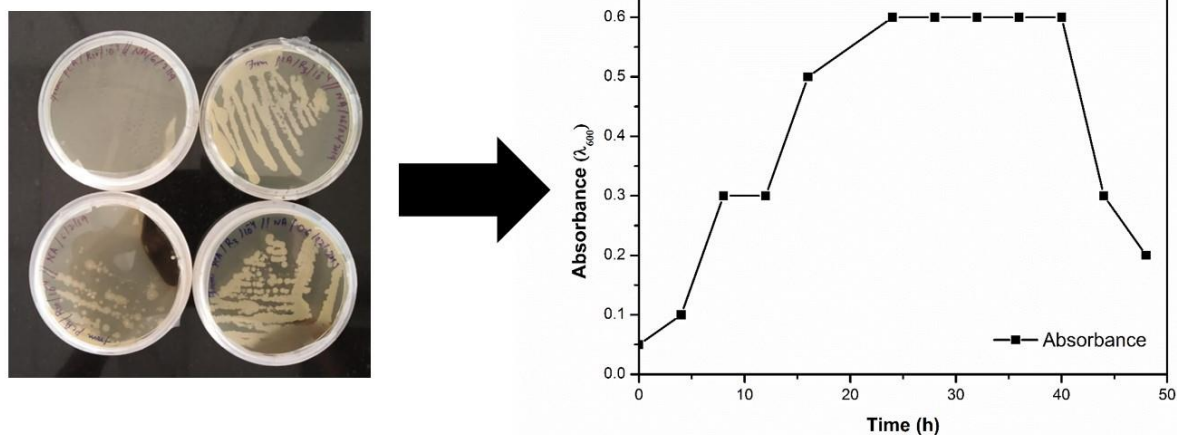


Figure 6. 3. Growth absorbance curve of the isolated microbial strains observed at 600 nm.

Furthermore, to identify the organisms responsible for biodegradation, three different kinds of media were used, namely eosin methylene blue agar (selective medium for Gram negative bacteria) at 37 °C , potato dextrose agar (medium for yeasts and molds) and plate count agar (medium for getting the total viable bacterial count) at 55 ± 5 °C (**Figure 6.4**).

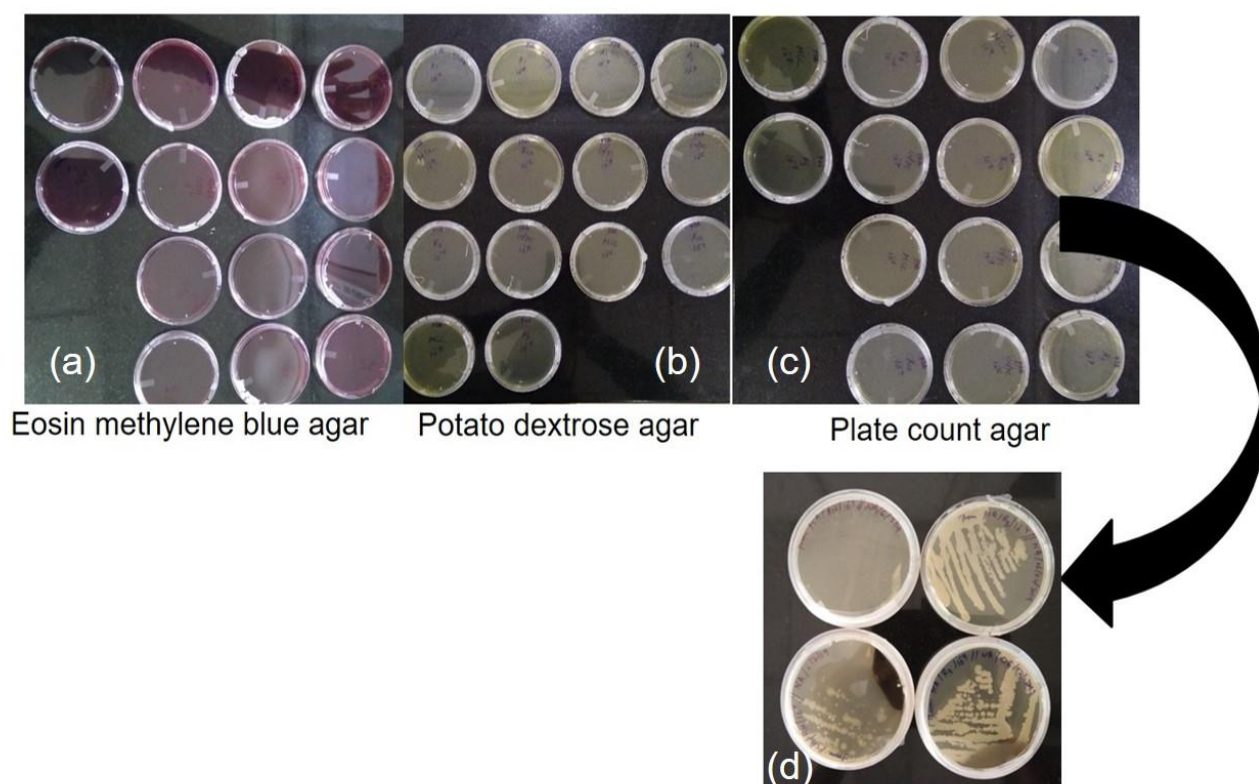


Figure 6. 4.: Growth of the isolated microbes on three different media plates: (a) Eosin methylene blue agar (b) Potato dextrose agar (c) Plate count agar (d) magnified representation of the plate count agar showing only bacterial growth.

No colony formation was observed on the eosin methylene blue agar plates, which suggested the absence of mesophilic Gram negative bacteria like coliforms during the composting process. Similarly, no microbial growth was observed in the potato dextrose agar media, suggesting the absence of fungus and yeasts during the biodegradation process. But growth was recorded on the plate count agar media, suggesting the presence of only bacteria, which clearly suggested that biodegradation occurring during the composting of the polymeric blends was solely due to bacterial presence. Gram staining of the bacterial population further revealed that the isolated bacteria were Gram-negative in nature.

6.2.3 Molecular weight analysis

Molecular weight analysis data are presented in **table 6.4**. It was observed that the M_n of all experimental samples decreased significantly after the 100th day. M_n of nPLA was found to decrease from 106 kDa to 24 kDa on the 100th day of the biodegradation process. Similar behavior was recorded for all the test samples. This chain cleavage in all the PLA/PCL based blends indicated conversion of ester bonds to hydroxyl groups. This conversion during biodegradation led to a loss in molecular weight of the test samples (Vieira et al., 2011). This behavior was modeled and the data are presented in **table 6.5**. It was observed that with a decrease in molecular weight, PDI of the degraded samples increased. This was due to chain fragmentation during the degradation process (Castro-Aguirre et al., 2018), which led to the cleavage of ester bonds. Molecular weight analysis of the test samples and their predicted data according to the first order reaction kinetics $-M_{n,t} = M_{n,0} \cdot e^{-ut}$ are presented in **table 6.5**. This indicated that the blends had different hydrolytic degradation rates (**Figure 6.5**). This behavior also triggered the biodegradation process as the cleavage of the bonds led to the formation of low molecular weight oligomeric or polymeric intermediates, which were further assimilated by the microbes during the biodegradation process.

Table 6. 4: Molecular weight analysis of the test samples before and after biodegradation.

<i>Sample designation</i>	<i>Before biodegradation</i>			<i>After biodegradation of 30 days</i>			<i>After biodegradation of 60 days</i>			<i>After biodegradation of 90 days</i>		
	M_n	M_w	PDI	M_n	M_w	PDI	M_n	M_w	PDI	M_n	M_w	PDI
<i>PLA/PCL/MCC:80/20/1</i>	88	128	1.5	41	89	2.1	21	67	3.2	3	17	5.7
<i>PLA/PCL/MCC:80/20/3</i>	78	127	1.6	21	56	2.6	14	42	3.0	4	24	6.3
<i>PLA/PCL:80/20</i>	89	148	1.6	43	108	2.5	40	89	2.2	35	94	2.6
<i>PLA/PCL:90/10/1</i>	87	109	1.3	37	81	2.1	21	56	2.6	7	18	2.5
<i>PLA/PCL/MCC:90/10/3</i>	79	124	1.6	21	56	2.6	10	31	3.1	2	3	1.5
<i>PLA/PCL:90/10</i>	96	150	1.6	61	102	2.1	52	96	1.8	40	94	2.3
<i>nPCL</i>	98	137	1.4	15	41	2.7	15	35	2.3	8	18	2.2
<i>nPLA</i>	106	177	1.6	53	99	1.8	29	68	2.3	24	39	1.6

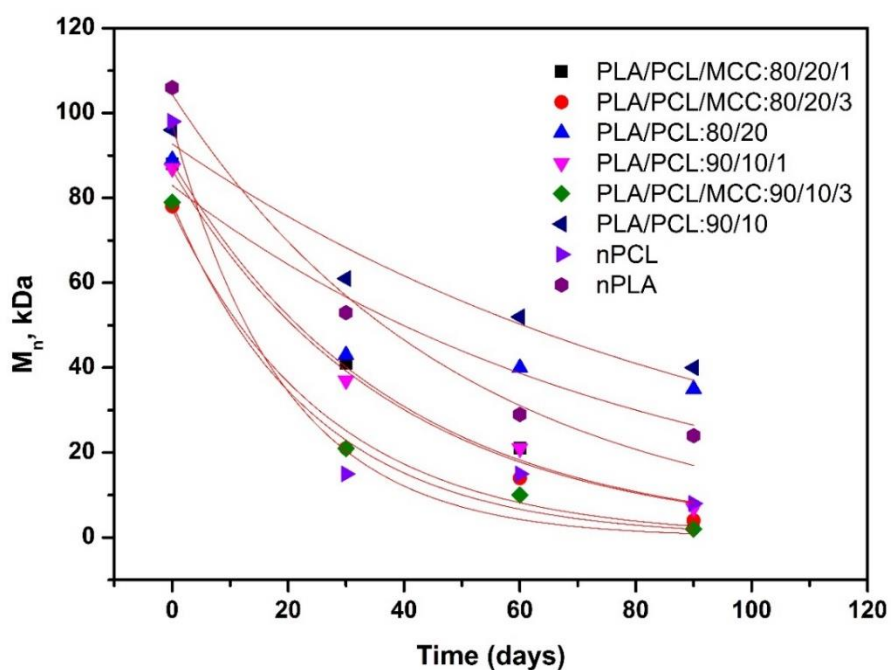


Figure 6. 5: Molecular weight analysis of the test samples and their predicted data according to the first order reaction kinetics.

Table 6. 5: Rate constant values of the samples before and after biodegradation.

Sample designation	Rate constant (u)	Regression (R^2) (%)
PLA/PCL/MCC:80/20/1	69.65565	95
PLA/PCL/MCC:80/20/3	47.60894	97
PLA/PCL:80/20	65.00408	79
PLA/PCL:90/10/1	67.50311	98
PLA/PCL/MCC:90/10/3	47.15398	99
PLA/PCL:90/10	75.92954	99
nPCL	46.26972	87
nPLA	104.3445	97

6.2.4 Spherulite growth analysis

Biodegradation of PLA is associated with its surrounding environment and its underlying degradation mechanism is hydrolytic degradation (Husarova et al., 2014b; Kalita et al., 2019). Chain mobility plays an essential role in PLA biodegradation, which is influenced by temperature as well as the type of compost used. POM analysis was done to understand degradation of the samples before and after biodegradation. There was a significant increase in spherulite size in nPLA after biodegradation. Spherulite size observed during the 15-minute study increased from $\sim 30\text{ }\mu\text{m}$ in the initial sample to $\sim 150\text{ }\mu\text{m}$ (**Figure 6.6**). This increase in spherulite size in the degraded samples might be attributed to the simultaneous lamellar growth and chain degradation in the amorphous region of the samples. After biodegradation of the amorphous portion, the crystalline region got more space for chain folding, chain mobility, and spherulite formation of bigger diameter than the initial samples. Formation of large spherulites after biodegradation could also be attributed to longer chains of higher molecular weight left behind as the crystalline portions due to the microbial assimilation of medium and shorter chains of the polymer (Owen, 2013).

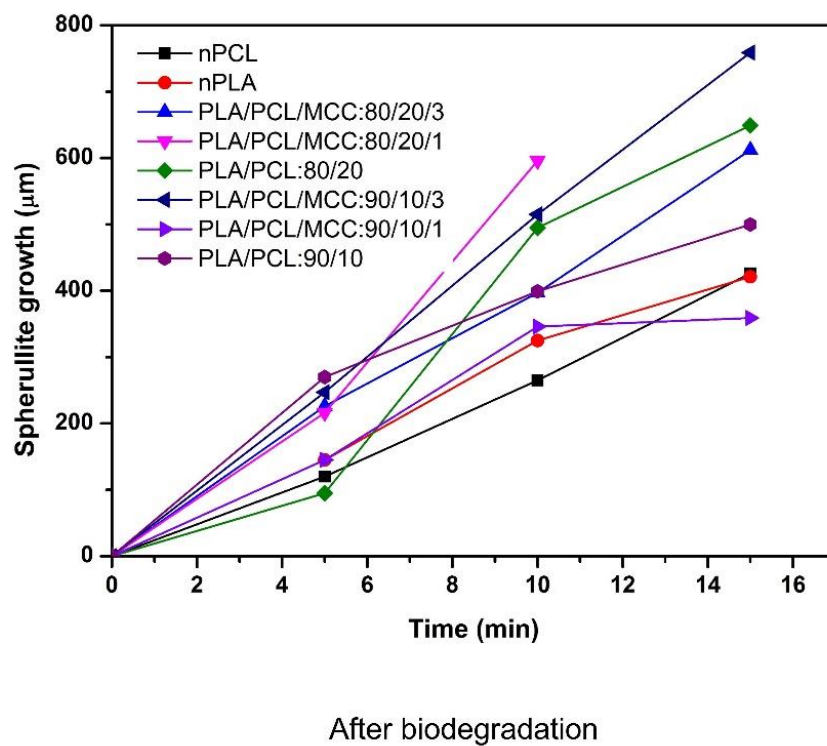
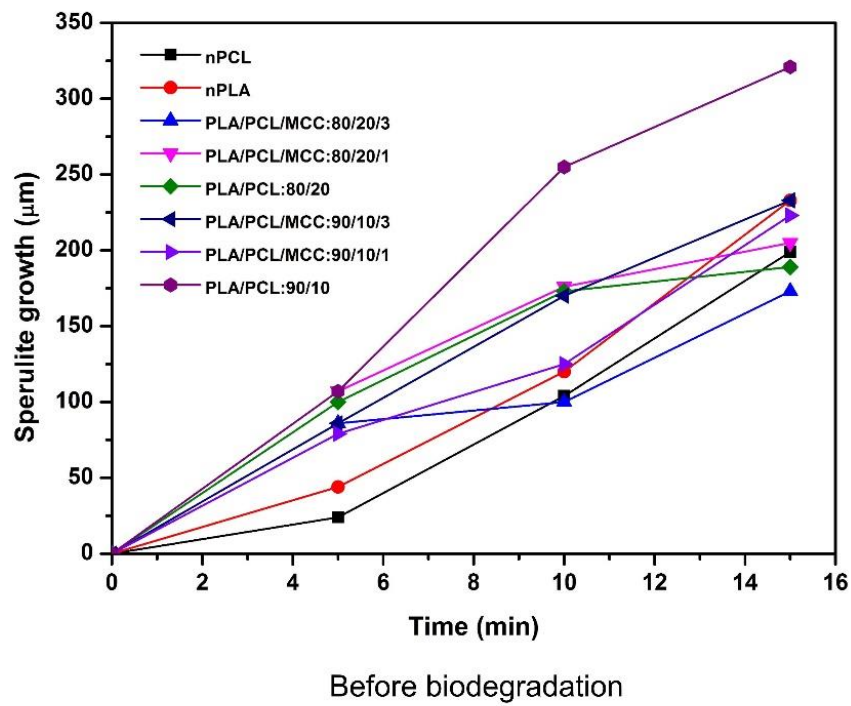


Figure 6. 6: Spherulite growth size of the samples before and after biodegradation.

It was also observed that the spherulite density of all the test samples decreased after biodegradation (**Figure 6.7**). This analysis revealed that the microbes were readily

degrading the amorphous regions of the samples, leaving behind the crystalline regions, which led to an increase in the spherulite size and a decrease in the spherulite density. This helped in clear recognition of the spherulite texture of the test samples. All the POM micrographs of the test samples are shown in **Figure 6.8** and **Figure 6.9**.

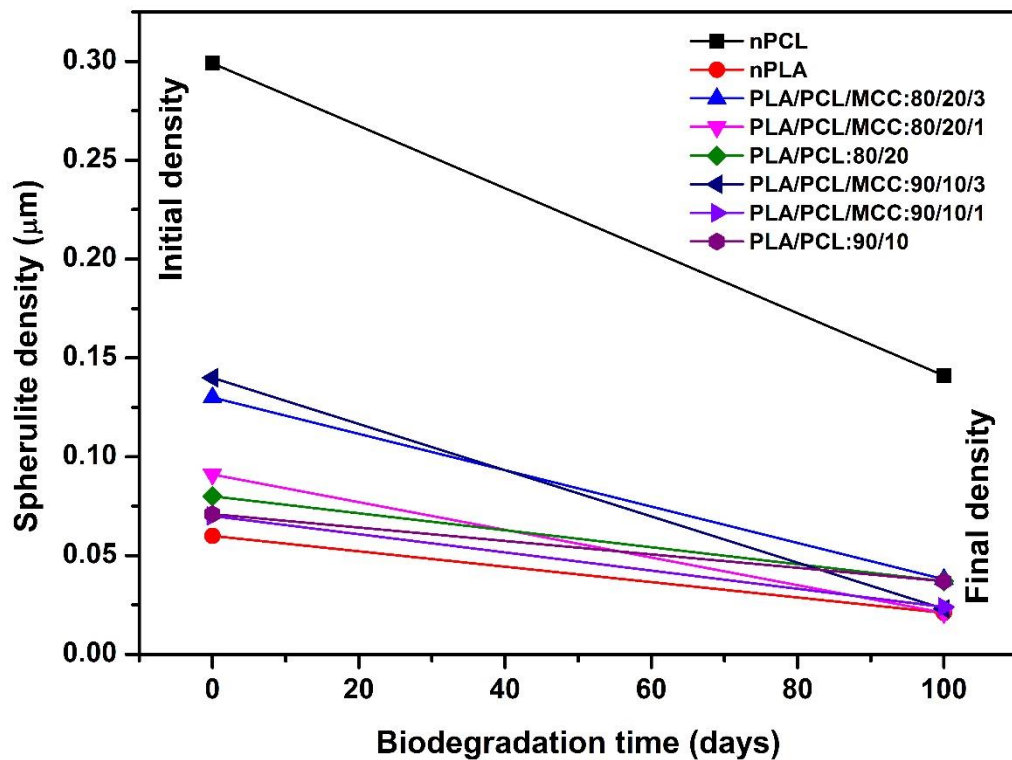


Figure 6. 7: Spherulite density of samples before and after biodegradation.

The initial density of samples represents the density of spherulite observed in the test samples before biodegradation, whereas, the final density represents the density of spherulite observed in the test samples after biodegradation.

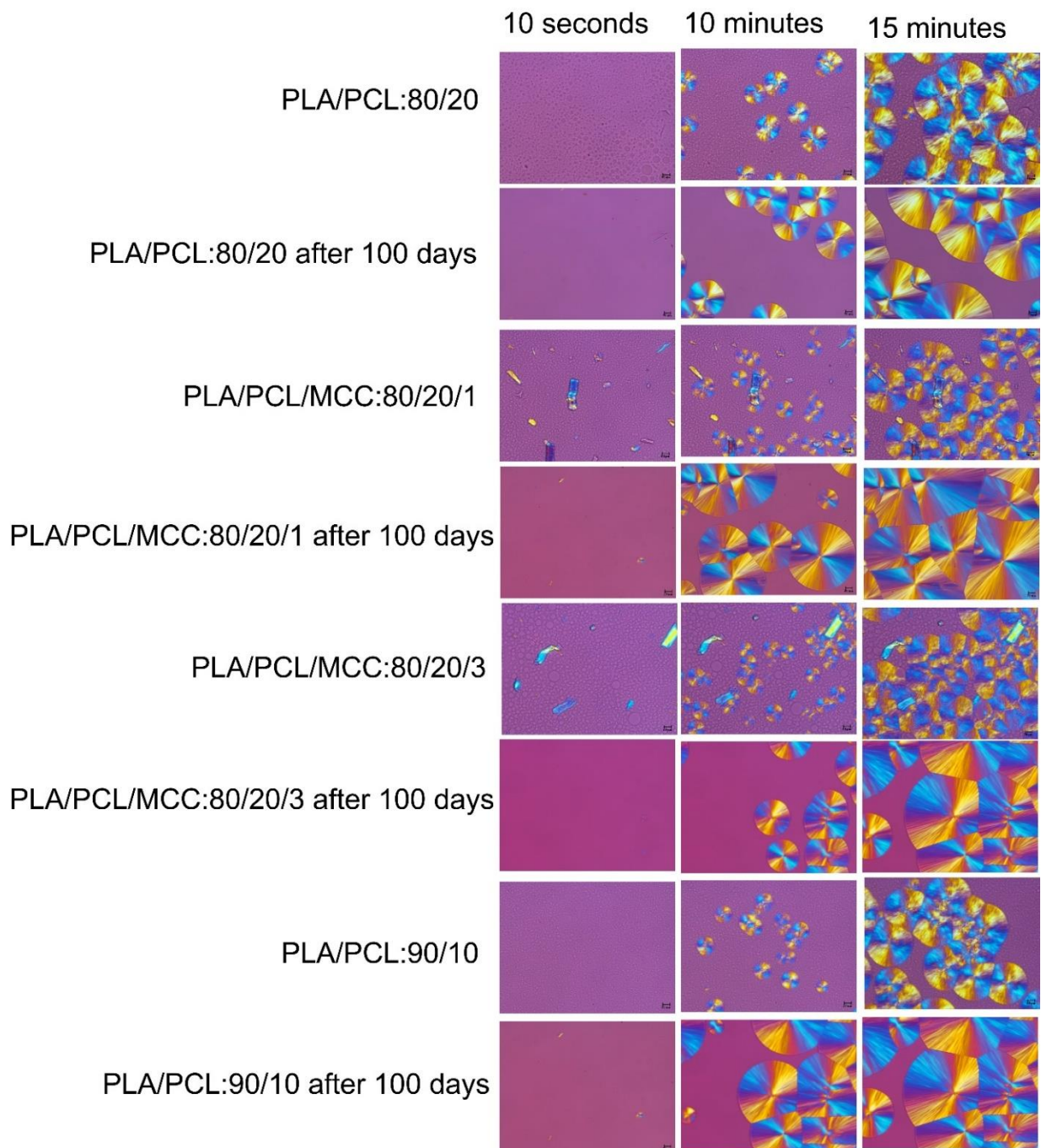


Figure 6. 8: Micrographs showing spherulite formation before and after biodegradation, captured at times 10 seconds (initial), 10 minutes and 15 minutes.

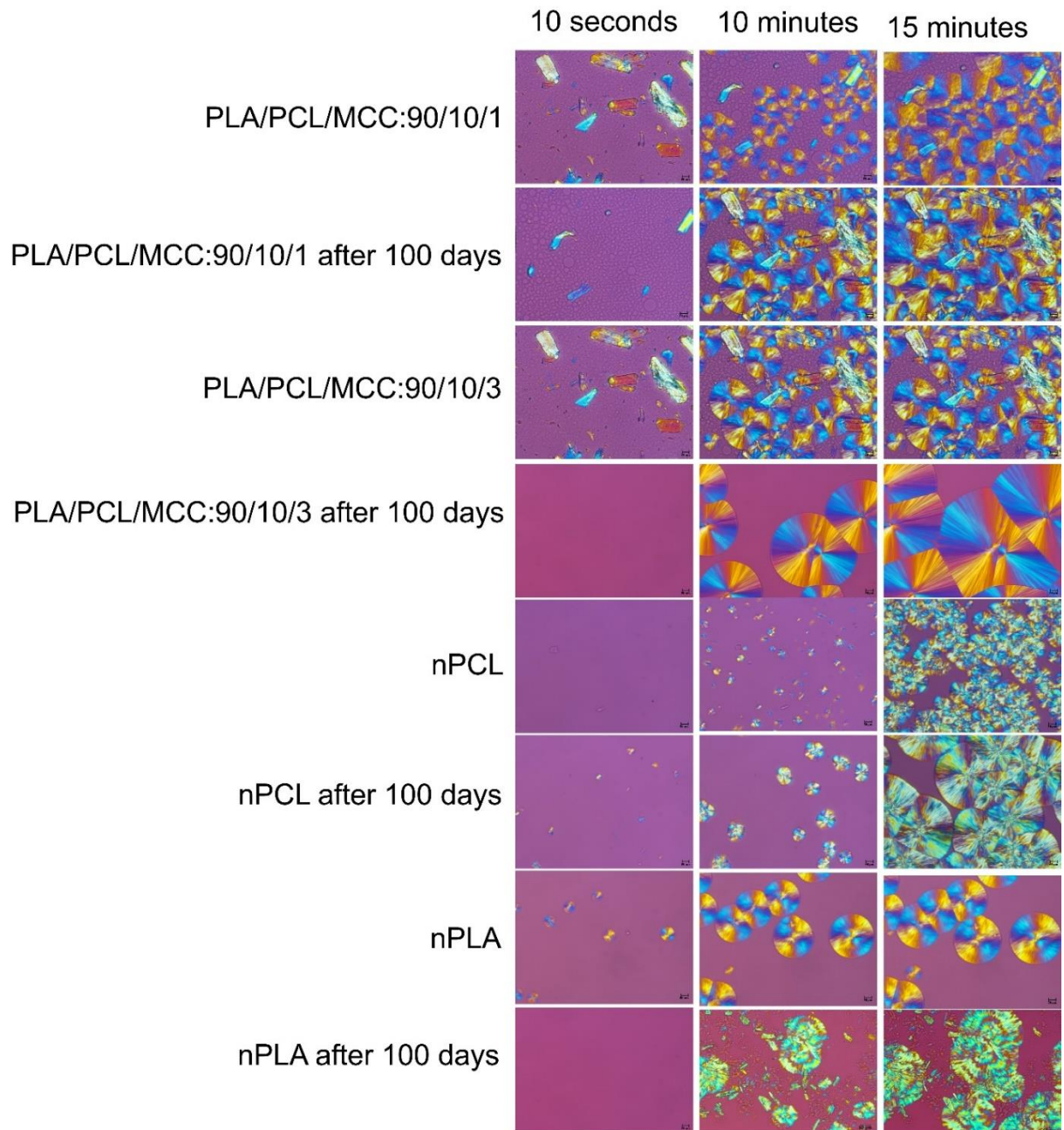


Figure 6. 9: Micrographs showing spherulite formation before and after biodegradation captured at times 10 seconds (initial), 10 minutes and 15 minutes.

6.2.5 Wettability analysis

The blend matrix was observed both before and after biodegradation for wettability changes occurring on the surface of the blends which underwent biodegradation. It was found that the hydrophilicity of all the test samples decreased with an increase in degradation time. This behaviour was attributed to the formation of carboxylic and hydroxyl group on the

blend surface as well as the presence of cavities on the PLA matrix leading to hydrophilicity and higher chain mobility, which ultimately led to microbial assimilation on the surface. The water contact angle of nPLA decreased from $(76 \pm 5)^\circ$ to $(45 \pm 10)^\circ$. A similar decrease in contact angle was also observed for all the test samples (**Table 6.6**). **Figure 6.10** depicts the decrease in contact angle of the samples during the initial 30 days of biodegradation, which was attributed to the formation of oligomeric components on the surface of the blends, leading to an increase in the hydrophilicity and lowering of the contact angle of the samples gradually. This phenomenon was also responsible for the formation of biofilm on the surface of the polymer because of the change in wettability behavior of the polyblended samples under composting conditions.

Table 6. 6: Water contact angle of the samples before and after biodegradation under composting conditions, measured initially, on the 30th day and the 60th day (note: It was difficult to measure the contact angle of the samples after 60th day as they became very disoriented).

Sample designation	Before biodegradation	After biodegradation	
	Water contact angle (°)	Water contact angle (°)	
	Initial	30 th day	60 th day
nPLA	76 ± 5	62 ± 10	45 ± 15
nPCL	81 ± 8	61 ± 15	53 ± 12
PLA/PCL:90/10	78 ± 7	59 ± 5	52 ± 13
PLA/PCL:80/20	74 ± 6	69 ± 8	51 ± 10
PLA/PCL/MCC:80/20/1	78 ± 10	60 ± 6	55 ± 9
PLA/PCL/MCC:80/20/3	75 ± 10	60 ± 5	54 ± 10
PLA/PCL/MCC:90/10/1	80 ± 9	58 ± 6	55 ± 12
PLA/PCL/MCC:90/10/3	79 ± 8	59 ± 5	51 ± 13

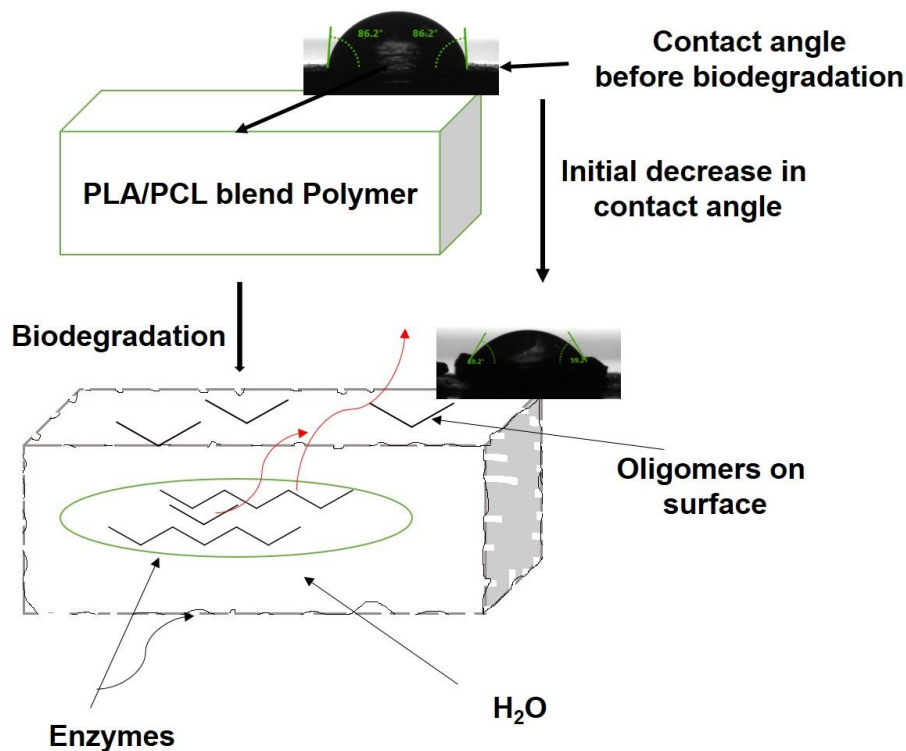
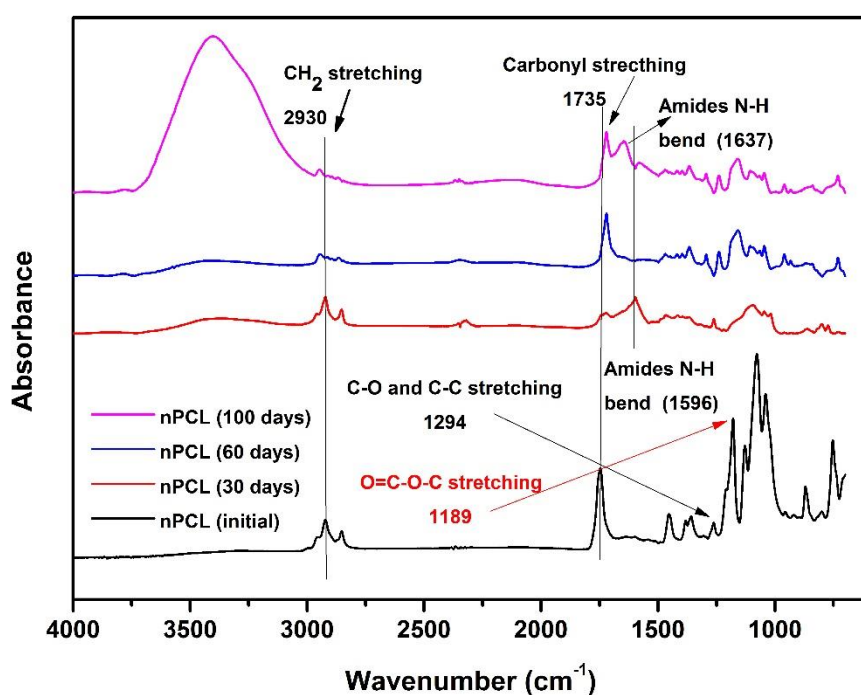


Figure 6. 10: Representative image highlighting the surface of the polymer during the initial stage and after the biodegradation process of 30 days, under composting conditions.

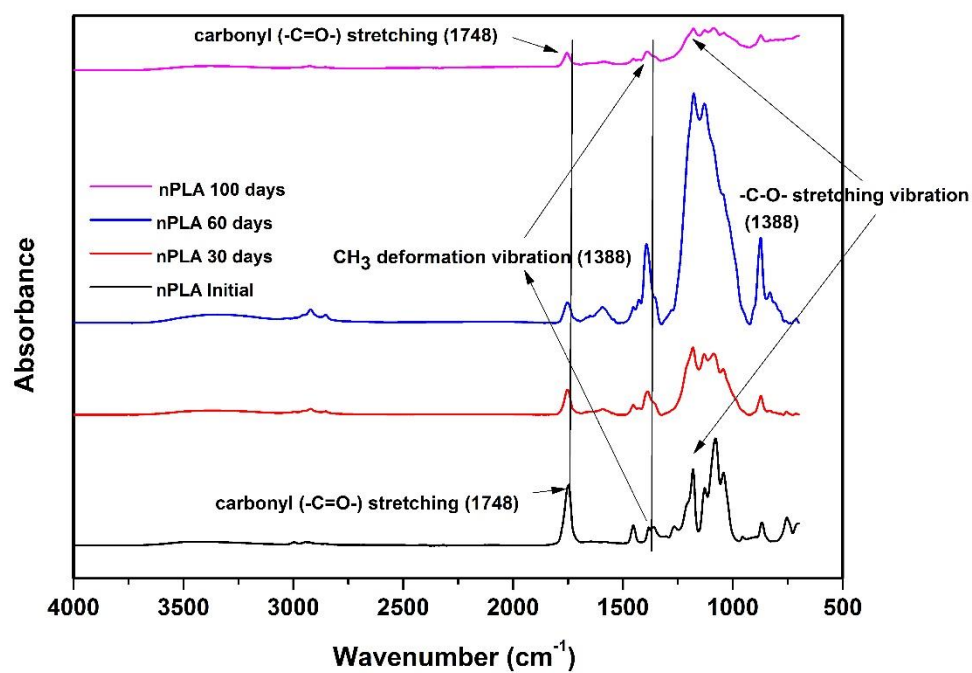
6.2.6 FT-ATR analysis

FT-ATR spectrum of all the test samples analyzed until the 100th day of biodegradation are shown in **figure 6.11 (a-h)**. In the nPLA sample, sample shifting of the peaks was observed from 1750 cm^{-1} to 1758 cm^{-1} . This corresponded to the carbonyl (-C=O-) stretching. Various authors (Kalita et al., 2019; Weng et al., 2013a, 2013b) have also reported the carbonyl peak shift during biodegradation of PLA in different environments. In the nPCL samples, CH_2 stretching shifted from 2929 to 2947 cm^{-1} on the 100th day, suggesting biodegradation. 30th day spectra of all the test samples showed the formation of amides bonds (N-H bending) at 1596 cm^{-1} due to the attachment of enzymes and diffusion of soil on the polymer surface. This enzyme attachment happened due to the formation of biofilms on the surface of the polymer during the biodegradation process. In the test samples

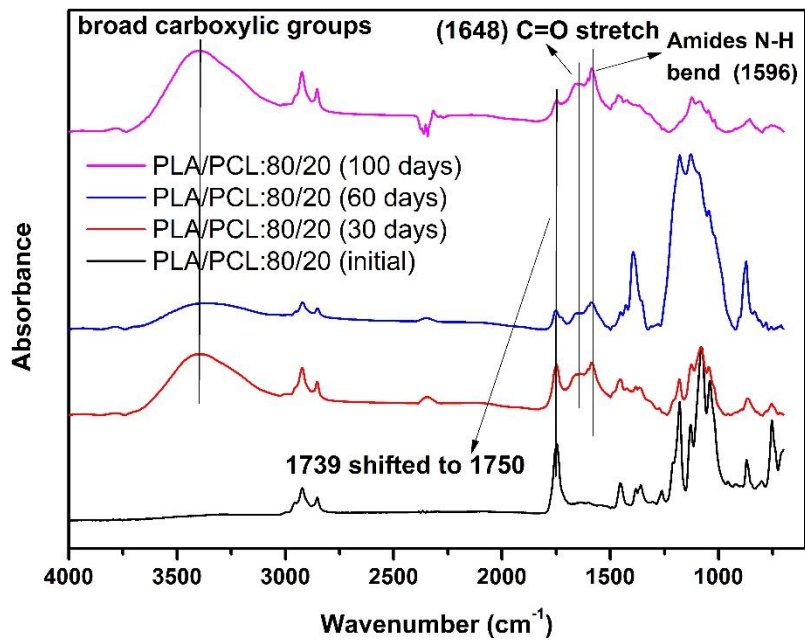
PLA/PCL/MCC: 80/20/1, PLA/PCL/MCC: 80/20/3, PLA/PCL/MCC: 90/10/1 and PLA/PCL/MCC: 90/10/3, there was a shift of the small peak from 1450 cm^{-1} to 1456 cm^{-1} with an increase in intensity. This suggested asymmetric bending vibration of C-H. A broad carboxylic peak was also observed for all the test samples till the 100th day due to the formation of more carboxylic groups. This also implied oligomer formation on the sample surface due to microbial attack on the polymeric chains. This kind of IR footprint shifting had been observed by various authors under different environment in various biopolymers and their blends (Karamanlioglu et al., 2017; Pantani and Sorrentino, 2013; Paul et al., 2005; Sedlarik et al., 2008).



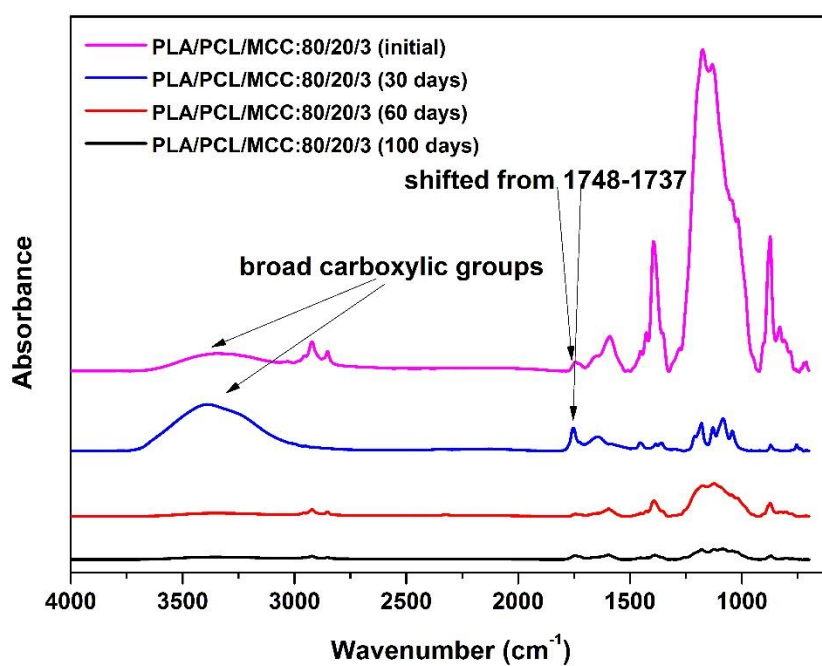
(a)



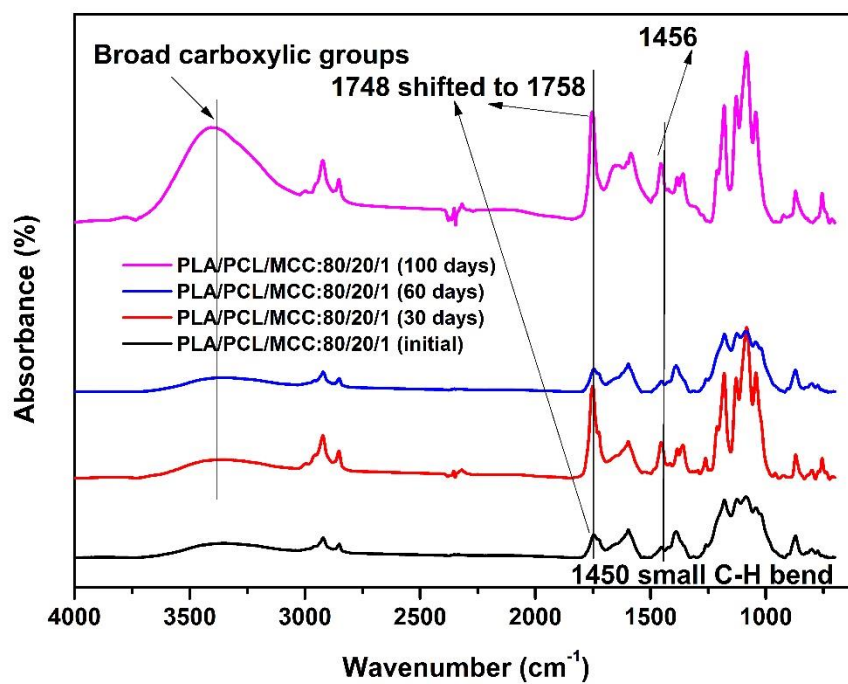
(b)



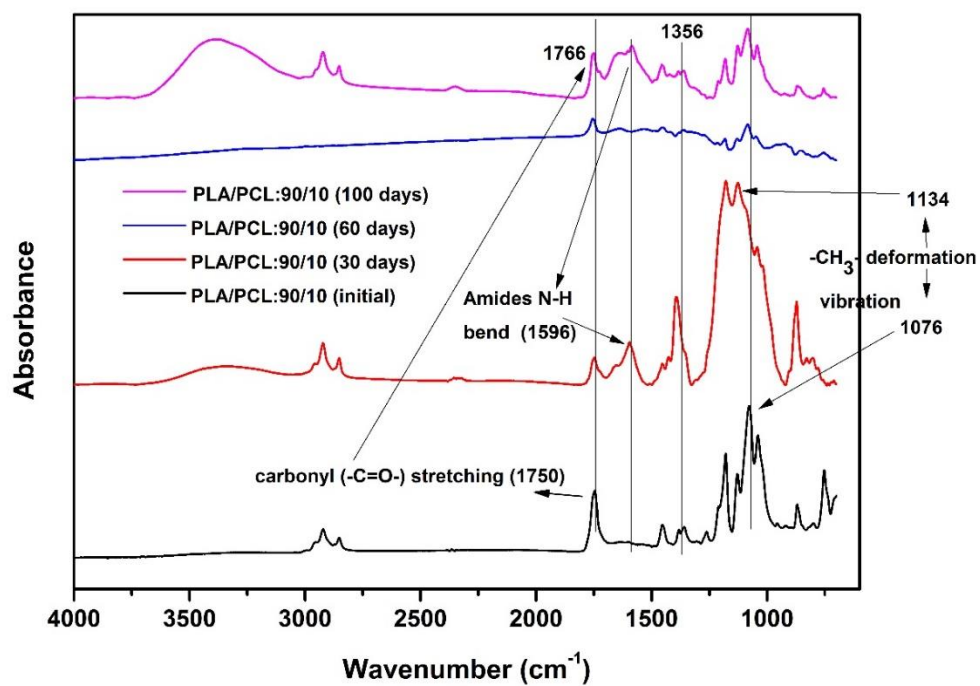
(c)



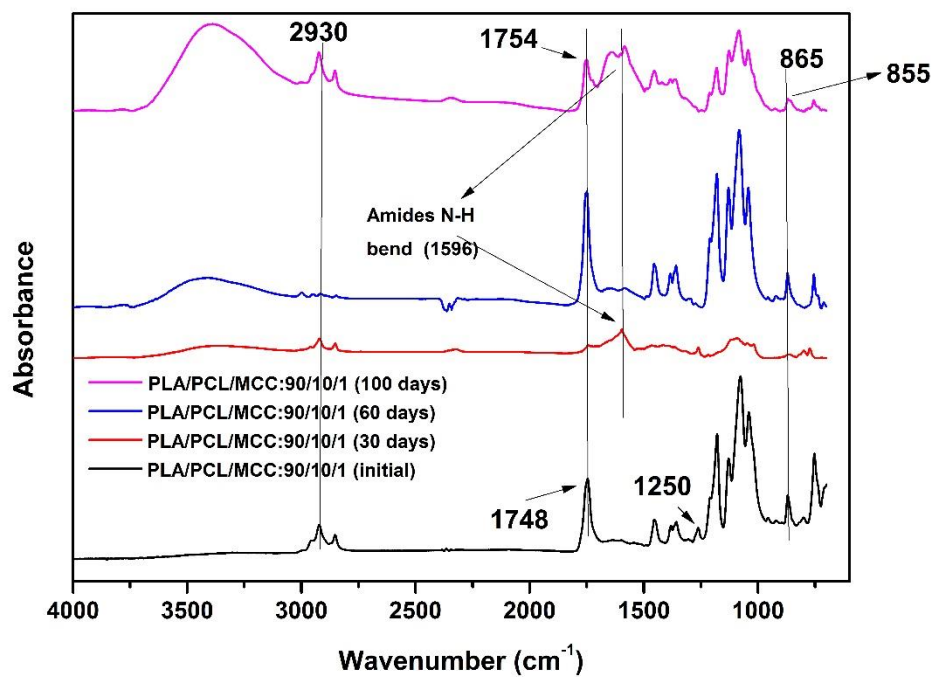
(d)



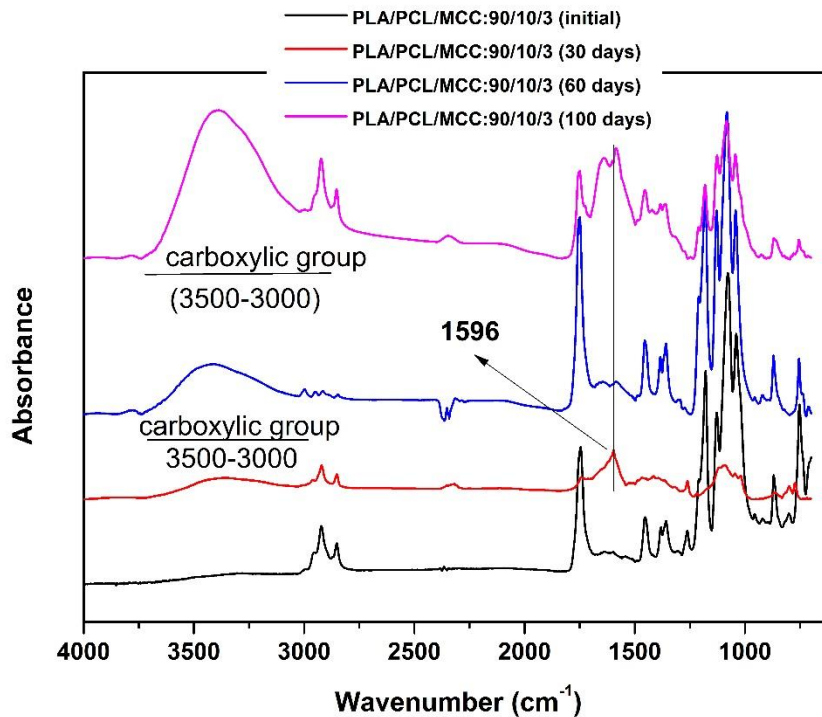
(e)



(f)



(g)

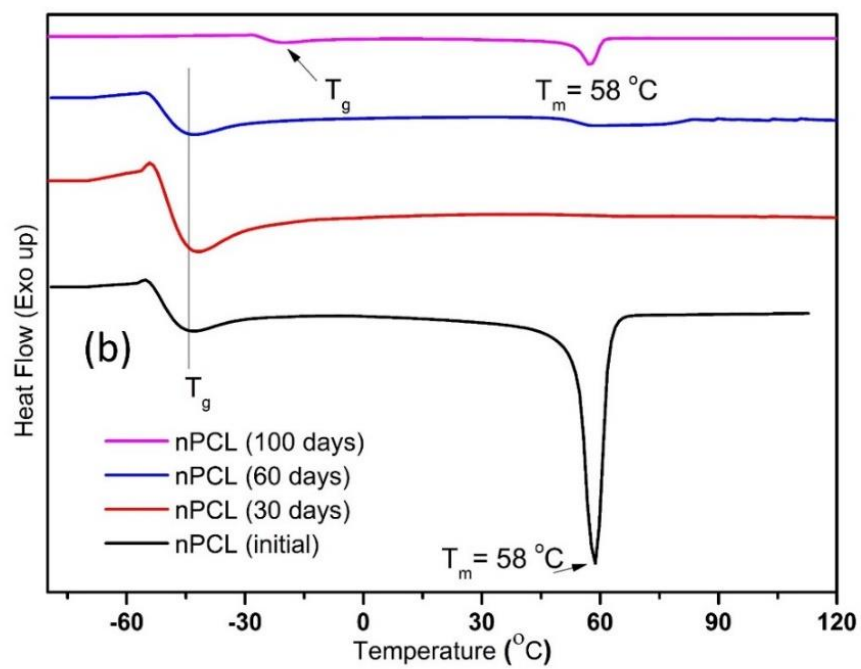
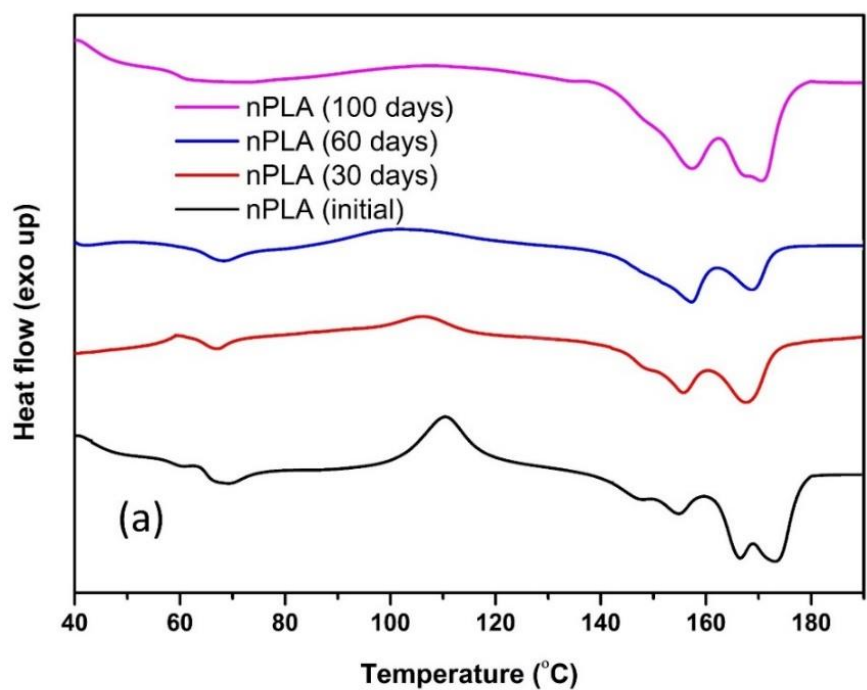


(h)

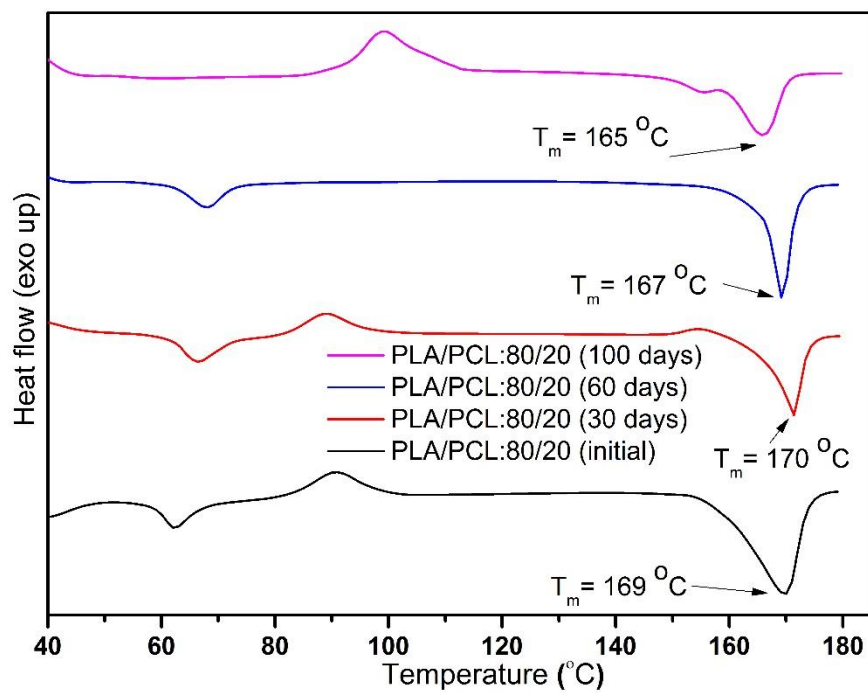
Figure 6. 11 (a-h): FTIR representation of the samples before and after biodegradation

6.2.7 DSC analysis

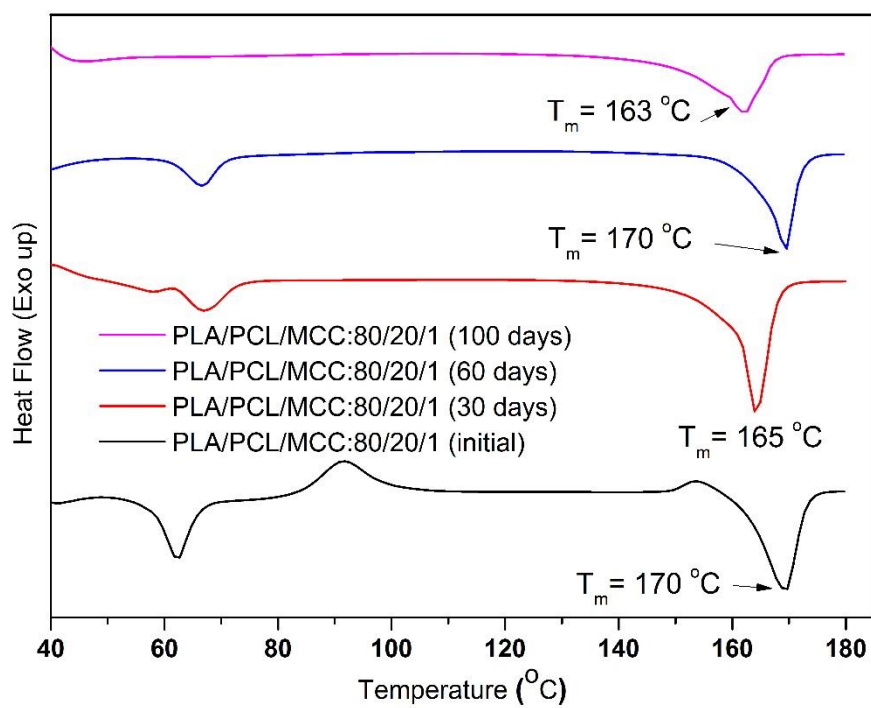
DSC thermographs of all the samples are shown in **Figure 6.12 (a-h)**. DSC thermographs of all test samples were compared during the biodegradation period of the initial day to the 100th day. The thermograms revealed a significant difference between the test samples before biodegradation and after biodegradation. nPLA and other polyblended samples lost their capacity to crystallize as shown in **Figure 6.12 (a)**. It was found that the percentage crystallinity X_c (%) of all the test samples increased significantly on the 30th day and then decreased gradually on the 60th day (**Table 6.7**). This signified degradation of the amorphous portions of the test samples, leaving behind only the crystalline portions (Pantani and Sorrentino, 2013). This occurred gradually s due to the chain scission of high molecular weight chains of the polymer into low and medium molecular weight chains.



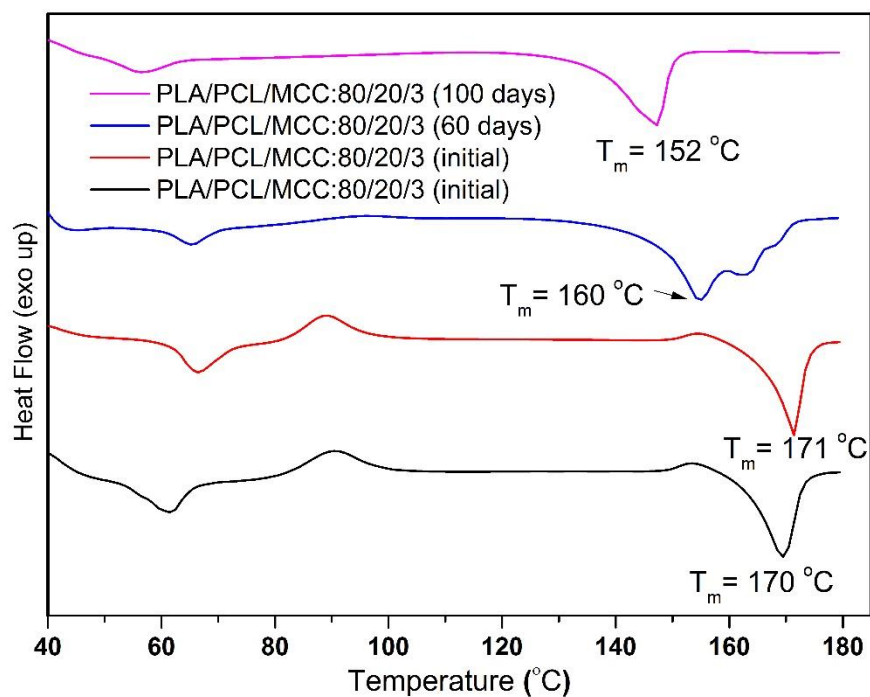
(a) nPLA (b) nPCL.



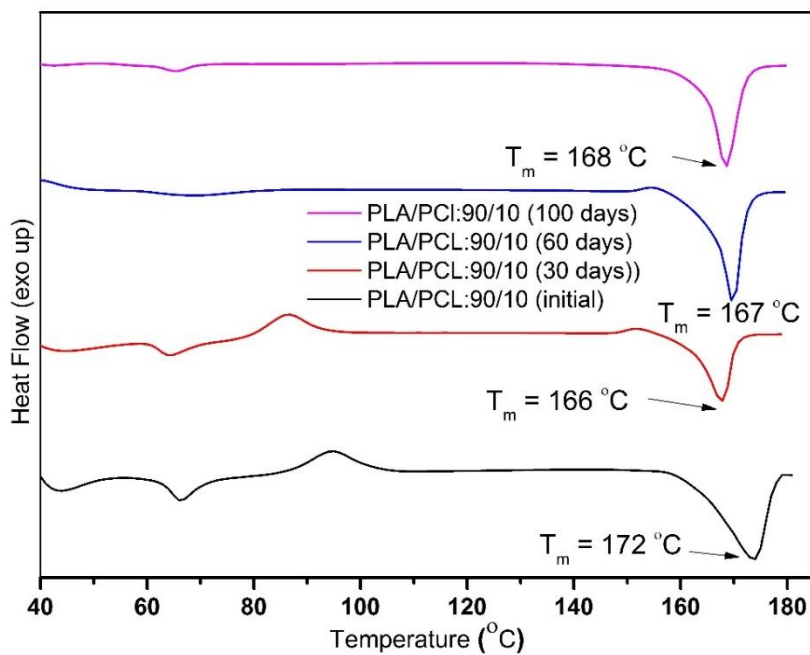
(c)



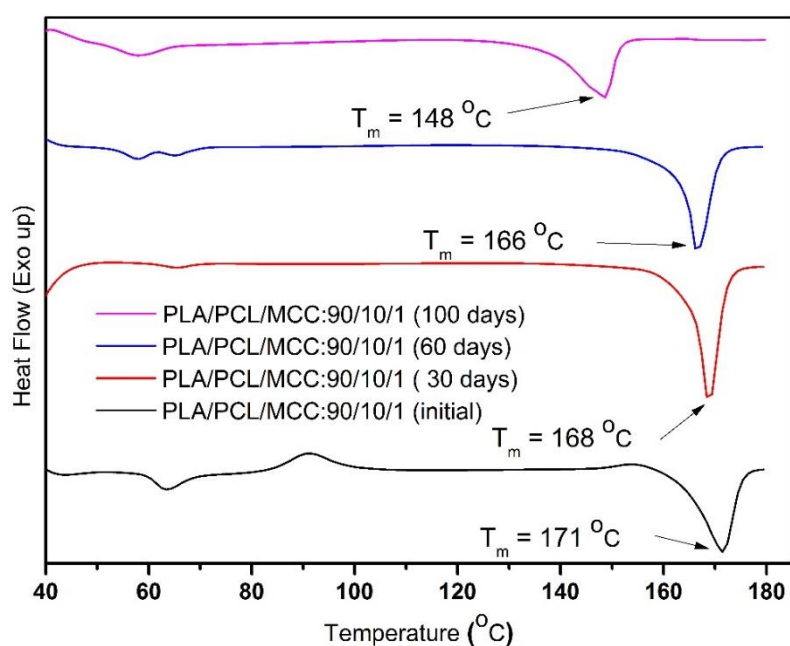
(d)



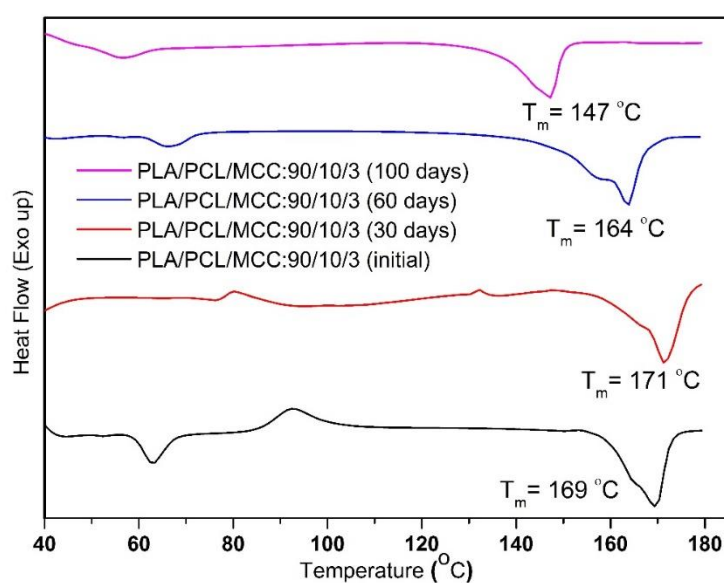
(e)



(f)



(g)



(h)

Figure 6. 12(a-h). DSC thermograms of the samples before and after biodegradation (a) nPLA (b) nPCL (c) PLA/PCL: 80/20 (d) PLA/PCL/MCC: 80/20/1 (e) PLA/PCL/MCC: 80/20/3. (f) PLA/PCL: 90/10 (g) PLA/PCL/MCC: 90/10/1 (h) PLA/PCL/MCC: 90/10/3.

Table 6. 7: Percentage crystallinity of the samples before and after biodegradation.

Sample designation	Percentage crystallinity X (%)			
	Before biodegradation	After biodegradation		
		30 th day	60 th day	100 th day
nPLA	24	65	67	nill
nPCL	Highly crystalline	Highly crystalline	Highly crystalline	Highly crystalline
PLA/PCL:90/10	28	47	63	nill
PLA/PCL:80/20	37	46	68	56
PLA/PCL/MCC:80/20/1	29	74	44	46
PLA/PCL/MCC:80/20/3	28	54	70	55
PLA/PCL/MCC:90/10/1	27	68	60	nill
PLA/PCL/MCC:90/10/3	28	60	51	nill

Samples having a higher percentage of PLA and MCC tended to degrade faster as seen from percentage biodegradation results, and which was further proved from the percentage crystallization values shown in **Table 6.7**. It could be observed from the DSC thermograms that the melting point of the samples decreased gradually, signifying biodegradation. It was also noticed from the melting peaks of all the test samples that they did not undergo a significant decrease in their melting temperature as the biodegradation progressed. With a decrease in molecular weight, the melting temperature also decreased significantly, again suggesting biodegradation of the test samples (Stloukal et al., 2015). Similarly, recrystallization temperature of all the test samples decreased significantly during biodegradation.

6.2.8 Morphological analysis

Surface morphology of the test samples before and after biodegradation is shown in **Figure 6.13**. The morphological changes were observed on the 100th day of the composting process. Crack formation was observed on the polymer surface after biodegradation was attributed to the action of microbes or biofilm formation on the surface of the polymers and diffusion of the cells inside the polymer. Water (moisture inside the CV) diffused into the amorphous domains of the samples which initiated the hydrolytic degradation of the PLA portion. With progress in the hydrolytic degradation process, the fillers tried to move away from the matrix towards the compost resulting in holes, cavities, and cracks, observed on the 100th day of the biodegradation process. Surface morphology of the biodegraded samples revealed the formation of fibril-like structure in the PLA blends, again suggesting biodegradation through surface erosion. Contrary to this, the nPCL surface was rougher and no such fibril structure was observed in the biodegraded samples, suggesting bulk degradation.

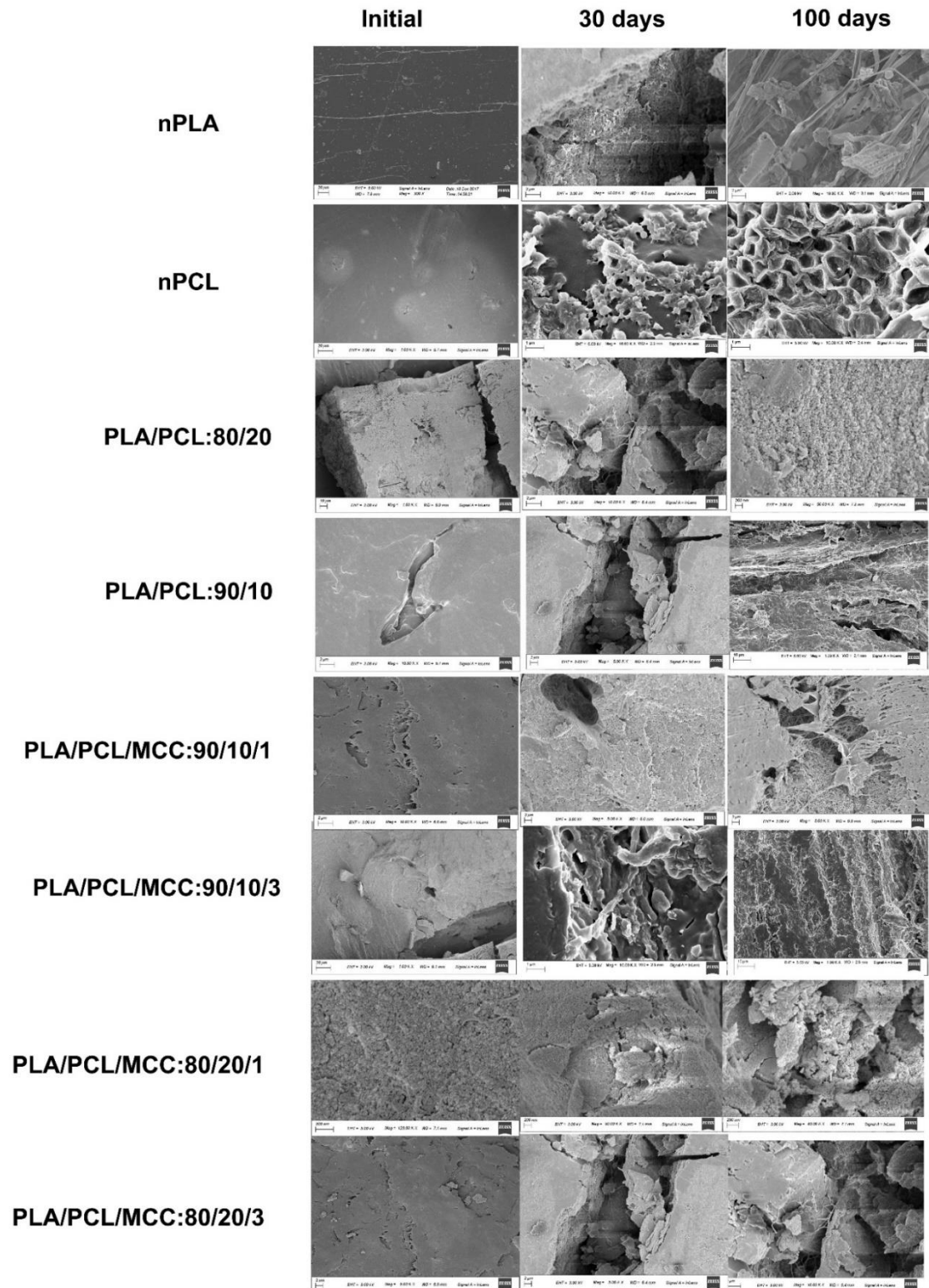


Figure 6. 13: FESEM images representing the test samples before biodegradation and after biodegradation under composting conditions.

6.3 Conclusion

In this study, extruded nPLA, nPCL, PLA/PCL: 80/20, PLA/PCL: 90/10, PLA/PCL/MCC: 80/20/1, PLA/PCL/MCC: 80/20/3, PLA/PCL/MCC: 90/10/1 and PLA/PCL/MCC: 90/10/3 biocomposite samples were analysed by the biodegradation study. Biodegradation percentage results showed that with higher MCC and PCL component in the test polyblends, biodegradation was higher, as compared to a lower percentage of PLA and MCC in the polyblended test samples. But, the addition of higher MCC amounts (greater than 1%) in PLA/PCL: 90/10 suppressed biodegradation in comparison to nPLA and nPCL. Percentage biodegradation of the test samples was found as follows: PLA/PCL/MCC: 80/20/3 > nPLA > PLA/PCL: 90/10 > nPCL > PLA/PCL/MCC: 80/20/1 > PLA/PCL: 80/20 > PLA/PCL/MCC: 90/10/1 > PLA/PCL/MCC: 90/10/3. The decrease in molecular weight analysis proved that chain scission mechanism occurred leading to the formation of intermediates, which further led to microbial assimilation. The new microbial biofilm formation assay done in this study showed that the absorbance of the test samples after biodegradation was due to microbial film formation on the test sample surface. Microbes attacked the amorphous regions of the blends, leaving behind the crystalline portion, which was proved by the optical spherulite micrograph studies that showed an increase in spherulite size and spherulite density due to the spaces formed after the erosion of the amorphous regions from the blend surface. The cavities thus formed were observed by FESEM analysis, which revealed that PLA formed fibril-like structures due to degradation under composting conditions. These disorientations formed due to biodegradation led to diminishing melting peaks in the blends and decreasing recrystallization temperature as observed from the DSC thermograms. DSC thermograms further revealed that the increase in percentage crystallinity of the test samples was due to microbial assimilation of the amorphous portions, leaving behind the crystalline portions

of the samples. Water contact angle study suggested breakage of the polar carbonyl group and formation of hydroxyl and carboxyl groups, thus increasing the hydrophilicity of the sample surface which further facilitated biodegradation. All the results obtained signified that the PLA based polyblends were almost 50-80% biodegradable in 100-120 days. These observations might open a new window for the fabrication and use of compostable biopolymeric blends, thereby reducing our carbon footprint. These tests might also help in material characterization and preparation of the test samples to achieve higher biodegradability.





Potential of algal biomass in accelerating the biodegradation of PLA based composites under composting condition and comparison of lab synthesized PCL with PLA

Algal biomass extract contains cellulose, lignin as well as various proteins and fats attached with it. Its highly nitrogenous contentwise. High amount amine content makes it hydrophilic and basic. Potential of algal biomass extract as a filler in PLA matrix is immense. PCL is biodegradable under thermophilic composting condition under short span of time (60-70 days). Biodegradation of PLA based biocomposites can be compared with lab synthesized PCL for better understanding. Biodegradation of PLA consists of sequential steps of hydrolytic degradation and further microbial assimilation under thermophilic composting condition. Hydrolytic degradation at composting temperature may throw some light on the intermediate release for microbial assimilation. Molecular weight analysis is the most important measuring technique for knowing the extent of degradation of the polymers.

Publications

- Naba Kumar Kalita, Doli Hazarika, Purabi Bhagabati, Ajay Kalamdhad and Vimal Katiyar Biodegradation Study of Biodegradable Polymers and Demonstrating Algae Biomass Extract as a Potential Filler for Compostable PLA Bioplastic. (To be submitted)

Abstract

The present study evaluates the degradation of PLA/algae biomass extract biocomposites under abiotic as well as composting condition and lab synthesized PCL based biocomposites under composting condition. The study was divided into two different case studies. In case study 1 PLA based biocomposites were studied at composting temperature under abiotic condition at 58 ± 5 °C. Hydrolytic degradation model is followed to predict and present the autocatalytic hydrolytic degradation process using molecular weight analysis. The study highlighted that chain scission alone cannot produce enough oligomers for microbial assimilation and provided information related to critical molecular weight which marks the sudden weight loss of the materials. In case study 2 biodegradation was studied for all the test samples similar to case study 1. Here, lab synthesized PCL was introduced to understand the biodegradation process more precisely using a different polymer. Addition of algae 5% extract in PLA increased the biodegradation of the PLA based materials. PLA/algae 5% extruded sheet (ES) also induced biofilm or microbial attachment on the surface more quickly than neat PLA ES due to nitrogen content. The degradation of the test materials is summarized by material characterization at different interval of time, which suggest biodegradation of the materials under composting condition using differential scanning calorimetry (DSC), Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM). Morphological studies revealed microbial attachment or biofilm formation on the films. Under both abiotic and biotic conditions PLA/algae 5% ES demonstrated excellent degradability. Furthermore, this study will help in designing PLA based biocomposites using algal biomass as well as other nitrogen rich fillers which might increase the biodegradability under different environment.

7.1 Introduction

Conventional plastics causes severe pollution and is tremendously affecting the day-to-day activity of various species in land and water. Many of the present one time use plastics takes years of time to degrade, which blocks drainage and clogs seas and rivers creating a mess for the organism living there. This plastics in landfill also increases toxicity of the land and water table of the nearby landfill area. Therefore, biodegradable polymers having similar properties as conventional plastics are promoted as their counter alternatives. These biopolymers get degraded under various environment (Kalita et al., 2019; Weng et al., 2013a). However, their degradation in managed and unmanaged environments is not well understood. Understanding degradation mechanism and secretion of plastics as non-biodegradable compostable and biodegradable is very tedious job in countries like India. Hence, development of plastics which can be thrown to the soil and which degrades within certain time period without managing for its degradation is the utmost necessity. This kind of strategy will be more effective in developing biopolymer in order to replace its conventional counterpart. Although non-biodegradable polymers can be recycled, but after recycle, various properties of the plastics decreases limiting its application (Song et al., 2009). Whereas, biodegradable plastics offers end-of-life management like composting which tremendously benefits the environment and non-biodegradable plastics lacks this kind of end-of-life management system (Gómez and Michel, 2013; Spaccini et al., 2016). Basically, biodegradable polymers are materials that can be decomposed into CO_2 , CH_4 , H_2O , inorganic compounds or biomass. Among the commonly used biopolymers Poly(lactic acid) PLA and polycaprolactone (PCL) can be biodegraded within 180 days of time under composting condition (Al Hosni et al., 2019a; Narancic et al., 2018; Vieira et al., 2010; Yagi et al., 2014). PLA one of the most commercially produced synthetic biopolymer which accounts for 25% of the global biopolymer production. PLA is

biodegradable in various environmental conditions and can be biodegraded both biotically or abiotically (Kale et al., 2007c; Kalita et al., 2019; Pradhan et al., 2010a). Literature suggests degradation of PLA at 58 °C under composting condition. Various microorganisms have been isolated from compost and have been shown to biodegrade PLA at different thermophilic temperatures (Jandas et al., 2013; Walczak et al., 2015). Biodegradation also depends upon various shapes and sizes of the plastics and processing types. Extruded sheets of PLA have a different biodegradation rate than extruded PLA strip of same grade (Qi et al., 2017). So, processing condition and type can influence biodegradation of PLA and PLA based composites. PCL another biopolymer derived from petrochemicals, is a thermoplastic. PCL is biodegradable and is known to be compatible with various other biopolymers and has been demonstrated in various applications. Like PLA, PCL is also biodegradable under various natural environments like soil, water and compost. These polymers are susceptible to biodegradation due to breakage of ester bonds and the enzymes like lipase and protease pathway helps in microbial assimilation (Al Hosni et al., 2019a). Various literatures suggest use of algal biomass in large quantity for biorefinery and other pharmaceutical purposes. Its use is immense from biotechnology to chemical industries (Bi and He, 2013). Application of agro wastes as fillers and as blends for preparing biocomposites has generated immense scientific interests due to its pragmatic environmental effects. These include (a) biodegradability (b) reduction in carbon footprint (c) for reducing cost of the biopolymer-based products etc. Use of algal biomass as filler in polymer preparation can prove to be a profitable as well as probable strategy for increasing biodegradability. Study used residual microalgal biomass in Poly(butylene adipate-co-terephthalate) for preparation of green biocomposites and found good mechanical properties (Torres et al., 2015).

Biodegradation of biopolymers under composting condition follows two simultaneous steps namely hydrolytic degradation acceded by biodegradation (microbial assimilation) (Kalita et al., 2019; R. Valapa et al., 2016). Relatively little is known about the abiotic degradation process of PLA based biocomposites under composting temperature and pH. Various techniques can be used for biopolymer biodegradation evaluation like CO₂ evaluation, decrease in molecular weight, calculation of residual weight analysis or weight loss measurement and mathematical modelling (Han and Pan, 2009; Kale et al., 2007c; Pradhan et al., 2010a; Qi et al., 2017). In this study two case studies were used to understand the biodegradation process as a whole. Moreover, case study 1 intended to evaluate the abiotic process of PLA based composites under composting pH and temperature. A hydrolytic degradation model and erosion of biodegradable polymers proposed elsewhere (Sevim and Pan, 2018) to predict the polymer degradation. This model helped in understanding molecular weight loss as mass loss of PLA based composites with respect to its autocatalytic action triggered by oligomer diffusion. The model data was finally compared with our experimental data. A code was developed for modelling the data and was used with little modifications as used by the previous authors (Pan, 2014). Finally, case study 2 was used to evaluate the biodegradation behavior of PCL and PLA based materials under composting condition. Molecular weight analysis and differential scanning calorimetry (DSC) were applied to understand the intensity of biodegradation under composting condition. Chapter 4 of the reference (Pan, 2014) provides the full detail about the model and its derivations. From both case study 1 and case study 2 algal biomass has proved to be a potential biofiller in PLA based system as it accelerates the biodegradation process by attracting microbes for biofilm formation on the film surfaces.

7.2 Results and Discussion

7.2.1 Algal biomass characterization

FTIR analysis of the algal biomass was done in order to observe amides and alcohols presence. It was revealed presence of alcohols (O-H) group (**Figure 7.1**) which will satisfy our biodegradability issue. Moreover, presence of amines also creates possibilities of its use as fillers in PLA based systems. Whole algal biomass constitutes of essential amino acids and non-essential amino acids which is also a source of nitrogen. Authors reported presence of fatty acids as well as carboxylic acids and hydroxylic acids in the biomass which makes it susceptible to biodegradation (Sudhakar and Premalatha, 2015). TGA analysis (**Figure 7.2**) revealed $T_{50} = 700\text{ }^{\circ}\text{C}$ which suggests it is thermally stable. Weight % of the samples from $110\text{ }^{\circ}\text{C}$ to $700\text{ }^{\circ}\text{C}$ basically represents volatile materials. Various literatures also reported algal biomass of containing high amount of cellulose and nitrogen making it a super waste (Bi and He, 2013). SEM analysis was used to understand the morphology of the algal biomass. It was found that the algal biomass has lumpy morphology with large particle size (**Figure 7.3**). Although various literatures showed absence of any kind of heavy metals and other impurities in the biomass (Tibbetts et al., 2015). This makes algal biomass a promising candidate for biopolymer biocomposite preparation.

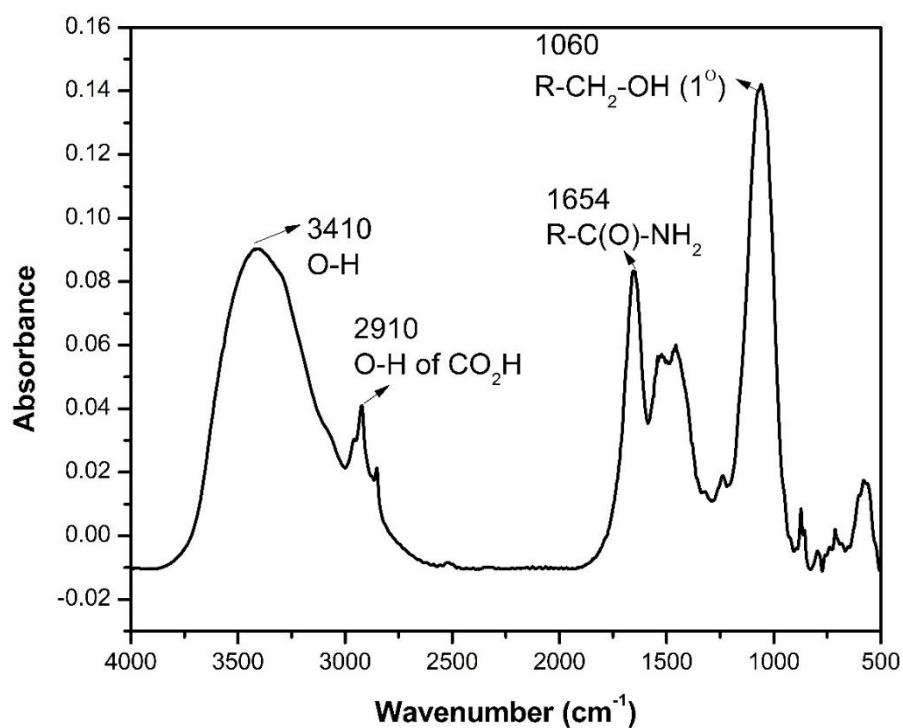


Figure 7. 1: FTIR spectra of the whole algal biomass.

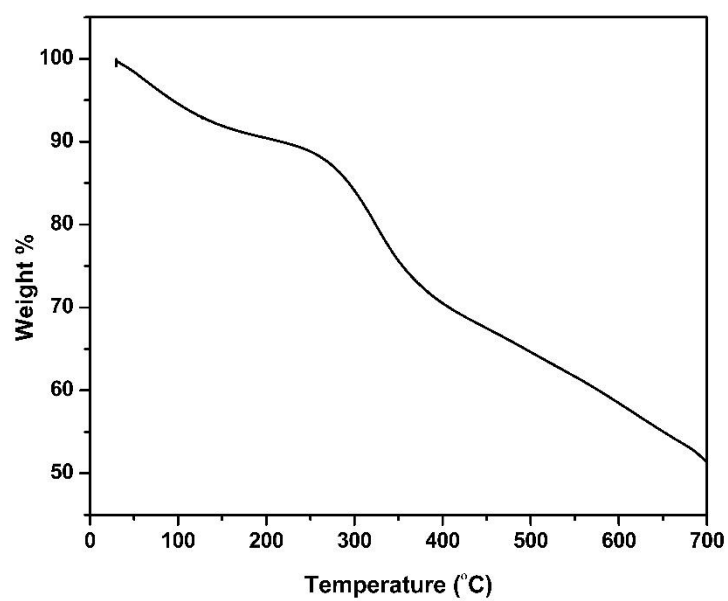


Figure 7. 2: TGA thermogram of the algal biomass representing T_{50%}.

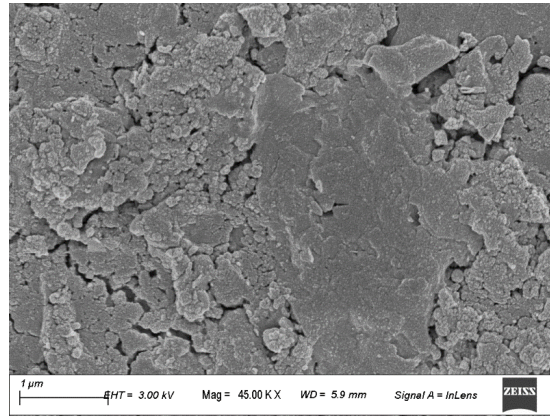


Figure 7. 3: SEM image representation the morphology of the algal biomass.

7.2.2 Case Study 1

7.2.2.1 Abiotic degradation

Abiotic degradation process of PLA and PLA based biocomposites involves hydrolysis of ester groups into either hydroxyl and carboxyl group. This step further increases the autocatalytic action of PLA based composites causing hydrolytic degradation. Manual observation of the test samples is shown in **figure 7.4** before and after abiotic degradation. Oligomers and fillers diffuse through the PLA matrix on to the buffer solution, which increases the autocatalytic activity. m (fixed) is the average degree of polymerization of all the short chains. For simplicity, m is assumed to remain constant during degradation and generally taken between 4-6. n (fixed) acid dissociation exponent of $-\text{COOH}$ groups, which should be 0.5 but may change empirically to fit experimental data; dimensionless. α and β are empirical parameters (fixed), here, the information about short chain production is taken in order to determine the values for α and β . $\overline{R_s}$ (change with time) is the total number of chain scissions per unit volume normalized by the initial number of ester units of all the chains per unit volume. Unit for all the concentrations: mol m^{-3} . $\overline{M_n}$ (change with time) is the number average molecular weight normalized by its initial value. Parameter N_{dp0} (fixed

parameter) is the initial degree of polymerization given by M_n/M_{unit} . C_{e0} is the initial concentration of ester bonds of the polymer is given by $\rho_{initial}/M_{unit}$, where M_{unit} is the molecular weight of a repeating unit. C_{chain0} represents the number of polymer chains per unit volume at the beginning of the degradation. k_1 and $\overline{k_2}$ (fixed) is the hydrolysis rate constant for non-catalytic reaction (1/time) and hydrolysis rate constant for autocatalytic reaction (1/time). Predicted and experimental data of the M_n is given in **figure 7.5** which depicts the $\overline{M_n}$ and values are presented in **table 7.1**.

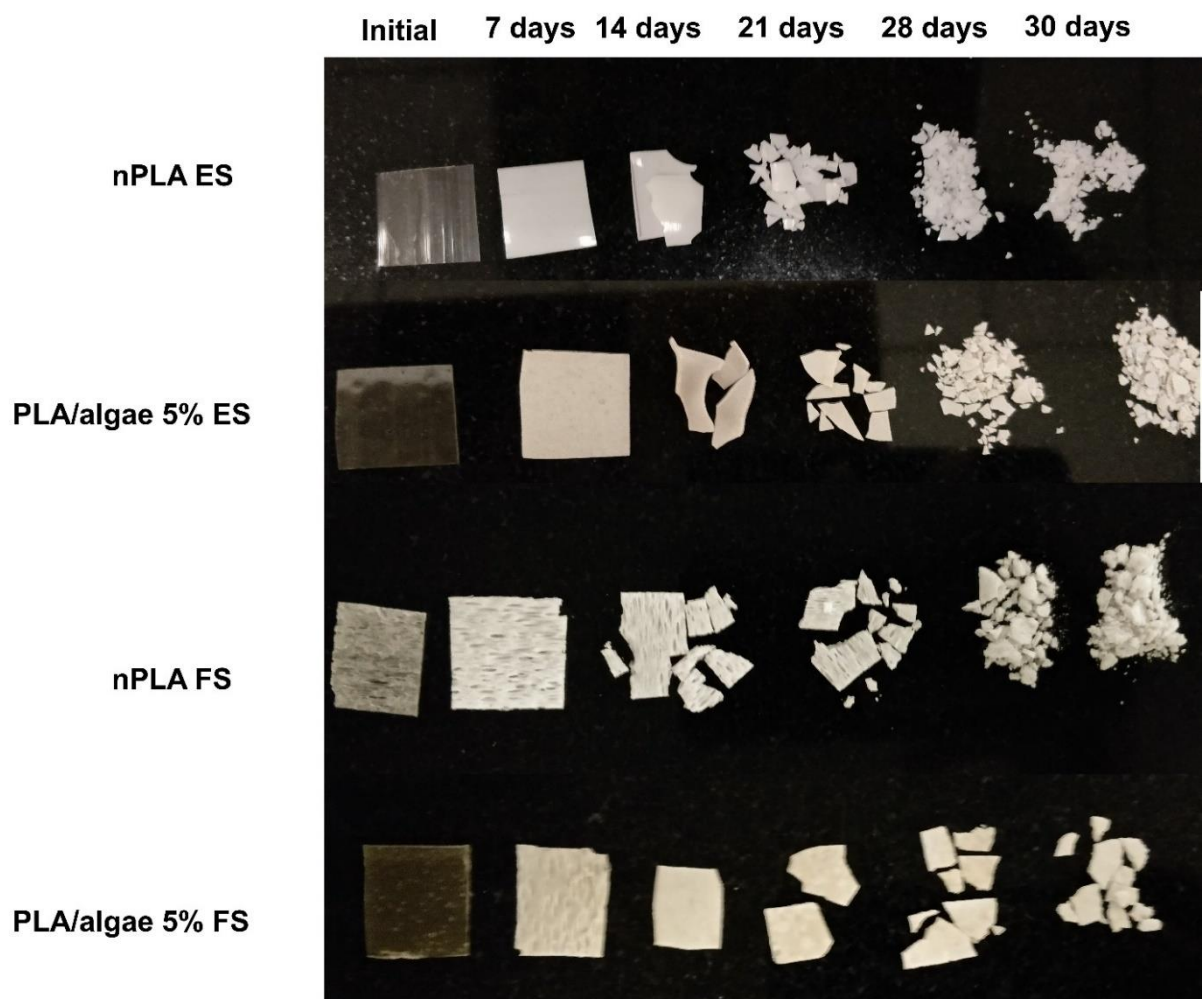


Figure 7. 4: Manual observation of the test samples during the abiotic degradation time interval (initial to 30 days)

Table 7. 1: Parameters used in the model to fit the experimental data of the abiotic degradation.

Parameters	Unit	nPLA ES	Pla/algae 5% Es	nPLA FS	PLA/algae 5% FS
C_{e0}	Mol m ⁻³	16805.5	16388.89	13194.44	15833.3
C_{chain0}		11.415	14.8807	7.8898	9.229
N_{dp0}		1472.22	1101.347	1672.33	1715.583
k_1	Day ⁻¹	0.000104974	0.000075085	0.0000908	0.000081955
$\overline{k_2}$	Day ⁻¹	0.003373	0.002242	0.003118	0.002879
α		0.5	0.5	0.5	0.5
β		0.5	1	1	1
n		0.5	0.5	0.5	0.55
m		4	4	4	4

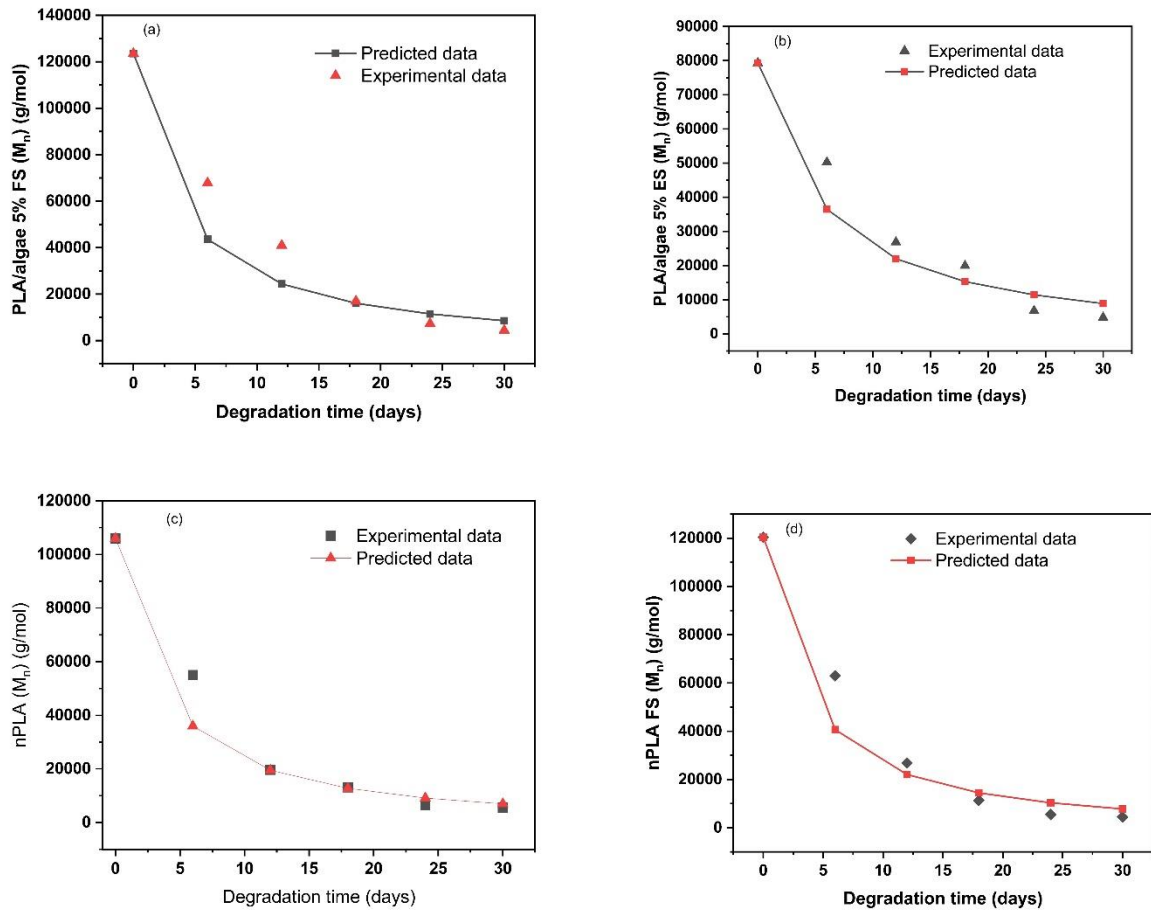


Figure 7. 5: $\overline{M_n}$ (change with time) is the number average molecular weight (M_n) normalized by its initial value. Straight line represents the predicted value and the discrete points represents the experimental data.

The discrete symbols in **figure 7.5** represents the experimental data of the number average molecular weight. The fit of the model data of the molecular weight loss suggest degradation of the samples due to autocatalytic action of the PLA based composites. For all the samples it was observed that there was a sudden molecular weight loss. The dissolution of the intermediates is the reason behind the sudden molecular weight loss. It can be observed from **figure 7.6** that pH of the solution changed drastically with time suggesting autocatalytic activity of PLA. This sudden molecular weight loss can also be attribute to surface erosion of the PLA matrix rather than the bulk degradation. The idea of bulk degradation was diminished here since the mass loss % of the samples were very less as compared with molecular weight loss of the samples as observed in **figure 7.5** and **figure 7.7**. Therefore, it can be confirmed that abiotic degradation facilitates surface degradation and mass loss and molecular weight loss cannot be correlated until a threshold number average molecular weight is reached (Pan, 2008). From study, it is clear that chain scission mechanism of PLA cannot dissociate enough oligomers to match the molecular weight loss at that corresponding mass loss. Sevim and Pan, 2018 found various surface and center differentiations during the degradation process. They found hollow centers with whitish layer on the top, which they attributed to the acid accumulation at the interior. This was proved from **figure 7.6** where we found decrease in pH of the buffer solution with time, leading to acid accumulation

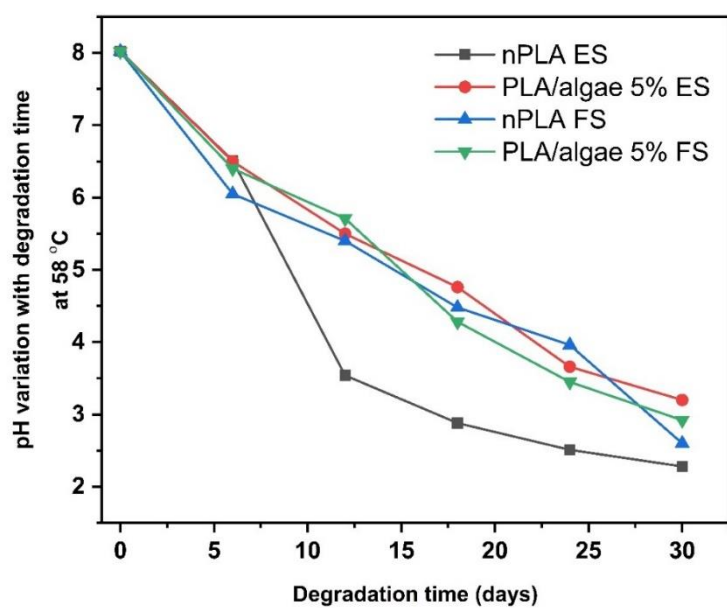


Figure 7. 6: pH variation of the buffer solution with time during the abiotic degradation process.

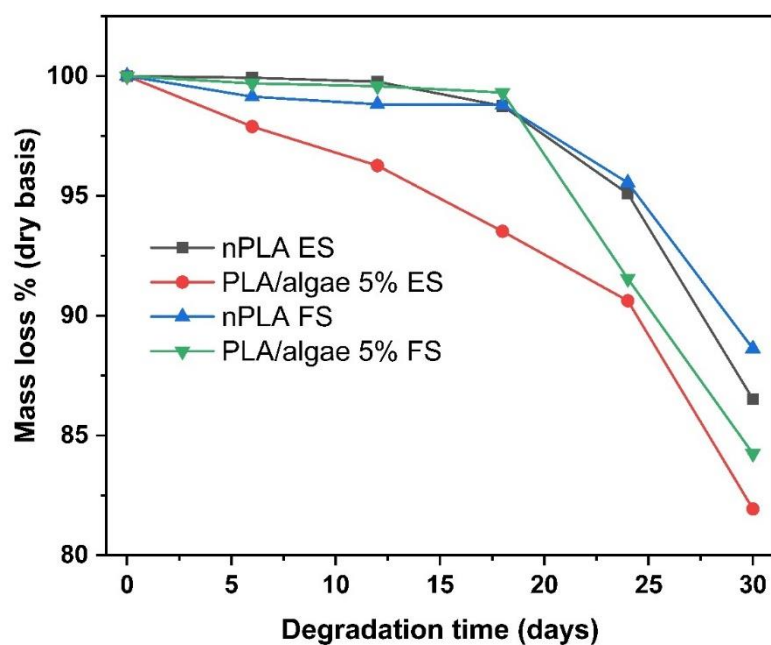
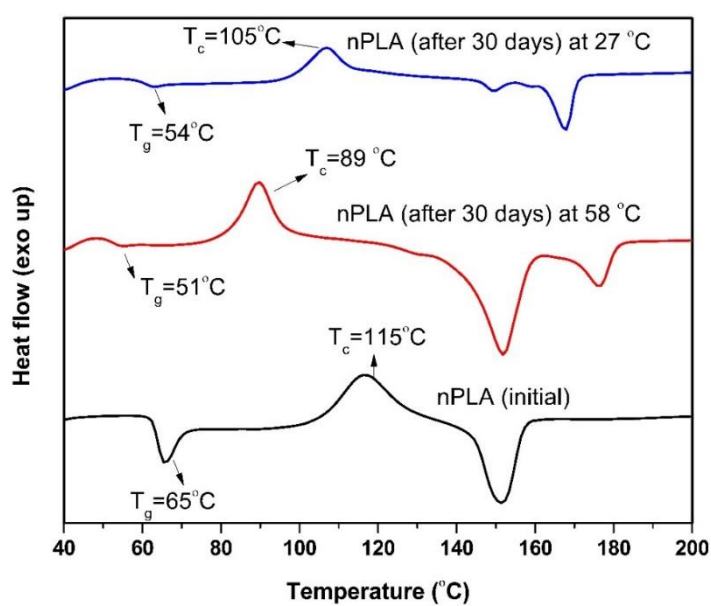
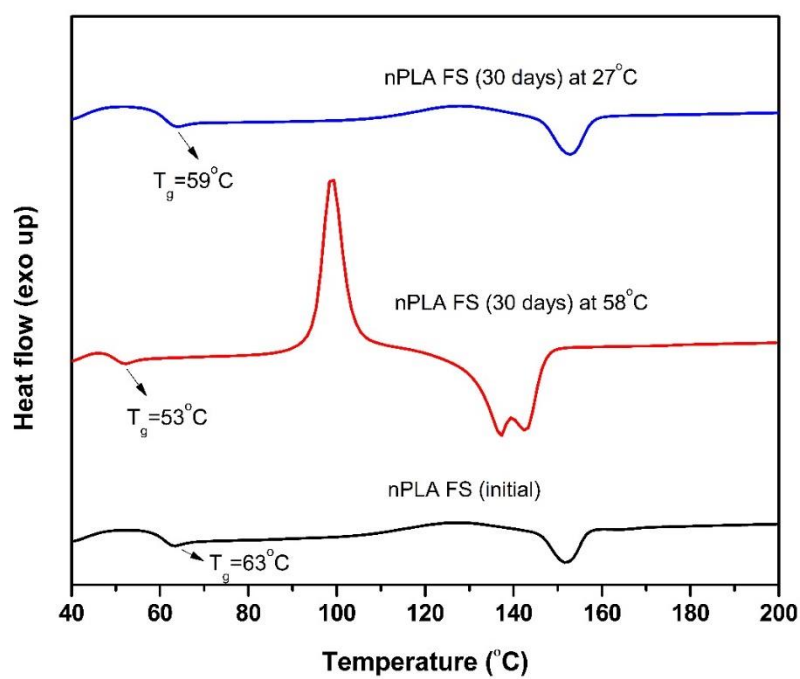


Figure 7. 7: Mass loss (%) of the test samples observed during the abiotic degradation process.

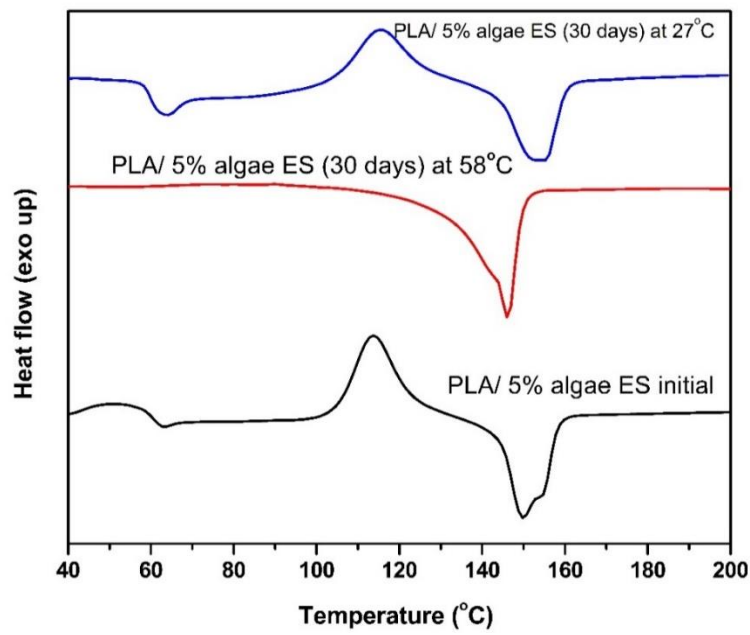
From the molecular weight analysis data, it is clear that our experimental datasets were following the predicted datasets. From the **table 7.1** data of predicted model suggest k_1 , $\overline{k_2}$ values predicting both autocatalytic as well as catalytic reaction taking place during the degradation of PLA based composites. It was found that nPLA ES and nPLA FS showing high $\overline{k_2}$ values, than PLA/algae 5% ES and PLA/algae 5% FS. This suggest addition of 5% algae into the PLA matrix either in extrude sheet or foamed sheet increased the autocatalytic action of the PLA based composites. From the rate constants it is clear that non-catalytic activity was prominent during the degradation period. At 27 °C not very significant changes were observed for test samples as it was observed at 58 °C. DSC data are presented in **figure 7.8 (a-d)**. It was observed that melting temperature of the samples treated at 58 °C decreased significantly with decrease in T_g suggesting degradation which happens due to segmental oscillations of macromolecules which rapidly change after degradation. Further degree of crystallization (**Table 7.2**) results showed increase in crystallinity percentage due to degradation of the amorphous portion leaving behind the crystalline region (Han and Pan, 2009).



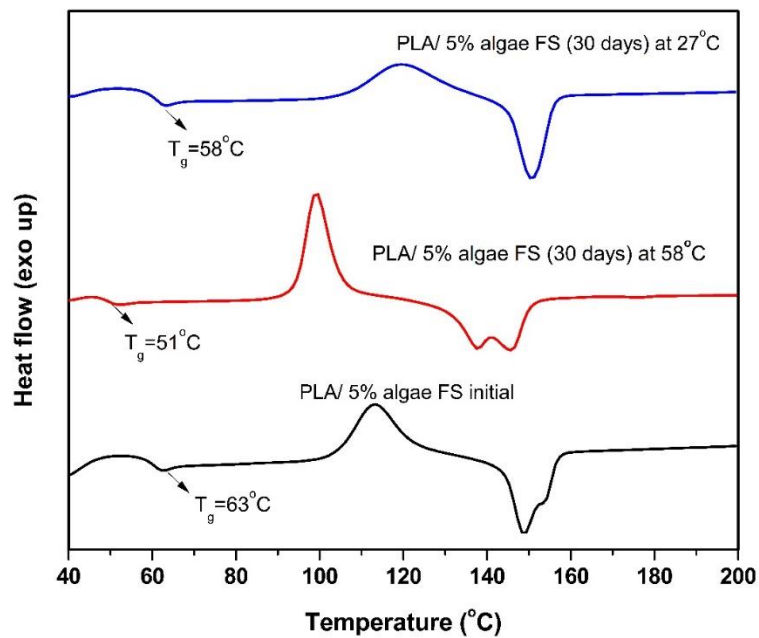
(a)



(b)



(c)



(d)

Figure 7. 8 (a-d): DSC thermograms of the test samples at different temperature on the 30th day of the abiotic degradation process.

It is often reported that fast crystallization retards biodegradation. It was observed in nPLA ES that crystallization percentage changed from 8% to 69% in 30 days whereas, in the case PLA/algae 5% crystallization percentage changed from 16% to 31 % which was slow as compared to nPLA ES. Our model also suggests the same, since, hydrolysis rate constant k_1 also supports the faster hydrolysis or degradation of the PLA/algae 5% samples. This is because faster crystallization retards hydrolysis whereas, slower crystallization gives more free space for the amorphous region to grow and degrade. From microscopic analysis it can be seen that during abiotic degradation of PLA based samples surface morphology changed leading to cracks and holes. These are mainly formed due to the degradation of amorphous region leaving behind the cracks and holes as shown in **figure 7.9**.

Table 7. 2: Percentage crystallization of samples before and after abiotic degradation at two different temperature.

Samples	At 58 °C		At 27 °C
	Before abiotic degradation (Xc %)	After abiotic degradation (Xc %)	After abiotic degradation (Xc %)
nPLA ES	8	69	10
nPLA FS	16	31	19
PLA/algae 5% ES	9	28	12
PLA/algae 5% FS	14	44	18

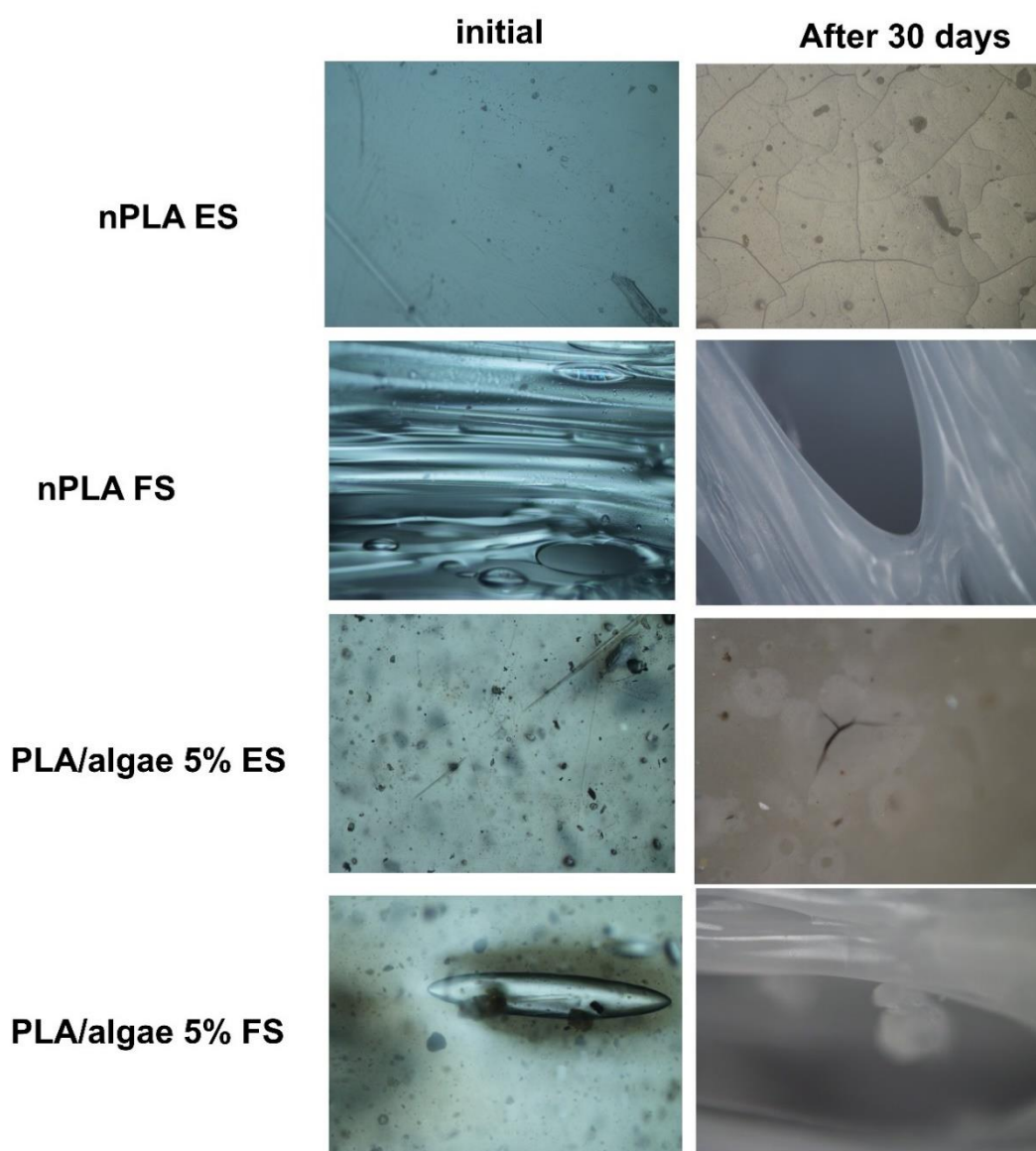


Figure 7. 9: Morphology of the test samples before and after abiotic degradation observed using POM.

7.2.3 Case study 2

7.2.3.1 Biodegradation under composting condition

Biodegradation test was performed accordingly (Kalita et al., 2019). Here, high molecular weight lab synthesized PCL was used in two different forms and shapes for biodegradation. PCL preparation and characterization are described elsewhere (Bhagabati et al., 2019). Rest of the test samples were kept the same as the abiotic degradation process. Extruded sheets

(ES) and Foamed sheet (FS) of nPLA, PLA/algae 5% ES, nPLA FS and PLA/algae 5% FS were used for the study. Molecular weight of the test samples revealed molecular weight loss of the samples during composting as shown in **table 7.3..** It was found that PLA/algae 5% ES molecular weight decreased from 77 kDa to 16 kDa on the 30th day itself which suggests significant amount of biodegradation as compared to nPLA. nPLA molecular on the 30th day decreased from 103 kDa to 80 kDa which is not significant in terms of molecular weight loss. Algae extract has caused chain fragmentation in PLA as high PDI of 2.2 was observed on the 30th day. After 60th day it's very difficult to measure and pick test film pieces from soil. So, on 90th day all the compost soil samples where the films were degraded were tested for its molecular weight analysis. For that soil samples were dipped in chloroform overnight and then filtered for GPC analysis. It was found that oligomers and polymers ranging from 300Da, 4 kDa-8 kDa respectively, were present in the compost soil which cannot be observed through naked eye, as shown in the flowchart of PCL biodegradation in **figure 7.10**. All the test samples follow the same flowchart shown in **figure 7.10** only differing in number of days of biodegradation at their respective stage. Initial stage is the preparation of the material in certain shape. Stage 2 represents the degradation environment type and the test sample underneath it. Stage 3 where the polymer starts to degrade partially with mass and molecular weight loss and assimilation under the selected environment. In this stage material characterization is possible for understanding the extent of biodegradation. In Stage 4 test materials gets completely disseminate under the selected environment, making it impossible to see through the naked eye, which makes physical characterization of the biopolymer almost impossible. In this stage the environment characterization is necessary to detect microbial assimilation or microplastics for proper understanding of the biodegradation process under that environment. In our representation flowchart we showed lab synthesized PCL whose molecular weight

decreased significantly from 44 kDa to 10 kDa. Molecular weight loss happens during biodegradation due to formation of intermediates and microbes in compost assimilates the intermediates into CO₂, water and biomass. Chain scission mechanism plays an important role in PLA biodegradation which converts the carbonyl groups into carboxyl and hydroxyl group (Kalita et al., 2019).



Table 7. 3: Molecular weight analysis of the test samples before and after biodegradation.

	Before biodegradation			After 30 th day			After 60 th day			After 90 th day (with compost soil)		
Sample	M _n (kdn)	M _w (kdn)	PDI	M _n (kdn)	M _w (kdn)	PDI	M _n (kdn)	M _w (kdn)	PDI	M _n (kdn)	M _w (kdn)	PDI
nPLA ES	103	169	1.6	80	139	1.6	46	96	2.0	18	27	1.5
PLA/algae 5% ES	77	156	2.0	16	41	2.2	8	13	1.8	3	3.5	1.1
nPLA FS	154	246	1.5	104	200	1.9	46	82	1.8	44	128	2.9
PLA/algae 5% FS	123	209	1.7	81	150	1.8	38	76	2.1	48	123	2.5
PCL pellet form	44	81	1.8	38	72	1.8	14	29	1.9	10	29	2.7
PCL dumbbell form	39	78	2.0	35	70	1.9	28	54	1.91	11	24	1.8

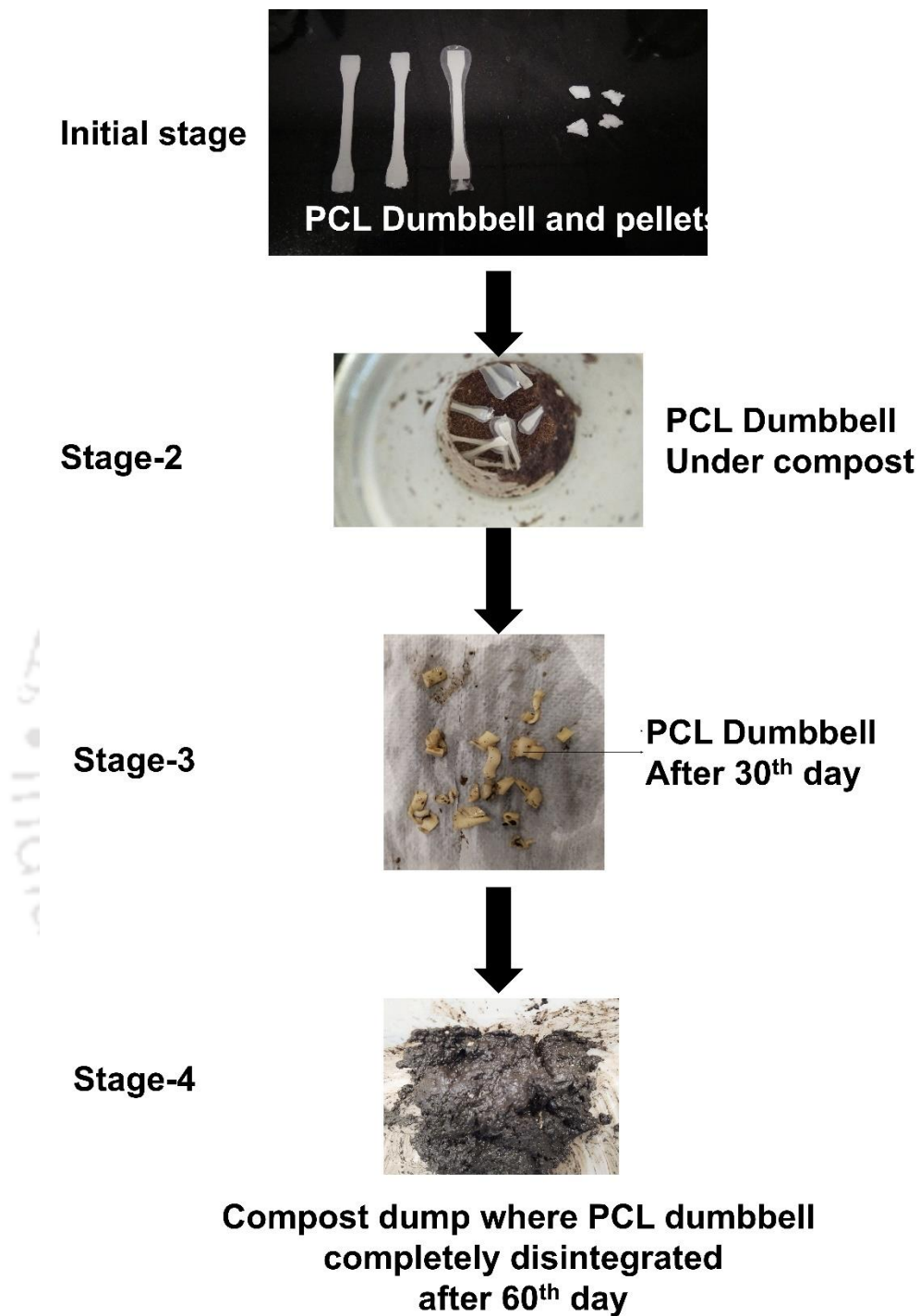
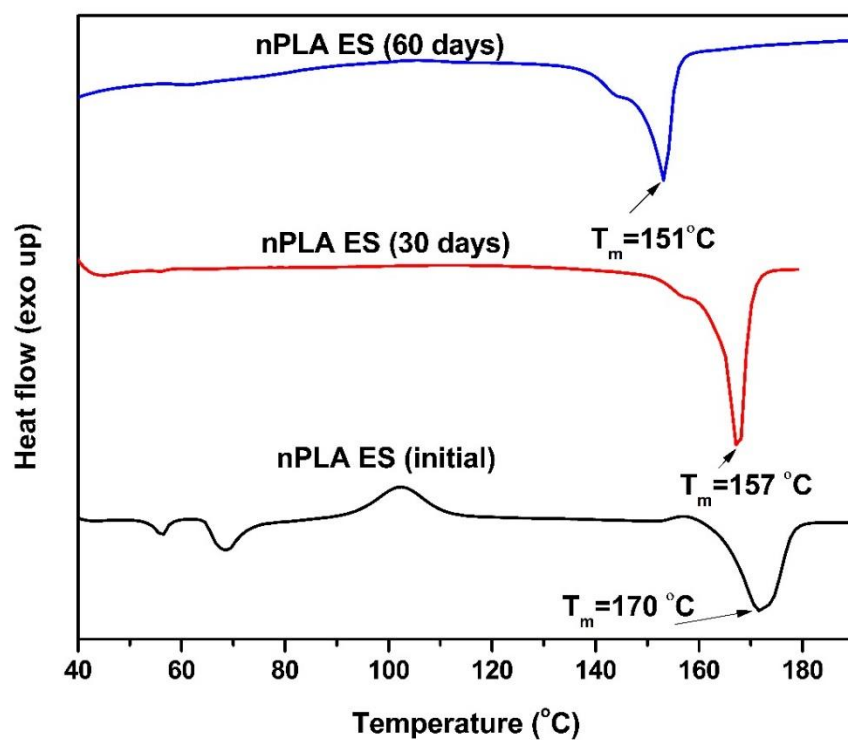
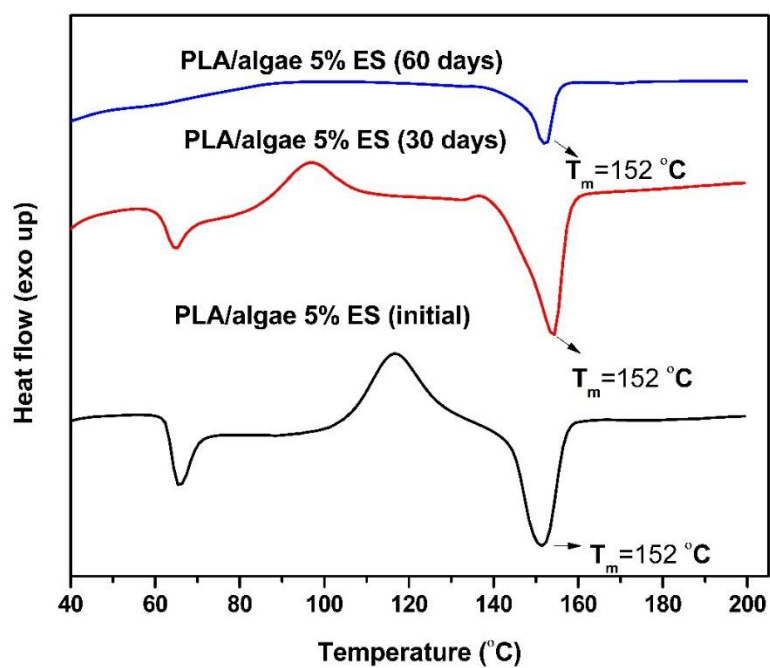


Figure 7. 10: Flowchart representing different stages of the biodegradation of a biopolymer (here PCL is used to understand the different stages).

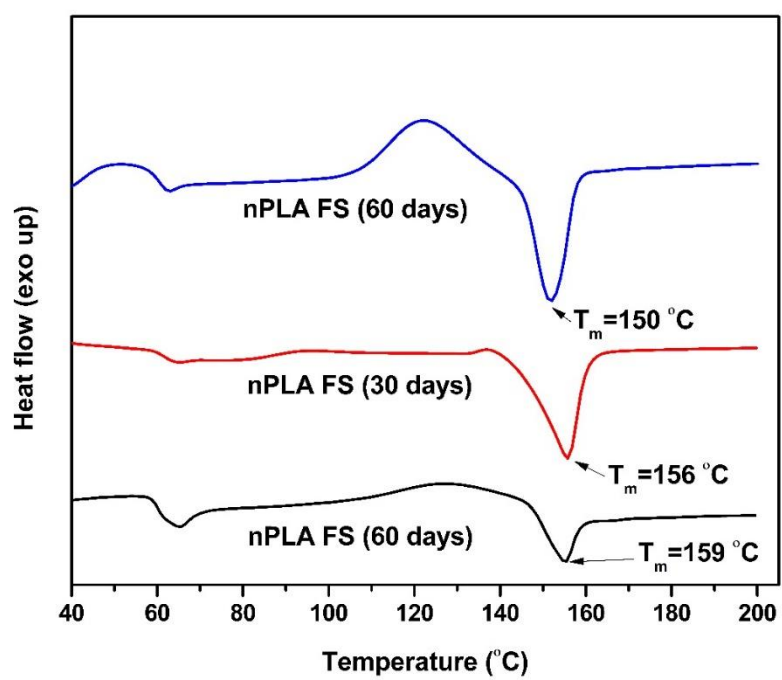
Changes in crystallinity percentage is very important since PLA undergoes preferential degradation of its amorphous region than crystalline part due to microbial assimilation property and easier disintegration of its amorphous part due to hydrolysis. From differential scanning calorimetry (DSC) thermograms shown in **figure 7.11 (a-f)**, it can be seen that PLA based samples melting points got decreased with biodegradation time as well as disappearance of T_c which suggests degradation and increase in percentage crystallization leading to decrease in T_g . This decrease in T_g and increase in crystallinity percentage can be attributed to decrease in macromolecular chain length leading to secondary crystallization as well as higher chain mobility (Pekařová et al., 2018). Although in PCL samples T_g increased from $-50\text{ }^{\circ}\text{C}$ to $-20\text{ }^{\circ}\text{C}$, which suggests degradation of PCL chains and making the PCL samples more brittle and eventually leading to fragmentation and molecular weight loss. Increase in T_g verifies that PCL is losing its flexibility, due to biodegradation. In case of PLA/algae 5% based samples its T_c got decreased from $115\text{ }^{\circ}\text{C}$ to $88\text{ }^{\circ}\text{C}$ on the 30th day of the biodegradation process. On the 60th day samples lose, its capacity to crystallize due to inherent presence of crystalline portion remained after biodegradation.



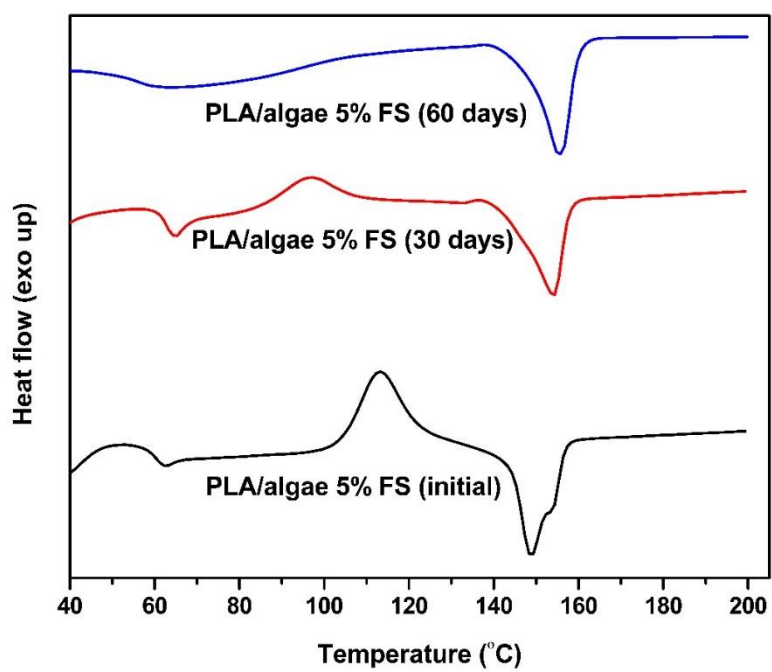
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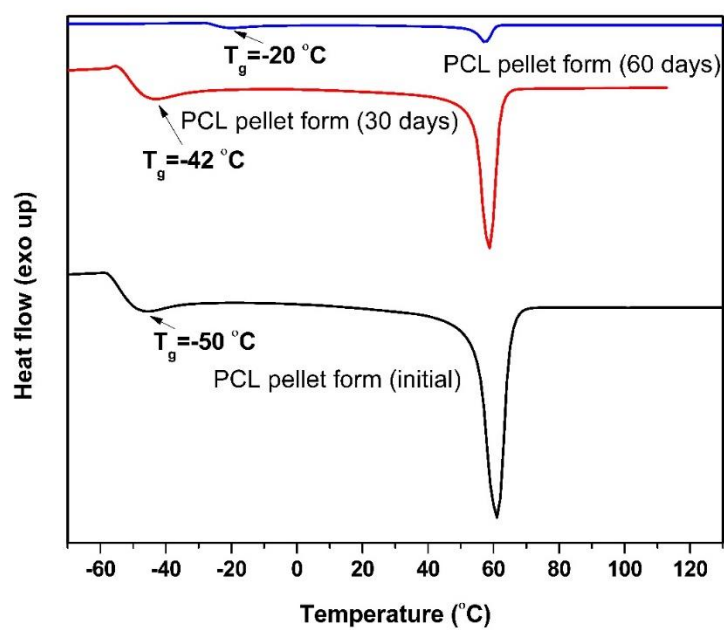
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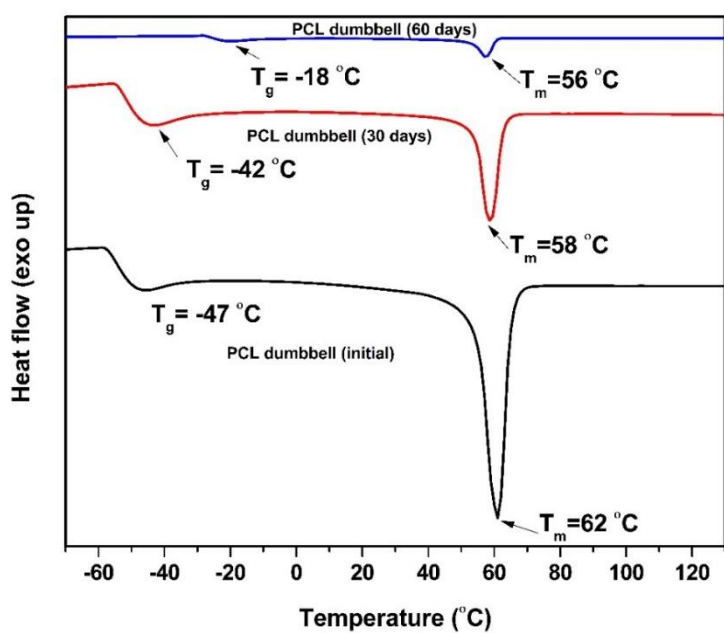
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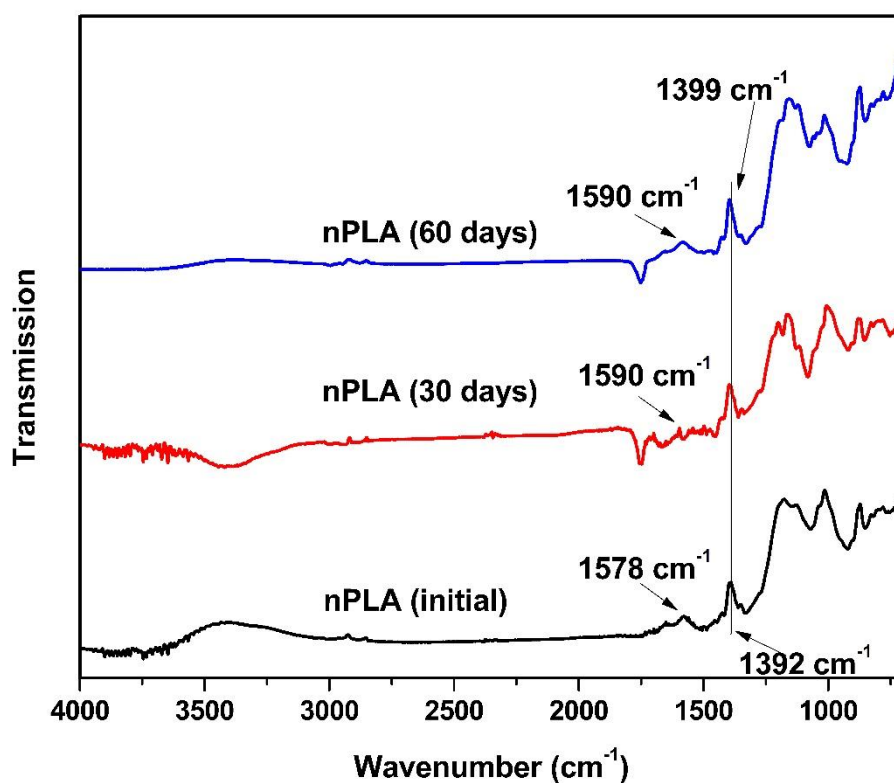
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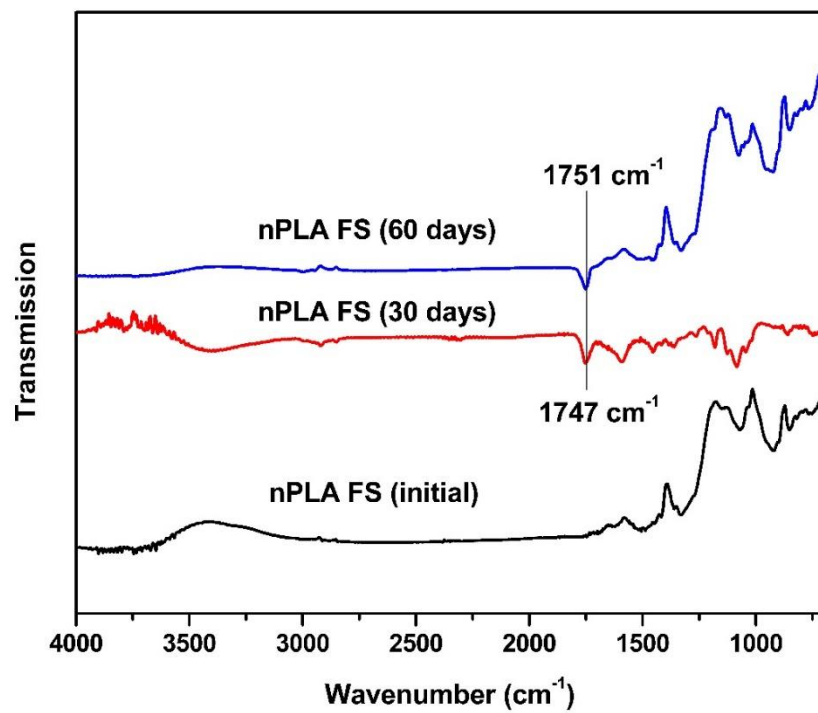
(f)

Figure 7. 11(a-f): DSC thermograms of the test samples before and after biodegradation under composting condition.

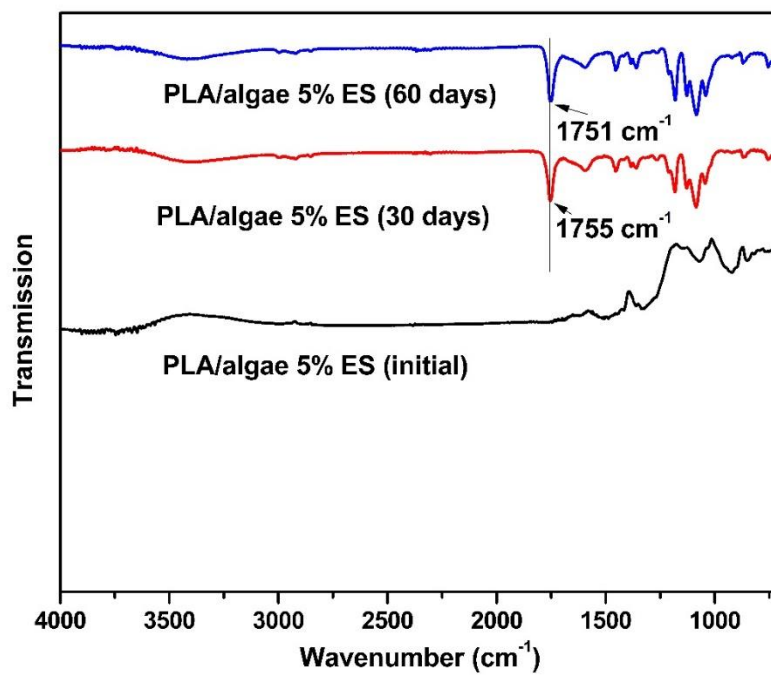
FTIR analyses are presented in **figure 7.12(a-f)**. From FTIR analysis it was observed that in most of the samples mostly in PLA/algae 5% based samples formation of R-C(O)-O-R which is a (C=O) stretching ester which was observed on the 30th day sample. This peak is small intensity peak formed due to breakage of aliphatic ester group. Mostly from FTIR analysis for all the test samples it can be observed that peak shifting was occurring in all the test samples. This is due to biodegradation, which causes breakage of bonds and functional groups (A. De Campos et al., 2011). Most of the test samples got high and medium intensity peaks at 1718 cm⁻¹ to 1751 cm⁻¹, suggesting formation of carboxylic groups, amides and ketones during the course of the biodegradation process. This footprint forms due to biofilm formation or enzymes released either by microbes or the polymer itself.



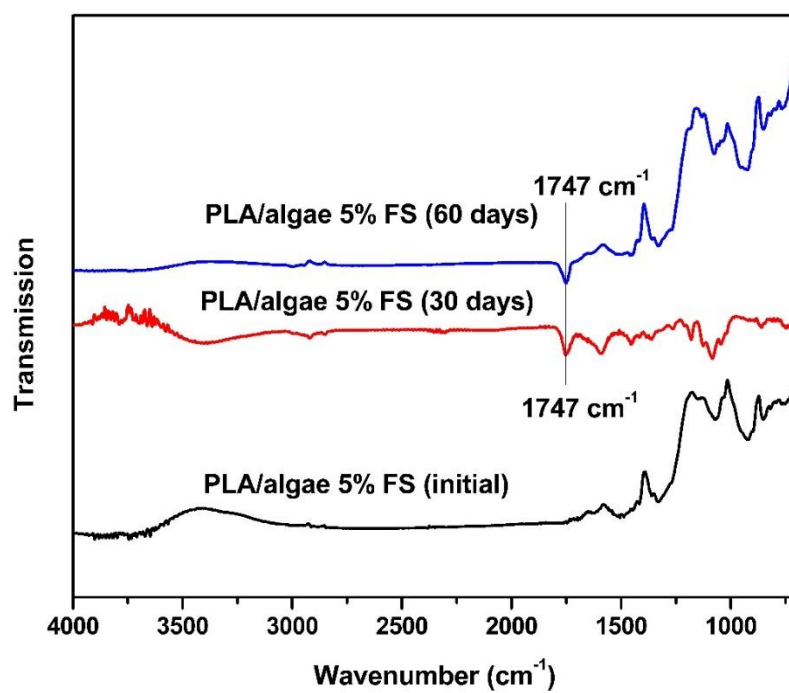
(a)



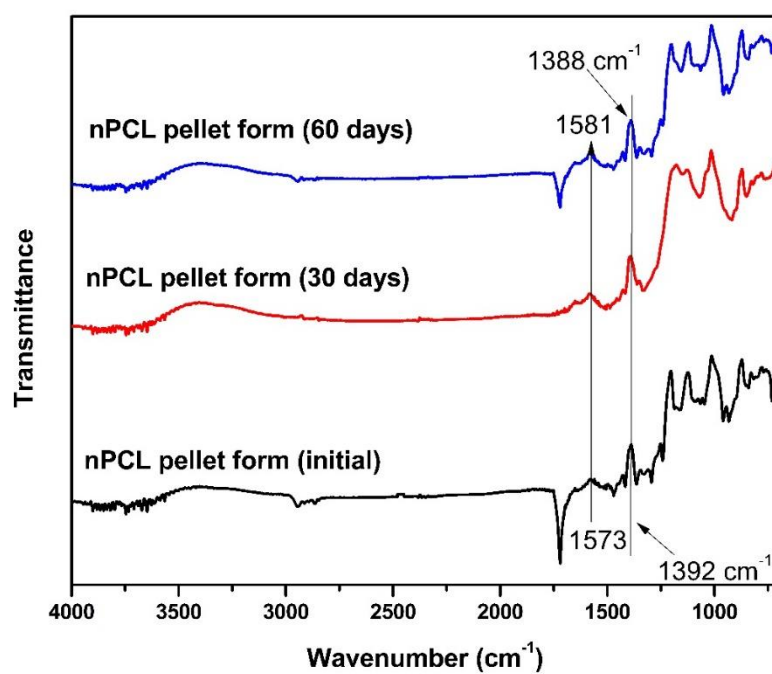
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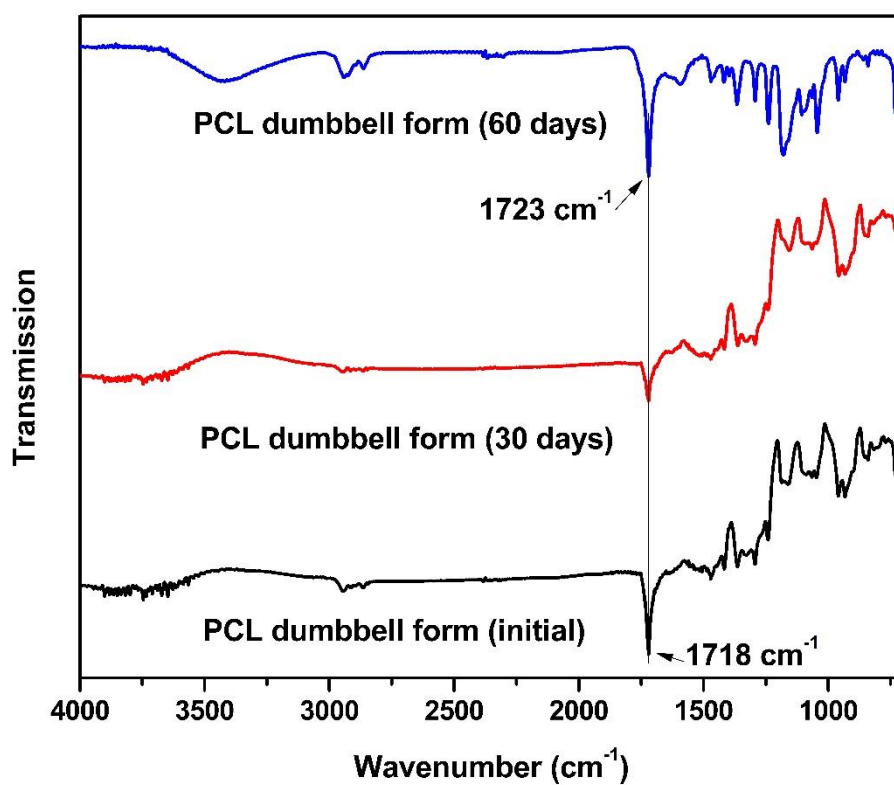
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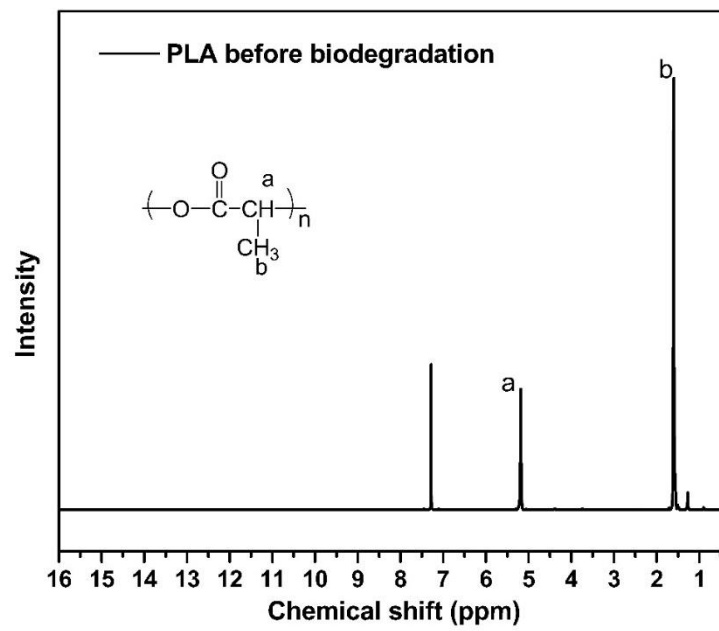
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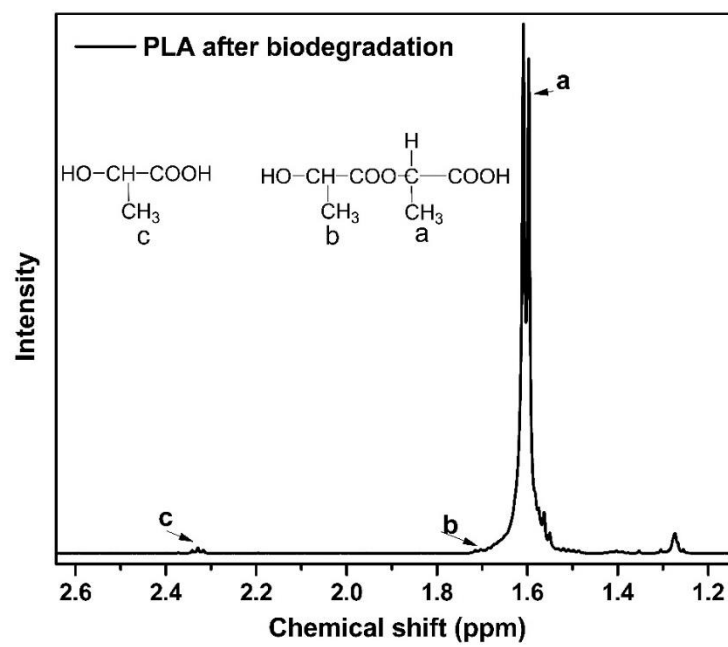
(f)

Figure 7. 12(a-f): FTIR analysis of the test samples before and after biodegradation representing peak changes during the biodegradation process.

^1H Nuclear magnetic resonance (NMR) spectroscopy was done to analyse the changes in the chemical composition during the degradation process. The chemical composition and changes that occurred in the PLA/algae 5% extruded samples and neat PLA samples over the biodegradation time presented in the **Figure 7.13 (a-d)**. The spectra were recorded after the films were dissolved in CDCl_3 and tetramethylsilane (TMS) was used as an internal standard. The biocomposite composition was determined based upon the protons of methyl group of PLA (at $\delta=1.58$ ppm). PLLA has $-\text{OH}$ and $-\text{COOH}$ groups at its two ends. The ^1H -NMR spectrum of PLLA is shown in **figure 7.13(a)**. The resonance signal at 1.3-1.6 (d, 3H, CH_3) is assigned to methyl protons ($-\text{CH}_3$) and 5.06–5.15 (q, 1H, CH) to methine protons ($-\text{CH}$) of the repeat unit of PLLA. In the spectrum of biodegraded PLA/algae biomass 5, all the other nonanomeric protons have peaks in the range of 3.5 to 4.0 ppm suggesting presence of nitrogen moiety. Because of the low concentration, degradation products were not detected by NMR spectroscopy before the 70th day of incubation. Similar conditions were reported elsewhere ([Musioł et al., 2016](#)).



(a)



(b)

For surface morphological studies three samples namely nPCL dumbbell form, PLA/algae 5% ES and PLA/algae 5% FS were studied as shown in **figure 7.14**. From the analysis it is clear that formation of cracks and holes were observed on all the samples. In PLA based samples cracks and holes were observed due degradation of the amorphous portion. Whereas, as observed in PCL based sample it forms rough patches, which concludes the degradation process. Since, both PLA and PCL follow different pathways of degradation, so formation of particles on the surface (Xu et al., 2016) of the PCL might indicate formation of acetyl-CoA which is utilized by the microbes for further assimilation as shown in **figure 7.15**. Acetyl-CoA forms as intermediate during degradation of PCL (Heimowska et al., 2017).

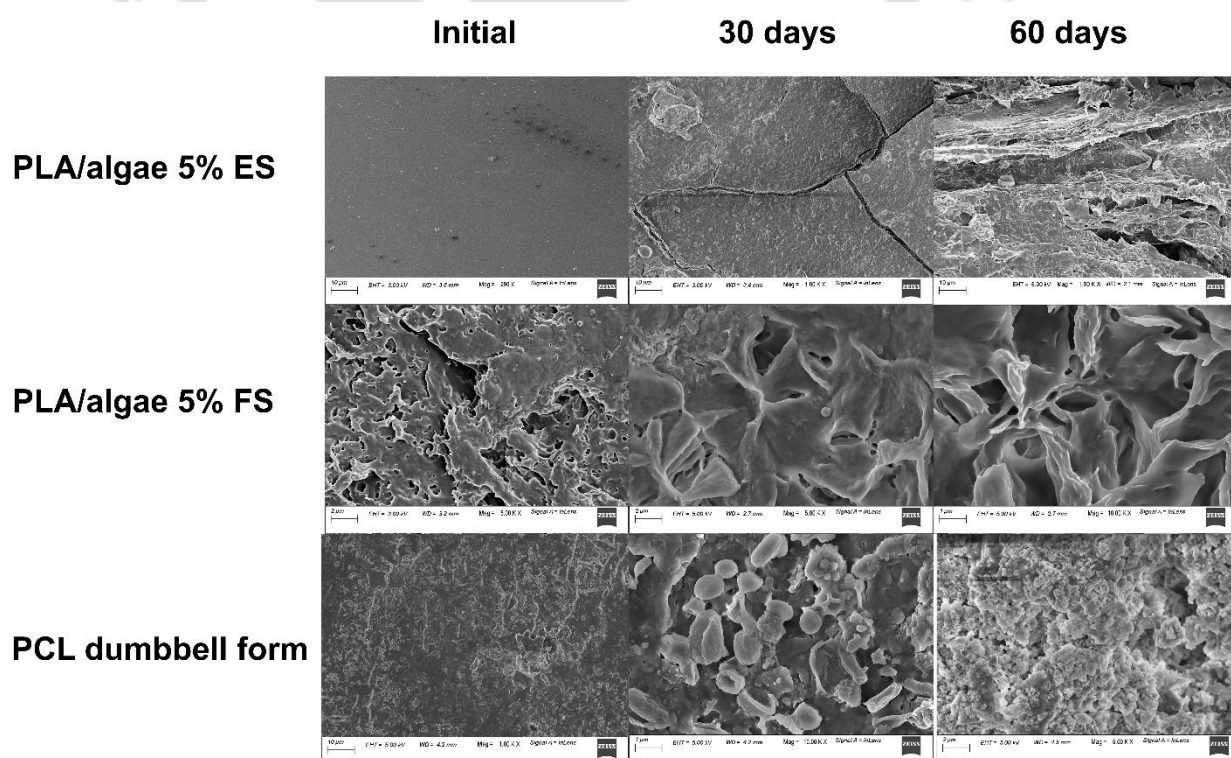
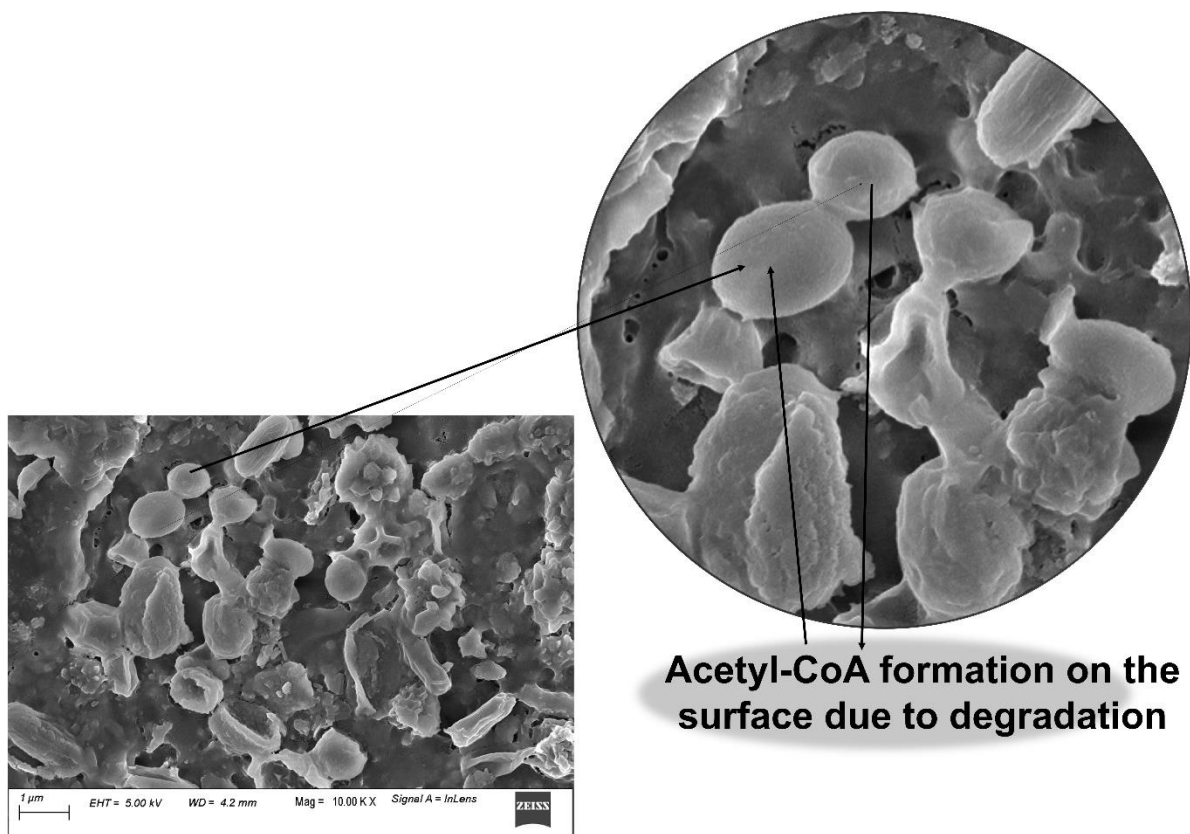


Figure 7. 14: Morphological changes observed using SEM during the biodegradation process.

From SEM analysis, algae extract induced microbial attachment on the surface and biofilm formation was observed as shown in **figure 7.16** for two samples.



Spherical morphology formation on the surface

Figure 7. 15: Surface morphology of PCL samples during the biodegradation process which represents formation of Acetyl-CoA and other intermediates on the surface.

It was clear that in PLA/algae 5% ES samples microbial attachment was much higher and clear in the 15th day of the degradation process. This was missing in case of nPLA film. This was also justified from our molecular weight loss data where, M_n of PLA/algae 5% decreased almost 80% as compare to nPLA on the 60th day. So, addition of algae extract which is a high nitrogen content material induced the nitrogen (N_2) atom bonded to one side of the carbonyl group of PLA forming amide groups. This increases the formation of biofilms faster on the PLA/algae 5% films, as amides are much liked by the microbes due to nitrogen source then the carbonyl/carboxyl/hydroxyl groups of neat PLA. This phenomenon has been documented (**Figure 7.16**), although a complete study till the end of

biodegradation can conclude whether how much time does nPLA takes for microbial attachment under composting condition.

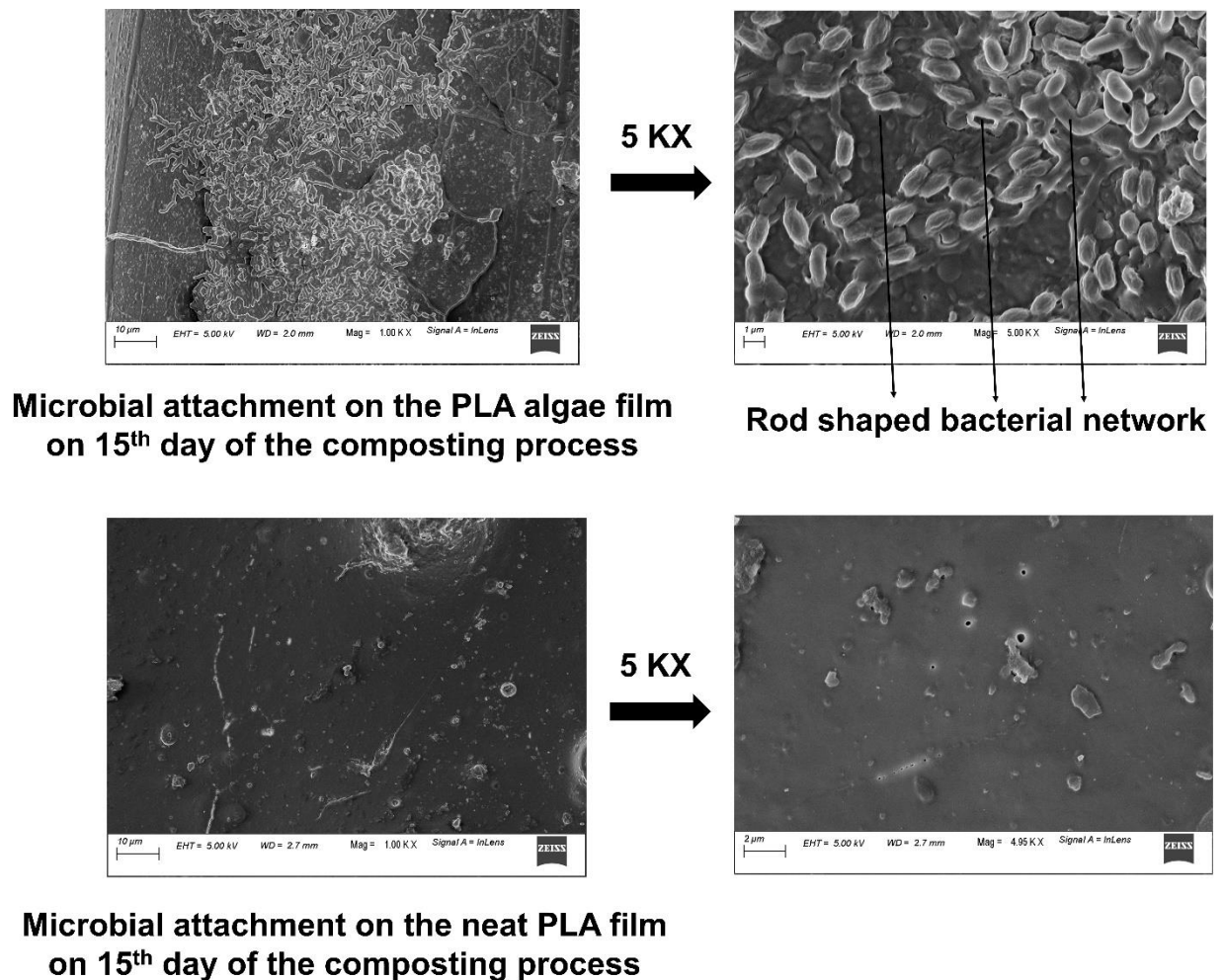


Figure 7. 16: Microbial attachment comparison on the PLA/algae 5% ES and neat PLA ES films on the 15th day of the composting process. This shows how algae as filler increasing the synergy of the degradation environment.

7.3 Conclusions

With the increase in demand of biodegradable plastics and consumption, its degradation behavior has to be understood well in order to prepare new material. In case study 1 the degradation time and the hydrolytic step of the biodegradation process at composting temperature of 58 °C was observed. The mathematical model presented to describe the autocatalytic hydrolytic degradation process. Our study highlighted that chain scission alone cannot produce enough oligomers for microbial assimilation. The modelled data provided information related to critical molecular weight, which marks the sudden weight loss of the materials. Molecular weight analysis showed degradation of the test's samples under abiotic condition but mass loss was not so significant as compared to molecular weight loss. Modelled data also revealed both autocatalytic and non-catalytic reaction takes place during abiotic degradation of PLA based materials. DSC analysis revealed degradation was happening in the test samples as crystallinity percentage of the test samples increased after 30 days. FTIR and morphological analysis also revealed degradation of the test samples. In case study 2 biodegradation was studied for all the test samples similar to case study 1. Here, lab synthesized PCL was introduced to understand the biodegradation process more precisely. It was found that addition of algae 5% extract in PLA increased the biodegradation of the PLA based materials. PLA/algae 5% ES also induced biofilm or microbial attachment on the surface more quickly than neat PLA ES due to nitrogen content. The degradation of the test materials was summarized by material characterization at different interval of time, which suggests biodegradation of the materials under composting condition. Morphological studies revealed biodegradation occurring through microbial attachment or biofilm formation in the films. Under both abiotic and biotic conditions PLA/algae 5%, ES demonstrated excellent degradability. Future studies will be aimed towards characterization of PLA based films using different compositional algae

biomass extract and thereby tuning its biodegradability under composting as well as soil conditions.





***Studies on various biopolymeric blends and composites under outdoor
natural soil condition***

Use of bioplastics is still in commercial debate table due to its biodegradability in natural soil or unmananged environmental condition. Although, industrial composting is highly suitable for developed countries due to their high waste seggregation ratio then countries like India. So setting up industrial composting setup exclusively for bioplastic end-life management is not economically feasible. Hence, search and research for biopolastics that can biodegradable under natural environmental condition is utmost necessity. Although use of fillers like algal biomass extract and modified chitosan has been proven to be good accelrator for biodegradation of the PLA based composites, its degradation in unmanaged soil conditon can have much more positive social impact in the developemnt of the developing countries of the globe thereby, reducing plastic pollution.

Publications:

- Naba Kumar Kalita, Doli Hazarika, Purabi Bhagabati, Ajay Kalamdhad and Vimal Katiyar, Biodegradation study of bioplastics under unmanaged natural soil burial condition.(To be submitted)

Abstract

This study is designed to understand the biodegradation behaviour of PLA and PCL based biocomposites, blends and polyblends of different shapes. Fillers like wood-dust, algal biomass and MCC were used in preparation of the test samples. Outdoor soil with descent growth of grass was selected for the test. Samples were buried 20 cm deep. Outdoor temperature profiling was done both for the environment as well as inside 20cm deep soil. Temperature has been daily monitored with outdoor temperature in order to observe environmental behaviour that might add as a parameter of degradation under outdoor soil condition in a particular latitude and longitude. An average of 33.5 °C of outdoor temperature and 23 °C was received during the period of June 2019 to December 2019 for 180 days. Molecular weight analysis revealed that nPCL (labmade), nPLA (labmade) and PCL/algaebiomass 20% samples underwent accelerated molecular weight loss under soil condition. Almost 90% molecular weight loss was observed for nPCL (labmade) and 95% molecular weight loss for nPLA (labmade) under soil condition. However, other samples failed the biodegradation test under soil condition as most of them did not underwent critical molecular weight loss threshold. Thickness of all the test samples increased after soil burial for 180 days except nPLA (extruded sheet), nPLA foamed sheet and PLA/algaebiomass 5% foamed sheet due to swelling, moisture uptake and soil adhesion on the test sample surface. Test samples were analysed using infrared spectroscopy during the biodegradation period. Hydrophilicity of the test samples decreased due to surface erosion and formation of hydrophilic end groups on the sample surfaces. Thus this study confirmed that most of the PLA and PCL based biopolymeric materials were not biodegradable under natural soil condition within a time period of 180 days.

8.1 Introduction

Increase in municipal waste generation which mainly constitutes plastics is creating havoc around the globe. Landfills cause land, water as well as water pollution, due to which landfilling is banned in many countries. Growing use of nonbiodegradable conventional petroleum based plastics and its effect is clearly visible on ocean surfaces and cities in developing countries. Nearly 30% of plastic waste is still landfilled in Europe, and the current European Union plan is to reduce landfilling of plastic to no more than 10% by 2030. In the USA, 53% of total municipal solid waste (MSW) is landfilled, with plastic waste representing 13% of municipal solid waste. Although plastic waste pollution is a global environmental problem, still certain countries are suffering more due to poor recycling rate. Recycling of the plastics is also masked by high price in comparison to relatively low quality of plastic. These issues are recently addressed by development of biodegradable polymers. Although these polymers are sometimes degradable only in manageable environmental condition. Most of the biopolymers like poly(lactic acid) PLA, polycaprolactone (PCL) can be used in certain applications like packaging etc. PLA which takes longer time in degrading under unmanaged natural soil condition is one of the world's most commercially viable bioplastic that can be used for packaging applications. PLA is compostable under industrial composting conditions and can biodegrade completely within 180 days of time. PLA is a linear aliphatic polyester which is biodegradable. PLA forms intermediates through chain scission induced by hydrolysis and these intermediates are further assimilated by the microbes for complete carbon mineralization. PCL is also another biodegradable linear polyester derived from petroleum precursors. It has low melting point and is highly compostable. It is also biodegradable under unmanaged condition taking shorter span of time than PLA. PCL is highly flexible due to low glass transition temperature (T_g), which makes it an ideal compostable bioplastic for various useful applications. PCL is more

biodegradable than PLA under industrial composting condition. So their blend can tune certain properties and biodegradability required for bioplastic production. Environmental problems are related to human behaviour, so for preventing further escalation of global plastic pollution, designing of bioplastics for packaging application based on PLA or PCL which can biodegrade under unmanaged natural soil condition is the necessity of the present world.

Various authors studied PLA/PCL and PLA/polyhydroxybutyrate (PHB) blends for understanding its biodegradability under soil condition. PCL was found to be completely biodegraded under industrial composting condition under 45 days of time. Whereas, PLA/PCL:80/20 showed 100% relative biodegradation under 75 days of time under industrial composting condition. Although, these blends took more than 265 days under home composting condition which suggests difficulty in degradation of these blends under unmanaged natural environments. Authors here observed PCL, PLA, PHB and PBS thick test samples under uncontrolled soil condition for 21 months and no significant degradation was observed for all the polymers except PCL where fungal growth was observed on the surface. Like the addition of fillers such as modified chitosan or functionalised CNC add on certain characteristics like high permeability and mechanical strength in PLA composites. Similarly addition of various fillers like starch and wood flour effects its biodegradability. Authors here blended starch and wood flour with PLA to study the biodegradation activity of the blend composites,. They found that addition of starch acted as biological fuel which initiated and accelerated the growth of microorganism in the soil as well as on the PLA surface thereby accelerating the biodegradation rate of the PLA samples under outdoor soil condition. Eventhough, biodegradation mechanism of PLA and PCL based composites has been studied under soil condition, still affect of various fillers is still ambiguous. And lifetime of the materials are not clearly established with respect to their biodegradation

under soil condition. This chapter will focus on the various PLA and PCL based blends, polyblends and biocomposites biodegradation behaviour under natural soil condition. Under natural unmanaged soil condition various methods can be used to evaluate the extent of degradation. In this chapter molecular weight analysis, fourier transform infrared (FTIR) spectroscopy and water contact angle analysis will be discussed briefly. In this chapter test samples of different shapes were used for the degradation study namely, nPLA (extruded sheet), nPLA (extruded tray), nPLA (pellet form, lab synthesized), nPCL (strip), nPCL pellet, nPCL (dumbbell form), PCL/20% algae (dumbbell form), PCL/30% algae (dumbbell form), PCL/30% wood-dust (dumbbell form), PLA/PCL:80/20 (strip), PLA/PCL/MCC:80/20/3 (strip), PLA/algae 5% (extruded sheet).

8.2 Results And Discussion

8.2.1 Outdoor temperature, humidity profiling and manual observation

Outdoor temperature was measured from 6th day of June 2019 to 6th day of December 2019 in order to have a idea about the amount of temeprature the soil surface received during a particular day. **Figure 8.1** represents the outdoor temperature profile. Temperature was measured two times a day representating the maximum and minimum teperature received during a day. Per day temperature was averaged basing on the maximum and minimum temperature recorded. Temperature was recorded for 180 days. These temeprature was again averaged for obtaining the average temperature received during the biodegadatation process. It was found that an average of 33.5 °C was received throught the 180 day timeline. Temperature of the soil under 20 cm depth was also measured for 180 days (**figure 8.2**). It was found that the temperature under soil was about 23 °C. This temperature was good enough for microbial flora to grow around the polymer during the degradation period. This kind of temperature is also suitable for certain kind of soil bacteria for reproduction (Weng et al., 2013a).

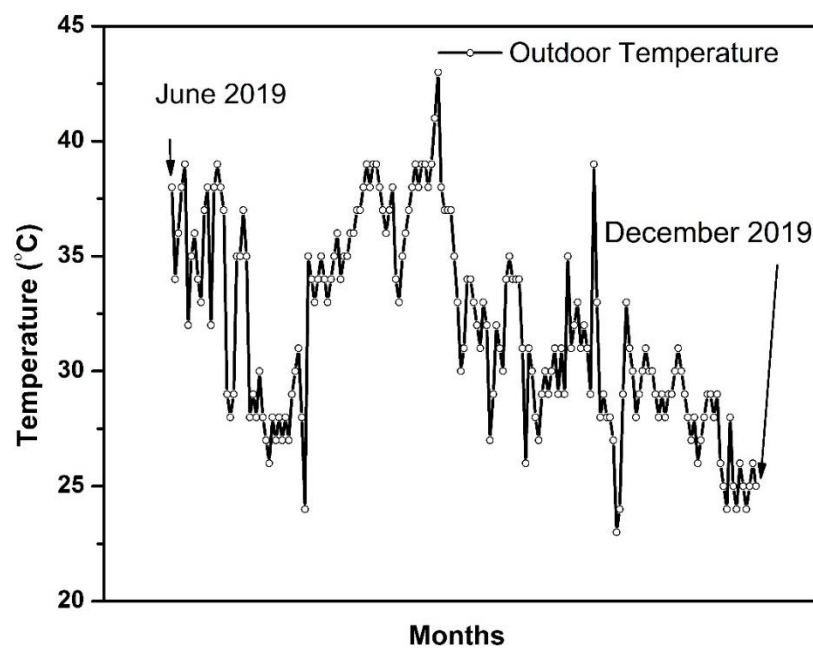


Figure 8.1: Graph representing outdoor temperature profile during the degradation period

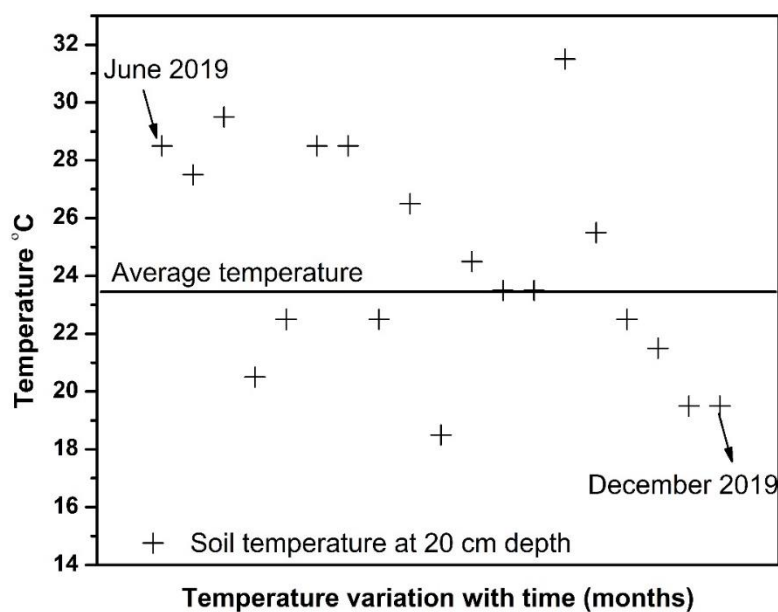


Figure 8. 2: Graph representing temperature profile of soil under 20 cm deep.

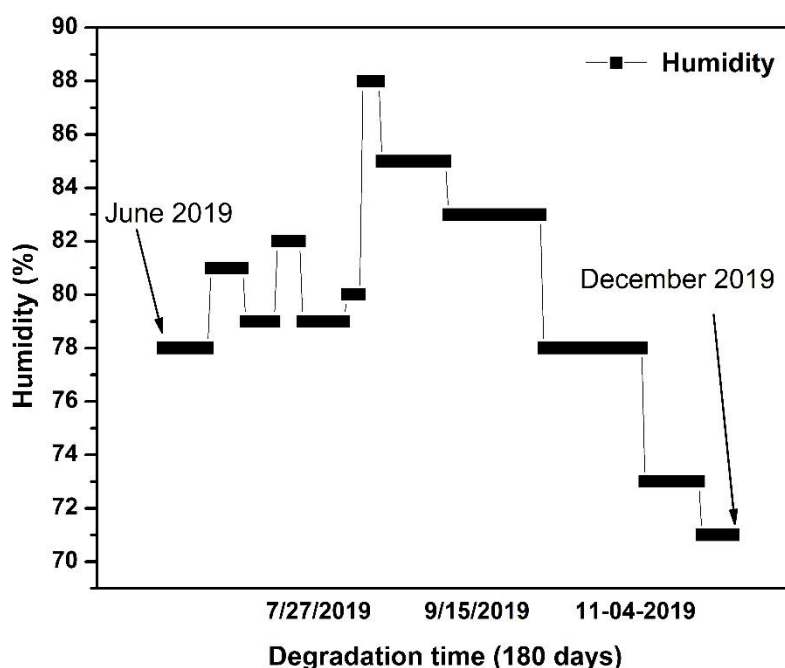
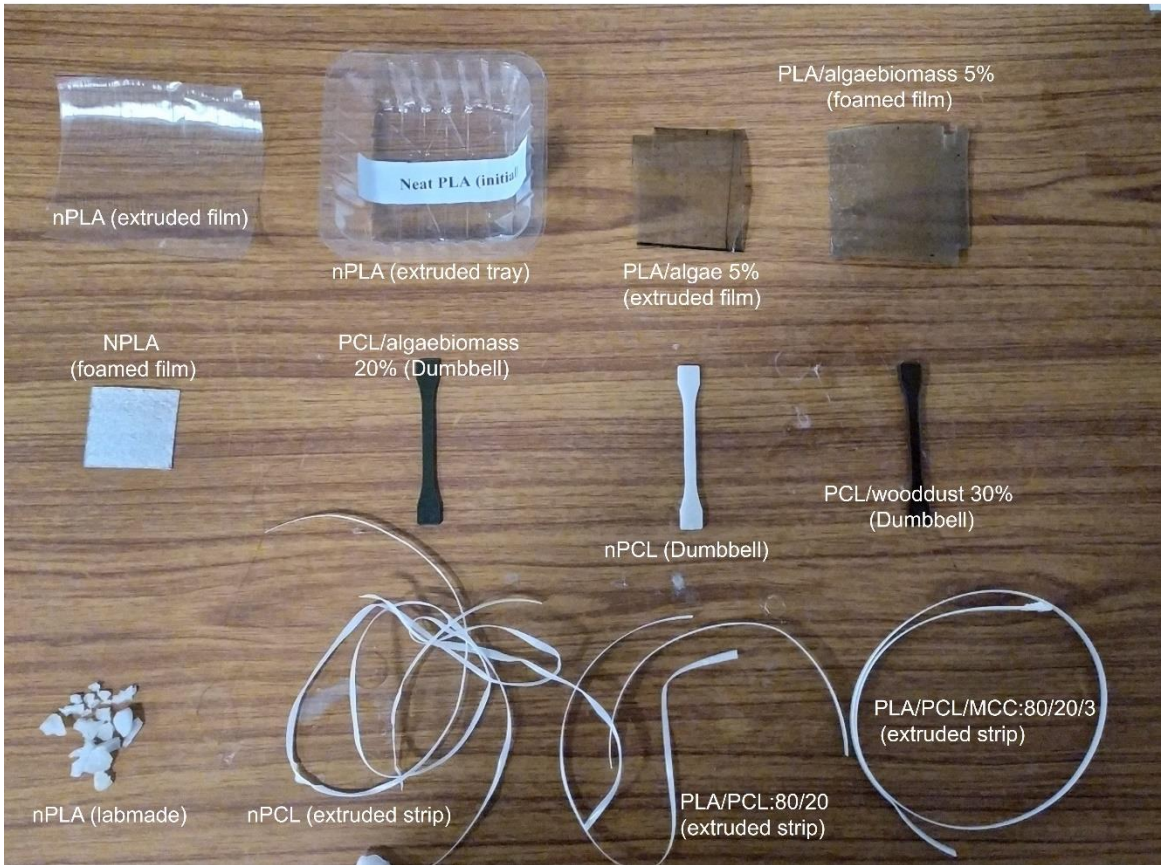


Figure 8. 3: Graph representing the humidity (%) of the degradation environment throughout the degradation period.

Humidity is an important parameter in biodegradation studies both in controlled or uncontrolled environment (Leejarkpai et al., 2011; Weng et al., 2013b) as this control the growth of the desired microorganisms as well moisture content. In our studies we measured humidity of the outdoor environment from June 2019 to December 2019 (Latitude 26°10'20"N Longitude 91°44'45"E) for the study conducted area. It was found that on average ~75 % humidity was in the environment throughout the degradation period (**Figure 8.3**).

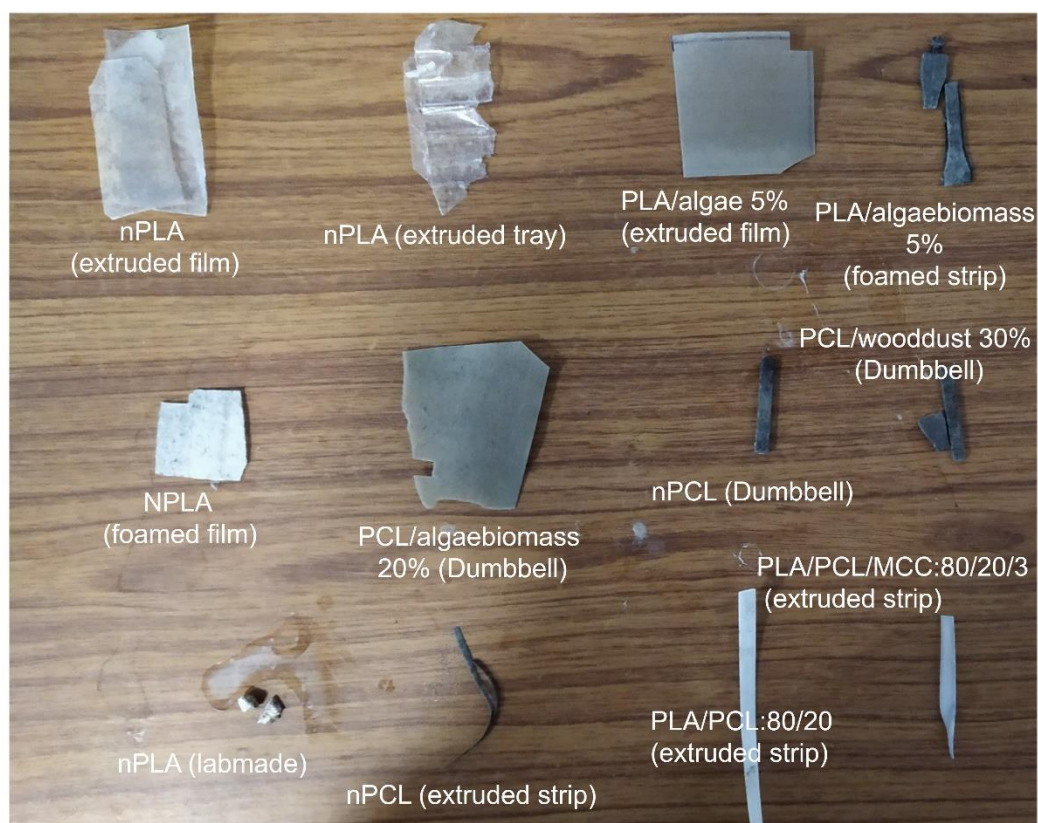
From the **figures 8.4. (a-c)** manual observation it has been clear that samles under natural soil condition changed its visibility from transparent to opaque after 180 days of degradation. This happens due to degradation of the amorphous regions from the polymers (Pekařová et al., 2018). The changes observed manually through cameras also represents degradation of the test samples (Rudeekit et al., 2008).

Soil Biodegradation test samples before biodegradation



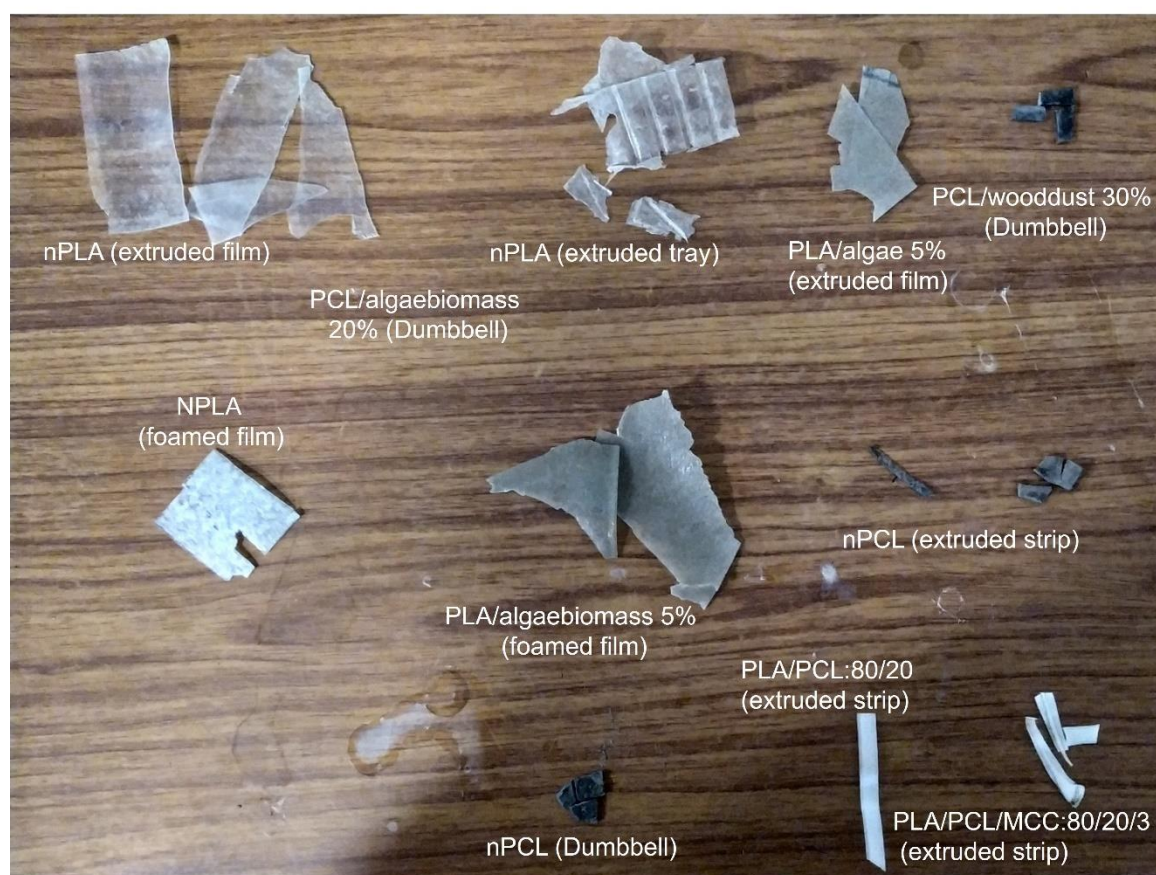
(a)

Soil Biodegradation test samples after 90 days of biodegradation



(b)

Soil Biodegradation test samples after 180 days of biodegradation



(c)

Figure 8. 4(a-c): Manual observation of test samples before and after biodegradation.

8.2.2 Molecular weight analysis

Molecular weight analysis of the samples were done before and after biodegradation. Number average and weight average molecular weight of the samples was calculated with Poly-dispersity index (PDI). **Table 8.1 (a)** represents measured molecular weight change data of the test samples before and after biodegradation under soil burial condition. Observation of molecular weight loss is another indication of degradation in polymer (Kalita et al., 2019; Pekařová et al., 2018). From **table 8.1 (a)** it can be seen that number average molecular weight (M_n) of PLA (extruded tray) was 101 kDa initially which got reduced to 56 kDa on the 180th day. Similarly, PCL lab-made number average molecular weight (M_n) reduced from 44 kDa to 10 kDa. This reduction in molecular weight is due to

chain scission mechanism at the polymer backbone triggered by water or moisture as a initial step of biodegradation (Gorrasi and Pantani, 2013; Kalita et al., 2019; R. babu Valapa et al., 2016). Moisture in soil initiate hydrolysis which leads to fromation of low molecular weight intermediates. This intermediates are at first remains intact on the surface of the polymer by forming water bridge a phenomenon known as solavation (Alex et al., 2018). After increase in degradation time these intermediates are released into the exposed enviroment, due to which the molecular weight of the polymer decreases. Thereafter, microbes in the soil releases enzymes which binds on the surface of the polymer leading to assimilation of the intermediates into various products like CO₂, CH₄, N₂, H₂O and biomass (Kalita et al., 2019; Zumstein et al., 2019). It was observed that most of the test samples underwent molecular weight loss during the degradation time period under soil burial condition. Samples with PLA/algaebiomass 5% and PCL/algaebiomass 20% showed significant amount of molecular weight loss under soil condition as compared to PLA and PCL based test samples. This may be due to presence of amine groups in algae biomass extract which are more susceptible towards hydrolysis which ultimately leads to biodegradation. Amino acids mostly contains carboxylic acids in their backbone (Sudhakar and Premalatha, 2015) and this protein concentration in the algal biomass extract also leads to degradation as this creates engroups in the polymers which leads to degradation faster than the other test samples. Algal biomass extract contains OH groups which are also another triggering agent for hydrolysis. From **table 8.1(a)** it can be observed that weight average molecular weight M_w of the test samples decreased substantially during the course of the degradation process after 180 days of soil burial, in accordance with the published data (Kalita et al., 2019; Pekařová et al., 2018; R. babu Valapa et al., 2016).

Polydispersity index (PDI) = M_w/M_n is another parameter which explains that random degradation occurs when PDI is ~ 2 or substantially over 2.0 (Abrusci et al., 2011). In our

results measured (**Table 8.1(a)**) PDI increased over 2.0 in many of the test samples over the 180 days degradation period. It can be seen that PDI value of commercial neat PCL and commercial neat PLA ranged between 1.4-1.7 respectively. When algaebiomass 5% was added in PLA its PDI got increased to 1.8 which suggests addition of algaebiomass introduced short chains leading to higher PDI. Similar behaviour was observed in case of PCL, where 20% algaebiomass was added, and its PDI increased to 2.03. Whereas, addition of wood-dust upto 30% didn't increase the PDI above 1.8, suggesting molecular weight distribution was not random but its accessibility of reactive bonds keeps it close to PDI value 2. This would make it more easier for PLA chains to undergo random chain scission during the degradation period. After 180 days of biodegradation PDI of neat PLA (extruded sheet) and nPLA (extruded tray) PDI values only increased upto 1.9 from 1.7. Number average molecular weight of these two polymers also decreased a little from 101 kDa to 58 kDa, suggesting very little degradation under soil burial condition under natural environment. Since the PDI values are >2 , its internal chain fragmentation was less, leading to less molecular weight loss and decreased chain scission activity. This low chain scission activity produces less intermediates and mass loss due to which microbial attachment as well as microbial assimilation becomes difficult to achieve under the natural environment. Whereas, this condition can be achieved for PLA during biodegradation under thermophilic phase of the composting process, due to its maintained temperature of 58 ± 5 °C during the biodegradation process (Kalita et al., 2019; Leejarkpai et al., 2011; Pradhan et al., 2010a). PDI value of PLA/algaebiomass 5% (extruded sheet) increased from 1.8 to 2.4 which clearly indicates action of random chain scission mechanism triggered by hydrolysis. This suggests that addition of algaebiomass as filler makes it easier for moisture and microbes to access the reactive bonds for degradation. Similar case was also observed in PCL/algaebiomass 20% where PDI value increased from 2 to 3.9 with maximum decrease

of number average molecular weight in the test sample during 180 days of soil burial test under natural soil condition. So algal biomass can act as a trigger filler for biodegradation to occur as it contains amino acids and high nitrogen content which makes it easier to access its reactive groups for the microbes under natural environment too. Similar results were also observed by (Narancic et al., 2018) where they found that PCL degraded under 136 days of time under unmanaged condition. Authors here also reported, when PLA was blended with 20 % PCL the blend was home compostable. This suggests that PLA can be made home compostable or degradable under natural soil condition of proper filler or blend is found which has more accessible sites and is compatible with PLA. Since compatibility of the blend is an important factor for characterization and marketing of a plastic blend (Balali et al., 2018; Narancic et al., 2018). However, when we mixed PLA/PCL:80/20 and PLA/PCL/MCC:80/20/3 for biodegradation study under soil condition we found that the blend is not degradable under soil natural condition under 180 days of time. Although, its PDI increased from 1.8 to 3.4 suggesting random scission mechanism taking place. Molecular weight also decreased significantly for the mentioned blends. But this blend will need more time to completely biodegrade under soil condition. This experiment was a step to understand biopolymer biodegradation behaviour under natural and unmanaged environment condition.

Table 8. 1 (a): Molecular weight of the test samples before and after biodegradation under natural soil condition

Sample	Initial			30 th day			60 th day			90 th day		
	M _n (kDa)	M _w (kDa)	PDI	M _n (kDa)	M _w (kDa)	PDI	M _n (kDa)	M _w (kDa)	PDI	M _n (kDa)	M _w (kDa)	PDI
PLA (tray)	101	179	1.7	77	136	1.7	73	130	1.7	73	175	1.9
PCL/algaebiomass 20% (Dumbbell)	37	75	2.0	34	74	2.1	33	71	2.1	25	59	2.4
PLA/algae foam sheet	125	214	1.7	119	228	1.8	115	222	1.8	72	167	2.3
PCL (dumbbell)	44	96	2.1	34	81	2.3	33	73	2.2	31	67	2.2
PLA/5% algae sheet	77	166	1.8	69	152	1.9	65	143	2.2	63	138	2.1
PLA/ foam sheet	133	211	1.7	115	257	1.7	123	214	1.7	111	217	1.8
nPCL (commercial)	126	157	1.4	104	159	1.5	84	174	2.0	68	141	2.4
NPLA (extruded sheet)	96	125	1.8	78	118	1.5	72	142	1.9	67	125	1.8
PLA (labmade)	160	290	1.8	35	85	2.3	20	63	3	18	63	3.5
PCL/wood dust 30%	50	87	1.7	39	80	2.0	36	77	2.1	34	72	2.1
PLA/PCL:80/20	89	148	1.8	71	143	2.0	70	158	2.3	49	112	2.2
PLA/PCL/MCC:80/20/3	78	127	1.6	72	148	2.0	71	152	2.1	57	127	2.2

Table 8.1 (continuation): Molecular weight of the test samples before and after biodegradation under natural soil condition

Sample	120 th day			150 th day			180 th day		
	M _n (kDa)	M _w (kDa)	PDI	M _n (kDa)	M _w (kDa)	PDI	M _n (kDa)	M _w (kDa)	PDI
PLA (tray)	70	140	2.0	60	128	2.0	56	110	1.9
PCL/algae biomass 20% (Dumbbell)	22	65	2.9	19	133	3.8	15	59	3.9
PLA/algae foam sheet	71	182	2.6	69	139	2.0	62	130	2.1
PCL (dumbbell)	30	73	2.6	21	53	2.6	10	54	5.4
PLA/5% algae sheet	60	125	2.1	54	131	2.2	31	76	2.4
PLA/ foam sheet	91	188	2.1	71	139	2.0	70	154	2.2
nPCL (commercial)	62	195	3.1	55	185	3.2	16	21	1.3
NPLA (extruded sheet)	80	140	1.8	60	128	2.0	56	110	1.9
PLA (labmade)	18	56	3.1						
PCL/wood dust 30%	34	70	2.1	22	65	3.0	14	72	5.1
PLA/PCL:80/20	50	117	2.6	40	105	2.7	39	133	3.4
PLA/PCL/MCC:80/20/3	44	133	2.3	39	102	2.7	34	118	3.4

It is well documented that biodegradation of biopolymer occurs through two sequential steps: (i) hydrolysis and (ii) microbial assimilation (Kalita et al., 2019; Leejarkpai et al., 2011; R. Valapa et al., 2016). Therefore, under natural soil condition understanding the abiotic degradation process is very much necessary in order to estimate the rate constant and the nature of degradation.

The evolution of molecular weight during abiotic hydrolysis was modelled. The analytical solution of the model is given by Eq. 1. (Han and Pan, 2009; Kalita et al., 2019; Tayal and Khan, 2000; Vieira et al., 2014)

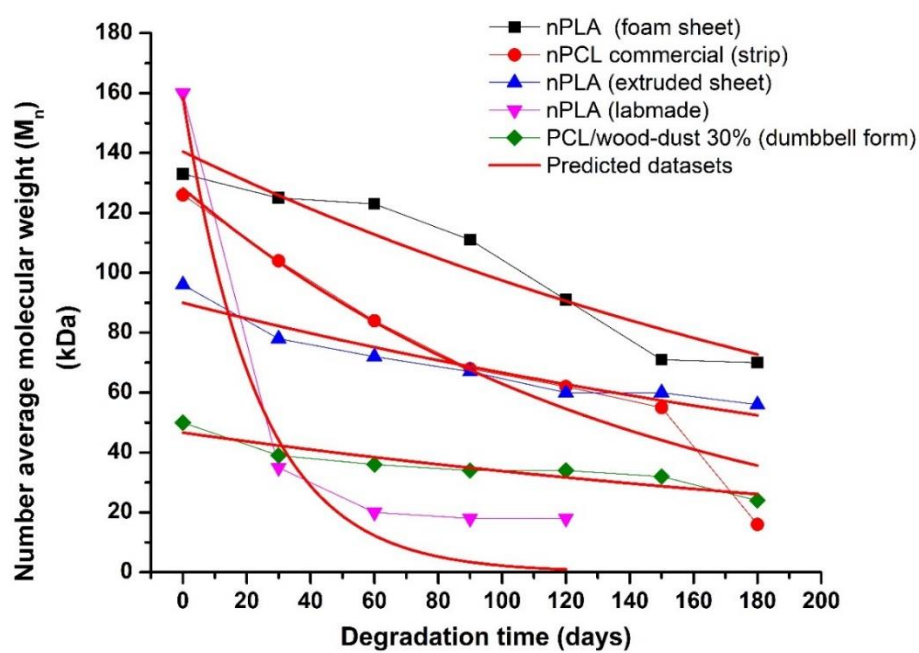
$$M_{n,t} = M_{n,0} \cdot e^{-ut}$$

where $M_{n,t}$ and $M_{n,0}$ (Dalton) are final and initial weight average MW at time t , and $t = 0$, respectively. Parameter u (day^{-1}) is the rate constant.

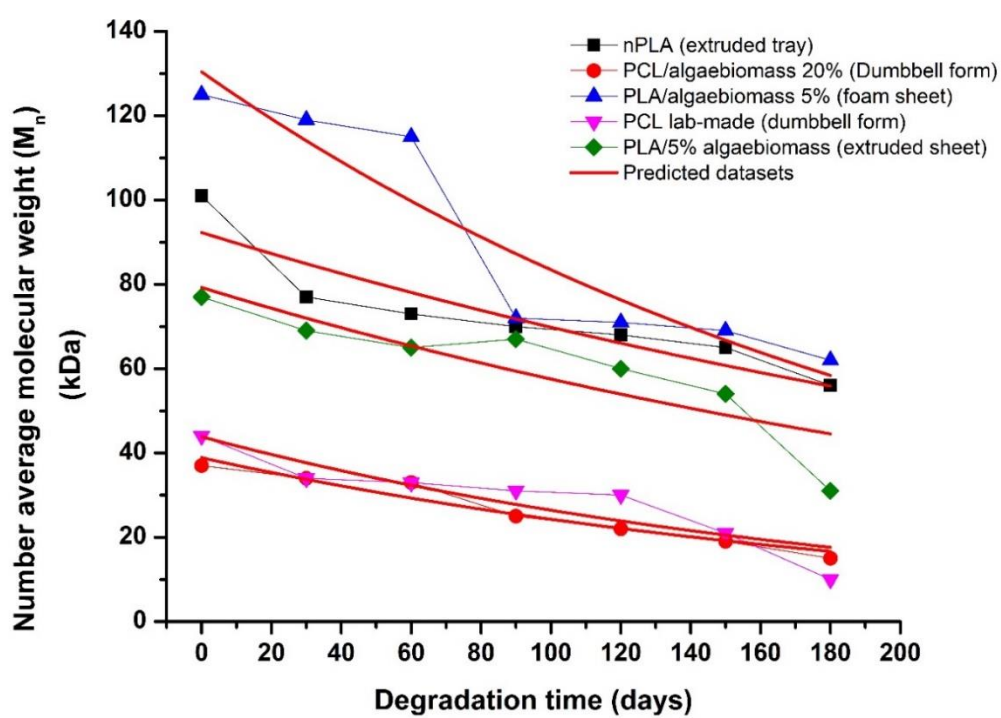
The M_n obtained from the GPC data were evaluated by applying the above equation from first-order kinetics describing the random chain scission of ester bonds. The parameters and coefficient of determination (R^2) are listed in **table 8.2**, and depicted in **figure 8.5(a-c)**. The calculated chain scission rate constant suggests that addition of algaebiomass in both PLA and PCL accelerated the random chain scission of ester bonds. For neat PLA extruded sheet and extruded tray, random chain scission constant was 0.0028 day^{-1} and 0.003 day^{-1} . When added with algaebiomass, 20% PCL and PCL/MCC:20/3 it was found to be 0.003, 0.00462 and 0.00469 day^{-1} respectively. Similar behaviour was also found by (Stloukal et al., 2015) where they reported that addition of nanoclay in PLA accelerated the random chain scission constant of the biopolymer. The rate constant for pure PLA and PLA/organo modified clay was found to be 0.0673 day^{-1} and 0.1063 day^{-1} . They reported this experiment at thermophilic temperature. Our data measure and reported are under natural soil condition. Our observed molecular weight data were in line with the predicted kinetics data.

Table 8. 2: Chain scission rate constants with regression coefficient for the test samples

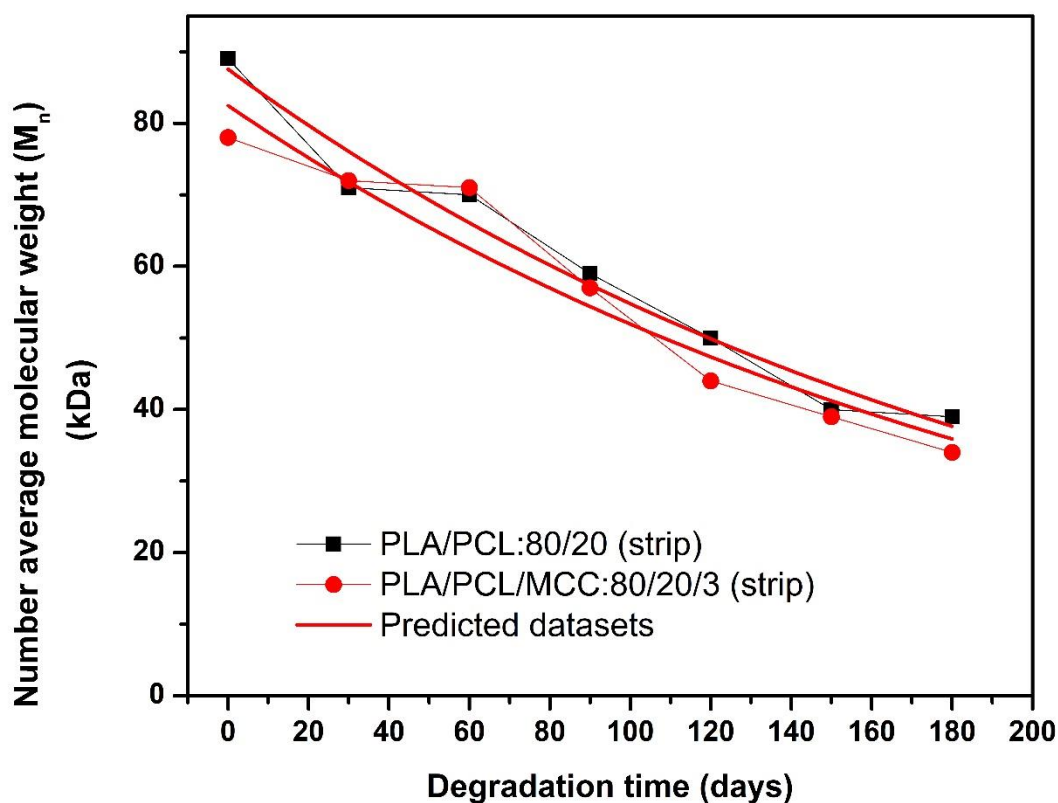
Samples	$(M_{n0}) \text{ day}^{-1}$	Rate constant (u) (day^{-1})	R^2 (%)
PLA (tray)	92.3	-0.00279	80
PCL/algae 20% (Dumbbell)	38.8	0.00471	94
PLA/algae foam sheet	130.4	-0.00446	85
PCL (dumbbell)	43.8	-0.00507	79
PLA/5% algae sheet	79.2	-0.0032	71
PLA/ foam sheet	140.4	-0.00365	89
nPCL (commercial)	128.2	-0.00711	91
NPLA (extruded sheet)	89.9	-0.003	90
PLA (labmade)	158.4	-0.04259	94
PCL/wood dust 30%	46.6	-0.00322	84
PLA/PCL/MCC:80/20/3	87.5	-0.00469	96
PLA/PCL:80/20	74.9	-0.00462	92



(a)



(b)



(c)

Figure 8. 5(a-c): Molecular weight analysis of the test samples and their predicted data according to the first order reaction.

8.2.3 Weight loss analysis and size (thickness) analysis of the test samples

The residual weight fraction (Φ) of all the test samples was measured as a function of degradation time, under natural soil condition. Although with time, Φ values observed followed a decreasing trend, it can be observed from **figure 8.6** that very less mass loss occurred in nPLA and PLA based blends. Whereas, mass loss was highest in nPCL labmade and PCL labmade based blends.

Various hydrolytic degradation studies observed that at elevated temperature like 55 °C, mobility of molecular chains intensifies and chain scission increases, resulting in mass loss

which happens under controlled environment (Gorrasi and Pantani, 2013; Kalita et al., 2019; R. babu Valapa et al., 2016; Vieira et al., 2014). Literatures reported very little or no evident mass loss of PLA products at 37 °C within 90 days, which suggests temperature under a controlled environment plays vital role in PLA degradation (Xu et al., 2016). In our study it was found that addition of algalbiomass in both PLA and PCL, Φ value, got decreased with degradation time. For nPLA (extruded sheet) and PCL (labmade) dumbbell Φ value decreased upto 81% and 38%, suggesting heavy mass loss in case of PCL (labmade). Whereas, nPLA (extruded sheet) showed very less degradation in terms of mass loss at outdoor temperature under uncontrolled and natural environment. These results are clear evidence that in outdoor natural soil condition degradation of PLA is very low with respect to mass loss analysis.

From our visual observation it was seen that with mass loss thickness of the test samples either increased or decreased with degradation time. Increase in thickness may be due to various factors like diffusion of moisture and soil leading to swelling. This creates voids inside the bulk, leading to weight loss with increase in thickness with increase in degradation time.

PCL is very crystalline material (Bhagabati et al., 2019) and have a very low melting point of 50-60 °C with low T_g of about -60 to -40 °C. Here, in our study mass loss of PCL based blends decreased significantly (**Figure 8.6**) with increase in size and significant molecular weight loss was also observed. This suggest degradation of the PCL based blends under soil condition except PLA/PCL based blends. Sometimes degradation of the amorphous portion leads to increase in crystallinity which retards the autocatalytic hydrolysis reaction due to diffusion during biodegradation but leads to weight loss (Han and Pan, 2009) which increases the thickness of the material before it gets complete disoriented and fragmented. Thickness of nPLA ES and PLA/algaebiomass 5% decreased significantly which suggests

surface erosion of the material in our study. This sudden molecular weight loss can also be attribute to surface erosion of the PLA matrix rather than the bulk degradation (Pan, 2014; Wang et al., 2008). This results are further evident from our thickness measurement data (Figure 8.7) and presented in table 8.3.

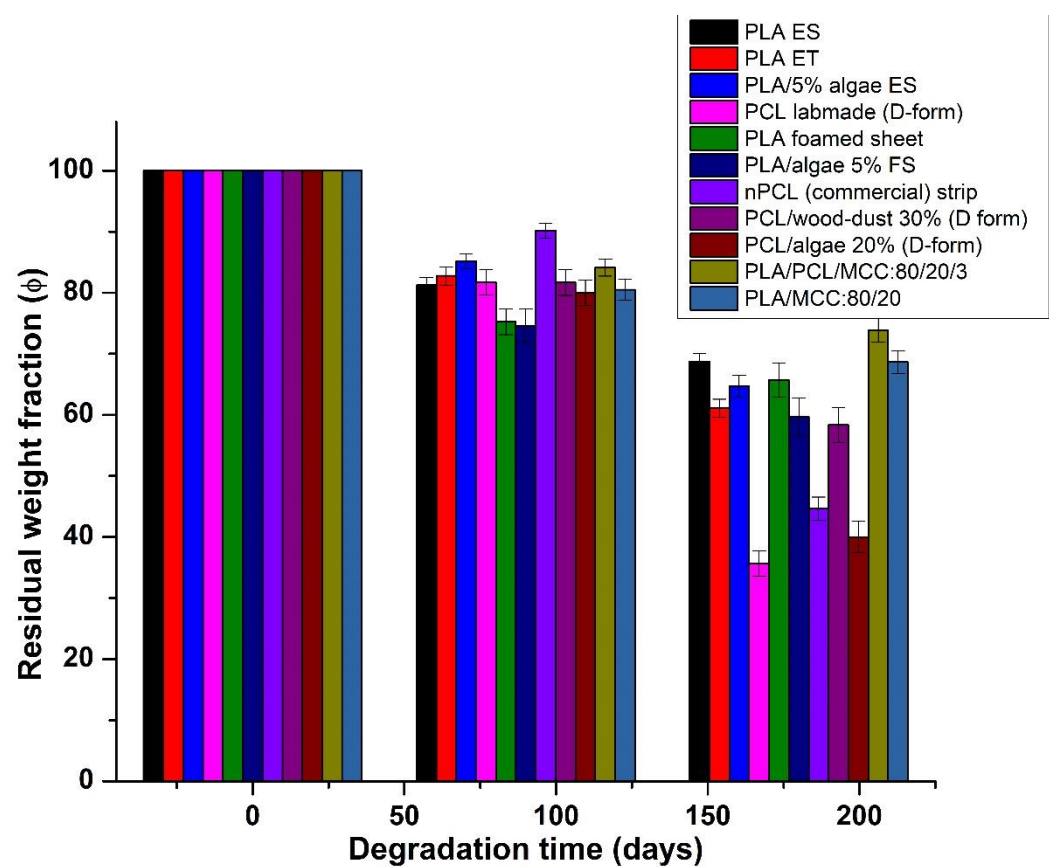


Figure 8. 6: Residual weight fraction of the test samples before and after biodegradation under soil condition

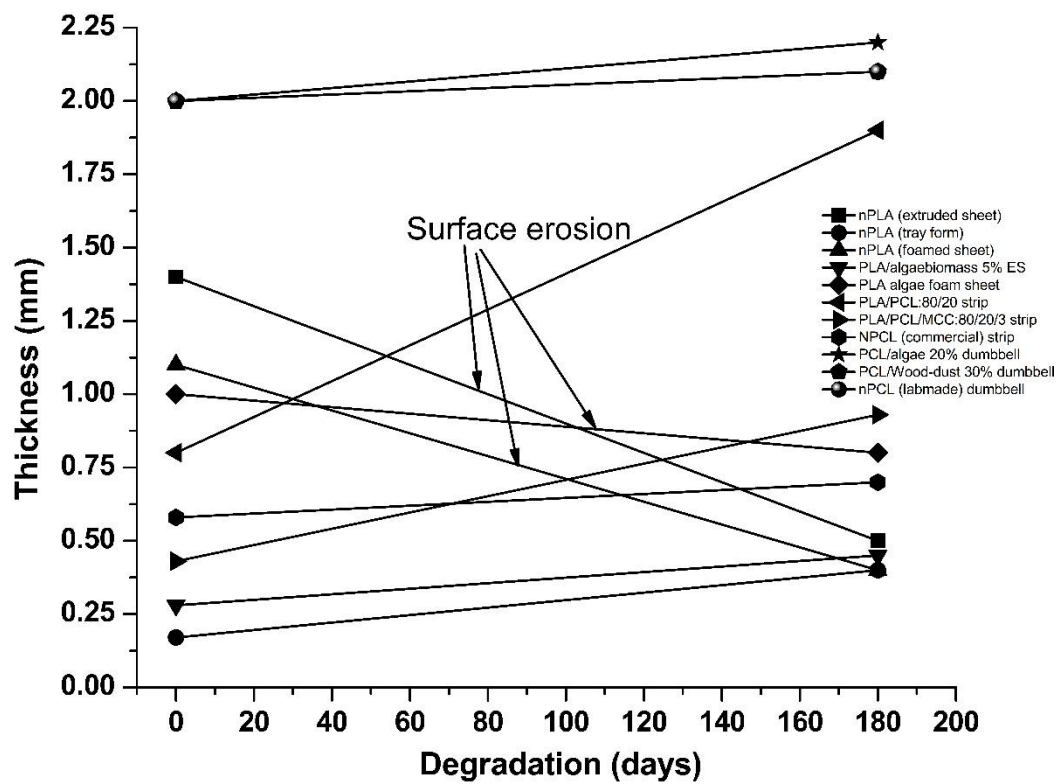


Figure 8. 7: Thickness changes in the test samples during the degradation period

Table 8. 3: Thickness measurement of test samples before and after degradation under soil condition

Sample	Thickness (mm) initial	Thickness (mm) after 180 days
NPLA (ES)	1.40	0.50
NPLA (tray form)	0.17	0.40
NPLA FS	1.10	0.40
PLA/algaebiomass 5% ES	0.28	0.45
PLA algae foam sheet	1.00	0.80
PLA/PCL:80/20 strip	0.80	1.90
PLA/PCL/MCC:80/20/3 strip	0.43	0.93
NPCL (commercial) strip	0.58	0.70
PCL/algae 20% dumbbell	2.00	2.20
PCL/Wood-dust 30% dumbbell	2.00	2.10
NPCL (labmade) dumbbell	2.00	2.10

8.2.4 Infrared spectroscopy analysis

Before degradation, the PLA IR spectra at the 3000-2900 cm^{-1} position represented O–H stretching vibration. For nPLA ES or ET (**Figure 8.8 (a-l)**), before burial, the absorption peak of C=O stretching vibration was found at 1748 cm^{-1} . After 6-month (180 days) burial in 20 cm of soil, the absorption peak of C=O stretching vibration was shifted to 1737 cm^{-1} . These phenomena demonstrated that the degradation occurs in PLA due to breakage of the carbonyl group.

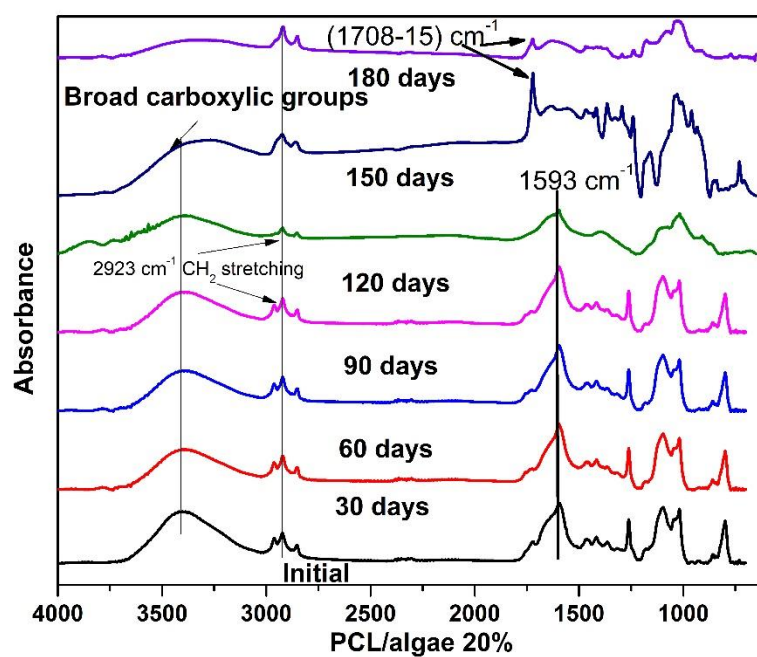
Authors here (Kalita et al., 2019) demonstrated that degradation of PLA in 20 cm of soil was mainly aerobic biodegradation, which justify our results too.

In the PCL/algae 20% samples, CH₂ stretching shifted from 2923 to 2947 cm⁻¹ on the 180th day, suggesting biodegradation. Carbonyl shifting occurred at 1708 to 1715 cm⁻¹.

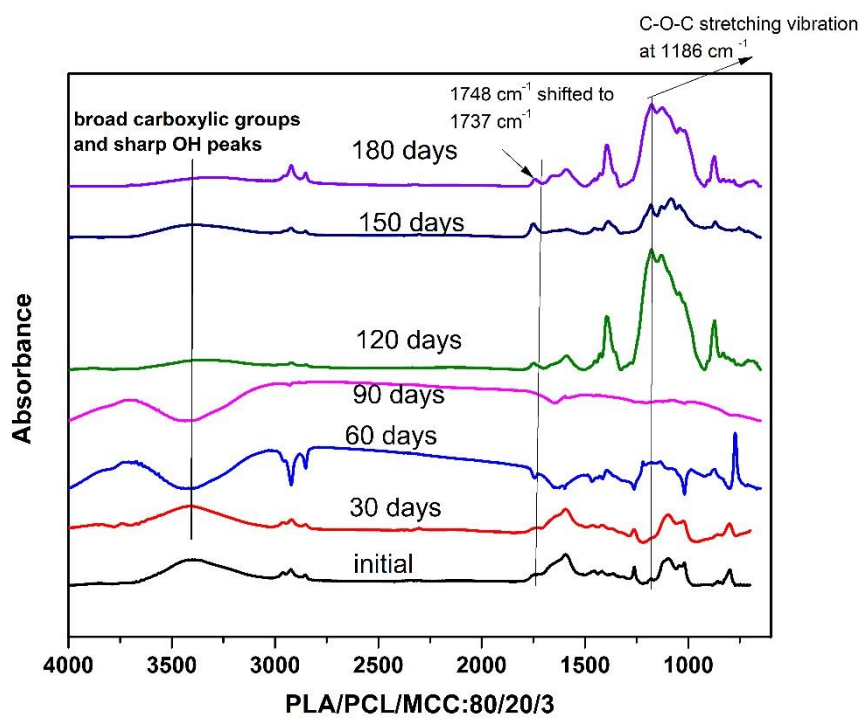
In PLA/algaebiomass 5% ES the spectra at 2995 cm⁻¹ represented the antisymmetric vibrations, and shifted to 2945 cm⁻¹ after biodegradation which represents the symmetric vibrations of CH₃. Here, the adjacent oxygen and carbonyl groups creates a higher stretching vibration frequency. Similar behaviour was observed by (Weng et al., 2013a) in case of PLA/PBAT blend.

In the test samples PLA/PCL/MCC: 80/20/3, there was a shift of the small peak from 1450 cm⁻¹ to 1456 cm⁻¹ with an increase in intensity. This suggested asymmetric bending vibration of C-H. A broad carboxylic peak was also observed for the test samples till the 180th day due to the formation of more carboxylic groups. This also implied oligomer formation on the sample surface due to microbial attack on the polymeric chains. This kind of IR footprint shifting had been observed by various authors under different environment in various biopolymers and their blends (Jayasekara et al., 2005; Kalita et al., 2019; Weng et al., 2013a, 2013b).

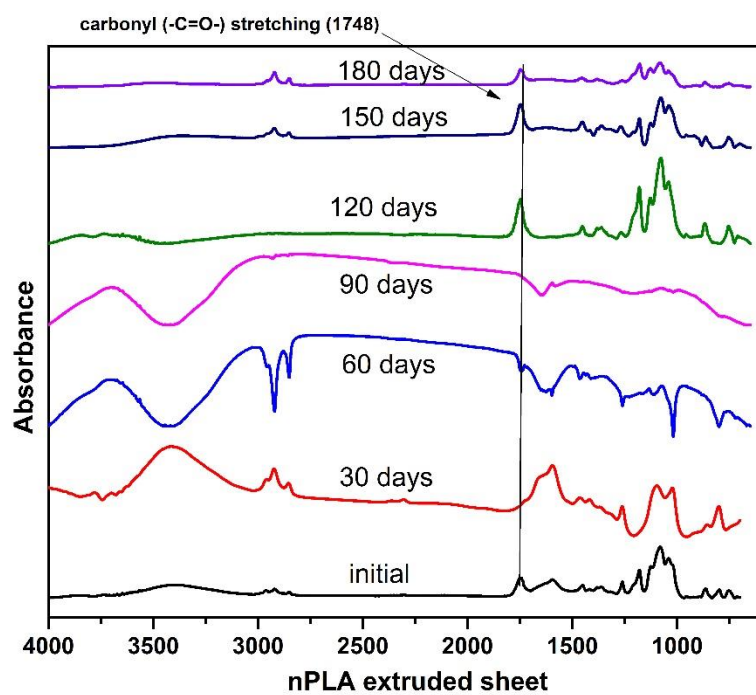
As can be seen from the IR spectrs analysis, there were slight changes in the positions of the sample peaks before and after degradation, but in general there were no major changes under natural outdoor soil condition.



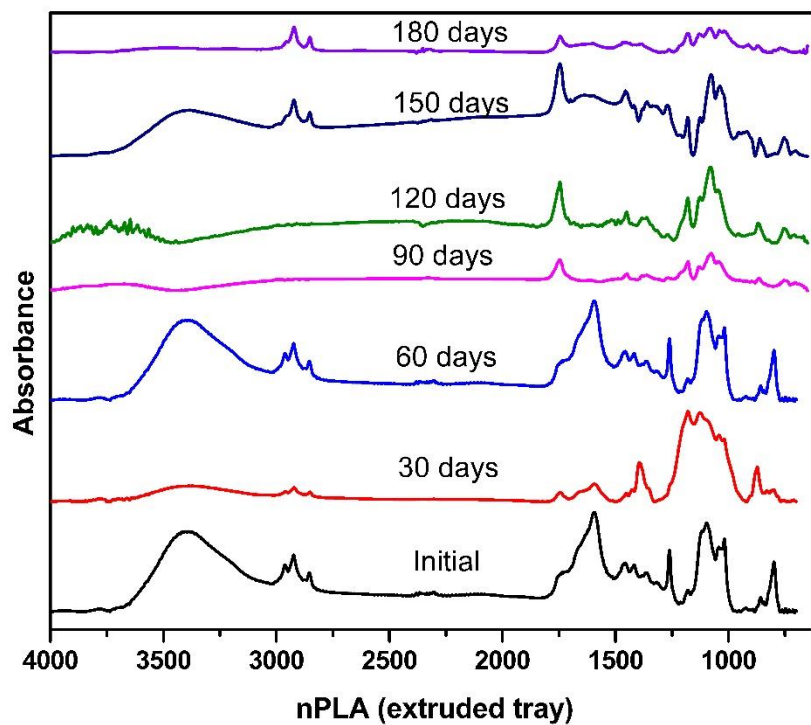
(a)



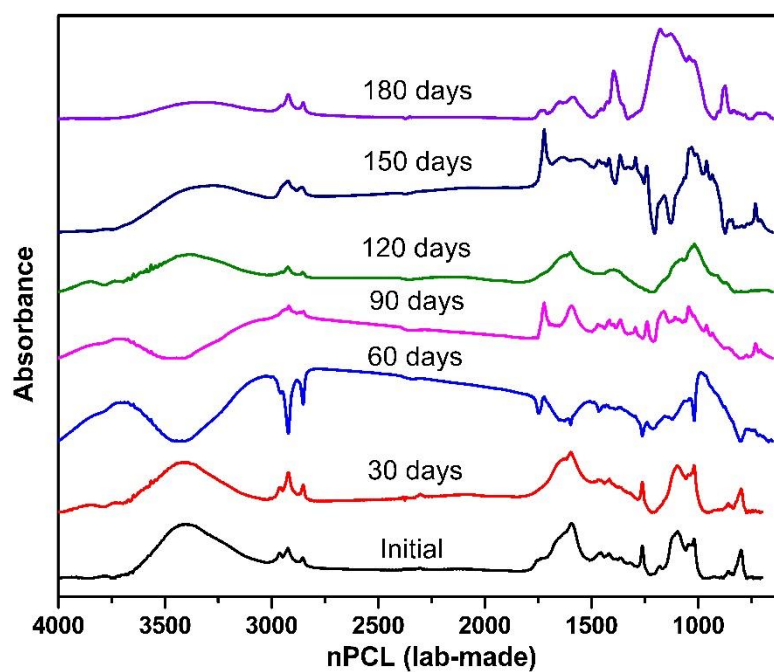
(b)



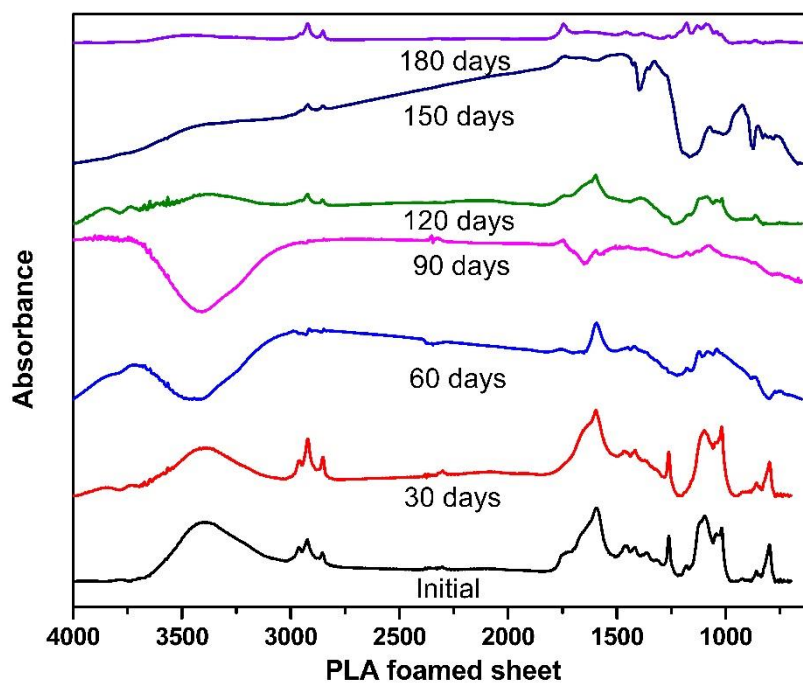
(c)



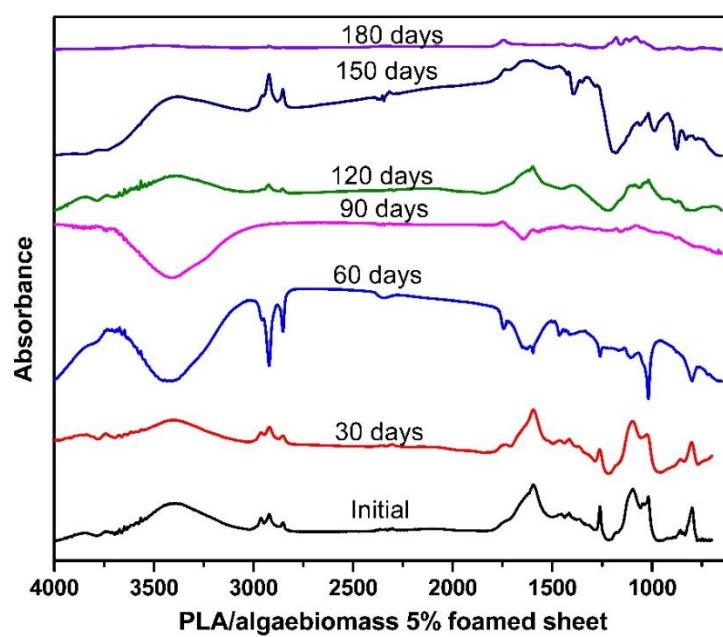
(d)



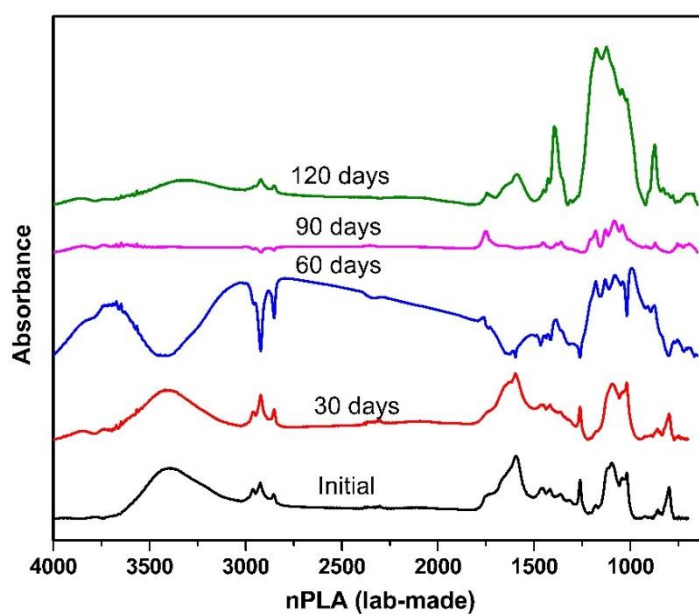
(e)



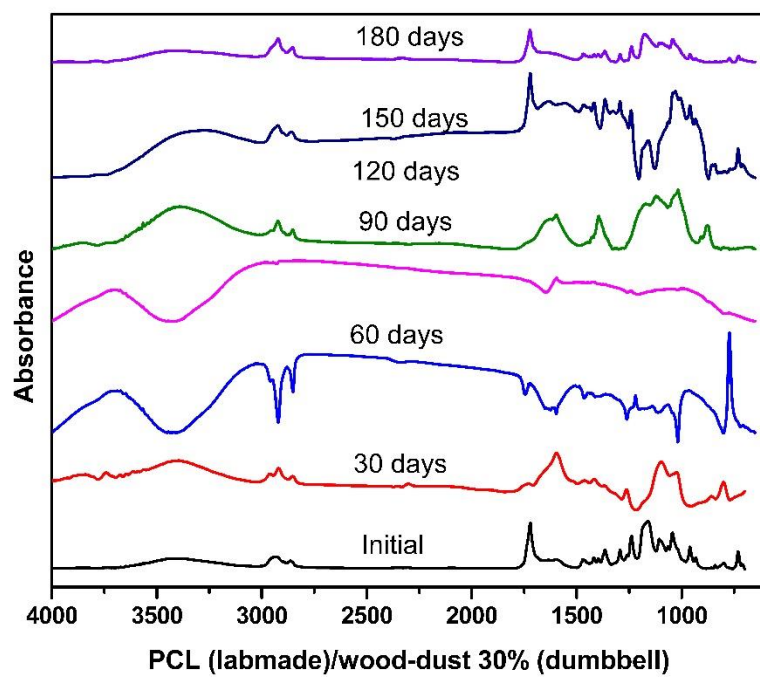
(f)



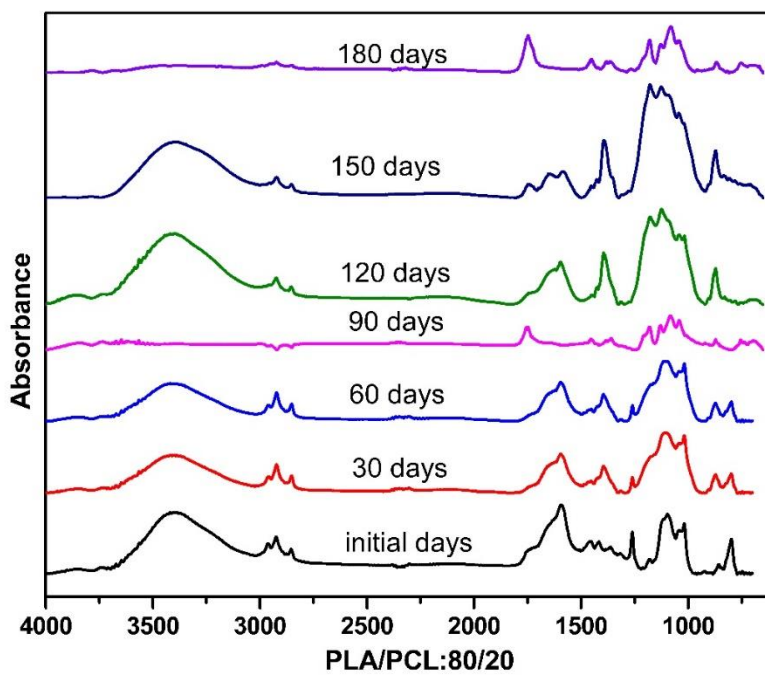
(g)



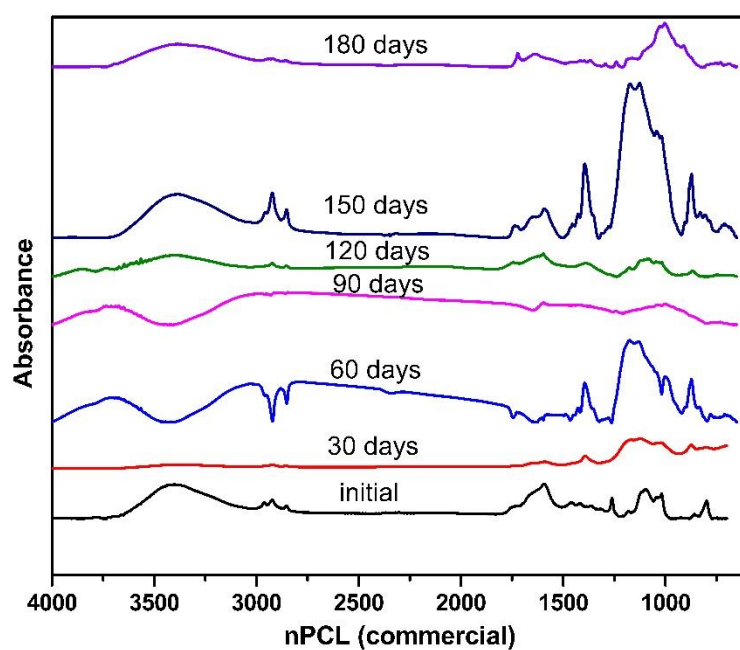
(h)



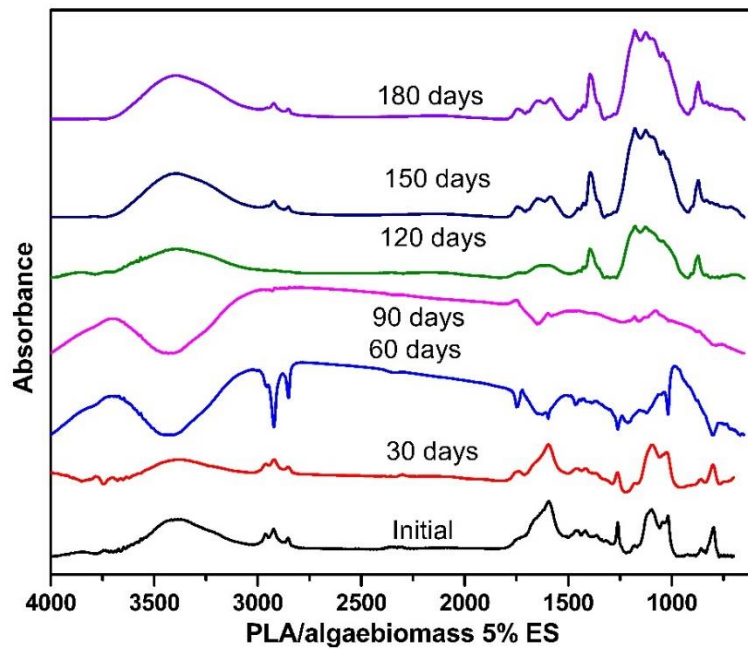
(i)



(j)



(k)



(l)

Figure 8. 8 (a-l): IR spectroscopy analysis of samples before and after biodegradation under soil condition

8.2.5 Contact angle analysis

The blends and biocomposites were observed both before and after biodegradation for wettability changes occurring on the surface of the blends and biocomposites, which underwent biodegradation under outdoor natural soil condition. It was found that the hydrophilicity of all the test samples decreased with an increase in degradation time. This behaviour was attributed to the formation of carboxylic and hydroxyl group on the test sample surface leading to hydrophilicity and higher chain mobility (Kalita et al., 2019). The water contact angle of nPLA decreased from $(74 \pm 5)^\circ$ to $(60 \pm 4)^\circ$. A similar decrease in contact angle was also observed for all the test samples (**Table 8.4**). **Figure 8.9 (a-b)** depicts the decrease in contact angle of the samples during the initial and 180 days of biodegradation. Decrease in contact angle was attributed to the formation of oligomeric components on the surface of the test samples, leading to an increase in the hydrophilicity and lowering of the contact angle of the samples gradually (Stloukal et al., 2015).

In our study, it was observed that addition of algaebiomass in PLA decreased water contact angle from 79 ± 5 to $69 \pm 3^\circ$ in 30 days, which was significant in comparison to $53 \pm 8^\circ$ after 180 days of soil biodegradation. This suggests degradation was not consistent throughout the degradation period. It may depend upon the temperature of the outdoor environment and soil temperature as previously profiled in section 8.2.1. Similar behaviour was observed in case of PCL based test samples too as sudden decrease in contact angle was observed in first 30 days. However, in PCL addition of algal biomass and wood-dust retarded surface degradation as compared to neat PCL. As in neat PCL water contact angle decreased from 93 ± 6 to $69 \pm 5^\circ$ in 30 days, whereas, PCL/algae 20% (dumbbell) and PCL/wood dust 30% samples contact angle decreased from 85 ± 3 to $68 \pm 5^\circ$ and 79 ± 4 to $72 \pm 2^\circ$ respectively. Similar behaviour were also observed in PLA/PCL based blends. This may be due to phase separation which increases water permeability and accelerates the degradation process

(Siparsky et al., 1998; Woodard and Grunlan, 2019). As authors (López-Rodríguez et al., 2006) found that these blends water permeability increases then their respective individual polymers.. Based on their results, a phase-separated morphology was concluded to have a significant role in the observed degradation of PCL–PLLA semi-IPNs (interpenetrating networks). Hence decrease in hydrophilicity was less in neat PLA based test samples as reactive sites were less on the carbon backbone or in other words reactive groups remain inaccessible for moisture to hydrolyse (Xu et al., 2016).

Fact can be drawn from our observed results that this water permeability also leads to hydrolysis under soil burial environment.

Table 8. 4: Water contact angle of samples before and after biodegradation under soil condition

Sample	Initial	30 days	60 days	90 days	120 days	150 days	180 days
PLA (tray)	74±5	70±6	60±6	58±5	61±5	60±4	60±4
PCL/algae 20% (dumbbell)	85±3	68±5	67±7	65±6	55±6	52±6	47±5
PLA/algae foam sheet	77±5	69±5	65±6	65±7	59±5	55±5	53±6
PCL (dumbbell)	93±6	63±6	51±8	50±4	55±4	55±2	53±7
PLA/5% algae sheet	79±5	69±3	65±5	63±5	56±4	54±5	53±8
PLA/ foam sheet	86±4	78±6	70±6	69±9	63±5	60±4	55±5
nPCL (commercial)	80±3	71±5	70±9	60±8	38±6	35±6	33±6
NPLA (extruded sheet)	78±5	72±4	69±4	68±6	66±5	61±5	60±8
PCL/wood dust 30%	79±4	72±2	63±8	55±7	51±6	46±6	38±4
PLA/PCL:80/20	82±7	71±5	70±6	59±8	55±8	52±4	51±3
PLA/PCL/MCC:80/20/3	81±8	65±8	63±5	56±6	54±9	52±8	49±9

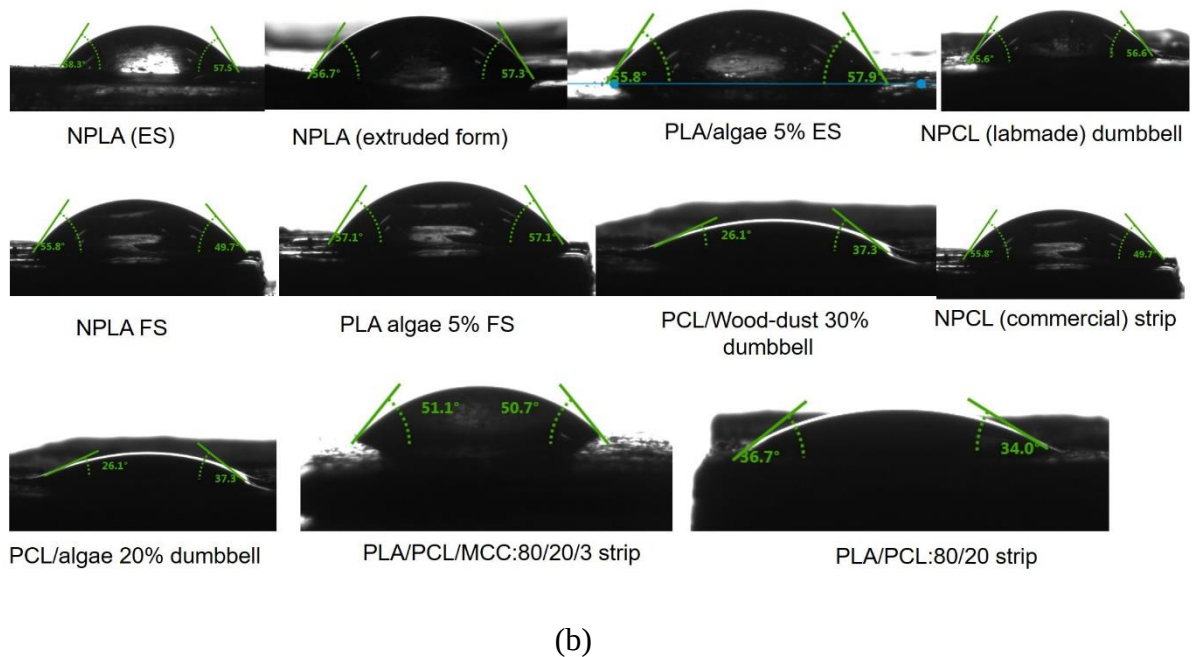
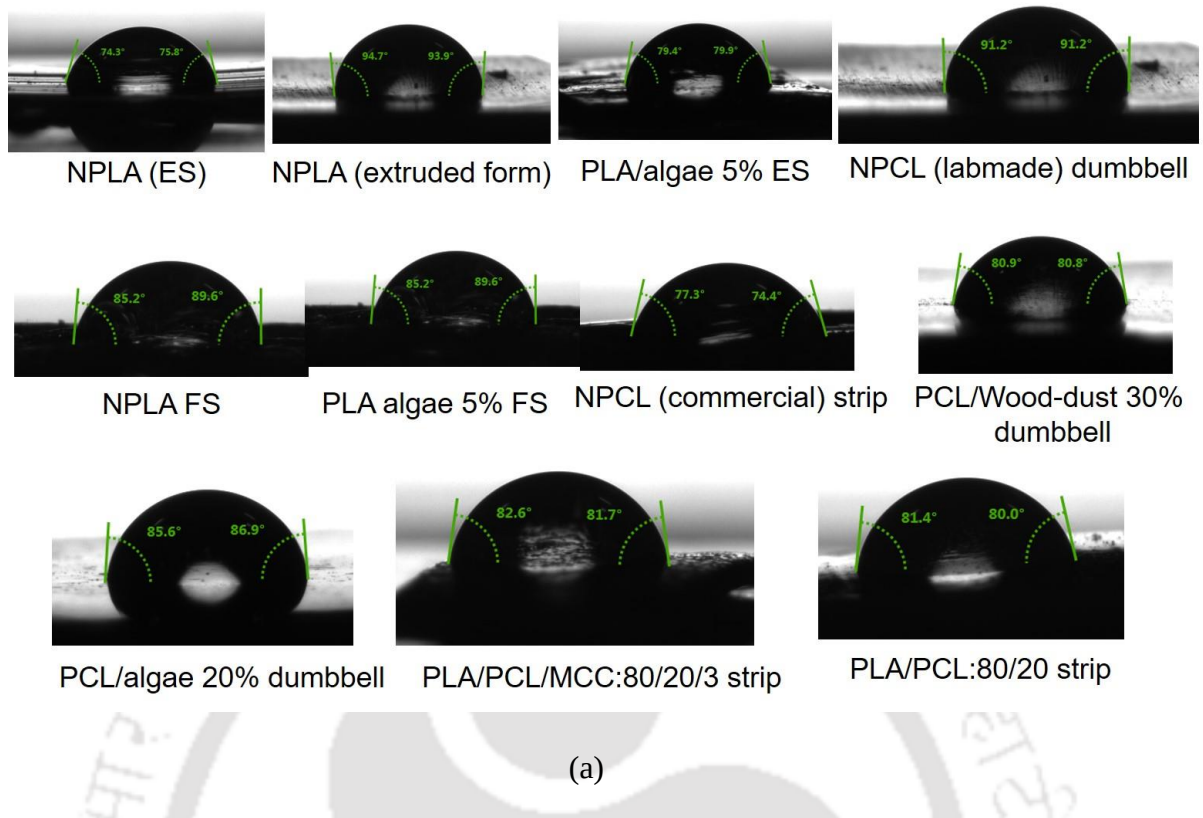


Figure 8. 9(a-b): Water contact angle of test samples under natural soil condition (a) initial contact angles (b) contact angle after 180th day of degradation

8.2.3 Conclusion

This study was designed to analyze the biodegradation behaviour of PLA and PCL based biocomposites, blends and polyblends of different shapes. Fillers like wood-dust, algal biomass and MCC used showed degradation behaviour according to their material property. Outdoor temperature and humidity profiling was done both for the environment as well as inside 20cm deep soil. An average of 33.5 °C of outdoor temperature and 23 °C was received during the period of June 2019 to December 2019 for 180 days. Molecular weight analysis revealed that nPCL (labmade), nPLA (labmade) and PCL/algaebiomass 20% samples underwent accelerated molecular weight loss under soil condition. Almost 90% molecular weight loss was observed for nPCL (labmade) and 95% molecular weight loss for nPLA (labmade) under soil condition. Mass loss, thickness measurement, IR studies and contact angle studies suggests samples got degraded after 180 days but not so significant as it does under controlled environment. Thus this study confirmed that most of the PLA and PCL based biopolymeric materials were not biodegradable under natural soil condition within a time period of 180 days. Although nPCL and PCL/algaebiomass 20% showed to disintegration under natural soil condition with heavy molecular weight loss and mass loss. The degradation of PCL was caused by bacteria catalyzed erosion from the surface to the interior, while PLA was hydrolytically degraded from the surface which is shown in our thickness measurement data. This is the reason that it had different degradation behaviour under uncontrolled natural soil condition, where there were different humidities and microbial strains throughout the degradation process and time as observed elsewhere (Weng et al., 2013b).

This study also opens up another window, that under outdoor natural soil condition fillers and biopolymer molecular weight would play a vital role in tuning the biodegradability under these unmanaged or uncontrolled condition. More fillers need to be explored for

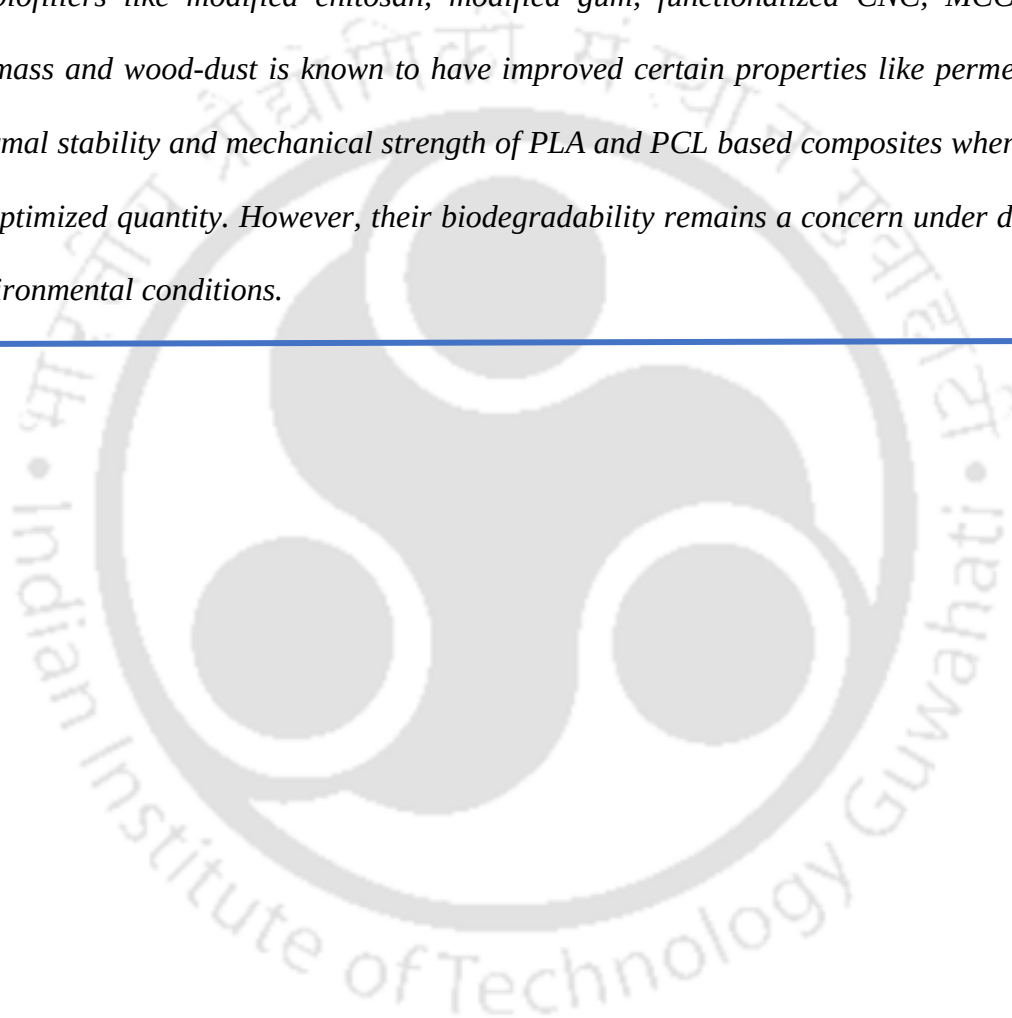
degrading these test samples within 180 days under uncontrolled natural condition in order to match the controlled environment results. This would help in sustainable development of materials in order to sustain our landfills for more years than usual.





Comparison of all the test materials under different environment

Biodegradation of biopolymers is an essential end-of-life parameter for a bioplastic for entering the market of biodegradable polymers. Bioplastics like PLA and PCL are one of the most extensively studied and used biopolymers for various applications. The addition of biofillers like modified chitosan, modified gum, functionalized CNC, MCC, algal biomass and wood-dust is known to have improved certain properties like permeability, thermal stability and mechanical strength of PLA and PCL based composites when added in optimized quantity. However, their biodegradability remains a concern under different environmental conditions.



Abstract

This chapter focuses on the overall comparison of the test materials used and different types of compost used in the whole thesis. All the test samples were compared by their carbon mineralization rate under aerobic composting conditions. Whereas several test samples were analyzed under unmanaged soil conditions and were compared according to decrease in their molecular weight analysis of the samples. Under composting condition it was found that neat PLA samples took around 95 to 110 days under different compost types at thermophilic composting condition. It was observed that with the addition of 5% modified chitosan the biodegradation rate increased and 90 % C—CO₂ was observed under 83 days period of time. Further addition of algal biomass extract to the PLA matrix increased the biodegradation rate and was found to be 90% C-CO₂ mineralization under 60 days of time. Both these results signify that presence of higher amount of nitrogenous content in the biofillers increases the biodegradation rate of the PLA based materials under thermophilic composting conditions. Since amine groups present in the modified chitosan and algal biomass helps in faster hydrolysis due to attachment of weakly bonded carboxylic groups on the amino acids present in the biomass as well in modified chitosan. However, most of the blends and composites used failed the outdoor soil biodegradation test under 180 days of time. This might be due to soil microflora and temperature are not suitable for the polymers to biodegrade and may require more time for the study like 365 days. So, biodegradation of PLA and PCL based polymeric composites and blends need more time to biodegrade under soil conditions completely as studied. Although lab synthesized PLA and lab synthesized PCL got biodegraded under soil condition and their molecular weight reduced significantly 90 folds within 180 days of time. This comparative throw light on the biodegradability of various PLA and PCL based blends and their biocomposites under both controlled and uncontrolled environments.

9.1 Introduction

Biodegradable polymers are gaining popularity around the globe due to their end-of-life management system when compared to conventional plastics which does not degrade upto hundreds of years, even destroy and pollute our land, ocean and fresh ground water sources (Kalita et al., 2019; Leejarkpai et al., 2011; Pekařová et al., 2018). Biodegradable plastics are quickly gaining popularity around the globe due to their easy processibility with existing processing units of conventional plastics (Bhasney et al., 2019). Although it is still costly and economically not viable for many developed countries. However biodegradable polymers are gaining foothold in the globe through the developed countries where there is understanding and popularity of the bioplastics among the masses (Narancic et al., 2018). According to European bioplastics market survey (www.bio-based.eu/markets) almost 22 million tonnes of bioplastics were produced in 2019. Among them, biobased/non-biodegradable plastics constitutes 56.8 % of the global market share, whereas, biodegradable plastics constitutes 43.2 % of the global market share. Among the biodegradable bioplastics starch blends constitutes 42.3%, PLA constitutes 23.8%, PBAT constitutes 16.8%, PBS constitutes 10.7%, PHA constitutes 3.3% and others constitutes 3.2% of the global biodegradable bioplastic production. These bioplastics were used mainly for rigid and flexible packaging systems, agriculture mulch films, textiles etc, (Dhar et al., 2017). Now studying the biodegradable polymers with their biodegradation activity is more necessary since biodegradation activity can be tuned with respect to their material characterization. Although biodegradation mechanism of various biopolymers like PLA and PCL are well documented in literature (Leejarkpai et al., 2011; Narancic et al., 2018; Pekařová et al., 2018, 2018b; Singh et al., 2014). Its biodegradation activity with various biofillers and blends can help in preparation of the material for acceleration of

biodegradation under different environment like aerobic composting condition and outdoor soil condition (Leejarkpai et al., 2011; Weng et al., 2013a).

Aerobic composting of bioplastics is a process in which organic carbon content of the bioplastics is converted into CO₂, H₂O and biomass through microbial assimilation (Kalita et al., 2019; Zumstein et al., 2019). Various standard organizations like ASTM International, ISO etc., have set protocols in order to define biodegradable polymers. According to ASTM International D5338 a bioplastic can be termed biodegradable or compostable only if 90 % of its organic carbon content is converted into CO₂, water and energy within 180 days of time, in aerobic composting condition at 58±5 °C (ASTM International, 2015b, 2004).

Generally various techniques are employed to study the evolved CO₂ during the biodegradation of the bioplastics like respirometry methods, titration etc. Studies suggest use and design of various composting reactors or vessels for the biodegradation of bioplastics under thermophilic temperature (Kale et al., 2007b; Leejarkpai et al., 2011; Narancic et al., 2018). Generally, biodegradation is a sequential step comprising of hydrolytic degradation step which allows the formation of oligomeric intermediates of low molecular weight which is further assimilated by the microbes in the next step into CO₂, H₂O and biomass (Kalita et al., 2019). For biodegradation of the bioplastics compost quality and type also matters according to the ASTM International D5338 standard. Biodegradable polymers take more time to biodegrade under landfill condition or outdoor soil condition, which is why aerobic composting is preferred since this process doesn't release any toxic substances and can be reused as a fertilizer for agricultural purposes.

Various authors tried to degrade PLA and PCL based blends and composites under soil condition and found that very less degradation occurred on those samples under uncontrolled outdoor soil condition (Arrieta et al., 2014; Narancic et al., 2018; Weng et al.,

2013b, 2013a). This kind of tests are very important for a country like India, where waste management is still not so popular and still most of the plastics are either dumped in the landfills or are found thrown in the drains and rivers due to lack of proper waste segregation methods and infrastructure in place. Therefore, development of bioplastics which can biodegrade under normal uncontrolled natural soil condition would help more to the plastic pollution of land, cities, and oceans (Bhasney et al., 2019; Dhar et al., 2017). In this chapter various PLA and PCL based biocomposites, blends and polyblends were studied under composting condition using food waste compost, paper mill sludge and water hyacinth compost. Further various fillers like modified chitosan, modified gum arabica, functionalized CNC, algal biomass extract and MCC were utilized as fillers in order to improve certain properties. So studying the impact of the fillers in the bioplastic matrix would help in tuning biodegradability of the test material. Various PLA and PCL based test samples of various shapes were also used to study their biodegradation under soil condition. This chapter highlighted and compared all the test samples used in the thesis under composting as well as under natural soil condition with their percentage biodegradation activity and extent of biodegradation.

9.2 Results and Discussions

9.2.1 Comparative biodegradation under controlled environment

Controlled biodegradation was carried out under compost as substrate. For this three different kinds of compost were used namely, food waste compost (Varma and Kalamdhad, 2014), paper mill sludge (Hazarika et al., 2017) and water hyacinth (Singh and Kalamdhad, 2012) based compost. For the biodegradation study under composting condition ASTM International D5338-15, D 6400 standard protocol were followed.

In the first study various PLA based biocomposites using biofillers were studied under food waste compost as shown in **figure 9.1**. From this study it was observed that PLA/chitosan

biocomposite showed almost 90% C-CO₂ evolution after 81 days of biodegradation. NPLA showed 90% C-CO₂ after 103 days of biodegradation. Whereas, PLA/CNC and PLA/Gum took more time to biodegrade than NPLA. This indicates that all biofillers not necessarily accelerates the biodegradation of PLA based biocomposites. It's the properties of biofillers that matters the most which would indeed help in tuning biodegradability like it does in improving certain properties.

Observing the results of the first study where PLA/Chitosan which having amine group creates more reactive sites for degradation to occur which results in accelerated biodegradation process. Keeping this in view we used algaebiomass 5% in PLA which showed almost 90% C-CO₂ evolution within 60±5 days under the controlled environment (**Figure 9.1**). Thus it was concluded that nitrogen content with amino acids presence in algae biomass (Torres et al., 2015), which also helped in faster biodegradation of PLA based biocomposites.

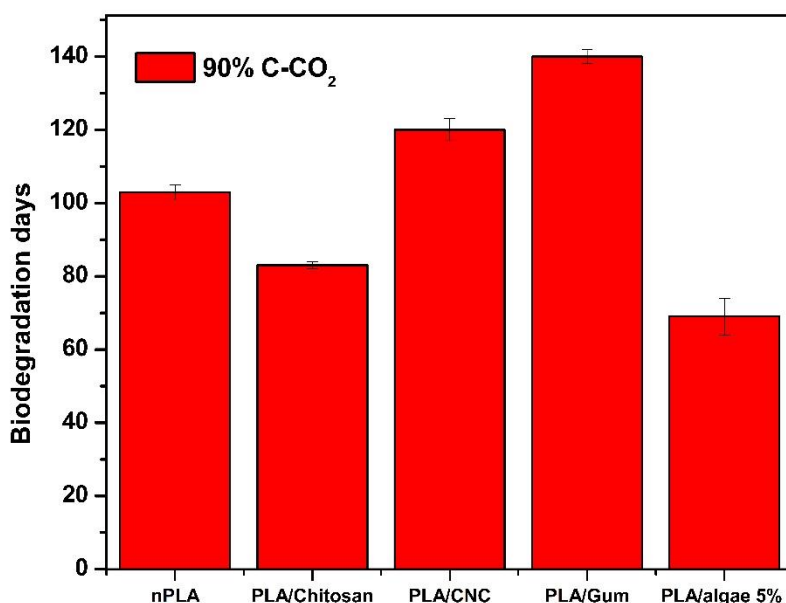


Figure 9. 1: Biodegradation of various PLA based biocomposites.

In another study PLA/LLDPE/MCC based polyblends were characterized for biodegradation study under paper mill sludge compost. In this study it was observed that 90 % C-CO₂ evolution was found in NPLA within 110 days of biodegradation period. It was found that when PLA/LLDPE/MCC:80/20/3 was used for biodegradation study, almost 55-60% biodegradation was observed in 180 days of time period (**Figure 9.2**). It is noteworthy to be mentioned that LLDPE was not biodegradable under composting condition. But when mixed with PLA and MCC it would help in disintegration into small fragments in compost without degradation. Similar results were obtained by authors under different environment (Dubey et al., 2016; Goheen and Wool, 1991) So this kind of blending can be a good alternative candidate keeping bioplastics economics in view. Further development can be made using PLA and other non-biodegradable polymers for tuning biodegradability and by retaining certain properties required for specific application like packaging etc.

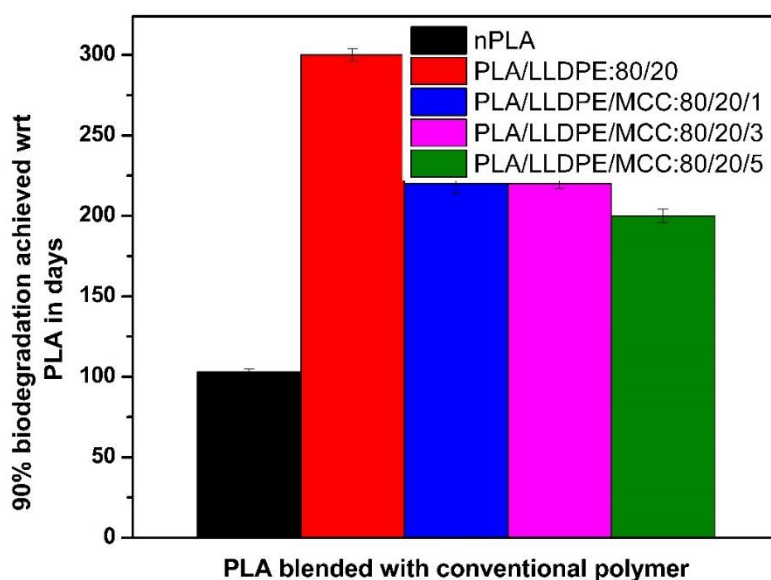


Figure 9. 2: Biodegradation of PLA based biobased polyblends done under thermophilic composting condition.

For understanding biodegradability of biopolymeric polyblends PLA/PCL/MCC was used. Both PLA/PCL blend is compatible if blended specifically for its use like packaging and drug delivery systems (Narancic et al., 2018; Siparsky et al., 1998; Vieira et al., 2011). In our study it was found that PLA/PCL/MCC:80/20/3 showed the best biodegradability under composting condition (**Figure 9.3**). 90% C-CO₂ evolution for this blend was achieved within 80 days of biodegradation under controlled thermophilic composting condition. Therefore, biopolymeric polyblends can be a good option for utilization as bioplastics keeping biodegradability a major goal to achieve.

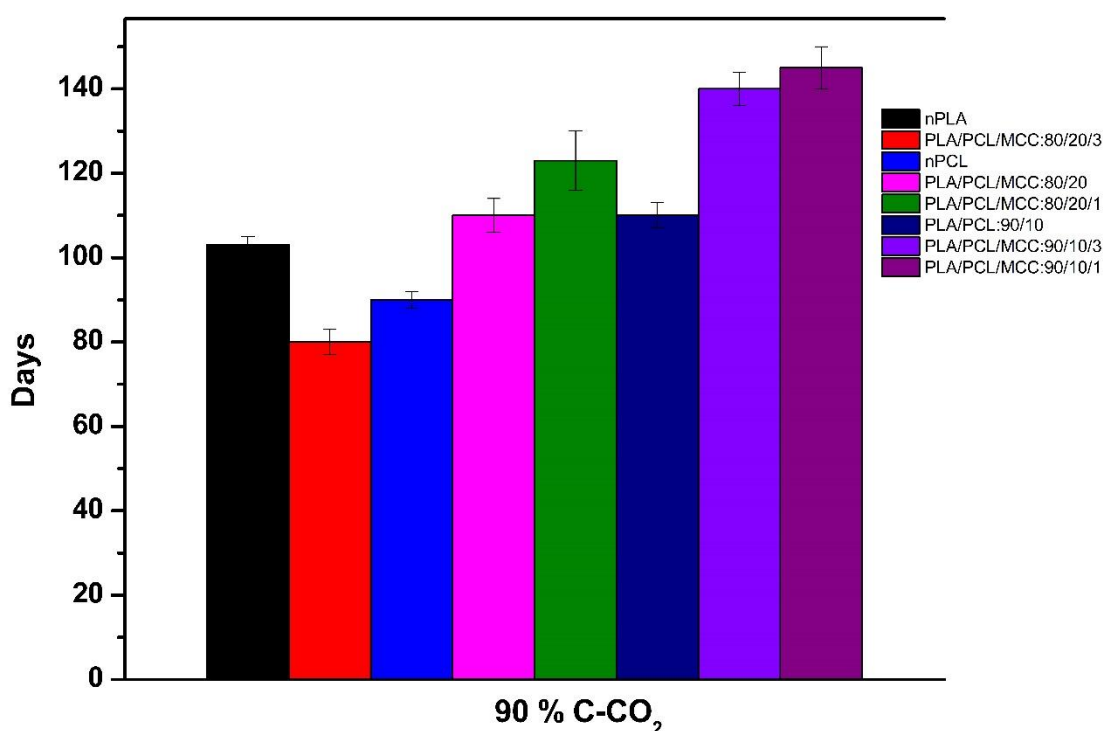


Figure 9. 3: Biodegradation of PLA/PCL based polyblends under thermophilic composting condition.

9.2.2 Degradation of NPLA under different types of compost

Three different kinds of compost was used for the biodegradation study namely; food waste compost, papermill sludge compost and water hycinth compost. Biodegradation of NPLA was studied using the ASTM D5338-15 standard protocol. Under food waste compost NPLA showed 90% C-CO₂ evolution within 98±5 days of the biodegradation period (**Figure 9.4**). Biodegradation of NPLA under compost type is as followed: food waste compost > water hycinth compost > papermill sludge compost. Lot of understanding has to be generated and studies need to be conducted in understanding the biopolymer compost compatibility and properties for best results.

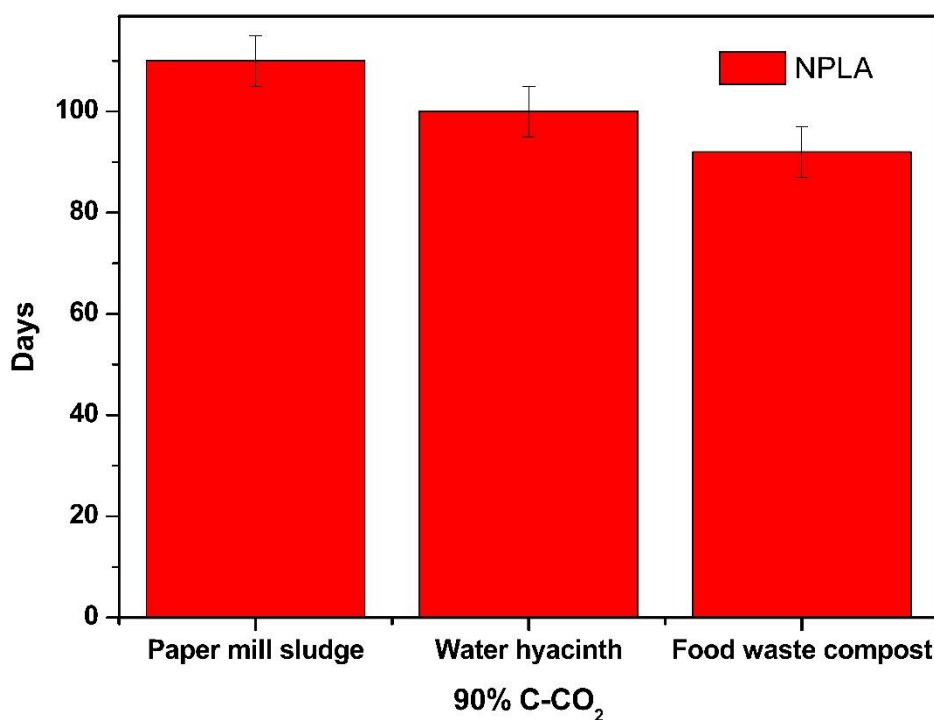


Figure 9. 4: Graph representing the best compost used for the biodegradation of PLA under thermophilic composting condition

9.2.3 Comparative biodegradation under natural outdoor soil (uncontrolled) environment

This study was designed to focus on the biodegradation behaviour of PLA and PCL based biocomposites, blends and polyblends of different shapes. Fillers like wood-dust, algal biomass and MCC were used in preparation of the test samples. Outdoor temperature and humidity profiling was done both for the environment as well as inside 20cm deep soil. Daily outdoor temperature was monitored daily in order to observe environmental behaviour that might add as parameter of degradation under outdoor soil condition in a particular latitude and longitude. An average of 33.5 °C of outdoor temperature and 23 °C was received during the period of June 2019 to December 2019 for 180 days. Molecular weight analysis revealed that nPCL (labmade), nPLA (labmade) and PCL/algaebiomass 20% samples underwent accelerated molecular weight loss under soil condition. Almost 90% molecular weight loss was observed for nPCL (labmade) and 95% molecular weight loss for nPLA (labmade) under soil condition. Based upon mass loss, thickness measurement, IR studies and contact angle studies suggests samples got degraded after 180 days but not so significantly as it does under controlled environment (**Figure 9.5**).

The degradation of PCL was caused by bacteria catalyzed erosion from the surface to the interior, while PLA was hydrolytically degraded from the surface only as shown in our thickness measurement data. This is the reason that it had different degradation behaviour under uncontrolled natural soil condition, where there were different humidities and microbial strains throughout the degradation process and time as observed elsewhere (Weng et al., 2013b).

This study also opens up another pathway, that under outdoor natural soil condition fillers and biopolymer molecular weight can play a virtual role in tuning the biodegradability under these unmanaged or uncontrolled condition. More fillers needs to be explored for degrading these test samples within 180 days under uncontrolled natural condition in order to match

the controlled environment results. This would help in sustainable developemnt in order to sustain our landfills for more years than usual.

Sample (shape)	Compost Biodegradation (180 days)	Soil Biodegradation (180 days)
nPLA (commercial)		
nPCL (commercial)		
nPCL (lab synthesized)		
PLA/Chitosan (sheet)		
PLA/CNC (sheet)		
PLA/Gum (sheet)		
PLA/algae 5% (sheet)		
PLA/PCL:80/20 (strip)		
PLA/PCL:90/10 (strip)		
PLA/PCL/MCC:80/20/3% (strip)		
PLA/PCL/MCC:80/20/1% (strip)		
PLA/PCL/MCC:90/10/3% (strip)		
PLA/PCL/MCC:90/10/1% (strip)		
PLA/LLDPE:80/20 (strip)		
PLA/LLDPE/MCC:80/20/1% (strip)		
PLA/LLDPE/MCC:80/20/3% (strip)		
PLA/LLDPE/MCC:80/20/5% (strip)		
nPLA (tray form)		
nPLA (lab synthesized)		
PLA/algae 5% (foamed sheet)		
nPLA (foamed sheet)		
PCL/algae 20% (dumbbell)		
PCL/algae 30% (dumbbell)		
PCL/wood-dust 30% (dumbbell)		
PCL/wood-dust 40% (dumbbell)		

PASS	FAIL	N/A
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Figure 9. 5: Biodegation of test samples done under natural soil environment

9.3 Conclusions

Composting of bioplastics and bioplastics under natural uncontrolled environment is very much different in terms of the biopolymeric degradation behaviour. Controlled

biodegradation was done at 58 ± 5 °C whereas, soil under outdoor condition totally depended upon the weather of that latitude and longitude.

It was observed that under controlled composting environment NPLA and its biopolymeric biocomposites and blends were completely biodegradable within 180 days time period as per standard ASTM D5338-15 protocol. PLA/Chitosan and PLA/algaebiomass 5% biocomposites showed very accelerated biodegradation like C-CO₂ evolution of 90% within 81 and 60 days of biodegradation respectively under simulated composting condition. Although, PLA/PCL/MCC:80/20/3 showed 90 % C-CO₂ evolution within 83 days of the biodegradation period. Whereas, PLA/LLDPE/MCC:80/20/3 showed almost 75% biodegradation under composting condition, leaving behind LLDPE completely undegradable. This blend disintegrated during the degradation period, suggesting biopolymer/conventional plastics disintegrability which would reduce choking of drains and landfills in future.

Biodegradation under natural outdoor soil showed an average of 33.5 °C of outdoor temperature and 23 °C was received during the period of June 2019 to December 2019 for 180 days. Molecular weight analysis revealed that nPCL (labmade), nPLA (labmade) and PCL/algaebiomass 20% samples underwent accelerated molecular weight loss under soil condition. Almost 90% molecular weight loss was observed for nPCL (labmade) and 95% molecular weight loss for nPLA (labmade) under soil condition. Mass loss, thickness measurement, IR studies and contact angle studies suggests samples got degraded after 180 days but not so significant as it does under controlled environment. Thus this study confirmed that most of the PLA and PCL based biopolymeric materials were not biodegradable under natural soil condition within a time period of 180 days. Although nPCL and PCL/algaebiomass 20% showed to disintegration under natural soil condition with heavy molecular weight loss and mass loss. This study also reveals that under outdoor

natural soil condition fillers and biopolymer molecular weight would play a vital role in tuning the biodegradability under these unmanaged or uncontrolled conditions. More fillers needs to be explored for degrading these test samples within 180 days under uncontrolled natural condition in order to match the controlled environment results. This would play a key role in building up sustainable development by exploiting bioplastics as a candidate to sustain our landfills for more years than usual.





Study of plant growth using the compost used in biodegradation and inducing biopolymers in both soil as well as natural soil condition

Biopolymer assimilated in compost during the biodegradation process, should not leave any kind of toxic material as per various standards. Utilizing this compost as fertilizer or as growth substrate would definitely enhance soil quality, since biopolymers contains organic carbon. In other words, biopolymers can be considered as a source of fertilizer or substrate to enhance compost or soil quality. However, biopolymers effect on crops or used compost for biodegradation can be studied to have better picture, to prove that end-of-management technique of biopolymer composting can be far better in terms of economics related to biopolymers as well the biopolymers “nature-to-nature” sustainability.

Publications:

- Naba Kumar Kaita, Doli Hazarika, Ajay Kalamdhad and Vimal Katiyar, Study of plant growth using the compost used in biodegradation and inducing biopolymers in both soil as well as natural soil condition (To be submitted).

Abstract

This study demonstrated the plant growth study of potato (*Solanum tuberosum L*) on used (biopolymer biodegradation biomass) compost as fertilizer or growth substrate under controlled greenhouse condition. The tuber crop growth condition inside the green house was maintained at 20 °C and 70% relative humidity (RH). Samples were combined as follows: soil/potato, compost/soil/potato, used compost/soil/potato, soil/compost/induced biopolymer, and soil/compost/biopolymer/LLDPE and their growth was monitored and analyzed throughout a period of 120 days. Germination %, salt uptake capacity and molecular weight of the samples after growth is studied, and, electrical conductivity (μS) and pH of soil monitored throughout the growth period. Molecular weight of the samples with biopolymer and without biopolymer was found to be 400 Da and 430 Da signifying zero uptake of any oligomers from the biopolymers. Despite of high pH of 7.8, the growth was good, assuming null effect of high pH on plant growth. Biodegradation of the polymers during the composting did not increase the conductivity of the tested growth medium which showed good growth in the potato plant having good salt uptake. This study revealed current understanding to identify the knowledge gaps and future research priorities related to biopolymers as well as biopolymers tremendous role for “nature-to-nature” sustainability.

10.1 Introduction

Advancement of bioplastics, or more specifically annually with increase of utilization of renewable resources would reduce the negative influence of plastic waste on the environment. Now a day's biodegradable materials are of prime interest for environmental reasons. Biodegradability is the most important property due to which production of bioplastics has received considerable attention. Therefore, study of the biodegradability plays a significant role in development of biopolymers (Kalita et al., 2019; Katiyar and Nanavati, 2011; Pradhan et al., 2010a; Zumstein et al., 2019). The objective is to set up a sustainable environment and restrict potential deposits of inappropriate plastic waste in the environment. The term bioplastics can be linked to biologically-based plastics synthesized from biomass and renewable resources such as polylactic acid (PLA) and polyhydroxyalkanoate (PHA) or plastics obtained from fossil fuels including aliphatic plastics such as polybutylene succinate (PBS) can be utilised as substrate for microorganisms. The most studied biodegradable polymers are polysaccharides (cellulose derivatives primarily starch) (Ramani and Charles, 1999; Shi et al., 2011; Siracusa et al., 2008).

Most studied plants and Terrestrial emissions of microplastics in industrialized countries predominantly originate from the application of sewage sludge in farmlands (Nizzetto et al., 2016). Sewage sludge, also referred to as biosolids, is applied to improve and maintain productive soils, and stimulates plant growth in many areas of the world (Singh and Agrawal, 2008). The application of agricultural sewage sludge alone results in a major input of plastic particles into agricultural soils, estimated to be between 63,000 and 430,000 and 44,000-300,000 tons per year of microplastics (<5 mm) in the EU and North-America farmlands, respectively (Nizzetto et al., 2016), and between 2800 and 19,000 tons per year in Australian agroecosystems (Ng et al., 2018). In addition, there are also new technological

innovations in which plastics are added as a nano-coating to seeds in combination with an active ingredient, such as fertilizers or pesticides. As a result, there is an accumulation of plastic particles in terrestrial systems. To date, most work on the distribution and toxic potential of nano- and microplastics has focused on aquatic environments, and there is an urgent need for research on potential adverse effects of nanoplastics (<100 nm) and microplastic pollution in terrestrial ecosystems (Horton et al., 2017). From the above information gathered, we can see that most of the work distribution and toxic potential of microplastics related to conventional plastics has been studied under aquatic, sewage sludge or either in laboratory conditions.

Fewer have studied the impact of compost used for biodegradation study of biopolymers and reusing that compost as fertilizer or as soil for plant growth (Adamcová et al., 2019; Tuominen et al., 2002). In this study, authors have complete biodegradation of all tested lactic acid polymers and their biodegradation was confirmed. Biotests clearly showed that poly(esterurethane) where lactic acid prepolymers were chain linked with 1,4-butane diisocyanate did not exhibit any ecotoxicological effect, and neither did poly(ester-amide) or poly-(lactide). However, they discussed about the importance of composting process, in order to understand the degradation products of polymers in compost and its effect in plant growth (Tuominen et al., 2002). Authors here carried out experiments suggesting materials should also be tested in real conditions that differ from those in a laboratory, as these samples are intended for clients who can compost them in actual conditions. This study opens new doors for materials manufacturers who should focus on testing under real conditions (Adamcová et al., 2019).

Few studies were conducted on microplastics as well as plastics exposure to terrestrial non-vascular plants like cress (*Lepidium sativum* L.), a terrestrial plant species frequently used in ecotoxicological assessments, to assess sites of adsorption, uptake and impact on

germination, growth, and chlorophyll production (Bosker et al., 2019). Authors also studies ecotoxicological effect of bioplastics composted under composting condition and reutilizing it as fertillizer. Here authors (Tuominen et al., 2002) studied cress, radish and barley which are common crops for studying plant growth on exposure to bioplastics as well as used compost after biodegradation study. PLA and PU were used as bioplastic source material. In this study one material radish is used which is a tuber crop.

Among tuber crops potato is the most important food crops in India after rice, wheat and maize (Borah et al., 2016). Therefore, in this study we examine the influence of the resultant compost after the decomposition process and checked in the terms of its phytotoxic effect on selected plants potato (*Solanum tuberosum* L). Potato was selected as it is one of the most common tuber crop used around the world. Composting of PLA based materials and their biodegradation/ disintegration under laboratory condition using ASTM International D5338-15 would be used as compost source for plant growth.

10.2 Results and Discussions

10.2.1 Germination rate of the samples under different medium

Potato seed germination was found to be highest in used compost (after biodegradation study biomass) of about 75% within 7 days. This proves that used compost can be reutilized as fertilizer that would be helpful for creating wealth from waste. Whereas, LLDPE/PLA/soil/compost sample showed the least germination rate of 10%. This result showed that LLDPE inhibited the growth rate of the potato seeds. This indicates that conventional plastics inhibits plant growth under controlled environment condition. From these result it can be concluded that future work can be done on effect of bioplastics on potato germination with detailed analysis.

Figure 10.1 showing the plant growth on different media throughout the growth period. Crop was cultivated on the 120th day and sampling was done as shown in **figure 10.2**, samples are small in size due to their native to North-east region of India. Potato in this region is grown during November to middle of March and germination data presented in **table 10.1**.

Table 10. 1: Germination % of the test samples grown under various soil conditions

Germination % of test samples	
PLA/potato/soil	40%
LLDPE/PLA/soil/potato/compost	10%
Used compost(after biodegradation study)/potato	75%
soil/potato	50%
compost/potato	60%
PLA/compost/potato	45%

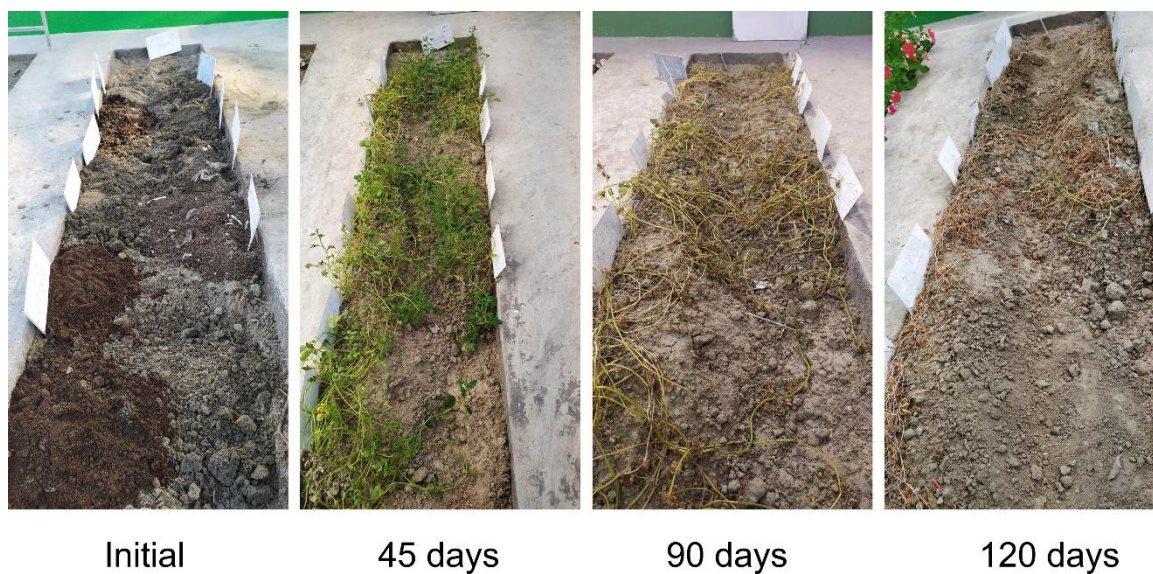


Figure 10. 1: Showing the plant growth on different media throughout the growth period

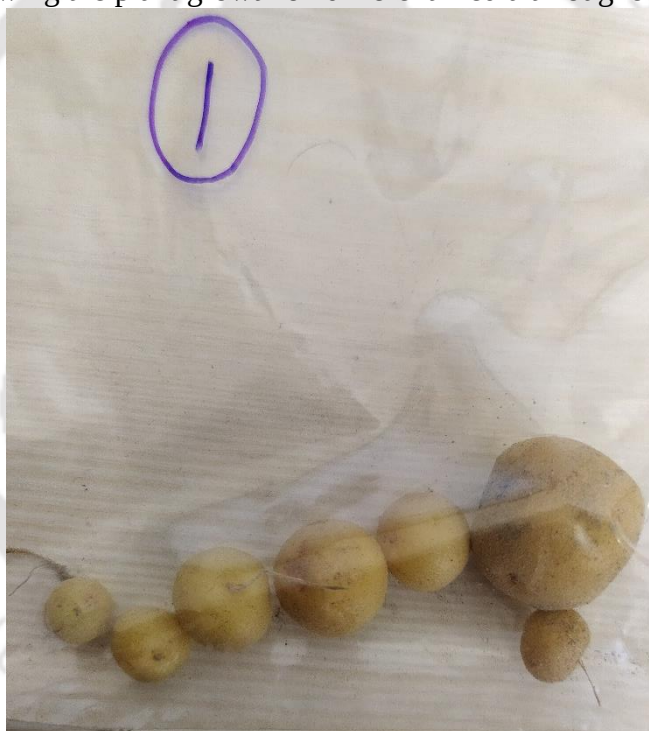


Figure 10. 2: Representative image of the potato samples cultivated after 120th day

10.2.2 Molecular weight analysis of the test samples

Molecular weight of the test samples were studied using gel permeation chromatography (GPC). Molecular weight of the potato samples was done as any oligomers from PLA or PCL will be reflected or could be identified from the molecular weight data. In order to understand this phenomenon 10 mg of chopped potato samples were dipped in 10 ml of

chloroform overnight. Molecular weight of the samples revealed that there was no molecular weight difference in samples without biopolymers and samples grown in biopolymeric or polymeric medium. However, LLDPE was not considered for molecular weight analysis. Molecular weight of all the test samples varied from 400 Da to 450 Da., signifying null effect of biopolymeric oligomers or monomeric unit on the test sample growth.

10.2.3 Electrical conductivity and pH of soil

One measurement commonly made on compost samples is salinity, which is estimated by measuring electrical conductivity (VanderGheynst et al., 2004). High levels of salt in a compost can result in a reduction in crop yields when salt concentrations near the root zone accumulate to a level at which the root can no longer extract sufficient water from the soil-compost solution. Salt tolerance varies among crops and is affected by cultural conditions under which the crop is grown. Electrical conductivity didn't changed among the samples which revealed that biopolymers had negligible effect on the electrical conductivity of the soil as shown in **table 10.2**. Used compost electrical conductivity was found to be 0.22 $\mu\text{S}/\text{mm}$ to 0.18 and had negligible effect on the plant growth. This suggests used biomass (biodegradation of polymers under composting condition) can be reused as soil or fertilizer for plant growth.

For plant growth pH was measured to understand the suitability of the test medium or soil medium for the plant growth. The optimal range of pH for most plants and crops is 5.5 (Tuominen et al., 2002). High pH effects the growth of plants. In this study pH varied from 6.5 to 7.8 (**Table 10.3**), had negligible effect on the plant growth. Similar results were obtained during the growth of cress, barley and redish plants (Tuominen et al., 2002).

Table 10. 2: Electrical conductivity of soil measured initially and after 120 days of growth period

Electrical conductivity ($\mu\text{S}/\text{mm}$)	Initial	120 th day
PLA/potato/soil	0.20	0.21
LLDPE/PLA/soil/potato/compost	0.13	0.12
Used compost(after biodegradation study)/potato	0.22	0.18
soil/potato	0.12	0.16
compost/potato	0.10	0.12
PLA/compost/potato	0.14	0.15

Table 10. 3: pH measurement of the soil before and after 120 days of plant growth

pH	Initial	120 th day
PLA/potato/soil	6.5	7.0
LLDPE/PLA/soil/potato/compost	7.1	7.6
Used compost(after biodegradation study)/potato	8.0	7.6
soil/potato	6.8	7.0
compost/potato	7.4	7.2
PLA/compost/potato	7.6	7.3

10.2.4 Potato stem length and thickness measurement

Potato stem length and stem thickness also signifies good growth quality (Gao et al., 2014). Stem growth was measured using scale and callipers as shown in **figure 10.3 (a-b)**. Growth was measured for first 30 days and presented in **table 10.4**. It can be seen that all the test samples showed early growth and from the day 3 onwards measurement was done. Stem length of 63.8 ± 10 and 176 ± 10 mm was observed on day 3 and day 30 respectively for samples grown on compost biomass. This data was clearly higher than other test conditions (**table 10.4**), suggesting sustainability of the biodegradation of polymer under compost biomass can be reused as fertilizer for plant growth. Similar behaviour was observed in the thickness measurement data (**table 10.4**). This indicates good plant growth under several test conditions. This work opens window for more work related to plant growth under

biopolymer biodegradation studied compost or used compost as fertilizers, like role of microbes and soil behaviour etc.



Figure 10. 3(a): Stem growth was measured using scale and callipers as shown (representative image)



Figure 10. 4 (b): Representative image during stem length measurement

Table 10. 4: Stem length (mm) and stem thickness (mm) measurement of the crop measured for 30 days

Samples	Stem length (mm)			Stem thickness (mm)		
	Day 3	Day 15	Day 30	Day 3	Day 15	Day 30
Plain soil	18.8 ± 2	23 ± 4	90 ± 8	1.9 ± 0.2	2.0 ± 0.1	2.4 ± 0.1
Only Compost	21 ± 4	38 ± 7	120 ± 7	2.1 ± 0.8	2.5 ± 0.5	2.8 ± 0.3
Compost after biopolymer biodegradation	63.8 ± 10	140 ± 8	176 ± 9	5.9 ± 1	6.0 ± 1	6.8 ± 1
Soil/compost/biopolymer /LLDPE	7 ± 2	15 ± 8	39 ± 6	1.3 ± 1.2	1.9 ± 0.1	2.1 ± 0.3
Compost/biopolymer	46.8 ± 12	100 ± 2	149 ± 5	3.1 ± 0.4	3.2 ± 0.3	3.4 ± 0.5
Soil/biopolymer	43 ± 10	110 ± 9	160 ± 2	2.1 ± 0.6	2.8 ± 0.1	2.7 ± 0.1

10.2.5 Salt uptake capacity of the test samples measured using inductively coupled plasma mass spectrometry (ICP-MS).

ICPMS was done for all the test medium as well as test samples grown on that particular medium. Salt concentration and few heavy metals were analysed for soil and test samples and presented in **table 10.5(a)** and **table 10.5(b)**.

These minerals like Fe and other salts like Na, Mg and P are mostly acquired by the potatoes from soil (Subramanian et al., 2011). Therefore, we used more sophisticated method like ICPMS for testing the samples in order to know if the biopolymer/biomass/soil had any effect on the plant growth. Even heavy metals like Pb was also analysed for both in the testing media and test samples. In the testing media or the test samples it was found to be under permissible limits in India. In the compost biomass or used compost 24 ppb level of Pb was detected, whereas, nill amount of Pb was detected in the test samples. Similarly salt concentrations in the test samples shows that plant growth was good (**Table 10.5(a-**

b)). However, future detailed analysis can reveal more details related to reuse of compost biomass after biopolymer biodegradation in more sustainable approach.



Table 10.5 (a): Salt and mineral concentration in soil medium used for potato cultivation

Composition	Plain soil /potato	Only Compost /potato	Compost after biopolymer biodegradation /potato	Soil/compost/biopolymer/LLDPE /potato	Compost/biopolymer /potato	Soil/biopolymer /potato
Na	216	154	309	264	65	89
Mg	105577	50615	157345	137961	23736	20987
Si	8017	4860	12344	8014	2173	1789
K	9676	3584	8801	7005	1754	1908
Fe	25886	13466	49627	35313	6610	7890
Pb	9	1.7	24	12	0	0
P	682	468	1111	1349	330	480
Cd	0	0	0	0	0	0

Table 10.5 (b): Salt and mineral concentration in test samples or whole potato

Composition	Plain soil /potato	Only Compost /potato	Compost after biopolymer biodegradation /potato	Soil/compost/biopolymer/LLDPE /potato	Compost/biopolymer /potato	Soil/biopolymer /potato
Na	203	153	323	147	259	65
Mg	96342	55199	175000	11950	15109	23725
Si	7823	5021	12760	966	825	2173
K	7505	4148	8801	2798	7563	1754
Fe	25886	13093	48682	2488	3513	2000
Pb	9	7	4	10	19	10
P	890	865	568	491	900	600
Cd	2633	0	0	0	0	0

10.3 Conclusion

This study demonstrated the plant growth study of potato (*Solanum tuberosum* L) on used (biopolymer biodegradation biomass) compost as fertilizer or growth substrate under controlled greenhouse condition. Molecular weight of the samples with biopolymer and without biopolymer was found to be 400 Da and 430 Da signifying zero uptake of any oligomers from the biopolymers. Despite of high pH of 7.8, the growth was good, assuming null effect of high pH on plant growth. Biodegradation of the polymers during the composting did not increase the conductivity of the tested growth medium which showed good growth in the potato plant having good salt uptake. This study revealed current understanding to identify the knowledge gaps and future research priorities related to biopolymers as well the biopolymers “nature-to-nature” sustainability. Future detailed analysis can reveal more details related to reuse of compost biomass after biopolymer biodegradation and its relations with plant growth.



Conclusion and Future Prospects

This chapter summarizes the key findings and inferences drawn from the research work, and provides the perspectives for future studies on biodegradation of certain class of biopolymers.

11.1 Conclusions

The main objective of the research work was to understand the extent of biodegradation of certain class of poly(lactic acid) PLA and polycaprolactone (PCL) based bioplastics under controlled composting condition as well as under uncontrolled natural soil condition. The major findings and diverse phenomenon of biodegradation of PLA and PCL based green biocomposites presented in this thesis, includes: (i) the composting setup was built indigenously with accordance to ASTM International D5338 connecting a gas chromatography system with a 16 port gas valve, (ii) biodegradation of the biopolymers without addition of any external inoculum under composting condition at thermophilic temperature, (iii) addition of nitrogeneous based biofillers which accelerates the biodegradation rate of PLA based biocomposites under thermophilic composting condition (iv) addition of MCC as biofiller for increasing the biodegradation rate of PLA/PCL based blends (v) biodegradation behavior can be studied for tuning the biodegradability of a bioplastic (vi) identification of a class of bioplastic that is biodegradable under uncontrolled outdoor soil condition. This thesis has addressed some fundamental issues related to the biodegradation of biopolymers under composting as well as natural soil condition.

The key achievements reported in this regard are summarized next

- (i) Biodegradation of PLA and PCL based biocomposites under thermophilic composting condition can be studied without addition of municipal waste or any

kind of external inoculum if proper compost quality is used for the biodegradation study.

- (ii) PLA based green biocomposites using modified chitosan; modified gum; and functionalized CNC were prepared for understanding the biodegradation behavior of the films. These green biocomposites were found to have good permeability and mechanical strength than neat PLA. So understanding its biodegradability would help in understanding its tuneability. These samples were tested in the indigenously developed composting setup. It was found that PLA/Chitosan test samples showed better biodegradability than neat PLA. Whereas, PLA/CNC and PLA/Gum showed less percentage biodegradation than neat PLA within 140 days of time. This study also suggests that it is not necessarily for every biofiller to increase the biodegradation rate of PLA based biocomposites. So biofiller choice and property is essential for attaining fast biodegradation within 180 days of time. Biodegradation kinetics was studied which successfully demonstrated the biodegradation rate of the samples by analyzing the experimental datasets with the predicted values.
- (iii) PLA based polyblends namely, PLA/LLDPE: 80/20 was prepared and blended with 1%, 3% and 5% MCC. These polyblends were tested for their biodegradation behavior under paper mill sludge compost. These polyblends were prepared to give PLA elasticity and LLDPE biodegradability. From the results, it was observed that with increase amount of addition of MCC biodegradation of PLA was faster in the polyblend. Naturally, PLA only gets degrade but this helps in disintegration of the blend, which might help in decreasing plastic pollution. Fundamentally, it was found that these kind of blends inhibits the biodegradability of PLA after a period of time during the

biodegradation time interval under composting condition. These results and observation has helped in characterization of the test samples during the biodegradation time interval. It was found that spherulite growth analysis could be studied to know the extent of biodegradation in the polymeric sample under composting condition during the biodegradation time interval.

- (iv) PLA/PCL/MCC polyblends of different concentration were used as test samples to understand the biodegradation behavior under water hyacinth compost. Biodegradation percentage results showed that with higher MCC and PCL component in the test polyblends, biodegradation was higher, as compared to a lower percentage of PLA and MCC in the polyblended test samples. The new microbial biofilm formation assay done in this study showed that the absorbance of the test samples after biodegradation was due to microbial film formation on the test sample surface. All the results obtained signified that the PLA based polyblends were almost 50-90% biodegradable in 100-120 days. These observations might open a new idea for the fabrication and use of compostable biopolymeric blends, thereby reducing our carbon footprint.
- (v) Algal biomass extract was demonstrated as a potential biofiller, which accelerates the biodegradation of the bioplastics under composting condition. Molecular weight analysis showed degradation of the test samples under abiotic condition but mass loss was not so significant as compared to molecular weight loss. Modelled data also revealed both autocatalytic and non-catalytic reaction takes place during abiotic degradation of PLA based materials. It was found that addition of algae 5% extract in PLA increased the biodegradation of the PLA based materials. PLA/algae 5% ES also induced biofilm or microbial attachment on the surface more quickly than neat PLA ES due to nitrogen content. The

degradation of the test materials was summarized by material characterization at different interval of time, which suggests biodegradation of the materials under composting condition. Morphological studies revealed biodegradation occurring through microbial attachment or biofilm formation in the films. Under both abiotic and biotic conditions PLA/algae 5% ES demonstrated excellent degradability.

- (vi) Biodegradation study conducted under natural outdoor condition (uncontrolled) for 180 days revealed that PLA based biocomposites is not degradable under natural soil condition within the study period. Whereas, PCL labmade and PCL commercial was found to be degradable under the uncontrolled environment. This may be due to various reasons like PCL having low T_g from $-60\text{ }^{\circ}\text{C}$ to $-40\text{ }^{\circ}\text{C}$ which means short chains. These short chains are more susceptible to hydrolysis than longer or high molecular weight chains. Since PCL containing algaebiomass as filler has amine groups which are polar and ionizable and makes it basic (amines). This makes them easily dissociate like the carboxyl and amines. Nitrogen source also plays an important role in the degradation process. This study also directs, that under outdoor natural soil condition fillers and biopolymer molecular weight would play a vital role in tuning the biodegradability under these unmanaged or uncontrolled condition. More fillers needs to be explored for degrading these test samples within 180 days under uncontrolled natural condition in order to match the controlled environment results. This would help in sustainable development in order to sustain our landfills for more years than usual.
- (vii) The plant growth study of potato (*Solanum tuberosum* L) on used (biopolymer biodegradation biomass) compost as fertilizer or growth substrate under

controlled greenhouse condition was conducted. Samples were combined as follows: soil/potato, compost/soil/potato, used compost/soil/potato, soil/compost/induced biopolymer, and soil/compost/biopolymer/LLDPE and their growth was monitored and analyzed throughout a period of 120 days. This study revealed current understanding to identify the knowledge gaps and future research priorities related to biopolymers as well the biopolymers “nature-to-nature” sustainability.

11.2 Future Prospects

Given that the majority of neat biopolymers and blends do not meet test standards one can expect even slower degradation in soil, oceans and rivers with lower temperatures and this needs to be carefully considered when shaping plastic waste policies and plastic product design. Analysis of the profile of microorganisms communities present in inocula could clarify the different performance of tested materials in different environments. If society is to replace nondegradable with biodegradable plastics we need to ensure that we understand the biodegradability of these plastics and in particular, their blends as the latter are the most likely form that biodegradable plastics will take ahead to the marketplace.

This knowledge can inform the design and application areas of these materials so that they have a positive impact on end-of-life management options and low impact on the environment if inadvertently released; e.g., biodegradable plastics that degrade in a wide variety of unmanaged environments could be prioritized for applications where they have a higher chance of ending up in the environment (for example, packaging used in coastal areas and plastics used in marine applications).

11.2.1 Scope for future works

The work presented in the thesis can be further advanced in several directions as listed below:

- Identification of potential fillers or additives that can increase biodegradation of high molecular weight PLA and PCL under composting condition. Future studies can be aimed towards characterization of PLA and PCL based films using different compositional algae biomass extract and tuning its biodegradability under composting as well as soil condition.
- Design of biopolymers based upon PLA and PCL that can biodegrade under outdoor soil condition. This can be achieved by tuning molecular weight of the samples and addition of more organic nitrogenous compounds.
- Identification of proper quality outdoor soil through degradation study and analyzing its microbial flora under a specific area of states or districts. This would help in proper disposal of bioplastics under landfill condition for complete assimilation of these plastics.
- Studies on identification of various biodegradable strains that can be used in bioremediation of the bioplastics wastes under controlled environment can be done.
- Design of composting setup that can biodegrade bioplastics under natural composting condition. These tests might also help in material characterization and preparation of the test samples to achieve higher biodegradability.
- Future detailed analysis related to biopolymers as fertilizers would open new window for developing biopolymers and which can be thrown in the fields for crop growth etc.

11.3. Research Outputs

Research article:

Naba Kumar Kalita, Nagar, M. K., Mudenur, C., Kalamdhad, A., & Katiyar, V. (2019). Biodegradation of modified Poly (lactic acid) based biocomposite films under thermophilic composting conditions, *Polymer Testing*, 76, 522-536. (IF: 3.2)

Naba Kumar Kalita, Siddharth Mohan Bhasney, Chethana Mudenur, Ajay Kalamdhad and Vimal Katiyar, (2020) End-of-life evaluation and biodegradation of Poly (lactic acid)(PLA)/Polycaprolactone (PCL)/Microcrystalline cellulose (MCC) polyblends under composting conditions, *Chemosphere*, 247, 125875. (IF: 5.7)

Naba Kumar Kalita, Siddharth Mohan Bhasney, Ajay Kalamdhad and Vimal Katiyar, (2020), Biodegradable Kinetics and Behavior of Bio-based Polyblends under Simulated Aerobic Composting Conditions, *Journal of Environmental Management*, 261, 110211. (IF: 5.6)

Submitted and prepared research articles:

Naba Kumar Kalita, Anubhav Sharma, Siddharth Mohan Bhasney, Ajay Kalamdhad and Vimal Katiyar Demonstrating an Ideal Compostable Plastic using Biodegradability Kinetics of Poly(lactic acid) based Green Biocomposite Films under Aerobic Composting (AC) Conditions. (Submitted)

Naba Kumar Kalita, Doli Hazarika, Purabi Bhagabati, Ajay Kalamdhad and Vimal Katiyar Biodegradation Study of Biodegradable Polymers and Demonstrating Algae Biomass Extract as a Potential Filler for Compostable PLA Bioplastic. (To be submitted)

Naba Kumar Kalita, Doli Hazarika, and Vimal Katiyar, Biodegradation of biopolymers under natural outdoor soil condition and ecotoxicological effect of biopolymers on plant growth after composting of bioplastics. (To be submitted)

Book Chapter:

Naba Kumar Kalita, Melakuu Tesfaye, Purabi Bhagabati and Vimal Katiyar, 2017, book chapter titled as 'Trends of end-of-life Options: Recycling, Reusing and Composting of Waste Food Packaging' in book 'Bio-based Plastics for Food Packaging Applications'. (Smithers Rapra, ISBN: 9781910242582).

Naba Kumar Kalita, Ajay Kalamdhad and Vimal katiyar, Recent Trends and Advances in the Biodegradation of Conventional Plastics, (2019), Advances in Sustainable Polymers, Synthesis, Fabrication and Characterization ISBN: 978-981-15-1251-3. Doi: https://doi.org/10.1007/978-981-15-1251-3_17.

Oral Presentations:

Naba Kumar Kalita, Ajay Kalamdhad and Vimal Katiyar, Enzymatic degradation behavior of distinct PLLA/PDLA blends through thermostable enzymes, International Symposium on Sustainable Polymers & National Symposium on Chemistry Education for Sustainable Engineering, August 23-25, 2019, IIT Guwahati.

Naba Kumar Kalita, Ajay Kalamdhad and Vimal Katiyar, Biodegradation Studies of Polylactide based Biocomposites under Controlled Composting Conditions, (Recycle 2018), IIT Guwahati, 22nd to 24th Feb, 2018.

Naba Kumar Kalita, Ajay Kalamdhad and Vimal katiyar, Comparative biodegradation evaluation of various bioplastics and compost type under industrial composting condition, (Recycle 2020), IIT Guwahati, 13-14 February, 2020.

Poster Presentations:

Naba Kumar Kalita, Ajay Kalamdhad and Vimal Katiyar, Biodegradation and morphological study of PLA based biocomposites under composting condition, (SPSI-Macro 2018), IISER Pune, 19th to 22nd Dec, 2018.

Naba Kumar Kalita, Ajay Kalamdhad and Vimal Katiyar, Biodegradation behaviour of Polylactide based Biocomposites under Composting Conditions, (Research Conclave'18), IIT Guwahati, 8th to 11th Mar, 2018.

Naba Kumar Kalita, Ajay Kalamdhad and Vimal Katiyar, Biodegradation study of Poly (lactic acid) based films under composting conditions, (IJBS 17), IIT Guwahati, 1st to 4th Feb 2018.

Naba Kumar Kalita, Ajay Kalamdhad and Vimal Katiyar, Aerobic biodegradation study of PLA based biocomposites under composting conditions at thermophilic temperature, (ASP 17), IIT Guwahati, 8th -11th Jan, 2018.

Naba Kumar Kalita, Anubhav Sarmah, Ajay Kalamdhad and Vimal Katiyar, Aerobic biodegradation kinetic study of PLA based biocomposites under composting conditions, (ASP 17), IIT Guwahati, 8th -11th Jan, 2018.

Naba Kumar Kalita and Vimal Katiyar, Biodegradation of Poly(Lactic Acid) Extruded Films under Soil Conditions, Materials Research Society of India Symposium on Advance Materials for Sustainable Applications & 27th Annual General meeting of MRSI jointly organized by CSIR-NEIST Jorhat, IIT Guwahati and Tezpur University at CSIR-North East Institute of Science and Technology, Jorhat on 18-20 February 2016.

Naba Kumar Kalita, Rahul Patwa, Prodyut Dhar, Amit Kumar and Vimal Katiyar, Effect of hydrolytic degradation on the wettability behaviour of bio-filler reinforced poly(lactic acid) extruded bionanocomposites, Conference: Proceedings of International Conference on Polymer Science and Technology, MACRO-2017, January 2017; At: Thiruvananthapuram, Kerala, India.

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Japan Student Services Organization (JASSO), Kyoto Institute of Technology (KIT)
and IIT Guwahati awarded me the opportunity for attending student exchange programme
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REFERENCES

- Abou-Zeid, D.M., Miller, R.J., Deckwer, W.D., 2004. Biodegradation of aliphatic homopolyesters and aliphatic-aromatic copolyesters by anaerobic microorganisms. *Biomacromolecules* 5, 1687–1697. <https://doi.org/10.1021/bm0499334>
- Abrusci, C., Pablos, J.L., Corrales, T., López-Marín, J., Marín, I., Catalina, F., 2011. Biodegradation of photo-degraded mulching films based on polyethylenes and stearates of calcium and iron as pro-oxidant additives. *International Biodeterioration and Biodegradation* 65, 451–459. <https://doi.org/10.1016/j.ibiod.2010.10.012>
- Adamcová, D., Zloch, J., Brtnický, M., Vavrková, M.D., 2019. Biodegradation/Disintegration of Selected Range of Polymers: Impact on the Compost Quality. *J Polym Environ* 27, 892–899. <https://doi.org/10.1007/s10924-019-01393-3>
- Al Hosni, A.S., Pittman, J.K., Robson, G.D., 2019a. Microbial degradation of four biodegradable polymers in soil and compost demonstrating polycaprolactone as an ideal compostable plastic. *Waste Management* 97, 105–114. <https://doi.org/10.1016/j.wasman.2019.07.042>
- Alex, A., Ilango, N.K., Ghosh, P., 2018. Comparative Role of Chain Scission and Solvation in the Biodegradation of Polylactic Acid (PLA). *The Journal of Physical Chemistry B* 122, 9516–9526. <https://doi.org/10.1021/acs.jpcc.8b07930>
- Arkatkar, A., Juwarkar, A.A., Bhaduri, S., Uppara, P.V., Doble, M., 2010. Growth of *Pseudomonas* and *Bacillus* biofilms on pretreated polypropylene surface. *International Biodeterioration and Biodegradation* 64, 530–536. <https://doi.org/10.1016/j.ibiod.2010.06.002>
- Arrieta, M.P., Lopez, J., Rayon, E., Jiminez, A., 2014a. Disintegrability under composting conditions of plasticized PLA-PHB blends. *Polymer Degradation and Stability* 108, 307–318. <https://doi.org/10.1016/j.polymdegradstab.2014.01.034>
- Astm, 2014. Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in. *Astm D5988 – 12* 1–6. <https://doi.org/10.1520/D5988-12.2>
- ASTM International, 2015a. ASTM D5338-15 : Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials under Controlled Composting Conditions, Conditions, Incorporating Thermophilic Temperatures. *ASTM Standards*. 1–7. <https://doi.org/10.1520/D5338-15.2>

- ASTM International, 2015b. ASTM D5338-15 : Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials Under Controlled Composting Conditions, Conditions, Incorporating Thermophilic Temperatures. ASTM Standards. 1–7. <https://doi.org/10.1520/D5338-15.2>
- ASTM International, 2004. ASTM D6400 standard specification for compostable plastic.
- ASTM Standard D 5338, 1998, 2003. “Test Method for Determining Aerobic Biodegradation of Plastic Materials Under Controlled Composting Conditions.” ASTM International, West Conshohocken, 98, 1–6. <https://doi.org/10.1520/D5338-11>. Copyright
- Balali, S., Davachi, S.M., Sahraeian, R., Shiroud Heidari, B., Seyfi, J., Hejazi, I., 2018. Preparation and Characterization of Composite Blends Based on Polylactic Acid/Polycaprolactone and Silk. *Biomacromolecules* *acs.biomac.8b01254*. <https://doi.org/10.1021/acs.biomac.8b01254>
- Bhagabati, P., Hazarika, D., Katiyar, V., 2019. Tailor-made ultra-crystalline, high molecular weight poly(ϵ -caprolactone) films with improved oxygen gas barrier and optical properties: a facile and scalable approach. *International Journal of Biological Macromolecules* *124*, 1040–1052. <https://doi.org/10.1016/j.ijbiomac.2018.11.199>
- Bhardwaj, H., Gupta, R., Tiwari, A., 2013. Communities of Microbial Enzymes Associated with Biodegradation of Plastics. *Journal of Polymers and the Environment* *21*, 575–579. <https://doi.org/10.1007/s10924-012-0456-z>
- Bhasney, S.M., Bhagabati, P., Kumar, A., Katiyar, V., 2019. Morphology and crystalline characteristics of polylactic acid [PLA]/linear low density polyethylene [LLDPE]/microcrystalline cellulose [MCC] fiber composite. *Composites Science and Technology* *171*, 54–61. <https://doi.org/10.1016/j.compscitech.2018.11.028>
- Bi, Z., He, B.B., 2013. Characterization of microalgae for the purpose of biofuel production. *Transactions of the ASABE* *56*, 1529–1539. <https://doi.org/10.13031/trans.56.10090>
- Bitinis, N., Verdejo, R., Bras, J., Fortunati, E., Kenny, J.M., Torre, L., Lopez-Manchado, M.A., 2013. Poly(lactic acid)/natural rubber/cellulose nanocrystal bionanocomposites Part I. Processing and morphology. *Carbohydrate Polymers* *96*, 611–620. <https://doi.org/10.1016/j.carbpol.2013.02.068>
- Borkotoky, S.S., Chakraborty, G., Katiyar, V., 2018. Thermal degradation behaviour and crystallization kinetics of poly (lactic acid) and cellulose nanocrystals (CNC) based

- microcellular composite foams. *International Journal of Biological Macromolecules* 118, 1518–1531. <https://doi.org/10.1016/j.ijbiomac.2018.06.202>
- Borah, S., Bowmick, B.C., Hazarika, C., 2016. Production behaviour of potato in Assam- A critical analysis across zones and size groups of farms. *Econ. Affa.* 61, 23. <https://doi.org/10.5958/0976-4666.2016.00004.8>
- Bosker, T., Bouwman, L.J., Brun, N.R., Behrens, P., Vijver, M.G., 2019. Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant *Lepidium sativum*. *Chemosphere* 226, 774–781. <https://doi.org/10.1016/j.chemosphere.2019.03.163>
- Cadar, O., Paul, M., Roman, C., Miclean, M., Majdik, C., 2012. Biodegradation behaviour of poly(lactic acid) and (lactic acid-ethylene glycol-malonic or succinic acid) copolymers under controlled composting conditions in a laboratory test system. *Polymer Degradation and Stability* 97, 354–357. <https://doi.org/10.1016/j.polymdegradstab.2011.12.006>
- Campos, A.D., Marconato, J.C., Martins-franchetti, S.M., 2011. Biodegradation of Blend Films PVA / PVC , PVA / PCL in Soil and Soil with Landfill Leachate. *Brazilian Archives Biology and Technology* 54, 1367–1378. <https://doi.org/10.1590/S1516-89132011000600024>
- Castro-Aguirre, E., Auras, R., Selke, S., Rubino, M., Marsh, T., 2018. Enhancing the biodegradation rate of poly(lactic acid) films and PLA bio-nanocomposites in simulated composting through bioaugmentation. *Polymer Degradation and Stability* 154, 46–54. <https://doi.org/10.1016/j.polymdegradstab.2018.05.017>
- Castro-Aguirre, E., Auras, R., Selke, S., Rubino, M., Marsh, T., 2017. Insights on the aerobic biodegradation of polymers by analysis of evolved carbon dioxide in simulated composting conditions. *Polymer Degradation and Stability* 137, 251–271. <https://doi.org/10.1016/j.polymdegradstab.2017.01.017>
- Chinaglia, S., Tosin, M., Degli-innocenti, F., 2018. Biodegradation rate of biodegradable plastics at molecular level. *Polymer Degradation and Stability* 147, 237–244. <https://doi.org/10.1016/j.polymdegradstab.2017.12.011>
- Dagnon, K., Pickens, M., Vaidyanathan, V., D’Souza, N., 2014. Validation of an Automated Multiunit Composting System. *J Polym Environ* 22, 9–16. <https://doi.org/10.1007/s10924-013-0596-9>
- De Jong, S.J., Arias, E.R., Rijkers, D.T.S., Van Nostrum, C.F., Kettenes-Van Den Bosch, J.J., Hennink, W.E., 2001. New insights into the hydrolytic degradation of

- poly(lactic acid): Participation of the alcohol terminus. *Polymer* 42, 2795–2802.
[https://doi.org/10.1016/S0032-3861\(00\)00646-7](https://doi.org/10.1016/S0032-3861(00)00646-7)
- Dhar, P., Bhasney, S.M., Kumar, A., Katiyar, V., 2016a. Acid functionalized cellulose nanocrystals and its effect on mechanical, thermal, crystallization and surfaces properties of poly (lactic acid) bionanocomposites films: A comprehensive study. *Polymer* (United Kingdom) 101, 75–92.
<https://doi.org/10.1016/j.polymer.2016.08.028>
- Dhar, P., Kumar, A., Katiyar, V., 2016b. Magnetic Cellulose Nanocrystal Based Anisotropic Polylactic Acid Nanocomposite Films: Influence on Electrical, Magnetic, Thermal, and Mechanical Properties. *ACS Applied Materials and Interfaces* 8, 18393–18409. <https://doi.org/10.1021/acsami.6b02828>
- Dhar, P., Gaur, S.S., Soundararajan, N., Gupta, A., Bhasney, S.M., Milli, M., Kumar, A., Katiyar, V., 2017. Reactive Extrusion of Polylactic Acid/Cellulose Nanocrystal Films for Food Packaging Applications: Influence of Filler Type on Thermomechanical, Rheological, and Barrier Properties. *Industrial and Engineering Chemistry Research* 56, 4718–4735. <https://doi.org/10.1021/acs.iecr.6b04699>
- Dhar, P., Tarafder, D., Kumar, A., Katiyar, V., 2015. Effect of cellulose nanocrystal polymorphs on mechanical, barrier and thermal properties of poly(lactic acid) based bionanocomposites. *RSC Advances* 5, 60426–60440.
<https://doi.org/10.1039/c5ra06840a>
- Dřimal, P., Hoffmann, J., Družbík, M., 2007. Evaluating the aerobic biodegradability of plastics in soil environments through GC and IR analysis of gaseous phase. *Polymer Testing* 26, 729–741. <https://doi.org/10.1016/j.polymertesting.2007.03.008>
- Dubey, P., Chand, N., Mathur, S., Upadhyaya, P., 2016. Photodegradation Effect on LLDPE/LDPE/PLA Blend Films. *European Journal of Advances in Engineering and Technology* 3, 54–59.
- Dulnik, J., Denis, P., Sajkiewicz, P., Kołbuk, D., Choińska, E., 2016. Biodegradation of bicomponent PCL/gelatin and PCL/collagen nanofibers electrospun from alternative solvent system. *Polymer Degradation and Stability* 130, 10–21.
<https://doi.org/10.1016/j.polymdegradstab.2016.05.022>
- Fontanella, S., Bonhomme, S., Koutny, M., Husarova, L., Brusson, J.M., Courdavault, J.P., Pitteri, S., Samuel, G., Pichon, G., Lemaire, J., Delort, A.M., 2010. Comparison of the biodegradability of various polyethylene films containing pro-oxidant additives.

- Polymer Degradation and Stability 95, 1011–1021.
<https://doi.org/10.1016/j.polymdegradstab.2010.03.009>
- Fukushima, K., Abbate, C., Tabuani, D., Gennari, M., Camino, G., 2009. Biodegradation of poly(lactic acid) and its nanocomposites. *Polymer Degradation and Stability* 94, 1646–1655. <https://doi.org/10.1016/j.polymdegradstab.2009.07.001>
- Funabashi, M., Ninomiya, F., Kunioka, M., 2009. Biodegradability evaluation of polymers by ISO 14855-2. *International Journal of Molecular Sciences* 10, 3635–3654. <https://doi.org/10.3390/ijms10083635>
- Furuike, T., Nagahama, H., Chaochai, T., Tamura, H., 2015. Preparation and Characterization of Chitosan-Coated Poly(l-Lactic Acid) Fibers and Their Braided Rope. *Fibers* 3, 380–393. <https://doi.org/10.3390/fib3040380>
- Gao, Y., Jia, L., Hu, B., Alva, A., Fan, M., 2014. Potato Stolon and Tuber Growth Influenced by Nitrogen Form. *Plant Production Science* 17, 138–143. <https://doi.org/10.1626/pps.17.138>
- Garlotta, D., 2002. A Literature Review of Poly (Lactic Acid). *Journal of Polymers and the Environment* 9, 63–84. <https://doi.org/10.1023/A:1020200822435>
- Ge, J., Huang, G., Huang, J., Zeng, J., Han, L., 2016. Particle-Scale Modeling of Methane Emission during Pig Manure/Wheat Straw Aerobic Composting. *Environmental Science and Technology* 50, 4374–4383. <https://doi.org/10.1021/acs.est.5b04141>
- Goheen, S.M., Wool, R.P., 1991. Degradation of polyethylene-starch blends in soil. *Journal of Applied Polymer Science* 42, 2691–2701. <https://doi.org/10.1002/app.1991.070421007>
- Gómez, E.F., Michel, F.C., 2013. Biodegradability of conventional and bio-based plastics and natural fiber composites during composting, anaerobic digestion and long-term soil incubation. *Polymer Degradation and Stability* 98, 2583–2591. <https://doi.org/10.1016/j.polymdegradstab.2013.09.018>
- Gorrasi, G., Pantani, R., 2013. Effect of PLA grades and morphologies on hydrolytic degradation at composting temperature: Assessment of structural modification and kinetic parameters. *Polymer Degradation and Stability* 98, 1006–1014. <https://doi.org/10.1016/j.polymdegradstab.2013.02.005>
- Gu, J.D., 2003. Microbiological deterioration and degradation of synthetic polymeric materials: Recent research advances. *International Biodeterioration and Biodegradation* 52, 69–91. [https://doi.org/10.1016/S0964-8305\(02\)00177-4](https://doi.org/10.1016/S0964-8305(02)00177-4)

- Gupta, A., Pal, A.K., Woo, E.M., Katiyar, V., 2018. Effects of Amphiphilic Chitosan on Stereocomplexation and Properties of Poly(lactic acid) Nano-biocomposite. *Scientific Reports* 8, 1–13. <https://doi.org/10.1038/s41598-018-22281-1>
- Han, X., Pan, J., 2009. A model for simultaneous crystallisation and biodegradation of biodegradable polymers. *Biomaterials* 30, 423–430. <https://doi.org/10.1016/j.biomaterials.2008.10.001>
- Hazarika, J., Ghosh, U., Kalamdhad, A.S., Khwairakpam, M., Singh, J., 2017. Transformation of elemental toxic metals into immobile fractions in paper mill sludge through rotary drum composting. *Ecological Engineering* 101, 185–192. <https://doi.org/10.1016/j.ecoleng.2017.02.005>
- Heimowska, A., Morawska, M., Bocho-Janiszewska, A., 2017. Biodegradation of poly(ϵ -caprolactone) in natural water environments. *Polish J. Chem. Technol.* 19, 120–126. <https://doi.org/10.1515/pjct-2017-0017>
- Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. *Science of The Total Environment* 586, 127–141. <https://doi.org/10.1016/j.scitotenv.2017.01.190>
- Husorova, L., Pekarova, S., Stloukal, P., Kucharczyk, P., Verney, V., Commereuc, S., Ramone, A., Koutny, M., 2014a. Identification of important abiotic and biotic factors in the biodegradation of poly(l-lactic acid). *International Journal of Biological Macromolecules* 71, 155–162. <https://doi.org/10.1016/j.ijbiomac.2014.04.050>
- Husorova, L., Pekarova, S., Stloukal, P., Kucharczyk, P., Verney, V., Commereuc, S., Ramone, A., Koutny, M., 2014b. Identification of important abiotic and biotic factors in the biodegradation of poly(l-lactic acid). *International Journal of Biological Macromolecules* 71, 155–162. <https://doi.org/10.1016/j.ijbiomac.2014.04.050>
- Jandas, P.J., Mohanty, S., Nayak, S.K., 2013. Sustainability, Compostability, and Specific Microbial Activity on Agricultural Mulch Films Prepared from Poly(lactic acid). *Industrial & Engineering Chemistry Research* 52, 17714–17724.
- Jarerat, A., Tokiwa, Y., Tanaka, H., 2006. Production of poly(L-lactide)-degrading enzyme by *Amycolatopsis orientalis* for biological recycling of poly(L-lactide). *Applied*

- Microbiology and Biotechnology 72, 726–731. <https://doi.org/10.1007/s00253-006-0343-4>
- Jayasekara, R., Harding, I., Bowater, I., Lonergan, G., 2005. Biodegradability of a selected range of polymers and polymer blends and standard methods for assessment of biodegradation. *Journal of Polymers and the Environment* 13, 231–251. <https://doi.org/10.1007/s10924-005-4758-2>
- Jayasekara, R., Lonergan, G.T., Harding, I., Bowater, I., Halley, P., Christie, G.B., 2001. An automated multi-unit composting facility for biodegradability evaluations. *Journal of Chemical Technology and Biotechnology* 76, 411–417. <https://doi.org/10.1002/jctb.388>
- Kalamdhad, A.S., Kazmi, A.A., 2009. Rotary drum composting of different organic waste mixtures. *Waste Management & Research* 27, 129–137. <https://doi.org/10.1177/0734242X08091865>
- Kale, G., Auras, R., Singh, S.P., 2007a. Comparison of the degradability of poly(lactide) packages in composting and ambient exposure conditions. *Packaging Technology and Science* 20, 49–70. <https://doi.org/10.1002/pts.742>
- Kale, G., Auras, R., Singh, S.P., Narayan, R., 2007b. Biodegradability of polylactide bottles in real and simulated composting conditions. *Polymer Testing* 26, 1049–1061. <https://doi.org/10.1016/j.polymertesting.2007.07.006>
- Kale, G., Kijchavengkul, T., Auras, R., Rubino, M., Selke, S.E., Singh, S.P., 2007c. Compostability of bioplastic packaging materials: An overview. *Macromolecular Bioscience* 7, 255–277. <https://doi.org/10.1002/mabi.200600168>
- Kalita, N.K., Nagar, M.K., Mudenur, C., Kalamdhad, A., Katiyar, V., 2019. Biodegradation of modified Poly(lactic acid) based biocomposite films under thermophilic composting conditions. *Polymer Testing*. <https://doi.org/10.1016/j.polymertesting.2019.02.021>
- Kalita, N.K., Bhasney, S.M., Mudenur, C., Kalamdhad, A., Katiyar, V., 2020. End-of-life evaluation and biodegradation of Poly(lactic acid) (PLA)/Polycaprolactone (PCL)/Microcrystalline cellulose (MCC) polyblends under composting conditions. *Chemosphere* 247, 125875. <https://doi.org/10.1016/j.chemosphere.2020.125875>
- Kapanen, A., Venelampi, O., Ita, M., 2002. Biodegradation of Lactic Acid Based Polymers under Controlled Composting Conditions and Evaluation of the Ecotoxicological Impact 445–455.

- Karamanlioglu, M., Preziosi, R., Robson, G.D., 2017. Abiotic and biotic environmental degradation of the bioplastic polymer poly(lactic acid): A review. *Polymer Degradation and Stability* 137, 122–130. <https://doi.org/10.1016/j.polymdegradstab.2017.01.009>
- Katiyar, V., Nanavati, H., 2011. Solid state polymerization of poly(L -lactide): Multiple-fold increase in molecular weight via an efficient catalyst system. *Polym Eng Sci* 51, 2078–2084. <https://doi.org/10.1002/pen.22133>
- Kijchavengkul, T., Auras, R., Rubino, M., Ngouajio, M., Fernandez, R.T., 2008. Assessment of aliphatic-aromatic copolyester biodegradable mulch films. Part II: Laboratory simulated conditions. *Chemosphere* 71, 1607–1616. <https://doi.org/10.1016/j.chemosphere.2008.01.037>
- Kijchavengkul, T., Auras, R., Rubino, M., Ngouajio, M., Thomas Fernandez, R., 2006. Development of an automatic laboratory-scale respirometric system to measure polymer biodegradability. *Polymer Testing* 25, 1006–1016. <https://doi.org/10.1016/j.polymertesting.2006.06.008>
- Kijchavengkul, T., Kale, G., Auras, R., 2009. Degradation of biodegradable polymers in real and simulated composting conditions. *ACS Symposium Series* 1004, 31–40. <https://doi.org/10.1021/bk-2009-1004.ch003>
- Kim, H.S., Kim, H.J., Lee, J.W., Choi, I.G., 2006. Biodegradability of bio-flour filled biodegradable poly(butylene succinate) bio-composites in natural and compost soil. *Polymer Degradation and Stability* 91, 1117–1127. <https://doi.org/10.1016/j.polymdegradstab.2005.07.002>
- Kim, M.S., Seo, K.S., Khang, G., Lee, H.B., 2005. Ring-Opening Polymerization of ϵ -Caprolactone by Poly(ethylene glycol) by an Activated Monomer Mechanism. *Macromol. Rapid Commun.* 26, 643–648. <https://doi.org/10.1002/marc.200400650>
- Kitadokoro, K., Thumarat, U., Nakamura, R., Nishimura, K., Karatani, H., Suzuki, H., Kawai, F., 2012. Crystal structure of cutinase Est119 from *Thermobifida alba* AHK119 that can degrade modified polyethylene terephthalate at 1.76 Å resolution. *Polymer Degradation and Stability* 97, 771–775. <https://doi.org/10.1016/j.polymdegradstab.2012.02.003>
- Komilis, D.P., 2006. A kinetic analysis of solid waste composting at optimal conditions. *Waste Management* 26, 82–91. <https://doi.org/10.1016/j.wasman.2004.12.021>

- Komilis, D.P., Ham, R.K., 2003. The effect of lignin and sugars to the aerobic decomposition of solid wastes. *Waste Management* 23, 419–423. [https://doi.org/10.1016/S0956-053X\(03\)00062-X](https://doi.org/10.1016/S0956-053X(03)00062-X)
- Kontou, E., Anthoulis, G., 2007. The effect of silica nanoparticles on the thermomechanical properties of polystyrene. *Journal of Applied Polymer Science* 105, 1723–1731. <https://doi.org/10.1002/app.26409>
- Kucharczyk, P., Pavelkova, A., Stloukal, P., Sedlarek, V., 2016. Degradation behaviour of PLA-based polyesterurethanes under abiotic and biotic environments. *Polymer Degradation and Stability* 129, 222–230. <https://doi.org/10.1016/j.polymdegradstab.2016.04.019>
- Labet, M., Thielemans, W., 2009. Synthesis of polycaprolactone: a review. *Chem. Soc. Rev.* 38, 3484. <https://doi.org/10.1039/b820162p>
- Le Marec, P.E., Ferry, L., Quantin, J.C., Bénézet, J.C., Bonfils, F., Guilbert, S., Bergeret, A., 2014. Influence of melt processing conditions on poly(lactic acid) degradation: Molar mass distribution and crystallization. *Polymer Degradation and Stability* 110, 353–363. <https://doi.org/10.1016/j.polymdegradstab.2014.10.003>
- Lee, S., 2002. Microstructure, tensile properties, and biodegradability of aliphatic polyester/clay nanocomposites. *Polymer* 43, 2495–2500. [https://doi.org/10.1016/S0032-3861\(02\)00012-5](https://doi.org/10.1016/S0032-3861(02)00012-5)
- Leejarkpai, T., Suwanmanee, U., Rudeekit, Y., Mungcharoen, T., 2011. Biodegradable kinetics of plastics under controlled composting conditions. *Waste Management* 31, 1153–1161. <https://doi.org/10.1016/j.wasman.2010.12.011>
- Leja, K., Lewandowicz, G., 2010. Polymer biodegradation and biodegradable polymers - A review. *Polish Journal of Environmental Studies* 19, 255–266.
- Li, M., Witt, T., Xie, F., Warren, F.J., Halley, P.J., Gilbert, R.G., 2015. Biodegradation of starch films: The roles of molecular and crystalline structure. *Carbohydrate Polymers* 122, 115–122. <https://doi.org/10.1016/j.carbpol.2015.01.011>
- Lim, H.A., Raku, T., Tokiwa, Y., 2005. Hydrolysis of polyesters by serine proteases. *Biotechnology Letters* 27, 459–464. <https://doi.org/10.1007/s10529-005-2217-8>
- Lipsa, R., Tudorachi, N., Darie-Nita, R.N., Oprică, L., Vasile, C., Chiriac, A., 2016. Biodegradation of poly(lactic acid) and some of its based systems with *Trichoderma viride*. *International Journal of Biological Macromolecules* 88, 515–526. <https://doi.org/10.1016/j.ijbiomac.2016.04.017>

- López-Rodríguez, N., López-Arraiza, A., Meaurio, E., Sarasua, J.R., 2006. Crystallization, morphology, and mechanical behavior of polylactide/poly(ϵ -caprolactone) blends. *Polym. Eng. Sci.* 46, 1299–1308. <https://doi.org/10.1002/pen.20609>
- Lucas, N., Bienaime, C., Belloy, C., Queneudec, M., Silvestre, F., Nava-Saucedo, J.E., 2008. Polymer biodegradation: Mechanisms and estimation techniques - A review. *Chemosphere* 73, 429–442. <https://doi.org/10.1016/j.chemosphere.2008.06.064>
- Luo, Y.B., Wang, X.L., Wang, Y.Z., 2012. Effect of TiO₂ nanoparticles on the long-term hydrolytic degradation behavior of PLA. *Polymer Degradation and Stability* 97, 721–728. <https://doi.org/10.1016/j.polymdegradstab.2012.02.011>
- Matson, J.E., Huguenard, B.R., 2017. Evaluating Aptness of a Regression Model 1898. <https://doi.org/10.1080/10691898.2007.11889469>
- Miao, C., Hamad, W.Y., 2016. In-situ polymerized cellulose nanocrystals (CNC)—poly(L-lactide) (PLLA) nanomaterials and applications in nanocomposite processing. *Carbohydrate Polymers* 153, 549–558. <https://doi.org/10.1016/j.carbpol.2016.08.012>
- Murphy, C.A., Collins, M.N., 2018. Microcrystalline cellulose reinforced polylactic acid biocomposite filaments for 3D printing. *Polymer Composites* 39, 1311–1320. <https://doi.org/10.1002/pc.24069>
- Musioł, M., Sikorska, W., Adamus, G., Janeczek, H., Richert, J., Malinowski, R., Jiang, G., Kowalczyk, M., 2016. Forensic engineering of advanced polymeric materials. Part III - Biodegradation of thermoformed rigid PLA packaging under industrial composting conditions. *Waste Management* 52, 69–76. <https://doi.org/10.1016/j.wasman.2016.04.016>
- Narancic, T., Verstichel, S., Reddy Chaganti, S., Morales-Gamez, L., Kenny, S.T., De Wilde, B., Babu Padamati, R., O'Connor, K.E., 2018. Biodegradable Plastic Blends Create New Possibilities for End-of-Life Management of Plastics but They Are Not a Panacea for Plastic Pollution. *Environmental Science and Technology* 52, 10441–10452. <https://doi.org/10.1021/acs.est.8b02963>
- Ng, E.-L., Huerta Lwanga, E., Eldridge, S.M., Johnston, P., Hu, H.-W., Geissen, V., Chen, D., 2018. An overview of microplastic and nanoplastic pollution in agroecosystems. *Science of The Total Environment* 627, 1377–1388. <https://doi.org/10.1016/j.scitotenv.2018.01.341>
- Nieddu, E., Mazzucco, L., Gentile, P., Benko, T., Balbo, V., Mandrile, R., Ciardelli, G., 2009. Preparation and biodegradation of clay composites of PLA. *Reactive and*

<https://doi.org/10.1016/j.reactfunctpolym.2009.03.002>

- Nizzetto, L., Futter, M., Langaas, S., 2016. Are Agricultural Soils Dumps for Microplastics of Urban Origin? *Environ. Sci. Technol.* 50, 10777–10779. <https://doi.org/10.1021/acs.est.6b04140>
- Oda, Y., Yonetsu, A., Urakami, T., Tonomura, K., 2000. Degradation of polylactide by commercial proteases. *Journal of Polymers and the Environment* 8, 29–32. <https://doi.org/10.1023/A:1010120128048>
- Owen, K.L., 2013. Control of microstructure in poly-lactic acid and the effect on biodegradation. *School of Metallurgy and Materials m_rs*.
- Pal, A.K., Katiyar, V., 2016a. Nanoamphiphilic Chitosan Dispersed Poly (lactic acid) Bionanocomposite Films with Improved Thermal , Mechanical , and Gas Barrier Properties. <https://doi.org/10.1021/acs.biomac.6b00619>
- Pal, A.K., Katiyar, V., 2016c. Thermal degradation behaviour of nanoamphiphilic chitosan dispersed poly (lactic acid) bionanocomposite films. *International Journal of Biological Macromolecules*. <https://doi.org/10.1016/j.ijbiomac.2016.11.024>
- Pan, J., 2014. Introduction to computer modelling for the design of biodegradable medical devices, in: *Modelling Degradation of Bioresorbable Polymeric Medical Devices*. pp. 3–13. <https://doi.org/10.1533/9781782420255.1.3>
- Pantani, R., Sorrentino, A., 2013. Influence of crystallinity on the biodegradation rate of injection-moulded poly(lactic acid) samples in controlled composting conditions. *Polymer Degradation and Stability* 98, 1089–1096. <https://doi.org/10.1016/j.polymdegradstab.2013.01.005>
- Paul, M.A., Delcourt, C., Alexandre, M., Degée, P., Monteverde, F., Dubois, P., 2005. Polylactide/montmorillonite nanocomposites: Study of the hydrolytic degradation. *Polymer Degradation and Stability* 87, 535–542. <https://doi.org/10.1016/j.polymdegradstab.2004.10.011>
- Pekařová, S., Chodák, I., Omaníková, L., Johec-Mošková, D., Kucharczyk, P., Sedničková, M., Bočkaj, J., Alexy, P., Perd'ochová, D., Kleinová, A., Koutný, M., Janigová, I., Sedlařík, V., 2018a. Changes of physical properties of PLA-based blends during early stage of biodegradation in compost. *International Journal of Biological Macromolecules*. <https://doi.org/10.1016/j.ijbiomac.2018.02.078>
- Petinakis, E., Liu, X., Yu, L., Way, C., Sangwan, P., Dean, K., Bateman, S., Edward, G., 2010a. Biodegradation and thermal decomposition of poly(lactic acid)-based

- materials reinforced by hydrophilic fillers. *Polymer Degradation and Stability* 95, 1704–1707. <https://doi.org/10.1016/j.polymdegradstab.2010.05.027>
- Picard, E., Espuche, E., Fulchiron, R., 2011. Effect of an organo-modified montmorillonite on PLA crystallization and gas barrier properties. *Applied Clay Science* 53, 58–65. <https://doi.org/10.1016/j.clay.2011.04.023>
- Pradhan, R., Misra, M., Erickson, L., Mohanty, A., 2010a. Compostability and biodegradation study of PLA-wheat straw and PLA-soy straw based green composites in simulated composting bioreactor. *Bioresource Technology* 101, 8489–8491. <https://doi.org/10.1016/j.biortech.2010.06.053>
- Pradhan, R., Reddy, M., Diebel, W., Erickson, L., Misra, M., Mohanty, A., 2010b. Comparative compostability and biodegradation studies of various components of green composites and their blends in simulated aerobic composting bioreactor. *International Journal of Plastics Technology* 14. <https://doi.org/10.1007/s12588-010-0009-z>
- Pranamuda, H., Tokiwa, Y., Tanaka, H., 1997. Polylactide Degradation by an *Amycolatopsis* sp. *Applied and environmental microbiology* 63, 1637–1640. <https://doi.org/10.1128/AEM.63.4.1637-1640.1997>
- Qi, X., Ren, Y., Wang, X., 2017. New advances in the biodegradation of Poly(lactic) acid. *International Biodeterioration and Biodegradation* 117, 215–223. <https://doi.org/10.1016/j.ibiod.2017.01.010>
- Ramani, N., Charles, P., 1999. Help define and grow a new biodegradable plastic industry. *ASTM standardization news* 36–42.
- Ramos, M., Fortunati, E., Peltzer, M., Jimenez, A., Kenny, J.M., Garrigós, M.C., 2016. Characterization and disintegrability under composting conditions of PLA-based nanocomposite films with thymol and silver nanoparticles. *Polymer Degradation and Stability* 132, 2–10. <https://doi.org/10.1016/j.polymdegradstab.2016.05.015>
- Rudeekit, Y., Numnoi, J., Tajan, M., Chaiwutthinan, P., Leejarkpai, T., 2008a. Determining Biodegradability of Polylactic Acid under Different Environments. *Journal of Metals, Materials and Minerals* 18, 83–87.
- Sedlarik, V., Saha, N., Sedlarikova, J., Saha, P., 2008. Biodegradation of blown films based on poly(lactic acid) under natural conditions. *Macromolecular Symposia* 272, 100–103. <https://doi.org/10.1002/masy.200851214>

- Selke, S., Auras, R., Nguyen, T.A., Castro Aguirre, E., Cheruvathur, R., Liu, Y., 2015. Evaluation of biodegradation-promoting additives for plastics. *Environmental Science and Technology* 49, 3769–3777. <https://doi.org/10.1021/es504258u>
- Sevim, K., Pan, J., 2018. A model for hydrolytic degradation and erosion of biodegradable polymers. *Acta Biomaterialia* 66, 192–199. <https://doi.org/10.1016/j.actbio.2017.11.023>
- Shah, A.A., Hasan, F., Hameed, A., Ahmed, S., 2008. Biological degradation of plastics: A comprehensive review. *Biotechnology Advances* 26, 246–265. <https://doi.org/10.1016/j.biotechadv.2007.12.005>
- Shi, B., Topolkarayev, V., Wang, J., Corporation, K., 2011. *Biopolymers, Processing, and Biodegradation*.
- Sikorska, W., Musiol, M., Nowak, B., Pajak, J., Labuzek, S., Kowalczyk, M., Adamus, G., 2015a. Degradability of polylactide and its blend with poly[(R,S)-3-hydroxybutyrate] in industrial composting and compost extract. *International Biodeterioration and Biodegradation* 101, 32–41. <https://doi.org/10.1016/j.ibiod.2015.03.021>
- Singh, J., Kalamdhad, A.S., 2012. Concentration and speciation of heavy metals during water hyacinth composting. *Bioresource Technology* 124, 169–179. <https://doi.org/10.1016/j.biortech.2012.08.043>
- Singh, R.P., Agrawal, M., 2008. Potential benefits and risks of land application of sewage sludge. *Waste Management* 28, 347–358. <https://doi.org/10.1016/j.wasman.2006.12.010>
- Singh, S.K., Matta, A.K., Rao, R.U., Suman, K.N.S., Rambabu, V., 2014. 3rd International Conference on Materials Processing and Characterisation (ICMPC 2014) Preparation and Characterization of Biodegradable PLA/PCL Polymeric Blends. *Procedia Materials Science* 6, 1266–1270. <https://doi.org/10.1016/j.mspro.2014.07.201>
- Siparsky, G.L., Voorhees, K.J., Miao, F., 1998. Hydrolysis of Polylactic Acid (PLA) and Polycaprolactone (PCL) in Aqueous Acetonitrile Solutions: Autocatalysis. *Journal of environmental polymer degradation* 6, 31–41. <https://doi.org/10.1023/A:1022826528673>
- Siracusa, V., Rocculi, P., Romani, S., Rosa, M.D., 2008. Biodegradable polymers for food packaging: a review. *Trends in Food Science and Technology* 19, 634–643. <https://doi.org/10.1016/j.tifs.2008.07.003>

- Song, J.H., Murphy, R.J., Narayan, R., Davies, G.B.H., 2009. Biodegradable and compostable alternatives to conventional plastics. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364, 2127–2139. <https://doi.org/10.1098/rstb.2008.0289>
- Spaccini, R., Todisco, D., Drosos, M., Nebbioso, A., Piccolo, A., 2016. Decomposition of bio-degradable plastic polymer in a real on-farm composting process. *Chemical and Biological Technologies in Agriculture* 3, 4. <https://doi.org/10.1186/s40538-016-0053-9>
- Stloukal, P., Kalendova, A., Mattausch, H., Laske, S., Holzer, C., Koutny, M., 2015a. The influence of a hydrolysis-inhibiting additive on the degradation and biodegradation of PLA and its nanocomposites. *Polymer Testing* 41, 124–132. <https://doi.org/10.1016/j.polymertesting.2014.10.015>
- Subramanian, N.K., White, P.J., Broadley, M.R., Ramsay, G., 2011. The three-dimensional distribution of minerals in potato tubers. *Annals of Botany* 107, 681–691. <https://doi.org/10.1093/aob/mcr009>
- Sudhakar, K., Premalatha, M., 2015. Characterization of micro algal biomass through FTIR/TGA /CHN analysis: Application to *scenedesmus* sp. *Energy Sources, Part A: Recovery, Utilization and Environmental Effects* 37, 2330–2337. <https://doi.org/10.1080/15567036.2013.825661>
- Sukkhum, S., Tokuyama, S., Kitpreechavanich, V., 2009. Development of fermentation process for PLA-degrading enzyme production by a new thermophilic *Actinomadura* sp. T16-1. *Biotechnology and Bioprocess Engineering* 14, 302–306. <https://doi.org/10.1007/s12257-008-0207-0>
- Suyama, T., Tokiwa, Y., 1998. Phylogenetic Affiliation of Soil Bacteria That Degrade Aliphatic Polyesters Available Commercially as Biodegradable Plastics *Phylogenetic Affiliation of Soil Bacteria That Degrade Aliphatic Polyesters Available Commercially as Biodegradable Plastics* 64, 1–5.
- Tayal, A., Khan, S.A., 2000. Degradation of a water-soluble polymer: molecular weight changes and chain scission characteristics. *Macromolecules* 33, 9488–9493. <https://doi.org/10.1021/ma000736g>
- Tesfaye, M., Patwa, R., Gupta, A., Kashyap, M.J., Katiyar, V., 2017. Recycling of poly (lactic acid)/silk based bionanocomposites films and its influence on thermal stability, crystallization kinetics, solution and melt rheology. *International Journal*

- of Biological Macromolecules 101, 580–594.
<https://doi.org/10.1016/j.ijbiomac.2017.03.085>
- Thoorens, G., Krier, F., Leclercq, B., Carlin, B., Evrard, B., 2014. Microcrystalline cellulose, a direct compression binder in a quality by design environment—A review. *International Journal of Pharmaceutics* 473, 64–72.
<https://doi.org/10.1016/j.ijpharm.2014.06.055>
- Tibbetts, S.M., Whitney, C.G., MacPherson, M.J., Bhatti, S., Banskota, A.H., Stefanova, R., McGinn, P.J., 2015. Biochemical characterization of microalgal biomass from freshwater species isolated in Alberta, Canada for animal feed applications. *Algal Research* 11, 435–447. <https://doi.org/10.1016/j.algal.2014.11.011>
- Torres, A., Li, S.M., Roussos, S., Vert, M., 1996. Screening of microorganisms for biodegradation of poly (lactic-acid) and lactic These include : Screening of Microorganisms for Biodegradation of Poly (Lactic Acid) and Lactic Acid-Containing Polymers 62, 2393–2397. <https://doi.org/papers://590F92D9-0B76-4B88-8729-9AF064BE5AC8/Paper/p5836>
- Torres, A., Li, S.M., Roussos, S., Vert, M., 1999. Microbial Degradation of a Poly(lactic acid) as a Model of Synthetic Polymer Degradation Mechanisms in Outdoor Conditions 218–226. <https://doi.org/10.1021/bk-1999-0723.ch014>
- Torres, S., Navia, R., Campbell Murdy, R., Cooke, P., Misra, M., Mohanty, A.K., 2015a. Green composites from residual microalgae biomass and poly(butylene adipate-co-terephthalate): Processing and plasticization. *ACS Sustainable Chemistry and Engineering* 3, 614–624. <https://doi.org/10.1021/sc500753h>
- Trache, D., Hussin, M.H., Hui Chuin, C.T., Sabar, S., Fazita, M.R.N., Taiwo, O.F.A., Hassan, T.M., Haafiz, M.K.M., 2016. Microcrystalline cellulose: Isolation, characterization and bio-composites application—A review. *International Journal of Biological Macromolecules* 93, 789–804.
<https://doi.org/10.1016/j.ijbiomac.2016.09.056>
- Tripathi, N., Katiyar, V., 2017. Poly (Lactic Acid)/ Modified Gum Arabic Based Bionanocomposite Films : Thermal Degradation Kinetics.
<https://doi.org/10.1002/pen>
- Tripathi, N., Katiyar, V., 2016. PLA / functionalized-gum arabic based bionanocomposite films for high gas barrier applications 43458, 1–8.
<https://doi.org/10.1002/app.43458>

- Trongsatitkul, T., Chaiwong, S., 2017. In situ fibre-reinforced composite films of poly(lactic acid)/low-density polyethylene blends: effects of composition on morphology, transport and mechanical properties. *Polymer International* 66, 1456–1462. <https://doi.org/10.1002/pi.5449>
- Tsuji, H., Echizen, Y., Nishimura, Y., 2006. Enzymatic degradation of poly(L-lactic acid): Effects of UV irradiation. *Journal of Polymers and the Environment* 14, 239–248. <https://doi.org/10.1007/s10924-006-0023-6>
- Tsuji, H., Ikada, Y., 1999. Stereocomplex formation between enantiomeric poly(lactic acid)s. XI. Mechanical properties and morphology of solution-cast films. *Polymer* 40, 6699–6708. [https://doi.org/10.1016/S0032-3861\(99\)00004-X](https://doi.org/10.1016/S0032-3861(99)00004-X)
- Tuominen, J., Kylmä, J., Kapanen, A., Venelampi, O., Itävaara, M., Seppälä, J., 2002. Biodegradation of lactic acid based polymers under controlled composting conditions and evaluation of the ecotoxicological impact. *Biomacromolecules* 3, 445–455. <https://doi.org/10.1021/bm0101522>
- Valapa, R. babu, Pugazhenth, G., Katiyar, V., 2016. Hydrolytic degradation behaviour of sucrose palmitate reinforced poly(lactic acid) nanocomposites. *International Journal of Biological Macromolecules* 89, 70–80. <https://doi.org/10.1016/j.ijbiomac.2016.04.040>
- Valapa, R.B., Pugazhenth, G., Katiyar, V., 2015. RSC Advances PAPER Effect of graphene content on the properties of poly (lactic acid) nanocomposites. *RSC Advances* 5, 28410–28423. <https://doi.org/10.1039/C4RA15669B>
- VanderGheynst, J.S., Pettygrove, S., Dooley, T.M., Arnold, K.A., 2004. Estimating Electrical Conductivity of Compost Extracts At Different Extraction Ratios. *Compost Science & Utilization* 12, 202–207. <https://doi.org/10.1080/1065657X.2004.10702184>
- Varma, V.S., Kalamdhad, A.S., 2014. Stability and microbial community analysis during rotary drum composting of vegetable waste. <https://doi.org/10.1007/s40093-014-0052-4>
- Vieira, A.C., Guedes, R.M., Tita, V., 2014. Constitutive modeling of biodegradable polymers: Hydrolytic degradation and time-dependent behavior. *International Journal of Solids and Structures* 51, 1164–1174. <https://doi.org/10.1016/j.ijsolstr.2013.12.010>
- Vieira, A.C., Vieira, J.C., Ferra, J.M., Magalhães, F.D., Guedes, R.M., Marques, A.T., 2011. Mechanical study of PLA-PCL fibers during in vitro degradation. *Journal of*

- the Mechanical Behavior of Biomedical Materials 4, 451–460.
<https://doi.org/10.1016/j.jmbbm.2010.12.006>
- Vieira, A.C., Vieira, J.C., Guedes, R.M., Marques, A.T., 2010. Experimental Degradation Characterization of PLA-PCL, PGA-PCL, PDO AND PGA fibers. Materials Science Forum 636, 825–832.
- Walczak, M., Swiontek Brzezinska, M., Sionkowska, A., Michalska, M., Jankiewicz, U., Deja-Sikora, E., 2015a. Biofilm formation on the surface of polylactide during its biodegradation in different environments. Colloids and Surfaces B: Biointerfaces 136, 340–345. <https://doi.org/10.1016/j.colsurfb.2015.09.036>
- Wang, Y., Pan, J., Han, X., Sinka, C., Ding, L., 2008. A phenomenological model for the degradation of biodegradable polymers. Biomaterials 29, 3393–3401.
<https://doi.org/10.1016/j.biomaterials.2008.04.042>
- Way, C., Wu, D.Y., Dean, K., Palombo, E., 2010. Design considerations for high-temperature respirometric biodegradation of polymers in compost. Polymer Testing 29, 147–157. <https://doi.org/10.1016/j.polymertesting.2009.10.004>
- Webb, H.K., Arnott, J., Crawford, R.J., Ivanova, E.P., 2013. Plastic degradation and its environmental implications with special reference to poly(ethylene terephthalate). Polymers 5, 1–18. <https://doi.org/10.3390/polym5010001>
- Weng, Y.X., Jin, Y.J., Meng, Q.Y., Wang, L., Zhang, M., Wang, Y.Z., 2013a. Biodegradation behavior of poly(butylene adipate-co-terephthalate) (PBAT), poly(lactic acid) (PLA), and their blend under soil conditions. Polymer Testing 32, 918–926. <https://doi.org/10.1016/j.polymertesting.2013.05.001>
- Weng, Y.X., Wang, L., Zhang, M., Wang, X.L., Wang, Y.Z., 2013b. Biodegradation behavior of P(3HB,4HB)/PLA blends in real soil environments. Polymer Testing 32, 60–70. <https://doi.org/10.1016/j.polymertesting.2012.09.014>
- Weng, Y.X., Wang, Y., Wang, X.L., Wang, Y.Z., 2010. Biodegradation behavior of PHBV films in a pilot-scale composting condition. Polymer Testing 29, 579–587.
<https://doi.org/10.1016/j.polymertesting.2010.04.002>
- Williams, D.F., 1981. Enzymic Hydrolysis of Polylactic Acid. Engineering in Medicine 10, 5–7. https://doi.org/10.1243/EMED_JOUR_1981_010_004_02
- Woodard, L.N., Grunlan, M.A., 2019. Hydrolytic Degradation of PCL-PLLA Semi-IPNs Exhibiting Rapid, Tunable Degradation. ACS Biomaterials Science and Engineering 5, 498–508. <https://doi.org/10.1021/acsbiomaterials.8b01135>

- Xu, H., Yang, X., Xie, L., Hakkarainen, M., 2016. Conformational Footprint in Hydrolysis-Induced Nanofibrillation and Crystallization of Poly(lactic acid). *Biomacromolecules* 17, 985–995. <https://doi.org/10.1021/acs.biomac.5b01636>
- Yagi, H., Ninomiya, F., Funabashi, M., Kunioka, M., 2014. Mesophilic anaerobic biodegradation test and analysis of eubacteria and archaea involved in anaerobic biodegradation of four specified biodegradable polyesters. *Polymer Degradation and Stability* 110, 278–283. <https://doi.org/10.1016/j.polymdegradstab.2014.08.031>
- Yoshida, S., Hiraga, K., Takanaha, T., Taniguchi, I., Yamaji, H., Maeda, Y., Toyohara, K., Miyamoto, K., Kimura, Y., Oda, K., 2016. A bacterium that degrades and assimilates poly(ethyleneterephthalate). *Science* 351, 1196–1199. <https://doi.org/10.1126/science.aad6359>
- Zenkiewicz, M., Malinowski, R., Rytlewski, P., Richert, A., Sikorska, W., Krasowska, K., 2012. Some composting and biodegradation effects of physically or chemically crosslinked poly(lactic acid). *Polymer Testing* 31, 83–92. <https://doi.org/10.1016/j.polymertesting.2011.09.012>
- Zhang, Q., Li, D., Zhang, H., Su, G., Li, G., 2018. Preparation and properties of poly(lactic acid)/sesbania gum/nano-TiO₂composites. *Polymer Bulletin* 75, 623–635. <https://doi.org/10.1007/s00289-017-2059-2>
- Zhang, X., Hua, H., Shen, X., Yang, Q., 2007. In vitro degradation and biocompatibility of poly(l-lactic acid)/chitosan fiber composites. *Polymer* 48, 1005–1011. <https://doi.org/10.1016/j.polymer.2006.12.028>
- Zumstein, M.T., Narayan, R., Kohler, H.E., McNeill, K., Sander, M., 2019a. Dos and Do Nots When Assessing the Biodegradation of Plastics. *Environmental Science & Technology* 53, 9967–9969. <https://doi.org/10.1021/acs.est.9b04513>

