Degradability Studies of Linear Low Density Polyethylene and Its Blends with Polylactide

Thesis submitted in partial fulfillment of the requirement for the award of degree of

Master of Technology
in
Environmental Science & Technology

By:
Navleen Kaur
(Roll No. 600801004)

Under guidance

Dr. Pramod K. Bajpai
Distinguished Professor
Department of Chemical Engineering

Dr. Haripada Bhunia
Assistant Professor
Department of Chemical Engineering

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

June 2010

CERTIFICATE

This is certified that the thesis entitled ***"Degradability Studies of Linear Low Density Polyethylene and Its Blends with Polylactide",*** *is an authentic record of my own work carried out as requirements for the award of degree of M.Tech (Environmental Science & Technology) at Thapar University, Patiala, under the guidance of Dr. Pramod K. Bajpai (Distinguished Professor, ChED) and Dr. Haripada Bhunia (Assistant Professor, ChED) during January to June 2010.*

Navleen

Date:- 11th June, 2010

Navleen Kaur
(Roll no. 600801004)

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

Dr. Pramod K. Bajpai
Distinguished Professor
Department of Chemical Engineering
Thapar University, Patiala

11/6/10

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

Countersigned by:

11/06/2010

Dr. N. Das
Head and Associate Professor
Department of Biotech & Env Sciences
Thapar University, Patiala

23/7

Dr. R.K. Sharma
Dean, Academic Affairs
Thapar University, Patiala

ACKNOWLEDGMENTS

The real spirit of achieving a goal is through then way of excellence and austere discipline. I would have never succeeded in completing my task without the cooperation, encouragement and help provided to me by various personalities

With deep sense of gratitude I express my sincere thanks to my esteemed supervisor, Dr. Pramod K. Bajpai, Department of Chemical Engineering, Thapar University, Patiala, for his valuable guidance for his able guidance, valuable advice and helpful suggestions at times of difficulties which I faced pursuing this thesis work. He guided me and given me full time to understand the minute details and all the basic concepts necessary for the successful completion of thesis. His feedback and editorial comments were also invaluable for the writing of this thesis.

I express my sincerest regards and gratitude to my supervisor Dr. Haripada Bhunia, Assistant Professor, Thapar University, Patiala, for his valuable guidance in carrying out this work under his effective supervision, encouragement, enlightenment and cooperation. His enthusiasm and optimism made this experience both rewarding and enjoyable.

I feel privileged to offer my sincere thanks and owe an enormous deal of gratitude to Dr. N. Das, Head of Biotechnology & Environmental Science and Technology for helping me to move ahead with this work at every stage and without his invaluable support it would have been impossible to accomplish anything.

I shall be failing in my duties if I do not express my deep sense of gratitude towards Dr. Anita Rajor, Assistant Professor, Department of Biotechnology and Environmental Sciences, Thapar University ,Patiala and Gursewek Singh (Ph.D scholar) who have been a constant source of inspiration for me throughout this work.

I am also thankful to all the staff members of the department for their full cooperation and help.

My greatest thanks to all those who wished me success especially my parents and friends. Above all I render my gratitude to the ALMIGHTY who bestowed self-confidence, ability and strength in me to complete this work.

Place: Thapar University, Patiala *Navleen Kaur*

ABSTRACT

Important information concerning polymer's final fate in the environment can be achieved in biodegradation studies performed under certain environmental conditions. In this context, the focus of the present work was to evaluate the degradability level of blends containing aliphatic polyesters using standard methods.

In this Thesis, the degradability of linear low density polyethylene (LLDPE) and poly(l-lactic acid) (PLLA) blend films under controlled composting conditions was investigated according to modified ASTM D 5338 (2003). PLLA can have a major impact on the biodegradation on LLDPE films. LLDPE 80 (80 wt % LLDPE & 20 wt % PLLA) degraded faster than M-g-L 80/4 (80 wt % LLDPE, 20 wt % PLLA and 4 parts compatibilizer per hundred parts of resin) and LLDPE 100. Weight changes, tensile strength, DSC parameters of melting and changes in morphology of polymer surface (SEM) were tested after composting. The possible reason of observed changes was also discussed. Tensile strength of LLDPE 100, LLDPE 80 and M-g-L 80/4 decreased by 20 %, 54 % and 35 % respectively. Surface morphological changes of composting blend films were observed by scanning electron microscopy. Six kinds of microbes were isolated from the compost. These microbes were observed by gram staining technique under the microscope. In the case of pure culture (polyethylene degrading bacteria), plastic films of LLDPE100 were found to be more biodegradable than its blends.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ASTM	American Society for Testing and Materials
ATR-FTIR	Attenuated Total Reflectance- Fourier Transform Infrared spectroscopy
CSF	Control Sample Film
DSC	Differential Scanning Calorimetry
FTIR	Fourier Transform Infrared spectroscopy
ISO	International Standard Organization
LLDPE	Linear Low Density Polyethylene
MW	Molecular Weight
NA	Nutrient Agar
NB	Nutrient Broth
OECD	Organization for Economic Cooperation & Development
PLLA	Poly(l-lactic acid)
SEM	Scanning Electron Microscopy
TOC	Total Organic Carbon
$\mathbf{T_g}$	Glass Transition Temperature
$\mathbf{T_m}$	Melting Temperature
XRD	X-Ray Diffraction

Chapter-1

Introduction

Plastics are man-made long chain polymeric molecules (Scott, 1999). More than half a century ago synthetic polymers started to substitute natural materials in almost every area and nowadays plastics have become an indispensable part of our life (Shah *et al*., 2008). Plastics have been used worldwide because of their lightweight, inertness and low cost. Over 200 million tons of plastic are manufactured annually around the world, according to the Society of Plastics Engineers. Of those 200 million tons, 26 million are manufactured in the United States. The EPA reported in 2003 that only 5.8% of those 26 million tons of plastic waste are recycled, although this is increasing rapidly.

Much of the reason for disappointing plastics recycling goals is that conventional plastics are often mixed with organic wastes (food scraps, wet paper, and liquids), making it difficult and impractical to recycle the underlying polymer without expensive cleaning and sanitizing procedures. On the other hand, composting of these mixed organics (food scraps, yard trimmings, and wet, non-recyclable paper) is a potential strategy for recovering large quantities of waste and dramatically increases community recycling goals. Food scraps and wet, non-recyclable paper comprises 50 million tons of municipal solid waste. Biodegradable plastics can replace the non-degradable plastics in these waste streams, making municipal composting a significant tool to divert large amounts of otherwise nonrecoverable waste from landfills.

If even a small amount of conventional plastics were to be commingling with organic materials, the entire batch of organic waste is "contaminated" with small bits of plastic that spoil prime-quality compost humus. Composters, therefore, will not accept mixed organic waste streams unless they are completely devoid of nondegradable plastics. So, because of a relatively small quantity of nondegradable plastics, a significant waste disposal strategy is stalled.

However, proponents of biodegradable plastics argue that these materials offer a solution to this problem. Certified biodegradable plastics combine the utility of plastics

(lightweight, resistance, relative low cost) with the ability to completely and fully biodegrade in a compost facility. Rather than worrying about recycling a relatively small quantity of commingled plastics, these proponents argue that certified biodegradable plastics can be readily commingled with other organic wastes, thereby enabling composting of a much larger position of nonrecoverable solid waste. Commercial composting for all mixed organics then becomes commercially viable and economically sustainable. More municipalities can divert significant quantities of waste from overburdened landfills since the entire waste stream is now biodegradable and therefore easier to process. The use of biodegradable plastics, therefore, is seen as an enabler for the complete recovery of large quantities of municipal solid waste (via aerobic composting) that were heretofore unrecoverable by other means except land filling or incineration.

Traditional applications of synthetic polymers are mostly based on their inertness to environmental degradations (hydrolysis, oxidation, biodegradation, and so on). The rapid increase in the volume of use of synthetic polymers has contributed to the solid waste management problems in recent years. Total management of polymer wastes requires complementary combinations of recycling, incineration for energy, and biodegradation. Polymers prepared from renewable and sustainable resources can be designed, synthesized, and engineered by environmentally compatible routes and can be disposed after use by biodegradation (composting, etc.). Biodegradable polymers are necessary in the design, synthesis and applications of biomedical implants, drug release systems and packaging.

Biodegradable polymers are used and studied in an increasingly large number of mass produced applications such as packaging, paper coating, fibers, films, and other disposable articles, as well as in biomedical applications, (resorbable surgical sutures, implants, and controlled drug delivery devices). These application sectors bring special requirements to the polymers and monomers which must be fulfilled before applications can be successfully launched on the market. These polymers need to be biodegradable and non-toxic in the biomedical applications, bioresorbable and biocompatible. On the other hand, polymers should have good chemical, mechanical, thermal and rheological properties. In addition, in the packaging sector, the raw materials should be renewable and the end products should be compostable to reduce the use of fossil resources. Furthermore, the raw materials and the end products should be low cost and the production processes on an

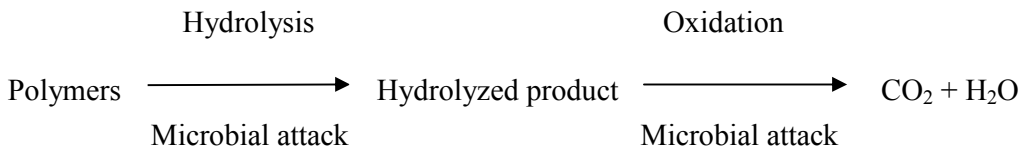
industrial scale should be efficient, environmentally friendly and economically competitive.

Biodegradation is gradual decomposition and deterioration of a material caused by its assimilation by living micro-organisms such as fungi, bacteria and actinomycetes. These microbial agents accelerate the physical aging process or the biotic degradation. Biodegradation is the breakdown of materials through the action of living organisms as defined by ASTM D5338-03 (ASTM Designation D5338-03, Raghavan, 1995). The polymers are generally prone to hydrolysis through the inter unit ester or amide linkages. Moreover, polymers have higher hydrophilic character, longer repeat units and smaller crystalline structure. As a result, the flexible step polymers fit into the active site of enzymes. This enhances biodegradation. Besides carbon, hydrogen, oxygen and nitrogen are the basic nutrients for the growth of any micro-organism and such hydrolysable step growth polymers having C-C, C-N and C-O linkages provide the nutrient source for the microorganism splits the high molecular weight polymer randomly to low molecular weight degraded products.

Environmentally degradable polymers and plastics (EDPs) are a group of polymeric materials experiencing a rapid growth in number as well as in their applications and quantities used. The assessment of their key characteristic degradability, including eventually biodegradability as the ultimate stage, is scientifically and technically a challenging issue and has led to differing interpretations in the past. In order to standardize techniques and criteria, a number of standards, were established by different standardization bodies, which are also used as a basis for certification schemes. In the past 10 years, several biodegradable plastics have been introduced into the market. However, none of them is efficiently biodegradable in landfills. For this reason, none of the products has gained widespread use.

Biodegradation of polymers may be achieved by two major paths (1) design of a polymer from monomers which are vulnerable to micro-organisms and (2) incorporation of biodegradable additives in the non-biodegradable polymer (Maiti, 2005). Polymers obtained from biodegradable monomers such as poly-l-lactic acid or poly-b-hydroxybutyrate linkages contain hydrolysable linkages. The biodegradable component in the partial biodegradable polymer contains some hydrolysable groups or linkages such as

ester, hydroxyl, amide and urea etc. Such hydrolysable groups are hydrolysed under microbial attack as shown below.



Since, the biodegradable components are finally converted to CO_2 and water, the integrity of the biodegradable polymer matrix is destroyed as a result of the non-biodegradable portion becomes first porous, then brittle and finally mixes with the dust. Depending on the evolution of the synthesis process, different classifications of the biodegradable polymers have been proposed. Fig. 1.1 shows the classification of biodegradable polymers.

Only, three categories (a–c) are obtained from renewable resources:

(a) polymers from biomass such as agro-polymers from agro-resources (e.g. starch, cellulose),

(b) polymers obtained by microbial production, e.g. the polyhydroxyalkanoates (PHAs),

(c) polymers conventionally and chemically synthesized whose monomers are obtained from agro-resources, e.g. the polylactic acid (PLA),

(d) polymers whose monomers and polymers are obtained conventionally, by chemical synthesis.

These different biodegradable polymers can also be classified into two main families: agro-polymers (category a) and biodegradable polyesters (categories b, c, d).

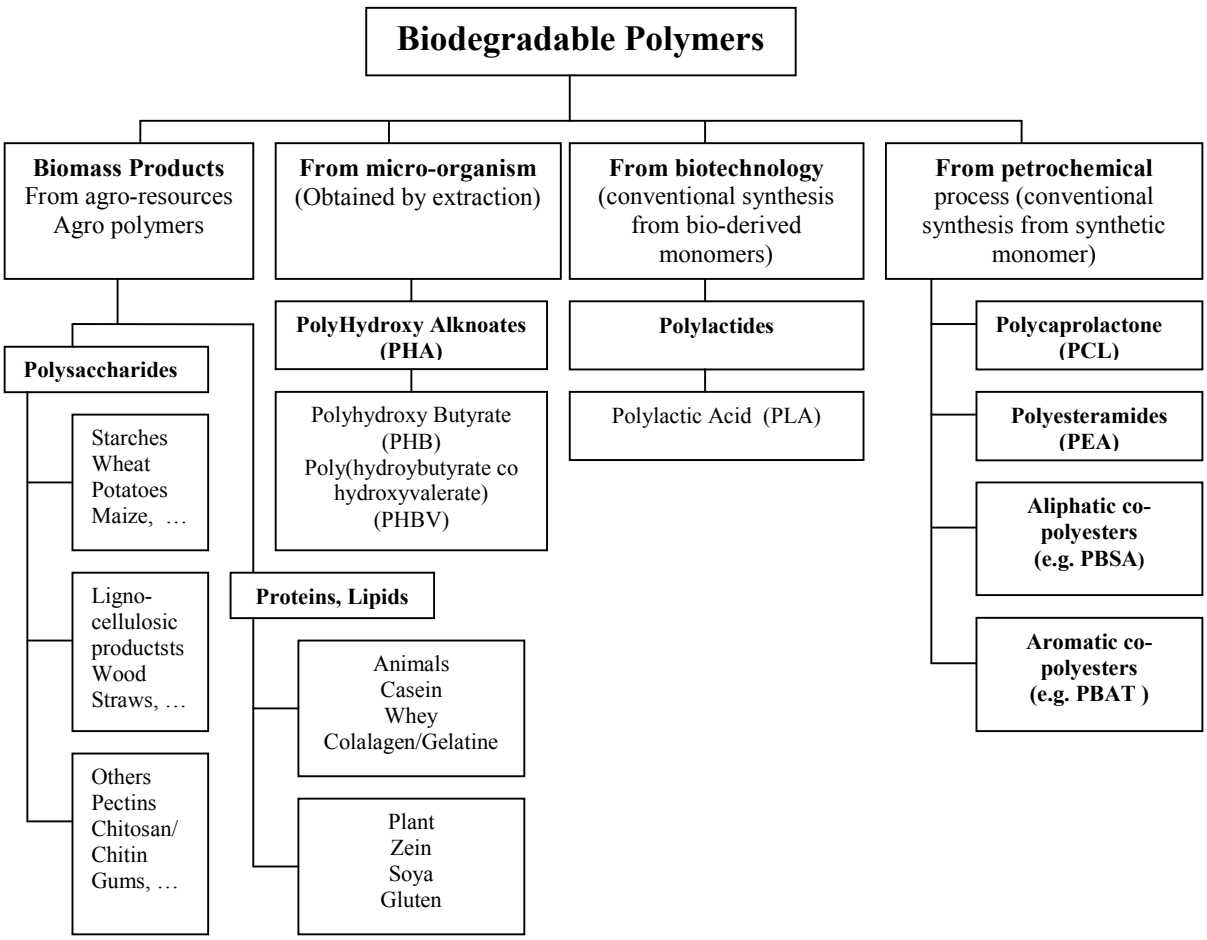


Fig 1.1 Classification of biodegradable polymers.

There is a worldwide research effort to develop biodegradable polymers for agricultural applications or as a waste management option for polymers in the environment. Most of the efforts were synthesis oriented, and not much attention was paid to the identification of environmental requirements for, and testing of biodegradable polymers. Consequently, many unsubstantiated claims to biodegradability were made, and this has damaged the general acceptance of biodegradable polymer.

This research looks for the degradation of commercial polythene. It was investigated in two stages namely, by abiotic conditions and secondly in the presence of selected microorganisms. Biodegradation of polythene by a pure microbial culture contributes to our understanding of the process and the factors affecting polythene biodegradation.

Chapter-2

Literature Review

Nondegradable plastic materials are widely used in industry and agriculture. Because of their high durability, they accumulate in the environment at a rate of 25 million tons per year (Orhan and Buyukgungor, 2000). Polyethylene, particularly as thin films, has found widespread use as a packaging material primarily because of its excellent mechanical properties, barrier properties against water borne microorganisms, low-cost and high energy effectiveness (Briassoulis *et al*., 2004). However, this property of recalcitrance to microorganisms, which had once made polyethylene a popular choice as a packaging material, has now made it a subject of much criticism. Most of the polyethylene, after serving its useful life as a packaging film, finds its way to the landfill sites, where it simply refuses to degrade because of its non-biodegradable nature. The inherent resistance of polyethylene to biological attack can be attributed to its hydrophobic nature (carbon-only backbone), high molecular weight and the absence of functional groups recognizable by microbes. The major strategy to facilitate the disintegration and subsequent degradation of the polymeric polyethylene chain is focused on direct incorporation of carbonyl groups within the backbone or its in-situ generation by introduction of pro-oxidant at the processing stage. Typical pro-oxidants include UV activators like aromatic ketones and/or transition metal based complexes (Roy *et al.,* 2008). Polyethylene appears to be one of the most inert plastic materials. Indeed, in a long-term study on the biodegradation of ^{14}C-labelled polyethylene, Albertsson and Karlsson (1990) found that after 10 years of incubation in soil, <0.5% carbon (as CO_2) by weight was evolved from an UV-irradiated polyethylene sheet. Nonirradiated polyethylene emitted <0.2% carbon dioxide during the same time. Furthermore, no signs of deterioration could be observed in a polyethylene sheet that had been incubated in moist soil for 12 years and only partial degradation was observed in a polyethylene film buried in soil for 32 years (Otake *et al*., 1995).

Synthetic plastics accumulate at a rate of 25 million tons per year in the terrestrial and marine coastal environment. Polyethylene represents 64% of the synthetic plastics produced and they are mainly used for manufacturing plastic bags, bottles, disposable containers, which are discarded within a short time (Byuntae *et al.,* 1991). The degradation

of polymers involves several physical and chemical processes accompanied by small structural changes which lead nevertheless to significant deterioration of the quality of the material (Brown *et al.,* 1974; Doi *et al*., 1992).

It is frequently asserted by environmental pressure groups that the polyolefins cannot biodegrade since the molecular weight must be less than 500 for this to occur. However, it was demonstrated by an interdisciplinary group at the University of Aston over 25 years ago that, whilst normal commercial polyethylenes do not biodegrade, polyethylenes formulated with transition metal prooxidants (notably iron complexes), after ageing or weathering by exposure to UV light, support microbial growth. At that time, plastics litter was becoming a major problem and it was suggested by these workers that this provided ‘‘for the first time the possibility of a combined photo- and bio-degradation process in which degradation commenced by the former can be completed by the latter, ultimately converting the plastic to useful humus, water and CO_2’’ (Eggins *et al.,* 1971). Pre-ageing by either light or heat was shown to be essential abiotic precursors to biodegradation and low molar mass oxidation products are rapidly bioassimilated by thermophilic microorganisms. Albertsson and co-workers have since shown that a wide range of biodegradable oxidation products are formed in the abiotic peroxidation process (Albertsson *et al.,* 1993). It was shown in an earlier study of a commercial photo-biodegradable polyethylene (Plastor1) that low molar mass products are removed from the surface of the polymer by bioerosion without significant effect on the molar mass of the bulk polymer. The biodegradation of photodegradable polyethylene begins at MW 40,000 and it was concluded that photo-initiated peroxidation is the rate-determining step in the biodegradation of the polyolefins in sunlight. However, in recent years the emphasis in polymer biodegradation has shifted from simply protecting the environment from unwanted plastics packaging litter to recovering value from the plastics used. In the case of agricultural plastics, once they have fulfilled their purpose, for example as mulching films, they are required to be bioabsorbed into the soil as humic material (Scott, 1999) but composting of biodegradable plastics requires a rather different solution. The European Union Waste Framework Directive put forward by the EU in March 1991 defines ‘‘recycling/reclamation of organic substances’’ as ‘‘spreading on land resulting in benefit to agriculture or ecological improvement including composting and other biological transformation processes’’ (Scott, 1999). Rapid conversion to carbon dioxide is not ‘‘recovery’’ since carbon dioxide has a negative influence on the environment. Conversion of degradable polyolefin packaging to cell biomass on the other hand provides added value

to the compost. It is now recognised that biodegradation can occur by two different mechanisms; namely hydro-biodegradation and oxo-biodegradation (Scott 1999). The former is much more important in the case of hydrolysable natural polymers such as cellulose, starch and polyesters whereas the latter predominates in the case of other natural polymers such as rubber and lignin. Lignin normally requires the presence of enzymes that initiate peroxidation (Scott, 2002). The synthetic hydrocarbon polymers do not hydrolyse under normal environmental conditions but it was shown in a study discussed earlier that, after transition metal catalysed thermal peroxidation, they biodegrade readily in the presence of a variety of thermophilic microorganisms. It was subsequently shown that lignin biodegrading organisms are particularly effective (Lee *et al.,* 1991).

From a chemical perspective, we would expect polyethylene to be biodegradable, as linear alkenes are usually subject to biodegradation. However, for polyethylene there is an inverse relationship between molecular weight and biodegradability. Linear hydrocarbon oligomers with molecular weights lower than 620 support microbial growth, while those having higher molecular weights are not utilized (Potts, 1995; Haines and Alexander, 1974). It is widely accepted that the resistance of polyethylene to biodegradation stems from its high molecular weight, its three-dimensional structure and its hydrophobic nature, all of which interfere with its availability to micro-organisms. Nevertheless, several studies have demonstrated partial biodegradation of polyethylene after UV irradiation (Cornell *et al*., 1984), thermal treatment (Albertsson *et al.,* 1998; Sepulveda *et al.,* 2002) or oxidation with nitric acid (Brown *et al.,* 1974). Furthermore, a synergistic effect has been found between photo-oxidation and biodegradation of polyethylene (Albertsson, 1987). Apparently, the biodegradation of polyethylene is enhanced by oxidation pretreatment, which increases surface hydrophilicity by the formation of carbonyl groups that can be utilized by microorganisms (Albertsson, 1978; Albertsson, 1980; Cornell *et al*., 1984).

The degradation of plastics in nature is a very slow process which is first initiated by environmental factors followed by wild micro-organisms. The environmental factors include temperature, humidity, pH and UV. Biodegradation is the ability of micro-organism to influence abiotic degradation through physical, chemical or enzymatic action (Albertsson *et al*., 1987; Lee *et al.,* 1991; Erlandsson *et al.,* 1997; Chiellini *et al*., 2003). Interplay between biodegradation and different factors in the biotic and abiotic

environments are very important. The micro-organisms reported for the biodegradation of the polyethylene include fungi (*Aspergillus niger, Aspergillus flavus, Aspergillus oryzae, Chaetomium globusum, Penicillium funiculosum, Pullularia pullulan), bacteria (Pseudomonas aeruginosa, Bacillus cereus, Coryneformes bacterium, Bacillus sp., Mycobacterium, Nocardia, Corynebacterium, Candida and Pseudomonas)* and Actinomycetales.

Biodegradation resulting from the utilization of polyethylene as a nutrient (i.e. a carbon source) may be more efficient if the degrading micro-organism forms a biofilm on the polyethylene surface. However, the hydrophobicity of the polyethylene interferes with the formation of a microbial biofilm. Attempts to facilitate colonization of polyethylene by adding nonionic surfactants to the culture medium promoted the biodegradation of polyethylene (Albertsson *et al.,* 1993; Ehara *et al.,* 2000). Presumably, the surfactant increased the hydrophilicity of the polyethylene surface and thus facilitated the adhesion of bacteria to the polymer.

The ability of this bacterium to form a biofilm on polyethylene was attributed to the hydrophobicity of its cell surface (Gilan *et al*., 2004). Addition of a small amount (0.05%) of mineral oil to the culture medium increased both biofilm formation and the subsequent biodegradation of the polyethylene, presumably by increasing the hydrophobic interactions between the bacterial biofilm and the polymer (Gilan *et al*., 2004). A thermophilic bacterium *Brevibaccillus borstelensis* strain 707 (isolated from soil) utilized branched low-density polyethylene as the sole carbon source and degraded it (Hadad *et al.,* 2005).

The rate of biodegradation of polyethylene, even after prolonged exposure (10–32 years) to microbial consortia of soil, was found to be very low, thus accounting for carbon mineralization of less than 1% (Albertsson and Karlsson, 1990; Otake *et al.,* 1998). More recently, it has been demonstrated in soil burial tests that the use of suitable additives in polyethylene films induced substantial oxidation with consequent fragmentation, drop in molecular weight, increase in wettability, ultimately followed by high mineralization (60–70%) and fixation of about 8–10% of carbon into cell biomass (Chiellini *et al.,* 2003; Jakubowicz, 2003). The low rate of biodegradation of plastics is usually due to lack of water solubility and due to the size of the polymer molecules which prevents it to get

transported directly into the cells (Gilan *et al.,* 2004; Sivan *et al.,* 2006). The two major problems with polyethylene are its high hydrophobicity (due to the presence of only $–CH_2$ groups) and its high molecular weight (more than 30 kDa). The biotic mechanism reported for the degradation of high molecular weight polymers are due to the extra-cellular enzymes produced by micro-organisms which degrade the main polymeric chain and result in intermediates of lower molecular weight with modified mechanical properties, making it more accessible for the microbial assimilation (Palmisano and Pettigrew, 1992). Thermal or radiation treatments on polyethylene reduce the polymeric chain size and form oxidized groups such as carboxyl, carbonyl and hydroxyl. These treatments modify the properties (crystallinity level, morphological changes) of the original polymer and facilitate the polymer biodegradation (Lee *et al*., 1991).

Abiotic degradation in the presence of pro-oxidants generally leads to the formation of functional macromolecules, which can thermally or photochemically cleave repeatedly to low molecular weight oxygenated fragments. These include aliphatic carboxylic acids, alcohols, aldehydes and ketones which can support microbial growth and in turn get consumed by microorganisms (Chellini *et al*., 2006; Chellini *et al.,* 2006). There have been some studies on the biodegradation of prooxidant activated polyethylene in soil, wastewater, sludge and compost (Albertsson et al., 1987, Abd- El- Rehim *et al.,* 2004, Orhan, 2001). This approach offers several advantages, like diverse microbial inoculum or close relation to the real conditions in the nature and in waste treatment processes.

The degradation of abiotically aged low density polyethylene (LDPE) films containing trace quantities of a representative pro-oxidant (cobalt stearate) was investigated in the presence of well defined enriched microbial strains namely, *Bacillus pumilus, Bacillus halodenitrificans* and *Bacillus cereus* in nutrients medium. The films were initially subjected to an abiotic treatment comprising UV-B irradiation, and subsequently inoculated with the bacterial strains. The degradation in the polymeric chain was monitored by changes in the mechanical, morphological, structural and thermal properties (Roy *et al*., 2006 a; Roy *et al.,* 2006 b; Roy *et al*., 2007).

How polymers' degradation proceeds in a specific case depend on the environment the plastics are exposed to, during their useful lifetime and the environment the polymer wastes are disposed to, afterwards. The kinetics of polymer degradation depends on

whether the environment is dry air, humid air, soil, and a landfill, a composting environment, sewage, freshwater or a marine environment. Each environment has its own characteristic concentration profile of important factors: oxygen, water, other chemicals, daylight and degrading microorganism (Hadad *et al.,* 2005). According to the nature of the environment there may be a relatively more efficient or less efficient mechanism by which degradation can occur. In one environment a very efficient degradation mechanism may be available, whereas in another environment the same mechanism might not be available at all for lack of appropriate conditions. Also according to the nature of the environment, there may be a larger or a smaller concentration of chemicals that react with the plastic during the degradation process. More specifically, the environmental factors affecting the rate of degradation by microorganisms include temperature, moisture level, atmospheric pressure, and pressure of oxygen, concentrations of acids and metals, and the degree of exposure to light. Factors relating to microorganisms include their concentration, whether or not they have enzymes for which the polymer is a substrate, the concentration of enzymes, the presence of trace nutrients for the microorganisms and the presence of inhibitors or predators. If any of the required elements is absent, or if it is present at a level that falls below a critical threshold, biodegradation may not only slow down but may stop altogether until proper conditions are once again present.

During the past three decades, non-biodegradable plastic materials have replaced biodegradable products in a variety of applications. The drastic rise in the use of plastic materials has not been accompanied by a corresponding development of procedures for the safe disposal or degradation of these materials. As a consequence, plastic wastes accumulating in the environment pose an ever increasing ecological threat to terrestrial and marine wildlife. An important number of biodegradable polymers (biopolymers) exist that are derived from both synthetic and natural sources but most of them are quite costly. Growing environmental concerns have created an urgent need to develop new biodegradable materials that have comparable properties with today's polymeric materials at an equivalent cost. The most problematic plastic, in this regard, is probably polyethylene, which being resistant to microbial attack is one of the most inert synthetic polymers. Most studies on the biodegradation of polyethylene are based on natural soil as the biotic environment, but some studies have used bacterial or fungal cultures amended with polyethylene. In most habitats — both natural and artificial — the majority of microbial populations form biofilms on solid surfaces. In many cases, the metabolic

activity of the microbial populations that form the biofilm is higher than that of suspended bacteria. When the solid surface also serves as the substrate (as in the case of polyethylene challenged by microbial populations), it is clear that carbon availability is greater in a biofilm. However, the hydrophobicity of polyethylene constitutes an obstacle that interferes with colonization and biofilm formation.

Important information concerning polymer's final fate in the environment can be achieved in biodegradation studies. Environmental biodegradation concerns the complete conversion of organic chemicals to inorganic products mediated by microbial processes. The degree of polymer biodegradation can be measured according to the carbon dioxide mass and/or methane evolved, oxygen consumption, degradation products (e.g., monomers) released, and polymer carbon converted into biomass (Nageotte *et al.,* 2006). Standard test methods have been proposed by several international organizations to assess biodegradability of polymeric materials (ASTM, ISO, OECD).

Chapter-3
Materials & Methods

3.1 Materials

3.1.1. PLLA (Polylactide)

Commercial-grade poly (L-lactic acid) (trade name Biomer L 9000, weight-average molecular weight = 20 kDa, number average molecular weight = 10.1 kDa, MFI 3.0 gm/10 min with 2.16 kg standard die at 190^0C) was supplied by Biomer Forst-Kasten-Str Kailling, Germany.

3.1.2. Plastic films

In the present investigation, pure linear low density polyethylene (LLDPE 100), LLDPE 80 (80 wt % LLDPE & 20 wt % poly(l-lactic acid) (PLLA), M-g-L 80/4 (80 wt % LLDPE, 20 wt % PLLA and 4 parts compatibilizer [grafted low density polyethylene maleic anhydride (M-g-L)] per hundred parts of resin) films of size 80×25 mm with thickness 0.125 mm were made by melt blending of LLDPE and PLLA in an extrusion mixer with post extrusion blown film attachment with and without a compatibilizer which were reported of our earlier work (Singh *et al*., 2010). The market available biodegradable polymer films (controlled sample films, CSF) of size 80×25 mm with thickness 0.11 mm were kindly provided by M/s Balson Industries, Pune, Maharashtra, India (www.balsonindustries.com).

3.1.3. Compost

The mature compost (municipal solid waste) was obtained from a compost plant, New Delhi Municipal Council, Okhla, New Delhi, India. The compost inoculum was well-aerated coming from the organic fraction of municipal solid waste and sieved on a screen of < 10 mm. The compost inoculum was free from larger inert materials (glass, stones, metals, etc.) as much as possible. These items are removed manually as much as possible to produce a homogenous compost inoculum. The compost had the following basic

properties: total solids (TS%) 81%; volatile solids at 550^0C (VS%)18%; pH 7.2; C/N ratio 15.3.

3.1.4. Pure bacterial culture

Brevibacillus borstelensis (MTCC number 1952) was procured from Microbial type Culture & Gene bank, Institute of Microbial Technology, Chandigarh. This bacterium is a thermophilic, gram-positive, spore forming rod with a growth optimum at 50^0C.

3.2 Methodology

3.2.1 Abiotic Treatment

3.2.1.1 Thermal Aging [ASTM D5510 (2001)]

The plastic films, LLDPE 100 & its blends (LLDPE 80, M-g-L 80/4) were exposed to 90^0 C for different time intervals in an oven and estimated the failure time interval for the selection of the heat aging temperatures. Determine the relationship between a defined property change and temperature using ASTM D5510 (2001).

3.2.2 Biotic Treatment

3.2.2.1 Controlled Composting (Modified ASTM 5338(2003)

The compost inoculum was well-aerated coming from the organic fraction of municipal solid waste and sieved on a screen of < 10 mm. The compost inoculum was free from larger inert materials (glass, stones, metals, etc.) as possible. The plastic films, CSF, LLDPE 100 & its blends (LLDPE 80, M-g-l 80/4) were embedded in the compost and incubated in incubator Model NSW-152 of Narang Scientific Works Pvt. Ltd., India for a period of 28 days initially, kept at 37^0C for a period of one day to stimulate a mesophilic start-up phase. Subsequently, the temperature was raised to 50^0C for a period of four days for optimum composting conditions. Then the temperature was reduced to 37^0C for the remainder of the test period to stimulate a mesophilic curing phase and maintained the

moisture content and aerobic conditions manually as much as possible for controlled composting.

3.2.2.1.1. Isolation of compost microorganisms

Culture isolation from compost was performed using nutrient broth at 37° C on shaker incubator at 150 rpm for 24 hrs. Then, the cultures were serially diluted and surface spread on nutrient agar (NA) plates and incubated at 37° C for 24 hrs. The different isolated colonies were streaked (surface plating) on NA plates and were preserved to maintain its suitability and for further studies.

3.2.2.2. Biotic treatment as per ASTM D5338 (2003) standard for aerobic treatment using municipal solid compost

The plastic films, CSF, LLDPE 100 & its blends (LLDPE 80, M-g-l 80/4) were treated under controlled composting conditions according to ASTM standard D5338-98. Four composting vessels (one blank, one positive, one negative and one test sample) of 3 L capacity were used for the study. Each composting vessels contained 600g of compost, three samples of 2 g each and 1 L of distilled water. For CO_2 trapping, three 5000-mL bottles fitted with gas sparging and containing $Ba(OH)_2$ solution were used. The composting vessels were incubated for a period of 45 days. Initially, the incubation temperature was maintained at 35^0C (± 2^0C) for a period of one day to stimulate a mesophilic start-up phase. Subsequently, the temperature was raised to 58^0C (± 2^0C) and maintained for a period of four days. After this sanitizing period, the temperature was reduced to 50^0C (± 2^0C) for optimum composting conditions and maintained until day 28. Temperature was then reduced to 35^0C (± 2^0C) for the remainder of the test period to simulate a mesophilic curing phase. The incubation time of 45 days may be extended until no significant CO_2 production in excess of the inoculum is recorded for a period of one week.

3.2.2.3 Biodegradation of polyethylene by a thermophilic bacterium Brevibacillus borstelensis.

3.2.2.3.1 Culture maintenance and growth

Nutrient broth (NB) media was used to maintain the bacterial culture (*Brevibacillus borstelensis*, polyethylene-degrading bacterium strain). Liquid cultures (100 ml) were incubated in flasks (250 ml) on a shaker incubator (150 rpm) at 50^0 C for a period of 2 days which was then preserved for further use.

3.2.2.3.2. Treatment

Liquid cultures (100 ml) were incubated in flasks (250 ml) on a shaker incubator (150 rpm) at 50^0 C for a period of 30 days. Bacterial strains assayed for their ability to utilize polyethylene as the sole source of carbon and energy were grown in VB medium (Vogel and Bonner, 1956) modified as follows (g/l): KNO_3, 2.0; KH_2PO_4, 5.8; K_2HPO_4, 3.7; $MgSO_4.7H_2O$, 0.25; yeast extract, 0.1; and 1 ml of trace-elements stock solution containing (g/l in 1 mol HCl/l solution): $FeSO_4.7H_2O$, 2.78; $MnCl_2.4H_2O$, 1.98; $CoSO_4.7H_2O$, 2.81; $CaCl_2.2H_2O$, 1.67; $CuCl_2.2H_2O$, 0.16; $ZnSO_4.7H_2O$, 0.29, pH 7.8. Biodegradation tests were performed with polyethylene films CSF, LLDPE 100 & its blends (LLDPE 80, M-g-l 80/4) that had been dried overnight at 60^0 C, weighed, disinfested (30 min in 70% ethanol) and added to flasks, each containing 100 ml of mineral medium (*ca* 300 mg of film per flask). Each test consisted of three flasks (triplicates).

3.3. Analytical/ Testing Procedures

3.3.1. Tensile testing

Physical properties such as tensile strength and elongation at break were measured according to ASTM D 882-91 procedure on Zwick Universal testing machine (model

Z010 Zwick/Roell, Germany) at room temperature (25°C), 50% relative humidity, and a cross-head speed of 50 mm/min. Five replicates were run for each composition and the average values have been reported. Relative elongation and relative tensile strength of the samples treated with compost were compared with untreated control samples.

3.3.2. Fourier transform infrared (FTIR) and attenuated total reflectance (ATR) spectroscopy

FTIR analysis is a useful tool to determine the formation of new or disappearance of functional groups. So degradation products, chemical moieties incorporated into the polymer molecules such as branches, co-monomers, unsaturation and presence of additives such as antioxidants can be determined by this technique. Fourier transform-attenuated total reflectance (FT-ATR) infrared spectroscopic studies were carried out on film samples using a Perkin Elmer FT-IR spectrophotometer (Model BX-II, Shelton, USA) in the horizontal ATR mode, using a zinc-selenide crystal. A total of 16 scans were taken with a resolution of 4 cm^{-1}. The spectrum was analyzed using spectrum software (LX100627-I, Shelton, USA).

3.3.3. Thermal analysis

Thermal analysis was carried out with differential scanning calorimetry (DSC; PerkinElmer DSC-2). All measurements were performed under nitrogen. DSC measurements were carried out with heating from room temperature to 300^0C at a rate of 10^0C/min and were controlled by a compatible computer running the PerkinElmer (STAR SW900) instrument software. The software-collected data and provided graphical analysis tools were used to determine the transition temperatures and peak areas. DSC studies revealed the significant thermal properties of the samples, such as T_g and T_m. Thermogravimetric analysis (TGA) is a helpful tool to characterize thermal degradation (amount and rate of mass loss), thermal stability, and the lifetime behavior of polymeric materials. Such characterizations provide valuable information for selection of material, prediction of product performance, and product quality. The thermogravimetric behavior

of the blends was determined by using TGA (Perkin Elmer Pyris, diamond TG/DTA) under a nitrogen flow of 50 ml/min. Samples weighing 3 mg ($\pm$ 1 mg) were heated from 50 to 500^0C, at a heating rate of 10^0C/min.

3.3.4. X-ray diffraction analysis

All of the samples were characterized with a X- ray diffractometer with X'Celerator (X'Pert PRO, PANalytical, Netherlands). During the experiment, the scanning speed and diffraction angle were 5^0 /min and $5–60^0$ (2θ) at 45 kV and with a current of 40 mA.

3.3.5. Morphological evaluation using scanning electron microscopy (SEM)

The films were washed in 70 % ethanol to remove cell mass from the residual film as much as possible and then dried at 45^0C for 24h. These films were used to evaluate the surface and bio-deterioration. Scanning electron micrographs of the films were taken with a scanning electron microscope (JEOL, Model JSM 6510 LV). The accelerating voltage was 15 kV. The specimens were coated with 50 μm of thick gold film in an automatic sputter coater (Polaron) to avoid charging under an electron beam prior to SEM studies.

3.3.6. Weight loss

A simple and quick way to measure the biodegradation of polymers is by determining the weight loss. Microorganisms that grow within the polymer lead to an increase in weight due to accumulation, whereas a loss of polymer integrity leads to weight loss. Weight loss is proportional to the surface area since biodegradation usually is initiated at the surface of the polymer. This method cannot be used on polymers that absorb water. Multiple samples were weighed with an accurate four-digit balance and average values are reported here.

3.3.7. Dry weight of residual polythene for pure culture

To facilitate accurate measurement of the weight of the residual polyethylene, the bacterial biofilm was removed from the polyethylene surface by incubating the polyethylene samples in flasks containing 2% (v/v) aqueous sodium dodecyl sulphate (SDS) solution for 4 h. The polyethylene samples were collected on a filter paper, rinsed with distilled water and then dried overnight at 60°C before they were finally weighed. The initial weights of the pre-incubated polyethylene samples were also measured following the same procedure as mentioned above.

3.3.8. Microbial examination

Microbes were isolated by serial dilution techniques and morphologically observed by microscope (Zeiss-axiotron dual photo) under 8x. Further, these microbes were observed by gram staining technique under the microscope (Nikon Eclipse (50i) Nikon Corporation, Japan) 100x.

3.3.9. Total carbon content of the sample with TOC analyzer

The T.O.C. (Total Organic Carbon) was measured using TOC-V_{CPH} (solid module) from Shimadzu, Japan. The TOC-V_{CPH} analyzer utilizes 680°C heat and platinum catalyst to provide a lower maintenance instrument with a detection limit of 0.0040 mg per liter.

3.3.10. Determination of the cumulative CO_2 production from the test substances

The amount of CO_2 produced by the test substances were determined titrimetrically by the difference, in milliliters of titrant, between the test substances and the blank $Ba(OH)_2$ traps. The titration was performed with 0.05 N HCl. The mmole of CO_2 produced were calculated from the following equation:

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

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

$$\% \text{ biodegradation} = \frac{\text{mean } C_g \text{ (test)} - \text{mean } C_g \text{ (blank)}}{C_i} \times 100$$

where,

C_g = amount of gaseous - carbon produced, g, and

C_i = amount of carbon in test compound added, g.

Chapter-4

Results & Discussion

4.1. Abiotic Treatment

In general degradation is the process where the deterioration in the properties of the polymer takes place due to different factors like, light, heat, mechanical etc. As a consequence of degradation, the resulting smaller fragments do not contribute effectively to the mechanical properties and the article becomes brittle and the life of the material becomes limited. Thus, any polymer or its composite which is to be used in outdoor applications must be highly resistant to all the environmental conditions. Sum of the polymeric materials are biodegradable like polylactic acid, polycapralactone, polyhydroxybutyrate, polyhydroxyvalerate, polyvinyl alcohol so they can be degraded very easily. However the polymers like polyethylene, polyvinyl alcohol, polypropylene, polystyrene are not biodegradable or easily degradable so they need some pre-treatment like photo-degradtion, thermal degradation, hydrolytic degradation, mechanical, oxidative degradation before biotic environment.

4.1.1. Thermal aging

The mechanical properties of LLDPE 100 and its blends before and after thermal aging are shown in Table 4.1 and Figures 4.1 & 4.2. Tensile strength as well as elongation at break of LLDPE 100 does not show any change in the mechanical properties but its blending with PLLA in the case of LLDPE 80, it shows decrease in the mechanical properties, as PLLA is brittle in nature and acts as filler when it is dispersed in LLDPE. In the case of M-g-L 80/4, the addition of compatibilizer decreases the tensile strength as well as elongation at break, probably due to high melt flow index of the compatibilzer and its polymeric nature. It also acts as a plasticizer which increases or decreases the elongation at break. The blends LLDPE 80 and M-g-L 80/4 show the maximum degradation after 48 hours at 90^0C after which there is no significant change, but in this case only time selection for the thermal degradation has been achieved which needs further investigation for the selection of the degradation under different temperatures according to ASTM D5510.

Table 4.1 Heat aging of oxidatively degradable plastics at 90^0 C

Time (in h)	LLDPE 100		LLDPE 80		M-g-L 80/4	
	Tensile strength (MPa)	**Elongation at break (%)**	**Tensile strength (MPa)**	**Elongation at break (%)**	**Tensile strength (MPa)**	**Elongation at break (%)**
0	20.1	926.0	16.1	702.0	8.55	446.80
10	20.2	930.2	16.8	712.8	7.16	413.8
24	19.9	907.8	12.2	648.5	6.95	378.8
48	22.4	944.6	9.42	499.3	5.50	313.6
96	20.8	954.7	9.68	504.5	5.57	319.8
192	20.5	949.6	9.48	501.7	5.82	320.6

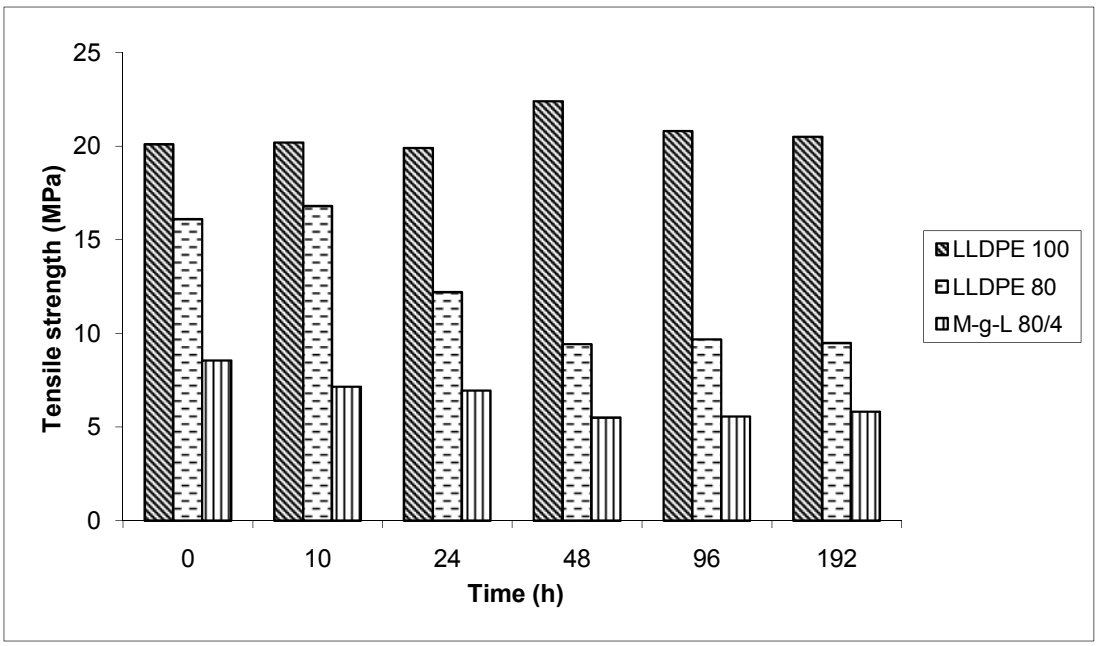


Fig. 4.1 Tensile strength (MPa) of plastic films after thermal aging

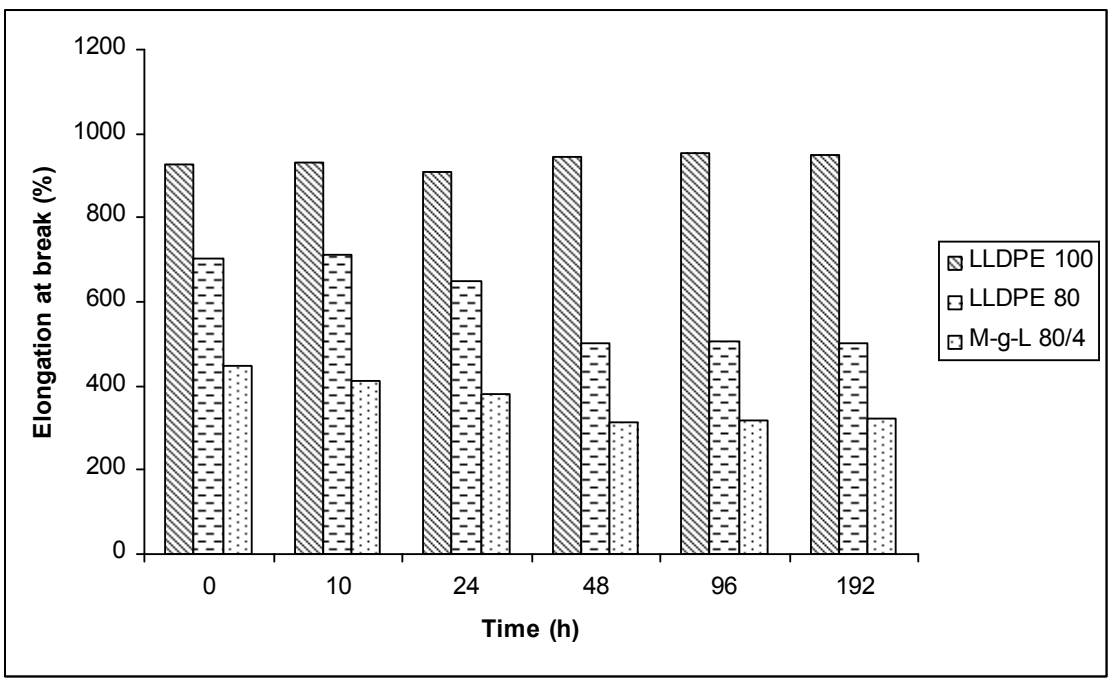


Fig. 4.2 Elongation at break (%) of plastic films after thermal aging

4.2. Biotic treatment

Biodegradation or biotic degradation is chemical degradation of materials (e.g. polymers) brought about by the action of naturally occurring microorganisms such as bacteria, fungi and algae (chemical degradation that does not involve biological activity is defined as abiotic degradation) (Weiland *et al.,* 1995). As biodegradation proceeds it produces carbon dioxide and/or methane and water. If oxygen is present, the biotic degradation that occurs is aerobic degradation and carbon dioxide is produced. If there is no oxygen available, the biotic degradation is anaerobic degradation, and methane is produced instead of carbon dioxide. Under some circumstances both gases are produced. Mineralization is defined as the conversion of biodegradable materials or biomass to gases (like carbon dioxide, methane, and nitrogen compounds), water, salts and minerals, and residual biomass. Mineralization is complete when all the biodegradable materials or biomass is consumed and all the carbon in it is converted to carbon dioxide. Complete mineralization represents the rendering of all chemical elements into natural biogeochemical cycles. Usually, there are two steps involved in the biodegradation of the polymer (Bonhomme *et al.,* 2003)

• Mechanical (grinding), chemical (irradiations by ultraviolet rays; e.g. photodegradation), or thermic degradation. During this stage, microscopic fungi and bacteria, or other biological agents (earthworms, insects, roots of plants, even rodents), can also fragment the product (biofragmentation). This first phase is very useful, because it leads to the increase of the surface of

the material exposed to the microbodies occurring in the second phase.

• The second phase corresponds to the biodegradation. Microbodies attack and digest the product, which is transformed in to by-products which are assimilated by the microbodies, the final result being CO_2 or CH_4, water and biomass production. This second phase is often concomitant of the first one.

4.2.1. Controlled composting as per D5338 (2003)

4.2.1.1. Mechanical properties

The mechanical properties of the plastic films before and after composting are shown in Table 4.2 and Fig 4.3 & 4.4. After composting, there is a decrease in the tensile strength and elongation at break of LLDPE and its blends which shows that after composting there is considerable loss of mechanical properties. A maximum loss of tensile strength 19.9 % was observed with LLDPE 100 54.11% LLDPE 80, 34.3% with M-g-L 80/4 and 56.6 % with CSF respectively under composting which shows that after composting there is a loss of mechanical properties even in LLDPE 100 and its blends with PLLA in the case of LLDPE 80 shows the decrease in the mechanical properties, as PLLA is brittle in nature and acts as filler when it is dispersed in LLDPE. In the case of M-g-L 80/4 due to the addition of compatibilizer, decreases the tensile strength as well as elongation at break, probably due to high melt flow index of the compatibilizer and its polymeric nature. It also acts as a plasticizer which increases or decreases the elongation at break.

Table 4.2 Mechanical properties of plastic films before and after composting

Sample	Before composting		After composting	
	Tensile Strength (MPa)	**Elongation at break (%)**	**Tensile Strength (MPa)**	**Elongation at break (%)**
LLDPE 100	20.1	926.0	16.1	698.0
LLDPE 80/20	19.7	702.0	9.04	473.9
M-g-L 80/4	8.55	446.80	5.61	342.5
CSF	8.32	558.4	3.86	78.0

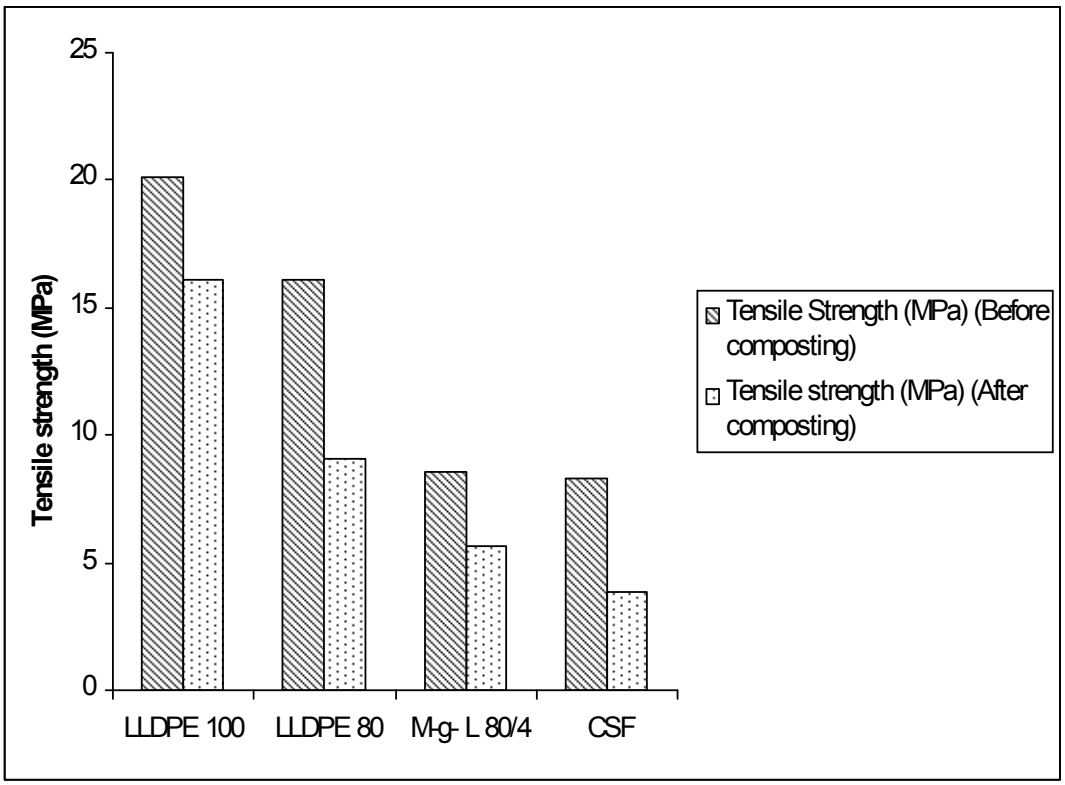


Fig. 4.3 Tensile strength (%) of plastic films before and after treatment

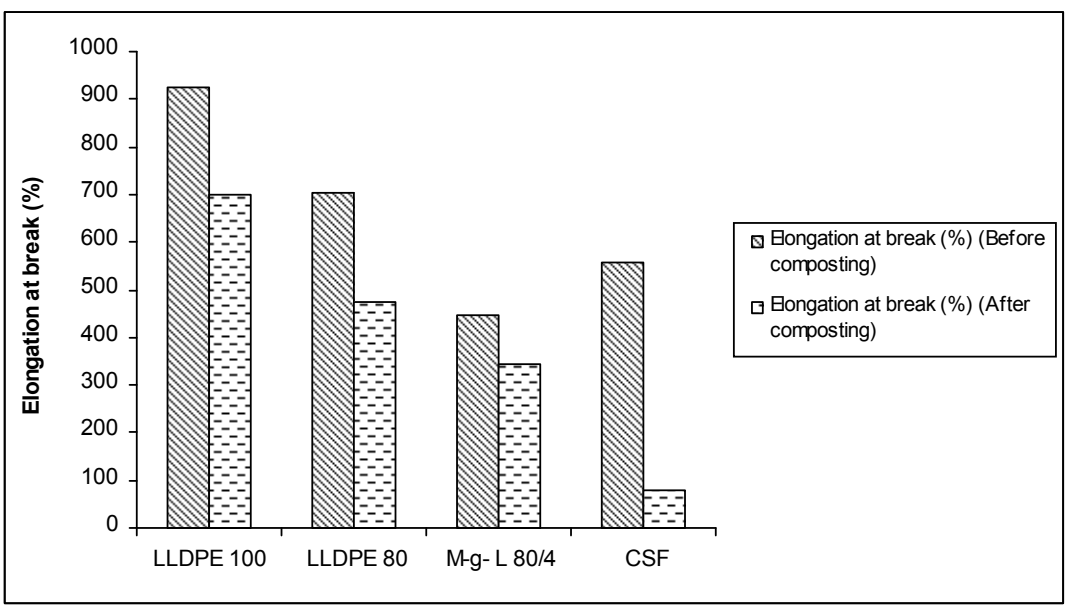


Fig. 4.4 Elongation at break (%) of plastic films before and after composting

4.2.1.2 FTIR spectroscopic analysis

Fourier transform infrared spectroscopy (FTIR) analysis is a useful tool to determine the formation of new or disappearance of functional groups. So degradation products, chemical moieties incorporated into the polymer molecules such as branches, co-monomers, unsaturation and presence of additives such as antioxidants can be determined by this technique. Initial spectra were in agreement with the chemical structures of the LLDPE 100 before and after composting which shows that there is no significant difference after incubation. Transmission results show that there is no significant change in the bulk. ATR results mean that if biodegradation is only superficial, it does not significantly change the chemical structures (Nageotte *et al*., 2006). In case of LLDPE 80 and M-g-L 80/4, it revealed a reduction in carbonyl groups after incubation with the bacteria as shown in Table 4.3. The FTIR spectrum showed a typical carbonyl peak at 1712 cm^{-1}. Composting of LLDPE 80, M-g-L80/4 photo-oxidized showed a marked reduction in the amount of carbonyl residues. The reduction in carbonyl residues was also estimated in terms of a carbonyl index, which is the ratio between the absorbance peaks of carbonyl to that of CH_2 at 1462–1463 cm^{-1}.

Table 4.3 Carbonyl index obtained from FTIR spectra of the plastic sample before and after composting

Samples	**Carbonyl index** ($A_{C=O}$:A_{CH2})*	
	Before composting	**After composting**
LLDPE 100	Nil	Nil
LLDPE 80	1.10	1.017
M-g-L 80/4	1.00	0.99
CSF	Nil	Nil

*The carbonyl index expresses the ratio between the absorbance peak of the carbonyl (1712 cm^{-1}) and that of the CH_2 groups at 1462– 1463 cm^{-1}.

4.2.1.3 XRD (X-Ray Diffraction) analysis

The X-ray diffraction shows that there is an increase in crystallanity of LLDPE 80 by 4.13% and CSF by 10.16% Table 4.4 & fig. 4.5 this is due to the assimilation of the amorphous part of the polymer by the bacteria and thus only crystalline region of the plastic film is left behind leading to increase in the crystallanity. However in the case of M-g-L 80/4, there is no increase in the crystallanity probably due to the fact that the degradation of the fibers occuring from outside surface of the fibers. The compatibilzer maleic anhydride has higher resistance to microorganisms than PLA and LLDPE in the fibers and can wrap PLLA and LLDPE and protect it from degradation. Therefore, LLDPE in the polyblends with a compatibilizer has much lower degradation than in the pure fibers. But there is no change in the crystallanity of LLDPE 100 after composting it for 28 days.

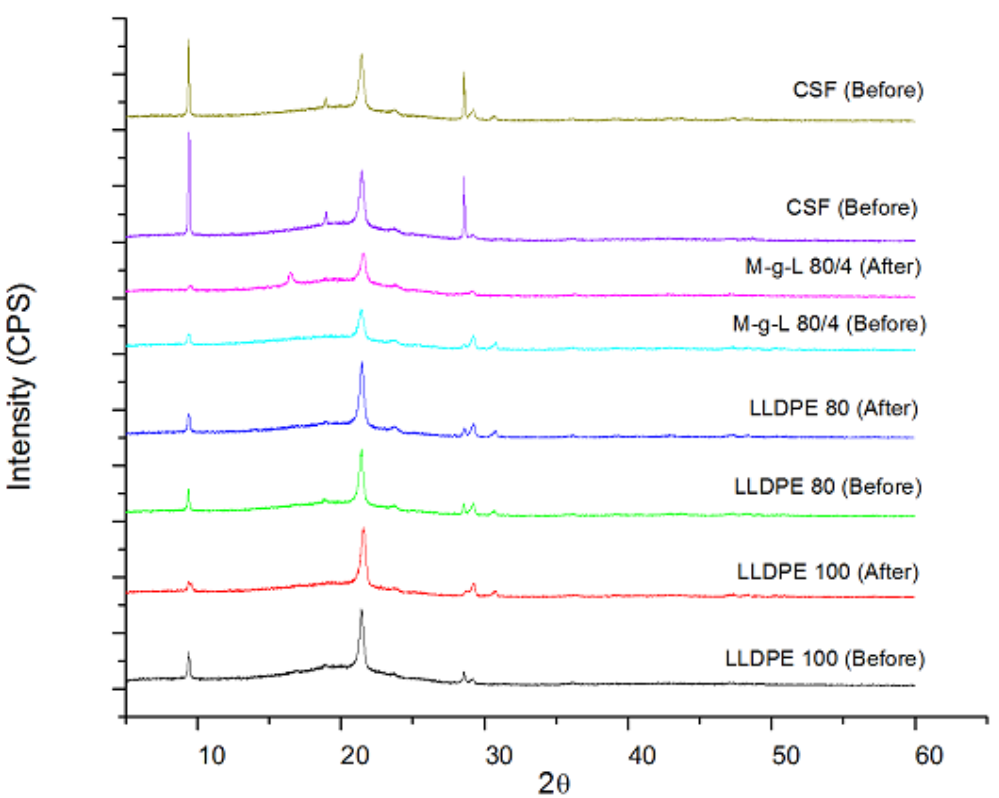


Fig 4.5 XRD patterns of the plastic films before and after composting

Table 4.4 Crystallanity (%) before and after composting

Sample	Crystallanity (%) (Before composting)	Crystallanity (%) (After composting)
LLDPE 100	70.61	69.79
LLDPE 80	71.06	75.19
M-g-L 80/4	75.78	75.59
CSF	60.29	70.45

4.2.1.4. DSC

The change of crystallinity after composting was confirmed by DSC measurements. Melting peaks occuring at 125°C on DSC curves were due to the melting of crystallites of LLDPE100 and its blends. The changes of melting enthalpy indicated changes of crystalline degree of plastic samples. In Table 4.5, melting enthalpies of crystalline phase is presented. The changes of melting enthalpy ΔH [J/g] of plastic material after composting can be seen very clearly. The increase in crystallinity was confirmed by an increase of melting enthalpy of the crystalline phase of plastic samples after 28 days of composting, because the amorphous phase was degraded first (Rutkowska *et al.,* 2002).

Table 4.5 The changes of melting enthalpy ΔH (J/g) of plastic films before and after composting

Samples	Changes of melting enthalpy ΔH (J/g)	
	Before composting	After composting
LLDPE 100	61.63	98.85
LLDPE 80	52.07	65.33
M-g-L 80/4	61.78	64.07

4.2.1.5. Thermal properties

The TGA of LLDPE 80, M-g-L 80/4 films before and after the biotic exposure are presented in Fig. 4.6 Surprisingly, an increase in the T_{20} from 266.47 ^{0}C to 328.66 ^{0}C for LLDPE 80 and T_{20} from 326.07 ^{0}C to 394.920 ^{0}C for M-g-L 80/4 was observed. This increase could be attributed to the preferential bioerosion of the low molecular weight fragments generated during the biotic exposure of the films as they are recognizable by the microbial enzymes (Sepulveda *et al.,* 2002).

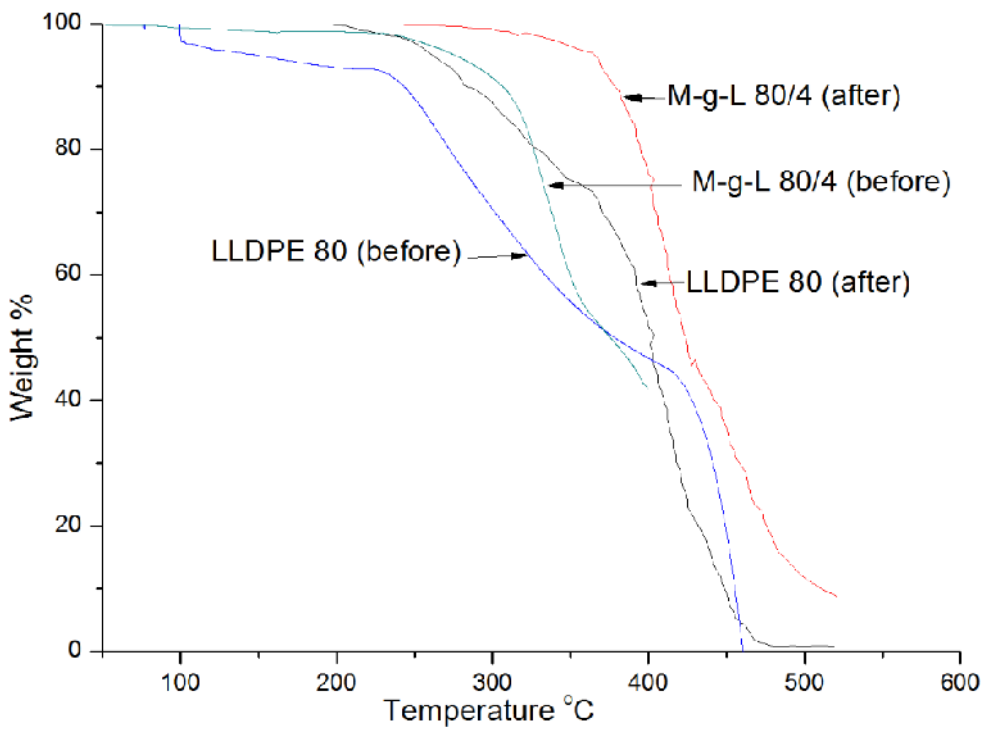


Fig.4.6. Change in the thermal behaviour of LLDPE 80 and M-g-L 80/4 before and after composting

4.1.6. Morphological characterization

Figures 4.7(a) and 4.7(b) show the SEM images of the LLDPE 100, LLDPE 80, M-g-L 80/4, CSF, before and after composting respectively. The before composting image is shown for the reference. There is a uniform dispersion of different phases before the

degradation. The change in the uniformity after the degradation can be seen by the white spots, indicating that the surface erosion has taken place due to degradation which would loosen the grip within the matrix resulting in loss of mechanical properties.

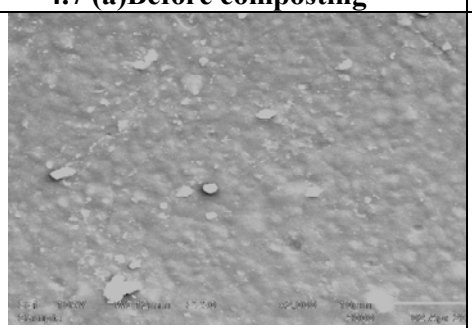
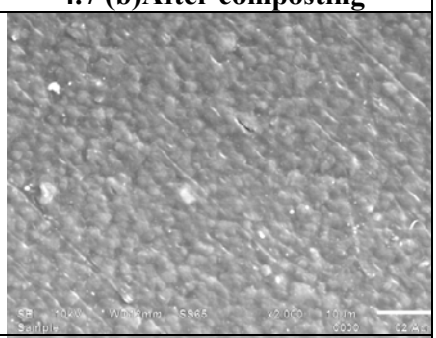
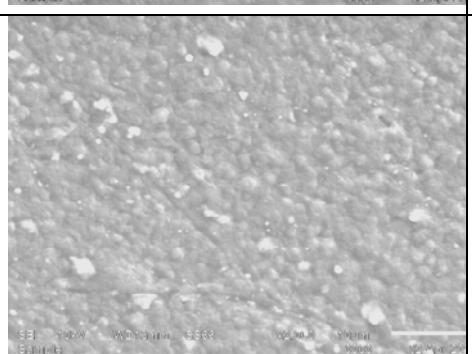
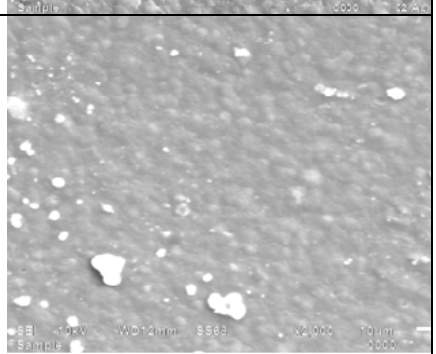
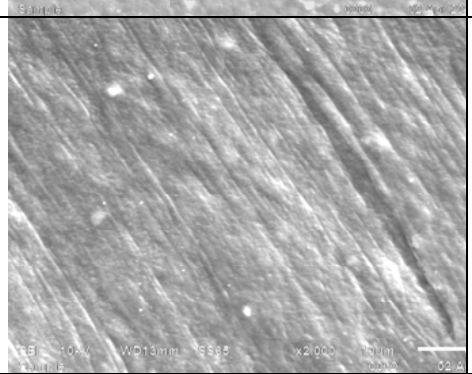
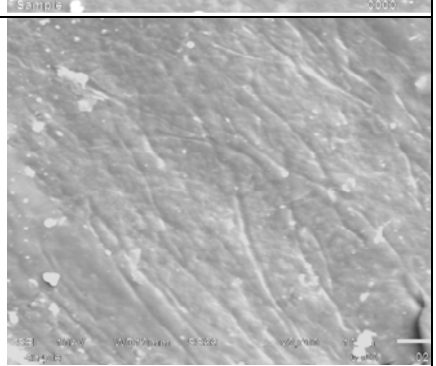
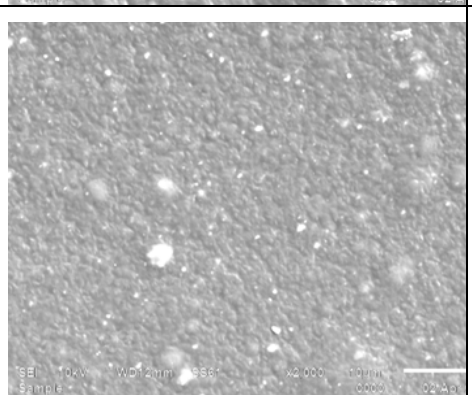
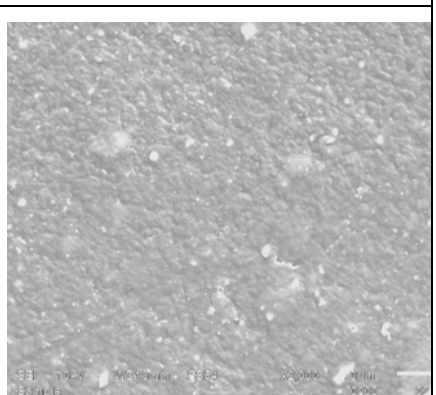
Magnification(x2000)		
Sample	**4.7 (a)Before composting**	**4.7 (b)After composting**
LLDPE 100		
LLDPE 80/20		
M-g-L 80/4		
CSF		

Fig. 4.7 SEM images of the plastic films before and after composting

4.2.1.7. Weight loss

A maximum weight loss of 17 % was observed with CSF under compost within 28 days. Weight loss of 11.6 % with LLDPE 80, 1.39 % with LLDPE 100 and 0.36 % with M-g-L 80/40 under compost was observed (Table 4.3 and Fig. 4.7). Karlsson and Albertsson (1998) have also reported that the total weight loss during degradation was 16 % and in addition to H_2O and CO_2, shorter hydrocarbons, alcohols, organic acids, ketones, aldehydes, etc. are also formed.

Table 4.6 Percentage weight loss of plastic films under compost

Sample	Before compost weight (g)	After compost weight (g)	Weight loss (%)
LLDPE 100	0.5154	0.5082	1.39
LLDPE 80	0.4594	0.4061	11.6
M-g-l 80/4	0.4180	0.4165	0.36
CSF	0.8833	0.7329	17

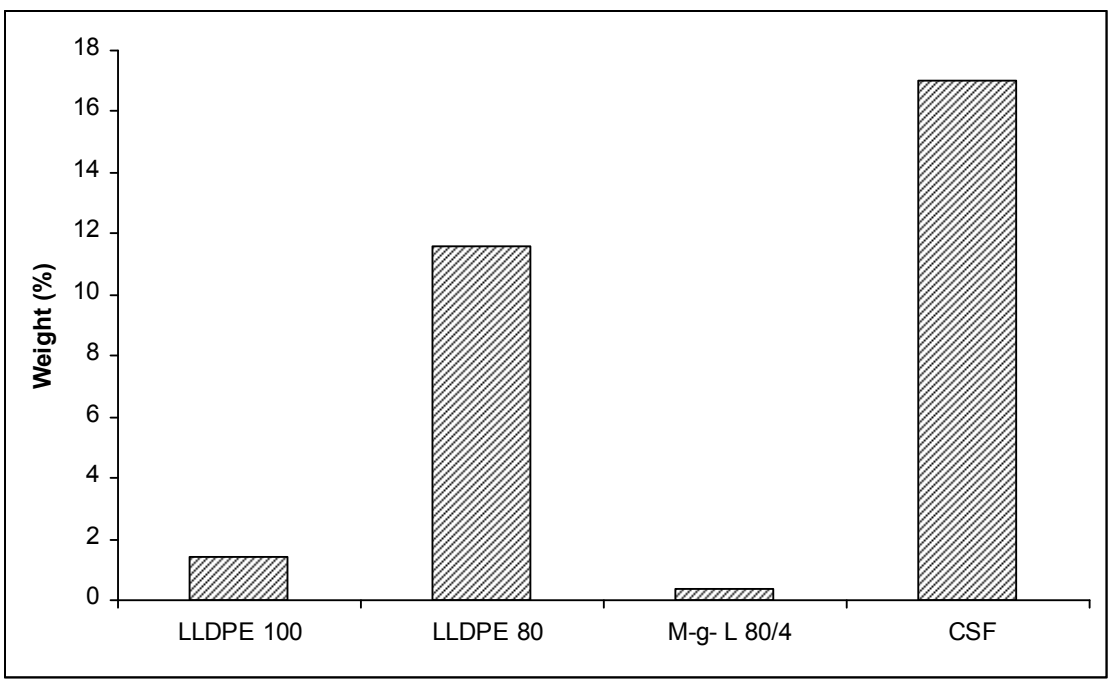
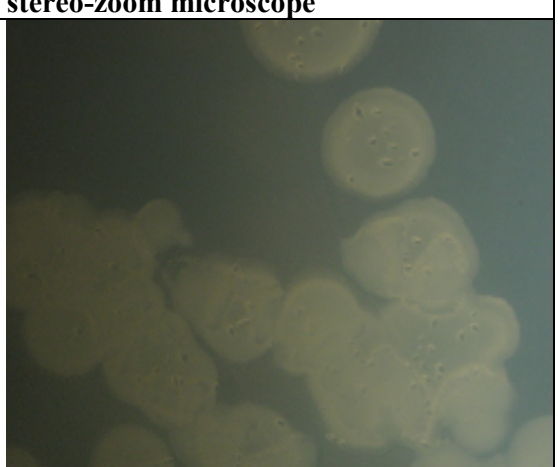
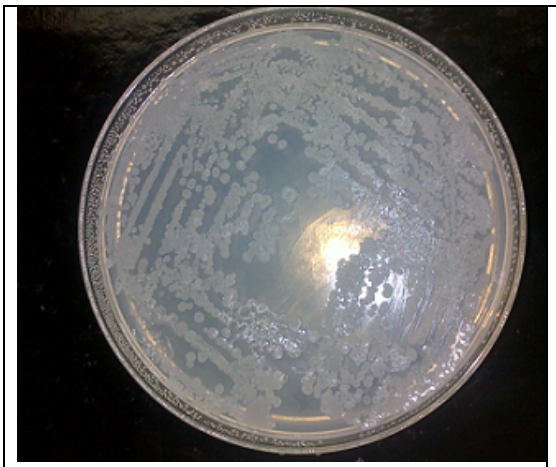


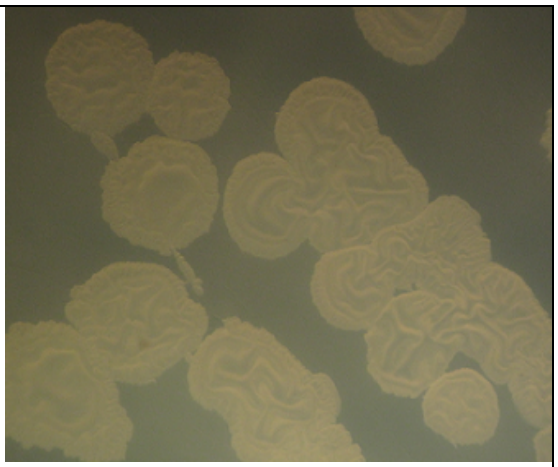
Fig 4.8 Percentage weight loss after composting

4.1.8. Microbial examination

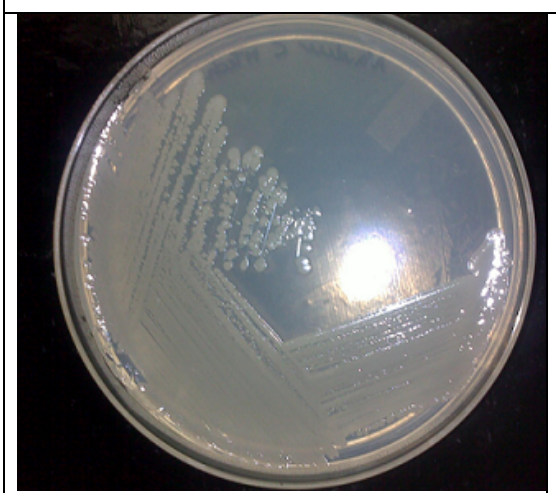
The biodegradation of plastic films under controlled composting conditions was the result of synergistic effect of microorganisms in the compost. Isolation of the microorganisms from the compost can be helpful for further understanding the biodegradation of the plastic films. Six different strains were isolated from the compost, namely TU1, TU2, TU3, TU4, TU5 and TU6 (Fig. 4.9 & 4.10). Strain TU1 was a convex shape, whitish opaque, regular, pin head type colony, gram negative rod shape bacteria. Strain TU2 was a flat surface, wavy in shape, filamentous type colony formations and whitish, gram-positive, rod shape bacteria. Strain TU3 was a flat surface, irregular, wavy, whitish colony formations, spherical gram-negative cocci in shape. Strain TU4 was a flat surface, whitish, circular colony formations, gram-negative rod shape bacteria. Strain TU5 circular, regular, flat surface, spherical, gram positive cocci. Strain TU6 was irregular, wavy, whitish, flat surface, spherical, gram-positive cocci. Detailed characterization of these strains are still needed to exactly indentify the bacteria.

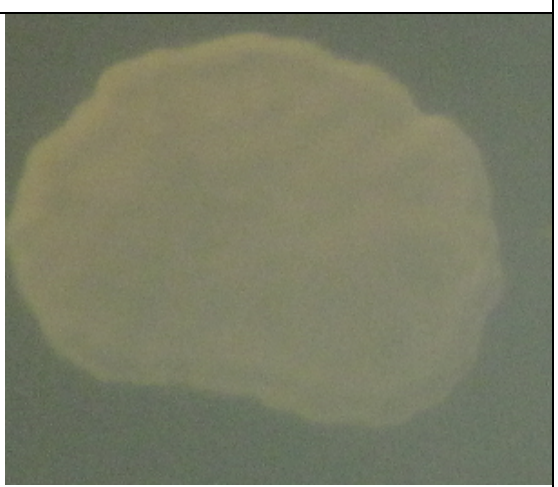
Images of petriplates showing the isolated colonies.	**Images of the isolated colonies under stereo-zoom microscope**
	
Strain TU1	



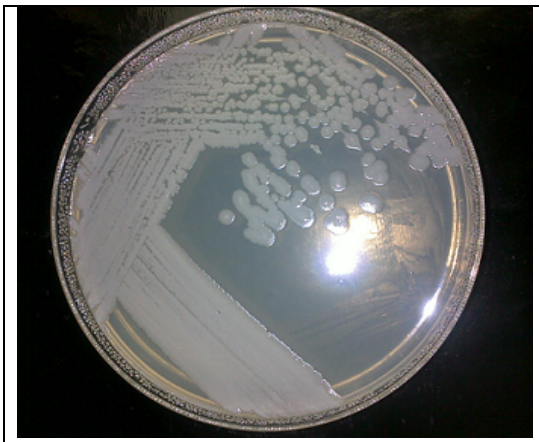


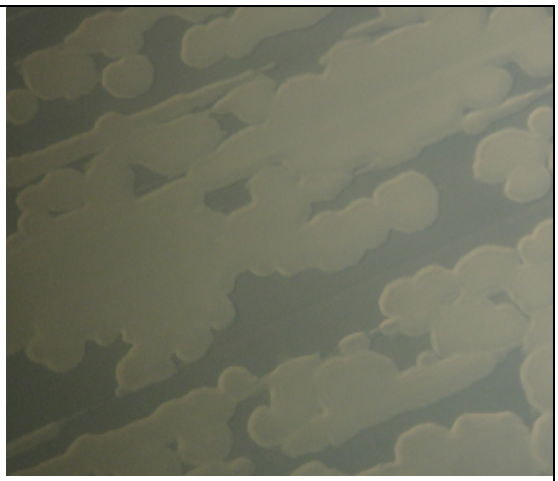
Strain TU2



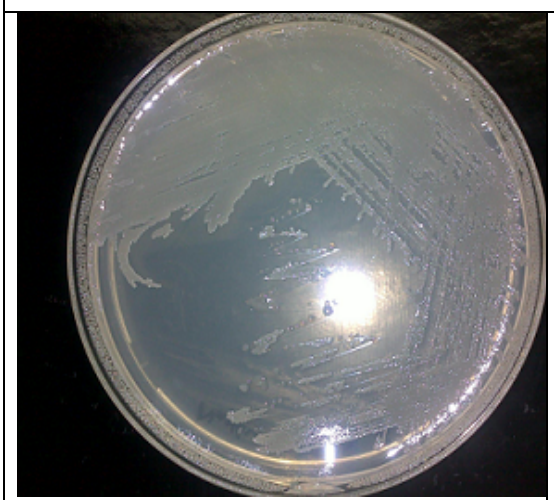


Strain TU3

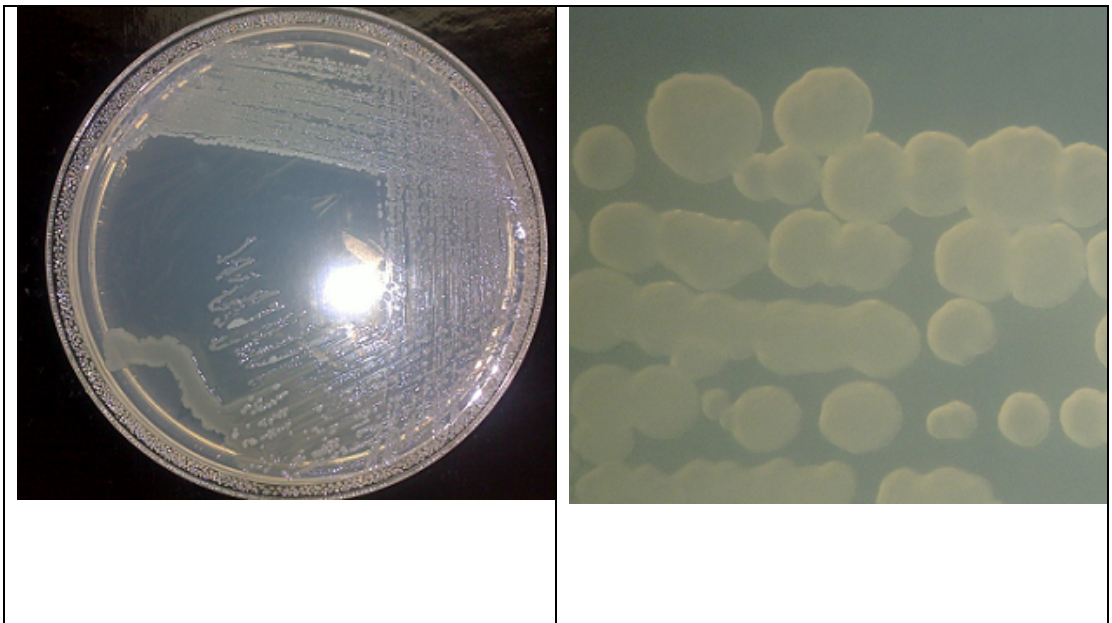




Strain TU4



Strain TU5



Strain TU6

Fig 4.9 Photographs of the petriplates showing the isolated colonies and the isolated colonies under stereo-zoom microscope from the compost.

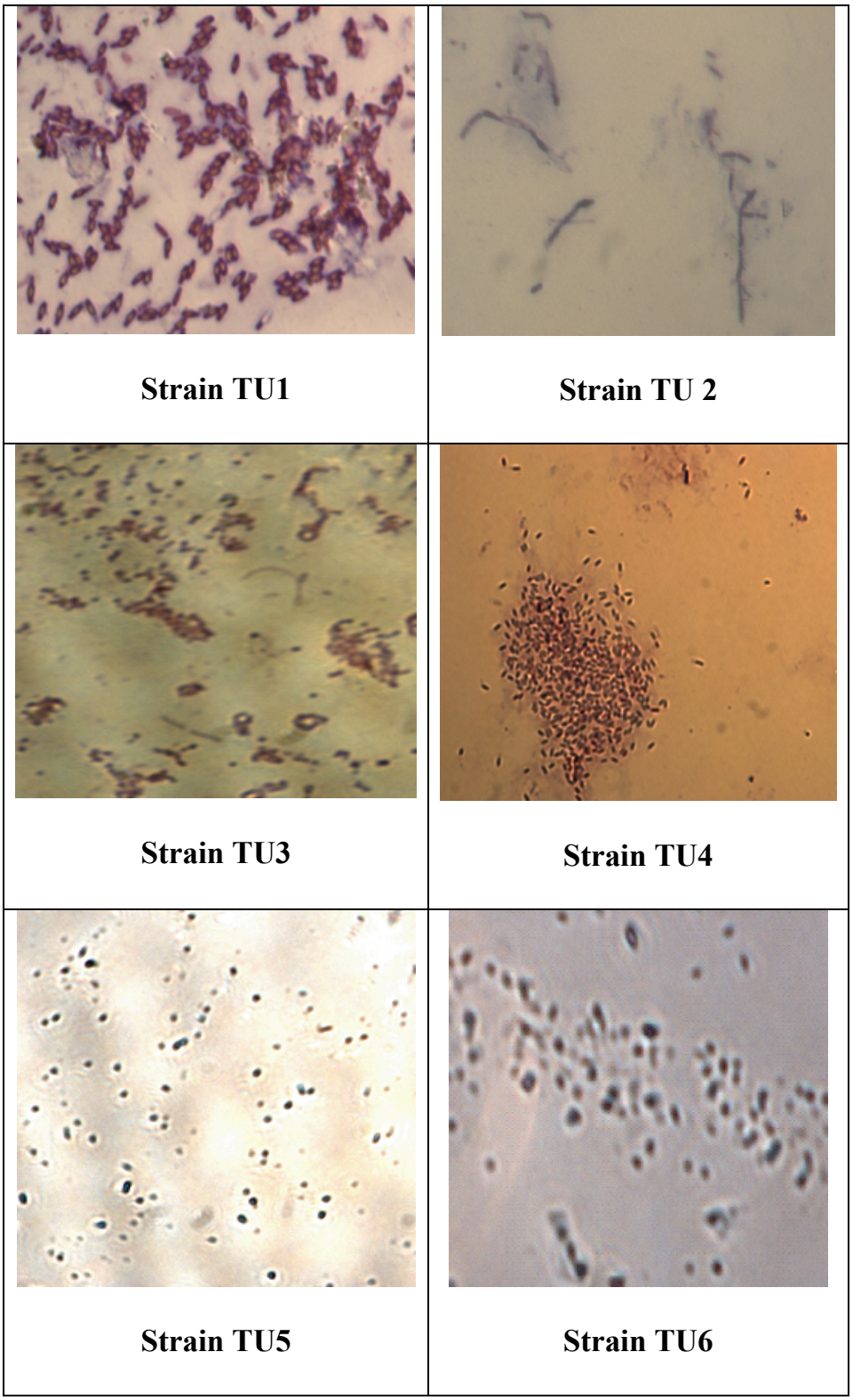


Fig 4.10 Optical microscopic studies of the microorganisms isolated from the compost

4.2.2. Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis.*

4.2.2.1. Weight loss (%)

In this current study, a maximum weight loss of 10.7 % was observed with LLDPE under incubation with *B. borstelensis* within 30 days of incubation which shows that LLDPE 100 is degradable with *B. borstelensis*. Weight loss of 1.22 % with LLDPE 80, 0.74 % with M-g-L and 2.17% with CSF under incubation was observed Table 4.7 and Fig.4.11 show that these blends when mixed with PLLA do not undergo degradation. Hence this culture seems to degrade pure LLDPE and not PLLA.

Table 4.7 Weight loss (%) of the plastic films before and after treatment

Sample	Before treatment (weight in mg)	After treatment (weight in mg)	Weight loss (%)
LLDPE 100	309.2	276.1	10.7
LLDPE 80	278.8	268.8	3.58
M-g-L 80/4	390.2	387.3	0.74
CSF	291.4	285.2	2.12

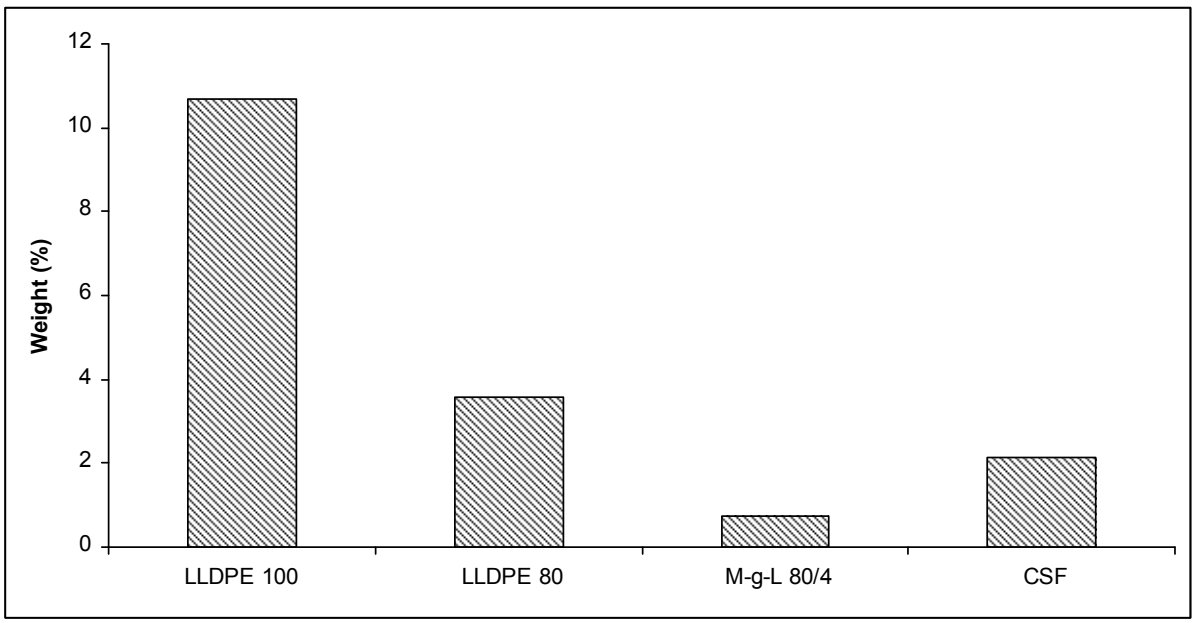


Fig 4.11 Percentage weight loss of plastic films after treatment

4.2.2.2. XRD analysis

The X-ray diffraction shows that there is an increase in crystallanity of LLDPE 100 28.18%, LLDPE 80 by 22.66% and of CSF by 2.11% (Table 4.8 and Fig. 4.12) probably due to assimilation of the amorphous part of the polymer by the bacteria and thus only crystalline region of the plastic film is left behind leading to increase in the crystallanity but in the case of M-g-L 80/4, there is no increase in the crystallanity. However, be due to the fact that the degradation of the fibers occurred from the outside surface of the fibers. The compatibilzer maleic anhydride has higher resistance to microorganisms than PLA and LLDPE in the fibers probably wraps LLLA and LLDPE and protects it from degradation. Therefore, LLDPE in the polyblends with a compatibilizer has much lower degradation than in the pure fibers.

Table 4.8 Crystallanity (%) of the plastic films before and after treatment

Sample	Crystallanity (%) (Before treatment)	Crystallanity (%) (After treatment)
LLDPE 100	70.61	98.79
LLDPE 80	75.06	97.72
M-g-L 80/4	71.78	69.60
CSF	60.29	80.4

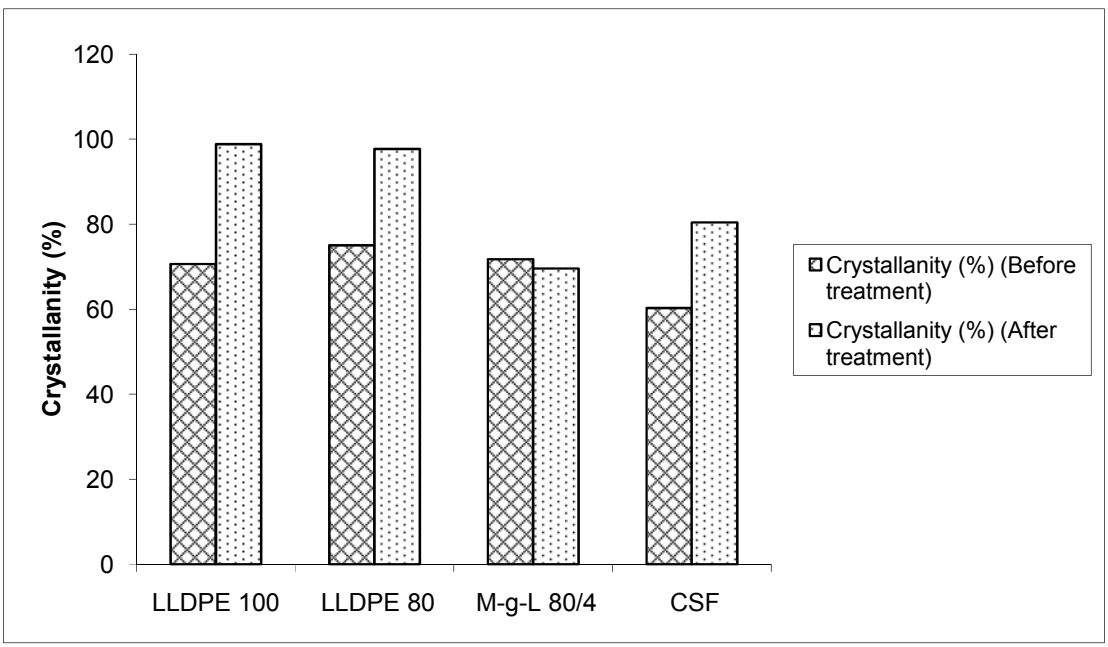


Fig 4.12 Crystallanity (%) of the plastic films before and after treatment

4.2.2.3. FTIR spectroscopic analysis

Fourier transform infrared spectroscopy (FTIR) analysis is a useful tool to determine the formation of new or disappearance of functional groups. So degradation products, chemical moieties incorporated into the polymer molecules such as branches, co-

monomers, unsaturation and presence of additives such as antioxidants can be determined by this technique.

The FTIR spectrum of LLDPE 100 when incubated with *B. borstelensis* for 30 days showed a typical carbonyl peak at 1712 cm^{-1} which showed a marked reduction in the amount of carbonyl residues (Hadad *et al.* 2005) as shown in Table 4.9. The reduction in carbonyl residues was also estimated in terms of a carbonyl index, which is the ratio between the absorbance peaks of carbonyl at 1712 cm^{-1} to that of CH_2 at 1462–1463 cm^{-1}. It was found that the incubation of LLDPE 80 and M-g-L 80/4 with *B. borstelensis* reduced the carbonyl index but there was no change in case of CSF.

Table 4.9 Carbonyl index obtained from FTIR spectra of the plastic sample before and after composting

Samples	**Carbonyl index** ($A_{C=O}$:A_{CH2})*	
	Before treatment	**After treatment**
LLDPE 100	Nil	1.05
LLDPE 80	1.10	1.03
M-g-L 80/4	1.00	0.97
CSF	Nil	Nil

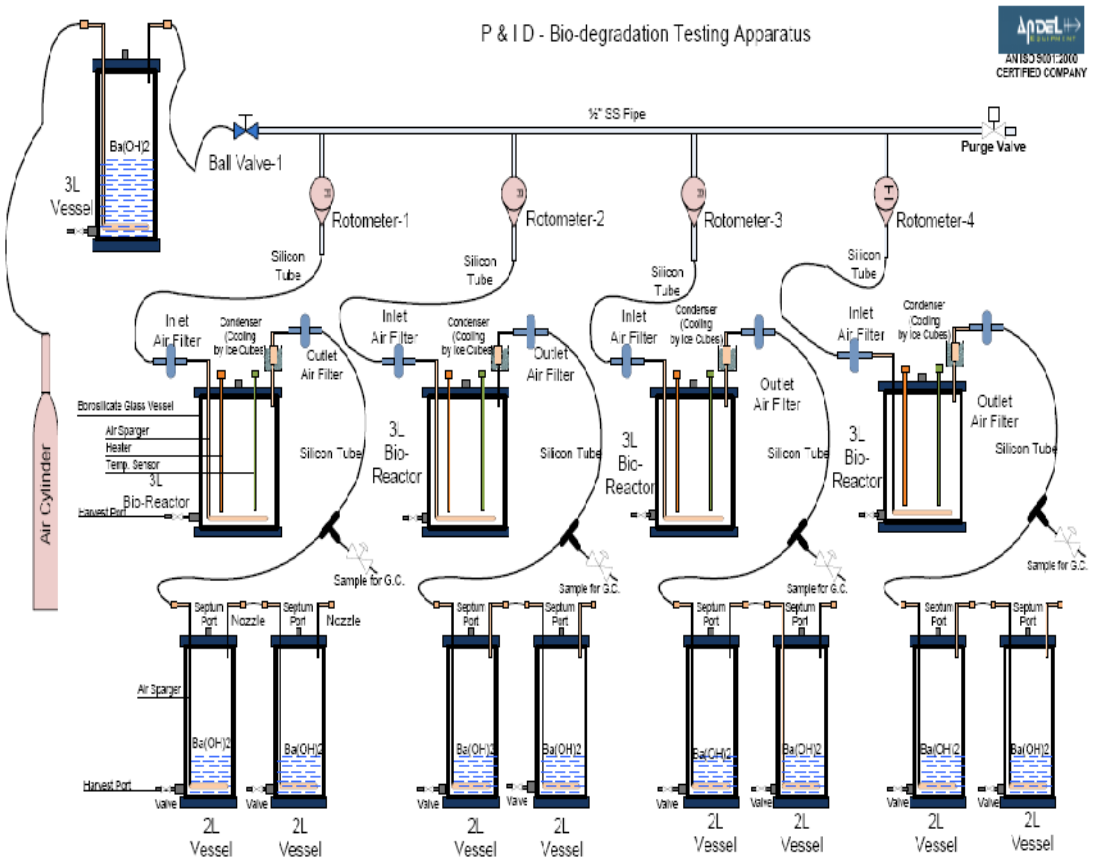
*The carbonyl index expresses the ratio between the absorbance peak of the carbonyl (1712 cm^{-1}) and that of the CH_2 groups at 1462– 1463 cm^{-1}.

4.2.3 Biotic treatment as per ASTM D5338 (2003) standard for aerobic treatment using municipal solid compost

Important information concerning polymer's final fate in the environment can be achieved in biodegradation studies performed in the composting environment. Biodegradable plastics that will be compostable in an appropriate composting infrastructure has been designed according to Standard Test Method for Determining the Aerobic Biodegradation of Plastic Materials under Controlled Composting Conditions as per ASTM D5338 (2003) (Fig.4.13 & 4.14). The fate of these organic polymers in the environment and the time required for their total mineralization to CO_2 have yet to be fully understood. A

preliminary test had been done but the results were not appreciably reliable because the time period for which composting had been done was for very short duration and it needs much more time for composting to occur which can be studied further.

As reported by Albertsson and Karlsson (1990) that polyethylene subjected to 26 days of artificial UV irradiation before being buried in soil evolved less than 0.5% carbon (as CO_2) by weight after 10 years. Without prior irradiation, less than 0.2% carbon dioxide was produced. For polymer degradation in soil, CO_2 detection proved to be more complicated than in compost because of slower degradation rates that led not only to long test durations (upto 2 years) but also low CO_2 evolution as compared to that from the carbon present in soil. One means of overcoming problems with background CO_2 evolution from the natural matrices compost or soil is to use an inert, carbon-free and porous matrix, wetted with a synthetic medium and inoculated with a mixed microbial population. This method proved practicable for stimulating compost conditions (degradation at~60 °C) (Bellina *et al.,* 2000), but has not yet been optimized for soil conditions.



TITLE	P & ID Bio-degradation Testing Apparatus			CLIENT Thapar University, Patiala	
	NAME	SIGN	DATE	PROJ. CODE	NA
DRAWN	Ajay Banga			DRAWG. NO.	NA
APPRV'D	Karthik			PART NO.	NA
Q.A				SCALE	NA
ANDEL EQUIPMENT (P) Ltd # 298, Industrial Area, Ph-5, Mohaliph 0172-2213851					

Fig 4.13 Design of biodegradability test apparatus for the according to standard ASTM D5338 (2003)

Fig 4.14 Biodegradability test apparatus according to standard ASTM D5338 (2003)

Table 4.10 Total carbon content in the plastic samples

Samples	Weight of the sample (mg)	Total carbon (mg)
LLDPE 100	8.7	7.360
LLDPE 80	9.1	7.542
M-g-L 80/4	8.9	6.972
PLLA 100	10.7	5.492

Chapter-5
Conclusions

To reduce the impact of plastic waste on the environment, two main efforts have generally been pursued: one is to synthesize biodegradable plastics, and the other to isolate selected microorganisms to biodegrade plastic wastes. Polyethylene is widely used as a packaging material. Traditionally, the polyethylene is non-biodegradable. To develop biodegradable polyethylene, biodegradable polymers such as PLLA, a natural biodegradable polymer, are added to synthetic plastics to make them biodegradable.

By heat degradation (thermal aging), high molecular mass materials are broken down to low molecular mass intermediates which can be seen by the loss in mechanical properties of LLDPE blends that can be continuously biodegraded by microorganisms.

Important information concerning polymer's final fate in the environment can be achieved in biodegradation studies performed in the composting environment. Biodegradable plastics that will be compostable in an appropriate composting infrastructure which has been designed according to Standard Test Method for Determining the Aerobic Biodegradation of Plastic Materials under Controlled Composting Conditions D5338 (2003) has been done. The fate of these organic polymers in the environment and the time required for their total mineralization to CO_2 have yet to be fully understood.

Biodegradation of LLDPE and its blends in compost was very fast. The film was assimilated by the microorganisms upto some extent over the period of 28 days. The obtained results indicate that LLDPE and its blends were very sensitive to enzymatic attack of microorganisms in living environment. DSC studies of LLDPE and its blends incubated in compost lead to the degradation of amorphous phase, resulting in an increase in crystallinity of the polymer. Blending LLDPE and PLLA improves the resistance of LLDPE to hydrolysis and biodegradation of LLDPE occurs. A maximum loss of tensile strength 19.9 % was observed with LLDPE 100, 54.11% with LLDPE 80, 34.3% with M-g-L 80/4 and 56.6 % with CSF respectively under composting for 28 days which shows that after composting there is a loss of mechanical properties even in LLDPE 100 and its

blends with PLLA. The X-ray diffraction shows that there is an increase in crystallanity of 4.13% with LLDPE 80, 10.16% with CSF this is due to the assimilation of the amorphous part of the polymer by the bacteria and thus only crystalline region of the plastic film is left behind leading to increase in the crystallanity but in the case of M-g-L 80/4 there is no increase in the crystallanity may be due to the fact that the degradation of the fibers should occur from the outside surface of the fibers. The compatibilzer maleic anhydride has higher resistance to microorganisms than PLA and LLDPE in the fibers probably wraps PLLA and LLDPE and protects it from degradation. Therefore, LLDPE in the polyblends with a compatibilizer has much lower degradation than in the pure fibers. But there is no change in the crystallanity of LLDPE 100 after composting it for 28 days. In addition to improving the properties of LLDPE, the LLDPE/PLLA polyblend fibers will have relatively low price and can be engineered to have good dyeability and controlled degradability for medical and textile applications.

Microbial degradation of a solid polymer, such as polyethylene, requires the formation of a biofilm on the surface of the polymer to enable the microorganism to efficiently utilize the non-soluble substrate. Indeed, *Brevibacillus borstelensis* effectively colonized the polyethylene surface. This may explain the relatively rapid biodegradation of polyethylene, which was evident (as measured by weight loss, XRD and FTIR) as early as 30 days after inoculation.

It is seen that polyethylene—considered to be inert—can be biodegraded if the right microbial strain is isolated. They used enrichment culture methods which were found to be effective for isolating a thermophilic bacterium (*Brevibaccillus borstelensis*) capable of utilizing polyethylene as the sole carbon and energy source. In this current study, a maximum weight loss of 10.7 % was observed with LLDPE under incubation with *B. borstelensis* within 30 days of incubation which shows that LLDPE 100 is degradable with *B. borstelensis* which showed that these blends when mixed PLLA does not undergo degradation. Hence this culture is purely degrades LLDPE and not PLLA.

Incubation of LLDPE and its blends (LLDPE 80, M-g- L 80/4 and CSF) with B. borstelensis (30 days, at 50 ^{0}C) showed weight loss of 3.58 % with LLDPE 80, 0.74 % with M-g-L and 2.17% with CSF under incubation. The FTIR spectrum of LLDPE 100 when incubated with *B. borstelensis* for 30 days showed a typical carbonyl peak at 1712

cm^{-1} which showed a marked reduction in the amount of carbonyl residues (Hadad *et al.* 2005) as shown in Table 4.9. The reduction in carbonyl residues was also estimated in terms of a carbonyl index, which is the ratio between the absorbance peaks of carbonyl at 1712 cm^{-1} to that of CH_2 at 1462–1463 cm^{-1}. It was found that the incubation of LLDPE 80 and M-g-L 80/4 with *B. borstelensis* reduced the carbonyl index but there was no change in case of CSF.

There is a growing interest in the development of degradable plastics to enhance the biodegradability of the plastics in landfills and composts. The degradable plastic must still retain all of the physical properties expected by the consumer and, then, when placed in the appropriate environment, degrade more rapidly than conventional disposable plastics.

Recommendations for Future Work

- To increase our knowledge about biodegradation of LLDPE, we need to put more efforts into identifying LLDPE-degrading microorganisms isolated from the compost. An understanding of the mechanisms of both natural and synthetic polymer degradation by microorganisms and enzymes will open new prospects in the field of biodegradable plastics.
- Composting according to ASTM D5338 needs more detailed study to know the fate of these organic polymers in the environment and the time required for their total mineralization to CO_2 have yet to be fully understood. A preliminary test had been done but the results were not appreciably reliable because the time period for which composting had been done was for very short duration and it needs much more time for composting to occur which can be studied further.
- Composting under controlled conditions gave appreciablly very good results in a very short duration ie. 28 days. May be if the time interval for composting conditions is increased it could produce better results.
- Abiotic treatment is done here by thermal aging and only time interval is selected which is best suited but still the temperature has to be selected. Moreover, numeber of other abiotic treatments are available like UV treatments, hydrolytic aging, photo-oxidative aging before it is subjected to biotic treatment.

- In the case of pure culture, other cultures which are available for the degardation of polyethylene can be tested upon and the results can be compared with *Brevibacillus borstelensis*.

References

Albertsson A C. Biodegradation of synthetic polymers. Limited microbial conversion of C-14 in polyethylene to (CO-2)-C-14 by some soil fungi. Journal of Applied Polymer Science 1978;22:3419–3433.

Albertsson A C. The shape of the biodegradation curve for low and high density polyethylenes in prolonged series of experiments. European Polymer Journal 1980;16:623-630.

Albertsson A C, Karlsson S. The influence of biotic and abiotic environments on the degradation of polyethylene. Progress in Polymer Science 1990;15:177–192.

Albertsson A C, Andersson S O, Karlsson S. The mechanism of biodegradation of Polyethylene. Polymer Degradation and Stability 1987;18:73-87.

Albertsson A C, Sares C, Karlsson S. Increased biodegradation of LDPE with nonionic surfactants. Acta Polymerica 1993;44:243-246.

Albertsson A C, Erlandsson B, Hakkarainen K, Karlsson S. Molecular weight changes and polymeric matrix changes correlated with the formation of degradation products in biodegraded polyethylene. Journal of Environmental Polymer Degradation 1998;6:187-195.

Abd-El-Rehim H A, Hegazy EI-Sayed A, Ali A M, Rabie A M. Synergistic effect of combining UV-sunlight-soil burial treatment on the biodegradation rate of LDPE. Photochem Photobiol A Chem 2004;163:547-556.

ASTM Designation D5338-03 Standard Test Method for Determining Aerobic Biodegradation of Plastics Materials Under Controlled Composting Conditions, American Society for Testing and Materials , Philadelphia.

Bonhomme S, Cuer A, Delort A-M, Lemaire J, Sancelme M, Scott G. Environmental biodegradation of polyethylene. Polymer Degradation and Stability 2003;81:441.

Bellina G, Tosin M, Degli-Innocenti F. The test method of composting in vermiculite is unaffected by the priming effect. Polymer Degradation and Stability 2000;69:113–20.

Briassoulis D, Aristopoulou A, Bonora M, Verdolt I. Degradation characterization of agricultural low density polyethylene films. Biosystem Engineering 2004;88:131-143.

Brown B S, Mills J, Hulse J M. Chemical and biological degradation of plastics. Nature 1974;250:161-163.

Byuntae L, Anthony L P, Alfred F, Theodore B B. Biodegradation of degradable plastic polyethylene by *Phanerocheate and Streptomyces species*. Applied Environmental Microbiology 1991;57: 678–688.

Chiellini E, Corti A, Swift G. Biodegradation of thermally-oxidised. Fagmented low density polyethylenes. Polymer Degradation and Stability 2003;81:341-351.

Chiellini E Corti A, D'Antone S, Baciu R. Oxo-biodegradable carbon backbone polymers oxidative degradation of polyethylene under accelerated test conditions. Polymer Degradation and Stability 2006;91:2739-2747.

Cornell J H, Kaplan A M, Rogers M R. Biodegradation of photooxidized polyalkanes. Journal of Applied Polymer Science 1984;29:2581-2597.

Doi Y, Kanesawa Y, Tanahashi N, Kumagai Y. Biodegradation of microbial polyesters in the marine environment. Polymer Degradation and Stability 1992;36:173–177.

Eggins HOW, Mills J, Holt A, Scott G. In: Sykes G, Skinner FA, editors. Microbial Aspects of Pollution. London: Academic Press;1971:267–77.

Erlandsson B, Karlsson S, Albertsson A C. The mode of action of corn starch and a pro-oxidant system in LDPE: influence of thermooxidation and UV-irradiation on the molecular weight changes. Polymer Degradation and Stability1997;55:237–245.

Ehara K, Lioyshi Y, Tsutsumi Y, Nishida T. Polyethylene degradation by manganese peroxidase in the absence of hydrogen peroxide. Journal of Wood Science 2000; 46:180-183.

Gilan I, Hadar Y, Sivan A. Colonization, biofilm formation and biodegradation of polyethylene by a strain of *Rhodococcus ruber.* Applied Microbiology and Biotechnology 2004;65:97-104.

Hadad D, Vanesh S, Sivan A. Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*. Journal of Applied Microbiology 2005;98:1093–1100.

Haines J R, Alexander M. Microbial degradation of high molecular-weight alkanes. Applied Microbiology and Biotechnology 1974; 28 1084.

Jakubowicz I. Evaluation of degradability of biodegradable polyethylene (PE). Polymer Degradation and Stability 2003; 80:39–43.

Lee B, Pometto III A L, Fratzke A, Bailey T B. Biodegradation of degradable plastic polyethylene by *Phanerochaete* and *Streptomyces* species. Applied and Environmental Microbiology 1991;57:678–685

Maiti S, Jana T. Biodegradable polymers. Polymer Recycle and Waste Management, Anusandhan Prakashan, Midnapore, 2005.

Nageotte V, Pestre C, Cruard-Pradet T, Bayard R. Aerobic and anaerobic biodegradability of polymer films and physic- chemical characterization. Polymer Degradation and Stability 2000;620-627.

Orhan Y, and Buyukgungor, H. Enhancement of biodegradability of disposable polyethylene in controlled biological soil. International Biodeterioration and Biodegradation 2000;45:49-55.

Otake Y, Kobayashi T, Ashabe H, Murakami N, Ono K. Biodegradation of low-density polyethylene, polystyrene, polyvinyl-chloride, and urea-formaldehyde resin buried under soil for over 32 years. Journal of Applied Polymer Science 1995;56 1789-1796.

Otake Y, Kobayashi T, Murakami N, Ono K. Oxidative degradation and molecular weight change of LDPE buried under bioactive soil for 32–37 years. Journal of Applied Polymer Science 1998;70:1643–1648.

Palmisano A C, Pettigrew C A. Biodegradability of plastics. Bioscience 1992;42:680–685.

Pinchuk L S, Makarevich A V, Vlasova G M, Kravtsov G A, Shapovalov V A. Electret Thermal analysis to assess biodegradation of polymer composites. International Biodeterioration & Biodegradation 2004;54:13–18.

Potts J E, Biodegradation. In: Aspects of Degradation and Stabilization of Polymers ed. Jelinek, H.H.G. pp. 617-658. New York: Elsevier. 1978.

Raghavan D. Characteristics of biodegradable plastics. Polymer Plastic Technology 1995.;34(1): 41-63.

Roy P K, Surekha P, Rajagopal C, Choudhary V. Effect of cobalt carboxylates on the photo oxidative degradation of low-density polyethylene. Part-I. Polym Degrad Stab 2006a;91:1980-1988.

Roy P K, Surekha P, Rajagopal C, Raman R, Choudhary V. Study on the degradation of LDPE in the presence of cobalt stearate and benzyl. Journal of Applied Polymer Science 2006b;99:236-243.

Roy P K Surekha P, Rajagopal C, Chatterjee S N, Choudhary V. Studies on the photo-oxidative degradation of LDPE films in the presence of oxidised polyethylene. Polymer Degradation and Stability 2007;92:1151-1160.

Rutkowska M, Krasowska K, Heimowska A, Steinka, Janik H, Haponiuk J, Karlsson S. Biodegradation of modified poly(ε-caprolactone) in different environments. Polish Journal of Environmental Studies 2002;11:413-420.

Saha N, Zatloukal M, Saha P. Modification of polymers by protein hydrolysate—a way to biodegradable materials. Polymers for Advanced Technologies 2003;14:854–860.

Scott G. In: Scott G, editor. Degradable Polymers: Principles and Applications. 2nd ed. Netherlands: Kluwer; 2002,Chapter 3.

Scott G. Polymers in Modern Life. Polymers and the Environment. Cambridge, UK: The Royal Society of Chemistry 1999.

Sepulveda V, S-Castaneda T, G-Rojas M, Manzur A, F-Torres E. Thermally treated low density polyethylene biodegradation by *Pencillium pinophilum* and *Aspergillus niger*. Journal of Applied Polymer Science 2002;83:305-314.

Sivan A, Szanto M, Pavlov V. Biofilm development of the polyethylenedegrading bacterium *Rhodococcus ruber*. Appl Microbiol Biotechnol 2006;72(2):346–52.

Shah A A, Hasan F, Hameed A, Ahmed S. Biological degradation of plastics: A comprehensive review. Biotechnology Advances 2008;26:246-265.

Singh G, Bhunia H, Rajor A, Jana R N, Choudhary V. Mechanical properties and morphology of polylactide, linear low-density polyethylene and their blends. Journal of Applied Polymer Science. DOI 10.1002/app.32305 2010

Volke S T, Saucedo C G, Gutierrez R M, Manzur A, Favela T E. Thermally treated low density polyethylene biodegradation by Penicillium pinophilum and Aspergillus niger. Journal of Applied Polymer Science 2001;83:305–314.

Vogel H J, Bonner M D. Acetylornithinase of *E. coli* partial purification and some properties. Journal of Biological Chemistry 1956;218:97–106.

Weiland M, Daro A, David C. Biodegradation of thermally oxidised polyethylene. Polymer Degradation and Stability 1995;48:275–289.

Yamada O K, Mukumoto H, Katsuyaya Y, Saiganji A, Tani, Y. Degradation of polyethylene by a fungus, *Penicillium simplicissimum* YK. Polymer Degradation and Stability 2001;72:323.

Publications

1) Kaur N, Rajor A, Singh G, Bhunia H and Bajpai P K. Advances in biodegradability of plastics – Process and Standards. In: Proc. International Conference on Recent Trends in Management, Technology and Research. Published by Macmillan Press (2009), p.129-136.

2) Kaur N, Singh G, Bajpai P K and Bhunia H. National Conference on Chemical Engineering : Opportunities and Challenges, SLIET, Longowal, Distt. Sangrur. March 5 & 6, 2010.