UNIVERSITY OF PISA

Galileo Galilei School

PhD Course in Chemical Science- XXIII cycle (2008-2010)

Oxo-Biodegradation of Full Carbon Backbone Polymers under Different Environmental Conditions

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CERTIFICATE

This is to certify that this thesis entitled **"Oxo-biodegradation of Full Carbon Backbone Polymers under Different Environmental Conditions"** has been submitted by **M. Sudhakar** (Reg no: 423877) for the degree of Doctor of Philosophy (Ph.D) in Chemical Science, to University of Pisa, Pisa - 56126, Italy. This work or any part of thereof has not been submitted elsewhere for any other degree.

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DECLARATION

I do hereby declare that this work was carried out by me under the guidance and supervision of Prof. Dr. Emo Chiellini, and day-by-day tutoring by Dr. Andrea Corti, INSTM Laboratory of Bioactive Polymeric Materials for Biomedical and Environmental Applications (BIOlab), Department of Chemistry & Industrial Chemistry, University of Pisa, via Risorgimento 35, 56126 Pisa, Italy. This work or any part of thereof has not been submitted elsewhere for any other degree.

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Dedicated to my Parents
&
Teachers

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ACKNOWLEDGEMENTS

I am indepted with respect to my supervisor Prof. Emo Chiellini, for giving me an opportunity to work under him. His able guidance and constructive criticism helped me to progress in research pursuit. I am thankful for his constant encouragement and valuable contribution of his time throughout the course of my thesis. It has been a great honor for me to work under his inspiring guidance.

My sincere thanks to my tutor Dr. Andrea Corti for sharing his academic experience for the successful completion of the objective undertaken. His valuable suggestions and timely discussions enabled me to achieve this work with precision and build up a solid background in the performance of the tests for the assessment of biodegradation of polymeric materials and relevant items under different environmental conditions.

I am very thankful to Prof Syed H. Imam, USDA, Agricultural Research Services, USA for his suggestions and discussions during the part of my research work.

I am very thankful to financial support by Galileo Galilei PhD school, and Department of Chemistry, University of Pisa, Italy.

I would like to thank the following industries - Ciba Chemicals (Italy), Polymeri Europa (Italy), Symphony Polymers (UK), and EPI (Canada), which provided polymeric materials and support throughout the course of my work.

I am very much thankful to Dr. Federica Chiellini, Dr. Vassilka IIieva, Dr. Elisabeth Grillo Fernandas, Dr. Stefania Cometa, and Dr. Marcella Ferri during laboratory work.
I also thankful to Dr. Narducci, Dept of Chemical Engineering, University of Pisa – Italy, who performed SEM analysis of samples.

My special thanks to Maria Viola, Maria Caccamo and Michela Bianchi. Persons that help me in all situations!

I would like also to thank several people from the BIOLAB for all the time we spent together in these years, for share the good times and for the help that they gave me in the moments I needed. Great colleagues like Dario, Anna Maria Piras, Antonella, Carlos, Cesare, Dino, Gastone, Matteo, Marco, Mohamed, Alberto, Mamoni, Catherine and all the others that are too long to list...

Finally, I am greatly indebted to my family. My family encouraged me in all moments providing emotional support and love for my carrier.

LIST OF ABBREVIATIONS

AL:	Alkali Lignin
CO_i:	Carbonyl Index
C_S:	Amount of carbon in the sample
DSC:	Differential Scanning Calorimetry
Ea:	Activation Energy
EC:	European Community
EEA:	European Environmental Agency
EDP:	Environmentally Degradable Plastics
EPI:	Environmental Technologies Inc.
EU:	European Union
FDA:	Food and Drug Administration
FTIR:	Fourier Transform Infrared Spectroscopy
GPC:	Gel Permeation Chromatography
HDPE:	High-Density Polyethylene
HPLC:	High Pressure Liquid Chromatography
ID:	Molecular Weight Distribution
k:	Rate Constant
LDPE:	Low-density polyethylene
LLDPE:	Linear low-density polyethylene
MFI:	Melt Flow Index
Mn:	Number-Average Molecular Weight
MSM:	Mineral Salt Medium
MW:	Weight-Average Molecular Weight
NR:	Natural rubber
PE:	Polyethylene
PP:	Polypropylene
PS:	Polystyrene
PNSL:	Pine Nut Shells Lignin
PLA:	Poly Lactic Acid
PDA:	Potato Dextrose Agar
ROO_i:	Hydroperoxide Index

SB:	Soil Burial
SEC:	Size Exclusion Chromatography
SEM:	Scanning Electron Microscopy
Tm:	Melting temperature
T_c:	Crystallization Temperature
Tonset:	Decomposition Temperature
TDPA®:	Totally Degradable Plastics Additives
TD:	Thermally Degraded
T_g:	Glass Transition Temperature
TGA:	Thermogravimetric Analysis
$ThCO_2$:	Theoretical Amount of CO_2
TOC:	Total Organic Carbon
UV:	Sunlight Exposed
W_S:	Total Sample Amount
X_c:	Crystallinity Degree

SUMMARY

The end-of-life management of post-consumer plastic materials plays an important role in the development of sustainable polymer products. Over the last few years the growing effort to find environmentally more friendly solutions led to the support of biodegradable materials as an alternative to poly(hydrocarbon) (PE, PP, PS). In parallel PE, PP, PS full-carbon backbone thermoplastic polymers have been re-engineered by addition of pro-oxidants able to promote the carbon backbone oxidation eventually followed by backbone breakdown.

The major strategies in order to overcome the intrinsic recalcitrance of polyolefins to biological attack have been focused on the introduction of functional groups and chemical components (pro-oxidants) able to promote the formation of free radicals at carbon backbone susceptible to uptake oxygen with the formation of hydroperoxides. These last give raise to a free radical chain reaction leading to an abiotic, thermally and/or photophysical assisted breakdown of the polymer backbone with formation of oxidized groups. The oxidized fragments are vulnerable to microorganisms leading to a biotic phase with digestion of the chain fragments to CO_2, H_2O and cell biomass.

The production and consumption of plastics, in the last decade has recorded a remarkable increase in the scientific and industrial interest in environmentally degradable polymers and relevant plastic items (EDPs). Since the ultimate fate of EDPs has to be their conversion by microorganisms into metabolites such as CO_2, H_2O and new cell biomass (i.e. mineralization). The requirement of two steps, abiotic and biotic, in the degradation mechanism of oxo-biodegradable plastic items has recently inspired the definition and approval by the American Society for

Testing and Materials (ASTM) of a Standard Guide ASTM D6954-04 "*Standard guide for exposing and testing plastics that degrade in the environment by a combination of oxidation and biodegradation*". Analogous initiative was undertaken soon after the approval in 2002 of the EN 13432 norm on "*Requirements for packaging recoverable through composting and biodegradation – Test scheme and evaluation criteria for the final acceptance of packaging*" by British Standard Institute (BSI) as it was considered too discriminatory toward large consume plastic commodities. The norm BSI 8472 is in progress and when approved should give the input for an extend approval to EC- Countries.

The new strategic vision aiming at reengineering polymeric formulations based on well known biostable full carbon backbone polymers convertible to eco-compatible plastic items imply the following steps:

- *Abiotic treatment meant to promote and assist the oxidative degradation under different environmental conditions.*
- *Biotic digestion of the oxidized polymer fragments.*
- *Assessment of ultimate environmental fate of the analyzed samples and their impact on toxicity.*

Abiotic degradation studies, carried out under different test conditions, were performed in order to establish the role of pro-oxidant additives in enhancing the rate and extent of oxidation and evaluation of full-carbon backbone chain scissions as a prerequisite to promote the attack by microorganisms and finally to end up with biodegradation.

The propensity to oxidation in terms of rate and extent was found to be dependant upon the following abiotic parameters:

1) Type and amount of pro-oxidant.
2) Temperature at which the samples are exposed.
3) Outdoor exposure, time, temperature and light dose.

4) Exposure under static or dynamic conditions in oven in air atmosphere at controlled temperatures.

5) Exposure in air environment at controlled humidity level.

Combined effects, were also found to be dependent upon the cross-action of abiotic parameters and structural characteristics of the analyzed samples. Poly(ethylene) (LDPE, LLDPE, HDPE), Poly(propylene) (PP, BOPP), Poly(styrene) (HIPS, CPS).

Thermal oxidation was particularly effective in the case of PE and PP samples, whereas only minor effects were ascertained in the case of PS. The rate and extent of oxidation of PE/PP samples were positively affected by both temperature and oxygen partial pressure, whereas a slight drop in rate and extent of oxidation was found to be associated to the humidity level in the case of PE, but not in the case of PP samples. On the other hand, sunlight outdoor exposure (3 months late spring/early summer) resulted less efficient in promoting oxidation of the analyzed LLDPE samples. This behavior can be attributed in a first instance to the ambient temperature monitored during the test that was in any case below 35°C. On the contrary, thermal degradation behavior of samples previously submitted to outdoor sunlight exposure appeared to be different from that exhibited by the pristine samples submitted only to thermal degradation tests. The absence of induction phases in the oxidation processes of light exposed samples was evident during the tests. These observations may suggest that the initiation of the oxidation process, as promoted by light irradiation, positively affected the rate of oxidation once the samples were submitted to a thermal treatment. It can be therefore suggested that the combination of UV radiation and temperature was capable to promote oxidative degradation of the tested LLDPE samples containing pro-oxidant additives. In particular, the level of oxidation, is promoting the increase of the amount of the solvent

extractable fraction as well as a significant decrease of the relevant molecular weight that was found to correlate with the extent of oxidation (carbonyl index), as determined by FT-IR spectroscopy. This holds true particularly in the case of PP and PE samples, thus providing evidence on the statistical random scission of the polymer chains, according to Norrish I and/or Norrish II, which was accompanied by the formation of substantial amounts of low molecular weight fractions extractable by different solvents. On the contrary, in the case of PS samples, the random chain scissions does not seems to occur in spite of the presence of tertiary carbon atoms in the main chain in 1-3 positions. Instead sub-terminal oxidation and relevant release of oxidized polyaromatic moieties might be the main degradation mechanism occurring for the outdoor exposed PS samples.

In addition, GPC determinations showed that the molecular weights of solvent extractable fractions from abiotically degraded PE and PP samples are fairly low (0.4-1.9kD) and compatible with their potential vulnerability by natural occurring microorganisms. The results obtained during thermal and photo degradation tests are therefore demonstrating that the polyolefin matrices can be effectively oxidized by using pro-oxidant additives based on transition metal organic salts, as well as that the rate and extent of the oxidation processes is depending upon the environmental conditions.

A study was undertaken on the oxo-biodegradable materials preparation of LDPE/alkali lignin (AL) blends in abscnce or presence of pro-oxidant additives. The obtained results, even within the limits of the number of the samples investigated, are therefore ultizing natural auto-oxidazable and biodegradable components. This may represent an useful intriguing implementation in affecting the propensity to oxo-degradation of the re-engineered polyolefins composites.

The biodegradation propensity of abiotically pre-aged (thermal and outdoor exposed) and pristine polyolefin samples have been ascertained in aqueous and soil burial conditions as aimed at establishing the mineralization rate and extent of several polymeric materials, as well as to ascertain the progress of polymer oxidation and degradation of full carbon backbone polymers by natural occurring microorganisms. The microbial consumption of oxidized fractions present in abiotically degraded PE and PP films was confirmed by the decrease (30-35%) in the CO*i* values of the films submitted to the biodegradation test with respect to the starting pre-treated samples. The microbial degradation and assimilation was particularly effective in the case of solvent extracted fractions from PE and PP degraded samples. Nevertheless the higher propensity to microbial assimilation of linear oxidized fractions coming from PE with respect to fairly high branched PP fractions was observed in accordance to the role of sterical effects of side chains in refraining the microbial attacks.

During soil burial respirometric tests it was also ascertained the potential for the ultimate biodegradability of polyolefins (LLDPE, PP and PS) previously exposed to abiotic degradation tests (thermal and/or outdoor). Finally it has been interestingly found that **single soil borne microbial species are capable to promote the oxidation of pro-oxidant loaded LLDPE samples** once the process has been initiated by relatively mild degrading conditions to which the samples have been exposed, such as those related to a few months outdoor exposure. The information pertaining the level of thermal and photo-oxidation required to achieve an effective and sustained biodegradation of full-carbon backbone polymers is critical for the design of polyolefin-based products and predicting their environmental fate. The research activity undertaken during the present PhD thesis provides important information with respect to synergistic effects of microbial/enzymatic attack and physical-chemical parameters in

promoting the degradation of partially oxidized full-carbon backbone polymers, thus allowing for a better design of oxo-biodegradable materials to be really and ultimately biodegraded under different natural environments.

1. INTRODUCTION

Synthetic and semi-synthetic polymeric materials were developed for their versatility, easy processability, durability and resistance to all forms of degradation as promoted by physical, chemical and biological means or combinations thereof. Enhanced durability is achieved when required by including stabilizing additives and by processing under conditions that maximize the maintenance of molecular weight and functionality during fabrication and under subsequent service conditions. Macromolecular materials have been widely accepted for their costs, effectiveness to provide large variety of items including comfort and quality of life both in modern industrial societies and in developing countries. Moreover the demand in the next two decades for polymeric materials is expected to increase by two to three fold primarily as a consequence of an increase in plastics consumption in developing countries, with an annual growth rate worldwide of 7-10%. It was estimated a worldwide demand of plastics of about 200 million tons in 2000, 275 million tons have been indeed produced in 2010 [1].

Plastics are ubiquitous because the commercially available plastic items and commodities span a very wide range of useful properties and hence applications. It is commonly claimed that approximately more than one third of all commodity plastics are used for packaging purposes. The main reasons being associated to peculiar characteristics of plastic materials. They are inexpensive, easy to fabricate, strong, tough, and stretchy, have good barrier properties and are re-usable and recyclable, among other characteristics. The polyethylene (PE) shopping bag is an example of a common plastic article that is used in very large quantities because it provides exactly what it is supposed to do at very low cost. It has supplanted the alternatives, e.g., the brown paper bag, almost completely at check out stations because it has overall superior properties and, most

importantly, it is much less of environmental burden relevant to production and transport [2]. One criticism that is leveled at commodity plastics in short lived applications, however, is that they persist too long after they are used and discarded. It has been therefore estimated that they accumulate in the environment at a rate of 25 million tons per year. In general it has been suggested that the environmental pollution of these materials that often has been claimed as "White Pollution" can be attributable to the excessive and improper use, as well as to the lack of degradability of the post-consume items. The banning or taxing of PE shopping bags and analogous products is not the answer, however, because consumer requirements need to met, and there is no acceptable substitute: innovative technology is required.

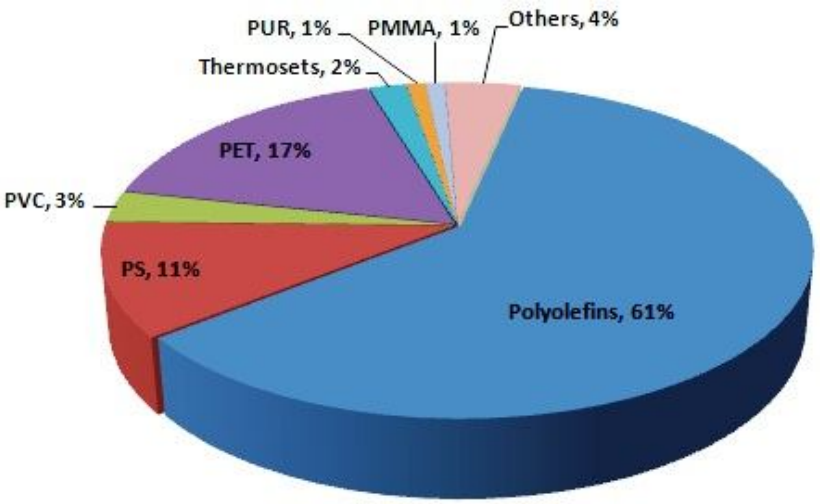


Figure 1. Typology of plastic shares in packaging market.

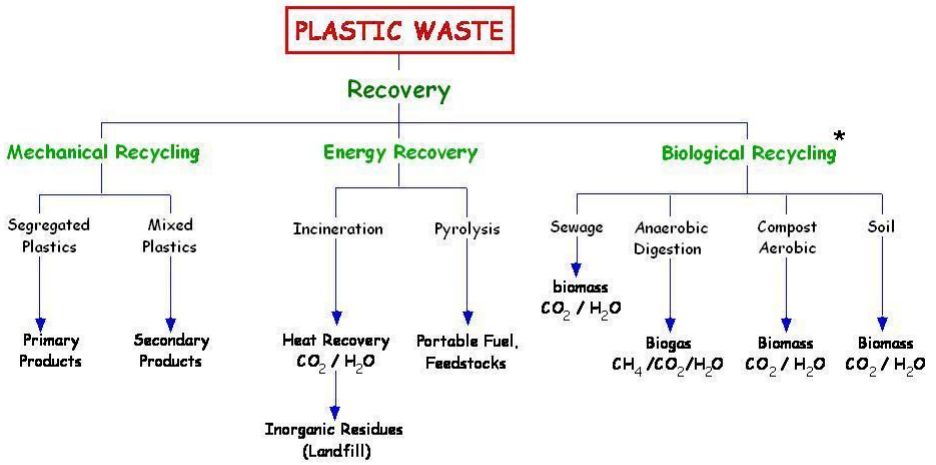
1.1. Waste Disposal Issues and Legislative Background

The greatest environmental pressure for the packaging chain comes from legislation. According to the European Environmental Agency, packaging waste is the major and growing waste stream [3]. Its amounts have

increased in most European countries despite the agreed objective of waste prevention. The rapid growth of specific waste, such as plastic waste, requires a pertaining accurate plastic waste management and recycling with the help of finding appropriate environmentally sound technologies. Although disposal in landfill is set to decrease over the next ten years, the technology is not yet available for the recovery of all the products that at present go into landfill and a major objective is to reduce the volume of active landfills. Landfill is normally composed of alternating layers of mixed domestic wastes, some of which are biodegradable and of soil to protect the surrounding environment from gaseous emissions and percolate due to putrefaction of the organic waste. The prevention in the use of soil leads to a desirable reduction in landfill as waste disposal strategy, thus stimulating the development of more environmentally acceptable technological solutions [4].

Generally, all the plastics will degrade and biodegrade, but time is too long with respect to the waste disposal requirements, due to their chemical characteristics and fabrication conditions. Scheme 1 represents the options of plastic waste recovery in solid waste management stream.

In a ideal situation all the plastic would be collected or re-used or recycled or submitted to incineration by energy recovering. The reprocessing of industrial wastes organic material, contrary to that occurring, for waste items such as glass and metals that can be recycled into products with similar properties to primary materials, does not is general apply to plastic items. The energy recovery by incineration to obtain high caloric value is ecologically acceptable way of utilising carbon-based polymer wastes, but there is the possibility of toxic emission from some chlorinated polymers, particularly PVC which may produce dioxins during combustion [5].



(*) The biological recycling tends to end up in a bioincineration process if negligible part of the organic carbon present in the plastic waste is converted to cell biomass

Scheme 1. Options of plastic waste management.

On the other hand, the increase of the waste, plastics have to be regarded as resources to be re-used or biodegraded at the end of their service life in order to mitigate their negative environmental impact. The reprocessing of individual polymers in mechanical recycling generally leads to the production of downgrade products. Consequently recycled mixed plastics are normally unsuitable for secondary applications. In particular, reprocessed polyolefins each time leads to a loss of mechanical and physical properties due to free radical oxidation and peroxide formation. Furthermore, reprocessing itself requires almost one third of oil-based energy for secondary based products (example PE). By considering that the energy utilized during reprocessing has to be added to the energy spent in transportation and waste treatment, and the additives used to provide a serviceable product, the ecological benefits of recycling is frequently lost. However, all the plastics can be pyrolysed to give fuels,

petrochemical feedstocks and in a few selected cases monomers [6]. In the meantime, growing importance of municipal composting, there is an increasing interest in polymeric materials that can be converted to the so-called compostable plastics ending, however, to a "bioincineration" without any energy or matter reserve. They can be polymers from either renewable or petrochemicals feedstocks. Biological recycling should be considered as an alternative to the more traditional recycling procedures and this has stimulated researches around the world to modify existing polymers or to synthesize new polymers that can be returned to the biological cycle after use, wherever possible to convert at least part of the carbon of the plastic waste into cell biomass.

1.2. Importance of Polyolefins and Waste Disposal Problems

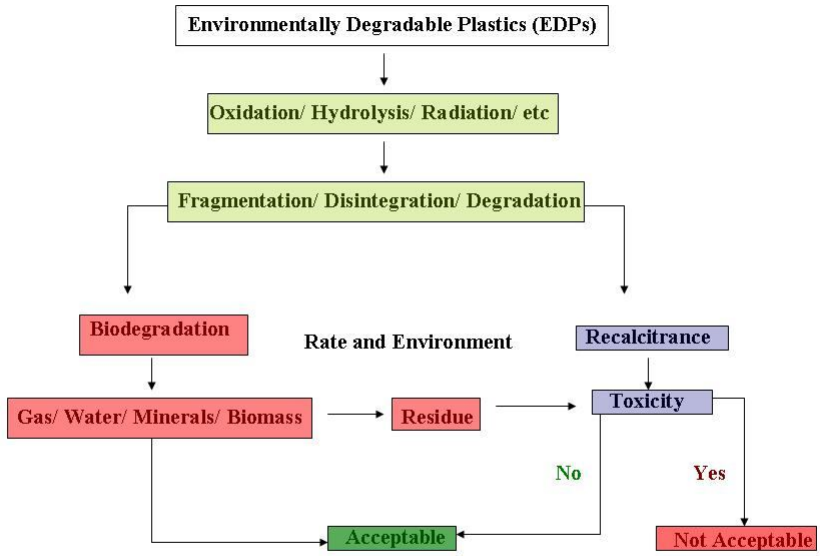
Among the numerous applications of commodity thermoplastics, polyolefins occupy a major role in films for packaging and agriculture purposes. Polyethylene (PE) and polypropylene (PP) are commonly used in these areas because of their low cost, easy processability and good mechanical properties. Mainly, the use of plastics in agriculture is increased 2-3 fold due to the necessity of a increasing yields of produces, earlier harvests, less reliance on herbicides and pesticides, better protection of food products and more efficient water conservation [7]. Plastic films can improve products quality and yield by mitigating extreme weather changes, optimizing growth conditions, extending the growing season and reducing plant diseases. Almost half of this amount is used in protected cultivation (greenhouses, mulching, small tunnels, temporary coverings of structures for fruit trees, etc.). The vast majority of the protected cultivations area covered by plastic materials is dominated by the use of plastic made out of polyethylene (PE) [8]. In particular, low-density polyethylene (LDPE) is the most widely used

polyethylene grade, due to its relatively good mechanical and optical properties, combined with a competitive market price. A major concern bound to the plastic waste disposal after use in packaging and agriculture segments, is represented by the collection, and clearing problems and the final disposal problems are recognized as real-life situations where biodegradability would be a very useful property. Unfortunately polyolefins (and most other man-made polymers) are recalcitrant to biodegradation. Specifically for the case of agricultural plastic wastes, one of the alternative ways of disposal is represented by *in situ* biodegradation, upon post harvesting of the culture. Biodegradation has to do with specially designed polymeric materials. Most experts and acceptable standards [9] define a fully biodegradable polymer and hence derived plastic, as a polymer (plastic) that is completely converted by microorganisms to carbon dioxide, water, minerals and biomass (or in the case of anaerobic biodegradation, carbon dioxide, methane and humic material) without leaving any potentially harmful substances. Definitions given by the standardizations bodies (eg, ISO, ASTM, CEN etc.,), establish that the biodegradation processes of materials must proceed at specified rate in test conditions up to completion in proper time, without any accumulation of constituents with unknown fate and risk.

1.3. Environmentally Degradable Polymers and Plastics

Environmentally degradable plastics (EDPs), based on the term rather than on a specific definition, can be considered to include a wide group of natural and synthetic polymeric materials that undergo chemical change under the influence of environmental factors. The chemical change must be followed by complete microbial assimilation of degradation products resulting in carbon dioxide and water [10, 11]. The process of EDPs degradation comprises two phases, fragmentation and mineralization

(Scheme 2). During the initial phase, disintegration is significantly associated with the deterioration in physical properties, such as discoloration, embrittlement and fragmentation. The second phase is assumed to be the ultimate conversion of plastic fragments, after being broken down to molecular sizes, to CO_2, water, cell biomass (aerobic conditions), and CH_4, CO_2 and cell biomass in the case of anaerobic conditions. The EDPs degradation and assimilation must be complete and occur at a sufficiently rapid rate so as to avoid accumulation of materials in the environment [12, 13].



Scheme 2. Environmental degradation and biodegradation of EDPs.

EDPs can be synthesized from renewable or non-renewable feedstocks. Examples of EDPs from renewable feedstocks are cellulose, starch, starch esters, collagen, viscose, cellulose acetate, polyhydroxy alkanoates, polylactic acid etc, whereas from non-renewable feedstocks are poly(vinyl alcohol) (PVA), poly(ε-caprolactone) (PCL), aliphatic-

aromatic copolyesters, blends of starch and biodegradable polyesters etc. Renewable feedstocks used for EDPs production can be simple natural compounds (such as amino acids, sugar, resources of vegetal, aquatic, and animal origins) or can be derivatives from natural compounds that have undergone chemical transformation to give appropriate building blocks for EDPs. EDPs can also be produced from non-renewable feedstocks, most commonly from natural oil and gas. EDPs are often used as blends or composites in which two or more biodegradable materials are combined to provide optimal performance while maintaining or enhancing complete biodegradability [10, 14].

The formulation of environmentally sound degradable polymers and plastics will constitute a key for the management of plastic waste [15]. The competition with the presently adopted technologies such as burial in landfill sites, incineration with energy recovery and mechanical or chemical recycling is expected to be strengthen, even though one may predict that all of them will coexist with an appreciable decrease of landfilling practice and the introduction of the new concept of prevention that should help to rationalize the production and management of plastic waste. The technologies based on recycling including also the energy recovery by incineration will be flanked by the increasing option of environmentally degradable plastics.

These should be designed to replace the conventional commodity plastics in those segments in which recycling is difficult and labor-intensive with hence a heavy penalization on the cost-performance of “recycled” items.

The global vision of environmental protection and sustainability [16], and criteria for the future industrial development, are to be connected with a number of actions all over the world aimed at providing adequate solutions and suggestions for minimizing the negative impact of the increasing production and consumption of polymeric materials and

plastics. This holds particularly true in the case of merceological segments such as packaging, kitchenware, detergency, and disposables that all together may reach levels of 40-50% of the worldwide plastic manufacturing. As a consequence of that new vision in the production and consumption of plastics, in the last decade remarkable increase in the scientific and industrial interest in EDPs can be envisaged.

1.3.1. *Definitions*

The American Society for Testing and Materials (ASTM) and the International Organization for Standardization (ISO) define:

a) ***Degradation*** as *"An irreversible process leading to a significant change of the structure of a material, typically characterized by a loss of properties(e.g. integrity, molecular weight, structure or mechanical strength) and/or fragmentation. Degradation is affected by environmental conditions and proceeds over a period of time comprising one or more steps"* [17, 18]. According to the ASTM definition [19],

b) ***Biodegradable plastic*** is *"A degradable plastic in which the degradation results from the action of naturally occurring microorganisms such as bacteria, fungi and algae"*

Two principal types of commercially viable biodegradable plastics have been developed and are finding a variety of applications in many mercantile segments and consumer products:

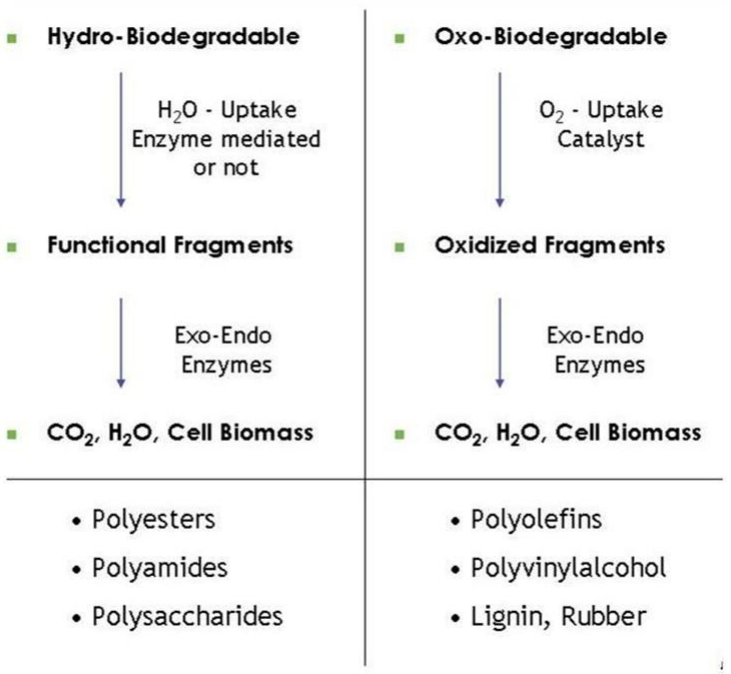
1) ***Oxo-biodegradable*** polymers and hence relevant plastic items, for which degradation is the result of oxidative and cell mediated phenomena, either simultaneously or successively;

2) ***Hydro-biodegradable*** polymers and hence relevant plastic items, for which degradation is the result of hydrolytic and cell-mediated phenomena, either simultaneously or successively.

Both types of biodegradable polymers feature a two-stage sequential molar mass reduction in the environment with the first stage being basically abiotic (Scheme 3).

Since the objective is to reduce the amount of plastic with minimum effect on the environment, the second stage is bioassimilation of the molecular fragments that are generated in the first stage [14].

Abiotic mechanisms are generally regarded as too slow by themselves to be adequate in a variety of disposal environments.



Scheme 3. General features of environmentally degradable polymeric materials and plastics.

There are several applications in which really quite rapid degradation of plastics after use is required. For example, plastics that end of in water or sewage treatment systems are an example of situations in which they need to loose integrity relatively so as to avoid plugging pumps, filters and the like. Hydrolytically unstable biodegradable plastics can provide an

answer here. In many other uses (e.g., food packaging) however, hydrolytic instability is a disadvantage. Overall stability is required during shelf storage and use but this should be followed by relatively rapid abiotic degradation within a specific time, depending on the disposal environment. The avoidance of the accumulation of plastic fragments requires that these be consumed through biodegradation by microorganisms in virtually all disposal environments. Effective biodegradation of such residues can be achieved when originally hydrophobic plastics acquire water wettable (hydrophilic) surfaces and a relatively low molecular weight so that there is a significant number of molecular ends accessible at the surface. The science and technology of the development of oxo-biodegradable plastics can meet these criteria.

1.4. Oxo-Biodegradation of Full Carbon-Backbone Polymers

Of the current worldwide production of synthetic polymers, nearly 90% is represented by full carbon-backbone macromolecular systems (polyvinyls and polyvinylidenics [20], and 35-45 % production is for one time user items (disposables and packaging). Therefore it is reasonable to envisage a dramatic environmental impact attributable to the accumulation of plastic litter and other plastic waste from discarded full carbon-backbone polymers, which are conventionally recalcitrant to physical, chemical and biological degradation processes. In contrast to the "hydro-biodegradation" process of natural and synthetic polymers containing hetero atoms in the main chain (polysaccharides, proteins, polyesters, polyamides, polyethers), the mechanism of biodegradation of full-carbon-backbone polymers requires an initial oxidation step, mediated or not by enzymes, followed by fragmentation, again mediated or not by enzymes, with substantial reduction in molecular weight. The functional fragments then become vulnerable to microorganisms present in different

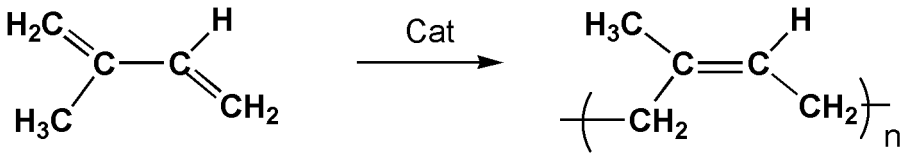
environments, with production (under aerobic conditions) of carbon dioxide, water, and cell biomass. Scheme 3 outlines the general features of environmentally degradable polymeric materials, which are classified as hydro-biodegradables and oxo-biodegradables. Typical examples of the so called oxo-biodegradable polymers are represented by poly(ethylene), poly(vinyl alcohol) [21], natural rubber [poly (*cis* 1,4 isoprene)] and lignin (a natural complex heteropolymer) [22].

1.4.1. *Natural rubber (cis-polyisoprene)*

Full carbon-backbone polymers are normally associated with synthetic polymers obtained by the polymerization of vinyl compounds with a few cases of naturally occurring polymers. The most studied and best understood of these last is natural cis-poly(isoprene) (Natural rubber, NR) (Scheme 4), identical to synthetic counterpart (UR). NR was one of the earliest industrial polymers to be developed commercially and it was recognized to be degraded in the environment even before it reached the industrialized countries. In addition, rubber latex products were rapidly attacked by microorganisms leading to more general loss of mechanical properties and to eventual bioassimilation in the soil environment.

The synthetic polyolefins are more environmentally stable than polydiene rubbers, that are less resistant to environmental stress than that might can be expected on the basis of formal structures. The abiotic peroxidation of NR occurs at ambient temperature as long oxygen is present in the system. A similar mechanism of oxidation occurs in synthetic polyolefins generating free radicals due to heat/light in the polymer chains, which immediately reacts with oxygen to form peroxides and hydroperoxides groups. However, oxo-biodegradation also proceeds in parallel in microbially active environments. It has been demonstrated experimentally [23,24], that pure strains of bacteria (in particular actinomycetes) and

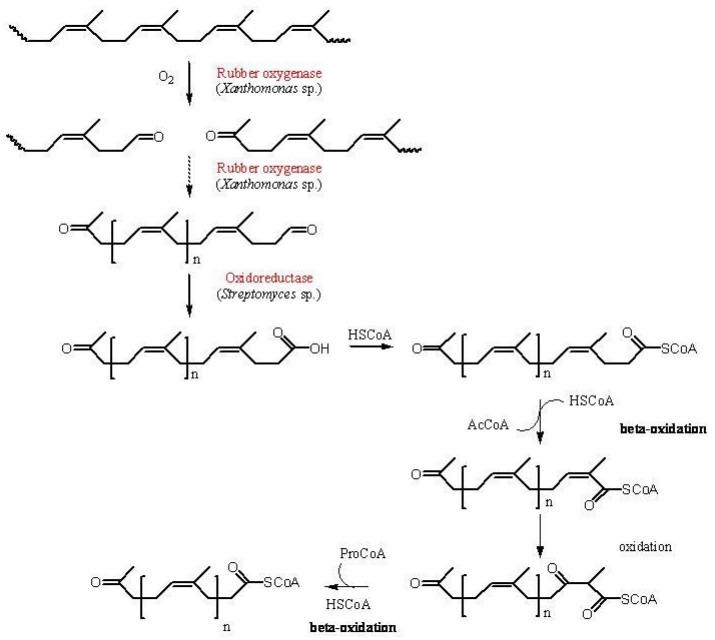
fungi cause up to 55% loss of mass of rubber sheets in 70 days. The actinomycete, *Nocardia* (sp. St835A) was found by Tsuchii and co workers [25] to be particularly effective in degrading NR gloves in the absence of any other source of carbon. A mass loss of 75% was achieved in two weeks and the same strain in laboratory bioreactors led to complete degradation of NR in 45 days [26].



Scheme 4. Structure of *cis*-polyisoprene (natural rubber, NR)

Ikram and co-workers [27] have shown that in normal soils at 25°C, NR gloves showed 54% loss of thickness after 4 weeks and 94% mass loss after 48 weeks. Commercial nitrile and neoprene rubbers showed insignificant loss in this time and plasticized PVC showed a smaller mass loss (11.6%) due to the biodegradation of the plasticizer and not to the biodegradation of the polymer itself. Bacterial populations on the NR gloves (12317 cell/mg) were higher than for fungi (441 cell/mg), which were in turn significantly higher than actinomycetes (297 cell/mg). Nevertheless, Heisey and Papadatos [28] isolated 10 actinomycetes (seven strains of *Steptomycetes*, two strains of *Amycolatopsis* and one stain of *Nocardia*) from soil that reduced the mass of NR gloves from 10-18% in 6 weeks. Steinbuchel and co-workers [29], using rubbers as the sole source of carbon, found that NR and IR (synthetic polyisoprene rubber) biodegrade at a similar rate in the presence of *Pseudomonas aeruginosa*. NR gloves were 26% mineralized in 6 week compared with 21% for IR gloves. This slight difference may well have been due to the difference in the antioxidants used in the formulation, although these were

not identified. It is clear, however that, contrary to the views of some environmentalists [30], there is no intrinsic difference between natural and synthetic polymers provided the same structure and comparable molecular weight. It has been pointed out that some actinomycetes can utilize CO_2 as a source of carbon [31]. It is therefore necessary to equate microbial growth and associated formation of protein to loss of weight of the substrate. Delort ad co-workers[32] have shown that loss of carboxylic acids formed during abiotic peroxidation of PE correlates with the formation of protein and polysaccharides, almost certainly associated with the cross-linked bacterial cell wall structure. *Nocardia* and *P. aeruginosa* [29,33], were shown to break the *cis*-poly(isoprene) chain by an oxidative mechanism since aldehyde groups were found to accumulate during microbial degradation. This is always the first product formed during the abiotic peroxidation of *cis*-poly(isoprene) and the evidence suggests that bacteria initiate a radical-chain peroxidation (Scheme 5).



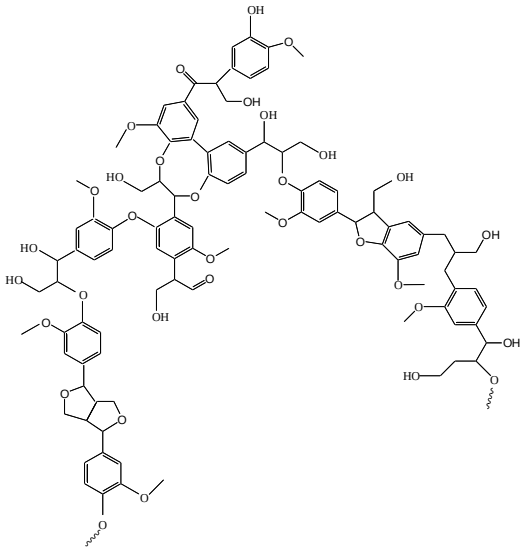
Scheme 5. Suggested biodegradation mechanism of NR [34]

1.4.2. *Lignin*

Lignin is another example of natural polymer that, like *cis*-poly(isoprene), cannot biodegrade by a hydrolytic process, but biodegrades slowly by the oxidative attack due to extracellular peroxidases produced by fungi and actinomycetes [35]. Lignin is a cross-linked polymer containing benzene rings (Scheme 6). it is formed in chemical association with cellulose (lignocellulose) and constitutes the tough cell wall structure of plants. The aromatic structures contain alkoxy and hydrocarbon substituents that link the basic unit below into a macromolecular structure through carbon-carbon and carbon-oxygen bonds. Both chemical and physical properties of lignin resemble those of the synthetic phenol-formaldehyde (PF) resin. Like the PF resin, lignin provides physical, chemical and biological protection to the growing plant wood, straw and husks etc. Lignin, due to its physical (hydrophobic) and chemical inertness, does not readily degrade abiotically of biotically and when it does occur, the lignin tends to accumulate. However, lignin does biodegrade slowly under composting conditions. Lignin in grass and straw were found to biodegrade to the extent of 17-53% in 100 days. In laboratory studies, thermophilic composting grass straw showed 45% degradation in 45 days but the process is slowing down at more extended times. A number of peroxidases have been isolated that remove lignin from ligno-celluloses without affecting cellulose itself. *Manganese Peroxidase* (MnP) in particular has been implicated as an important enzymes formed by white rot fungus during deligninfication of kraft pulps [36].

The chemical degradation of lignin is occurring during the pulping and bleaching process due to the reaction of chemicals including sodium hydroxide, sodium sulphide, chlorine dioxine and oxygen or ozone [37]. However, vast amounts of lignin derivatives from pulping and bleaching process where chemical degradation of lignin happen are created and

these compounds are a threat to the environment if not treated intelligently. Consequently, biodegradation of lignin in the environment is not only a scientific research interest but also a necessary solution to the environmental threat created by the pulp and paper industry.



Scheme 6. Lignin structure

Until now, white-rot fungi are the only organisms able to mineralize lignin efficiently to carbon dioxide and water through a process initially catalyzed by extracellular enzyme [38]. Researches on the lignin biodegradation and white-rot fungi is an interdisciplinary scientific projects. It is also a systematic research concerning basic research and applied research. The basic research includes degradation of lignin by white-rot and litter-decomposing fungi, production, properties of ligninolytic and cellulosolytic enzymes and catalytic mechanisms of peroxidases secreted by white-rot fungi. The applied researches cover the following main research scope:

1) Use of fungi to treat wood chips to decrease chemical or energy consumption in pulping and to remove pitch (extractives) from wood chips for biopulping.

2) Degradation of polycyclic aromatic hydrocarbons by litter-decomposing fungi in soil.

3) Removal of organic material from soil contaminated with dioxins and dibenzofurans.

4) Degradation of lignin and lignocellulose in compost [39].

Interestingly, in the nature, white-rot fungi posses the unique ability to degrade lignin completely to carbon dioxide, while this is not their end purpose. White-rot fungi degrade lignin in order to have an access to the cellulose molecule which is a carbon source for them. Only recently, investigators began to understand the mechanism by which degradation of lignin is accomplished.

The lignin degradation enzyme system of white-rot fungi is extracullular and has relatively low substrate specificity. Two enzymes i.e. peroxidases and hydrogen peroxide secreted by the fungi catalyze reactions of the highly reactive and non-specific free radicals, resulting in the depolymerization and degradation of lignin. Consequently, though lignin is naturally a highly oxidized polymer, it can eventually be completely oxidized to carbon dioxide by white-rot fungi.

Furthermore, ^{14}C-labelled techniques had been used to better understanding the new features in lignin biodegradation [40-42]. These results may give explanation to the questions that have puzzled researchers for many years.

Besides white-rot fungi, there are many genera of actinomycetes and eubacteria which can degrade extracted lignin. Many bacterial strains, especially actinomycetes, can solubilize and modify the lignin structure extensively, but their ability to mineralize lignin is limited. Although aerobic microorganisms are primarily lignin degraders in most environments, it has been shown that anaerobic rumen microorganisms are cabable of degrading plant fibre cell walls [43, 44]. It has been

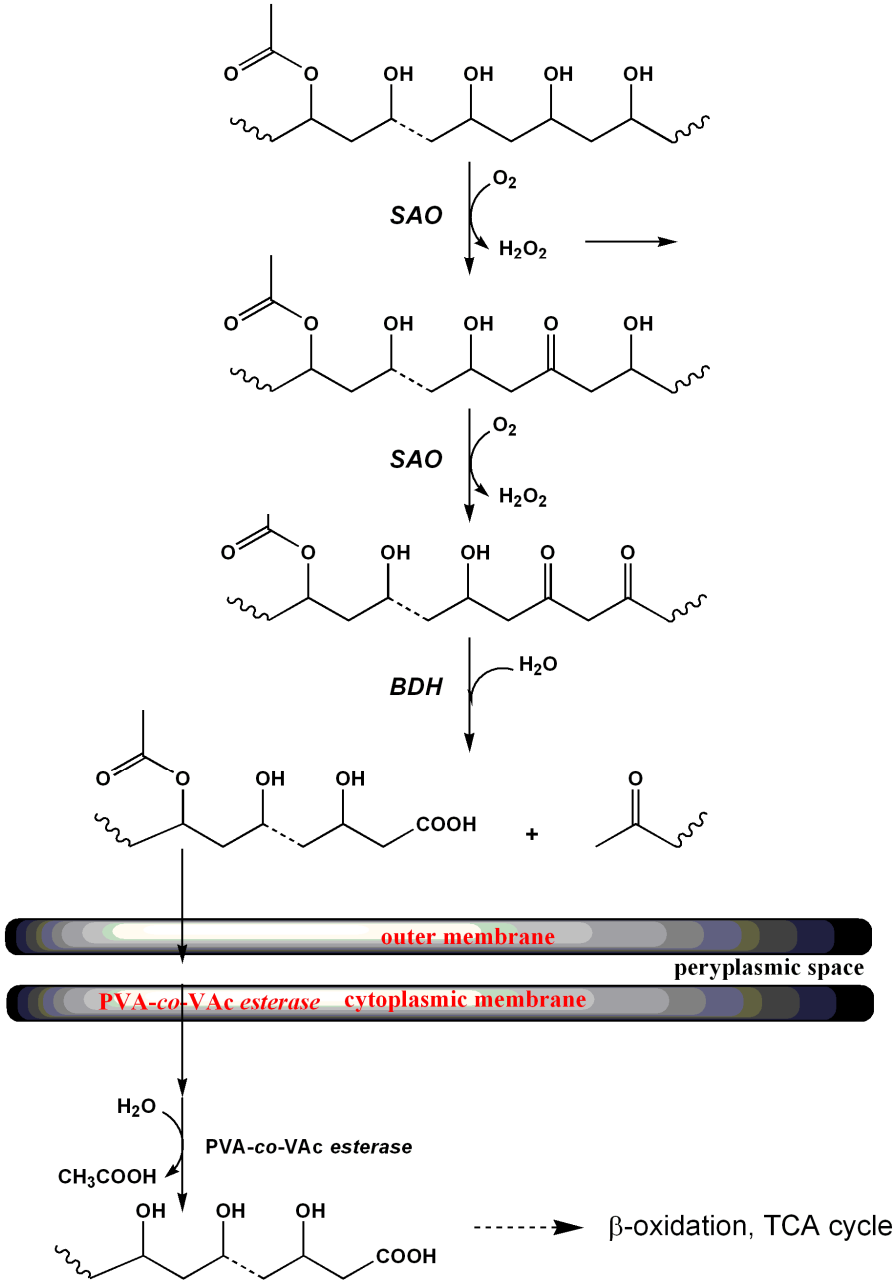
reviewed the anaerobic microbial degradation of lignin compounds and concluded that the intermediate metabolic products called oligolignols, released during aerobic degradation, may be partially degraded to CO_2 and CH_4 by anaerobic microorganisms.

1.4.3. *Poly(vinyl alcohol) (PVA)*

Among the synthetic full carbon backbone polymers, PVA can be considered as truly biodegradable. Several microbial strains responsible of PVA oxidation and assimilation have been isolated.

The major biodegradation mechanism of PVA in aqueous media is represented by the oxidative random cleavage of the polymer chains, the initial step being associated with the specific oxidation of methylene carbon bearing the hydroxyl group, as mediated by *Oxidase*- and *Dehydrogenase* types enzymes, to give β-hydroxy ketone as well as 1,3-diketone moieties. The latter groups are susceptible to carbon-carbon bond cleavage promoted by specific *β-diketone hydrolase*, leading to the formation of carbonyl and methyl ketone end groups. [45, 46]. The ultimate biochemical fate of partially hydrolyzed PVA samples has been recently described by using *Pseudomonas vesicularis* PD strain, a specific PVA assimilating bacterium [47]. This bacterium metabolites PVA by a *Secondary Alcohol Oxidase* (SAO) throughout the oxidation of the hydroxyl groups followed by hydrolysis of the formed β-diketones by a specific hydrolase (*β-Diketone Hydrolase*, BDH). Both enzymes are extracellular, and the polymer are cleaved by repeated enzyme-mediated reactions outside the cell into small fragments, which are further incorporated and assimilated inside the bacterial cytoplasm and metabolized up to carbon dioxide (Scheme 7,). The initial oxidation step of PVA macromolecules can also be promoted by ligninolytic enzymes (*lignin peroxidase* [Lip] and *laccase*) produced by white-rot fungal

species such as *Phanerocheate crysporium* [48, 49]. The monoelctronic enzymatic oxidation reaction leads to formation of free radicals along with the formation of carbonyl groups as well as double bonds, thus increasing the macromolecules unsaturation [48].



Scheme 7. Biodegradation pathway of partially acetylated PVA [49]

1.4.4. *Oxidation, degradation and biodegradation of poly(ethylene) (PE)*

Since the synthesis and during all the following manufacturing processes, as well as during their shell life (use and disposal), polyolefins are sensitive to oxidative degradation. In fact, the presence of sensitizing "impurities" capable to promote the oxidation of macromolecules can be recognized during the compounding and processing of polyolefins based plastic items.

For instance, it has been stated that carbonyl [50, 51] and hydroperoxide groups [50, 52-55] represent the major sensitizing impurities formed during the processing of PE. At this stage, chemical structure of polyolefins is considered to play the most important role in influencing the oxidative degradation process, whereas during use and disposal steps, the oxidation of both PP and PE appear to be mainly affected by structural parameters such as the degree of polymerization, chain conformation, degree of cristallinity and geometry [56].

In the case of PE, the poor reactivity of non-polar C-C and C-H bonds strong constrains to the degradation processes by free radical pose reactions. These are generally initiated by bond-breaking processes promoted by energy input in the form of heat, radiation or mechanical stress. Of course, the susceptibility of bond scission is depending upon the bond energy, therefore the initial radical reactions in PE are mostly restricted to the defects of the chemical primary structure such as tertiary carbon atoms at branching sites and double bonds. Afterwards, a secondary complex series of radical reactions may lead to the total degradation of the polymer chains throughout further bond scissions, recombinations and substitutions.

The overall sequence of reactions that are at the basis of PE, and more in general of polyolefins oxidation, have been traced during several decades

of active studies producing an huge amount of original papers and reviews.

In accordance it has been widely accepted that the starting point of the degradation process can be mainly recognized in the homolytic bond cleavage in the carbon backbone as occurring during the polymer processing, , in response to shear stresses during extrusion [57]. In the presence of oxygen, like in most of the industrial processes, the carbon-centered radical is converted to a peroxy radical which is thought to be further converted to hydroperoxide radical by hydrogen abstraction from a vicinal methylene groups.

The high reactivity of hydroperoxide groups when exposed under both heat and/or UV radiations promote a further series of reactions leading to chain scission (molar mass reduction) and formation of several different oxidized groups.

In the overall peroxidation process of PE, the hydroperoxide groups decomposition is therefore considered as the rate-determining step [56]. This starts a radical chain reaction among oxygen and C-H bonds in the polymer chains, where hydroperoxides are the key intermediates, in fact their formation and decomposition promote an autoaccelerating cycle of interlocking reactions [58].

Even though this chapter is not aimed at reviewing the paramount literature on the polyolefins degradation, a general overview on the mechanism of PE oxidation and kinetics thereof could be useful for a better understanding of the ultimate environmental fate of PE.

In this connection, the basic mechanism proposed by Bolland and Gee [59] that comprises the classic steps consisting of 1) Initiation, 2) Chain propagation, 3) Termination and 4) Evolution of alkoxy radicals, can be considered as actually valid. In the case of conventional polyolefins it can be described as a several step branching, promoted by free radical chain

reactions, the slowest of which is the homolytic cleavage of hydroperoxide groups attached to the main-chain carbon atoms, as shown in Scheme 8.

As already mentioned, the key intermediates in the proposed mechanism are hydroperoxides, whose decomposition give further free radical groups and most of the deriving oxidation products.

Many of the kinetic studies of thermal oxidation of polyolefins are relative to the polymers in the melt state. In order to identify the reactions that promote the oxidative degradation, several investigations have been also carried out in polymer solution. Nevertheless, by considering that the practical use of polyolefin is in the solid state, several studies have been devoted to the kinetics of thermal and photooxidation of polymer films and sheets. In accordance, different oxidation products have been identified and several parameters capable to influence the oxidation processes, such as oxygen pressure, temperature, sample thickness, have been considered in the investigations.

1.	Initiation	$RH + \text{heat/shear stress} \Rightarrow R\bullet + \bullet H$
		$R\bullet + O_2 \Rightarrow RO_2\bullet$
2.	Propagation	$RO_2\bullet + RH \Rightarrow ROOH + R\bullet$
		$ROOH + \text{heat and/or UV light} \Rightarrow RO\bullet + \bullet OH$
3.	Termination	$2\ R\bullet \Rightarrow R\text{-}R$
		$R\bullet + \bullet OH \Rightarrow ROH$
		$RO\bullet + \bullet R \Rightarrow ROR$
4.	Evolution of alkoxy peroxide	$RO\bullet \Rightarrow$ ketones, alcohols, acids, esters

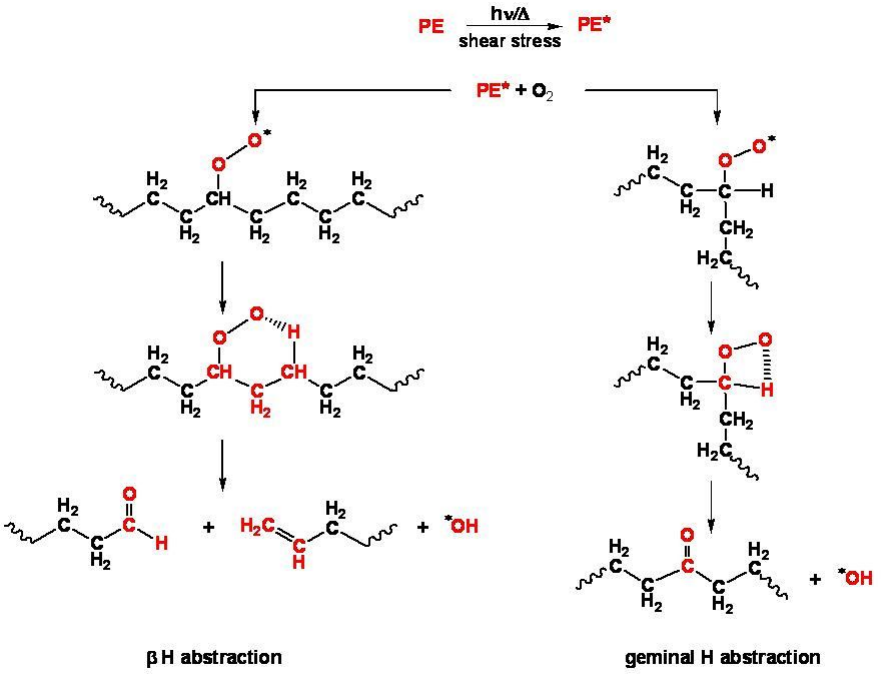
Scheme 8. Polyolefin oxidation mechanism.

From an analytical point of view, the FT-IR spectroscopy is considered one of the most powerful technique to be used in the kinetic studies of

thermal and photooxidation of polyolefins, particularly polyethylene, in the solid state (films and sheets). Considerable changes can be easily monitored in various regions of the FT-IR absorption spectra during the thermal and photo exposure of PE films. In particular, the formation of associated hydroperoxides whose maximum absorption band is recorded between 3400 and 3200 cm^{-1}, as well as the absorption increase in the carbonyl region (1650-1850 cm^{-1}) are currently utilized to evaluate the rate and extent of the oxidative degradation of polyeolefins. In the mean time, also the absorbance variation of peaks related to vibrations of the double bonds, as well as the single carbon-oxygen absorption band, may provide valuable information on the mechanism and oxidation products formation during thermal and photo exposure of PP and PE films.

Under not-limiting oxygen concentrations during primary initiations it can be assumed that all the macroradicals as they are produced (e.g. by shear stresses) they are oxidized to peroxy radicals and by intra- or intermolecular hydrogen abstraction converted to hydroperoxides. A fairly complex series of chain reactions involving formation/decomposition of peroxy radicals and more frequently hydroperoxides, constitutes the propagation step leading to oxidized products formation and chain scissions. It has been estimated that, in oxidized solid polyethylene, more than 80% of the oxygen-containing products are represented by carbon chains bearing carbonyl and carboxyl groups [56]. Carbonyl groups along the carbon backbone are produced by hydroxyl radicals and hydroperoxides decomposition implying their conversion to alkoxy macroradicals. These last peroxy radicals may be transformed *via* hydrogen β abstraction to produce a carbonyl group and a chain-end radical throughout chain scission (Scheme 9). Accordingly, it has been ascertained a straightforward relationship between the amount of chain scission and the number of carbonyl groups. For this reason,

quantitative FT-IR analysis can be currently and effectively used for monitoring and predicting the rate and extent of oxidative degradation of polyethylene.



Scheme 9. Hydrogen abstraction and chain scission in PE.

As a consequence of the radical oxidation processes and relevant chain scissions, a fairly high number of degradation products containing functional groups, have been recorded in several investigations. In particular, two different classes represented by low-medium molecular weight fractions and volatile intermediates, respectively can be detected in kinetic studies of thermal and photooxidation of PE. As a consequence, the oxidation processes of PE and in particular of low density polyethylene (LDPE) can be effectively also monitored by gravimetric analysis showing the weight variation as a function of the thermal aging time and temperature [60]. In a case study, carried out on a LDPE sample

containing thermal pro-degradant additives, the time profile of weight variation was showing a sigmoid profile [60], thus accounting for the exponential accumulation of oxidized low-medium molecular weight fractions followed by the progressive weight decrease as a consequence of the loss of volatile intermediates.

The formation of , low-medium molecular weight products containing carbonyl and hydroxyl groups has been identified in various studies [61]. It has been also ascertained that the relative amounts of these products account for at least 80% of products containing carbonyl and carboxyl groups [56]. Carboxylic acids tend to accumulate particularly during prolonged exposure times, being other oxidized products such as alcohols, ketones and aldehydes susceptible to further oxidation to carboxylic acids. In classic studies most of the low molecular weight degradation products from both thermally and photo-oxidized PE have been isolated and identified by solid phase extraction coupled with gaschromatography mass-spectrometry [62-64]. Accordingly, several semi-volatile compounds including alkanes, alkenes, ketones, aldehydes, alcohols, mono- and dicarboxylic acids, lactones, keto-acids and esters have been identified. In addition, fast volatile organic products (C2-C6), even though in a very small proportion, have been also detected. Among these acetaldehyde represents the most abundant component [56, 65].

Monocarboxylic and dicarboxylic acids have been found to be the most abundant products during prolonged aging under aerobic conditions. Primary alcohol and aldehydes derived from hydroperoxide decomposition are susceptible to be further oxidized during thermal aging. Also the photolytical cleavage of keto groups through Norrish I & II mechanisms may lead to the formation of carboxylic groups.

The presence of fairly large amounts of carboxylic groups suggests the severe breakdown of PE matrix. This may be qualitatively evidenced by

the FT-IR spectra of PE samples recorded after different times of thermal treatment by monitoring the variation of both shape and intensity of the broad absorption band in the range 3600-2800 cm^{-1} typically associated to aliphatic carboxylic acids.

Due to the relative stability to thermal oxidation in opposition to the instability to photo-oxidation, keto groups are considered as typically associated to the thermal degradation processes of PE.

Other products, recognizable in the volatile and semi-volatile fractions of oxidized PE, such as keto-acids have been identified during low temperature thermal degradation [66], whereas lactones are usually generated under very severe conditions or when extensive degradation is taking place.

Nevertheless, since almost the totality of the oxidation products results from the decomposition of polymer hydroperoxides, they are formed and trapped within the bulk, and only small fractions can escape thereof.

In this connection, it has been suggested that also the estimation of the fractions exctractable with organic solvents might provide useful information on the level of the oxidative degradation of PE as well, and allowing for the determination of the amount of low-medium molecular weight oxidized components. Further information about the either statistic or sequential formation of functional groups can be achieved by the assessment of extent of oxidation of soluble and precipitated fractions, as well as by their molecular weight distribution. Oxidized PE fractions, soluble in a thrichlorobenzene-methanol solution, showed the largest amount of oxidized functional groups present on relatively low molecular weight chain, whereas a substantial portion of the polymer was represented by macromolecules characterized by a low level of oxidation [56].

In a recent study [60], the amount of extractable fraction from thermally oxidized LDPE samples containing pro-oxidant additives, have been evaluated as a function of the level of polymer matrix oxidation as assessed by the CO*i* (Figure 2).

In this study, it has been evidenced that the amount of acetone extractable fractions is positively correlated to the level of oxidation induced by the thermal treatment in oven, thus reaching fairly high amount corresponding to 25-30% of the original weight of the analyzed sample at a CO*i* value around 5 (Figure 2). The acetone extracted fractions resulted to be characterized by low molecular weight (0.90-1.50 kDa), and a high level of oxidation , as demonstrated by NMR and FT-IR analysis. Furthermore it was also observed that the progress of the oxidation level, as related to the CO*i*, increased the quantity of soluble fractions characterized progressively by lower molecular weight values.

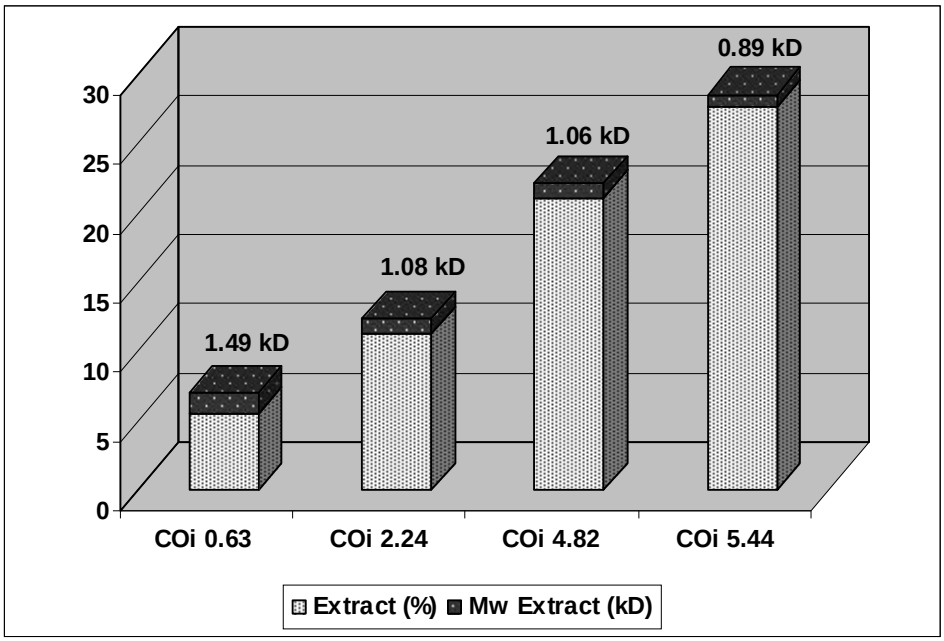


Figure 2. Percentage of fractions extractable with acetone and relevant molecular weight in thermally treated LDPE film containing a pro-oxidant at various level of oxidation as determined by carbonyl index (CO*i*).

The large amount of different oxidation products, as well as their relative concentration, accounts for the occurrence of a substantial number of interrelating elementary reactions and consequently of rather complex oxidation kinetics. In spite of the interest for these complex series of chain reactions that has attracted a great deal of attention over the past 50 years, only a little agreement on the kinetic models and values of specific rate constants has been achieved by both theoretical and experimental approaches. One of the most controversial fact is the basic hypothesis whether the oxidation mechanism should be considered as an homogeneous phenomenon or a heterogeneous process involving the spreading of degradation from localised sites. This latter interpretation is taking into account the semi-crystalline nature of solid polyolefins, where amorphous regions, more prone to oxygen diffusion and hence oxidation, coexist with crystalline regions that appear more impervious to oxidation processes.

Even though of this chapter is not intended to review this topic, some basic and simplified information on kinetic oxidation processes could be useful to predict the ultimate oxo-biodegradation propensity of polyethylene. In particular the effect of physical parameters such as aging time, temperature and radiation intensity may provide the basic factors affording for a following extensive biodegradation step.

Indeed, one of the most important feature allowing for a prediction of the oxidative behavior of polyolefin based films is needing the correlation between the experimental kinetics of oxidation with the chemical reactions meant to occur in the polymer bulk.

In several studies, it has been ascertained that the early stage of thermo-oxidative degradation of polyethylene as monitored by the formation of carbonyl groups is apparently in agreement with a typical self-accelerated mechanism [67].

Nevertheless, in order to simplify the real mechanism, it has been suggested that the kinetic data should be treated by employing some basic assumptions, which are however compatible with the general scheme of reactions previously described. In particular, it has to be considered that:

1) Only the reactions of macroradicals (R*) and peroxymacroradicals (RO_2*) are the rate detemining steps.

2) The macroradical concentration may reach a steady state after a short initial step.

3) The hydrocarbon concentration (e.g. the sites of possible oxidation) should be considered as costant.

4) The decomposition of polymer hydroperoxides is unimolecolar or pseudo-unimolecolar.

5) During the propagation step the only recations of R* is the oxygen uptake, whilst macroperoxyradicals promote hydrogen abstraction.

6) The termination rate constant of step is independent of the type of the radicals.

In addition the not-limiting concentration of oxygen could be also considered. In fact, it has been repeatedly reported that limited oxygen concentration may shift the chain scissions (e.g. molecular weight decrease) toward the cross-linking reactions through radical chain termination, thus inducing the apparent molecular weight increase [68].

The kinetics of carbonyl and hydroperoxide group formation reported in the literature to describe the thermal oxidation of low density PE are currently the object of a deep revision by many researchers, with some of them even disputing their validity. It is however accepted that the overall mechanism is complicated and the proposed kinetics does not yield a satisfactory model to account for many of the experimental data obtained from the thermal degradation studies of unstabilized, as well as stabilized solid LDPE samples. In particular it has been suggested that the main

source of discrepancy and conflicting interpretations may rise from the preferential fitting of the experimental results to power laws such as: [Carbonyl] = $h \cdot t^b$ t= aging time, h = rate costant of polymer oxidation, b= rate constant of aging . These attempts were reported in early studies suggesting that the oxygen absorption, carbonyl group formation and hydroperoxide concentration were increasing according to a quadratic or a biquadratic law in thermally aged solid polyolefin [69].

Indeed, more recently, it has been suggested that the formation of associated carbonyl groups and associated hydroperoxides may occur according to autoaccelerating kinetics in the early stages of the process, thus resembling the exponential type. When the oxidation on the contrary is proceeding to prolonged aging times, the increase of hydroperoxides and associated carbonyl groups are thougth to fit in a better mode a linear increase with time (i.e. constant rate) [67].

Other studies, carried out by spotting several FT-IR measurements over an LPDE film surface, have also suggested, that in this case the thermal oxidation can be considered as a fairly homogeneous process, rather than to proceed according to a heterogeneous mechanism that should be expected to occur by considering the semy-crystalline nature of the polymer [69].

Several studies also suggested that the initiation of thermal oxidation of polyolefins and in particular LDPE, at moderate temperatures (60-80°C) is promoted to the largest extent by the oxidation products (free and associated hydroperoxides, carbonyl groups) initially formed onto the surface layers [69, 70]. These products can diffuse more or less rapidly, depending upon their molar mass into the bulk, where they may induce, especially in the amorphous regions, further oxidation chains. Hence, it does appear that the thermal oxidative degradation may spread rather slowly from the surface within the whole solid sample according to a

complex behavior typical of both homogeneous and heterogeneous processes [69]. It is worth noting, however, that the above mentioned behavior can be considered as valid in the case of fairly high (80°C) temperature, whereas at lower oxidation temperature the effect of sample thickness, as barrier to diffusion, is prevalent. In other words, at lower temperature the oxidation rate was found to increase with the sample thickness, thus indicating the preferential oxidation of the surface layers in accordance with the activation energy of oxygen diffusion (40 kJ mol^{-1}) [71, 72]. Even though also the rate of diffusion of the initiation products from the surface layers into the bulk is likely to affect the procees [70].

Despite the relatively good interpretation of the experimental data as in agreement with an homogeneous model of thermal oxidation of solid LDPE, some evidences such as the leveling-off of the hydroperoxides, could be better interpretated according with the heterogeneous nature of semy-crystalline LDPE. The proposed model has been therefore based on the spreading of the initiation products from amorphous domains, after they reached a maximum amount, to other adjacent amorphous domains until the whole solid polymer be oxidized, when the spreading rate is a function of the oxidation time [73].

In conclusion, it has been suggested that in the case of LDPE there is an exponential-type increase in the early stages of the oxidation process, followed by a linear increase at the later stages. However, the heterogeneous oxidation model and the corresponding kinetics developed for LDPE are thought to be also valid for other polyethylenes, including LLDPE, as well as Ziegler and Phillips-type HDPEs. The differences in the oxidative processes among the different PE polyethylene types seems therefore to concern mainly with the rate of oxidation, which seem to be most heavily affected by the nature of the PE. In this respect, polymer density/crystallinity as well as the manufacturing process are important.

Indeed, the increasing density and hence crystallinity degree lead to decreasing rates of oxidation spreading. The catalyst residues appear to be even important. If small amounts of Ti-catalyst residues have only a slightly accelerating effect, Cr-catalyst residues increase considerably the rate of spreading from one amorphous domain to the next of the polymer bulk [74].

1.4.4.1. *Oxo-biodegradable polyethylenes*

The feasibility of producing environmentally degradable and low cost plastic items from polyolefins is dating back to the last decades of the 20th century. In this period, in fact, the potential abiotic degradability and eventually the ultimate biodegradability started to be considered as a positive attribute for several applications, particularly in packaging and agricultural market segments [75, 76].

On the other hand, in the meantime the recalcitrance of commercial high molecular weight polyolefins to environmental degradation and biodegradation was generally accepted. This was in particular recognized by observing the extremely low degradation rate of polyethylene in natural environments. It was therefore assumed that the resistance of PE to biological attack resides in its peculiar structural features, as well as in the presence of antioxidants and UV stabilizers that refrain macromolecules from abiotic oxidation and following fragmentation to oxygenated moieties . High molecular weight, hydrophobicity and lacking of functional groups recognizable by microbial (i.e. hydrolytical) enzymes represents structural parameters which greatly hamper the action of microorganisms. In solid PE items, macromolecules are densely aligned in semycrystalline structures, so very limited free chain ends are available for enzymatic oxidation eventually only at the surface. The slow rate of biodegradation can be at least partially attributed also to the

presence of antioxidants. In accordance, it was observed that antioxidants free polyethylene films were at least susceptible to bio-erosion, whereas under the same conditions control films containing the antioxidant resulted completely inert to the action of microorganisms [77, 78].

Taking into account these evidences, major strategies to enhance the environmental degradation and biodegradation of PE have been (historycally) focused on copolymerisation with functional monomers including carbon monoxide [79], blending or grafting with functional polymers and compounds respectively, and ultimately addition of pro-oxidant additives.

1.4.4.1.1. Copolymerisation

Copolymerisation has been traditionally carried out in order to introduce UV-absorbing groups capable to enhance the photo-oxidation process. In this connection carbonyl groups can be incorporated into the PE main chains by the copolymerization of ethylene with carbon monoxide [80, 81], or in the side chain as by copolymerization with vinyl ketones which are commercialized under the Ecolyte® trade name [82, 83]. In this last case it has been demonstrated that a great variety of side chains may be attained when the copolymerization is carried out at high pressures, conditioning as a result of the so-called “backbiting” mechanism in polyethylene [84]. Moreover, it has been also demonstrated that side chain carbonyl groups are more photoactive than those contained in the main chains because of the higher quantum yields for the formation in the solid phase of free radicals by both Norrish Type I and II processes. Additionally ethylene copolymers containing side chain acyl groups can be used as masterbatches in order to induce photodegradation of PE, thus allowing to control under a certain extent the degradation rates. This strategy is the basis of commercial Ecolyte process.

Even though, these materials usually have no induction periods and can be used mainly in short term applications didn't find so far any substantial commercial exploitation.

1.4.4.1.2. Prodegradant systems.

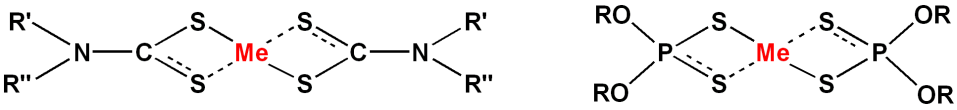
More recently, a strategy has been introduced as aimed at using pro-oxidant additives for a controlled oxidative breakdown of polyhydrocarbons into fragments vulnerable to microorganism. This is based on the use of pro-oxidants additives and it has been suggested that this alternative may provide a more efficient control of the shelf life, service life and degradation rate of the reengineered polyolefins in several applications [57].

- Organic salts of transition metals

Most of these compounds are based on organic salts of transition metals active in "one electrontransfer" process between two oxidation states. Several polymer soluble metal carboxylates and acetylacetonates of Co^{3+}, Fe^{3+} and $Mn^{+3}$are very effective photo-prooxidants capable to initiate the degradation process through the metal salts photolysis and/or thermolysis to give the reduced form of the metal ion and a free radical under UV irradiation [FeX_3, $h\upsilon > FeX_2 + X^*$]. The anion radical promote a fast hydrogen abstraction from the polymer and the relevant formation of hydroperoxide. Afterward the general radical oxidation mechanisms of the polyeolefins is thought to proceed being enhanced by the usual redox reactions between hydroperoxides and metal ions [FeX_2, + ROOH > RO* + FeX_2OH].

In an other case the properties of many metal complexes containing sulphur as a ligand play an opposite role acting either as photo or thermal stabilizer and as sensitizer after an induction period. Dithiocarbamates and dithiophosphates (Scheme 10) are the principal representatives of this

class of additives that exert the antioxidant effect by decomposing hydroperoxides by an ionic mechanism [85, 86]. After that, however, the ligand is destroyed, thus releasing free transition metal ions which start to behave as pro-oxidant according to the above cited mechanism. Hence antioxidant and photosensitizer properties are both present in the same compound.



Scheme 10. Structural formulas of dithiocarbamates and dithiophosphates

This evidence constituted the basis of the development of a well known class of photodegradable polyethylenes having a defined and controlled induction period initially started by Scott and further refined in collaboration with Gilead in order to finely control the lifetime before the photooxidation commences. This has been accomplished by using two component systems in which the length of the induction period is controlled by one metal thiolate and the rate of photooxidation by a second [87, 88]. The most representatives of this class of "delayed action" photosensitizer are the Fe(III) dithiocarbamates and dithiophosphates. The so-called "Scott-Gilead" technology led to the commercialization of several photodegradable polyethylenes especially devoted to applications in agricultural segments, such as mulching films, which requires a well defined induction period before the starting of the photodegradation process.

A different system, even constituted by a combination of a photosensitizer and a photoantioxidant was developed by Allen, by using anthraquinone and Tinuvin 770, respectively [89].

Photodegradable PE samples produced according to the Scott-Gilead technology have been extensive tested under both photo and thermal

exposure, as aimed at establishing the mechanism of polymer degradation and the effect of different type of pro-oxidants and additives.

In a case study, three different photodegradable PE samples containing iron dimethyldithiocarbamate (sample SG1), iron dimethyldithiocarbamte and 0.8% carbon (sample SG2) and iron dimethyldithiocarbamate and nichel dibuthyldithiocarbamate (sample SG3) were submitted to UV irradiation between 280 and 359 nm, both at room temperature and at 50°C for 300 h, in comparison with an additive-free LDPE film. The oxidative degradation of samples was also studied during 5 weeks under thermal aging at 80°C [90]. Degradation rate was assessed by monitoring molecular weight changes and structural analysis of the degradation products.

Depending upon type and combination of photo pro-degradants, different oxidative behaviours were recorded during UV exposure . In particular, it was ascertained that Mw and Mn of the samples additivated with iron dimethyldithiocarbamate (sample SG1) only decreased after a few hours of UV irradiation. Longer induction times were instead observed in the samples containing both iron and nichel dimethyldithiocarbamates. In addition, molecular weight analyses were also suggesting that the scissions of main chains was the dominant process, whereas cross-linking resulted in a fairly low extent. A complex behaviour was also observed by submitting the UV exposed samples to thermal aging. For instance, iron dimethyldithiocarbamate was inducing the drop of both Mw and Mn during both UV irradiation and under following thermal exposure. On the contrary, the same catalyst was promoting a slight increase of the molecular weight when the SG1 LDPE samples were submitted to thermal degradation only (Table 1).

Different degradation products were also recorded. Dicarboxylic acids were found as the main components in the photoexposed samples, whilst

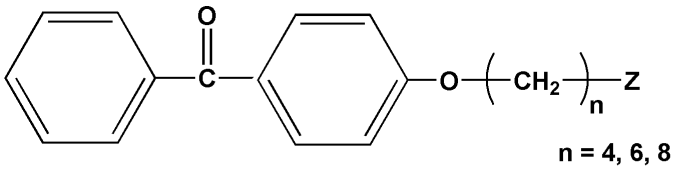
mono and dicarboxylic acids, as well as ketones and ketoacids were recorded in higher relative amounts in post thermally oxidized specimens with respect to the analogous sample submitted only to UV exposure.

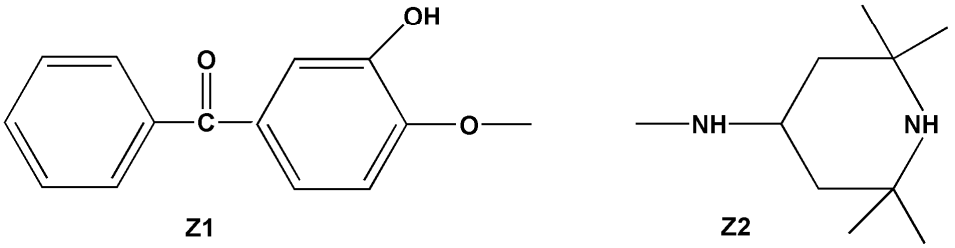
Table 1. Molecular weight analysis of iron dimethyldithiocarbamate containing LDPE samples submitted to different aging treatment [90]

Sample aging	**Mw**	**Mn**	**ID**
	kD	kD	
none	190.0	32.7	5.9
UV 100 hours	52.3	6.0	8.8
UV 100 hours + 80°C 5 weeks	23.1	4.8	4.8
80°C 5 weeks	202.0	49.8	4.4

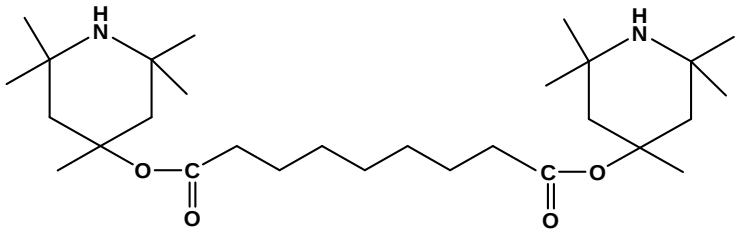
The strategy to control the life time of polyolefin under UV irradiation by mixing a photo-antioxidant and a photosensitizer constituted the basis of the studies performed by Acosta and co-workers [91]. In particular the feasibility to obtain life time controlled photodegradable polyethylene, was investigated by connecting photostabilizer and photosensitizer moieties in the same compound. Sterically hindered piperidine or ortho-hydroxy-benzophenone were assayed as photostabilizer moieties, whereas benzophenone was chosen as photosensitizer. Two types of additives were then synthesized in which the stabilizer component was a substituted benzophenone or the hindered piperidine (Scheme 11).

The synthesized compounds were tested in comparison with mixtures of benzophenone with well known commercial photostabilizers with similar structures such as Tinuvin 770 and Cyasorb 531 (Scheme 12).

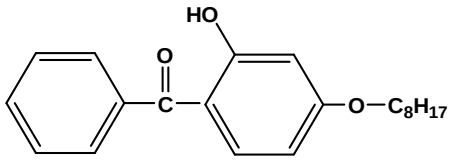




Scheme 11. Structural formulas of mixed photosensitizers and photostabilizers based on and substituted benzophenone or hindered piperidine.



Tinuvin 770 Bis(2,2,6,6,-tetramethyl-4-piperidyl)sebacate



Cyasorb 531 2-hydroxy-4-octyloxy benzophenone

Scheme 12. Structural formula of commercial photostabilizers

In order to compare the activity of the synthesized compounds, HDPE additivated samples were exposed to both thermal degradation at 110°C and UV-exposure and the oxidation rates monitored by the evaluation of non-volatile carbonyl groups by FT-IR spectroscopy [91].

The recorded embrittlement times, during the photodegradation tests, that were arbitrarily fixed at a concentration of 0.06 carbonyl units, were found to be depending upon the concentration of the additives in the HDPE films, as well as by their chemical structure and in particular by the number of methylene groups of the alkyl substituent. It was therefore observed that in the case of coupled chromophores system the photosensitizing effect played by benzophenone was dominant by increasing the concentration from 0.05 to 0.25 %, with corresponding decrease of the time of embrittlement. In addition the photoactivation was greater than that of benzophenone itself.

A more complex behaviour was observed in the case of the systems bearing the hindered pyridine as photostabilizer moiety. At a methylenes residues number as low as 4 in the aliphatic bridging chains a small increase in stabilization was observed with the increasing of the additive concentration, by contrast that photostabilization and photosensitizing effects were effectively competing. Whilst, by increasing the length of the aliphatic chains, the photosensitizing effect became dominant especially at low concentration. Whereas, the photostabilisation effect was higher at higher concentration in the case of systems bearing long aliphatic chains.

Very different behaviours were indeed recorded during the thermal degradation tests in oven. In this case the coupled chromophores systems even with different alkyl chain lengths turned to be powerful thermal pro-oxidants at very low concentration (0.05%), whereas the systems coupling benzophenone and hindered piperidine the thermal antioxidation was preavailing and icreased with increasing concentration and the alkyl chains length.

The reported investigation is therefore suggesting that antioxidant/pro-oxidant properties where constrained in the same compound can be modulated by the type of additive, the final concentration in the

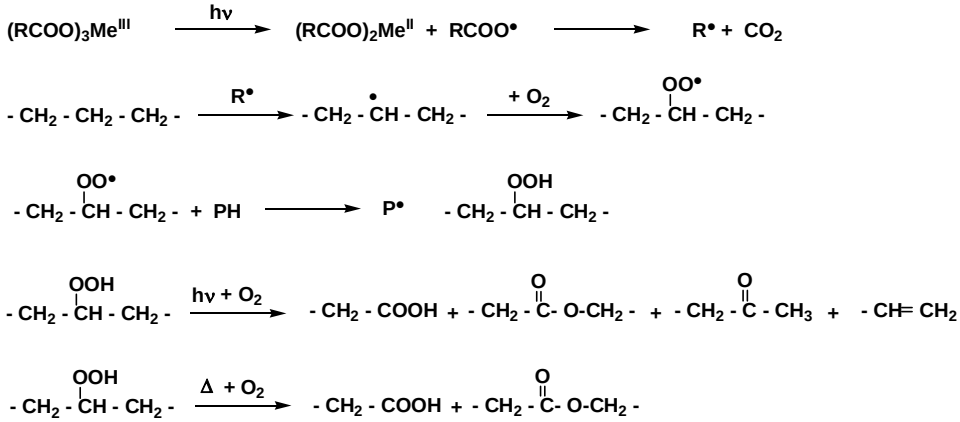
polyolefin films, as well as by their chemical structures. For instance in coupled chromophore systems of aromatic ketones residues, the photosensitizing properties of benzophenone moiety were dominating over the stabilizing effect played by 2-hydroxybenzophenone, very likely because the influence of the excited state properties by hydroxyl group with consequence on the proton transfer rate. On the other hand the systems including benzophenone and hindered pyridine behave principally as thermal stabilizer, the antioxidant effect being increased by the concentration and the alkyl chains length which allows for a more effective compatibilization and hence dispersion into the polymer matrix.

- Transition metal carboxylates pro-oxidant additives.

An other class of pro-oxidant additives is represented by compounds capable to induce the oxodegradative process of polyolefins by absorbing energy as heat. Also this class of additives is based on the activity exerted by transition metal ions typically added to the final product in the form of fatty acid salts or acetylacetonate complexes. The most employed cations are Mn^{2+} [92] and Co^{2+} [93]. Instead of Fe^{3+} complexes which play a major role in photo-oxidation processes, Mn^{2+} and Co^{2+} are needed to accelerate the radical chain reactions of polyolefin oxidation through the formation and decomposition of hydroperoxides and peroxides as induced by energy (heat and/or light) absorption.

The mechanism of oxidative degradation of polyethylene catalysed by transition metal ions has been recognized as a sequence of free radical chain reactions [94, 95]. As a typical example, cobalt stearate in carbon chain polymers containing when exposed to energy absorption is susceptible to transfer one electron in the 3d cobalt subshell of atoms leading to the formation of carboxylic acid free radicals that easily decarboxylates to form alkyl radicals. These latter does react with carbon backbone macromolecules, thus promoting the formation of

macroradicals especially in the presence of tertiary carbon atoms, susceptible to produce hydroperoxides in the presence of oxygen (Scheme 13).



Scheme 13. Radical chain reactions in polyolefins catalyzed by metal carboxylates

Nowadays, several prooxidant formulations are sold under different trade marks.Master batches marketed. containing either photo- or thermal or both pro-oxidants constituted by organic ligands with transition metal ions.

The effectiveness of Cobalt stearate in promoting the accelerated thermal oxidation of LDPE films has been also recently confirmed [96]. Indeed, an huge increase of Melt Flow Index (MFI) as a consequence of massive chain scissions was observed after 100 hours thermal ageing at 70°C of LDPE films containing 0.1-0.3% Cobalt stearate. Significant decay of mechanical properties, such as elongation at break, and MFI increase were also recorded when the same films were submitted to UV exposure. In accordance, cobalt stearate can be considered to be effective in promoting both photo and thermal oxidation of LDPE. Nevertheless, largest degree of oxidation were observed during the thermal aging, thus suggesting once more that Cobalt organic salts are more suitable thermal pro-oxidants for polyolefins.

In addition, rate and extent of oxidative degradation were positively correlated with the content of Cobalt stearate.

The effectiveness of Cobalt organic complexes has been also investigated either as a function of the type of organic ligand, as well as of the aging conditions (photo or thermal exposure). It is known, in fact, that the catalytic activity is depending upon the valence and ionic bonding, as well as on the possibility to be intimately miscible blending with polymer chains at molecular level [97].

In other studies, the effect of carboxylate chain lengths (laurate, palmitate and stearate) as organic ligands for Cobalt ions as photo-sensitizers was investigated. LDPE films containing 0.05-0.2% of each type of cobalt carboxylates were prepared and tested under UV exposure, by using FT-IR spectroscopy, mechanical testing and molecular weight determinations as analytical tools [98]. It were therefore monitored to show that the oxidative degradation increased with the increase of the chain lengths of carboxylates residue. Therefore, beside the content of cobalt, during the photo exposure higher levels of degradation as detected by the MFI assessment were obtained with cobalt stearate, followed by palmitate and laurate. It was therefore suggested that the efficacy of Cobalt metal complexes in affecting the rate and extent of photo-oxidation of LDPE films is related to the length of carboxylate residues. This feature can be attributed either to the higher thermal stability of stearate during the LDPE processing, as well as to the better miscibility of longer carboxylate groups within the LDPE matrix.

Taking into account the intrinsic capability of carboxylic acid polymers, such as Ethylene-*co*-Acrylic Acid (EAA), as well as those of styrene based polymers in inducing the photo/thermal oxidation of polyethylene, the capability to accelerate the oxidative degradation of LDPE films as mediated by synergistic effect of cobalt/polymeric complexes has been

investigated. In particular a cobalt complex with styrene-*co*-maleate copolymer (CSMA) was prepared with the aim of having in the same compound the pro-oxidant activity of transition metal ions, the capability of hydrogen abstraction from acidic groups by peroxy radicals, as well as the light absorbing cromophores of styrene [98]. The synthesized cobalt/polymer complex (CSMA), was tested as pro-oxidant in LDPE films in comparison with cobalt stearate during thermal degradation tests at 70°C and photodegradation tests carried out by exposing the test films to light in the 280 - 350 nm wavelength range.

Despite its theoretical capability to promote the radical oxidation of LDPE matrix, the CSMA additive did not induce any larger degradation of LDPE films neither after 100 h thermal aging or 600 h UV exposure, with respect to that recorded in the case of a not-additivated control. On the contrary, films containing Cobalt stearate pro-oxidant underwent to an extensive oxo-degradation under both thermal and UV-exposure under analogous conditions. It was also suggested by MFI determination that crosslinking side reactions may occur in the case of UV exposed LDPE films containing CSMA Cobalt Complex.

Cobalt stearate, was found to promote the fragmentation of LDPE films even in soil burial tests carried out at ambient temperature for 12 months, whereas pro-oxidant free LDPE- and LDPE containing CSMA did not exhibit any physical changes within the same environmental and timeframe conditions.

The collected results were indeed showing the inability to induce the oxidation of LDPE by CSMA complex, although it was containing three different components each individually capable of initiating polyolefin degradation. It was therefore suggested that in spite of oxidation number and ionic bonding of Cobalt ions in CSMA complex, equivalent to the situation in Cobalt stearate, the ineffectiveness of the CSMA complex

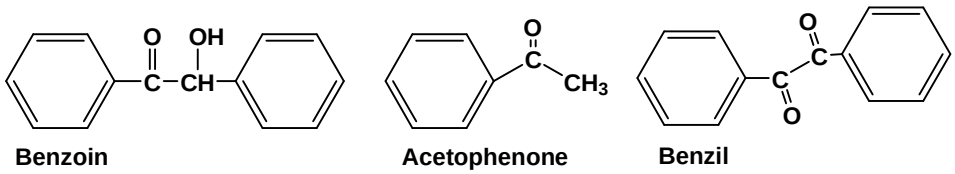
might be attributed to the inherent crosslinked structure of CSMA hindering the Cobalt ion availability in single electron transfer reaction connected also to the intimate blending of CSMA with PE chains at molecular level during the processing. On the contrary, Cobalt stearate, was shown to maintain its redox activity, due to the intimate miscibility with PE which appears to be a key factor for photo and thermal degradation initiation by pro-oxidant additives.

In the case of Manganese stearate, the effectiveness in promoting the oxidation process of polyolefin matrix as a function of aging conditions has been also evidenced [90]. In particular, Mn organic salts were found to induce a fast and substantial drop in the LDPE molecular weight when submitted to thermal aging. By contrast, if the same samples were preliminary exposed to UV irradiation no longer promotion of oxidation occurred during the following heat exposure step. This behavior was attributed to the deactivation of Mn stearate catalyst by UV irradiation [90].

- Aromatic ketones.

Aromatic ketones (Scheme 14), such as benzophenone, represents active photoinitiators for several polymers. Since the early ninetysixties, it was established that benzoin and benzophenone can be used to sensitize the formation of singlet oxygen through different steps including light absorption by carbonyl groups. Norrish type II cleavage involving n-π* excited states of carbonyl groups and formation of singlet oxygen molecules by quenching of the n-π* triplet state of carbonyl groups is taking place [99].

Recently, the role of benzyl has been investigated as photo-prooxidant for LDPE films in combination or not with cobalt stearate under exposure to sun light, artificial wheatering and thermal aging [100, 101].



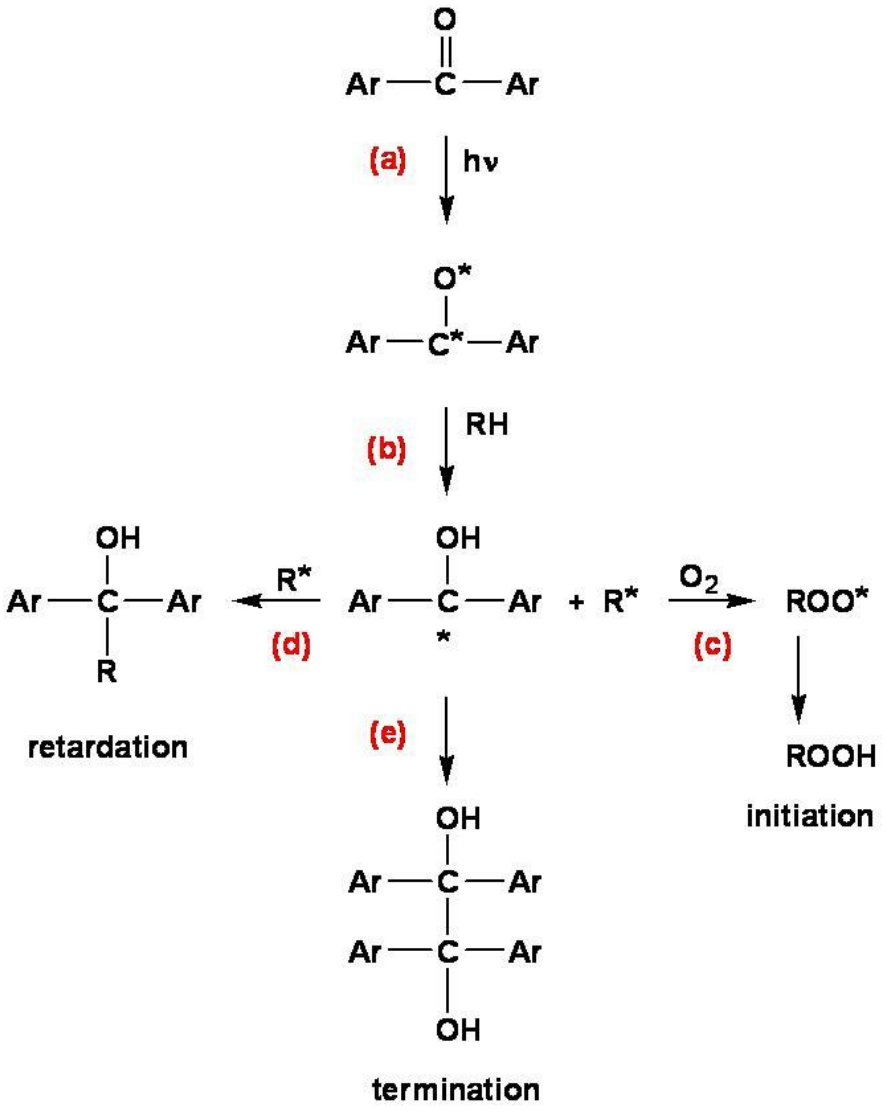
Scheme 14. Aromatic ketones as photosensitizers

During this latter aging test, benzyl did not induce any appreciable increase in the level of oxidation, as determined by carbonyl index assessment, with respect to the control film even after 1000 h of heating at 70°C in air. Only minor variation in the oxidation levels were indeed appreciated in LDPE films containing benzyl only during natural and UV weathering tests. On the contrary the effectiveness of cobalt stearate alone or in combination with benzyl in promoting the oxidation and fragmentation of LDPE films was observed during both thermal and light exposure treatments.

These observations are apparently in contrast with previous studies showing the effectiveness of aromatic ketones in inducing photo and thermal degradation of HDPE [91]. However, the inability of benzyl alone, even though containing two keto groups, to promote the oxidative degradation of LDPE, can be tentatively explained by considering the mechanism of photo-initiation induced by aromatic ketones [102] (Scheme 15).

It has been suggested that the rate constants of formation and termination by recombination of aromatic ketone radicals as sketched in b and d reactions, respectively (Scheme 15) are depending upon the polymer structure. In the presence of highly branched LDPE the reaction with the polymer chains is much faster than linear polyethylene such as HDPE, because of the higher stability of the resulting macroradicals R*, thus accelerating also the termination reaction. In addition, it has been also proposed a relatively fast recombination of aromatic ketone free radicals

leading to resonance stabilized oxetane ring structures. Therefore the above mentioned features might explain why benzyl alone does not promote the degradation of LDPE.



Scheme 15. Photooxidation of polyolefins when exposed to the light in the presence of aromatic ketones

- Polymeric additives

The feasibility to enhance or at least to control the effect of metal complexes (e.g. pro-oxidant catalyst) on the rate and extent of polyolefin oxidation, by employing polymeric additives, constitutes an other topic which has been investigated for the preparation of oxo-degradable full carbon backbone polymers.

Polymeric additives, based on matrices of synthetic and natural origin, have been used and their role has been investigated. It is known that the most frequently used pro-oxidants are aliphatic salts of transition metals such as Zn, Cu, Ag, Co, Ni, Fe, Mn, Cr [103]. Usually, the final formulation of pro-oxidant additives also contains an auto-oxidable substance such as unsaturated or polyunsaturated compounds. These last materials are thought to facilitate the oxidation of less reactive saturated carbon chain polymers, being very prone to auto-oxidation. In addition, they may improve the mechanical properties of environmentally degradable polyolefins. As an example, styrene-butadiene (SB) copolymers have been extensively utilized in polyethylene/starch blends with the aim to compensate the deterioration of mechanical properties provoked by the addition of the natural filler [104].

In a case study, methylmethacrylate-butadiene-styrene copolymer (MBS) has been investigated as autooxidable compound capable to accelerate the thermal oxidation of LDPE containing cobalt stearate as pro-oxidant catalyst [105]. Ethylene-acrylic acid copolymer (EAA) was utilized as compatibilizer and plasticizer for the preparation of LDPE/starch blends [105].

It was indeed ascertained, during thermal degradation tests, that the addition of small amounts (2.5-5.0% by weight) of MBS to LDPE films additivated with Co-stearate (0.1% by weight) greatly enhanced the film degradation. In particular, both the rate and extent of oxidation, as

determined by the carbonyl index, were increased by increasing the amount of MSB used in the preparation of LDPE blends, thus obtaining materials that were heavily disintegrated in short time at 70°C.

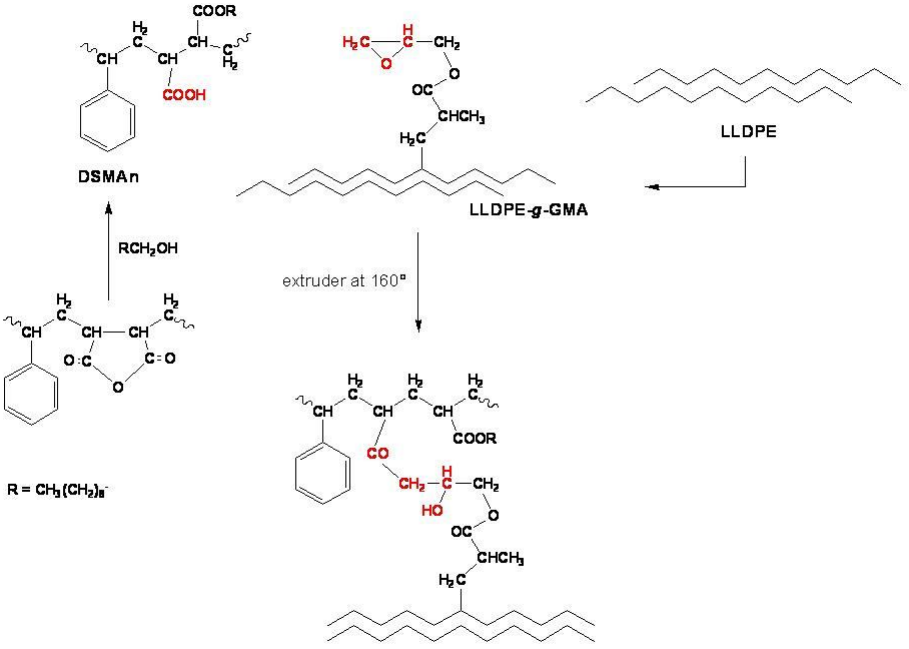
As a degradation polymeric promoter, partially oxidized polyethylene (OPE) has also been recently utilized and tested in the preparation and characterization of photo-degradable LDPE films [106]. Films containing different amounts (0.5-5% by weight) OPE were prepared by blown extrusion, by using a thermally preoxidized (100°C for 12 h) LDPE sample containing 0.1% cobalt stearate as pro-oxidant. LDPE films containing OPE were exposed to UV-B irradiation up to 600 h in comparison with an additive-free sample. During the aging test, the effectiveness of OPE to promote the oxidative degradation of the virgin LDPE matrix was ascertained by monitoring of different parameters such as MFI, intrinsic viscosity and carbonyl index. A relationship between the amount of OPE and the extent of degradation of LDPE film samples was then established. It was therefore suggested by the authors that the initiation of photooxidation might be attributed to the presence of oxidized functional groups, particularly carbonyl groups, present in OPE. These groups were thought to promote the oxidation of virgin LDPE polymer matrix in accordance with Norrish type I and II reactions, involving the oxidative cleavage of macromolecular chains after UV absorption by carbonyl chromophores. In spite of these suggestions, it can not be excluded at all also the activity exerted by the residual content of cobalt stearate in an active state coming from OPE. In this connection, it has to be evidenced that the specimens containing the highest amount of OPE, which is corresponding to a 0.5% theoretical content of CS, underwent a notably lower extent of photo-oxidation, as assessed by Carbonyl index, with respect to LDPE films additivated with 0.5% CS

based on the same resin batch and photo-aged under the same conditions and time frame [101].

1.4.4.1.3. Blending and modification with biodegradable polymers and additives.

Another strategy to address the environmental degradation of polyolefins comes from the preparation of blends with heteropolymers bearing functional groups susceptible to photo-oxidation. Among these styrene/maleic anhydride copolymers (SMAn) have been ones of the most investigated [107]. In particular, the effect of decanol grafted onto SMAn (DSMAn) utilized for the preparation of LLDPE blends in the presence of LLDPE grafted with glycidyl methacrylate (LLDPE-g-GMA), was studied with the aim to assess the propensity to photodegradation, and to understand the mechanism of the process [108]. During the preparation (e.g. blow extrusion) of LLDPE-based films containing 20-40 weight % DSMAn and 5-15% LLDPE-g-GMA a reactive processing has been thought to occur leading to a crosslinking of the reactive polymers (Scheme 16).

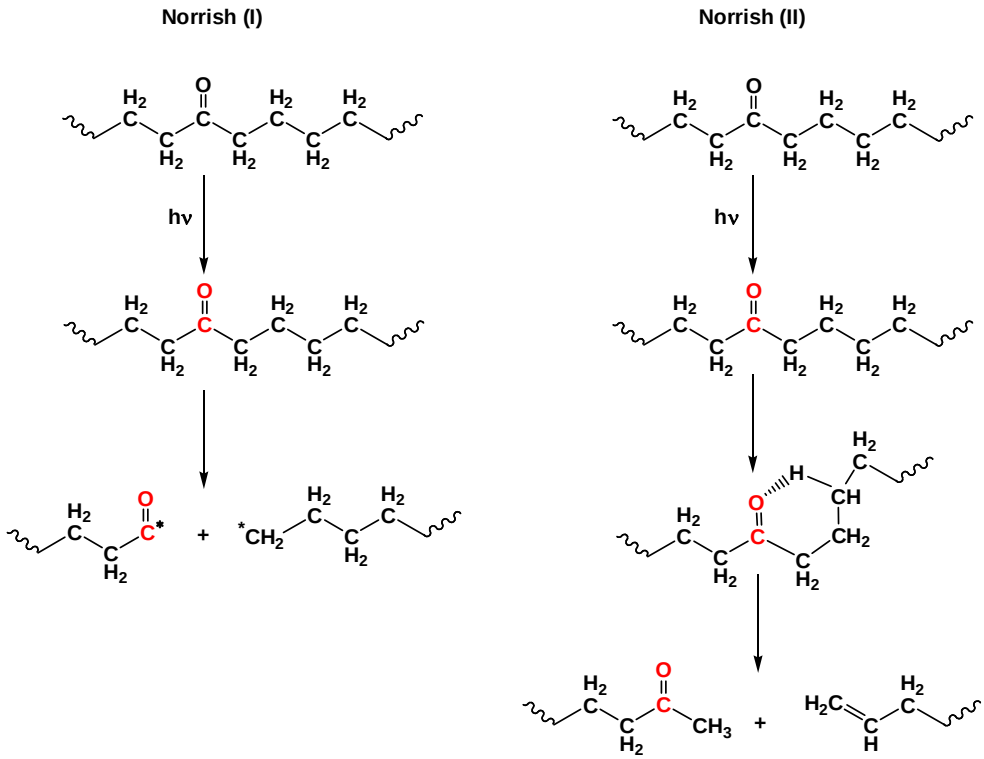
In accordance, the resulting blends are containing an hydrophilic component with a carboxylic acid and ester carbonyl group in the side chain, being theoretically more susceptible to environmental degradation as mediated prevailingly by photo-oxidation. It has been therefore ascertained that such type of polymers bearing carboxyl groups in the side chains undergo chain scission by Norrish Type II reaction (Scheme 17) [109].



Scheme 16. Scheme of the reactive blending of LLDPE, ESMA and LLDPE-g-GMA [108].

DSMA/LLDPE blends compatibilized or not with LLDPE-g-GMA were submitted to aging test carried out either in a Xenon arc lamp weatherometer and in outdoor exposure to direct sunlight. In both cases the films undergo an embrittlement in shorter times in the case of compatibilized sample. It was therefore suggested that DSMAn induces photooxidation in the blends and the effect can be intensified by the addition of LLDPE-g-GMA as compatibilizer. The results also indicated that the photooxidation takes place first in DSMAn phase and the radical herein produced can migrate to the LLDPE phase, thus inducing the oxidative cleavage of the full carbon backbone. It was therefore hypotesized that a mechanism of radical transport across the phase boundaries is responsible for the initiation of oxidation in LLDPE phase.

This type of radical transfer from one phase to an other has been evidenced in blends of poly(styrene) and poly(vinyl acetate) [110, 111].



Scheme 17. Norrish I and II mechanisms of photophysical breakdown of oxidized polyethylene.

Pal and co-workers therefore hypothesized that DSMAn deriving radicals, once produced in the first stage of photo-degradation, migrated to the LLDPE phase, thus reacting with atmospheric oxygen to produce unstable hydroperoxyde groups capable to undergo a further decomposition (by light absorption) with the generation of carbonyl groups in the main chain. As a result the further degradation leading to a relatively fast chain scissions and fragmentation of the blend was thought to occur according Norrish I and II type reactions (Scheme 17).

Since the original idea from Griffin to blend polyethylene with starch, a vast research activity was devoted to produce blends and composites of polyolefins (PE, PP, PS) with natural polymers, as well as to modify and grafting them with the aim of enhancing the compatibility with functional polymers and eventually with the environmental acceptance.

The partial replacement of synthetic polymers with renewable resources, jointly with the hypothetical stimulation of the full carbon backbone polymer degradation and biodegradation, constituted the guiding ideas of this strategy. In fact, once the natural components are consumed by microorganisms, pores and voids formation, increasing the surface area, are thought to make the synthetic matrix more vulnerable to either abiotic (e.g. oxygen) and biotic degrading agents.

One of the major problems encountered in the blends preparation has been undoubtedly the very poor compatibility between hydrophobic polyeolefins and polar natural polymers. Hence several technologies, have been developed to improve the compatibility between natural polymers and polyolefins. The use of coupling agents such as maleic anhydride, methacrylic anhydride and maleimide, starch gelatinization and the use of compatibilizers, represents only a part of the approaches employed in order to mix high amount (more than 30-40% by weight) of natural polymers with polyethylene. In accordance an enormous amount of contributions has been produced, whose critical revision is not in the aims of this thesis.

Nevertheless, it is important to note that the interest of this topic is still very high and that a well definite borderline between other strategies such as those using pro-oxidant additives does not exist. As an example, since from 1988 a process involving the use starch, an unsaturated polymer as compatibilizer and a pro-oxidant was patented by Griffin [112].

Moreover, several articles dealing with the blending of polyethylene with lignin and with biodegradable aliphatic polyesters from both natural and synthetic origin have been published. Nonetheless, most of the commercial available environmentally degradable polyethylenes are still based on the original ideas developed by Guillet [83], Scott [87] and Griffin[113] in the 1970s.

In the following paragraphs the results obtained on the environmental fate of pristine and degradable polyethylenes belonging to above cited classes will be reviewed.

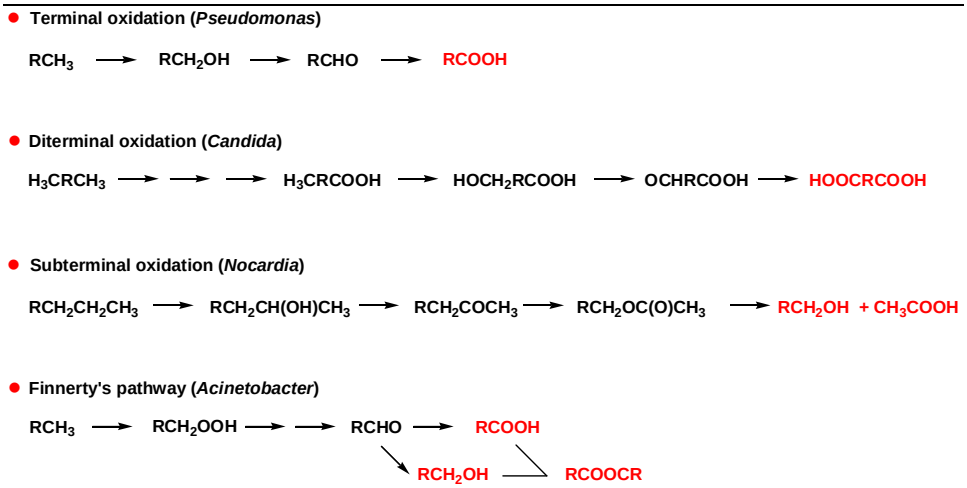
1.4.4.2. *Biodegradation of pro-oxidant-free polyethylene by natural occurring microorganisms*

The contemplation of the inherent capabilities of microorganisms to promote the biodegradation and eventually the utilization of polyethylene (PE) as carbon source seems to be more controversial nowadays that in fairly recent past years. Conflicting results have been indeed obtained that are suggesting from one side the ability of specific microbial strains to degrade high molecular weight PE, whilst many studies are yet claiming the inertness to biological attack of this polyolefin and structurally alike polymers also when exposed to very physical-mechanical stressing and active microbial consortia such as those applying to composting windrows. Degradation of PE by microorganisms has been generally monitored in terms of microbial growth, biometry (e.g. biochemical oxygen demand or carbon dioxide emission, including $^{14}CO_2$ generation from radio-labelled samples), sample weight loss, mechanical properties, variation of structural features (e.g. molecular weight, spectroscopic features, mechanical properties).

- Biodegradation of polyethylene by bacteria

Preliminary investigations are dating back to the early 1960s. At that time, the increase of microbial cell numbers was taken as an indication of PE assimilation by bacteria in comparison with low molar mass paraffins [114]. From these studies it was suggested that several bacteria can utilize as carbon source low molecular mass PE fractions of MW slightly below 5000 (DPn ~ 180), whereas no microbial activity was detected on higher Mw fractions. Potts and co-workers assessed that linear paraffin with Mw below 700 ($C_{50}H_{102}$) were utilized by different microorganisms [115]. Similar results were obtained later by Albertsson and Banhidi that recorded the utilization of short oligomeric fraction of HDPE by microorganisms after 2 years biodegradation experiments [116].

Indeed, it can be suggested from a theoretical point of view that since PE is a nominally straight-chain hydrocarbon, it should be metabolized according to the biochemical pathways holding true for linear alkanes (Scheme 18). On the other hand, it has been established that there is a molecular weight upper limit for the utilization of *n*-alkanes as a carbon source by microorganisms. Haines and Alexander established that linear hydrocarbons with more than 44 carbon atoms (tetracontane) cannot be metabolized by soil micro-organisms [117]. More recently in a study carried out by using single bacterial strains [118] this dimensional limit has been extended to 720 Dalton corresponding to an hydrocarbon chain constituted by 50 carbon atoms. In any case these limits are thought to be related to the bacterial metabolism of n-alkanes that needs the accessibility to methyl chain ends by extra cellular oxidizing enzymes to start the biodegradation process.



Scheme 18. Microbial oxidation pathways of *n*-alkanes.

The first step is known as hydroxylation (ω-oxidation) which give rise to the corresponding primary alcohols which are further enzymatically oxidized to aldehyde and hence to carboxylic acids. The resulting carboxylated *n*-alkanes can be metabolized according to the β-oxidation process in analogy with the catabolism of fatty acids. Thus, the rate and eventually the ultimate extent of biodegradation of solid n-alkanes can be strongly affected by the availability of CH_3 chain ends susceptible to enzymatic oxidation. It follows that the number of chain ends present at the surface of a solid *n*-alkane decreases with the molecular weight increase and hence with extremely low values in the case of high molecular weight polyethylene.

On the other hand, it can not be at least hypothetically ruled out that oxidizing biological processes other than the ω-oxidation of terminal methyl groups, such as the random chain cleavage as mediated by dehydrogenation/oxidation leading to carbonyl groups, might play an important role in the biodegradation of PE.

Taking into account these suggestions Kawai and her collaborators tried to establish a numerical simulation model for the biodegradability of PE

starting from experimental data relevant to the biodegradation of PE-wax having Mw 2900 (C200) and Mn 1100 (C71), respectively [119, 120]. The computational model for the numerical simulation was set up regarding two main factors:

a). Sample weight loss due to β-oxidation.

b). Fast consumptions of low molecular weight fractions.

Both factors were substantiated by experimental data.

In particular the increase of both Mw and Mn of PE wax was observed after cultivation as an indication of the microbial consumption, in the meanwhile the time profile of molecular weight variation suggested the fast assimilation of the lower Mw fractions, as well as the gradual decrease of assimilation rates with increase in molecular weight. By applying the numerical model, which was validated by experimental results, to the biodegradation of PE, the authors provide suggestions supporting the terminal oxidation and β-oxidation as the main processes involved in the microbial degradation of PE. In addition, by using this approach it was possible to distinguish between the process of biodegradation as mediated by bacteria such as *Sphingomonas* or *Aspergillus* sp. and *Penicillium* sp. fungi. It was therefore evidenced that bacteria exhibited higher biodegradation rates that were attributed to the higher affinity toward PE of Gram-negative bacteria cell walls with respect to the more hydrophilic chitin walls of fungi [120]. *Sphingomonas* species were thought to metabolize PE wax, preferentially with Mw below 2000, throughout primary terminal oxidation followed by β-oxidation with enzymatic systems located in the cell membrane fraction, thus suggesting for the transport of oxidized PE wax into the periplasmic space through outer membranes [121].

Regarding the capabilities of single microbial species to attack PE as carbon substrate, only a few reports are so far available. Nevertheless, in

the recent years evidences in the occurrence of soil microorganisms directly involved in the biodegradation processes of PE have been reported by Ohtake and collaborators [122, 123]. These researchers claimed their assumption by analyzing LDPE films and bottles buried in soil for many (up to 32) years, thus being negligible when exposed if any to physical degrading factors such as heat and light. They found discoloration and traces of microbial growth, as well as significant molecular weight decrease in the portion of LDPE items directly exposed to soil contact, whereas other portion less contaminated by soil did not showed any appreciable traces of degradation. In addition, the microscopic FT-IR spectroscopic analysis clearly evidenced that the degraded and microbial colonized part of LDPE mulch films were characterized by the presence of intense absorption bands relevant to -C=C- double bonds, carbonyl and hydroxyl groups [122]. Furthermore, starting from various specimen as microbial inoculum, including soil adherent to LDPE scattered films and surrounding vegetable soil samples, the authors isolated and identified three different *Bacillus* species (*B. circulans, B. brevies, B. sphaericus*) clearly involved in the biodegradation processes of LDPE. As a confirmation, the isolated strains were tested in pure culture supplemented with outdoor weathered antioxidant-free LDPE powder as sole carbon source. Even though neither molecular weight analysis and biometry (e.g. carbon dioxide) evaluation were performed, FT-IR characterization confirmed the formation of hydroxyl groups as an evidence of the microbial attack. Much more convincing were the SEM pictures collected after the lysis of the bacterial cells adhering to the oxidized portions of LDPE, which revealed the presence of “body footh print” as degraded portion of the polymer matrix in the correspondence of bacterial cells (Figure 3) [123].

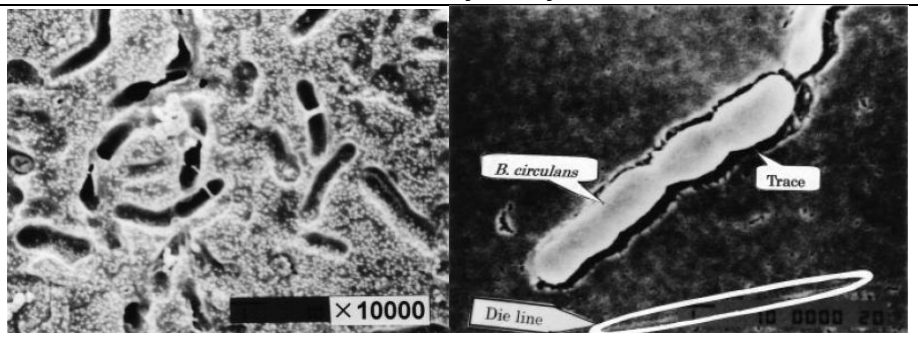


Figure 3. SEM pictures of Bacillus cells and relevant "body footh prints" on LDPE sample [123].

A fungal strain, identified as *Penicillium simplicissimum* YK capable to growth in the presence of high molecular weight PE as sole carbon source was isolated from soil and leafage samples [124]. The fungal strain growth was tested in the presence of untreated PE, UV-irradiated and PE treated with hot nitric acid as carbon sources. The course of PE samples degradation was examined by molecular weight analysis (HT-GPC) and FT-IR spectroscopy. It was therefore recognized that the isolated fungal strain utilized PE fractions in the molecular weight range of 4,000 up to 28,000 of untreated sample. Indeed, *P. simplicissimum* YK exhibited higher growth in the presence of UV irradiated than untreated PE, in addition when the fungus was grown in the presence of PE treated with nitric acid a significant decrease of the starting molecular weight (100,000) was recorded. It is interesting to note that FT-IR characterization clearly showed a marked reduction after the fungal cultivation in the intensity of carbonyl and double bond absorption band resulting from the pre-ageing of PE by UV irradiation and nitric acid treatment, respectively. These results are confirming the increasing susceptibility to biodegradation of PE containing functional groups, and at the same time evidenced the occurrence in natural environments of microorganisms capable to metabolize at least partially high molecular weight virgin PE samples. This latter suggestion was evidenced by

Wasserbauer *et al* [125]. They found that PE which was extracted to remove antioxidants can be oxidized by *Pseudomonas putida* with the production of CO_2, thus suggesting that the oxidation of the polyolefin was induced by monooxygenase hydroxylation enzymes produced by the bacterial cells.

Other investigation as aimed at finding microorganisms capable of degrading high molecular weight PE provided the isolation of a strain of *Rhodococcus ruber* after an enrichment procedure carried out on soil samples collected from PE waste burial sites [126]. Due to the high cell hydrophobic character the bacterial strain was recognized to be able to heavily colonize PE surface by biofilm formation and degrading up to 8% mass of the starting material in a few weeks of incubation. Indeed, the formation of bacterial biofilm is considered as a positive feature to make microorganisms capable to be assimilated as carbon source non-soluble substrate. In the same investigation was in fact ascertained that the addition of small concentration of mineral oil enhanced either the biofilm formation as well as the extent of PE weight loss. The same authors also suggested that the biofilm development by *R. ruber* cells is increased under carbon-starved culture condition such as that containing PE as sole carbon source. The increased hydrophobic interaction between the bacterial cells and PE surface were therefore considered as positively able to improve the biodegradation of the synthetic polymer [127].

The significance of microbial biofilm formation as a factor to enable microorganisms to efficiently utilize solid hydrophobic substrates such as PE films, enhancing their enzymatic activity, has been recently confirmed in a study carried out in the presence of dual cultures of *Penicillium frequentans* and *Bacillus mycoides* [128]. Both the fungal and bacterial strains were isolated after 2-4 years soil buried PE pieces and cultivated in the presence of either intact or thermally oxidized PE as sole carbon

source. It was recognized that efficient biodegradation of both unmodified and thermally treated samples, as recorded by sample weight loss (7%) with concomitant CO_2 emissions was appreciable only after the micelyum of *P. frequentans* was colonized by the *B. mycoides* cells to form a packed biofilm at the PE surface. On the contrary each microorganism when tested in axenic mode did not provide any significant PE biodegradation. It is also interesting to note that both *P. frequentans* and *B. mycoides* are recognized as hydrocarbon degrading microorganisms, being alkane monoxygenase the enzymatic system found in the genomes of several *Bacillus* spp.

An other bacterial strain was selected from a group of bacterial isolated from soil samples collected from a PE waste disposal site after an enrichment technique by using intermediate size PE oligomres in the form of liquid waxes. The microorganisms was identified as a thermophilic *Brevibacillus borstelensis* strain [129]. The selected strain was found to be able to utilize PE as sole carbon source. Nevertheless, the higher biodegradation process as recorded by either 17% sample mass loss and 34% molecular weight decrease was achieved by combining UV irradiation followed by 3 months incubation with *B. borstelensis* at 50°C. Indeed, it has been repeatedly suggested that oxidized fragments formed during thermal or photo-oxidation of PE can be readily assimilated by various microorganisms. In some cases this “preferential” utilization as carbon source has been found to induce a little increase of the average molecular weight, such as in the case of the investigation carried out by Albertsson *et al* in the presence of *Arthrobacter paraffineus* [130]. One of the most important issue rising from this indication is strictly correlated to the occurrence of further biodegradation process once the oxidized fragments of PE have been depleted by microorganisms. In the case of the studies carried out with *Brevibacillus borstelensis* it was indeed observed

that the biodegradation step last for 30 days during which a fast consumption of carbonyl-containing intermediates was recorded. Consequently, two consecutive 30 day incubation steps in the presence of the isolated strain, were carried out on the same PE sample with the aim of assessing if the microorganism would have been able to metabolize full carbon backbone; that was significantly deprived of carbonyl groups. The obtained results, even though not supported by FT-IR characterization, showed that the PE biodegradation rate (e.g. weight loss and Mw decrease) was almost the same during the two 30 day incubation steps, thus suggesting that the assimilation of carbonyl bearing fragments might have induced further degradation of full carbon backbone. In conclusion the reported study was claiming that *B. borstelensis* is capable of degrading fairly high molecular weight PE either in a pre-oxidized or untreated form.

Even though the few reports regarding single microorganism capability to efficiently attack virgin (e.g. untreated PE), at the end of this paragraph, some remarks can be drawn. First of all it has to be mentioned the renewed interest in recent years in the search of effective microorganisms for biodegradation studies of recalcitrant or poorly degradable synthetic polymers. In addition some general features regarding the physiology of the isolated species can be highlighted. In particular most of them have been recognized as alkane-degrading species and were isolated from PE contaminated soil sites, whereas many are also producing oxidizing enzymes such as the monooxygenase-cytochrome P450 system of *R. ruber*. The biofilm formation has been also considered as a positive factor helping the viability of microorganisms as well as their degradative efficiency. In this latter case a suggestion regarding the modification of environmentally degradable polyethylene to be colonized by biofilm-forming microorganisms should be taken into account.

- Biodegradation of polyethylene by ligninolytic microorganisms and related enzymes

Since early studies by Lee et al. [131] a great interest in the exploitation of lignin-degrading microorganisms for biodegradation of polyethylene and other synthetic polymers has been recognized. In their study Lee *et al.* evidenced the role of the *Streptomyces* species in the significant reduction, after a few days of incubation, of average molecular weight of thermally treated LLDPE containing transition metal ions (Fe, Zn, Ni and/or Mn), lipids and 6% starch. In the case of Streptomyces species (*S. basidius, S. setonii and S. viridosporus*) the presence of extracellular enzymes capable to directly attack the polyethylene matrix was ascertained [132, 133]. The investigations were carried out in the presence of the culture filtrates of each actinomycete species, thus evidencing the presence of active enzymes capable of promoting a further oxidation of thermally aged PE/starch containing pro-oxidant agents. In particular, the presence of primary and secondary alcohols functionalities in the PE chains was evidenced that was accompanied by significant decrease of mechanical properties (tensile strength, percent elongation and strain energy) and molecular weight. It was also evidenced that the most effective species was *S. viridosporus* and the nature of ligninolytic systems in the bacterial culture filtrates. On the contrary the ligninolytic fungus *Phanerochaete crysosporium* did not exhibit any degrading activity even in the presence of heat (70°C) or UV pretreated samples. In this regard, conflicting results were however obtained by Nishida and collaborators that reported the degradation of high molecular weight polyethylene membrane by lignin degrading fungi, including *P. chrysosporium* [134]. The reported studies were carried out with as aimed at establishing the effectiveness of well known lignin-degrading fungi such as *P. chrysosporium* and *Trametes versicolor* with respect to a new

white-rot strain (IZU-154) capable to biodegrade nylon membrane under ligninolytic cultural conditions [135]. Comprehensive studies were also undertaken to establish the effects of cultural conditions in terms of both source and concentration of carbon and nitrogen substrates, as well as to evaluate the effects played by different ligninolytic enzymes, manganese peroxidase (MnP) and laccase [136, 137]. Among the tested fungi, the nylon degrading white rot strain IZU-154 and *P. chrysosporium* were the most effective, thus promoting the 100% relative elongation decrease of PE membrane (HIPORE 1100, Asahi Kasei) after 4 days incubation under carbon and nitrogen sources starvation conditions. It is well known that fungal ligninolytic activity can be considered as a secondary metabolic activity which is stimulated under nutritional carbon and/or nitrogen limitations [138]. In accordance Nishida *et al* firstly suggested that the PE membranes degradation was strictly related to the ligninolytic activity of the investigated fungi. During the course of their studies they also demonstrated the role of *MnP*. The secretion of oxidative lignin-degrading enzymes *laccase*, *MnP* and *lignin peroxidase* (*LigP*) was checked during the treatment of PE membranes with the selected fungi, thus recording the production of laccase and *MnP* only. Further investigations, carried out by isolating and purifying *MnP* from fungal cultures, finally suggested that in the case of *P.chrysosporium* and white-rot IZU-154 strain the most effective enzyme in the oxidation and biodegradation of PE was *MnP*, an heme oxidoreductase acting on a peroxide as acceptor. This finding inspired new investigations as aimed at ascertaining the enzymatic degradation of PE membranes, as well as the better conditions in terms of co-factors and co-substrate. As expected a positive effect was exerted by the addition of Mn(II) salts in the incubation medium, whereas the effects of other transition metal salts such as $FeSO_4$, $ZnSO_4$ and $CuSO_4$ were negligible. It was also ascertained

that PE degradation occurred in malonate buffered media, whilst in acetate buffer MnP was ineffective most likely because Mn(III) generated from Mn(II) is salified by this last buffering solution that hinders the propensity of Mn (III) to enter in the electron transfer process at the basis of CH_2 bond oxidation. This observation was therefore suggesting that Mn(III) is directly involved in the PE oxidation. Indeed in further studies Nishida and collaborators reported that Mn(III) was generated by the oxo-reductase activity of *MnP*, also evidencing that the presence of unsaturated fatty acid or Tween 80 surfactant improved the PE membrane degradation by the radicals generated from the peroxidation of these latter substrates [136].

An other lignin-degrading enzymatic system that has been investigated for the biodegradation of PE is represented by *laccase*. *Laccase* was found to be the only secreted oxidizing enzyme by *Trametes versicolor* when growth on PE membranes, even though it was less effective with respect to other ligninolytic fungi [134]. Laccases are multicopper polymeric oxidases of broad specificity produced by plant and fungi, that carry out one-electron oxidation of phenolic and related compounds, and reduce O_2 to water. The oxidizing activity toward non-phenolic compound is strictly related to the laccase redox potential. In this connection it has been repeatedly observed that the non-phenolic compound oxidation, including non-phenolic lignin residues, by *laccases* can greatly increase by the presence of redox mediators such as 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) or 1-hydroxybenzotriazole (HBT) [128, 129]. In accordance Nishida and collaborators investigated the ability of *T. versicolor laccase* to degrade PE membrane with and without the addition of HBT [139]. The complete failure of relative elongation as witnessed by the PE membrane disintegration (Figure 4) and the dramatic Mw drop from 242 kDa to 28.3

kDa was recorded in a few days, thus also evidencing that the addition of 0.2 mM HBT greatly enhanced the laccase activity. This latter observation was therefore suggesting that the radicals coming from the laccase-mediated oxidation of HBT can strongly contribute to the PE degradation. Indeed, it is well known that the redox potential is one of the most important factor in lignin degradation, because the most predominant structures in lignin are non-phenolic subunits which are characterized by high redox potential. In addition, the dimension of the enzymes involved in lignin oxidation, being either *LigP*, or *MnP* or *laccase*, too large to provide an effective penetration and oxidation of polymeric systems such as unaltered wood. For these reason veratryl cation, Mn(III) and HBT represents helpful radical mediators for *LigP*, *MnP* and *laccase*, respectively.

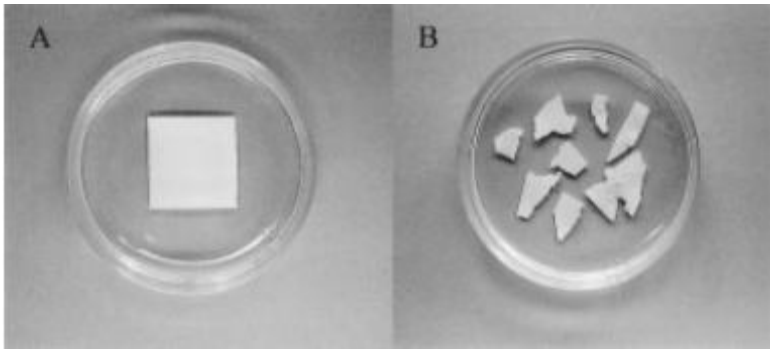


Figure 4. PE membrane disintegration by laccase, A untreated control, B PE membrane after 5 days laccase/HBT system treatment [139].

Lignin degrading fungi such as *P. chrysosporium* have been utilized in order to test the biodegradability of PE/lignin blends [140] in which the natural polymer would be expected to act as an initiator of radical reactions [141]. The fungus was grown under cultural conditions suitable for the optimal production of lignolytic enzymes in the presence of antioxidant-free LDPE blended with 10-30% (w/w) beech wood lignin.

Under the adopted conditions significant degradation of LDPE/lignin blend was observed as evolution of neat CO_2, as well as decay of tensile strength. By assessing the structural features (e.g. FT-IR spectroscopic characteristics and molecular weight) of solvent extractable fractions, coming from the samples after biodegradation it was observed that they were almost represented by heavily oxidized low molecular weight PE intermediates. In particular the presence of diene, carbonyl hydroxyl groups, and hydroperoxide radicals was detected, thus suggesting that the radical oxidation of lignin in the blends could activate the degradation/biodegradation of the polyolefin counterpart. It was also supposed that the lignin component may affect the environmental degradation of polyolefins during outdoor exposure by initiating the catalytic oxidation of the full carbon backbone polymers as mediated by light and/or by lignin-degrading microorganisms.

1.4.5. *Oxidation, degradation and biodegradation of poly(propylene) (PP)*

The general oxidation process described in Scheme 8 is typical for all polyolefins, but polypropylene, because of the “tertiary” carbon breaks down with some additional specific reactions. Because of the greater reactivity of the tertiary carbons, polypropylene is intrinsically lest stable and all commercial polypropylenes need to be stabilized against oxidation to render them processable.

As with polyethylene the compounds formed from the oxidative breakdown of polypropylene have been determined by mass spectrometry and FTIR and the physical effects are similar – reduction in molecular weight, formation of carbonyl groups and loss of physical properties. Another interesting discovery is that oxidation can be transferred via

infectious species, which means that degradation can “migrate” from one layer of a multi-layer film to another [142]

The initiation of PP photooxidation occurs by the abstraction of hydrogen atom, preferably methyne hydrogen, by free radical produced impurities such as hydroperoxide formed during processing. Various studies showed that the photo and thermal initators such as iron carboxylates, cobalt carboxylates and manganese stearate can accelerate the photodegradation of PP and PE [143-145].

The most probable mechanism of PP degradation is induced by heat or light and undergo an electron transfer with formation of free radicals. These alkyl free radicals abstract tertiary hydrogen from PP and form propyl macroradicals. These propylmacro radicals reacts with oxygen and generate peroxy macroradicals, which are converted to hydroperoxide. Alkoxy radicals formation from the decomposition of hydroperoxide is an important step because the resulting macroradicals leads to main chain scission with formation of carbonyl groups. Finally, the keto groups undergo Norish type I and type II reactions and liberate, as a consequence of chain scission, different kinds of products such as aldehydes, carboxylic acids, esters, lactones, peracids and peresters [146]

Evidence about the biodegradation of PP came from the increasing concentrations of the methylene chloride extractable products from the incubated PP, together with the contemporary weight loss of the sample [147]. Spectral analysis revealed that the extraction products were mainly hydrocarbons. As such metabolites were absent in the extracts obtained either from the uninoculated controls or from cultures grown without polypropylene, we confirmed that microbial attack of the polymer occurred. The finding that enzymatic attack of polyethylene occurs [77, 148] like enzymatic attack of trypsin on poly(ether urethane) [149] suggests that synthetic polymers may be recognized by natural metabolic

machineries and then transformed into lower-molecular-weight compounds. Polypropylene was more susceptible than high-density polyethylene to microbial attack in neat and sterilised samples. The higher weight losses of sterilised samples with lower intrinsic viscosity suggested that chain scission and radio-oxidized functional groups were important units in the biodegradation of polymers [150]. It is well suggested that the well-known metabolic flexibility and adaptability of microorganisms and mycelia can result in the biodegradation of isotactic polypropylene and polyethylene, two macromolecules that supposedly are highly recalcitrant to biological metabolism.

1.4.6. *Oxidation, degradation and biodegradation of poly(styrene) (PS)*

1.4.6.1. *Thermal and photo-oxidation of Crystal Poly(styrene) (CPS)*

Heat or irradiation at short wavelengths may cause the formation of macroradicals by hydrogen abstraction mainly from tertiary carbon atom of the PS backbone. Tertiary polystyryl radicals have been therefore repeatedly identified by ESR spectroscopy. Once formed, the macroradicals in the presence of oxygen are converted to peroxyradicals and furthermore to hydroperoxy group by hydrogen abstraction from the polymer chain. The decomposition of hydroperoxy groups, either by photolysis or thermolysis, leads to the formation of alkoxy macroradicals that may react in several ways (Scheme 19):

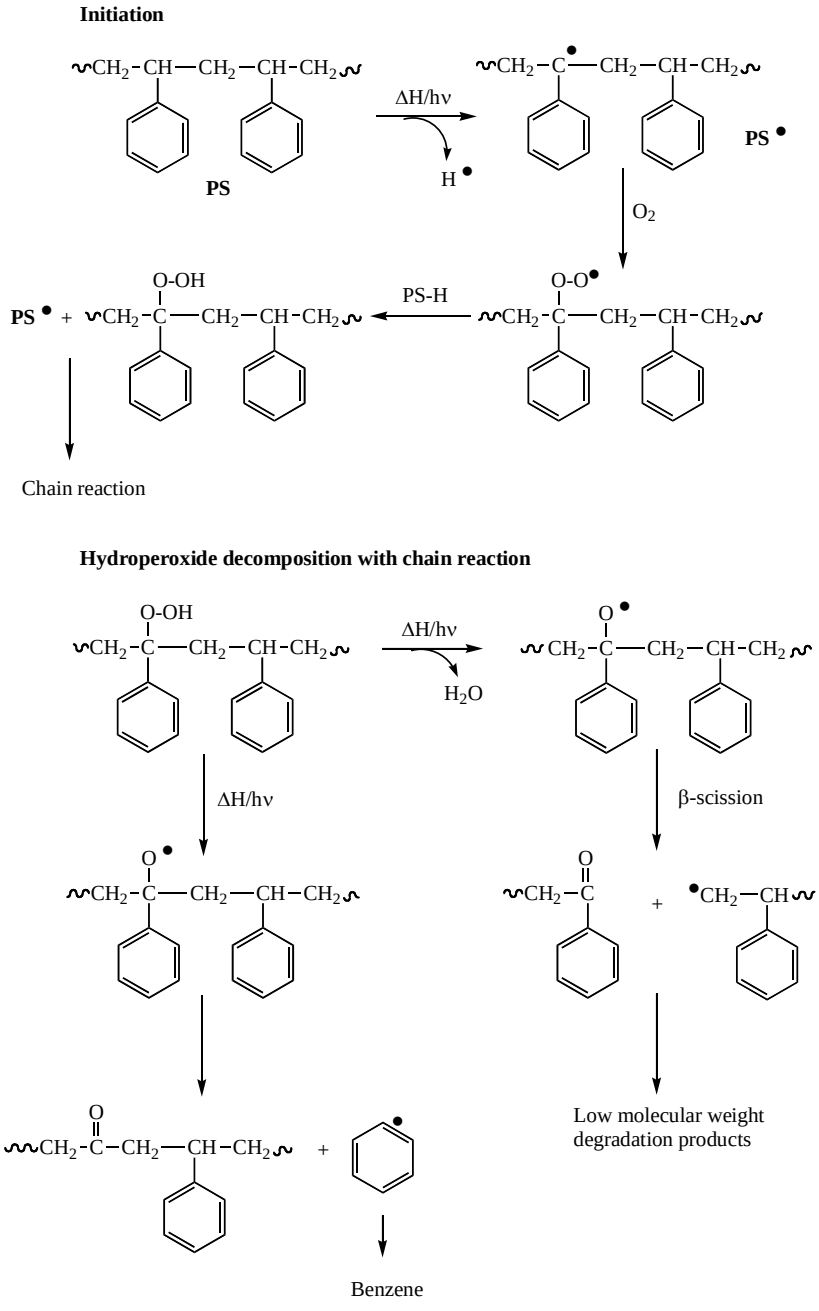
a. Formation of hydroxyl group by hydrogen atom abstraction

b. β-Scission of the macroradicals.

Two types of scissions have been hypothesized:

i) Scission at the C-Ph ring bond

ii) Scission of the C-CH_2 bond.



Scheme 19. Thermal and photo-oxidation mechanisms of PS.

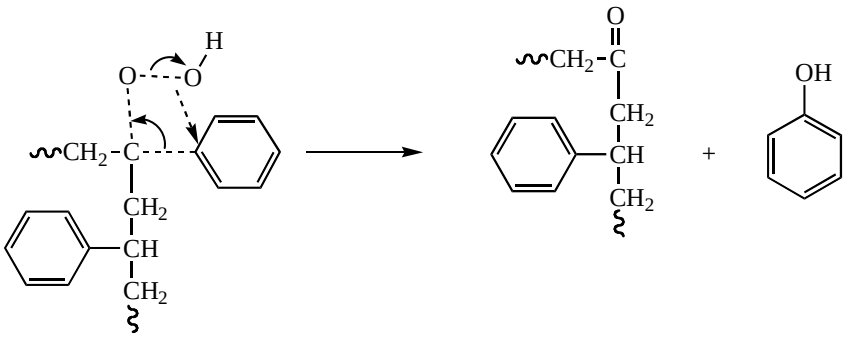
In the first case the bond scission may produce a phenyl radical that has been considered as the precursor of benzene whose formation has been found in several studies of thermal and photoxidation of PS. The C-CH_2 bond scission leads to an acetophenone type end group. A complex series of reactions are thought to be involve these groups, leading to the formation of several low molecular weight degradation products including acetophenone, benzaldehyde, benzoic acid, formic acid, acetic acid, benzene, dibenzoylmethane, benzoic anhydride produced by a depolymerization mechanism [151, 152].

It has been also suggested that most of the oxidation reactions are confined to the very surface layers of solid PS leading to the appreciation of weight losses, whereas the polymer chain scissions could be fairly limited.

It has however hypothesized that hydroperoxides might also decompose without chain scission, thus producing a carbonyl group along the main chain and phenol as low molecular weight oxidation product (Scheme 20).

High-impact polystyrene (HIPS) consists of a blend of PS and polybutadiene in low content (2-8 mol%) where polybutadiene is introduced before the free radical polymerization of styrene. In the case of HIPS the abiotic radical degradation leading to oxidation and chain scission are thought to occur primarily at two sites namely the styrene-butadiene phase boundary and in the olefin blocks.

Hydroperoxide decomposition without chain scission



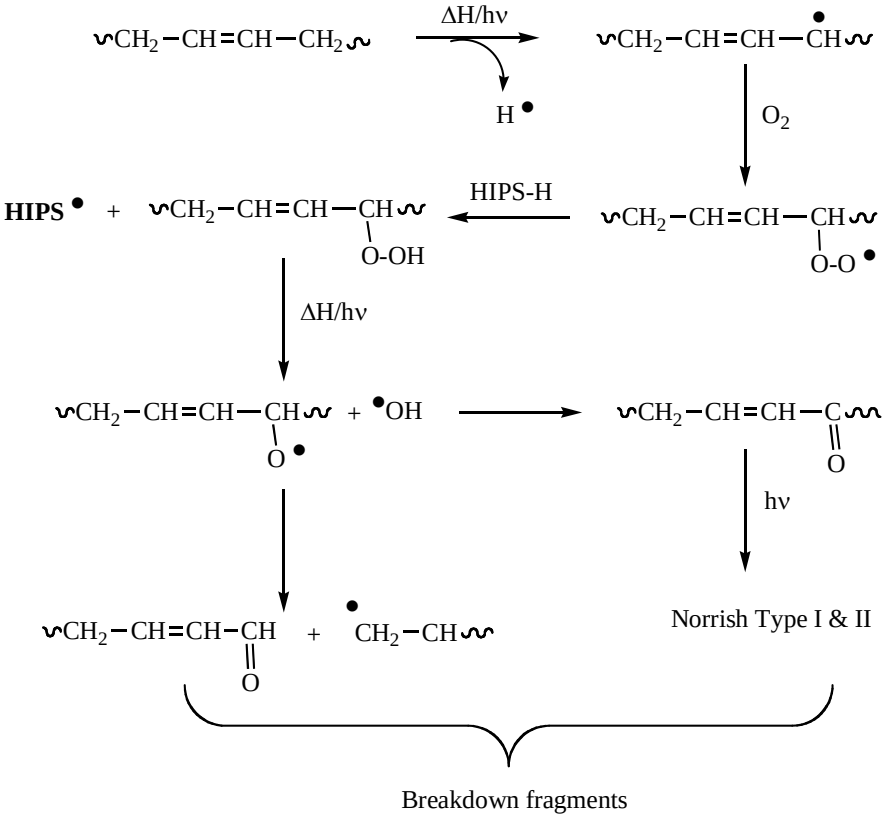
Scheme 20. Hyroperoxide decomposition in oxidized PS without macromolecule chain scission

1.4.6.2. *Thermal and photo-oxidation of High Impact poly(styrene) (HIPS)*

In accordance, aging of HIPS, as known from the literature, proceeds as a two-phase oxidation, in which the rate depending upon the content of the polybutadiene (PB) component .

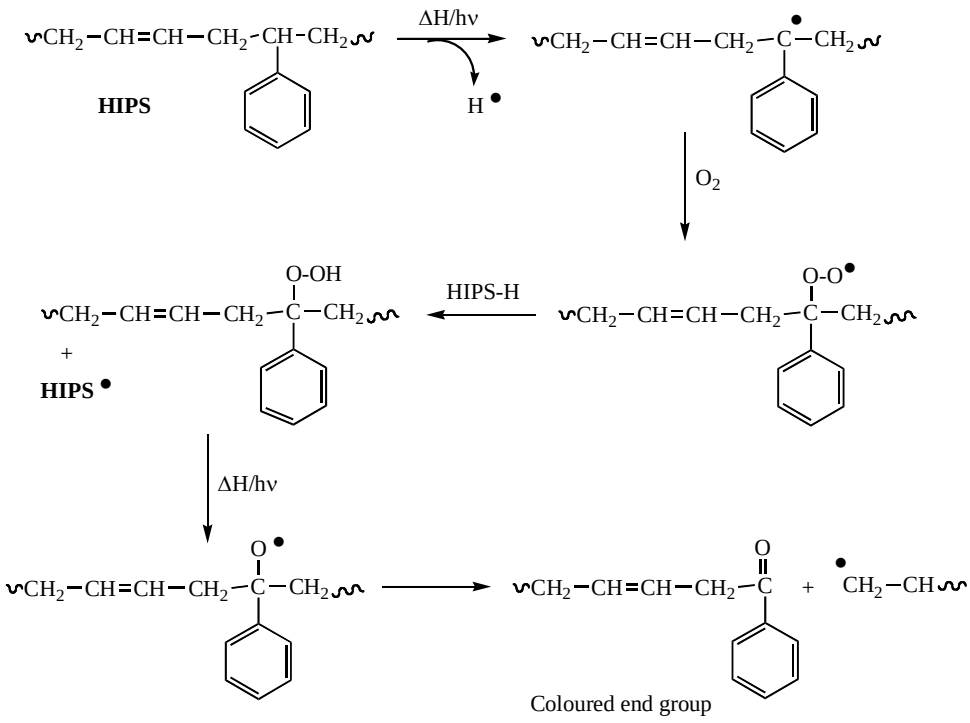
Nevertheless, hydroperoxidation, at least during photoexposure, does occur primarily in the polybutadiene nodules, essentially in the α position to the double bonds (Scheme 21) [153]. In any case, breakage of the olefin-styrene boundary linkage usually leads to benzyl radicals that can be further oxidized to hydroperoxides. Furthermore the decomposition of the hydroperoxides at the chain ends is producing acetophenone. Phenylpropene or stilbene end groups can also be formed whose decomposition further release acetophenone as degradation product (Scheme 22) [153].

Hydroperoxide formation and decomposition with chain reaction in the olefin blocks of HIPS



Scheme 21. Thermal and photo-oxidation mechanisms of HIPS in the olefin blocks.

Hydroperoxide formation and decomposition with chain reaction at the olefin-PS boundary blocks of HIPS



Scheme 22. Thermal and photo-oxidation mechanisms of HIPS in the PS blocks.

1.4.6.3. *Biodegradation of polystyrene (PS) by natural occurring microorganisms*

The thermal and photo-oxidation of styrenic polymers has attracted the attention of the academic research since mid sixthies of the last century [154]. In several studies the formation of hydroperoxides as a consequence of the photo-irradiation and thermal degradation in air has been established [155-158]. Afterwards, many secondary reactions including photolysis, decomposition by energy transfer and intramolecular decomposition of hydroperoxides may lead to the

polymer chain scissions as well as to the production of low molecular weight intermediates. The radical chain scission of PS has been also established in thermal degradation studies carried out under nitrogen atmosphere, thus continuing through the β-scission of the macroradicals (e.g. unzipping depolymerization) at the ceiling temperature [159].

In photooxidized PS, aromatic and aliphatic ketones, peroxyesters, volatile products such as water, carbon dioxide, benzaldehyde and acetophenone, have been detected. The oxidation of the aromatic ring has been also suggested. In addition, benzoic acid, methyl benzoate and styrene have been found in the solvent extract of the photooxidized PS by HPLC analysis [151].

Benzene, toluene, ethyl benzene dimethyl benzene, styrene, distyrene and tristyrene have been recognized as the major low molecular weight degradation products of thermally treated PS under both air and nitrogen atmosphere, even though on a ppm scale concentration [159].

It is however interesting to note that no indications are reported in the literature regarding the generation of recalcitrant polynuclear aromatic pollutants (PAC) like antracene, fenantrene, pyrene etc.

Most of these compounds can be found in the Volatile Organic Compounds (VOCs) in the gaseous emissions of industrial and municipal solid waste (MSW) treatment processes. In this connection, recently it has been hypothesized that styrene in VOC from aerobic biological treated MSW, often occurring at relatively high temperatures, could be derived from polystyrene [160]. It is however worth noting that either benzene and substituted benzene, as well as styrene constitute the naturally

occurring aromatic hydrocarbons, the latter compound being formed by the enzymatic decarboxylation of cinnamic acid [161].

On the other hand, an huge amount of information are available on the microbial degradation of benzene-related hydrocarbons. As is the case of aliphatic hydrocarbons, aerobic biodegradation of aromatic hydrocarbons involves the participation of molecular oxygen as a direct reactant as well as the terminal electron acceptor. In addition, many important aromatic hydrocarbons can sustain the growth of bacteria when they are present as the sole source of carbon and energy.

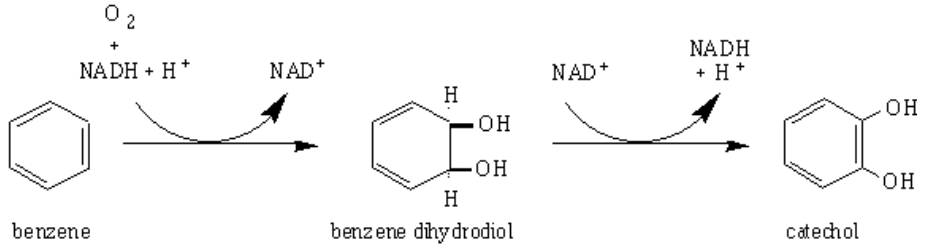
1.4.6.3.1. Biodegradation of aromatic hydrocarbons

- Biodegradation of Benzene

The reaction that is common to all pathways leading to the mineralization of aromatic substrates is the cleavage of the benzene ring. Molecular oxygen serves like a reactant in two steps in the pathways for benzene catabolism. In each of these reactions, both atoms from molecular oxygen become incorporated into the substrate. Enzymes that catalyze such reactions are identified as *dioxygenases* [162].

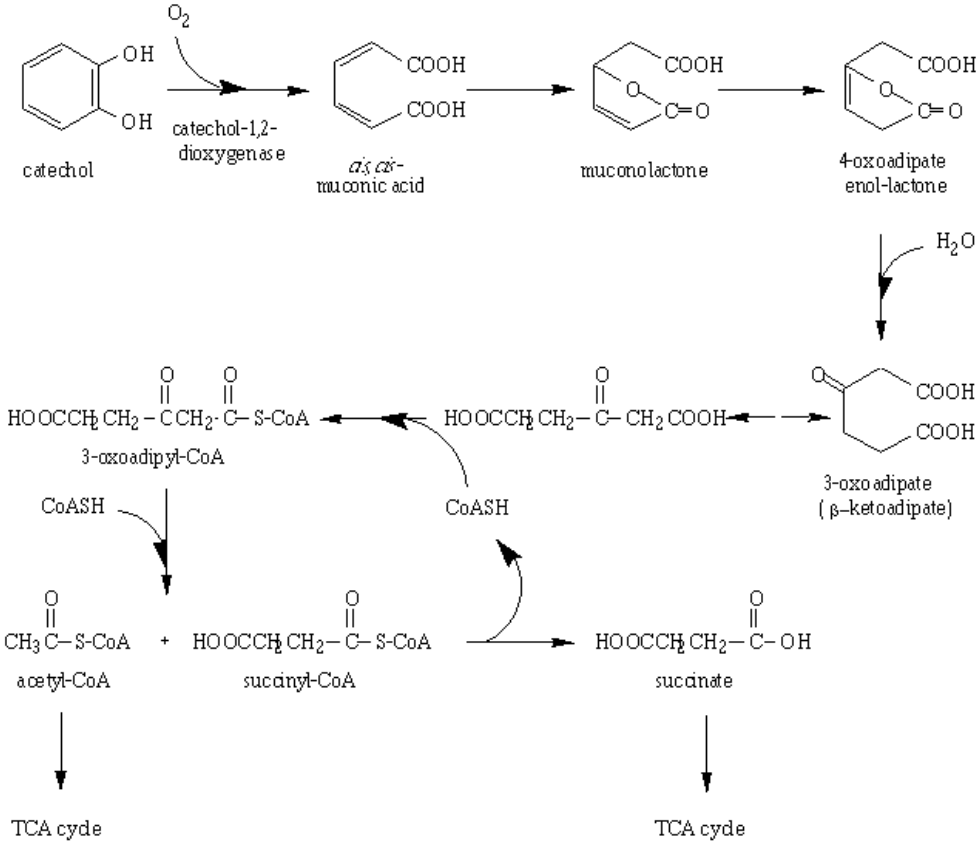
Ring cleavage and subsequent bacterial metabolism of benzene requires that the aromatic ring be destabilized, that is partial loss of its resonance energy (140-160 KJ/mol), then more reactive. This is accomplished by a *dioxygenase*-catalyzed reaction between benzene and molecular oxygen, that lead to the formation of benzene dihydrodiol (i.e., *cis* -1,2-dihydroxycyclohexa-3,5-diene) [163, 164]. Aromaticity is restored by a *dehydrogenase*-catalyzed conversion of benzene dihydrodiol to catechol (1,2-dihydroxybenzene), which is the ring cleavage substrate. The

reactions pathway leading to catechol form benzene is shown in Scheme 23.

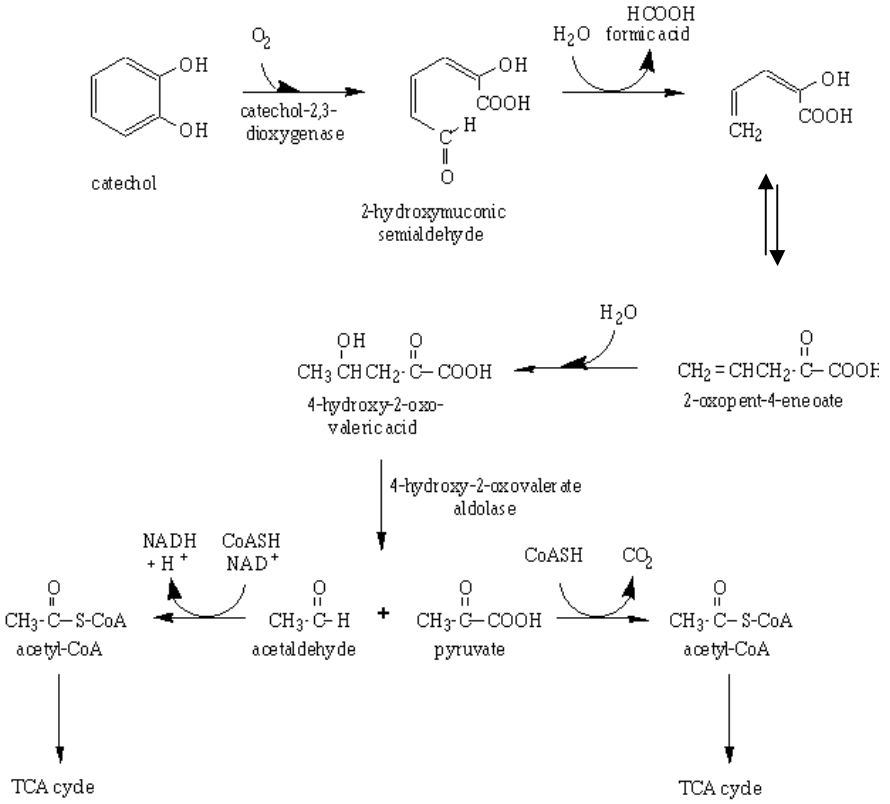


Scheme 23. Oxidation of benzene to catechol

Catechol is susceptible to be catabolized by ring cleavage, in which the aromatic ring is broken-up by further oxidation. Ring cleavage can occur by either one of two pathways. The ortho-cleavage pathway, in which the aromatic ring is split between the two carbon atoms bearing hydroxyl groups, or the meta-cleavage pathway, in which the ring is broken between a hydroxylated carbon atom and an adjacent unsubstituted carbon atom [164]. Each of these ring-cleavage reactions is catalyzed by a *dioxygenase*. The subsequent metabolic pathways are quite different, but they both lead to tricarboxylic acid (TCA) cycle intermediates (acetate and succinate) or to substrates that can be easily converted to TCA cycle intermediates (pyruvate and acetaldehyde). The ortho-cleavage pathway (also called the β-ketoadipate pathway) is shown in Scheme 24, and the meta-cleavage pathway is represented in Scheme 25.



Scheme 24. *Ortho*- cleavage pathway for catabolism of catechol.

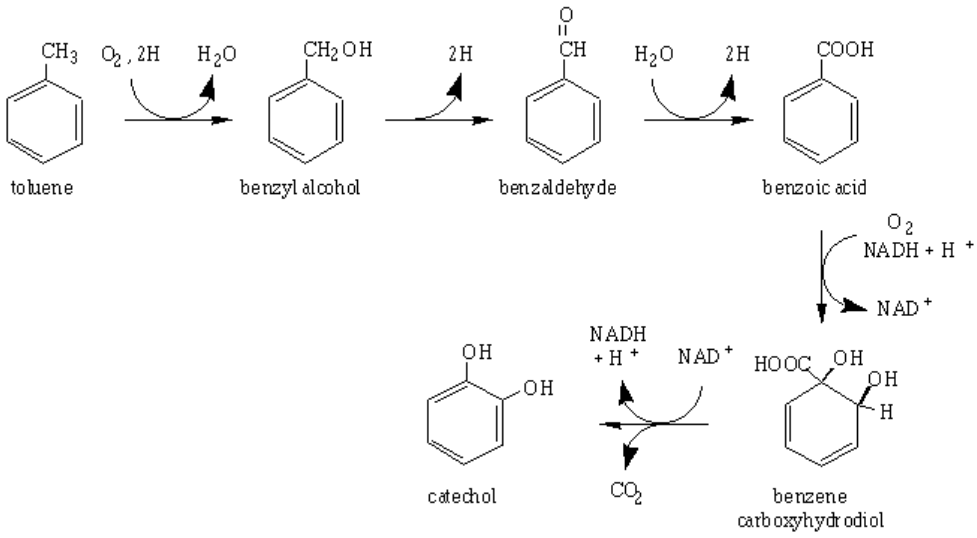


Scheme 25. *Meta*- cleavage pathway for catechol catabolism.

- Biodegradation of alkylbenzenes

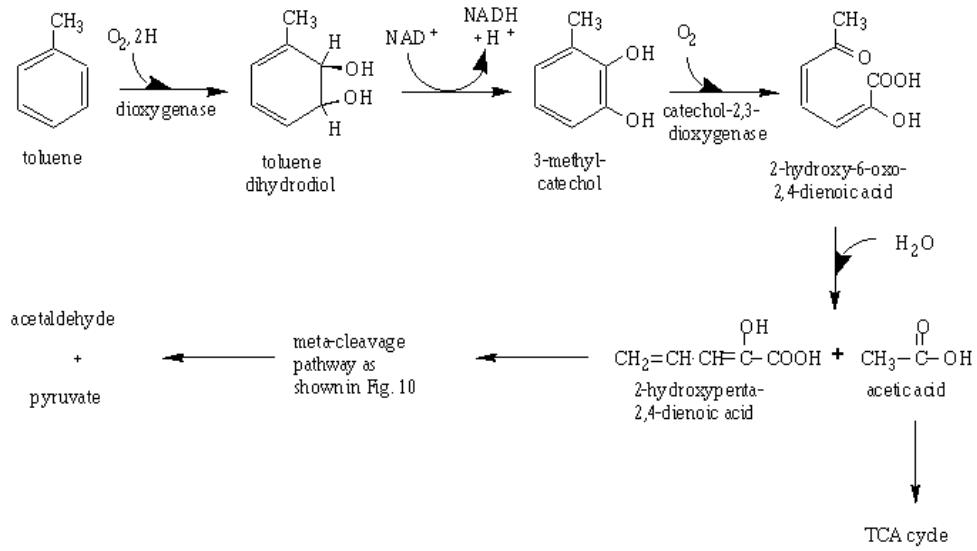
Alkyl-substituted benzenes, such as toluene, ethylbenzene, and the xylenes, are common environmental contaminants. These compounds can serve as the sole sources of carbon and energy for a variety of bacteria, including members of the *Pseudomonas* , *Achromobacter*, and *Nocardia* genera [165]. Metabolism of alkylbenzenes may be initiated by oxidation of either the alkyl side chain or the aromatic ring. Growth of *Pseudomonas aeruginosa* on toluene is an example of a catabolic pathway that is initiated by side-chain oxidation [163, 165]. In a

monooxygenase-catalyzed reaction, toluene is converted to benzyl alcohol, which is further oxidized to benzoic acid by dehydrogenation. Benzoic acid is the substrate for insertion of oxygen into the aromatic ring, leading to production of catechol. The reactions leading to catechol are shown in Scheme 26. Catechol cleavage proceeds as was described above.



Scheme 26. Oxidation of toluene to catechol by *Psuedomonas aeruginosa.*

Oxidation of toluene and ethylbenzene by *Pseudomonas putida* provides an example of the other pathway by which alkyl-substituted benzenes are degraded. Initiation occurs by *dioxygenase*-catalyzed ring hydroxylation, leading to 3- or 4-methylcatechols (or the analogous ethylcatechols) [163, 165] (Scheme 27). The alkylcatechols are further oxidized by meta-cleavage. Similar pathways for toluene oxidation are observed in *Pseudomonas mildenbergii*, *Achromobacter* sp., and *Nocardia corallina*. The reactions involved are outlined in Scheme 27.



Scheme 27. Toluene metabolism by *P. putida* : Ring hydroxylation pathway.

- Biodegradation of styrene

Other than benzene and alkylbenzenes, also styrene can be metabolized by microorganisms. In the literature several original papers on the biodegradation of styrene by *Pseudomonas putida*, *P. fluorescens*, *Xanthobacter* sp. and *Rhodococcus rhodochrous* can be found along with studies concerning the genetic and the key enzymes such as *styrene monooxygenase*, *epoxystyrene isomerase* and *phenylcetaldehyde dehydrogenase* involved in the bacterial metabolism of styrene [166, 167 168, 169, 170, 171].

It has been repeatedly supposed that styrene is oxidized to styrene oxide by the *styrene monooxygenase* and subsequently isomerized to phenylacetaldehyde by the specific isomerase. This latter compound can be therefore metabolized in the bacterial cells trough the general pathway of aromatic hydrocarbons biodegradation [170].These studies have been

recently contributed to the development of a biofiltration reactor inoculated with a *P. putida* styrene degrading strain for the treatment of VOCs in industrial waste gases. A styrene removal efficiency of 75% in a few days has achieved at loading rates corresponding to 100 g/ m^3, without observing any accumulation of intermediate products, thus confirming the final mineralization of the aromatic compound [172].

2. OBJECTIVES

The durable properties of plastics such as those based on polyolefins make them the ideal material for a large number of applications including agriculture, packaging and disposables items and it accounts for 60% share among all the plastics consumption.

However, as the concern on environmental preservation is growing, this kind of material represents a relevant drawback due to their recalcitrance to microbial attack and hence tend to accumulate in the environment. In this regard, a great deal of research activity on the environmentally degradable polymeric materials and plastics (EDPs) based on re-engineered polyolefins is meeting an increasing attention to overwhelm the criticism that is leveled at commodity plastics in many short-lived applications. In fact they may persist in the environment too long once they are used and discarded in a controlled or uncontrolled manner.

The major strategies in order to overcome the intrinsic recalcitrance of polyolefins (PE, PP and PS) to biological attack were focused on the introduction of functional groups by copolymerization or substances capable to promote the formation of free radical precursors (e.g. hydroperoxides) by photophysical and thermal exposure leading to a fragmentation of the polymer backbone into oxidized lower molar mass fragments (abiotic step). This step will be followed by microbial attack of the oxidized and fragmented products (biotic step). In accordance, these materials are identified and classified as ***oxo-biodegradable polymers and plastics*** for which degradation is the result of oxidative and cell-mediated phenomena, either simultaneously and/or successively.

The present study has been focused on the mechanism of physical-chemical processes (abiotic) that promote as ultimate stage the biodegradation of full carbon backbone polymers, thus transforming

these polymers in eco-compatible materials. In particular, the investigations undertaken in the present PhD thesis work was the evaluation of the rate of biodegradation of oxidized and degraded oxo-biodegradable polyolefin samples (PE, PP, PS) under aqueous, soil and compost conditions. Based on these criteria polyolefin (PE, PP and PS) film samples formulated with different pro-oxidant (type and content) based on transition metal organic salts able to promote oxidative degradation/biodegradation by abiotic and/or biotic actions have been ascertained into three tiers.

Tier 1 Acceleration of samples aging in standard tests for both thermal and photo-oxidation processes and determination of the degree of abiotic degradation.

Tier 2 Monitoring of the biodegradation of the pre-treated samples in respirometric apparatus.

Tier 3 Assessment of interactions between polymeric materials and microbes and of any toxicity effect of the metabolized polymer fragments.

The following studies have been focused to understand the degradation and biodegradation mechanism of full carbon backbone polymeric materials and relevant plastic items and approaching the minimization of the problems ongoing during plastic waste disposal under natural environmental conditions.

3. EXPERIMENTAL

3.1 Reagents and Solvents

The reagents and solvents employed in the experiments and their respective source provider are listed below. Potassium hydroxide (KOH) pellets, technical grade; Hydrochloric acid (HCl) volumetric standard, 0.1N, analytical grade; Barium chloride ($BaCl_2$), technical grade; Acetone (C_3H_6O) HPLC grade; Chloroform ($CHCl_3$) HPLC grade; Ethanol absolute (C_2H_5OH); o-Xylene chromasolv plus (C_8H_{10}) for HPLC 98%; Dichloromethane (DCM) analytical grade; Tetrahydrofuran (THF) analytical grade; Di-potassium monohydrogen phosphate (K_2HPO_4); Potassium dihydrogen phosphate (KH_2PO_4); Ammonium nitrate (NH_4NO_3); Magnesium sulphate ($MgSO_4 \cdot 7H_2O$); Manganese sulphate ($MnSO_4 \cdot H_2O$); Calcium chloride ($CaCl_2$); Zinc chloride ($ZnCl_2$); Potassium dichromate ($K_2Cr_2O_7$), Sulphuric acid (H_2SO_4) d= 1,84 g/l; Potassium sulphate (K_2SO_4); Mohr salt soultion ($FeSO_4(NH_4)_2 \cdot 6H_2O$); Phenolphthalein indicator; were from Carlo Erba chemicals Ltd. Sodium fluoride (NaF); Diphenyl alanine indicator; Dimethyl sulfoxide (DMSO) from J.T. Baker; and poly(1,4-*cis*-isoprene) $[CH_2CH=C(CH_3)CH_2]n$, as natural rubber was purchased from Aldrich.

Potato Dextrose Agar (PDA); Bacto agar; Yeast extract; Nutrient broth; were purchased from Difco laboratories, USA.

3.2. Test Materials. Origin, Treatment

The environmental fate of the following polymeric materials has been investigated.

3.2.1. *Full carbon backbone polymeric materials*

The following test materials were used for abiotic degradation and biodegradation studies.

- Low Density Polyethylene (LDPE)

Riblene® FL20R I. 3114931 pellets having 2.2 MFI and 0.921 g/cm^3 density was supplied by Polimeri Europa, Mantova-Italy.

- Linear Low Density polyethylene (LLDPE)

LLDPE films containing different types of pro-oxidants additives and control films were supplied by Ciba Chemicals Italy. In Table 3.1 the composition and characteristics features of LLDPE films submitted to oxo-biodegradation studies, are reported.

Table 3.1. Linear Low Density Polyethylene (LLDPE) films submitted to oxo-biodegradation studies.

Test Sample a)	**code**	**Thickness** (μm)
LLDPE Dowlex NG 5056-G + Research product[b)]	LLDPE-TD1	11.7
LLDPE Dowlex NG 5056-G + Envirocare AG1000[c)]	LLDPE-TD2	10.3
LLDPE Dowlex NG 5056-G not additivated[d)]	LLDPE-TD0	8.8

a) Samples stored at 4°C in the dark; b), c) Pro-oxidant additivated and d) not additivated.

- High Density Polyethylene (HDPE)

Untreated and thermally degraded HDPE samples with degradable plastic additives (Table 3.2) were kindly by Symphony Polymers UK.

- Totally Degradable Plastic Additives (TDPA®)

TDPA® DCP 579, is a proprietary formulation containing pro-degradants, stabilizers and fillers in PE resin matrix developed by Environmental Technologies Inc., Vancouver, Canada.

Table 3.2. High Density Polyethylene (HDPE) films submitted to oxo-biodegradation studies.

Test Sample [a)]	code	Shape
HDPE	HUA	Film
HDPE + d2w additivated	HT1	Fragment

a) samples stored at 4°C in dark.

- Polystyrene (PS)

Crystal and High Impact Poly(styrene) based (CPS/HIPS) blown extruded film samples incorporating different EPI pro-oxidant additives and the corresponding additive-free control film have been investigated (Table 3.3).

Table 3.3. Polystyrene (PS) films submitted to oxo-biodegradation studies.

Test Sample [a)]	code	Thickness (μm)
PS film withTDPA additive 1	PS-TD1	10-30
PS film withTDPA additive 2	PS-TD2	10-30
PS film without additive (control)	PS-TD0	10-30

a) samples stored at 4°C

- Polypropylene (PP)

Different PP based films containing different types of proprietary pro-oxidant additives and the corresponding additives-free samples have been also investigated. The PP films were received as such and in the form of pre-aged (e.g. abiotically oxidized and fragmented) as well. Samples are listed in Table 3.4.

Table 3.4. Polypropylene (PP) submitted to oxo-biodegradation studies.

Test Sample [a)]	code	Thickness (μm)	Shape
PP film withTDPA additive 1	PP-TD1	30-40	Film
PP film with TDPA additive 2	PP-TD2	n.d.	Fragments

a) samples stored at 4°C; n.d – not detectable

3.2.2. *Oxo-biodegradable natural polymers*

- Alkali Lignin

Alkali lignin (AL) is a type of lignin produced by acid treatment of the black liquor of wood extraction with soda process with acid. The AL material with low sulphonate content used in this study was purchased from Aldrich as a fine powder having 60 kD Mw and 10 kD Mn (Figure 3.1).

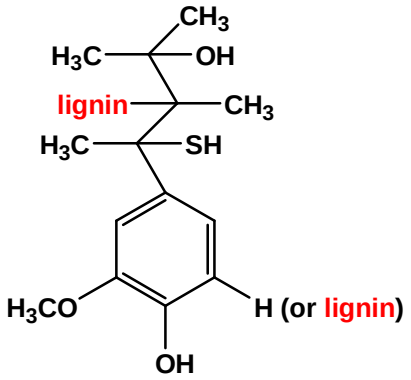


Figure 3.1. Schematic representation of lignin structure

- Natural Rubber

Poly(1,4-*cis*-isoprene) (PIP) (38 kD Mw, 350 poise viscosity at 37°C), from natural rubber was purchased from Aldrich, and used as a reference material for oxidative degradable polymer. Raw synthetic PIP latex rubber (NR) does not contain any non-rubber constituents, except antioxidants, that are usually added during the manufacture to prevent ageing of the materials. For many commercial applications, raw rubber is subjected to a vulcanization process in which the PIP chains are cross linked either by heating in the presence of sulfur, as in case of tires, or by irradiation and peroxidation, as in case of NR latex gloves. Therefore, further substances are added in these two cases. To extract antioxidants from NR, the material was treated with acetone as follows: 3 g of NR

were extracted with 100 ml of acetone for 2 days at room temperature. During this period acetone was repeatedly (approximately 3-5 times) replaced by fresh acetone until no colored substances were extracted from NR. [173]. The purified material was coated onto teflon membranes and left to dry and subsequently used as reference material in soil burial biodegradation tests.

- Pine Nut Shells Lignin (PNSL)

Local production of pine nut shells deriving from the pine forests located in the Regional Natural Park of San Rossore-Migliarino Massaciuccoli (SRMM), Tuscany-Italy, constitute the source of pine seed shells as an useful waste to be converted to valuable raw material.

The pine nut shells have been grinded in fine powder and chemically treated to separate lignin from the other components (cellulosics & terpenics).

To extract the lignin component from PNS, the extraction procedure reported in Figure 3.2 was utiized. [174-176].

- Wheat Straw

A wheat straw (WS) sample was obtained from, the Agriculture Faculty, of the University of Pisa. Italy. Basically, WS contains three main constituents namely cellulose, hemicellulose and lignin, which undergoes different enzyme mediated degradation and biodegradation by uptake of water or oxygen. Accordingly, WS has been exploited as reference materials in biodegradation tests, being representative of different hydrolytic and oxidative biochemical pathway.

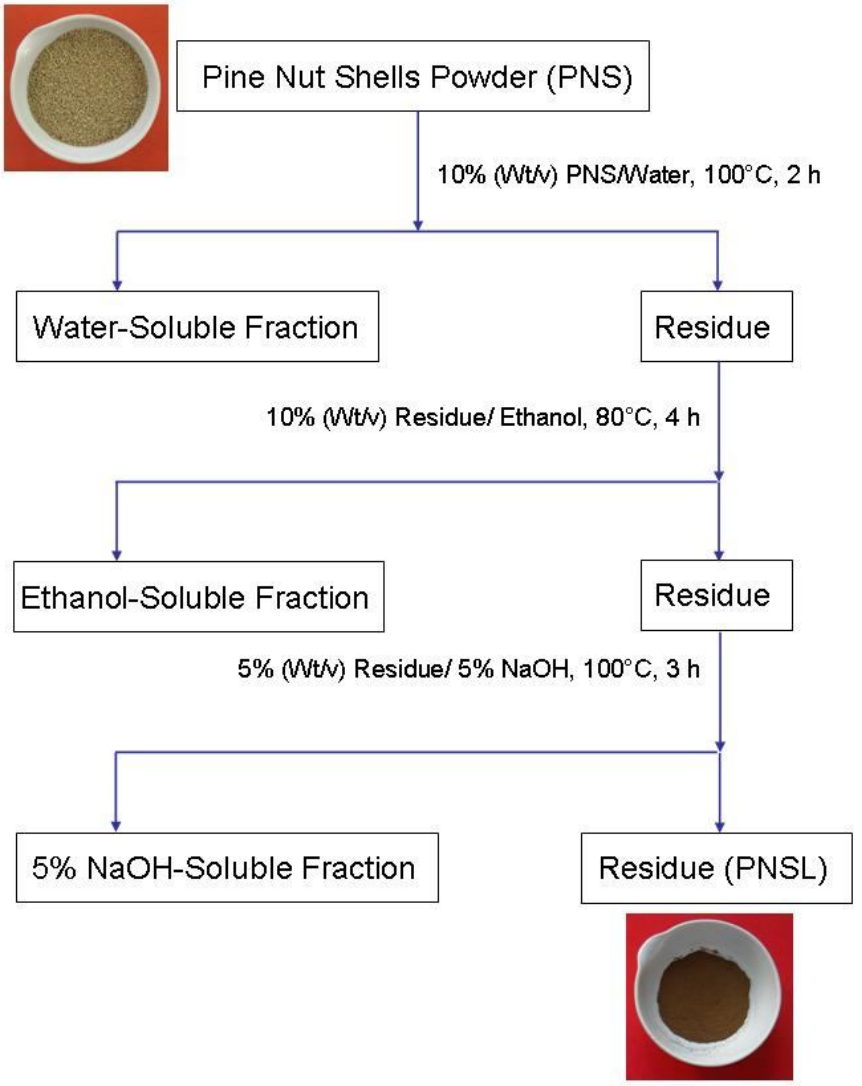


Figure 3.2. Flow sheet of the extraction procedure of lignin (PNSL) from pine nut shells.

Before to use the material was finely chopped and submitted to purification by 24 hours soaking in cold water. After removing excess water, the material was pasteurized at 120°C ,1 atm, for 30 minutes, and

dried at 60°C until they reached constant weight. After drying WS sample was grinded into tiny pieces before to be used in soil burial biodegradation tests (Figure 3.3).

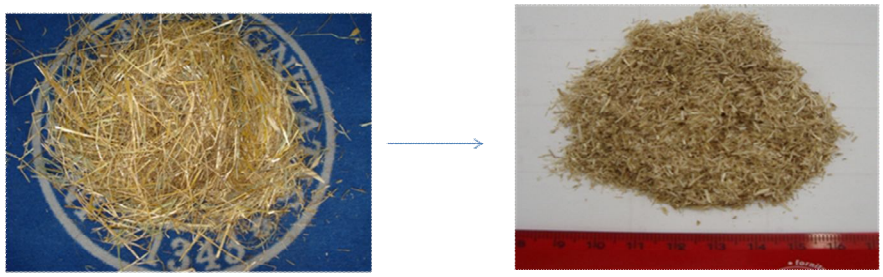


Figure 3.3. Wheat straw (WS) sample for soil burial biodegradation tests a) collected WS b) chopped.

3.2.3. *Natural, synthetic and hybrid hydro-biodegradable polymeric materials*

- Cellulose Filter Paper

An ashless Whatman 42 grade cellulose filter paper sample(CFP) was employed as positive control material in biodegradation studies.

- Poly(lactic acid) (PLA) plastic items

A disposable plastic cup made from PLA polymeric material, collected from commercial source (retail shop) Pisa, Italy was used in biodegradability testing under soil burial conditions.

- Ecoflex plastic items

A plastic bag made by the synthetic polyester Ecoflex® was kindly supplied by BASF, Germany. Ecoflex® is an aliphatic-aromatic copolyester based on terephthalic acid, adipic acid, 1,4-butanediol and modular units . The mechanical properties of Ecoflex® are comparable with those of low density poly(ethylene) and the most common

processing methods for Ecoflex® is the film extrusion using the conventional blown film lines for LDPE.

Ecoflex based plastic bag was employed in the soil burial biodegradation test to compare the biodegradation behaviour of oxo-biodegradable polymeric materials.

- Mater-Bi based plastic items

Trade names Mater-Bi containing biodegradable starch-based hybrid blend polymeric material was supplied Novamont, Italy. A commercial plastic bag made with Mater-Bi was also employed in soil burial biodegradation tests in order to analyze and bring a comparison with the biodegradability of oxo-biodegradable polymeric materials.

3.2.4. *Polymeric composites*

Two different series of polymeric composites represented by Ecoflex synthetic polyester mixed with pine seed lignin (Ecoflex/PNSL) and LDPE mixed with alkali lignin (LDPE/AL) have been formulated and processed by melting procedures.

3.2.4.1. *Preparation of lignin (PNSL)/Ecoflex® composites*

The lignin fraction from pine seed shells (PNSL), was blended in the melt with Ecoflex® FBX 7011 pellets. It has a mass density of 1.25 – 1.27 g/cm^3. According to the DSC testing, its Glass Transition Temperature (T_g) and Melting Temperature (T_m) is -30^{o}C and 117^{o}C, respectively.

The following calculation was done before Brabender processing.

Mass density of PNSL 0.75g/cm^3; Mass density of Ecoflex FBX 7011 1.25 - 1.27g/cm^3; Volume of the Brabender 44 cm^3.

$$M_pD_f + M_fD_p = 44D_pD_f \qquad \text{(Eq. 1)}$$

V_p. (Volume of Ecoflex); V_f. (Volume of Fillers); M_p. (Mass of Ecoflex); M_f. (Mass of Fillers); D_p. (Density of Ecoflex); D_f. (Density of PNSL).

The final weights needed for the Brabender processing was summarized in Table 3.5. according to the Eq. 1.

Table 3.5. Composition of Ecoflex/PNSL composites

Ecoflex/PNSL		**Code**
Weight ratios	Weight for Brabender	
(w/w)	(g)	
100/0	55/0	E100
90/10	46.44/5.16	E90
80/20	38.84/9.71	E80
70/30	32.08/13.75	E70
60/40	26.06/17.37	E60

Before processing, both Ecoflex and lignin filler powder were dried in oven at 60°C for 48 h. PNSL with Ecoflex® composites were prepared in a torque rheometer W50 EHT roller blades connected to a Plastograph Can-Bus Brabender under the following processing conditions: The polyester was added into the Brabender at 150°C with a rotor speed of 50 rpm and residential time of 2 min. Subsequently, the PNSL filler was added and the processing temperature was set at 140°C with a rotor speed of 75 rpm and residential time of 6 min. Finally, the composites mixture was collected and cut into pellets for the hot compression moulding.

The homogenous mixtures of Ecoflex® and lignin filler pellets were compressed into thin film by using a P200E Collin type laboratory hot compression moulding equipment. One portion of 1.2 g of pellets were placed between two teflon coated stainless steel plates. The plates were

set into the hot compression moulding machine with both upper and lower plate heated at 140°C for 4 min under 90 bar compression. After heating, the samples were cooled by circulating water at ambient temperature for 4 min at 90 bar compression. The two plates containing the composite films were then transferred into a -20°C refrigerator for several minutes. Finally, the thin films were collected and the thickness of the films ranged from 130-150 µm was measured by micron metrology.

3.2.4.2. *Alkali Lignin/LDPE/pro-oxidant additives (LDPE/AL/P) blends*

Antioxidant-free LDPE: Riblene FL20R I. 3114931 – (LDPE) supplied by Polimeri Europa, having 2.2 MFI and 0.921 g/cm^3 density, has been utilized as continuous matrix for the preparation of blends with alkali lignin (AL) with and without the addition of EPI-TDPA DCP 579 master batch (P) pro-oxidant system.

LDPE was melt blended with the other components in a torque rheometer W50 EHT roller blades connected to a Plastograph Can-Bus Brabender having a mixing head with a volumetry capacity of 50 cm^3. Prior to mixing all the components were dried in oven at 60°C for 12 h. Mixing was performed at 160°C, rotor speed 30 rpm and 10 min residence time. The composition of the blends, as prepared according to the [Eq. 1] calculation are reported in Table 3.6.

Immediately after the melt blending all the samples (0.7 g) were hot pressed in thin sheet into a Collin P 200E under the following conditions: 160°C temperature, 150 bar compression, 3 min residence time, subsequently, the films were cooled to ambient temperature for 5 min maintaining the same pressure of the isothermal step.

Table 3.6. Composition of the LDPE/AL blends submitted to compression molding.

code	AL	LDPE	P	AL	thickness
	(g)	(g)	(g)	%	(μm)
AL0	0.0	40.0	0.0	0.0	46-52
AL1	0.4	39.6	0.0	1	46-48
AL3	1.2	38.8	0.0	3	42-46
AL5	2.0	38.0	0.0	5	52-55
AL1/P	0.4	38.4	1.2	1	46-52
AL3/P	1.2	37.6	1.2	3	56-58
AL5/P	2.0	36.8	1.2	5	42-49
AL0/P	0.0	38.8	1.2	0	40-43

3.3. Abiotic Degradation Experiments

The following section describe the test methods utilized for abiotic degradation (photo, thermal), which influence the oxidation process of full carbon backbone polymers.

3.3.1. *Thermal degradation tests in static oven*

Thermal oxidations of test samples were carried out at different temperatures in the 28°C - 65°C range in 5.4 l mini incubators (Falc Instruments s.r.l., Italy) having 15x18x19 inner dimension. Test sample films (2 x 4 cm dimension) were placed in plastic holders allowing for direct exposure with the local environmental conditions. At time intervals of thermal aging, the samples were analyzed by using FT-IR spectroscopy. The FT-IR spectra were recorded in the same spot region during analysis (Figure 3.4).

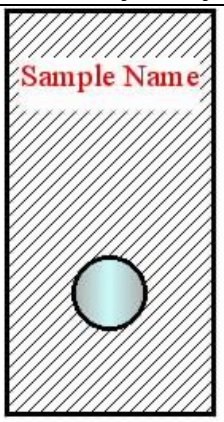


Figure 3.4. Plastic holder for FT-IR analysis used during the ageing tests in outdoor exposure and/or heating in oven.

3.3.2. *Thermal degradation test in air-ventilated oven*

Approximately 20x30 cm specimen of each film samples corresponding to 1.5 g were placed in glass Petri dishes (Figure 3.5) and were submitted to thermal degradation at 65°C under constant air flow in a STF-F120Lt oven having 45x32x45cm inner dimension (Falc Instruments s.r.l., Italy). At regular time intervals the samples were analysed by FT-IR spectroscopy by recording relevant spectra from different regions of each test specimen. Sample weight variation was also recorded in an analytical balance during the ageing test.

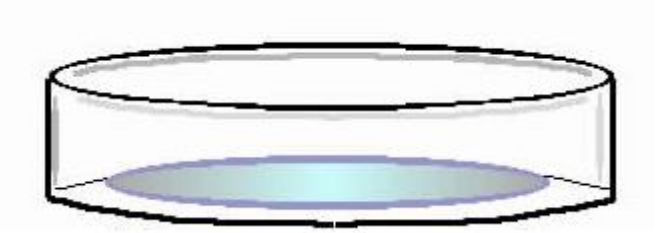


Figure 3.5. Test sample assemblage in glass Petri dishes for thermal aging in air ventilated oven

3.3.3. *Thermal degradation test with relative humidity*

Degradation test was carried out in 4 litre closed glass vessels under constant relative humidity (RH) as generated by 75% saturated NaCl

solution at 55°C. Test samples films were placed in plastic holders allowing direct contact to the environmental conditions (Figure 3.6). At time intervals the test samples were withdrawn, conditioned at room temperature in a desiccator and analyzed by FT-IR spectroscopy and recording the FT-IR spectra on the same spot region.

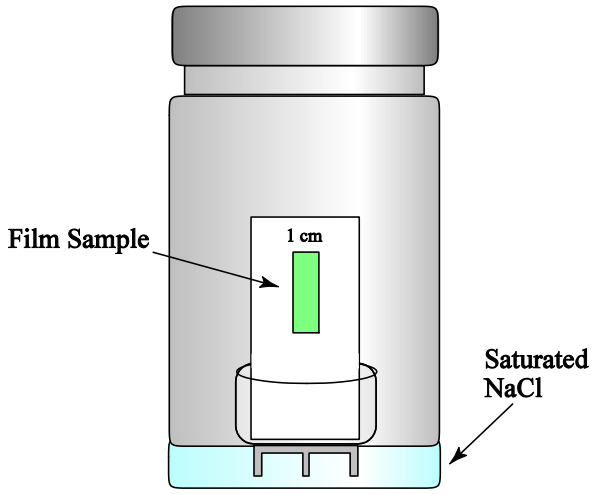


Figure 3.6. Schematic representation of thermal degradation tests at 75% relative humidity.

3.3.4. *Sunlight exposure degradation tests*

The tests were carried out in accordance with ASTM D5272-08 *standard practice for outdoor exposure testing of photodegradable plastics* [177].

Each sample (7 x 7 cm dimension) was allocated in a stainless steel rack exposed at 30° in the south-west direction in outdoor conditions (Figure 3.7). During the exposure time, the minimum and maximum temperatures were recorded daily. At time intervals, the samples were analysed by FT-IR spectroscopy by taking relevant spectra from 5 different regions of each test specimen.

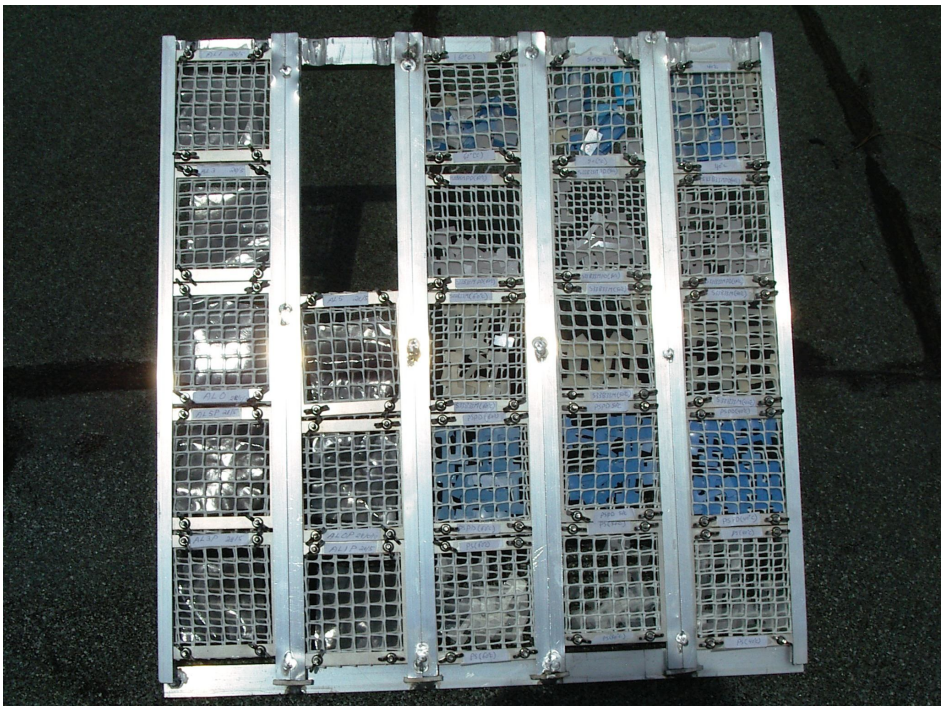


Figure 3.7. Set up used for outdoor exposure degradation of plastic film test specimens.

3.3.5. *Thermal degradation test of LLDPE films after outdoor exposure treatment, in oven under static conditions and air-ventilated oven at different temperatures.*

Outdoor exposed LLDPE film samples were also submitted to further thermal treatments at various temperatures (45, 55 and 65°C) in order to verify if, after the outdoor exposure, the reached level of oxidation could be further increased by thermal treatment carried out in the dark in oven under static conditions. Data of oxidation level in air-ventilated oven were collected only at 65°C. At time intervals, test specimens were analysed by FT-IR spectroscopy and recording the FT-IR spectra on the same spot region thus allowing a comparative monitoring of the progress of the carbonyl group formation.

3.3.6. *Degradation tests at ambient temperature of pristine and abiotically pre-aged test samples.*

Degradation tests were carried out at room temperature as aimed at establishing the rate and the extent of oxidative degradation of pristine abiotically pre-aged test materials once maintained under normal environmental conditions. The test film samples relevant to the pristine, outdoor exposed and thermally aged having different level of oxidation were exposed to room temperature in the dark, while monitoring the progress of oxidation by FT-IR spectroscopy.

3.4. Biodegradation Tests

The following biodegradation experiments were carried out under both aqueous and solid media conditions.

3.4.1. *Respirometric biodegradation tests in aqueous medium*

Aerobic biodegradation tests were carried out in aqueous medium by using as respirometric apparatus 300 ml Erlenmeyer flasks, containing 100 ml of mineral salt medium having the following composition per liter of distilled water: KH_2PO_4 85 mg, K_2HPO_4 218 mg, Na_2HPO_4 334 mg, $(NH_4)_2SO_4$ 10 mg, NH_4NO_3 10 mg, $CaCl_2$ 36 mg, $MgSO_4 \cdot 7H_2O$ 23 mg, and $FeCl \cdot 6H_2O$ 0.3 mg, pH 7.4±0.2. Each flask was equipped with silicone rubber stoppers hanging 40 ml capacity plastic vials filled with CO_2 absorbing 0.05 N KOH solutions (Figure 3.8).

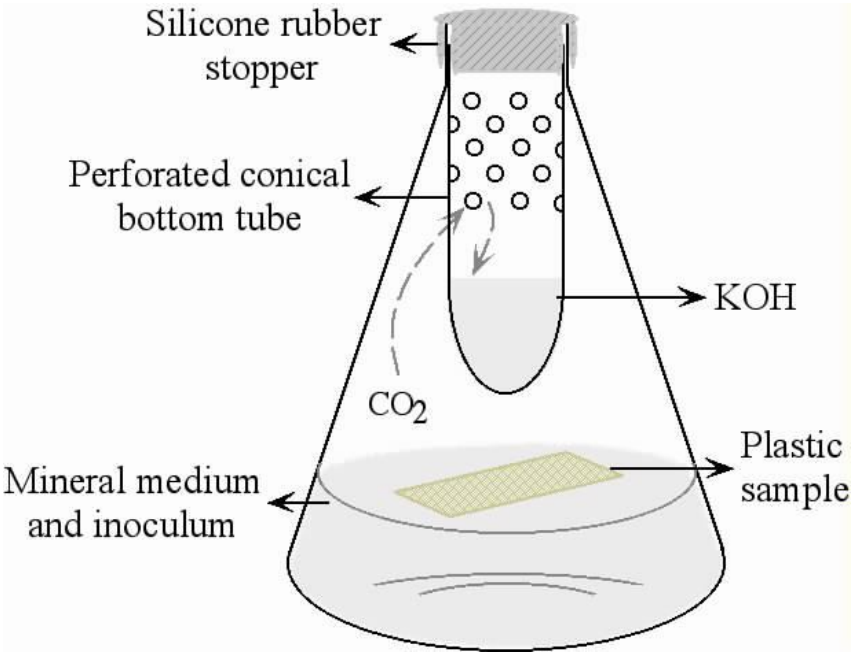


Figure 3.8. Schematic representation of aqueous medium Biodegradation tests

The microbial inoculum was prepared by cultivating 1 ml of a forest soil suspension (1g/10 ml 0.9% NaCl solution) in 300 ml mineral salt medium containing 100 mg of the sample fraction of thermally oxidized LDPE-TDPA sample as sole carbon and energy source. This soil culture was used as microbial source in 10% by volume ratio to inoculate each test flask once the stationary phase, as determined by the optical density at 660 nm in a UV/Vis410 Jasco spectrophotometer, was reached. All the test materials were supplied to the microbial cultures as sole carbon sources at approximately 0.1 % by weight concentration level.

Test flasks were incubated at room temperature (25°C) in the dark on a rotatory shaker (120 rpm). All the runs were carried out in triplicate. Every 5 -7 days CO_2 absorbers, containing 20 ml of N KOH solution were retrieved and replaced with fresh KOH solution in known aliquots. The

retrieved KOH solutions were back-titrated with 0.1 N HCl and the amount of adsorbed CO_2 was computed.

The head space of flasks was sufficiently large to provide the cultures with oxygen; moreover the flasks and vials were opened weekly so that the head-space air could be refreshed. Gas-tight sealing of the vessels was necessary to prevent water evaporation during the long incubation. At the end of the test all the test materials were withdrawn from aqueous cultures, carefully washed and characterized by gravimetric analysis (e.g. weight variation), FT-IR spectroscopy and thermal analysis (TGA and DSC).

3.4.2. *Respirometric biodegradation tests in soil medium*

Soil burial biodegradation tests were carried out in cylindrical glass vessels (Biometer Flask 500 ml capacity) containing a multilayer substrate in which defined amounts of forest sandy soil (20 g) were placed (Figure 3.9). Soil samples sieved at 0.6 mm, mixed with 10 g perlite and supplemented with 10 ml of 0.1% $(NH_4)_2HPO_4$ solution, were sandwiched between two layers of 15 g perlite wetted with 15 ml of distilled water. Accordingly perlite was used to ensure satisfactory incubation conditions, whereas soil samples were used mainly as microbial inoculum. This arrangement guarantees more favorable and reliable signal-to-noise ratio resulting in improved test accuracy, particularly when limited carbon dioxide emissions are expected from the test samples [178].

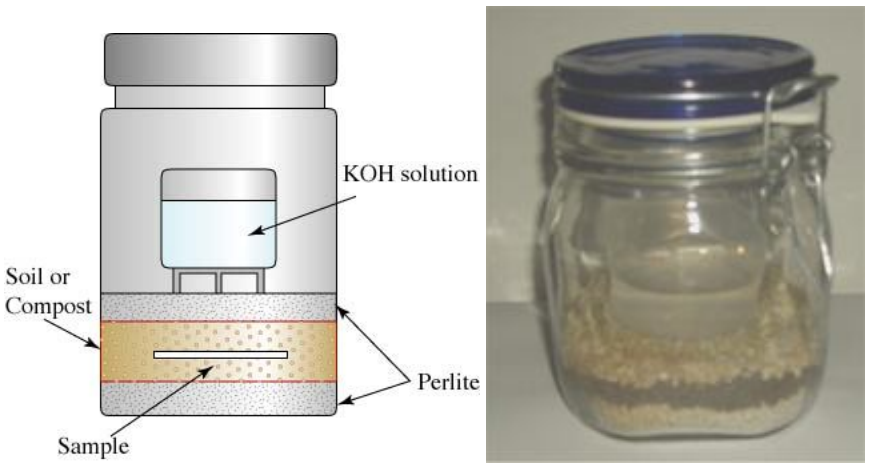


Figure 3.9. Schematic & real Biometer flask set up for simulated soil burial biodegradation tests.

Polymeric test specimens and reference materials were placed in the core of a soil middle layer at 20 mg/g ratios and the culture vessels were kept in the dark and incubated at room temperature. The CO_2 evolved from samples and blanks was trapped in each test vessel by means of 40 ml of 0.05 N KOH solution contained in a beaker set in the test vessel. The absorbing solution was back-titrated with 0.1 N HCl at time intervals by adding 0.5 N $BaCl_2$ solution before the titration in one-tenth proportion with respect to the overall volume of the absorbing alkaline medium. Phenolphthalein was used as indicator. All the tests were carried out in triplicate.

Every 3 months of incubation each culture was carefully aerated by turning up the middle soil layer, re-wetted and added with other 5 g of fresh soil/perlite mixture in order to mimicking the situation of a real agricultural soil. At the same time small fragments of the analyzed test samples were withdrawn from the relevant culture, carefully washed with distilled water an submitted to morphological, spectroscopic and thermal characterization in order to evaluate the progress of chemical degradation

of the test samples as a consequence of the incubation in an microbiologically active soil sample.

3.4.3. *Respirometric biodegradation tests with single fungal strain in aqueous medium*

The biodegradation tests were carried out in 300 ml Erlenmeyer flasks equipped with silicone rubber stoppers hanging 40 ml capacity plastic vials filled with 20ml CO_2 absorbing KOH solutions (0.05 N). Each flask contained 100 ml mineral salt medium having the following composition per liter of distilled water: KH_2PO_4 85 mg, K_2HPO_4 218 mg, Na_2HPO_4 334 mg, $(NH_4)_2SO_4$ 10 mg, NH_4NO_3 10 mg, $CaCl_2$ 36 mg, $MgSO_4.7H_2O$ 23 mg, $MnSO_4$ H_2O 0.021 g, $CuSO_4$ $5H_2O$ 0.006 g, $CoCl_2$ $6H_2O$ 0.006 g, pH 7.4±0.2.

The microbial inoculum represented by single fungal strain was prepared by cultivating the microorganism on Potato Dextrose Agar (PDA) plates. A 0.5 cm agar disk taken from PDA plates was used to inoculate each test flask. All the test materials were supplied to the microbial cultures as sole carbon sources at approximately 0.05 % by weight concentration.

Test flasks were incubated at room temperature (25°C) in the dark on a rotatory shaker (120 rpm). All the runs were carried out in triplicate. Every 7 -15 days the CO_2 absorbers, containing 20 ml of 0.05 N KOH solution were retrieved and replaced with fresh KOH solutions in known aliquots. The retrieved KOH solutions were back-titrated with 0.1 N HCl and the amount of CO_2 adsorbed was computed.

3.4.4. *Respirometric biodegradation tests with single fungal strains onto solid media under co-metabolic conditions*

Mineralization tests aimed at evaluating the capability of fungal strains to degrade and assimilate LLDPE samples under co-metabolic conditions (e.g. in the presence of an easily assimilable carbon source) have been

carried out onto thermally oxidized, outdoor exposed and pristine LLDPE specimens.

Tests were carried out in 300 ml flasks containing 50 ml of a solid medium having the following composition: K_2HPO_4 2.2g, KH_2PO_4 0.8 g, $MgSO_4$ $7H_2O$ 0.2 g, NH_4NO_3 0.25 g, $MnSO_4$ H_2O 0.021g, $CuSO_4$ $5H_2O$ 0.006 g, $CoCl_2$ $6H_2O$ 0.006 g, Glucose 1.0 g, distilled water 1000 ml, Bacto Agar 20.0g. Each biometer flask was equipped with silicone rubber stoppers hanging 40 ml capacity plastic vials filled with 20 ml CO_2 absorbing KOH solutions (0.05 N). The cultures were run in triplicate and incubated at room temperature. Every 5 -10 days proximal CO_2 absorbers, containing 20 ml of 0.05 N KOH solution were retrieved and replaced with fresh KOH solutions in known aliquots. The retrieved KOH solutions were back-titrated with 0.1 N HCl and the amount of CO_2 adsorbed was computed.

Previously isolated F2 fungal strain and *P. chrysosporium* were utilized as degrading microbial species. The microbial inoculum was prepared by cultivating the microorganisms on Potato Dextrose Agar (PDA) plates. A 0.5 cm agar disk taken from PDA plates was used to inoculate each test flask

In all the respirometric tests, the biodegradation extent of polymeric materials was calculated as the percentage of the overall CO_2 production [Theoretical CO_2, (Th.CO_2)] calculated on the basis of the overall organic carbon content of the samples. The values were corrected for the inoculum endogenous emissions obtained from the control (flasks containing all components, except polymer samples). All tests were conducted in triplicate, and the standard errors were calculated. Cellulose filter paper was used as the positive standard.

3.4.5. *Biodegradation tests with single microbial species in agar plates*

The lignin degrading *Phanerochaete chrysosporium* ATCC 34541 strain purchased from Deutsche Sommlung von Mikroorganismen und Zellkulturen (DSMZ)and four different fungal strains isolated from LDPE fragments retrieved after 2 years soil burial degradation tests, were utilized in pure cultures.

To isolate the fungal strains, the LDPE fragments were incubated in solid agar plate containing 20 ml sterilized low concentration salt agar medium having the following composition per liter of distilled water: K_2HPO_4 2.2 g, KH_2PO_4 0.8 g, $MgSO_4$ $7H_2O$ 0.2 g, NH_4NO_3 0.25 g, $MnSO_4$ H_2O 0.021 g, $CuSO_4$ $5H_2O$ 0.006 g, $CoCl_2$ $6H_2O$, pH 7.4. After 3 days incubation at 25°C in the dark, single fungal colonies were identified and repeatedly streaked on fresh PDA (Bacto- Difco, USA) agar plates for the isolation in single pure culture the fungal strains.

P. chrysosporium and four different fungal strains, isolated according to the above reported procedure (F1,F2, F3 and F4) were used separately in Petri dishes containing agar to inoculate LLDPE test specimens. Approximately 20 ml of agar medium having the following concentration per liter of distilled water : K_2HPO_4 2.2 g, KH_2PO_4 0.8 g, $MgSO_4$ $7H_2O$ 0.2 g, NH_4NO_3 0.25 g, $MnSO_4$ H_2O 0.021 g, $CuSO_4$ $5H_2O$ 0.006 g, $CoCl_2$ $6H_2O$ 0.006 g, Glucose 1.0 g, distilled water 1000 ml, Bacto Agar 20.0 g, was poured in 9.0 cm diameter petri plates after sterilization.

The test specimens relevant to outdoor exposed LLDPE films, were sterilized under UV lamp for 48 hours before to be aseptically transferred onto the agar surface, after that each plate was inoculated with a single fungal strain. Tests were carried out in triplicate.

The effect of the microbial activity was monitored after 20, 45, 65 and 180 days of incubation, film specimens were withdrawn from the agar plates and characterized by FT-IR spectroscopy and relevant

determination of the level of oxidation by means of Carbonyl index (CO*i*). Un-inoculated control samples of outdoor exposed specimens were incubated aseptically under the same conditions.

At the end of the biodegradation tests and prior to the analytical characterization all the sample specimens were submitted to a cleaning procedure carried out in order to remove any microbial components from the film sample surfaces. In particular, the specimen were carefully washed with distilled water. After that treatment, the specimens were suspend in distilled water (40 ml) containing 25 mg of sodiumdodecyl sulphate surfactant (SDS) and agitated at 120 rpm for two hours, in order to remove any traces of biofilm. The specimens were therefore washed twice with distilled water and recovered on nylon membrane having 0.45 μm porosity by vacuum filtration. Finally all the specimen were dried over night under vacuum in a freeze dryer apparatus.

3.5. Combined Aiotic and Biotic Degradation Tests

Studies aimed at recognize the synergistic effect of abiotic (thermal) and biotic (microbial active environment) conditions, were also carried out on pro-oxidant containing LLDPE samples in air-ventilated oven at 50°C kept in contact with forest soil and mature compost. Furthermore, experiments were carried out by evaluating thermal degradation rate and extent of outdoor exposed LLDPE samples after their incubation with single fungal strain.

3.5.1. *Thermal degradation test of LLDPE samples onto soil and mature compost at 50°C*

The degradation tests were carried out in 1 liter cylindrical vessels (Figure 3.9) containing two layers represented by 20 g silicate (bottom layer) and 25 g forest soil or mature compost sieved at 0.6 mm (upper layer), respectively.

The humidity level was adjusted at the 50% by weight of the used forest soil and mature compost samples. The test LLDPE samples were then placed at the surface of the natural matrices in the closed vessels.

At time interval (2-4 days) the film specimens were withdrawn, carefully washed with distilled water and dried in a desiccator at room temperature prior to the determination of carbonyl index by FT-IR spectroscopy. The humidity level of soil and compost was kept at constant regime during the experiment. The oxidation behavior of each test sample in contact with soil and compost was estimated according to the calculation of carbonyl index.

3.5.2. *Thermal degradation tests of LLDPE samples previously incubated with single fungal strain*

The specimens withdrawn after 65 days incubation in the presence of the selected fungal strains were further exposed to a thermal treatment in static oven at medium temperatures (45°C) with the aim to ascertain the influence of the microbial metabolism on the propensity of the LLDPE matrix to be further oxidized under abiotic conditions. Test specimens were placed in plastic holder allowing direct contact to the ambient temperature. At time intervals of thermal aging the test samples were analyzed by FT-IR spectroscopy and recording the FT-IR spectra on the same spot region.

3.6. Analytical Characterization

The following analytical characterization have been undertaken to follow the oxidative degradation and biodegradation of the investigated polymeric materials as aimed at understanding their ultimate environmental fate.

3.6.1 *Thermal characterization*

All the polymeric materials and film samples, before and after degradation and biodegradation experiments were characterized by both Differential Scanning Calorimetry (DSC) and Thermal Gravimetric Analysis (TGA).

- Differential Scanning Calorimetry (DSC)

The thermal analysis of of polymeric materials was carried out by a DSC822^e (700°C) module with FRS5 sensor and operated by means of STAR^e software. Measurements were performed under nitrogen flow rate of 80 ml/min according to the following protocol:

1. First heating scan from -20°C to 140°C at 10°C/min and 2 min of isotherm at the end;
2. First cooling scan from 140°C to -20°C at -10°C/min and 4 min of isotherm at the end;
3. Second heating scan from -20°C to 140°C at 10°C/min.

All the thermal transitions [Melting Temperature, ™; Glass Transition Temperature, (T_g); Crystallinity (%)] were taken from DSC traces recorded during both first and second heating. In order to assess the degree of crystallinity of samples, the enthalpy of fusion of100 % crystalline PE was taken as 293 J/g [179] In accordance, the degree of crystallinity of each analyzed sample was calculated as the ratio between the integration of the melting enthalpy of the sample referred to 293 J/g.

- Thermal Gravimetric Analysis (TGA)

TGA experiments were performed in the thermogravimetric analyzer series Q500 of the TA Instruments. Generally, sample amount was between 10-15 mg. TGA experiments were performed in the thermogravimetric analyzer under nitrogen or air atmosphere at 60 ml/min flow rate in the following conditions:

1. Heating from 25°C up to 610°C, heating rate 10°C/min.

Degradation temperature onsets at 2% weight loss (T_{ON}) and residue weight at 600°C were recorded during the TGA courses.

3.6.2. *FT-IR analysis and Carbonyl index determination*

Fourier transformed infrared (FT-IR) spectroscopic characterization of the analyzed materials was carried out with a Jasco model 410 spectrophotometer. The spectra were taken as an average of 16 scans with 2 cm^{-1} resolution. The carbonyl index (CO*i*) was calculated as the ratio of the optical density of the absorption band comprised between 1830-1650cm^{-1} (carbonyl peak), and the optical density of the absorption band at 1463 cm^{-1} (CH_2 scissoring vibration peak) [178].

3.6.3. *Gravimetric analysis*

Weight analyses of samples from degradation studies were carried out by recording the samples weight in an analytical laboratory balance with ± 0.1 mg precision. Before any determinations the samples were pre-conditioned at room temperature in a desiccator.

3.6.4. *Solvent fractionation of test samples*

To analyze the low molecular weight fractions produced during the abiotic degradation tests, exposed samples were submitted to an extraction procedure by using in sequence boiling distilled water, acetone and dichloromethane under reflux conditions for 2 hours in a Kumagawa apparatus. The obtained extracts were dried to constant weight under vacuum and the relevant molecular weight (Mw) and molecular weight distribution (ID) were evaluated by means of GPC analysis .

3.6.5. *Gel content determination*

The determination was carried out in accordance with ASTM D2765 - 01(2006*) Standard test methods for determination of gel content and swell ratio of crosslinked ethylene plastics* [180]. Gel content was evaluated as the percentage of insoluble material after extraction [Eq. 2] in a Kumagawa extractor for 13 hours in boiling o-xylene solvent using Whatman cellulose extraction thimbles. After extraction, the material was dried in a vacuum oven until constant weight. For each sample, four replicates were used for statistical validation.

Gel content (%) = Sample Wt after Extraction /Initial Sample Wt * 100 [Eq.2]

3.6.6. *Gel Permeation Chromatography (GPC)*

Molecular weight (Mw) and molecular weight distribution (ID) of the PS based samples and solvent extracted fractions from PE based samples have been analyzed by gel permeation chromatography (GPC) determinations with a Jasco PU-1580 liquid chromatograph equipped with a Jasco 830RI refractive index detector and Perkin Elmer LC-75 UV Vis detector, using a PLgel guard column and two PLgel Mesopore (30 cm, 10 mm) columns. Chloroform was used as mobile phase at 1 ml/min flow rate. Relative calibration has been obtained by analyzing monodisperse polystyrene standards.

3.6.7. *Scanning Electron Microscopy (SEM)*

The surface morphologies of analyzed polymeric films were recorded by using a JEOL (JSM-5600LV) scanning electron microscope (SEM) at the required magnification and with accelerating voltage of 14kV. The film samples were sputtered with gold before SEM observation.

3.6.8. *Organic carbon determination*

The amount of organic carbon in the test samples was determined according to an oxidation procedure. Approximately 60 mg of samples were put in a 100 ml round bottom flask. 20 ml of 2N $K_2Cr_2O_7$ were then added, followed by the drop wise addition of 20 ml concentrated H_2SO_4. The mixture was then heated until complete dissolution of the test sample. After cooling the overall volume of the mixture was adjusted to 100 ml with distilled water. 25 ml of test solution were put in a beaker, diluted up to 100 ml of distilled water and back titrated with 0.2 N $(NH4)_2Fe(SO4)_2 \cdot 6H_20$ solution (Mohr solution), after the addition of a few drops of diphenylamine (0.5 % in H_2SO_4) and 3.5 g NaF. A blank run without any organic substrate was carried in parallel. The organic carbon content was expressed from the following calculation.

$$\text{Organic carbon (\%)} = (a-b) \cdot N \cdot 0.012 / Wt \cdot 100 \qquad \text{[Eq. 3]}$$

Where:
a – volume of Mohr solution used in the titration of the test solution
b – volume of Mohr solution used in the titration of the blank solution
N- normality of Mohr solution
Wt – weight of the test sample in grams

3.6.9. *Carbon dioxide determination and evaluation of the degree of biodegradation in respirometric tests.*

The evaluation of the degree of biodegradation in the respirometric tests is based on the determination of CO_2 in the headspace of the culture vessels (biometer flask). CO_2 evolved inside a sealed biometer as a consequence of the microbial assimilation of the test sample, is trapped by an absorber containing alkaline solution and measured by back titration with HCl solution. At specific times, biometers were opened and aliquots of 10 ml of 0.05 N KOH, containing soluble inorganic carbon, were transferred to a beaker. To this solution, a volume of 1 ml of 0.5 N $BaCl_2$ was added to precipitate K_2CO_3 as insoluble $BaCO_3$. In this way,

KOH was back titrated by using 0.1N HCL up to phenolphthalein end point. Subsequently, biometer was recharged with a fresh KOH solution and immediately closed. This cycle of procedure was repeated periodically up to the end of experiment. The above procedure is based on the following reactions:

Carbon dioxide uptake by KOH solution

$$CO_2 + 2\ KOH \rightarrow K_2CO_3\ (soluble) + H_2O \qquad [Eq.\ 4]$$

Insoluble barium carbonate formation

$$K_2CO_3 + BaCl_2 \rightarrow BaCO_3\ (insoluble) + 2\ KCl \qquad [Eq.\ 5]$$

To determine complete or ultimate aerobic biodegradability (mineralization to CO_2), it is necessary to know the initial amount of organic carbon (TOC) in the sample and the relevant theoretical amount of CO_2 ($ThCO_2$).

It is possible to estimate the $Th.CO_2$ of the test plastic sample, knowing the amount of TOC as illustrated by Equations 6- 7.

$$C_S = TOC_S * Wts \qquad [Eq.\ 6]$$

Where C_S is the amount of carbon in the sample, TOC_S the relative amount of total organic carbon in the sample at time 0 and Wt_S the weight of sample. As to evolve 44 g of CO_2 it is needed 12 g of carbon (C), Equation 7 calculates the amount of $ThCO_2$.

$$ThCO_2 = C_S * 44/12 = TOC_S * W_S * 3.67 \qquad [Eq.\ 7]$$

The extension of biodegradation defined as mineralization percent is calculated by subtracting the accumulated CO_2 content evolved into the biometers of sample $(CO_2)S$ from that of blank $(CO_2)B$. The difference found is divided by the theoretical amount of CO_2 ($ThCO_2$) as calculated on the basis of the carbon content of the test sample (Eq. 8)

$$Biodegradation\ (\%) = 100*[(CO_2)S - (CO_2)B]/ThCO_2 \qquad [Eq.\ 8]$$

3.6.10. *Arrhenius plot*

In order to estimate the time required for the beginning of oxidation and fragmentation of the polymer matrix of oxo-biodegradable full carbon backbone samples, a diagram corresponding to Arrhenius plot was drawn for each test samples. The diagram was drawn by plotting (ordinate axis) the log of time corresponding to a certain CO*i* values and the reverse of absolute temperature (°K) (abscissa axis) corresponding to the same CO*i* values as recorded at three different temperature (e.g. 45, 55, and 65°C). According to the straight line so far obtained the time required for starting oxidation process at 35°C was calculated for each oxo-biodegradable full carbon backbone samples.

4. RESULTS AND DISCUSSION

Since the ultimate environmental fate of "degradable" polyethylenes has to be considered as the results of the combining action of abiotic factors and microorganisms, the definition of "oxo-biodegradable" materials in keeping with the processes of biodegradation of lignin and natural rubber was suggested [57].

The requirement of two steps, abiotic and biotic, in the degradation mechanism of oxo-biodegradable plastic items has recently inspired the definition and approval of Standard Guide ASTM D6954-04 "*Standard guide for exposing and testing plastics that degrade in the environment by a combination of oxidation and biodegradation*"[181].

This Standard Guide provides a framework to assess and compare the degree of degradation attainable under controlled thermal and photo-oxidation tests with the degree of biodegradation and ecological impacts in defined disposable environments after abiotic degradation. In accordance the ASTM D6954-04 guide is divided in three Tiers:

i) Accelerate aging in standard tests for both thermal and photo-oxidation processes and determination of the degree of abiotic degradation (Tier 1),
ii) Measuring biodegradation (Tier 2),
iii) Assessing the ecological impact after this last processes (Tier 3).

In order to exploit the Tier 1, the Standard Guide suggests to use test conditions for thermal or photo-oxidation likely to occur in application and disposal environment for which the test material is committed. In other words, accelerated oxidation should be carried out at temperatures and humidity ranges typical of test material application and disposal environment.

The evaluation of the extent and rate of degrdative oxidation of re-engineered polyolefins containing pro-oxidants, represents indeed a

powerful tool to predict their potential ultimate biodegradation. Several studies have been therefore carried out with the aim of investigating the mechanisms of photo and thermal oxidation of polyolefins containing pro-oxidant additives. Nevertheless, most of these studies have been carried out under strictly controlled laboratory conditions, as well as under extreme accelerating stressing conditions that can not be considered as representative of the natural environments. In particular, only a few investigations have been carried out by assessing the synergic effects of temperature, humidity and/or sunlight exposure that usually are jointly operative in outdoor exposure conditions.

In any case, by considering the general mechanisms of radical oxidation of full carbon backbone polymers, the following parameters can be currently monitored during the abiotic degradation step sketched for a material designed to undergo oxo-biodegradation: **1) *Weight variation***;

2) *Carbonyl index* (CO*i*); 3) *Wettability*; 4*) Molecular weight variation*; 5) *Fractionation by solvent extraction*.

In particular, gravimetric analysis can be effectively used to appreciate the weight variation as a consequence of the oxygen uptake during the early stage of oxidation, as well as the weight loss due to the volatilization of low molecular weight fragments at prolonged stage of thermal and photo degradation.

An other powerful tool for the quantitative and qualitative evaluation of the oxidation processes is constituted by the carbonyl index (CO*i*) as determined by FT-IR spectroscopy. It has been repeatedly reported that the most of the degradation intermediates of polyolefin peroxidation are bearing carbonyl groups, therefore their concentration, as determined by CO*i* can be used to monitor the progress of degradation [69]. In general these determinations are carried out on test films by recording the ratio of the optical density of the carbonyl absorption band at 1640 - 1840 cm^{-1}

range and the optical density of the absorption band at 1463 cm^{-1} [(CH_2 in plane bending vibrations (scissoring)]. In addition, FT-IR analyses provide also information on the presence during the oxygen uptake phase of carbonyl groups present in different moieties such as carboxylic acids (1712 cm^{-1}), ketones (1723 cm^{-1}), aldehydes (1730 cm^{-1}) and lactones (1780 cm^{-1}) [182].

The determination of wettability of film surfaces during the degradation tests by contact angle measurements, may provide useful information about the increasing polarity of the film surfaces as a consequence of oxidation and formation of functional groups. These information are also useful in order to predict the feasibility of microbial attack of polyolefins, by taking into account that one of the reason that have been suggested to explain the intrinsic recalcitrance of PE to biodegradation is the hydrophobic character hindering the adhesion of microbial cells.

In terms of potential ultimate biodegradation (e.g. conversion to CO_2 and H_2O, and cell biomass, mineralization), the assessment of molecular weight variation is a fundamental task. Indeed, it is suggested that from a theoretical point of view, PE in force of its structure as a straight chain hydrocarbon, should be metabolized according to the biochemical pathway of linear alkanes. On the other hand, it has been established that there is a dimensional limit (i.e molecular weight) for the n-alkanes utilization as carbon source by microorganisms. In this connection, Haines and Alexander established that linear hydrocarbons with more than 44 (tetratetracontane) carbon atoms can not be metabolized by soil microorganisms [117]. More recently, in a study carried out with single bacterial strains, this dimensional limit has been moved up to 0.72 kDa corresponding to 60 carbon atoms chain length [118].

Other information about the relationship between the level of oxidation as reached during the abiotic stage of degradation of “degradable”

polyolefins, the molecular weight reduction as well as the potential to be ultimately biodegraded in the environment can be effectively achieved by fractionation of pre-aged specimens submitted to solvent extraction. This practice may also provide, especially if carried out by using solvents with different polarity, additional information on the relative amount of different classes (e.g. carboxylates, alkanes etc.) of degradation products deriving from peroxidation and cleavage of macromolecular chains characterized by a full carbon backbone.

By taking into account these consideration, during the research activity carried out in the present PhD thesis, the oxidative behaviours of different synthetic polyolefin matrices (PE, PP, PS) containing pro-oxidant additives have been investigated under different test conditions as a meaningful task aimed at understanding the mechanism and extent of abiotic degradation. As a case study, also the preparation and degradation behaviour of LDPE matrix containing lignin have been undertaken. Finally, the ultimate environmental fate as mediated by complex microbial population or single microbial species of all the analyzed polymeric materials has been investigated in comparative biodegradation tests carried out by analyzing hydro-biodegradable polymers such as synthetic and semi-synthetic polyesters claimed as biodegradable [183].

4.1. Abiotic Degradation Tests & Characterization of Degraded Samples

In a series of degradation tests, the effect of different abiotic factors such as heat, light irradiation and their combination, have been investigated in order to ascertain the propensity to environmental degradation of re-engineered polyolefin plastic items. In these studies, also the structural parameters of the analyzed polymeric materials have been taken into consideration. In this connection different polymeric materials

represented by different PE grades (LDPE, HDPE, LLDPE), PP and PS have been utilized, by considering the different content and type of tertiary carbon atoms in the main chain.

During the tests the progress of the degradation process taking place in the polymer matrix has been monitored by FT-IR spectroscopy (e.g. CO*i* determination), thermal analysis by using differential scanning calorimetry (DSC) and thermal gravimetric analyses (TGA). The analysis were performed as aimed at evaluating the effect of the different abiotic test conditions to promote chain scission of the polyolefin matrix, as well as to analyze the structural characterization of the low molecular weight intermediates.

4.1.1. *Thermal degradation test in static oven at different temperatures of PE, PS and PP based film samples*

The oxidation propensity of polyolefin (PE, PP, PS) samples containing pro-oxidant additives, as induced by heat was assayed in static oven at different temperatures in the 28 – 65°C range. The indicated temperatures were selected with an intention of mimicking the thermophilic phase of a composting process and agriculture field conditions.

The oxidation kinetics of the thermally aged polyolefin samples were recorded by means of CO*i* evaluation by FT-IR spectroscopy. In this regard, the carbonyl index (CO*i*) of PE and PP samples was calculated on the basis of the relative intensities of the carbonyl absorption band between 1650 and 1830 cm^{-1} to that of methylene scissoring bending at 1465 cm^{-1}. In the case of PS based samples, CO*i* values were evaluated by the ratio of the optical density of the carbonyl region absorption band between 1690 and 1800 cm^{-1} and the overtone band at 1940 cm^{-1} of the aromatic ring. The CO*i* determinations were therefore used to compare

the thermal oxidation propensity of different test samples under various temperature conditions.

The characteristics of the samples analyzed in thermal degradation tests at different temperatures in static oven are reported in Table 4.1.

Table 4.1. Test samples relevant to static oven experiment at different temperatures.

Test Samples	Code	Description
LLDPE	LLDPE-TD1	LLDPE + TD1 pro-oxidant
	LLDPE-TD2	LLDPE + TD2 pro-oxidant
	LLDPE-TD0	LLDPE + no additivated (control)
PP	PP-TD1	PP + TDPA1 additive
	PP-TD2	PP + TDPA2 additive
PS	PS-TD1	PS + TDPA3 additive
	PS-TD2	PS+ TDPA4 additive
	PS-TD0	PS + no additivated (control)

In the case of LLDPE, three different blown films represented by two samples additivated with pro-oxidant systems and one pro-oxidant free control were submitted to thermal aging in station oven at 45, 55 and 65°C, thus recording the COi profiles sketched in Figures 4.1 and 4.2.

LLDPE-TD1 sample was susceptible to undergo a significant oxidation under all the temperatures used in the thermal degradation test (Figure 4.1). In particular, the polymer matrix oxidation was shown to start after 18 days at 65°C, 40 days at 55°C and 80 days at 45°C, respectively. As expected, longer induction times were recorded at lower temperatures (Figure 4.1). In any case a fairly high oxidation was achieved in relatively short times. The film sample started also to fragment when CO*i* approached 0.30 value. It is however worth noting that the level of

oxidation after 170 days of thermal aging was lower at 45°C with respect to the values recorded at 55 and 65°C (Figure 4.1).

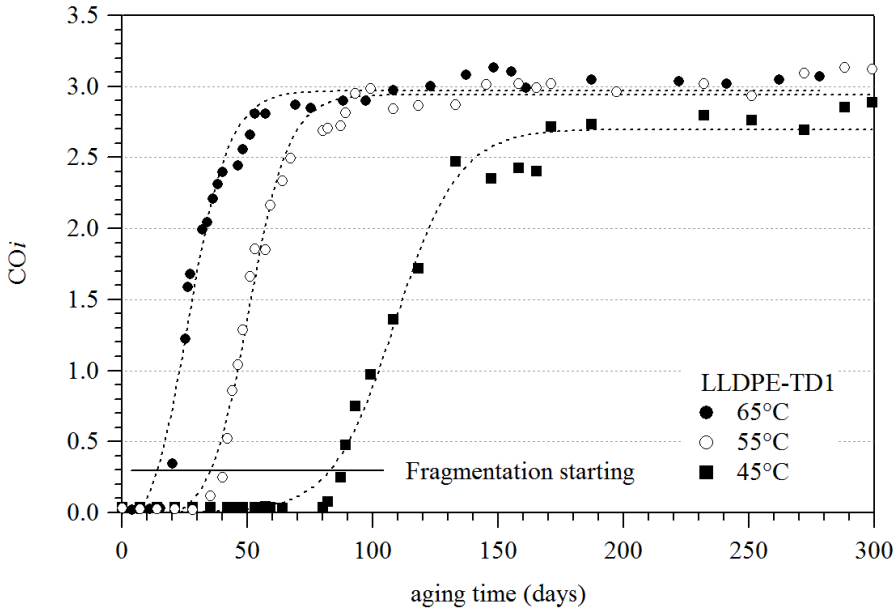


Figure 4.1. CO*i* profiles of LLDPE-TD1 sample recorded at different temperatures in static oven in air atmosphere.

In particular, a maximum level of oxidation corresponding to approximately 3.2 CO*i* value was approached at 55 and 65° C with only minor increase recordable even after very long treatment period (up to 300 days), whereas the highest CO*i* leveled off at 2.7 in the LLDPE-TD1 film specimen treated at 45°C for 180 days, thus approaching fairly higher values only at the end of test (Figure 4.1).

In the case of LLDPE-TD2 film sample, containing a different type of pro-oxidant additive, the oxygen uptake started after 53 days at 65°C, after 87 days at 55°C and after 160 days at 45°C (Figure 4.2). Also in this case the highest levels of oxidation were recorded at 55 and 65°C. Furthermore, a marked lower formation of carbonyl groups was observed at 45°C. In fact at this temperature the CO*i* profile started to increase

appreciably only after 160 days thermal degradation, thus approaching however 1.7 CO*i* value after 300 days of thermal treatment (Figure 4.2).

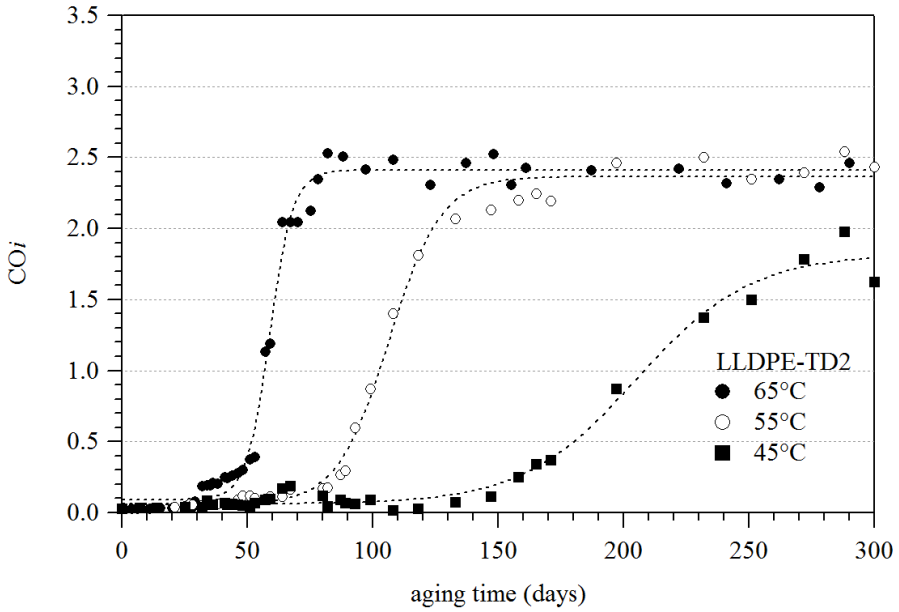


Figure 4.2. CO*i* profiles of LLDPE-TD2 sample recorded at different temperatures in static oven in air atmosphere.

In any case, however, the lower propensity to oxidation under thermal treatment of LLDPE-TD2 sample, with respect to LLDPE-TD1 film, has been observed in the same time frame and degradation temperatures.

LLDPE-TD0 pro-oxidant-free sample, incubated at the same temperatures and for the same aging time did not show any significant formation of carbonyl groups (data not shown).

By using the recorded CO*i* data of LLDPE samples, the estimation of the time required to reach a definite level of oxidation, as expectable in the correspondence of theoretical CO*i* values attainable at 35°C, was calculated out by means of the Arrhenius plot [184] (Figures 4.3 a) and b), Table 4.2).

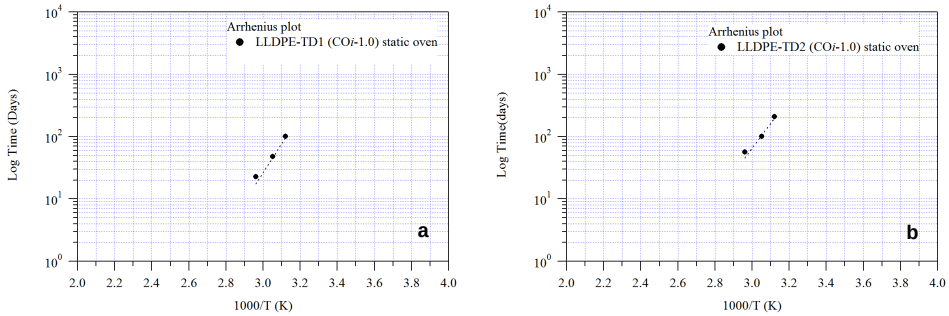


Figure 4.3. Arrhenius plot of LLDPE-TD1 (a) and LLDPE-TD2 (b) samples drawn according to the time required to reach 1.0 CO*i* value at 45, 55 and 65°C.

Table 4.2. Time (days) required for reaching different level of oxidation at 35°C as determined by the Arrhenius plot.

Sample	**Carbonyl index**				
	time	0.5	1.0	1.5	2.5
LLDPE-TD1	(days)	300	375	400	500
LLDPE-TD2	(days)	400	600	800	1050

In particular, it has been recorded that the time required for reaching an appreciable level of thermal oxidation (1.0-1.5 CO*i* values) is significantly higher in the case of LLDPE-TD2 sample (approximately 600-800 days), whereas the LLDPE-TD1 can reach the same oxidation extent theoretically after 12-13 months exposure at 35°C under static conditions.

An analogous test set up was utilized to analyze the oxidative thermal behaviour of two PP samples containing different pro-oxidant additives. The CO*i* profiles recorded at different temperatures are reported in Figures 4.4 and 4.5.

As expected even in the case of PP samples, the rate of the oxidation process was noticeably influenced by the temperature, thus proceeding much faster at 65°C. Nevertheless, even though after very prolonged

aging time (6 months) appreciable oxidation was observed also in the film sample specimens thermally treated at 45°C.

A different oxidative behaviour, depending upon the TDPA additive used in the PP formulation was also recorded during this test. In particular, under static condition the PP-TD2 additive appeared to be more effective in inducing the oxidative degradation of the PP matrix as revealed by the shorter induction times observed under different applied temperatures.

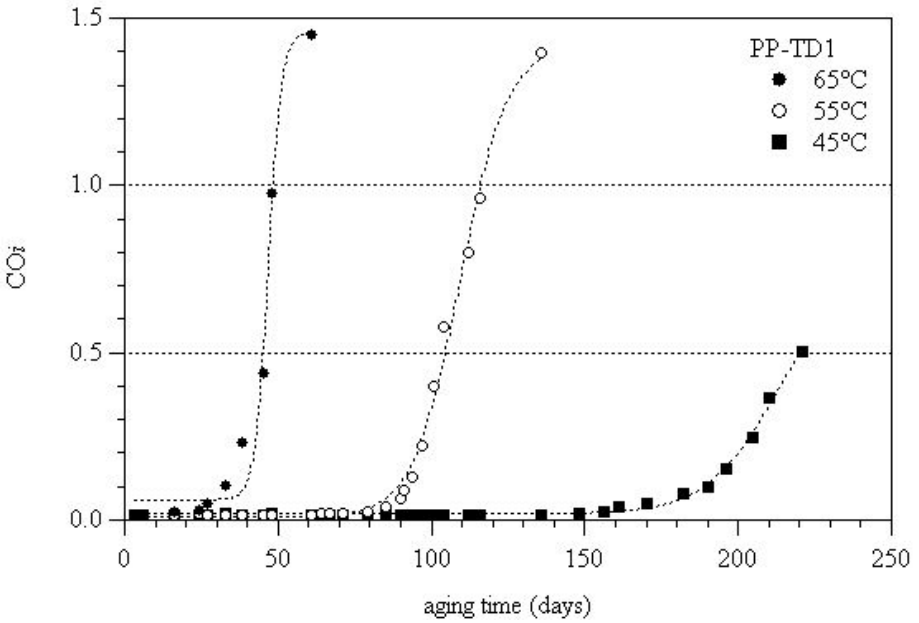


Figure 4.4. CO*i* profiles of PP-TD1 sample recorded at different temperatures in static oven in air atmosphere.

It is also worth noting that the recorded CO*i* values in the case of PP samples can not be considered to be fully representative of the overall extent of oxidation due to the complexity of the FT-IR spectra in the methylene vibrations region (1400 – 1500 cm^{-1}), once the samples approached a fairly high fragmentation which hindered a reliable CO*i* calculation.

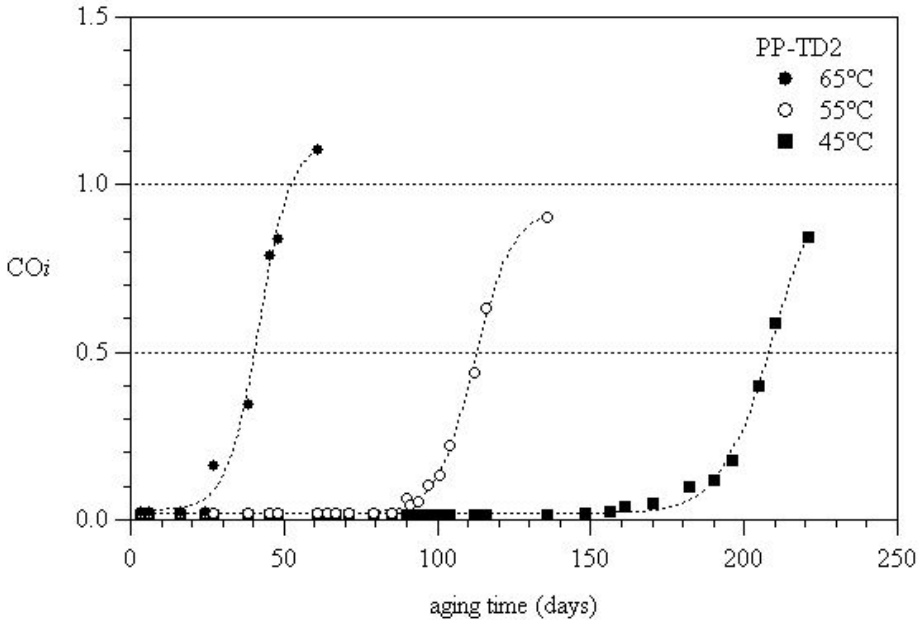


Figure 4.5. CO*i* profiles of PP-TD2 sample recorded at different temperatures in static oven in air atmosphere.

Finally, two PS based samples, PS-TD1 and PS-TD2 sheet specimens of approximately 100 μm thickness, were submitted to thermal degradation at 45, 55, and 65°C in static oven in air atmosphere. During the aging time, the oxidation of the PS matrices were monitored by measuring the relative increase of the carbonyl absorption band by FT-IR spectroscopy. In particular the oxidation index (Carbonyl index – CO*i*) was calculated by the ratio between the optical density of the band of the carbonyl group between 1690 and 1780 cm^{-1} and the overtone band at 1940 cm^{-1} of the aromatic ring. In addition, the overall increase of the carbonyl absorption band was in any case markedly lower with respect to the absorbance recorded in thermally oxidized LLDPE and PP systems. In accordance, the CO*i* profiles reported in Figures 4.6 and 4.7 can not be compared with those recorded for LLDPE and PP samples at the same temperatures. In any case, an appreciable oxidation of both PS-based samples was recorded after a few days of thermal treatment. Nevertheless, the rate and

extent of the oxidation of PS matrix resulted less influenced by the adopted incubation temperature, and the overall extent of oxidation was shown to approach a plateau phase, at fairly limited level of carbonyl content and after a few days (15) of thermal aging at 65°C (Figure 4.8).

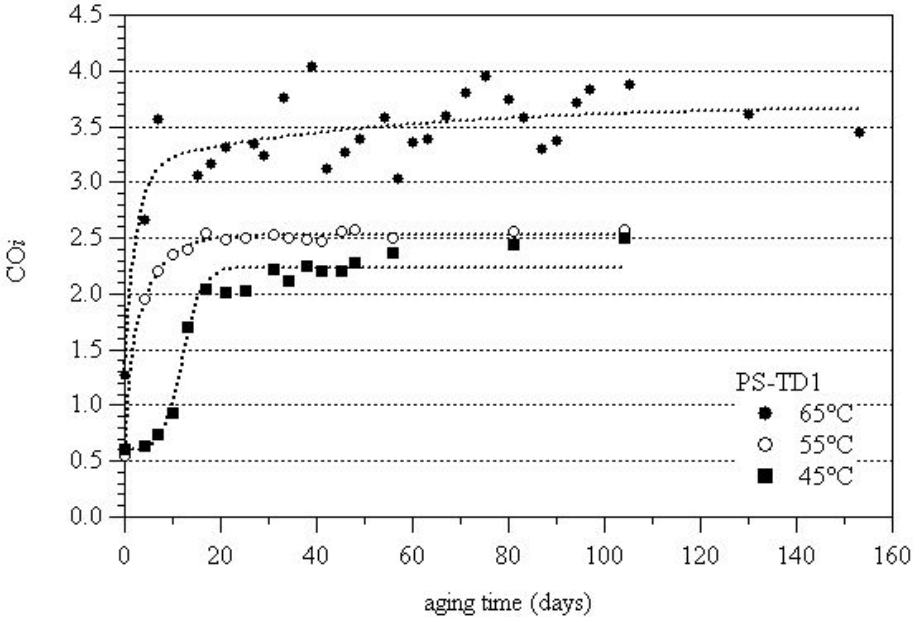


Figure 4.6. CO*i* profiles of PS-TD1 sample recorded at different temperatures in static oven in air atmosphere.

The results collected during thermal aging tests in static oven in air atmosphere are therefore demonstrating that all the investigated polyolefin matrices can be effectively oxidized by using pro-oxidant additives based on transition metals. The rate and extent of the oxidation processes is depending upon the temperature at least in the case of LLDPE and PP polymeric materials, whereas a different behaviour was observed when PS based samples were tested.

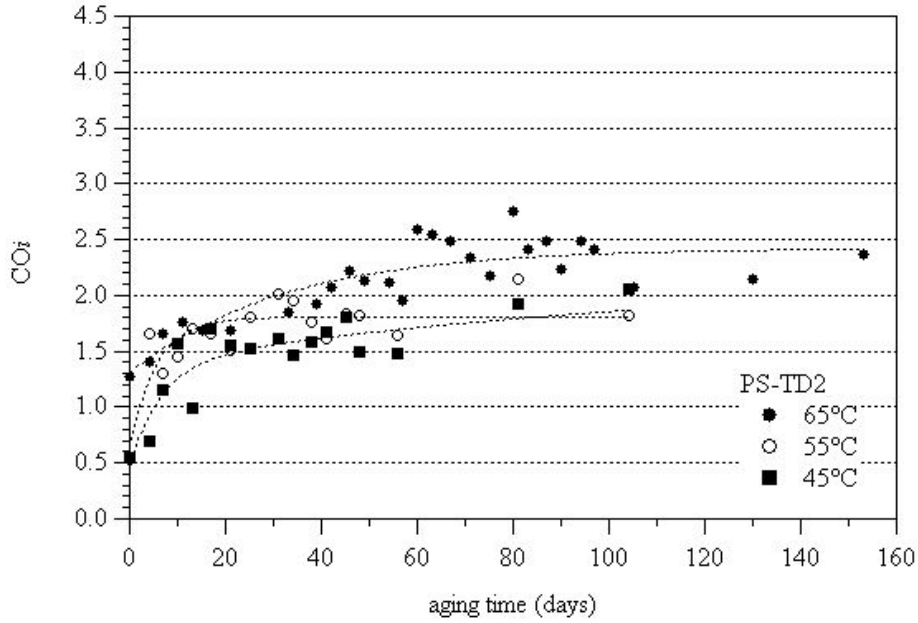


Figure 4.7. CO*i* profiles of PS-TD2 sample recorded at different temperatures in static oven in air atmosphere.

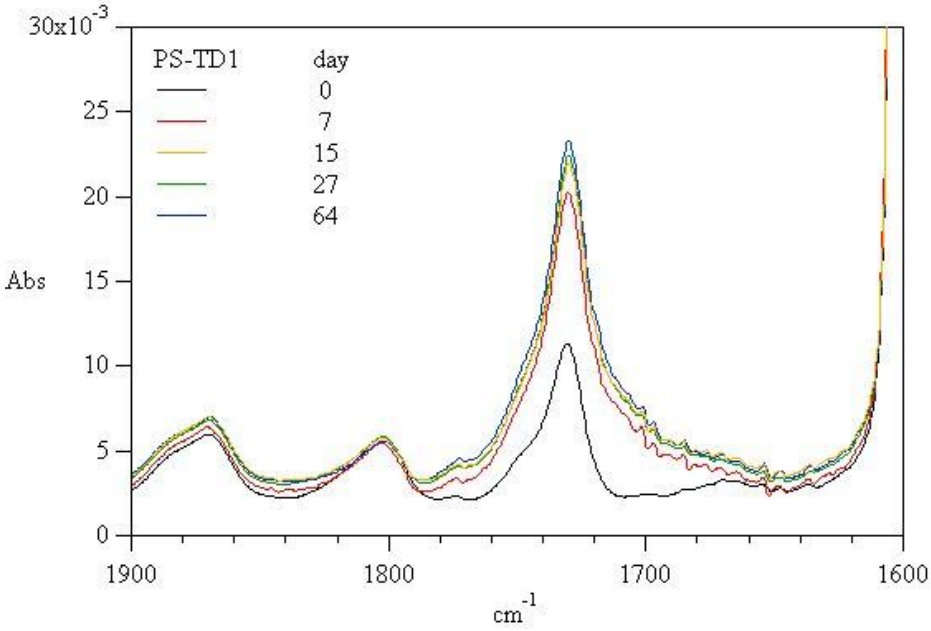


Figure 4.8. Carbonyl region of FT-IR absorption spectra of PS-TD2 sample thermally treated at 65°C in static oven in air atmosphere.

In the case of PS samples, the overall extent of polymer matrix oxidation was markedly lower despite the presence of easily oxidizable tertiary carbon atoms in 1-3 position in the main chain such as in the case of PP macromolecules. In addition, no significant variation of either molecular weight (Mw) or molecular weight distribution (ID) was recognized even after a prolonged (274 days) treatment was carried out under accelerated (forced air) condition at 65°C (Table 4.3).

Table 4.3 Molecular weight analysis of original and thermally treated PS-TD1 and PS-TD2 samples.

Test Sample	Treatment	Time	Mw	ID
		(days)	kDa	
PS-TD1	pristine	0	162.9	1.57
PS-TD1	45°C static oven	28	162.2	1.58
PS-TD1	55°C static oven	28	158.2	1.58
PS-TD1	65°C static oven	28	162.1	1.57
PS-TD1	65°C forced air oven	66	160.9	1.59
PS-TD1	65°C forced air oven	274	161.2	2.57
PS-TD2	pristine	0	162.9	1.57
PS-TD2	45°C static oven	28	157.5	1.57
PS-TD2	55°C static oven	28	159.1	1.58
PS-TD2	65°C static oven	28	160.6	1.58
PS-TD2	65°C forced air oven	66	162.1	1.58
PS-TD2	65°C forced air oven	274	157.3	3.03

The recorded data seem to suggest that the observed thermal oxidation of PS does not involve the random chain scission repeatedly established for LDPE and PP macromolecules [143-145], at least within the applied aging time.

Finally, it has to be considered that in the case of LDPE and PP film samples, the recorded differences in both the rate and extent of carbonyl group formation could be attributed to the presence of primary anti-

oxidant contained in the pro-oxidant master batch utilized for the film preparation.

A preliminary investigation, has been also undertaken, under the adopted conditions, as aimed at evaluating the propensity to thermal oxidation of antioxidant-free low density poly(ethylene) LDPE based film samples containing a potential pro-oxidant systems represented by an oxo-biodegradable natural polymers such as alkali lignin (AL) additivated or not with a proprietary pro-degradant master batch containing transition metals carboxylates.

The effectiveness of the pro-oxidant systems represented by 1.0, 3.0 and 5.0% by weight AL filler with and without EPI-TDPA pro-degradant (P), in promoting the oxidative degradation of the LDPE matrix was ascertained in thermal degradation tests carried out in static oven at different temperatures (28, 40, 50 and 60°) in the dark. Anti-oxidant free LDPE film was taken as a reference.

The progress of oxidation at different temperatures (40, 50 and 60°C), as monitored by CO*i* determinations, of the AL containing LDPE films are reported in Figures 4.9 - 4.11.

LDPE/AL blends (AL 0-5% w/w) additivated with 3% by weight pro-degradant master batch (P) showed a high propensity to thermal oxidation, whose rate and extent did not appeared to be much affected by the content of lignin within the investigated range of temperatures (40-60°C). On the contrary, a marked influence on the extent of oxidation have been recorded as depending upon the applied temperature. It was evident that the highest extent of oxidation (3.5-4.1 as CO*i*) was attained at 60°C (Figure 4.9), whereas at lower temperatures, the CO*i* profiles approached lowest values of 2.7 and 1.4 at 50° and 40 °C, respectively, under the same aging time frame (Figures 4.10 and 4.11).

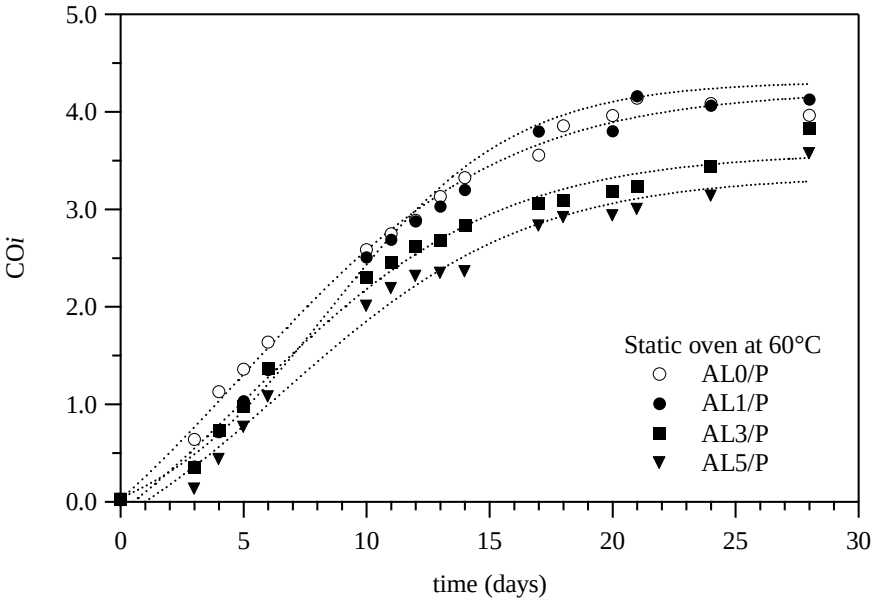


Figure 4.9. CO*i* profiles of LDPE/AL blends containing 3% pro-degradant and 0-5% w/w AL at 60°C in static oven in air atmosphere.

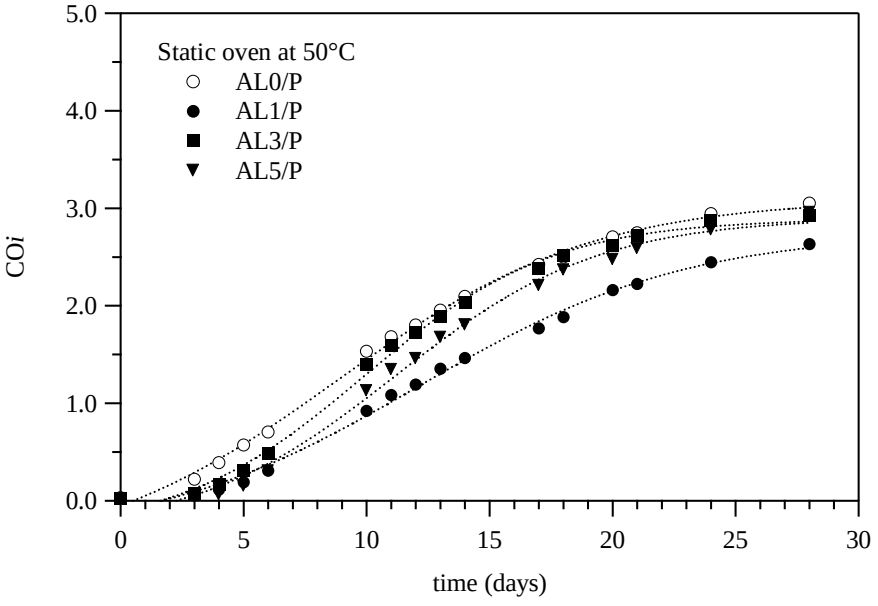


Figure 4.10. CO*i* profiles of LDPE/AL blends containing 3% pro-degradant and 0-5% w/w AL at 50°C in static oven in air atmosphere.

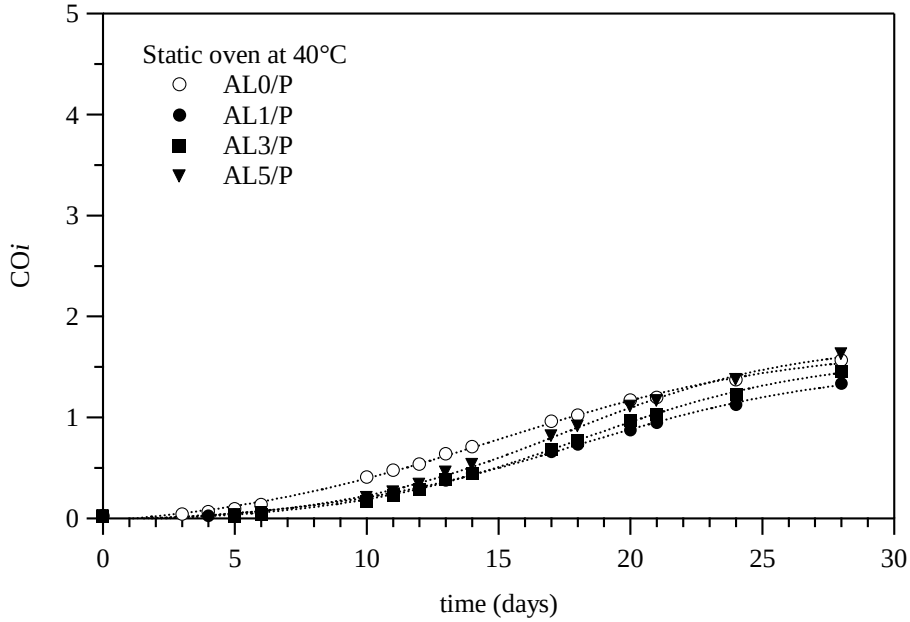


Figure 4.11. CO*i* profiles of LDPE/AL blends containing 3% pro-degradant and 0-5% w/w AL at 40°C in static oven in air atmosphere.

However, the presence of lignin which is recognized to have antioxidant properties due to phenolic groups [185] did not appeared to affect the process of thermal oxidation.

4.1.2. *Thermal degradation test in air-ventilated oven at 65°C of PE, PS and PP based film samples.*

Thermal degradation tests under very stressing conditions were carried out in order to collect more information on the structural changes of the polymer matrices in relatively short times. In accordance, most of the structural investigations (e.g., weight variation, thermal analysis, isolation and characterization of degradation intermediates) currently used in the investigation of oxidative processes of polyolefins have been also performed.

The test materials analyzed under the adopted conditions were the same specimens previously submitted to thermal degradation at different temperatures in static oven (Table 4.1).

In particular, approximately 1.5 g of each LLDPE-TD1, LLDPE-TD2 and LLDPE-TD0 (control) film samples were submitted to thermal degradation at 65°C in a oven under constant air flow (air-ventilated oven), thus recording the relevant CO*i* profiles as averaged values over 5 determinations each time (Figure 4.12). The higher propensity to thermal oxidation of LLDPE-TD1 sample, previously observed under thermal aging in oven under static conditions, was confirmed during this test. In particular LLDPE-TD1 film specimen was characterized by both a fairly short induction period, and by an higher level of oxidation corresponding to 5.1 CO*i* after 150 days aging. Under the adopted test conditions a 3.5 CO*i* value was recorded in the case of LLDPE-TD2 film sample, even though after longer (4 months) incubation time (Figure 4.12).

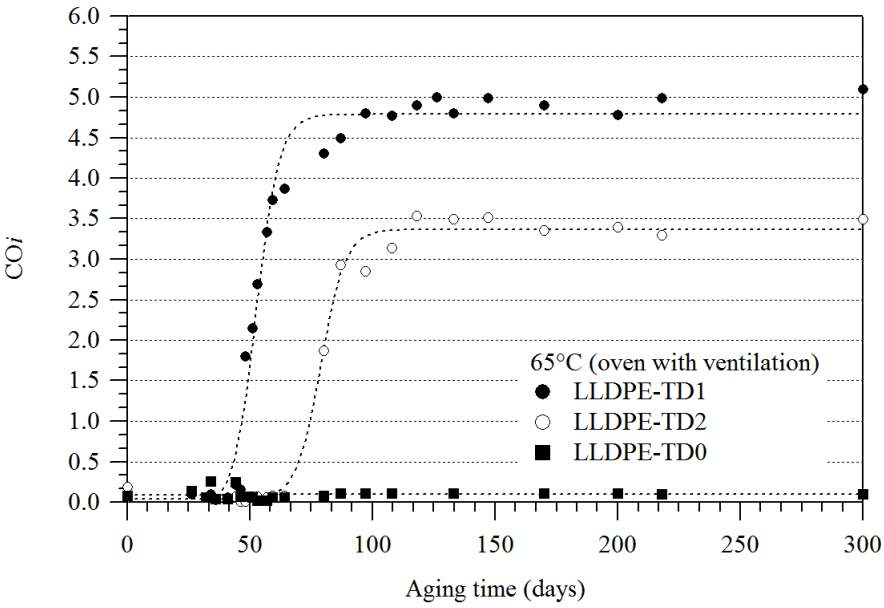


Figure 4.12. CO*i* profiles of LLDPE film specimens at 65°C in air-ventilated oven.

No significant formation of carbonyl group was observed in the control film sample (LLDPE-TD0).

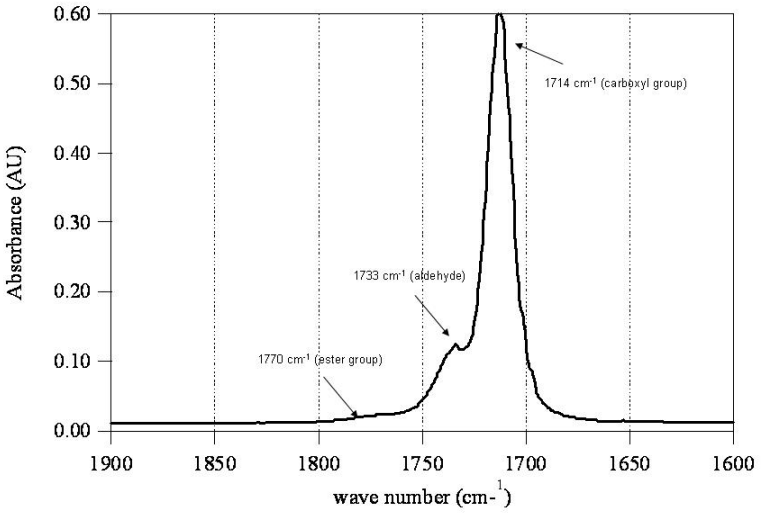
With respect to the test carried out at 65°C under static conditions, a higher level of oxidation in both LLDPE-TD1 and LLDPE-TD2 films was reached (Figure 4.12), thus indicating that the almost constant oxygen partial pressure at LLDPE sample-air interface (no oxygen diffusion control) is stimulating the overall oxidation process of the polymer matrix.

It is worth noting that the adopted conditions in term of oxygen diffusion can be considered as much more comparable with the environmental conditions the samples are eventually experiencing with respect to the static oven conditions. Nevertheless, once the oxidation profiles approached the stationary phase no further increase was observed even after very long aging time (Figure 4.12).

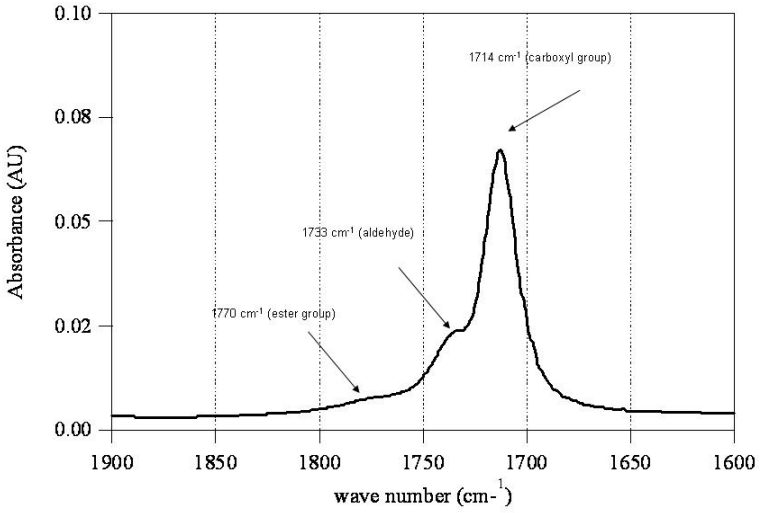
Indeed, the propensity of the different types of pro-oxidant contained in the two LLDPE based samples to induce different level of oxidation has to be mentioned.

The formation within the time of different types of carbonyl groups as relevant to ketone, carboxyl and ester moieties was also clearly recorded in both the pro-oxidant containing LLDPE-TD1 and LLDPE-TD2 samples (Figure 4.13).

Similar results were obtained in the case of pro-oxidant additives containing PP samples. At 65°C in forced air oven the induction period for the PP matrix oxidation was shown to correspond to 25 days in the case of PP-TD1 and 30 days in the case of PP-TD2 film sample, respectively. Therefore, in contrast with the results recorded at the same temperature in static conditions, it seems that TD1 additive is more effective in promoting the thermal oxidation process under the adopted conditions.



a



b

Figure 4.13. Profile of FT-IR absorption band in carbonyl region of thermally oxidized LLDPE-TD1 (a) and LLDPE-TD2 (b) samples

In any case fairly lower induction periods have been recorded under forced air condition rather than under static thermal treatment carried out

at the same temperature. In addition, also in the case of PP samples, higher levels of oxidation were reached in a fairly short time (35-48 days) (Figure 4.14).

It is, however to be observed that the relevant amount of different carbonyl groups is significantly different in the two polyolefin matrices, being the corboxilyc acids strongly prevailing in the oxidized LLDPE, whereas an higher content of aldehyde and ester carbonyl groups were detected in oxidized PP matrix (Figure 4.15).

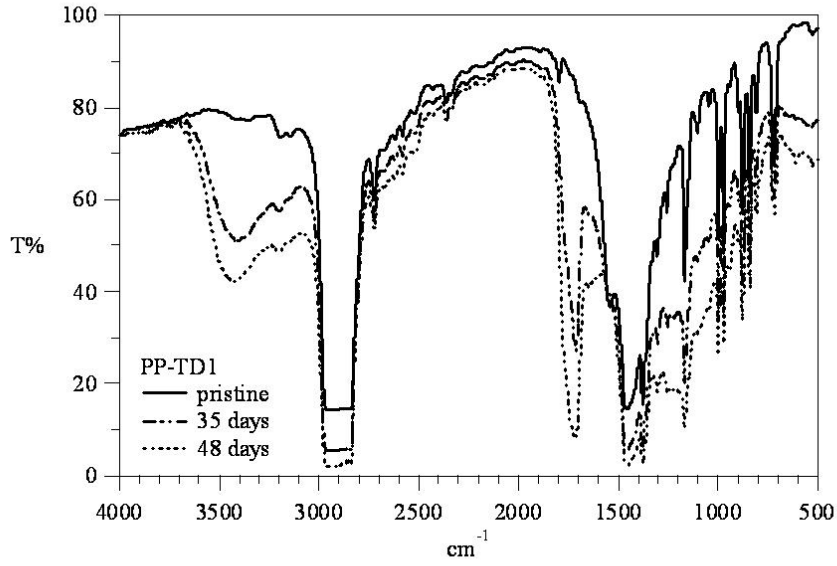


Figure 4.14. FT-IR spectra of PP-TD1 specimen submitted to thermal degradation at 65°C in air-ventilated oven.

PS based samples containing pro-oxidant systems submitted to thermal degradation under the same conditions showed after 5 months of thermal treatment only slight weight increases ranging between 0.9-1.2% and 0.7-0.9% in PS-TD1 and PS-TD2 samples, respectively. These results were in accordance with the fairly limited increase of oxidation as revealed by the

FT-IR analysis of the same PS samples treated in static oven at the same temperature (65°C).

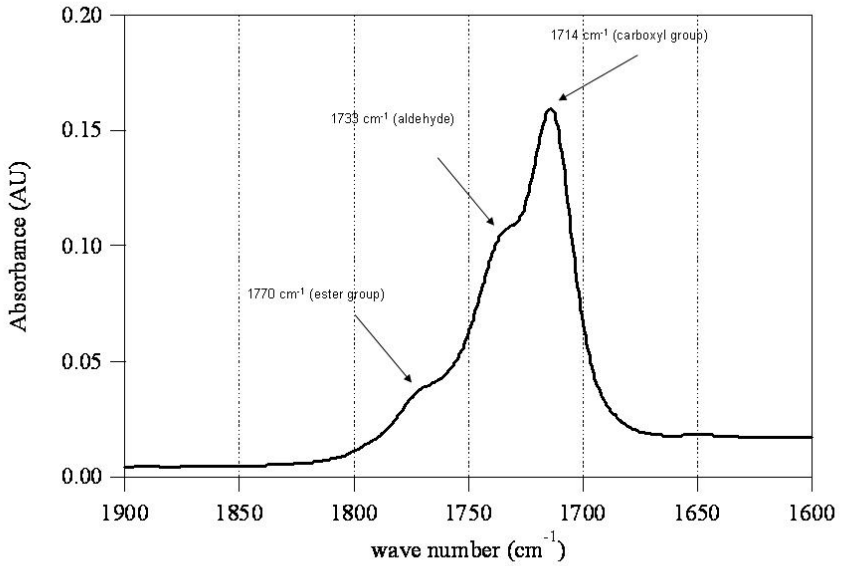


Figure 4.15. Profile of FT-IR absorption band in carbonyl region of thermally oxidized PP-TD2 sample.

At the end of the tests, the recovered test materials were submitted to fractionation by boiling solvent extraction in order to evaluate the propensity to the formation of lower molecular weight oxidized fractions, that were later submitted to a characterization by DSC and TGA analysis.

In the case of PS based films, no significant amount of extractable fraction (e.g. low molecular weight fraction) were obtained from the specimens withdrawn after 135 days of thermal aging. In accordance, no significant variation of the molecular weight and molecular weight distribution as induced by the thermal degradation was recorded by SEC.

PP-TD1 and PP-TD2 oxidized film specimens, characterized by CO*i* values of 0.50 and 1.012, respectively, were submitted after 35 days aging time, both to solvent extraction using boiling acetone. 93.2 mg of acetone

extract corresponding to 29.5 % by weight of the original specimen was recovered from PP-TD2 oxidized film. A slight lower amount (63.6 mg corresponding to 19.9 % by weight) of extractable fraction was instead recovered from PP-TD1 oxidized film. These results seems to suggest a positive correlation between the amount of acetone extractable fractions and CO*i* values. Similar results were obtained during the solvent extraction treatment of thermally aged LLDPE samples that was carried out by fractionating the oxidized samples with the following solvents: distilled water, acetone and dichloromethane (DCM), in that order. The results relevant to the thermally oxidized LLDPE film specimen fractionation are reported in Table 4.4, along with the CO*i* values of each fraction as determined by FT-IR. It is worth noting that the amounts of the extracted fractions are depending upon the type of solvent used in the extraction procedure.

Table 4.4. Solvent fractionation of thermally oxidized samples at 65°C in air ventilated oven.

Sample	**aging**	**Solvent**	**Extractable fraction[a)]**	**CO*i*[a)]**		
	(days)	(type	(%)	Starting specimen	Soluble fraction	Residue to extraction
LLDPE-TD1	118	Water	14.2	6.5	10.9	4.1
SD[b)]			*2.4*	*1.3*	*2.1*	*1.4*
LLDPE-TD1	118	Acetone	33.2	6.1	9.3	3.2
SD[b)]			*12.4*	*0.6*	*0.9*	*1.5*
LLDPE-TD1	118	DCM	32.3	5.4	9.3	3.5
SD[b)]			*5.3*	*2.2*	*2.2*	*1.9*
LLDPE-TD2	152	Water	4.5	4.2	5.7	2.3
SD[b)]			*1.1*	*0.6*	*1.9*	*0.2*
LLDPE-TD2	152	Acetone	22.9	3.4	9.0	3.0
SD[b)]			*6.2*	*0.3*	*1.0*	*0.9*
LLDPE-TD2	152	DCM	25.0	3.8	8.6	3.1
SD[b)]			*1.8*	*0.7*	*4.6*	*1.2*

a) Averaged over 5 replicates; b) Standard deviation

The CO*i* of all the solvent extracted fractions and relevant residues to extraction have been calculated after FT-IR characterization, thus recording higher values with respect to the corresponding CO*i* of film specimens in the case of the extractable fractions and lower values for the relevant residues to extraction (Table 4.4), thus demonstrating the formation of fairly high amount of heavily oxidized low molecular weight fractions. All the solvent fractions from PP and LLDPE samples were submitted to structural characterization with particular attention to molecular weight determination by GPC analysis. The recorded Mw and molecular weight distribution (ID) of the analyzed samples are reported in Tables 4.5 and 4.6. whereas the original GPC chromatograms relevant to LLDPE sample extracts are reported in Figures 4.16 and 4.17.

Table 4.5. Molecular weight (Mw) and molecular weight distribution (ID) of solvent extracted fractions from oxidized PP based films.

Sample	**Thermal aging**	**Solvent**	**Mw**	**ID**
	(days)	(type)	kDa	
PP-TD1	35	Acetone	3.4	3.66
PP-TD2	35	Acetone	1.1	2.82

Table 4.6. Molecular weight (Mw) and molecular weight distribution (ID) of solvent extracted fractions from oxidized LLDPE based films.

Sample	Thermal aging	Solvent	Mw	ID
	(days)	(type	(kD)	
LLDPE-TD1	118	Water	0.39	1.28
	118	Acetone	0.72	1.64
	118	DCM	0.74	1.58
LLDPE-TD2	152	Water	0.40	1.39
	152	Acetone	0.87	2.11
	152	DCM	1.23	2.31

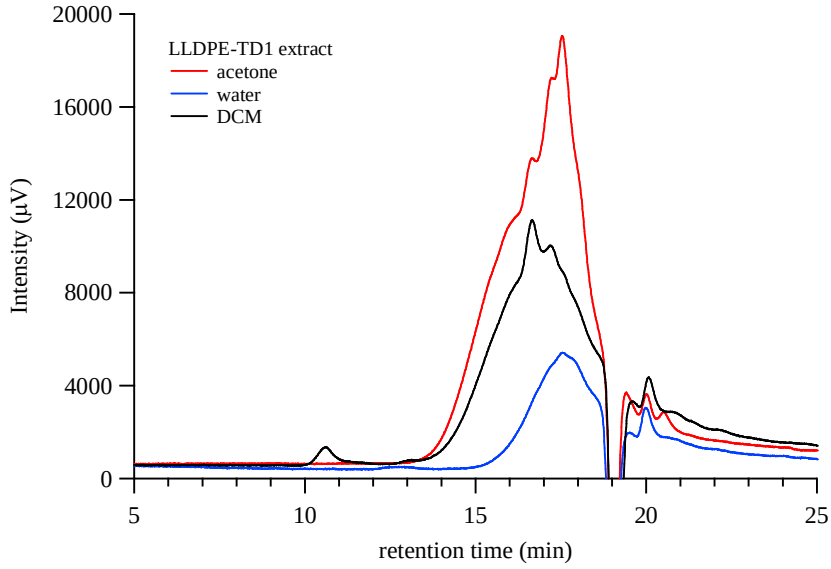


Figure 4.16. GPC chromatograms of solvent extracts of thermally oxidized LLDPE-TD1 sample.

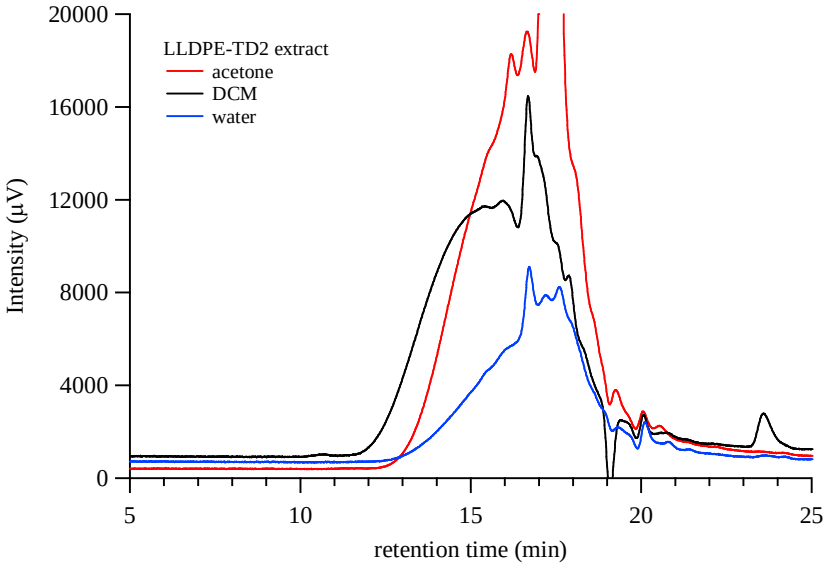


Figure 4.17. GPC chromatograms of solvent extracts of thermally oxidized LLDPE-TD2 sample.

The data recorded from GPC measurements performed on the solvent extracts from thermally aged LLDPE samples showed that all the types of extract were almost exclusively represented by low molecular weight fractions. It was also evident that the lowest molecular weight components are present in the water extracts of both samples, whereas fractions with similar molar mass dimensions were detected in the acetone and DCM extracts. Nevertheless, slight but significant differences were observed between the extracts obtained from the two LLDPE samples submitted to thermal oxidation. In particular it was evidenced that slight higher molecular weight fractions were extracted from LLDPE-TD2 sample, especially in the case of DCM extracts (Table 4.6).

FT-IR characterizations (Figure 4.18) provided some evidence of the higher level oxidation reached by the extractable fraction obtained from PP-TD1 sample, thus confirming the higher propensity of this sample to be oxidized under forced air conditions.

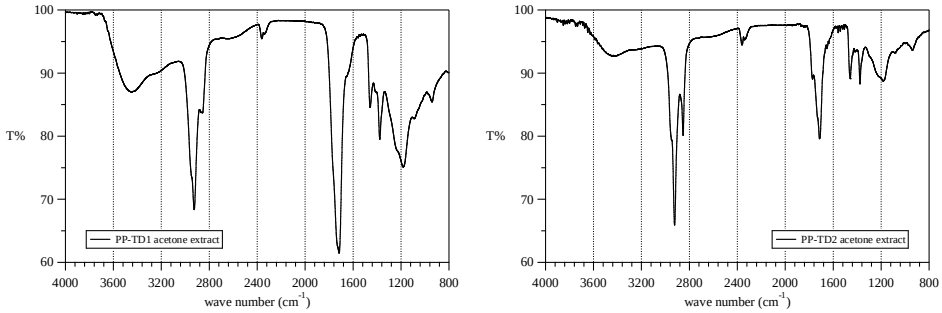


Figure 4.18. FT-IR spectra of PP-TD1 and PP-TD2 acetone extracts from film specimens submitted to thermal treatment in forced air oven at 65°C.

The fractionation of thermally oxidized samples with boiling solvents confirms the fragmentation of the polymer chains, whose extent is correlated to the degree of oxidation as determined by the carbonyl index (CO*i*). In addition, the GPC traces showed that the molecular weights of these fractions are fairly low (0.4-1.9kD) and compatible with their potential vulnerability by natural occurring microorganisms.

All the solvent fractions from thermally aged LLDPE samples were also characterized by FT-IR spectroscopy. The recorded FT-IR spectra are reported in Figures 4.19-4.21.

The FT-IR analysis showed that the solvent extract attainable from thermally aged LLDPE samples are characterized by the presence of oxidized carbon moieties, whose type and relevant amounts were depending upon the solvent used in the extraction procedure, and in a little extent, upon the kind of treated LLDPE sample. In particular, it was evident that both acetone and DCM extracts are characterized mostly by the presence of aliphatic ketones, (Figures 4.19, 4.20). The corresponding water extracts (Figure 4.21) being characterized by the presence of much more polar compounds, as evidenced by the appearance of strong absorption band in the hydroxyl regions (3300-3500 cm^{-1}) that expands 2500 cm^{-1} in agreement with the presence of carbonyl groups..

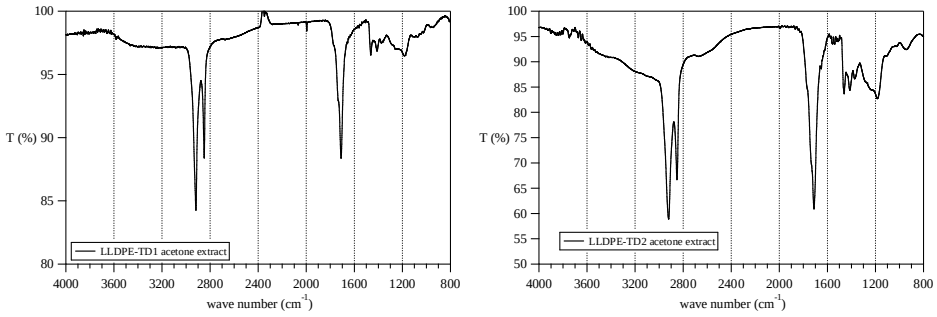


Figure 4.19. FT-IR spectra of acetone extracts from thermally oxidized LLDPE-TD1 and LLDPE-TD2 samples.

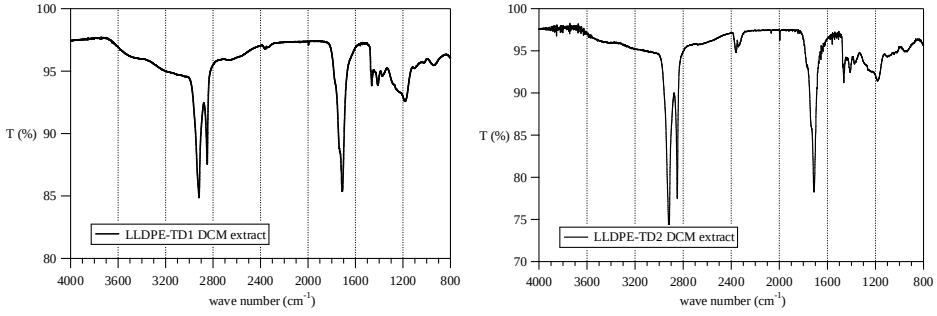


Figure 4.20. FT-IR spectra of dichloromethane (DCM) extracts from thermally oxidized LLDPE-TD1 and LLDPE-TD2 samples.

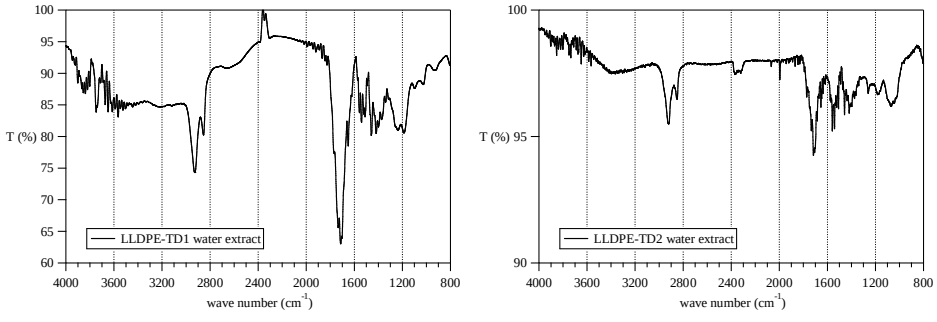


Figure 4.21. FT-IR spectra of distilled water extracts from thermally oxidized LLDPE-TD1 and LLDPE-TD2 samples.

The thermally oxidized LLDPE samples were also characterized by TGA measurements. In both cases it was found a marked decrease of the thermal stability of the treated samples after 118 and 152 days of thermal aging, respectively (Figure 4.22).

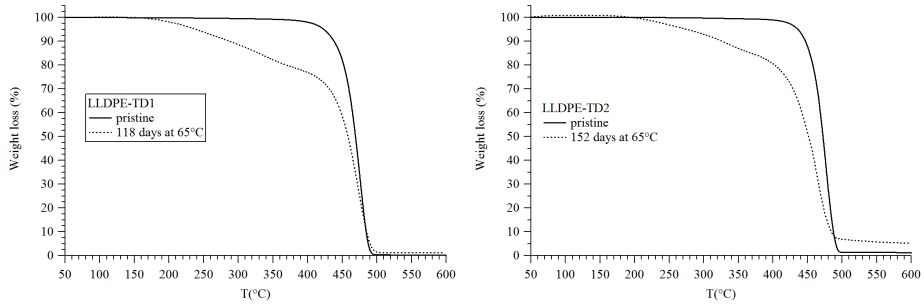


Figure 4.22. TGA traces of pristine and thermally oxidized LLDPE-TD1 and LLDPE-TD2 sample.

The starting degradation temperatures (T_{ON}) of the treated samples resulted to be approximately 200 °C lower than that recorded for the relevant pristine samples. Nevertheless, the progress of thermal aging did not seem to induce any further appreciable decrease of the thermal stability of both LLDPE-TD1 and LLDPE-TD2 samples (Table 4.7).

Table 4.7. Thermal properties of LLDPE-TD1 and LLDPE-TD2 samples submitted to thermal aging at 65°C in air ventilated oven, as recorded by TGA analysis.

Test Sample	Aging time	T_{ON} [a)]	Residue [b)]	CO*i*
	(days)	(°C)	(weight %)	
LLDPE-TD1	none	400.6	0.24	0.11
	118	200.8	1.19	4.71
	229	192.4	2.43	4.59
LLDPE-TD2	none	422.9	1.03	0.04
	152	206.0	3.12	3.52
	229	218.9	2.72	3.41

a) corresponding to 2% sample weight loss; b) recorded at 600°C

From the recorded data it was clearly evident that the analyzed samples underwent an extensive degradation of the polymer chains with the formation of low molecular weight – oxidized fractions. This observation was confirmed by DSC analysis of the aged LLDPE samples (Figure

4.23, Table 4.8). In particular a marked decrease of the melting temperatures of the thermally oxidized specimens was observed with respect to the corresponding untreated (pristine) specimen. Moreover, the aged specimens of both LLDPE-TD1 and LLDPE-TD2 were characterized by a single melting peak, whereas the original samples were characterized by two easily distinguishable, 110 and 120°C, melting temperatures, as recordable during the second heating in the DSC traces (Figures 4.23). These observations are in contrast with previous findings that report in the presence of an evident shoulder in the melting peak of degraded PE submitted to thermal and photo-oxidation treatments [186-190].

These results could be attributed to changes in crystallite sizes, molecular weight differences (due to chain breaking) and secondary re-crystallization. In accordance, the crystallinity degree of the analyzed LLDPE samples were found to increase during the first heating of the DSC analysis, thus maintaining similar values to that recorded in the case of pristine sample during the second heating of the DSC analysis (Table 4.8). These results, were therefore suggesting that the crystalline domains of the LDDPE samples were capable to reorganize as a consequence of the two heating steps carried out in the DSC measurements, thus evidencing a fairly low influence of the thermal aging on this behavior.

It can be then suggested that the oxidation of LLDPE samples does occur preferably in the amorphous phase in the semicrystalline materials, thus leading to an enrichment of the more structural regular macromolecules that are prone to crystallize.

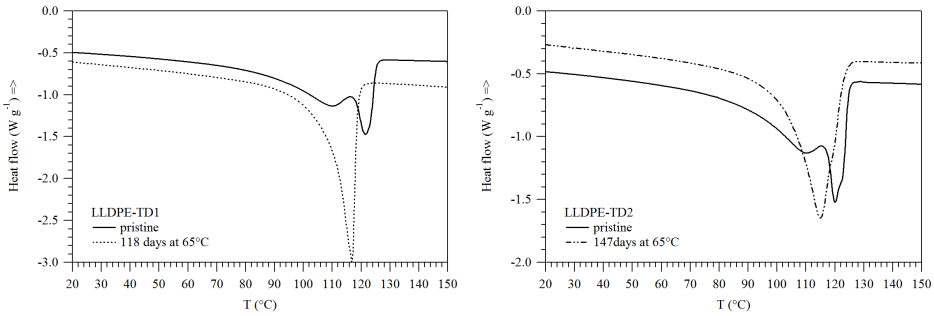


Figure 4.23. DSC traces (second heating) of pristine and thermally oxidized LLDPE-TD1 (a) and LLDPE-TD2 (b) samples.

Table 4.8. DSC parameters of LLDPE-TD1 and LLDPE-TD2 samples submitted to thermal aging at 65°C in air ventilated oven.

Test sample		**1st heating**		**2nd heating**	
	Aging time	Tm [a)]	Crystallinity	Tm [a)]	Crystallinity
	(days)	(°C)	(%)	(°C)	(%)
LLDPE-TD1	0	115.0, 121.8	42.7	110.9, 122.3	44.7
	118	117.4	59.1	116.8	47.4
LLDPE-TD2	0	121.7	41.9	110.0, 121.5	44.2
	152	118.0	51.2	115.0	47.5

a) melting peak

Thermally oxidized LLDPE-TD1 sample was also analyzed for the Oxidation Time Index (OIT) in a Mettler DSC instrument in comparison with the pristine sample in order to establish the potential behavior to further oxidation after the thermal degradation. The OIT characterization was carried out in accordance with the ASTM D6186-06 standard procedure. The test sample was heated for 300 s at 200°C under nitrogen atmosphere, after that the test was continued in high purity oxygen atmosphere, thus recording the relevant heat flow (Wg^{-1}) (Figure 4.24). The oxidation process started after 344 and 336 seconds in the case of

pristine and thermally oxidized LLDPE-TD1 sample, respectively. Different oxidation profiles were also observed, the thermally oxidized sample being oxidized at a lower rate (Figure 4.24). Nevertheless this analysis clearly showed that the thermally oxidized sample is susceptible to further oxidation, even though under very stressing condition (200°C temperature and pure oxygen atmosphere).

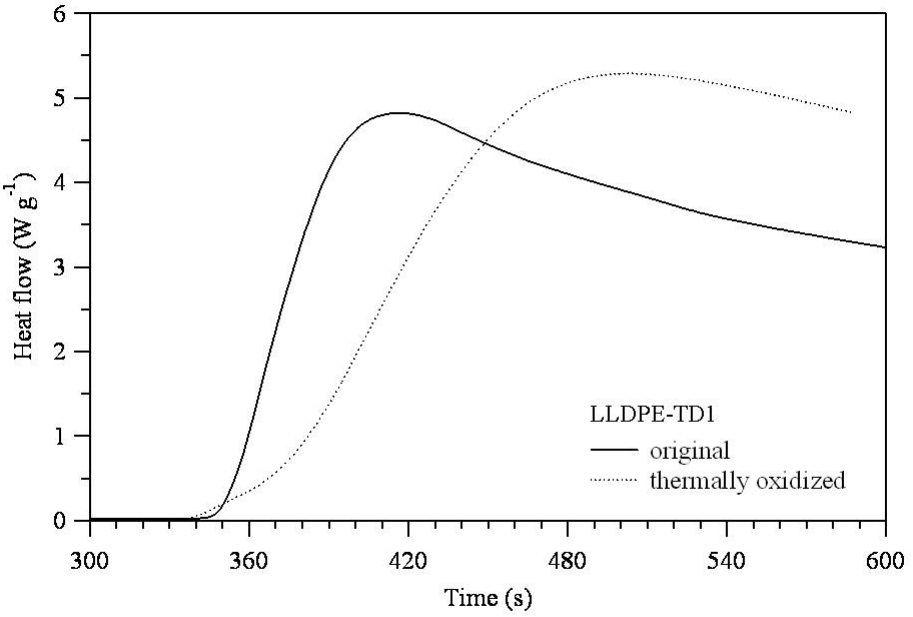
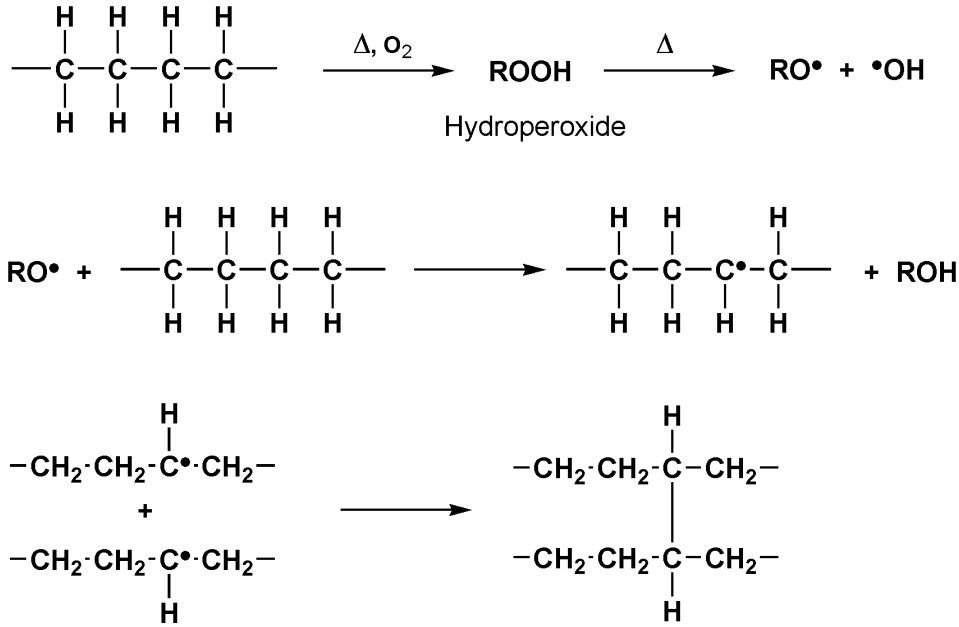


Figure 4.24. OIT index profiles of pristine and thermally oxidized LLDPE-TD1 sample.

Another important parameter to be check in order to establish the propensity to further oxidation and biodegradation of thermally aged polyolefins is the gel content. This structural feature is related to the degree of cross-linking as promoted by termination reaction in radical chain degradation mechanisms as recombination of two macroradicals. In accordance with Martins *et al*. [191] the crystallinity degree increase with increasing gel content. In these cases, the melting transition detected by DSC scan became broader proportionally to the PE cross-linking

increasing. It is well known that during processing and thermal aging, PE is subjected to different temperatures and shear stress allowing chemical reactions to occur. Degradation can be initiated by oxygen, shear, heat, catalyst, additives or any combination of these factors [192]. The gel content, or insoluble fraction, is produced in PE by recombination of macroradicals, thus leading to cross-linking. The thermal oxidation includes initiation, propagation, chain branching and termination steps [192, 193]. At the first degradation step, alkyl radicals are formed, but under oxygen deficient conditions not all alkyl radicals ($R^{\bullet}$) can be transformed into peroxide ($ROO^{\bullet}$) radicals. Depending on thermal conditions and the type of additive used, PE can undergo different radical reactions such as chain scission and chain branching, leading to cross-linking as previously described and illustrated in the Scheme 4.1. Cross-linking via an oxidation reaction is due to the recombination of alkyl radicals $R^{\bullet}$ with each other, with $RO^{\bullet}$ or $ROO^{\bullet}$ radicals [192].



Scheme 4.1. General mechanism of PE cross-linking.

Gel content determination was carried out in accordance with the ASTM D2765-01 standard procedure [194], by using *o*-xylene at boiling temperature as extracting solvent in a Kumagawa apparatus. The extraction procedure was carried out at reflux for 7 hours on both pristine and thermally degraded (229 days) LLDPE-TD1 sample, thus collecting 2.19 % and 4.27% insoluble residue (gel), respectively. These results, showing only a minor increase of the gel content in the thermally oxidized LLDPE-TD1 sample seem to suggest that fairly low cross-linking reactions are taking place during the thermal degradation process under the adopted test conditions, in keeping with the results collected by DSC analysis.

A degradation test was carried out, as aimed at establishing if the oxidation process of the LLDPE matrix once initiated after heat exposure may proceed at lower temperature (e.g. room temperature).

In accordance, LLDPE-TD1 and LLDPE-TD2 film specimens were previously maintained in static oven at 65°C and the CO*i* oxidation levels determined by FT-IR spectroscopy. At time intervals, in the correspondence of different CO*i* values, film specimens were withdrawn from the oven and kept at room temperature in the dark, while monitoring the progress of oxidation by FT-IR spectroscopy.

In particular, LLDPE-TD1 specimens were withdrawn from the oven after 28, 38 and 53 days aging time at 65°C, thus having 0.12, 1.57 and 2.74 CO*i* values, respectively. LLDPE-TD2 film specimens were withdrawn after 49 and 57 days, thus recording for each specimen 1.18 and 2.34 CO*i* values, respectively (Table 4.9).

The CO*i* profiles recordable at room temperature of the test samples previously treated at 65°C in static oven for different times are reported in Figures 4.25 and 4.26.

Table 4.9. Carbonyl index (CO*i*) value of partially oxidized LLDPE film specimens before to be exposed at room temperature condition.

Test Sample	Starting oxidation level	
	Exposure time (days)	CO*i*
LLDPE-TD1	28	0.12
	38	1.57
	53	2.74
LLDPE-TD2	49	1.18
	57	2.34

After a fairly long time at room temperature (120 days), the oxidation levels of the pre-aged films were not showing a notably increase with the sole exception of LLDPE-TD1 specimen having 2.74 CO*i* at the beginning of the test (Table 4.9). In all the other cases only a very small increase (less than 0.1%) of the CO*i* values could be detected after approximately 10 months maintenance in air at room temperature.

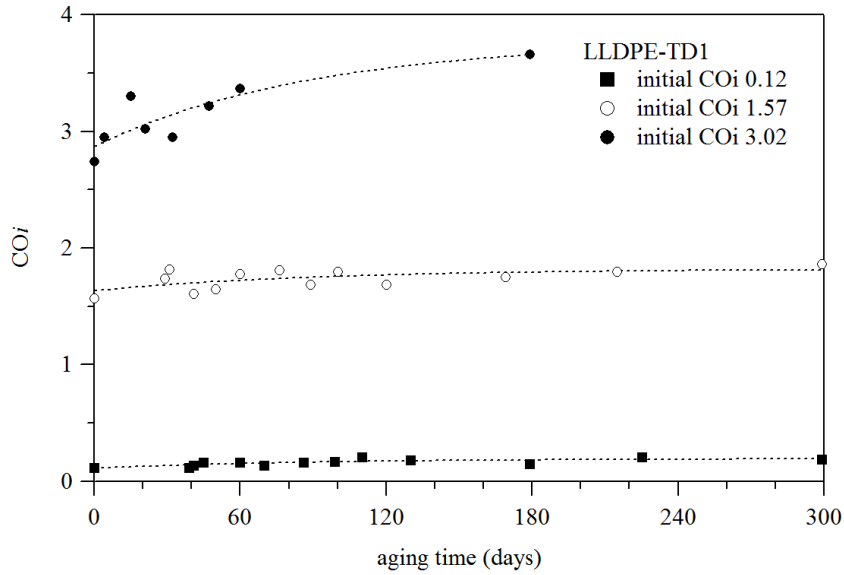


Figure 4.25. CO*i* profiles at room temperature of pre-oxidized at different level of oxidation LLDPE-TD1 test specimens.

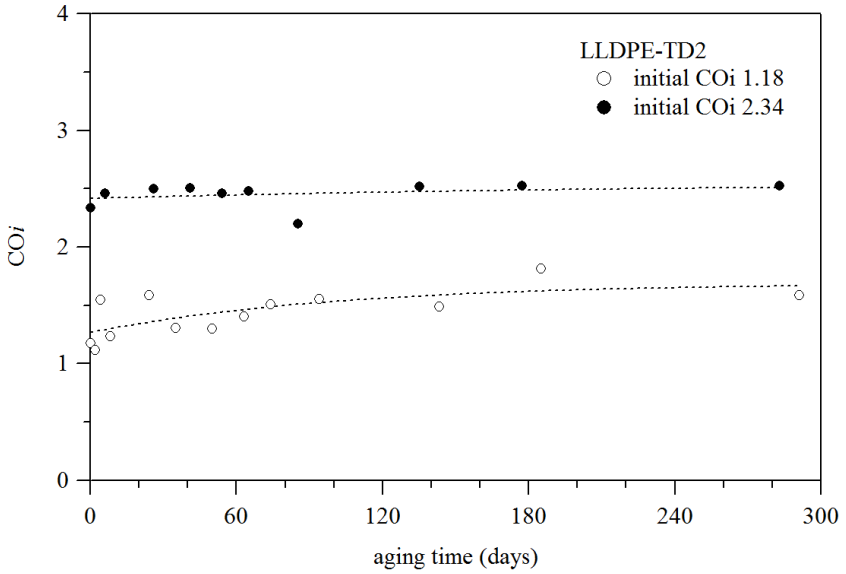


Figure 4.26. CO*i* profiles at room temperature of pre-oxidized at different level of oxidation LLDPE-TD2 test specimens.

Within the limits of the time frame recorded in holding the pre-oxidized samples at room temperature, it has been observed that the oxidation process may proceed only when the thermal pre-oxidation level reached a fairly high degree of oxidation. On the contrary it seems that the oxidation behavior of the analyzed sample at room temperature may continue only at extremely low rate.

4.1.3. *Photodegradation tests.*

Polyolefins (PE, PP and PS) containing different pro-oxidant catalyst or polymeric additives (from synthetic or natural origin) and relevant not additivated control film samples were also submitted to outdoor exposure [177] with the aim to study their photo-oxidative degradation behavior and to compare the obtained data with those recorded during the thermal degradation tests. These investigations were carried out by taking into account that most of the analyzed samples could be utilized for agricultural application purposes and specifically as mulching films in the

case of LLDPE based samples. In addition, by considering the UV absorption properties of PS aromatic ring in the main chain, it was considered to be useful to compare the oxidative degradation propensity under different test conditions as represented by heat exposure in the dark and sun light irradiation. During the exposure time both maximum and average temperature were daily recorded (Figure 4.27). The progress of oxidation of the analyzed samples was firstly investigated by means of FT-IR spectroscopy and relevant calculation of Carbonyl index (CO*i*). After that the characterization of the structural properties of the exposed samples was performed by thermal analysis (TGA and DSC), fractionation by solvent extraction and relevant spectroscopic and molecular weight analysis in accordance with the experimental protocol used for the characterization of thermally degraded samples.

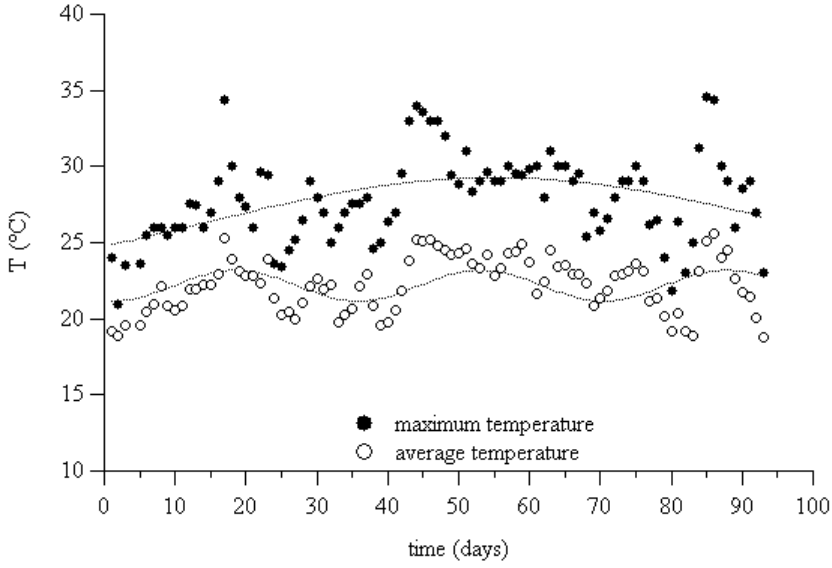
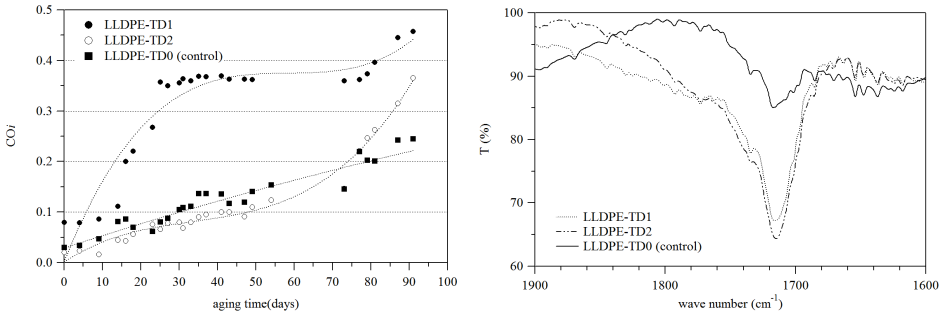


Figure 4.27. Maximum and average daily temperatures recorded during the outdoor exposure of the LLDPE samples.

Different oxidation behaviors were detected between the two pro-oxidant additive loaded LLDPE film specimens. In the case of LLDPE-TD1 sample, in fact, a plateau of the CO*i* profile was approached after three

weeks of outdoor exposure, after that a new exponential step was observed, whereas the CO*i* profile of LLDPE-TD2 film was maintaining a positive trend during the aging time, thus exhibiting an acceleration of the oxidation process during the last ten days of outdoor exposure (Figure 4.28). A comparable level of oxidation was detected in the case of LLDPE-TD0 control film sample with respect to LLDPE-TD2 sample at least in the first 75 days aging.

In any case, the levels of oxidation recorded after 93 days outdoor exposure of the pro-degradant loaded LLDPE film samples were fairly below those reached in the same time frame at 55 and 65°C even in oven under static conditions. It is also to be observed that comparable CO*i* values (0.4) were reached by LLDPE-TD1 sample only after 90 days of thermal aging at 45°C, whereas under outdoor exposure it takes only 20 days aging at significantly lower ambient temperature (Figure 4.28). These results were therefore suggesting that this LLDPE film does appear to be more susceptible to both photo- and thermal oxidation with respect to LLDPE-TD2 sample.



(a) **(b)**

Figure 4.28. Outdoor exposed LLDPE film samples a) CO*i* profile b) comparison spectra of carbonyl absorption regions after 93days exposed.

A comparison of the carbonyl absorption region recorded in the FT-IR spectra of the analyzed samples after 93 days out door exposure is reported in Figure 4.28 b.

After 93 days outdoor exposure, an appreciable formation of carbonyl absorption groups was observed in the test samples containing the pro-oxidant additives, and the shape of the carbonyl band was almost identical (Figure 4.28b). A lower intensity of carbonyl absorption band was instead observed in the case of LLDPE-TD0 control film sample within the same aging time (Figure 4.28 b).

4.1.3.1. *Poly(styrene) samples*

The outdoor exposure test according to the ASTM D5272 Standard Practice has been also carried out to investigate the photo-oxidation behaviour of two series of PS-based cast films [PS-TD1 pro-degradant loaded, and PS-TD0 pro-degradant-free (control)]. During the test, both FT-IR spectroscopy and GPC analysis have been used in order to monitor the progress of the degradation behaviour of the tested samples.

FT-IR characterization of the analysed samples revealed the formation of oxidized functional group (e.g. carbonyl groups at 1690-1800 cm^{-1} spectral region) in the polymer chains only after 10 months exposure (Figure 4.29 – 4.30).

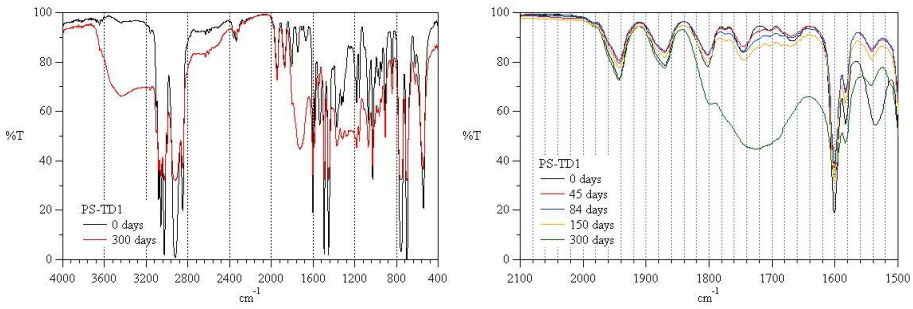


Figure 4.29. FT-IR whole spectrum (a) and limited to carbonyl and aromatic ring spectral regions (b) of PS-TD1 sample at various time of outdoor exposure.

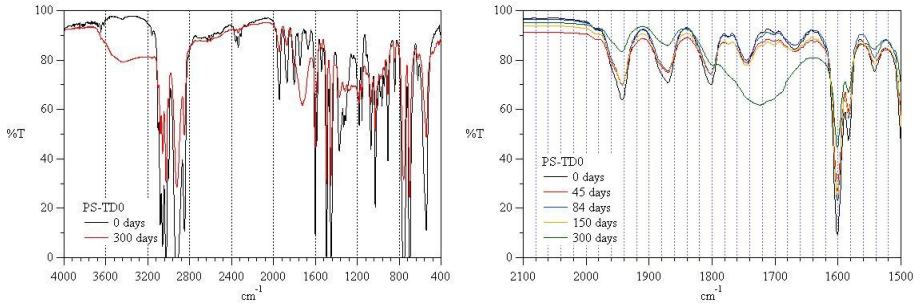


Figure 4.30. FT-IR whole spectrum (a) and limited to carbonyl and aromatic ring spectral regions (b) of PS-TD0 (control) sample at various time of outdoor exposure.

At the end of the test (10 months outdoor exposure), the intensity of the absorption peaks in the carbonyl region was significantly high particularly in the case of PS-TD1 samples. A similar behaviour was also recorded in the case of PS-TD0 control sample. Moreover, in all cases a marked discoloration, accompanied by the fragmentation of the exposed films, was observed.

It is also to worth to notice the broadening of the carbonyl absorption band, which might be attributed to presence of highly conjugated chromophores of both aliphatic and aromatic type.

The analyzed PS-based samples were also characterized by GPC analysis carried out at time intervals of the outdoor exposure test. GPC chromatograms, as recorded both by UV (254 nm) and Refractive Index (RI) detectors are reported in Figures 4.31 and 4.32.

GPC determinations, evidenced that the light irradiation seems to be much more efficient in initiating and stimulating the progress of the degradation of the PS matrix with respect to the thermal treatment, thus revealing a marked variation of the molecular weight and molecular weight distribution (Table 4.10) which was accompanied by the formation of significant amount of low molecular weight components in all the tested samples. In addition the recorded profiles of variation of molecular

weights in the analyzed samples does not seem to be in keeping with a degradation mechanism based on random chain scission of the polymer matrix.

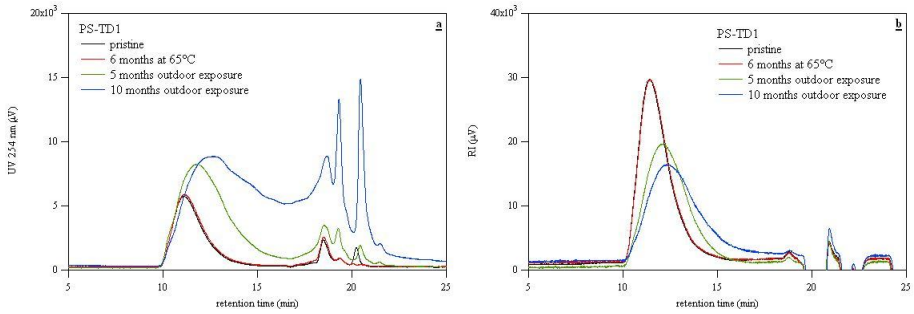


Figure 4.31. GPC chromatograms, UV detector (a), RI detector (b) of pristine and outdoor exposed PS-TD1 pro-degradant loaded sample.

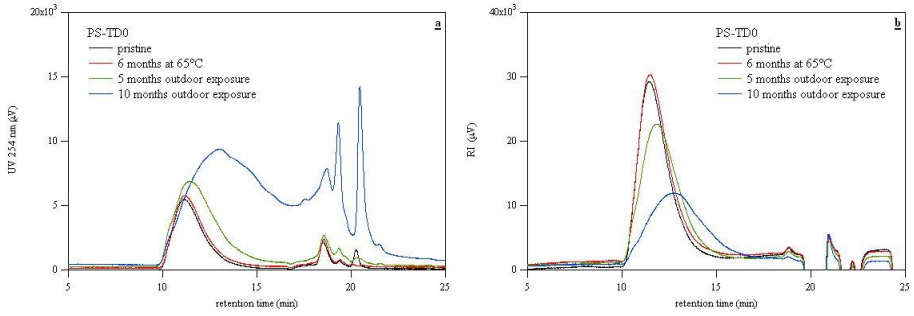


Figure 4.32. GPC chromatograms, UV detector (a), RI detector (b) of pristine and outdoor exposed PS-TD0 pro-degradant-free control sample.

The results collected during the present test, even though requiring further confirmation, are suggesting that the two PS film samples are exhibiting different degradation behaviours depending upon the abiotic test conditions. In particular, it has been ascertained that PS cast films when exposed at the sun light radiation showed a marked decrease of the Mw with a significant production of low mass compounds. On the contrary, the Mw of PS blown films aged in oven under fairly stressing conditions

(65°C) did not decrease significantly, even tough the partial oxidation of the polymer chains formation was suggested by the presence of carbonyl groups in the relevant FT-IR spectra.

The results herewith collected are therefore suggesting that the abiotic degradation process of PS based samples is different to the random chain scission usually recorded in the case of oxo-biodegradable poly(ethylene) and poly(propylene) samples.

It also worth nothing that in the GPC traces recorded on samples at the same concentration (0.3% w/v), one may observe a strong increase of the UV absorbance in the sample exposed outdoor with respect to the sample thermally aged in oven at 65°C (Table 4.10).

This behaviour is indicative of the formation of conjugate structures in the case of the PS samples aged outdoor with exposition to sun light.

Table 4.10. Molecular weight analysis (MW) of outdoor exposed PS samples.

Test Sample	**Aging Time**	**Mw**	**ID**
	(months)	kDa	
PS-TD1	0	228.1	1.85
PS-TD1	5	151.2	2.55
PS-TD1	10	119.6	3.64
PS-TD1	Thermal aging[a)]	222.6	1.89
PS-TD0	0	222.3	1.82
PS-TD0	5	180.7	2.32
PS-TD0	10	108.9	4.34
PS-TD0	Thermal aging [a)]	214.4	1.90

a) 6 months at 65°C in air ventilated oven.

4.1.3.2. *Structural characterization of poly(ethylene) samples*

In order to evaluate the propensity of the LLDPE films, aged under different test conditions (outdoor exposure), to be fragmented into low molecular weight oxidized fractions, a solvent extraction procedure was carried out by using the following series of solvents: distilled water, acetone and dichloromethane (DCM), in that order.

The results relevant to the thermally oxidized LLDPE film fractionations are reported in Table 4.11, along with the CO*i* values of each fraction. It is worth noting that the amounts of the extracted fractions are depending upon the type of solvent used in the extraction procedure.

Table 4.11. Solvent fractionation of LLDPE samples after 93 days outdoor exposure.

Sample	**Solvent**	**Extractable fraction[a)]**	**CO*i* [a)]**		
	(type)	(%)	Starting specimen	Soluble fraction	Residue to extraction
LLDPE-TD1	Water	0.4	0.46	1.15	0.40
LLDPE-TD1	Acetone	1.8	0.46	0.97	0.43
LLDPE-TD1	DCM	6.9	0.46	0.73	0.41
LLDPE-TD2	Water	0.2	0.37	0.89	0.30
LLDPE-TD2	Acetone	0.4	0.37	1.27	0.30
LLDPE-TD2	DCM	0.8	0.37	1.36	0.34

a) Averaged over 5 replicates

The CO*i* of all the solvent extracted fractions and relevant residues to extraction have been calculated after FT-IR characterization, thus recording higher values with respect to the corresponding CO*i* of film specimens in the case of the extractable fractions and lower values for the relevant residues to extraction (Table 4.11), thus demonstrating the

formation of fairly high amount of heavily oxidized low molecular weight fractions.

All the solvent extracted fractions were submitted to structural characterization with particular attention to molecular weight determination by GPC analysis. The original GPC chromatograms are reported in Figure 4.33, whereas recorded Mw and molecular weight distribution (ID) of the analyzed samples are reported in Table 4.12.

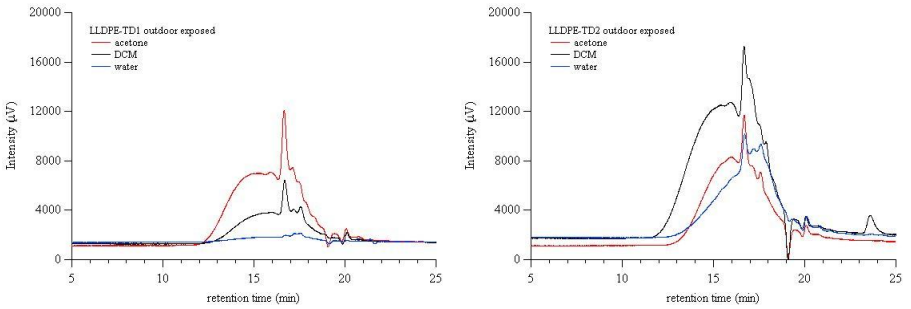


Figure 4.33. GPC chromatograms of solvent extracted fractions from outdoor exposed LLDPE-TD1 and LLDPE TD2 specimens.

The data recorded from GPC measurements performed on the solvent extracts from abiotically aged samples were showing that all the types of extract contained low molecular weight oxidized fractions. It was also evident that the lowest molecular weight compounds are present in the water extracts of both samples, whereas fractions with similar molar mass dimensions were detected in the acetone and DCM extracts. Nevertheless, slight but significant differences were observed between the extracts obtained form the two LLDPE samples submitted to both thermal oxidation and outdoor exposure. In particular it was evidenced that slight higher molecular weight fractions were extracted from LLDPE-TD2 sample, especially when DCM was used as extracting solvent.

Table 4.12. Molecular weight (Mw) and molecular weight distribution (ID) of solvent extracted fractions from oxidized LLDPE films.

Sample	Outdoor exposure	Solvent	Extractable fraction	Mw	ID
	(days)	(type)	(%)	(kD)	
LLDPE-TD1	93	Water	0.4	1.55	3.02
LLDPE-TD1	93	Acetone	1.8	1.82	2.69
LLDPE-TD1	93	DCM	6.9	1.39	2.47
LLDPE-TD2	93	Water	0.2	0.90	2.27
LLDPE-TD2	93	Acetone	0.4	1.18	2.14
LLDPE-TD2	93	DCM	0.8	1.86	2.89

All the solvent fractions were also characterized by FT-IR spectroscopy. The recorded FT-IR spectra are reported in Figures 4.34 – 4.36.

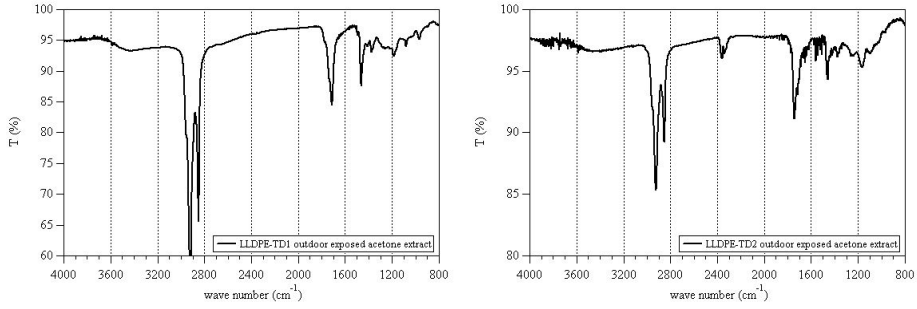


Figure 4.34. FT-IR spectra of acetone extracts from outdoor exposed LLDPE-TD1 and LLDPE-TD2 samples.

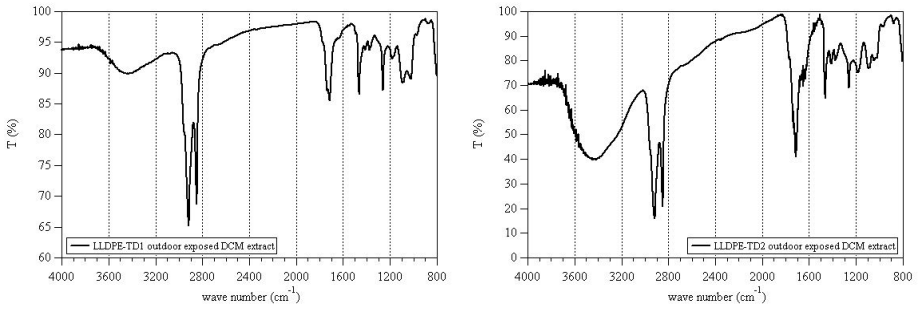


Figure 4.35. FT-IR spectra of dichloromethane (DCM) extracts from outdoor exposed LLDPE-TD1 and LLDPE-TD2 samples.

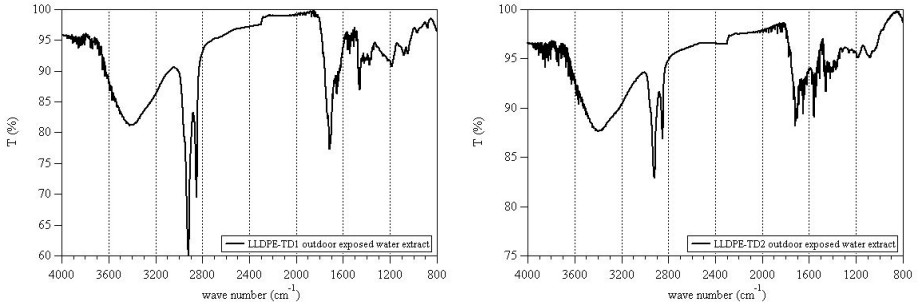


Figure 4.36. FT-IR spectra of distilled water extracts from outdoor exposed LLDPE-TD1 and LLDPE-TD2 samples.

The FT-IR analysis showed that the solvent extracts attainable from abiotically aged LLDPE samples are characterized by the presence of oxidized carbon moieties, whose type and relevant amounts were depending upon the solvent used, and in a little extent, upon the kind of treated LLDPE sample.

In the first case it was evident that both acetone and DCM extracts are characterized mostly by the presence of aliphatic ketones (Figures 4.34, 4.35). The corresponding water extracts (Figure 4.36) being characterized by the presence of much more polar compounds, as evidenced by the appearance of strong absorption band in the hydroxyl regions (3300-3500 cm^{-1}). The relative intensity of OH stretching bands was also higher in the solvent extracts obtained from outdoor exposed samples, especially in the case of LLDPE-TD2 specimen, with respect to the fractions extracted

from heavily oxidized thermally aged specimens (see Figure 4.21 for comparison). It can be therefore suggested that slightly different mechanisms in the oxidation of the polymer chains is taking place as a consequence of the prevailing abiotic degradation factors (heat or light irradiation), as well by the kind of pro-oxidant added to the LLDPE matrix.

At the end of the outdoor exposure test LLDPE-TD1 and LLDPE-TD2 aged samples were characterized by both TGA and DSC analysis. The thermal stability (TGA in nitrogen atmosphere) of the analyzed samples was shown to be reduced as consequence of the outdoor exposure, thus recording in both cases a significant drop of the onset temperatures (T_{ON}) corresponding to 2 % weight loss in the TGA profiles, with respect to the corresponding temperature values observed in the case of pristine specimens (Figure 4.37; Table 4.13). On the other hand, only minor changes were recorded in the case of the melting temperature and crystallinity degree, as determined by DSC analysis in comparison with the pristine specimen (Figure 4.38; Table 4.14).

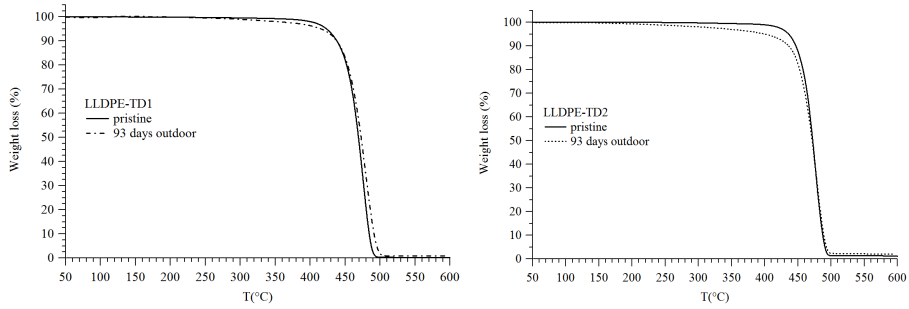


Figure 4.37. TGA traces of pristine and outdoor exposed LLDPE-TD1and LLDPE-TD2 specimens.

Table 4.13. Thermal properties of LLDPE-TD1 and LLDPE-TD2 samples submitted to the outdoor exposure, as recorded by TGA analysis.

Test Sample	Aging time	T_{ON} [a)]	Residue [b)]	CO*i*
	(days)	(°C)	(weight %)	
LLDPE-TD1	none	400.6	0.24	0.11
	93	353.5	0.88	0.46
LLDPE-TD2	none	422.9	1.03	0.04
	93	304.2	1.97	0.29

a) 2% weight loss; b) at 600°C

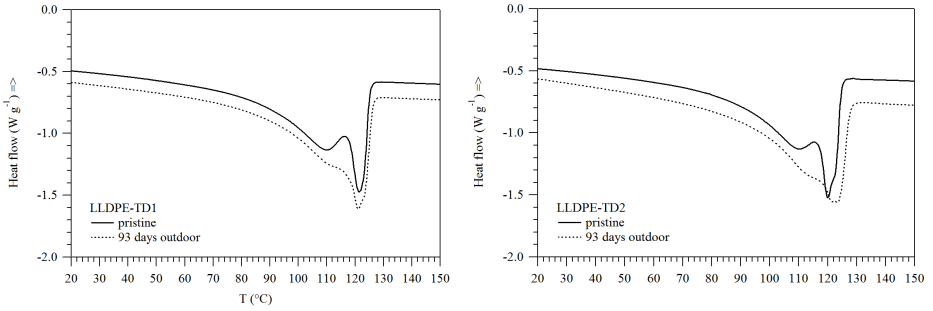


Figure 4.38. DSC traces (second heating) of pristine and outdoor exposed (a) LLDPE-TD1 and (b) LLDPE-TD2.

Table 4.14. DSC parameters of LLDPE-TD1 and LLDPE-TD2 samples submitted to outdoor exposure.

Test sample	Aging time	1st heating		2nd heating	
		Tm [a)]	Cristallinity	Tm [a)]	Cristallinity
	(days)	(°C)	(%)	(°C)	(%)
LLDPE-TD1	0	115.0, 121.8	42.7	110.9, 122.3	44.7
	93	116.0, 121.8	48.5	121.1	46.3
LLDPE-TD2	0	121.7	41.9	110.0, 121.5	44.2
	93	117.7, 122.0	52.4	121.5	47.5

a) melting peak

The propensity to oxidation of the LLDPE samples containing the pro-oxidant additives even under condition experiencing field scale situation was ascertained.

The extent of oxidation reached after the outdoor exposure during the summer season was lower than that recorded in thermal degradation tests carried out in oven for the same LLDPE samples. This behavior can be attributed in a first instance to the ambient temperature monitored during the outdoor test, which were almost during the test period below 35°C. It can be therefore suggested that the combination of UV radiation and temperature was capable to promote the initiation of the oxidative degradation of the tested LLDPE samples containing the pro-oxidant additives.

4.1.3.3. *Thermal characterization of poly(styrene) samples*

PS samples outdoor exposed up to 10 months aging time were also characterized by thermal analysis (TGA and DSC) in comparison with the pristine samples.

Analogously to the case of LLDPE samples the thermal stability of the outdoor exposed PS samples was reduced during the aging test. In this case however, a significant drop of the onset temperatures (T_{ON}) corresponding to 2 % weight loss in the TGA profiles was recorded under nitrogen atmosphere in both pro-degradant additivated PS-TD1 and PS-TD0 control sample, with respect to the corresponding temperature values observed in the case of pristine specimens (Figure 4.39 Table 4.15). In any case, by considering the slope of the TGA curves a slight higher thermal instability, as attributable to the outdoor exposure can be envisaged for the pro-degradant additivated sample. By contrast, the DSC analysis did not revealed any substantial difference in the melting temperature between outdoor exposed and pristine samples, whereas a

slight decrease of the glass transition temperatures, during the second heating DSC traces was observed independent of the presence of the pro-degradant additive (Table 4.15).

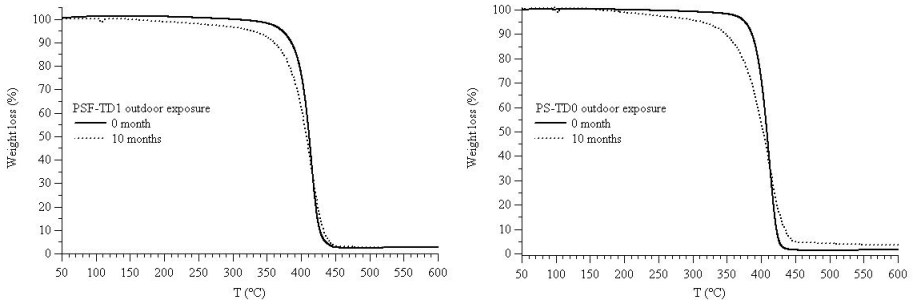


Figure 4.39. TGA traces of PS-TD1 and PS-TD0 samples before and after 10 months outdoor exposure.

Table 4.15. Thermal properties of PS-TD1 and PS-TD0 (control) samples submitted to 10 months outdoor exposure, as recorded by TGA analysis.

Test Sample	Aging time	T_{ON} [a)]	Residue [b)]	Tg [c)]
	(days)	(°C)	(weight %)	(°C)
PS-TD1	none	355.0	2.87	104.3
	300	294.7	2.67	100.8
PS-TD0	none	357.6	1.50	104.4
	300	234.9	3.64	97.6

a) 2% weight loss; b) at 600°C; c) Glass transition temperature.

4.1.3.4. *Thermal aging of outdoor exposed LLDPE samples*

In order to establish if the LLDPE samples once exposed to the direct sun light might be further oxidized by heat stress likewise what is thought to be occurring in real field conditions, LLDPE films with and without pro-oxidant additives previously exposed for 93 days in sunlight were subjected to further thermal aging in oven at 45, 55 and 65°C for a period

of 200 days. The oxidation process was monitored by measuring the increase in the CO*i* of the respective sample with time.

A rapid increase was observed in the CO*i* for both films (LLDPE-TD1 and LLDPE-TD2) that were pre-exposed to sunlight irradiations for 93 days and subsequently aged in oven at various temperatures (Figure 4.40). Interestingly, the increase in oxidation was immediate requiring little or no time for films to acclimatize. Also, the rates and extents of oxidation were proportional to the aging temperatures. A five to six-fold increase in oxidation was observed in films aged at 65^{o}C within less than 20 days (Figure 4.40). Thereafter, a plateau was reached and the rate of oxidation did not change very much. Films aged at 45 and 55^{o}C showed a similar trend, but the rates and extents of oxidation were considerably lower. An immediate and rapid increase in the CO*i* observed in LLDPE films with pro-oxidant suggest that the sunlight-induced oxidation followed by a thermal aging at moderate temperatures had a positive synergistic effect on the overall oxidation of these films that contributed towards the deterioration of its carbon backbone.

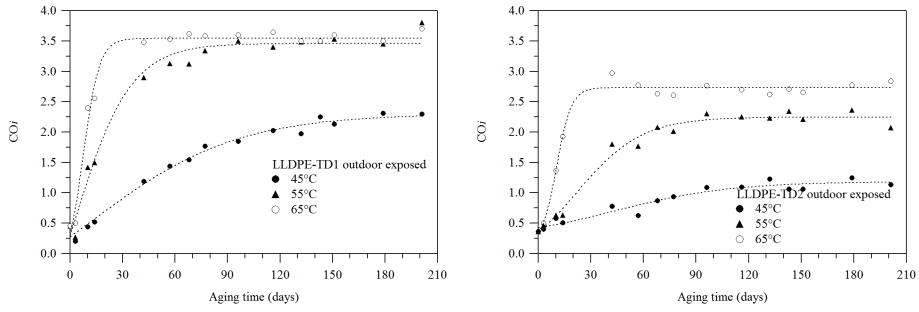


Figure 4.40. CO*i* profiles of LLDPE-TD1 and LLDPE-TD2 film specimens submitted to thermal degradation after 93 days outdoor exposure.

These results are in conformity with the work of other investigators. In this regard, it has been suggested that the overall "oxo-biodegradation

process" of full carbon backbone polymers is a two-stage process [11] that involves an abiotic oxidative degradation of polymer chains followed by the microbial assimilation of the oxidation products, where the oxidation step is a rate-determining step [57]. A previous study has also shown positive changes in the activation energy and susceptibility of LLDPE to biodegrade after photo-oxidation [195].

The results so far collected during the abiotic ageing of the analyzed PE, PP, and PS samples were suggesting the following remarks:

1) The propensity to thermal oxidation is significantly different in the two types of tested LLDPE-TD samples. LLDPE-TD1 sample showed a faster thermal oxidation with respect to LLDPE-TD2, and reacheed higher oxidation levels at the end of the test within the range of the adopted degradation temperatures. The degradation products (e.g. ketone, carboxylic acids and esters) and their relevant amount in thermally oxidized samples seem to be affected by the test temperature, with a significant increase of the amount of carboxylic and ester groups at higher temperature with respect to ketone groups.
2) The thermal oxidation profiles of LLDPE samples (CO*i* profiles) recorded at different temperatures reached a stationary phase without showing considerable further increase even after very prolonged aging time (300 days). It does appears, therefore, that under the adopted conditions the level of oxidation of the analyzed samples cannot be improved by the exposure time at least within the extended time frame.
3) The propensity to thermal oxidation of the analyzed PP samples showed, was depending upon the TDPA additives used in the PP formulations. In particular, TD2 additive appeared to be more effective in inducing oxidation in the PP matrix as revealed by the short induction times observed under different temperatures.

4) In the mean time, the fairly low propensity to thermal degradation of PS based film samples was established. The overall extent of PS polymer matrix oxidation was markedly lower despite the presence of easily oxidizable tertiary carbon atoms in 1-3 position in the main chain such as in the case of PP macromolecules. In addition, no significant variation either MW or ID even after prolonged thermal treatment (274 days) under forced at 65°C. The recorded data on thermal degradation of PS samples were suggesting that in this case the random chain scission is not involved.
5) The thermal degradation test carried out on the LDPE/alkali lignin/pro-oxidant (LDPE/AL/P) showed high propensity to thermal oxidation, whose rate and extent did not appeared to be much affected by the content. Moreover, the presence of lignin which is recognised to have antioxidant properties due to phenolic groups did not appear to affect the thermal oxidation.
6) The higher propensity to thermal oxidation of LLDPE-TD1 previously observed in static oven condition has been confirmed also during degradation in air ventilated oven.
7) The oxygen partial pressure is stimulating the oxidation processes of the analyzed samples, whose level of oxidation resulted significantly higher with respect to those reached for the same sample in the same aging time and temperature under static conditions. It is worth noting that the adopted conditions in term of oxygen diffusion can be considered as much more comparable with the environmental conditions the samples are eventually experiencing with respect to the static oven conditions.
8) The determination of the gel content in the thermally oxidized sample seems to suggest that fairly low cross-linking reactions are tough to occur during the thermal degradation process.

9) The fractionation of thermally oxidized samples with boiling solvents confirms the fragmentation of the polymer chains, whose extent is correlated to the degree of oxidation as determined by carbonyl index (CO*i*). In addition, the GPC determinations showed that the molecular weights of these fractions are fairly low (0.4-1.9kD) and compatible with their potential vulnerability by natural occurring microorganisms.
10) Within the limit of the exposure time adopted in the present thermal degradation test, it can be however suggested that in the case of PS based samples, the presence of pro-oxidant additives, at the adopted concentration, does not seems to promote a thermal oxidation process with rate and extent higher than those recorded in the case of a control sample without pro-oxidants. In addition, no significant amounts of extractable fraction (low molecular fraction) were obtained after thermal aging treatment.
11) The extent of LLDPE oxidation reached after the outdoor exposure during the summer season was lower than that recorded in thermal degradation tests carried out in oven for the same LLDPE samples. This behaviour can be attributed in a first instance to the ambient temperatures monitored during the test, that were almost below 35°C. It can be therefore suggested that the combination of UV radiation and temperature was capable to promote the initiation of the oxidative degradation of the tested LLDPE samples containing the pro-oxidant additives.
12) The thermal oxidation behavior of test samples previously submitted to outdoor exposure appeared to be different from that exhibited by the pristine samples submitted only to thermal degradation. The absence of induction phases in the oxidation processes of the analyzed samples was evident during this test. These observations

may suggest that the initiation of the oxidation process, as promoted by the light irradiation, positively affect the rate of oxidation when the samples are submitted to thermal degradation.

13) The results obtained outdoor exposed PS based samples oxidation behaviour are therefore suggesting that the abiotic degradation process of PS based samples is different to the random chain scission usually recorded in the case of oxo-biodegradable poly(ethylene) and poly(propylene) samples. They showed a marked discoloration, accompanied by the films fragmentation. It has been also ascertained that PS based films when exposed at the sun light radiation showed a marked decrease of the Mw with a significant production of low mass compounds. It also worth nothing by the GPC analysis, when carried on samples at the same concentration (0,3% w/v), that a strong increase of the UV absorbance in the sample exposed outdoor with respect to the sample thermally aged in oven at 65°C does occur. This behaviour is indicative of the formation of conjugate structures in the case of the PS samples aged outdoor.

4.2. Biotic Degradation (Biodegradation) of Polyolefins (PE, PP and PS) Samples Recovered from Abiotic Degradation Tests.

Further to the evaluation of the abiotic oxidation of "degradable" polyolefins, an other step to be investigated in order to envisage the ultimate environmental fate of these materials is the estimation of the extent of biodegradation under different conditions. The requirement of two steps, abiotic and biotic, in the degradation mechanism of oxo-biodegradable plastic materials has recently led to the preparation and approval of ASTM D6954-04 "*Standard guide for exposing and testing plastics that degrade in the environment by a combination of oxidation and biodegradation*" [181]. This standard provides a framework to assess and compare the degree of oxidation and degradation which can be obtained under controlled thermal and photo-oxidation tests as well as the degree of biodegradation and ecological impacts in defined environments after abiotic degradation. Evaluation scheme in ASTM D6954-04 are divided into three tiers relevant to:

i) Accelerated aging in standard tests for both thermal- and photo-oxidations and determination of the degree of abiotic degradation (*Tier 1*).

ii) Measuring biodegradation (*Tier 2*).

iii) Assessing the ecological impact after these processes (*Tier 3*).

Test materials resulting from the abiotic oxidation attack are therefore exposed to appropriate disposal environments (soil, landfill, compost) in standard respirometric (biometric) tests in order to assess the rate and degree of biodegradation (*Tier 2*). Finally, any residues of the materials under test, deriving from both the abiotic oxidation stage and from the biodegradation tests must be submitted to ecotoxicity tests to demonstrate their ultimate environmental compatibility (*Tier 3*). In this connection, all the test materials previously submitted to controlled abiotic degradation

tests, and relevant low molecular weight fraction recovered by boiling solvent extraction were exposed to microbial populations pertaining different appropriate disposal or use environments (soil, water stream) under standard respirometric (biometric) test methods in order to assess the rate and degree of biodegradation .

4.2.1. *Biodegradation tests in aqueous medium.*

A preliminary investigation aimed at evaluating the biodegradation propensity of the analyzed LLDPE film samples LLDPE-TD1 and LLDPE-TD2 was carried out in a respirometric test in aqueous medium by using original, thermally oxidized and outdoor exposed samples as sole carbon source.

The cumulative CO_2 emissions profiles, expressed as average value of three replicates, recorded during 90 days of incubation of the pristine, thermally oxidized and outdoor exposed LLDPE-TD1 film specimens and blanks are reported in Figure 4.41.

As expected, the higher CO_2 emissions with respect to the blank were recorded in the culture flasks supplemented with thermally oxidized LLDPE-TD1 specimens having also the highest CO*i* values. The biodegradation process of the thermally oxidized specimen was also found to start without an appreciable induction time. On the contrary no significant differences were recorded in the carbon dioxide production from original film, outdoor exposed specimens and blanks. These results are therefore suggesting that the level of oxidation achieved during the pre-aging steps is strongly influencing the biodegradation propensity of LLDPE samples containing prodegradant systems.

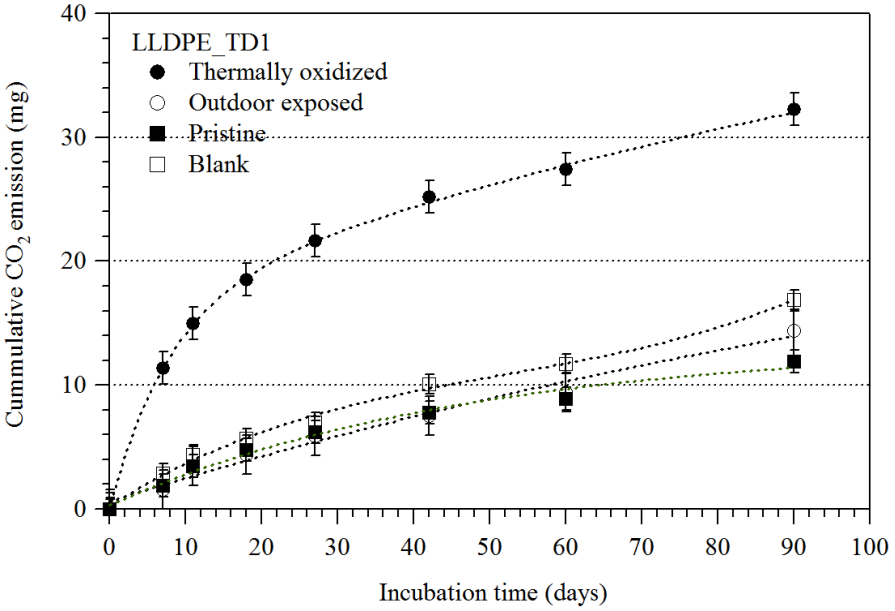


Figure 4.41. CO_2 emissions profiles of pristine, thermally oxidized and outdoor exposed LLDPE-TD1 film specimens and blanks in aqueous medium respirometric test.

Similar results were obtained in the case of LLDPE-TD2 film specimens (Figure 4.42). In this case, however, a slowest rate of CO_2 cumulative emissions from the cultures supplemented with thermally oxidized LLDPE-TD2 specimens was observed.

At the end of the test all the analysed materials were withdrawn from the aqueous cultures, carefully washed and characterized by gravimetric analysis (e.g. weight variation), FT-IR spectroscopy and thermal analysis.

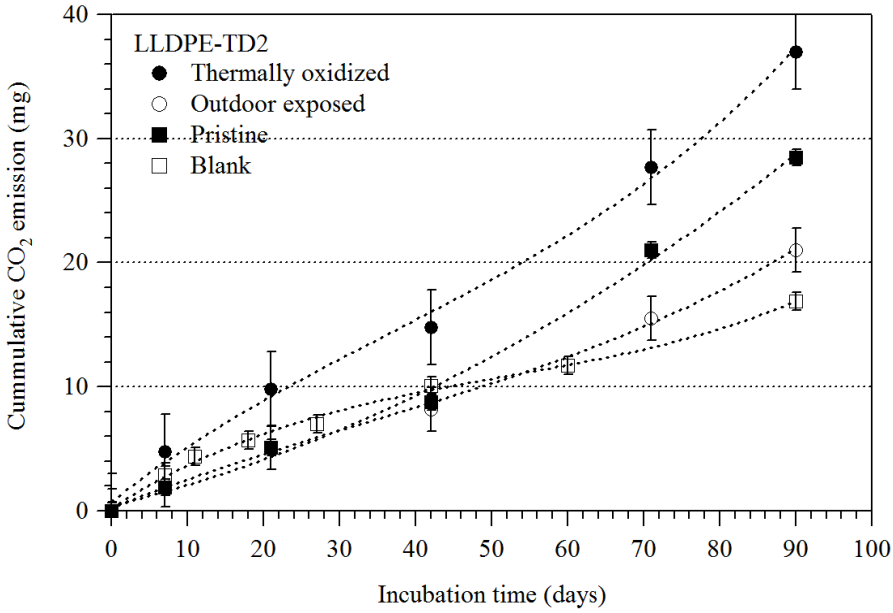


Figure 4.42. CO_2 emissions profiles of pristine, thermally oxidized and outdoor exposed LLDPE-TD2 film specimens and blanks in aqueous medium respirometric test.

In the case of thermally oxidized samples, after biodegradation in aqueous cultures, a significant decrease of both specimen weight and relevant CO*i* were recorded (Table 4.16), thus demonstrating that during the test the microbial population assimilated in a preferential way the oxidized fractions of the polyolefin samples.

On the contrary, an increase of the CO*i* values was detected in the case of outdoor exposed specimen submitted to biodegradation (Table 4.17). In this connection also an increase of the specimens weight was recorded that can be attributable either to the oxygen uptake and/or to the formation of microbial biofilms onto the specimens surface.

Table 4.16. Carbonyl index and weight assessment of thermally oxidized LLDPE samples submitted to biodegradation in aqueous medium respirometric test.

Sample	**CO*i***		**Starting weight**	**Final weight**	**Weight loss**
replicate	Initial	Final	(mg)	(mg)	(%)
LLDPE-TD1	4.70	3.09	113.1	86.3	11.2
LLDPE-TD1	4.68	2.82	113.2	105.2	7.1
LLDPE-TD1	4.71	3.46	109.3	96.1	12.1
Average	*4.69*	*3.15*	*118.9*	*95.9*	*10.1*
LLDPE-TD2	3.52	3.35	108.1	78.1	27.7
LLDPE-TD2	3.52	2.61	117.7	78.0	33.7
LLDPE-TD2	3.52	2.24	116.9	94.0	19.6
Average	*3.52*	*2.73*	*114.2*	*83.4*	*27.0*

Table 4.17. Carbonyl index and weight assessment of LLDPE outdoor exposed samples submitted to biodegradation in aqueous medium respirometric test.

Sample	**CO*i***		**Starting weight**	**Final weight**	**Weight loss**
replicate	Initial	Final	(mg)	(mg)	(%)
LLDPE-TD1	0.46	1.10	108.8	108.7	0.0
LLDPE-TD1	0.46	1.05	102.2	107.4	-5.1
LLDPE-TD1	0.46	0.69	103.8	109.8	-5.7
Average	*0.46*	*0.95*	*104.9*	*108.6*	*-3.6*
LLDPE-TD2	0.29	1.31	114.8	123.2	-7.3
LLDPE-TD2	0.28	1.34	118.5	121.4	-2.4
LLDPE-TD2	0.29	1.13	101.9	85.0	16.6
Average	*0.29*	*1.26*	*111.7*	*109.9*	*2.3*

Finally, a different behavior was observed in the case of pristine samples submitted to biodegradation (Table 4.18). In this latter case, no significant increase of the level of oxidation was detected, even though a marked weight increase did occur, that however could be attributed almost exclusively to biofilms formation onto the specimen surfaces.

Table 4.18. Carbonyl index and weight assessment of pristine LLDPE samples submitted to aqueous biodegradation test.

Sample	**CO*i***		**Starting weight**	**Final weight**	**Weight loss**
replicate	Initial	Final	(mg)	(mg)	(%)
LLDPE-TD1	0.11	0.01	115.2	117.5	-2.0
LLDPE-TD1	0.11	0.01	113.1	123.9	-9.5
LLDPE-TD1	0.11	0.01	111.9	126.2	-12.8
Average	*0.11*	*0.01*	*113.4*	*122.5*	*-8.0*
LLDPE-TD2	0.04	0.01	102.7	98.2	4.4
LLDPE-TD2	0.04	0.01	103.1	110.5	-7.2
LLDPE-TD2	0.04	0.01	122.3	135.5	-10.8
Average	*0.04*	*0.01*	*109.4*	*114.7*	*-4.8*

The recorded observations were confirmed by TGA analysis carried out on the test samples before and after the biodegradation process in the aqueous medium. In particular it was observed that the thermal stability of thermally oxidized LLDPE-TD1 and LLDPE-TD2 specimens increased after the incubation in the aqueous cultures. In accordance the temperatures corresponding to 2% weight loss (T_{ON}) of these two samples increased from 200.8 and 206.0 °C to 245.5 and 253.3 °C before and after biodegradation, respectively (Table 4.19, Figures 4.43, 4.44).

Table 4.19. Thermal properties of abiotically oxidized LLDPE-TD1 and LLDPE-TD2 samples submitted to biodegradation in aqueous medium, as recorded by TGA analysis.

Test Sample	Abiotic degradation	Biodegradation	T_{ON} [a)]	Residue [b)]	CO*i*
			(°C)	(weight %)	
LLDPE-TD1	none	none	400.6	0.24	0.11
	118 days at 65°C	none	200.8	1.19	4.71
	118 days at 65°C	aqueous test	245.5	6.05	3.46
	93 days outdoor	none	353.5	0.88	0.46
	93 days outdoor	aqueous test	218.0	9.07	1.05
	none	aqueous test	403.8	2.47	0.01
LLDPE-TD2	none	none	422.9	1.03	0.04
	152 days at 65°C	none	206.0	3.12	3.52
	152 days at 65°C	aqueous test	253.3	3.82	2.61
	93 days outdoor	none	304.2	1.97	0.29
	93 days outdoor	aqueous test	226.0	2.71	1.34
	none	aqueous test	378.0	4.56	0.01

a) 2% weight loss; b) at 600°C

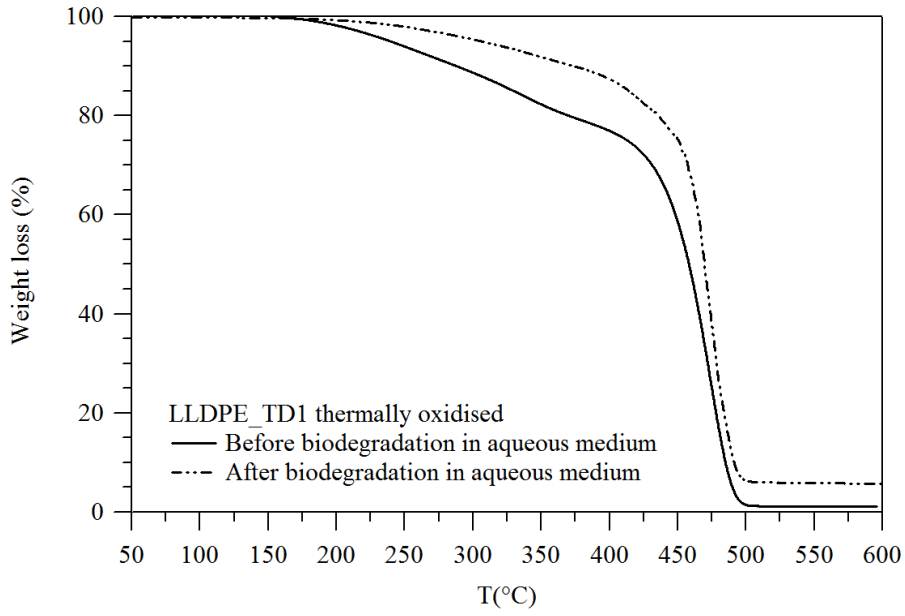


Figure 4.43. TGA traces in nitrogen atmosphere of thermally oxidized LLDPE-TD1 specimen before and after aqueous biodegradation.

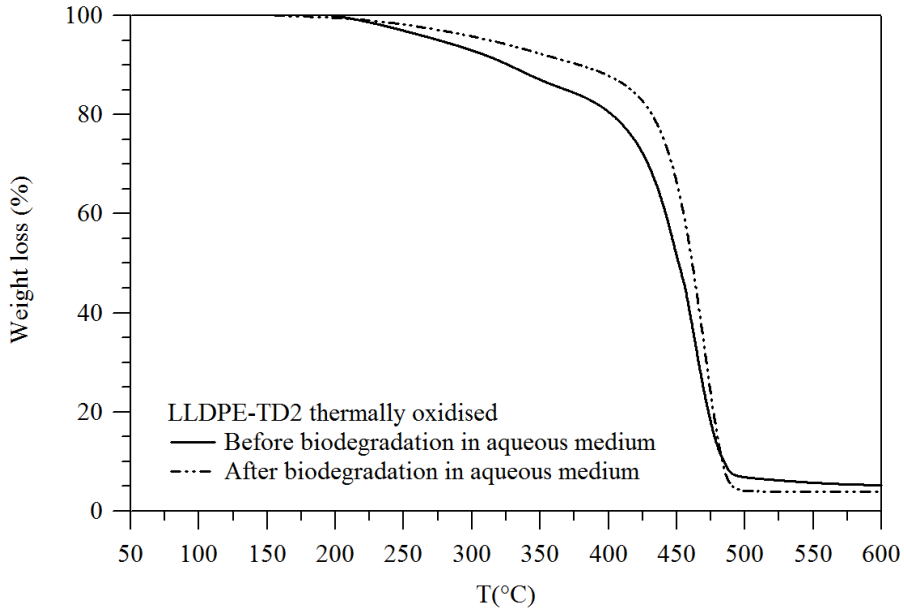


Figure 4.44. TGA traces in nitrogen atmosphere of thermally oxidized LLDPE-TD2 specimen before and after aqueous biodegradation.

On the contrary, the thermal stability of outdoor exposed samples was shown to decrease after the incubation in the aqueous microbial cultures. In accordance the T_{ON} temperatures of these samples decreased from 353.5 and 304.2°C to 218.0 and 226.0 °C before and after biodegradation, respectively (Table 4.19, Figures 4.45 and 4.46).

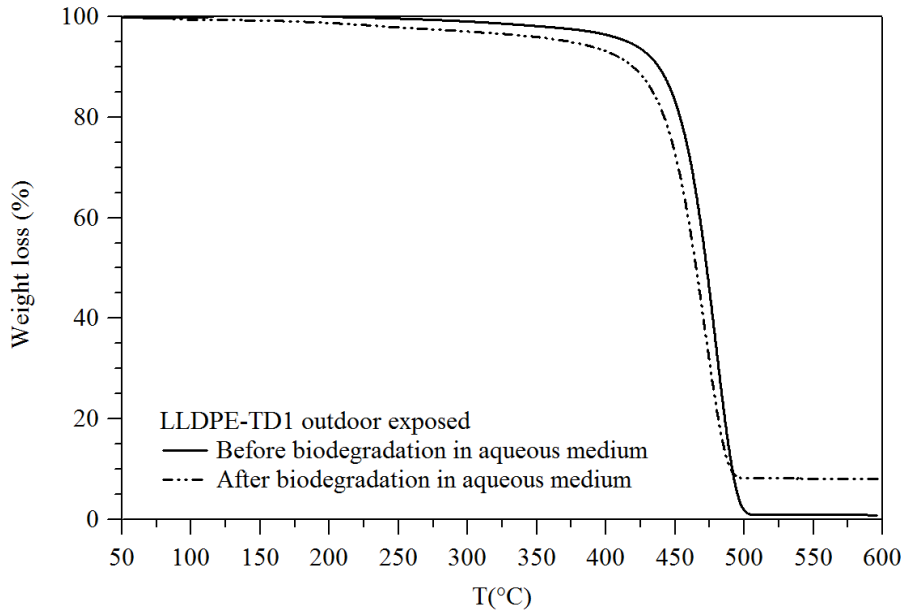


Figure 4.45. TGA traces in nitrogen atmosphere of outdoor exposed LLDPE-TD1 specimen before and after aqueous biodegradation.

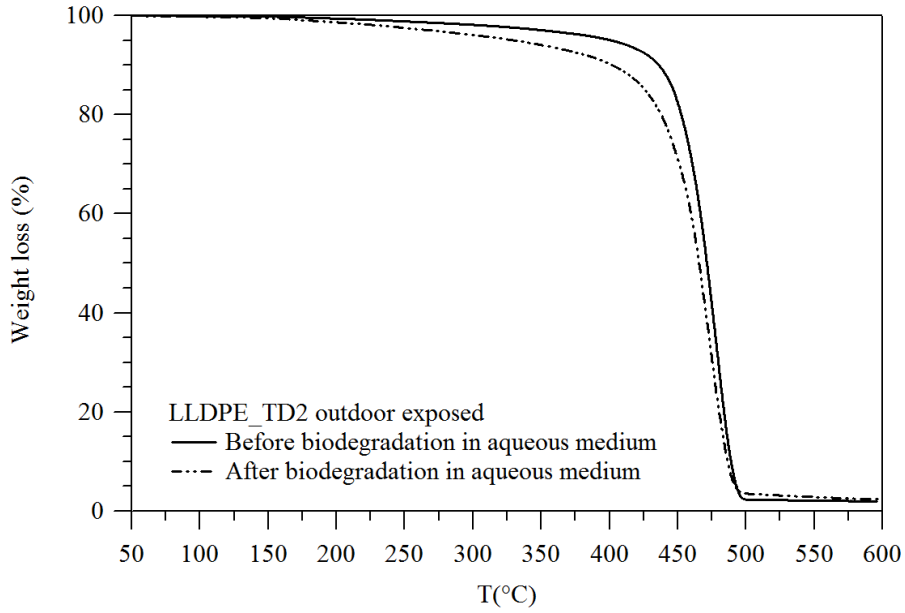


Figure 4.46. TGA traces in nitrogen atmosphere of outdoor exposed LLDPE-TD2 specimen before and after aqueous biodegradation.

Different results were finally recorded in the case of pristine samples submitted to the aqueous biodegradation test. In this case, in fact, the thermal stability did not change significantly in the case of LLDPE-TD1 specimens, whereas a decreased thermal stability was detected in the case of LLDPE-TD2 sample specimen (Table 4.19, Figures 4.47, 4.48).

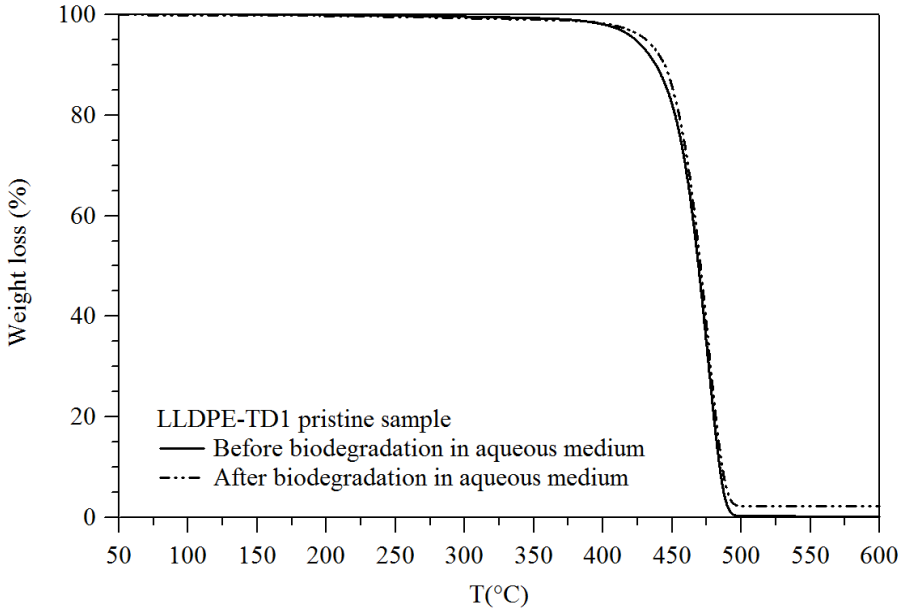


Figure 4.47. TGA traces in nitrogen atmosphere of original LLDPE-TD1 sample specimen before and after aqueous biodegradation.

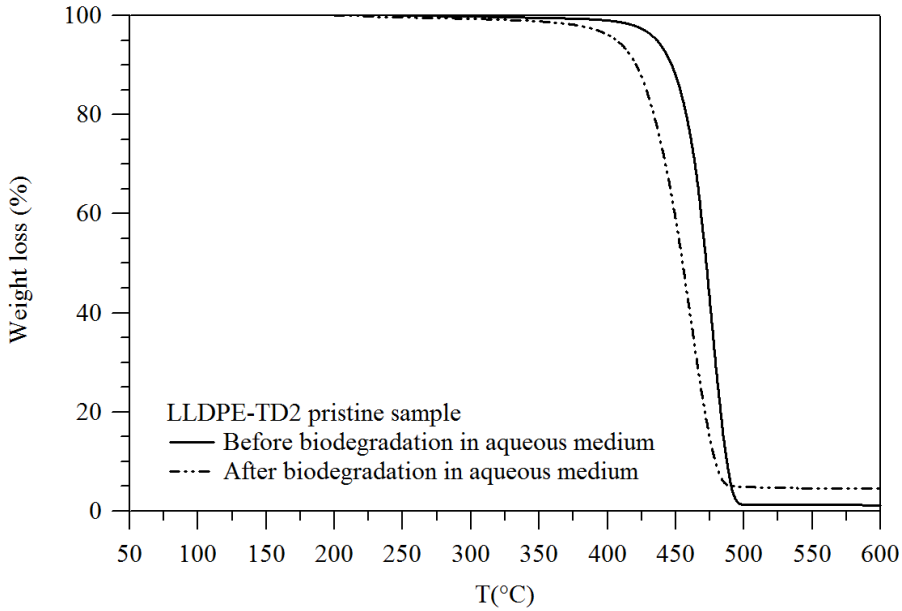


Figure 4.48. TGA traces in nitrogen atmosphere of original LLDPE-TD2 sample specimen before and after aqueous biodegradation.

All the samples submitted to the aqueous biodegradation tests were also analyzed by DSC, the data relevant to the melting temperatures and crystallinity are reported in Table 4.20. DSC traces, relevant to the second heating, reported in Figures 4.49-4.54 did not reveal, however, substantial variation in the thermal transitions before and after the incubation in aqueous microbial cultures. Nevertheless it was observed a slight decrease of the crystallinty of pristine samples submitted to the biodegradation test, whereas in the case of pre-oxidized sample specimens this parameter was found to slight increase after the incubation in the aqueous medium within the same incubation time (Table 4.20).

Table 4.20. DSC parameters of LLDPE-TD1 and LLDPE-TD2 sample specimens submitted to biodegradation in aqueous medium.

Test sample	Abiotic degradation	Biodegradation	1st heating		2nd heating	
			Tm a)	Crystallinity	Tm a)	Crystallinity
			(°C)	(%)	(°C)	(%)
LLDPE-TD1	none	none	115.0, 121.8	42.7	110.9, 122.3	44.7
	none	aqueous	114.1, 121.1	36.9	109.1, 120.5	39.5
	118 days at 65°C	none	117.4	59.1	116.8	47.4
	118 days at 65°C	aqueous	119.0	69.6	117.6	55.1
	93 days outdoor	none	116.0, 121.8	48.5	121.1	46.3
	93 days outdoor	aqueous	119.7	54.7	112.0	50.1
LLDPE-TD2	none	none	121.7	41.9	110.0, 121.5	44.2
	none	aqueous	120.7	39.7	120.7	39.6
	152 days at 65°C	none	118.0	51.2	115.0	47.5
	152 days at 65°C	aqueous	118.9	60.2	117.4	51.6
	93 days outdoor	none	117.7, 122.0	52.4	121.5	47.5
	93 days outdoor	aqueous	120.4	61.8	120.7, 112	53.7

a) Melting peak

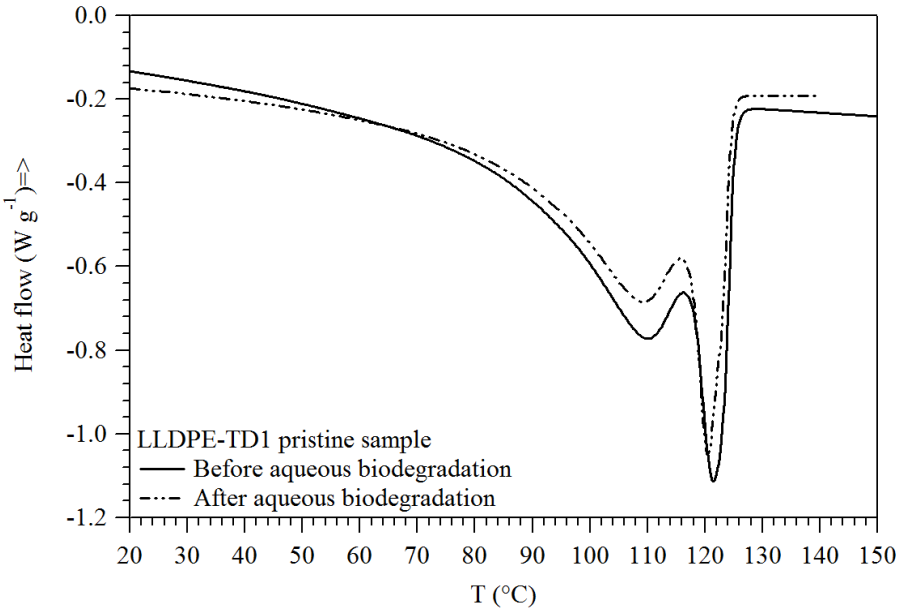


Figure 4.49. DSC traces in nitrogen atmosphere of original LLDPE-TD1 sample before and after biodegradation respirometric test in aqueous medium.

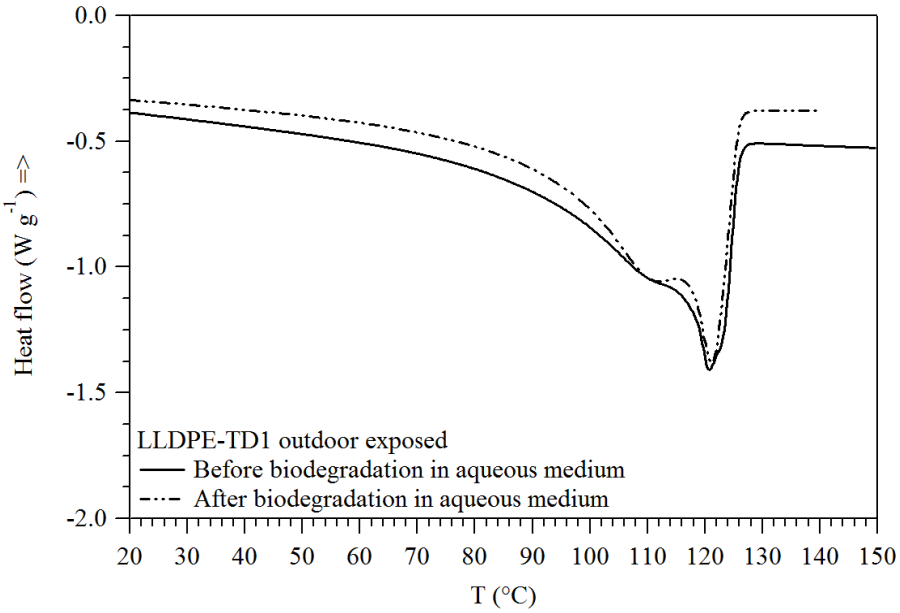


Figure 4.50. DSC traces in nitrogen atmosphere of sunlight exposed LLDPE-TD1 sample before and after biodegradation respirometric test in aqueous medium.

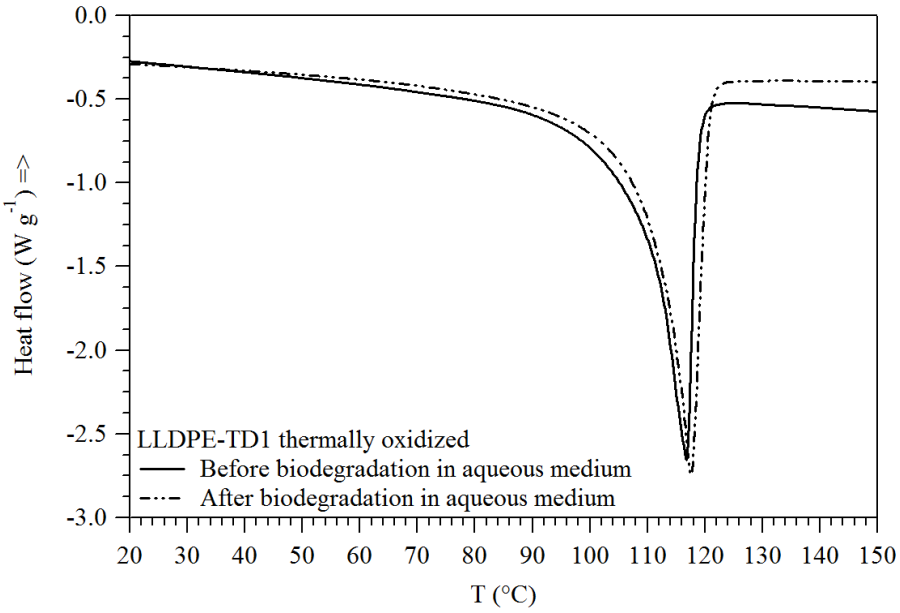


Figure 4.51. DSC traces in nitrogen atmosphere of thermally oxidised LLDPE-TD1 sample before and after biodegradation respirometric test in aqueous medium.

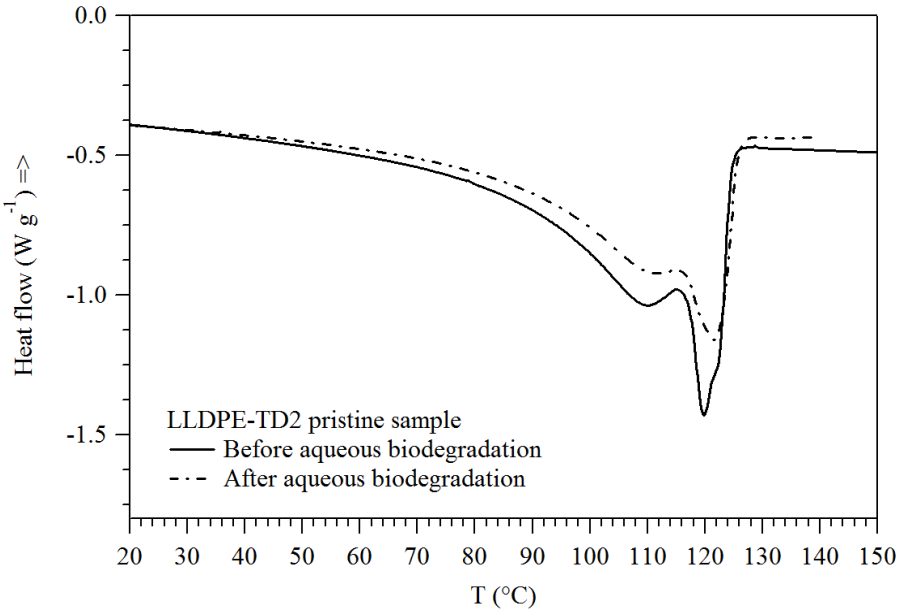


Figure 4.52. DSC traces in nitrogen atmosphere of original LLDPE-TD2 sample before and after biodegradation respirometric test in aqueous medium.

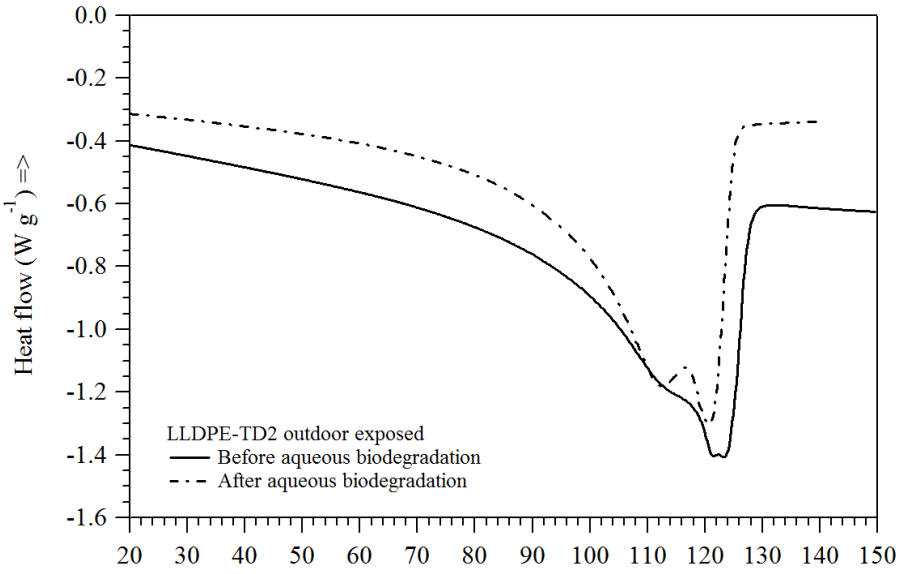


Figure 4.53. DSC traces in nitrogen atmosphere of sunlight exposed LLDPE-TD2 sample before and after biodegradation respirometric test in aqueous medium.

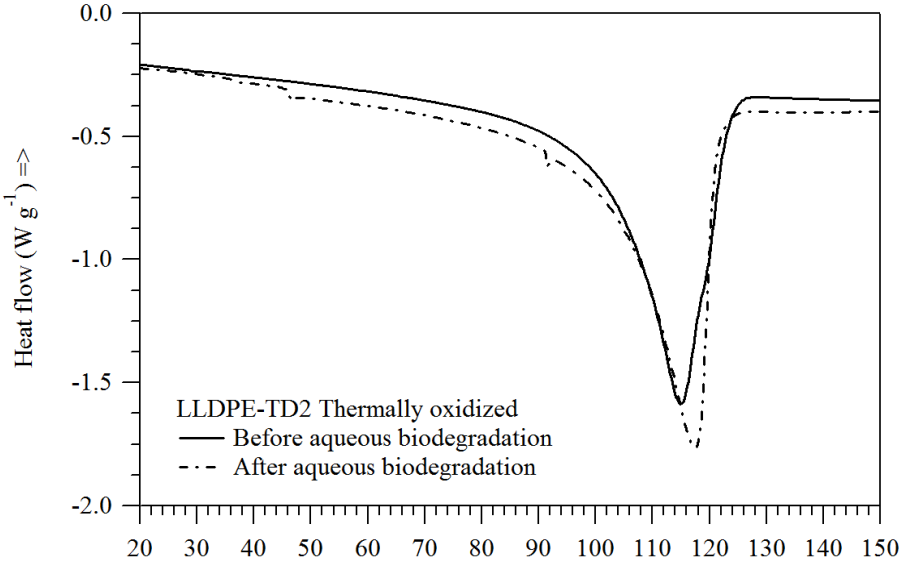


Figure 4.54. DSC traces in nitrogen atmosphere of thermally oxidized LLDPE-TD2 sample before and after biodegradation respirometric test in aqueous medium.

During the test it was therefore observed that the mineralization of the pre-oxidized LLDPE samples, do experience a mineralization level depending upon the CO*i* value, higher is the CO*i* value higher is the mineralization extent.

A second point to be remarked is that the LLDPE sample characterized by higher CO*i* level is experiencing a drop of the oxidation level accompanied by an increase of thermal stability, whereas in the case of LLDPE samples characterized by an initial lower CO*i* value, an increase of the oxygen content (CO*i*) and a corresponding lower thermal stability were observed. These data, along with the slight differences recorded in the crystallinity degree of biodegraded samples in aqueous medium, are also suggesting that the microbial population present in the aqueous medium may stimulate directly a certain level of oxidation of the polymer matrix.

In an other aqueous biodegradation test, carried out by using river water as microbial inoculum, the propensity to inherent biodegradability of the fractions extracted with boiling acetone from thermally oxidized in forced air convention oven at 65°C of LLDPE-TD1, PP-TD1 and PP-TD2 film specimens, having comparable molecular weight (1.2 kDa), was evaluated.

As positive reference material Docosane, a linear aliphatic solid hydrocarbon with 22 carbon atoms (Mw 0.3 kDa), was used. The test was aimed at comparing the rate and extent of biodegradation of low molecular weight fractions deriving from oxidized PE and PP matrix, which are characterized by different molecular structures. Almost straight chains in the case of LLDPE and highly branched (methyl groups in 1-3 position) in the case of PP extracts, respectively were compared.

The mineralization profiles recorded within 73 days of incubation are reported in Figure 4.55.

A fairly high biodegradation extent approaching 50% biodegradation was recorded in the case of the acetone extract obtained from the thermally oxidized LLDPE-TD1 sample after 73 days incubation. In the same test period also Docosane undergoes an extensive biodegradation process, thus reaching approximately 40% mineralization with a positive slope in the biodegradation curve (Figure 4.55).

It is worth noting that whereas the LLDPE samples not submitted to thermal aging, whatsoever the prior and post treatment (outdoor exposure, biotic degradation in aqueous medium), are characterized by a broad two peaks – DSC traces, whereas in the case of thermally aged either submitted to biodegradation test in aqueous medium, it was observed a single peak-DSC trace with an increase of crystallinity extent. This fact can be attributed to the annealing treatment undergoing in the case of thermal aging.

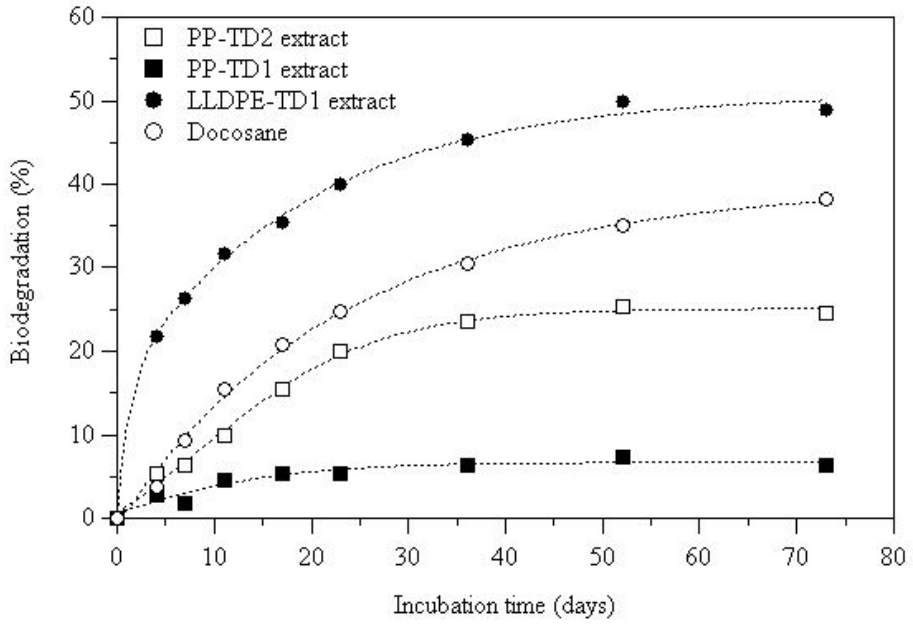


Figure 4.55. Mineralization profiles of PP-TD1, PP-TD2, LLDPE-TD1 acetone extracts and Docosane recorded in the river water biodegradation test.

On the contrary the acetone extracts obtained from the thermally oxidized PP-based samples were showing to approach a plateau phase after 35 days of incubation in the correspondence of fairly lower values of biodegradation. In particular the acetone extract of PP-TD2 sample exhibited a maximum level of biodegradation of 25%, whereas only 7% mineralization was recorded in the case of the acetone extract obtained from the thermally oxidized PP-TD1 film specimen (Figure 4.55). The recorded differences in the biodegradation extent of the two extracts of PP-based samples might be attributed to the different molecular weight as well as to the different degree of oxidation. In fact, lower (1.1 kDa) Mw and higher level of oxidation were ascertained in the case of PP-TD2 acetone extract with respect to the same characteristic of the acetone extract obtained from the oxidized PP-TD1 sample.

In any case, the lower propensity of PP acetone extracts with respect to the LLDPE-TD1 extract and Docosane to be assimilated by river water microorganisms can be attributed to the presence of low molecular fractions in PP sample having a fairly high branching degree that usually hinder the enzymatic attack of branched hydrocarbons [196].

4.2.2. *Soil burial Biodegradation tests*

Soil environment can be considered as one of the most probable throwing away natural habitat where plastic materials, such as in the case of agricultural items, or indirectly after composting may ultimate their degradation and biodegradation processes. Consequently, soil biodegradation tests have been carried out as aimed at establishing the mineralization rate and extent of several polymeric materials, as well as to ascertain the progress of polymer oxidation and degradation with a particular attention for full carbon backbone polymeric materials.

In this connection, soil burial respirometric tests have been set up to assess the potential biodegradation of polyolefins (LLDPE, PP and PS samples) previously exposed to abiotic degradation tests (thermal or outdoor). Moreover, the microbial assimilation (e.g. mineralization) of these materials was evaluated in comparison with oxo-biodegradable polymers from natural origin, as well as with and hydro-biodegradable materials of both synthetic or semi-synthetic origin.

In a soil biodegradation test, the mineralization of the samples reported in Table 4.22, was evaluated.

LLDPE samples utilized in this test were retrieved after 230 days thermal aging at 65°C, as well as after 3 months outdoor exposure, thus being characterized by different level of oxidation and polymer chain fragmentation (Table 4.21). The biodegradation behaviour of oxo-biodegradable LLDPE film samples was evaluated in comparison with hydro-biodegradable commercial grade materials tepresented by Ecoflex (BASF), Mater Bi (Novamont) and poly(lactic acid) (PLA), by using filter paper as positive control.

Table 4.21. Organic carbon content and theoretical carbon dioxide ($Th.CO_2$) of test film samples and reference materials analyzed in the soil burial respirometric test.

Run	Test sample	Treatment	Amount	Organic C	$Th.CO_2$
			(mg)	(%)	(mg)
A1	LLDPE-TD1	TD[a)]	253.0	77.2	716.2
A2	LLDPE-TD1	TD[a)]	248.3	77.2	702.9
A3	LLDPE-TD1	TD[a)]	251.8	77.2	712.8
B1	LLDPE-TD2	TD[a)]	251.8	n.d.[c)]	n.d.[c)]
B2	LLDPE-TD2	TD[a)]	254.3	n.d.[c)]	n.d.[c)]
B3	LLDPE-TD2	TD[a)]	254.3	n.d.[c)]	n.d.[c)]
C1	LLDPE-TD1	UV[b)]	258.5	n.d.[c)]	n.d.[c)]
C2	LLDPE-TD1	UV[b)]	255.1	n.d.[c)]	n.d.[c)]
C3	LLDPE-TD1	UV[b)]	260.8	n.d.[c)]	n.d.[c)]
D1	LLDPE-TD2	UV[b)]	250.7	n.d.[c)]	n.d.[c)]
D2	LLDPE-TD2	UV[b)]	249.6	n.d.[c)]	n.d.[c)]
D3	LLDPE-TD2	UV[b)]	247.2	n.d.[c)]	n.d.[c)]
E1	PLA	none	251.5	49.7	458.3
E2	PLA	none	251.8	49.7	458.9
E3	PLA	none	249.0	49.7	453.8[)]
F1	Mater Bi	none	249.5	42.8	391.0
F2	Mater Bi	none	253.0	42.8	397.4
F3	Mater Bi	none	249.9	42.8	392.5
G1	Filter paper	none	251.5	43.2	398.4
G2	Filter paper	none	251.1	43.2	397.6
G3	Filter paper	none	250.5	43.2	396.8
H1	Ecoflex	none	252.0	54.7	505.4
H2	Ecoflex	none	252.4	54.7	506.2
H3	Ecoflex	none	251.9	54.7	505.2

a) After 230 days at 65°C in ventilated oven; b) After 93 days outdoor exposure; c) not yet determined

The average cumulative CO_2 emissions detected from the test cultures and blanks during the incubation time are reported in Table 4.22.

Table 4.22. Average CO_2 emissions (mg) from test materials and blanks recorded during the soil burial biodegradation test.

Time	LLDPE-TD1	LLDPE-TD1	LLDPE-TD2	LLDPE-TD2	PLA	Mater Bi	Ecoflex	FP	Blank
days	TD[a)]	UV[b)]	TD [a)]	UV [b)]					
5	14.4	6.2	11.1	6.2	7.6	10.6	7.9	7.3	6.5
12	41.9	28.2	34.0	28.2	29.0	34.3	30.5	33.4	27.3
19	54.9	35.5	44.6	36.4	34.9	43.4	40.8	51.3	36.7
26	64.2	40.8	53.1	41.9	41.4	50.5	48.7	64.8	41.9
33	77.4	50.2	65.4	50.5	50.5	61.3	60.7	82.1	51.3
40	90.3	59.0	78.3	59.5	59.5	75.7	72.7	100.6	61.3
48	103.8	69.2	91.8	70.7	68.6	93.9	90.1	121.1	71.9
56	114.1	77.7	101.2	78.3	75.4	108.5	107.1	137.6	79.5
68	130.5	91.2	116.2	91.5	86.8	136.1	140.8	164.3	91.2
75	143.4	102.7	128.2	104.1	97.1	159.6	168.1	185.4	103.3
84	157.2	114.4	140.5	115.3	108.2	185.4	201.2	211.5	114.4
93	168.1	125.0	151.1	125.8	117.3	204.7	227.3	233.2	125.3
98	184.2	137.3	164.0	136.7	128.5	234.7	254.6	264.3	137.3
106	195.9	148.7	177.2	147.5	138.7	266.9	296.0	305.4	147.3
113	208.6	159.9	189.8	159.9	148.4	293.0	331.8	337.3	157.2
126	229.0	179.1	205.8	178.7	166.4	331.0	387.0	388.1	175.2
134	239.3	190.8	224.0	188.4	174.9	357.4	416.4	412.4	183.7
147	251.9	204.3	242.5	200.1	185.8	389.0	454.8	441.7	196.1
157	263.7	218.4	258.3	212.7	195.8	412.8	482.1	463.4	206.0
168	282.7	238.9	279.5	232.1	212.5	441.0	514.1	490.4	225.1
182	295.6	253.0	294.4	246.2	224.8	463.0	533.7	511.6	237.7
219	320.3	275.6	319.9	269.0	243.0	494.4	570.1	542.7	255.3
248	331.4	289.4	334.9	280.5	254.7	514.0	589.4	560.0	267.9
276	340.8	298.8	348.1	289.6	263.8	532.2	603.2	573.2	277.0
304	354.3	311.4	363.6	300.7	276.1	550.7	619.9	593.4	289.9
346	368.4	326.1	378.0	313.6	288.5	571.2	639.0	612.2	303.1
376	405.0	346.0	407.3	333.9	306.4	606.1	679.2	638.9	330.4
406	433.2	366.9	430.2	356.7	319.9	626.4	698.0	653.8	350.6
432	459.0	384.7	451.9	372.2	347.8	675.2	729.3	683.3	367.0
467	490.2	411.1	473.0	390.8	373.8	705.6	767.6	710.6	392.3
574	513.2	438.2	496.4	408.7	408.7	731.2	794.0	737.2	419.6
634	527.3	450.5	508.7	418.9	414.5	744.8	808.1	750.4	432.2
675	538.3	459.9	519.2	427.2	424.2	755.4	816.9	760.5	442.5
795	560.7	481.9	540.7	446.6	444.4	784.4	838.9	785.6	466.5

a) After 230 days at 65°C in ventilated oven; b) After 93 days outdoor exposure

The mineralization profiles recorded within the incubation time in the case of the hydro-biodegradable materials, including the positive control (filter paper) are reported in Figure 4.56. The test conditions were validated by the mineralization profile observed in the case of Filter paper, whose biodegradation extent approached 80 % in 19 months of incubation, thus approaching the plateau level (Figure 4.56). Fairly high biodegradation extents were also recorded and reached 80% in the case of Mater Bi and Ecoflex samples, that were showing a typical sigmoidal shape of the mineralization curves strictly similar to that observed for the filter paper profile. On the contrary no significant trace of mineralization by soil microorganisms was observed in the cultures fed with PLA specimens as carbon source (Figure 4.56).

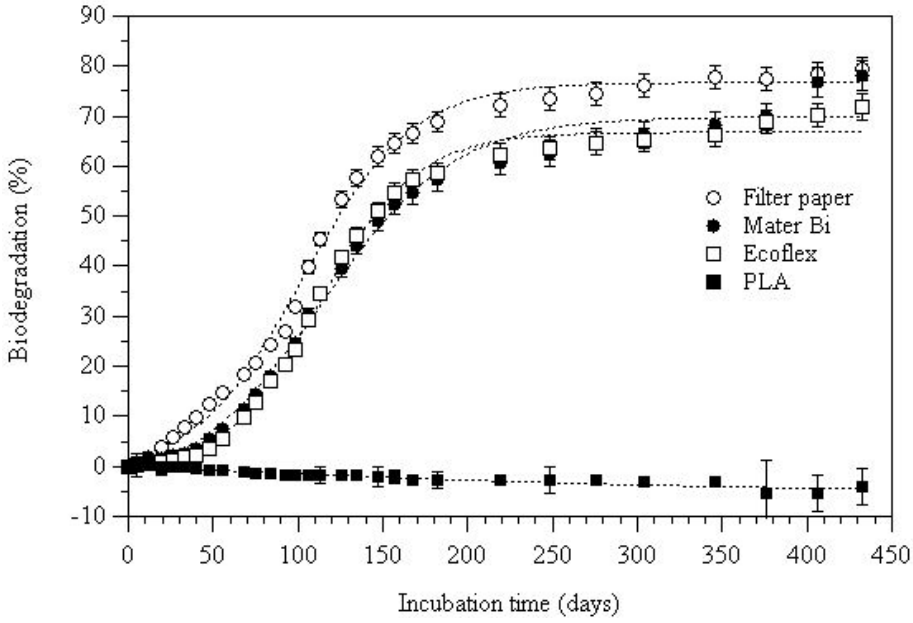


Figure 4.56. Mineralization profiles of hydro-biodegradable plastic materials and filter paper in respirometric soil burial tests.

In the case of oxo-biodegradable LLDPE based LLDPE-TD1 and LLDPE-TD2 samples significantly higher CO_2 emissions with respect to

the blank, as a clear indication of their utilization as carbon source by soil microorganisms, were recorded from the soil cultures supplemented with samples specimen heavily pre-oxidized by thermal treatment (Figures 4.57 and 4.58). The net CO_2 productions from thermally oxidized specimens were also found to further increase after 19 months of incubation. In accordance the approaching of a second exponential step in the biodegradation profile of LLDPE-TD1 sample was observed, thus approaching 14 % mineralization in 27 months incubation (Figure 4.59). The recorded behaviour of the mineralization process in soil, which is characterized by the presence of an exponential step since the beginning, followed by a prolonged dormant phase before a second exponential step took off, was repeatedly observed in soil burial tests carried out with thermally oxidized low density polyethylene (LDPE) samples [178].

On the contrary, the soil cultures supplemented with outdoor exposed specimen of both LLDPE samples, which were characterized by a fairly low initial level of oxidation, exhibited only minor differences in the CO_2 emissions with respect to the blanks (Figures 4.57 and 4.58), with corresponding very few, if any, mineralization of these samples by soil microorganism within the same time frame.

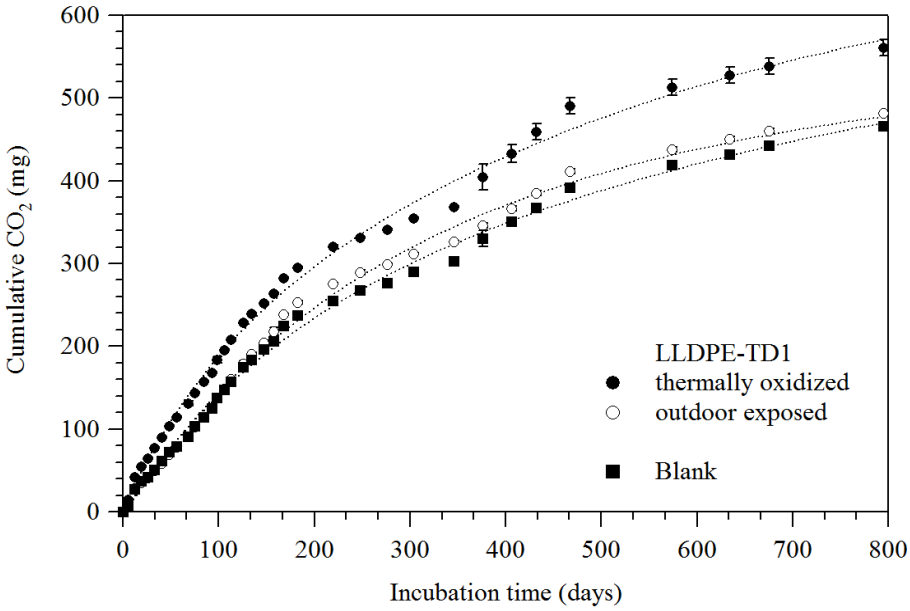


Figure 4.57. CO_2 average cumulative emissions from soil cultures supplemented with themally oxidized and outdoor exposed LLDPE-TD1 specimens.

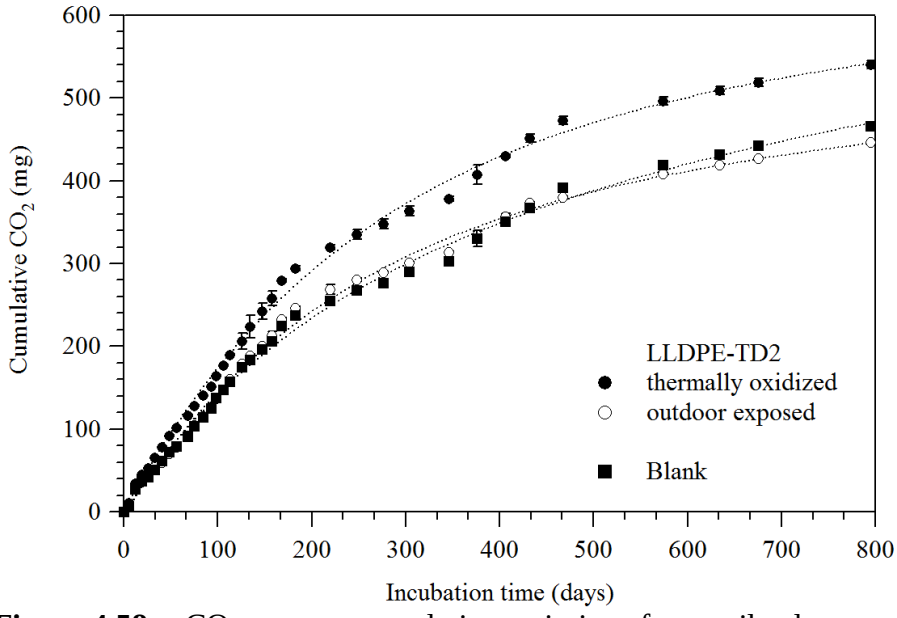


Figure 4.58. CO_2 average cumulative emissions from soil cultures supplemented with themally oxidized and outdoor exposed LLDPE-TD2 specimens.

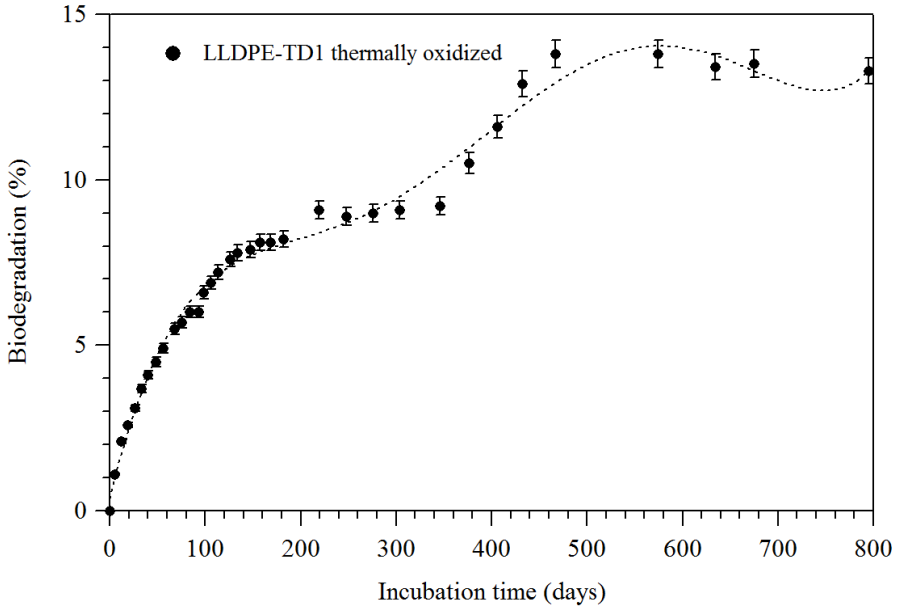
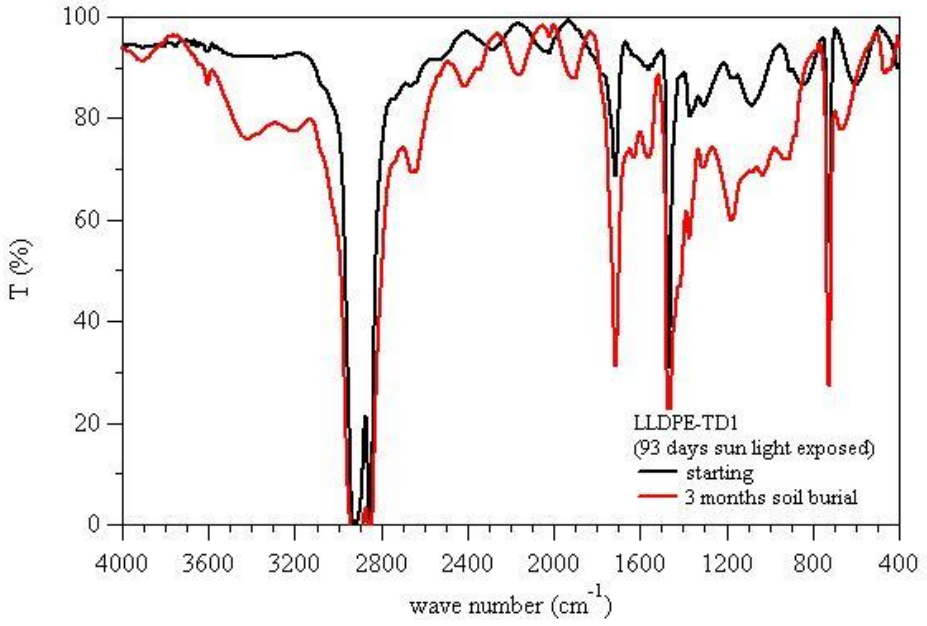


Figure 4.59. Mineralization profiles of thermally oxidized LLDPE-TD1 specimen in soil burial test.

In spite of the fairly low net CO_2 emissions, it is interesting to note that the oxidation degree, as determined by CO*i*, of both LLDPE-TD1 and LLDPE-TD2 outdoor exposed specimen, increased as a consequence of the incubation in soil cultures (Figures 4.60 and 4.61). This trend was confirmed after 12 months of incubation in soil, after a dormant period occurred between the 3rd and 6th months (Table 4.23). **These findings were therefore suggesting that the oxidative degradation of these materials once started by relatively mild abiotic treatment, such as 3 months outdoor exposure, still proceed once they are confined in active microbiological environment, even at room temperature and in the absence of light irradiation**.



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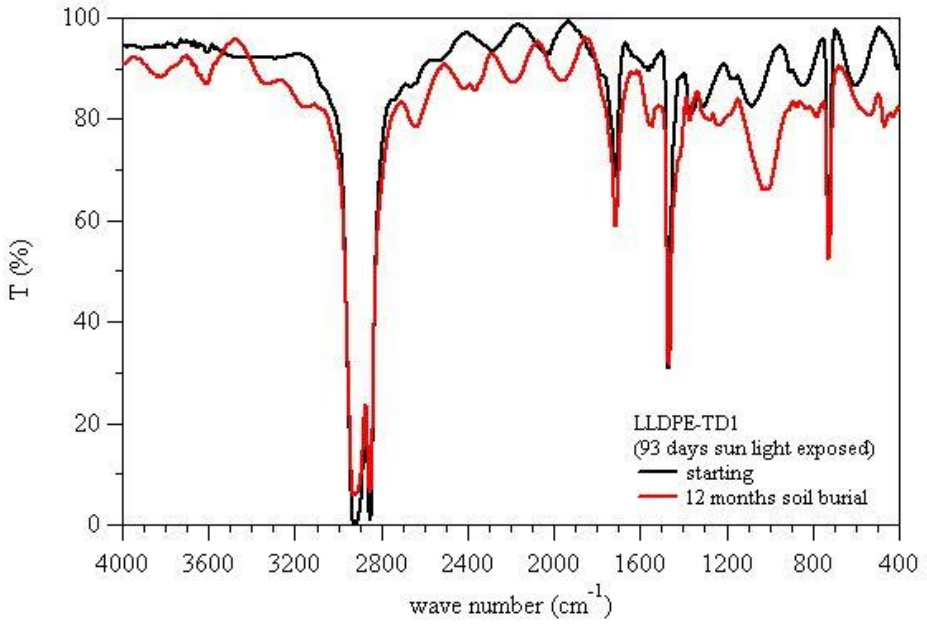
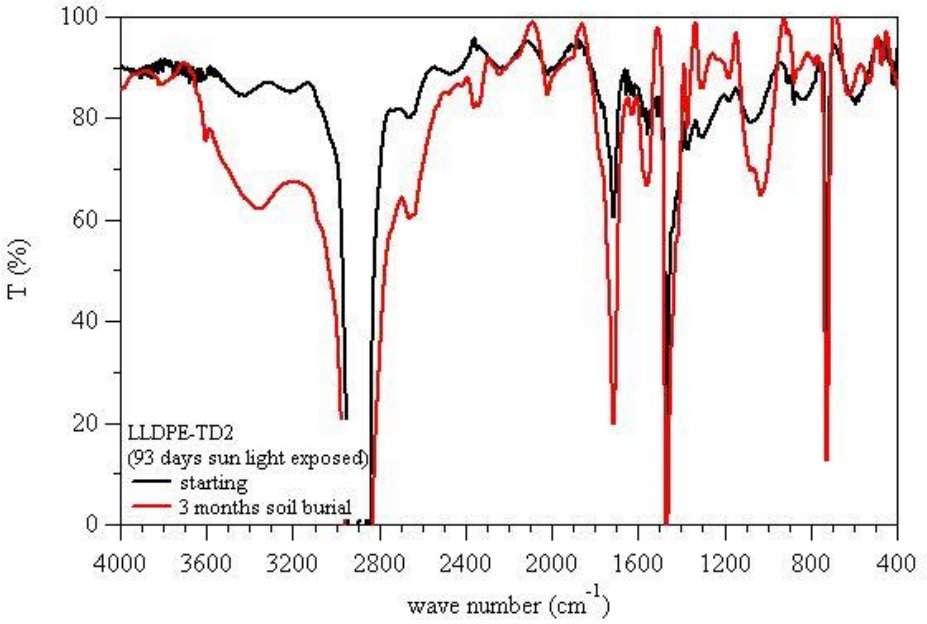


b

Figure 4.60. FT-IR spectra of outdoor exposed LLDPE-TD1 specimen before and after 3 (a) and 12 (b) months soil burial.



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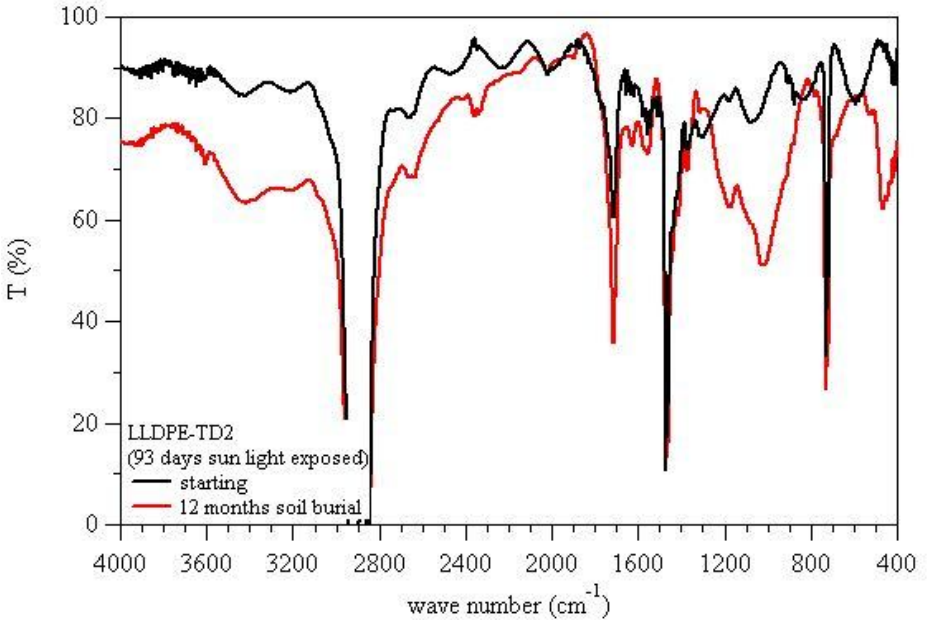

b

Figure 4.61. FT-IR spectra of outdoor exposed LLDPE-TD2 specimen before and after 3 (a) and 12 (b) months soil burial.

In accordance with these data, also the thermal stability, as determined by TGA, of the LLDPE-TD1 and LLDPE-TD2 outdoor exposed specimen,

withdrawn from the soil cultures after 3 months incubation, was significantly lower with respect to the corresponding values recorded before the biodegradation test (Figure 4.62; Table 4.24). Nevertheless, at longer incubation times (6 and 12 months) the onset temperatures (T_{ON}) corresponding to the beginning of thermal degradation were found to further increase, most likely because the assimilation of degraded LLDPE fractions by soil microorganisms.

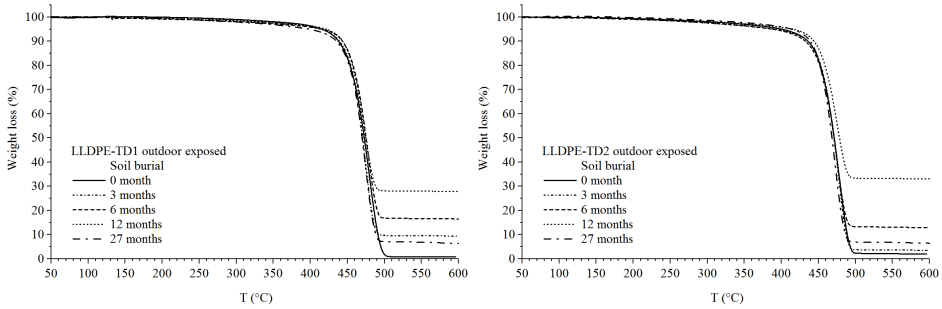

Figure 4.62. TGA traces of outdoor exposed LLDPE-TD1 and LLDPE-TD2 samples submitted to biodegradation in soil.

An opposite behavior was instead observed for the thermally oxidized samples. In that case, in fact a decrease of the CO*i* values was recorded after 3 and 6 months soil burial most likely attributable to the assimilation of oxidized fractions by soil microorganisms (Table 4.23). As a consequence, a corresponding increase in thermal stability was therefore observed in the thermally oxidized LLDPE specimen analyzed by TGA after 3 months of incubation in soil cultures (Figure 4.63; Table 4.24).

Thermally oxidized specimen were not further characterized either by both FT-IR spectroscopy and thermal analysis after 6 months of incubation in soil cultures because in the correspondence of the 12th month of the biodegradation tests, no noticeable fragments of the tested samples were recognizable, even microscopically, in the soil matrix.

Table 4.23. Evaluation of Carbonyl index (CO*i*) of thermally and outdoor exposed LLDPE samples under soil burial biodegradation test.

Test sample	**Abiotic aging**	**CO*i***									
		Soil burial incubation (months)									
		0	*Ave*	3	*Ave*	6	*Ave*	12	*Ave*	27	*Ave*
LLDPE-TD1	TD[a)]	4.43		3.45		1.64		n.d		n.d	
LLDPE-TD1	TD[a)]	4.65	*4.59*	3.47	*3.42*	1.65	*1.65*	n.d	-	n.d	-
LLDPE-TD1	TD[a)]	4.70		3.35		n.d		n.d		n.d	
LLDPE-TD2	TD[a)]	3.43		2.96		1.64		2.39	2.39	n.d	
LLDPE-TD2	TD[a)]	3.25	*3.41*	3.10	*3.03*	2.13	*1.91*	n.d.		n.d	-
LLDPE-TD2	TD[a)]	3.54		3.01		1.96		n.d.		n.d	
LLDPE-TD1	UV [b)]	0.41		0.75		0.55		0.83		1.47	
LLDPE-TD1	UV [b)]	0.43	*0.43*	0.69	*0.70*	0.71	*0.63*	0.76	*0.84*	1.28	1.53
LLDPE-TD1	UV [b)]	0.45		0.66		0.62		0.94		1.85	
LLDPE-TD2	UV [b)]	0.35		0.87		0.89		0.83		1.35	
LLDPE-TD2	UV [b)]	0.34	*0.36*	0.75	*0.75*	0.71	*0.78*	0.91	*1.05*	1.58	1.47
LLDPE-TD2	UV [b)]	0.39		0.63		0.74		1.40		-	

a) After 230 days at 65°C in ventilated oven; b) After 93 days outdoor exposure; n.d. not detectable

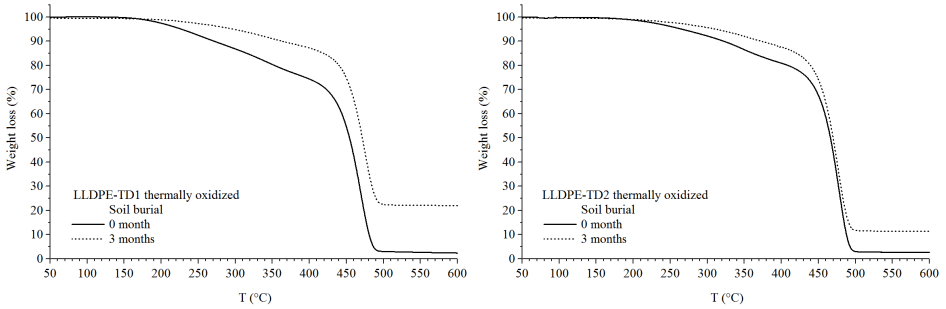

Figure 4.63. TGA traces in nitrogen atmosphere of original LLDPE-TD1 and LLDPE-TD2 samples specimen before and after 3 months of soil burial biodegradation test.

Table 4.24. Thermal properties of abiotically oxidized LLDPE-TD1 and LLDPE-TD2 samples submitted to biodegradation in soil, as recorded by TGA analysis.

Test Sample	Abiotic degradation	Soil burial incubation	T_{ON}	Residue	CO*i*
		(months)	(°C)	(weight %)	
LLDPE-TD1	none	0	401	0.24	0.11
LLDPE-TD1	TD [a)]	0	192	2.43	4.59
LLDPE-TD1	TD [a)]	3	233	22.03	3.42
LLDPE-TD1	UV [b)]	0	354	0.88	0.46
LLDPE-TD1	UV [b)]	3	322	9.41	0.69
LLDPE-TD1	UV [b)]	6	303	16.53	0.63
LLDPE-TD1	UV [b)]	12	336	27.8	0.84
LLDPE-TD1	UV [b)]	27	298	6.39	1.53
LLDPE-TD2	none	0	423	1.03	0.04
LLDPE-TD2	TD [a)]	0	219	2.72	3.41
LLDPE-TD2	TD [a)]	3	243	11.36	3.03
LLDPE-TD2	UV [b)]	0	304	1.97	0.29
LLDPE-TD2	UV [b)]	3	278	3.48	0.75
LLDPE-TD2	UV [b)]	6	276	12.84	0.78
LLDPE-TD2	UV [b)]	12	314	33.07	1.05
LLDPE-TD2	UV [b)]	27	336	6.45	1.47

a) After 229 days at 65°C in ventilated oven; b) After 93 days outdoor exposure

LLDPE samples submitted to biodegradation in soil were also characterized by DSC analysis, thus evidencing in all the analyzed specimens the overall increase of the degree of crystallinity during the progress of soil incubation (Table 4.24). On the contrary, the melting temperature (Tm) was not significantly affected by the incubation in the soil medium (Table 4.25; Figure 4.64), with the sole exception of the melting peak shape of LLDPE-TD2 specimen recovered after 3 months soil incubation (Figure 4.65).

Table 4.25. DSC parameters of LLDPE-TD1 and LLDPE-TD2 pre-aged specimens submitted to soil burial biodegradation tests.

Test sample	Abiotic degradation	Soil burial incubation	1st Heating		2nd Heating	
			Tm [c]	Crystallinity	Tm [c]	Crystallinity
		(months)	(°C)	(%)	(°C)	(%)
LLDPE-TD1	none	0	115, 122	42.7	111, 122	44.7
LLDPE-TD1	TD [a]	0	117	59.1	117 [d]	47.4
LLDPE-TD1	TD [a]	3	n.d.	n.d.	n.d.	n.d.
LLDPE-TD1	UV [b]	0	116, 122	48.5	121[d]	46.3
LLDPE-TD1	UV [b]	3	116, 120	56.3	113, 121	50.2
LLDPE-TD1	UV [b]	6	116	51.6	113, 121	46.5
LLDPE-TD1	UV [b]	12	116	56.6	114, 121	51.9
LLDPE-TD1	UV [b]	27	115	62.8	112, 120	53.8
LLDPE-TD2	none	0	122	41.9	110, 122	44.2
LLDPE-TD2	TD [a]	0	118	51.2	115[d]	47.5
LLDPE-TD2	TD [a]	3	119	63.8	117[d]	56.1
LLDPE-TD2	UV [b]	0	118, 122	52.4	122 [d]	47.5
LLDPE-TD2	UV [b]	3	116	57.0	113, 121	51.3
LLDPE-TD2	UV [b]	6	118, 123	60.8	114, 123	54.2
LLDPE-TD2	UV [b]	12	115, 122	60.0	113, 121	53.6
LLDPE-TD2	UV [b]	27	116	54.7	113, 121	50.2

a) After 230 days at 65°C in ventilated oven; b) After 93 days outdoor exposure; c) Melting peak; d) Broad Peak

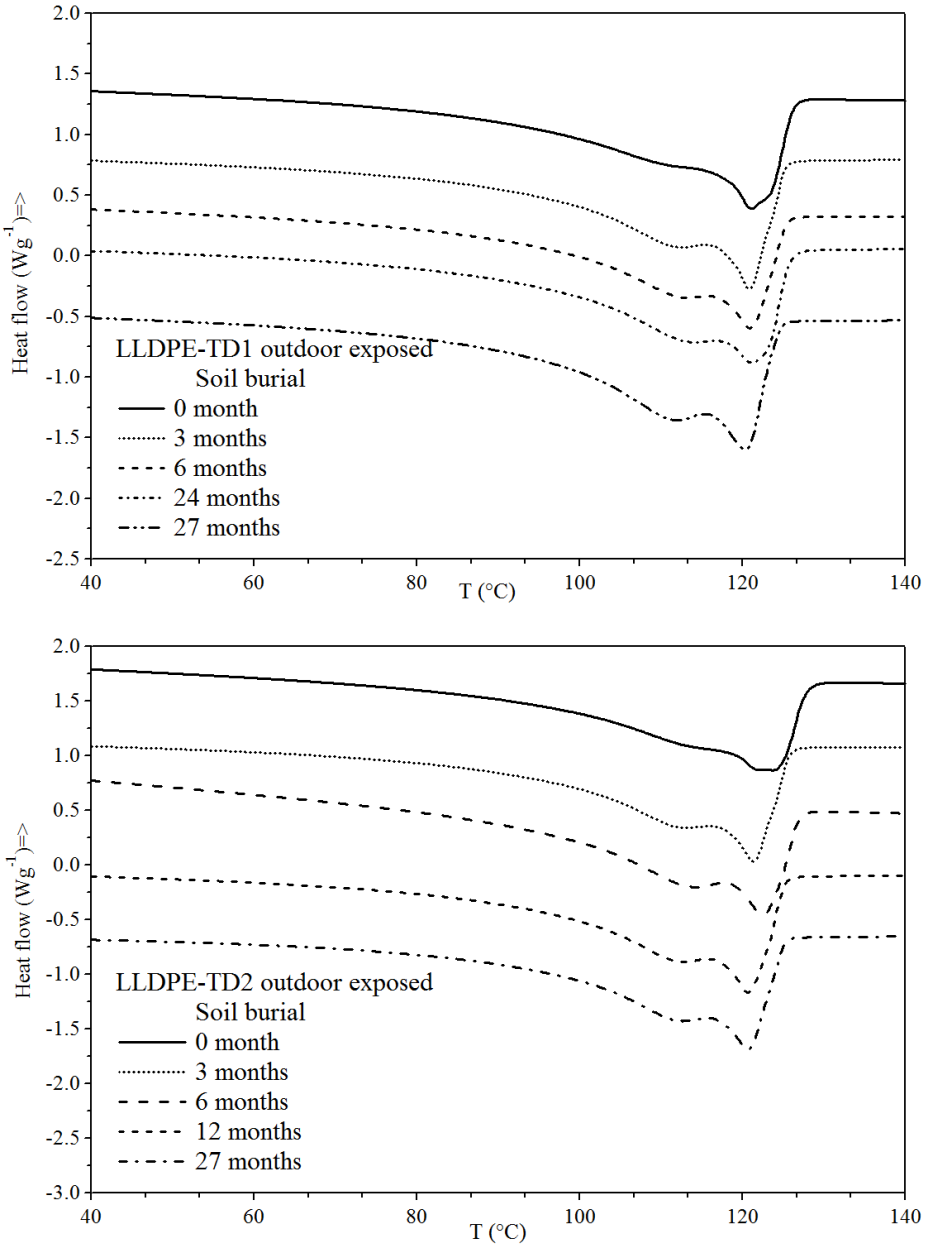

Figure 4.64. DSC traces in nitrogen atmosphere of outdoor exposed LLDPE-TD1 and LLDPE-TD2 samples specimen before and after different months of soil burial biodegradation test.

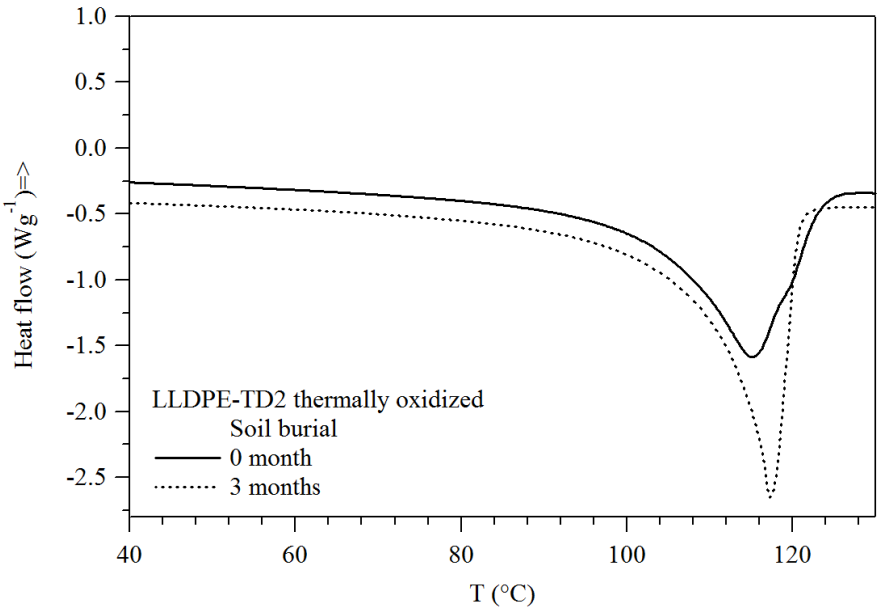

Figure 4.65. DSC traces in nitrogen atmosphere of outdoor exposed LLDPE-TD2 sample before and after 3 month of soil burial biodegradation test.

During an other soil burial respirometric test, the propensity to be assimilated by soil microorganisms of different polymeric materials was also ascertained. In particular, the biodegradation behaviours of LLDPE-TD1 and LLDPE-TD2 samples corresponding to the original untreated (e.g. pristine) materials, residues after acetone extraction of thermally oxidized specimen and specimen retrieved after thermal degradation in contact with soil, were compared with the soil biodegradation rate and extent of natural oxo-biodegradable polymeric materials. These latter were represented by natural rubber (NR), lignin extracted from pine seed shells (PNSL) and wheat straw (WS), which were utilized as potential reference compounds to be used in standard biodegradation tests. Most of these tests are, in fact, based almost exclusively on the determination of net evolved carbon dioxide and normally utilize hydro-biodegradable polymers such as cellulose and starch, as reference materials. Nevertheless, it has been repeatedly ascertained that the rate of

conversion to CO_2 of a carbon substrate is depending upon its chemical structure and formal level of oxidation. In the case of polysaccharide, for instance, the huge increase in the soil respiration rate has been evidenced, thus leading often to the overestimation of the biodegradation extent of glucosidic-like materials. This holds true by considering the relationship between the free-energy content of a carbon substrate (expressed as the standard free-energy of combustion) and its propensity to conversion to new microbial biomass rather than mineralization to CO_2, such as in the case of hydrocarbon materials [197]. Taking into account these considerations, it can be therefore suggested that standard soil biodegradation tests should utilize different reference materials representative of the two principal classes, hydro- and oxo-biodegradable, of carbon substrates. For that reason, cellulose or starch can be considered as appropriate reference materials for many hydro-biodegradable polymers, whereas NR and lignin should be utilized once the materials to be analyzed are requiring a preliminary oxidation step before to be utilized as carbon sources by soil microorganisms, such in the case of oxo-biodegradable polyolefins.

In the reported test, other than LLDPE, outdoor exposed PS-TD1 and thermally treated PP-TD1 samples were analyzed as representative of different oxidized full carbon backbone structures. In addition, hybrid materials constituted by LDPE/lignin blends (LDPE/L) and Ecoflex/PNSL blends were also investigated.

The average cumulative emissions recorded within 185 days of incubation at 28°C, from soil cultures supplemented with the analyzed polymeric materials and blanks are reported in Tables 4.26.

Table 4.26. Cumulative CO_2 emissions from soil cultures supplemented with LLDPE materials and blanks.

	Aged in soil		Pristine		ACE residue		Blank
Day	**LLDPE-TD2**	**LLDPE-TD1**	**LLDPE-TD2**	**LLDPE-TD1**	**LLDPE-TD2[(a)]**	**LLDPE-TD1[(a)]**	
5	9.7	7.9	7.9	7.0	8.8	7.9	8.8
10	15.0	14.1	14.1	13.2	15.0	14.1	12.3
15	21.1	19.4	20.2	21.1	22.0	19.4	17.6
21	31.7	29.9	31.7	32.6	33.4	29.9	29.0
27	38.7	35.2	37.0	37.8	39.6	36.1	35.2
35	45.8	41.4	42.2	43.1	44.9	41.4	41.4
42	49.3	44.9	44.9	46.6	48.4	44.9	44.9
50	60.7	54.6	55.4	55.4	57.2	52.8	53.7
60	62.5	60.7	58.1	57.2	59.8	57.2	56.3
69	68.6	68.6	62.5	62.5	66.0	63.4	61.6
81	77.4	78.3	70.4	69.5	72.2	70.4	67.8
95	88.9	88.0	79.2	77.4	80.1	79.2	77.4
110	96.8	103.8	87.1	83.6	87.1	88.9	86.2
125	107.4	121.4	97.7	95.9	101.2	98.6	101.2
152	120.6	145.2	117.0	126.7	119.7	123.2	118.8
185	133.8	165.4	132.9	142.2	136.4	135.2	135.5

(a) – Acetone extracted residue of thermally oxidized LLDPE.

From the data reported in Table 4.26 it was evident that thermally aged LLDPE-TD1 sample only experienced a significant conversion to CO2 by soil microorganisms, whereas the pristine materials and the residues to acetone extraction showed CO_2 emissions strictly comparable to the blanks. In this latter case it is to remark that the extraction procedure selectively removed the low molecular weight oxidized fraction from thermally aged materials, thus hindering the microbial attack of the LLDPE matrix residue to the extraction.

The higher propensity to mineralization in soil of hydro-biodegradable materials was confirmed by the data reported in Table 4.27. In particular an high extent of mineralization was ascertained in the case of filter paper

in a relatively short tiem of incubation. High level of CO_2 emission was also recorded in the case of the wheat straw sample which is containing fairly high amount of cellulose, as well as in the case of synthetic polyester Ecoflex. In this latter case, however is remarkable to note that the addition of PNSL lignin seems to induce acceleration in the mineralization rate most likely because the higher surface to volume ratio typically induced by the presence of fairly high amount of fillers in immiscible hybrid materials. Significantly high levels of conversion to CO2 by soil microorganisms were also recorded in the case of thermally oxidized PP-TD1 sample, whereas photodegraded PS-TD1 sample was recalcitrant to the microbial attack at least within the 185 days of incubation.

Table 4.27. Cumulative CO_2 emissions from soil cultures supplemented with different oxo-biodegradable materials, filter paper (FP) and blanks.

Day	Ecoflex	Ecoflex/ PNSL	LDPE/L	PP-TD1	PS-TD1	PNSL	NR	WS	FP	Blank
5	9.7	8.8	21.1	13.2	9.7	14.1	8.8	28.2	9.7	8.8
10	17.6	16.7	32.6	22.9	17.6	22.9	17.6	59.8	22.9	12.3
15	24.6	25.5	40.5	31.7	23.8	30.8	25.5	84.5	40.5	17.6
21	37.0	39.6	53.7	47.5	33.4	44.0	37.8	111.8	59.0	29.0
27	44.0	49.3	61.6	57.2	38.7	51.0	45.8	128.5	73.9	35.2
35	51.0	60.7	72.2	66.9	44.0	58.1	57.2	147.8	95.9	41.4
42	55.4	70.4	79.2	71.3	46.6	62.5	65.1	162.8	110.9	44.9
50	66.0	86.2	92.4	84.5	55.4	72.2	80.1	186.6	130.2	53.7
60	70.4	96.8	97.7	92.4	57.2	74.8	88.0	203.3	148.7	56.3
69	77.4	110.9	106.5	100.3	63.4	81.8	98.6	217.4	168.1	61.6
81	88.9	130.2	117.0	110.9	72.2	89.8	112.6	234.1	198.0	67.8
95	102.1	151.4	128.5	122.3	81.8	102.1	128.5	270.2	233.2	77.4
110	116.2	170.7	140.8	135.5	90.6	115.3	144.3	306.2	268.4	86.2
125	133.8	191.8	154.9	149.6	106.5	130.2	166.3	344.1	301.0	101.2
152	173.4	219.1	173.4	169.0	128.5	172.5	200.6	382.8	342.3	118.8
185	202.4	244.6	191.0	187.4	151.4	207.7	237.6	426.8	381.0	135.5

Finally, it is interesting to note that the CO_2 emission profiles of NR and PNSL natural oxo-biodegradable materials were similar to that recorded in the case of LLDPE and LDPE/L based materials (Figure 4.66), thus revealing overall slower rates of conversion to CO_2. These evidences were therefore suggesting that oxidized polyolefins undergoes biodegradation processes in soil that are involving microbial populations and biochemical pathways similar to those that are responsible for lignin and natural rubber biodegradation, as previously suggested in the case of untreated LDPE [34].

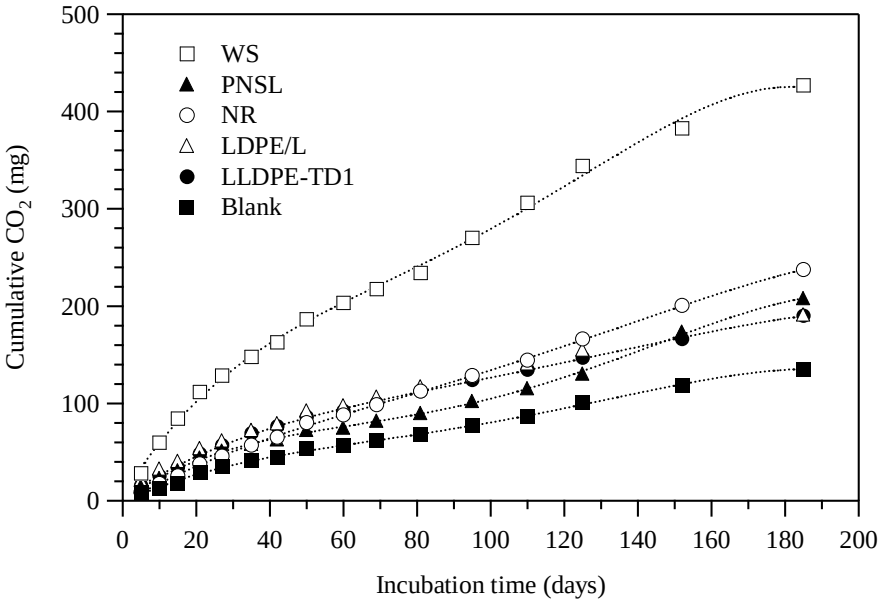

Figure 4.66. CO_2 average cumulative emissions from soil cultures supplemented with oxo-biodegradable materials.

4.3. Studies on the Interaction of Microbes and Polymeric Materials

The final part of the research activity within the doctorate course has been dedicated on the investigations of the role of microorganisms in the direct or synergistic degradation activity that can be exerted on the structural properties of full carbon backbone polymers. During the biodegradation tests carried out in soil, it was evidenced that the analyzed oxo-biodegradable polyolefins, particularly LLDPE samples, underwent further oxidation other than microbial mineralization in the case of heavily oxidized specimens. In accordance, investigations were carried out by using single microbial (e.g. fungal species) previously isolated from soil cultures amended with thermally oxidized LDPE samples [198]. The isolated strains were therefore utilized in single cultures with the aim to assess their capability to directly attack the polyolefin matrix.

4.3.1. *Preliminary biodegradation tests with single fungal strain in agar plates*

In a preliminary investigation, LLDPE sample specimens collected from the outdoor exposure test were submitted to a screening test undertaken in order to asses the effect of the microbial activity on the LLDPE samples characterized by a moderate level of oxidation. The specimens were aseptically transferred onto the agar surface in Petri dishes, after that the plates were inoculated with four different fungal strains (F1, F2, F3, F4) previously isolated from oxo-degradable LDPE fragments withdrawn after two years of soil burial incubation. Each outdoor exposed specimen, as well as the unaged specimen (control specimens of both LLDPE-TD1 and LLDPE-TD2 samples), were inoculated separately with a single fungal strain and incubated under the same conditions.

Different growing behaviors of the selected fungal strains were thus recorded, as ranging between the entirely colonization of LLDPE specimen surface, to the growth almost exclusively concentrated onto the agar surface free from the LLDPE specimens (Figure 4.67 a and b).

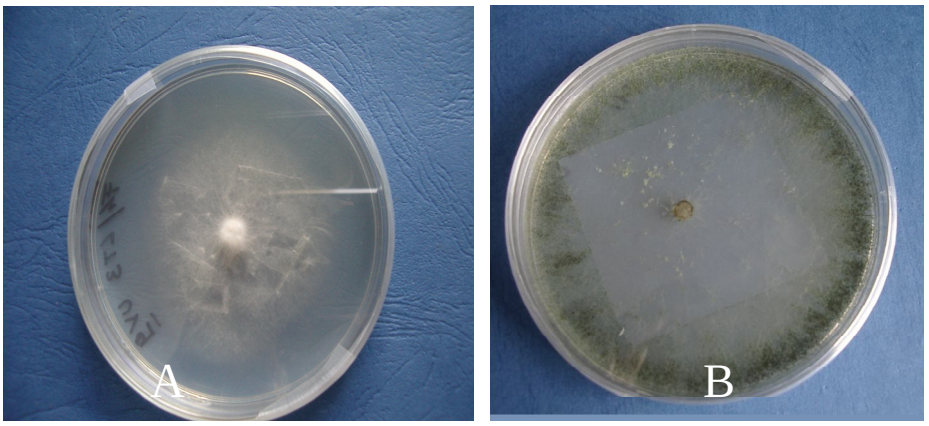

Figure 4.67. F2 (left picture) and F3 (right picture) fungal growth on the LLDPE-TD1 films pre-exposed to sunlight. The incubation period comprised of 20 days.

In order to verify if the different growing behaviors were related to different metabolic activity, after 20, 45 and 65 days of incubation, the film specimens were withdrawn from the agar plates and characterized by FT-IR spectroscopy and relevant determination of the level of oxidation by means of carbonyl index. The recorded data evidenced an appreciable decrease of the CO*i* values, thus suggesting that some of the selected fungal strains, particularly F2 and F4 strains, were capable to growth and assimilate the oxidized fractions present in the outdoor treated LLDPE samples (Figure 4.68). Nevertheless, in some cases also a slight increase of the CO*i* value was recorded within the incubation time, thus suggesting that the metabolic activity of the fungal strains under the adopted test conditions, may affect the structural properties of the LLDPE matrix. In particular, as reported in Figure 4.69, the fragmentation of the LLDPE

matrix in the proximity of the F2 fungal mycelium growing onto the film specimen was observed.

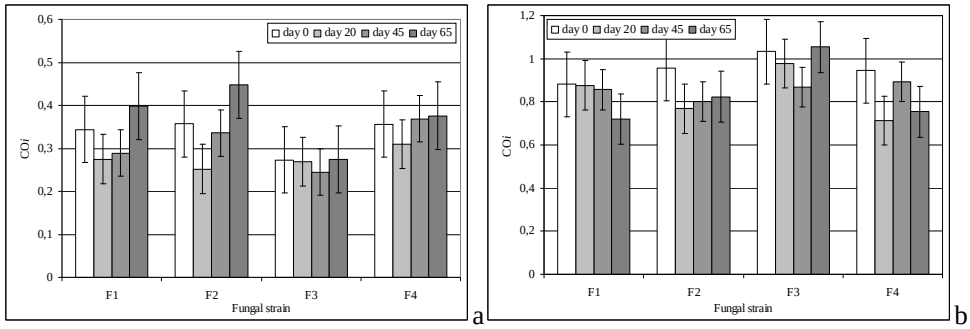

Figure 4.68. CO*i* profiles of outdoor exposed LLDPE-TD1 (a) and LLDPE-TD2 (b) films incubated with fungal F1, F2, F3 and F4 in agar plates.

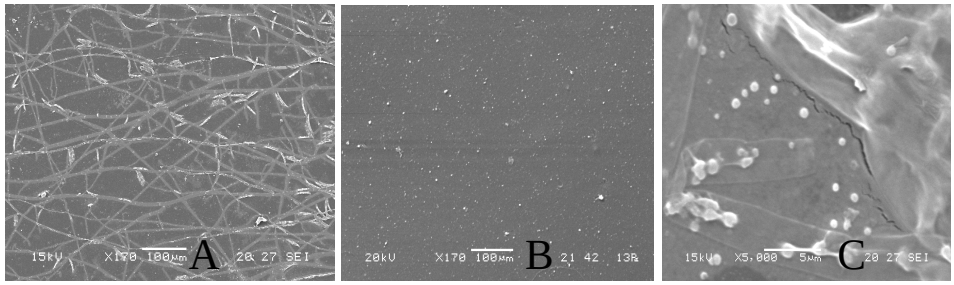

Figure 4.69. SEM micrographs of the F2 (A) mycelium growing on the surface of outdoor exposed LLDPE-TD2 specimens. F3 spore-forming fungus which preferred not to colonize the surface (B) but grew around the periphery of the film. The C is a higher magnification of A, showing fungal hyphae colonizing the film surface.

These observations have been further substantiated by the FT-IR characterization, carried out after the careful cleaning of the treated samples. The presence of absorption bands attributable to the formation of double bonds (ν C=C 1640-1580 cm^{-1})within the polymer chains was indeed appreciated as in the case of LLDPE-TD2 specimens incubated with the F2 and F4 fungal strains (Figure 4.70 and 4.71). In addition, new

absorption bands most likely attributable to the presence of oxidized functional moieties, such as hydroxyl groups, were recorded in the FT-IR spectra of the specimen incubated with F2 and F4 fungal strains. In this latter case the overall oxidation of the specimen after 65 days of incubation was particularly evident, thus indicating a sharp oxidizing metabolic activity of F4 fungal strain toward the LLDPE matrix.

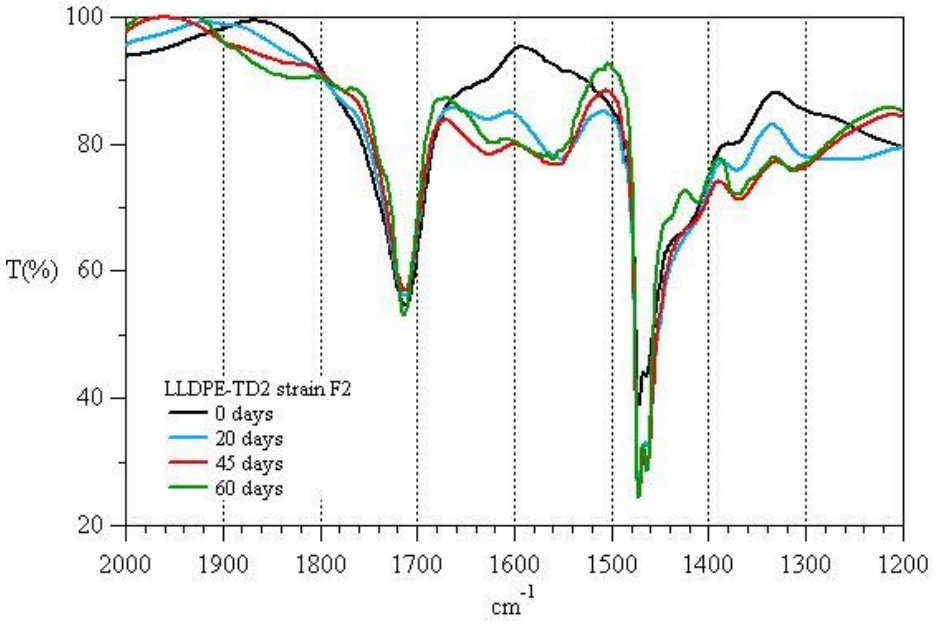

Figure 4.70. FT-IR spectral region of carbonyl and vinylidene groups of LLDPE-TD2 sunlight exposed specimen incubated in the presence of F2 fungal strain.

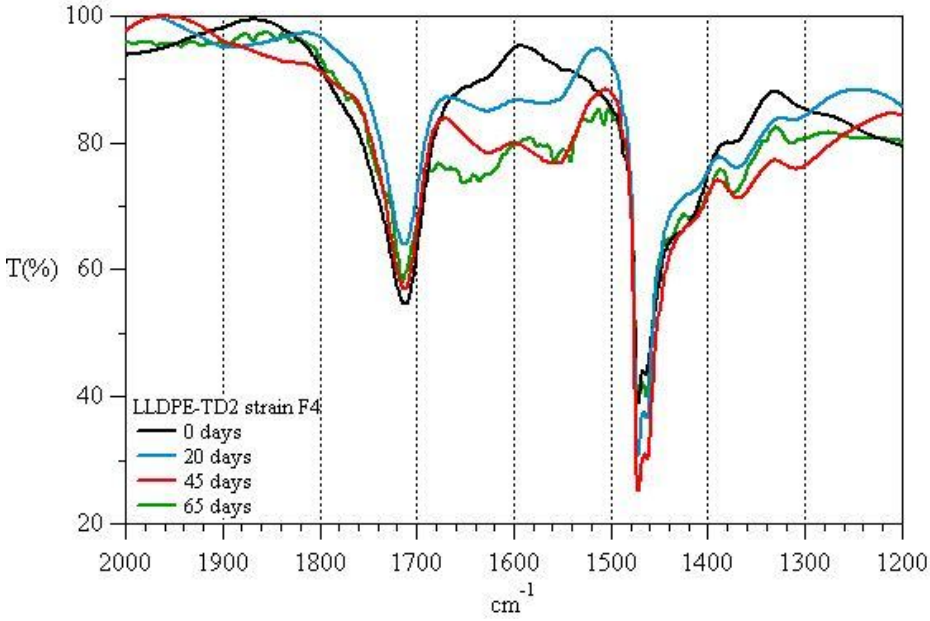

Figure 4.71. FT-IR spectral region of carbonyl and vinylidene groups of LLDPE-TD2 sunlight exposed specimen incubated in the presence of F4 fungal strain.

The whole FT-IR spectra of the tested samples in the presence of the selected fungal strains recorded at the beginning and after 65 days of incubation are reported in Figures 4.72-4.75.

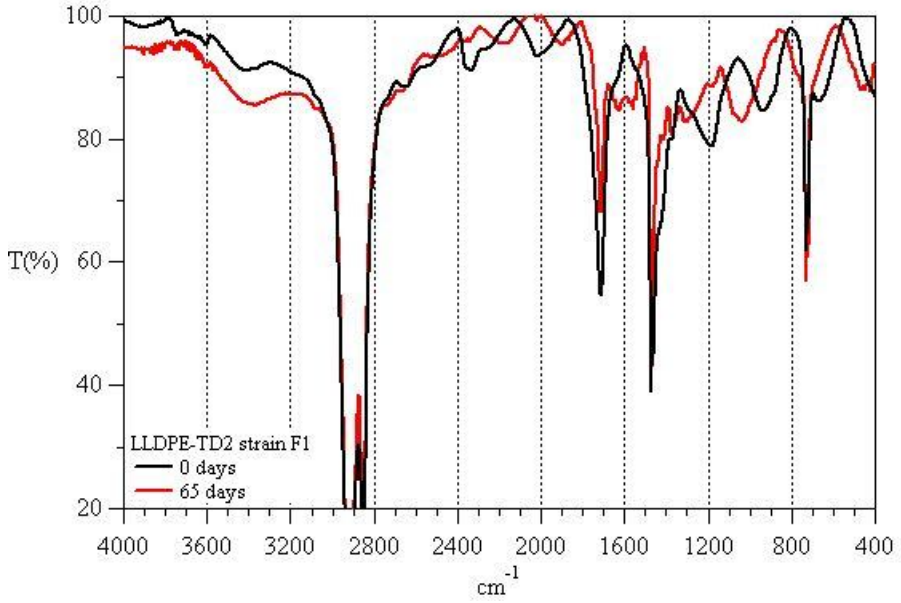

Figure 4.72. FT-IR spectra of LLDPE-TD2 specimen incubated with F1 strain.

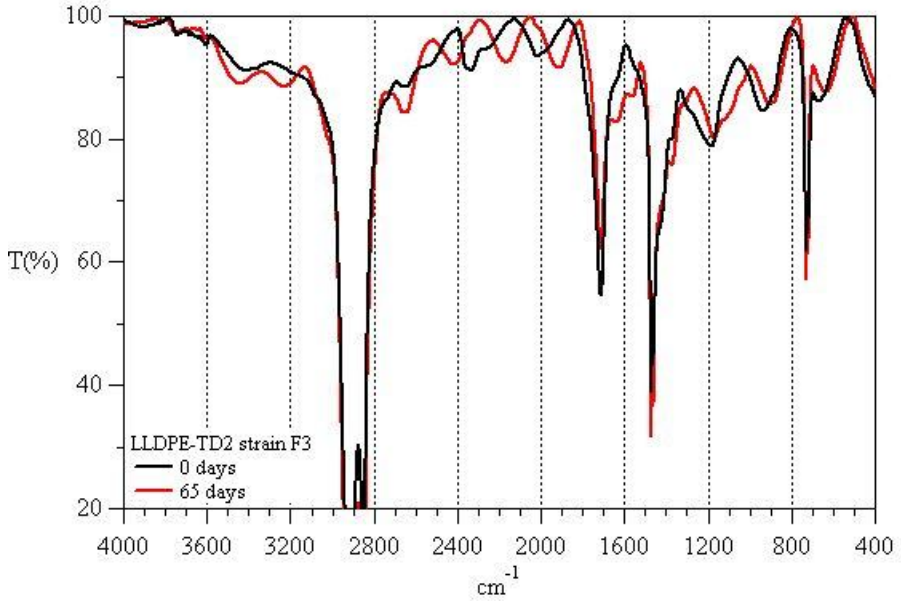

Figure 4.73. FT-IR spectra of LLDPE-TD2 specimen incubated with F3 strain.

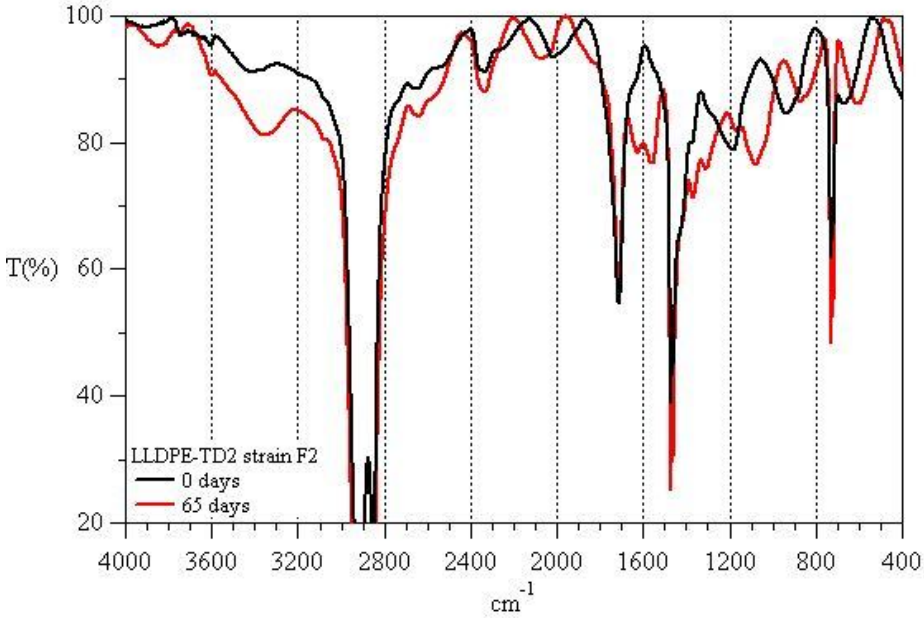

Figure 4.74. FT-IR spectra of LLDPE-TD2 specimen incubated with F2 strain.

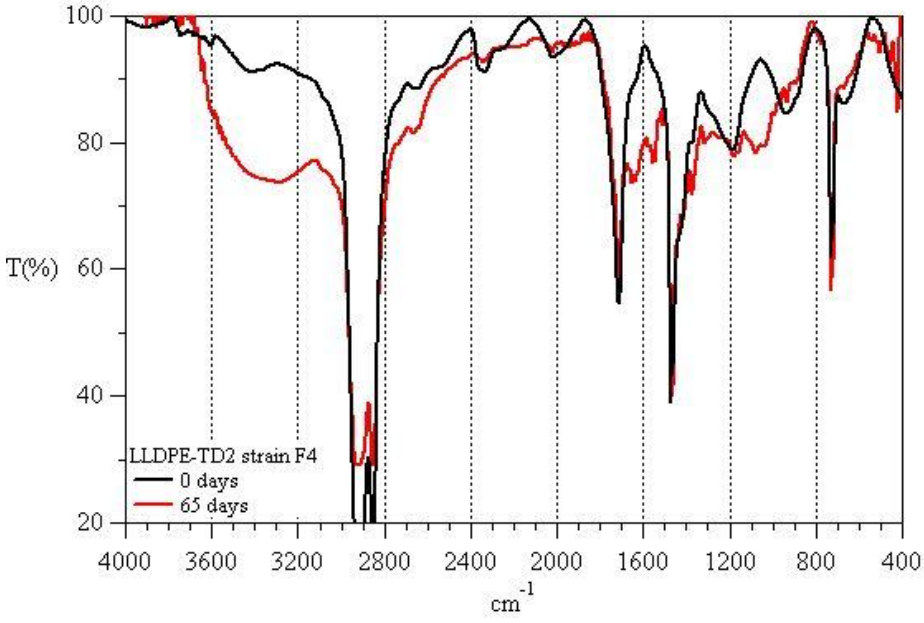

Figure 4.75. FT-IR spectra of LLDPE-TD2 specimen incubated with F4 strain.

The reported observation was confirmed in the case of LLDPE-TD1 outdoor exposed specimen (Figure 4.76-4.79). It was indeed observed that only in the FT-IR spectra of the LLDPE-TD1 specimen incubated with F4 strain an appreciable absorption in the OH region can be detected after 60 days of incubation (Figure 4.79). These results evidenced that within the tested microorganisms, those capable to a plentiful colonization of the film surfaces were also capable to promote a further oxidation of the polymer chains.

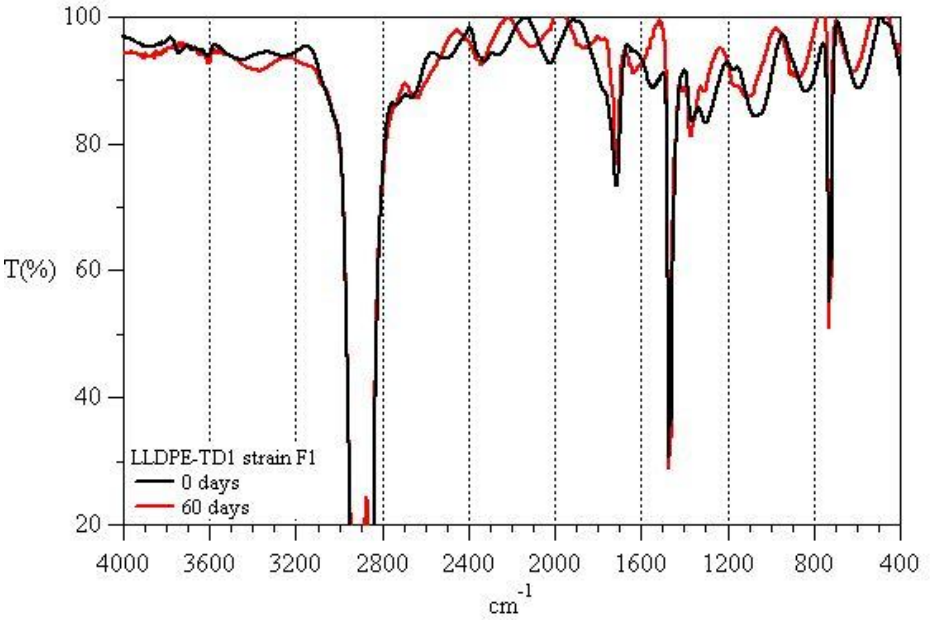

Figure 4.76. FT-IR spectra of LLDPE-TD1 specimen incubated with F1 strain.

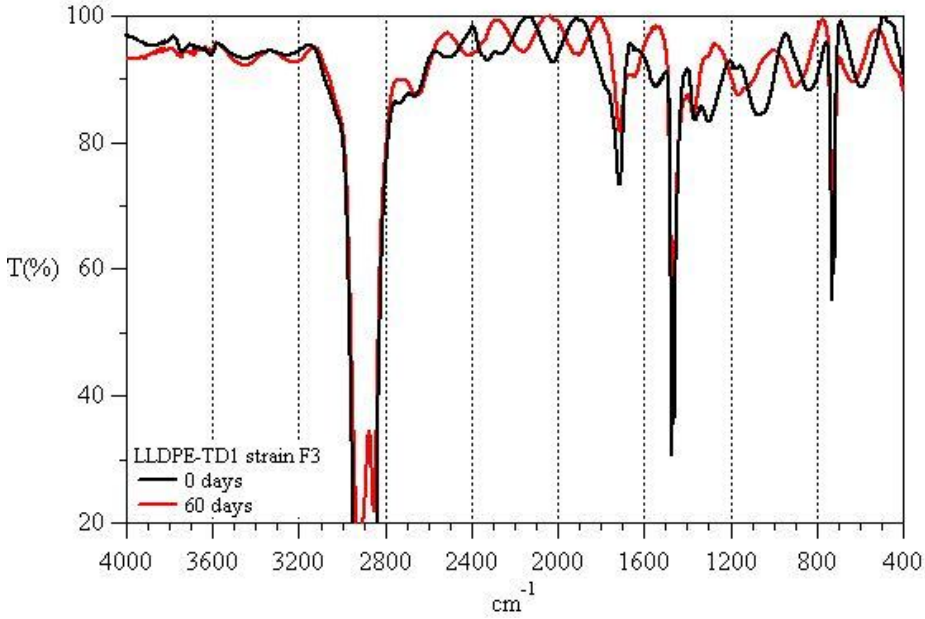

Figure 4.77. FT-IR spectra of LLDPE-TD1 specimen incubated with F3 strain.

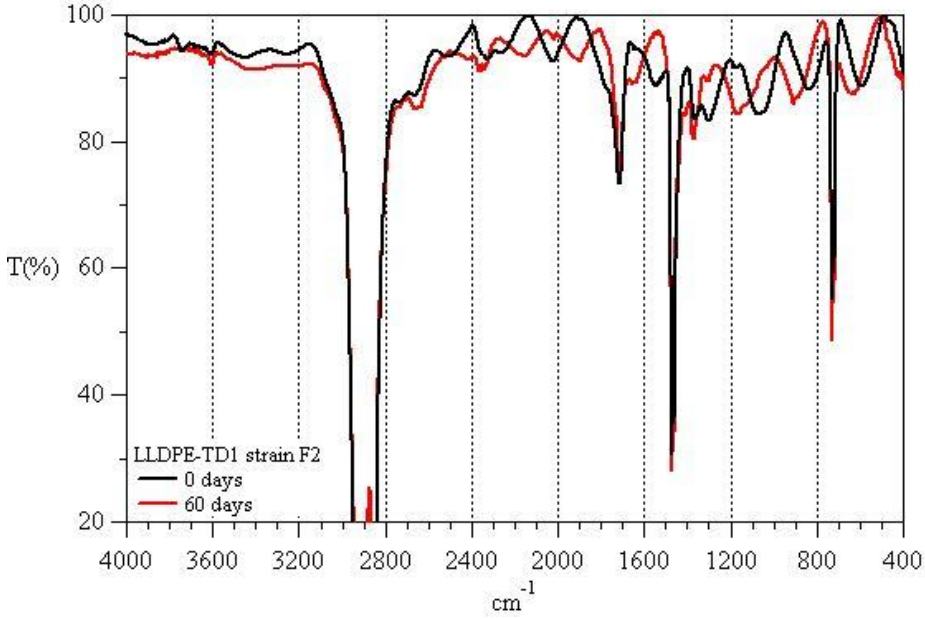

Figure 4.78. FT-IR spectra of LLDPE-TD1 specimen incubated with F2 strain.

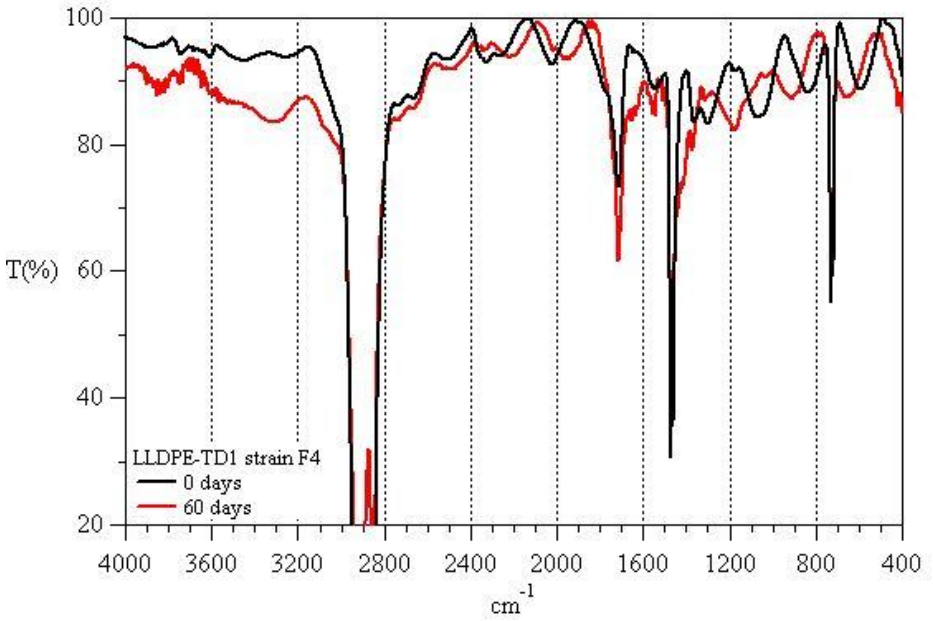

Figure 4.79. FT-IR spectra of LLDPE-TD1 specimen incubated with F4 strain.

All the tested specimen submitted to the incubation on agar plates in the presence of the selected fungal strains and the corresponding un-inoculated controls were also characterized by thermal gravimentric analysis (TGA) and DSC at the end of the test.

TGA profiles of outdoor exposed LLDPE-TD2 specimens treated with different fungal strains are reported in Figures 4.80-4.87 along with the relevant un-inoculated control incubated up to 65 days under the same conditions. The thermal decomposition temperatures corresponding to 2 % weight loss and the corresponding weight residues at 600°C, calculated from the TGA profiles are reported in Table 4.28.

It was ascertained, in accordance with the FT-IR characterization, that the thermal stability of outdoor exposed LLDPE-TD2 sample specimen clearly decreased as a consequence of the degrading activity of the selected fungal strains with respect to the un-inoculated control. It was

also confirmed by the TGA analysis that F2 and F4 fungal strains resulted the most effective in promoting the ongoing biotic degradation of the analyzed specimens (Table 4.28, Figures 4.80-3.87).

TGA characterization, also confirmed that outdoor exposed LLDPE-TD1 specimens were less prone to the fungal metabolism under the adopted incubation conditions. In these latter case, the thermal degradation profiles of the specimen withdrawn after the incubation with the selected microbial strains were indeed much more comparable with the corresponding un-inoculated controls (Figures 4.85-4.87, Table 4.28).

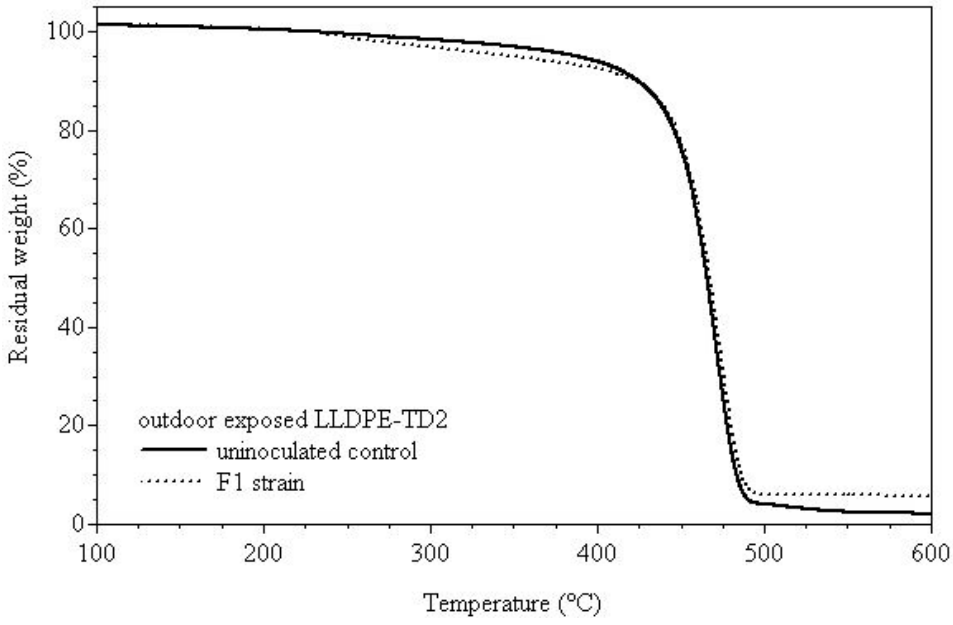

Figure 4.80. TGA traces of LLDPE-TD2 specimen incubated with F1 strain.

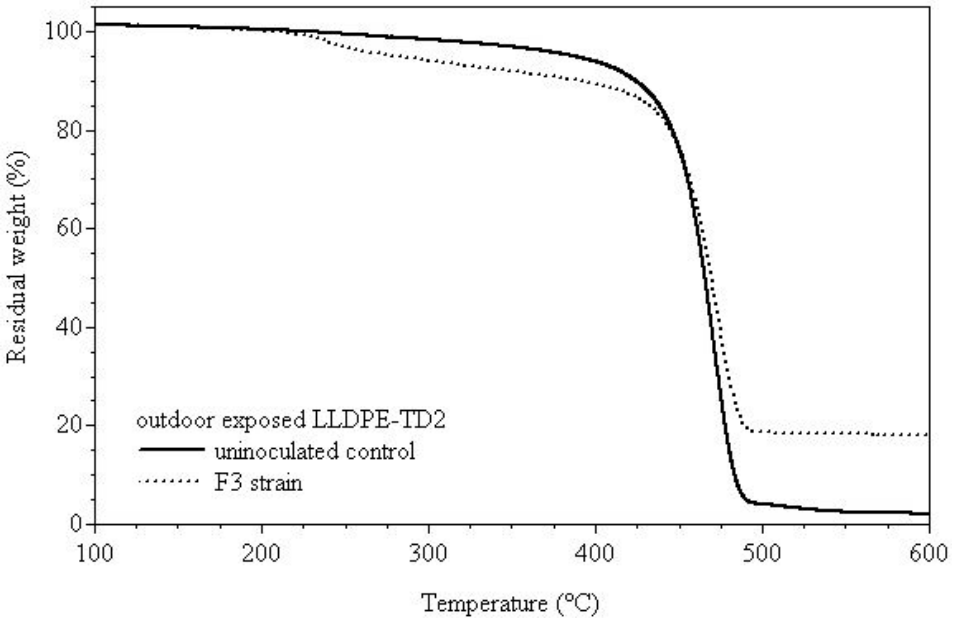

Figure 4.81. TGA traces of LLDPE-TD2 specimen incubated with F3 strain.

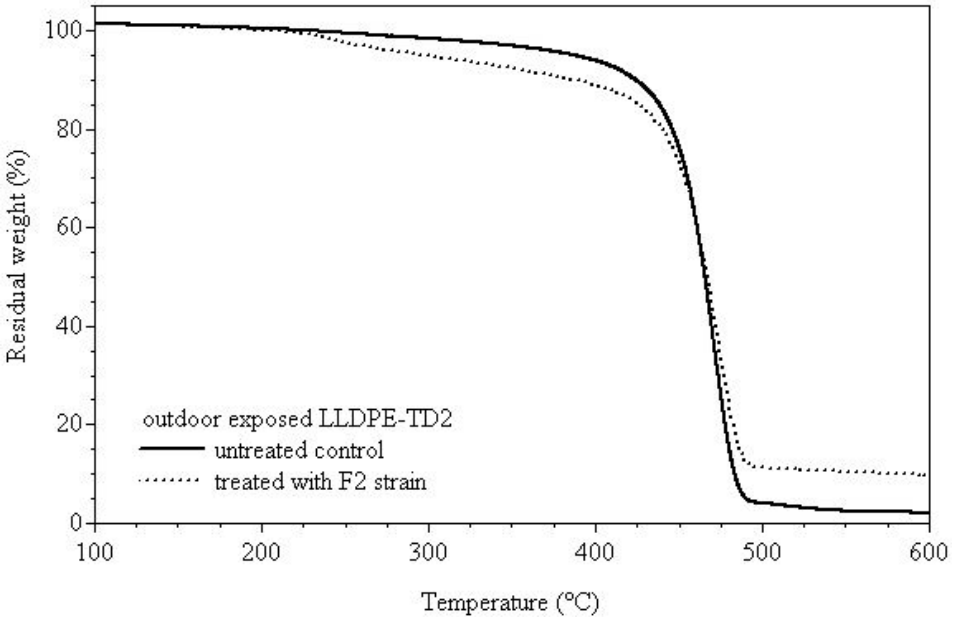

Figure 4.82. TGA traces of LLDPE-TD2 specimen incubated with F2 strain.

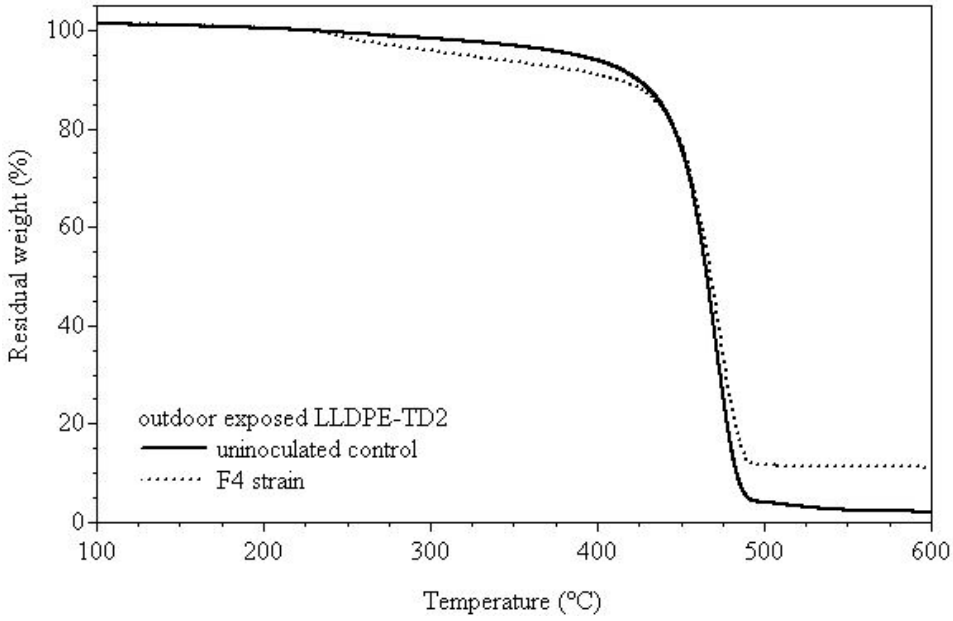

Figure 4.83. TGA traces of LLDPE-TD2 specimen incubated with F4 strain.

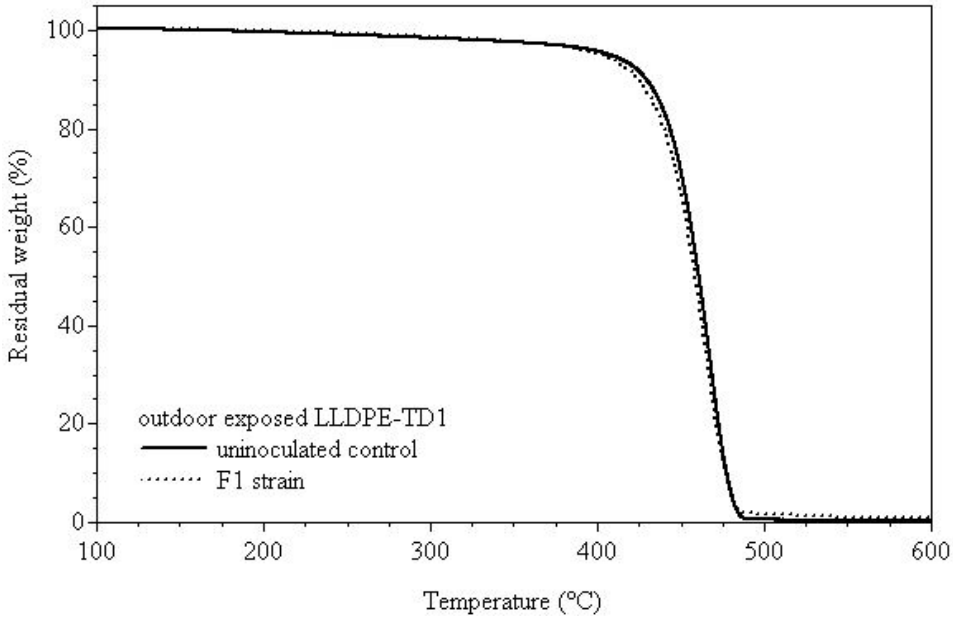

Figure 4.84. TGA traces of LLDPE-TD1 specimen incubated with F1 strain.

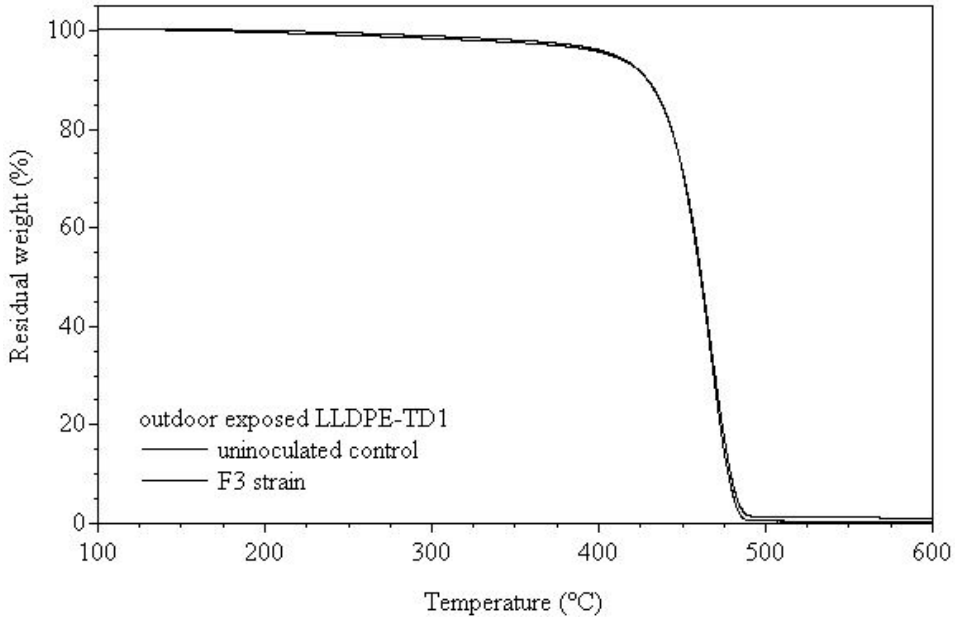

Figure 4.85. TGA traces of LLDPE-TD1 specimen incubated with F3 strain.

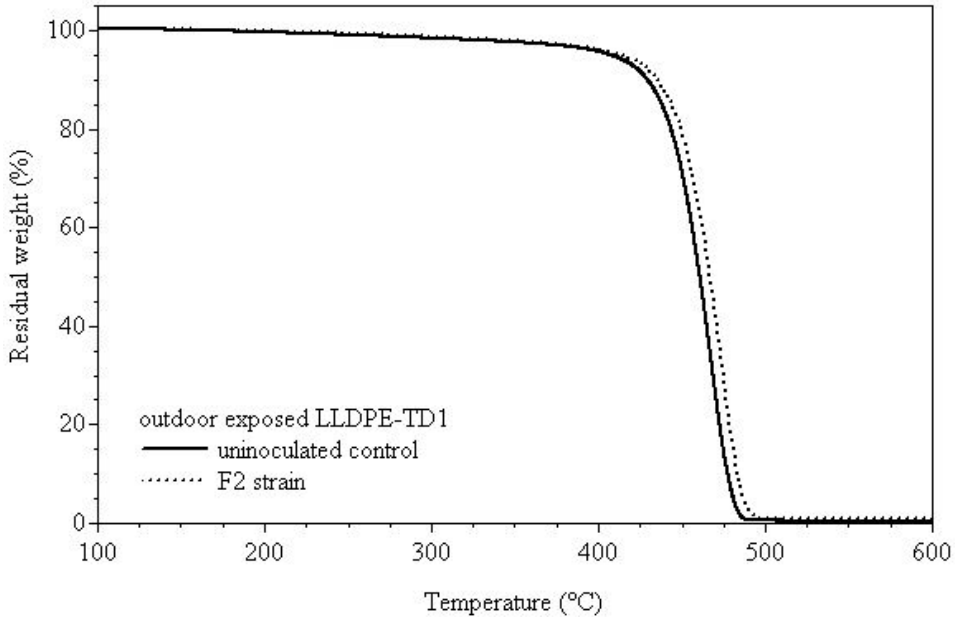

Figure 4.86. TGA traces of LLDPE-TD1 specimen incubated with F2 strain.

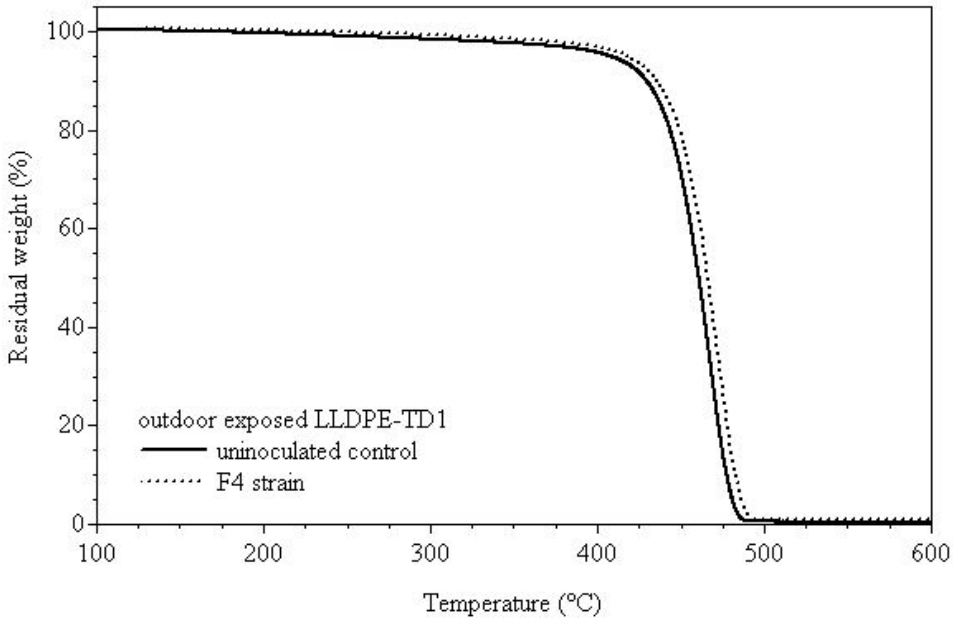

Figure 4.87. TGA traces of LLDPE-TD1 specimen incubated with F4 strain.

Table 4.28. Thermal properties of outdoor exposed LLDPE-TD1 and LLDPE-TD2 samples submitted to biodegradation in agarized medium in the presence of selected fungal strains, as recorded by TGA analysis.

specimen	LLDPE-TD2		LLDPE-TD1	
	T_{ON}	Residue at 600°C	T_{ON}	Residue at 600°C
	(°C)	(%)	(°C)	(%)
pristine	422.9	1.0	400.6	0.2
UV[a)]	304.1	1.9	353.5	0.8
UV[a)]-Control	263.8	0.7	325.8	0.3
UV[a)]-F1	245.7	5.6	335.9	0.8
UV[a)]-F2	227.4	7.2	327.2	1.0
UV[a)]-F3	233.6	12.5	378.7	1.1
UV[a)]-F4	229.5	9.4	369.5	0.9

a) After 93 days outdoor exposure

4.3.2. *Biodegradation respirometric test in aqueous medium in the presence of pure culture of F2 fungal strain*

In order to confirm the evidences relevant to the microbial oxidative attack of LLDPE matrix exerted particularly by F2 fungal strain, biodegradation tests were carried out in aqueous cultures of the chosen strain, in mineral salt medium, supplemented with thermally oxidized, sunlight exposed and pristine specimens of LLDPE-TD1 samples as sole carbon and energy sources.

The microbial inoculum represented by F2 fungal strain was prepared by cultivating the microorganisms on Potato Dextrose Agar (PDA) plates. A 0.5 cm diameter agar disk, taken from PDA plates, was used to inoculate each test flask. All the test materials were supplied to the microbial cultures as sole carbon sources at approximately 0.05 % by weight concentration.

Test flasks were incubated at room temperature (25°C) in the dark on a rotatory shaker (120 rpm).

The average cumulative CO_2 emissions profiles recorded from test cultures and blanks are reported in Figure 4.88.

The attained results confirmed the capability of F2 fungal strain to assimilate as sole carbon source the oxidized fraction of abiotically pre-aged LLDPE-TD1 specimens in aqueous mineral salt medium, the highest CO_2 emissions being recorded in the fungal cultures supplemented with thermally oxidized specimens, thus showing a positive slope in the relevant profile after 160 days of incubation (Figure 4.88). The propensity to be assimilated as carbon source of outdoor exposed LLDPE-TD1 specimen with respect to the thermally aged specimens by the F2 fungal strain was also observed, even though in a lower extent, whereas no appreciable differences in the overall CO_2 emissions from pristine and blanks samples were observed (Figure 4.88).

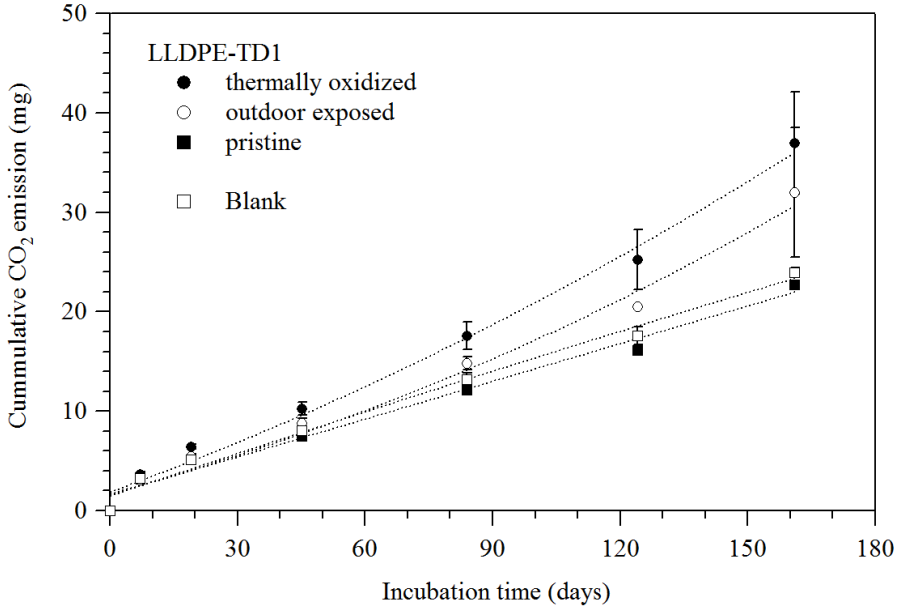

Figure 4.88. Average cumulative CO_2 emission from F2 fungal strain aqueous cultures supplemented with pristine and abiotically pretreated LLDPE-TD1 specimens.

With respect to other biodegradation tests carried out previously within the present research activity, where the assimilation of the oxidized fraction from abiotically aged samples was usually accompanied by a slight decrease of the starting CO*i* values, in this test the overall level of oxidation does appear to increase as a consequence of the incubation in the presence of F2 strain. In particular an average increase of the CO*i* from 4.92 at the beginning to 5.08 after 161 days of incubation was observed in the case of thermally oxidized specimen. This effect was much more evident in the case of outdoor exposed LLDPE-TD1 specimens, thus recording an average increase of the relevant CO*i* from 0.40 to 1.03 as a consequence of the incubation in F2 strain aqueous cultures (Table 4.29). The results collected in Table 4.29 evidenced that the metabolic activity of the selected fungal strain was promoting significant differences in the thermal stability of thermally oxidized, outdoor exposed and pristine LLDPE-TD1 specimens. In particular, the

temperatures corresponding to 2% weight loss (T_{ON}) in the thermally oxidized specimens increased from 192.4 to 250.2°C before and after biodegradation, respectively (Table 4.29, Figures 4.89). On the contrary, the thermal stability of outdoor exposed samples was shown to decrease after the incubation in the aqueous culture of F2 fungal strain. In accordance the T_{ON} temperatures of sample decreased from 353.5 to 344.2°C before and after biodegradation, respectively (Table 4.30, Figures 4.89). Finally, in the case of pristine samples submitted to the aqueous culture with single F2 fungal strain that thermal stability did not change significantly (Table 4.30, Figure 4.90).

Table 4.29. Weight variation and carbonyl index data of pristine, thermally oxidized and outdoor aged LLDPE-TD1 specimens exposed to F2 fungal strain in aqueous medium.

Run	**Test sample**	Aqueous biodegradation test with F2 fungus				
		Initial weight	Final weight	Weight loss	CO*i*	
		(mg)	(mg)	(mg)	Starting	Final
A1	LLDPE-TD1_TD[a)]	51.6	42.4	9.2	4.78	4.74
A2	LLDPE-TD1_TD [a)]	53.7	34.0	19.7	5.1	4.92
A3	LLDPE-TD1_TD [a)]	57.5	37.3	20.2	5.1	5.08
	Average	***54.3***	***37.9***	***16.4***	***4.92***	***4.91***
B1	LLDPE-TD1_UV [b)]	61.4	59.8	1.6	0.4	1.03
B2	LLDPE-TD1_UV [b)]	54.8	54.0	0.8	0.44	0.63
B3	LLDPE-TD1_UV [b)]	50.8	56.5	-5.7	0.66	0.48
	Average	***55.7***	***56.8***	***-1.1***	***0.5***	***0.7***
C1	LLDPE-TD1_pristine	56.2	56.8	-0.6	0.09	0.01
C2	LLDPE-TD1_pristine	51.7	51.7	0.0	0.01	0.01
C3	LLDPE-TD1_pristine	54.2	56.5	-2.3	0.02	0.01
	Average	***54.0***	***55.0***	***-1.0***	***0.0***	***0.0***

a) After 230 days at 65°C in ventilated oven; b) After 93 days outdoor exposure

Table 4.30. TGA results of pristine, thermally oxidized and outdoor aged LLDPE-TD1 specimens exposed to F2 fungal strain in aqueous medium.

LLDPE-TD1	Incubation time	T_{ON}	Residue at 600°C	CO*i*
specimen	(days)	(°C)	(%)	
pristine	0	400.6	0.24	0.11
pristine	160	416.0	1.23	0.01
TD[a)]	0	192.4	2.43	5.00
TD[a)]	160	250.2	5.64	4.90
UV [b)]	0	353.5	0.88	0.50
UV [b)]	160	344.2	4.58	0.70

a) After 230 days at 65°C in ventilated oven; b) After 93 days outdoor exposure

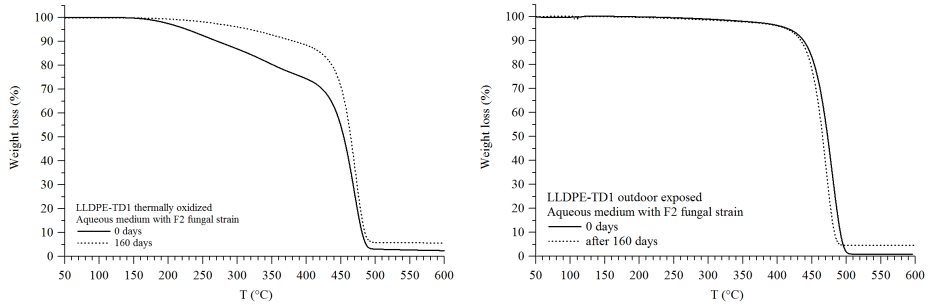

Figure 4.89. TGA traces of thermally oxidized (a) and outdoor exposed (b) LLDPE-TD1 specimens before and after incubation in aqueous medium with F2 fungal strain.

In any case, the structural changes in the polymer matrix as a consequence of the incubation with fungal strain, suggested by TGA characterization, were also evidenced by DSC analysis carried out on the same specimens (Table 4.31).

Noteworthy increase of the degree of crystallinity in the second heating was recorded in all the abiotically aged specimens exposed to F2 fungal strain metabolism, most likely because the preferential assimilation of low molecular weight compounds formed during thermal and outdoor exposure (Table 4.31, Figure 4.90). On the contrary, no significant

differences were recorded in the case of pristine specimens before and after the biodegradation test.

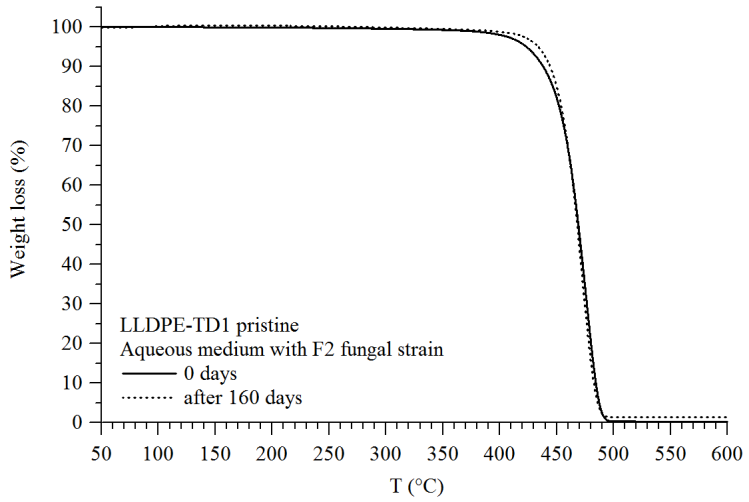

Figure 4.90. TGA traces of pristine LLDPE-TD1 specimens before and after incubation in aqueous medium with F2 fungal strain.

Table 4.31. DSC results of pristine, thermally oxidized and sunlight treated samples exposed single F2 fungal strain in aqueous medium.

LLDPE-TD1	**Incubation time**	**1st heating**		**2nd heating**	
specimen	(days)	Tm[c)]	Cristallinity	Tm[c)]	Cristallinity
		(°C)	(%)	(°C)	(%)
pristine	0	115.0, 121.8	42.7	110.9, 122.3	44.7
pristine	160	115.9; 121.7	42.9	111.2; 122.9	44.9
TD[a)]	0	118.2	54.9	115.9	52.6
TD[a)]	160	117.2	59.5	116.2	55.5
UV[b)]	0	116.0, 121.8	48.5	121.1	46.3
UV[b)]	160	115.3; 121.1	58.0	110.6; 121.2	57.7

a) After 230 days at 65°C in ventilated oven; b) After 93 days outdoor exposure; c) Melting peak

It is worth noting that the thermally oxidized samples before and after incubation onto F2 culture showed a single peak of melting whereas in the pristine and outdoor exposed samples, two distinct peaks appeared. As formerly commented, the aging at 65°C of the LLDPE samples resulted in an annealing treatment leading to an increase in the crystallinity level. The increase of crystallinity of the outdoor exposed samples with respect to the pristine specimens is not easily understandable. However, by considering the exposure of the samples during the summer season, occurring peaks of temperature might be considered responsible of an annealing effect (Figure 4.91).

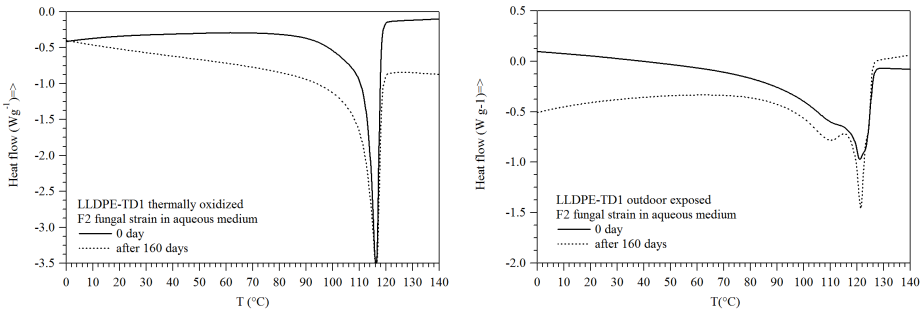

Figure 4.91. DSC traces of thermally oxidized (a) and outdoor exposed (b) LLDPE-TD1 specimens before and after incubation in aqueous medium with F2 fungal strain.

Finally, the progress in the polymer chain degradation of abiotically pre-aged and pristine LLDPE-TD1 sample incubated with F2 fungal strain in aqueous medium was also substantiated by FT-IR analysis. The presence of absorption bands as attributable to oxidized functional groups were therefore observed particularly in thermally oxidized, as well as in outdoor exposed specimens after the incubation with the fungal strain (Figure 4.92).

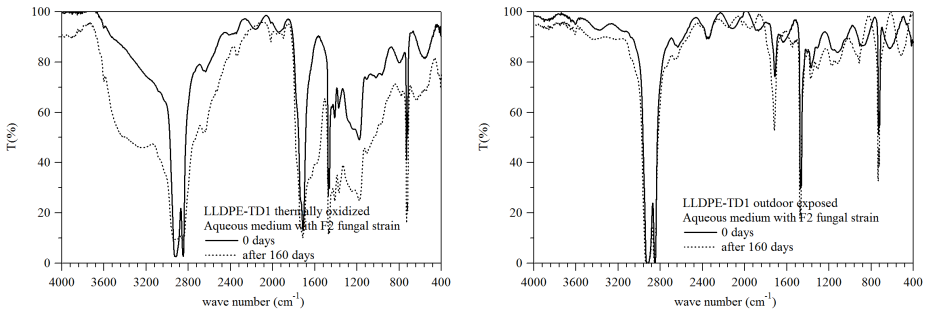

Figure 4.92. FT-IR spectra of thermally oxidized (a) and outdoor exposed (b) LLDPE-TD1 specimens before and after incubation in aqueous medium with F2 fungal strain.

The collected results were therefore substantiating that soil-borne fungal F2 strain is capable to assimilate as sole carbon source, the oxidized fractions of abiotically aged LLDPE LLDPE-TD1 samples, thus approaching mineralization level as depending upon the starting CO*i* value. At higher CO*i* values, a corresponding higher mineralization level was recorded. Some suggestions on the ongoing oxidative degradation of LLDPE polymer matrix as induced by the action of the selected microorganism were also collected.

4.3.3. *Mineralization test onto solid media in the presence of axenic cultures of isolated microorganisms under co-metabolic conditions.*

It is well known that the degrading activity of several microorganisms toward hardly metabolizable organic compounds, such as lignin, can be improved by the presence of small amounts of easily assimilable carbon substrate (e.g. glucose) while limiting other nutrients such as nitrogen or phosphorous compounds [199]. In this connection a mineralization test aimed at evaluating the capability of a fungal strain to degrade and assimilate LLDPE samples under co-metabolic conditions was carried out by using the F2 fungal strain and thermally oxidized, outdoor exposed

and pristine LLDPE-TD1 specimens. The microbial strain was chosen by considering the results attained in previous biodegradation tests.

During the test, the CO_2 productions from the test cultures were monitored within the time (Figure 4.93) whereas at the end the test, samples were submitted to structural characterizations by means of FT-IR spectroscopy and thermal analysis.

Considerable differences in the CO_2 emission from agar cultures were recorded as depending upon the LLDPE-TD1 specimen (Figure 4.93). The higher respiration values were indeed observed in the F2 cultures supplemented with abiotically pre-treated (thermally oxidized and outdoor exposed) specimens, whereas strictly similar CO_2 emission profiles were recorded in cultures containing pristine specimens and blanks (Figure 4.93).

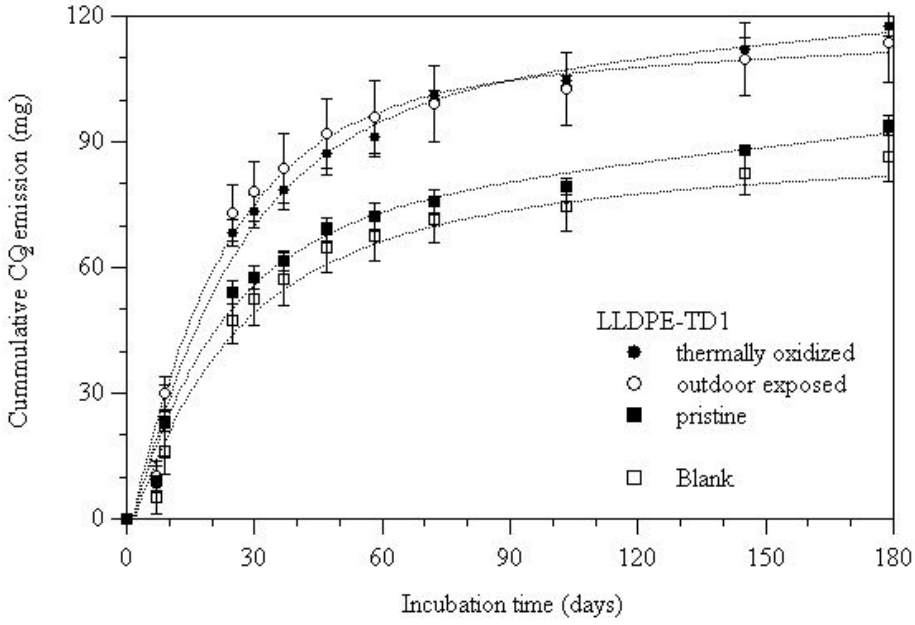

Figure 4.93. Average cumulative CO_2 emission from F2 fungal strain agar cultures supplemented with pristine and abiotically pre-treated (thermally and outdoor exposed) LLDPE-TD1 specimens under co-metabolic conditions.

The collected results were therefore once more demonstrating that abiotically pre-oxidized LLDPE-TD1 specimens can be utilized as carbon source by fungal species isolated from soil, even under co-metabolic conditions represented by the presence of an easily assimilable organic compound such as glucose.

After 6 months of incubation with the F2 fungal strain, LLDPE-TD1 specimens were carefully cleaned up and characterized by means of TGA and DSC thermal analysis. In Table 4.32 the thermal properties as recorded by TGA of the test specimens before and after the incubation with the fungal strain, are reported.

Table 4.32. Thermal properties of pristine, thermally oxidized and outdoor exposed LLDPE-TD1 specimens submitted to biodegradation in agarized medium supplemented with glucose in the presence of F2 selected fungal strain, as recorded by TGA analysis.

LLDPE-TD1	**Incubation time**	**T_{ON}**	**Residue at 600°C**	**CO*i***
specimen	(days)	(°C)	(%)	
pristine	0	400.6	0.24	0.11
pristine	178	381.5	1.49	0.21
TD[a)]	0	192.4	2.43	4.59
TD[a)]	178	74.1	21.13	3.20
UV [b)]	0	353.5	0.88	0.46
UV [b)]	178	235.7	3.33	0.70

a) After 229 days at 65°C in ventilated oven; b) After 93 days outdoor exposure

The results collected in Table 4.32 evidenced that the microbial activity of the selected fungal strain under co-metabolic conditions was promoting a marked decay in the thermal stability of thermally oxidized and outdoor exposed LLDPE-TD1 specimens. In accordance, strong decrease of onset temperatures corresponding to 2% sample weight loss were observed after incubation with the fungal strain in the abiotically pre-treated specimens.

This trend was dramatic in the case of thermally oxidized specimen (Table 4.32, Figure 4.94), even though the effect of sample contamination with agar residues can not be excluded at all.

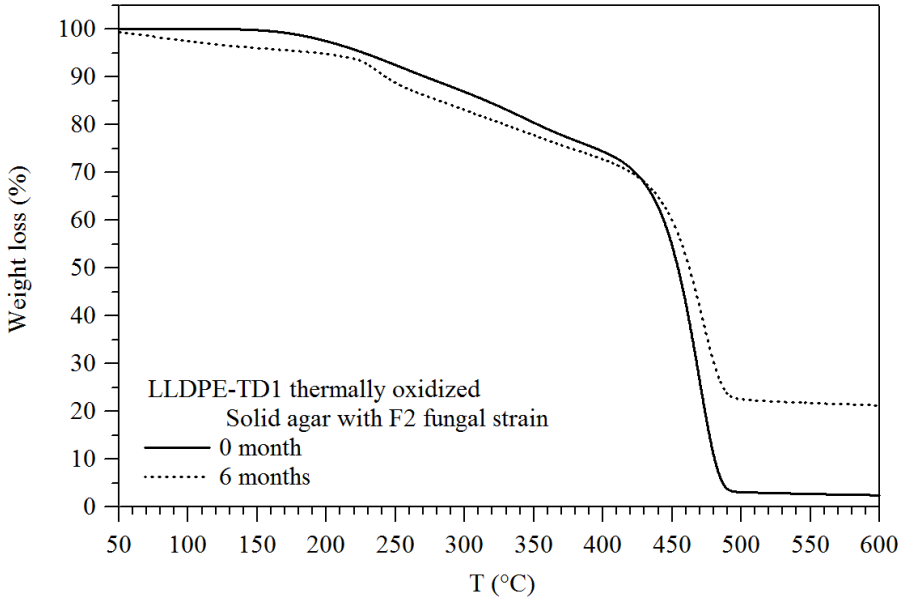

Figure 4.94. TGA traces of thermally oxidized LLDPE-TD1 specimen before and after incubation on agarized medium with F2 fungal strain.

In any case, the polymer chains degradation as a consequence of the incubation with the fungal strain under co-metabolic conditions, suggested by TGA characterization, was also evidenced by the DSC analysis carried out on the same sample specimens (Table 4.33).

Noteworthy increase of the degree of cristallinity was indeed recorded during the first heating of DSC characterization, as attributable to the assimilation of low molecular weight-oxidized fractions by the fungal strain. Nevertheless, during the second heating in the DSC characterization, the degree of cristallinity of thermally oxidized specimen was found to drop down from 47.4 to 30.6 % after the incubation period. This observation seems therefore to suggest that the

microbial activity was promoting a further oxidation and degradation of the polymer chains capable to hinder the crystallization behaviour after the first heating of the sample (Table 4.33).

Table 4.33. DSC parameters of pristine, thermally oxidized and outdoor exposed LLDPE-TD1 specimens submitted to biodegradation in agarized medium in the presence of F2 selected fungal strain.

LLDPE-TD1	**Incubation time**	**1st Heating**		**2nd Heating**	
specimen	(days)	Tm	Cristallinity	Tm	Cristallinity
		(°C)	(%)	(°C)	(%)
pristine	0	115.0, 121.8	42.7	110.9, 122.3	44.7
pristine	178	113.8, 120.6	42.1	111.2, 122.6	43.4
TD[a)]	0	117.4	59.1	116.8	47.4
TD[a)]	178	118.3	78.8	117.1	30.6
UV[b)]	0	116.0, 121.8	48.5	121.1	46.3
UV[b)]	178	117.5, 121.4	55.4	111.5, 121.4	51.2

a) After 229 days at 65°C in ventilated oven; b) After 93 days outdoor exposure

Finally, the progress in the polymer chains degradation of abiotically pre-aged LLDPE-TD1 sample specimen incubated under co-metabolic conditions with the selected F2 strain was also substantiated by FT-IR analysis. The presence of strong absorption bands as attributable to oxidized functional groups (e.g. hydroxyl and vinyl groups) were therefore observed in both thermally oxidized and outdoor exposed specimens after the incubation with the fungal strain (Figure 4.95).

The biodegradation test carried out onto solid medium under co-metabolic conditions was confirming the ability of selected fungal strains to assimilate as carbon source the oxidized low molecular weight fractions as produced during the abiotic pretreatments of LLDPE-TD1 sample. Furthermore, the fungal metabolism was also inducing the progress of

oxidative degradation of thermally oxidized and outdoor exposed specimens, thus confirming the occurrence of synergistic effects, as mediated by both physical and biochemical factors in the environmental degradation process of oxo-degradable LLDPE samples once exposed to preliminary abiotic treatments.

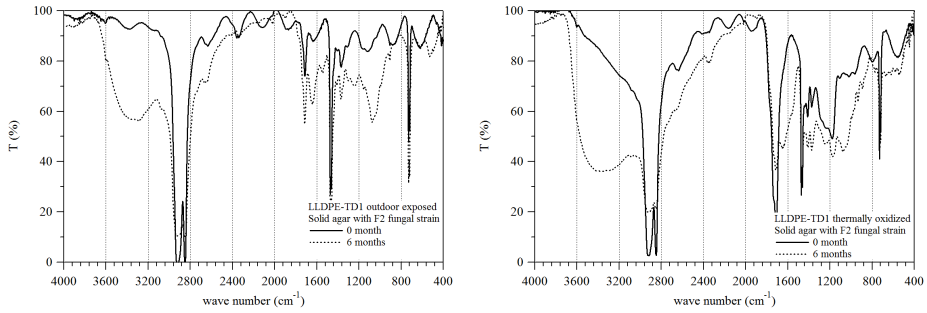

Figure 4.95. FT-IR spectra of outdoor exposed (a) and thermally oxidized (b) LLDPE-TD1 specimen before and after incubation on agarized medium with F2 fungal strain.

4.3.4. *Biodegradation of LLDPE films in agarized medium in the presence of Phanerocheate chrysosporium fungal strain.*

In order to confirm the ability of the soil-borne F2 fungal strain to promote the oxidation of LLDPE specimens, analogous tests were carried out by using the lignin-degrading fungi *Phanerocheate chrysosporium*. This strain has been utilized to study the degradation of a wide variety of recalcitrant organic pollutants because of the powerful oxidizing enzymatic tool associated to lignin-degrading or wood-rotting activities [199].

The biodegradation test was carried out onto solid media under co-metabolic conditions (e.g. in the presence of an easily assimilable carbon source such as glucose) by using LLDPE-TD1 and LLDPE TD2 thermally oxidized, outdoor exposed and pristine samples specimens. After 6 months of incubation on solid cultures inoculated with *P. chrysosporium*, LLDPE-TD1 and LLDPE -TD2 test specimens were

carefully cleaned up and characterized by means of TGA, DSC thermal analysis and determination of CO*i* by FT-IR spectroscopy (Tables 4.34 and 4.35).

Table 4.34. Thermal properties of pristine, thermally oxidized and outdoor exposed LLDPE-TD1 specimens submitted to biodegradation in agarized medium in the presence of *P. chrysosporium* fungal strain, as recorded by TGA analysis

Test Sample	Abiotic degradation	Incubation time	T_{ON}	Residue at 600°C	CO*i*
		(days)	(°C)	(weight %)	
LLDPE-TD1	none	0	400.6	0.24	0.11
LLDPE-TD1	none	180	389.9	1.25	0.20
LLDPE-TD1	TD [a)]	0	192.4	2.43	5.00
LLDPE-TD1	TD [a)]	180	245.07	6.041	3.54
LLDPE-TD1	UV [b)]	0	353.5	0.88	0.50
LLDPE-TD1	UV [b)]	180	283.6	2.31	0.72
LLDPE-TD2	none	0	422.9	1.03	0.04
LLDPE-TD2	none	180	424.78	1.75	0.22
LLDPE-TD2	TD [a)]	0	219	2.72	3.41
LLDPE-TD2	TD [a)]	180	251.44	4.815	2.80
LLDPE-TD2	UV [b)]	0	304	1.97	0.29
LLDPE-TD2	UV [b)]	180	236.7	2.19	1.23

[a)] After 229 days at 65°C in ventilated oven; [b)] After 93 days outdoor exposure

The results so far collected once more demonstrated that fungal species are able to utilize as carbon source the low molecular weight fractions produced during the abiotic pre-treatment of LLDPE samples additivated with pro-oxidants. In addition, the progress of oxidation of the samples submitted to outdoor exposure, as a consequence of the metabolic activity of *P. chrysosporium*, was also ascertained, thus demonstrating that a moderate level of oxidation in the LLDPE matrix is sufficient to induce further microbial attack as previously recorded in the tests carried out in the presence of the selected F2 fungal strain.

Table 4.35. DSC parameters of pristine, thermally oxidized and outdoor exposed LLDPE-TD1 specimens submitted to biodegradation in agarized medium in the presence of *P. chrysosporium* fungal strain.

Test sample	Abiotic Test	Incubation time (days)	1st Heating		2nd Heating	
			Tm [c] (°C)	Cristallinity (%)	Tm [c] (°C)	Cristallinity (%)
LLDPE-TD1	none	0	115, 122	42.7	111, 122	44.7
LLDPE-TD1	none	180	114, 121	42.2	111, 123	43.4
LLDPE-TD1	TD [a]	0	117	59.1	117 [d]	47.4
LLDPE-TD1	TD [a]	180	116	50.1	116	50.1
LLDPE-TD1	UV [b]	0	116, 122	48.5	121 [d]	46.3
LLDPE-TD1	UV [b]	180	113, 125	50.2	122	49.2
LLDPE-TD2	none	0	122	41.9	110, 122	44.2
LLDPE-TD2	none	180	115, 118	44.1	111, 121	46.4
LLDPE-TD2	TD [a]	0	118	51.2	115 [d]	47.5
LLDPE-TD2	TD [a]	180	119	59.0	116	51.6
LLDPE-TD2	UV [b]	0	118, 122	52.4	122 [d]	47.5
LLDPE-TD2	UV [b]	180	116.3	61.07	112, 122	55.3

a) After 230 days at 65°C in ventilated oven; b) After 93 days outdoor exposure; c) Melting peak; d) Broad peak

At the end of the biodegradation tests carried out in the presence of heterogeneous microbial populations, as well as in the presence of single microbial species, the following considerations can be drawn:

1) It has been observed that the mineralization in aqueous medium of the pre-oxidized LLDPE samples, do experience a mineralization level depending upon the CO*i* value, higher is the CO*i* value higher the mineralization extent.
2) A second point to remark is that the LLDPE sample characterized by higher CO*i* level is experiencing a drop of the oxidation level

accompanied by an increase of thermal stability, whereas in the case of LLDPE samples characterized by an initial lower CO*i* value, an increase of the oxygen content (CO*i*) and a corresponding lower thermal stability were observed.

3) The aqueous biodegradation carried out on the extracted fractions (oxidized low molecular weight) of PP and LDPE were showed a fairly high mineralization of straight chain compounds, such as in the case of LLDPE and Docosane with respect to highly branched PP extracts.

4) During soil burial tests, fairly high CO_2 emissions were recorded from the soil cultures supplemented with thermally oxidized specimens, whereas only negligible microbial assimilation was recorded in the case of outdoor exposed specimens. Indeed, the CO*i* tended to increase within the time. The biodegradation degree of thermally oxidized LLDPE films approached 14% in 27 months of soil burial with a positive trend of biodegradation processes.

5) Thermal characterizations of the oxo-biodegradable specimens submitted to biodegradation in soil, confirmed that a synergistic degradation process, as probably mediated by both abiotic process and microbial activity take place during the progress of the soil biodegradation test.

6) The undertaken biodegradation tests clearly indicates that some fungal species isolated from soil buried LDPE specimens, are capable to be used as carbon source the oxidized products of LLDPE samples submitted to outdoor exposure. Moreover, the metabolic activity of some of the isolated fungal species, may also promote a further oxidation of the LLDPE polymer matrix.

7) Nevertheless, different behaviors were recorded depending upon the type of pro-oxidant utilized in the sample preparation. All the structural characterizations together with the oxidation profiles recordable at 45°C of fungal treated specimens were therefore suggesting that outdoor exposed LLDPE-TD2 sample is more susceptible to fungal metabolism.
8) The capability of the isolatedF2 fungal strain to assimilate the oxidized fraction of abiotically pre-aged LLDPE-TD1 sample was evidenced.
9) Some suggestions on the ongoing oxidative degradation of LLDPE polymer matrix as induced by the action of the selected microorganism were also collected
10) The biodegradation test carried out on solid medium under co-metabolic conditions was confirming the ability of selected fungal strains to assimilate as carbon source the oxidized low molecular weight fractions as produced during the abiotic pretreatments of LLDPE-TD1 sample. Furthermore, the fungal metabolism was also inducing the progress of oxidative degradation of thermally oxidized and outdoor exposed specimens, thus confirming the occurrence of synergistic effects, as mediated by both physical and biochemical factors in the environmental degradation process of oxo-degradable LLDPE samples once exposed to preliminary abiotic treatments.

4.3.5. *Degradations tests carried out under a combination of abiotic and aiotic factors*

4.3.5.1. *Thermal degradation test of LLDPE samples onto soil and mature compost at 50°C.*

In order to verify the synergistic effect exerted by either abiotic (thermal) and biotic (microorganisms) conditions on the rate and extent of oxidative degradation of LLDPE samples containing pro-oxidant additives, LLDPE-TD1 and LLDPE-TD2 samples were maintained in oven at 50°C in direct contact with wet soil and mature compost samples (Figure 4.96). At time interval (2-4 days) the film specimens were withdrawn, carefully washed with distilled water and dried in a desiccator at room temperature before measuring the carbonyl index by FT-IR spectroscopy. The humidity level of soil and compost was kept constant during the experiment course. The oxidation behavior of each test sample in contact with soil and compost was estimated according to the calculation of carbonyl index (CO*i*). In the case of LLDPE-TD1 sample, the oxidation process started after 55 days in both specimens in contact with soil and compost. Nevertheless the highest rate and extent levels of oxidation, recordable as CO*i* degree, were exhibited by the LLDPE-TD1 specimen in soil contact incubation, whereas the corresponding specimen incubated in contact with mature compost experienced a noticeable lower degree of oxidation within the same time frame (Figure 4.96). On the contrary, appreciable oxidation level in the case of LLDPE-TD2 specimens were recorded only in soil cultures, whereas the specimen in contact with mature compost did not show any formation of carbonyl groups within 140 days of incubation (Figure 4.97).

The recorded results once more confirmed the higher propensity to oxidation of LLDPE-TD1 sample. Even the influence of the natural matrices on the oxidation process of the analyzed LLDPE samples was

evidenced during the test. In particular, it can be suggested that mature compost most likely because of the wealthy in organic compounds capable to interact with pro-oxidant additives, can affect negatively the oxidative degradation of the LLDPE chains.

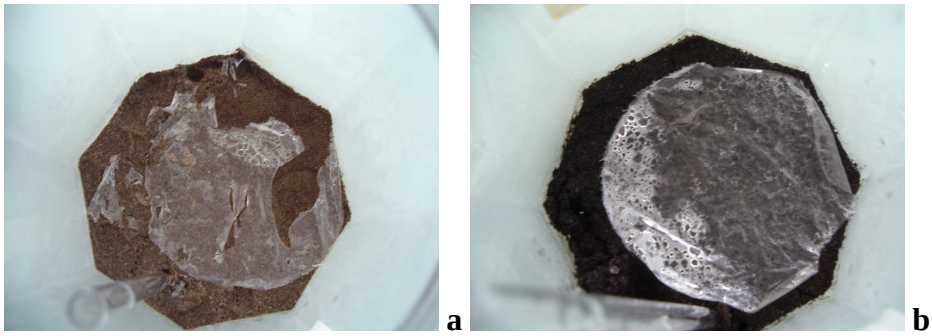

Figure 4.96. Pictures of LLDPE-TD1 specimens after 68 days thermal aging in contact with soil (a) and compost (b) media.

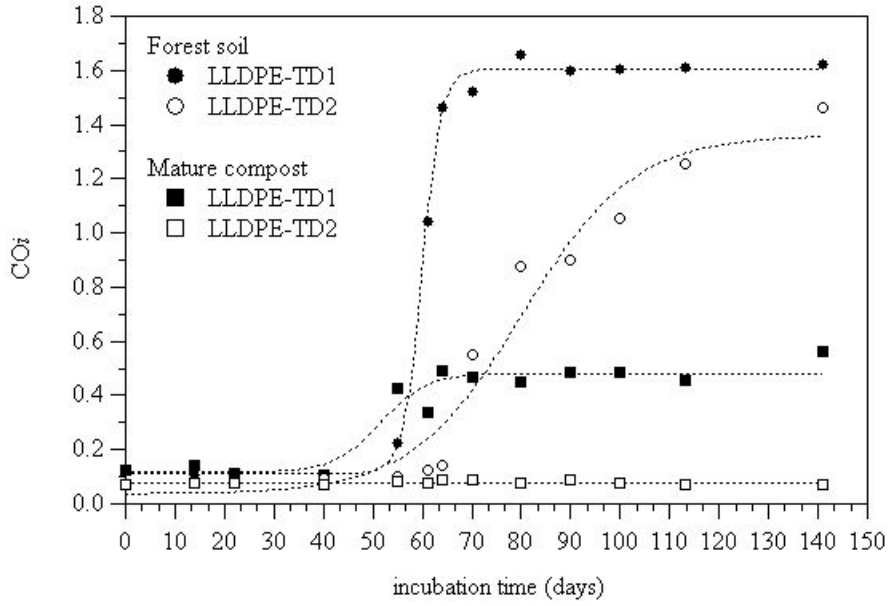

Figure 4.97. CO*i* profiles of LLDPE samples thermally aged at 50°C in contact with soil and compost media.

Different oxidative behaviors were then recorded during this test as depending upon the incubation medium (soil or compost) and LLDPE sample. In particular it was evidenced that under the adopted conditions

the LLDPE-TD1 sample showed the higher propensity to oxidation and the soil medium was much more effective in inducing the oxidation processes.

4.3.5.2. *Thermal degradation upon the exposure to fungal metabolic activity*

The specimens withdrawn after 65 days incubation in the presence of the selected fungal strains were further exposed to a thermal treatment in static oven at medium temperatures (45 and 55°C) as aimed at the evaluation of the influence of the microbial metabolism on the propensity of the LLDPE matrix to be further oxidized under abiotic conditions. The motivation of the adopted procedure was the purpose to mimicking the fate of a degradable mulching film under field scale conditions. It can be assumed, in fact, that after the crop season the debris of oxo-degradable films exposed to the sun light, can be buried in the soil, thus being submitted to the action of soil microorganisms. After that in concomitance with the soil preparation for the new crop season, some fragments can be further exposed to abiotic factors such as heat and light.

In accordance, during the thermal treatment in oven at 45 and 55°C, the level of oxidation, as determined by CO*i*, of the LLDPE samples specimen treated with each fungal strains and un-inoculated controls was monitored within the time. The relevant CO*i* profiles are reported in Figures 4.98-4.105.

The differences in the propensity of the tested film specimens was evaluated by the comparison of the best fitting as attainable from the experimental data, as well as on the basis of the highest level of oxidation recordable during the thermal treatments in oven.

It was therefore observed that, slight but significant differences in both the rate and extent of oxidation of fungal treated specimen with respect to the relevant un-inoculated specimens can be recorded only at 45°C,

whereas at 55° the abiotic stress as induced by heat is predominant, thus not allowing to discriminate between the influence of the preliminary biotic treatment and the thermal stress.

On the contrary, the CO*i* data recorded at 45°C, once more demonstrated the progress of degradation of LLDPE-TD2 specimens exposed to fungal strains, as well as the higher effectiveness of F4 strain in promoting the enzymatic degradation of the polymer matrix. The sample specimen preliminary incubated with this fungal strain, showed the reaches the highes level of oxidation once thermally aged at 45°C in oven, thus showing an exponential trend in the CO*i* profile with respect to the un-inoculated control (Figure 4.101).

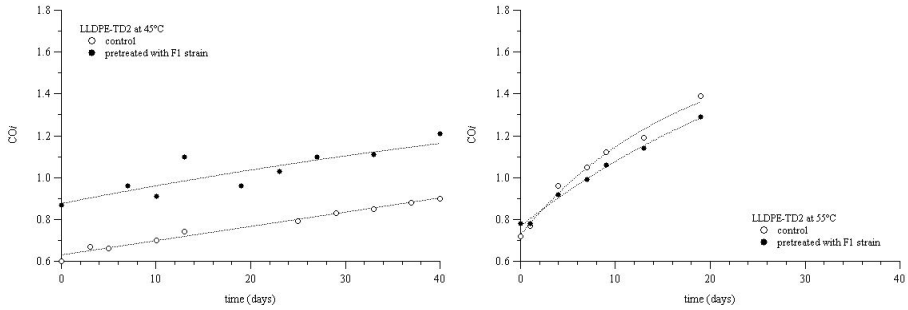

Figure 4.98. CO*i* profiles of LLDPE-TD2 specimen pretreated with F1 fungal strain and relevant un-inoculated control during thermal aging at 45 and 55°C in static oven.

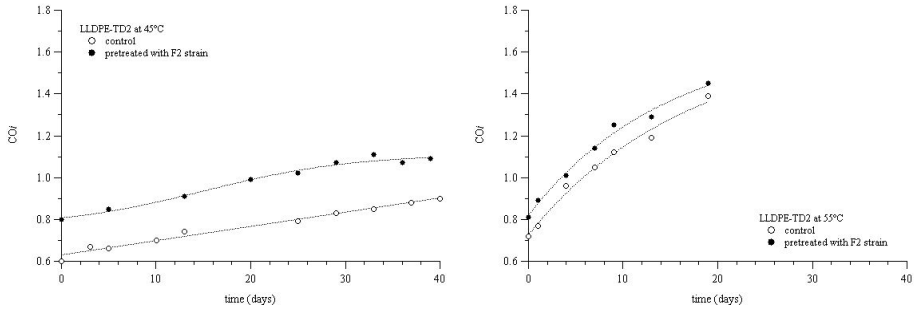

Figure 4.99. CO*i* profiles of LLDPE-TD2 specimen pretreated with F2 fungal strain and relevant un-inoculated control during thermal aging at 45 and 55°C in static oven.

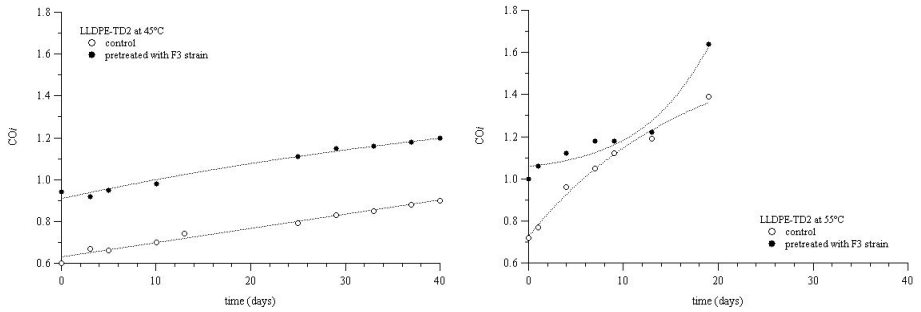

Figure 4.100. CO*i* profiles of LLDPE-TD2 specimen pretreated with F3 fungal strain and relevant un-inoculated control during thermal aging at 45 and 55°C in static oven.

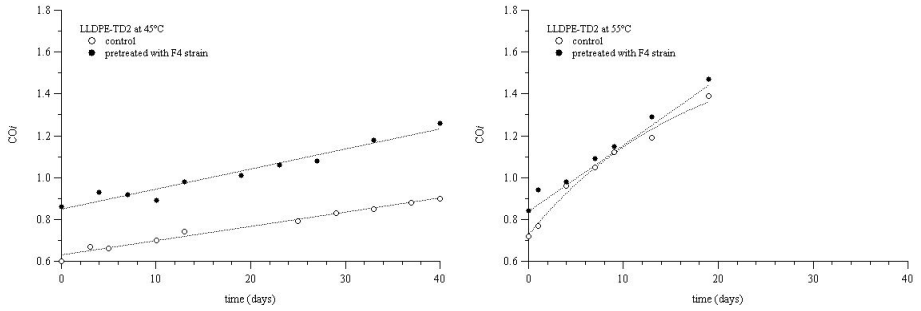

Figure 4.101. CO*i* profiles of LLDPE-TD2 specimen pretreated with F4 fungal strain and relevant un-inoculated control during thermal aging at 45 and 55°C in static oven.

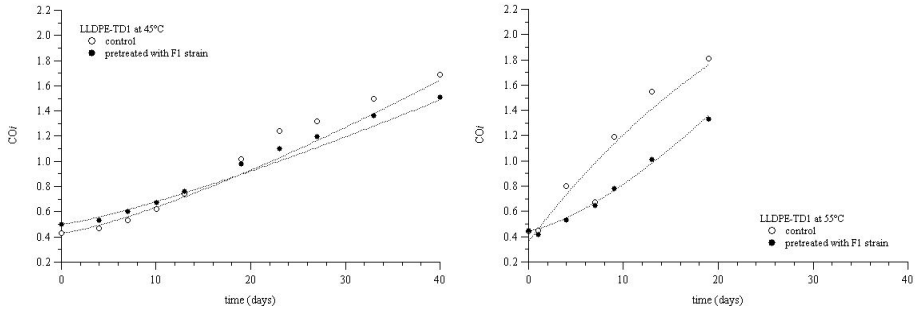

Figure 4.102. CO*i* profiles of LLDPE-TD1 specimen pretreated with F1 fungal strain and relevant un-inoculated control during thermal aging at 45 and 55°C in static oven.

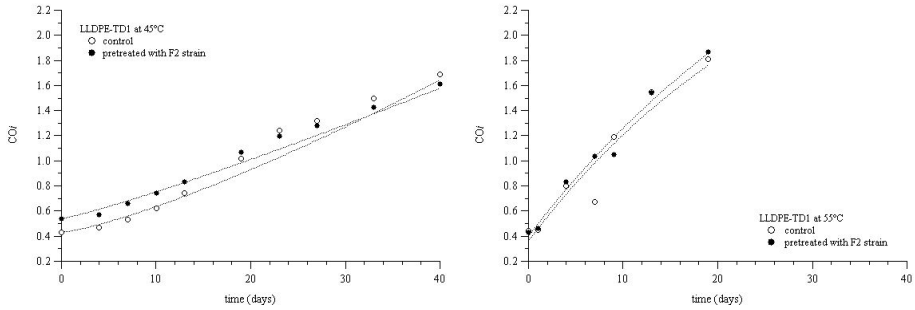

Figure 4.103. CO*i* profiles of LLDPE-TD1 specimen pretreated with F2 fungal strain and relevant un-inoculated control during thermal aging at 45 and 55°C in static oven.

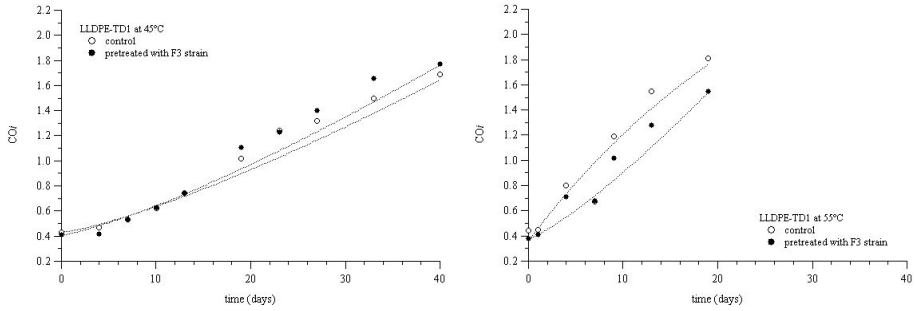

Figure 4.104. CO*i* profiles of LLDPE-TD1 specimen pretreated with F3 fungal strain and relevant un-inoculated control during thermal aging at 45 and 55°C in static oven.

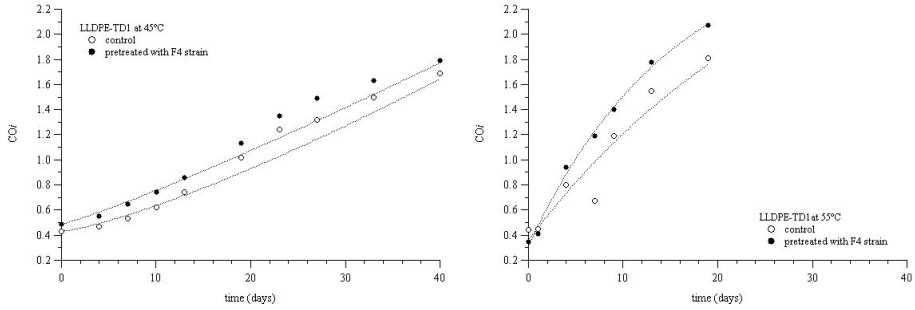

Figure 4.105. CO*i* profiles of LLDPE-TD1 specimen pretreated with F4 fungal strain and relevant un-inoculated control during thermal aging at 45 and 55°C in static oven.

While both tested and control films showed a steady increase in the CO*i* during the 40 day period of aging, the test sample exhibited a significantly higher rate and extent of oxidation throughout. By day 40, the average rate of increase in the CO*i*/day for LLDPE-TD2 test films incubated with F4 fungal strain was 0.0096 compared to 0.0068 for the control samples, which represents an excess of over 20% oxidation in the test samples over control (Figure 4.101).

The undertaken biodegradation tests clearly indicates that some fungal species isolated from soil buried LDPE specimens, are capable to growth on the oxidized products of LLDPE samples submitted to outdoor exposure as sole carbon source. Moreover, the metabolic activity of some of the isolated fungal species, may also promote a further oxidation of the LLDPE polymer matrix.

Nevertheless, different behaviors were recorded depending upon the type of pro-oxidant utilized in the sample preparation. All the structural characterizations together with the oxidation profiles recordable at 45°C of fungal treated specimens were therefore suggesting that outdoor exposed LLDPE-TD2 sample is more susceptible to fungal metabolism.

In order to further substantiate that the fungal metabolism may improve the propensity to oxidation of the LLDPE samples, a thermal degradation test will be carried out in static oven under relatively mild conditions (45°C) by treating the specimen retrieved from the fungal cultures in comparison with those submitted to sunlight exposure only.

4. CONCLUSIVE REMARKS

The mechanisms of physico-chemical processes that promote the degradation of full carbon backbone polymers such as PE, PP and PS as a gate to the biodegradation are assessed. Such processes allow the relevant converted plastic items to experience at the end of their service life the potential for a guided oxidative breakdown to functional fragments vulnerable to microorganisms present in solid and aquatic environments. The new strategic vision aiming at reengineering polymeric formulations based on well known biostable full carbon backbone polymers convertible to eco-compatible plastic items imply the following steps:

- *Abiotic treatment meant to promote and assist the oxidative degradation under different environmental conditions.*
- *Biodegradation experiments on oxidatively degraded semifinite and finite plastic items.*
- *Assessment of the interactions between microorganisms and samples sample aimed at understanding the ultimate environmental fate of the analyzed samples.*

4.1 Abiotic Degradation of Polyolefins (PE, PP and PS) Based Film Samples

Abiotic degradation studies, carried out under different test conditions, were performed in order to establish the role of pro-oxidant additives in enhancing the rate and extent of oxidation and evaluation of full-carbon backbone chain scissions as a prerequisite to promote attack by microorganisms and finally to end up with biodegradation.

The propensity to oxidation in terms of rate and extent was found to be dependent upon the following abiotic parameters: 1) Type and amount of pro-oxidant, 2) Temperature to which the samples are exposed,

3) Outdoor exposure 4) Air atmosphere exposure under static or dynamic conditions 5) Humidity level to which the samples are exposed.

Combined effects, were also found to be dependent upon the cross-action of abiotic parameters and structural characteristics of the analyzed samples (PE various grades, PP, HIPS and CPS).

In accordance with the performed activities the following general conclusions can be drawn.

- Thermal oxidation was particularly effective in the case of PE and PP samples loaded with pro-oxidants (prodegradant), whereas only minor effects were ascertained in the case of PS. The rate and extent of propensity to oxidation in case of the polyolefin films resulted PP>LDPE>LLDPE>HDPE
- The rate and extent of oxidation of PE samples were positively affected by both temperature and oxygen partial pressure, whereas a slight drop in rate and extent of oxidation was found to be associated to the humidity level in the case of PE, but not in the case of PP samples.
- The induction period was found to be dependent upon the type of pro-degradant, exposure temperature and type and grade of samples (LDPE<LLDPE<HDPE).
- Outdoor exposure of the samples resulted effective in promoting the oxidation of the analyzed samples in that order PS>PP>LLDPE
- Substantial drops in the molecular weight were found to be correlated to the extent of oxidation, as determined by FT-IR spectroscopy. This holds true particularly in the case of PP and PE samples, thus providing evidence on the statistical random scission of the polymer

chains, according to Norrish I and/or Norrish II, which was accompanied by the formation of fairly high low molecular weight fractions extractable by different solvents. On the contrary, in the case of PS samples, the random chain scissions does not seems to be involved in spite of the presence of tertiary carbon atoms in the main chain in 1-3 positions. Instead sub-terminal oxidation and relevant release of oxidized polyaromatic moieties might be the main degradation mechanism of the outdoor exposed PS samples

- GPC determinations showed that the molecular weights of solvent extractable fractions from abiotically degraded PE and PP samples are fairly low (0.4-1.9kD) and compatible with their potential vulnerability by natural occurring microorganisms.

4.2 Biotic Treatment of the Abiotically Treated Samples

The biodegradation propensity of abiotically pre-aged (thermal and outdoor exposed) and pristine polyolefin (PE, PP, PS) samples have been ascertained in aqueous and soil burial conditions as aimed at establishing the mineralization rate and extent for several polymeric materials, as well as at ascertaining the progress of oxidation and degradation of full-carbon backbone polymers by natural occurring microorganisms . The following conclusive remarks can be drawn:

- The microbial consumption of oxidized fractions present in abiotically degraded PE and PP films was confirmed by the decrease (30-35%) in the CO*i* values of the films submitted to the biodegradation test with respect to the starting pretreated samples.
- The microbial degradation and assimilation was particularly effective in the case of solvent extracted fractions from PE and PP degraded

samples. Nevertheless the higher propensity to microbial assimilation of linear oxidized fractions coming from PE with respect to fairly high branched PP fractions was observed in accordance to the sterical effects in refraining the enzymatic attack in the presence of highly branched hydrocarbons.

- During soil burial respirometric tests it was also ascertained the potential for the ultimate biodegradability of polyolefins (LLDPE, PP and PS) previously exposed to abiotic degradation tests (thermal or outdoor).
- Finally it has been found that **single soil borne microbial species are capable to promote the oxidation of pro-oxidant loaded LLDPE samples** once the process has been initiated by relatively mild degrading conditions to which the samples have been exposed, such as those related to a few months outdoor exposure.
- The information pertaining to the level of thermal and photo-oxidation required to achieve an effective and sustained biodegradation of full-carbon backbone polymers is critical for the design of polyolefin-based products and predicting their environmental fate.
- The research activity undertaken during the present PhD thesis provides important information with respect to synergistic effects of microbial/enzymatic attack and physical-chemical parameters in promoting the degradation of partially oxidized full-carbon backbone polymers, thus allowing for a better design of oxo-biodegradable materials to be really and ultimately biodegraded in different natural environments.

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APPENDIX

Publications

Andrea Corti, **Sudhakar Muniyasamy**, Manuele Vitali, Syed Imam and Emo Chiellini, Oxidation and biodegradation of polyethylene films containing pro-oxidant additives: Synergistic effects of sunlight exposure, thermal aging and fungal biodegradation, ***Polym. Degrad. Stab*** 2010, 95, 1106-1114.

Contributions in International Conferences

M. Sudhakar, Andrea Corti, Manuele Vitali, and Emo Chiellini, Environemental Biodegradation of Polyethylenes, **International polymer congress (APA 2009)** held on 17-20th December 2009, New Delhi, India.

M. Sudhakar, Andrea Corti, Manuele Vitali, and Emo Chiellini, Studies on the Environmental Fate of Eco-compatible Full Carbon Backbone Polymers, **Polymer Degradation and Discussion Group 28th Meeting** held on 6-10th, September 2009, Sestri levante, Italy.

M. Sudhakar, Andrea Corti, Manuele Vitali, and Emo Chiellini, Oxidative Degradation and Biodegradation Studies of Oxo-Biodegradable Polyethylene samples, **VII Convegno nazionale INSTM Sulla Scienza e Technologia Dei Materiali,** 9-12 June 2008, Tirrenia(Pi), Italy.

Attended an International meeting on **Composite materials (COMPOTEC – 2008)**, held on October 29-31in Marina di Carrara – Italy.