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Biodegradability of Polymers: Regulations and Methods for Testing

Dr. Rolf-Joachim Müller
Gesellschaft für Biotechnologische Forschung mbH, Braunschweig, Germany;
Tel.: +49 (0)531 6181 610; Fax: +49 (0)531 6181 175; E-mail: rmu@gbf.de

ASTM	American Society for Testing and Materials
AFM	atomic force microscopy
CEN	European Committee for Standardization (Comité Européen de Normalisation)

DIN	German Institute for Standardization (Deutsches Institut für Normung)
DOC	dissolved organic carbon
ISO	International Standardization Organization
JIS	Japanese Institute for Standardization
MITI	Ministry of International Trade and Industry (Japan)
OECD	Organization for Economic Co-operation and Development (Europe)
PHB	poly(β-hydroxybutyrate)
SEM	scanning electron microscopy
UNI	Italian National Standards Body (Ente Nationale Italiano di Unificazione)

1
Introduction

Since the first developments of polymeric materials, scientists and engineers have made intensive efforts to increase the stability of these materials with regard to their diverse environmental influences. As a result, polymeric materials (plastics) are now used in all sectors of life as very durable products with tailor-made properties. During the past decade the intense use of modern plastics, combined with their enormous stability, has created serious problems with plastic waste, with the main problems being caused by plastic packaging. As possible alternative waste management strategies to landfilling, incineration or plastics recycling are not optimal and remain the subject of much controversy and discussion among both scientists and the public. On the basis of these problems, an intensive activity has been undertaken since the early 1990s to develop novel plastics which have performance comparable with that of conventional polymers, but are also susceptible to microbial degradation. The intention was that these materials would reduce waste deposit volume while undergoing degradation in a landfill, or alternatively they could be treated in composting plants. These technologies offered a new approach to the management of plastics waste. Moreover, when this waste management system is combined with the use of renewable resources to produce the polymers initially, it is likely that biodegradable plastics may simply become part of a natural cycle.

The biodegradability of plastics provides these materials with novel and additional properties which may also be beneficial during their use. For example, in agriculture biodegradable mulch films would not need to be collected after use (a procedure which is highly labor-intensive) and then landfilled or incinerated, but would decompose with time and could simply be ploughed into the soil, where they would biodegrade. Hence, it is not surprising that this concept of using biodegradable plastics has become of major interest during recent years.

Biodegradable plastics, as novel materials, make claims to be environmentally friendly. Consequently, it must be proved by using scientifically based and generally accepted methods that this is indeed the case. A first generation of biodegradable plastics consisted simply of polyethylenes blended with starch. Initially, these were sold as biodegradable plastics, but in practice they did not fulfill the expectations of the users. Arguments for claiming these blends as biodegradable included the growth of microorganisms on the material's surface, or a certain loss in mechanical properties (e.g., tensile strength) when they were exposed to the environment. However, the evaluation methods used – which had originated from the field of plastics biocorrosion – proved unsuitable to characterize biodegradable materials. At the time, the failure of these polyethylenes led to a generally negative image of biodegradable plastics; however, the subsequent development of suitable testing methods and evaluation criteria for biodegradable plastics has resulted in the definition of standards by various national and international standardization bodies during the past 10 years. Indeed, this process is ongoing, as the number of different environments in which plastics may be degraded has made necessary the establishment of a complex and extensive battery of test methods and evaluation criteria for these materials.

2
General Mechanism of Biodegradation, and Definitions

The term "biodegradable plastics" normally refers to an attack by microorganisms on nonwater-soluble polymer-based materials (plastics). This implies that the biodegradation of plastics is usually a heterogeneous process. Because of a lack of water-solubility and the size of the polymer molecules, microorganisms are unable to transport the polymeric material directly into the cells where most biochemical processes take place; rather, they must first excrete extracellular enzymes which depolymerize the polymers outside the cells (Figure 1). As a consequence, if the molar mass of the polymers can be sufficiently reduced to generate water-soluble intermediates, these can be transported into the microorganisms and fed into the appropriate metabolic pathway(s). As a result, the end-products of these metabolic processes include water, carbon dioxide and methane (in the case of anaerobic degradation), together with a new biomass. The extracellular enzymes are too large to penetrate deeply into the polymer material, and so act only on the polymer surface; consequently, the biodegradation of plastics is usually a surface erosion process.

Although the enzyme-catalyzed chain length reduction of polymers is in many cases the primary process of biodegradation, nonbiotic chemical and physical processes can also act on the polymer, either in parallel or as a first stage solely on the polymer. These nonbiotic effects include chemical hydrolysis, thermal polymer degradation, and oxidation or scission of the polymer chains by irradiation (photodegradation). For some materials, these effects are used directly to induce the biodegradation process [e.g., poly(lactic acid); pro-oxidant modified polyethylene], but they must also to be taken into account when biodegradation is caused predominantly by extracellular enzymes. Because of the coexistence of biotic and nonbiotic processes, the entire mechanism of polymer degradation could – in many cases – also be referred to as environmental degradation.

Environmental factors not only influence the polymer to be degraded, they also have a crucial influence on the microbial population and on the activity of the different microorganisms themselves. Parameters such as humidity, temperature, pH, salinity, the presence or absence of oxygen and the supply of different nutrients have important effects on the microbial degradation of polymers, and so these conditions must be considered when the biodegradability of plastics is tested.

Another complicating factor in plastics biodegradation is the complexity of the plastic materials with regard to their possible structures and compositions. In many cases plastics do not consist simply of only one chemical homogeneous component, but

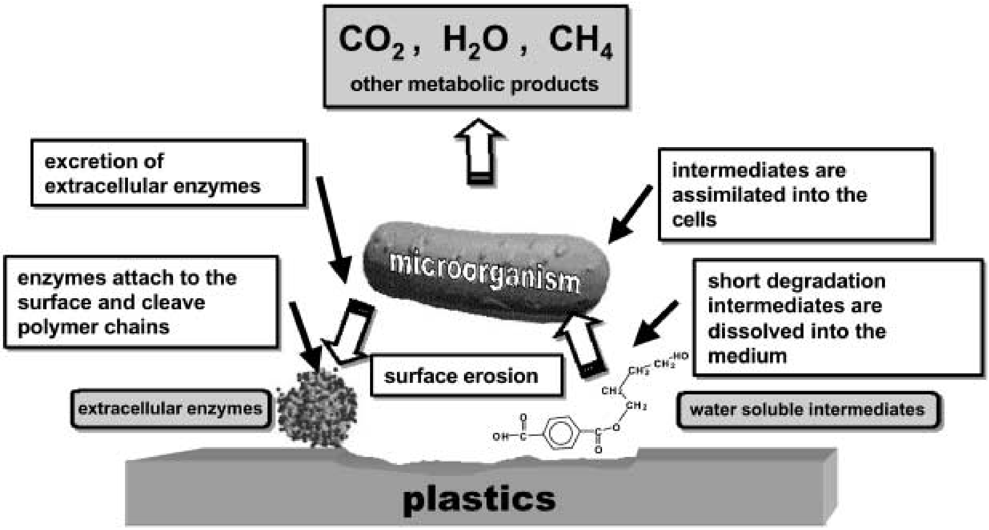

Fig. 1 General mechanism of plastics biodegradation.

contain different polymers (blends) or low-molecular weight additives (e.g., plasticizers). Moreover, within one polymer itself different structural elements can be present (copolymers), and these may either be distributed statistically along the polymer chains (random copolymers) or distributed alternately (alternating copolyesters); they may also be used to build longer blocks of each structure (block-copolymers). Another structural characteristic of a polymer is the possible branching of chains or the formation of networks (cross-linked polymers). These different structures of a polymer, despite having the same overall composition, can directly influence accessibility of the material to the enzyme-catalyzed polymer chain cleavage, and also have a crucial impact on higher-ordered structures of the polymers (crystals, crystallinity, glass transition) which have been shown predominantly to control the degradation behavior of many polymers (Marten, 2000). Additionally, the crystallinity and crystal morphology is dependent upon the processing conditions, and can change with time.

All of the above-described factors must be considered when measuring the biodegradation of plastics and interpreting the results, and this makes the testing of plastics biodegradability a highly interdisciplinary process.

The standardized evaluation of biodegradable plastics should always be based on definitions, and what biodegradation with regard to plastics actually means. Several different definitions have been published by national and international standardization bodies and organizations (Table 1).

Whilst in the ISO definition of biodegradable plastics only a chemical change of the material (e.g., oxidation) by microorganisms is requested, the CEN and DIN, in contrast, demand in their definitions the conversion of plastics into microbial metabolic products. Other definitions such as inherent biodegradability or ultimate biodegradability are adapted from the area of degradation of low molecular- weight chemicals, but these can also be applied to polymers. Generally, the definitions do not specify any environment or time frames; this must be carried out according to corresponding standards.

Based on these definitions, biodegradable plastics (or packaging materials) are not necessarily suitable for composting. In the definition of compostability, biodegradation of the material is only one requirement, and further demands such as good compost quality after composting plastics are also included.

Tab. 1 Definitions used in correlation with biodegradable plastics

DIN FNK 103.2	Biodegradable plastics[1] A plastic material is called biodegradable if all its organic compounds undergo a complete biodegradation process. Environmental conditions and rates of biodegradation are to be determined by standardized test methods. Biodegradation[3] Biodegradation is a process, caused by biological activity, which leads under change of the chemical structure to naturally occurring metabolic products.
ASTM sub-committee D20-96	Biodegradable plastics[1] A degradable plastic in which the degradation results from the action of naturally occurring microorganisms such as bacteria, fungi and algae.
Japanese Biodegradable Plastics Society	Biodegradable plastics[1] Polymeric materials which are changed into lower molecular weight compounds where at least one step in the degradation process is though metabolism in the presence of naturally occurring organisms.
ISO 472	Biodegradable plastics[1] A plastic designed to undergo a significant change in its chemical structure under specific environmental conditions resulting in a loss of some properties that may vary as measured by standard test methods appropriate to the plastic and the application in a period of time that determines its classification. The change in the chemical structure results from the action of naturally occurring microorganisms.
CEN	Biodegradable plastics[1] A degradable material in which the degradation results from the action of microorganisms and ultimately the material is converted to water, carbon dioxide and/or methane and a new cell biomass. Biodegradation[2] Biodegradation is a degradation caused by biological activity, especially by enzymatic action, leading to a significant change in the chemical structure of a material Inherent biodegradability[2] The potential of a material to be biodegraded, established under laboratory conditions. Ultimate biodegradability[2] The breakdown of an organic chemical compound by microorganisms in the presence of oxygen to biodegradability carbon dioxide, water and mineral salts of any other elements present (mineralization) and new biomass or in the absence of oxygen to carbon dioxide, methane, mineral salts and new biomass. Compostability[2] Compostability is a property of a packaging to be biodegraded in a composting process. To claim compostability it must have been demonstrated that a packaging can be biodegraded in a composting system as can be shown by standard methods. The end-product must meet the relevant compost quality criteria.

[1] Pagga (1998);[2] Calmon-Decriaud et al. (1998);[3] DIN V 94900 (1998)

However, despite these apparently inhomogeneous definitions, the different standards and evaluation schemes are surprisingly congruent.

3
Testing Methods

Test methods to determine biological action on man-made materials have been available for many years, and for different classes of materials. Nowadays, the evaluation of the degradability of chemicals in the environment (and especially in waste water) as one important aspect of the ecological impact of a compound has become very important when attempting to bring a new chemical product to the marketplace. For this reason, a large number of standardized tests have been developed for different environments, and with the use of different analytical methods (Pagga, 1997). An overview of existing international standards in this area is provided in Table 2.

Also for the evaluation of the influence of microorganisms on polymer, test methods have been established long before biodegradable plastics were first developed. Although conventional plastics are relatively resistant against environmental influences, in some cases microorganisms can partially attack the plastics and cause undesired changes in the material properties, e.g. in the color or in mechanical properties such as flexibility or mechanical strength (Pantke, 1977; Seal and Eggins, 1981) (Table 3).

Although this wide range of degradation tests already existed, it was necessary to develop special test methods when dealing with biodegradable plastics. The standards listed in Table 3 do not consider the special aspects (see above) of plastics materials, and for biocorrosion phenomena the question is not whether the plastic is degraded or not, but rather whether changes in the material properties may be caused by minor chemical changes in the polymers (e.g., extraction of plasticizer, oxidation, etc.).

The test methods which have been developed especially for biodegradable plastics during the past decade (Itävaara and Vikman, 1996) are predominantly based on principles used for the evaluation of low molecular-weight substances, but have been modified with respect to the particular environments in which biodegradable plastics might be degraded. The methods also consider the fact that plastics often have a complex composition and are degraded mainly by a heterogeneous surface mechanism.

Tab. 2 Standard test methods for biocorrosion phenomena on plastics

ASTM G21-96	Standard practice for determining resistance of synthetic polymer materials to fungi
ASTM G29-96	Standard practice for determining algal resistance of plastic films
DIN IEC 60068–2-10-1991	Elektrotechnik; Grundlegende Umweltprüfverfahren; Prüfung J und Leitfaden: Schimmelwachstum; (Identisch mit IEC 60068–2-10: 1988)
EN ISO 846–1997	Plastics – Evaluation of the action of microorganisms
IEC 60068–2-10-1988	Elektrotechnik; Grundlegende Umweltprüfverfahren; Prüfung J: Schimmelwachstum
ISO 846–1997	Plastics: Determination of behaviour under the action of fungi and bacteria. Evaluation by visual examination or measurement of changes in mass or physical properties

Tab. 3 Standard test methods for biodegradability of chemicals

OECD Guidelines (OECD, 1993)	
301	Ready Biodegradability
301 A–1992	DOC Die-Away Test
301 B–1992	CO_2 Evolution Test
301 C–1992	Modified MITI Test
301 D–1992	Closed Bottle Test
301 E–1992	Modified OECD Screening Test
301 F–1992	Manometric Respirometry Test
302	Inherent Biodegradability
302 A-1981	Modified SCAS Test
302 B-1992	Zahn-Wellens Test
302 C-1981	Modified MITI Test
302 D B draft (2002)	Inherent biodegradability-concave test
303	Simulation Test
303 A-2001	Aerobic Sewage Treatment: Activated Sludge Units
304	Biodegradation in Soil
304 A-1981	Inherent Biodegradability in Soil
306-1992	Biodegradability in Seawater
307 B draft (2000)	Aerobic and anaerobic transformation in soil
308 B draft (2000)	Aerobic and anaerobic transformation in aquatic sediment systems
309 B draft (2001)	Aerobic mineralisation in surface water-simulation biodegradation test
301 B draft (2002)	Ready biodegradability B CO_2 in sealed vessels (Headspace test)
311 B draft (2002)	Ready anaerobic biodegradability: Gas production from diluted anaerobic sewage sludge
ISO 7827–1994	Water quality – Evaluation in an aqueous medium of the “ultimate” aerobic biodegradability of organic compounds – Method by analysis of dissolved organic carbon (DOC)
ISO 9439–1999	Water quality – Evaluation in an aqueous medium of the “ultimate” aerobic biodegradability of organic compounds – Method by analysis of released carbon dioxide
ISO 9408–1999	Water quality – Evaluation in an aqueous medium of the “ultimate” aerobic biodegradability of organic compounds – Method by determining the oxygen demand in a closed respirometer
ISO 9887–1992	Water quality – Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium – Semi-continuous activated sludge method (SCAS)
ISO 9888 B 1999	Water quality – Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium – Static test (Zahn-Wellens method)
ISO 10634–1995	Water quality – Guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium
ISO 10707–1994	Water quality – Evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds – Method by analysis of biochemical oxygen demand (closed bottle test)
ISO 10708–1997	Water quality – Evaluation in an aqueous medium of the ultimate aerobic biodegradability of organic compounds – Method by determining the biochemical oxygen demand in a two-phase closed – bottle test
ISO 11733–1995	Water quality – Evaluation of the elimination and the biodegradability of organic compounds in an aqueous medium. Activated sludge simulation test
ISO 11734–1995	Water quality – Evaluation of the ultimate anaerobic biodegradability of organic compounds in digested sludge. Method by measurement of the biogas production

Tab. 3 (cont.)

ISO/FDIS 14592–1	Water quality – Evaluation of the aerobic biodegradability of organic compounds at low concentrations – Part 1: Shake-flask batch test with surface water or surface water/sediment suspension
ISO/DIS 14592–2	Water quality – Evaluation of the aerobic biodegradability of organic compounds at low concentrations – Part 2: Continuous flow river model with attached biomass
ISO 14593–1999	Water quality – Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium – Method by analysis of inorganic carbon in sealed vessels (CO_2 headspace test)
ISO/TR 15462–1997	Water quality – Selection of tests for biodegradability
ISO 16221 B 2001	Water quality – Guidance for determination of biodegradability in the marine environment
EN ISO 7827–1995	Water quality – Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds – Method by analysis of dissolved organic carbon (DOC)
EN ISO 9439–2000	Water quality – Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium – Carbon dioxide evolution test
EN ISO 9408–1999	Water quality – Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium by determination of oxygen demand in a closed respirometer
EN ISO 9887–1994	Water quality – Evaluation of the aerobic biodegradability of organic compounds in an aqueous medium – Semi-continuous activated sluge method (SCAS)
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EN ISO 11734–1998	Water quality – Evaluation of the "ultimate"; anaerobic biodegradability of organic compounds in digested sludge – Method by measurement of the biogas production
Inhibition tests	
ISO 8192 B 1986	Water quality – Test for inhibition of oxygen consumption by activated sludge
ISO 9509 B 1989	Water quality – Method for assessing the inhibition of nitrification of activated sludge microorganisms by chemicals and waste waters
ISO 10712 B 1995	Water quality – *Pseudomonas putida* growth inhibition test (*Pseudomonas* cell multiplication inhibition test)
ISO 11348 Part 1, 2, 3 – 1998	Water quality – Determination of the inhibitory effect of water samples and the light emission of *Vibrio fischeri* (Luminescent bacteria test)
EN ISO 8192 – 1995	Water quality – Test for inhibition of oxygen consumption by activated sludge
EN ISO 9509 – 1995	Water quality – Method for assessing the inhibition of nitrification of activated sludge microorganisms by chemicals and waste water
EN ISO 10712 – 1996	Water quality – *Pseudomonas putida* growth inhibition test (*Pseudomonas* cell multiplication inhibition test)
EN ISO 11348 Teil 1, 2, 3 – 1998	Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test)

3.1
General Principles in Testing Biodegradable Plastics

When testing the degradation phenomena of plastics in the environment, there is a general problem concerning the type of tests to be applied, and the conclusions which can be drawn. In principle, tests can be subdivided into three categories: field tests; simulation tests; and laboratory tests (Figure 2).

Although field tests, such as burying plastics samples in soil, placing it in a lake or river, or performing a full-scale composting process with the biodegradable plastic, represent the ideal practical environmental conditions, there are several serious disadvantages associated with these types of test. One problem is that environmental conditions such as temperature, pH, or humidity cannot be well controlled; secondly, the analytical opportunities to monitor the degradation process are limited. In most cases it is only possible to evaluate visible changes on the polymer specimen, or perhaps to determine disintegration by measuring weight loss. The latter approach is problematic however if the material breaks into small fragments that must be quantitatively recovered from the soil, compost or water. The analysis of residues and intermediates is complicated by the complex and undefined environment. Since the pure physical disintegration of a plastic is not regarded as biodegradation in the sense of most definitions (see above), these tests alone can never prove whether a material is biodegradable, or not.

As an alternative to field tests, various simulation tests in the laboratory have been used to measure the biodegradation of plastics. Here, the degradation might take place in compost, soil or sea-water placed in a controlled reactor in a laboratory. Although the environment is still very close to the field-test situation, the external parameters (temperature, pH, humidity, etc.) can be controlled and adjusted, and the analytical tools available are better than would be used for field tests (e.g., for analysis of residues and intermediates, determination of CO_2

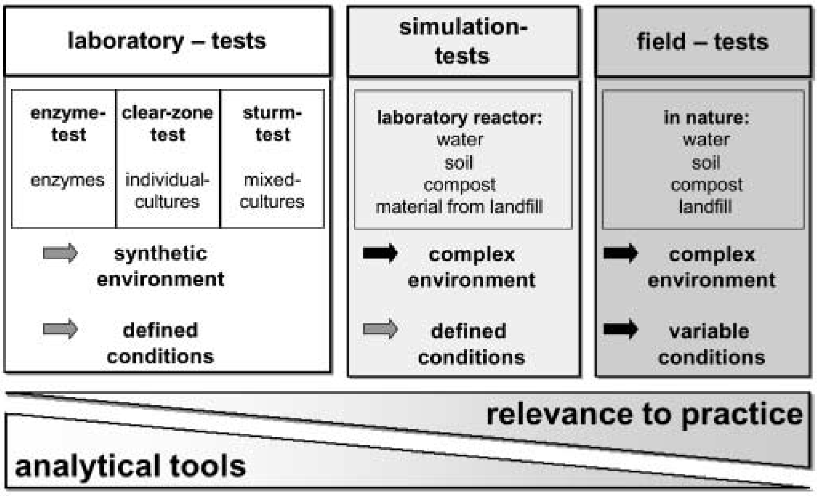

Fig. 2 Schematic overview on tests for biodegradable plastics.

evolution or O_2 consumption). Examples of such tests include the soil burial test (Pantke and Seal, 1990), the so-called "controlled composting test" (Pagga et al., 1995; Tosin et al., 1996; Degli-Innocenti et al., 1998; Ohtaki et al., 1998; Tuominen et al., 2002), test simulating landfills (McCartin et al., 1990; Smith et al., 1990; McCarthy et al., 1992) or aqueous aquarium tests (Püchner et al., 1995). On occasion, in order to reduce the time taken to conduct the tests, nutrients are added to increase the microbial activity and accelerate degradation.

The most reproducible biodegradation tests are the laboratory tests, where defined media are used (in most cases synthetic media) and inoculated with either a mixed microbial population (e.g., from waste water) or individual microbial strains which may have been especially screened for a particular polymer. In such tests, which may be optimized for the activity of the particular microorganisms used, polymers often exhibit a much higher degradation rate than would be observed under natural conditions. This can be regarded as an advantage when studying the basic mechanisms of polymer biodegradation, but in laboratory tests it is only possible to derive limited conclusions on the absolute degradation rate of plastics in a natural environment. However, for many systematic investigations these tests are widely used.

A move towards more reproducible and controlled degradation tests involves the use of systems where only those extracellular enzymes known to depolymerize a particular group of polymers are used. Comparable to weight loss measurements, this method cannot be used to prove biodegradation in terms of metabolism by a microorganism, but the system is valuable when carrying out

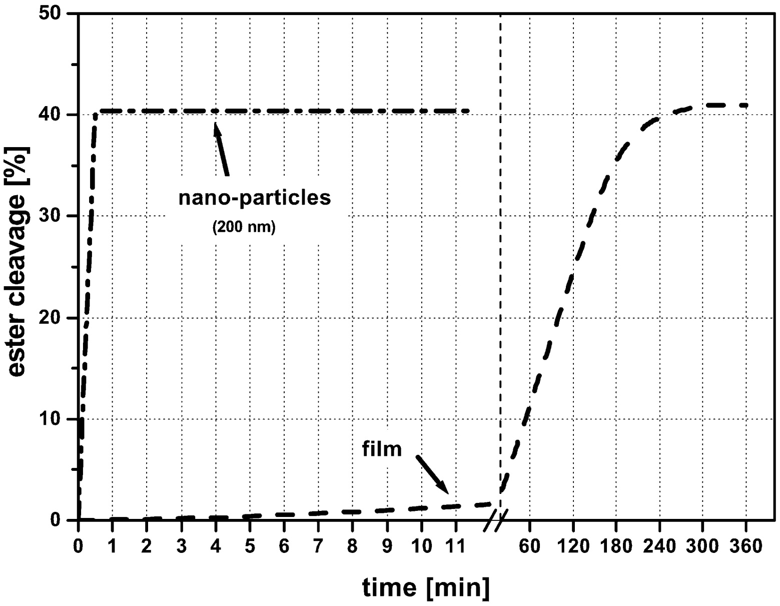

Fig. 3 Comparison of the enzymatic degradation of the polyester poly(tetramethylene adipate) with a lipase from *Pseudomonas* sp. for a polymer film and polymer nanoparticles at pH 7 and 40°C. Degradation expressed as percentage of cleaved ester bonds (maximum ester cleavage of ~40% results from the dissolution of low molecular-weight esters which are not accessible to attack by the lipase). (From Welzel et al., 2002.)