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Evaluation of Biodegradation of Plastics

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Abstract:
Currently, our world is facing many challenges such as increase in waste flows, energy depletion and climate changes. In addition, global plastics production exceeds 180 million tons per year with yearly increase in supply and demand (Cutter, 2006). These plastics turn to solid waste after their end of life and will accumulate in the environment. Hence, the production of biodegradable plastics is important from an environmentally friendly point of view to reduce the accumulation of plastic waste in the environment (Lovino et al., 2008). Petroleum-based plastic products are characterized as not easily degradable because of their relatively high stability and hydrophobic characteristics (Lovino et al., 2008).
Biodegradable plastics offer a lot of advantages such as increased soil fertility, low accumulation of bulky plastic materials in the environment (which invariably will minimize injuries to wild animals), and reduction in the cost of waste management. Furthermore, biodegradable plastics can be recycled to useful metabolites (monomers and oligomers) by microorganisms and enzymes. A second strategy involves degradation of some petroleum-derived plastics by biological processes. A typical example can be seen in the case of some aliphatic polyesters such as Polycaprolactone (PCL) and Polybutylene succinate (PBS) that can be degraded with enzymes and microorganisms [Tokiwa et al.,1976, Tokiwa and Suzuki, 1977a; Tokiwa and Suzuki, 1977b].

1. Biodegradation - Standard Definitions

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

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

Table 1: Definitions used in correlation with biodegradable plastics
[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.

2. 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). Although this wide range of degradation tests already existed, it was necessary to develop special test methods when dealing with biodegradable plastics. [Table 2]. The test methods which have been developed especially for biodegradable plastics during the past decade (Itavaara 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.

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 Umweltprufverfahren; Prufung J und Leitfaden: Schimmelwachstum; (Identisch mit IEC 60068-2-10: 1998)
EN ISO 846 – 1997	Plastics - Evaluation of the action of microorganisms
IEC 60068 – 2-10-1988	Elektrotechnik; Grundlegende Umweltprufverfahren; Prufung J und Leitfaden: 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

Table 2: Standard test methods for biocorrosion phenomena on plastics

3. 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 1).

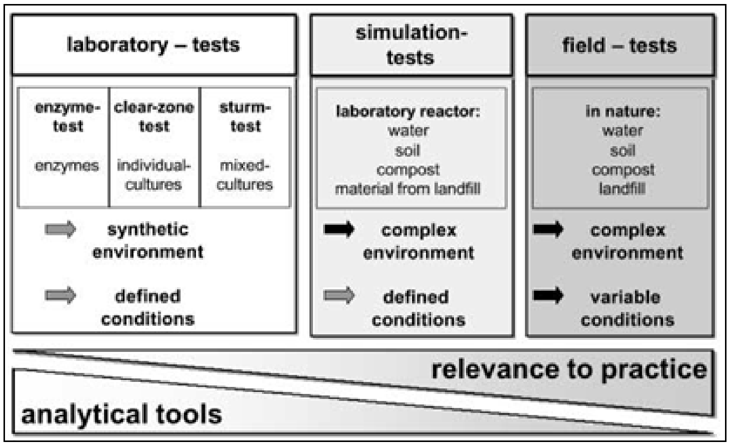

Figure 1: Schematic overview on tests for biodegradable plastics

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 plastics, 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. 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 for field tests (e.g., for analysis of residues and intermediates, determination of CO2 evolution or O2 consumption). 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.

4. Standard Testing Methods for Bio Degradation of Plastics

4.1. Visual Observations

The evaluation of visible changes in plastics can be performed in almost all tests. Effects used to describe degradation include roughening of the surface, formation of holes or cracks, de-fragmentation, changes in color, or formation of bio-films on the surface. These changes do not prove the presence of a biodegradation process in terms of metabolism, but the parameter of visual changes can be used as a first indication of any microbial attack. To obtain information about the degradation mechanism, more sophisticated observations can be made using either scanning electron microscopy (SEM) or atomic force microscopy (AFM) (Ikada, 1999). A number of other techniques can also be used to assess the biodegradability of polymeric material. These include; Fourier transform infrared spectroscopy (FTIR), differential scanning colorimetry (DSC), nuclear magnetic resonance spectroscopy (NMR), X-ray photoelectron spectroscopy (XPS), X-ray Diffraction (XRD), contact angle measurements and water uptake.

4.2. Weight Loss Measurements: Determination of Residual Polymer

The mass loss of test specimens such as films or test bars is widely seen in degradation tests (especially in field- and simulation tests), although again no direct proof of biodegradation is obtained. By combining a structural analysis of the residual material and the low molecular weight intermediates, detailed information regarding the degradation process can be obtained, especially if a defined synthetic test medium is used (Witt *et al.,* 2001).

4.3. Changes in Mechanical Properties and Molar Mass

As with visual observations, changes in material properties cannot be proved directly due to metabolism of the polymer material. However, changes in mechanical properties are often used when only minor changes in the mass of the test specimen are observed. Properties such as tensile strength are very sensitive to changes in the molar mass of polymers, which is also often taken directly as an indicator of degradation (Erlandsson *et al.,* 1997).

4.4. CO_2 Evolution / O_2 Consumption

Under aerobic conditions, microbes use oxygen to oxidize carbon and form carbon dioxide as one of the major metabolic end product. Consequently, the consumption of oxygen (respirometric test) (Hoffmann *et al.,* 1997) and the formation of carbon dioxide (Sturm test) are good indicators for polymer degradation, and are the most often used methods to measure biodegradation in laboratory tests. Due to the normally low amount of other carbon sources present in addition to the polymer itself when using synthetic mineral media, only a relatively low background respiration must be identified, and the accuracy of the tests is usually good.

4.5. Radio Labeling

In contrast to residue analysis, net CO_2 and $14CO_2$ evolution measurements are simple, non-destructive and measure ultimate biodegradation. If appropriately 14C labelled test material is available, the measurements and their interpretations are relatively straightforward. Biodegradability investigations using this technique for polymeric materials in different microbial environments show a high degree of precision and consistency (Sharabi and Bartha, 1993). However, labeled materials are expensive and not always available. The licensing and the waste disposal problems connected with radioactive work may also be a drawback.

4.6. Clear-Zone Formation

A very simple semi-quantitative method is the so-called clear-zone test. This is an agar plate test in which the polymer is dispersed as very fine particles within the synthetic medium agar; this gives the agar having an opaque appearance. After inoculation with microorganisms, the formation of a clear halo around the colony indicates that the organisms are at least able to depolymerize the polymer, which is the first step of biodegradation. This method is usually applied to screen organisms that can degrade a certain polymer (Nishida and Tokiwa, 1993; Abou-Zeid, 2001), but it can also be used to obtain semi-quantitative results by analyzing the growth of clear zones (Augusta *et al.,* 1993).

4.7. Enzymatic Degradation

The enzymatic degradation of polymers by hydrolysis is a two step process: first, the enzyme binds to the polymer substrate then subsequently catalyzes a hydrolytic cleavage. PHB can be degraded either by the action of intracellular and extracellular depolymerases in PHB degradation is the hydrolysis of an endogenous carbon reservoir by the accumulating bacteria themselves while extracellular degradation is the utilization of an exogenous carbon source not necessarily by the accumulating microorganisms (Tokiwa and Calabia 2004). During degradation, extracellular enzymes from microorganisms break down complex polymers yielding short chains or smaller molecules, e.g., oligomers, dimers, and monomers that are smaller enough to pass the semi-permeable outer bacterial membranes. The process is called depolymerization. These short chain length molecules are then mineralized into end products like CO_2, H_2O, or CH_4 (methane) and the degradation is called mineralization, which are utilized as carbon and energy source (Gu, 2003).

4.8. Controlled Composting Test

The treatment of solid waste in controlled composting facilities or anaerobic digesters is a valuable method for treating and recycling organic waste material (Biological Waste Management Symposium, 1995; OECD, 1994). Composting of biodegradable packaging and biodegradable plastics is a form of recovery of waste which can cut the increasing need of new landfilling sites. The test method is based on the determination of the net CO_2 evolution, i.e. the CO_2 evolved from the mixture of polymer compost minus the CO_2 evolved from the unamended compost (blank) tested in a different reactor (Bellina *et al.,* 1999).

5. Analytical Techniques used in Biodegradation Studies

5.1. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is useful to elucidate chemical and physical structure, hydrogen bonding, end group detection, degradation reactions, crosslinking behavior of molecules and copolymer composition in liquid and solid form of chemicals and polymers. FTIR technique is employed in the biodegradation studies of polymers to assess the chemical changes due to microbial activity (Milstein *et. al*., 1994; Galgali, *et. al.,* 2002; Mohamed *et al.,*, 2007; Singh and Sharma, 2008; Elashmawi *et al.,* 2008).

5.2. Gel Permeation Chromatography (GPC)

GPC or size exclusion chromatography (SEC) is used for the determination of molecular mass distribution of polymers to determine changes in molecular weight after biodegradation (Walter *et al*., 1995; Peng and Shen, 1999; Kale *et al.,* 2006). Polystyrene resins of known molecular weight are used to calibrate the instrument. GPC is a conventional technique that separates molecules according to their molecular size.

5.3. Nuclear Magnetic Resonance Spectroscopy (NMR)

Determination of soluble fraction, tensile strength, molecular weight changes is indirect evaluation method as they do not give any idea of chemistry of polymer changes. NMR spectroscopy (1H and 13C NMR) is the most versatile method that can be used as analytical tool for biodegradation in many studies (Massardier-Nageotte *et al.,* 2006; Shah *et al.,* 2008; Schlemmer *et al.,* 2009).

5.4. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) has diverse applications in studies of polymers. Surface morphology of the films of polymers can be studied by SEM. The samples are generally sputter-coated with gold or some metal ions before SEM examination. The variable pressure SEM technique is helpful in direct observation without any surface metallization at suitable magnification (Pinzari *et al.,* 2006).

5.5. High Pressure Liquid Chromatography (HPLC)

High pressure liquid chromatography (HPLC) is widely used to separate molecules on the basis of their size. The principle is that the larger molecules will elute earlier as they pass through the spaces in the column, while smaller ones elute later because they will take longer time to pass through the pores in the gel. HPLC is used to analyze the metabolic degradation products of xenobiotics. Styrene, epoxystyrene, phenylacetaldehyde, and 2-phenylethanol biotransformation products were detected by reverse-phase high pressure liquid chromatography (Marconi *et al.,* 1996; Beltrametti *et al.*, 1997).

5.6. Thermo Gravimetric Analysis (TGA)

TGA implies the heat treatment under controlled condition to record mass changes in the sample due to heating. But it does give any information about the evolved gases produced during thermal degradation of the sample (Bhandare *et al.,*, 1997). The dynamic thermogravimetry can be used to study the temperature at the maximum decomposition rate, rate of decomposition and the activation energy (Carrasco and Pages, 1996).

5.7. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Gas chromatography (GC) is a common chromatography method that relies on differences in partitioning behavior between a flowing mobile phase and a stationary phase in order to separate volatile or semi-volatile organic or inorganic compounds. The GC technique is used for the identification and quantification of residual monomers or solvents in resins and coatings, for analysis of volatile components or impurities, for verification of additive levels in polymer formulations and finished products, for quantification of trace contaminants, for analysis of gas mixtures, and for on-line or at-line monitoring of reactions. A number of specialized detectors are available, including FID (flame ionization), AED (atomic emission), FPD (flame photometric) and TCD (thermal conductivity). In addition, mass spectrometry (MS) is available, allowing great flexibility in both qualitative and quantitative evaluation of materials containing species that are difficult to separate. (www.arkema-inc.com/.../gas-chromatography-mass-spectroscopy analysis laboratory)

6. Molecular Techniques used in Biodegradation Studies

Once the biodegradation pathway is well established the organism could be genetically modified so that the desired enzyme is made to be secreted more or, the process could be directed to follow a specific pathway. The main drawback of biodegradation process is that the polymer takes longer time to degrade. Reducing the time period required for biodegradation can be achieved by either making the polymer more susceptible to microbial attack or by enhancing the capability of the microorganism to degrade the polymer (Genetic engineering). The latter possibility can be attempted by utilizing genetic engineering tools including DNA sequencing, protein sequencing, production of recombinant organism, etc. [Wackett, 2001].

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