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New perspectives in plastic biodegradation

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During the past 50 years new plastic materials, in various applications, have gradually replaced the traditional metal, wood, leather materials. Ironically, the most preferred property of plastics – durability – exerts also the major environmental threat. Recycling has practically failed to provide a safe solution for disposal of plastic waste (only 5% out of 1 trillion plastic bags, annually produced in the US alone, are being recycled). Since the most utilized plastic is polyethylene (PE; ca. 140 million tons/year), any reduction in the accumulation of PE waste alone would have a major impact on the overall reduction of the plastic waste in the environment.Since PE is considered to be practically inert, efforts were made to isolate unique microorganisms capable of utilizing synthetic polymers. Recent data showed that biodegradation of plastic waste with selected microbial strains became a viable solution.

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Introduction

The discovery of the chemical process for manufacturing synthetic polymers (plastics) from crude oil was a breakthrough, in Chemistry and in Material Sciences, and paved the way to the production of one of the most versatile group of materials ever produced. These new materials combined features exhibiting strength, flexibility, light-weight, easy and low-cost production. However, these materials were found to be extremely durable and were considered among the most non-biodegradable synthetic materials. These traits facilitated the application of plastics to almost any industrial, agricultural or domestic market. For example, current soil mulching with PE in Agriculture is a common practice (Figure 1).

The most consumed synthetic polymer is PE with a current global production of ca. 140 million tons per year. In the absence of efficient methods for safe disposal of plastic waste these synthetic polymers accumulate in the environment posing an ever increasing ecological threat to terrestrial and marine wild life [1,2•].

Until a few years ago the environmental pollution by plastic wastes had been considered merely as an aesthetic interference demonstrated by the plastics dispersion in the wind and thereby pollute large terrestrial and marine environments. However, when plastic debris is exposed to u.v. irradiation from sunlight it undergoes photo oxidation. Consequently, the plastic deteriorates, lose its tensile strength, becomes brittle and crumbles to small fragments and particles called microplastics. This physical fragmentation of the polymer exhibits real degradation in terms of molecular weight. One of the most ubiquitous and long-lasting changes to the environment is the accumulation polyethylene (PE), mulation of fragmented plastics. Within just a few decades, since mass production of plastic products has initiated, plastic debris has accumulated in terrestrial and marine environments. These microplastics can be ingested by various marine animals that, by mistake, identify the microplastics as plankton. Thus, the ingested plastic debris is likely to penetrate and accumulate in the food chain, exerting multiple hazards that their outcome still have to be elucidated [3,4••].

Abiotic degradation of plastics may affect biodegradation

Abiotic degradation includes the physical and/or chemical processes that exerts intramolecular modifications in the polymer. Biodegradable polymers are comprised of two types: a) Polymers that are intrinsically biodegradable; whose chemical structure enables direct enzymatic degradation (e.g. starch, cellulose, chitin, etc.) and b) Polymers that undergo photo oxidation or thermo oxidation upon exposure to u.v. or heat, respectively. Often, the synthetic polymers will contain pro-oxidant (a photo sensitizer) compounds; these are known as oxo-biodegradable polymers. For example, photodegradation of low density polyethylene (LDPE) and polypropylene (PP) films can be activated using metal oxides as catalysts [5–8]. Those materials require oxidative degradation in order to reduce the molar mass and to form oxygenated groups (such as carbonyl), which are more easily metabolized by microorganisms. Pro-oxidants may also be incorporated into the polymer chain (1–5%, w/w) [9]. Saturated humidity increases abiotic oxidative degradation and biodegradation, compared to natural humidity. The PE samples mineralized about 12% of the original carbon in compost at 58 °C for three months after being exposed for one year to natural weathering. Exposure periods longer than three

Figure 1

'Sea' of plastic. Soil mulching with polyethylene, of agricultural crops, has become a major source of polyethylene waste.

months and environmental moisture exert little influence on the degradability of cobalt-activated PE [10].

Microbial degradation of plastics

During the past two years two comprehensive reports on the degradation of plastic had been published [11••,12••]. These studies reported on plastic biodegradation (i.e. exerted by the aid of microorganisms) and on biodegradability (i.e. the potential of a synthetic polymer to be degraded by microorganisms). The degradation process of PE, provided as the sole source of carbon and energy in soil microorganisms specifically, showed that small fragments were consumed faster than larger ones [13]. However, this cannot serve as an indication for biodegradation that requires reduction in molecular weight.

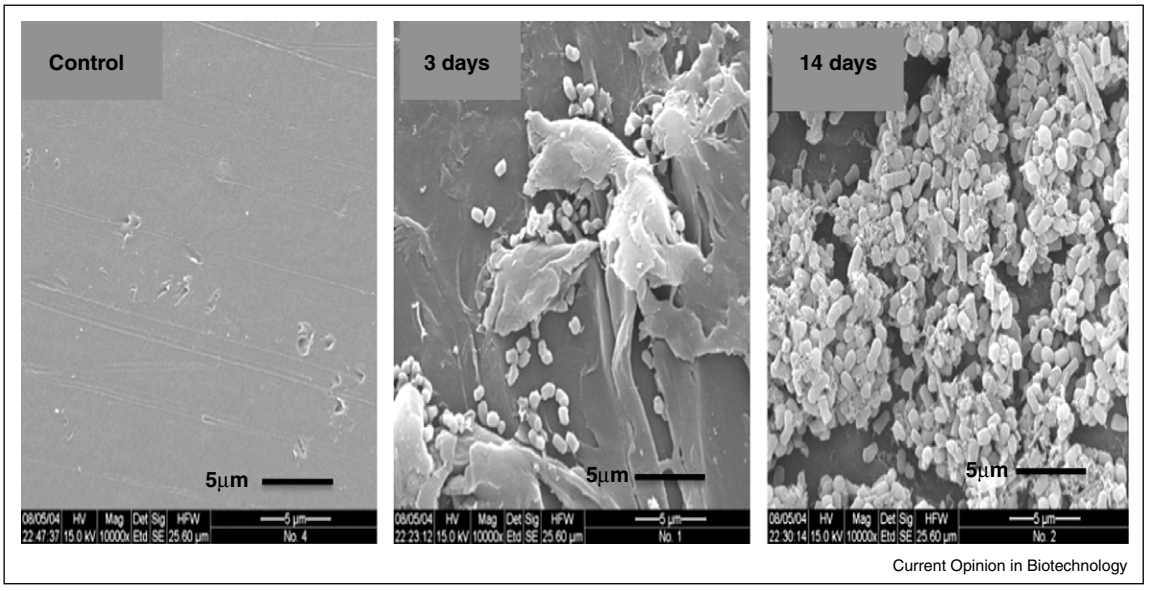
The oxidizing effect of u.v. irradiation on polyolefins degradation is well documented [11••]. For example, biodegradation in compost was investigated for irradiated ethylene propylene copolymers, low-density PE (LDPE) and isotactic-polypropylene (PP) films. As expected, the tests showed that degradation increased with increasing irradiation time. However, after 6 months of incubation, LDPE was still the slowest sample to be degraded [14]. The ability to degrade or modify PE films was also demonstrated with two *Actinomycetes* sp., showing that extracellular enzymes detected were able to degrade the polymer, although at a slow rate [15]. LDPE modified with starch was tested for biodegradation in soil microcosms for 6 months. It was shown that inoculation of soil with *P. chrysosporium* enhanced the degradation and biomass increased much more than in non-inoculated soil [15]. Heat-treated degradable films of starch-PE were incubated with extracellular concentrates of *S. viridosporus* T7A, *S. badius* 252 and *S. setonii* 75Vi2 and degradation by active enzymes was observed. The study confirmed a modification of the polymer structure and of the material as well as material-property changes [16]. Presumably, this was owing to the fact that many of these polymers served as nutrient reservoir that can be utilized after being depolymerized to short oligomers. Shah *et al.* [11••] reported various natural polyesters such as: polyhydroxybutirate [17] and polycaprolactone [18] that can also be degraded and assimilated by various microbial populations. Furthermore, a number of PHA depolymerases and their genes have been isolated [19,20].

Similarly, degradation of polyurethane was obtained with several fungal strains: *Fusarium solani*, *Curvularia senegalensis* and *Aureobasidium pullulans* sp. [21] and that of polyvinyl chloride (PVC) by the bacterium *Pseudomonas putida* [22].

The relative high number of reports exhibiting the biodegradability of a wide range of plastics may lead to a wrong conclusion that most of the plastic polymers can be readily biodegraded. In fact, in terms of amounts, the production of the polymers PE and PS is, by far, greater than that of the rest of the other plastic compounds that are considered biodegradable. Furthermore, not all types of degradable plastics are destroyed completely in natural environments, raising the question of the definition of biodegradable. Biodegradation is defined as the capacity of one or more cultures of microorganisms to utilize the synthetic polymer as a sole source of carbon. In contrast to natural polymers that exhibit a relatively high level of biodegradability, synthetic polymers such as PE and PS are considered to be non-biodegradable. To facilitate biodegradation of these polymers a preliminary step of photo-oxidation or thermo-oxidation has routinely been employed. This oxidation of the polymer results in the formation of carbonyl residues that can be consumed by non-specific microbial populations. Thus far, only a few microbial strains are capable of degrading standard non-oxidized PE [11••]. These include the actinomycete *Rhodococcus ruber* (strain C208), [23–25]. The thermophylic bacterium *Brevibacillus borstelensis* (strain 707)[26] and the fungus *Penicillium simplicissimum* [27]. Interestingly, even a large screening of 200 soil samples from various locations, yielded only five PE degrading strains [23]. In liquid culture, the degradation of PE by C208 resulted in a reduction of ca. 8% in the dry weight of the polyolefin within 30 days of incubation. Owing to its high durability PE is often photo-oxidized and/or thermo oxidized as a pretreatment before the incubation with the degrading culture [23].

Formation of biofilm on the plastic surface seems to be the favorite mode of growth of plastic degrading bacteria (Figure 2). Since plastic polymers such as PE and PS are hydrophobic, forming a stable biofilm requires that the

Figure 2



SEM photomicrographs of biofilm formed by *Rhodococcus ruber* C208 on the surface of UV photo oxidized polyethylene. Initiation of biodegradation was detecte as early as after 3 days. UV irradiated but not inoculated served as control.

bacterial surface will also be hydrophobic. Indeed, there is evidence in the literature demonstrating a correlation between carbon starvation and bacterial hydrophobicity [28]. Similarly, Sanin *et al.* (2003) reported changes in bacterial surface hydrophobicity in response to carbon starvation [29]. It was shown that with carbon starved culture bacterial isolates, including *R. corallinus*, became more hydrophobic and more adhesive than with non-starved cells. These findings may explain the high affinity of C208 cells for the polystyrene and raise the possibility that the low carbon availability in srain C208 cultures may also enhance hydrophobic interactions and biofilm development. Similarly, in a recent study, polyethylene, the biofilm of *R. ruber* formed on polyethylene showed high viability and even after 60 days of incubation adhered to the polyethylene without any supplementation of external carbon [24]. These findings are supported by biofilms of many other bacteria. For example, some plastic polymers (natural or synthetic) that are readily degraded by the general marine and soil microflora. This method relies on introducing non-specific chemical bonds into the polymer that increase its degradability by the general microbial population [11••]). Specific biodegradation is based on one or more axenic cultures that are used to inoculate the plastic [23,24,26,27] or as a consortium [30].

Despite of their high durability and non-biodegradability, synthetic polymers are not inert. Indeed, when C208 were grown on PS as the sole carbon source. The carbon limitation resulted in death of planktonic cells while biofilms exhibited high adhesion and durability [25]. Furthermore, prolonged incubation (of up to 8 weeks) of C208 with polystyrene resulted in a dense biofilm on the polystyrene surface that may have led to a partial degradation (about 0.8% weight loss) of the polymer [25]. Evaluation of bacterial cell-surface hydrophobicity can be carried out by assessing the bacterial adhesion to hydrocarbons (BATH) assay [31] or by the salt aggregation test (SAT) [32]. Both methods confirmed the high level of cell surface hydrophobicity in strain C208 compared that of three other isolates that were obtained from the same consortium. Interestingly, these strains were less efficient than C208 in the degradation of polyethylene. Indeed, mineral oil, but not nonionic surfactants, enhanced the colonization of polyethylene and increased biodegradation by about 50% [23].

Direct biodegradation of polyethylene by extracellular enzymes

In search for depolymerases that could serve as candidates for oxidizing durable synthetic polymers we have recently isolated a putative laccase produced by the actinomycete *R. ruber* that is involved in polyethylene biodegradation. Laccases are best known in lignin-biodegrading fungi, where they catalyse the oxidation of aromatic compounds. However, there is evidence of laccase activity on non-aromatic substrates [33]. Since laccase is a copper-binding enzyme with 4 binding sites that may contribute to its oxidizing activity [34], we have postulated that copper might affect laccase activity and contribute to the biodegradation of polyethylene. Indeed, the data obtained from RT-PCR, showed a 13-fold increase in laccase mRNA levels, confirmed that copper is involved in the induction of laccase in strain C208. Similarly, the addition of

400 μM of copper resulted in an 18-fold increase in the laccase activity of *Trametes versicolor* [35]. Likewise, Palmieri *et al.* (2000) reported that addition of $CuSO_4$ increased the laccase activity of *Pleurotus ostreatus* by up to 50-fold [36]. Nevertheless, laccase from *T. versicolor* did not affect polyethylene (Santo and Sivan, unpublished). This may be due to differences in enzyme activity and/or the presence of effective mediators in strain C208 laccase preparation.

Partial sequencing of strain C208 laccase enabled us to quantify the induction of laccase mRNA by copper. This is supported by the fact that a variety of species of the actinomycete *Streptomyces* also produced elevated levels of laccase in the presence of copper [37]. Similarly, southern blot analysis of laccase cDNA produced by the fungi *P. ostreatus* and *T. pubescens* revealed that copper has an inductive effect on laccase cDNA production [38].

One of the main features of the laccase produced by C208 is its relative high level of stability, with optimal activity at 70 °C. This temperature is markedly higher than that of other thermo stable laccase producers [39]. The high thermostability of the strain C208 laccase may pave the way for large scale reactors where degradation of polyethylene will be carried out at high temperature, facilitating high kinetcs reactions. By contrast, *S. lavendulae* and *T. versicolor* laccases reached their optimum at around 50 °C [39,40]. In accordance with the stimulation of laccase expression, copper enhanced polyethylene biodegradation revealed by FTIR spectra indicated an increase of more than 40% in the carbonyl index ($A_{C=O}$:A_{CH2}). Thus, laccase may play a role in oxidation of the hydrocarbon backbone of polyethylene. One of the most important indicators of polyethylene biodegradation is molecular weight reduction. We have demonstrated (by gel permeation chromatography) that cell-free laccase incubated with polyethylene mediated a reduction of ca. 20% and 15% in the average molecular weight and average molecular number of polyethylene, respectively. Modification of the surface of high density polyethylene films was achieved by treatment with soybean peroxidase [41]. Moreover, our findings advocate that copper and copper-binding enzymes may play a major role in biodegradation of polyethylene and, possibly, of other plastics.

Conclusions

Safe disposal of plastic waste via biodegradation should focus on the most consumed polymers (i.e. polyethylene, polypropylene and polystyrene). Unfortunately, these polymers are also the most durable plastics. In view of these obstacles, several tasks should be addressed in order to obtain safe waste disposal. These include a) photo and/or thermo oxidation applied before exposure to the biotic environment b) selection and isolation of a strain (or a consortium) that produce high levels of oxidative enzymes. c) Increase induction of intrinsic and external oxidative enzymes. d) Increase of cell surface hydrophobicity by the use of non-ionic surfactants that will enhance biofilm formation.

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