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An Overview of Methods for Biodegradability Testing of Biopolymers and Packaging Materials[1]

Merja Itävaara[2] and Minna Vikman[2]

This paper gives an overview of the methods used at the Technical Research Centre of Finland (VTT) for the biodegradability testing of solid polymers and packaging materials. Biodegradability of each polymer included in the packaging material should be separately tested. Aquatic aerobic and anaerobic tests and, in specific cases, enzymatic tests are used for screening purposes. The application of aquatic aerobic tests—an automated Sturm test (OECD 301B; ASTM D5209) and a VTT headspace test as well as an anaerobic test (ASTM D5210)—is discussed. Three composting tests and their applications are summarized. These tests are regarded as important because they can be used to simulate the biodegradability under real-life conditions. Several tests are needed to determine the fate of the polymer under real conditions and to study its biodegradability in different environments. The time needed for complete biodegradation of polymers in nature is impossible to predict with laboratory tests and should be studied *in vivo*.

KEY WORDS: Biodegradation; methods; biopolymers.

INTRODUCTION

The demand for testing biodegradability has come about as result of industry-produced polymers and packaging materials claimed to be biodegradable. In Finland, the Biopolymers (1992–1996) research program was started with financial support from TEKES (Technology Development Centre, Finland) in order to develop new kinds of polymers. Projects on thermoplastic starch, biodegradable polyesters, and polysaccharide/surfactant interactions, processing studies, and the development of biodegradability testing methods are included.

In nature, biodegradation is affected by several environmental factors, e.g., temperature, light, nutrients, pH, oxygen, and water content. Abiotic hydrolysis often initiates biodegradation [1]. Side chains and ring substituents such as -OH and -Cl groups are generally removed first [2]. Biodegradation is caused mainly by enzymes produced by microorganisms, but other mechanisms may also be involved. Biosurfactants which act as emulsifiers are supposed to play an important role in the biodegradation of hydrophobic compounds such as oil [3]. Microbial activity may also change the structure of the compounds in the medium, thus causing chemical degradation. Increasing acidity of the medium is a normal occurrence because organic acids are excreted by microbes. However, the pH may also rise, e.g., during aerobic composting, where the pH rapidly increases to 8–9 [4]. Owing to the great variation in natural conditions, the biodegradability of polymers varies tremendously on a global scale, as well as within smaller ecosystems. This environmental variation also results in differences in the prevailing microbial species, which means that the prevailing enzymatic environments are different. When testing, however, the conditions should be kept as constant as possible in order to ensure the repeatability of the results. This may be difficult even when using standard tests, because the media are not sterilized and the microbial population is derived from nature.

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[2] VTT Biotechnology and Food Research, P.O. Box 1504, Tietotie 2, FIN-02044 VTT, Finland. Fax: +358 0 4552028.

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The biodegradability of the first "biodegradable" plastics was tested by a method developed for studying the resistance of plastics to microbial growth [5]. This test has since been replaced by tests which measure the end products of microbial metabolism. Most tests are intended for studying the biodegradability of water-soluble chemicals in an aquatic environment. However, polymers are usually insoluble and their fate is terrestrial. Much attention has been paid to the development of testing methods which provide information about the true biodegradability after disposal. This paper gives an overview of the testing methods which we have used to evaluate the biodegradability of polymers and packaging materials.

SCHEME OF TESTS FOR BIODEGRADABILITY OF POLYMERS

Our approach for testing the biodegradability of polymers consists of screening tests and tests which simulate *in situ* conditions (Fig. 1).

The biodegradabilities of all the compounds included in the product are first tested separately using screening tests. However, biodegradability and the behaviour of the commercial product must also be studied under real conditions.

Although aquatic tests simulate conditions under aerobic or anaerobic municipal sewage sludge conditions, they are also suitable for screening purposes. The value of these tests is that they are inexpensive to perform and several polymers can be studied at the same time. Because the fate of packaging material will be soil or compost, it is recommended that compost or soil microbes are added into the inoculum in addition to sewage sludge when testing the biodegradability of these materials [6].

Aquatic biodegradability tests can be approximately divided into aerobic and anerobic tests. Carbon dioxide is a metabolic end product in aerobic degradation. Only tests based on the measurement of oxygen consumption (BOD) or carbon dioxide evolution are considered suitable for testing the aerobic biodegradation of solid polymers [7]. *In vivo* aerobic degradation takes place in the surface layers of soil and water. Wastewater treatment and composting plants are examples of controlled natural aerobic biodegradation processes. Anaerobic degradation occurs in lake sediments, landfill, anaerobic digestion plants, etc.

According to the standard tests (ASTM D 5209, D5210, D5271, D5338, D5511, D5526), the degree of biodegradability is determined from the evolution of metabolic end products and their proportion (%) of the theoretical value. The theoretical amount of metabolic end products that can be formed from the sample is calculated on the basis of the molecular composition or determined analytically.

The end products of biodegradation that are considered in biodegradation evaluations are as follows:

Aerobic	Anaerobic
$CO_2 + H_2O$	$CH_4 + CO_2 + H_2O$

Enzymatic tests can be used as screening tests when the structure of the polymer is known and enzymes degrading these polymers are available. Starch-based materials have been studied by enzymatic treatment and the results compared to those of other tests, e.g., composting [8, 9]. Cellulose modifications have also been studied

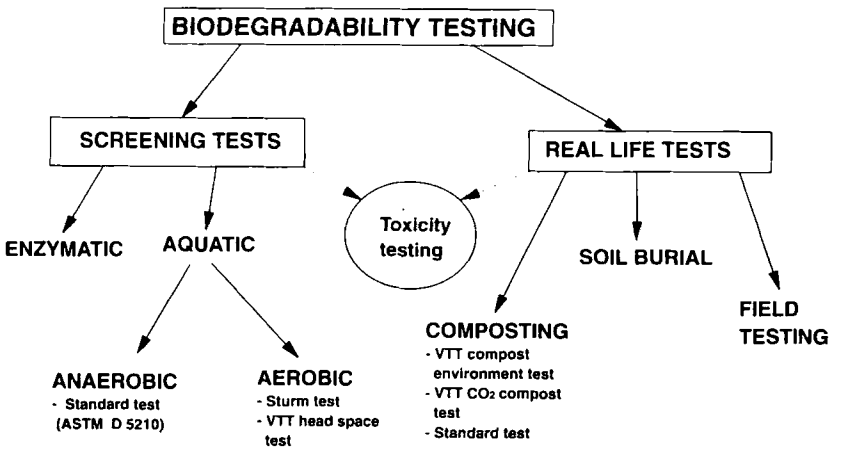

Fig. 1. Biodegradability tests for solid polymers.

using purified enzymes and unpurified *Trichoderma reesei* culture medium (Itävaara *et al.*, unpublished data).

Prediction of environmental polymer degradation is difficult and compost, soil burial, and field tests, which simulate real conditions, have therefore been introduced. We have been working on three composting tests [10]. One of these is a standard test (ASTM 5338, CEN draft) and two are "inhouse" tests. Field tests in Finnish forest soil have also been carried out in cooperation with the Finnish Forest Research Institute.

Ecotoxicological tests are required before the registration of pesticides and new chemicals [11]. Toxicity screening should also be performed at the end of the biodegradability tests because additional toxic compounds may be formed during biodegradation as a result of biotransformation. From the toxicological point of view the intermediate and end products of polymers should also be studied. The limitations of *in vitro* tests, e.g., the *Photobacter* test [12] are that toxicity in higher vertebrates as in man involves the complex interactive influences of different types of cells. Nor do the tests predict interactions and synergistic mechanisms between different chemicals. Toxicity may also vary depending on the physicochemical conditions. Toxicity may be species specific, acute, or chronic. Single-species tests such as the earthworm acute toxicity test (OECD 207) and the terrestrial plant growth test (OECD 208), which are used for toxicity evaluation of chemicals, are very limited and multispecies tests with model ecosystems give better information on the real fate of the compounds. However, they are time-consuming and expensive to perform [11].

SCREENING TESTS

Sturm Test

The Sturm test (OECD 301B; ASTM D5209) is a highly suitable method for measuring biodegradability of both water-soluble and insoluble compounds in an aquatic environment. Continuous aeration ensures a sufficiency of oxygen inside the bioreactor. The measurement of carbon dioxide evolved during degradation also gives more direct information on the bioconversion of the carbon backbone of the polymer to metabolic end products than the tests based on oxygen consumption. The measurement of oxygen consumption may result in erroneous results owing to nitrification [13] or chemical oxidation.

There are several problems associated with the Sturm test, e.g., leakages in the complicated system may result in erroneously low carbon dioxide values during the test. The carbon dioxide evolved during degradation is trapped in the base solution, usually barium hydroxide, which is then titrated as a function of time. The labor intensity of the method was the major reason for developing automated equipment for carbon dioxide measurement. The automated VTT Sturm test (Fig. 2) is based on measuring changes in the electrical conduc-

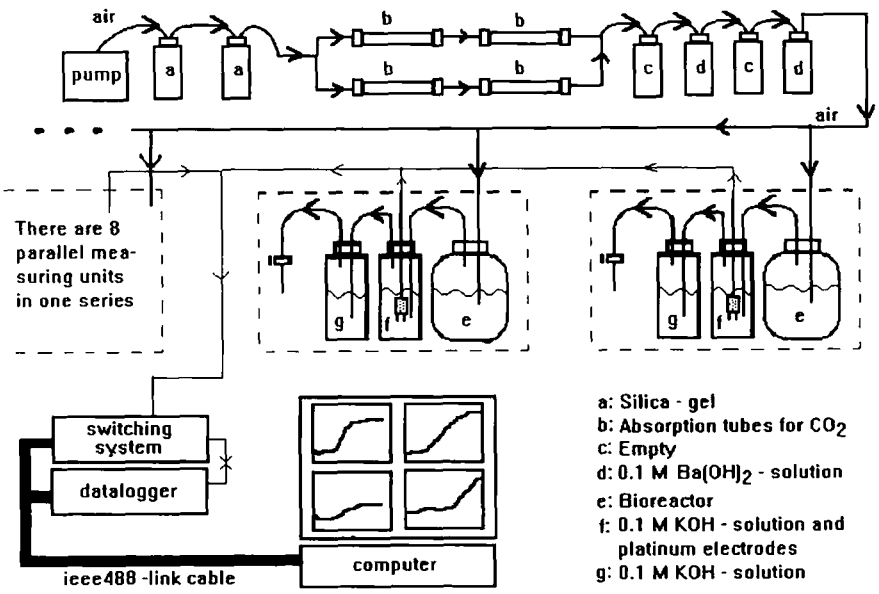

Fig. 2. A schematic diagram of the equipment used in the automated VTT Sturm test.

tivity of the base solution. Conductivity is calibrated to give the amount of CO_2 evolved and the results are recorded with a computer. Twenty-four bioreactors can now be connected simultaneously to the equipment, and if necessary, it can be expanded to cover 80 bioreactors. Before starting the test the theoretical amount of carbon dioxide that can be formed from the sample is recorded in the computer program, which then calculates cumulative carbon dioxide evolution as a percentage of the theoretical value.

VTT Headspace Test

Owing to the need for statistical evaluation of the results, a sufficient number of repetitive cultures has to be run in parallel. In the Sturm test, only two repetitive studies are usually performed. The Sturm test is also relatively demanding and is laborious to perform if CO_2 evolution is measured by titration. The VTT headspace test, which is also based on carbon dioxide evolution under aerobic conditions, was developed to overcome these problems. The benefits of the VTT headspace test are its simplicity and ability to test extensive series and hence perform statistical evaluation. No expensive equipment is needed and it is also easy to perform the test at different temperatures. The test is carried out in headspace bottles (volume 125 ml) containing 50 ml mineral nutrient medium and sewage sludge inoculum. The carbon dioxide evolved during biodegradation is determined from the gas and liquid phase at weekly intervals. The distribution of inorganic carbon between the gas and the liquid phases in the headspace test when native barley starch is used as the sample is presented in Fig. 3.

Biomass represents an important part of the organic carbon which has already been assimilated by microorganisms [14]. During the biodegradation of solid polymers microbes grow on the surface of the polymer as well as in the liquid, and separation of the sample from the microbial biomass is difficult. Indirect methods, involving the determination of, e.g., dehydrogenase activity [15], ATP [16], DNA [17], phospholipids [18], and ergosterol [19], have been used to evaluate the amount of biomass. A good correlation has been found between the intracellular protein concentration and the biomass dry weight in the VTT headspace test [20]. ATP activity during the biodegradation of some starch-based samples in the headspace test is presented in Fig. 4. The luciferin–luciferace assay is used to determine ATP (Biorbit, Finland), and the intensity of the light emitted is directly proportional to the ATP concentration. Starch-based Biopac and native barley starch were completely

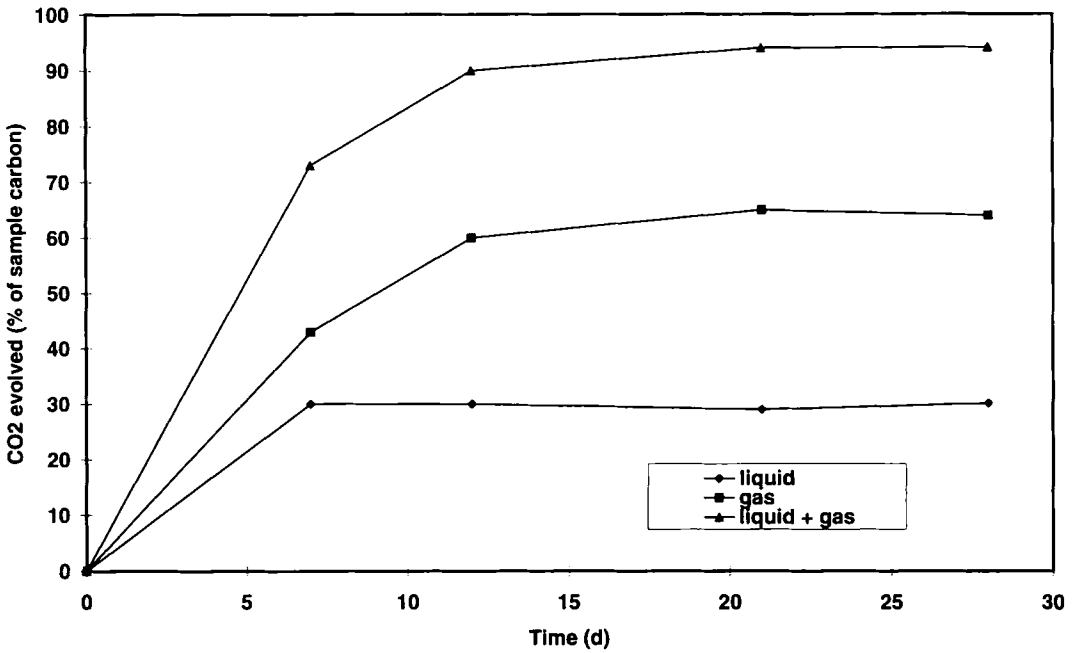

Fig. 3. Distribution of carbon dioxide between the liquid and the gas phase during biodegradation of native starch in the headspace test.

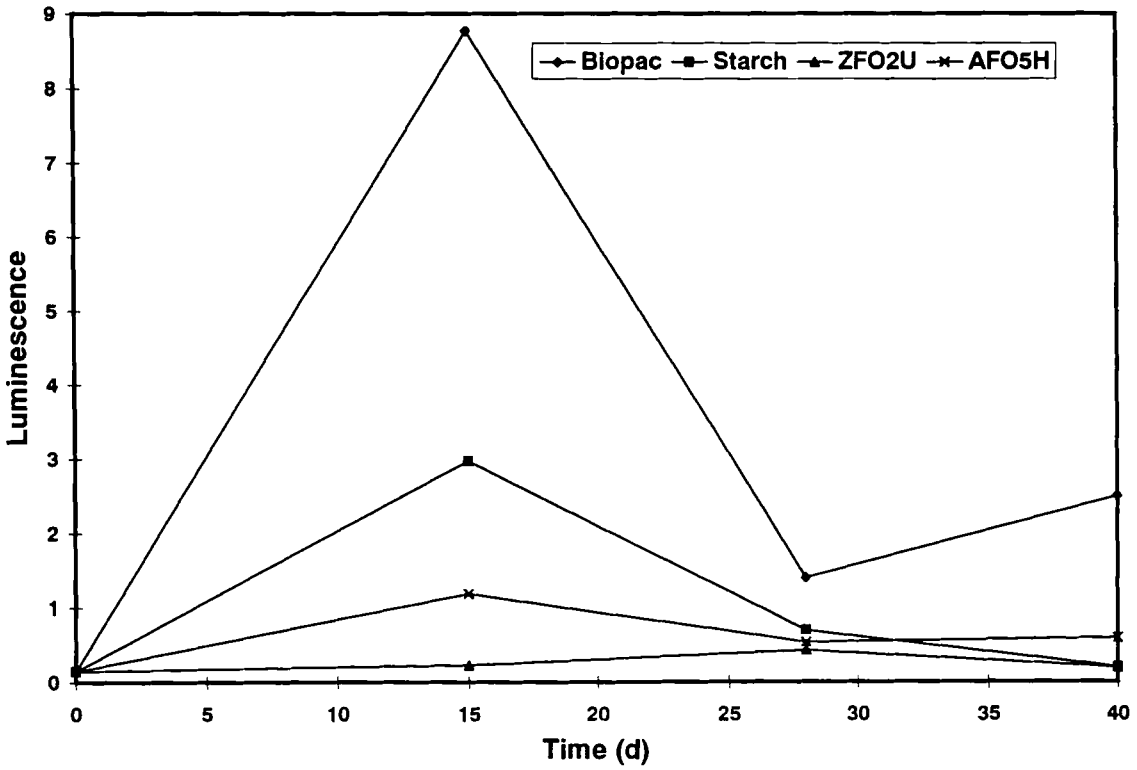

Fig. 4. ATP measurement as an indirect method for determining biomass during biodegradation of some starch-based materials. Samples: native barley starch, Biopac (Austrian Biologische Verpackungssysteme GmbH), and Mater-Bi films ZFO2U and AFO5H (Novamont North America).

degraded in this test, resulting in a larger amount of biomass and higher ATP activity compared to the starch-based Mater-Bi films. It should be noted, however, that some microorganisms exhibit tremendous changes in their activity when nutritional or other physiological conditions change [21].

Anaerobic Degradation (ASTM D5210)

Anaerobic degradation is biodegradation in the absence of oxygen. Initially acidogenic bacteria convert organic substances into lower molecular metabolites such as alcohols and short-chain fatty acids. Acetogenic bacteria further degrade these substances to acetate, carbon dioxide, and molecular hydrogen. A mixed population of microorganisms is needed for complete degradation of the polymer. In the final phase methane and carbon dioxide are formed as a result of anaerobic degradation, and these parameters can be used to study the degree of anaerobic degradation of the compounds [22]. Equal molar quantities of both CO_2 and CH_4 are formed and thus the volume of the gas produced over time is used as a measure of anaerobic biodegradability.

REAL-LIFE TESTS

Composting as a Biodegradation Process

Composting is a biooxidative process in which organic compounds are biodegraded into carbon dioxide, water, mineral salts, and humus [23, 24]. There is a succession of different species of microorganisms during the degradation of organic compounds, and heat is released as a result of the high metabolic activity [25]. The pH generally increases to above 8 during aerobic composting and then gradually decreases to below 7. The carbon/nitrogen ratio is important for the growth of microbes and should be adjusted if not within the optimal range of 20–40 [26]. Particle size and the moisture content of the biowaste are important because the particle size of biowaste determines the surface area ex-

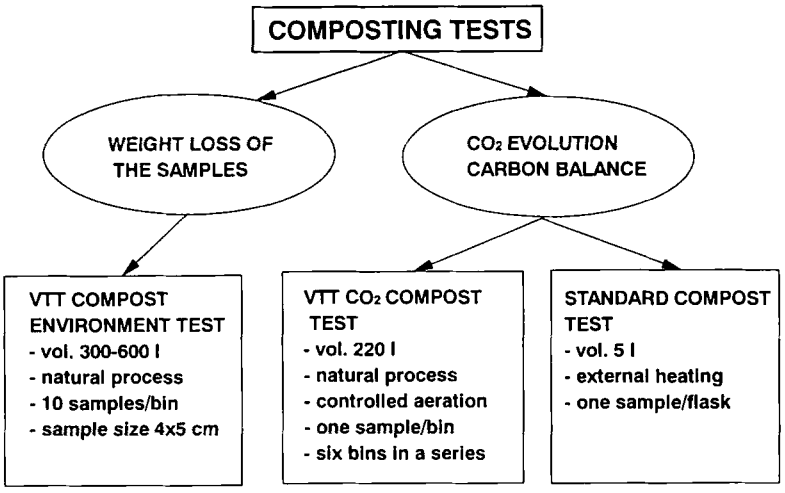

Fig. 5. Composting tests used at VTT Biotechnology and Food Research.

posed to the microorganisms. It also has an effect on oxygen availability inside the compost.

The capacity of microbes to degrade organic compounds is dependent on their ability to produce the extracellular enzymes involved in breaking down complex compounds. More complex polymers will be composted at a slower rate. Substances containing lignin or other aromatic structures break down slowly. The degradation of aromatic compounds is supposed to be related to the formation of humic acids [27].

The Fate of Packaging Materials in Composting

Composting is regarded as the most favored fate of biodegradable packaging materials and polymers [28]. Three composting tests developed for studying the compostability of packaging materials have recently been described in detail [10] and are summarized in Fig. 5.

The compost environment test and CO_2 compost test are performed in household waste composter bins under natural composting conditions. The standard compost test (ASTM D5333, CEN draft) is a laboratory compost test in which small amounts of mature compost (2-4 months old) are mixed with the sample and externally heated to maintain an elevated temperature (+58°C). There is probably no natural succession of microorganisms because the sample is mixed into the already composted, homogeneous substrate.

Compost Environment Test

The compost environment test has been developed for the comparative investigation of different packaging materials and evaluation of their degradability in the same composting environment. Compostability of the materials is determined as the weight loss of the samples. The composter bin (SEPE, Biolan Oy; volume 300, 600 L) is filled with biowaste consisting mainly of vegetable and fruit waste mixed with bark to provide better aeration. Composting activity parameters (temperature, pH, relative oxygen, and CO_2) are determined during composting to confirm that decomposition has been properly carried out [29]. The samples are supported by a frame system in which 10 samples with four repetitions can be studied in the same environment. A known biodegradable positive control sample has to be included in the test. Other physical and chemical tests can also be performed on the samples afterward when needed. Evaluation of the compostability of the samples is performed visually at weekly intervals in connection with turning the biowaste, and weight loss is measured at the end of the test when the positive control sample has been completely degraded and the temperature decreased to the outdoor temperature.

If the sample is hydrolyzed at elevated temperatures and moisture concentrations, it will be degraded into fragments during composting. The pH also increases to over 8 during aerobic composting, which may increase sample degradation. Therefore the effect of temperature and high pH are studied in a buffer solution to simulate the effect of physicochemical changes in the environment.

Standard Compost Test (ASTM D5338, CEN Draft)

This standard compost test is based on CO_2 evolution from the sample. Five-liter flasks were filled with

mature compost and the sample (dry weight ratio, 6:1) and externally heated. In the ASTM standard (ASTM D5338) there is a temperature profile, and in the CEN draft version the temperature is kept at a constant level of +58°C. Differences in the biodegradation of kraft-paper and polyhydroxybutyrate/polyhydroxyvalerate copolymer (PHB/PHV) were studied by comparing the two methods. The microorganisms did not display any shock reaction as a result of direct incubation at constant temperature (+58°C) [10].

VTT CO_2 Compost Test

The VTT CO_2 compost test is also based on CO_2 evolution, but the increase in temperature appears as a result of natural microbial activity during composting. The equipment was developed to compare the standard compost test to the natural composting. The equipment consists of six parallel bins (volume, 220 L) connected to automatic CO_2 and temperature measurement and controlling equipment, and the data are collected by the computer. Aeration can be controlled via temperature and CO_2 evolution. Two composter bins are for the blank (biowaste without the sample), two bins for the positive control (kraft paper), and two bins for the sample. The amount of CO_2 evolved from the sample during composting is determined by subtracting the CO_2 evolved from the blank. Residual carbon is measured after composting, and the carbon balance determined. Other composting activity parameters such as temperature, pH, carbon/nitrogen ratio, NH_4, NO_3, NO_2, volatile solids, dry weight, etc., have to be determined to follow the composting process. In the future humification will also be studied.

CONCLUSIONS

Real-life tests are laborious and expensive, and hence there is a demand for inexpensive screening tests. Aquatic and enzymatic tests are used for screening the biodegradability of each component included in the commercial product. Composting tests are used to verify the compostability of the complete product containing plastizers and other additives. The VTT compost environment is used to study the biodegradation time and the behavior or materials under real composting conditions. The test is a preliminary test and should be performed before CO_2-based compost tests. The standard compost test (ASTM 5338, CEN Draft) is a laboratory test in which a small amount of mature compost is mixed with the sample and incubated at elevated temperature. However, this test does not simulate real composting conditions and therefore the true compostability is confirmed in a household waste composter bin test based on CO_2 evolution in the VTT CO_2 compost test.

A limitation of the standard biodegradation tests is that they are based solely on the measurement of metabolic degradation end products. Biomass, volatile compounds, and the proportions of dissolved and undissolved parts of the polymer should be determined to study the carbon balance. Dissolved inorganic and organic carbon are generally determined, but the other parameters, e.g., biomass and the amount of insoluble residues, are difficult to analyze. Most tests give only a quantitative result, which cannot be used for calculating the carbon balance. Cometabolic degradation cannot be taken into consideration in the aquatic tests where the sample is the sole carbon source. However, computing tests based on CO_2 measurement are significant improvements to this. Carbon-14-labeled compounds should provide more precise information on the fate of new polymeric materials and their degradation route. Nonbiodegradability in aquatic tests does not mean that the polymer would not degrade in some other test, e.g., composting. Although all these tests should be performed, the biodegradability or cumulative properties in natural ecosystems are difficult to predict. We have compared the biodegradability of the same materials in different environments and found that completely biodegradable materials such as sausage casing, which consists solely of cellulose, degrade very fast during composting (in 28 days) but, when buried in forest soil, are not completely degraded during the first growing season. The time limit for degradation of biodegradable polymers is a difficult question and should be carefully studied if the products are intended to be disposed in nature. Biodegradable packaging materials are generally intended to be handled by municipal waste management companies and should be included in the organic waste stream. The biodegradability of these materials should also be studied in full-size composting processes or in anaerobic digestion plant. Even in controlled waste management systems there are different processing methods for biowaste, e.g., aeration and turning of biowaste during composting. The type of organic matter and its moisture content affect the decomposition of biowaste, as well as the packaging materials included. Despite these problems, the polymers should be designed for degradation within a certain time under specific conditions (e.g., controlled composting) to be acceptable for composting.

REFERENCES

1. A.-C. Albertsson and S. Karlsson (1990) in S. A. Barenberg, J. L. Brash, R. Narayan, and A. E. Redpath (Eds.), *Degradable Materials, Perspectives, Issues, and Opportunities*, CRC Press, Boca Raton, FL, pp. 263–286.
2. L. Y. Young and M. M. Häggblom (1989) in D. Kamely, A. Chakrabarty, and G. S. Omenn (Eds.), *Biotechnology and Biodegradation, Advances in Applied Biotechnology Series 4*, Portfolio Publishing, The Woodlands, pp. 3–20.
3. R. K. Hommel (1994) in C. Radledge (Ed.), *Biochemistry of Microbial Degradation*, Kluwer Academic, Dordrecht, The Netherlands, pp. 63–87.
4. C. G. Golueke (1991) in *The Art and Science of Composting*, JG Press, Emmaus, PA, pp. 14–39.
5. K. J. Seal and M. Pantke (1986) *Mater. Organismen* **21**(2), 151–156.
6. R. J. Müller, J. Augusta, and M. Pantke (1992) *Material Organismen* **27**(3), 179–189.
7. M. van der Zee, L. Sijtsman, G. B. Tan, H. Tournois, and D. de Wit (1994) *Chemosphere* **28**, 1757–1771.
8. M. Vikman, M. Itävaara, and K. Poutanen (1995) *J. Macromol. Sci. Pure Appl. Chem.* **A32**, 863–866.
9. M. Vikman, M. Itävaara, and K. Poutanen (1995) *J. Environ. Polym. Degrad.* **3**, 23–29.
10. M. Itävaara, O. Venelampi, and S. Karjomaa (1995) in J. Barth (Ed.), Proceedings of Biological Waste Management, "Wasted Chance," BWM Infoservice.
11. P. Bourdeau, G. C. Butler, D. Calamari, J. W. Gillett, V. Landa, D. Peakall, P. Sheehan, G. Persoone, R. Truhaut, and P. Wells (1990) in P. Bourdeau, E. Somers, G. M. Richardson, and J. R. Hickman (Eds.), *Short-Term Toxicity Tests for Non-genotoxic Effects*, Scope 41, IPCS Joint Symposia 8, SGOMSEC 4, John Wiley & Sons, Surrey, UK, pp. 49–55.
12. D. I. Stom, T. A. Geel, A. E. Balayan, G. I. Shachova, A. M. Kuznetsov, and S. E. Medvedeva (1992) *Arch. Environ. Contam. Toxicol.* **22**, 203–208.
13. C. G. van Ginkel and C. A. Stroo (1992) *Ecotoxicol. Environ. Saf.* **24**, 319–327.
14. D. Raghavan, G. C. Wagner, and R. P. Wool (1993) *J. Environ. Polym. Degrad.* **1**, 203–211.
15. J. M. Lopez, B. Koopman, and G. Bitton (1986) *Biotech. Bioeng.* **17**, 1080–1085.
16. O. Holm-Hansen (1973) *Bull. Ecol. Res. Comm.* **17**, 215–222.
17. K. Hund, U. Grolms, and T. Bünemann (1994) *Chemosphere* **28**, 813–823.
18. Å. Frostegård, A. Tunlid, and E. Bååth (1991) *J. Microbial. Methods* **14**, 151–163.
19. R. M. Gardner, G. W. Tindall, S. M. Cline, and K. L. Brown (1993) *J. Microbial Methods* **17**, 49–60.
20. M. Itävaara and M. Vikman (1995) submitted for publication.
21. A. Tunlid and D. C. White (1992) in G. Stotzky and J.-M. Bollag (Eds.), *Soil Biochemistry* **7**, Marcel Dekker, New York, pp. 229–262.
22. U. Pagga and D. B. Beimborn (1993) *Chemosphere* **27**, 1499–1509.
23. C. Garcia, T. Hernandez, and F. Costa (1991) *Environ. Manage.* **15**, 433–439.
24. K. Nakasaki, H. Yaguchi, Y. Sasaki, and H. Kubota (1990) *J. Fermen. Bioeng.* **70**, 431–433.
25. K. Iiymana, B. A. Stone, and B. J. Macauley (1994) *Appl. Environ. Microbiol.* **60**, 1538–1546.
26. M. T. Baca, F. Fornasier, and M. de Nobili (1992) *J. Fermen. Bioeng.* **74**, 179–184.
27. J. Peuravuori (1992) *Finnish Humus News* **4**, 1–99.
28. R. Narayan (1994) in M. Vert, J. Feijen, A. Albertsson, G. Scott, and E. Chiellini (Eds.), *Biodegradable Plastics and Polymers*, Redwood Press, Melksham, Wiltshire, UK, pp. 261–273.
29. J. S. Jeris and R. W. Regan (1973) *Compost Sci.* **14**, 10–15.