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Marek Koutny, Jacques Lemaire, A.M. Delort

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Marek Koutny [a,b,c,*], Jacques Lemaire [a], Anne-Marie Delort [b]

[a] *Centre National d'Evaluation de Photoprotection (CNEP), Universite Blaise Pascal, 63 177 Aubiere cedex, France*
[b] *Laboratoire de Synthese Et Etude de Systemes a Interet Biologique (SEESIB), UMR 6504 CNRS, Ensemble Universitaire des Cezeaux, Universite Blaise Pascal, 63 177 Aubiere cedex, France*
[c] *Tomas Bata University in Zlin, Faculty of Technology, Department of Environmental Protection Engineering, T.G.M. sq. 275, 76272 Zlín, Czech Republic*

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Abstract

Prooxidant additives represent a promising solution to the problem of the environment contamination with polyethylene film litter. Prooxidants accelerate photo- and thermo-oxidation and consequent polymer chain cleavage rendering the product apparently more susceptible to biodegradation. The question not fully resolved remains the biodegradation itself, its mechanism and especially the factors influencing the time-frame in which it can occur. The presented review is aimed to provide comprehensible information for both microbiologists and polymer scientists, who need participate in the research leading to an understanding of the microorganism action on the oxidized polyethylene and to design of new materials.

Keywords: Polyethylene; Prooxidant additives; Biodegradation; Biodegradable; Biofilm

Contents

* Corresponding author. Address: Tomas Bata University in Zlin, Faculty of Technology, Department of Environmental Protection Engineering, T.G.M. sq. 275, 76272 Zlín, Czech Republic. Tel.: +420 576 031 409; fax: +420 577 210 722.
E-mail address: mkoutny@ft.utb.cz (M. Koutny).

1. Introduction

A very visible portion of municipal and industrial waste consists of polyethylene (PE) films utilized on a massive scale as wrapping material, a typical example for the end-consumer being shopping bags. Polyethylene is also used in large quantities in agriculture for green-house construction or directly applied on the soil surface as mulching films, and there is, therefore a growing concern as to whether the plastic litter does not compromises soil quality or not.

Due to the exceptional mechanical properties of the material enabling production of films of thickness from 8 μm upwards and its low cost, the PE film products are often used for a short time and once only, and then become a waste. However the short service time is in sharp contrast with the remarkable resistance of PE to biotic degradation. The result of this disproportion is clearly visible all around us. Plastic litter has become an omnipresent part of our environment.

A frequent source of misunderstanding between the polymer scientists and microbiologists originates from the fact that for polymer scientists, degradation mainly concerns the loss of mechanical or other physical properties, whereas microbiologists are interested in the ultimate transformation of the material to carbon dioxide and biomass.

Even with some content of stabilizing additives, PE film loses its mechanical properties rather fast, especially when exposed to sun-light (during several months or at the most, a few years) and disintegrates into fragments. Although there are almost no data about the environmental fate of the fragments, it seems that their biodegradation is extremely slow and currently it is hardly possible to make even a rough estimation regarding the time necessary for their biodegradation to some substantial extent.

It is to be emphasized that the problem is to some degree only esthetic and psychological. PE is as a highly inert material, and to our knowledge, does not represent any ecotoxicological risk. The only known adverse environmental effects of PE films are when they are swallowed by wild animals and encapsulation of material on landfills and in the soil, thus altering microbial processes towards anaerobiosis. For this type of contamination the term "macropollutants" is sometimes used.

Searching for a solution is an ongoing process. Earlier, two principal strategies were suggested, i.e., waste separation and recycling of plastics and/or utilization of biodegradable materials. Unfortunately both strategies raise processing and economical problems and currently the global production of wrapping materials can be neither recycled nor replaced by biodegradable polymers.

In the 1970s two principal new approaches to solve the problem of macropollution by PE litter were developed (Arnaud et al., 1994).

The first is based on the introduction of a certain content of carbonyl groups directly into the main PE chains or on α positions of a short branches (Guillet process), during PE production by co-polymerisation with a suitable compound. Carbonyl groups then serve as reactive centers for the photolytic cleavage of the polymer backbone.

The approach that best respects current production and processing technologies consists in the use of special additives called prooxidants. These substances can be various complexes of transition metals particularly Fe, Co (Weiland et al., 1995) and Mn (Jakubowicz, 2003), and can increase the rate of oxidation by air oxygen and cleavage of PE chains under the influence of light and/or heat. Finally, the above process also results in PE film fragmentation and resolves the problem of visible pollution. But the question as to whether PE oxidized in this manner can be ultimately degraded by microorganisms, still remains to be clarified.

In some preparations part of the PE matter, e.g., up to 40%, is replaced by a biodegradable filler, typically starch. Although this type of filler can be relatively rapidly degraded it is now well accepted that it does not accelerate biodegradation of the PE matrix itself (Arnaud et al., 1994).

The ambition of the present review is to bring together and critically evaluate information concerning the possible ultimate biodegradability of PE with prooxidant content.

2. PE is remarkably resistant to microbial attack

Not very far in the past it was broadly accepted that the poor biodegradability of some synthetic compounds is a consequence of their novelty in the environment, so that the specific enzyme systems necessary to their degradation were not available. However, the research into xenobiotic degradation showed that microorganisms are equipped with substantial spectra of enzyme activities, especially various oxidases and peroxidases with broad substrate specificity, and moreover that the evolution of these activities can be relatively fast. More basic sources of the recalcitrance of some xenobiotics thus should be traced back to their physical and chemical properties that limit their chemical reactivity in general.

PE consists of molecules with an extremely high molecular weight (MW), typically several hundreds of thousands Da assembled from uniform $-CH_2-$ units. The molecular weight itself represents a serious problem because, as a molecule of this size cannot enter the cell, it is inaccessible to intracellular enzyme systems. For other macromolecular substrates, in general microorganisms often find the solution in the production of extracellular enzymes, which cut macromolecules to smaller fragments that can finally cross a cell wall and a cytoplasmic membrane. The PE molecule contains only non-polar C–C and C–H bonds which do not provide centers for nucleophilic or electrophilic attack, and the possibilities for its chemical reactivity are strongly limited, mainly to radical reactions. The most reactive, in fact, are the rare defects in the structure like the tertiary carbons of branching and double bonds, or oxygen-containing

groups incidentally present, but because of the low frequency of such defects the result is that their influence on the overall process could be limited, with the exception of vinylidene groups which were shown to be important in the photo-oxidation mechanism (Arnaud et al., 1984).

In the solid state PE molecules are densely aligned, form semicrystalline structures, and are highly hydrophobic so that only the surface with a limited number of free chain ends is available for enzymatic action. Diffusion of water and possible reactive molecules produced by microorganisms is very limited; there is practically no water diffusion and even no diffusion of oxygen in the crystalline zones. Due to above obstacles and in line with our everyday experience, PE is considered as being essentially a non-biodegradable material.

3. Photo- and thermo-oxidation of PE with prooxidants (prooxPE)

Another reason why industrially produced PE is stable in the environment is because it contains stabilizers (Briassoulis et al., 2004). These substances are present, even in a minimal concentration, in all commercial preparations to prevent PE oxidation during its processing, because molted PE at increased temperatures is sensitive to oxidation with air–oxygen. The residues of antioxidant stabilizers subsequently inhibit oxidation in the solid material also and prolong its lifetime enormously. The situation could be changed radically with the addition of prooxidant additives, which, unlike stabilizers, contribute to the initiation and the propagation of radical reactions. With a balanced combination of the amounts and the types of both antioxidant and prooxidant additives, PE film can be prepared that maintains all its mechanical and processing properties during the preset period and then, when all the antioxidant capacity has been used up, relatively fast loss of mechanical properties and consequent fragmentation occur (Dabin, 1993; Arnaud et al., 1994). Such features comply with the definition of so-called "materials with time-programmed mechanical properties".

The basis of the prooxidants are transient metal ions, typically added in form of stearate or other organic ligand complexes, most often stearates of Fe^{3+}, Mn^{2+} (Jakubowicz, 2003) or Co^{2+} (Weiland et al., 1995). Whereas Fe^{3+} complex plays a role in photo-oxidation process as a source of radicals for reaction initiation, the Mn^{2+} or Co^{2} are necessary for oxidation without the influence of light, when they catalyze decomposition of peroxides associated with chain cleavage. Under light the peroxides can be decomposed and chain cleavage occurs after absorption of a photon and without the need for metal ion catalysis (Fig. 1).

Photo- and thermo-oxidation processes are controlled by light intensity and temperature, hence can be accelerated artificially for laboratory testing, and result in a dramatic shift of the whole MW distribution and a decrease of the weight-average MW from several hundreds of thousands to several thousands. Cleaved chains are the most frequently terminated by carboxylic groups but other functionalities like esters, ketones, alcohols and double bonds can also be found. A broad spectrum of low MW compounds is formed as well as mainly, again, various carboxylic acids which can diffuse to the environment and eventually be extracted to aqueous media (Albertsson et al., 1993, 1995; Khabbaz et al., 1999).

Because the prooxidants and molecular oxygen are present exclusively in amorphous regions of the polymer the oxidation take place there predominantly whereas the crystalline zones remain intact.

Macroscopically oxidation manifests itself as loss of mechanical properties and fragmentation of the film, which at microscopic level is caused by the disruption of connecting chains between semicrystalline regions (Eyenga et al., 2002), and as increase of hydrophilicity and wetability of the film surface.

Apparently, the resulting material seems to be much more suitable for microbial attack than the initial PE film.

As follows from described features of the material it is necessary for the laboratory biodegradation testing to perform accelerated oxidation first, exposing samples to light radiation and/or increased temperature. The way of the laboratory treatment should be thoroughly set down and controlled in order that the exact relation between artificial photo- and thermo-oxidation and environmental weathering could be determined (Koutny et al., 2006). In principle

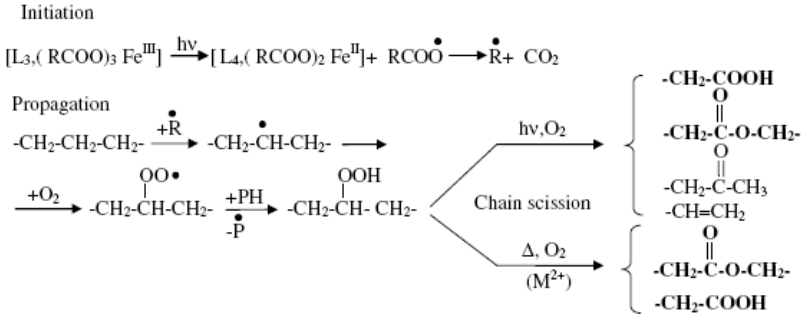

Fig. 1. Simplified scheme of abiotic degradation of PE with prooxidant content by action of air oxygen, light and/or heat. PH, polymer chain; L, suitable ligand.

such relation can differ for various materials but for example in the study by Jakubowicz (2003) 11 and 18 days of exposure at 60 °C in the dark was assigned to correspond to 2.5 and 4.5 years, respectively, in outdoor environment in case of two PE films with different prooxidant contents. Unfortunately in many studies the exact relation of abiotic sample treatments to natural conditions is not implicitly stated.

4. Biodegradation of oxidized PE

As already noted, a significant amount of low MW compounds is released to aqueous media from oxidized PE film. It was shown that the compounds could be consumed by microorganisms. Koutny et al. (2006) followed release of low molecular compounds to water media from thermo- and photo-oxidized HDPE and LDPE samples by NMR. These substances were subsequently completely consumed by *Rhodococcus rhodochrous* strain during 4 days of cultivation. The same samples without oxidation pretreatment did not release any substances. In another study (Albertsson et al., 1995) extractable compounds up to 12 carbon length were completely removed by a culture of *Arthrobacter paraffineus* as demonstrated with the GC–MS technique. After cultivation a new series of signals produced by alkanes with twenty to twenty six carbon atoms appeared on the chromatogram, indicating that by the bacterial action, some compounds with higher MW and lower solubility could also be extracted.

In this context the existence of microbial surface-active compounds enabling utilization of insoluble substrates could be of interest (Larkin et al., 2005). Such compounds were investigated for example with *Rhodococcus eryrthropolis* DSM 43215 growing on higher alkanes (Lang and Philp, 1998). They are relatively firmly associated with the bacteria surface, increase its hydrophobicity, and mediate adhesion of the bacteria on the substrate surface and passive transport of the substrate molecules. This could be related with the very low critical micelle concentration of the biosurfactants compared to the common synthetic surface-active compounds. For another poorly soluble substrate, phenantrene, it was shown that the phase transfer between the solid substrate and aqueous medium was the rate-controlling process of biodegradation (Bouchez et al., 1995). In the case of oxidized PE the microbial surface-active compounds can play a very important part also.

It seems that an addition of a synthetic detergent with physico-chemical properties different from the biosurfactants can affect biodegradation, more likely in a negative way, because it can probably increase mobility of poorly soluble compounds, but at the same time it can also compromise microbial adhesion on the material surface (Orr et al., 2004).

Two approaches exist in principle for biodegradation experiments. The first utilizing natural complex media, with established mixed microbial communities with a broad range of microbial strains and activities, enable to mimic biodegradation in situ, like in soil or compost. The second working with defined microbial strains in a synthetic medium where the experiments can be controlled and reproduced precisely, giving the possibility to compare experiments from different laboratories and to deduce information concerning the mechanism of biodegradation.

4.1. Biodegradation with defined microbial strains

The selection of suitable strains, which were tested for PE degradation, was based in principle on three ideas: (i) Collection strains of bacteria belonging to the *Streptomyces* genera and strains of fungi both producing lingolytic enzymes were used. The authors followed the idea that lignin as well as PE is an insoluble macromolecular substrate, during its biodegradation a broad range of oxidizing enzymes with unfocused substrate specificity is excreted which eventually could attack PE also. (ii) Collection strains of especially Gram-positive bacteria growing on higher *n*-alkanes were tested. In such strains we can expect the ability to utilize oxidized PE as a substrate of similar chemical structure; these strains can also produce biosurfactants necessary for mobilization of insoluble hydrophobic substrate molecules. (iii) Strains isolated from soil environment contaminated regularly over many years with PE, a classical approach in biodegradation studies.

An overview of published results together with brief subjective comments is presented in Table 1. Despite the number of experiments with different microorganisms and PE samples treated in different ways, it has to be admitted that not once a clear loss of some substantial part of PE matter was demonstrated. It was shown that microorganisms could grow on the surface and consume low molecular compounds generated by abiotic oxidation (Albertsson et al., 1998; Bonhomme et al., 2003; Koutny et al., 2006). Some authors also claim bioerosion on the samples observed after the biofilm removal (Arnaud et al., 1994; Weiland et al., 1995). Often the possibility is disregarded that at least some part of the microbial growth could be assigned to the consumption of additives like starch utilized in many preparations, or stearates from prooxidants, which are present in small but indispensable quantities in the material (Albertsson et al., 1998; Orr et al., 2004). The growth of microorganism on the PE film surface should not be interpreted as the sufficient proof of polyethylen biodegradation.

In most of the studies the authors observed a period of fast growth on the beginning of incubation caused by consumption of eventual additives and/or low molecular oxidation products of PE. After this fast initial phase the metabolic activity dropped down and further progress of biodegradation became very uneasy to detect. With the help of adenosine triphosphate and adenosine diphosphate determination it was shown that during many months after the initial fast growth period microorganisms still gained energy from oxidized PE film, however, apparently at rather low rate (Koutny et al., 2006).

Table 1
Overview of the polyethylene biodegradation studies with defined microbial strains and complex microbial communities

Microorganism	Source	Reference	*T*, month	Sample type	MW, kDa	Brief conclusion of the experiments by authors of the review
Aspergilus niger ATCC 9642 *Gliocladium virens* ATCC 9645 *Penicillium pinophilum* ATCC 11797 *Phanerochaete chrysosporium* H289	Collection strains	Manzur et al. (2003), Volke-Sepulveda et al. (1999, 2002)	>9	LDPE without prooxidants thermally and/or UV pretreated	Nd	Minor changes ATR-FTIR, CO_2 evolution equivalent to 0.5–1% mineralization, marked changes in crystallinity in disagreement with minor level of mineralization. Authors claim positive impact of ethanol as a co-metabolite on biodegradation
Cladosporium cladosporioides ATCC 20251 *Rhodococcus rhodochroust* ATCC 29672 Nocardia asteroides isolate	Collection strain Collection strain Rubber degrading	Arnaud et al. (1994), Bonhomme et al. (2003)	6	LDPE, Fe prooxidant TDPA® prooxidants from EPI Thermal and radiation pretreatment	14	Biofilm formation, bioerosion, no changes in MW
Arthrobacter paraffineus	Nd	Albertsson et al. (1995, 1998)	15 42	LDPE + starch/Fe stearate Thermal pretreatment and LDPE + starch, Mn stearate, + Styrenbutadien co-polymer Thermal pretreatment	~20	Consumption of the low MW compounds
Rhodococcus rubber isolate	Contaminated soil	Orr et al. (2004)	1	LDPE + unknown photosensitizer Thermal and UV pretreatment	Nd	Biofilm formation, mineral oil used as a co-metabolite
Brevibacillus borstelensis	Contaminated soil	Hadad et al. (2005)	3	LDPE + unknown photosensitizer Thermal and UV pretreatment	~100	Authors claim the weight loss and changes in molecular weight of the sample
Penicillium simplicissimum YK	Soil and leaves	Yamada-Onodera et al. (2001)	3	HDPE UV and thermally treated, treated with nitric acid	15	Authors claim growth on solid agar medium with PE (even with non-oxidised) as a sole carbon source Minor changes in MW distribution
Phanerochaete chrysosporium ME 446 *Streptomyces viridosporus* ATCC 39115 *Streptomyces badius* ATCC 39117 *Streptomyces setonii* ATCC 39116	Collection strains	Pometto et al. (1992)	2	LLDPE + 6% starch + prooxidants POLYCLEAN® UV and/or thermally treated	97–16	Changes in percent elongation
Streptomyces sp. isolate	Nd	El-Shafei et al. (1998)	1	PE + 6% starch	Nd	Changes in tensile strength and elongation
Aspergilus flavus *Mucor rouxii* 1835	Collection strain Nd			Thermal treatment		Incubation in complete microbiological medium

(*continued on next page*)

Table 1 (*continued*)

Microorganism	Source	Reference	*T*, month	Sample type	MW, kDa	Brief conclusion of the experiments by authors of the review
Fungi consortium of *A. niger* ATCC 6275, *G. virens* ATCC 9645, *Paecilomyces variotii* 10121 and *Penicillinum funiculosum* ATCC 19010	Collection strain	Weiland et al. (1995)	>8	LDPE and LLDPE + cobalt acetylacetonate	100–1	Biofilm formation, bioerosion, biodegradation of the low MW fraction
				Thermal pretreatment		
Streptomyces strains	Collection strain					
Microorganisms from compost	Mature compost					
Bacterial consortium KH-12	Nd	Kawai et al. (2004)	0.7	PE wax	2.9	Significant degradation and profound changes in MW distribution
Aspergillus sp. AK-3					1.2	
Composting (55 °C)	Mature compost	Jakubowicz (2003)	6.5	LDPE + Mn stearate	<5	CO_2 production corresponding to 60% mineralization
Soil microorganism	Forest soil	Chiellini et al. (2003), Chiellini (2004)	17	LDPE + TDPA™ additives Thermal treatment	6.7	CO_2 production corresponding to 50% mineralization in soil and 80% in compost
Composting (55 °C)	Mature compost and forest soil					

T, duration of the experiment; MW, weight-average molecular weight after the abiotic pretreatment; Nd, the data are unknown.

Isolation of active strains for prooxPE degradation represents a particular problem. Some of the authors made attempts to isolate potentially efficient microorganisms from the environment contaminated with plastics (Orr et al., 2004) or from the environment where they expected strains with favorable enzyme activities (El-Shafei et al., 1998; Yamada-Onodera et al., 2001). The question is whether in the environment contaminated with ordinary PE without prooxidants, some higher content of potential PE degrading microorganisms can be expected. As it was demonstrated and also according to our everyday experience PE, without prooxidant additives and moreover with some stabilizer content undergoes extremely slow biodegradation. Ohtake et al. (1998) studied one PE bottle without prooxidants buried in soil for 32–37 years and observed signs of some minimal degradation on its surface however they did not prove clearly their biotic nature. The idea also is well acceptable that the degradation could be mostly abiotic.

As the gain of energy from PE without prooxidants is apparently very low we must anticipate that the putative PE degrading bacteria would probably be some slow-growing strains referred to as oligothrophic, whose isolation and laboratory cultivation is often problematic or impossible. In this case a real danger exists that during the standard isolation procedure with oxidized prooxPE as a sole source of carbon and energy, faster growing strains utilizing low MW products of oxidation are isolated instead of some potentially more efficient strain which may be able to biodegrade substances with a much higher MW.

4.2. Biodegradation in the complex environment

Whereas the experiments with the defined strains in synthetic media did not bring undisputable quantitative proof of biodegradation, some results obtained during experiments in soil environments or under composting conditions are encouraging. Chiellini et al. (2003, 2004) followed carbon dioxide production during biodegradation of LDPE film with prooxidants. Before the biodegradation test the material was incubated 44 days at 55 °C and this preliminary abiotic thermo-oxidation caused decrease of weight-average MW to 6.7 kDa. The samples were then mixed with inert material, forest soil or mature compost as sources of microbial strains, moisturized and incubated at room temperature for soil and at 55 °C for compost inoculum. At the beginning very fast period of biodegradation about 30 days long was recorded at the end of which carbon dioxide production reached a plateau corresponding to about 4% mineralization and stagnated at this value. This phase without significant biodegradation progress lasted about 160 days and then the authors tried to revitalize the microbial community by a new inoculation with a small amount of fresh forest soil, agitation and moistening. The same manipulation was done also with blank cultures. After this treatment the beginning of biodegradation was observed. During approximately one more year of incuba-

tion the extent of mineralization reached 50–60% in the case of soil conditions and more than 80% in the case of composting conditions. It should be emphasized that abiotic thermo-oxidation was also still going on, which is especially important in the experiment under composting conditions at 55 °C.

Another composting experiment was performed by Jakubowicz (2003). His LDPE film with prooxidants was also thermally pre-treated in so far that the average MW dropped to under 5000 Da. Immediately when the experiment was started CO_2 evolution was recorded, without any lag-phase or steps on the CO_2 production course, and during the following six month reached a level corresponding to 60% mineralization. The results by Chiellini et al. (2003,2004) and Jakubowicz (2003) provide significant evidences, that support the idea of prooxPE biodegradability, also because the experiments are well documented and thoroughly compared with blank incubations.

Although the results of the studies cited above are positive, still we should be careful before accepting them as a sufficient and definitive proof of oxidized prooxPE biodegradability. In those experiments, in addition to PE the sample compartment contained also large quantities of other potential carbon substrates and especially during such unusually long experiments, some deviations cannot be fully excluded, even if the protocol is rigorously designed and the blank correction is done, because we cannot distinguish the CO_2 fraction originating purely from the oxidized prooxPE. It was shown for example that the incorporation of a sample can sometimes change the background CO_2 production significantly (Shen and Bartha, 1996).

Biodegradation in a complex environment like soil or compost can encompass some phenomena which cannot be easily simulated in experiments with the defined strains. These environments contain a certain portion of degradable carbon substrate and a high number of microorganisms equipped with a broad spectrum of enzyme activities establishing the potential for co-metabolic and/or symbiotic degradation. In co-metabolism carbon and energy derived from a co-metabolite, i.e., co-substrate, are utilized for the synthesis of enzymes, which then can attack and facilitate degradation of the recalcitrant substrate in question. Another possibility is that the substrate itself has limited capacity to induce the enzymes necessary for its degradation and in this case the presence of a co-metabolite as enzyme inducer can be also helpful. Apparently from a substrate like PE energy and carbon can be derived only at a very slow rate, therefore some form of co-metabolism possibly could be necessary. More convincing results of oxidized prooxPE degradation in complex environments with mixed microbial communities can originate also from the need for some not very common enzyme activity and collaboration between more microorganisms, as observed for another vinyl-type polymer, polyvinyl alcohol, which generally is considered as biodegradable, although in fact the competent strains are relatively rare (Shimao, 2001).

Despite the fact that some results of biodegradation experiments in complex media are encouraging for progress in the understanding of the mechanism, principal influencing factors and the time-frame of PE biodegradation, the development of a fully controlled system with one or several defined strains in synthetic medium, possibly with a chemically defined co-metabolite appears to be essential.

5. Mechanism of biodegradation

Currently there is very little data giving any clue that would make it possible to estimate the mechanism and contribution of microbial action on PE degradation (Fig. 2).

It was proved that low MW oxidation products are readily consumed by microorganisms but the exact meaning of low molecular in the case of PE remains to be clarified. Concerning longer *n*-alkanes, earlier studies showed that molecules up to about 500 Da can be decomposed (Haines and Alexander, 1974) but some more recent studies brought some evidence that even longer molecules could be degraded. In the experiment with PE wax where its MW distribution peaked at about 1000 Da, the bacterial consortium was able to consume quite rapidly molecules that were even bigger than 1000 Da (Kawai et al., 2004) as it was apparent from MW distribution curves for samples before and after the incubation. In another experiment soil microorganisms showed the capacity to degrade rapidly the acetone extractable fraction from the thermooxidized prooxPE film (Chiellini et al., 2003). The weight-average MW of the extracted fraction was determined to be 1500 Da. Again the authors measured carbon dioxide production and found about 70% mineralization during approximately one year of incubation.

It is not clear whether such big molecules are directly assimilated, possibly with the help of biosurfactants produced by microorganisms, and enter the pathway known for longer alkanes comprising intracellular beta oxidation (Albertsson and Banhidi, 1980; Kawai et al., 2002; Kawai et al., 2004) or must first be shortened by an unknown mechanism or cleaved by abiotic processes.

Previously discussed soil and especially composting experiments (Chiellini et al., 2003, 2004; Jakubowicz, 2003) showed that pre-thermooxidised prooxPE could be biodegraded to a great extent with a time horizon of about one year. This could suggest that microorganisms present do not wait passively for the lower MW products of abiotic oxidation and contribute in some way to PE oxidation and chain cleavage or at least that the biotic environment accelerates abiotic oxidation processes.

Some authors anticipate that the microorganism producing extracellular lingolytic enzymes may play an important role in the process (Pometto et al., 1992). Fungi and some bacteria produce various peroxidases and other enzymes which are able, as a consequence of their common

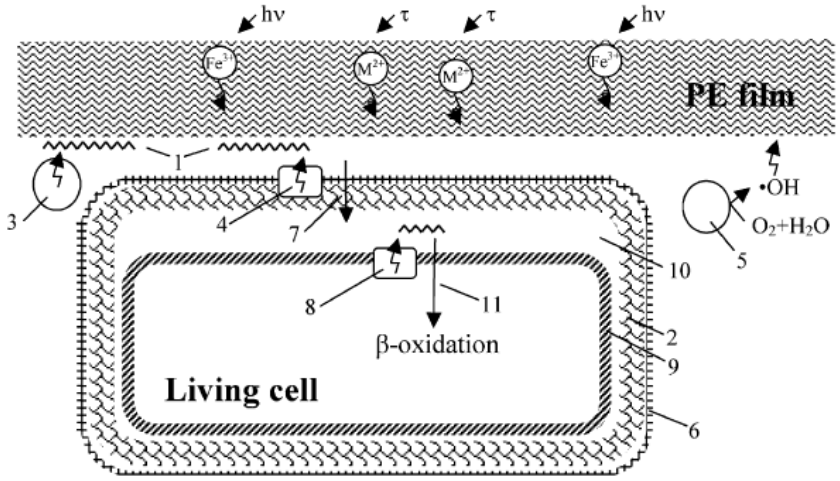

Fig. 2. Possibilities in PE biodegradation mechanism. By abiotic oxidation molecules with lower MW terminated with carboxylic groups are produced (1). The molecules still can be too big to get across the cell wall (2); so only the soluble extracellular enzymes (3); or cell wall associated enzymes (4); can mediate their further oxidation. Some enzymes can act indirectly via production of diffusible radicals (5). Biosurfactants (6); on the cell wall surface ensure adhesion of cells on the material and mobilize smaller water insoluble PE degradation products that can pass through the cell wall (7); and can be transformed by enzymes (8); in the cytoplasmic membrane (9); and/or in the periplasmic space (10) eventually. Molecules with probably even more limited size can be transported (11); across the cytoplasmic membrane and can be completely assimilated in the β-oxidation pathway.

action, to oxidise and break the structure of normally very recalcitrant insoluble high molecular lignin (Kirk et al., 1984). Lignolytic enzymes are produced in conditions of nutrient limitation (Cancel et al., 1993) and thus may be present in a PE degrading culture. However lignin as a polymer, consisting of aromatic benzene rings connected by oxygen and carbon containing bridges, is very distant from PE both structurally and in its reactivity. To our knowledge no transformation of aliphatic compounds by lignolytic enzymes has been observed. To disrupt the lignin structure the microorganism and their enzymes do not only interact directly with the substrate but also produce reactive radicals like superoxide (Morpeth, 1985), peroxide radical (Shen and Bartha, 1996), hydroxyl radical (Tanaka et al., 1999) and radicals derived from compounds of their metabolism (Kapich et al., 1999; Ruiz-Duenas et al., 2001; Watanabe et al., 2002) which serve as easily diffusible mediators of the oxidative action. It is possible that during PE biodegradation those small molecules can penetrate into the material and accelerate further radical oxidation with the catalysis of transient metals from prooxidants or from the environment.

The confrontation of the results from different studies reveals that probably the higher abiotic oxidation level and consequent decrease of the average MW to under about 5000 Da is the most important factor if some significant extent of biodegradation in a reasonable time period is desired (Table 1). In such samples another mechanism of microbial contribution could be considered. When the MW distribution peaks about 5000 Da or less a substantial part, e.g., 20% of the polymer matter, is present in the fraction with MW under 1000 or 2000 Da and, as it was pointed out previously (Chiellini et al., 2003; Kawai et al., 2004), this fraction can be relatively rapidly biodegraded. The vacancies produced can then cause swelling and relaxation of the whole material structure and facilitate diffusion of water and soluble compounds inside therefore substantially accelerating abiotic oxidation (Fig. 3).

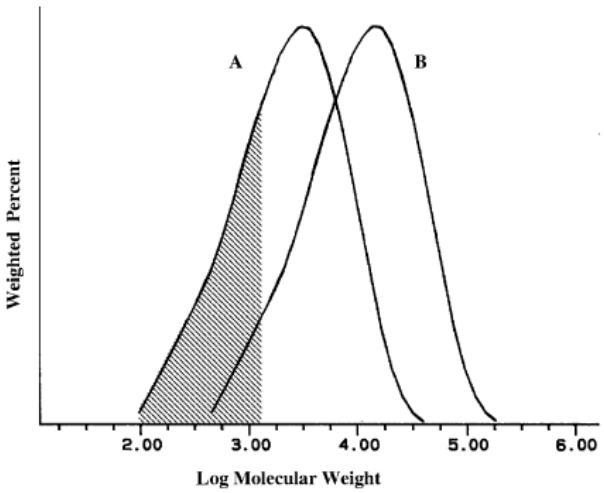

Fig. 3. MW distribution curves for two theoretical samples of PE with prooxidant additives after weathering. The shadowed part is the putative easily biodegradable fraction. Curves A represent theoretical sample where the abiotic oxidation reached a higher extent (peak at MW 3000) and where consumption of the easily degradable fraction can produce profound change in the material structure and subsequent acceleration of degradation processes. For curve B (peak at MW 16000) the consumption of easily degradable fraction need not have dramatic effect on the material integrity.

6. Conclusion

The presented review was aimed at concentrating and providing a context for information concerning biodegradation of PE with prooxidant additives. Although this phenomenon has been studied for more than ten years, some central questions remain unanswered. We cannot be sure if the microorganisms contribute actively to the process or only passively consume the low molecular products of the abiotic oxidation. Nor it is known what groups of microorganism participate in biodegradation and what enzyme systems they use. But most of all we are still not able to estimate the time-frame for the whole process and principal factors affecting the material decomposition. Only one larger scale field experiment was published, where non-preoxidised PE bags with prooxidant content were treated in a high scale composting plant in a mixture with normally processed material, which represented 99% of the mixture (Billingham et al., 2003). The positive conclusion was that the resulting compost did not exhibit ecotoxicity in the whole organism tests applied. Unfortunately the authors did not mention if the PE film disappeared or if the PE fragments still could be found in the final compost matter.

Study of PE biodegradation is confronted with methodological problems, because of the necessity to monitor slow processes on the surface of the material, and also management problems because of the long-term experiments that interfere with the established research funding system and the tendency of industry to launch new products in the shortest time possible. Due to the circumstances PE with prooxidants probably will be produced in a mass quantities before satisfactory knowledge has been acquired of its environmental fate.

7. Conclusive remarks

What has been proven

- By the catalytic action of prooxidants the average MW of polyethylene is dramatically reduced from several hundreds thousands to several thousands.
- Lower MW products of oxidation are consumed by microorganisms.
- Some microorganisms can form biofilms on the surface of oxidised PE films.
- In soil or compost environment highly preoxidised PE film was degraded to a substantial extent with a time horizon of about one year according to two recent studies (Chiellini et al., 2003; Jakubowicz, 2003).

What remains to be discovered?

- What must the minimal level of abiotic oxidation be in order to make PE film ultimately biodegradable during a laboratory test of about one year?
- Do microorganisms participate directly or indirectly in the polymer chain cleavage?
- What groups of microorganisms and what enzyme systems participate in PE biodegradation?

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