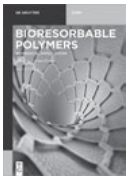
**Handbook of Biodegradable Polymers**

## Also of Interest


*Sustainable Polymers for Food Packaging.*
*An Introduction*
Katiyar, 2019
ISBN 978-3-11-064453-1, e-ISBN 978-3-11-064803-4




*Bioresorbable Polymers.*
*Biomedical Applications*
Devine (Ed.), 2019
ISBN 978-3-11-064056-4, e-ISBN 978-3-11-064057-1


*Polymeric Composites with Rice Hulls.*
*An Introduction*
Defonseka, 2019
ISBN 978-3-11-063968-1, e-ISBN 978-3-11-064320-6


*Advanced Composites Vol. 10*
*Biodegradable Composites.*
*Materials, Manufacturing and Engineering*
Kumar, Davim (Eds.), 2019
ISBN 978-3-11-060203-6, e-ISBN 978-3-11-060369-9

# Handbook of Biodegradable Polymers

Edited by
Catia Bastioli

3rd Edition

DE GRUYTER

**Editor**
Catia Bastioli
Novamont SpA
Via G.Fauser 8
28100 Novara NO
Italy

ISBN 978-1-5015-1921-5
e-ISBN (PDF) 978-1-5015-1196-7
e-ISBN (EPUB) 978-1-5015-1198-1

**Library of Congress Control Number: 2019953907**

**Bibliographic information published by the Deutsche Nationalbibliothek**
The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; Detailed bibliographic data are available on the Internet at http://dnb.dnb.de.


Cover image: Misha Kaminsky / iStock / Getty Images Plus
Typesetting: Integra Software Services Pvt. Ltd.
Printing and binding: CPI books GmbH, Leck

www.degruyter.com

# Acknowledgments

I would like to thank all the contributors to this volume for their time, patience and effort in producing this book.

Special and sincere thanks go to Federica Mastroianni for her extensive effort, which went beyond the handling of the considerable correspondence associated with the book and organisation of the reviewing process. Thanks are also extended to Gian Tomaso Masala for his reviewing of the chapter references.

Finally, I wish to dedicate this volume to the memory of Raul Gardini, who was a pioneer of Bioeconomy, and the unwitting origin of my interest and dedication to renewable raw materials and bioplastics.

https://doi.org/10.1515/9781501511967-202

# Preface

> '*The great problem of packaging, which every experienced chemist knows, was well known to God Almighty, who solved it brilliantly, as he is wont to, with cellular membranes, eggshells, the multiple peel of oranges, and our own skins, because after all we too are liquids. Now, at that time there did not exist polyethylene, which would have suited me perfectly since it is flexible, light and splendidly impermeable: but it is also a bit too incorruptible, and not by chance God Almighty himself, although he is a master of polymerisation, abstained from patenting it: He does not like incorruptible things*.'

I find this extract from Primo Levi's book '*The Periodic Table*', the best introduction to biodegradable polymers.

The durability of conventional plastics is a serious environmental drawback when these materials are used in applications with little probability of recycling, when recycling happens to be too expensive, or when plastics have a high probability of contaminating the natural environment or organic waste. In these, and only in these applications, biodegradable polymers really make the difference.

Biodegradable polymers must not be a simple replacement for traditional plastics. They must be used as an opportunity to redesign applications by focusing on the efficient use of resources and tending towards the elimination of waste, by transforming local issues into business opportunities and by developing a systemic vision to counterbalance the management culture that has contributed to the dissipative growth model we are now living in.

The fundamental criterion needed to avoid any aggravation of this situation, and indeed to reverse the trend, is the efficient use of resources, being aware that only a type of growth which could restore its central focus on local areas, a knowledge economy, the cascading model, and the absence of waste and rejects, will lead to continuous and harmonious growth.

A similar approach requires the selection of standards which have to go beyond products and towards systems. The objective should not be to maximise market volumes but to boost local regeneration from an environmental, social and economic viewpoint, promoting a cultural leap towards a system-based economy and shared trust among the different stakeholders.

For this purpose, the quality rules for biodegradable polymers have to be strict and guarantee, besides compostability and biodegradability in different environments, nontoxicity of products and additives, as well as a low environmental impact throughout the life cycle, with improving targets in terms of raw material quality and renewability level, feedstock sustainability, inuse efficiency and end of life options to close the loop.

Over the past 30 years, increasing effort has been dedicated to developing polymers designed to be biologically degraded in selected environmental conditions. In particular, industrial research has focused on discovering and developing biodegradable polymers that are, at the same time, easily processable, exhibit good per-

https://doi.org/10.1515/9781501511967-203

formance and are cost-competitive (considering both internal and external costs) with conventional polymers. When bioplastics are biodegradable according to European Norms 13432 – the European reference for the technical material manufacturers, public authorities, composters, certifiers and consumers – or its equivalents the American Society for Testing and Materials D6400, or International Organization for Standardization 14855, they can, besides other disposal options, be organically recycled through composting. Such characteristics, when composting infrastructures are available, may therefore represent a significant advantage in sectors like waste collection, catering or packaging which have a high probability of being contaminated by food, or ending up in organic waste or nature: in such cases, organic recycling must be preferred to mechanical recycling. The property of a plastic to biodegrade in household compost permits its disposal in widespread composting infrastructures, at the same time optimising the quality of organic waste and maximising its diversion from landfill. The ability of a plastic to biodegrade *via* composting is also proof that its chemical structure is intrinsically biodegradable.

Significant literature shows that the most widespread compostable bioplastics, currently available on the market, are also able to fully biodegrade in soil and even in the marine environment or through home composting. A range of standards are also available to certify the behaviour of these bioplastics in many different environments.

The present volume reviews the most important achievements, the programmes and approaches of institutions, the private sector and universities to develop biodegradable polymers, and it explores their potential in depth. The volume covers: the most relevant biodegradable polymers of renewable and nonrenewable origin, the present business situation, a review of the main studies on their environmental impact and a critical analysis of the methodologies involved, the potential of new areas such as biocatalysis in the development of new renewable building blocks for biodegradable polymers, the expansion of the biorefinery concept towards integrated biorefineries, and the main policy and funding initiatives recently undertaken at the European Union (EU) level to foster the innovation capacity in Europe and to favour the market entry of innovative biobased and biodegradable products. It also takes into consideration aspects related to the biodegradation of these polymers in different environments and the related standards and case studies (including the interactions of biodegradable items with different anaerobic digestion technologies), showing their use in helping to solve specific solid waste problems.

The demand for biodegradable polymers has steadily grown over the last 10 years, at an annual rate of between 20–30%, in regions where composting infrastructures are well developed and the separate collection of organic waste is well established. Wherever the separate collection of biowaste is in place (and this is an unwavering trend in the EU), all the traditional short life pollutants of organic waste (when it is with polyethylene, renewable or not) are critical, because they are not biodegradable. The *organic recycling of biowaste requires plastic-free streams* in order to assure high recycling rates.

The existing link between the increasing use of biodegradable polymers and the efficient infrastructures for organic recycling and the separate collection of organic waste can be perceived as a limitation to the fast growth of this class of material. Instead it represents a unique opportunity to reconnect the solution of long-lasting environmental problems to local growth and regional regeneration putting into practice the knowledge-based economy.

The Italian case study presented in the book curated by Walter Ganapini '*Bioplastics: A Case Study of Bioeconomy in Italy*' shows how biodegradable polymers can be a powerful catalyst for the activation of local area regeneration. The book is dedicated to the Italian approach for resolving the problem of disposable carrier bags. It is all about transforming a category of waste which presents extremely critical issues (high surface area-volume ratio, large number of articles produced, the fact that the bags, if dispersed, cannot be reabsorbed into the environment, and marine pollution) into an opportunity, in order to solve an even more pressing problem; that of organic waste being sent to landfill.

A small number of disposable carrier bags, if coupled with reusable bags, and made from biodegradable, compostable plastics, can be reused as valuable resources in organic waste collection. This makes them a powerful and important means of intercepting organic waste, with no expense required from local councils, helping to achieve the objective of improving the quantity and quality of organic waste: a feedstock that is important for the future development of the bioeconomy, and for the fertility and quality of soil. Being able to count on a niche market (which is already of significant size), facilitates achieving economies of scale for biodegradable polymers, resulting in increased possibilities to build integrated local biorefineries dedicated to medium-high value-added products, demonstrators and flagships required not only for biodegradable polymers but also for a range of related building blocks and agricultural chains as a whole. This will also generate new opportunities for traditional chemistry, by laying down the foundations for the redevelopment and environmental upgrading of deindustrialised chemical plants.

The change in the perception of biodegradable polymers is evident by simply considering the trends from 1989 to 2012 in the fields of 'biodegradable' (+2,800% for scientific literature and +1,100% for the sum of World Intellectual Property Organization (WIPO) patents, European patents (EP) and United States (US) patents) and 'biodegradable plastics' (+1,400% for scientific literature and +4,200% for the sum of WO, EP and US patents).

The opportunity to utilise renewable raw materials (RRM) in the production of some of these biodegradable polymers and to reduce the dependency on foreign petroleum resources, along with the exploitation of new functional properties in comparison with traditional plastics, has significant benefits. Besides biodegradability, the technical developments made during the research process could have significant advantages for the final consumers and could contribute to the solution of technical, economic and environmental issues in specific market areas.

RRM as industrial feedstocks for the manufacture of chemical substances and products, such as oils from oilseed crops, starch from cereals and potatoes, and cellulose from straw and wood, as well as organic waste, have therefore been given more and more attention over the last few years. By employing physical, chemical and biochemical processes, these materials can be converted into chemical intermediates, polymers and speciality chemicals able to replace fossil feedstocks, thus implying less energy involved during production and a wider range of disposal options resulting in a lower environmental impact.

Legislative attention able to properly address this issue could become a further incentive to the development of products from RRM and maximise the environmental, social and industrial benefits.

Biobased products were, in fact, one of the six sectors included in the 2007 'Lead Market Initiative' of the European Commission (EC), with the aim of fostering the emergence of such lead markets with high economic and societal value, focusing on areas where coordinated policymaking can speed up market development [1]. More recently, in February 2012, the EC launched a new 'Bioeconomy Strategy' [2], focusing resources and investments in the strategic sector of biobased products, in order to shift Europe towards a greater and more sustainable use of renewable resources.

In addition, in July 2013, the EC encouraged the creation of a public-private partnership of Biobased Industries. It includes approximately 70 full members (EU large and small companies, clusters and organisations) and more than 100 associated members (universities, research and technology organisations, associations, European trade organisations and European technology platforms) from the fields of technology, industry, agriculture and forestry, with the shared commitment to invest in collaborative research, development and demonstration of biobased technologies.

A supportive and coordinated European strategy for an increased market uptake would help many biobased products, among them biodegradable biopolymers, to accelerate reaching economies of scale, in order to attract investments and generate sustainable economic growth. The EU bioeconomy already has a turnover of nearly €2 trillion and employs more than 22 million people, 9% of the total employment in the EU. Each euro invested in EU-funded bioeconomy research and innovation, with a coherent and incentivising framework, is estimated to trigger €10 of added value in bioeconomy sectors by 2025 [2]. It is also estimated that this growth will be enhanced with the development of the model of integrated biorefineries for the production of high value-added products, such as biobased chemicals and materials. Biorefineries will process a variety of biomass-based feedstocks, and the necessary growth in biomass production is expected to increase the turnover and employment of the seed sector by 10%, resulting in 5,000 extra jobs [3].

The significant increase in the importance of innovative biopolymers is linked to the achievement of high-quality standards.

The quality of biodegradable products is assured not only by the control of the biodegradability parameters but also by the assessment of real functionality. A biodegrad-

able product is useless if it does not perform as a traditional product or better in terms of mechanical resistance, duration and so on. For this reason, the commitment of producers of biodegradable biopolymers in the creation of a quality network able to guarantee the standards of the product, in all the steps of the life cycle, becomes very relevant.

The elaboration and diffusion of best practices in the field of organic waste collection, where the use of biodegradable compostable bags is a tool to improve the quality of the system, has for example, permitted thousands of municipalities all over Europe to implement the proposed model. In fact, it has been demonstrated that, despite the heterogeneity of anaerobic digestion technologies and processing conditions, an efficient and optimised treatment of municipal biowaste, collected with compostable bioplastic bags, allows preserving the advantages given by the bags in the collection phase and to secure the most efficient treatment of the collected feedstock enabling the highest input and minimum production of residues.

The cooperation with public bodies is also a key factor in the success of biodegradable biopolymers, because the topics under discussion are strictly related to public interest, such as safety, environment and health.

The implementation of appropriate environmental policies in key areas (like waste collection) can become a further incentive to the development of products from RRM and can maximise the environmental, social and industrial advantages. Together with the intensification of investment, as well as research and development actions in the biodegradable polymers sector, it would be possible to create a network of partnerships among stakeholders of the entire supply chain, from agriculture to waste management, and thereby promote new models of development towards higher levels of sustainability and cultural growth.

Today, biodegradable biopolymers are available on the market, at different levels of development, and are mainly carbohydrate-based materials. Starch can be physically modified and used alone or in combination with other polymers, or it can be used as a substrate for t h e fermentation and production of polyhydroxyalkanoates or lactic acid, is then transformed into polylactic acid through standard polymerisation processes. An alternative option is represented by vegetable oil-based polymers.

Despite the constant growth of the market, the land use for bioplastics currently represents just 0.006% of the global agricultural area (which means around 300,000 ha out of 5 billion ha) and it is expected to rise to 0.022% by 2016 (that is, 1.1 million ha). Meanwhile, the increase in the efficiency of feedstock and agricultural technology is continuously enhancing good agricultural practices [4]; moreover, recent trends have focused on the use of marginal lands or contaminated soils and residues.

The increasing use of bioplastics has opened entirely new generations of materials with new performances in comparison with traditional plastics. The possibility offered by physically modified starch to create functionalised nanoparticles able to modify the properties of natural and synthetic rubbers and other synthetic polymers, the naturally high oxygen barrier of starch and its derivatives, and their high permeability to water vapour already offer a range of completely new solutions to the plastic industry.

The use of RRM, however, is not by itself a guarantee of low environmental impact. Aspects such as the production processes, the technical performance and the weight of each final product, and its disposal options, have to be carefully considered along all the steps of the product's life. The engineering of biobased materials for specific applications using life cycle analysis in a cradle-to-grave approach is therefore a critical aspect.

The involvement of upstream players, that is farmers and their associations, is a very important prerequisite. In agriculture, new agronomical approaches and the development of new genotypes for nonfood applications should be taken into consideration. Agricultural crops and processes associated with lower environmental impact and lower costs are important factors in the development of new biobased products.

Effort must also be made at the industrial level in order to develop less expensive and higher performance products and low- impact technologies. Policies should therefore be focused more on supporting innovation and scale up of new technologies which can create solid added value and are capable of responding to the societal challenges faced by our planet.

The involvement of specific stakeholders can be achieved if a communication programme is launched and operated in parallel with industrial activities. The success of the project is very much linked to the diffusion of a new environmental awareness, at all levels: politicians, public administrators, investors, associations, customers, non-governmental organisations (NGO), citizens and society at large, all of them must be reached by specific communications, in order to initiate a comprehensive and coherent sustainable strategy, with positive effects in the local areas involved.

This, in turn, must give rise to specific legislative actions in order to quantify the social and environmental benefits linked to the nonfood use of agricultural and natural raw materials, and to the bioconversion of waste materials into industrial products.

## References

[1] *A Lead Market Initiative for Europe – COM(2007) 860 Final*, Communication from the Commission to the Council, the European Parliament, the European Economic and Social Committee and the Committee of the Regions.

[2] *Innovating for Sustainable Growth: A Bioeconomy for Europe – COM(2012) 60 Final*, Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions.

[3] *The Bio-based Industries Vision – Accelerating Innovation and Market Uptake of Bio-based Products*, Vision Document of the Bio-based Industries Consortium – European Public-Private Partnership on Bio-Based Industries. *http://biconsortium.eu/sites/default/files/downloads/BIC_BBI_Vision_web.pdf*, July 2012, p. 15.

[4] European Bioplastics, *Bioplastics – Facts and Figures*. *http://en.european-bioplastics.org/wp-content/uploads/2013/publications/EuBP_FactsFigures_bioplastics_2013.pdf, 2013, p. 5.*

# Contents

Maarten van der Zee

# 1 Methods for evaluating the biodegradability of environmentally degradable polymers

## 1.1 Introduction

This chapter presents an overview of the current knowledge on experimental methods for monitoring the biodegradability of polymeric materials. The focus is, in particular, on the biodegradation of materials under environmental conditions. Examples of *in vivo* degradation of polymers used in biomedical applications are not covered in detail, but have been extensively reviewed elsewhere, e.g., [1–3]. Nevertheless, it is important to realise that the degradation of polymers in the human body is also often referred to as biodegradation.

A number of different aspects of assessing the potential, rate and degree of biodegradation of polymeric materials are discussed. The mechanisms of polymer degradation and erosion are reviewed, and factors affecting enzymatic and nonenzymatic degradation are briefly addressed. Particular attention is given to the various ways of measuring biodegradation, including complete mineralisation to gases (such as carbon dioxide ($CO_2$) and methane ($CH_4$)), water and possibly microbial biomass. Finally, some general conclusions are presented with respect to measuring the biodegradability of polymeric materials.

## 1.2 Background

There is a worldwide research effort to develop biodegradable polymers for agricultural applications or as a waste management option for polymers in the environment. Until the end of the 20$^{th}$ century, most of the efforts were synthesis oriented and not much attention was paid to the identification of environmental requirements for, and testing of, biodegradable polymers. Consequently, many unsubstantiated claims of biodegradability were made, which has damaged the general acceptance.

An important factor is that the term biodegradation has not been applied consistently.

In the medical field of sutures, bone reconstruction and drug delivery, the term biodegradation has been used to indicate degradation into macromolecules that stay in the body but migrate (e.g., ultrahigh molecular weight (MW) polyethylene (PE) from joint prostheses), or hydrolysis into low MW molecules that are excreted from the body (bioresorption), or dissolving without modification of the MW (bioabsorption) [4, 5]. On the other hand, for environmentally degradable plastics, the

https://doi.org/10.1515/9781501511967-001

term biodegradation may mean fragmentation, loss of mechanical properties, or sometimes degradation through the action of living organisms [6]. Deterioration or loss in physical integrity is also often mistaken for biodegradation [7]. Nevertheless, it is essential to have a universally acceptable definition of biodegradability to avoid confusion as to where biodegradable polymers can be used in agriculture or fit into the overall plan of polymer waste management. Many groups and organisations have endeavoured to clearly define the terms 'degradation', 'biodegradation' and 'biodegradability'. But there are several reasons why establishing a single definition among the international community has not been straightforward, including:

- The variability of an intended definition given the different environments in which the material is to be introduced and its related impact on those environments.
- The differences of opinion with respect to the scientific approach or reference points used for determining biodegradability.
- The divergence of opinion concerning the policy implications of various definitions.
- Challenges posed by language differences around the world.

As a result, many different definitions have officially been adopted, depending on the background of the defining organisation and their particular interests. However, of more practical importance are the criteria for calling a material 'biodegradable'. A demonstrated potential of a material to biodegrade does not say anything about the time frame in which this occurs, nor the ultimate degree of degradation. The complexity of this issue is illustrated by the following common examples.

Low-density PE has been shown to biodegrade slowly to $CO_2$ (0.35% in 2.5 years) [8] and according to some definitions can thus be called a biodegradable polymer. However, the degradation process is so slow in comparison with the application rate that accumulation in the environment will occur. The same applies for polyolefin- starch blends which rapidly lose strength, disintegrate and visually disappear if exposed to microorganisms [9–11]. This is due to utilisation of the starch component, but the polyolefin fraction will nevertheless persist in the environment. Can these materials be called 'biodegradable'?

## 1.3 Defining 'Biodegradability'

In 1992, an international workshop on biodegradability was organised to bring together experts from around the world to achieve areas of agreement on definitions, standards and testing methodologies. Participants came from manufacturers, legislative authorities, testing laboratories, environmentalists and standardisation organisations in Europe, USA and Japan. Since this fruitful meeting, there is a general agreement concerning the following key points [12]:

- For all practical purposes of applying a definition, material manufactured to be biodegradable must relate to a specific disposal pathway such as composting, sewage treatment, denitrification and anaerobic sludge treatment.
- The rate of degradation of a material manufactured to be biodegradable has to be consistent with the disposal method and other components of the pathway into which it is introduced, such that accumulation is controlled.
- The ultimate end products of the aerobic biodegradation of a material manufactured to be biodegradable are $CO_2$, water and minerals, and the intermediate products should include biomass and humic materials. (Anaerobic biodegradation was discussed in less detail by the participants).
- Materials must biodegrade safely and not negatively impact the disposal process or use of the end product of the disposal.

As a result, specified periods of time, specific disposal pathways and standard test methodologies were incorporated into definitions. Standardisation organisations such as the European Committee for Standardization, International Organization for Standardization (ISO) and American Society for Testing and Materials (ASTM) were consequently encouraged to rapidly develop standard biodegradation tests so these could be determined. Society further demanded nondebatable criteria for the evaluation of the suitability of polymeric materials for disposal in specific waste streams such as composting or anaerobic digestion. Biodegradability is usually just one of the essential criteria, besides ecotoxicity, effects on waste treatment processes and so on.

In the following sections of this chapter, the biodegradation of polymeric materials is looked upon from the chemical perspective. The chemistry of the key degradation process is represented by Equations 1.1 and 1.2, where $C_{POLYMER}$ represents either a polymer or a fragment from any of the degradation processes defined earlier. For simplicity, the polymer or fragment is considered to be composed only of carbon, hydrogen and oxygen; other elements may, of course, be incorporated in the polymer, and these would appear in an oxidised or reduced form after biodegradation depending on whether the conditions are aerobic or anaerobic, respectively:

*Aerobic biodegradation*:

$$C_{POLYMER} + O2 \rightarrow CO_2 + H_2O + C_{RESIDUE} + C_{BIOMASS} \quad (1.1)$$

*Anaerobic biodegradation*:

$$C_{POLYMER} \rightarrow CO2 + CH_4 + H_2O + C_{RESIDUE} + C_{BIOMASS} \quad (1.2)$$

Complete biodegradation occurs when no residue remains and complete mineralisation is established when the original substrate, $C_{POLYMER}$ in this example, is completely converted into gaseous products and salts. However, mineralisation is a very slow process under natural conditions because some of the polymer undergoing

biodegradation will initially be turned into biomass [13, 14]. Therefore, complete biodegradation and not mineralisation is the measurable goal when assessing removal from the environment.

## 1.4 Mechanisms of polymer degradation

When working with biodegradable materials, the obvious question is why some polymers biodegrade and others do not. To understand this, one needs to know about the mechanisms through which polymeric materials are biodegraded. Although biodegradation is usually defined as degradation caused by biological activity (especially enzymatic action), it will usually occur simultaneously with – and is sometimes even initiated by – abiotic degradation such as photodegradation and simple hydrolysis. The following section gives a brief introduction to the most important mechanisms of polymer degradation.

### 1.4.1 Nonbiological degradation of polymers

A great number of polymers are subject to hydrolysis, such as polyesters, polyanhydrides, polyamides, polycarbonates, polyurethanes (PU), polyureas, polyacetals and polyorthoesters. Different mechanisms of hydrolysis have been extensively reviewed; not only for backbone hydrolysis, but also for the hydrolysis of pendant groups [15–17]. The necessary elements for a wide range of catalysis, such as acids and bases, cations, nucleophiles and micellar and phase transfer agents, are usually present in most environments. In contrast to enzymatic degradation, where a material is degraded gradually from the surface inwards (primarily because macromolecular enzymes cannot diffuse into the interior of the material), chemical hydrolysis of a solid material can take place throughout its cross-section, except for very hydrophobic polymers.

Important features affecting chemical polymer degradation and erosion include: a) the type of chemical bond, b) the pH, c) the temperature, d) the copolymer composition and e) water uptake (hydrophilicity). These features will not be discussed here, but have been covered in detail by [4].

### 1.4.2 Biological degradation of polymers

Polymers represent major constituents of living cells which are most important for metabolism (enzyme proteins, storage compounds), genetic information (nucleic acids) and the structure (cell wall constituents, proteins) of cells [18]. These polymers have to be degraded inside cells in order to be available for environmental changes

and to other organisms upon cell lysis. It is therefore not surprising that organisms, during many millions of years of adaptation, have developed various mechanisms to degrade naturally occurring polymers. However, for the many new and varied synthetic polymers that have found their way into the environment only in the last 70 years, these mechanisms may not as yet have been developed.

There are many different degradation mechanisms that combine synergistically in nature to degrade polymers. Microbiological degradation can take place through the action of enzymes or by-products (such as acids and peroxides) secreted by microorganisms (bacteria, yeasts, fungi and so on). In addition, macroorganisms can eat and, sometimes, digest polymers and cause mechanical, chemical or enzymatic ageing [19, 20].

Two key steps occur in the microbial polymer degradation process: first, a depolymerisation or chain cleavage step, and second, mineralisation. The first step normally occurs outside the organism due to the size of the polymer chain and the insoluble nature of many of the polymers. Extracellular enzymes are responsible for this step, acting in either an endo (random cleavage on the internal linkages of the polymer chains) or exo (sequential cleavage on the terminal monomer units in the main chain) manner.

Once oligomeric or monomeric fragments of a sufficiently small size are formed, they are transported into the cell where they are mineralised. At this stage the cell usually derives metabolic energy from the mineralisation process. The products of this process, apart from adenosine triphosphate (ATP), are gases (e.g., $CO_2$, $CH_4$, nitrogen ($N_2$) and hydrogen ($H_2$)), water, salts and minerals, and biomass. Many variations of this general view of the biodegradation process can occur, depending on the polymer, the organisms and the environment. Nevertheless, there will always be, at one stage or another, the involvement of enzymes.

Enzymes are biological catalysts which can induce enormous ($10^8$–$10^{20}$ fold) increases in reaction rates in an environment otherwise unfavourable for chemical reactions. All enzymes are proteins, i.e., polypeptides with a complex three-dimensional structure, ranging in MW from several thousand to several million g/mol. Enzyme activity is closely related to the conformational structure, which creates certain regions at the surface, forming an active site. The interaction between an enzyme and substrate takes place at the active site, leading to the chemical reaction, eventually giving a particular product. Some enzymes contain regions with absolute specificity for a given substrate while others can recognise a series of substrates. For optimal activity most enzymes must associate with cofactors, which can be of inorganic (e.g., metal ions) or organic origin (such as coenzyme A, ATP and vitamins such as riboflavin and biotin) [18]. Different enzymes can have different mechanisms of catalysis. Some enzymes change the substrate through some free radical mechanism, while others follow alternative chemical routes. When assessing the biodegradability of polymeric materials, it is important to realise that there are an enormous amount of different enzymes – each catalysing its own unique reaction on groups of substrates or on very specific chemical bonds; in some cases acting complementarily, in others synergistically.

## 1.5 Measuring the biodegradation of polymers

As can be imagined from the various mechanisms described above, biodegradation does not only depend on the chemistry of the polymer, but also on the presence of the biological systems involved in the process. When investigating the biodegradability of a material, the effect of the environment cannot be neglected. Microbial activity, and hence biodegradation, is influenced by:

- The presence of microorganisms.
- The availability of oxygen.
- The amount of available water.
- The temperature.
- The chemical environment (pH, electrolytes and so on).

In order to simplify the overall picture, the environments in which biodegradation occurs are basically divided in two: a) aerobic (with oxygen available) and b) anaerobic (no oxygen present). The availability of oxygen greatly affects the composition of the microbial community that is active in the environment, and thus its ability to biodegrade particular polymers. This division can in turn be subdivided into 1) aquatic and 2) high solids environments. Figure 1.1 schematically presents the different environments, with examples in which biodegradation may occur [21, 22].

| | 1) Aquatic | 2) High solids |
|---|---|---|
| a) Aerobic | – Aerobic wastewater treatment plants<br>– Surface waters; e.g., lakes and rivers<br>– Marine environments | – Surface soils<br>– Organic waste composting plants<br>– Littering |
| b) Anaerobic | – Anaerobic wastewater treatment plants<br>– Rumen of herbivores | – Deep-sea sediments<br>– Anaerobic sludge<br>– Anaerobic digestion/biogasification<br>– Landfill |

**Figure 1.1:** Schematic classification of different biodegradation environments for polymers.

The high solids environments will be the most relevant for measuring environmental biodegradation of polymeric materials, since they represent the conditions during biological municipal solid waste treatment, such as composting or anaerobic digestion (biogasification). However, possible applications of biodegradable materials other than in packaging and consumer products, e.g., in fishing nets at sea, or undesirable exposure in the environment due to littering, explain the necessity of aquatic biodegradation tests.

Numerous ways for the experimental assessment of polymer biodegradability have been described in the scientific literature. Because of slightly different definitions or interpretations of the term ‘biodegradability’, the different approaches are

therefore not equivalent in terms of information they provide or practical significance. Since the typical exposure environment involves incubation of a polymer substrate with microorganisms or enzymes, only a limited number of measurements are possible:those pertaining to the substrates, to the microorganisms or to the reaction products. Four common approaches available for studying biodegradation processes have been reviewed in detail by Andrady [13, 14]:

- Monitoring the accumulation of biomass.
- Monitoring the depletion of substrates.
- Monitoring the reaction products.
- Monitoring the changes in substrate properties.

In the following sections, different test methods for the assessment of polymer biodegradability are presented. Measurements are usually based on one of the four approaches given above, but combinations also occur. Before choosing an assay to simulate environmental effects in an accelerated manner, it is critical to consider the closeness of fit that the assay will provide between substrate, microorganisms or enzymes, and the application or environment in which biodegradation should take place [23].

## 1.5.1 Enzyme assays

### 1.5.1.1 Principle

In enzyme assays, the polymer substrate is added to a buffered or pH-controlled system, containing one or several types of purified enzymes. These assays are very useful in examining the kinetics of depolymerisation, or oligomer or monomer release from a polymer chain under different assay conditions. The method is very rapid (minutes to hours) and can give quantitative information. However, enzyme assays are not suitable to determine mineralisation rates.

### 1.5.1.2 Applications

The type of enzyme to be used, and quantification of degradation, will depend on the polymer being screened. For example, Mochizuki and co-workers [24] studied the effects of the draw ratio of polycaprolactone fibres on enzymatic hydrolysis by lipase. The degradability of polycaprolactone fibres was monitored by dissolved organic carbon (DOC) formation and weight loss. Similar systems with lipases have been used for studying the hydrolysis of broad ranges of aliphatic polyesters [25–30], copolyesters with aromatic segments [26, 31–33] and copolyesteramides [34, 35].

Other enzymes such as α-chymotrypsin and α-trypsin have also been applied to these polymers [36, 37]. The biodegradability of polyvinyl alcohol (PVA) segments,

with respect to block length and stereo chemical configuration, has been studied using isolated PVA-dehydrogenase [38]. Cellulolytic enzymes have been used to study the biodegradability of cellulose ester derivatives as a function of the degree of substitution and substituent size [39]. Similar work has been performed with starch esters using amylolytic enzymes such as α-amylases, β-amylases, glucoamylases and amyloglucosidases [40]. Enzymatic methods have also been used to study the biodegradability of starch plastics or packaging materials containing cellulose [41–46].

#### 1.5.1.3 Drawbacks

Caution must be used in extrapolating enzyme assays as a screening tool for different polymers since the enzymes have been paired to only one polymer. The initially selected enzymes may show significantly reduced activity towards modified polymers or different materials, even though more suitable enzymes may exist in the environment. Caution must also be used if the enzymes are not purified or appropriately stabilised or stored, since inhibition and loss of enzyme activity can occur [23].

### 1.5.2 Plate tests

#### 1.5.2.1 Principle

Plate tests were initially developed in order to assess the resistance of plastics to microbial degradation. Several methods have been standardised by standardisation organisations such as the ASTM and the ISO [47–49]. They are now also used to see if a polymeric material will support growth [23, 50]. The principle of the method involves placing the test material on the surface of a mineral salts agar in a Petri dish containing no additional carbon source. The test material and agar surface are sprayed with a standardised mixed inoculum of known bacteria and/or fungi.

The test material is examined, after a predetermined incubation period at constant temperature, for the amount of growth on its surface and a rating is given.

#### 1.5.2.2 Applications

Potts [51] used the method in his screening of 31 commercially available polymers for biodegradability. Other studies, where the growth of either mixed or pure cultures of microorganisms is taken to be indicative of biodegradation, have been reported [6]. The validity of this type of test and the use of visual assessment alone, for all plastics,

has been questioned by Seal and Pantke [52]. They recommended that mechanical properties should be assessed to support visual observations. Microscopic examination of the surface can also give additional information.

A variation of the plate test is the 'clear zone' technique [53], sometimes used to screen polymers for biodegradability. A fine suspension of polymer is placed in an agar gel as the sole carbon source and the test inoculum is placed in wells bored into the agar. After incubation, a clear zone around the well, detected visually or instrumentally, is indicative of utilisation of the polymer. The method has, for example, been used in the case of starch plastics [54], various polyesters [55–57] and PU [58].

#### 1.5.2.3 Drawbacks

A positive result in an agar plate test indicates that an organism can grow on the substrate, but does not mean that the polymer is biodegradable, since growth may be on contaminants, on plasticisers which are present, on oligomeric fractions still present in the polymer and so on. Therefore, these tests should be treated with caution when extrapolating the data to field situations.

### 1.5.3 Respiration tests

#### 1.5.3.1 Principle

Aerobic microbial activity is typically characterised by the utilisation of oxygen. Aerobic biodegradation requires oxygen for the oxidation of compounds to its mineral constituents, such as $CO_2$, $H_2O$, sulfur dioxide ($SO_2$), phosphorous pentoxide ($P_2O_5$) and so on. The amount of oxygen utilised during incubation, also called the biological oxygen demand (BOD), is therefore a measure of the degree of biodegradation. Several test methods are based on measurement of the BOD, often expressed as a percentage of the theoretical oxygen demand (TOD) of the compound. The TOD, which is the theoretical amount of oxygen necessary for completely oxidising a substrate to its mineral constituents, can be calculated by considering the elemental composition and the stoichiometry of oxidation [13, 59–62] or based on experimental determination of the chemical oxygen demand (COD) [13, 63].

#### 1.5.3.2 Applications

The closed bottle BOD tests were designed to determine the biodegradability of detergents [61, 64]. These have stringent conditions due to the low level of inoculum (in the

order of $10^5$ microorganisms/l) and the limited amount of test substance that can be added (normally between 2 and 4 mg/l). These limitations originate from the practical requirement that the oxygen demand should not exceed half the maximum dissolved oxygen level in the water at the temperature of the test, to avoid the generation of anaerobic conditions during incubation.

For nonsoluble materials, such as polymers, less stringent conditions are acceptable and alternative ways for measuring BOD were developed. Two-phase (semi) closed bottle tests enable a higher oxygen content in the flasks and permit a higher inoculum level. Higher test concentrations are also possible, encouraging higher accuracy with the direct weighing in of samples. The oxygen demand can alternatively be determined by periodically measuring the oxygen concentration in the aquatic phase by opening the flasks [60, 65–67], by measuring the change in volume or pressure in incubation flasks containing $CO_2$-absorbing agents [59, 68, 69], or by measuring the quantity of oxygen produced (electrolytically) to maintain a constant gas volume/pressure in specialised respirometers [59, 62, 65, 66, 68].

#### 1.5.3.3 Suitability

BOD tests are sensitive and relatively simple to perform, and are therefore often used as screening tests. However, the measurement of oxygen consumption is a nonspecific, indirect measure for biodegradation and is not suitable for determining anaerobic degradation. The requirement for test materials to be the sole carbon/energy source for microorganisms in the incubation media eliminates the use of oxygen measurements in complex natural environments.

### 1.5.4 Gas ($CO_2$ or $CH_4$) evolution tests

#### 1.5.4.1 Principle

The evolution of $CO_2$ or $CH_4$ from a substrate represents a direct parameter of mineralisation. Therefore, gas evolution tests can be important tools in the determination of the biodegradability of polymeric materials. A number of well- known test methods have been standardised for aerobic biodegradation, such as the (modified) Sturm test [70–75] and the laboratory controlled composting test [76–79]; as well as for anaerobic biodegradation, such as the anaerobic sludge test [80, 81] and the anaerobic digestion test [82, 83]. Although the principle of these test methods is the same, they may differ in medium composition, inoculum, the way substrates are introduced, and in the technique for measuring gas evolution.

### 1.5.4.2 Applications

Anaerobic tests generally follow biodegradation by measuring the increase in pressure and/or volume due to gas evolution, usually in combination with gas chromatographic analysis of the gas phase [84, 85]. Most aerobic standard tests apply continuous aeration; the exit stream of air can be directly analysed continuously using a $CO_2$ monitor (usually infrared detectors) or titrimetrically after sorption in dilute alkali. The cumulative amount of $CO_2$ generated, expressed as a percentage of the theoretically expected value for total conversion to $CO_2$, is a measure of the extent of mineralisation achieved. A value of 60% carbon conversion to $CO_2$, achieved within 28 days, is generally taken to indicate ready degradability. Taking into account that in this system there will also be incorporation of carbon into the formation of biomass (growth), the 60% value for $CO_2$ implies almost complete degradation. While this criterion is meant for water-soluble substrates, it is probably applicable to very finely divided moderately degradable polymeric materials as well [13]. Nevertheless, most standards for determining the biodegradability of plastics consider a maximum test duration of 6 months.

Besides the continuously aerated systems, described above, several static respirometers have been described. Bartha and Pramer [86] describe a two-flask system; one flask, containing a mixture of soil and the substrate, is connected to another chamber holding a quantity of $CO_2$ sorbant. Care must be taken to ensure that enough oxygen is available in the flask for biodegradation. Nevertheless, this experimental set-up and modified versions thereof have been successfully applied in the assessment of the biodegradability of polymer films and food packaging materials [87–89].

The percentage of carbon converted to biomass instead of $CO_2$ depends on the type of polymer and the phase of degradation. Therefore, it has been suggested to regard the complete carbon balance to determine the degree of degradation [90]. This implies that besides the detection of gaseous carbon, the amount of carbon in soluble and solid products also needs to be determined. Soluble products, oligomers of different molecular size, intermediates and proteins secreted from microbial cells can be measured as COD or as DOC. Solid products, biomass, and polymer remnants require a combination of procedures to separate and detect different fractions. The protein content of the insoluble fraction is usually determined to estimate the amount of carbon converted to biomass, using the assumptions that dry biomass consists of 50% protein and that the carbon content of dry biomass is 50% [90–92].

### 1.5.4.3 Suitability

Gas evolution tests are popular test methods because they are sensitive and relatively simple to perform. A direct measure for mineralisation is determined and

water-soluble or insoluble polymers can be tested as films, powders or objects. Furthermore, the test conditions and inoculum can be adjusted to fit the application or environment in which biodegradation should take place. Aquatic synthetic media is usually used, but also natural seawater [93, 94] or soil samples [86, 88, 89, 95] can be applied as biodegradation environments. A prerequisite for these media is that the background $CO_2$ evolution is limited, which excludes the application of real composting conditions. Biodegradation under composting conditions is therefore measured using an inoculum derived from matured compost with low respiration activity [76–78, 96, 97].

A drawback of using complex degradation environments, such as mature compost, is that the simultaneous characterisation of intermediate degradation products and determination of the carbon balance is difficult due to the presence of a great number of interfering compounds. To overcome this, an alternative test was developed based on an inoculated mineral bed-based matrix [98, 99].

## 1.5.5 Radioactively labelled polymers

### 1.5.5.1 Principle and applications

Some materials tend to degrade very slowly under stringent test conditions without an additional source of carbon. However, if readily available sources of carbon are added, it becomes impossible to tell how much of the evolved $CO_2$ can be attributed to decomposition of the plastic. The incorporation of radioactive $^{14}C$ in synthetic polymers gives a means of distinguishing between $CO_2$ or $CH_4$ produced *via* metabolism of the polymer, and that generated by other carbon sources in the test environment. By comparison of the amount of radioactive $^{14}CO_2$ or $^{14}CH_4$ to the original radioactivity of the labelled polymer, it is possible to determine the percent by weight of carbon in the polymer which was mineralised during the exposure period [51, 100–102]. Collection of radioactively labelled gases or low MW products can also provide extremely sensitive and reproducible methods to assess the degradation of polymers with low susceptibility to enzymes, such as PE [8, 103] and cellulose acetates [104, 105].

### 1.5.5.2 Drawbacks

Problems with handling the radioactively labelled materials and their disposal are issues on the down side of this method. In addition, in some cases it is difficult to synthesise the target polymer with the radioactive labels in the appropriate locations, with representative MW, or with representative morphological characteristics.

## 1.5.6 Laboratory-scale simulated accelerating environments

### 1.5.6.1 Principle

Biodegradation of a polymer material is usually associated with changes in the physical, chemical and mechanical properties of the material. It is indeed these changes, rather than the chemical reactions, which make the biodegradation process so interesting from an application point of view. These useful properties might be measured as a function of the duration of exposure to a biotic medium, to follow the consequences of the biodegradation process on material properties. Biotic media can be specifically designed at laboratory scale to mimic natural systems whilst allowing maximum control of variables such as temperature, pH, microbial community, mechanical agitation and supply of oxygen. Regulating these variables improves the reproducibility and may accelerate the degradation process. Laboratory simulations can also be used for the assessment of long-term effects, achieved by continuous dosing, on the activity and environment of the disposal system [50].

### 1.5.6.2 Applications

The Organisation for Economic Co-operation and Development's 'Coupled Unit' test [106] simulates an activated sludge sewage treatment system, but its application for polymers would be difficult as DOC is the parameter used to assess biodegradability. Krupp and Jewell [107] described well-controlled anaerobic and aerobic aquatic bioreactors to study the degradation of a range of commercially available polymer films. A relatively low loading rate of the semicontinuous reactors and a long retention time were maintained to maximise the biodegradation efficiency. Experimental set-ups have also been designed to simulate marine environments [108], soil burial conditions [108–110], composting environments [111–116], and landfill conditions [117–119] at laboratory scale, with controlled parameters such as temperature and moisture level, and a synthetic waste to provide a standardised basis for comparing the degradation kinetics of films.

A wide choice of material properties can be followed during the degradation process. However, it is important to select one which is relevant to the end-use of the polymer material or provides fundamental information about the degradation process. Weight loss is a parameter frequently followed because it clearly demonstrates the disintegration of a biodegradable product [120–122]. Tensile properties are also often monitored, due to interest in the use of biodegradable plastics in packaging applications [54, 123, 124]. In those polymers where biodegradation involves a random scission of the macromolecular chains, a decrease in the average MW and a general broadening of the MW distribution provide initial evidence of a breakdown process [86, 125, 126]. However, no significant changes in material characteristics may be observed in recovered material if the mechanism of biodegradation involves

bioerosion, i.e., enzymatic or hydrolytic cleavage at the surface. Visual examination of the surface with various microscopic techniques can also give information on the biodegradation process [115, 127–130]. Likewise, chemical and/or physical changes in the polymer may be followed by (combinations of) specific techniques such as infrared [10, 131] or ultraviolet (UV) spectroscopy [84, 132], nuclear magnetic resonance measurements [115, 126–133], X-ray diffractometry [115, 134, 135] and differential scanning calorimetry (DSC) [115, 136, 137].

#### 1.5.6.3 Drawbacks

An inherent drawback in the use of mechanical properties, weight loss, MW, or any other property which relies on the macromolecular nature of the substrate is that in spite of their sensitivity, these can only address the early stages of the biodegradation process. Furthermore, these parameters give no information on the extent of mineralisation. Especially in material blends or copolymers, the hydrolysis of one component can cause significant disintegration (and thus loss of weight and tensile properties) whereas other components may persist in the environment, even in a disintegrated form [13]. Blends of starch, poly(3-hydroxy butyrate) or poly(ε- caprolactone) with polyolefins are examples of such systems [11, 43, 138].

### 1.5.7 Natural environments, field trials

Exposure in natural environments provides the best true measure of the environmental fate of a polymer, because these tests include a diversity of organisms and achieve a desirable natural closeness of fit between the substrate, microbial agent and the environment. However, the results of that particular exposure are only relevant to the specific environment studied, which is likely to differ substantially from many other environments. An additional problem is the timescale for this method, since the degradation process, depending on the environment, may be very slow (months to years) [23]. Moreover, little information on the degradation process can be gained other than the real time required for weight loss or total disintegration. Nevertheless, field trials in natural environments are still used to extrapolate results acquired in laboratory tests to biodegradation behaviour under realistic outdoor conditions [115, 116, 127, 139, 140].

## 1.6 Conclusions

The overview presented above makes clear that there is no such thing as a single optimal method for determining the biodegradation of polymeric materials. First of

all, the biodegradation of a material is not only determined by the chemical composition and corresponding physical properties, but the degradation environment, to which the material is exposed, also affects the rate and degree of biodegradation. Furthermore, the method or test to be used depends on what information is requested; especially as the biodegradation concept is very important in relation to the end of life of a material, while it could be just one aspect of health and environmental safety in other cases.

It is fairly obvious, but often neglected, that one should always consider why a particular polymeric material should be (or not be) biodegradable when contemplating how to assess its biodegradability. After all, it is the intended application of the material that governs the most suitable testing environment, the parameters to be measured during exposure and the corresponding limit values. For example, investigating whether biodegradation of a plastic material designed for food packaging could facilitate undesired growth of (pathogenic) microorganisms requires a completely different approach from investigating whether its waste can be discarded *via* composting (i.e., whether it degrades sufficiently rapidly to be compatible with existing biowaste composting facilities).

It is important to state that it will not be sufficient to ascertain macroscopic changes, such as weight loss and disintegration, or growth of microorganisms, to define a material as biodegradable because these observations may originate from a partial biodegradation or from the degradation of a component of the material itself. In order to study the real biodegradation of a material in the environment (composting, anaerobic digestion, soil and water) it is necessary to determine the mineralisation, which is the transformation of the material into: $CO_2$, $CH_4$ (anaerobic condition), water and new biomass. Furthermore, it is important to evaluate the eventual toxic effects that the addition of the material could have on the environment, in order to avoid introducing dangerous substances. In this way we will be sure that no harm will be caused to the environment itself. This is the same approach followed by the principal standardisation bodies with standards regarding the compostability of plastics and packaging. These standards (European Norms 13432, ISO 17088 and ASTM D6400) describe the specifications for compostable plastics and packaging.

## References

[1] T. Hayashi, *Progress in Polymer Science*, 1994, **19**, 663.
[2] D.F. Williams and S.P. Zhong, *International Biodeterioration & Biodegradation*, 1994, **34**, 95.
[3] F. Buchanan in *Degradation Rate of Bioresorbable Materials – Prediction and Evaluation*, Woodhead Publishing Ltd, Cambridge, UK, 2008.
[4] A. Göpferich, *Biomaterials*, 1996, **17**, 103.
[5] G. Mabilleau and A. Sabokbar in *Degradation Rate of Bioresorbable Materials – Prediction and Evaluation*, Ed., F. Buchanan, Woodhead Publishing Ltd, Cambridge, UK, 2008, p.145.

[6] A-C. Albertsson and S. Karlsson in *Degradable Materials – Perspectives, Issues and Opportunities*, Eds., S.A. Barenberg, J.L. Brash, R. Narayan and A.E. Redpath, CRC Press, Boston, MA, USA, 1990.
[7] A.C. Palmisano and C.A. Pettigrew, *Bioscience*, 1992, **42**, 680.
[8] A-C. Albertsson and B. Rånby, *Journal of Applied Polymer Science: Applied Polymer Symposium*, 1979, **35**, 423.
[9] R.G. Austin in *Degradable Materials – Perspectives, Issues and Opportunities*, Eds., S.A. Barenberg, J.L. Brash, R. Narayan and A.E. Redpath, CRC Press, Boston, MA, USA, 1990, p.209.
[10] S.M. Goheen and R.P. Wool, *Journal of Applied Polymer Science*, 1991, **42**, 2691.
[11] V.T. Breslin, *Journal of Environmental Polymer Degradation*, 1993, **1**, 127.
[12] Anonymous in *Towards Common Ground – Meeting Summary of the International Workshop on Biodegradability*, Annapolis, Maryland, USA, 20–21st October, 1992.
[13] A.L. Andrady, *Journal of Macromolecular Science: Reviews in Macromolecular Chemistry & Physics*, 1994, **C34**, 25.
[14] A.L. Andrady in *Handbook of Polymer Degradation*, 2nd Edition, Ed., S.H. Hamid, Marcel Dekker Inc., New York, NY, USA, 2000, p.441.
[15] T. St.Pierre and E. Chiellini, *Journal of Bioactive and Compatible Polymers*, 1986, **1**, 467.
[16] T. St.Pierre and E. Chiellini, *Journal of Bioactive and Compatible Polymers*, 1987, **2**, 4.
[17] R.E. Cameron and A. Kamvari-Moghaddam in *Degradation Rate of Bioresorbable Materials – Prediction and Evaluation*, Ed., F. Buchanan, Woodhead Publishing Ltd, Cambridge, UK, 2008, p.43.
[18] L. Stryer in *Biochemistry*, 2nd Edition, W.H. Freeman and Company, New York, NY, USA, 1981.
[19] T.A. Anderson, R. Tsao and J.R. Coats, *Journal of Environmental Polymer Degradation*, 1993, **1**, 301.
[20] P.J. Whitney, C.H. Swaffield and A.J. Graffam, *International Biodeterioration and Biodegradation*, 1993, **31**, 179.
[21] M. Van der Zee, J.H. Stoutjesdijk, P.A.A.W. Van der Heijden and D. De Wit, *Journal of Environmental Polymer Degradation*, 1995, **3**, 235.
[22] G. Eggink, M. Van der Zee and L. Sijtsma in *International Edition of the IOP on Environmental Biotechnology*, IOP-Milieubiotechnologie, The Hague, The Netherlands, 1995, p.7.
[23] J.M. Mayer and D.L. Kaplan in *Biodegradable Polymers and Packaging*, Eds., C. Ching, D.L. Kaplan and E.L. Thomas, Technomic Publishing Co. Inc., Lancaster, PA, USA, 1993, p.233.
[24] M. Mochizuki, M. Hirano, Y. Kanmuri, K. Kudo and Y. Tokiwa, *Journal of Applied Polymer Science*, 1995, **55**, 289.
[25] Y. Tokiwa and T. Suzuki, *Journal of Applied Polymer Science*, 1981, **26**, 441.
[26] Y. Tokiwa, T. Suzuki and K. Takeda, *Agricultural and Biological Chemistry*, 1986, **50**, 1323.
[27] I. Arvanitoyannis, A. Nakayama, N. Kawasaki and N. Yamamoto, *Polymer*, 1995, **36**, 2271.
[28] A. Nakayama, N. Kawasaki, I. Arvanitoyannis, J. Iyoda and N. Yamamoto, *Polymer*, 1995, **36**, 1295.
[29] T. Walter, J. Augusta, R-J. Müller, H. Widdecke and J. Klein, *Enzyme and Microbial Technology*, 1995, **17**, 218.
[30] M. Nagata, T. Kiyotsukuri, H. Ibuki, N. Tsutsumi and W. Sakai, *Reactive and Functional Polymers*, 1996, **30**, 165.
[31] H.S. Jun, B.O. Kim, Y.C. Kim, H.N. Chang and S.I. Woo, *Journal of Environmental Polymer Degradation*, 1994, **2**, 9.
[32] E. Chiellini, A. Corti, A. Giovannini, P. Narducci, A.M. Paparella and R. Solaro, *Journal of Environmental Polymer Degradation*, 1996, **4**, 37.
[33] M. Nagata, T. Kiyotsukuri, S. Minami, N. Tsutsumi and W. Sakai, *Polymer International*, 1996, **39**, 83.

[34] M. Nagata and T. Kiyotsukuri, *European Polymer Journal*, 1994, **30**, 1277.
[35] M. Nagata, *Macromolecular Rapid Communications*, 1996, **17**, 583.
[36] I. Arvanitoyannis, E. Nikolaou and N. Yamamoto, *Polymer*, 1994, **35**, 4678.
[37] I. Arvanitoyannis, E. Nikolaou and N. Yamamoto, *Macromolecular Chemistry and Physics*, 1995, **196**, 1129.
[38] S. Matsumura, Y. Shimura, K. Toshima, M. Tsuji and T. Hatanaka, *Macromolecular Chemistry and Physics*, 1995, **196**, 3437.
[39] W.G. Glasser, B.K. McCartney and G. Samaranayake, *Biotechnology Progress*, 1994, **10**, 214.
[40] C. Rivard, L. Moens, K. Roberts, J. Brigham and S. Kelley, *Enzyme and Microbial Technology*, 1995, **17**, 848.
[41] A.A. Strantz and E.A. Zottola, *Journal of Food Protection*, 1992, **55**, 736.
[42] V. Coma, Y. Couturier, B. Pascat, G. Bureau, J.L. Cuq and S. Guilbert, *Enzyme and Microbial Technology*, 1995, **17**, 524.
[43] S.H. Imam, S.H. Gordon, A. Burgess-Cassler and R.V. Greene, *Journal of Environmental Polymer Degradation*, 1995, **3**, 107.
[44] S.H. Imam, S.H. Gordon, R.L. Shogren and R.V. Greene, *Journal of Environmental Polymer Degradation*, 1995, **3**, 205.
[45] M. Vikman, M. Itävaara and K. Poutanen, *Journal of Macromolecular Science, Part A: Pure and Applied Chemistry*, 1995, **A32**, 863.
[46] M. Vikman, M. Itävaara and K. Poutanen, *Journal of Environmental Polymer Degradation*, 1995, **3**, 23.
[47] ASTM G21-09, *Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi*, American Society for Testing and Materials, Philadelphia, PA, USA, 2009.
[48] ASTM G22-76, *Standard Practice for Determining Resistance of Plastics to Bacteria*, American Society for Testing and Materials, Philadelphia, PA, USA, 1996. {Withdrawn in 2002}
[49] ISO 846:1997, *Plastics – Evaluation of the Action of Microorganisms*, International Organization for Standardization, Genève, Switzerland, 1997.
[50] K.J. Seal in *Chemistry and Technology of Biodegradable Polymers*, Ed., G.J.L. Griffin, Blackie Academic and Professional, London, UK, 1994, p.116.
[51] J.E. Potts in *Aspects of Degradation and Stabilization of Polymers*, Ed., H.H.G. Jellinek, Elsevier Scientific Publishing Co., Amsterdam, The Netherlands, 1978, p.617.
[52] K.J. Seal and M. Pantke, *Material und Organismen*, 1986, **21**, 151.
[53] F.P. Delafield, M. Doudoroff, N.J. Palleroni, C.J. Lusty and R. Contopoulos, *Journal of Bacteriology*, 1965, **90**, 1455.
[54] J.M. Gould, S.H. Gordon, L.B. Dexter and C.L. Swanson in *Agricultural and Synthetic Polymers – Biodegradability and Utilization*, Eds., J.E. Glass and G. Swift, ACS Symposium Series 433, American Chemical Society, Washington, DC, USA, 1990, p.65.
[55] J. Augusta, R-J. Müller and H. Widdecke, *Applied Microbiology and Biotechnology*, 1993, **39**, 673.
[56] H. Nishida and Y. Tokiwa, *Chemistry Letters*, 1994, **3**, 421.
[57] H. Nishida and Y. Tokiwa, *Chemistry Letters*, 1994, **7**, 1293.
[58] J.R. Crabbe, J.R. Campbell, L. Thompson, S.L. Walz and W.W. Schultz,International Biodeterioration and Biodegradation, 1994, 33, 103.
[59] ISO 9408:1999(E), *Water Quality – Evaluation of Ultimate Aerobic Biodegradability of Organic Compounds in Aqueous Medium by Determination of Oxygen Demand in a Closed Respirometer*, International Organization for Standardization, Genève, Switzerland, 1999.
[60] ISO 10708:1997(E), *Water Quality – Evaluation in an Aqueous Medium of the Ultimate Aerobic Biodegradability of Organic Compounds – Determination of Biochemical Oxygen Demand in a Two-phase Closed Bottle Test*, International Organization for Standardization, Genève, Switzerland, 1997.

[61] OECD 301D, *Ready Biodegradability: Closed Bottle Test*, Guidelines for Testing of Chemicals, Organisation for Economic Co-operation and Development, Paris, France, 1992.
[62] OECD 302C, *Inherent Biodegradability: Modified MITI Test (II)*, Guidelines for Testing of Chemicals, Organisation for Economic Co-operation and Development, Paris, France, 2009.
[63] ISO 6060:1989(E), *Water Quality – Determination of the Chemical Oxygen Demand*, International Organization for Standardization, Genève, Switzerland, 1989.
[64] ISO 10707:1997(E), *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)*, International Organization for Standardization, Genève, Switzerland, 1997.
[65] ISO 14851:2004(E), *Determination of the Ultimate Aerobic Biodegradability of Plastic Materials in an Aqueous Medium – Method by Determining the Oxygen Demand in a Closed Respirometer*, International Organization for Standardization, Genève, Switzerland, 2004.
[66] ASTM D5271-02, *Standard Test Method for Determining the Aerobic Biodegradation of Plastic Materials in an Activated-Sludge-Wastewater- Treatment System*, American Society for Testing and Materials, Philadelphia, PA, USA, 2002. {Withdrawn in 2011}
[67] EN 14048:2003, *Packaging – Determination of the Ultimate Aerobic Biodegradability of Packaging Materials in an Aqueous Medium – Method by Measuring the Oxygen Demand in a Closed Respirometer*, European Committee for Standardization, Brussels, Belgium, 2003.
[68] OECD 301F, *Ready Biodegradability: Manometric Respirometry Test*, Guidelines for Testing of Chemicals, Organisation for Economic Co- operation and Development, Paris, France, 1992.
[69] L. Tilstra and D. Johnsonbaugh, *Journal of Environmental Polymer Degradation*, 1993, **1**, 247.
[70] ASTM D5209-92, *Standard Test Method for Determining the Aerobic Biodegradation of Plastic Materials in the Presence of Municipal Sewage Sludge*, American Society for Testing and Materials, PA, USA, 1992. {Withdrawn in 2004}
[71] ISO 9439:2000(E), *Water Quality – Evaluation of Ultimate Aerobic Biodegradability of Organic Compounds in Aqueous* Medium – *Carbon Dioxide Evolution Test*, International Organization for Standardization, Genève, Switzerland, 2000.
[72] ISO 14852:2004(E), *Determination of the Ultimate Aerobic Biodegradability of Plastic Materials in an Aqueous Medium – Method by Analysis of Evolved Carbon Dioxide*, International Organization for Standardization, Genève, Switzerland, 2004.
[73] EN 14047:2003, *Packaging – Determination of the Ultimate Aerobic Biodegradability of Packaging Materials in an Aqueous Medium – Method by Analysis of Evolved Carbon Dioxide*, European Committee for Standardization, Brussels, Belgium, 2003.
[74] OECD 301B, *Ready Biodegradability: Modified Sturm Test*, Guidelines for Testing of Chemicals, Organisation for Economic Co-operation and Development, Paris, France, 1992.
[75] ASTM D6691-09, *Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in the Marine Environment by a Defined Microbial Consortium*, American Society for Testing and Materials, PA, USA, 2009.
[76] ASTM D5338-11, *Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials under Controlled Composting Conditions. Incorporating Thermophilic Temperatures*, American Society for Testing and Materials, PA, USA, 2011.
[77] ISO 14855-1:2007(E), *Determination of the Ultimate Aerobic Biodegradability of Plastic Materials under Controlled Composting Conditions – Method by Analysis of Evolved Carbon Dioxide – Part 1: General Method*, International Organization for Standardization, Genève, Switzerland, 2007.
[78] ISO 14855-2:2009(E), *Determination of the Ultimate Aerobic Biodegradability of Plastic Materials under Controlled Composting Conditions – Method by Analysis of Evolved Carbon Dioxide – Part 2: Gravimetric Measurement of Carbon Dioxide Evolved in a Laboratory-Scale Test*, International Organization for Standardization, Genève, Switzerland, 2009.

[79] EN 14046:2003, *Packaging – Evaluation of the Ultimate Aerobic Biodegradability of Packaging Materials under Controlled Compositing Conditions – Method by Analysis of Released Carbon Dioxide*, European Committee for Standardization, Brussels, Belgium, 2003.
[80] ASTM D5210-92(2007), *Standard Test Method for Determining the Anaerobic Biodegradation of Plastic Materials in the Presence of Municipal Sewage Sludge*, American Society for Testing and Materials, PA, USA, 2007.
[81] ISO 11734:1998(E), *Water Quality – Evaluation of the 'Ultimate' Anaerobic Biodegradability of Organic Compounds in Digested Sludge – Method by Measurement of the Biogas Production*, International Organization for Standardization, Genève, Switzerland, 1998.
[82] ASTM D5511-11, *Standard Test Method for Determining Anaerobic Biodegradation of Plastic Materials under High-Solids Anaerobic-Digestion Conditions*, American Society for Testing and Materials, PA, USA, 2011.
[83] ASTM D5526-94(2011)e1, *Standard Test Method for Determining Anaerobic Biodegradation of Plastic Materials Under Accelerated Landfill Conditions*, American Society for Testing and Materials, PA, USA, 2011.
[84] M. Day, K. Shaw and J.D. Cooney, *Journal of Environmental Polymer Degradation*, 1994, **2**, 121.
[85] P. Puechner, W-R. Mueller and D. Bardtke, *Journal of Environmental Polymer Degradation*, 1995, **3**, 133.
[86] R. Bartha and A. Yabannavar in *Proceedings of the* 4th *International Workshop on Biodegradable Plastics and Polymers and Fourth Annual Meeting of the Bio-environmentally Degradable Polymer Society*, Durham, NH, USA, 1995, p.28.
[87] A.L. Andrady, J.E. Pegram and Y. Tropsha, *Journal of Environmental Polymer Degradation*, 1993, **1**, 171.
[88] A. Yabannavar and R. Bartha, *Soil Biology and Biochemistry*, 1993, **25**, 1469.
[89] A.V. Yabannavar and R. Bartha, *Applied Environmental Microbiology*, 1994, **60**, 3608.
[90] S. Urstadt, J. Augusta, R-J. Müller and W-D. Deckwer, *Journal of Environmental Polymer Degradation*, 1995, **3**, 121.
[91] M. Itävaara and M. Vikman, *Chemosphere*, 1995, **31**, 4359.
[92] B. Spitzer, C. Mende, M. Menner and T. Luck, *Journal of Environmental Polymer Degradation*, 1996, **4**, 157.
[93] A.L. Allen, J.M. Mayer, R. Stote and D.L. Kaplan, *Journal of Environmental Polymer Degradation*, 1994, **2**, 237.
[94] R. Courtes, A. Bahlaoui, A. Rambaud, F. Deschamps, E. Sunde and E. Dutriex, *Ecotoxicology and Environmental Safety*, 1995, **31**, 142.
[95] P. Barak, Y. Coquet, T.R. Halbach and J.A.E. Molina, *Journal of Environmental Quality*, 1991, **20**, 173.
[96] U. Pagga, D.B. Beimborn, J. Boelens and B. De Wilde, *Chemosphere*, 1995, **31**, 4475.
[97] U. Pagga, D.B. Beimborn and M. Yamamoto, *Journal of Environmental Polymer Degradation*, 1996, **4**, 173.
[98] M. Tosin, F. Degli-Innocenti and C. Bastioli, *Journal of Environmental Polymer Degradation*, 1998, **6**, 79.
[99] G. Bellia, M. Tosin, G. Floridi and F. Degli-Innocenti, *Polymer Degradation and Stability*, 1999, **66**, 65.
[100] ASTM D6340-98, *Standard Test Methods for Determining Aerobic Biodegradation of Radiolabeled Plastic Materials in an Aqueous or Compost Environment*, American Society for Testing and Materials, PA, USA, 2007.
[101] ASTM D6692-01, *Standard Test Method for Determining the Biodegradability of Radiolabeled Polymeric Plastic Materials in Seawater*, American Society for Testing and Materials, PA, USA, 2001. {Withdrawn in 2010}

[102] ASTM D6776-02, *Standard Test Method for Determining Anaerobic Biodegradability of Radiolabeled Plastic Materials in a Laboratory-Scale Simulated Landfill Environment*, American Society for Testing and Materials, PA, USA, 2002. {Withdrawn in 2011}
[103] A-C. Albertsson, C. Barenstedt and S. Karlsson, *Journal of Environmental Polymer Degradation*, 1993, **1**, 241.
[104] R.J. Komarek, R.M. Gardner, C.M. Buchanan and S. Gedon, *Journal of Applied Polymer Science*, 1993, **50**, 1739.
[105] C.M. Buchanan, D. Dorschel, R.M. Gardner, R.J. Komarek, A.J. Matosky, A.W. White and M.D. Wood, *Journal of Environmental Polymer Degradation*, 1996, **4**, 179.
[106] OECD 303A, *Simulation Test – Aerobic Sewage Treatment: Coupled Units Test*, Guidelines for Testing of Chemicals, Organisation for Economic Co-operation and Development, Paris, France, 2001.
[107] L.R. Krupp and W.J. Jewell, *Environmental Science and Technology*, 1992, **26**, 193.
[108] D.L. Kaplan, J.M. Mayer, M. Greenberger, R. Gross and S. McCarthy, *Polymer Degradation and Stability*, 1994, **45**, 165.
[109] R. Dale and D.J. Squirrell, *International Biodeterioration and Biodegradation*, 1990, **26**, 355.
[110] K.J. Seal and M. Pantke, *Material und Organismen*, 1990, **25**, 87.
[111] R.M. Gardner, C.M. Buchanan, R. Komarek, D. Dorschel, C. Boggs and A.W. White, *Journal of Applied Polymer Science*, 1994, **52**, 1477.
[112] C.M. Buchanan, D.D. Dorschel, R.M. Gardner, R.J. Komarek and A.W. White, *Journal of Macromolecular Science, Part A: Pure and Applied Chemistry*, 1995, **A32**, 683.
[113] R.A. Gross, J-D. Gu, D. Eberiel and S.P. McCarthy, *Journal of Macromolecular Science, Part A: Pure and Applied Chemistry*, 1995, **A32**, 613.
[114] EN 14045:2003, *Packaging – Evaluation of the Disintegration of Packaging Materials in Practical Oriented Tests under Defined Composting Conditions*, European Committee for Standardization, Brussels, Belgium, 2003.
[115] S. Maiti, D. Ray, D. Mitra, S. Sengupta and T. Kar, *Journal of Applied Polymer Science*, 2011, **122**, 2503.
[116] E. Rudnik and D. Briassoulis, *Industrial Crops and Products*, 2011, **33**, 648.
[117] ASTM D7475-11, *Standard Test Method for Determining the Aerobic Degradation and Anaerobic Biodegradation of Plastic Materials under Accelerated Bioreactor Landfill Conditions*, American Society for Testing and Materials, PA, USA, 2011.
[118] ASTM D5526-94(2011)e1, *Standard Test Method for Determining Anaerobic Biodegradation of Plastic Materials Under Accelerated Landfill Conditions*, American Society for Testing and Materials, PA, USA, 2011.
[119] G.P. Smith, B. Press, D. Eberiel, S.P. McCarthy, R.A. Gross and D.L. Kaplan, *Polymeric Materials Science and Engineering*, 1990, **63**, 862.
[120] V. Coma, Y. Couturier, B. Pascat, G. Bureau, S. Guilbert and J.L. Cuq, *Packaging Technology and Science*, 1994, **7**, 27.
[121] C.M. Buchanan, C.N. Boggs, D. Dorschel, R.M. Gardner, R.J. Komarek, T.L. Watterson and A.W. White, *Journal of Environmental Polymer Degradation*, 1995, **3**, 1.
[122] D. Goldberg, *Journal of Environmental Polymer Degradation*, 1995, **3**, 61.
[123] G. Iannotti, N. Fair, M. Tempesta, H. Neibling, F.H. Hsieh and M. Mueller in *Degradable Materials – Perspectives, Issues and Opportunities*, Eds., S.A. Barenberg, J.L. Brash, R. Narayan and A.E. Redpath, CRC Press, Boston, MA, 1990, p.425.
[124] J. Mergaert, A. Webb, C. Anderson, A. Wouters and J. Swings, *Applied Environmental Microbiology*, 1993, **59**, 3233.
[125] L. Tilstra and D. Johnsonbaugh, *Journal of Environmental Polymer Degradation*, 1993, **1**, 257.
[126] D.S.G. Hu and H.J. Liu, *Journal of Applied Polymer Science*, 1994, **51**, 473.

[127] H.B. Greizerstein, J.A. Syracuse and P.J. Kostyniak, *Polymer Degradation and Stability*, 1993, **39**, 251.
[128] L.V. Lopez-Llorca and M.F. Colom Valiente, *Micron*, 1993, **24**, 457.
[129] H. Nishida and Y. Tokiwa, *Journal of Environmental Polymer Degradation*, 1993, **1**, 227.
[130] C. Bastioli, A. Cerutti, I. Guanella, G.C. Romano and M. Tosin, *Journal of Environmental Polymer Degradation*, 1995, **3**, 81.
[131] M.J. Kay, R.W. McCabe and L.H.G. Morton, *International Biodeterioration and Biodegradation*, 1993, **31**, 209.
[132] N.S. Allen, M. Edge, M. Mohammadian and K. Jones, *Polymer Degradation and Stability*, 1994, **43**, 229.
[133] A. Löfgren and A-C. Albertsson, *Journal of Applied Polymer Science*, 1994, **52**, 1327.
[134] A-C. Albertsson and S. Karlsson, *Macromolecular Symposia*, 1995, **98**, 797.
[135] J. Schurz, P. Zipper and J. Lenz, *Journal of Macromolecular Science, Part A: Pure and Applied Chemistry*, 1993, **A30**, 603.
[136] A-C. Albertsson, C. Barenstedt and S. Karlsson, *Journal of Applied Polymer Science*, 1994, **51**, 1097.
[137] J.P. Santerre, R.S. Labow, D.G. Duguay, D. Erfle and G.A. Adams, *Journal of Biomedical Materials Research*, 1994, **28**, 1187.
[138] A. Iwamoto and Y. Tokiwa, *Polymer Degradation and Stability*, 1994, **45**, 205.
[139] K.K. Leonas, M.A. Cole and X-Y. Xiao, *Journal of Environmental Polymer Degradation*, 1994, **2**, 253.
[140] P. Halley, R. Rutgers, S. Coombs, J. Kettels, J. Gralton, G. Christie, M. Jenkins, H. Beh, K. Griffin, R. Jayasekara and G. Lonergan, *Starch – Stärke*, 2001, **53**, 362.

Rolf-Joachim Müller

# 2 Biodegradation behaviour of polymers in liquid environments

## 2.1 Introduction

According to many definitions [1], the biodegradation of plastics is usually primarily induced by the action of various microorganisms, although often nonbiotic effects such as irradiation, thermal degradation or chemical hydrolysis contribute to the degradation process. The activity of microorganisms is closely connected to the presence of water. The supply of nutrients to microorganisms and the transportation of excreted enzymes and metabolic products occur *via* diffusion in the aqueous environment surrounding the cells. (Thus, it can be said that an aqueous environment is actually the natural one for a microbe.) However, in environments regarded as non-liquid such as soil, compost or surfaces of solids, microbes can also be active as long as a specific aqueous microenvironment allows the transportation processes necessary for biological activity. For example, in soil, microbial life takes place in the thin water-films located between the particles or in water-filled cavities in the soil components. A soil-humidity of around 50–60% is optimal for aerobic biological processes, where the humidity is given as a percentage of the maximum water-holding capacity, which also takes into account the structural elements of the soil (actually it reflects the filling of the cavities in the material).

Although water is a basic component of the microbial world, many organisms need or prefer the contact to a solid matrix. For example, many fungi exhibit better growth on surfaces than in agitated liquids, which can, besides other effects, be attributed to the sensitivity of the fungal mycelium to mechanical forces. Differences in the optimal living conditions of different microorganisms results in the presence of very specialised microbial communities in various environments and thus, lead to specific degradation behaviours of substances (which act as energy and/or nutrient sources).

Discussing the biodegradation of plastics in a liquid environment usually means natural degradation in freshwater (lakes, rivers), in a marine environment, or in aerobic and anaerobic sludges (wastewater treatment). However, many degradation studies of plastics in laboratories have been performed in defined synthetic or in complex liquid nutrient broths, which can also be regarded as degradation in a liquid environment. While studies using real natural aqueous systems enable information regarding the behaviour of biodegradable plastics in a distinct natural environment to be obtained, laboratory studies with special aqueous media are used for fundamental studies of biodegradation processes or to optimise the evaluation of the intrinsic biodegradability of a plastic, e.g., under typical conditions. Special liquid media provide defined and optimal living conditions for many organisms and thus, in many cases,

https://doi.org/10.1515/9781501511967-002

increase degradation rates leading to reduced test durations. Furthermore, analytical procedures to characterise the degradation process or to detect degradation intermediates are facilitated in a homogeneous and well- defined liquid medium.

This chapter covers the biodegradation in real liquid environments as well as in especially designed laboratory test systems, and also reviews the role of aqueous test environments of national and international standards in evaluating the biodegradability of plastics.

## 2.2 Degradation in real liquid environments

In most cases biodegradability is a property which is related to the behaviour of the plastic items after they become waste. The biodegradation of plastics in landfills was discussed at an early stage of the development of such materials to reduce the waste volume and thus, save deposit space. Nowadays, composting as an alternative waste treatment system to landfilling, incineration or recycling is of major interest. However, the biodegradability of plastics can also contribute to the property profile of a product during its application. In the agricultural field, mulching films made from biodegradable plastics are now being commercialised; in addition, controlled release formulations with fertilisers or agrochemicals are being developed. In this context, degradation testing of plastics in soil has been intensified over the last few years. Generally speaking, it can be stated that most of the investigations on biodegradable plastics in the past focused on solid environments such as landfills, compost or soil.

However, the aspect of avoiding waste is not the only issue that needs to be addressed when considering the fate of biodegradable plastics in liquid environments; biodegradability as a novel property for special applications of plastics is also an important consideration.

The prevention of marine environment pollution, for instance, is regulated by the MARPOL Treaty. This international convention prohibits the disposal of any plastics waste in the oceans, e.g., from ships or from offshore platforms. The international convention generated activities to check if biodegradable plastics, used as an alternative to conventional polymers, are suitable to be degraded in a marine environment [2]. A further problem exists from littering, where plastic items are washed away to the sea by rivers or blown by wind from the shores and can cause the death of numerous marine animals [3].

Notably in Japan, the Fisheries Agency is active in developing fishery equipment (e.g., fishery nets), which are biodegradable and do not cause permanent harm to sea life when lost during fishing [4].

Besides these aerobic environments, the biodegradability of plastics under anaerobic conditions should also be considered; especially with the collection and biological treatment of green waste from households (kitchens), anaerobic digestion

(normally followed by an aerobic composting stabilisation) becomes more and more important, particularly in certain European countries. In addition to this waste management aspect, the introduction of biodegradable plastics to natural anaerobic environments (e.g., sediments in lakes, rivers or oceans), may occur and therefore the biodegradation behaviour of plastics in the absence of $O_2$ is also of practical interest.

Most of the investigations reported in the literature concerning biodegradation in natural liquid environments consider natural polyhydroxyalkanoates (PHA) such as polyhydroxybutyrate (PHB) or the copolyester containing valerate units poly (hydroxybutyrate-*co*-hydroxyvalerate) (PHBV). These biodegradable materials were of outstanding interest in the past; however, nowadays the commercial relevance of these materials is limited. For commercially important biodegradable plastics, mainly synthetic polyesters, not much data regarding degradation in natural liquid environments is available.

## 2.2.1 Degradation in freshwater and marine environment

### 2.2.1.1 Polyhydroxyalkanoates

Doi and co-workers [5] exposed PHBV of different copolymer compositions to seawater (1.5m depth) at temperatures between 14–25 °C (depending on the season). There was no clear detectable influence of the degradation rate on the hydroxyvalerate (HV) content of the copolymer.

Erosion rates (removal of polymer material from each surface of the film sample) were in the order of magnitude of 2.5 μm/week (at approximately 22 °C). A significant influence of temperature was found for the degradation of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) polymers. Increasing the temperature from approximately 14 to 24 °C nearly doubled the degradation rate of the polymers (erosion rate at 24 °C was approximately 3.8 μm/week). Imam and co-workers [2] tested the degradation of PHBV (12 mol% HV) and PHBV/starch- blends in tropical coastal waters (in baskets at 0.5 m depth and temperatures of 25–32 °C) and stated a significant weight loss for both materials (approximately 500 μm sheets). While pure PHBV degraded quite slowly (10–40% weight loss within 400 days), the starch-blends were totally disintegrated in less than 150 days. From these data an erosion rate of about 0.4–1.7 μm/week can be estimated for PHB and >11 μm/week for PHBV.

The degradation of a PHBV (Biopol) in freshwater at a depth of 20–85 m was investigated by Brandl and Püchner [6] at temperatures ranging only from 6–8 °C. Despite the low temperatures and the reduced $O_2$ concentration in the deeper water layers, 17 μm films of PHBV (8 mol% HV) were totally disintegrated within 254 days. Erosion data on PHBV bottles demonstrated that the degradation rate significantly decreased with increasing water depth, although even at a distance of 85 m from the surface a clear biological degradation could be observed.

#### 2.2.1.2 Synthetic polyesters

Besides the work on natural PHA-polyesters, degradation experiments in seawater with synthetic polymers such as poly(ε-caprolactone) (PCL) and modified polyethylene (PE) are also reported in the literature.

Rutkowska and co-workers reported a complete defragmentation of PCL samples in seawater (Baltic Sea) at temperatures between 9–21 °C [7] within 8 weeks; temperature was stated to be a major influencing factor for degradation. For PCL, chemical hydrolysis and enzymatic surface erosion are responsible in parallel for the polymer degradation. The same research group, using polyester urethanes, found a significant weight loss in seawater (Baltic Sea) within 12 months, while a polyether urethane was not biologically attacked under the same experimental conditions [8].

Polyolefins such as PE and polypropylene (PP) are usually not accessible to direct microbial attack. For such polymers, biological degradability is achieved by the addition of starch, prooxidant additives or photosensitive components. Starch, a natural polymer, can be degraded by microorganisms which enhances the defragmentation of the polyolefins (if the starch is accessible to the microbes). The additives increase the initial reduction of polymer chain length by chemical processes to form short chain length oligomers which can finally be metabolised by microorganisms.

However, no significant changes in material properties nor any reliable weight loss of differently modified PE and PP could be observed by Gonsalves and co-workers [9, 10] upon exposure to seawater (1–9 m depth, temperatures of 13–30 °C) for 5–12 weeks. The primary (chemical) degradation depends on the exposure temperature and the normal temperatures (maximum and minimum) found in seawater, the reaction rate is probably too slow to observe any changes in the materials within the duration employed.

Photodegradable PE proved to be degraded slower under seawater and freshwater floating conditions compared with environmental exposure to air [11]. The temperatures (12–28 °C), a shielding from sunlight by the water and biofouling were stated as reasons for the slower loss of physical properties in water. However, the disintegration of some samples could be observed within a period of 30–66 days. Similar observations were made by Leonas and Gorden in a laboratory simulation test [12].

Recently, much interest has arisen regarding the behaviour of biodegradable and compostable carrier bags in the marine environment. While compostable bags are designed to be reused at home as bin liners to collect biowaste and hence be composted together, characterisation of the biodegradability under environmental conditions is necessary due to littering of this product, it is therefore of interest to characterise the potential residence time in sensitive environments such as the sea.

Recent studies show that compostable carrier bags can undergo a strong physical degradation when exposed in the open sea [13]. Degradation is not just due to a mechanical effect, but it is the consequence of true biodegradation as shown by [14] in a study where the marine environment is subdivided into its different habitats and a specific test method is proposed or required for each habitat.

The activated sludge stage of a wastewater treatment plant is a liquid environment exhibiting a high microbial activity. Gilmore and co-workers tested the behaviour of different polymers in this environment [15]. Sheets of PHBV (500 µm; 26.5 mol% HV) were disintegrated within 60 days (at 22 °C), corresponding to an approximate erosion rate of 30 µm/week; at lower temperatures (12–19 °C) an erosion rate of approximately 6 µm/week was observed. Starch-filled polyolefins (without prooxidants) and blends of polyolefins with the degradable polyester PCL exhibited no hint of any biological attack (weight loss or changes in mechanical properties), even in this active environment.

## 2.3 Degradation in laboratory tests simulating real aquatic environments

Field tests in real environments have a number of limitations and problems. Parameters such as temperature or water quality can vary during the test period, and monitoring of the biodegradation process is usually limited to visual changes or at least to the determination of the weight loss of the samples. To overcome these deficiencies, controlled laboratory tests simulating natural (aquatic) environments are often used to investigate biodegradation processes.

### 2.3.1 Aerobic liquid environments

Investigations of Tsuji and Suzuyoshi [16] on PHB, PCL and polylactic acid (PLA) (films of 50 mm thickness) in a laboratory test with seawater at 25 °C resulted in erosion rates of 0.6 µm/week for PHB and 0.2 µm/week for PCL. This data are comparable to findings in field tests. In contrast to both these polyesters, PLA did not show any significant weight loss in this experiment; this can obviously be attributed to the different types of degradation mechanisms. While PHB and PCL are primarily attacked by enzymes (PHB depolymerases, lipases) at the surface, PLA is known to be mainly degraded initially by a nonenzymatically catalysed hydrolysis mechanism, which is strongly temperature dependent. While, for instance, in compost (at temperatures up to 70 °C) PLA has been shown to be quite rapidly chemically depolymerised and then metabolised by microorganisms, this reaction mechanism is much slower at 25 °C, where PLA is in the glassy state below its glass transition temperature ($T_g$). Thus, it can be expected that PLA is, despite the presence of various polyester-degrading microorganisms, only degraded very slowly in liquid environments such as seawater or freshwater.

A direct comparison of degradation rates in different liquid and non-liquid environments at different temperatures is given in a publication by Manna and Paul [17]

using PHB as the degradable polyester (250 µm sheets). It is quite surprising that no significant general differences in the degradation rate between liquid environments (freshwater and sewage sludge) and solid environments (compost and soil) at the same temperature could be detected (Figure 2.1).

A pronounced temperature dependence of degradation was observed, where in most cases the highest weight losses were obtained at 30 °C (except with freshwater where a maximum degradation was observed at 40 °C). Compared with freshwater, microbial attack is somewhat higher in sewage sludge, an environment of high microbial activity. Erosion rates estimated from the weight loss data in freshwater are comparable to those presented previously for field tests (0.7 µm/week at 20 °C; 1.3 µm/week at 30 °C and 1.5 µm/week at 40 °C).

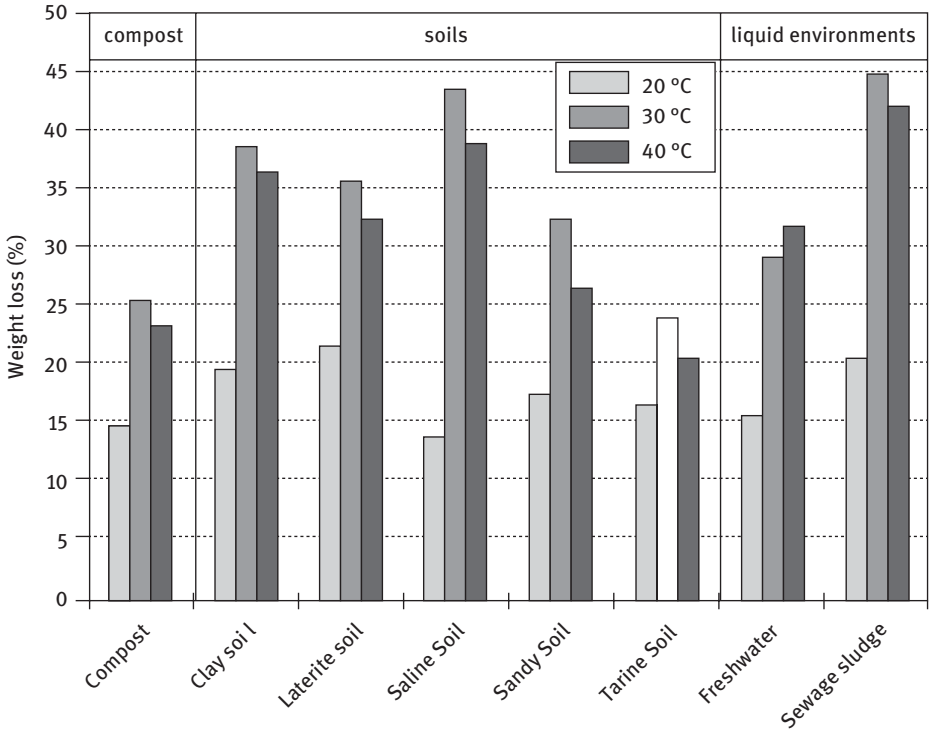



**Figure 2.1:** Weight loss of 250 µm sheets of PHB in different environments after 200 days of incubation. Reproduced with permission from A. Manna and A.K. Paul, *Biodegradation*, 2000, 11, 5, 323. ©2000, Springer.

A direct comparison of microbial degradation of PHBV (14 mol% HV; fibres of approximately 213 and 493 µm diameter) in fresh and seawater was performed by Ohura and co-workers [4], using natural water supplemented with some mineral salts to increase

microbial activity. In this accelerated simulation test, a complete degradation (weight loss measurement and determination of the biological oxygen demand (BOD)) of the 213 µm fibres could be achieved within 2 weeks in freshwater (two sources) and within 4 weeks in seawater (two sources); furthermore, for the 493 µm fibres, the degradation in freshwater was almost twice as high as in seawater.

Some years previously, the same research group had investigated the degradation of a number of PHA-copolymers and a number of synthetic polyesters under similar test conditions using water from a river [18] (100 µm films at 25 °C, weight loss and BOD). Again quite rapid degradation was achieved with the accelerated test system used. A 100 µm film of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) P(3HB-*co*-3HV) (14% HV) was almost totally degraded within one week. The erosion rates obtained were in a range of 30–50 µm/week and much higher than observed in field tests under natural conditions.

The test system used allowed comparison of the biodegradation of different polymers in freshwater. The PHBV-copolymer degradation rate increased with the copolyester composition up to a HV content of 14 mol%, and then decreased to nearly zero for P(3HB-*co*-3HV) (80% HV).

Copolyesters of 3-hydroxybutyrate with 4-hydroxybutyrate and a pure poly(4-hydroxybutyrate) homopolyester were completely degraded within 4 weeks. For copolymers of 3HB with 3-hydroxypropionate (3HP) the copolyesters proved to be rapidly degradable, while pure 3HP was not attacked. Among a number of different synthetic aliphatic polyesters, PE succinate degraded at approximately the same rate as the PHBV copolymers. PE adipate, polybutylene adipate and polybutylene sebacate also exhibited a weight loss within 4 weeks of incubation, but degradation was slower than for the other materials. For PE sebacate, polybutylene succinate and polyhexylene succinate, no significant weight loss could be observed under the test conditions applied. Interestingly, there is no correlation between the degradation rate and the melting points of the substances, a finding which has been made *via* enzymatic degradation tests [19, 20].

For synthetic polyesters, the degradation behaviour in seawater compared with freshwater is different from that of natural PHA [21]. While PHB and PHBV copolymers are quite rapidly degradable in both liquid environments, seawater and freshwater, synthetic polyesters seem to be less degradable in seawater. PCL (100 µm films) degrades completely in freshwater within approximately 2 weeks. In seawater (from the ocean) a BOD of 50–60% could be observed after only 4 weeks. In seawater from a bay, degradation is surprisingly high, reaching the maximum BOD level after only one week. PE succinate is more rapidly degradable than PCL in freshwater, but exhibits no microbial degradation in seawater, from the ocean or from a bay. This behaviour can probably be attributed to the occurrence of different microorganisms able to degrade the natural and synthetic polyesters. PHA are natural polymers and nature developed many organisms to degrade and utilise this carbon and energy source. Thus, PHA degraders are present in many environments. PCL is a synthetic polyester, but its

structure resembles cutin, a natural polyester from plants. Thus, it is likely that many organisms do exist which are able to degrade PCL. In contrast, other synthetic polyesters have been shown to be degraded, more or less by accident, by microorganisms producing lipase-like enzymes with a broad substrate spectrum; the enzymes, and corresponding organisms, are specific to a particular polyester. It is suggested that for many synthetic polyesters, the number of organisms able to attack these polymer structures is much smaller than for degraders of natural or natural-like polyesters. As a consequence, the probability of synthetic polyesters being degraded depends, in particular, on the absolute number of organisms present in a particular environment. As a general conclusion, it can be supposed that the degradation of synthetic, nonnatural polyesters is more dependent on the microbial population present in a distinct environment than is the case for natural materials such as PHB.

From this viewpoint, the natural environments should be better identified, in terms of ecological habitats, and test approaches should in turn be based on the typical conditions experienced by the plastic products when entering each habitat, since the microbial population (and thus biodegradation activity) could be quite different. Tosin and co-workers [13] have identified 6 habitats where plastic waste can reside when littered in the marine environment: 1) pelagic domain (the plastic products float freely in estuaries and the open ocean water), 2) eulittoral zone (tides and storm waves bring great quantities of plastic waste to the shoreline, where plastic products get partly buried and kept wet by tidal inundation and waves), 3) supralittoral zone (the plastic products are washed onto the beach, exposed to a sandy soil with a low moisture level), 4) sublittoral zone (plastic products settle on marine sandy sediment where they are exposed to the seawater/sediment interface), 5) plastic products can otherwise sink to the bottom of the deep sea and 6) plastic products can be slowly buried within sediments on the sea-floor.

A further specialised test system was used by Allen and co-workers [22], and Gonda and co-workers [23]. In these tests completely synthetic media, inoculated with individual microbial strains or defined microbial consortia, were used to investigate the biodegradation of polymers.

Honda and Osawa [24] used a simulation test with synthetic wastewater inoculated with mud from a lake to investigate the behaviour of PCL for the denitrification of wastewater (at 25 °C); they found a remarkable degradation of the PCL plates used (erosion rates approximately 10–15 μm/week).

### 2.3.2 Anaerobic liquid environments

Compared with investigations of polymer degradation under aerobic conditions, very little information is available in the literature regarding the degradation of plastics under anaerobic conditions. Again, most of the investigations published are focused on PHA.

It can be expected that the degradation characteristics of polymers in an anaerobic environment are different from those observed in the presence of $O_2$, since anaerobic microorganisms have a much more limited set of enzymes and thus, are more specialised with regards to substrates. Additionally, the energy benefit for the organisms is lower without having $O_2$ as an electron acceptor, resulting mostly in slow growth of anaerobic microorganisms.

In 1992, Budwill and co-workers published a paper on the degradation of PHB and PHBV copolyesters in an anaerobic mineral medium inoculated with sewage sludge [25]. It could be shown that PHB powder, as well as PHBV (13% HV and 20% HV) powder, degraded almost completely in the laboratory simulation test (35 °C, degradation monitored *via* $CH_4$ production) over a period of less than 3 weeks. No clear difference in degradation behaviour was observed between the homopolyester PHB and the PHBV-copolyester. In a later study, the same authors extended the investigation to other conditions [26]. The degradation of PHB and PHBV with a microbial consortium from an anaerobic pond sediment at 15 °C was significantly slower than that with sewage sludge at 35 °C (6 week lag-phase; complete degradation after 14 weeks). Again PHB and PHBV exhibited nearly the same degradation behaviour.

The anaerobic degradation of PHB and PHBV (8.4 mol% HV) in a mineral medium with sludge from a wastewater plant of the sugar industry at 35 °C was tested by Reischwitz and co-workers [27]. In this case, a significant degradation of the polyester powders (approximately 50 µm diameter) was also observed within 3 weeks, with no significant differences between PHB and PHBV.

Urmeneta found an almost complete degradation of PHBV powder (7 mol% HV) in a liquid anaerobic slurry from a freshwater sediment within 6 weeks (at 15 °C) up to an amount of 0.5 mg PHB/$cm_3$ sediment [28].

Shin and co-workers extended anaerobic biodegradation tests to other materials [29] (a synthetic mineral medium inoculated with anaerobic sludge from a municipal waste treatment plant at 35 °C). They found a rapid degradation of PHBV (8 wt% HV) and cellophane films (50–75 µm) within 3 weeks, whereas no degradation (*via* biogas formation) was observed for the synthetic polyesters PLA and polybutylene succinate. A corresponding finding was made by Gartiser and co-workers [30]. While a PHBV copolymer (60 µm film) was degraded under similar conditions to those used by Shin and co-workers in less than 3 weeks, no biogas formation could be observed for the synthetic polyester PCL over a period of 11 weeks. In this paper it was also demonstrated that cellulose acetate polymers (approximately 2.5 degrees of OH-substitution) are in principle degraded under anaerobic conditions, however, the rate of metabolisation is significantly slower than that of PHA. A detailed investigation on the anaerobic degradability of various starch- and cellulose-esters is given by Rivard and co-workers [31]. The dependence of the anaerobic degradation rate on the degree of substitution and the type of substituent is discussed.

An extensive investigation on the anaerobic degradability of a number of natural and synthetic polyesters was performed by Abou-Zeid [32]. Along with PHA, PHB

and PHBV (10 mol% HV), Abou-Zeid also tested PCL, the aliphatic homopolyester polybutylene adipate (SP4/6) and the copolyester polybutylene adipate-*co*- butylene terephthalate where about 40% of the diacid component consists of the aromatic terephthalic acid (BTA 40:60). Weight loss measurements of polymer films (40–74 µm thickness) in two different anaerobic sludges from wastewater treatment plants and an anaerobic river sediment demonstrated that both natural PHA were rapidly eroded (up to 100% within 14 weeks at 35 °C) compared with the synthetic polyesters. While PCL exhibited a low but significant weight loss, SP4/6 and the aliphatic/aromatic copolyester BTA 40:60 exhibited no clear indication of microbial attack under these test conditions (Figure 2.2).

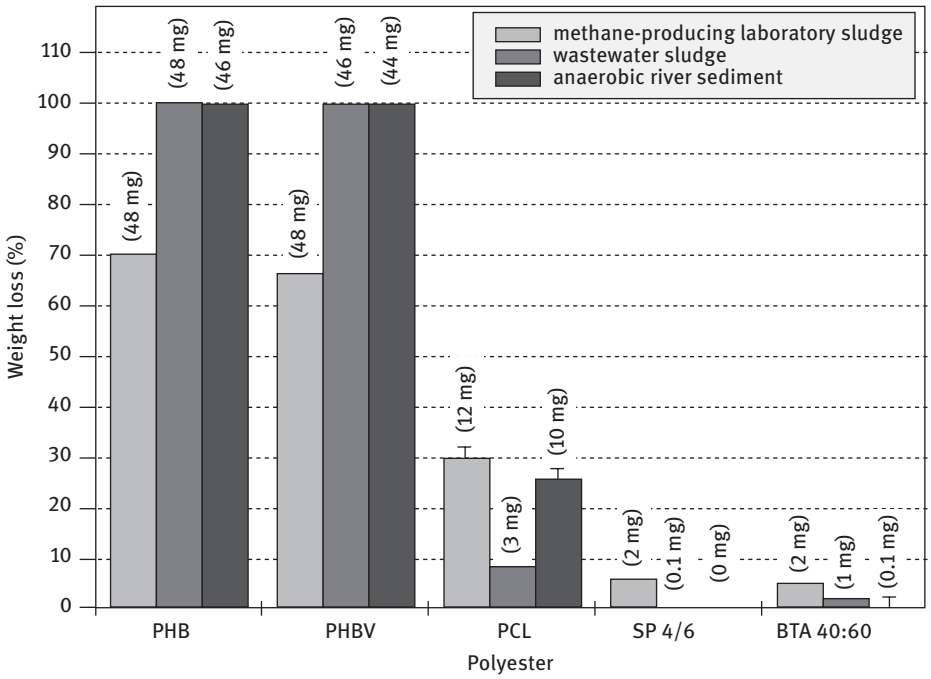



**Figure 2.2:** Weight loss of various polyesters in different anaerobic environments after 14 weeks at 35 °C. (Polyester films: diameter = 25 mm; surface area: 39.3 $cm^2$; initial film weights = 39–49 mg; 3 films per test.) Reproduced with permission from D.M. Abou-Zeid, *Anaerobic Biodegradation of Natural and Synthetic Polyesters,* Technical University Braunschweig, Braunschweig, Germany. ©2000, Technical University Braunschweig.

Similar results were obtained by Abou-Zeid when monitoring anaerobic biodegradation *via* biogas formation in a synthetic medium inoculated with anaerobic sludge (Figure 2.3). From these measurements it could be clearly demonstrated that PHB, in contrast to aerobic conditions, degraded faster than the copolyester PHBV. Again, the

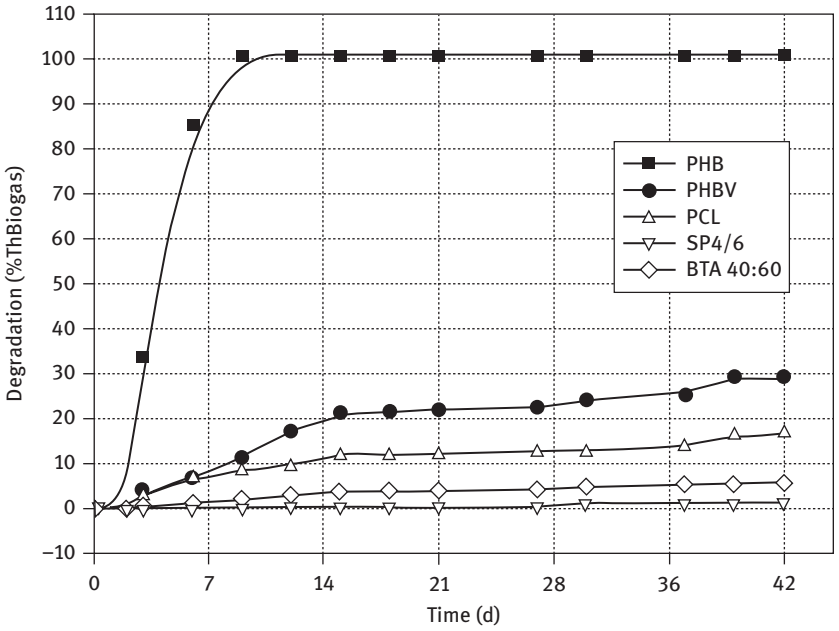



**Figure 2.3:** Time-dependent mineralisation (percentage of the theoretical biogas volume) of various polyesters in anaerobic conditions and wastewater sludge at 37 °C over a period of 42 days. (Polyester films: Ø = 19 mm; surface area: 22.7 $cm^2$; initial film weights: 35–40 mg; 2 films per test). Reproduced with permission from D.M. Abou-Zeid, *Anaerobic Biodegradation of Natural and Synthetic Polyesters*, Technical University Braunschweig, Braunschweig, Germany. ©2000, Technical University Braunschweig.

synthetic polyesters exhibited a significantly slower anaerobic degradation rate. Finally, Abou-Zeid showed that synthetic aliphatic polyesters are in principle degradable, but for aliphatic-aromatic copolyesters of technical relevance (approximately 40 mol% aromatic component in the acids) no clear indication of an anaerobic attack could be found. The different rates observed in the biodegradation of the analysed synthetic aliphatic polyesters calls for further studies. The reason why such differences in rate are recorded is still unknown, and further specific research is therefore needed in order to better understand the microbiology and the enzymology involved in the process.

These observations may have some significance for the biological treatment of biodegradable plastics, as anaerobic digestion becomes more and more established alongside aerobic composting. It cannot be supposed that synthetic polyesters will be significantly degraded in an anaerobic process with typical residence times of about 3 weeks. However, in most cases, anaerobic digestion processes contain a final aerobic step for the stabilisation of the anaerobic compost. If the polyester materials disintegrate sufficiently in the anaerobic step and will not disturb the technical process, the final biodegradation can take place during the aerobic stabilisation.

## 2.4 Degradation in laboratory tests with optimised and defined liquid media

For many investigations on biodegradable plastics reported in the literature, degradation tests in defined synthetic media, inoculated with mixed microbial populations or with individual strains, have been used. These kinds of tests have significant advantages when investigating the basic biological degradation processes of polymers. Due to the usage of defined, in most cases synthetic media, and the possibility of controlling the environmental parameters (such as temperature, pH, salinity, nutrient supply and so on), these tests give better reproducible results than degradation tests under natural conditions. Compared with laboratory tests in a solid matrix, (e.g., soil burial and controlled composting tests), analytical procedures aimed at the analysis of intermediates or persistent residues are facilitated [33] in defined aqueous media.

Monitoring of the biodegradation process in such tests can be performed with various methods. Weight loss measurements of films or formed items are the easiest way, but do not necessarily prove microbial metabolisation of the material. However, in combination with a detailed analysis of the intermediates and the residual polymer (also possible by quantitative chromatographic analysis when using fine polymer powders), useful information can be gained regarding the degradation mechanism and possible residual components, as demonstrated by Witt and co-workers, for the degradation of the aliphatic-aromatic copolyester Ecoflex with a thermophilic actinomycete (*Thermobifidia fusca*), previously isolated from compost [34].

The methods most frequently used to measure the biodegradation process in laboratory tests with liquid media determine the consumption of $O_2$ (e.g., Sapromat test) [35, 36], or the release of $CO_2$ (Sturm-test) caused by the metabolic activity of the microorganisms (respirometric tests) [37]. Due to the usually low amount of other carbon sources being present, in addition to the polymer itself, when using synthetic mineral media, only a fairly low background respiration has to be accounted for and the accuracy of the tests is usually good. These kinds of tests have already been used for a long time to evaluate the degradability of diverse substances and chemicals in water, (e.g., in the Organisation for Economic Co-operation and Development (OECD) guidelines (Table 2.1)) and have now been adapted to the application of nonwater-soluble polymeric materials; in particular, the type of analytical methods, especially for the determination of $CO_2$, have been modified. The OECD guidelines describe the trapping of $CO_2$ in barium hydroxide solution in combination with manual titration. More sophisticated methods use the detection of $O_2$ and $CO_2$ concentration in the air stream (used for aeration) with infrared detectors and paramagnetic $O_2$ detectors. However, despite the advantage of an automated and continuous measurement, there are also some disadvantages with these methods. The exact air flow has to be measured, the signals of the detectors must be stable for quite a long period of time and,