Leonardo da Vinci Program

Environmentally Degradable Plastics

CONTRACT No: I/98/2/05261/PI/II.1.1.b/CONT

PROJECT CONTRACTOR: Consorzio per l'AREA di ricerca scientifica e tecnologica di Trieste – AREA Science Park - Trieste, Italy

PROJECT COORDINATOR: ICS – UNIDO - International Centre for Science & High Technology - Trieste, Italy

PARTNERS: CNRS-UM, University of Montpellier, Montpellier (France)

KTH, Royal Institute of Technology, Stokholm (Sweden)

UT, University of Twente, Enschede (The Netherlands)

TUG, Technical University of Graz, Graz (Austria)

EC OFFICER: Ms Marta Ferreira
DGXXII.B.I. - Leonardo da Vinci Section
200 Rue de la Loi (JECL 7/34), B-1049 Brussels
Tel: +32-2-2962658 / Fax: +32-2-2995325

STARTING DATE: 05.04.1999 **DURATION: 24 Months**

PROJECT FUNDED BY EC UNDER THE LEONARDO DA VINCI PROGRAM - DGXXII

PREFACE

On December 6, 1994, the Council of Ministers of the European Union adopted the Leonardo da Vinci program for the implementation of a Community vocational training policy (Official Journal L 34O, 29 December 1994, pages 8 to 24). This program, adopted as the first phase had a key objective of supporting the development of policies and innovative action in the Member States, by promoting projects in the context of transnational partnerships which involve different organizations with an interest in training.

The adoption of the Leonardo da Vinci program also represented a rationalization of Community action in the area of vocational training, providing the basis to enhance the value of the acquis. The program came at a time when the White Paper on "Growth, Competitiveness and Employment" forcefully emphasized the crucial importance of vocational training as a key factor in combating unemployment and strengthening the competitiveness of European enterprises. The program aimed at responding to the demand for new skill needs which are generated by the evolution of our society and the problem of employment in Europe.

The Leonardo da Vinci Community vocational training action program, introduced in 1994, entered second phase, which runs from 1 January 2000 to 31 December 2006. Promoting a Europe of knowledge is central to the implementation of the program, which seeks to consolidate a European co-operation area for education and training. Within the framework of LDV program, the project entitled “Managers of Innovation in Environmentally Degradable Plastics-MEDP” has been proposed by five recognized European institutions and approved by European Commission. The duration of the project was two years, from April 5th, 1999 to April 5th, 2001.

Main objectives of the program were:

- Creation of an Information-Package (INFO-Pack) aimed at building up decision-makers well aware of all the issues relevant to plastic waste management (PWM) with capability of suggesting sound solutions to minimizing the environmental impact of plastic wastes.
- Developing a training package (T-Pack) for technologists working in the field environmentally degradable plastics (EDPs).
- Creation of a Database (DB) on the EDPs technologies, market opportunities and legislation amenable to an easy and continuous up dating.
- Development of a logical inventory framework of EDPs producers and technologies to be used for implementation of the T-Pack.

The project enjoyed the contributions of 5 European institutions from 5 different countries, namely Austria (Technology University of Graz, TUG), France (University of Montpellier I, UM), Italy (International Center for Science and High technology, ICS-UNIDO), The Netherlands (University of Twente, UT) and Sweden (Royal Institute of Technology, KTH) under the administrative management of Area Science Park (Trieste, Italy) and scientific coordination of ICS, an autonomous body of UNIDO as its specialized center in initiatives favored the technology transfer and promotion in emerging countries and countries in transition. The partners hold a worldwide recognized expertise in the field of environmentally degradable polymeric materials.

The present book summarizes the materials of Training Package, while Info-Pack and database is published separately.

I would like to express my gratitude to all project partners group leaders, Prof. A. Albertsson, Prof. G. Braunegg, Prof. P. Dijkstra and Prof. M. Vert, and all contributors within each group. My appreciation is also due to Prof. Emo Chiellini (ICS-UNIDO expert) for his scientific management and substantial contribution, to Dr. R. Ferretti and Ms. A. Galvagna for their careful administration, and to Ms. P. Volpi for her secretarial support. Particular appreciation is due to Ms. Xin Ren (ICS fellow) for her work at completing, finalizing and editing of the Training Package.

Stanislav Miertus
ICS-UNIDO Area Coordinator
Trieste, Italy

PROJECT PARTNERS AND CONTRIBUTORS

ICS — ICS-UNIDO – International Centre for Science and High Technology
AREA Science Park, Bldg. L2, Padriciano 99, 34012 Trieste (Italy)
Tel: +39-040-9228114 / Fax: +39-040-9228122 e-mail: miertus@ics.trieste.it

Prof. Stanislav Miertus

Ms. Xin Ren (Chapter 7, 8 and Chapter 10), **Prof. Ramani Narayan**(Chapter 10)

Dr. Vladimir Frecer(Chapter 9),**Mr. Chang Bae Jang** (Chapter 12), **Ms. Paola Volpi**

Prof. Emo Chiellini (ICS-UNIDO and University of Pisa, Pisa , Italy)

Dr. Attilio Lombardi (Chapter 11)**,**

Andrea Corti, Patrizia Cinelli, Ilieva Vassilka Ivanova and Maria Viola

(Chapter 1, Chapter 3, section 6.4 and 6.5 of Chapter 6, and Chapter 11)

Tel: +39-050-918299 / Fax: +39-050-28438 e-mail: chlmeo@dcci.unipi.it

CNRS-UM — University of Montpellier I, CRBA (URA CNRS 1465), 15 Ave. Charles Flauhault, 34060 Montpellier Cedex (France)
Tel: +33-467418260 / Fax: +33-467520898 e-mail: crba@pharma.univ-montp1.fr

Prof. Michel Vert

Ms. Xiaoling Leclercq, Xavier Garric

(Chapter 2)

KTH — Royal Institute of Technology, Department of Polymer Technology,
S-10044 Stockholm (Sweden)
Tel: +46-8-7908274 / Fax: +46-8-100775 e-mail: aila@polymer.kth.se

Prof. Ann-Christine Albertsson

Dr. Farideh Khabbaz

(section 6.1 and 6.2 of Chapter 6)

TUG — Technische Universität Graz, Institut für Biotechnologie, Petergass 12, A-8010 Graz (Austria)
Tel: +43-316-8738412 / Fax: +43-53-4893823 e-mail: Braunegg@biote.tu-graz.ac.at

Prof. Gerhart Braunegg

Dr. Florian Schellauf

(Chapter 4)

UT — Department of Chemical Technology, University of Twente, 7500 AE Enschede (The Netherlands)
Tel: +31-53-4893004 / Fax: +31-53-4893823 e-mail: p.j.kijkstra@ct.utwente.nl

Prof. Pieter Dijkstra

Mrs. H.W.M. ten Hoopen

(Chapter 5, 6.3 of Chapter 6, and 8.2.3 of Chapter 8)

Consorzio per l'AREA di ricerca scientifica e tecnologica di Trieste – AREA Science Park

Training and Human Resources Development Unit
Area Science Park, A, Padriciano 99, 34012 Trieste (Italy)
Tel: +39-40-3755291 / Fax: +39-40-266376
e-mail: roberto.ferretti@area.trieste.it, antonia.galvagna@area.trieste.it

Dr. Roberto Ferretti and Antonia Galvagna

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

1.1 Background EDPs

Synthetic and semi-synthetic polymeric materials were originally developed for their durability and resistance to all forms of degradation including biodegradation. Special performance characteristics are achieved in items derived therefrom through the control and maintenance of their molecular weight and functionality during the processing and under items operative conditions. The polymeric materials had been and are currently widely accepted because of their ease of processability and amenability to provide a large variety of cost effective items that helped enhance the comfort and quality of life in the modern industrial society. However the above quoted features, that make the polymeric materials so convenient and useful to the human life, have contributed to create a serious plastic waste burden, sometimes unfairly oversized by media because of the visible spreading of plastic litter in the environment and the heavy contribution to landfill depletion due to the unfavorable weight to volume ratio of plastic items that is in average 1 to 3 [2,3].

On the other hand the expectations in the 21st century for polymeric materials demand are in favor of a 2 to 3 fold increase production[4], thus overcoming the world-wide annual production of paper (250 Mil/tons) as a consequence of the increase of the plastics consumption in developing countries and countries in transition. Indeed a one-two order of magnitude jump in the plastics consumption with respect to the present annual level of 1 Kg (India) –15 Kg (China) pro-capite can be envisaged for those countries once the living standards of industrialized countries with an annual average consumption pro-capite of 100 kg will be approached.

The design, production and consumption of polymeric materials for commodity and specialty plastic items have certainly to face all the constraints and regulations already in place or to be issued in the near future, dealing with the management of primary and post-consume plastic waste. In this connection the formulation of environmentally sound degradable polymeric materials and relevant plastic items will constitute a key option among those available for the management of plastic waste[5-7]. The competition with the presently adopted technologies such as burial in landfill sites, incineration with energy recovery and mechanical or chemical recycling is expected to be strengthen, even though one may predict that all of them will coexist with an appreciable decrease of landfilling practice and the introduction of the new concept of prevention that should help to rationalize the production and management of plastic waste. The technologies based on recycling. including also the energy recovery by incineration, will be flanked by the increasing option of environmentally degradable plastics. These should be designed to replace the conventional commodity plastics in those segments in which recycling is difficult and labor-intensive with hence a heavy penalization on the cost-performance of “recycled” items. A downgrading of the original material properties is indeed occurring both during the lifetime of the items meant to be recycled and their reprocessing stages once they reached the recyclable item rank.

An overview on environmentally degradable polymers and plastics cannot therefore be treated outside of the framework of the global issue related to the waste production and relevant management. The position held by environmentally degradable plastics would be outlined in terms of the development levels so far reached and of the future perspectives. It is worth mentioning that a major aspect that has attracted the attention of plastic manufacturers, polymer scientists, and public officers, is represented by the establishment of definitions comprising all the possible categories of environmentally degradable polymers and plastics, together with suitable standards and testing protocols. The nature and fate of the degradation products constitute another crucial point for the acceptance of environmentally sound synthetic polymeric materials undergoing degradation under specific environmental conditions.

Issues bound to plastic waste has promoted, within the global vision of environment protection and sustainability[8], criteria for the future industrial development, a number of actions all over the world aimed at providing adequate solutions and suggestions for minimizing the negative impact of the

increasing production and consumption of polymeric materials and plastics.

Since the early nineties[9], academic and industrial scientists started to consider the potential in minor added-value utilization of the up-to-then specifically and exclusively designed biodegradable-bioerodibble polymeric materials for biomedical and pharmaceutical applications[10-13].

In particular these comprised merceological segments such as packaging, kitchenware, detergency, and disposables that all together may reach levels of 40-50% of the worldwide plastic manufacturing.

As a consequence of that new vision in the production and consumption of plastics, in the last decade we assisted to a remarkable increase in the scientific and industrial interest on Environmentally Degradable Polymers and Plastics (EDPs) as nicely documented by the exponential growing trend of the number of publication in open literature and in patens (Fig. 1.1).

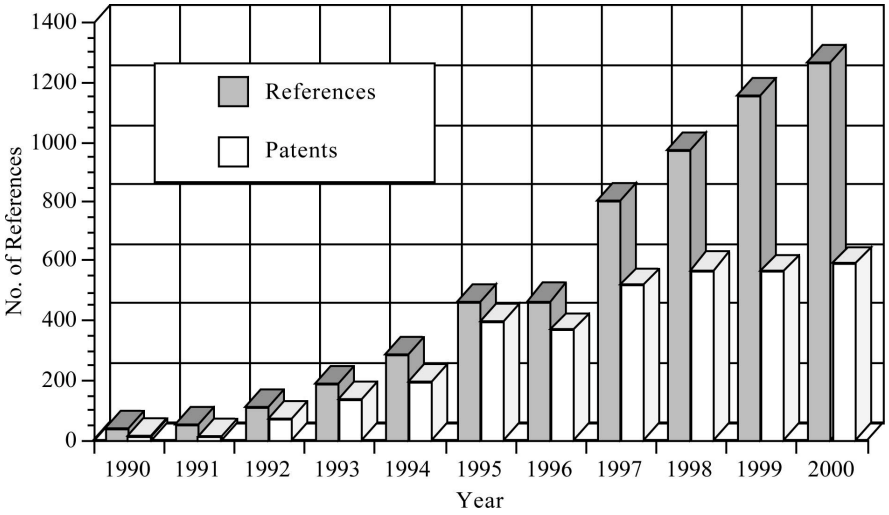


Fig. 1.1 Trend of overall references and patens relevant to EDPs

In order to build up a common understanding background on issues bound to plastic waste management and avoid misuse of some fundamental concepts, it is useful for a fair appreciation of EDPs, to provide some general definitions that had been amply debated and basically accepted on a common consensus ground.

1.2 Definitions

Degradation

Degradation is an irreversible process leading to a significant change of the structure of the material, typically characterized by a loss of properties (e.g. integrity, molecular weight or structure, mechanical strength) and/or fragmentation. Environmental conditions and proceeds affect Degradation over a period of time comprising one or more steps.

Biodegradable

Definition ISWA: Capable of being broken down chemically by the action of microorganisms.

Definition CEN: Potential of a material to be degraded caused by biological activity especially by enzymatic action leading to a significant change of the chemical structure of the material.

Definition ASTM and ISO: Degradation results from the action of naturally occurring micro-organisms such as bacteria, fungi and algae.

Testing the biodegradability is one of the necessary steps in the testing strategy for materials to define ultimate compostability and a good indicator of ultimate compostability. However, biodegradability is NOT THE SAME as compostability. For example, a big potato is fully biodegradable but will not compost 'as such' in a composting environment. To assure compostability other factors such as size, thickness, shape etc. play an important role. In a certain context, even the legal or geographical context may influence the definition of compostable, to make fully biodegradable products "not-compostable".

Compostable

Several standardization committees such as ISO, ASTM, CEN, DIN and UNI have been working hard on compostability testing and acceptance criteria for several years now. As a result the general guidelines and principles regarding testing and basic characteristics have been defined and are universally accepted although for some aspects discussion is still going on. Keywords are material characteristics, biodegradation, disintegration and compost quality. CEN, DIN and UNI so far have only elaborated distinct pass levels and criteria. Official standards and criteria are considered a necessity for any successful breakthrough of bioplastics into the market. Further standardisation activities are needed and going on in the field of ecotoxicity tests, anaerobic biogasification tests and biodegradation in natural environments.

Definition ISWA: ISWA does define composting but not 'compostability'

Definition ORCA: for a product to be degraded and disintegrated in a composting or anaerobic environment followed by further mineralisation in the soil.

The following four criteria present the basic framework for evaluating the acceptability of *waste products* for recovery in either (aerobic) composting or (anaerobic) biogasification facilities designed to process organic household waste beyond simple gardenwaste. The criteria take into account the influence of biowaste components on the following key issues: facility operations and composting technologies, environmental safety and biodegradation, compost quality, and landfill diversion.

Processing of a *waste product* must be compatible with the physical operations in a composting facility. For existing facilities, technologies in use differ widely, and may need "plant-by-plant" assessment. For new plants the planned technology should take into full account the definition and implications of the envisaged feedstock.

All materials (organic and inorganic) in the considered *waste product* must be safe for the environment when composted, meaning they will neither adversely affect biological activities during the composting process, nor will they deteriorate the physical and chemical properties of compost-amended soil, nor adversely affect biota in compost-amended environments.

Processing of the considered *waste product* in a composting facility does not adversely affect the quality of compost routinely produced, such that national or international quality standards or any specific local demands are not compromised. Including the considered *waste product* in the composting bio-waste definition must contribute positively to diversion of waste from landfill at an overall cost locally justifiable versus potential alternative waste management options.

Definition CEN: Property of a packaging to be biodegraded in a composting process.

Definition ASTM and ISO: Degradation by biological processes during composting to yield carbon dioxide, water, inorganic compounds and bio-mass at a rate consistent with other known compostable materials and leave no visually distinguishable or toxic residues.

To claim compostability it must have been demonstrated that a packaging can be biodegraded and disintegrated in a composting system (as can be shown by standard test methods) and completes its biodegradation during the end-use of the compost. The compost must meet the relevant quality criteria which includes: heavy metal content, no eco-toxicity, no obviously distinguishable residues.

Chemically and physically degradable

American Society for Testing and Materials (ASTM) and International Standard Organization (ISO) have defined chemical and physical degradability, see the Appendix 1.

Environmentally degradable plastics (EDPs) therefore can be defined as follows:

- Polymeric materials that retain the same formulation as conventional plastics during use.
- Polymeric materials that degrade after use into low molecule weight compounds by combination of the above biological, chemical and physical stimulus in the environment.
- Polymeric materials that ultimately degrade into CO2 and H2O.

Self-check questions

1. What problems are occurring from the use of fossil fuel based polymers?
2. Why are they still produced in ever increasing quantities?
3. What are the advantages of EDPs ?

Hints for Answers

See section 1.1.

References

1. E. Chiellini, S. Miertus, R. Narayan, X. Ren *to be submitted* to *EPF e-Magazine* (2001)
2. R.D. Leaversuch, *Mod. Plast. Intern*., **8**, 50 (1995)
3. M. Farrell and N. Goldstein, *Biocyle*, **11**, 74 (1995)
4. O. Vogl, J. *Macromol. Sci. Pure. Appl. Chem*, **A33**, 963 (1996)
5. ISO TC61 – SC5/WG22. "*International Technical Committee for "Plastics Standards. Biodegradable Plastic International Standards*"
6. ASTM Technical Committee D20 SC20.96 on "*Environmentally Degradable Plastics*"
7. CENT TC 261 – Technical Committee on "*Plastic and Plastic Waste";* CEN TC249-WG9 *on Plastics, Characterization of Plastics Degradability*"
8. *Chem. Eng. News*, April 8, 1991, p. 4
9. *The First International Scientific Consensus Workshop on Degradable Materials – Perspective Issues and Opportunities*, S.A. barenberg, J.L. Brash, R. Narayan, A.E. Redpath (Eds), CRC Press, Boca Rota (1990)
10. *Polymers in Medicine: Biomedical & Pharmaceutical Applications*, E. Chiellini and P. Giusti (Eds), Plenum Press, New York - USA (1983)
11. *Polymers in Medicine: Biomedical & Pharmaceutical Applications*, E. Chiellini, P. Giusti, C. Migliaresi, & L. Nicolais (Eds), Plenum Press, New York - USA (1987)
12. *Biomaterials Science and Engineering*, J. Bu Park (Ed), Plenum Press, New York – USA (1984)
13. *Polymers as Biomaterials*, S. W. Shalaby, A. S. Hoffman, B. D. Ratner, T. A. Horbett (Eds.), Plenum Press, New York – USA (1984)

CHAPTER 2

POLYMERS OF NATURAL AND SYNTHETIC ORIGINS

Objectives

- Students will get to know some basic concept regarding EDPs from a general viewpoint and regardless of their natural or synthetic origin.
- They should be able to assign a grade taken among degradable, biodegradable, bio-resorbable, bio-absorbable, bio-assimulable, etc. to qualify the subgroups appearing in the InfoPack
- Combining the information contained in this chapter with those in the InfoPack and in other chapters of this Training Pack, students will be able to bridge methods, processes, structures, differences between EDPs of natural and synthetic origins, properties of the derived materials and which properties make up a usable or potentially usable polymer.

Introduction

EDPs can be of natural or of synthetic origins. The full list can be found in the InfoPack. Natural polymers, also named biopolymers, are produced by living systems and serve either as scaffolding or contribute to living processes and belong to biochemical pathways. Among the living systems that produce natural polymers are animals, plants and micro-organisms. Although it is believed that what nature makes can be degraded by natural processes, some natural polymeric compounds can be quite resistant to biodegradation. It is the case for lignin or for the wood of some trees that are currently used outdoor thanks to their bio-resistance. In contrast, most synthetic or man-invented polymers are bio-stable, i.e. they cannot be degraded rapidly by biological processes. Usually they have been selected for this reason, beside low cost, versatility and ease to be produced and processed at high rates and to identical devices, using extrusion, injection-molding or similar processing techniques. For the last thirty years, scientists have been looking for synthetic polymers that exhibit properties similar to those of commodity or specialty polymers but are degradable or biodegradable.

Basically, biodegradable polymers and polymeric devices should be made of biopolymers that are able to biodegrade rapidly so that they can return to bio-mass through biological pathways. Biopolymers have been used as unique sources of polymeric materials until synthetic polymers take over them in many of their applications such as textiles, packaging, etc. . One of the major problems raised by the use of biopolymers as polymeric materials is that they cannot be easily processed by the techniques set up for synthetic polymers. An alternative is to modify biopolymers to make them processable by the techniques of plasturgy. However, chemical modifications usually leads to the loss of biodegradability, specific enzymes becoming unable to recognize their substrate. It is the reason why one of the most attractive alternative to biopolymers as sources of degradable or biodegradable polymers is offered by synthetic polymers that can be biodegraded (the number is limited to a few of the currently available industrial polymers such as poly (vinyl alcohol) and polycaprolactone) or that can be first degraded by abiotic chemical processes, the degradation by-products resulted from cleavage of macromolecules then being biodegraded, or even better, bioassimilated.

Finally, the ideal EDPs should be synthetic to allow easy processing and versatility insofar as properties are concerned. It should degrade or biodegrade to yield degradation by-products that can be bioassimilated and thus lead to bio-mass formation. If chemicals that are necessary to elaborate synthetic bioassimilable EDPs can be generated from this bio-mass, one should be able to deal with real biorecyclable polymers.

2.1 Making Up One's Mind Regarding EDPs

Today, there are several sectors of the human activities that are relevant of a beneficial use of degradable and biodegradable polymeric materials and compounds, namely the sectors of biomedical,

pharmaceutical, agricultural and environmental applications. Although they appear very much different at first glance, these applications have some common characteristics, especially when one consider:

- the necessity to eliminate the polymeric wastes when the macromolecular material or compound is requested for a limited period of time
- the fact that living systems have some similarities in the sense that they function in aqueous media, they involve cells, membranes, proteins, ions, etc…
- the fact that living systems can be dramatically perturb by toxic products, especially low molar mass ones,
- the fact that natural wastes were designed to be degraded or biodegraded, biocompatible and biorecycled.

Another characteristic of the relevance of degradable and biodegradable compounds with respect to various sectors of the human activities is that each of these sectors has developed its own science and thus its own terminology. For instance, surgeons, pharmacists and environmentalists do not use the same word to reflect a well-defined phenomenon. On the other hand, “biomaterial” means “therapeutic material” for health people and material of natural origin for specialists of material issued from renewable compounds such as crops. Last but not the least, the elimination (excretion) of biostable oligomeric degradation by-products is possible from a human body. It is not possible from the earth globe where any product or chemical is stored unless it is recycled or bioassimilated in one way or another.

Because the human health and the environmental sustainability are more and more interdependent, and because science, applications and norms are developed in each sector, it is urgent to harmonize the terminology or to define special terminology whenever a general one is not possible.

The field of the norms is an enlightening source of examples. Each scientist or so interested in degradation and/or biodegradation has introduced definitions independently, thus resulting in mismatching and sometimes improper uses that can lead to misunderstanding and confusion.

2.2 The Problems

There are different levels of degradation when one goes from a polymeric device up to insertion into a living organism regardless of its type: an isolated organism or the environment itself. These various levels are schematized in Fig. 2.1.

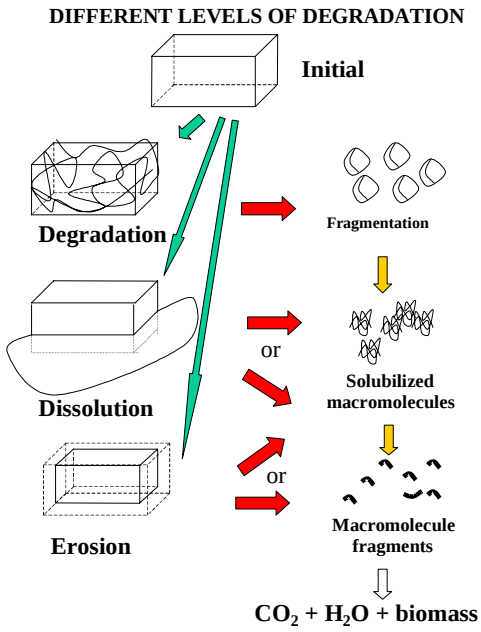


Figure 2.1 The various levels of degradation for a polymeric device

From this schematic presentation it appears that fragmentation and dissolution do not correspond to macromolecule breakdown. Actually they reflect the disappearance of the visible device and thus leave biostable macromolecular compounds as residues. In the human body, the fragments or the dissolved macromolecules will be either retained unless they are rejected through abscess or by filtration if molar masses are lower than the kidney filtration threshold (10,000 to 40,000 daltons depending on the compound). In the environment, fragments or dissolved macromolecules can be stored as organic sand or get up to running water or to the underground water after dissolution.

Macromolecule breakdown to biostable small molecules is another stage where toxic compound might be generated and thus only biocompatible biostable degradation by-products are acceptable. Here the concept of biocompatibility is essential. It is well admitted in medicine and pharmacology. It is not yet defined as such insofar as the outdoor environment is concerned.

The last (ultimate) stage of degradation is multiple in the sense that it includes mineralisation and bio-mass formation with some residual material occasionally.

Therefore, this scheme shows the need for specific terms to distinguish these different stages and distinguish the particularities of the various sectors of the human activity that are concerned.

Another fundamental discussion has to be made to distinguish the possible routes leading from the device to the ultimate stage, namely mineralisation + bio-mass formation.

There are two main routes to degrade a polymeric device up to mineralisation and bio-mass formation. These routes are schematized on Figure 2.2.

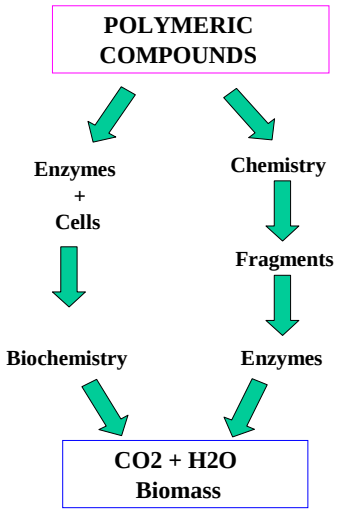


Figure 2.2 The two general routes leading to ultimate degradation and bio-assimilation

The left-hand side route corresponds to the attack of the device or compound by enzymes followed by an enzymatic processing of the degradation products through biochemistry. This route requests the presence of proper enzymes and thus of specific cells under viable conditions (atmosphere, water, nutrients). No life-allowing conditions, no degradation. The right hand side route differs in the sense that the breakdown of device and macromolecule depends on chemical processes, only the elimination of the small molecules generated proceeds through biochemical pathways. Here the reagents (light, water, heat…) are required to trigger the degradation. No triggering phenomenon, no degradation.

If one combines the several levels of degradation with these two different mechanisms of polymer breakdown, it is again obvious that the number of specific words required to distinguish the various possibilities is rather large.

Regardless of the existing definitions, let us consider each possibility and let us try to introduce one word or a choice of words (in bold) able to reflect specifically and conceptually this possibility:

Chemical or unknown mechanism of polymer chain cleavage: **Degradation**

Enzyme mediated polymer chain cleavage: **Biodegradation**

Enzymatic degradation of a macromolecular structure going up to demonstrated mineralisation + bio-mass formation: **Ultimate biodegradation**. At this point one should consider the ultimate biodegradation as reflecting the sum of **Mineralisation** + **Bioassimilation.**

Breakdown of a device to fragments with no breakdown of the constituting macromolecules due to external forces: **Fragmentation;** due to internal stresses**: disintegration**

Breakdown of a device to fragments due to external microorganisms or enzymes: **Bio-fragmentation**.

Breakdown of a device to fragments due to enzymes present within the matrix**: Bio-disintegration**

Breakdown of a device by dissolution without polymer chain degradation or biodegradation: **Dissolution**

Breakdown of a device via surface degradation because dissolution by the solvent is faster than diffusion of the solvent within the matrix: **Erosion or surface erosion**

Breakdown of a device via surface biodegradation**: Bio-erosion** (this case is typical of enzymes that cannot penetrate the matrix macromolecule network with the possible exception of highly swollen hydrogels)

Breakdown of macromolecules can sometimes be faster inside than outside, either because of simple chemistry (diffusion-reaction phenomena) or because of entrapped enzymes or living cells : **heterogeneous degradation** and more precisely : **degradation in the bulk**

Conversion of carbon + hydrogen + oxygen + nitrogen to CO_2 + H_2O + ammonium salts: **Mineralisation**

Conversion of carbon + hydrogen + oxygen + nitrogen to bio-mass**: Bioassimilation**

Mineralization + Bioassimilation + Bio-mass formation: **Ultimate biodegradation**
(In the case of animal bodies, partial degradation or biodegradation is acceptable if the remnants can be eliminated through excretion. A specific term is needed: **Bio-resorption** is very often used for therapeutic devices even if it is not accepted worldwide. In this field, the term bio-absorption was first introduced. If this term reflects well the fact that a device disappears visually within a living system, it does not reflect the fact that the disappearance is due to biodegradation nor those degradation by-products are eliminated through one way or another. Behind bio-absorption, storage is possible because skin and mucosa are closed insofar as high molar mass compounds are concerned)

Partial degradation: **Degree of degradation** (volume or weight fraction Vt/Vo or Wt/Wo, Vt stands for volume at time t; Vo stands for volume at time zero. W stands for weigh with same subscripts.) or **percentage of degradation** (volume or weight percentage 100.Vt/Vo or 100.Wt/Wo) (The initial values can be replaced by the sum of the disappeared material + the remnants at time t)

Partial biodegradation: **Degree of biodegradation** or **percentage of biodegradation**

The property of being degradable**: Degradability** (one should not talk in terms of percentage of degradability but use the degree or the percentage of degradation, i.e. a measured quantity)

The property of being biodegradable: **Biodegradability** (the previous remark applies to biodegradability).

Several important remarks have to be assimilated to fully understand the need for so many distinct situations:

- According to the above remarks and analysis, a biodegradable compound will be always degradable but a degradable compound is not necessarily biodegradable;
- A given compound can be qualified as degradable or biodegradable depending on the living system that is involved (for instance, PCL is degradable and not biodegradable in an animal body. It is degradable and biodegradable in the presence of microorganisms. In contrast, PLA is degradable in animal bodies and in the environment but not biodegradable, except in the presence of some exotic enzymes. It is actually compostable rapidly but at high temperature only)
- Bio-assimilation can be used for assimilation by animal bodies or by outdoor microorganisms. For animal bodies, one must consider bio-resorption.

Under these conditions, active compost must be regarded as another kind of living system, aside animal bodies and the environment. The following terms have to be considered:

Medium undergoing solid fermentation with temperature increase: **Compost**

Degradation process carried out under the conditions typical of compost formation via solid fermentation with temperature increase: **Composting**

Partial degradation or biodegradation during composting: **degree of composting**

The property of being compostable: **Compostability**

Self-check Questions

1. What are the differences between EDPs of natural and synthetic origins?
2. Why naturally occurring polymers or bio-polymers are no longer biodegradable when they have been chemical modified? Looking to the InfoPack, try to find one or two examples of polymeric compounds that are derived from bio-polymers and are no longer biodegradable after chemical modification
3. What are the differences between degradability and biodegradability?
4. Is cellulose acetate derived from a bio-polymer? Which one ? Is it biodegradable?

Hints for Answers

See Introduction, section 2.1 and the other chapters

Exercise

Elaborate the scheme of the two basic routes by which a polymeric compound can be bio-assimilated (for correction, see Fig. 2.2 above). Find some other definitions for biodegradability (CE: http://www.cenorm.be/, DIN: http://www.din.de/ ISO, ASTM: http://www.astm.org/) and compare them. Try to find your own definition.

Reading Materials

See InfoPack for more details.

2.3 Naturally Occurring Polymers

There are many kinds of bio-polymers. The list is given in the InfoPack. The three main groups of environmentally degradable polymers produced by nature are: polysaccharides, polyesters and proteins.

Polysaccharides are polymers containing a large variety of carbohydrate monomers linked together by glycosodic links and often containing other substances as well. The types most abundantly found in nature are surely cellulose and starch from plants and chitin produced by insects and marine organisms. But also other polysaccharides like dextrane, xanthan, fructanes, agar-agar and alginates produced by bacteria and fungi are already used for technical applications mostly in food industry. Well-known

strains are *Leuconostoc mesenteroides* for the production of Dextran and *Azotobacter vinelandii* and several algae for Alginate.

Naturally occurring polyesters are polyhydroxyalkanoates produced by bacteria as carbon reserve material. The most important type is the poly-3-hydroxybutyrate homopolymer, which was also detected in eucaryotic cells in small quantities. Many other types of PHAs can be produced by modification of the basic production process. Important strains for PHA production are *Ralstonia eutropha*, *Alcaligenes latus* and genetically modified strains of *Escherichia coli*. Most recently genetically modified plants for PHA production were developed.

In contrast to the first two groups of polymers, the proteins are not thought to be useful as substitutes for oil-derived plastics. Some experiments are going on to crosslink cheap protein wastes or blend them with other EDPs but no result has been obtained so far. But there are other economically more interesting fields of application for proteins: the enzymes with their numerous and interesting applications belong to this group. Mostly, genetically modified organisms are used for the production of pharmaceutical products like interferon, insulin, virus proteins and vaccines.

Some of these polymers are produced by nature in such large amounts, that a technical production process is not necessary. They can be obtained from plants or other source in large quantities:

The most common polymer is cellulose, an important structural polymer in plants but also found in fungi. Up to 14.000 molecules of β-glucose are linked together by 1-4 bonds and the molecular weight can reach over $2x10^6$ Dalton (Da). The recovery of cellulose for paper production is a well-established process and a big industry has developed. Recently interest has focused also on the hemicelluloses like Xylane occurring together with the cellulose and new applications for materials are found.

Starch is the second very important polysaccharide produced by plants as reserve material. Two different types of starch can be distinguished: Amylose is a linear macromolecule consisting of 200 to 1000 α-glucose units reaching a molecular weight between 50.000 and 200.000 Da. Amylopectin is a branched molecule with a significant higher molecular weight than amylose reaching $1x10^6$ Da. There is a vast number of different methods for the modification of starch and a lot of different applications ranging from food industry to packaging material.

Other polymers belonging to this group are alginates produced in large quantities by algae and used in food industry. Chitin is produced by insects and marine organisms in huge amounts as structural component of their exoskeleton and as part of the cell wall of some fungi.

Self-check Questions

1. Name some of the most important naturally occurring EDPs.
2. Name two bio-polymeric compounds that take a long time to biodegrade?
3. Which class of bio-polymers cellulose and starch and chitin belong to?
4. Give the name of some compounds resulting from then chemical modification of cellulose, starch and chitin.

Hints for Answers

See Chapter 3 of InfoPack and Section 2.3.

Exercise

Are poly (β-hydroxyl acids) also designed by the acronym PHA bio-polymers?
Name the two main PHA that have been industrialized. To which class of polymers does PHA belong?
What are the characteristics of Pullulan, Xanthan and Alginates? (See Chapter 3 of InfoPack and Chapter 4 of this book.)

2.4 Synthetic EDPs

The list of synthetic EDPs is also provided in the InfoPack. Detailed information on the various synthetic EDPs can be found in the Chapter 3 of InfoPack and also in Chapter 5 of this book.

Self-check Questions

1. Write the chemical function that is used to link repeating units in the following synthetic EDPs:
    - Aliphatic polyesters
    - Poly(orthoesters)
    - Polyanhydrides
    - Polyamides
2. Are polylactides biodegradable or degradable and bioassimilable?
3. What are the differences between poly (L-lactide) and poly (DL-lactide)?
4. Is EcoPLA useable as biomedical EDP?
5. Is poly(vinylalcohol) biodegradable ?

Hints for Answers

See InfoPack, Chapter 2 and Chapter 4 of this book.

Exercise

Try to list all the potential or real applications of lactic acid derived polymers.
What is the general formula of polyphosphazenes?

Try to assign a qualification taken among the terms listed in part 2.2 to synthetic polymers listed in the Chapter 2 of InfoPack, considering the given information.

CHAPTER 3

BIODEGRADABLE BLENDS AND COMPOSITE MATERIALS --- FORMATION AND PROCESSING

3.1 Introduction

A composite material is described as a macroscopic combination of two or more materials in order to achieve a performance from the composite that was not available from the separate constituents (1). There are several examples of this synergism in nature such as wood, leaves, teeth, bones. For example wood contains an oriented hard phase which provides strength and stiffness and a softer phase which provides toughness (2).

Composite materials represent one of the fastest growing commodities in the world market. The market for conventional composite materials is based on construction, industrial, transportation and aerospace. Aerospace are low volume, high performances composites, industrial are intermediate in volume and performances whereas automotive (transportation) are recognized as high volume lower performances. Table 3.1 reports the market share evaluated on the shipment of thermoplastic and thermoset based composites by market as reported by Young at the first international conference on lignocellulosic plastic composites (3).

Table 3.1 Thermoplastic and Thermoset Composites by Market in 1995

Market	Shipments(Ktons)	Market share(%)
Transportation	283	25.0
Construction	227	20.1
Marine	180	15.9
Corrosion Resistant Equipment	145	12.8
Electrical/Electronic	98	8.7
Consumer Products	73	6.5
Appliances/Business	67	5.9
Aerospace/military	19	1.7
Other	39	3.5
Total	1,131	100

In the last years a growing importance has been devoted to the research of suitable alternatives for those composite materials that are produced with materials not degradable and hard to be recycled. This refers in particular to single-use consumer products (4). These items pose a genuine environmental concern, as most of the plastic used to produce them is recalcitrant and does not degrade when disposed in the environment after their useful life is over (5). Degradable-plastic composites are emerging materials that offer benefit to the environment. This results in minimizing waste which would be otherwise disposed of landfills.

To improve degradability, natural polymers have been introduced in different kind of composites. Thus it has been supposed that the presence of a certain amount of natural polymers in blends with synthetic polymers can promote the degradation of the synthetic component. Since enzyme based reactions that involve the formation of reactive free radicals could be expected to be relatively indiscriminate and non-selective of their substrate (6).

Natural polymers, or bio-polymers, are produced in nature living organism, and by plants through biosynthetic processes that involve carbon dioxide consumption. Arguments used in favor of "natural" polymers are: biodegradability, renewable resources, bio and mechanical recyclability, non waste producing etc. Moreover natural polymers can be combined either with recycled plastic resins or paper/plastic waste with thus conversion into valuable composites. Indeed in some cases natural polymers present a slow rate of biodegradation, but because they are produced in nature there is no concern about it, contrary to synthetic polymers.

The most widespread natural polymers are polysaccharides, such as starch and cellulose. Other important classes include polyesters, such as poly(hydroxyalkanoate)s, proteins, like wool, silk and gelatin, and hydrocarbons, such as natural rubber.

In the attempt to produce biodegradable composite materials natural polymer have been widely used both in particulate composites and in fibrous composite. Natural polymers are considered suitable to replace synthetic ones in specific applications where a long span life is not required even if natural polymer price is a disadvantage on the cost of the final products (7). In this regards materials such as renewable crops, agricultural waste and/or by-products are a good source of natural polymers as they are comparatively less expensive (8).

The cost of finished composite items depends not only on the price of the composite materials but also on labor costs and energy required to process the materials. In this regards the first approach to produce biodegradable composite is that of using common processing procedures. In some cases the technology has to be adapted to the characteristic of the biodegradable materials. This is particularly true when natural polymers are going to be used.

3.2 Description of Composite and Blends

3.2.1 Particulate Composite and Blends

In particulate composites the reinforcing phase is often spherical or at least has dimensions of similar order in all directions. Polymeric materials particles have been used as fillers to improve strength, toughness, processability, dimension stability, frictional wear and lubrication properties and in some cases degradation rate. The filler can improve some properties while degrading others.

When a mixture of two amorphous materials form a single phase of intimately mixed segments of the two macromolecular components, a blend is formed and the components are considered miscible. In a homogeneous polymer blend, a single and composition dependent glass transition temperature is observed. Whereas an immiscible blend has separate glass transitions associated with each phase (9). The most common techniques for preparing blends are mechanical mixing, melting mixing, and solution casting. Mechanical blending is an economically convenient technique. It is important to optimize the size of the dispersed phase considering the final performance of the blend. In melting mixing the polymer components have to experience a shearing deformation process in the molten state (10). Thermoplastic resins when heated during processing soften and flow as viscous liquids and when cooled they solidify. Extrusion is the most diffused shaping method to process thermoplastic polymers by the melt followed by injection molding (11). In extrusion a molten material is forced by means of advancing screw(s) into a shaping device. A schematic representation of a single screw extruder is reported in Fig. 3.1.

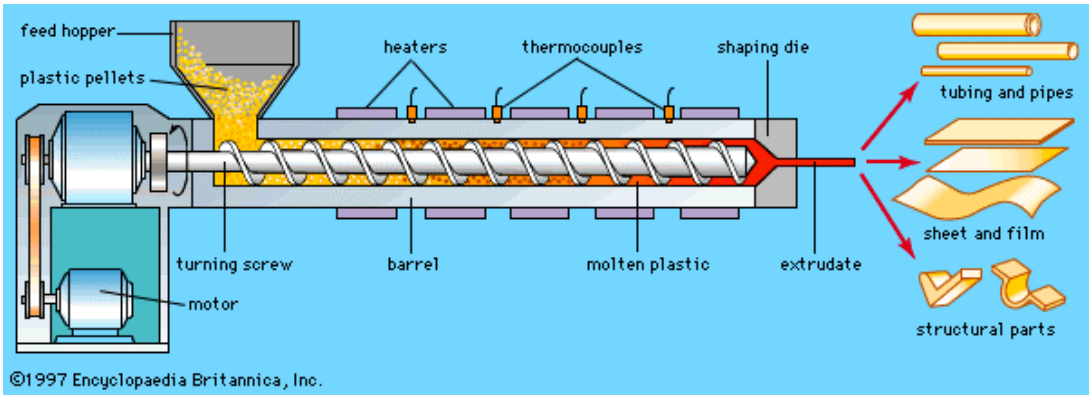


Fig. 3.1 Schematic Representation of a Screw Extruder

Because the viscosity of most plastic melts is high, extrusion requires the development of pressure to force the melt through the die. In order to have a homogeneous product the incorporation of the additives such as plasticizers, antioxidants, colorants and fillers requires mixing them into the plastic when it is in the molten state. This can be done in specific mixer equipped with a heating system, usually hot oil circulating system. In extrusion processing, the extruder melts the plastic by a combination of heat transfer through the barrel and dissipation of work energy from the extruder drive motor. In the act of melting, and in subsequent sections along the barrel the required amount of mixing is usually achieved. Extruders usually accept dry solid feed. For blends and composites production twin-screws extruders are also utilized. Twin-screws extruders can be tangential or intermeshing, the latter can be counter or co- rotating. This type of extruder is characterized by improved mixing and pumping versatility.

Miscible polymer blends present one phase and process much like a homo-polymer or random copolymer (12). Two phase blends have unique processing characteristics. Multiphase blends can exhibit phase segregation and orientation under high shear processing conditions. For immiscible pairs, the details of the mixing process determine the morphology of the resulting composite.

In co-extrusion, very thin layers of high-performance materials can be co-extruded with other less expensive materials to incorporate specific properties and optimize the contribution of each component. Co-extrusion is a one step solvent-free process offering economic and environmental advantages over various laminating and coating technique.

Natural polymers started to be used in composite materials as fillers in order to improve the degradation rate. Starch has been extensively used for this purpose due to its low cost and large availability on the market (13). Starch is a polysaccharide of repeating glucose unit. The two major components of starch are mostly linear chains of a-D-glucopyranosyl units joined by a-1-4 linkages termed amylose and a highly branched component termed amylopectin in which branches are formed by joining linear chains with a 1-6 linkages (14). Amylose molecules have molecular weights of 200,000-700,000, while branched amylopectin have molecular weight as high as 100-200 million. Amylopectin constitutes the highest component in common starch (up to 100% in waxy starches, 72% in normal maize starch, and 80% in potato starch). Starch is the major form of carbohydrate storage in green plants and is considered the second largest bio-mass, next to cellulose, produced on earth. It is the principal component of most seeds, tubers, and roots and is produced commercially from corn, wheat, rice, tapioca, potato, sago, and other sources. Most commercial starch is produced from corn which is comparatively cheap and abundant throughout the world. Wheat, tapioca, and potato starch are produced on smaller scale and at higher prices.

Starch occurs in plants in the form of granules which may vary in diameter from 2 to 150 mm. Rice starch has the smallest granules and potato starch the largest ones. Native starch granules are insoluble in cold water but imbibe water reversibly and swell slightly. In hot water a larger irreversible swelling occurs producing conversion from crystalline, granular starch to dispersed and amorphous state, this process is known as gelation. Gelation takes place over a temperature range that depends on the starch type (15).

Composite materials containing starch have been prepared in a first time by using starch as a filler (16-21). These formulations containing granular starch were generally limited to starch contents of approximately 10% by weight or less due to mechanical properties deterioration for higher filler content. Composite materials prepared by blending gelatinized starch with water soluble or water dispersible polymers were first developed in the late 1970s (17-19) and have been object of continuous research. Composite films were prepared by casting of water solution of starch and water soluble polymers such as poly(vinyl alcohol) (PVA). In the process generally used (20-23), the casting dope has been prepared by mixing starch, PVA, plasticizers and other chemicals followed by heating, under stirring, the mixture at about 90 ˚C for some hours. The resulting solution has been cast in trays or plates, dried in oven and then equilibrated at 50% relative humidity. To improve moisture resistance in some cases water-resistant coating layers have been applied on the films. These layers, composed of poly(vinyl chloride) (20) and poly(vinyl acetate) (22) were going to compromise the real biodegradability of the material. Thus studies have been developed also on biodegradable coatings (24) for real biodegradable composite films. Further studies to improve water resistance without compromising bio-degradability have been performed on the use of cross-linking agents (23). These cast films have the advantage of starch contents up to 50-60% with good properties, even if they use

relatively high cost raw materials. Thus solution processing is an interesting method to produce biodegradable composites and to study materials properties and interactions but is not economically acceptable for high processing costs and low efficiency in comparison with thermoplastic processing.

In the United States, Otey and co-workers developed a process for extrusion compounding and blowing of starch as a thermoplastic component in films at 5-10% moisture content (25). Subsequently processing of starch to have thermoplastic starch was developed (26-28) and further improved by specific studies on the melt rheology and degradation during the extrusion procedures (31, 32). The development of the technology for the extrusion of starch offered a route to composite materials with high starch content, relatively low raw materials cost, and inherent biodegradability (33).

Thermoplastic starch can be produced out of native starch using a swelling or plasticizing agent while applying a dry starch in compound extruders without adding water. When a starch with a water content higher than 5% is plastified or pasted under pressure and temperature, a de-structured starch is always formed. In the production procedure of thermoplastic starch, the mainly water-free raw material is homogenized and melted in an extrusion process with a plastifing material (34). Usually the extruder temperature is maintained in the range 120-220 ˚C, and the used plasticizers are chosen among polyols such as glycerol, sorbitol etc. Thermoplastic starch does not contain crystalline structure and no longer re-crystallizes, in contrast with de-structured starch.

Starch has been extruded by using single screw extruder with standard 19 mm, 3/1 compression ratio screw. Studies have been performed to optimize the extrusion processes and different kind of screws have been proposed such as fluted spiral mixing section screws (35).

Ethylene acrylic acid copolymer or polyvinyl alcohol have been used together with other ingredients to produce films (36-40). Montedison produced processable materials by compounding starch and ethylene vinyl acetate or ethylene vinyl alcohol (41). These approaches stimulated industrial activities, several patents were filed as dealing with starch-plastic composites that were reputed to be totally degradable (4, 13). Very often in order to achieve a practical and cost-effective material and mostly to limit moisture susceptibility of starch based materials a large amount of conventionally non-degradable polymers has been added.

In its gelatinized form, starch is readily accessible to natural enzymes, amylases, and it is available from several renewable plant resources. Composite materials prepared with starch and synthetic polymer were proposed for packaging and agricultural applications where deterioration of physical properties as the starch biodegraded, was an advantage in terms of collection cost saving. The degradation of starch by microorganism, when the starch-plastic composites were placed in suitable environments, was extensively demonstrated experimentally (17, 26, 42, 43).

The degradation of starch was however not sufficient to classify the composite material "biodegradable" since the synthetic polymer resulted very often to be recalcitrant to degradation (44). Especially for starch-polyethylene composites the fragments, resulting from composite deterioration, may require decades to completely biodegrade. Moreover, the toxicity of degradation products is largely unknown (45). Thus composites materials prepared by starch and HDPE or LDPE, initially claimed as biodegradable, are nowadays classified as fragmentable not biodegradable composite materials. To improve polyolefins degradation, auto-oxidizable chemicals were added in the composites (18).

To achieve an effective degradability, blends or composite materials have been produced by processing of starch with biodegradable polymers such as: poly(vinyl alcohol), poly(lactic acid), poly(e-caprolactone), poly(hydroxybutyrate-co-valerate), polyesteramide, etc (46-53). Thus the low cost of starch makes it attractive to be blended with high cost biodegradable polymers such as poly(hydroxybutirate-co-valerate) (PHBV) (54).

These approaches stimulated industrial activities, several patents have been filed dealing with starch-plastic composites that are reputed to be totally degradable (55-67). Materials known as MATER-BI from Novamont(68), Degra-Novon, and Aquanovon from NOVON (69) and ECOSTAR from National Starch and Chem. Co. (70) have been introduced into the market and used as molding compounds, films, foams etc.

Table 3.2 Biodegradable Composites Based on Starch and synthetic Biodegradable Polymers

Synthetic-Semi-synthetic Polymers
Polyesteramide
Copolyamide
Polybutylene succinate adipate(Bionolle)
Polycaprolactone
Poly(vinyl alcohol)
Polylactic acid
Adipic acid PHEE/poly(lactic acid)
Cellulose acetate
Hydroxy-functionalized polyester
Bionolle 3003

In composites based on synthetic or semi-synthetic polymers and natural polymers the effect of the natural polymers addition on the properties of the final material follows the same general trends as other fillers. As the natural polymer volume fraction increases, yield strength, tensile strength, and elongation to break decrease while the modulus generally increases. Most of the commonly used natural polymers (starch, cellulose, gelatin etc) are hydrophilic while most of synthetic polymers of commercial interest are hydrophobic. The resulting high interfacial energy results in poor adhesion between the continuos matrix phase and the natural fillers. Various techniques have been explored to improve interfacial adhesion and thereby the properties of this type of composite materials. One approach is to utilize compatibilizer, which act as polymeric surfactants. Thus the compatibilizer is going to be located at the matrix/filler interface, and thereby improves stress transfer across the interface itself (71).

The research on biodegradable composites materials based on synthetic and natural polymers is still attracting a lot of attention as documented by the increasing number of publications and patents on that topic.

A huge variety of natural polymers, other than starch, have been blended with biodegradable synthetic or semi-synthetic polymers to produce biodegradable composite materials. Cellulose (72), pectin (73), chitosan (74-76), lignin (77, 78), soy protein (78), wheat gluten (80), gelatin (81), silk fibroin (82) are just a few examples of a large variety of available natural materials which are growing in consideration for biodegradable composites production.

Particular attention is given to aliphatic polyesters which have excellent mechanical properties and biodegradability and are well suited to disposable applications. A drawback is that they currently have a high cost. In an effort to reduce their cost, blending with low cost natural polymers has been pursued (83). Modified polyester and powdered cellulose, sodium alginate and chitosan in lyophilized form have been used as fillers. The samples were prepared in the form of films of different thickness and contained various amounts of natural components (84).

Table 3.3 presents some examples of composite materials based on biodegradable synthetic polymers and natural polymers collected on the 1999 and 2000 chemical abstract on the subject biodegradable polymers composite.

Natural polymers have been not only the most used additive in composites production together with synthetic polymers but also with a huge variety of other natural polymers such as blends based on starch and pectin (85), starch and gelatin (86). This particular type of natural polymer/natural polymer blends and/or composite has been investigated particularly for the realization of edible films and items (87, 88). Table 3.4 reports Some examples of recent composite materials based on biodegradable natural polymers (1999-2000).

Table 3.3 Examples of Composite Materials Based on Biodegradable Synthetic and Semi-synthetic Polymers and Natural Polymers[a]

Synthetic Polymers	Natural Polymers
PHB	Chitin and Chitosan
PLA	Bacteria Cellulose Gel
PVA	Gelatin
PVA	DNA sodium salt from salmon testes
PVA	Chitosan
PCL	Wheat Gluten
Polyester	Gluten
PCL	Soy Protein
PEG	Chitosan
Cellulose acetate butyrate	Lignin
Starch-CPL copolymer	Lignin
PHB	lignin

[a] Data referred to year 1999,
PHB=Polyhydroxybutirate, PLA=Polilactic acid, PVA=Poly(vinyl alcohol), PCL=Polycaprolactone, PEG=Poly(ethylene glycol),

Table3.4 Example of Biodegradable Composites Based Totally on Natural Polymers

Natural Polymer (Continuous phase)	**Natural Polymer (Filler)**
Starch	Cellulose fibers
Starch	Pectin
Starch Cellulose	
Gelatin	Sugarcane Bagasse
Gelatin	Starch
Casein	Starch

Composite materials also consider the use of biodegradable polymers with inorganic fillers such as polyesters blended with inorganic oxide powders (89). This type of composite materials need an appropriate processing. For example, to prepare blends based on PCL and aragonite (33% w/w aragonite), the polycaprolactone/aragonite composite was heated and molded into a 2-mL polyethylene syringe barrel and allowed to harden on cooling. The plunger was replaced and the filled syringe immersed in hot water for 5 min. On removal from the water, the composite was easily injected from the syringe until it hardening on cooling (90).

3.2.2 Fibrous Composites

Fibrous composites consist of fibers in a continuous matrix where the fibers may be short or discontinuous and randomly arranged or continuous filaments arranged in parallel to each other. In this last case the fibers may be in the form of woven roving, collections of bundle of continuous filaments, or braided. Structural materials require strength, stiffness, and toughness. Other properties such as resistance to corrosion, creep, fatigue, temperature, or moisture, are also needed in most structural materials. These properties are important too because of their effect on strength, stiffness (dimensional stability), and toughness. In continuos fibers composites the fibers must support all main loads and limit deformations acceptably. From 1920, glass fibers have been selected to be used as a reinforcement in plastic composites due to glass fibers strength (4 GPa). Glass fibers strength increases when the fibers is drawn to smaller diameters. Chopped strand mat consisting of short lengths of glass fibers (25 to 75 mm) randomly arranged, have been used in marine and automotive applications. From 1960, also ceramic, boron and carbon fibers started to be used for composite materials production. Materials realized with continuous aligned stiff fibers such as carbon (graphite), boron, aramid, glass or aromatic polyamides (Kevlar) have been defined "advanced composite" to distinguish them from composites realized by filling the plastic matrix with chopped-fibers or other fillers. These fibers possess the desirable properties of low density (1.4-2.7 g/cm3) and extremely high strengths (3-4.5 Gpa) and modulus (80-550 Gpa) (91). In advanced composites the fibers are typically 50 times stronger

and 20-250 times stiffer than the matrix polymer. The role of the plastic matrix is primarily that of a glue or binder keeping them separated and transferring load to the fibers so that they resist bending and compression, and protects the fiber from surface damage. These materials found applications in aerospace industry, military and civil aircraft, sport items such as tennis rackets and golf club shafts.

Technique used to fabricate continuous fiber-composites are filament winding and pultrusion.(2). In filament winding a traversing carriage lays down impregnated fibers on a revolving, lathe like mandrel as reported in Fig.3.2 a. In wet winding, the fibers are impregnated with the resin (most frequently a thermoset) just before being wound onto the mandrel. The wrap angle, that is the angle between the fiber and the axis of the mandrel , can be varied between 0 and almost 90˚. In this way multidirectional components can be realized relatively easily. After the winding process, the whole fixture is cured in an oven and the mandrel is removed by respectively dissolving (salt, sand based), melting (low melting point alloys), or collapsing and dismantling (steel). Items such as cylinder and spherical shells can be manufactured economically by this technique with up to 60% of fiber volume.

Pultrusion is used to produce constant cross-sectional shapes such as rods and I beams. In this process, reported in figure 3.2b, the fibers pass through a resin tank and are then pulled through a heated die to cure and form the final product. On leaving the die the material is sufficiently hard and strong to be pulled mechanically by pull rollers to a collecting drum with large diameter or to a cutter wheel. Both thermosetting and thermoplastic matrix are commonly processed with this technique.

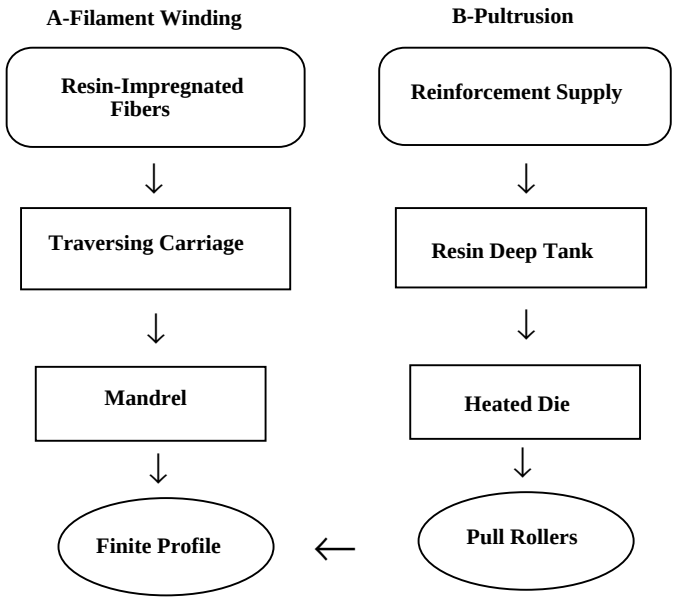


Fig.3.2. (a) Filament Winding Process, and (b) Pultrusion Process

Fibers based composite are also produced by compression molding. The press is composed of two plates, one moving and one fixed (base), on which respectively the male and female mold are placed (Fig.3.3). A heating system is connected with the molds allowing to reach the desired temperature. A desired number of layers, pre-impregnated composite or mixture are placed on the female mold, then the moving plate is moved until the required pressure between the male and female mold is achieved. The material is kept under heating and pressure for the required time. Some press are provided with a cooling system (water cooling) in order to cool the composite to room temperature before of removing from the mold.

Fibrous composite can also be produced by resin transfer molding. In this technique dry fibers are placed in a closed mold and the resin is introduced into the mold under external pressure or vacuum. The resin may be cured under the action of its own exothermal heat or an external heating is applied. By this technique it is possible to produce economically high quality composites.

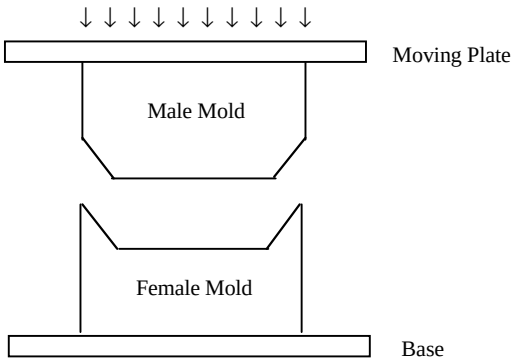


Fig. 3.3. Schematic Representation of a Molding Press

Fiber composite laminates can also be produced and they often consist of un-directional (parallel) continuous fibers in a polymer matrix with the individual layers, plies or laminae, stacked with selected fiber angles so as to produce specific laminate stiffness and strength values.

The search for environmental friendly composite material has led to a dramatic increase of interest in using natural fibers as fillers and reinforcement in plastic composites. Natural fibers seem to have little resistance towards environmental influences. This can be recognized in the composite and can be advantageously utilized for the development of biologically degradable composites with good physical properties. The use of natural fibers in composite materials has an ancient origin. Straw was already used by the Egyptians in the pharaonic period for the fabrication of clay composites. Plant fibers were added into pottery as a reinforcement by Incas and Mayans.

In modern technology, short cellulose fibers have been used as a filler in Bakelite molding compounds to give products which were strong and tough and found applications in automotive components industry since native cellulose fibers are among the strongest and stiffest fibers available. Cellulose is the most abundant organic polymer on Earth, is widely used together with synthetic polymers in a variety of materials ranging from coated paper to laminates for packaging. Cellulose fibers offer the advantages of low density, high modulus, low price and biodegradability. The theoretical value of stiffness of a single crystal of cellulose is more than 130 Gpa. The stiffness of a typical wood fiber is between 20-40 Gpa.

Natural fibers present the advantages to be inexpensive, strong, lightweight, environmentally compatible (92-94). However, the poor dimensional stability, low biological resistance and lack of thermo-plasticity of lignocellulosic fibers have limited the use of these materials to produce single-use articles. For these reasons fibrous materials have been blended with thermoplastic matrices in composites containing various percentages of the fibers (95).

The physical properties of natural fibers are mainly determined by the chemical and physical composition such as the structure of fibers, cellulose content, angle of fibrils, cross-section etc. Only a few characteristic values, but especially the specific mechanical properties, can reach comparable values of traditional reinforcing fibers.

Nowadays there are considerable quantities of agro-based fibers available on a worldwide basis for a variety of applications (96). Table 3.5 shows the estimated annual availability of agro-based resources as reported by Rowell at the First International Symposium on Lignocellulosic-Plastic Composites held in Brazil in 1996 (97).

Natural fibers require low processing temperature and result incompatible with hydrophobic polymers. The limiting processing temperatures when using lignocellulosic materials with thermoplastic is important to determine processing techniques. High melting temperatures (200 ˚C) that reduces melt viscosity and facilitate good mixing cannot generally be used (except for short periods) and other routes are needed to facilitate mixing of fibers and matrix in natural fibers based composites. In short fibers composites fibers dispersion, fiber length distribution, fiber orientation and fiber-matrix adhesion

control composite properties. When mixing the polar and hydrophilic fibers with non-polar hydrophobic matrix, difficulties in dispersing the fibers are observed. Clumping and agglomeration of the fibers compromise composite mechanical properties.

Table 3.5. Estimated World Annual Availability of Fibers Sources

Fiber Source	Dry Metric Mtons
Wood	1,750
Straw	1,145
Stalks	970
Sugar Cane Bagasse	75
Reeds	30
Bamboo	30
Cotton Staple	15
Core (jute, kenaf, hemp)	8
Papyrus	5
Bast (jute, kenaf, hemp)	3
Cotton Linters	1
Esparto Grass	0,5
Leaf	0,48
Sebai Grass	0,2
Total	**4,033**

Thus for most polymers, it is thermodynamically unfavorable to form homogeneous mixtures with each other. The reason is that the combinatorial entropy of mixing of two polymers is dramatically smaller than that for two low molecular weight compounds while the enthalpy of mixing is often positive or zero (98). In these cases polymers are not miscible. Such multi-component materials present at the same time many advantages which are direct consequence of this incompatible nature. In fact immiscibility is desired in some polymer-polymer composites in which each phase can contribute its own characteristics to the product. Anyway in the solid state good mechanical behavior requires efficient transfer of stress between the component phases, which depends on the adhesion at the interface. Thus the application of natural fibers as reinforcements in composite materials requires, just as for glass-fiber reinforced composites, a strong adhesion between the fiber and the matrix, regardless of whether a traditional polymer (thermoplastics or thermosets) matrix, a biodegradable polymer matrix or cement is used. Natural fibers physical structure can be modified by alkali treatment and acetylation processes. These different treatments change among others the hydrophilic character of the natural fibers, so that moisture effects in the composite are reduced (99).

The effectiveness of this method is strongly influenced by the treatment conditions used. The mechanical and other physical properties of the composite are generally dependent on the fiber content, which also determines the possible amount of coupling agents in the composite.

The processing conditions play, next to the mechanical properties of natural fibers, an important role for the industrial use of these materials. Several types of compounding equipment such as batch and continuos equipment, have been used for blending lignocellulosic fibers and plastics. The ultimate fiber length present in the composite depends on the type of compounding and molding equipment used. Factors such as shearing forces generated in the compounding equipment, retention time, screw geometry, temperature and viscosity of blends contribute to fibers attrition.

In the past natural fibers have been processed with plastics by molding after grinding of the fibers together with the resin such as for fiber-phenol formaldehyde composite. This method is very limited in the draw-depth for the molded products.

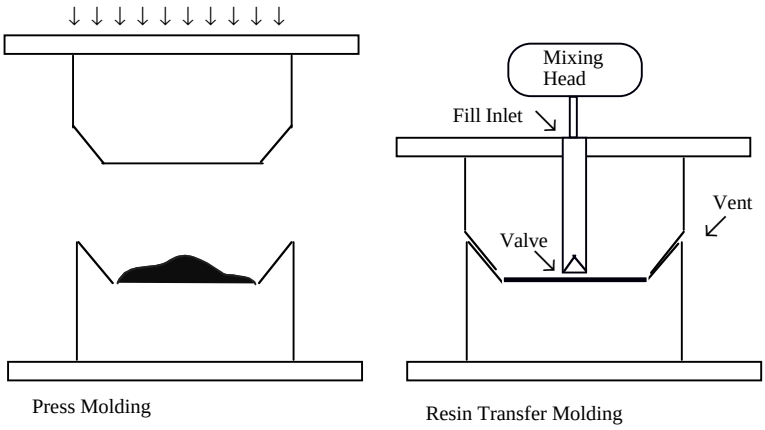


Fig. 3.4. Schematic Representation of Resin Transfer Molding.

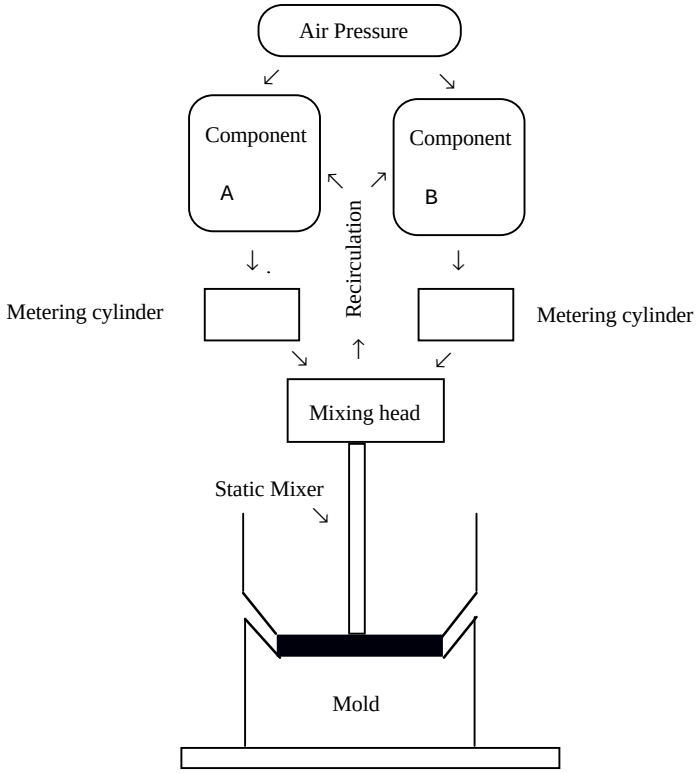


Fig. 3.5 Schematic Representation of Structural Reaction Injection Molding

Fibrous materials can be blended with thermoset resins by the use of a Randoweb machine (91). This equipment allows the formation of non-woven webs from short fiber materials such as wood and agro-based ultimate fibers in combination with long fiber stock, such as bast fiber strands or synthetic fibers. The long fibrous material imparts mechanical integrity to the web after needle-punching. In this method a thermosetting resin is added to the web either by addition of a powdered resin or by spraying of a solution of the resin. The web is then compression molded at elevated temperatures to a variety of rigid shapes. Non-woven webs can also be prepared on the Randoweb machine with various percentages of thermoplastic fibers which act as the binding agent when the product is compression molded at elevated temperature. A variation on the use of non-woven webs of natural fiber should be through application of resin transfer molding and structural reaction injection molding. When the composites are formed by a thermoplastic resin as the major component with various percentage of natural fibers the processing follows the sequence shown in Fig. 3.6.

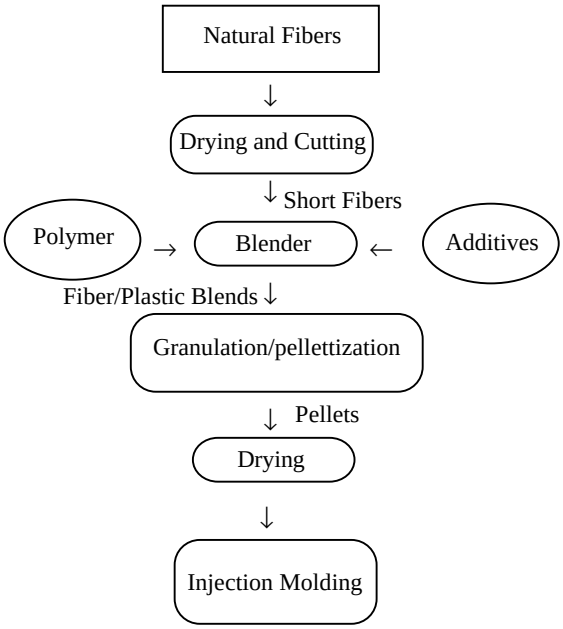


Fig. 3.6 Schematic Representation of Laboratory Injection Molding of Bio-based Materials

Another technique used to blend plastic and natural fibers is the high intensity compounding using a turbine mixer (thermokinetic mixer). The plastic/natural fiber mixing can be improved by adding dispersing aids or coupling agents. The high shearing action developed in the mixer decreases the length of the fibers in the final composites. Anyway, the improved fiber dispersion resulted in improved composites' properties. With this technique, no pre-drying of the fibers is needed (97).

Natural fibers seem to have little resistance towards environmental influences. This can be recognized in the composites and can be advantageously utilized for the development of biodegradable composites with good physical and mechanical properties. Thus agro-based fibers are a viable alternative to inorganic/mineral based reinforcing fibers in commodity fiber thermoplastic composite materials as long as the right processing conditions are used and for applications where water absorption is not critical (98). Several type of biodegradable composite materials with natural fibers as fillers have been prepared in the past . Some typical examples with the relevant references are collected in Table 3.6.

Table 3.6 Some example of Fibrous Biodegradable Composites

Polymer	**Fibers**	**Ref.**
Poly(ester amide)	Flax and Cotton Fibers	98, 101
Poly(hydroxybutyrate-co-valerate)	Pineapple Fibers	102, 103
Biopol	Jute	104,105
Poly(vinyl alcohol)	Lignocellulosic Fibers	106
Poly(vinyl alcohol)/gelatin	Bagasse	107
Gelatin	Bagasse	108
Starch	Cellulose Fibers	109
Poly(vinyl alcohol), Protein Hydrolyzate	Wood Flour	110

3.2.3. Other Composites

Two or more layers of material bonded together form a laminate composite. Laminates are common in automotive industry such as automobile windshields (laminated glass) where homogeneous isotropic layers of material are bonded together to form non homogeneous composite laminates. Fibrous laminated composites are formed by laying up a number of pre-impregnated mats or tapes; the pre-impregnated laminate is then cured in an oven. Lamination can be un-directional which consists of fiber-impregnated tapes laminated with fibers running in the same direction for strength along one axis.

In quasi isotropic lamination, impregnated tapes or mats are laminated in three, four, or more directions to produce isotropic properties in the plane of the fibers.

In 1913 the Formica Products Company was formed and in 1931 decorative laminated were introduced on the market. These materials were made by a layer of urea-formaldehyde on a Kraft paper core impregnated with phenolic resin to be compressed and heated between polished steel platens (111). Wood is an example of a natural laminated composite. Plywood is a man made laminate composite consisting of thin sheets of wood arranged with the grain in alternate sheets at right angles. At the beginning of plywood production in the mid-nineteenth century natural adhesive were used. Nowadays phenolic resins and urea-formaldehyde based adhesive are commonly used even if these substances have a negative impact on the environment and on workers health safety. Recently an environmentally friendly adhesive for wood and plywood has been developed by blending starch with poly(vinyl alcohol) crosslinked by hexamethoxymethylmelamine showing performances comparable with urea-formaldehyde based adhesive (112).

Apart from particulate, fibrous and laminate composites, other type of materials have been also developed such as foamed items based on potato starch in blends with poly(vinyl alcohol) (113). The particular foaming process is based on baking a butter based on starch and PVA. The butter was prepared by premixing the ingredients in a kitchen Aid mixer with a wire whisk attachment and adding water to reach a total solid content of 33%. Foam trays were prepared using a lab model-baking machine (model LB TRO) that essentially consists of two heated steel molds, the top of which can be hydraulically lowered to mate with the bottom half for a set amount of time. Baking temperature and time were set for the different butters. Earthshell has started to market composite foamed items prepared with this processing based on potato starch, PVA and a limited amount of wood fibers (114).

The research on biodegradable composites and blends is still in an embryonic stage with a particular attention to low cost materials such as agricultural or industrial cuttings, by-products or wastes.

Reference (Reading Materials)

(1). J. Morton, W.J. Cantwell, Composite Materials, Chapter in Kirk Othmer Encyclopedia of Chemical Technology, 4th ed., Vol.7, John Wiley & Sons, NY, p.1 (1994.)

(2) F. P. Gerstle Jr, Composites Chapter in Encyclopedia of Polymer Science and Engineering, Vol. 3, John Wiley & Sons, NY p. 776, (1994)

(3) R.A.Young, First International Lignocellulosics-Plastics Composites, March 13-15 1996, Sao Paolo Brazil, Ed. A.L.Leao, F.X.Carvalho, E.Frollini, p.1 (1997)

(4) W. M. Doane, J. Polym. Mater. 11, 229, (1994)

(5) H.Feil, Macromol. Symp., 127, 7 (1998)

(6) G.M.Chapman, in ACS Symposium Series 575, Polymers from Agricultural Coproducts, M.L.Fishman, R.B.Friedman and S.J.Huang Eds., American Chemical Society, Washington DC, p. 29 (1994)

(7) P.Galli, A.Addeo, Macrom. Symp, 127, 59 (1998)

(8) R. Narayan, in Polymers from Agricultural Coproducts, M.L.Fishman, R.B.Friedman and S.J.Huang Eds., American Chemical Society, Washington DC, 1994, p. 2

(9) H. Keskkula, D.R. PAul, J.W. Barlow, Polymer Blends, Chapter in Kirk Othmer Encyclopedia of Chemical Technology, 4thed., Vol.19, John Wiley & Sons, NY, p. 837 (1994.)

(10) S. Danesi, Polymer Blends, M Kryszewski, A. Galeski, E. Martuscelli Eds., Plenum press, NY, p.35 (1984)

(11) M. Xanthos, B.D. Todd Plastic Processing Chapter in Kirk Othmer Encyclopedia of Chemical Technology, 4th ed., Vol.19, John Wiley & Sons, NY, p.290 (1994.)

(12) D.W. Fox, R.B. Allen, Compatibility, Chapter in Poymer Science and Technology, 4th ed, Vol. 3, p. 758 (1994)

(13) C.L.Swanson, R.L.Shogren, G.F.Fanta, S.H.Imam, J.Environ.polym.Degrad., 2, 155 (1993)

(14) J.N. Be Miller, carbohydrate, Chapter in Kirk Othmer Encyclopedia of Chemical Technology, 4th ed., Vol.4, John Wiley & Sons, NY, p.911 (1994)

(15) R.L.Whisltelr, J.R.Daniel, Starch, Chapter in Kirk Othmer Encyclopedia of Chemical Technology, 3rd ed., Vol.21, John Wiley & Sons, NY, p.492 (1983)

(16) G.J.L.Griffin , Am.Chem.Soc.Div.Org.Coat.Plast.Chem. 33, 88 (1973)

(17) G.J.L. Griffin U.S.Patent 4,016,117 (1977)

(18) G.J.L.Griffin U.S.Patent 4,021,388 (1977)
(19) G.J.L.Griffin U.S.Patent 4,125,495 (1978)
(20) F. H. Otey, A. M. Mark, C. L. Mehltretter, C. R. Russell, Ind. Eng. Chem. Res, 13, 90 (1974).
(21) R.S.Lenk, R.E. Merrall, Polymer, 22, 1279 (1981)
(22) S.M. Lahalih, S.A. Akashah, F.H. Al-Hajjar, J.Appl.Polym.Sci., 26, 2366 (1987)
(23) L. Chen, S. H. Imam, S. H. Gordon, R. V. Green, J. Environ. Polym. Degr., 5, 111 (1997).
(24) J.W.Lawton, Cereal-Novel Uses and Processes, Campbell Ed., Plenum Press, NY, p.43 (1997)
(25) F. H. Otey, R. P. Westhoff, C. R. Russell, Ind. Eng. Chem. Res, 16, 305 (1977).
(26) F.H. Otey, R.P.Westhoff, U.S.Patent 4,337,181 (1982)
(27) F.H. Otey, R.P.Westhoff, W.M. Doane, Ind. Eng. Chem. Res, 19, 592 (1980).
(28) R. P. Westhoff, R. H. Otey, C. L. Mehltretter, and C. R. Russell, Ind. Eng. Chem. Prod. Res. Dev. 13, 123 (1974).
(29) I.Tomka, A.Troesch, PCT Int.Pat.Appl.WO 90/05161 (1990)
(30) G.J.L. Griffin , PCT Int. Pat.Appl.WO 91/04286 (1991)
(31) J.L.Willett, M.M. Millard, B.K. Jasberg, Polymer, 38, 5983 (1997)
(32) J.L.Willett, B.K. Jasberg, C.L. Swanson, Polym.Eng.Sci. 35, 202 (1995)
(33) J.L.Willett, B.K.Jasberg, C.L.Swanson, in Polymers from Agricultural Coproducts, M.L.Fishman, R.B.Friedman and S.J.Huang Eds., American Chemical Society, Washington DC, 1994, p. 50
(34) J. Lorcks, Polym. Degrad. Stab. 59, 145 (1998)
(35) K.Jaseberg, J.L.Willett, ANTEC '96, Indianapolis (USA), 5-10 MAy, Society of Plastic Enginners, Vol. II-Materials (1996)
(36) F.H.Otey, A.M.Mark, US Patent 3,949,145 (1976)
(37) L. Zhiqiang, F. Yi, Y. Xiao-Su, J.Appl.Polym.Sci., 74, 2667 (1999)
(38) F.H.Otey US Patent 4,133,784, (1979)
(39) G.F.Fanta, C.L.Swanson, W.Doane, J.Appl.Polym.Sci. 40, 811 (1990)
(40) R.S. Lenk, R.E.Merrall, Polymer, 22, 1279 (1981)
(41) PCT Patent WO 91/02025 (1991)
(42) F.H. Otey, A.M. Mark, U.S. PAtent 3,949,145 (1976)
(43) R.P. Westhoff, F.H, Otey, C.L. Mehltretter, C.R.Russell, Starch, 31, 163-165 (1979)
(44) K. Arevalo-Nino, C.F. Snadoval, L.J.Galan, S.H.Imam, S.H. Gordon, R.V. Greene, Biodegradation, 7, 231 (1996)
(45) A.C. Albertsson, S. Karlsson, J. Appl. Polym. Sci. 35, 1289-1302 (1988)
(46) J.A. Ratto, P.J. Stenhouse, M. Auerbach, J. Mitchell, R. Farrell, Polymer, 40, 6777, (1999)
(47) G. Biresaw, C. J. Carriere, Polym. Prepr, 41, 64 (2000)
(48) L. Averous, N. Fauconnier, L.Moro, C.Fringant, J. Appl. Polym. Sci., 76, 1117 (2000)
(49) L.Averous, L.Moro, P. Dole, C. Fringant, Polymer, 41, 4157 (2000)
(50) M. Avella, M. E. Errico, P. Laurienzo, E. Martuscelli, M. Raimo, R. Rimedio, Polymer, 41, 3875 (2000)
(51) B.A.Ramsay, V.Langlade, P.J.Carreau, J.A.Ramsay, Appl.Environm.Microbiol.59,1242 (1993)
(52) R.L.Shogren, J.Environm.Polym.Deg., 3, 75, (1995)
(53) M.Yasin, S.H. Holland, A.M. Jolly, B.J. Tighe, Biomaterials, 10, 400 (1989).
(54) M.A. Kotnis, G.S.O'Brien, J.L.Willett, J.Environm.Polym.Deg. 3, 97 (1995)
(55) C. Bastioli, R.Lombi, G. del Tredici, I. Guanella, Eur. Pat. Appl. 0,400,531 (1990)
(56) C. Bastioli, V.Bellotti, G.del Tredici, L.Del Giudice, PCT Int. Pat. Appl. WO 91/02024 (1991)
(57) C. Bastioli, V. Bellotti, L. Del Giudice, R.Lombi, A. Rallis, PCT Int. Pat. Appl. WO 91/02023 (1991)
(58) G. Lay, J. Rehm, R.F.T. Stepto, M. Tomka Eur. Pat. Appl. 0,327,525 (1989)
(59) J.P. Sacchetto, D.J. Lenz, J. Silbiger, Eur. Pat. Appl. 0.404,723 (1990)
(60) J.P. Sacchetto, J.Rehm, Eur. Pat. Appl. 0,407,350 (1991)
(61) D.J. Lenz, J.P. Sacchetto, J. Silbiger, Eur. Pat. Appl. 0,409,788 (1991)
(62) J. Silbiger, D.J. Lenz, J.P. Sacchetto, Eur. Pat. Appl. 0,409,789 (1991)
(63) J.J. Vanderbit, C.M. Neeley, PCT Int. Pat. Appl. WO 90/15843 (1990)
(64) W.M. Doane, W. Xu, M. Mang, N. Michael et al, PCT Int. Appl. WO 2000011064 A1(2000)
(65) PCT Int. Appl. WO 90/05161/US 5,362,777?EP 0,397,819
(66) Yoshihara, Toshinobu PCT Int. Appl. WO 2000009609 A1 (2000)
(67) J.L. Willett, W.M. Doane, W. Xu, M. Mang, N. Michael , J.E. White, U.S. US 6025417 A (2000)
(68) http://www.materbi.it/welcomeSi.html, May 2001.

(69) http://www.novoinc.com, May 2001.
(70) http://www.ecostar.inc, May 2001.
(71) J.L. Willett, R.L. Shogren, "Woodfiber-Plastic Composites" Madison, Winsconsin, 1-3 May, 1995, Proceedings No.7293, p. 7644.
(72) Y. Nishio, T. Hratani, T. Takahashi, R. S. J. Manley, Macromolecules, 22, 2547 (1989).
(73) D. R. Coffin, M. L. Fishman, T. V. Ly, J. Appl. Polym. Sci., 57, 71 (1996).
(74) T. Ikejima, K. Yagi, Y.Inoue Macromol. Chem. Phys., 200, 413 (1999)
(75) T.Ikejima, ; Inoue, Y. Carbohydr. Polym., 41 351 (1999)
(76) M. Mucha, J. Piekielna, A. Wieczorek, Macromol. Symp., 144, 391 (1999)
(77) I. Ghosh, R.K.Jain, W.G. Glasser, ACS Symp. Ser., 742, 331 (2000)
(78) H. Nagele, J. Pfitzer, N. Eisenreich, P. Eyerer, P. Elsner, W. Eckl, PCT Int. Appl. WO 2000027923 A1 (2000)
(79) J.John, M.Bhattacharya, Polym. Int., 48, 1165 (1999)
(80) J.John, J.Tiang, M.Bhattacharya, Polymer, 13, 2883, (1998)
(81) E. Chiellini, P. Cinelli, A. Corti, E.R.Kenawy, E. Grillo Fernandes, R. Solaro, Macromol. Symp., 152, 83 (2000)
(82) T. Tanaka, T. Tanigami, K. Yamaura, Polym. Int, .45, 175 (1998)
(83) S. W. Lim, IK Jung, KH Lee, BS Jin, Eur.Polym.J. 35 , 1875 (1999)
(84) M. Ratajska, S.Boryniec Polym. Adv. Tech., 10, 625 (1999)
(85) D. R. Coffin, M. L. Fishman, J. Appl. Polym. Sci., 54, 1311 (1994).
(86) I. Arvanitoyannis, E.Psomiadou, A.Nakayama, S.Aiba, N.Yamamoto, Food Chem., 60, 593 (1997)
(87) I. Arvanitoyannis, E.Psomiadou, A.Nakayama, Carbohydr. Polym., 31, 179 (1996)
(88) E.Psomiadou, I. Arvanitoyannis, N.Yamamoto, Carbohydr. Polym., 31, 193 (1996)
(89) Ko, Young Kwan PCT Int. Appl. WO 2000031166 A1 (2000
(90) Stainton, Neil Mcvean PCT Int. Appl. WO 2000018443 (2000)
(91) D.V.Rosato, G.Lubin, ed., Handbook of Composites, Van Nostrand Reinhold Co., New York, pp.1-14 (1982)
(92) J.M. Felix, P. Gatenholm J.Appl.Polym.Sci., 50, 699 (1993)
(93) J.M. Felix, P.Gatenholm, H.P. Schreiber, J.Appl.Polym.Sci., 51, 285 (1994)
(94) A. J. Michell, J.E. Vaughan, D.Willis, J.Appl.Polym.Sci., 22, 2047 (1978)
(95) R.A.Young, First International Lignocellulosics-Plastics Composites, March 13-15 1996, Sao Paolo Brazil, Ed. A.L.Leao, F.X.Carvalho, E.Frollini,p.1 (1997)
(96) R.A.Young, Vegetable Fibers, Chapter in Kirk Othmer Encyclopedia of Chemical Technology, 4th ed., Vol.10, John Wiley & Sons, NY (1994)
(97) R.M. Rowell , A.R. Sanadi , D.F. Caulfield , R.E. Jacobson , First International Lignocellulosics-Plastics Composites, March 13-15 1996, Sao Paolo Brazil, Ed. A.L.Leao, F.X.Carvalho, E.Frollini, p.23 (1997)
(98) H.W. Kammer, J.Piglowski , in Polymer Blends Processing, Morphology and Properties, M.Kryszewski, A.Galeski, E.Martuscelli, Eds., Plenum Press NY, pp.19-34 (1984)
(99) A. K. Bledzki, J. Gassan, Prog. Polym. Sci., 24, 221 (1999)
(100) L. Jiang, G. Hinrichsen, Angew. Makromol. Chem., 268, 13 (1999)
(101) L. Jiang, G. Hinrichsen, Angew. Makromol. Chem., 268, 18-21 (1999)
(102) S. Luo, A. N. Netravali, J. Mater. Sci., 34, 3709 (1999)
(103) S. Luo, A. N. Netravali, Polym. Compos., 20, 367 (1999)
(104) Khan, Mubarak A.; Ali, K. M. Idriss; Hinrichsen, G.; Kopp, C.; Kropke, S. Polym.-Plast. Technol. Eng., 38(1), 99 (1999)
(105) Mohanty, A. K.; Khan, Mubarak A.; Hinrichsen, G. Compos. Sci. Technol., 60, 1115 (2000)
(106) E. Chiellini, P. Cinelli, S.H. Imam, Biomacromolecules in press. (2001)
(107) Bledzki, A. K.; Gassan, J. Prog. Polym. Sci., 24, 221 (1999)
(108) E. Chiellini, P. Cinelli, E. Grillo Fernandes, A.Lazzeri Biomacromolecules in press (2001)
(109) Bergthaller, W. J.; Funke, U.; Lindhauer, M. G.; Radosta, S.; Meister, F.; Taeger, E. ACS Symp. Ser., 723, "Biopolymers: Utilizing Nature's Advanced Materials", 14 (1999)
(110) A. Pavol, D. Bakos, K. Kolomaznik, M. Sedlak, Michal, E. Sedlakova, Eva PCT Int. Appl. WO 2000061660 A1 2000, 16 pp. (1999)
(111) G. Lubin, ed., Handbook of Composites, Van Nostrand Reinhold Co., Princeton, N.J., (1982).
(112) S.H.Imam, L.Mao, L. Chen, R.V. Greene, Starch/Starke, 6, 225 (1999)
(113) R.L.Shogren, J.W.Lawton, K.F. Tifenbacher, L.Chen J.Appl.Polym.Sci, 68, 2129 (1998)
(114) http://www.earthshell.com, May 2001.

CHAPTER 4

PRODUCTION OF EDPS BY BIOLOGICAL METHODS

Objectives

- Students will get to know the most important EDPs, especially polyhydroxyalkanoates and polysaccharides and their various subgroups.
- They will learn how these subgroups differ in structure and properties and for which applications they are used.
- Students will find out about the different strains used for EDP production, their physiological differences, advantages and disadvantages.
- Students will study the methods and processes for the production of these EDPs and the differences between them.
- Students will learn about the properties of the materials and which properties make up a usable polymer.
- Students will also learn about the downstream processing and why it is such an important part of the EDP production.

Summary

The most important EDPs are introduced and the crucial points in EDP production with bacteria and fungi are stated. The two main parts deal with the different aspects of the production of polyhydroxyalkanoates (PHAs) and polysaccharides like the different metabolic possibilities of the production strains, process development, downstream processing and product properties. Some background knowledge from the bio-sector might be useful for the understanding of this material.

In 1993, the annual world production of fossil fuel-based, or conventional, polymers (which will here often be vernacularly referred to as 'plastics') was 100 million tons, and the output may reach 150 million tons by 2000 [1].

In developed countries, most goods made of plastics end up after their useful life as discarded waste (69% of them in the United States), accounting of 20% by volume of U.S. landfills [4]. It has for many years been recognized that reducing plastic refuse could go a long way in preventing a landfill crisis. When discarded in nature, conventional polymers can persist for many decades, at best a mere eyesore, at worst posing a threat to wildlife. Consequently, proposed or already passed legislation in the U.S. and Europe aims at reducing the use of polymers [5]. However, the remarkable usefulness of polymers probably precludes any serious slowdown in their production.

Along with photolysis, biodegradation is one of the two principal ways by which some polymers can break down. Biodegradability is defined as the capacity of to be broken down, especially into innocuous products, by the action of living things - as micro-organisms. Bacteria and fungi are the main participants in the process of biodegradation in the natural world. The breakdown of materials provides them with precursors for cell components and energy for energy-requiring processes. Biodegradation is thus nothing more than catabolism. One important type of such biologically produced, biodegradable plastics are polyhydroxyalkanoates (PHA), the main subject of this chapter.

Note: The references cited in this chapter refer to the literature index of PHAs in the InfoPack.

4.1 Generals of Biological Methods for Polymer Production

It is not only biodegradability that makes EDPs so fascinating. It is as well their synthesis from renewable carbon sources, based on agriculture, even on industrial wastes, allowing to come to a sustainable closed cycle process for production and use of such polyesters instead of the end-of-the-pipe technologies connected to production and use of classical plastics. One important type of such

biologically produced, biodegradable plastics are polyhydroxyalkanoates (PHA), the main subject of this chapter.

4.1.1 The Bioreactor

Many other interesting polymers cannot be obtained directly from nature. Either the sheer amount of polymer produced in the natural environment is too low for commercial exploitation or special conditions are needed to trigger the production of the polymers. These special conditions can be established in a so-called bioreactor. Also quite often polymers obtained from nature do not have the required properties needed for an application because of ever-fluctuating conditions in a natural environment. In a bioreactor certain conditions can be kept up over the whole production process and a polymer with optimal properties can be obtained.

In a bioreactor micro-organisms grow under controlled conditions and form the relevant product. Since biological systems are very sensitive towards changes of the environmental conditions, temperature, pH, dissolved oxygen concentration in aerobic processes and other parameters have to be controlled very carefully and regulation has to be very precise. Usually the micro-organisms grow at temperatures around 30°C, but some extremophiles are able to grow at much higher temperatures. The pH in bacterial fermentation should in general be kept around seven, fungi can prefer a much lower pH.

Two general types of media can be fed in the bioreactor: synthetic media contain defined carbon sources like glucose, fructose or other carbohydrates, sometimes even CO_2. $(NH_4)_2SO_4$ or ammonia water are used as nitrogen source. Several other elements like phosphor, magnesium, calcium and a variety of trace elements are also needed by the micro-organisms. The advantage of such a medium is, that the exact content of the compounds at any given moment during the fermentation can easily be determined by simple analytical methods. A major drawback is the high costs of the compounds used. Therefore these types of medium are mostly used in laboratory scale to study kinetics, for optimisation of growth and production and in the pharmaceutical industry.

Complex media often contain undefined carbon and nitrogen sources like meat extract, yeast extract or cheap and impure waste and surplus materials like molasses, starch hydrolysate, whey and corn steep liquor. Usually the exact composition of these materials is unknown and impurities can inhibit growth of the micro-organisms. These media are used for cheap production of large quantities of bulk materials. Accordingly when producing EDPs for mass market applications one will have to use these types of cheap production methods.

From this follows that growth conditions and nutritional status of the micro-organisms can be controlled very effectively. It is also possible to change the conditions with a distinct shift to trigger the production after a growth phase like in PHA production.

A crucial point in aerobic fermentation processes is the oxygen supply of the cells. The solubility of oxygen in water at these temperatures is quite low and accordingly mass transfer has to be enhanced by dispersing the gas in the bioreactor very well with the help of a special design of the reactors and vigorous stirring. Especially when working with high concentrations of bio-mass, which uses a lot of oxygen, sometimes pure oxygen has to be supplied to the fermenter instead of pressurised air.

Especially when producing exopolysaccharides the viscosity of the medium will increase dramatically and oxygen transfer will be limited. But in some cases a low oxygen concentration in the reactor is even desired. PHA and polysaccharide production for instance can be triggered by low oxygen concentrations. But this does of course not mean, that oxygen supply can be completely turned off, because in this case the cells would inevitably die.

When comparing a bioreactor with a chemical reactor there are several significant differences:

- Conditions in a bioreactor are much milder than in a chemical reactor since one is working with living organisms or parts thereof. Usually no pressure and temperatures around 30°C are applied. Nevertheless production under sterile conditions needs a lot of expensive apparatus.

- In bioreactor the product concentrations are sometimes very low. The medium is a complex mixture of different soluble and non-soluble substances and product recovery is quite difficult.

- In chemical syntheses pure and therefore expensive chemicals have to be used, while biological systems can use cheap and complex sources, which are often simply waste or surplus materials.

- Compared to chemical reactions bio-reactions are rather slow.

- The "bio-catalysts" used (cells, enzymes) are extremely selective and cheap and the product is usually formed in one step. With the help of genetic engineering "tailor-made" catalysts can be produced. Fresh catalyst can be produced simply by cell growth. Catalysts in chemical reactions are expensive and can only be regenerated to a certain extent. They very often contain toxic elements, which leads to high disposal costs. The strains however are submitted to mutations and genetically constructs may not be stable enough, especially in long time continuous fermentation.

4.1.2 Downstream Processing

Downstream processing summarizes all steps necessary to recover the product from the fermentation broth after the fermentation is finished. The broth is usually very complex consisting of water, proteins, salts, bio-mass, sometimes polysaccharides, which are not the product and several low molecular weight compounds. The recovery of the product can sometimes be the most difficult and expensive step in the whole production process.

Recovery of the product should be fast to prevent degradation of the product by hydrolytic enzymes and as selective as possible to avoid unnecessary steps and costs in downstream processing. For optimal use of the capacity of the equipment a continuous process is necessary. This is not always possible and often several reactors finishing the production at different times are linked to the same downstream equipment in order to use it more efficiently.

In general the first step is separation of bio-mass and broth by centrifugation. A broad range of different centrifuges is available. Which one is used depends on the viscosity of the broth, diameter of the cells, the flow and other parameters.

If the product is inside the cells centrifugation is already a major separation and concentration step. Either the product can be extracted from the cells (like PHAs) or the cells have to be ruptured by one of the many methods and the product has to be isolated from the cell homogenisate. The latter method is often used for the recovery of enzymes, which have to be separated from all the other proteins by chromatography, a very selective but also very expensive separation method.

If the product is in the supernatant of the centrifugation other components can often be removed by precipitation. Recovery of the product is the accomplished by extraction, adsorption, chromatography or a second precipitation step.

During all this steps it is very important to keep thermal and mechanical stress for the product as low as possible. E. g. extraction of PHAs with chloroform can lead to severe decreases of the molecular weight of the polymer due to formation of radicals and breaking of the polymer chains. Higher temperatures, shear stress and many organic compounds easily denature proteins. After its recovery the product is purified and dried. Of course the purification of a pharmaceutical product is much more extensive than that of a bulk product like EDPs.

Self-check Questions

1. Why are polysaccharides and PHAs so interesting?
2. What are the main differences between a bio-reactor and a chemical reactor?
3. Give a brief outline of a typical downstream process.

Hints for Answers

See section 4.1 and also Chapter 2.

Exercise

Discussion: "Can all fossil-fuel based polymers be substituted by EDPs? Why? Why not?"

Reading Materials:

1. http://europa.eu.int/comm/environment/index_en.htm
Here you can inform yourself about existing EU policies on environmental issues. Have a look at the chapters about waste, especially packaging, landfills and waste management statistics.

2. http://www.biomaterials.org/
The Society For Bio-materials is a professional society which promotes advances in all phases of materials research and development by encouragement of cooperative educational programs, clinical applications, and professional standards in the bio-materials field.

3. http://www.plasticsresource.com/
Nice site of the American Plastics Council about all things concerning plastics and environment in general.

4.2 Polyhydroxyalkanoates

Polyhydroxyalkanoates, or PHAs, are homo- or heteropolyesters synthesized and intracellularly stored by numerous prokaryotes. They can be produced in large quantities from renewable resources by means of well known fermentation processes and the imposition of particular culture conditions. A number of physical or chemical methods are known to extract them from the producing bio-mass. Production processes such as batch, semi-batch and continuous fermentation are all known to work. PHAs have properties similar to those of some polyolefins. This, combined with the fact that they are fully and rapidly biodegraded under the appropriate conditions, has generated a high interest in them as substitutes to petroleum-based polymers in many applications [10].

The majority of PHAs are aliphatic polyesters. Their general formula is shown in Figure 4.1. The composition of the side chain or atom R and value of x determine together the identity of a monomer unit. For poly(3-hydroxyalkanoates) (or poly(β-hydroxyalkanoates)), the most common PHAs, x is equal to 1. Pure Polyhydroxybutyrate [P(3HB)] is composed of monomers with a methyl group as side chain, and the Polyhydroxyvalerate [P(3HV)] units of Polyhydroxybutyrate-co-valerate (P(3HB-*co*-3HV))s contain an ethyl group on carbon number 3. A large number of PHAs other than P(3HB) and P(3HB-*co*-3HV)s are now known.

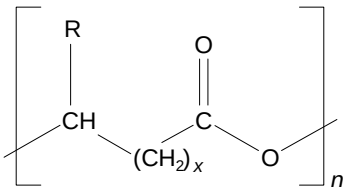


Figure 4.1 General formula of PHAs

PHAs are vastly distributed in the natural world. In addition to its and other PHAs' occurrence in numerous genera of eubacteria [reviewed in 90 and 91], low-molecular-mass P(3HB) has been found, as a short-chain oligomer (n = 100 to 200) complexed with other large molecules, in the cytoplasmic membrane of enterobacteria like *Escherichia coli* and in eukaryotes from plants to humans[92, 93].

PHAs are stored in the form of granules by bacteria. The observation of the granules as refractile bodies in bacterial cells under the microscope goes back at least to Beijerinck in 1888 [reported in 11]. The first determination of the composition of a PHA had to wait until 1927 and the work of Lemoigne [12]. During the following thirty years, interest in P(3HB) was scant and nearly restricted to the description of detection and cell-content estimation methods and to culture conditions that lead to its synthesis and degradation inside *Bacillus* cells [cited in 13]. A convincing proposal for a functional role for P(3HB) first came from Macrae and Wilkinson in 1958: The authors concluded that P(3HB) was a carbon- and energy-reserve material that slowed down cell autolysis and death, and correctly speculated on the involvement of acetate and coenzyme, a complexes in the pathway of P(3HB) synthesis [13]. The field of PHAs was well developed by the end of 1973, but interest in the

biopolymers remained directed almost solely at their physiological significance as microbiological substances.

Pure P(3HB) is brittle and has a low extension to break [63]. This lack of flexibility limits its range of applications, and if P(3HB) were the only existing polyhydroxyalkanoate, it is dubious that a large market niche could be found for PHAs. However, Wallen and Davis reported in 1972 the isolation from activated sludge of a polyester with physical and chemical properties not identical (but similar) to those of P(3HB) [63]. Analysis later revealed the presence of 3-hydroxyvaleric-acid (3HV) and 3-hydroxybutyric-acid units as major components, and 3-hydroxyhexanoic-acid, and possibly 3-hydroxyheptanoic-acid units as minor components of the new compound [64]. This was the first report of a heteropolymeric PHA. The potential significance of the existence of PHAs other than pure P(3HB) was recognized virtually right away, so that when ICI filed patents in the early 1980's for the production by various processes [68-70], extraction from producing cells [71-76], and blending with other organic polymers [77], of P(3HB), the company also claimed a process for the production by fermentation of bacterial copolyesters of 3HB and a range of other monomers, including 3HV units, from a variety of substrates, including carbohydrates such as glucose, and organic acids such as propionic acid [78].

Interest in copolymers, in particular in copolymers of 3HB and 3HV (i.e., P(3HB-*co*-3HV)s), stemmed from the fact that they have melting points much lower, and are less crystalline, more ductile, easier to mold and tougher, than pure P(3HB) [79], and are thus better candidates for commodity materials. Variation in their 3HV content leads to a range of properties spanning a wide variety of thermomechanical properties.

But the fact is that biodegradables have not yet replaced conventional plastics in a significant way. The cost of polyolefins like polyethylene and polypropylene is less than US \$1 kg^{-1} [reported in 85]. BIOPOL®, whose price has been drastically reduced since its production began [79], still sells at about seventeen times the price of synthetic plastics [86]. This impediment to the marketability of PHAs can only partly be alleviated by the willingness of the public to pay more for products that are considered environmentally friendly.

Self-check Questions

1. What are PHAs ? Explain their physiological role and their usefulness for mankind.
2. Draw the formulas of Poly(3-hydroxybutyrate), Poly(3-hydroxybutyrate-co-3-hydroxyvalerat) and Poly(3-hydroxybutyrate-co-4-hydroxybutyrate).
3. What are the drawbacks of pure PHB ? What types of PHAs are better and why?
4. Compare the costs of PHAs and polyolefins.

Hints for Answers

1, 3, 4: see section 4.2;
2 : see Figure 4.1

Exercise

Experiments:
Have a look at some bacterial cells containing PHB under the microscope. What do you see?
Compare some articles made of different types of polymers (PET, PS, PHB, PHBV). Do they have different properties? Are there differences between the two types of PHAs? Can you use all the polymers for all applications?

Reading Materials

http://www.metabolix.com/index.html
Homepage of the Metabolix Company with a technology profile of PHAs.

4.3 Recently Discovered Polyhydroxyalkanoates

4.3.1 Novel PHAs from *Ralstonia eutropha* and *Alcaligenes latus*

In addition to P(3HB) and P(3HB-*co*-3HV)s [75], *R. eutropha* has been shown to produce P(3HB-*co*-4HB) [94, 95], P(3HB-*co*-3HV-*co*-5HV) [96], and P(3HB-*co*-4HB-*co*-3HV) [97] polymers in the past. *A. latus* has also already been reported to synthesize P(3HB) [98] and P(3HB-*co*-3HV) [99, 100]. Nakamura et al. [101] reported the production of a pure P(4HB) homopolymer by *R. eutropha*. Valentin et al. [104] were able to obtain a P(3HB-*co*-3HV-*co*-4HV) terpolyester with up to 8.8 mol% 4HV from 4-hydroxyvaleric acid or 4-valerolactone as sole carbon sources in batch, fed-batch or two-stage fed-batch cultures of various strains of *R. eutropha*. A poly(3-hydroxybutyrate-*co*-3-hydroxypropionate) copolyester has been produced by R. eutropha in a nitrogen-free medium containing 3-hydroxypropionic (3HP) acid, 1,5-pentanediol or 1,7-heptanediol [105].

4.3.2 Novel PHAs from the *Pseudomonas* genus

Pseudomonads are undoubtedly the most versatile accumulators of PHAs. The syntheses by *P. oleovorans* of four- to twelve-carbon monomers (R = CH_3 to $(CH_2)_8CH_3$) from n-alkanes, n-alkanoates and n-alcohols, unsaturated monomers from n-alkenes, and of branched-side-chain units from branched substrates, have been reviewed [87], along with PHA accumulation from n-alkanoic acids by other pseudomonads. Huisman et al. [108] advanced that the capacity to accumulate a wide range of PHAs with very little or no 3HB units was a distinguishing trait of fluorescent pseudomonads. The composition of PHA monomers synthesized by pseudomonads is related to that of their substrates, with most units containing 2 carbon atoms less than the carbon source.

More recent investigations by Huijberts et al. [109] with *P. putida* revealed the synthesis by this micro-organism growing on glucose of PHAs composed of seven different monomers, including units of 3-hydroxydecanoate (3HD; the major constituent), 3-hydroxyhexanoate (3HHx), 3-hydroxyoctanoate (3HO), and saturated and mono-unsaturated monomers of 12 and 14 carbon atoms.

Other unsaturated, medium-side-chain (MSC) PHAs from pseudomonads have been lately reported. Lee and colleagues [106] used *Pseudomonas* sp. A33 and other related organisms isolated by Schirmer et al. [107] to produce various copolyesters. *Pseudomonas* sp. A33 in the presence of 1,3-butanediol stored a PHA of 3HB units and nine other different constituents, including the saturated, 16-carbon 3-hydroxyhexadecanoate (3HHD. The authors used several techniques to demonstrate that this PHA was a real copolymer and not a blend of polymers, but did not determine whether it had a random distribution of monomers or consisted of block structures.

Poly(3-hydroxyalkanoates) with phenyl units as part of the functional group have been produced by *P. oleovorans*. Kim et al. [114] fed the organism with mixtures of 5-phenylvaleric acid and either n-nonanoic acid or n-octanoic acid, to obtain two different polymers, one of 3-hydroxyalkanoate units corresponding to the fed alkanoate, the other of 3-hydroxy-5-phenylvalerate (3H5PV). 3H5PV made up to 40.6 mol% of the total polymer, which reached 31.6% in mass of the dry cell matter (CDM).

PHAs with halogenated functional groups can be synthesized by *P. oleovorans*. In addition to the chlorinated and fluorinated polymers reported [116 - 118], poly(3-hydroxyalkanoate) copolymers containing brominated repeating units have been produced. Kim et al. [119].

Recently, Bear et al. were able to produce a copolyester containing up to 37 % terminal epoxy groups in the side chains, when *P. oleovorans* was fed with a mixture of 10-epoxyundecanoic acid and sodium octanoate [120].

4.3.3 Novel PHAs from Other Microorganisms

R. rubrum has recently been used by Ulmer et al. [121] to synthesize unusual polymers containing 3HB, 3HV, and 3-hydroxy-4-pentenoate (3H4PE) repeating units. A poly(3HV) homopolymer has been synthesized by three strains of *Chromobacterium violaceum* fed on sodium valerate [125].

Self-check Questions

1. What new types of PHAs were found recently ?
2. Are the bacteria able to accumulate large amounts of these special PHAs ?
3. What are the advantages of polymers with side chains containing functional groups ?

Hints for Answers

Read through section 4.3 again

Exercise

Discussion: "Think of advantages and fields of application for these new polymers."

4.4 Intracellular Aspect of PHA Granules

The number of P(3HB) granules in the cytoplasm of *R. eutropha* has been observed to remain constant at eight to twelve during cultivation in nitrogen limitation [129]. Accommodation of additional polymer was done through increases in the diameter of the granules, gradually forcing the cells to change their shape from cylindrical to spherical. The cells invariably stopped increasing their P(3HB) content at around 80% of the CDM in polymer, and as PHA synthase activity and intracellular monomer concentration remained high at this point, the authors concluded that physical constraints at the level of cell geometry were the limiting factor for polymer accumulation. At the same time, the molecular mass (MM) of the polymer decreased gradually during a fermentation. In phosphate-limited culture, the MM of P(3HB) in *R. eutropha* went down from 2×10^6 to 6×10^5 Da as the polymer content of the cells increased [129]. Similar results were obtained under different culture conditions.

Studies with numerous organisms have shown that *in vivo* P(3HB) granules have diameters of 0.2 to 0.7 µm and are surrounded by a membrane coat composed of lipid and protein about 2 nm thick [27, 30, 131 - 133]. The polymer chains generally form helices, and each granule probably contains a minimum of 1000 molecules [87]. X-ray-diffraction analysis of solid P(3HB) led initially to the belief that the core of the inclusion bodies was crystalline [22, 23], but more recent ^{13}C-NMR spectroscopy of whole cells of *Methylobacterium* and *R. eutropha* by Barnard and Sanders showed that the bulk of the P(3HB) homopolymer and P(3HB-*co*-3HV) copolymer is in fact in a very labile state, well above its glass-transition point [134, 135]. In a recombinant strain of *E. coli* harboring the PHA genes of *R. eutropha*, however, Hahn et al. [136] have deduced that the accumulated P(3HB) was in a quasicrystalline form, possibly due to hydrogen bonding to other molecules or cations.

The activities of PHA synthase and PHA depolymerase are closely related to the membrane protein layer of the granules [32, 45, 49, 140 - 142]. P(3HB) extraction methods that damage or destroy the membrane lead to loss of synthase activity and increased susceptibility to depolymerization.

Self-check Questions

1. Describe the approximate size and structure of PHA granules
2. What upper limit of PHA content can be reached in cells of *B. megaterium* and why?
3. Where are the PHA synthase and depolymerase enzymes located?

Hints for Answers

See Chapter 4

4.5 Polyhydroxyalkanoate Metabolism in R. eutropha and A. latus

The pathways and enzymology of PHA synthesis and degradation have been studied in many organisms. P(3HB) synthesis and degradation were shown to be the complementary parts of a cycle in *R. eutropha* and *Azotobacter beijerinckii* more than twenty years ago [38, 41]. Since then, synthesis of PHAs other than P(3HB) has been partly or completely elucidated in *R. eutropha*. In contrast, much less is known about *A. latus*.

4.5.1 Metabolism During Balanced Growth

R. eutropha and *A. latus* catabolize carbohydrates via the Entner-Doudoroff pathway to pyruvate, which can then be converted through dehydrogenation to Acetyl-Coenzyme A (acetyl-CoA). During reproductive growth, acetyl-CoA enters the tricarboxylic acid (TCA) cycle with the release of free Coenzyme A (CoASH) and is terminally oxidized to CO_2 generating energy in the form of Adenosinetriphosphate (ATP) reducing equivalents in the form of Nicotinamidadenindinucleotide (NADH), phosphorylated Nicotinamidadenindinucleotide (NADPH) and Flavinadenindinucleotide ($FADH_2$), and biosynthetic precursors (2-oxoglutarate, oxaloacetate) [8]. Direct amination or transamination of the oxaloacetate leads to the synthesis of amino acids, which are incorporated into the polypeptide chains of nascent proteins. Oxidation of the TCA-produced pyridine nucleotides in the respiratory chain generates additional phosphate-dependent ATP, which can support the endergonic requirements of protein biosynthesis. The rate of admission of acetyl-CoA into the TCA cycle is thus contingent upon the availability of sources of nitrogen, phosphorus and other elements, as well as on the oxidative potential of the environment.

Synthesis of P(3HB) from acetyl-CoA condensation (see below) during cell reproduction is never completely non-existent - and can be substantial in *A. latus* - reflecting the availability of acetyl-CoA to be used for purposes other than oxidation even in these conditions.

4.5.2 Triggering Mechanism for Increased Polymer Accumulation

The rate of P(3HB) synthesis can however increase significantly when cells encounter growth-limiting conditions other than a limitation in the carbon source. In *R. eutropha* deficiencies in nitrogen, phosphorus, oxygen [18, 43, 143], magnesium, or sulfate [144] are known to work. Cessation of protein synthesis leads to high concentrations of NADH and NADPH. These in turn inhibit citrate synthase and isocitrate dehydrogenase, resulting in a slowdown of the TCA cycle and the channeling of acetyl-CoA towards P(3HB) biosynthesis [21]. The potential role of citrate synthase in the regulation of P(3HB) production via its ability to control carbon flux into the tricarboxylic acid cycle is discussed by Henderson and Jones [145]. This can result in massive accumulation of the polymer. Metabolic flux analysis for P(3HB) synthesis from various carbon sources have shown that the maximum P(3HB) yield may be limited by the available NADPH [146]. In recombinant *E. coli*, the level of NADPH and/or the NADPH/NADP ratio seem to be the most critical factor regulating the activity of acetoacetyl -CoA reductase and, subsequently, P(3HB) synthesis (NADP = oxidized form of NADPH) [147].

When carbon sources other than strictly acetyl-CoA precursors (e.g. valeric acid) are also present under these conditions, they can be incorporated into the polymer chain, leading to monomers other than 3HB units.

Self-check Questions

1. What are the conditions triggering the accumulation of PHAs ?
2. Which limitations are possible ? Why not a carbon limitation ?
3. What is happening on the coenzyme level, while PHA accumulation is triggered ?

Hints for Answers

See section 4.5.1 and 4.5.2

Exercises

Draw a general scheme of a bacterial metabolism (Entner-Doudoroff, TCA-cycle and maybe NADPH regeneration + ATP synthesis) with the help of textbooks. Where are reactions or “junctions” important for PHA synthesis? Which parts of the metabolism are theoretically inactive during PHA-synthesis?

Reading Materials

http://www.wsu.edu/~hurlbert/pages/Chap7.html

Excellent online text book chapter about bacterial metabolism and enzymology from the Washington State University home page. Includes links to other great metabolism sites.

4.5.3 Pathways of PHA Synthesis

The pathways of PHA synthesis in *R. eutropha* from various substrates in nitrogen-free conditions are shown in figure 4.2.

P(3HB) is produced from acetyl-CoA by the sequential action of three enzymes, 3-ketothiolase, acetoacetyl-CoA reductase and PHA synthase (Pathway I) [38]. 3-Ketothiolase reversibly combines two acetyl-CoAs into acetoacetyl-CoA and is competitively inhibited by high concentrations of CoASH, which is released when acetyl-CoA enters the TCA cycle [148]. NADPH-dependent acetoacetyl-CoA reductase reduces its substrate to R-3-hydroxybutyryl-CoA, and this is incorporated by PHA synthase into the polymer chain. 3HB units can also be synthesized form butyric acid directly via acetoacetyl-CoA without its prior degradation to acetyl-CoA (Pathway II) [148]. In this sequence of reactions featuring β-oxidation of the substrate, both NADH-linked and NADPH-linked acetoacetyl-CoA reductases effect the epimerization of S-3-hydroxybutyryl-CoA to the R isomer, and 3-ketothiolase is not involved.

When propionic acid is present in the medium, 3-ketothiolase condenses one propionyl-CoA with one acetyl-CoA to form 3-ketovaleryl-CoA, which is polymerized after reduction to 3-hydroxyvalerate monomers by PHA synthase (Pathway III) [149]. Acetyl-CoA can be provided by a second substrate, but elimination of the carbonyl carbon of propionyl-CoA always takes place to some extent, so that both 3HB and 3HV units are synthesized when propionic acid is the sole carbon source. Still, the 3HV fraction in the copolymer rises with increasing ratio of propionic acid to acetyl-CoA-generating substrate [149].

Valeric acid can also serve as a precursor for 3-hydroxyvalerate units (Pathway IV) [148]. Similarly to 3HB synthesis from butyric acid, this does not involve the catabolism of the acid to a shorter alkyl-CoA, but rather its direct incorporation into the polymer via valeryl-CoA and its β-oxidation to 3-hydroxyvaleryl-CoA. Use of valeric acid leads thus to higher 3HV contents in the polymer in *R. eutropha* than propionic acid, as decarboxylation of propionyl-CoA and loss of units with an odd number of carbons is reduced - but not totally eliminated, as the intermediary S-3-hydroxyvaleryl-CoA can be degraded to propionyl-CoA and acetyl-CoA [150].

Figure 4.2 also shows the pathway of 4-hydroxybutyrate synthesis from 4-hydroxybutyric acid (Pathway V) [90, 151]. 4-Hydroxybutyryl-CoA is first formed, and part of it is polymerized by PHA synthase. A portion of the hydroxyacyl-CoA is however dehydrated to the corresponding enoyl-CoA, which enters the pathway of 3HB synthesis via R-3-hydroxybutyryl-CoA. According to Valentin et al. [144] it is more likely that formation of 3-hydroxybutyryl-CoA occurs via succinate semialdehyde, succinate, pyruvate, and acetyl-CoA from 4-hydroxybutyrate. A copolymer of 3HB and 4HB is thus usually produced from 4HB acid.

Nakamura et al. [105] have proposed a pathway for the synthesis of 3-hydroxypropionate in *R. eutropha* (Pathway VI). When 3HP acid is present as the sole carbon source in nitrogen-free medium, its metabolization into 3-hydroxypropionyl-CoA is largely followed by a decarboxylation to acetyl-CoA and 3HB synthesis. But a portion of the 3-hydroxypropionyl-CoA is also directly polymerized into 3HP, presumably under the action of PHA synthase, producing random copolyester of 3HB and 3HP units. In contrast, *A. latus* DSM 1124 cannot grow on 3HP acid.

Synthesis of P (3HB-*co*-3HV) from 2-hydroxyoctanoic (2HO) acid or 12-hydroxystearic acid (18 carbon atoms) by *Alcaligenes* AK 201 has been tentatively explained by Akiyama and Doi [153] (Pathway VIII). When 2HO acid is used as the sole carbon source, β-oxidation of the compound would yield CO_2 and n-heptanoate, which could be further incorporated as acetyl-CoA and valeryl-CoA in the copolyester through pathways I and IV in Fig. 4.2. 12-Hydroxystearate would first be degraded to 2HO by a five successive β-oxidative cleavages, producing acetyl-CoAs in the process.

Self-check Questions

1. Which different cosubstrates do you know? What types of polymers are formed from these cosubstrates?
2. What are the differences between the pathways?
3. Why are only the R isomers incorporated into the polymer?

Hints for Answers

See section 4.3 and 4.5.3

Exercise

Find the prices of glucose and as many cosubstrates as possible (fine chemicals). Judging from these prices do you think that heteropolymers are cheaper or more expensive than the homopolymer PHB?

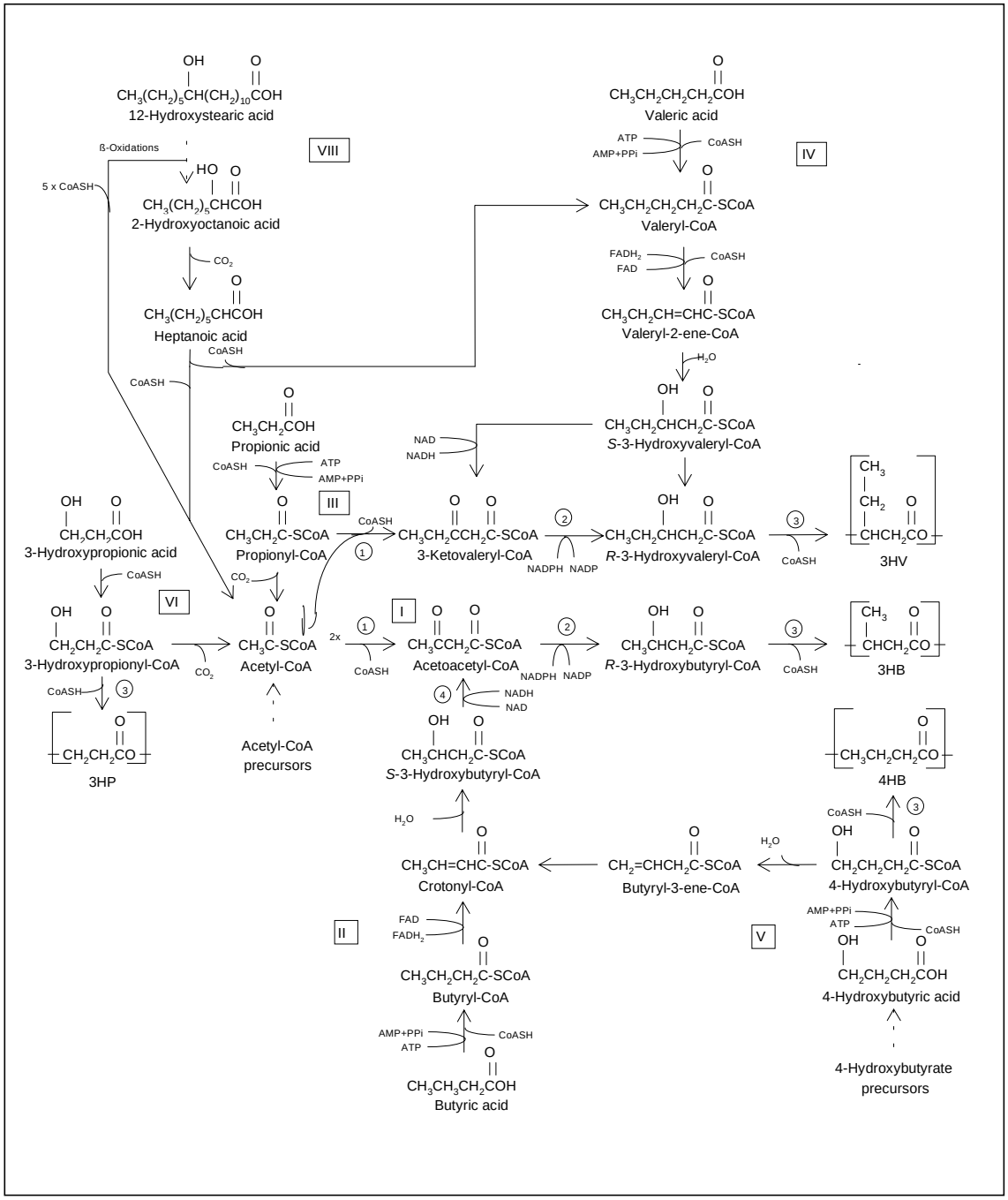


Figure 4.2 Pathways of PHA synthesis in *R. eutropha*, except for Pathway VII (*Alcaligenes* AK 201) [38, 90, 105, 146, 149, 150, 151, 153]. Enzymes: ①, 3-ketothiolase; ②, NADPH-dependent acetoacetyl-CoA reductase; ③, PHA synthase; ④, NADH-dependent acetoacetyl-CoA reductase. See text for details.

4.5.4 Enzymology of PHA Synthesis in *R. eutropha*

3-Ketothiolase

Haywood et al. purified 3-ketothiolase (acetyl-CoA acetyltransferase) from a glucose-utilizing strain of *R. eutropha* [155]. They found it to consist of two distinct constitutive isoenzymes, 3-ketothiolase A and B, each with its own substrate specificity. 3-Ketothiolase A is active with only four- or five-carbon 3-ketoacyl-CoAs and must solely be responsible for PHA synthesis in *R. eutropha*, as this micro-organism does not accumulate PHAs with hydroxyacid repeating units of more than five carbon atoms. 3-Ketothiolase B has a broader specificity (four- to ten-carbon 3-ketoacyl-CoAs), and Haywood and colleagues have speculated that it may have a function other than PHA accumulation.

The authors observed that the condensation reaction effected by 3-ketothiolase was strongly inhibited by free coenzyme A, as mentioned above. This accounts for the low P(3HB) levels in *R. eutropha* during unrestricted growth on acetyl-CoA-generating substrates, when high amounts of CoASH are released by the TCA cycle. The involvement or not of 3-ketothiolase can thus be a key factor in P(3HB)-metabolism regulation in *R. eutropha*. The use of pentanoic acid as 3HV precursor also limits the role of 3-ketothiolase.

NADPH- or NADH-dependent acetoacetyl-CoA reductases

Two acetoacyl-CoA reductases were found by Haywood et al. in *R. eutropha* [cited in 87], each with a distinct substrate and coenzyme (NADH or NADPH) specificity. NADPH-dependent reductase mediates the reversible reduction of four- to six-carbon 3-ketoacyl-CoAs to only the R isomers of hydroxyacyl-CoAs.

PHA synthase

The PHA synthase of *R. eutropha* is capable of polymerizing 3-hydroxy-, 4-hydroxy-, and 5-hydroxyalkanoates from R isomers of four- to five-carbon hydroxyacyl-CoAs [140, 104], although its activity is markedly higher with four-carbon substrates [140]. If Nakamura et al. [105] (see above) are right in their speculation on the pathway of 3HP synthesis, the enzyme must also effect the polymerization of 3-carbon compounds. It has been isolated in two forms, a soluble one predominating during unrestricted growth of the cells, and a granule-associated one when culture conditions favored P(3HB) accumulation [140].

Self-check Questions

1. Draw a scheme of the PHA synthesis, name the enzymes and explain their function.
2. How many different forms of each enzyme are known?
3. Which enzymes are important for the regulation of PHA synthesis?

Hints for Answers

See section 4.5.4 and figure 4.2

4.5.5 Genes for PHA Synthesis

The three genes for PHA biosynthesis in *R. eutropha* have been characterized and cloned in *E. coli* [160 - 163]. The resulting recombinant strains were able to accumulate large amounts of the polymer. The hypothesis by Schubert et al. [162] that the genes for 3-ketothiolase, acetyl-CoA reductase and PHA synthase are clustered has been confirmed, they form a single operon (combination of promoter, operator and structural genes) [164], and Peoples and Sinskey [161] have shown that the three enzymes are coded in the order synthase-thiolase-reductase. They could not determine whether one or more promoters were involved, but Schubert et al. identified the promoter for the PHA synthase gene and noted that it is probably that of the whole operon [164]. PHA synthesis is subject to transcriptional control [163], as is usual for metabolic pathways affected by environmental conditions.

Genser and co-workers [165] have isolated, sequenced, and expressed in *E. coli* the three PHA genes of *A. latus*. Sequencing revealed high respective homologies (71 to 80%) to the *R. eutropha* genes and the same orientation and organization, suggesting a single-operon arrangement here also. A functional promoter, of different structure and possibly more active than that from *R. eutropha*, was located

upstream of the PHA synthase gene. Based on the nature of the homologies, the authors concluded that *R. eutropha* and *A. latus* inherited their PHA genes by horizontal transfer from a common ancestor.

4.5.6 Intracellular PHA Degradation, Cyclic Nature of the PHA Metabolism

The intracellular degradation of P(3HB) has been studied in detail in a number of organisms [21]. P(3HB) hydrolysis is effected by the sequential action of PHA depolymerase, R-3-hydroxybutyrate dehydrogenase and acetoacetyl-CoA synthetase, to form R-3-hydroxybutyric acid and acetyl-CoA [90]. In *R. eutropha*, the sole product of P(3HB) hydrolysis is R-3-hydroxybutyric acid, but a mixture of dimers and monomers of the acid can be obtained in other organisms [45]. In nitrogen-free cultures, Doi et al. [156] have studied the kinetics of P(3HB) accumulation and degradation in *R. eutropha* during carbon excess and limitation, respectively. They found that the rate of polymer hydrolysis (degradation) was about 10 times lower than that of its synthesis, and proposed a reaction scheme for the depolymerization process.

Studies with *B. megaterium* showed that, unlike PHA synthase, PHA depolymerase is in a soluble form, not bound to the granule [29]. There may however be a protein component of the granule outer-membrane that inhibits depolymerase activity [84]. This would explain the increase in polymer hydrolysis upon damage on granule structure by solvent action.

PHA metabolism in *R. eutropha* was shown to be a cyclic process during P(3HB) and P(3HB-*co*-3HV) synthesis in nitrogen-free medium. In shake-flask experiments, Doi et al. [166] submitted *R. eutropha* cells containing P(3HB) from butyric acid to P(3HB-*co*-3HV)-accumulating conditions (pentanoic acid as the sole carbon source). By analysis of the change in polymer composition with differential-scanning-calorimetry melting curves, they could show that the P(3HB) homopolymer was gradually replaced by a copolymer at a relatively constant 3HV fraction (50 ± 5 mol%). Almost complete replacement of the homopolymer was achieved after 96 h. In the reverse experiment, P(3HB-*co*-3HV) was similarly replaced by P(3HB). These investigations showed that polymer synthesis is concomitant with its degradation in *R. eutropha* under nitrogen starvation, resulting in cyclic PHA metabolism.

4.5.7 Enzymology of Extracellular PHA Degradation

Although a number of micro-organisms have long been known to be capable of extracellular PHA degradation [21], until recently only the extracellular P(3HB)-depolymerase systems of *Pseudomonas lemoignei* and the activated-sludge isolate *Alcaligenes faecalis* T1 had been purified and characterized, and the P(3HB) depolymerase gene of the latter cloned and sequenced [48, 178 – 180]. The enzyme's main products of P(3HB) degradation were dimeric and trimeric esters of 3-hydroxybutyrate. Jendrossek et al. [183], however, isolated a bacterium identified as *Comamonas* sp. whose extracellular depolymerase degraded P(3HB) to monomeric 3-hydroxybutyrate, indicating a mechanism of hydrolysis not reported so far.

The depolymerase system and genes of *P. lemoignei* have been further investigated by Jendrossek's group [177, 184, 185]. The authors identified, cloned and sequenced five PHA depolymerase genes from this organism, including one for the degradation of pure P(3HV) in addition to that of P(3HB) and P(3HB-*co*-3HV)s. An extra-cellular P(3HO) depolymerase from a new *P. fluorescens* isolate was also characterized by Jendrossek and colleagues [111], and its gene was cloned in *E. coli*, sequenced and characterized [186]. The enzyme's main product was the dimeric ester of 3HO.

Self-check Questions

1. Complete the scheme you made in section 4.5.4 with the steps for PHA degradation.
2. Why has the PHA metabolism cyclic nature?
3. What would happen if the polymerization rate would be lower, equal or higher than the depolymerization rate?
4. Why is extra-cellular depolymerization of PHAs so important?

Hints for Answers

See Figure 4.2, section 4.5.4 and 4.5.6.

4.6 Detection and Analysis of PHAs

PHAs can be detected and their content in cells determined by a number of methods [21, 87]. The most common detection technique *in vivo* is the fluorescent staining of granules [188]. Many methods for the analysis of cell content, structure and composition of P(3HB) and other PHAs have been reported, including gas chromatography (GC) after solvent extraction and hydrolytic esterification of the polymer [189, 190], pyrolysis under nitrogen of extracted PHAs followed by GC-mass spectrometry [191], and a variety of ^{1}H- and ^{13}C-NMR (Nuclear magnetic resonance spectrometry) techniques [192, 193]. P(3HB) can also be determined by IR-spectrometry (Infrared spectrometry) after extraction in chloroform [194]. Molecular-mass determinations are now typically performed by gel-permeation chromatography, which has mostly replaced the earlier calculations from the intrinsic viscosity of P(3HB) solutions [see 21]. Glass transition and melting temperatures of solid-state PHAs are estimated by differential scanning calorimetry [87].

Zeiser [reported in 195] modified the conditions of the gas-chromatographic detection of poly(3-hydroxyalkanoates) developed in 1978 [189] to adapt the method for the determination of P(3HB-*co*-4HB). Measurement of P(3HB) in live cells of *R. eutropha* by flow cytometry and spectrofluorometry after Nile-Red staining of granules has been recently investigated [196]. The authors noted that the rapidity of the methods could be useful for process control during cultivation.

Self-check Questions

1. Name some of the methods for determining the structure and composition of PHAs.
2. Why is it important to know the composition of PHAs ?

Hints for Answers

See section 4.2 and 4.6.

Exercises

Find the characteristic peaks of PHAs in IR, NMR and GC spectra (for advanced trainees)

4.7 Some Physical Properties of PHAs

4.7.1 Solid-state Conformation

Solid-state P(3HB) is a compact right-handed helix with a two-fold screw axis (i.e. two monomer units complete one turn of the helix) and a fiber repeat of 0.596 nm [204]. The forces underlying this conformation are mainly van-der-Waals interactions between the carbonyl oxygens and the methyl groups. The stereoregularity of P(3HB) makes it a highly crystalline material. It is optically active, with the chiral carbon always in the R absolute configuration in biologically produced P(3HB). Its melting point is around 177 °C [79], close to that of polypropylene, with which it has other similar properties, although the biopolymer is stiffer and more brittle [60, 205].

P(3HB-*co*-3HV) chains also have crystalline conformations [204]. The properties of copolymers of 3HB and 3HV vary with their content in 3HV. The melting temperature of P(3HB-*co*-3HV) has a minimum (ca. 80°C) at a 3HV molar fraction of about 30% (pseudo-eutectic point). Below this 3HV content, the P(3HB) lattice is the sole crystalline phase, while above 30 mol % 3HV, P(3HB) units are embedded in a P(3HV) crystalline matrix. The distribution of the two monomers is statistically random [206]. Their overall lower crystallinity and glass-transition temperatures confer on P(3HB-*co*-3HV)s enhanced mechanical properties, such as toughness and softness, that make them more interesting thermoplastics than pure P(3HB) [205]. Random copolymers of 3HB and 4HB also display lower crystallinity and glass-transition points than P(3HB) [94], resulting in mechanical behaviors close to those of elastic rubbers when the 4HB content exceeds 40 mol % [cited in 87]. Similarly, the crystallinity of P(3HB-*co*-3HP) copolymers decreases with increasing fraction of 3HP units [105].

4.7.2 Viscoelastic Relaxation and Thermal Properties of PHAs

3-Hydroxybutyrate-3-hydroxyvalerate (3HB-3HV) as well as 3-hydroxybutyrate-4-hydroxy-butyrate (3HB-4HB) copolyesters have been investigated by differential scanning calorimetry, thermogravimetric analysis and dynamic mechanical spectroscopy, over a wide range of compositions (0-95 mol% 3HV; 0-82 mol% 4HB). Both series of isolated copolyesters are partially crystalline at all compositions. Quenched samples show a glass transition temperature (Tg) that decreases linearly with increasing co-monomer molar fraction, more markedly when the co-monomer is 4HB. Above Tg, all copolyesters, rich in 3HB units, show a cold crystallization phenomenon followed by melting, while at the other end crystallization on heating is observed only in 3HB-3HV copolymers. The viscoelastic spectrum, strongly affected by thermal history, shows two relaxation regions: the glass transition, whose location depends on copolymer type and composition, and a secondary dispersion region at low temperatures (-130/-80°C). The latter results from a water-related relaxation analogous to that of P(3HB) and, in 3HB-4HB copolymers, from another overlapping absorption peak centered at -130°C, attributed to local motion of the methylene groups in the linear 4HB units [208].

4.7.3 Molecular Mass and Molecular-mass Distribution of Extracted PHAs

The molecular-mass distribution of a polymer is an indicator of the distribution of its individual molecules' molecular mass (MM) around the average molecular mass; a narrow distribution around a high average is usually desired. In addition to being a function of the producing organism and the strategy of production (duration of fermentation, growth rate, carbon-source concentration, etc.) [87], the average MM of PHAs is affected by the method of extraction. Values have up to recently typically ranged between 2 x 10^5 to 2 x 10^6 Da [87]. Zeneca considered MMs of about 600 000 Da acceptable for the thermoplastic applications of its BIOPOL® [79].

More recently, Page's group [210] used the same strain of *A. vinelandii* growing on beet molasses to produce a 4-million-Da P(3HB), quite possibly the highest MM PHA reported so far. The authors studied the effects of the substrate on the degree of polymerization.

Self-check Questions

1. What structure has P(3HB) in solid state ?
2. Explain why the properties like melting temperature ™, Tg and others change with increasing comonomer content. How do they change?
3. Which MM is considered to be acceptable for thermoplastic applications? Do bacteria produce PHAs with MM in that range?

Hints for Answers

See section 4.7

Reading Materials

http://www.psrc.usm.edu/macrog/index.htm

Learn more about the glass transition point and other important polymer properties and how to determine them in this on line training course from the University of Southern Mississippi.

4.7.4 Biodegradability

P(3HB) and P(3HB-*co*-3HV)s are degraded in both aerobic and anaerobic environments by the action of extracellular enzymes from microbial populations [79]. Doi et al. [151] have further pursued their early studies on the hydrolytic and enzymatic degradation of films of P(3HB), P(3HB-*co*-3HV)s and P(3HB-*co*-4HB)s in various environments, studies that found that the presence of 4HB units enhances the rates of both types of erosion. Nakamura et al. [101] exposed P(3HB-*co*-4HB) films to extracellular PHA depolymerase isolated from *Alcaligenes faecalis*. Enzymatic degradation as measured by weight loss was accelerated by 4HB contents up to 28 mol%, but depolymerization was inhibited at 4HB fractions above 85 mol% of the copolyester. In another set of similar experiments [217], the critical 4HB fraction was 13 mol%, at which point the rate of degradation was about 10 times faster than that of the homopolymer P(3HB). Doi and colleagues [218] have speculated that this acceleration could be attributed to the decreased crystallinity of 4HB copolymers relative to P(3HB) and P(3HB-*co*-3HV)s,

offering the degradative enzymes better access to the polymer chains. Nishida and Tokiwa [219] confirmed that crystallinity depressed the microbial degradability of P(3HB). A P(3HB-*co*-4-mol% 3HP) copolyester was found to enzymatically degrade faster than P(3HB) [105].

Nishida and Tokiwa's [219] observations suggested two different methods of microbial attack on P(3HB): a preferential degradation of amorphous regions of the polymer by extra-cellular depolymerase, followed by colonization by bacteria of the film surface and subsequent localized degradation.

Although the conditions in conventional municipal landfills are reputed to be unfavorable to biodegradation [220], P(3HB-*co*-3HV) was observed to lose weight in a simulated landfill environment, albeit at slower rates than those estimated in anaerobic sewage conditions and for P(3HB) in certain types of soils [79]. Mergaert and co-workers [221] investigated the decomposition of P(3HB), P(3HB-*co*-10-mol% 3HV) and P(3HB-*co*-20-mol% 3HV) in household compost heaps. After 150 days, a substantial mass loss was observed for the P(3HB-*co*-20-mol% 3HV) only, but the authors noted that degradation rates depend strongly on the microbial population involved, the substrate specificity of the extra-cellular enzymes and the temperature, as has been mentioned elsewhere [219].

Self-check Questions
What influences besides microbial attack lead to degradation of polymers?

Hints for Answers
See section 4.1 and 4.7.4

4.8 Strategies of PHA Production

Since the early fermentation descriptions by Baptist [52, 53] and others [68-70, 78], an important amount of research has looked into the optimization of PHA production processes. While not directly concerning the matter of process improvement, much literature on PHAs has potential impacts on existing production technologies: the use of novel substrates, the utilization of new organisms, and the better understanding of known ones (and the role PHAs play in their lives). Other research efforts have specifically addressed the issue of productivity. Examples of these are reports on the obtainment of better substrate-to-product yields and production rates through improved control of conventional systems, and on the development of innovative fermentation techniques.

4.8.1 Investigations and Variations of the Conventional Strategy
PHA concentrations of greater than 80 g l^{-1} with productivity of greater than 2 g PHA l^{-1} h^{-1} can be routinely obtained by fed-batch cultivation of several bacteria. Metabolic engineering approaches have been used to expand the spectrum of utilizable substrates and to improve PHA production. These advances will lower the price of PHA from the current market price of ca. US$ 16 kg^{-1}, and will allow PHA to become a leading biodegradable plastic material in the near future [238].

4.8.1.1 Discontinuous regime
Kim et al. [114, 239] have used on-line glucose control to obtain high-cell-density cultures of *R. eutropha* with high concentrations of P(3HB) and P(3HB-*co*-3HV). Following the observation that growth of *R. eutropha* was maximized at glucose concentrations between 10 and 20 g L^{-1}, the authors kept the sugar content of a 2.5-L culture within these limits during both growth and P(3HB)-accumulation phases [114]. Close monitoring of the glucose concentration in the mineral-salts medium was achieved by either exit-gas analysis by mass spectrometry and stoichiometric deduction of glucose content from CO_2-evolution rate, or with automatic glucose assay of filtered broth samples. At its most productive, the culture produced in 50 h 164 g CDM L^{-1} containing 121 g P(3HB) L^{-1} (76%). Overall polymer productivity was thus 2.42 g L^{-1} h^{-1}.

In similar experiments [239], these authors added propionic acid to the glucose solution to produce P(3HB-*co*-3HV) during the accumulation phase. The effect of the ratio of propionic acid to glucose in

the solution on the final concentration, 3HV content, and productivity of copolymer was studied. Very high CDM and polymer concentrations were achieved here also. As the propionic acid-to-glucose feed ratio was increased from 0.17 to 0.52 mol mol^{-1}, the copolymer 3HV fraction went up from 4.3 to 14.3 mol%, but its productivity decreased from 2.55 to 1.64 g L^{-1} h^{-1}. Similarly, the yield of 3HV from propionic acid decreased from 0.33 to 0.28 mol mol^{-1}. These experiments by Kim and colleagues have yielded the highest copolymer productivity reported so far for PHA-producing fermentation.

Doi's group [241] has appropriately brought some of their previous work one step further by showing that P(3HB-*co*-3HV) synthesis from butyric and pentanoic acids can be exploited for the production of substantial amounts of the copolymer in a fermentor. In fed-batch cultures at high carbon-to-nitrogen ratio, the presence of the two carbon sources during the growth and accumulation phases produced after 30 h up to 13.5 g P(3HB-*co*-27-mol% 3HV) L^{-1} (72% of cell dry weight (CDW)) with high yields. Decreasing the carbon to nitrogen ratio (C/N ratio) led to a gradual inhibition of polymer synthesis simultaneous to an increase in its 3HV fraction. One possible significance of this work, not mentioned by its authors, is the effect of small quantities of nitrogen source during the accumulation phase. PHA-production processes usually feature the total exhaustion of an element essential for growth. In this case residual bio-mass (all non-polymer cell material) stays constant during polymer storage. In the aforementioned cultures of Koyama and Doi, the supply of small amounts of nitrogen during the accumulation period supported a slight but constant growth of the cells. This may have played a role in the high amounts of polymer produced. These results were confirmed by Aragao et al. maintaining a controlled residual growth capacity by feeding sub-optimal amounts of NH_4OH during PHA accumulation phase.

The ability of *Alcaligenes eutrophus* to grow and produce polyhydroxyalkanoates (PHA) on plant oils was evaluated by Fukui and Doi [245]. When olive oil, corn oil, or palm oil was fed as a sole carbon source, the wild-type strain of *A. eutrophus* grew well and accumulated poly(3-hydroxybutyrate) homopolymer up to approximately 80% (w/w) of the cell dry weight during its stationary growth phase.

In 1985 Braunegg and Bogensberger [175] have shown for the first time, that PHA production can as well occur associated to the growth of microorganisms. Dry bio-mass of *A. latus* DSM 1123 when grown on sucrose as a sole carbon source showed a PHB content between 58 % and 70% without any special growth limitation applied. Culture conditions for the optimum growth and biosynthesis of PHB in *Alcaligenes latus* DSM 1123 were investigated by Cho et al [246]. Optimum carbon and nitrogen sources and their concentrations for growth were detected, and batch and fed-batch fermentation were performed in a 2.5 L jar type aerobic fermentor with various pH control solutions. Sucrose and $(NH_4)_2SO_4$ were the most effective carbon and nitrogen sources for the growth of *A. latus*. The optimum C/N ratio varied with the concentrations of carbon and nitrogen sources. The maximum specific growth rate was obtained at the sucrose concentration of 30 g/L and C/N ratio of 30. The specific growth rate increased more than two times and lag time was reduced when yeast extract and polypeptone were added. PHB could be synthesized in the logarithmic growth phase. By using NH_4OH and NaOH solutions in the first and second stage as pH control solutions, significant increases in the specific growth rate, bio-mass and PHB concentrations were observed. Under optimal conditions, the maximal bio-mass and PHB accumulation yield($Y_{P/X}$) attained after 40 h were 17.6 g/L and 46%, respectively.

A two-stage fed-batch method employing two different micro-organisms growing on two substrates in complex medium was reported by Tanaka et al. [247] to produce P(3HB). In the first stage, the pentose xylose was converted by a strain of *Lactococcus lactis* to a mixture of lactic and acetic acids. After removal of the cells by (presumably aseptic) centrifugation, *R. eutropha* was used to inoculate the supernatant in the same 1-L fermentor. No nutrient deficiency was present to favor polymer synthesis, but the cells accumulated P(3HB) to up to 55% of their CDM during growth on lactate. In 24 h, 4.7 g homopolymer L^{-1} were produced.

Enzymatically hydrolyzed potato processing waste has been studied as a possible source of a fermentable substrate for the production of P(3HB) by *R. eutropha*. The results indicated that potato starch waste could be converted with high yield to a concentrated glucose solution. The most economical process used barley malt as a source of amylase enzyme with an optimal ratio of 10:90 g g^{-1} of potato waste. A conversion efficiency of 96% of the theoretical value was obtained with a final

glucose concentration of 208 g L^{-1}. After dilution and addition of mineral salts the hydrolysate was converted by a batch culture to 5.0 g L^{-1} of P(3HB), comprising 77% of the cell dry weight [248].
Ishizaki and colleagues [249, 250] have perfected the operation of autotrophic cultures of *R. eutropha* for the production of high quantities of P(3HB). They addressed the two major difficulties of this strategy, namely poor utilization of gases and danger of explosion, by using a gas-recycling system and keeping the oxygen concentration in the gas feed below the lower explosion limit (approx. 7%), respectively. The authors noted however that the very high oxygen-transfer requirements of the fermentor, owing to the low O_2 content of the gas feed, would be problematic for the scaling-up of this system necessary for commercial exploitation.

Park et al. [258, 259] used a mutant strain of *R. eutropha* capable of using alcohol as a carbon source for the production of P(3HB) and P(3HB-*co*-3HV) in fed-batch fermentors. With phosphate limitation as inducing factor, ethanol was used for the production in 7 L of 46.6 g P(3HB) L^{-1} (74% of the CDM) in 50 h [232]. When 1-propanol was added to the medium, up to 15.1 mol% in 3HV units were incorporated to the polymer, and when propanol was the sole carbon source, the cells accumulated about 85% of their CDM in P(3HB-*co*-35.2-mol% 3HV). Both alcohol were completely consumed.

An interesting approach to PHA production using a mixed culture is shown by Katoh et al. [252]. The mixed culture system was considered to be effective when sugars such as glucose are converted to lactate by *Lactobacillus delbrueckii* and the lactate is converted to poly (ß-hydroxy-butyrate) (PHB) by *Alcaligenes eutrophus* in one fermentor. For the modeling of the effect of NH_3 concentration on the cell growth of *A. eutrophus* and PHB production rates, metabolic flux distributions were computed at two culture phases of cell growth and PHB production periods. The model may be used for several purposes such as control, optimization, and understanding process dynamics.

4.8.1.2 Continuous culture

Ramsay et al. [100] were the first to investigate P(3HB) and P(3HB-*co*-3HV) production in one- and two-stage continuous cultures. In a one-stage chemostat, *R. eutropha* DSM 545 accumulated 33% of its dry mass as P(3HB) when fed with a nitrogen-limited medium of glucose and mineral salts. P(3HB-*co*-3HV) was produced in similar experiments with *A. latus* when propionic or valeric acid was added to the feed mixture containing sucrose as main carbon source. In single-stage chemostat, feed propionic-acid concentrations of up to 5 g L^{-1} yielded a copolymer with a 3HV molar fraction reaching 20% without affecting the polymer productivity obtained with sucrose only. Substitution of the three-carbon acid with valeric acid led to higher 3HV contents in the copolymer. At high concentrations of propionic acid in the feed (8.5 g L^{-1}), assimilation of sucrose was inhibited. In this case, transfer of the reactor's effluent into a second chemostat led to complete consumption of the sugar and obtainment of P(3HB-*co*-11-mol% 3HV) representing 58% in mass of the CDM.

Koyama and Doi [213] also investigated P(3HB-*co*-3HV) production in chemostat by *R. eutropha* growing on fructose and pentanoic acid. By varying the dilution rate and ammonium sulfate concentration of the feed, they obtained a maximum productivity of 0.31 g of a 41-mol%-3HV copolymer L^{-1} h^{-1} (42% of CDM). Large amounts of unused fructose were detected in the culture broth.

Incomplete utilization of substrates, resulting from high carbon-to-nitrogen ratios in feeds, is often encountered in single-stage continuous PHA-producing processes. Unless the extra carbon can easily and cheaply be recycled back into the process, such losses entail a lower production profitability. The use of a second stage downstream from the first, as shown by Ramsay et al. [97], can advantageously increase the time of exposure for the organisms to conditions favorable for polymer accumulation, leading to higher yields and productivity. Attempts to develop continuous processes for a profitable production of PHAs will most probably be successful only when multi-stage arrangements are considered. Ramsay et al. [257] have argued that in the first stage, 50 to 60 g high-protein bio-mass L^{-1} would have to be produced. Braunegg et al. [195] have presented theoretical evidence that the use of a plug-flow tubular reactor for the second stage allows a maximal productivity for a number of organism/substrate systems, including *R. eutropha* and *A. latus* synthesizing PHAs from carbohydrates.

Self-check Questions

1. Which substrates have been used for the production of PHAs?
2. Why is the use of waste materials extensively studied?
3. Which are the two most important strains used for the production of the standard PHAs?

4. Which regimes were used? Which one most often?

Hints for Answers

See section 4.8.1

Exercise

Discuss the advantages and disadvantages of discontinuous and continuous reactors for the production of PHAs. Why are discontinuous systems used more often? Discuss the differences of costs for running the two systems. Substrate costs, equipment (especially working volume needed) etc. (for advanced trainees).

Excursion: Visit a biotechnological plant or laboratory to see bacteria "in action".

4.8.2 The Use of *Pseudomonads*

Ramsay's group has made extensive investigations of PHA production in a fermentor by members of the genus *Pseudomonas*. *P. pseudoflava* was grown in batch fermentation on glucose, xylose or arabinose, and accumulated P(3HB) from these, and was grown in chemostat on the hydrolysate from the hemicellulosic fraction of poplar-wood (mostly xylose as carbon source) [264]. Although Ramsay and colleagues did not specifically mention this, the use of this inexpensive hydrolysates to support the growth of *P. pseudoflava* in chemostat could quite possibly fulfill the first-stage requirements of a two-stage continuous process. *P. oleovorans* was used in chemostat to produce medium-side-chain PHAs from sodium octanoate [265].

PHA synthesis cultures by *P. putida* KT2442 growing on long-chain fatty acids in continuous cultures was studied by Huiberts and Eggink. The effects of growth rate on bio-mass and polymer concentration were determined, the highest volumetric productivity was 0.13 g PHA L^{-1} h^{-1} at a specific growth rate of 0.1 h^{-1}. The molecular mass of the polymer remained constant at all growth rates but changes in the monomeric composition of the copolymer synthesized were observed. Optimal PHA formation was observed at a C/N ratio of 20 mol mol^{-1}.

A two-step fed-batch cultivation of *P. putida* was performed with glucose and octanoate as the main carbon source for cell growth and PHA accumulation, respectively. Under nitrogen-and oxygen-limiting conditions 18.6 g L^{-1} PHA were obtained with a yield of about 0.4 g PHA g^{-1} octanoate. By supplying octanoate in the first step, production of mcl-PHAs was significantly enhanced; it yielded 35.9 g PHA L^{-1} (65.5% of cell dry mass) after 39 h of the fed-batch operation. This indicated that octanoate addition during growth stimulated quite efficiently the biosynthesis of mcl-PHAs [268].

When cultivated in chemostat with octanoate as sole carbon source and nitrogen limitation [265], *P. oleovorans* produced a PHA with a 3HB/3HHx/3HO/3HD ratio of 0.1:1.7:20.7:1.0 which was relatively independent of the octanoate concentration of the feed. The maximum productivity in copolymer was approx. 0.14 g $L^{-1}h^{-1}$ from 7 g octanoate L^{-1} (all used) at the dilution rate investigated (0.24 h^{-1}).

4.8.3 The Use of *Burkholderia cepacia*

B. cepacia (the new designation for *P. cepacia*) was used by Ramsay's group to produce P(3HB) from fructose in batch fermentation [257]. *B. cepacia* accumulated in about 80 h 2.6 g P(3HB) L^{-1} (47% of the CDM) in nitrogen-limited cultures. However, since about 35 g fructose L^{-1} were consumed by the micro-organism to achieve this, the polymer-from-sugar yield was only slightly above 0.074 g g^{-1}. Although this is low, the authors argued that since *B. cepacia* is likely to be capable of utilizing a variety of industrial or food wastes for growth, profitable PHA-producing processes might be conceivable with this organism.

4.8.4 The Use of *Azotobacter vinelandii UWD*

A. vinelandii UWD has grown from a microbiological curiosity to a serious contender for the most interesting potential organism for the production of PHAs from inexpensive carbon sources. Early on, Page's group detected a very low NADH oxidase activity in the new strain. They concluded that this defect could explain the bacterium's habit of accumulating P(3HB) in high quantities (65 to 75% of the

CDM) during exponential growth: the polymer-synthesizing process is one (but not the only) way NAD can be regenerated [270]. This was the beginning of the work that eventually inspired Genser et al. [165] to search for a similar cause for the constitutive PHA accumulation in *A. latus.* Page and Cornish [271, 203] also found that while nitrogen-fixation in strain UWD interfered with P(3HB) synthesis from glucose during nitrogen-depleted accumulation conditions, the addition to the medium of fish peptone, a complex nitrogen source, restored even greater polymer production rates and yields. Since *A. vinelandii* UWD can utilize unrefined, complex carbon sources, such as beet molasses, for PHA production [272], Page et al. have investigated the potential of the micro-organism for profitable biopolymer-producing processes. After establishing that valerate was the best 3HV precursor (and that propionate was no precursor), Page and co-workers [273] used the salt in combination with beet molasses to produce P(3HB-*co*-3HV) from strain UWD in a 2.5-L fermentor. Thirty-eight to 40 h of fed-batch regime with various valerate-addition strategies yielded 18 to 22 g copolymer L^{-1} (59 to 71% of the CDW) containing 8.5 to 23 mol% 3HV. In the absence of valerate, the cells produced only P(3HB) to 23 g L^{-1} (66% of the CDW).

Self-check Questions

1. What type of PHA is produced by the pseudomonads?
2. How do these polymers differ from the scl-PHAs?
3. Why are *Burkholderia cepacia* and *Azotobacter vinelandii* UWD interesting for PHA production?

Hints for Answers

See section 4.7, 4.8.2, 4.8.3 and 4.8.4.

4.8.5 Recombinant Strains for PHA Production

As early as 1988 the PHB biosynthetic pathway from *Alcaligenes eutrophus* H16 has been cloned and expressed in *Escherichia coli* [276]. PHB was produced in the cosmid clones at approximately 50% of the level found in *A. eutrophus*. One cosmid clone was subjected to subcloning experiments, and the PHB biosynthetic pathway was isolated on a 5.2-kilobase KpnI-EcoRI fragments. This fragment, when cloned into small multicopy vectors, can direct the synthesis of PHB in *E. coli* to levels approaching 80% of the bacterial cell dry weight.

Alcaligenes eutrophus transformant AER3, AER4 and AER5 harboring cloned phbCAB, phbAB and phbC genes (from *A. eutrophus* encoding acetyl-CoA-acetyltransferase (EC-2.3.1.16), aceto-acetyl-CoA-reductase and poly-hydroxybutyrate-synthase introduced via shuttle vector plasmid pKT230) were cultured under various different culture conditions to elucidate the optimal culture conditions for accumulation of poly-beta-hydroxybutyrate (PHB). The transformants showed increased total cell growth and PHB accumulation due to the recombinant enzymes. In batch culture, the transformant synthesized PHB more effectively at the high C/N molar ratio compared to the parent strain. Fed-batch culture was more effective for maximizing PHB biosynthesis compared to the batch culture mode. The plasmid stability was maintained at about 85% after 36 h and elongated morphological changes of transformant at the early growth stage was noticed. The gene amplification through the transformation of cloned PHB biosynthesis genes in *A. eutrophus* appears to be an excellent method for strain improvement to achieve an effective accumulation of PHB [278].

The increase of gene dosage of the poly(3-hydroxybutyrate) biosynthesis operon in *Ralstonia eutropha* to test whether PHB synthesis rates may be increased by recombinant methods was studied by Jackson and Srienc [279]. The native *R. eutropha* phbCAB operon was inserted into the broad-host-range vector pKT230. This PHB operon-containing plasmid, and a control plasmid containing the identical broad-host-range replicon but not the PHB genes, were transferred to *R. eutropha* H16. Analysis of whole-cell lysates indicated that the strain harboring the operon-containing plasmid possessed ß-ketothiolase and acetoacetyl-CoA reductase specific activities that were 6.0 and 6.2 times elevated, respectively, as compared to the control strain with a single operon. After growth on fructose, PHB synthesis rates were sharply dependent on the type of carbon source offered during the PHB accumulation phase under nitrogen limitation. In the case of the strain harboring the control plasmid, and in comparison to fructose as carbon source, PHB accumulation was 2.15, 2.83, and 2.60 times faster when resuspended in nitrogen-free medium with lactate, acetate, or 3-hydroxybutyrate, respectively. The strain harboring the PHB operon-containing plasmid synthesized PHB at a lower specific rate in each case. During exponential growth on fructose, the strain harboring the control

plasmid was again more efficient at forming PHB. These results suggest that increasing the intracellular concentration of PHB precursors may be a superior alternative to raising the levels of PHB enzymes for enhancing PHB productivity in *R. eutropha*.

Recombinant PHA producers seem to have several advantages as PHA producers compared with wild-type PHA-producing bacteria. However, the PHA productivity (amount of PHA produced per unit volume per unit time) obtained with these recombinant *E. coli* strains has been lower than that obtained with the wild-type bacterium *Alcaligenes latus*. To endow the potentially superior PHA biosynthetic machinery to *E. coli*, the PHA biosynthesis genes from *A. latus* have been cloned [280]. Recombinant *E. coli* strains harboring the *A. latus* PHA biosynthesis genes accumulate PHB more efficiently than those harboring the *R. eutropha* genes. With a pH-stat fed-batch culture of recombinant *E. coli* harboring a stable plasmid containing the *A. latus* PHA biosynthesis genes, final cell and PHB concentrations of 194.1 and 141.6 gL^{-1}, respectively, were obtained, resulting in a high productivity of 4.63 g of PHB/liter/h. This improvement should allow recombinant *E. coli* to be used for the production of PHB with a high level of economic competitiveness.

In order to scale up medium-chain-length polyhydroxyalkanoate (mcl-PHA) production in recombinant microorganisms, Prieto et al. [282] generated and investigated different recombinant bacteria containing a stable regulated expression system for phaC1, which encodes one of the mcl-PHA polymerases of *Pseudomonas oleovorans*. The mini-Tn5 system was used as a tool to construct *Escherichia coli* 193MC1 and *P. oleovorans* POMC1, which had stable antibiotic resistance and PHA production phenotypes when they were cultured in a bioreactor in the absence of antibiotic selection. The molecular weight and the polydispersity index of the polymer varied, depending on the inducer level. *E. coli* 193MC1 produced considerably shorter polyesters than *P. oleovorans* produced.
Interesting results where published by Dennis et al. [283] on the formation of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate). The acetoacetyl-CoA reductase and the polyhydroxy-alkanoate (PHA) synthase from *Ralstonia eutropha* were expressed in *Escherichia coli, Klebsiella aerogenes*, and PHA-negative mutants of *R. eutropha* and *Pseudomonas putida*. While expression in *E. coli* strains resulted in the accumulation of PHB, strains of *R. eutropha, P. putida* and *K. aerogenes* accumulated poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) when even chain fatty acids were provided as carbon source, and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) when odd chain fatty acids were provided as carbon source. This suggests that fatty acid degradation can be directly accessed employing only the acetoacetyl-CoA reductase and the PHA synthase. This is also the first proof that the PHA synthase from *R. eutropha* can incorporate 3-hydroxyhexanoate (3HHx) into PHA and has, therefore, a broader substrate specificity than previously described.

Self-check Questions

1. Which strain is the standard host organism for the cloning the PHA genes?
2. Are the genetically modified strains able to compete with wild type or mutant strains?
3. Is it possible to produce mcl-PHAs with these organisms?

Hints for Answers

See section 4.8.5.

4.8.6 In Vitro Production of PHAs

Beside the studies of PHA production in fermentation processes applying living microorganisms also in vitro systems may be used in future. A combined chemical and enzymatic procedure has been developed to synthesize macroscopic PHB granules in vitro. The granule form in a matter of minutes when purified polyhydroxyalkanoate (PHA) synthase from Alcaligenes eutrophus is exposed to synthetically prepared (R)-3-hydroxybutyryl-CoA, thereby establishing the minimal requirements for PHB granule formation. The artificial granules are spherical with diameters of up to 3 µm and significantly larger than their native counterparts (0.5 µm). The isolated PHB was characterized by ^{1}H and ^{13}C NMR, gel-permeation chromatography, and chemical analysis. The in vitro polymerization system yields PHB with a molecular mass > 1.10^{7} Da, exceeding by an order of magnitude the mass of PHBs typically extracted from microorganisms. It was demonstrated that the molecular mass of the polymer can be controlled by the initial PHA synthase concentration. Preliminary kinetic analysis of de novo granule formation confirms earlier findings of a lag time for the enzyme but suggests the involvement of an additional granule assembly step. Minimal requirements for substrate recognition

were investigated. Since substrate analogs lacking the adenosine 3',5'-bisphosphate moiety of (R)-3-hydroxybutyryl CoA were not accepted by the PHA synthase, the authors provide evidence that this structural element of the substrate is essential for catalysis [287].

Additional work in this field was performed by Lenz et al. [288], showing the effectiveness of glycerol on stabilizing the polymerase after purification and on eliminating the lag phase in vitro polymerization reactions of 3-hydroxybutyryl CoA (HBCoA), and 3-hydroxyvaleryl CoA (HVCoA). K_M values were determined for the activity of the polymerase with both HBCoA and HVCoA, and the rates of propagation for both monomers were estimated. With a racemic mixture of HBCoA, the enzyme polymerized only the [R] monomer.

4.8.7 Production of PHAs with Transgenic Plants

The obtainment of polyhydroxyalkanoates from genetically modified crop plants represents a drastic change in methodology. With this strategy, the steps necessary to procure the substrates used in a fermentative process are no longer required, as naturally occurring carbon dioxide and sunlight serve as carbon and energy sources, respectively. While this field of research is still in its infancy, progress since the initial trials has shown the concept to be promising. The first investigations reported on the use of the plant *Arabidopsis thaliana* harboring the PHA genes of *R. eutropha*. [289] The successful expression of the *R. eutropha* genes encoding acetoacetyl-CoA reductase and PHA synthase in the cytoplasm of *A. thaliana* were reported. The 3-ketothiolase gene is endogenous in plant cytoplasm. These experiments resulted in P(3HB) synthesis in the cytoplasm, nucleus and vacuoles of all plant tissue, but in low amounts and at the cost of stunted growth and poor seed production. This was attributed to the diversion toward polymer accumulation of acetyl-CoA normally channeled into essential metabolic pathways.

The second phase of research [290] has focused on the targeting of the PHA pathway to a specific sub-cellular compartment, the plastid, where biosynthesis of triglycerides from acetyl-CoA normally occurs. All three genes needed to be cloned in this case, and this led to the accumulation of high levels of P(3HB) with few deleterious effects on the growth or fertility of the hosts. The homopolymer was stored within plastids to up to 14% of the dry mass of the plants (a 100-fold increase from expression in the cytoplasm) in the form of granules of size and appearance similar to those of bacterial PHA inclusions.

The genes encoding acetoacetyl-CoA reductase and PHA synthase from *R. eutropha* were also expressed in cotton (*Gossypium barbadense* L. cv Sea Island) fibers. Transgenic plants containing both enzymes produced PHA in the fibers, since β-ketothiolase activity is present in cotton fibers [291]. The presence of P(3HB) granules in transgenic fibers resulted in measurable changes of thermal properties, the fibers exhibited better insulating characteristics. The rate of heat uptake and cooling was slower in transgenic fibers, resulting in higher heat capacity [292].

Attempts to demonstrate the feasibility of profitable production on an agricultural scale are the next step [85]. Poirier's group have proposed a number of oilseed crops that could be targeted for seed-specific PHA production, like rapeseed (closely related to *A. thaliana*), sunflower and soybean. Some of these are already under investigation by major companies. Depending on whether accumulation levels can be further increased, PHAs stored in plants have any deleterious effects on crop value in other respects, synthesis of PHAs other than P(3HB) can be induced, and extraction of the biopolyesters is feasible at reasonable costs, the cost of PHAs produced in plants might be lowered enough to make them competitive with conventional plastics. But the tendency of arable land to become one of the most precious commodities on Earth [251] will present a formidable obstacle to applications in this field.

Self-check Questions

1. Do you think in vitro production of PHAs is a cheap method?
2. What types of transgenic plants are used for PHA production?

Hints for Answers

See section 4.8.7.

Exercise

Discussion:

Lately the production of a copolymer by transgenic plants was reported. The comonomer content and the properties are closely linked to the growth-conditions of the plant. Do you think one can harvest plants containing PHAs with constant properties from the same field? Or over the years? In different countries? What measures have to be taken to ensure a constant polymer quality?

4.9 Extraction and Purification of PHAs

While physical methods have been described [72], PHAs are usually extracted from the producing cells with solvents or mixtures thereof. Mild polar compounds like acetone and alcohols [73] weaken or break down non-polymer cell material (NPCM), leaving P(3HB) granules intact, although some longer-side-chain PHAs are soluble in acetone [199]. NPCMs mostly consist of nucleic acids, lipids and phospholipids, peptidoglucan and proteinaceous materials. In contrast, chloroform [194] and other chlorinated hydrocarbons [75] dissolve all PHAs. Methods employing both types of solvents (i.e., lipid extraction with PHA non-solvent followed by polymer dissolution) are therefore usually applied. The dissolved polymer is then separated from the solvent, usually by evaporation or precipitation with acetone or an alcohol, such as methanol or ethanol. Drying the cells prior to the extraction steps [74] can facilitate the subsequent polymer recovery, as can changing the pH [76] or temperature [71, 76] of the polymer-solvent mixture.

Non-solvent processes have been developed in answer to the high cost of large-scale solvent extraction. Holmes and Lim [70] described the enzymatic process used at Zeneca for the recovery of P(3HB) and P(3HB-*co*-3HV). First, a high-temperature (100 to 150 °C) treatment of the cells provokes cell lysis and denaturation of nucleic acids, which could otherwise interfere with the subsequent steps. Non-PHA bio-mass is then solubilized with proteolytic enzymes (pepsin, trypsin, papain, others, and mixtures thereof) and anionic surfactants. Concentration of PHA by centrifugation is finally followed by bleaching with H_2O_2.

PHB can also be separated from the bio-mass by heating to above 100°C under pressure, releasing the pressure, and separating PHB granules from the cell debris [298] or by drying a finely divided stream or spray of an aqueous suspension of the cells with a gas heated to above 100°C. Then extracting the PHB, preferably after a lipid extraction step with a solvent such as a partially halogenated hydrocarbon such as 1,2-dichloroethane or chloroform [299]. Brake used heating under pressure in the presence of a C1-C6 alcohol, and optionally also water[300].

Hypochlorite digestion of bacterial bio-mass from intracellular poly-β-hydroxybutyrate (PHB) has not been used on a large scale since it has been reported to severely degrade PHB. In their study Berger et al., to minimize degradation, the initial *Alcaligenes eutrophus* bio-mass concentration, digestion time, and pH of NaOCl solvent were optimized to minimize degradation of PHB. Consequently, a PHA of 95% purity with a Mw of 600,000 and polydispersity index (PI) of 4.5 was recovered from bio-mass initially containing a polymer with Mw of $1.2*10^6$ and a PI of 3 [302].

Hahn et al. studied the recovery of PHB from *Alcaligenes eutrophus* and a recombinant *Escherichia coli* strain harboring the *A. eutrophus* PHA biosynthesis genes. The amount of PHB degraded to a lower-molecular-weight compound in *A. eutrophus* during the recovery process was significant when sodium hypochlorite was used, but the amount degraded in the recombinant *E. coli* strain was negligible. However, there was no difference between the two microorganisms in the patterns of molecular weight change when PHB was recovered by using dispersions of a sodium hypochlorite solution and chloroform.

Another method for recovering PHA compounds produced by fermentation of microorganisms comprises mechanical breakdown of the cells, followed by removal of the cell fragments and dissolved components, then drying of the PHA-containing bio-mass and extraction of the PHAs with acetic acid. The novelty is that after drying, the bio-mass is treated for five minutes to two hours with the acetic acid at a temperature below the extraction temperature, before raising the temperature to allow extraction to take place [304].

Self-check Questions

1. What different methods can be used to recover the PHAs from bio-mass?
2. Explain the drawbacks of PHA recovery with organic solvents.
3. Is it possible to recover PHAs in intact granules?

Hints for Answers

See section 4.9.

4.10 Polysaccharides

High molecular mass polysaccharides [306] are formed by condensation of large numbers of activated sugars. If they are always the same, *homopolysaccharides* are formed (e.g. dextran, curdlan), if they differ from each other, *heteropolysaccharides* are the polymerization reaction results (e.g. xanthan). Starch, a widely used plant reserve polymer consists of amylose (long unbranched chains of α [1→4] linked D-glucose, molecular weight $2x10^5$ to $2x10^6$) and amylopectin (α [1→4] linked D-glucose in the backbone and α [1→6] linked D-glucose in the branch points, molecular weight up to $4x10^8$) is a raw material that can be made thermoplastic for production of compostable items, or can be complexed with ethylen-vinyl alcohol copolymers [306]. Cellulose, produced by plants or microorganisms is another important natural polysaccharide consisting of β[1→4] linked D-glucose residues.

This link (http://www.genome.ad.jp/kegg/catalog/cpd_polysacch.html)gives the structures of some important polysaccharides. (From the GenomeNet WWW Server)

4.10.1 Dextran

Even though dextran can be prepared by polymerizing 1,6-anhydro-2,3,4-tri-*O*-benzyl-ß-D-glucopyranose using phosphorous pentachloride as a catalyst and subsequently removing benzyl groups, all commercially available dextrans are biotechnological products. 96 strains have been described to form the polysaccharide, but only *Leuconostoc mesenteroides* and *Leuconostoc dextranicum* are used commercially. These microorganisms produce the enzyme complex dextransucrase, responsible for the dextran formation according to the overall equation

$$(1,6\text{–}\alpha\text{–D-Glucosyl})_n + C_{12}H_{22}O_{11} \text{ ----------> } (1,6\text{–}\alpha\text{–D-Glucosyl})_{n+1} + nC_6H_{12}O_6$$

sucrose — *Sucrase* — dextran — fructose

The enzyme glycoprotein releases fructose from sucrose and transfers the glucose residue to the reducing end of the growing dextran chain on an acceptor molecule, which is bound to the enzyme. During polymerization, the growing dextran chain is always bound to the enzyme. The degree of polymerization increases until an acceptor molecule releases the polymer chain from the enzyme.

Dextran can be produced either directly by batch fermentation or indirectly by the use of the enzyme complex dextransucrase mentioned above. Direct production is of course the simpler process, but molecular weights of dextrans are varying. A mixed-culture fermentation system was designed by Kim et al. [308] for the production of size-limited dextrans. This process was simpler and more economical than traditional methods. It required the establishment of microbial consortia of *Lipomyces starkeyi* ATCC 74054 and *Leuconostoc mesenteroides* ATCC 10830. Controlling initial conditions, growth, and enzyme production by both organisms controlled the product size. In this process, both strains were grown separately and then mixed. Dextran fermentation was then allowed to proceed. At the desired time (and molecular size), the fermentation was harvested. The optimum pH and temperature for production of clinical dextran (75,000 MW) were 5.2 (+/-0.1) and 28 (+/-0.5) °C, respectively. Varying the ratio of *L. mesenteroides* to *L. starkeyi* in the inoculum did not significantly affect either the final cell ratios or dextran production.

Maintenance of an adequate dissolved oxygen concentration is problematic due to the non-Newtonian fluid behavior of the nutritional broth becoming more and more viscous during fermentation. During dextran production phase no more oxygen is needed, and bioreactors are stirred only, fermentation

control proceeds by measuring fructose concentration in the broth. From 0.5 g of bacterial bio-mass about 80 g of dextran are formed during the process.

Table 4.1 Further Uses of Dextrans [307]

Product	**Function**
Pharmaceutical grade	
Cryoprotective	Inhibits cell damage on freezing
X-ray opaque compositions	Suspending agent
Water-insoluble vitamin preparations	Stabilizing agent
Tablets	Binding agent
Sustained-action tablets	Protracts dissolution
Chloral-dextran complex	Supresses taste and stomach irritant action
Microcapsules of kerosene, menthol, aspirin, etc.	Methylcellulose-dextran, encapsulating agent
Cosmetic preparations	Wrinkle smoothing
Food grade	
Syrups and candies	Improves moisture retentivity and body and inhibits crystallization
Gum and jelly confections	Gelling agent
Ice cream	Prevents shrinkage and ice formation
Icing compositions	Stabilizing agent
Pudding compositions	Bodying agent
Industrial grade	
Oil drilling fluids	Dextran-aldehyd complex, inhibits water loss and coats well wall
Solution for flooding underground reservoirs	Increases viscosity of water
Drilling muds	Protective colloid
Olefinically polymerizable resins	Filler and modifier
Alumina manufacture	Sedimentation agent
Purification of caustic soda	Iron-dextran complex precipitates
Metal powder production	Gel precipitation suppresses crystal growth
Nuclear fuel production	Complexing agent

The enzymatic process permits better reaction control leading to a more uniform material with molecular weights depending on the nature of the glucosyl acceptor employed, very often a dextran with a molecular weight of about 75.000 is produced. The process consists of two stages, the aerobic production of the extracellular enzyme complex typically at low sugar concentrations (2 % sucrose) at 25 ^{o}C and a pH of 6.7, and the enzymatic dextran synthesis under reducing conditions. Ammonium ions depress the yield of enzyme produced, pH adjustment is made with caustic alkali. The enzyme is separated from the bacterial cells by centrifugation (pH = 5.0) and can be stored at 15 ^{o}C for as long as 30 days. The expected enzyme yield is about 40 dextransucrase units per ml, converting 40 mg of sucrose to dextran in 1 hour under standard conditions.

For dextran production 10% w/v sucrose is supplied as substrate, the incubation is conducted at 15 ^{o}C or below in the presence of about 30 units/ml of dextran sucrase. Molecular weight of dextran produced can be controlled by sucrose concentration, enzyme concentration, and by the temperature and time used for incubation.
After its production dextrans are precipitated by addition of either methanol or acetone (1^{o}C), and the supernatant is decanted. Precipitated dextran is resolubilized in distilled water at 60 to 70 ^{o}C and precipitated again for cleaning purposes. The typical yield for dextran is about 60-70% of the glucose part of sucrose. About 2000 tons of dextranes are consumed worldwide each year. Depending on product qualities they are sold for a price of 35 – 2800 $US/kg.

Self-check Questions

1. Describe the key reaction leading to dextran.
2. Name some of the applications of dextran.
3. Which strains are used for the commercial production of dextran?
4. Why is it so difficult to keep up good aeration during the process? Is oxygen needed during the whole process?

Hints for Answers

See section 4.10.1 and 4.1.

4.10.2 Xanthan

Xanthan gum, an extracellular heteropolysaccharide synthesized by *Xanthomonas campestris* is another biopolymer produced commercially in quantities above 20.000 tons per year. Properties of xanthans used in foods are shown in table 4.2. Xanthan gum has a high molecular weight ($2x10^6 – 5x10^7$) and contains D-glucose (2.8 mol), D-mannose (3.0 mol), D-glucuronic acid (2.0 mol), acetic acid (approximately 4.7%) and pyruvic acid (approximately 3%). The structure of xanthan is a pentasaccharide repeating unit consisting of a β[1→4]-linked D-glycosyl backbone ("cellulosic backbone") with α [1→3]-linked trisaccharide side chains (D-mannose-β [1→2]-D-glucuronic acid-β [1→4]mannose). Most commercial xanthans are fully acetylated on the internal D-mannose residue and carry pyruvate ketals on about 30% of the side chain terminal mannose residues.

Table 4.2 Different properties of Xanthan used in foods

Function	**Application**
Adhesive	Icings and glazes
Binding agent	Pet foods
Coating	Confectionery
Emulsifying agent	Salad dressing
Encapsulation	Powdered flavors
Film Formation	Protective coatings, sausage casings
Foam stabilizer	Beer
Stabilizer	Ice cream, salad dressings
Swelling agent	Processed meat products
Syneresis inhibitor	Cheeses, frozen foods
Thickening agent	Jams, sauces, syrups, and pie fillings

The article by Becker et al. [309] outlines aspects of the biochemical assembly and genetic loci involved in its biosynthesis, including the synthesis of the sugar nucleotide substrates, the building and decoration of the pentasaccharide subunit, and the polymerization and secretion of the polymer. An overview of the applications and industrial production of xanthan is also covered.

Xanthan gums are produced aerobically in bio-reactors (200 m^3 reactor volume) in a strictly monoseptic batch process at 28 ^oC and pH equal to 7.0 from carbon sources like flours, starch, starch hydrolyzates, glucose or sucrose. Initial carbohydrate concentrations may vary from 2 to 5% depending on the substrate type. Dissolved oxygen concentration is kept above 20 % of air saturation. Often organic nitrogen sources are used (meat-peptone, soy peptone dried distillers'soluble, urea). Although available kinetic data provide a useful insight into the effects of medium composition on xanthan production by *Xanthomonas campestris*, they cannot account for the synergetic effects of carbon (glucose) and nitrogen (yeast extract) substrates on cell growth and xanthan production. In their work Lo et al. studied the effects of the glucose/yeast-extract ratio (G/YE) in the medium on cell growth and xanthan production in various operating modes, including batch, two-stage batch, and fed-batch fermentations. In general, both the xanthan yield and specific production rate increased with increasing G/YE in the medium, but the cell yield and specific growth rate decreased as G/YE increased. A two-stage batch fermentation with a G/YE shift from an initial low level (2.5% glucose/0.3% yeast extract) to a high level (5.0% glucose/0.3% yeast extract) at the end of the exponential growth phase was found to be preferable for xanthan production [310] Xanthan yields can be increased if the pH value of the nutritional broth is controlled during fermentation by addition of ammonium hydroxide. Rheology of the increasing viscous fermentation broth is a rather complex problem, because xanthan solutions exhibit pseudoplastic behavior and display yield stress and visco-elasticity.

The final fermentation broth is diluted with water in order to decrease viscosity and centrifuged for partial removal of the cells. Then the product is precipitated by addition of methanol or i-propanol in

the presence of 2%w/w potassium chloride. Other recovery methods proposed are drum-drying or spray-drying for a technical grade product. 10-20x10^3 tons of xanthan are produced worldwide and sold for a price of 10 – 14 $US/kg.

Self-check Questions

1. Which strain is used for Xanthan production?
2. Describe the structure of Xanthan. Is it a homopolymer or heteropolymer?
3. Give an overview over the production process.

Hints for Answers

See section 4.10.2.

Reading Materials

http://helios.bto.ed.ac.uk/bto/microbes/xanthan.htm
Picture page from the University of Edinburgh with photos of *Xanthomonas campestris* colonies, recommended Reading Materials and lots of links to other pages dealing with fungi and excretion of exopolysaccharides.

4.10.3 Alginates

Alginate is a linear polymer of beta-1,4-linked L-guluronic acid and D-mannuronic acid. Although all commercial alginates are today of algal origin, there is interest in the production of alginate-like polymers from bacteria. The species *Azotobacter vinelandii* seems to be the best candidate for the industrial production of alginate molecules characterized by a chemical composition, molecular mass and molecular mass distribution suited to a well defined application, especially required in the biotechnological, biomedical and pharmaceutical fields. The production of alginate by *A. vinelandii* has been to date widely investigated both in batch (mainly in the shaken flask scale) and in continuous cultures. The article by Clementi summarizes current knowledge on the structure and properties of alginates and their applications and presents an overview of up-dated research on the physiology, genetics and kinetics of the production of alginate by *Azotobacter vinelandii* and its rheology, including the results of recent studies[313]. Another review was prepared by Rehm and Valla [315].

Recovery of high-purity alginate from the medium comprises: (a) extracting algal material or crude alginate with a solution of a complexing agent; (b) sedimenting cell components and particles from the solution with a porous binder, (c) filtering the solution; (d) precipitating alginate from the solution; and (e) collecting the precipitate. [317]

Pseudomonas aeruginosa is an opportunistic pathogen causing severe infections, especially in lungs of patients with cystic fibrosis. Environmental conditions induce the production of alginate, which is one of the most important factors of virulence of *P. aeruginosa*. [318] In the article by Schmitt-Andrieu and Hulen [319] a scheme of alginate biosynthetic pathway and a model for the alg genes regulation are described from results published in literature. Purified alginate added to bacterial suspensions caused a decrease in growth, suggesting that alginate contributes to oxygen limitation for the organism and likely for patients afflicted with the inherited autosomal disease cystic fibrosis. [320]

Besides for applications in food industry Alginates are very interesting as matrices for immobilization of enzymes and microorganisms. Using a model system, a concept for the immobilization of microbial cultures within alginate beads directly in a 1500-L fermentor with a height to diameter ratio of 1.85 is described by Champagne et al. [326] The system is comprised of a 60-cm diameter bowl fixed to the top of an agitation shaft, where calcium-ion-rich media is continuously recirculated from the bulk solution to the bowl. The rotation of the shaft and bowl creates a climbing film (vortex) of solution. An atomizing disk centrally recessed within the bowl sprays an alginate solution into the climbing film where the droplets harden into beads. The effect of heat treatment on the alginate solution on resulting bead properties was examined. The sterilization operation did not appear to have a major effect on the alginate bead mechanical properties of firmness and elasticity which was much more a function of alginate concentration. Beads of various sizes were produced by the unit. The system was characterized by the dimensionless numbers $Re,\omega = (\omega \times \rho \times D(2))/\mu$ a.

Small diameter alginate beads (microspheres) were formed by Poncelet et al. via internal gelation of alginate solution emulsified within vegetable oil. Gelation was initiated by addition of an oil-soluble acid thereby reducing the pH of the alginate solution and releasing soluble Ca^{2+} from the citrate complex. Smooth, spherical, micron-sized beads were formed. The mean diameter ranged from 200 to 1000 microns, controlled by the reactor impeller design and rotational speed. The technique has potential for large-scale and continuous applications in immobilization. [327]

Alginate is also used as matrix for immunoisolation of cells and tissues in vivo. Klock et al. have demonstrated previously that commercial alginates contain various fractions of mitogenic impurities and that they can be removed by free flow electrophoresis. The use of purified material is a necessity in order to reveal the parameters that control biocompatibility of the implanted material (such as stability, size, surface charge and curvature, etc.). In this study, they present a protocol for the chemical purification of alginates on a large-scale. Beads made from alginates purified by this multi-step chemical extraction procedure did not induce a significant foreign body reaction when implanted for 3 weeks either intraperitoneally or beneath the kidney capsule of Lewis or non-diabetic BB/Gi rats. [329]

Self-check Questions

1. Which organisms are used to produce alginates in industrial scale?
2. Are bacteria suited for the production of alginates?
3. Some strains producing alginates are pathogenic. Explain the connections between the disease caused by this strains and the alginate production. (see also link below)
4. In what other field of application than in food industry can alginates be used?

Hints for Answers

See section 4.10.3

Exercise

Experiment: Encapsulation of yeast in alginate. Comparison of metabolic activity and stability of immobilized and free yeast.

Reading Materials

http://www.ecfsoc.org/pa_review/nh_lect.html
A summary of the role of various Pseudomonas strains in infections of patients suffering from cystic fibrosis.

4.10.4 Pullulan

Pullulan is an exopolysaccharide consisting of 1,6-linked maltotriose units and is excreted by the fungus *Aureobasidium pullulans.* The material is used as protective coating in food industry.

Pullulan production by *Aureobasidium pullulans* ATCC 201253 using selected nitrogen sources was studied by West et al. in a medium using corn syrup as a carbon source. Independent of the corn syrup concentration present, the use of corn steep liquor or hydrolysed soy protein as a nitrogen source instead of ammonium sulphate did not elevate polysaccharide production by ATCC 201253 cells grown in an aerated, batch bioreactor containing 4 litres of medium. Pullulan production on corn steep liquor or hydrolysed soy protein as a nitrogen source became more comparable as the concentration of corn syrup was increased. Cell weights after 7 days of growth on any of the nitrogen sources were similar. The viscosity of the polysaccharide on day 7 was highest for cells grown on ammonium sulphate and 12.5% corn syrup. The pullulan content of the polysaccharide elaborated by ammonium sulphate-grown cells on day 7 decreased as the corn syrup level rose in the medium. [331] Pullulan production by *Aureobasidium pullulans* strain RP-1 using thin stillage from fuel ethanol production as a nitrogen source was also studied in a medium using corn syrup as a carbon source. The use of 1% thin stillage as a nitrogen source instead of ammonium sulphate elevated polysaccharide production by strain RP-1 cells when grown on a concentration of up to 7.5% corn syrup, independent of yeast extract supplementation. Dry weights of cells grown in medium containing ammonium sulphate as the nitrogen source were higher than the stillage-grown cells after 7 days of growth. The viscosity of the polysaccharide on day 7 was higher for cells grown on thin stillage rather than ammonium sulphate as a nitrogen source. The pullulan content of the polysaccharide elaborated by ammonium sulphate-grown

cells on day 7 was higher than the pullulan content of polysaccharide produced by stillage-grown cells regardless of whether yeast extract was added to the culture medium. [332]

Ethanol-precipitated substances after fermentation of various agro-industrial wastes by *Aureobasidium pullulans* were examined for their pullulan content. Grape skin pulp extract, starch waste, olive oil waste effluents and molasses served as substrates for the fermentation. A glucose-based defined medium was used for comparison purposes. Samples were analysed by an enzyme-coupled assay method and by high-performance anion-exchange chromatography with pulsed amperometric detection after enzymic hydrolysis with pullulanase. Fermentation of grape skin pulp extract gave 22.3 g l^{-1} ethanol precipitate, which was relatively pure pullulan (97.4% w/w) as assessed by the coupled-enzyme assay. Hydrolysed starch gave only 12.9 g l^{-1} ethanol precipitate, which increased to 30.8 g l^{-1} when the medium was supplemented with NH_4NO_3 and K_2HPO_4; this again was relatively pure pullulan (88.6% w/w). Molasses and olive oil wastes produced heterogeneous ethanol-precipitated substances containing only small amounts of pullulan. [333]

Self-check Questions

1. Which strain is used for pullulan production?
2. In which industry is pullulan used?

Hints for Answers

See section 4.10.4

See section 4.10.4

4.10.5 Chitin and Chitosan

Besides cellulose chitin is the most common polymer on earth. It is the substance responsible for the stiffness of the exoskeletons of insects and shells of marine organisms like crabs and others from which chitin is obtained in an industrial process (see link below in Reading Materials). Chitin can also be found in certain fungi. Chitosan can be obtained from chitin by enzymatic deacetylisation. Chitosan is already produced in large quantities and is used as glue, wound dressing and as additive in soil conditioners and feed materials.

Accordingly a recombinant chitin-depolymerase was produced by Tokuyasu et al. With the aid of a signal sequence of a chitinase from *Streptomyces lividans*, a recombinant chitin deacetylase, whose gene originated from a Deuteromycete, *Colletotrichum lindemuthianum*, was produced in the culture medium of *Escherichia coli* cells, existing as a highly active form without the signal peptide. During the production of the recombinant chitin deacetylase, both a slight increase in the value of OD_{600} in the culture medium and a drastic decrease in viable cell number were observed. When penta-N-acetyl-chitopentaose was used as the substrate, the recombinant chitin deacetylase had comparable kinetic parameters to those of the original enzyme from the fungus. The addition of a C-terminal six histidine sequence to the recombinant enzyme caused a slight decrease in the k_{cat} value, and the further addition of a 12 amino acid sequence at its N-terminus caused a further decrease in the value. This production system allowed us to easily produce in the culture media the recombinant chitin deacetylases. [339]

A method for the lab-scale production and isolation of chitosan from hyphal walls of *Mucor rouxii* was developed by White et al. Hyphal wall yields were generally 16 to 22% on a dry cell weight basis, of which 35 to 40% was glucosamine. Chitosan was readily extracted from purified, mycelial walls with acetic, formic, and hydrochloric acids; the last named was the most efficient. The yield of chitosan isolated ranged from 4 to 8% of the dry weight of the cell wall material. [340]
The properties of the “microsomal” chitin synthase of *Mortierella vinacea* was investigated. The pH optimum was between 5,8 and 6,2, and the temperature optimum was between 31 and 33°C. The Km for UDP N-acetyl-D-glucosamine was 1.8 mM. The enzyme was stimulated by Mg^{2+} and a slight stimulation was also effected by N-acetyl-D-glucosamine. Soluble chitodextrins were inhibitory. A pH-dependent, heat-stable inhibitor of chitin synthase activity was present in the soluble cytoplasm from the mycelium. The effects of aeration and glucose concentration on enzyme production in growing cultures were also investigated; maximum specific activity of chitin synthase was associated with the cessation of exponential growth. [341]

Self-check Questions
1. Compare structure of chitin and chitosan. Which reaction is needed to obtain chitosan from chitin?
2. Which fields of application are there for chitosan?
3. Which is the most common polymer on earth?

Hints for Answers
See section 4.10.5 and 4.1.

Reading Materials
http://www.bae.ncsu.edu/bae/courses/bae465/1995_projects/bake/smith/index1.html
A fine summary of properties and fields of application of chitin and chitosan from NC State University.

4.10.6 Curdlan

Curdlan is a β-1,3 glucan produced by a strain of *Alcaligenes faecalis*. It is used as gelling agent in food industry and in low calorie products because it is not degraded in the human body.

As a guide to both strain and process improvement and based on certain assumptions concerning both glucose and energy metabolism of the process organism, *Alcaligenes faecalis* var. *myxogenes*, the theoretical "maximum" carbon (glucose) substrate to product conversion efficiency (i.e., product yield) has been estimated for "curdlan-type" beta(1→3)-glucan exopolysaccharide production in batch fermentations. Under nitrogen limitation, which promotes curdlan biosynthesis ($\mu = 0$), the rate of glucose consumption for cellular maintenance energy (grams of glucose per gram of cells per hour) was approximately five times higher than under carbon limitation. The decrease in the theoretical "maximum" curdlan conversion efficiency of 74% to the average value of 50-56% was due primarily to the high maintenance coefficient of the nitrogen-starved culture. [342]

The biosynthesis of curdlan has been studied in batch and continuous cultures of *Alcaligenes faecalis* var. *myxogenes*. Curdlan production is associated with the poststationary phase of a nitrogen-depleted, aerobic batch culture. Exopolymer is not detected in single-stage, carbon-limited continuous cultures but curdlan can be isolated from the effluent of a nitrogen-limited chemostat operating at a dilution rate (D) of less than 0.1 h^{-1}. A spontaneous variant of strain ATCC 21680 was isolated and found to be compatible with long-term, nitrogen-limited chemostat culture. The specific rate of curdlan production is approximately four times higher in poststationary batch cultures than in single-stage continuous fermentations. The product yield ($Y_{P/S}$) associated with batch processing (nongrowing cultures) is approximately 0.5 g curdlan/g glucose, with CO_2 being the only detectable by-product. [343]

Self-check Questions
Why is curdlan interesting for low-calorie products?

Hints for Answers
See section 4.10.6

Reading Materials
http://www.botany.utexas.edu/facstaff/facpages/mbrown/ongres/icheese.htm. Article from the University of Texas on the microscopic structure of Curdlan containing some fine photos.

4.10.7 Other Polysaccharides

A cell-bound polysaccharide (CBP) produced by the marine bacterium *Zoogloea sp*. (KCCM 10036) (and therefore called zooglan [345]) was used as the adsorbent of metal ions and as a new support for enzyme immobilization. The CBP gel beads showed highly effective adsorbing in Cr, Pb, and Fe ion in solutions. The adsorption rates were above 95% at pH 5.0, 25°C, in 10 mg/liter of each metal solution. The gel beads formed by the CBP were stable within the range of pH 4.0-7.0 and at a temperature of 40-55°C. The optimum pH and temperature of the immobilized glucoamylase by the CBP gel beads (poly-G) were 5.0 and 45°C, respectively. The immobilized glucoamylase produced 10.5 mg/liter of glucose from 10 mg/ml of soluble starch. [344]

The cyanobacterium *Aphanocapsa halophytia* MN-11, was immobilized in calcium alginate gel and coated on light-diffusing optical fibers (LDOF) for sulfated extracellular polysaccharide production. Results indicated that sulfated extracellular polysaccharide production depends on the number of immobilized cells and the light intensity. In addition, the production rate reached 116.0 mg (mg dry cells)$^{-1}$ day^{-1} when the cells that were immobilized on LDOF were incubated under a light intensity of 1380 cd sr m^{-2} at a cell concentration of 1.0×108 cells/cm^3 gel. Cells immobilized on LDOF produced about ten times more sulfated extracellular polysaccharide than those immobilized in calcium alginate beads only (11.7 mg(mg dry cells)$^{-1}$ day^{-1}). [346]

Cellulose production from sucrose by Acetobacter strains is accompanied by the accumulation of a water-soluble polysaccharide, called levan. To improve cellulose productivity, a levansucrase-deficient mutant, LD-2, was derived from Acetobacter strain 757 and used as a host for the construction of recombinant strains. An LD-2 mutant harboring a plasmid containing the sucrase gene, sucZE3, from *Zymomonas mobilis* together with zliS, a gene that encodes a secretion-activating factor under the control of the Escherichia coli lac promoter, had sucrase activity and produced much cellulose and little levan in a medium containing sucrose. In addition, a mutant levansucrase gene, mutant sacB, from *Bacillus subtilis*, which encodes a protein with little levan-forming activity, was generated by site-directed mutagenesis and introduced into the LD-2 mutant. This introduction also resulted in the higher cellulose productivity and little levan. [347]

The ability of casamino acids and vitamin-assay casamino acids to support gellan production by *Sphingomonas paucimobilis* ATCC 31461 was examined in a medium containing glucose or corn syrup as the carbon source relative to yeast extract supplementation. When glucose or corn syrup served as the carbon source, the presence of yeast extract in the growth medium stimulated gellan production by strain ATCC 31461 on casamino acids. Using vitamin-assay casamino acids as the nitrogen source, the addition of vitamins lowered gellan synthesis by glucose-grown cells regardless of yeast extract supplementation while gellan elaboration by corn syrup-grown strain ATCC 31461 cells could only be increased by supplementing vitamins into medium lacking yeast extract. Independent of carbon source, the absence of yeast extract in the medium reduced bio-mass production. Bio-mass production by the strain grown on either carbon source was increased by supplementing vitamins in the medium containing yeast extract. [348, 349]

A rhamnose-containing microbial polysaccharide has been produced by *Klebsiella sp*. I-714. strain. The polysaccharide has been used as a source for the obtention of L-rhamnose. Physiological conditions enhancing polysaccharide synthesis were studied in batch culture (0.3-l and 2-l bioreactors). The four carbon sources tested, sucrose, sorbitol, Neosorb and Cerelose, allowed exopolysaccharide production. Larger amounts of polymer were produced when high carbon/nitrogen ratios and complex nitrogen sources were used. Exopolysaccharide synthesis was greatest at 30 °C, which was a suboptimal growth temperature. A reduction in the phosphate content of the medium enhanced rhamnose-containing polysaccharide production. When the initial carbon source concentration was augmented, byproducts other than exopolysaccharide were formed. Rhamnose-containing polysaccharide rheology can be modulated by changing the phosphate content of the medium. [351] A biotechnological process has been developed to purify the hydrolyzed polysaccharide (EPSH), which contains rhamnose, galactose and glucuronic acid. Microbial removal of galactose is followed by a continuous chromatographic separation of glucuronic acid to render pure rhamnose. The technical feasibility of the process has been studied with special emphasis on microbial inhibitor's removal[352].

CHAPTER 5

MEDICAL, PHARMACEUTICAL AND COSMETIC APPLICATIONS

Objective

- Students will learn concepts used in the area of biodegradable polymers for medical and pharmaceutical applications.
- Students will get insight in materials that can be applied and the requisites put on materials.
- Students will learn about the properties of suitable materials that can be applied.
- Students will get an overview of the "players" in the field
- Students will learn the necessary steps to be taken to enter the market. This is evaluated by a case study.

Summary

Biodegradable polymers have about 30 years ago been developed mainly for medical and pharmaceutical applications. The high value of these products has been a bases for the success of these materials. The increased knowledge on these materials has led to the development of new materials and processes that resulted in a much wider area for applications. In this chapter we focus on the current status of biomedically applied biodegradable polymers.

5.1 Background

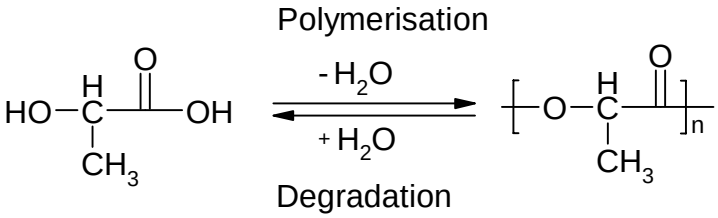
In medical practice implant materials are either applied for long term or temporary use. Whereas for long-term applications biostable materials are required, biodegradable materials may well be used in case of temporary applications. The advantages of the use of biodegradable materials are well recognized:

a) The ability of the body to regenerate functions can be supported by the use of an implant. The material will lose its function in time as a result of the degradation and a gradual restoration of the body's function can take place.
b) A second advantage in the application of these materials is that a second operation for the removal of the implant is not necessary.

Examples of biostable biomedical devices:
- Artificial hip or knee implant
- Intraocular lens
- Pacemaker
- Artificial hearth
- Breast implant
- Cohlear implant
- Mandibular titanium implant
- Dialysis-access graft
- Blood vessel prosthesis
- Adjustable femoral implant
- Spinal fixation

Biodegradable materials have a large potential in medical and pharmaceutical applications. The use of these materials started some thirty years ago with the development of surgical sutures based on aliphatic polyesters. These materials were build from monomeric units like glycolic acid and lactic acid, components that are also present in the human body. In the human body many metabolic pathways are present that may or will degrade the material. Oxidation, reduction, and (enzymatic hydrolysis) are examples of such pathways and amongst these hydrolysis is the most well known. It is therefore not surprising that hydrolysis has been adopted in the design of biodegradable polymers.

Figure 5.1 Lactic acid and poly(lactic acid)



Upon degradation of the materials these components are formed and released again and do not evoke adverse reactions in the body. They are eventually metabolized into carbon dioxide and water. As a result of these early findings research and development was mainly focused on the generation of patents on new materials and products. In the following years scientists became aware of the necessity to gain more fundamental insight in the structure property relationships of these materials. Still working closely with researchers in the medical and pharmaceutical industries resulted in a fast development of the science of biodegradable materials for biomedical technology and engineering. Major areas of applied research are nowadays to be found in drug delivery systems and tissue engineering.

Tissue Engineering

An exciting development is the use of degradable materials as matrices for the formation of tissue of the patient (tissue engineering). Tissue can be first generated outside the body by culturing cells on a porous matrix and then implanted, or the matrix can be implanted in the body, whereafter tissue is generated in the biological environment. Important aspects are the adhesion and growth of cells in the matrix, cellular interactions and signaling, cell differentiation and the influence of growth factors. Examples of current subjects are the development of a nerve grafts, repair of the central nerve system, endothelialized blood vessels, tissue engineered cartilage, substitutes for bone and skin and encapsulation of pancreatic islets for insuline delivery.

Pharmaceutical Applications

Currently a large research effort is directed to the design of systems for the controlled delivery of drugs. The aim is to provide the drug in the body in the right concentration at the desired location for the required time period. Many novel protein drugs are unstable and cannot be applied orally. Antitumor drugs have toxic side effects and should be released at the tumor site. Growth factors needed in tissue engineering should be delivered in a controlled way from the polymer matrix.
Tissue engineering and drug delivery thus represent significant growth areas for medical device manufacturers and pharmaceutical companies. They provide opportunities for new product development, and are commercially very attractive.

Exercises

Which type of materials can be used to construct biostable implants. What would be the requisites for these materials to be applied? Discuss these items with others.

Reading Materials

An introduction to the area can be found through the link given below. The index of the archives is indicated. http://www.devicelink.com/mpb/archive/98/03/002.html

5.2 Polymers

Biodegradable polymers may be divided into two groups. These comprise materials from synthetic and natural origin. Natural degradable materials that can be applied for the manufacturing of biomedical

devices are proteins and polysaccharides. Examples of proteins are collagen, fibrinogen, and albumin. Examples of polysaccharides are dextran, chitin and glucoseamineglycans (Figure 5.2)

Proteins comprise different amino acids (different R-groups) in the polymer chain. In structural proteins like collagen, the sequence of amino acids is (Gly-Pro-X) in which X are designated different amino acids. In polysaccharides, different alkohol, amine and carboxylic acid functional groups can be present.

In this overview we focus our attention to the polymers that can be synthetically prepared and have been or intended to be applied in medical devices. In the past years tremendous progress has been achieved in the controlled synthesis of these materials. Moreover industries now provide an easy access to the monomers these polymers can be build from. Designing the polymers also means that a certain control and prediction can be achieved in the way of degradation and the degradation products generated. This also enables a more easy and detailed study of the tissue reactions involved upon degradation. In Fig. 5.3, the structures of the most well known biodegradable materials for medical applications have been presented. These materials degrade under physiological conditions in products that are not harmful to the human body.

Besides the consideration that the degradation products are acceptable to the human body and can be metabolized the device implanted has to meet other requirements in order to be successfully applied. Following has been presented a summation of requisites to be fulfilled by biodegradable materials to be used as medical devices.

- Suitable mechanical properties. The decrease in mechanical properties upon degradation of the implant materials should preferentially be in line with and be taken over by the healing tissue.
- There should be no thrombogenic, carcinogenic, immunogenic, and allergenic reactions evoked by the material or its degradation products.
- There should be no adverse tissue reaction.
- The degradation products should be fully resorbed, metabolized and/or eliminated from the body.
- Processing of the material should not alter the material in such a way that the materials characteristics are changing.
- The effect of sterilization on the polymer properties and structure has to be known.

Exercises

Give the degradation products upon hydrolysis of the polymers given in Fig. 5.3.

Reading Materials

Handbook of Biodegradable Polymers, Domb, A.J.; Kost, J. Wiseman, D.M. Harwood Academic Publishers, 1997

Figure 5.2 Structure and examples of proteins and

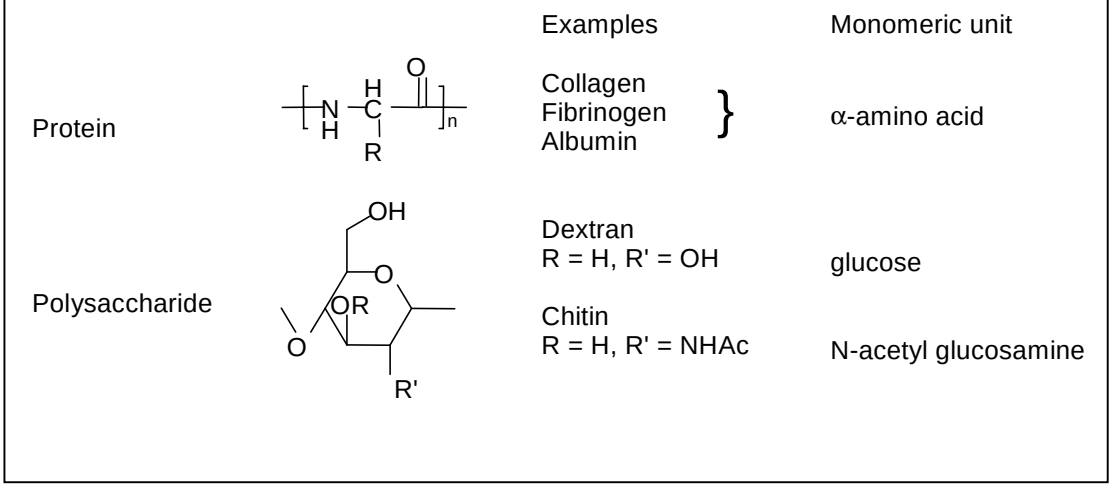
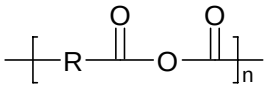
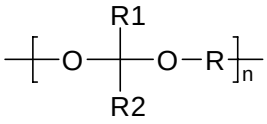


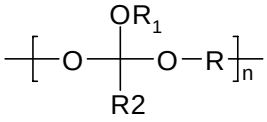
Fig. 5.3 Functional groups contained in degradable polymers



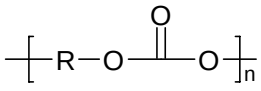
poly(anhydrides)



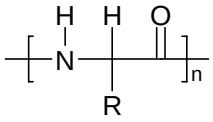
poly(ketals)



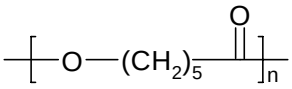
poly(ortho esters)



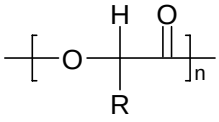
poly(carbonates)



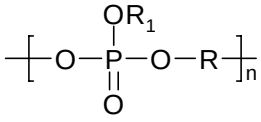
poly(peptides)



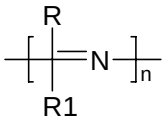
poly(ε-caprolactone)



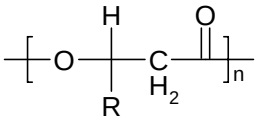
poly(α-hydroxy-esters)



poly(phosphate esters)



poly(phosphazenes)



poly(β-hydroxy-esters)

5.3 Polymer Synthesis

5.3.1 Monomers

The aliphatic polyesters are the most well-known and investigated polymers during the past three decades. They are generally built through ring-opening polymerization of lactide, glycolide, caprolactone, and p-dioxanone (figure 5.4). Other monomers investigated are derivates of these monomers and may contain (protected) functional groups. Other monomers used in homo and copolymerization reactions are e.g. trimethylene carbonate, cyclic depsipeptides and others providing materials with a wide range of properties.

Bicyclic, bifunctional monomers provide crosslinking and in this way biodegradable hydrogels have been prepared. Of the monomers used to prepare synthetic aliphatic polyesters only lactide is a chiral molecule. The monomer has two chiral centers and we distinguish L,L-lactide, D,D-lactide and Dl-lactide (or meso-lactide). Polymers prepared from these monomers afford polymers with different properties (figure 5.5). L-lactic acid is the naturally occurring molecule.

Figure 5.4 Monomers used in the synthesis of aliphatic polyesters.

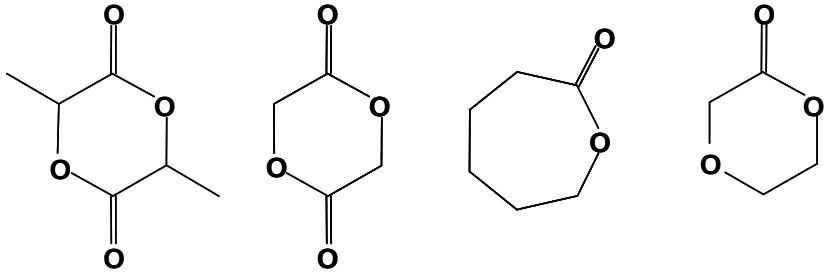
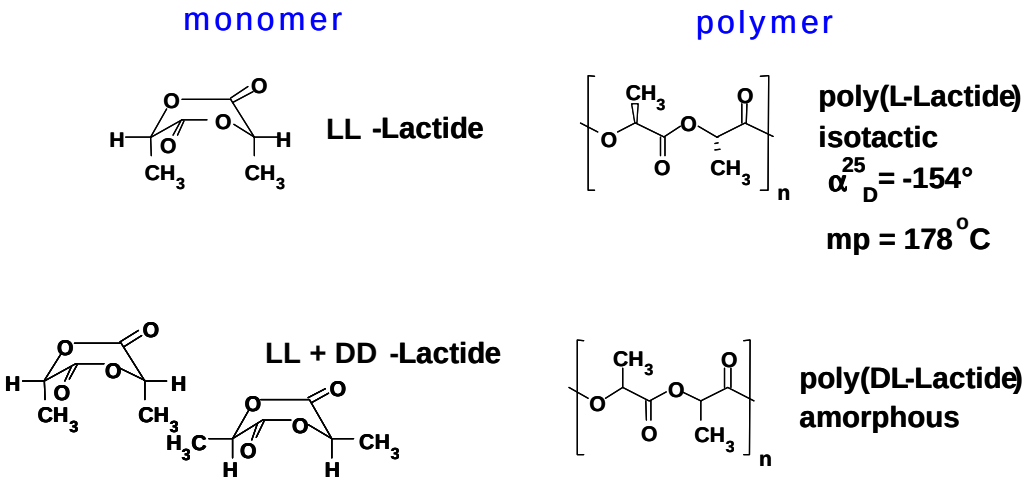


Figure 5.5



Exercises:

Why is the meso-lactide achiral? Draw structures of the polymer chain with at least 4-5 monomer units for Poly(L-lactide), Poly(D,L-lactide) and Poly(meso-lactide)

5.3.2 Polymer Synthesis

Synthetic polymer chemistry has evolved over the past decades to the point where both structure and properties can be controlled very accurately. This holds for bulk as well as for specialty polymers. The properties of polymeric materials are primarily determined by their chemical structure.

Considerable research effort has been directed towards the synthesis of macromolecules with complex structures. Examples include block copolymers, graft copolymers, star copolymers, dendrimers and other macromolecular architectures. The combination of advanced polymerization methods and efficient coupling methods has resulted in a broad array of chemical structures which can be prepared nowadays. In order to obtain a high molecular weight product, reactions are repeated many times, and thus a high selectivity, is required. The functionality used in coupling methods need to have a high reactivity, because the concentration of reacting groups is usually low. The synthesis of special macromolecular structures imposes severe restrictions on the polymerization process. Ultimately, the synthesis of polymers with control of molecular weight, molecular weight distribution and end group identity can only be achieved by a polymerization process which has a high selectivity in initiation and

propagation and termination. Also, the relative rates of these processes must be favorable. If these conditions are fulfilled, the polymerization is called a living polymerization.

Aliphatic polyesters are generally prepared from lactones in the bulk or in solution. The advantage of bulk polymerization is certainly that no organic solvents have to be used in the synthesis and thus will also not remain in the polymer or in the device prepared from it. Nowadays these polymers of low or high molecular weight can be prepared and control over the polymerization process has become possible with the development of various catalyst systems. Because the catalysts are not removed from the polymer, especially in bulk polymerizations, a non-toxic catalyst material is preferred.

Exercise

In synthesis many metal alkoxides are used for the preparation of polyesters by ring-opening polymerization. Give a reaction scheme of the initiation and propagation.
Which metals are preferably applied in catalyst systems.

Reading Materials

W. M. Stevels, P. J. Dijkstra, J. Feijen, *Trends Polym. Sci.* **5**, 300 (1997).
D. Mecerreyes, R. Jerome, P. Dubois, *Advances in Polymer Science* **147**, 1-59 (1999).

5.4 Polymer Producers

In this section the main polymer producers of biodegradable polymers to be applied in medical devices are presented. The reader may explore the websites for further reading of this section.

1. Absorbable Polymer Technologies, Inc.
2683 Pelham Parkway, Pelham, AL 35124; USA (http://www.absorbables.com)
Absorbable Polymer Technologies, Inc. (APT) is a research and development-based company specializing in the design, development, and manufacturing of biodegradable-polymer formulations for controlled-release pharmaceuticals, and medical devices for enhanced therapies.
The company provides: biodegradable-polymer-synthesis (Good Laboratory Practices (GLP), clean-room manufacturing facilities); applications-development; analytical laboratories products (standard and custom-synthesized materials, like polylactides etc.)

2. Birmingham Polymers, Inc.
756 Tom Martin Drive, Birmingham, AL 35211-4467, USA (http://www.birminghampolymers.com)
Birmingham Polymers Inc. (BPI) is a company that manufactures and sells biodegradable polymers for medical application. All polymers are produced under current Good Manufacturing Practice (GMP), and the company maintains both Drug and Device Master Files with the FDA. The company provides:
Synthesis of lactide, glycolide and ε-caprolactone polymers etc. ; Research, development and manufacturing Good Manufacturing Practices (cGMP); Sample Kits are also available.

3. Boehringer Ingelheim Pharma KG, Fine Chemicals, Binger Straße 173, D-55216, Ingelheim, Germany (http://www.boehringer-ingelheim.com/finechem/)
The Boehringer Ingelheim group of companies is one of the world's leading pharmaceutical corporations. It focuses on the human pharmaceutical as well as on the animal health business.
RESOMER® is the generic term used to describe the polymers that are produced on the basis of lactic and glycolic acid. Homopolymers of lactic acid (polylactides) are mainly used to produce resorbable implants in medical devices. Copolymers of lactic and glycolic acid are raw materials for the pharmaceutical industry, which serve mainly to encapsulate active ingredients for controlled release.

4. PURAC biochem, Arkelsedijk 46, P.O. Box 21, 4200 AA Gorinchem, The Netherlands (http://www.purac.com)
PURAC is the world's largest manufacturer of natural L (+)-lactic acid and lactates, with factories in Brazil, Spain and the Netherlands - plus a worldwide sales network. PURAC, a subsidiary of CSM - a renowned Dutch multinational specializing in the production and marketing of food ingredients and foodstuffs - is represented in more than 100 countries. PURAC works according to the GMP method and is ISO certified. For customers in highly sophisticated medical and pharmaceutical areas worldwide, the Biomaterials Business Unit offers lactide and glycolide monomers and biodegradable

polymers and copolymers. PURASORB lactide and/ or glycolide polymers and copolymers have been developed to provide materials for a variety of medical devices and pharmaceutical applications, like wound closure products, orthopaedic implants and controlled drug delivery systems.

5. SBU Caprolactones, Solvay Interox Ltd, Baronet Road, Warrington, CheshireWA4 6HB, UK (http://www.solvay.com/cap)
Solvay Caprolactones provides caprolactone monomer and polymers. CAPA® is the Solvay trademark for its range of caprolactones, comprising monomer and polymers of varying molecular weight. The main CAPA® R&D facilities are located in the UK but with significant support provided by Central R&D laboratories of Solvay in Brussels. Applications include: Biodegradable Bottles, Biodegradable Films, Controlled Release of Drugs, Pesticides and Fertilisers, Polymer Processing, Adhesives, Non Woven Fabrics, Synthetic Wound Dressings, Orthopaedic Casts

Exercise
Make an overview of the commercially available materials. Why is there a focus on a narrow spectrum of materials? Compare your results with those given in the literature as given in the references at the end of this chapter.

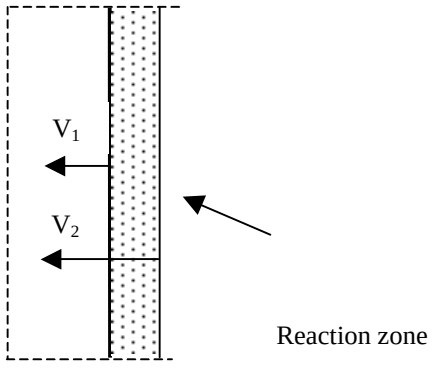
Reading Materials
Explore the websites of these polymer manufacturers.

5.5 Degradation

The degradation rate of biodegradable medical devices like sutures, bone plates or screws, matrix materials used in tissue engineering, gels and soluble materials and others depends on several factors:

- Chemical structure of the polymer
- Configuration of monomeric units in the polymer
- Molecular weight, Polydispersity
- Morphology (amorphous or semicrystalline materials)
- Glass transition temperature
- Additives
- Method of sterilization used
- Application site
- Degradation mechanism (enzymes vs. water)

V1 = rate of water intrusion
V2 = rate of polymer hydrolysis



V1 > V2: reaction zone increases in time leading to bulk hydrolysis
V1 < V2: reaction zone at surface leading to bulk hydrolysis

Fig. 5.6 Schematic representation of degradation by bulk hydrolysis or surface erosion
(J. Heller, R.V. Sparer, G.M. Zentner, in "Biodegradable Polymers as Drug Delivery Systems", Ed.: M. Chasin and R. Langer, Marcel Dekker Inc. NY, 1990)

The degradation of implant materials can be divided into two groups. Materials degrade by bulk degradation or surface erosion. Bulk degradation initially starts with the absorption of water and hydrolysis of labile bonds in the amorphous phase. There will be a reduction in molecular weight and subsequent loss in properties. The acid end groups generated catalyze the degradation. The degradation

may lead to fragmentation of the device depending on the rate of degradation of crystalline regions. Finally enzymatic degradation can take place during the course of hydrolytic degradation and will become more important during the final stages.

When the rate of water penetration in the polymer bulk is slow, as with hydrophobic materials surface erosion becomes the main mechanism of degradation. This mechanism can be achieved by combining a hydrophobic backbone with hydrolytically very labile bonds. An example is the polyanhydrides. A second method is the use of excipients that distributed in the polymers matrix will neutralize acid end groups generated during the degradation like in aliphatic polyesters or polyorthoesters.

Exercise

How will the release of large molecules like proteins depend on the geometry and degradation of the device. You may consider micro-spheres, films, rods in combination with a surface erodible or bulk degradable material.

Reading Materials

1. http://www.devicelink.com/mpb/archive/98/03/002.html
2. http://www.devicelink.com/mpb/archive/97/11/003.html
3. Handbook of Biodegradable Polymers, Domb, A.J.; Kost, J. Wiseman, D.M. Harwood Academic Publishers, 1997

5.6 Definitions

In the conferences of the European Society for Biomaterials of 1986 and 1991 discussions have been taken place to get consensus on the definitions on degradable materials for biomedical applications.
Later also on the European and Dialysis and Transplant Association (CCB) in 1993 definitions have been added.

In the 1986 meeting (ref) the following definitions have been set:

Biomaterial: A biomaterial is a non-viable material used in a medical device, intended to interact with biological systems.

In this respect a medical device has been defined as:

Medical device: A medical device is an instrument, apparatus, implement, machine, contrivance, in vitro reagent, or other similar or related article, including any component, part or accessory, which is intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment or prevention of disease in men.

In the ESB meeting of 1991 (ref) a more straightforward definition of a biomaterial has been given.

Biomaterial: A biomaterial is any material intended to interact with biological systems to evaluate, treat, augment or replace any tissue, organ or function in the body.

In the CCB meeting the term Biomaterial has been defined as:

Biomaterial: A non-viable material used in a medical device to interact with biological systems.

Other related definitions are:

Implant: An implant is a medical device made from one or more biomaterials, that is intentionally placed within the body, either totally or partially buried beneath an epithelial surface (ESB).

Biocompatibility: Biocompatibility is the ability of a biomaterial to perform with an

appropriate host response on a specific application (ESB 1986).

Biocompatibility: The ability of a material, device, or system to perform without a clinically significant host response in a specific application. CCB1993

Several definitions have been given on the terms degradation, biodegradation, and bioresorption. Chronologically the following have been presented:

Biodegradation: Biodegradation is defined as the gradual breakdown of a material mediated in or by a biological system (ESB 1991);
The breakdown of a material by biologic activity (CCB 1993).

Bioresorption: Bioresorption is defined as the process of removal by cellular activity and/ or dissolution of a material in a biological environment (ESB 1991);
The dissolution process of a material in a biological environment (CCB 1993)

Interesting is to see that working definitions adopted at the Second International Scientific Workshop on Biodegradable Polymers and Plastics (Montpellier, France Ref) resemble these definitions but also may slightly differ or may lead to differences in interpretation.

Polymer degradation: is a deleterious change in the properties of a polymer due to a change in chemical structure.

Controlled degradable polymer: is a polymer that by design degrades at a predictable rate.

Biodegradable polymer: is a polymer in which the degradation is mediated at least partially by a biological system.

Polymer erosion: is the process of dissolution or wearing away of a polymer surface.

Polymer fragmentation: is a form of polymer degradation in which the polymer molecule is broken up or segemented into lower molecular weight units.

Bioabsorbable polymer: is a polymer that can be assimilated by a biological system.

Exercises

Give definitions of the terms: degradation and biodegradation reflecting materials used for biomedical or environmental application. Are the requisites different? Discuss this with others.

Reading Materials

Part of this information can be found in the paper from A. Lendlein in “Chemie in unserer Zeit 33, 279, 1999” and references cited therein.

Case Study

In your organization you have developed a polymeric material that is easily extruded into fibers. The fibers show high mechanical strength and it is suggested to evaluate the market for biodegradable suture materials for medical use. An up to date inventory has to be made of commercially available suture materials. The chemical structure and properties of these materials has to be mapped and compared with the results of your own developed material.

The companies that are involved in biodegradable materials for medical devices has been listed below. However it may be necessary to perform a web search on sutures using keywords like medical, degradable, biodegradable and others to complete or update the overview. Furthermore you may have to use the books given in the reference list at the end of this chapter to compare and complement your web search. A biomedical related web site provide helpful information: http://www.biomat.net/.

Company information

1. Advanced Polymer Systems
123 Saginaw Drive, Redwood City, CA 94063, USA
http://www.advancedpolymer.com/

2. Advanced Tissue Sciences
10933 North Torrey Pines Road, La Jolla, California 920371005, USA
http://www.advancedtissue.com/

3. Alkermes, Inc.
64 Sidney Street , Cambridge, Massachusetts 02139 , USA
http://www.alkermes.com/

4. ARTHREX
2885 South Horseshoe Drive, Naples, FL 34104, USA
http://www.arthrex.com/flash.htm

5. Atrix Laboratories, Inc.
2579 Midpoint Drive, Fort Collins, CO 80525, USA
www.atrixlabs.com/

6. BIONX Implants, Inc.
1777 Sentry Parkway West, Gwynedd Hall, Suite 400, Blue Bell, PA 19422 USA
http://www.bionximplants.com/

7. BIONX Implants, Ltd.
PO Box 3, Hermiankatu 68L, FIN33720 Tampere, Finland

8. DePuy Orthopaedics
P.O. Box 988, 700 Orthopaedic Drive, Warsaw, IN 46581-0988, USA
http://www.depuy.com/index.cfm

9. Ethicon, Inc.
Sommerville, New Jersey, USA
http://www.ethiconinc.com/

10. Instrument Makar, Inc.
2950 East Mount Hope • , Okemos, MI 48864 , USA
http://www.instmak.com/

11. Linvatec Corporation
11311 Concept Boulevard, Largo; Florida 33773-4908, USA
http://www.linvatec.com/

12. MacroMed, Inc.
9520 South State Street, Sandy, Utah 84070, USA
http://www.macromed.com/

13. Samyang
263 YeonjiDong, ChongnoGu, Seoul 110725, Korea
http://www.samyang.com/english/index.html

14. Smith & Nephew, Inc.
Endoscopy Division, Andover, Massachusetts 01810, USA
http://www.sn-e.com/

15. Acufex Microsurgical
Smith & Nephew, 130 Forbes Blvd., Mansfield, MA 02048, USA
16. Southern BioSystems, Inc.
756 Tom Martin Drive, Birmingham, AL 352114467, USA

http://www.southernbiosystems.com/

17. Sulzer Medica Ltd.
Zurcherstrasse 12, CH8401 Winterthur, Switzerland
http://www.sulzer.ch

18. TESco Associates, Incorporated
4 Lyberty Way, P.O. Box 769, Westford, MA 01886 U.S.A. Tel: (978) 3920551

19. THM Biomedical, Inc.
325 South Lake Ave, Suite 608, Duluth, Minnesota 55802, USA
http://www.thmbiomedical.com

References

1 Biodegradable Polymers as Drug Delivery Systems, Chasin, M.; Langer, R., Marcel Dekker, Inc., 1990
2 Controlled Release of Drugs: Polymers and Aggregate systems, Rosoff,M., VCH Publishers, New York, 1988
3 Polymer Yearbook 16, Pethrick, R.A.; Zaikov, G.E.; Tsuruta, T.; Koide, N., Harwood Academic Publishers, 1999
4 Polymer Yearbook 15, Pethrick, R.A.; Zaikov, G.E.; Tsuruta, T.; Koide, N., Harwood Academic Publishers, , 1998
5 Controlled drug delivery, Park, K., ACS Professional Reference Book, 1997
6 Encyclopedic handbook of biomaterials and bioengineering: Part A Materials vol. I, Wise, D.L. et al., Marcel Dekker, Inc., 1995
7 Encyclopedic handbook of biomaterials and bioengineering: Part A Materials vol. II, Wise, D.L. et al., Marcel Dekker, Inc, 1995
8 Encyclopedic handbook of biomaterials and bioengineering: Part B Applications vol. I, Wise, D.L. et al., Marcel Dekker, Inc., 1995
9 Encyclopedic handbook of biomaterials and bioengineering: Part B Applications vol. II, Wise, D.L. et al., Marcel Dekker, Inc. 1995
10 Degradable Polymers: Principles and applications, Scott, G.; Gilead, D., Chapman & Hall, 1995
11 ACS Symposium Series: Polymers of Biological and Biomedical Significance, Shalaby, S.W.; Ikada, Y.; Langer, R.; Williams, J., American Chemical Society; Washington, 1992
12 ACS Symposium Series: Polymeric Delivery Systems, El-Nokaly, M.A.; Piatt, D.M.; Charpentier, B.A., American Chemical Society; Washington, 1993
13 Biomedical application of synthetic biodegradable polymers, Hollinger, J.O., CRC Press, 1995
14 Synthetic Biodegradable Polymer Scaffolds, Atala, A. and Mooney, D.J.; Vacanti, J.P. and Langer, R., Birkhauser Boston, 1997
15 Hydrogels and Biodegradable Polymers for Bioapplications, Ottenbridge, R.M.; Huang, S.J.; Park, K., ACS Washington D.C., 1996
16 Biodegradation and Biodeterioration of Polymers: Kinetical Aspects, Gumargalieva, K.Z.; Zaikov, G.E., Nova Science Publishers, Inc.; Commack, New York, 1998
17 Handbook of Biomaterials Evaluation: Scientific, Technical, and Clinical Testing of Implant Material, von Recum, A.F., Taylor & Francis, Philadelphia, 1999
18 Biodegradable polymers and plastics, Vert, M; Feijen, J.; Albertsson, A.; Scott, G. and Chiellini, E., Royal Society of Chemistry; Cambridge, 1992
19 Biomedical Applications of Polymeric Materials, Tsurata, T.; Hayashi, T.; Kataoka, K.; Ishihara, K.; Kimura, Y., CRC Press, 1993
20 The Biomedical Engineering Handbook, Bronzino, J.D., CRC Press in coop. with IEEE Press, 1995
21 Handbook of Biodegradable Polymers, Domb, A.J.; Kost, J. ; Wiseman, D.M., Harwood Academic Publishers, 1997
22 Biodegradable hydrogels for drug delivery, Park, K.; Shalaby, W.S.W.; Park, H., Technomic Publishing company, Inc., 1993

CHAPTER 6

DEGRADATION MECHANISM AND CHARACTERISATION

Objectives

- Students will learn the concepts of degradation, including environmental, photo- and bio-degradation.
- Students will learn and understand the degradation mechanisms of polymers, mainly polyolefins.
- Students will learn the techniques that are useful to study the degradation of polymers.
- Students will learn the advantages and disadvantages of different techniques and the need to combine two or more techniques to assess the degradation of polymers.
- Students will have a general idea about various methods used for surface analysis and GPC.

Summary

The general concepts of environmentally degradable polymers, the mechanisms for plastic degradation in a natural environment, and methods used for the evaluation of the degradability of polyolefins will be addressed in this lesson.

6.1 Mechanisms of Plastic Degradation

6.1.1 Degradation of Polymeric Materials

Polymeric materials are exposed to degradation during manufacturing, processing, and long-term use. Degradation is a destructive change in the chemical structure, in the physical properties, or in the appearance of a polymer.

Environmentally degradable polymers: are those polymers which degrade by a combined and cumulative effect of heat, sunlight, oxygen, water, pollution, micro-organisms (bacteria, fungi, alga, etc.), macroorganisms (insects, crickets, woodlice, snails, etc.), mechanical action, wind, and rain and so on. The main mechanisms of environmental degradation are photolysis, thermolysis, oxidation, hydrolysis and biological attack. The overall environmental degradation of polymers can thus be divided into biocatalytic processes involving enzymes (biodegradation) and pure chemical and radical processes (physical-chemical degradation) such as oxidation, irradiation and hydrolysis. Oxidative degradation and biodegradation are the most important processes involved in the environmental degradation of polyolefins.

6.1.2 Physical-Chemical Degradation Mechanisms of Polymers

Polymers contain bonds that are mainly of the C-C, C-H, C-Cl or C-O type. These bonds can be broken if the polymer is exposed to energy corresponding to their bond energy values. Exposure of polyolefins to oxygen, particularly at elevated temperatures or in sunlight will lead to degradation of the polymer. Degradation of polyolefins in the presence of oxygen is an autocatalytic process called auto-oxidation (Figure 6.1).

Auto-oxidation is a free radical reaction, where the initiating step occurs when the chemical bonds in the molecules are broken. The scission preferentially occurs in weak links where the bond energies are lower and leads to the formation of radicals (reaction 1). The cleavage can occur by e.g. exposure to UV-radiation, heat, ionising radiation and mechanical stresses. These radicals can react with atmospheric oxygen and start the auto-oxidation of the polymer (reactions 1-6).

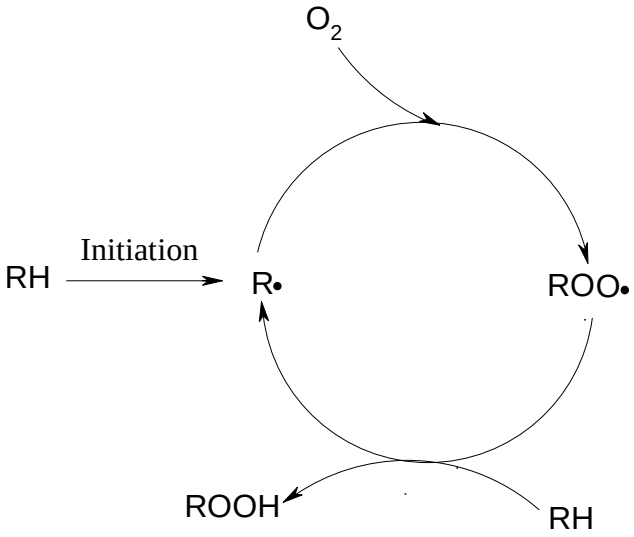


Figure 6.1. The auto-oxidation scheme.

Initiation:

$$RH \longrightarrow R\bullet + H\bullet \quad (1)$$

Propagation:

$$R\bullet + O_2 \longrightarrow RO_2\bullet \quad (2)$$

$$RO_2\bullet + RH \longrightarrow ROOH + R\bullet \quad (3)$$

Termination:

$$R\bullet + R\bullet \longrightarrow R - R \quad (4)$$

$$RO_2\bullet + R\bullet \longrightarrow ROOR \quad (5)$$

$$RO_2\bullet + RO_2\bullet \longrightarrow \text{Products} \quad (6)$$

The hyroperoxides have a key position in the auto-oxidation reactions, since they are very unstable compounds and decompose to new radicals when exposed to heat or irradiation (reactions 7-8).

$$ROOH \xrightarrow[\Delta]{h\nu} RO\bullet + \cdot OH \quad (7)$$

$$2\,ROOH \longrightarrow RO\bullet + ROO\bullet + H_2O \quad (8)$$

The decomposition of hydroperoxides is catalysed by transition metal ions, particularly cobalt, iron, manganese and copper. The effect of the metal ions is to reduce the activation energy of the hydroperoxide decomposition (reactions 9-10).

$$ROOH + M^n \longrightarrow RO\bullet + OH^- + M^{n+1} \quad (9)$$

$$ROOH + M^{n+1} \longrightarrow ROO\bullet + H^+ + M^n \quad (10)$$

Besides these reactions, further reactions take place during photo-oxidation. The carbonyl groups that are one of the strongest UV absorbing groups, undergo photolysis by the Norrish type I and type II reactions (Fig. 6.2), resulting in chain cleavage.

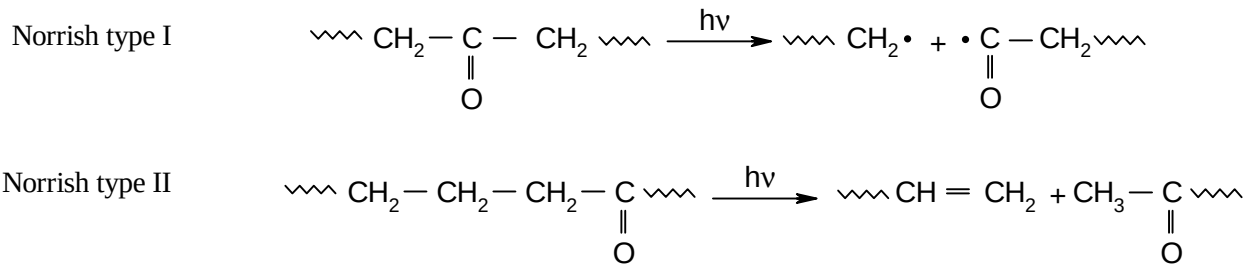


Fig.6.2 Norrish photoreactions.

The Norrish type I reaction is a free radical reaction where cleavage occurs at the carbonyl group to give two free radicals. The type II process is an intermolecular re-arrangement resulting in scission of the main chain to give a methyl ketone and a terminal double bond.

6.1.3 Biodegradation Mechanisms of Polymers

Biodegradation is defined as degradation which occurs by the action of enzymes and/or chemical decomposition associated with living organisms (bacteria, fungi etc.) or their secretion products. The rate of degradation is sensitive to microbial population, moisture, temperature, and oxygen in the environment. For inert polyolefins, oxidation is the initial step for biodegradation, and attack by micro-organisms is a secondary process.

Biodegradation of polyethylene is comparable to the biodegradation of paraffin. The biodegradation of paraffin starts with an oxidation of the alkane chain to a carboxylic acid, which undergoes β-oxidation (Fig. 6.3). A mechanism for the biodegradation of polyethylene was presented in 1987 which shows similarities with the β-oxidation of fatty acids and paraffins in man and in animals. In the biodegradation of polyethylene, an initial abiotic step involves oxidation the polymer chain, and this leads to the formation of carbonyl groups. During microbial assimilation, a decrease in carbonyl groups was noted. The carboxylic acids formed react with coenzyme A (CoA) and remove two carbon fragments, acetyl-CoA, which is metabolised by the citric acid cycle and produces carbon dioxide and water as final degradation products.

Photo-oxidation increases the biodegradation of polymers. Photo-oxidation leads to the scission of the main chain in polymer and leads to the formation of low molecular weight products, a larger surface area through embrittlement and a greater degree of hydrophilicity by the introduction of carbonyl groups and thus promotes the biodegradation of the polymer.

6.1.4 Hydrolysis of Polymers

Hydrolytic degradation takes place when polymers containing hydrolysable groups such as polyesters, polyanhydrides, polycarbonates, polyamides etc., are exposed to moisture. The hydrolysis proceeds by a random hydrolytic chain scission of these linkages. The low molecular weight degradation products that are formed are usually equivalent to the repeat unit of the polymer or the dimer thereof.

Self-check Questions

1. Describe the degradation mechanisms of polymers in an abiotic environment.
2. Describe the degradation mechanisms of polymers in a biotic environment.
3. Which parameters effect the rate of biodegradation?
4. What is the role of transition metal ions in the degradation of degradable polyolefins?

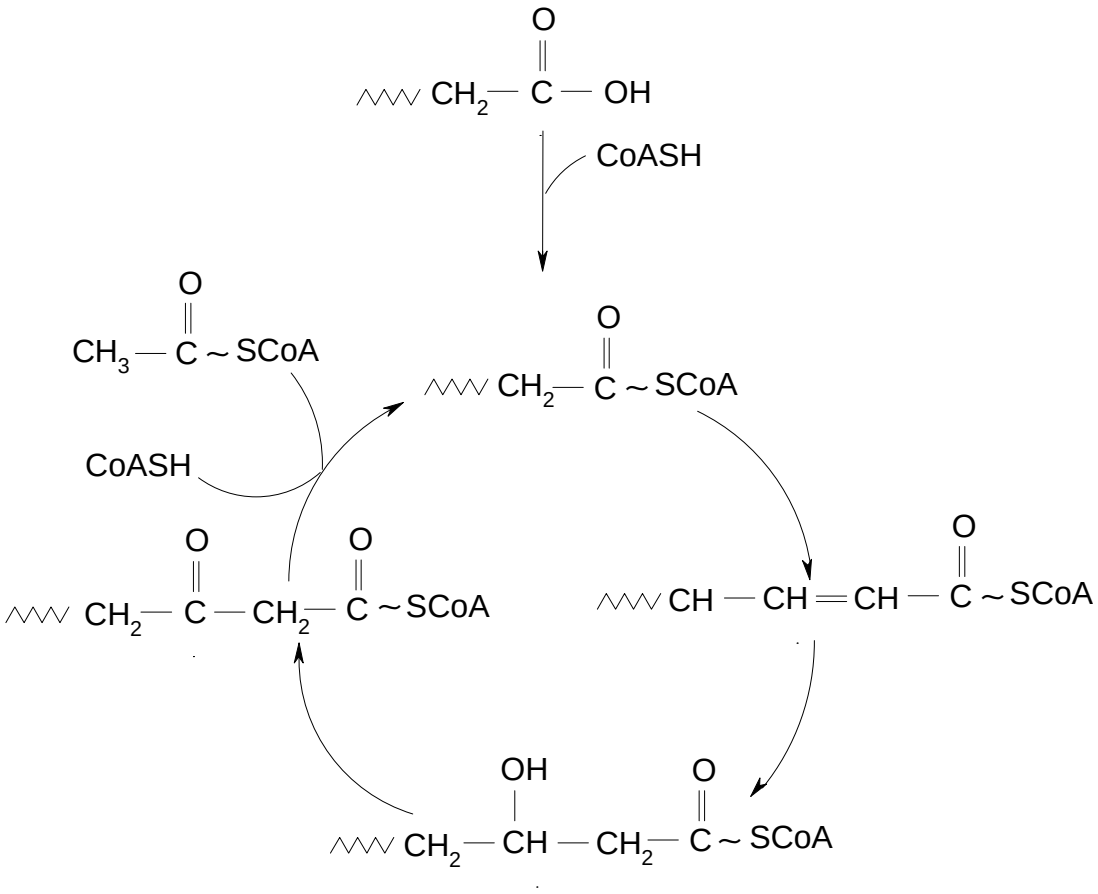


Fig. 6.3 The β-oxidation of carboxylic acids.

Hints of Answers

See section 5.1.1 and section 5.1.2

Exercise

Group discussion: Discuss the biodegradation of polyolefins, mechanisms of biodegradation, and factors which affect biodegradation.

6.2 Methods Used to Evaluate the Degradability of Polyolefins

The evaluation of polyolefin degradability may be divided into five parts: molecular weight characterisation, morphological characterisation, physical properties determination, mechanical properties determination and melt rheology analysis.

6.2.1 Molecular Characterisation

Molecular weight measurement is a very useful way of following degradation and degradation mechanisms in polymeric materials. The most frequently used method for the determination of molecular weight and molecular weight distribution is size exclusion chromatography (SEC). By combination of SEC with either light scattering or viscometry, the degree of long-chain branching can be assessed.

In the case of biodegradation, results obtained from molecular weight measurements do not always correlate with the other results such as weight loss, since biodegradation occurs initially at the surface of the material, whereas changes in molecular weight are apparent when the bulk of the polymer begins to deteriorate. However this method is a very significant technique for following the mechanism of degradation, e.g. where in the polymer chain the cleavage occurs.

6.2.2 Spectroscopy

Infrared Spectroscopy (IR) and Nuclear Magnetic Resonance Spectroscopy (NMR) are two spectroscopic methods which can be utilised to follow degradation in polymers. Spectroscopy techniques provide information about e.g. the type and concentration of chemical species, degradation products, chemical moieties incorporated into the polymer molecules (branches, co-monomers, un-saturation), additives such as antioxidants and catalyst residues.

6.2.3 Thermal Analysis

Thermal analysis of polyolefins generally involves heating or cooling a sample at a controlled rate while monitoring some of its physical characteristics. Changes in morphology (heat capacity, crystallinity, melting temperature) can be measured by Differential Scanning Calorimetry (DSC). Thermogravimetry (TGA) can be used to measure changes in weight.

6.2.4 Mechanical Properties

The measurement of mechanical properties such as elongation at break and tensile strength is usually a useful way of following the rate of degradation in polymeric materials. In the case of biodegradation, these parameters are not a direct measurement of biodegradability, since biodegradation on the surface of the polymer does not lead to changes in the polymer bulk. Therefore mechanical properties measurements must be used together with other methods to confirm the biodegradation.

6.2.5 Chemiluminescence Measurements

Chemiluminescence (CL) is very useful technique for measuring the very weak luminescence that is emitted as a result of oxidative reactions. The CL emitted during the oxidation of polymers correlates well with the oxygen uptake.

6.2.6 Identification and Quantification of Degradation Products

Degradation of polymers leads to the formation of low molecular weight products, and Mass Spectrometry is very sensitive and important method for the analysis and identification of such products. Using Gas Chromatography Mass Spectrometry (GC-MS) or High Pressure Liquid Chromatography Mass Spectrometry (HPLC-MS), degradation products from polymers and their additives can be identified. The nature of the degradation products provides information about the degradation mechanisms.

6.2.7 Methods Used to Evaluate Biodegradation

Different aspects have to be considered to characterise the biodegradation of polyolefins, among them: methods to evaluate biodegradation and specific test and conditions. Traditional tests methods for the testing of biodegradability are as follow:

Visual Inspection

Microbial colonies and mycelium growth on the polymer surface, which can generally be seen by the eye.

Weight Loss

A simple and quick way to measure the biodegradation of polymers is a weight loss determination. Micro-organisms grown within the polymer lead to an increase in weight, whereas a loss of polymer

integrity leads to a weight loss. Weight loss is proportional to the surface area since biodegradation usually initiated at the surface of the polymer. This method cannot be used on polymers that are water absorbing.

Oxygen Consumption or Carbon Dioxide Evolution

Measurement of the metabolic activity of the micro-organisms by oxygen uptake or CO_2 evolution is a direct method of biodegradation. Oxygen consumption measurement: respirometers are used and the oxygen uptake is measured by the change in pressure in the sealed bottles in which the soil and plastics samples are contained. The limitation of this method lies in the difficulty of distinguishing the oxygen consumption for the other metabolic pathways such as nitrification or chemical oxidation.

Changes in the Surface

Scanning electron microscopy (SEM) can be used to detect changes in the surface of the polymer during degradation. This method can be used only to estimate biodegradation and not for quantification. The limitation of this technique is that it is not possible to distinguish whether the changes in structure are due to the biodegradation or to chemical degradation. Furthermore the growth of micro-organisms can be due to the consumption of additives and not the polymer.

Self-check Questions

1. Which test methods provide information about crystallinity?
2. What kind of information can you obtain from SEC, IR, DSC?
3. What is the limitation of the oxygen uptake measurement?

Hints of Answers

See section 5.2.

Exercise

Group discussion: Discuss advantages and disadvantages of different test methods used to evaluate the degradability of polyolefins

Reading Materials

1. TITLE: Degradable polymers
AUTHORS: Albertsson A. C., Karlsson S., (Editors)
PUBLICATION INFORMATION: Oxford, Huthig & Wepf , April 1998.
PUBLICATION YEAR: 1998, ISBN: 3-85739-327-0

2. TITLE: Degradability, Renewability and Recycling-Key Functions for Future Materials
AUTHORS: Albertsson A. C., Chiellini E., Feijen J., Scott G., (Editors)
PUBLICATION INFORMATION: Weinheim, Germany, WILEY-VCH, October 1999.
PUBLICATION YEAR: 1999, ISBN: 3-527-29904-1

3. TITLE: Polymer Degradation and Stabilization
AUTHORS: Grassie N., Scott G., (Editors)
PUBLICATION INFORMATION: Cambridge: Cambridge U. P., 1985
PUBLICATION YEAR: 1985, ISBN: 0-521-24961-9

4. TITLE: Degradable polymers: Principles and Applications
AUTHORS: Scott G., Gilead D., (Editors)
PUBLICATION INFORMATION: London, Chapman & Hall, 1995.
PUBLICATION YEAR: 1995, ISBN: 0-412-59010-7

5. TITLE: Degradation and Stabilisation of Polyolefins
AUTHORS: Sedlacek, Blahoslav, (Editors)
PUBLICATION INFORMATION: New York, Cop. 1977.
PUBLICATION YEAR: 1977, ISBN: 99-0130972-7

6. TITLE: Degradation and Stabilization of Polyolefins

AUTHORS: Allan N. S. (Editor)
PUBLICATION INFORMATION: London, Applied Science, Cop., 1983.
PUBLICATION YEAR: 1983, ISBN: 0-85334-194-x

7. TITLE: Mechanisms of Polymer Degradation and Stabilization
AUTHORS: Scott G., (Editoe)
PUBLICATION INFORMATION: London, Elsevier Applied Science, Cop., 1990.
PUBLICATION YEAR: 1990, ISBN: 1-85166-505-6

6.3 Surface Analysis of Polymers

6.3.1 Introduction

In this chapter of the training pack different techniques for the analysis of polymeric surfaces are described. The techniques are divided in three sections: techniques for the analysis of surface structure and morphology, techniques for the analysis of the chemical composition and techniques for the analysis of physical surface characteristics.

All methods are described in a uniform manner: of each method, first the analysis principle is discussed followed by a concise description of the method and the sample information that is obtained by the method. Furthermore, a short literature list is given including available web-based databases that can be consulted for instance for the interpretation of results.
Of each method, a schematic figure shows the basic, physical principle of the analysis as well as the radial and lateral distance over which sample information is or can be obtained by the method. At the end of each section a few questions will be given in order to examine yourself.

Two important practical aspects of surface analysis are sample preparation and the conditions during analysis. In many cases samples need to be prepared in a certain way to make them suitable for the intended analysis. This may include sizing and drying of the specimen. Care should be taking that this sample handling does not lead to alteration of the surface. Furthermore the analysis technique itself may alter the surface during analysis due to the required conditions for analysis like vacuum or the impact of the analysis itself such as electron bombardment and X-ray irradiation. Therefore, while interpreting the results it should be carefully considered whether surface alteration might have occurred due to the analysis itself.

In the table all analysis methods that are discussed in this chapter are presented according to the characteristic measured, their depth and lateral resolution, and the conditions applied during analysis. This figure can be used for a quick selection of the analysis method once it is known what kind of information about the sample is required.

In this section, the techniques are categorized according to the information they yield, i.e. Surface structure and topography, Chemical surface properties, and Physical surface properties. If applicable, subdivision will be made with respect to the specific principle (e.g. scanning probes). The most commonly used analysis techniques will be summarized according to a uniform structure:

Principle:
Short description of the principle on which the technique is based. Furthermore, a schematic picture of the analysis technique will be given, which shows the principle of measurement and the lateral and depth resolution of the technique. This allows quick screening of the applicability of the technique for the surface you want to investigate.
Description: Detailed description of the technique.
Surface characteristic: Summary of characteristics measured.
Info: where to find extra information, including suppliers, books etc.

Table 6.4 Summary of surface analysis techniques

Technique	Characteristic measured	Information Depth (nm)	Lateral Resolution (nm)	Condition*
COM/CM	Structure, 3-D image	70	200	A
NSOM	Structure	70	10	A
STM	Topography	0.1	0.1	UHV/A
AFM	Topography, force maps	0.1	0.1	A/L/UHV
TEM	Morphology	1000	0.1	UHV
SEM	Topography, Morphology	1000	1-5	UHV/A/L
XPS	Elemental Composition	1-10	10^4	UHV
AES	Elemental Composition, depth	2	15	UHV
SIMS	Chemical structure, depth	5	10^3	UHV
TOF-SIMS	Chemical structure, depth	5	150	UHV
RBS	Elemental Composition, depth	2	2.10^6	UHV
LEIS	Elemental Composition	0.5	3.10^3	UHV
HFS	H content	500	2.10^6	UHV
PIXE	Elemental Composition	500	3.10^6	UHV
FTIR	Chemical structure	10^3	5.10^3	UHV
EELS	Chemical structure	10^3	10^6	UHV
TXRF	Elemental maps	3-20	2.10^5	UHV
EDX	Elemental composition	10^3	10^3	UHV
Contact Angle	Surface free energy	0.5	-	L
Zeta potential	Zeta potential	0.5	-	L

*A = Ambient, L=Liquid, UHV = Ultra High Vacuum

6.3.2 Analysis of Surface Structure and Topography

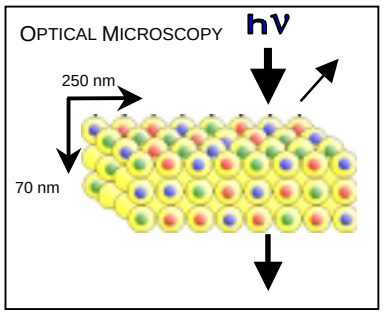
Optical Microscopy

In optical microscopy light is used to acquire an image of the sample under investigation. The way the light is directed at the sample and measured may differ per technique.

Info:
Optical Microstructural Characterization of Semiconductors, M. S. Ünlü, J. Piqueras, N. Kalkhoran, and T. Sekiguchi, (Proceedings of MRS, 2000).
http://www.mcbaininstruments.com/reflib.htm
http://nsm1.fullerton.edu/~skarl/EM/Instruction.html
http://www.mwrn.com/product/light_microscopy/optical.htm

Conventional Optical Microscopy (COM)

Principle:
Light that is cast on a sample is partly absorbed/reflected. Due to differences in transmission/reflection in the sample an image is obtained, which is magnified by a lens system.



Description:
In conventional microscopy light is used to see the sample under investigation. Light is either reflected or transmitted by the sample. Via several lenses magnification is achieved. If applicable, the view can be stored on film or computer. The method of operation is determined by the configuration of the microscope (stereo, upright, or inverted), the characteristics of the sample (transparent or not, fluorescence), and the light used (i.e. un-/polarised). With stereomicroscopes 3-D images are generated, which can be very useful for depth-analysis of samples. Using polarised light it is possible to distinguish different phases in the samples, for example crystalline and amorphous. Major advantages are the ease of operation, the versatility of samples that can be investigated and the low costs. A drawback is the relatively low magnification (500-2000x).

Surface characteristic:
Surface structure, phase differences, fluorescence, roughness.

Info:
Optical microscopy: emerging methods and applications, B. Herman, J.J. Lemasters, Academic Press, San Diego, 1993.
Handbook of Microscopy: applications in materials science, solid-state physics and chemistry, S. Amelinckx Ed., VCH, Weinheim, 1997.
http://www.leica-microsystems.com
http://www.zeiss.com
http://www.nikon.com
http://www.olympus.com

Confocal Microscopy (CM)

Principle:
"Confocal" means that distance from the focal plane to respectively the light source and the detector are the same. Laser light reflected/transmitted by the sample is cast onto a detector. Due to differences in transmission/reflection in the sample an image is obtained. Magnification is achieved by a lens system.

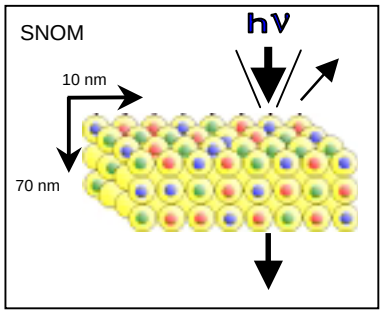
Description:
Confocallity is attained by an arrangement of diaphragms that act as a point source and as a point detector, respectively. Due to the confocality, in the final image the focus point corresponds to the point of focus in the object. Light from above and below the plane of focus of the object is eliminated from the final image by the detector pinhole. This gives rise to a high loss of light and limits the versatility. The emitted/reflected light passing through the detector pinhole is transformed into electrical signals by a photomultiplier and displayed on a computer monitor screen.
Moving the focal plane through the sample (optical slicing) creates a 3-D image of the real material. This eliminates the need for extensive sample preparation. Due to the optical set-up, all optical laws apply, limiting the resolution to 200 nm in the x-y-direction and 70 nm in the z-direction.

Surface characteristic:
3-D image, fluorescence.

Info:
Confocal scanning optical microscopy and related imaging systems, T.R. Coyle, Academic Press, San Diego, 1996.
http://www.confocal-systems.de
http://www.cs.ubc.ca/spider/ladic/confocal.html
http://swehsc.pharmacy.arizona.edu/exppath/old/conf_www.html

Scanning Near Field Optical Microscopy (SNOM/NSOM)

Principle:
A small spot of "light" is scanned over the specimen by an aperture (100 nm) close (25 nm) to the sample surface and the reflected (or transmitted) light is detected for image formation.



Description:
The fundamental limit on the size of viewable objects is imposed by the wavelength of the light used (diffraction limit 250 nm for visible light). To circumvent this diffraction limit, a small aperture is scanned very close (a fraction of a wavelength) to the surface of interest. Light cannot pass through such an aperture, however an evanescent field, the optical near-field, protrudes from it. Light can be shone down the fibre, or light can be detected via the fibre from a 'distant' light source. The optical near-field decays exponentially with distance, and is thus very surface sensitive. Furthermore, it is only detectable in the immediate vicinity of the tip. SNOM (also known as NSOM) can therefore detect objects down to the 10 nm regime.

Surface characteristic:
Topography, light images, orientation.

Info:
Near-field optics: theory, instrumentation, and applications, M.A. Peasler, P.J. Moyer, Wiley-Interscience, New York, 1996.
http://www.ukesca.org/tech/nsom.html
http://www.uni-muenster.de/Physik/PI/Fuchs/researchactivities/methods/snom
http://www.snom.omicron.de
http://www.thermomicro.com
http://www.witec.de/ (also for STM en AFM)

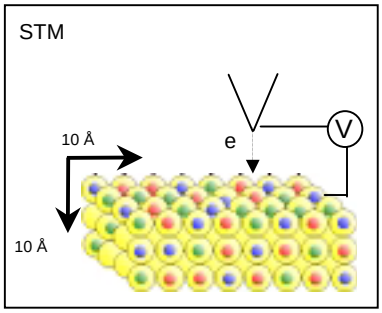
Scanning Probe Microscopy (SPM)

SPM is a general term that includes all techniques where a sharp tip is scanned over the surface to measure a specific surface characteristic (in this respect NSOM is also a SPM technique). Below, the respective techniques will be discussed in detail.

Info:
Scanning probe microscopy: analytical methods, R. Wiesendanger, Springer Verlag, Berlin, 1998.
http://www.thermomicro.com
http://www.witec.de
http://www.angstromtools.com
http://www.omicron.de
http://www.mwrn.com/product/scanning_probe/microscope.htm

Scanning Tunneling Microscope (STM)

Principle:
The "tunneling" current between the scanning tip and the sample is measured and can be converted into a surface topography image.



Description:
A sharp tip is scanned in close proximity (few Å) across the surface. A voltage bias is placed across the tip-sample gap, which induces a "tunneling" current to flow between the tip and the sample. This current is exponentially dependent upon the distance between the tip and the sample. Therefore, STM is very surface sensitive. Because the STM works by a current to or from the sample, the sample must conduct electricity. However, STM images of non-conducting polymers can be obtained when they are deposited on conducting substrates. STM was developed to be used under Ultra High Vacuum (UHV) conditions but nowadays images are also obtained under ambient conditions and even in liquid. An atomic resolution (<10 Å) is obtained.

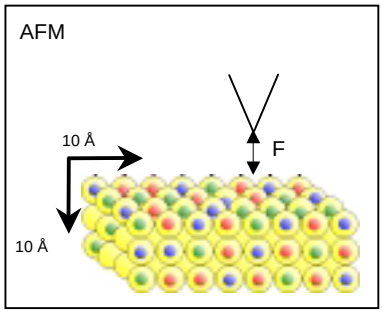
Surface characteristic: Topography.

Info:
Scanning tunneling microscopy and its applications, C. Bai, Springer Verlag Berlin, 1995.
Atomic force microscopy/scanning tunneling microscopy, S.H. Cohen, M.T. Bray, Plenum Press, New York, 1994.
Introduction to scanning tunneling microscopy, C.J. Chen, Oxford University Press, New York, 1993.
http://socrates.berkeley.edu/~davisgrp/stm/background/STMbasics.htm
http://nanowiz.tripod.com/stmbasic/stmbasic.htm
http://www.mines.edu/students/p/pejohnso/explain.html
http://www.ukesca.org/tech/stm.html

Atomic Force Microscopy

Principle:

The surface of a sample is probed with a sharp tip that is located at the free end of a cantilever. The molecular forces exerted by the surface against the tip cause the cantilever to bend or deflect. The measured cantilever deflections allow a computer to generate a surface map of measured force.



Description:
AFM measures forces between the tip and the surface. Depending on the mode of operation and the force measured, many surface characteristics can be imaged. The tip can be constantly in contact with the surface (contact mode), it can gently tap the surface while oscillating at high frequency (tapping mode), or it can be scanned just minutely above the surface (non-contact mode). A detector measures the cantilever deflection as the tip is scanned over the sample, or the sample under the tip. Image processing software allows easy extraction of useful surface parameters. Usually, no preparation of the sample is needed. Interpretation of the data is critical and one should be aware of the many artifacts that can be brought about by the measurement itself.

The resolution of AFM can be very high (atomic) in both lateral and depth, but is strongly dependent on the sample. On highly ordered surfaces atomic resolution can be achieved, whereas on poorly ordered surfaces the resolution is determined by the tip-sample contact area. AFM can be used to study insulators and semiconductors as well as electrical conductors. Furthermore, depending on the mode of operation, measurements can be carried out in various environments (UHV, Ambient, Liquid etc). The force measured is related to the mode of operation and also the tip characteristics (e.g. Magnetic Force Lateral Force, Electrostatic Force, Chemical Force etc.).

Surface characteristic:
A surface map is obtained of a specific characteristic of the sample. The specific name is usually referring to the surface characteristic measured. For instance, Electrostatic Force Microscopy (EFM) maps locally charged domains.

Info:
Scanning force microscopy: with applications to electric, magnetic and atomic forces, D. Sarid, Oxford University Press, New York, 1994.
http://www.gpec.univ-mrs.fr/friction/afmbasic.html
http://www.di.com
http://www.triple-o.de

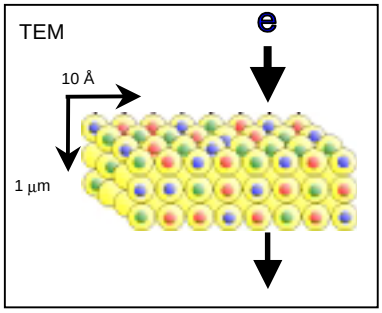
Electron Microscopy

Just as Light Microscopes use light, Electron Microscopes use electrons to acquire an image. Electrons are directed towards the sample by a voltage bias. Focussing and magnification are carried out by magnetic lenses. By interactions within the sample, the electron beam is altered. These alterations are transformed to an image. Because electron beams are used, UHV conditions apply.

Info:
Electron Microscopy and analysis, P.J. Goodhew, F.J. Humphreys, R. Beanland (Eds.), Taylor & Franchis, London, 2000.
High-resolution electron microscopy for materials science, D. Shindo, K. Hiraga, Springer-Verlag, Tokyo, 1998. http://www.nissei.com/
http://nsm1.fullerton.edu/~skarl/EM/Instruction.html

Transmission Electron Microscopy (TEM)

Principle:
An electron beam is directed onto a sample and partly absorbed. The transmitted beam is projected.



Description:
A TEM works much like a slide projector, now working with an electron beam instead of a light bulb. The electron beam is affected by the

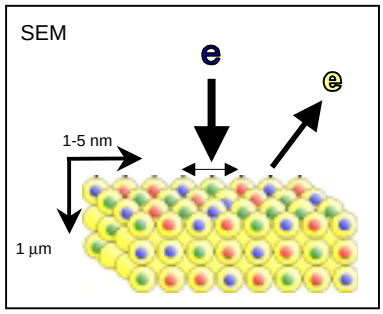
structures and objects in the sample. The beam is only transmitted through certain parts of the sample. This transmitted beam is then projected onto the viewing screen via magnetic lenses, forming an enlarged image of the sample with about 10-20 Å resolution. Due to the high absorption of electrons, the specimen must be thin (<1 µm). To enhance contrast in electron absorption/scattering, the sample may be stained with an agent that selectively reacts with part of the material. For instance, OsO_4 reacts selectively with unsaturated bonds in a sample and strongly absorbs electrons. After reaction, those parts that have reacted with OsO_4 stand out very clearly in the image.

Surface characteristic:
Morphology, Crystallographic Information, Compositional Information (if equipped with EELS).

Info:
http://www.mwrn.com/product/electron_microscopy/transmission/microscope.htm

Secondary Electron Microscopy (SEM)

Principle:
A focused beam of electrons is rastered across a sample surface thereby generating secondary electrons that are detected.



Description:
The secondary electrons coming from the sample are detected, which results in an image of the variation of secondary electron intensity with position on the sample. The variation is largely dependent on the angle of incidence of the focused beam onto the sample, thus yielding a topographical image with a high resolution (10-30 Å). This image is magnified by magnetic lenses. Using special electron sources (Field Emission) even higher resolutions can be obtained. To avoid charging, insulating samples must be coated with a conductive film. Furthermore, UHV conditions are required (with exception of environmental SEM).

Surface characteristic:
Morphology, Topography, Crystallographic and Compositional information (if equipped with EDX).

Info:
http://www.mwrn.com/product/electron_microscopy/scanning/microscope.htm
http://www.gatan.com
http://www.ebsciences.com/
http://www.emsdiasum.com/ems/

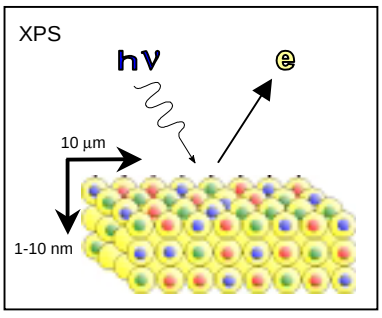
Self-check Questions:

1. Which technique is most suitable to determine the phase separation of a semi-crystalline polymer?
2. If you want to have a 3-D image of your sample, which techniques would you use?
3. To get a quick indication of the surface topography/structure, which techniques are the easiest?
4. Give two similarities between NSOM and STM.

6.3.3 Analysis of Chemical Surface Properties

Electron Spectroscopy

Electron Spectroscopy for Chemical Analysis (ESCA) or X-ray Photoelectron Spectroscopy (XPS)



Principle:
X-rays are directed towards the sample, causing the emission of photoelectrons from the sample. The kinetic energy of these photoelectrons is a measure of the binding energy.

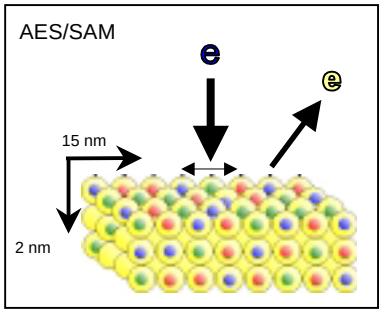
Description:
In an XPS measurement the sample under investigation is irradiated under UHV with a focused, monochromatic X-ray beam, which causes photoelectrons to be emitted from the sample. The binding energy (in the sample of course) of the emitted photoelectrons is calculated by subtracting the kinetic energy from the energy of the incoming X-rays. From the binding energy and intensity of a photoelectron peak, the elemental identity, chemical state (bonded to what), and quantity of an element can be determined. The depth of analysis is determined by the escape depth of the photoelectrons and the angle of the sample plane relative to the spectrometer (1-10 nm). Using angular-dependent XPS, depth profiles are obtained in a non-destructive manner. ESCA, also known as XPS, is the most widely used surface analysis technique because of its relative simplicity in use and data interpretation.

Surface characteristic:
Valence states and/or bonding environment of atoms, elemental composition (surface mapping can be done on most modern systems), depth profiling.

Info:
Practical Surface Analysis, Vol. 1. Auger and X-ray Photoelectron Spectroscopy, D. Briggs, M.P. Seah (eds.), John Wiley & Sons Ltd, Chichester, UK, 1990.
http://www.xpsdata.com/useful_books.htm
http://prins00.ethz.ch/abstracts/XPS.htm
http://goliath.inrs-ener.uquebec.ca/commerce/xps-tech.html
http://www.vgscientific.com/
http://www.kratos.com/
http://www.phi.com/
http://www.ukesca.org/data.html

Auger Electron Spectrometry (AES) or Scanning Auger Microscopy (SAM)

Principle:
The sample is irradiated with a focused electron beam producing Auger electrons, the energies of which are characteristic of the element from which they are generated.



Description:
As already guessed from the abbreviation, SAM is related to SEM because in both an electron beam is scanned over the surface. This results in a high lateral resolution. In fact, the SEM image that is inherently obtained is used for positioning of the beam in SAM. In SAM, the generated Auger electrons are detected. Similarly to XPS, the quantity of an element can be determined. However, for the elements usually found in polymers, the energy of Auger electrons has less differentiating power than photoelectrons. Therefore, only the elemental composition is obtained. Because of charging, the sample must be conductive, although polymers can be measured when deposited on conductive substrates. The main application AES is used for is depth profiling. With an ion beam a thin surface layer is removed and successively an AES measurement is performed. By repetitive sputter/measure cycles a compositional depth profile is obtained.

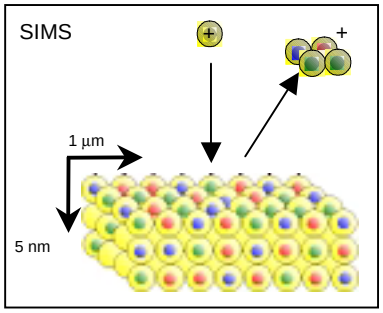
Surface characteristic:
Elemental composition, compositional depth profile.

Info:
Methods of Surface Analysis, A. W. Czanderna, ed., Elsevier, New York, 1975.
Photoelectron and Auger Spectroscopy, T. A. Carlson, Plenum Press, New York, 1975.

Mass Spectroscopy

Secondary Ion Mass Spectrometry (SIMS)

Principle:
A sample surface is bombarded with a primary ion beam and the emitted secondary ions are analysed by mass spectrometry.



Description:
An ion beam is directed onto a surface generating secondary ions. The resulting ion fragmentation patterns contain information useful for identifying molecular species. The secondary ions are analysed by a mass spectrometer. This allows detection of very low amounts of material. The lateral resolution of the ion beam is maintained through the spectrometer so that a mass resolved image of the secondary ions is obtained. Analogous to AES a depth profile can be generated by repetitive sputter/measure cycles. When operated in a continuous mode (Dynamic SIMS), the sample is continuously sputtered and measured, thereby automatically obtaining a depth profile.

Surface characteristic:
Elemental map, chemical structure, depth profiling.

Secondary ion mass spectroscopy of solid surfaces, V. Cherepin, VNU Science Press, Utrecht, 1987.
Secondary Ion Mass Spectrometry: Basic Concepts, Instrumental Aspects, Applications, and Trends; A. Benninghoven, F. G. Rüdenauer, and H. W. Werner, Wiley, New York, 1987.
http://epswww.unm.edu/simslab/basics.html
http://www.cea.com/cai/simstheo/caistheo.htm
http://www.simsworkshop.org/default.html
http://www.ukesca.org/data.html
http://www.vgscientific.com/
http://www.kratos.com/
http://www.phi.com/

Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS)

Principle:
The only difference with SIMS is that the analysis of the secondary ions is carried out by a specially designed time-of-flight mass spectrometer.

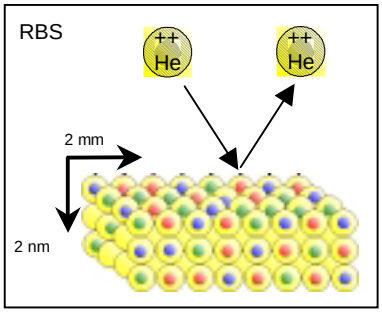
Description:
TOF-SIMS spectra are generated using a pulsed primary ion source (very short pulses of <1 ns). The emitted secondary ions are extracted into the TOF analyzer by applying a potential between the sample surface and the mass analyzer. Secondary ions travel through the TOF analyzer with different velocities, depending on their mass to charge ratio (m/z). For each primary ion pulse, a full mass spectrum is obtained by measuring the arrival times of the secondary ions at the detector and performing a simple time to mass conversion (ke=1/2mv2). The TOF-SIMS technique is capable of detecting secondary ions produced over a large mass range and performs this mass analysis at relatively high mass resolutions. The technique also is capable of generating an image of the lateral distributions of these secondary ions at spatial resolutions of better than 0.15 microns.

Surface characteristic:
Chemical composition, imaging, depth profiling.

Matrix Assisted Laser Desorption Ionisation TOF-SIMS (MALDI-TOF-SIMS)

Principle:
A very small amount of sample (about 1 pmole) is greatly diluted in a supporting matrix. By absorption of a pulse of UV laser light by the matrix, charged molecular ions are ejected into the vapour phase. These are then analysed similar to the way analysis is performed using TOF-SIMS.

Rutherford Backscattering Spectrometry (RBS)



Principle:
Helium ions are directed towards the sample and, after backscattering, analysed with respect to energy and number distribution.

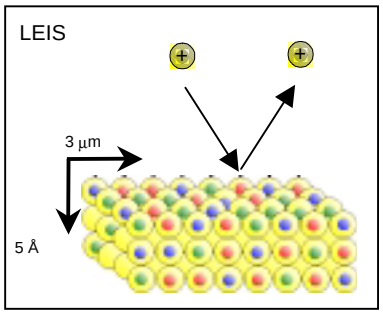
Description:
Rutherford Backscattering (RBS) involves measuring the number and energy of He^{++} ions in a beam that backscatter after colliding with atoms in the near-surface region of a sample at which the beam has been targeted. The energy of the backscattered ions provides information on both the composition and depth distribution of elements in the target. RBS is ideally suited for determining the concentration of trace elements heavier than the major constituents of the substrate. Its sensitivity for light masses is poor.

Surface characteristic:
Quantitative depth profile, elemental composition.

Info:
Backscattering spectrometry, W-K. Chu, J.W. Mayer, M-A. Nicolet, Academic Press, Boston, 1978.
http://www.pelletron.com/
http://www.danfysik.com/
http://www.cea.com/

Low/Medium/High Ion Scattering (L/M/HEIS)

Principle:
Mono-energetic ions are directed towards the sample and analysed after backscattering. The energy of the backscattered ions is characteristic of the mass of the target atoms from which they are scattered.

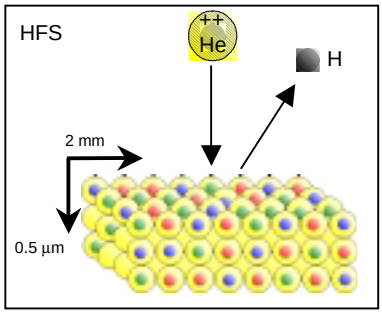


Description:
Incoming ions are scattered by the atoms in the surface. Ion scattering techniques are classified by the energy of the impinging ions used (High, Medium, and Low). According to the laws of conservation of energy and momentum ("billiard ball"), the energy after backscattering is related to the mass of the atoms from which they are scattered. The energy spectrum obtained can thus directly be interpreted as a mass spectrum of the surface atoms. The information depth is limited because the noble gas ions have a high neutralization probability. Furthermore, ions scattered from below the surface lose energy inelastically at a rate related to the ion's path length in the target. This extra energy loss is therefore directly related to the depth of the scattering atom.

Surface characteristic:
Elemental composition.

Info:
http://www.calipso.nl/

Hydrogen Forward Scattering Spectrometry (HFS)



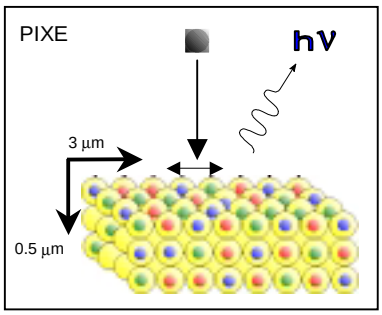
Principle:
Impinging He^{++} ions cause hydrogen (H) atoms to be scattered forward. The forward scattered H atoms are measured with respect to their number and energy.

Description:
Similar to RBS, He^{++} are directed onto the sample where they collide with surface atoms. This results in the emission of hydrogen (H) atoms that are collected by a solid-state detector, while the He atoms are stopped by a foil placed between the sample and the detector. The number of forward scattered H atoms provides information on the concentration of H in the sample, while the energy of the H provides depth information. Usually conducted together with RBS.

Surface characteristic:
Quantitative H content depth profile.

Particle Induced X-Ray Emission (PIXE)

Principle:
A high-energy particle beam (usually protons) is directed at the sample, causing electrons to be excited from their core shells. Upon decay, X-rays are emitted which are analysed.



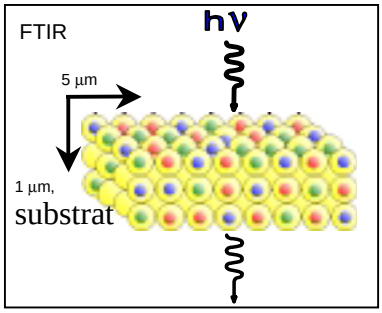
Description:
By impact of a particle, electrons are excited from their core energy levels to higher energy levels. When these excited electrons fall back, the surplus in energy is emitted in X-rays. The energy and the intensity of the X-rays are used to identify each element within the sample. By scanning of the particle beam, a map of the concentration of each element is obtained. PIXE measures the total presence of elements and cannot be used for depth profiling.

Surface characteristic:
Elemental composition map over the full depth of measurement.

Info:
http://www.supernet.net/~pixe/pixe.html
PIXE- A Novel Technique for Elemental Analysis, S.A.E. Johansson and J.L. Campbell; John Wiley and Sons, 1988.
Particle-Induced X-Ray Emission Spectrometry (PIXE): S.A.E. Johansson, J.L. Campbell, and Klaus G. Malmqvist (Eds.); John Wiley and Sons, 1995.

Vibrational Spectroscopy

Fourier Transform Infrared Spectrometry (FTIR)



Principle:
Fourier-Transformed infrared light is detected after being transmitted/reflected by the sample.

Description:
Infrared light is shone onto a sample, which partially absorbs/reflects it (cf. TEM). Therefore, the sample must be transparent to infrared light or have smooth reflecting surface. After Fourier transformation of the intensity changes an absorption spectrum (intensity vs. wavelength) is obtained. Analysis of the spectral absorption bands allows identification of the composition of the sample. An image showing the distribution of a particular IR frequency may be produced by having a motorised stepping stage connected to a microscope.

Surface characteristic:
Chemical structure of the material.

Info:
Fundamentals of Fourier transform infrared spectroscopy, B.C. Smith, CRC Press, Boca Raton, 1996.
Spectrometric identification of organic compounds, R. M. Silverstein, F.X. Webster, Wiley, New York, 1998.
http://infrared.als.lbl.gov/FTIRinfo.html
http://www.fdmspectra.com/
http://www.bio-rad.com/B2B/BioRad/br_start.jsp
http://www.bruker.com/
http://www.perkin-elmer.com
http://www.jeol.com
http://www.nicolet.com
http://www.bomem.com/

Related Techniques

Attenuated Total Reflection (ATR)

With the ATR technique, the infrared beam samples a surface by internal reflection of the light, thereby enhancing the signal and the surface sensitivity. The infrared light penetrates the surface via the evanescent field.

Multiple Internal Reflection (MIR)

The MIR approach can enhance the ATR technique by bouncing the light off the surface several times.

Diffuse Reflection

For highly scattering samples (e.g. powders) the scattered light has to be directed towards the IR detector. It is assumed that only diffuse scattered light has penetrated the sample surface.

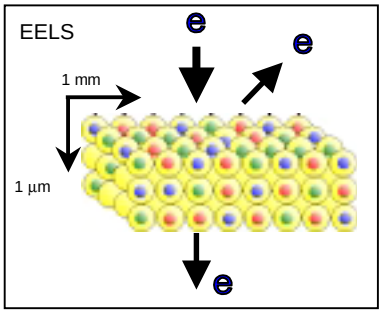
Raman Spectroscopy

Principle:
Laser light interacts with the molecular vibrations, causing a shift in frequency that is characteristic of chemical functionalities present in the sample.

Description:
Raman spectroscopy is often used together IR spectroscopy as it is sensitive to vibrational modes that are not or only weakly sensitive to the IR light. Furthermore, Raman Spectroscopy can be used in aqueous environments, whereas IR cannot. Monochromatic laser light is shone onto a sample and partly interacts with the molecular vibrations of material in such a way that either energy is added (Anti-Stokes process) or subtracted (Stokes process) to the photon energy. The energy loss due to molecular vibrations is brought about by the polarisation of the electron cloud of the chemical bond. Using a focused laser beam together with a microscope, a 3-D image of a specific Raman absorption is obtained.

Info:
Raman microscopy: developments and applications, G. Turrell, J. Corset, Academic Press, New York, 1996. http://www.lot-oriel.com/
http://www.jyinc.com/
http://www.bio-rad.com

Electron Energy Loss Spectroscopy (EELS)



Principle:
A monoenergetic electron beam interacts with the sample under investigation (transmission/backscatter) thereby transferring part of the kinetic energy by amounts corresponding to characteristic absorption frequencies.

Description:
EELS involves the bombardment of a sample with a monoenergetic beam of electrons. The electrons impinging on the sample may lose energy by a variety of mechanisms (e.g. plasmon and phonon loss). The amount of energy loss is characteristic for the vibrational motion of atoms and molecules on and near the surface. The technique is frequently used in association with Transmission Electron Microscopy (TEM). In TEM, the losses predominantly occur in the bulk of the sample, as the beam travels through the thin specimen to the EELS detector the other side. An electron passing through material can interact with electron clouds of the atoms present and transfer some of its kinetic energy to them. To get a higher surface sensitivity, the electron beam is usually reflected of the surface resulting in a sharp peak corresponding to elastically scattered electrons with a number of peaks at lower energy, which correspond to plasmon or other excitations. Electrons with energy in the range of a few electron volts sample only a few atomic layers.

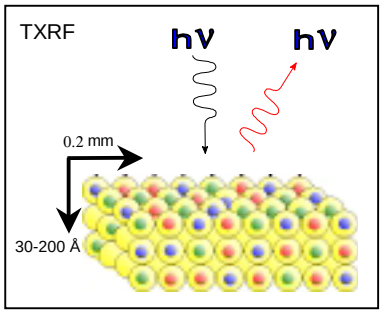
Surface characteristic:
Chemical structure.

Info:
Electron Energy-Loss Spectroscopy In The Electron Microscope, R. F. Egerton, Plenum Press, New York, 1989. www.chembio.uoguelph.ca/educmat/chm729/eels/eels0.htm

Others

Total Reflection X-Ray Fluorescence (TXRF)

Principle:
X-rays impinging the sample excite electrons of surface atoms, which on back-fall emit X-rays.

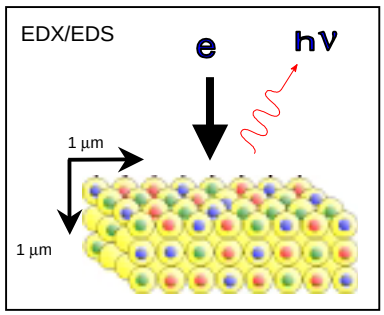


Description:
X-rays reach surface at a glancing angle, within the critical angle for total external reflectance, thereby exciting the electrons of atoms in the top few monolayers of the sample. On back-fall, photons (fluorescence) are emitted with energies that are characteristic of the particular element. Due to the glancing angle, samples must be smooth and flat.

Surface characteristic:
Elemental maps.

Energy Dispersive X-ray Spectrometry (EDS or EDX)

Principle:
An electron beam excites electrons of surface atoms, which on back-fall produce photons whose energy is characteristic of the element that emits it.



Description:
An EDS attachment uses the electron beam of a SEM to excite the atoms in the surface of a solid. On back-fall, these excited atoms produce characteristic X-rays that are readily detected. The detection and identification of the x-rays produced allows qualitative and quantitative elemental analysis. Due to the principle of SEM, a spatial distribution of elements can be obtained, although the spatial resolution is much less (1 µm). Due to the SEM principle, the sample requirements for SEM also apply here.

Surface characteristic:
Elemental composition map.

Info:
http://www.mwrn.com/product/electron_microscopy/scanning/microscope.htm

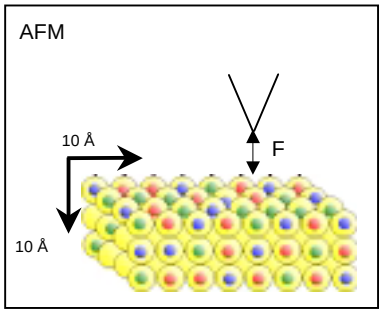
Self-check Questions

1. Which techniques can be used to determine the surface chemical composition of a polymer? And what if you want to know the depth profile of a specific element?
2. If you want to know the chemical structure of your sample, which techniques can be used and what is the quickest/easiest?
3. If information about both the surface structure and the chemical composition is important, which techniques save time due to a smart combination of techniques?

6.3.4 Analysis of Physical Surface Properties

Atomic Force Microscopy (AFM)

Principle:
As described earlier, AFM can be used to measure forces between the tip and the sample.



Description:
Using different modes of operation different characteristics of the surface are measured. The most commonly used modes are described below. One should keep in mind however that measuring may be easy, but image interpretation is the hardest part of the job. Artefacts such as tip imaging should not be mistaken for real surface features.

Tapping Mode AFM
Tapping mode was developed to be able to measure soft materials. In this mode the tip vibrates at its resonance frequency and is scanned over the surface. Due to the vibration, the tip touches the surface for very short times, minimizing inelastic deformation. The tip-sample interaction causes a change in the amplitude, the phase and the resonance frequency of the cantilever. The spatial variations of these changes can be imaged.

Force-Modulation
When operated in Force-Modulation mode, disconnection of the tip from the sample is prevented. Herewith a surface image of the (visco-) elasticity is obtained.

Magnetic Force
In this mode a magnetic tip is used to probe the variation of magnetic properties over the sample. Especially useful for storage media.

Lateral Force
Variations in the lateral force are assumed to be related to the tip-sample adhesion force. Using chemically modified tips it is possible to measure surface chemical states variations such as hydrophilicity/-phobicity. Until now mainly used in research.

Electrostatic Force
Electrostatic forces between the tip and the sample are measured thereby mapping locally charged domains.

Surface characteristic:
Surface map of specific tip-sample force.

Info:
Scanning force microscopy: with applications to electric, magnetic and atomic forces, D. Sarid, Oxford University Press, New York, 1994.

Surface Free Energy

Principle:
The angle between a droplet of a liquid or an air bubble and the surface is the result of an equilibrium of the forces that act on the droplet. The surface free energy is derived from this observed contact angle.

Description:
A droplet of a liquid with known surface tension (γ) is put onto the surface under analysis. Due to the surface tensions between the respective phases (solid, liquid, and vapour) a contact angle (Θ) between the liquid and the surface develops and can be measured. This angle is defined as: $\gamma_{LV} \cos\Theta = \gamma_{SV} + \gamma_{SL}$.

By using a series of liquids with different surface tensions, a so-called Zismann plot can be constructed ($\cos\Theta$ vs. γ_{LV}), from which the surface free energy can be calculated ($\cos\Theta \rightarrow 1$, $\Theta=0$). In addition, using polar and apolar liquids, the surface free energy can be divided in a polar and a dispersive (apolar) fraction. Due to the nature of the contact angle, the surface energy is a very surface sensitive technique (outermost 5Å).

Surface characteristic:
Surface free energy.

Info:
Chan, C.M., Contact Angle Measurement; Chan, C.M., Ed.; Hanser Publishers: Munchen, 1994.
http://www.extech.com.au/
http://www.dataphysics.de/

Zeta Potential

Principle:
The zeta potential of a surface is derived from the streaming potential that develops by a streaming medium between two parallel plates of the material under investigation.

Description:
A medium is flowed through two parallel plates of the material studied. By varying the pressure difference between the in- and outlet, from the corresponding potentials developed the zeta potential can be calculated. The zeta potential is dependent on the surface charge of the material.

Surface characteristic:
Zeta potential.

Info:
http://www.micromeritics.com/zeta.html
http://www.zeta-meter.com/
http://www.lavallab.com/eng/zeta-eng/streaming-potential.htm

Self-check Questions

1. Is AFM a suitable technique to measure the density of functional groups on a surface? If so, suggest an experiment to map COOH groups on a surface.
2. If your polymer swells in water, how can you still determine the surface free energy?

Literature

Standards

General methods for localization of standards regarding surface analysis are described on the following sites(www.astm.org/, http://www.din.de/, http://www.nen.nl/ . Subscription might be obligatory. Usually libraries of universities already have a subscription.

General References

1. Surface Characterization, A User's Sourcebook, D. Brune, R. Hellborg, H.J. Whitlow, O. Hunderi (eds.), Wiley-VCH, Weinheim, Germany, 1997.
2. Surface Analysis, The Principal Techniques, J.C. Vickerman (ed.), John Wiley & Sons Ltd, Chichester, UK, 1997.
3. Structural and chemical analysis of materials: X-ray, electron and neutron diffraction, X-ray, electron and ion spectrometry, electron microscopy, J.P. Eberhart, J. Wiley & Sons, Chichester, UK, 1991.
4. http://www.xpsdata.com/useful_books.htm

Specific References

1. Surface Analysis with STM and AFM, S.N. Magonov, M.-H. Whangbo (eds.), VCH Verlagsgesellschaft, Weinheim, Germany, 1996.
2. Practical Surface Analysis, Vol. 1. Auger and X-ray Photoelectron Spectroscopy, D. Briggs, M.P. Seah (eds.), John Wiley & Sons Ltd, Chichester, UK, 1990.
3. Practical Surface Analysis, Vol. 2. Ion and neutral spectroscopy, D. Briggs, M.P. Seah (eds.), John Wiley & Sons Ltd, Chichester, UK, 1991.
4. http://www.simsworkshop.org/www/internet/surfaceanalysis.html

Suppliers

http://www.vlifescience.com.au/BuySell/Product/Category/
http://www.uksaf.org/iandc.html
http://www.analyticon.com

6.4 GEL Permeation Chromatography (GPC)

6.4.1. Introduction

The Gel Permeation Chromatography (GPC) is a widely used technique that separates molecules according to their size (hydrodynamic volume) in solution. The principal application of this technique is the characterization of molecular weight (MW) and molecular weight distribution (MWD) of polymers, but it is also used in the analysis of oligomers and low molecular weight compounds. It complements other methodologies based on viscometry, osmometry, ultracentrifugation and light scattering. During the 1950's the use of GPC was started for the analysis of water-soluble polymers using cross-linked dextran gel columns. Those gels give excellent separation properties, but the time takes for analysis were very long because the gels tend to compact at high flow rates. During 1960's a rigid gel was developed for the use in organic solvent, accordingly.

GPC was then extended to the analysis of synthetic polymers. Moreover, the time required for the chromatographic experiment resulted significantly shorter with respect to the cross-linked dextran gels packed columns. In relatively recent years significant progresses have been made in the development of several rigid gels both for aqueous and non-aqueous systems so far extending the performance of GPC determinations. Moreover the recent instrumentation with high sensitive, stable detectors and better column technology has further reduced the analysis time and increased the number of information about the polymer characteristics.

6.4.2 GPC Theory

The GPC is an exclusion chromatography that separates the molecules with different size in solution. The molecules of polymer in solution (mobile phase) go throughout the pores of the gel (stationary phase) and are excluded therefrom on the basis of the hydrodynamic volume; this accounts for a shortest path (e.g. less elution volumes or retention times) in the case of largest molecules.

The theoretical models proposed in the elution mechanism based on size exclusion chromatography are based on equilibrium or flow conditions. While flow mechanism are important in some cases, there is abundant evidences indicating that most practical GPC separation are performed under equilibrium conditions.

The following equation is an empirical expression relating the GPC elution parameters:

$$V_R = V_0 + k_{GPC}\, V_i \qquad [1]$$

where V_R is the peak retention volume, V_0 is the total volume of the mobile phase, k_{GPC} is the distribution coefficient and V_i represents the volume of stationary phase (i.e. the solvent volume within the porous packaging). The k_{GPC} is often included between 0 and 1, 0 for excluded species and 1 for totally permeating species.

Equilibrium based theoretical models suggest that the distribution of macromolecules between the mobile and stationary phases is a consequence of the thermodynamic equilibrium, thus assuming that the retention volume is independent from the flow rate.
Finally, the steric exclusion based theoretical model assumes that both the macromolecule size and the distribution of pore sizes in the stationary phase determine the elution volumes.

Thermodynamic interpretation

Another approach based on the equilibrium model assumes that k_{GPC} represent a true equilibrium constant. The standard free energy change (9) ΔG^0 for the transfer of polymer molecules from the mobile phase to the stationary phase at constant temperature T is related to k_{GPC} by:

$$\Delta G^0 = - k\ T \ln k_{GPC} \qquad [2]$$

where k is Botzman constant.

GPC separation can be considered to be consisting of two (component) mechanisms. The first involving the steric exclusion phenomenon having a free energy change ΔG_D, the second one, if present, should have a free energy change ΔG_P resulting from the interaction of the polymer molecules with the stationary phase.

$$\Delta G^0 = \Delta G_D + \Delta G_P \qquad [3]$$

Accordingly the cited contributions quoted in Equation [1] - [3] provide the following comprehensive relationship:

$$V_R = V_0 + V_i \exp[(-\Delta G_D/kT)+(-\Delta G_P/kT)] \qquad [4]$$

Steric exclusion

In the presence of a true inert pore surface, the value of ΔG_P will be zero. This steric exclusion model is equivalent to the statistical mechanical treatment of the loss in conformational entropy when a macromolecule approaches an inert surface. The relevant thermodynamic theories (4, 9) compute the accessible pore volumes when a polymer molecule is transferred from a mobile phase to a pore within the packing. The distribution coefficient k_D at equilibrium is defined as the ratio of accessible conformation for polymer within the porous packing to that in the mobile phase. It is assumed that there are no changes in the free energy of mixing when the polymer molecules move from one phase to the other, and there is not interaction between the polymer sample and the inert porous packing. Consequently ΔG_D is given

$$\Delta G_D = - T\ \Delta S_D = - kT \ln k_D \qquad [5]$$

where ΔS_D is the standard entropy change. In the steric exclusion mechanism k_D becomes identical with k_{GPC}. The statistical-mechanical interpretation of k_D shows that the separation is determined by the mean molecular projection independent of molecular geometry(5, 8, 9). Giddings et al. (9) give

$$k_D = \exp(- s\ L/2) \qquad [6]$$

where L is the mean external length or molecular projection, e.g. L corresponds to the diameter of a sphere, and *s* is the surface area per unit pore volume.

6.4.3 GPC Practical Assembly

GPC analytical devices include a pump, capable of stable flow rate (range from 0.1 to 3.0 ml/min) equipped with high-pressure injection valve, a column (usually 2 columns) and a detector with high stability and sensitivity. Polyolefin based polymeric material often require operative conditions at high temperature (130°C), so a complex device aimed at maintaining column, connections and other parts at stable temperature is needed. The columns can be packed either with a porous cross-linked polymer, or with a porous inorganic material. Commercially available GPC columns are usually about 30 cm long and 0.7 cm outer diameter, and their packing particle size is less than 30 μm.

Most of GPC apparatus are equipped with two detectors working contemporary and consisting of a differential refractive index and an ultraviolet absorption based detectors. Both fixed wavelength and turnable devices, however require calibration for their response factors at low molecular weights in order to obtain quantitative concentration of oligomers and additives.

Another detector, which is receiving increasing attention, is the light scattering evaporative analyzer, where the effluent is passed throughout an atomizing head into heated evaporative column. The solvent is thus evaporated and the solute is formed into a small particle, detectable by light scattering at 120° to the incident beam. Its use is advantageous when mixed solvents are used or when low refractive index difference between solvent and polymer occurred.

6.4.4 Determination of Molecular Weight Distribution (MWD) by GPC

The data available from the GPC includes the determination of average molecular weight of a polymer and its integral and differential molecular weight distributions. Both these data require the instrument calibration.

6.4.4.1 Calibration

The calibration may be done in two different modes: by using a series of narrow MWD standards, or by the universal calibration approach.

Calibration with narrow MWD standards

This method involves the chromatographic analysis of some standards and plotting their MW versus elution volume or time. After calibration the data are treated mathematically so that MW at any elution volume or time is available either by interpolation or polynomial curve fitting. Theoretical solutions are obtained [11] for standards that have long-normal, Schulz or Stockmayer distributions. The use of linear or uncorrected polynomial expression may lead to serious errors.

Universal calibration

Experimental evidences that the polymer molecules size determines GPC separation was provided by Benoit *et al.*(10) who examined homo-polymers and copolymers with cross-linked poly(styrene) gels and tetrahydrofuran as eluent. They proposed the product $M[\eta]$ as universal parameter, where M is the molecular weight and $[\eta]$ is intrinsic viscosity. This universal parameter is proportional to the hydrodynamic volume of a polymer molecule, as well as to its size according to the Einstein and Flory-Fox equations respectively.

Equation [5] and [6] suggest that k_D is temperature independent, a characteristic of a mechanism controlled by entropy changes. Cooper and Bruzzone (11) have obtained an experimental calibration curve for poly(styrene) and poly(isobutene) in trichlorobenzene for porous glass columns at 25 and 150 °C. Their separations were depending on the hydrodynamic size and were independent on the polymer structure, polymer-solvent interaction, and temperature. Furthermore, the exponent *a* in the Mark-Houwink equation is in the range 0.7 - 0.8 for poly(styrene) (12) in this eluent, and the eluent is very compatible with the cross-linked poly(styrene) gel. For polymer in good solvents, these results support the view that the separation is controlled by entropy changes.

The equilibrium theories therefore predict that the behavior of all polymers can be represented by an universal size parameter, so a MW calibration curve for one polymer may be calculated by utilizing poly(styrene) standards of narrow MWD. The calibration are related by the following expression:

$$\log M_p - \log M_{ps} = \log [\eta]_{ps}/[\eta]_p \qquad [7]$$

where p refers to the polymer and ps to poly(styrene) standard.

Anderson and Stoddart (13) observed that in the middle of k_D range, theoretical plots of k_D versus the logarithm of polymer size are essentially linear. By following their procedure and assuming that hydrodynamic volume is the universal size parameter determining a steric exclusion separation, it can be write:

$$k_D = - A \ln [\eta] M + B \qquad [8]$$

where A and B are constants.

6.4.5 Average Molecular Weights and Distribution

Molecular weight averages may be calculated from a series of chromatogram heights, h_i measured at equal elution intervals. Molecular weight is assigned to those points from the correct calibration curve. The equations to calculate the number, weight, and Z average molecular weights are reported:

$$M_n = \frac{\sum h_i}{\sum (h_i / M_i)} \qquad [9]$$

$$M_w = \frac{\sum h_i M_i}{\sum h_i} \qquad [10]$$

$$M_z = \frac{\sum h_i M_i^2}{\sum h_i M_i} \qquad [11]$$

The differential distribution may be obtained from the chromatogram directly from the calibration curve. The differential distribution is obtained by drawing dw/dM versus M, where w is the weight fraction with MW below M. This may be written as:

$$\frac{dw}{dM} = \frac{dw}{dV_e}\frac{dV_e}{d(\log M)}\frac{d(\log M)}{dM} = \frac{1}{M}\frac{dw}{dV_e}\frac{dV_e}{d(\log M)} \qquad [12]$$

The ordinate of chromatogram is dw/dV_e. If the calibration curve is linear the latter term is constant, on the contrary if non-linearity occurs, the numerical differentiation of calibration curve must be performed at each point. Sometimes it is more convenient to plot the differential distribution in terms of log M:

$$\frac{dw}{d(\log M)} = \frac{dw}{dV_e}\frac{dV_e}{d(\log M)} \qquad [13]$$

Thus, in the case of a linear calibration curve the differential distribution plotted on a logM scale will be identical with elution curve plotted on a retention volume scale.

The two ordinates dw/dM and dw/d(logM) are related by:

$$\frac{dw}{d(\log M)} = \frac{dw}{dM}\frac{1}{2.303M} \qquad [14]$$

From the differential distribution the average molecular weights may be calculated from eqns. [15], [16] and [17]

$$M_n = 1\Big/\int \frac{1}{M}\left(\frac{dw}{dM}\right)dM \qquad [15]$$

$$M_w = \int M\left(\frac{dw}{dM}\right)dM \qquad [16]$$

$$M_z = \frac{\int M^2\left(\frac{dw}{dM}\right)dM}{\int M\left(\frac{dw}{dM}\right)dM} \qquad [17]$$

Similar expressions are available in terms of dw/d(log M). Several computer programs, which perform these calculations, have been widely distributed.

6.4.6 Experimental Set Up

There are considerable experimental evidences dealing with the interaction between solute and stationary phase in chromatographic separation of polystyrene with crosslinked polystyrene carried out by using eluents that are poor or theta solvents (9 - 11). A plot of log of hydrodynamic volume versus V_R for polystyrene is displaced to high V_R with respect to a plot for another polymer for which these same eluents are good solvents. Thus cyclohexane is a good solvent for polyisoprene and poly(dimethyl siloxane) which follow the same plot of log [n]M versus V_R, whereas the plot for polystyrene is displaced to much higher V_R values (9, 10).

Interactions between polymer and solvent and between eluent and stationary phase are the basis of serious errors in the molecular weight determinations.

As the eluent becomes a good solvent for the polymer, the solution tends towards an athermal mixture, i.e. a zero heat change. Then the polystyrene molecules will not display preferential affinity for the gel or the eluent, so that the tendency for polymer retention by interacting with the stationary phase is reduced considerably. This behavior has been confirmed in experimental GPC separations with poly(dimethyl siloxane) having Kp = 1 (steric exclusion) and with polystyrene in trans-decalin which is a good solvent for poly(dimethyl siloxane) (α = 0.72-0.76) and a poor solvent for polystyrene at 25°C (11). The displacement of the plot of log [n]M versus V_R for polystyrene is shown to decrease as the temperature is raised (11). The influence of temperature on the separation mechanism may be more important than what has been previously considered. Even for tetrahydrofuran that is a good solvent

with excellent compatibility with crosslinked polystyrene gels, Mori and Suzuki (12) demonstrated that Kp changed significantly over the temperature range 10 to 45°C.

Self-check Questions

1. What are the principles at fundament of GPC analytical methodology?
2. What kind of useful information is GPC providing on the polymer structures?
3. Identify role and function of mobile phase and stationary phase in GPC.
4. What kind of structural parameters can be obtained by GPC?
5. What is the function of calibration in GPC? Why is that needed and why mono-disperse standards are required?

Exercises

Exemplify how the average number (Mn) and weight average (Mw) molecular weight can be defined and calibrated from GPC traces. Given a definite GPC profile, calculate their molecular weight distribution (MWD).

Reading Material

1. Analysis of Polymer Systems, L.S. Bark and N.S. Allen Eds., Applied Science Publishers Ltd, London (1982)
2. Polymers: Chemistry & Physics of Modern Materials, J.M.G. Cowie Ed., International Textbook Company Limited, London (1973)

Bibliography

(1) J.C. Giddings, E. Kucera, C.P. Russell and M.N. Myers, *J. Phys. Chem.*, **72**, 4397 (1968)
(2) E.F. Casassa, *J. Phys.Chem.*, **75**, 3929 (1971)
(3) M.E. Van Kreveld and N. Van Den Hoed, *J. Chromatogr.*, **83**, 111 (1973)
(4) E.F. Casassa, *Macromolecules*, **2**, 14 (1969)
(5) Z. Grubisic, P. Rempp, and H. Benoit, *J. Polym. Sci.*, **B5**, 753 (1977)
(6) A.R. Cooper, and A.R. Bruzzone, *J. Polym. Sci.*, ***Polym. Phys.*, 11**, 1423 (1973)
(7) J.V. Dawkins, J.W. Maddock, and D. Coupe, *J. Polym. Sci.*, Part A-2, **8**, 1803 (1970)
(8) D.M.W. Anderson and J.F. Stoddart, *Anal. Chim. Acta.*, **34**, 401 (1966)
(9) J.V. Dawkins and M. Hemming, *Makromol. Chem.*, **176**, 1795 (1975)
(10) J.V. Dawkins and M. Hemming, *Makromol. Chem.*, **176**, 1777 (1975)
(11) J.V. Dawkins and M. Hemming, *Makromol. Chem.*, **176**, 1815 (1975)
(12) S. Mori and T. Suzuki, *Anal. Chem.*, **52**, 1625 (1980)

6.5 Testing the Biodegradation of Polymers and Plastics

6.5.1. Generalities on Biodegradation Procedures

The development of laboratory test methods aimed at evaluating the environmental fate of a polymeric material should consider in the first instance the kind of the various environments (i.e. natural soil, stream and surface waters, or tropically originated environments such as compost and landfill) in which the test materials could be discharged at the end of its life cycle. This issue has been actually considered as a fundamental guideline in the set-up of laboratory scale tests evaluating the biodegradation of polymers and plastics.

Accordingly in the last two decades, several techniques have been raised in order to at least partially mimicking the environmental conditions of natural habitats (soil, marine and surface water), and those associated to the most common waste water (sewage sludge, anaerobic digester) and organic refuse (compost and landfill) treatment methodologies. The composting process, in particular, is currently utilized for the fast treatment of the organic fraction of urban solid waste under controlled humidity and

aeration conditions throughout the action of strictly and facultatively thermophilic microorganisms. The semi-natural environment generated during the exothermic oxidation of the organic matter can be considered as the most stressing environment in terms of both chemical and thermal conditions (1). Accordingly the composting process has attracted increasing attention as environmental system in which the fate of slow and moderate degrading materials, such as many polymeric materials can be evaluated in a relatively short time (2-4).

Many of standard laboratory methodologies approved by international testing associations, such as ASTM (USA) (5), ISO (6) and CEN (Europe) (7) are mutated by taking into account the usual stressing conditions occurring during the aerobic composting of organic matter and are collected in Table 6.5.

Table 6.5 Laboratory official test methods for determining the biodegradation of polymer and plastic

Organization	**Number**	**Title of the standard**
ASTM	***D5209-92***	Standard test method for determining the aerobic biodegradation of plastic materials in the presence of municipal sewage sludge
ASTM	***D5210-92***	Standard test method for determining the anaerobic biodegradation of plastic materials in the presence of municipal sewage sludge
ASTM	***D5247-92***	Standard test method for determining the aerobic biodegradability of degradable plastics by specific microorganisms
ASTM	***D5271-93***	Standard test method for determining the aerobic biodegradation of plastic materials in an activated-sludge-wastewater-treatment system
ASTM	***D5338-98e1***	Standard test method for determining aerobic biodegradation of plastic materials under controlled composting conditions
ASTM	***D5511-94***	Standard test method for determining anaerobic biodegradation of plastic materials under high solids anaerobic-digestion conditions
ASTM	***D5525-94a***	Standard practice for exposing plastics to a simulated active landfill environment
ASTM	***D5526-94***	Standard test method for determining anaerobic biodegradation of plastic materials under accelerated landfill conditions
ASTM	***D5951-96***	Standard practice for preparing residual solids obtained after biodegradability standard methods for plastics in solid waste for toxicity and compost quality testing
ASTM	***D6002-96***	Standard guide for assessing the compostability of environmentally degradable plastics
ASTM	***D6003-96***	Standard test method for determining weight loss from plastic materials exposed to simulated municipal solid waste (MSW) aerobic compost environment
ASTM	***D6094-97***	Standard guide to assess the compostability of environmentally degradable non woven fabrics
ASTM	***D6340-98***	Standard test method for determining aerobic biodegradation of radiolabeled plastic materials in a aqueous or compost environment
ASTM	***D6400-99***	Standard specification for compostable plastics
ISO	***14851: 1999***	Determination of the Ultimate Aerobic Biodegradability of Plastic Materials in an Aqueous Medium - Method by measuring the Oxygen Demand in a Closed Respirometer
ISO	***14852: 1999***	Determination of the Ultimate Aerobic Biodegradability of plastic materials in an Aqueous Medium - Method by Analysis of evolved Carbon Dioxide
ISO	***14855: 1999***	Determination of the Ultimate Aerobic Biodegradability and Disintegration of plastic materials Under Controlled Composting Conditions - Method by Analysis of evolved Carbon Dioxide
CEN	*EN 13432*	Requirements for packaging recoverable through composting and biodegradation -Test scheme and evaluation criteria for the final acceptance of packaging

Several other laboratory scale methods have been developed as partial modification of standard tests in aqueous media traditionally used in the evaluation of the biodegradation of low molecular weight substance in the presence of microorganisms associated to waste water sewage sludge and anaerobic digester. In addition to the standard test methods adopted by the various international testing associations, several laboratory protocols have been proposed in the specialised literature. Some of these are conceptually associated to the incubation of test materials in soil matrices that represent the most common disposal environment namely for polymeric and plastic items utilized in the agricultural practices (8-11).

A typical experimental protocol aimed at the evaluating the biodegradability of a polymeric material is based on the incubation of the test samples in the presence of complex microbial consortia or single microbial species and enzymes, under established test conditions. Analytical characterizations are thus focused on the measurement of the chemical and physical properties of the original polymeric materials subject to the biochemical degradation, as well as to the evaluation of the microbial growth and to the qualitative and quantitative analysis of degradation intermediates and end products, accordingly to the general feature of the transformation of a polymeric material induced by microorganisms and enzymes (Fig. 6.4). The transformation of a polymer is a progressive reduction of its molecular weight accompanied by the release of low molecular fractions that can be directly assimilated by microbial cells as carbon and energy source.

Hence, in order to estimate the rate and extent of the biodegradation of a polymeric material, several methods are used for the evaluation of the increase of microbial bio-mass, to the determination of mass reduction of test material, as well as to the analysis of the biochemical end products and intermediates, or to the evaluation of changes of the chemical, physical and mechanical properties of the test materials subject to the biochemical reactions. Evidently, if many of the analytical methods are utilized simultaneously, the confidence and the reliability of the test results, as well as the hypothetical knowledge about the biochemist of the degradation process can be experimentally implemented and substantiated.

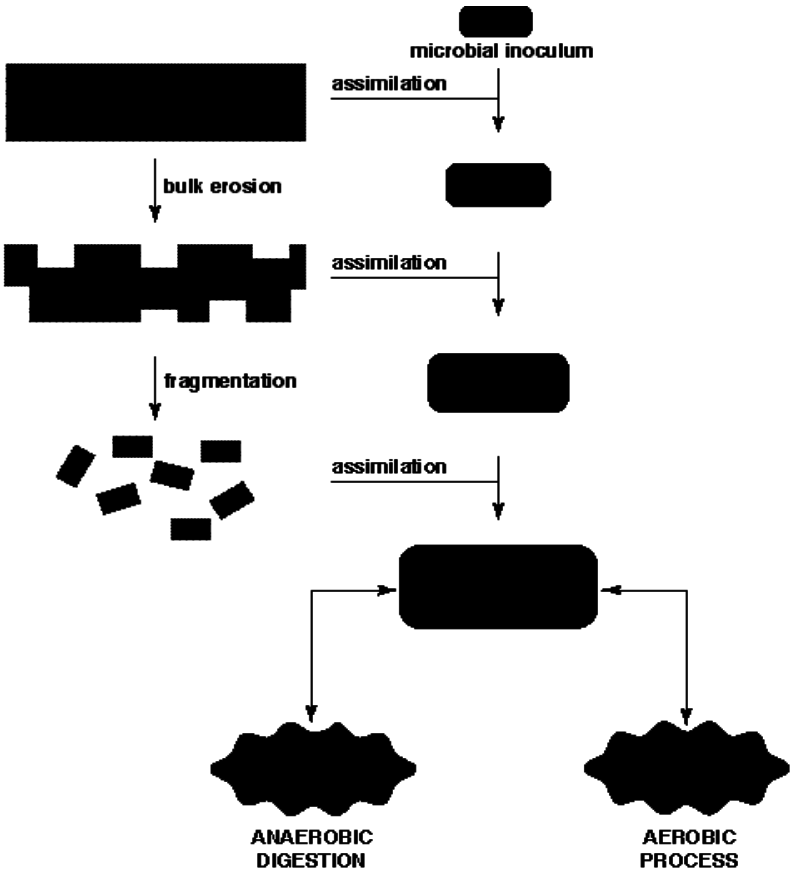


Fig. 6.4 Schematic representation of the biodegradation of a polymer matrix in aerobic conditions

Estimation of the potential biodegradation of a polymer sample throughout the microbial bio-mass.

A polymeric material could be utilized as carbon source by microorganisms. It is generally assumed that about one-third of the food consumed by microbial becomes the solid organic cell material of the organisms. The other two-thirds is oxidized to carbon dioxide and water by the biochemical action of the microorganisms in the presence of oxygen (Aerobic), or biogas (carbon dioxide and methane) under anaerobic conditions. Accordingly, at least hypothetically, a biodegradable polymer should promote an increase of microbial bio-mass within the incubation time as a consequence of the cell growth and microorganism proliferation.

The evaluation of the microbial bio-mass, however, can provide only partial information on the biodegradation extent of a test materials because a definite correlation between the biochemical transformation of its organic carbon and the conversion into new cell constituents does not exist. In many cases easily assimilatable organic substrates such as cellulose gives high percentage (60%) of carbon conversion into new microbial bio-mass, whereas truly biodegradable recalcitrant materials such as lignin can be converted at a very slow rate into cellular constituents (12).

On the other hand, many substrates, typically the monosaccharide and oligosaccharides deriving from the enzymatic hydrolysis of polysaccharides, are known to increase the activity of zymogen microflora in soil matrices (13), that is characterized by a very intense respiratory activity (burning) leading to a fast conversion of the glucidic carbon to carbon dioxide without a parallel high production of cellular bio-mass (14). Therefore in the presence of such substrates their potential biodegradation can be underestimate if the determinations are based only on the evaluation of microbial bio-mass.
The metabolic shift from intense cellular respiratory activity to the synthesis of cellular constituents can be also affected by the physiological conditions of microbial cell, generally also depending upon the relative amounts of carbon and nitrogen supplied in the cultivation media. From this point of view, it appears that to ensure the optimal physiological condition for the cell activity approximately 10 C/N ratio, such as that corresponding to the composition of most cellular constituents, should be guarantee in the set up of the incubation conditions of any biodegradation test (15).

6.5.2 Test Methods for Biodegradation

6.5.2.1 Evaluation of the degree of colonisation of test material surfaces by microbial growth structures

Different official test methods, originally utilized in the evaluation of the resistance of synthetic polymers to the microbial degradation, have been also proposed for a preliminary indication about the propensity of a plastic material to biodegrade (16). In these test methods the extent of biodegradation was correlated to the degree of colonization by microbial growth structures of the test material surfaces of standardized shape and areas, through definite indexes, once incubated onto solid culture media inoculated with different fungal and bacterial species. Such methodology could be useful for fast routine screening tests in the presence of large number of test samples (17) characterized by high purity degree and low oligomeric fraction contents. Otherwise, the observed microbial growth could be induced by the assimilation of additives (plasticizers, antioxidants etc.) and low molecular weight fractions present in the plastic item, instead of the utilization of the polymer carbon backbone.

6.5.2.2 Evaluation of the number of micro-organisms in an aqueous medium

The enumeration of the number of bacterial cells, directly correlated to the bio-mass, can be obtained by the relationship with the optical density of culture solution and relevant dilutions, detectable in the wave length region between 590 and 690 nm (18). This kind of analysis can be especially helpful in the monitoring of the time profiles of microbial growth (i.e. increasing bio-mass) in liquid cultures supplemented with a polymeric test material as sole carbon and energy source.

The microbial cell number in liquid media can be also detected through their count in definite volume chambers under the direct optical microscope observation on Petroff-Hausser slide (19).
Finally, the determination of Colonies Forming Units (CFU) parameter (19), obtained by seeding appropriate dilutions or suspensions on Petri dishes containing solid growth substrates, represents an alternative useful method for the evaluation of the microbial bio-mass. This latter procedure becomes

meaningful when the biodegradation test and relevant analysis have to be carried out by solid incubation media such as soil and compost, where the direct microscopic observation of microorganisms is not practicable.

6.5.2.3 Biochemical evaluation of microbial bio-mass

The microbial bio-mass, as well as its time profile during a biodegradation experiment, can be conveniently correlated to the measurement of the amounts of different cellular components such as ATP, DNA and protein (20,21). These procedures are particularly advantageous in the bio-mass estimation in complex environmental matrices such as soil and compost (22).

Estimation of the potential biodegradation of a polymer sample throughout the measurements of "reagents" concentration during the biodegradation reaction.

The mass consumption of a polymeric material in a biodegradation process (biochemical reaction) is accompanied by the production of mineral end products (mineralization) as a consequence of the biochemical activity of microorganisms, as well as by the formation of low molecular weight fractions in the presence of isolated enzymatic systems. Accordingly, both the analysis of the substrates concentration (i.e. amount of polymer matrix and molecular oxygen in aerobic conditions), as well as the evaluation of the reaction products, represent the conceptual basis for the set up of suitable procedures aimed at determining the biodegradation degree of a polymeric or plastic material (23).

6.5.2.4 Analysis of the biodegradation reaction substrates

The gravimetric analysis is one of the most common and simple methods in order to assess the mass variation of a solid test material in a biodegradation experiment. The weight of a test sample is periodically analyzed by retrieving, accurately cleaning and drying test specimen from the incubation media. The main sources of errors in this type of measurements are generally related to the difficulties in the cleaning procedure without the loss of small fragments and debries of test specimen, that usually occur in biodegradation experiment involving the use of soil and compost sample as incubation media.

The analysis of dissolved organic carbon by means of both automatical total organic carbon (TOC) analyzers and traditional dichromate oxidation (COD) represent also effective procedures in order to evaluate the mass losses of water soluble materials in biodegradation tests carried out in aqueous media, where the polymer samples are supplied as sole carbon sources. It has to be mentioned, however, that the recordable data can be significantly affected by the transformation of the original polymeric substrates in recalcitrant intermediates and products of microbial synthesis (23).

In the case of hydrosoluble polymers, such as poly(vinyl alcohol) (PVA) and poly(ethylene glycol) (PEG), specific analytical procedures aimed at determining the polymer concentration in liquid cultures within the time frame of the biodegradation test can be utilized. As an example, the PVA concentration in aqueous media is usually determined by spectrophotometric analysis of the PVA-iodine complex in the presence of boric acid (24).

The use of specific analytical determinations of certain polymeric substrates assumes a basic significance when the biodegradation experiments are carried out under co-metabolic conditions (25). This latter option is based on the simultaneous addition of an easily assimilable substrate(co-substrate) such as glucose, and recalcitrant test materials to the microbial cultures in order to achieve higher degradation rate and extents (26).

The biodegradation of water soluble polymers can be also monitored by means of viscometric analysis of the supernatant of microbial liquid cultures and enzymatic solutions supplemented with test materials. Accordingly both the relative solution viscosity (η_{rel}) and inherent viscosity (η_i) can be determined within the incubation time in the presence of suitable polymer concentrations. Molecular weight and molecular weights distribution analysis by means of both visometric and chromatographic (typically size exclusion chromatography - GPC) assume a key issue in investigations of the biodegradation mechanisms. Further information useful in the rebuilding of the biochemical routes leading to the biological degradation of a polymer compound have to be collected by using specific spectroscopic analysis (FT-IR, AT-IR, NMR).

During biodegradation tests carried out under aerobic conditions, the theoretical oxygen demand can be calculated on the basis of the elemental composition of test materials and by considering the

stoichiometry of the biochemical oxidation of test compounds to carbon dioxide and water. Therefore, the Biochemical Oxygen Demand (BOD) parameter is often used for monitoring the biodegradation rate and extent of polymer and plastic materials. BOD values correspond to the amount of oxygen (expressed in mg/l or parts per million, ppm) that microorganisms take from water when they oxidize organic matter. BOD values can be obtained either by measuring the amount of oxygen required to maintain a constant gas volume in a closed respirometric apparatus connected to the incubation vessels, or by measuring the volume or pressure changes both automatically or manually. Biodegradation degree is thus calculated as percentage of net absorbed theoretical oxygen demand (the theoretical maximum amount of oxygen required to oxidize a chemical compound completely, calculated form the chemical formula or determine by elemental analysis) of test materials corrected for the BOD measured in microbial cultures that are not supplemented with any carbon sources (blanks).

BOD determinations could be strongly affected by possible nitrification processes. Accordingly the recorded BOD values have to be corrected for serious errors on the calculation of biodegradation degrees based on carbon oxidation, a nitrogen containing test material shall be avoided. However, in the case of nitrogen-free materials the errors are normally negligible, because the oxidation of ammonia containing salts in the incubation media is considered by the subtraction of the BOD values recorded in the blanks.

As a general feature, in the set-up of experiments based on respirometric procedures, such as BOD measurements, the analysis of a truly biodegradable compounds utilized as positive reference material, is normally suggested in order to verify the optimal incubation conditions for the microbial *inoculi*, as well as in order to classify the test material as biodegradable in official standard test.

Respirometric Determination of the Biodegradation (Aerobic Degradation Mediated by Microorganism)

The total aerobic biodegradation (ultimate aerobic biodegradation) of an organic compound such as polymeric materials, is represented by its break down by microorganisms in the presence of oxygen into carbon dioxide, water and mineral salts of any other elements present (mineralization), and new bio-mass. Accordingly the evaluation of the carbon dioxide (respirometric activity) produced by microbial cultures fed with the polymeric test material as a side carbon source represents one of the most useful method for determining its biodegradation degree.

As in the case of BOD the biodegradation degree is expressed as percentage of the net theoretical carbon dioxide (the maximum amount of CO_2 potentially evolvable by the complete oxidation of the test material as calculated from the chemical formula or by elemental analysis) corrected for the CO_2 emission due to the microbial respiration in the blanks.

Usually, for the measurements of the CO_2 production simple titrimetric procedures can be adopted (27). In the case of materials that undergo biodegradation at moderate or slow rates the use of ^{14}C labeled compounds has been frequently suggested, such as in the case of prolonged biodegradation studies poly(ethylene) samples (28-30).

Respirometric tests based on the CO_2 measurements appears also to be very appropriate for the evaluation of the potential biodegradation in the presence of environmental solid incubation substrate such as soil (31,32) and compost (3); as well as in aqueous media (33,34).

Anaerobic Digestion Test for the Biodegradation Determination

The microbial degradation of polymeric material can also occur under anaerobic (absence of oxygen) methanogenic conditions, such as those typically associated to anaerobic sewage sludge digester. The breakdown of an organic compound under methanogenic conditions lead to the formation of carbon dioxide, methane, water and mineral salts (mineralization) as end products, and new bio-mass.

Accordingly the determination of methane and carbon dioxide productions, by using analytical methods suitable for the detection and quantification of these gases (such as gaschromatography or with devices for measuring gas components by infrared spectroscopy) can be utilized for assessing the potential biodegradation of polymeric materials in the presence of anaerobic microorganisms.

The biodegradation degree is thus calculated, as net percentage of theoretical amount of released biogas (Th biogas) that represents the theoretical maximum amount of biogas (assumed to confined to the formation of CH_4 and CO2), calculated from molecular formula, after the complete biodegradation of an organic compound under anaerobic conditions. Usually in the presence of test materials that are not containing other elements such as nitrogen or sulfur, but carbon, the theoretical biogas (CH_4+CO_2), can be calculated on the basis of chemical formula accordingly to the Buswell & Mueller equation:

$$C_cH_hO_o + (c - h/4 - o/2)\ H_2O \rightarrow (c/2 + h/8 - o/4)\ CH_4 + (c/2 - h/8 - o/4)\ CO_2$$

As in all types of respirometric tests, the values recorded in the cultures supplemented with the test materials have to be subtracted by those recorded in the blanks, in order to calculated the biodegradation percentage.

Evaluation of Biodegradation Extent on the Basis of Metabolites Monitoring

Finally the biodegradation of a polymeric material could be monitored by the analysis of intermediates deriving from the biochemical reactions of microorganisms and enzymes.

In the first case the formation of low molecular weight fractions have been used to monitoring the biodegradation of poly(ethylene) throughout the gaschromatographic detection of volatile compounds in the head space of soil containing flasks added with the test material (36,37).

In the case of enzymatic hydrolysis also the estimation of the oligomeric and monomeric end products, such as the analysis of reducing sugars from the hydrolytical degradation of polysaccharides mediated by glucosidic enzymes, can be consider a suitable methodology in the assessment of such polymer biodegradation.

6.5.3 Case Studies

6.5.3.1 Generalities

In the characterization of the environmental fate of polymeric materials one of the most important issues is represented by the evaluation of the ultimate biodegradation (e.g. mineralization), hence the conversion of the materials into the products of the microbial assimilation of the materials (CO_2 and water in the presence of molecular oxygen, or CO_2, CH_4, and water in anaerobic conditions) as well as the production of more microbial bio-mass itself. By considering that the life-cycle of micro-organisms, particularly bacteria, is relatively short, and consequently the cellular bio-mass undergo a continuos conversion into inorganic products of the organic matter oxidation (CO_2, CH_4, and H_2O), the production and determination of gaseous compounds deriving from the microbial attack of a polymer sample, can be itself utilized with a good approximation for the evaluation (measurements) of its ultimate biodegradability.

This latter instance become fairly unavoidable under laboratory scale situations where the experiment have to be carried out in closed vessel often in the presence of complex substrates (e.g. soil and compost), in which the evaluation of the increase of microbial bio-mass can be fairly complex methodologies.

As a general feature the experimental set-up of a respirometric test for the determination of the gaseous inorganic compounds produced by the microbial assimilation of a test material, is based on the comparison of the gaseous products detectable from test runs supplemented with the test compounds as sole energy and carbon sources for the microorganisms, eventually associated to complex environmental matrices such as soil, compost, and sewage sludge, and those recorded from the micro-organisms itself (endogenous respiration). In terms of CO_2 the correction of the productions in presence of the test sample by the amount of endogenous respiration of microbial cellular component lead to the determination of the net CO_2 production, in other words the CO_2 evolved only from the carbon contained in the test material.

The extent of mineralization or bio-degration is thus calculated as the percent of net CO_2 amount divided by the CO_2 deriving from the theoretical total oxidation (theoretical CO_2) of the organic carbon of the test material.

The test, can be also designed in order to evaluate the fate of the polymeric materials under particular environmental conditions mimicking the real disposal environments. Accordingly many tests can be carried out in the presence of the microflora associated to natural soils, compost, sewage sludge, anaerobic digester etc.

To determine the CO_2 evolved from the test cultures, automatically recording devices based on IR absorption or gas chromatographic sampling in the exhaust air flowing throughout the culture can be used. However the automatic instrumentation is usually fairly expensive in terms of capital investment and maintaining, thus limiting the number of test experiment, or samples number, or both. A simple back titration of alkali solutions used for trapping the CO_2 produced from the test cultures can be therefore utilized in the quantification of the CO_2 productions. Also many official test methods suggest this methodology.

In the case of detection of carbon dioxide throughout absorption in alkaline solution (e.g. $Ba(OH)_2$ or KOH), a particular attention has to be dedicated to the signal-to-noise ratio, represented by the comparison between the values obtained from the cultures added with test compounds and those in the blanks. This issue appears a fundamental in polymer samples that undergo biodegradation at slow or moderate rates. In these conditions, the accuracy of the test could be strongly affected by the high level of carbon dioxide production in the blanks, such as those organic-rich media, namely compost or organic soil sample, are used as microbial active environments. More reliable results could be obtained by using large amounts of samples. But in some cases, the large local concentration of both the samples itself, as well as their degradation products, could negatively interfere with the optimal microbial growth conditions, thus compromising the test reliability.

In order to increase the signal-to-noise ratio, large amounts of organic rich incubation media (compost and forest or garden soil samples), that are generally characterized by a very high microbial activity, can be replaced by hygroscopic chemically inert materials, such as perlite and vermiculite. In these conditions compost and soil samples acts mainly as sources of microbial inoculi, instead of true incubation substrates.

Recently, different methodologies, based on mineral beds have been proposed in the specialized literature, as well as official standard methods for testing polymers biodegradation under simulating soil burial (10) or composting (38) conditions. The reliability and accuracy of these biodegradation experiments can be also affected by the stimulation to an higher rate of mineralization of the organic matter associated to the substrates as induced by the addition of some testing materials, namely polysaccharides and polysaccharides deriving materials. This phenomenon is known as “priming effect” and can be attributed to a sort of co-metabolism promoted by easily degradable substrates such as the glucides deriving from the hydrolysis of polysaccharides (39). Consequently, the CO_2 production in polysaccharides supplemented cultures is enhanced by the higher microbial mineralization of the substrates organic matter. As a consequence, the mineralization rate and extent priming effect inducible materials can be overestimated, often reaching values above 100%.

In order to provide very reliable results in organic-rich media and poorly degradable or polysaccharides based materials, respirometric tests require the use of ^{14}C-labeled test samples, whose preparation as well as their testing for biodegradation are fairly expensive and very demanding on laboratory safety and operator skills. However, the suggested mineral bed is promising to overcome the “priming effect” also in testing starch-based materials (38). Accordingly, these methodologies can be utilized to assess the biodegradation of polymeric materials of both synthetic and natural origin with a high degree of confidence and well suited for the routine screening of several different samples under laboratory scale.

6.5.3.2 Sample preparation and characterisation

Polymer samples to be tested in a repsirometric biodegradation experiment have to be characterized for their carbon content, as well as for molecular weight and molecular weights distribution, and functional groups, particularly when carbonyl, esters or hydroxyl functionality are expected to be the preferential

sites of enzymatic attack. Also thermal, mechanical and thermo-mechanical properties should be evaluated prior to the tests in order to achieve the complete characterization of the materials that could be useful for a comparison of the same properties in the sample retrieved from the incubation media. Accordingly, the degree of mineralization could be useful, compared with the changes in sample properties to obtain either the respirometric test validation, or fundamental information about the biochemical routes. During the set-up of a biodegradation respirometric experiments the following steps may be considered.

(1) Sample conditioning

Polymer samples either as films or powders (of definite shape and size) have to be dehydrated before the carbon content analysis. In order to avoid changes in the polymeric material structure, as well as losses of low molecular weights fraction and additives, a suitable procedure replacing the traditional exsiccation in warm ovens, is the freezing-drying at reduced pressure (e.g. liophylization). Definite polymer amounts are thus liophylized up to constant weight and then maintained in a desiccator containing silica gel.

(2) Determination of the organic carbon content and related amount of Theoretical Carbon Dioxide ($ThCO_2$)of a test material

The carbon content of polymeric materials can be analyzed by automatic elemental analysis. Otherwise, a dichromate oxidation followed by titration of dichromate excess with NH_4FeSO_4 (Mohr salt) can be also utilized. As an example, the following procedure is suggested:

Reagents:
2 N $K_2Cr_2O_7$ solution
0.2 N $FeSO_4$•$(NH_4)_2SO_4$•$6H_2O$ (Mohr salt solution) containing 25 ml H_2SO_4 conc. (d=1.84 and 95 % by weight) per litre. The solution has to be standardized everytime.
0.1 N $K_2Cr_2O_7$ as standard
Ag_2SO_4, NaF
Diphenylamine solution (0.5 % in H_2SO_4 conc.)
H_2SO_4 conc.(d=1.84)

Procedure:
About 100 mg of dried, pulverized sample are placed in a 100 ml volumetric flask, afterwards 20 ml of 2 N $K_2Cr_2O_7$ and then 20 ml H_2SO_4 conc. are added drop-wise under stirring. At the same time a blank run is prepared. Small amount (10 - 20 mg) of silver sulfate is added as oxidation catalyst. Volumetric flasks are thus maintained in a boiling bath for at least 1.5 hours. Then fast cooled and filled with high purity grade distilled water up to 100 ml.

25 ml of clear liquid solution are withdrawn and diluted with 100 ml distilled water in a 400 ml capacity beker. This latter solution is added of 3 - 4 g di NaF and 3 - 4 drops of diphenylamine solution as redox indicator, then titrated with 0.2 N $FeSO_4$•$(NH_4)_2SO_4$•$6H_2O$. At the equivalent point the solution color turns from blue/violet to green. The oxidation reaction under the assumption of a number oxidation for the carbon equal to zero:

$$2\ K_2Cr_2O_7 + 8\ H_2SO_4 + 3\ C = 2\ Cr_2(SO_4)_3 + 2\ K_2SO_4 + 8\ H_2O + 3\ CO_2$$

Consequently

$$Corg.\% = \frac{(a - b)\cdot N \cdot 0.003 \cdot 4}{P}$$

Where *a* represents the amount (ml) of Mohr solution utilized in the blank run, *b* the amount utilized in the sample run, N the title (normality) of Mohr salt solution, and P the weight (g) of test sample.
In order to validate the adopted methodologies the analysis of control sample having a known carbon content is suggested. The suggested procedure can be also adopted for the evaluation of the carbon content of the incubation substrates (soil and compost) utilized in the respirometric biodegradation experiments.

6.5.3.3 Specific Examples

Simulated aerobic composting - ASTM D5338-92 test (5)

A respirometric biodegradation test aimed at simulating the aerobic composting process, as partially derived from ASTM D5338-92 standard test is described as following. Blanks and test cultures are assembled in 150 ml capacity cylindrical vessels equipped with rubber stoppers leading a glass pipe positioned at 1 cm from the bottom vessel. The bottom portion (about 3 cm length) is then filled with glass beads (5mm size) in order to avoid the pipes occlusion and guarantee the homogenous aeration of the incubation medium (Figure 6.5).

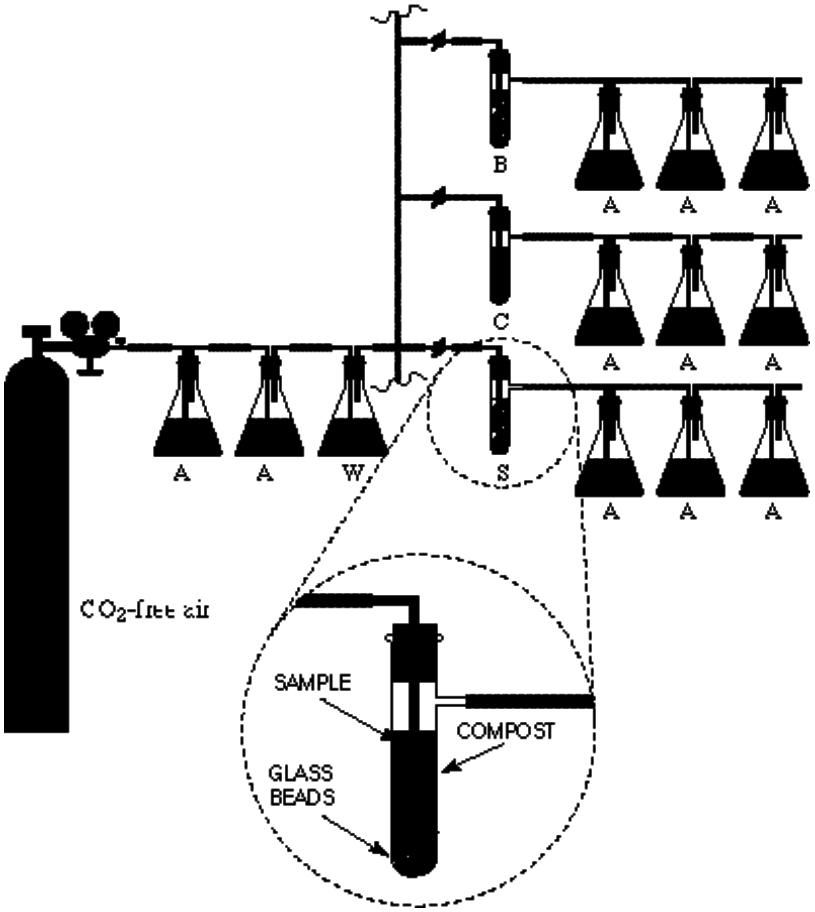


Fig. 6.5 Respirometric apparatus utilized in the simulating composting respirometric test

Each vessel is then filled with 30 g of mature compost (2.43 % nitrogen content, 9.62 C/N ratio) deriving from the aerobic digestion of the organic fraction of municipal solid waste. Test samples are added as shredded sheets by using a 0.7 ratio of the organic carbon deriving from the test material and those of the incubation (compost) medium. Culture vessels are then placed in a water bath and connected with a high purity CO_2-free air cylinder. The air flow is divided into the different test and blank vessels and regulated with brass needle valves at approximately 30 ml/min.

The amounts of carbon dioxide evolved from each cultures is measured directly every two-five days by bubbling the relevant exhaust air in a series of three 300 ml flasks containing 250 ml of 0.25N $Ba(OH)_2$ solution and back titration with 0.5 N HCl of 50 ml aliquotes of the alkaline solution contained in the proximal CO_2-absorbing flasks. At the mean time the remaining two absorbing flasks are shifted by one place, and the distal ones are replaced with flasks containing alkaline fresh solutions. The amount of absorbed CO_2 can be calculated by following:

$$CO_2\ (mg) = [(250 \cdot N_{Ba(OH)2}) - 5(ml_{HCl} \cdot N_{HCl})]$$

In order to simulate the thermophilic and mesophilic phases of field scale composting process, the following temperature program is suggested to be applied to the test cultures during the incubation time: 3 days at 35ºC, heating from 35 to 55ºC in 3 days, 10 days at 55ºC, cooling from 55 to 37ºC in 10 days, maintenance12 days at 37ºC, and finally 6 days at 35ºC. This latter step can be followed by a further incubation period at room temperature, in order to simulate the post-maturation conditions generally achieved in the field scale composting treatment.

Under the adopted incubation conditions the biodegradability of poly(vinyl alcohol) (PVA) blown film, high density poly(ethylene) (HDPE) film samples have been monitored along with a filter paper sample utilized as positive reference material (40). The mineralization profiles of test and reference materials are shown in Fig. 6.6, along with the temperature-time profile applied to the test cultures.

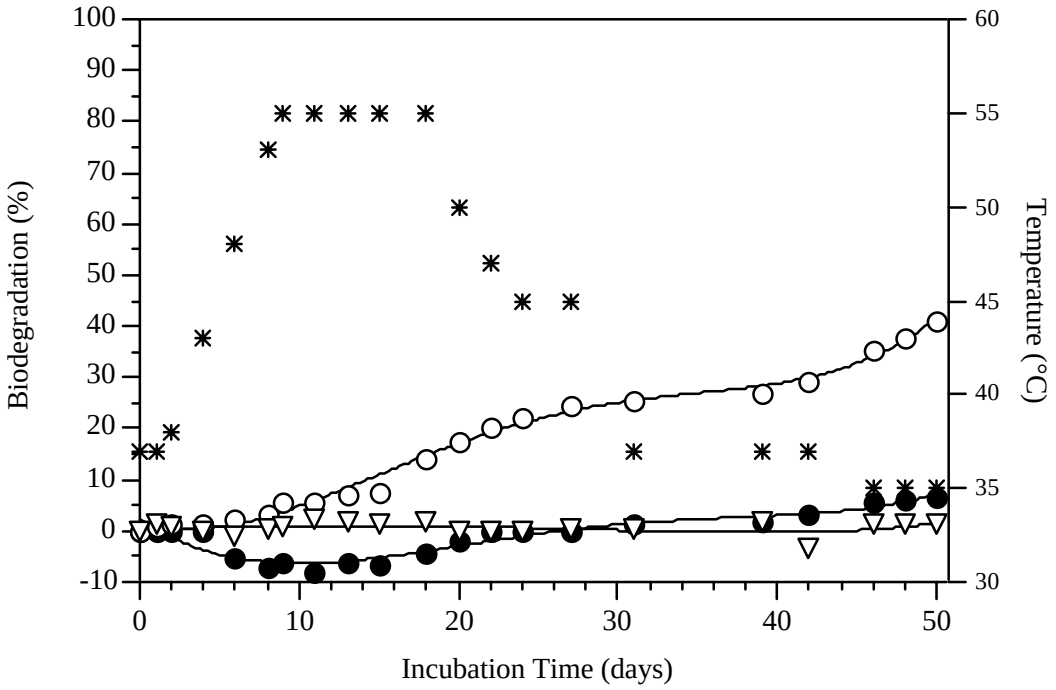


Fig. 6.6 Biodegradation profiles of sample analyzed in the simulating composting respirometric test

Biodegradation of PVA-based plastic films does not exceed 7% in 48 days of incubation. Little differences can be also detected with the biodegradation rate and extent of HDPE films that can be considered as a negative control. On the other hand, a fairly high bio-assimilation of paper is obtained during the same incubation time. Moreover, the still increasing degradation rate of this paper sample accounts for the reliability of such test procedure. However the very large CO_2 productions in the balnks may affect significantly the accuracy of test results.

It is worth noting that during the thermophilic phase of the suggested simulated composting procedure, CO_2 emissions from PVA supplemented cultures are lower than those recorded in the blank runs as revealed by the negative biodegradation values in Figure 6.6. This behavior can be attributed to a sort of noxious effect exerted by the polymer sample at the adopted concentration on the thermophilic microorganisms associated to the compost matrix.

Simulated soil burial - Biometer flask - CO_2 equilibrium condition

This procedure was originally proposed to assess the biodegradation of pesticides in soil (41). Recently the same methodology has been proposed as respirometric test for biodegradation of synthetic polymers in soil (32). Test materials are confined directly in definite amounts of soil as incubation medium, allocated inside flasks equipped with a side arm containing alkaline solutions for trapping the CO_2 produced by the microbial activity. In these conditions the accuracy of such tests could be affected by the high level of CO_2 deriving from the mineralization of the organic matter contained in the soil matrices.

As a partial modification of the original procedure, polymer biodegradation experiments have been carried out in glass vessels containing a multi-layer substrate in which a definite amount of a soil sample, mixed with 30-50% by weight perlite, is sandwiched between two layers of perlite. The polymer samples to be tested are placed within the soil (middle) containing layer. The role of perlite, a chemically inert, heat expended aluminum silicate, naturally occurring rock, is to reduce the overall amount of soil and hence the carbon dioxide production in the blanks.

Moreover, perlite is largely utilized in horticultural applications, as a component of soil-less growing mixes, where it helps to provide aeration and optimal moisture conditions for plant growth. Accordingly, perlite can ensure satisfactory incubation conditions while limiting the soil content to the function of microbial source (inoculum).

A typical example of a respirometric biodegradation test utilizing the modified "biometer flask" procedure is described in the following.

Experiment set-up

The test is carried out in 500 ml capacity cylindrical vessel sealed with a glass cap (8 cm ø) provided with a poly(ethylene) gasket, containing a multy-layer incubation medium. The bottom of each vessel is filled with 10 g perlite (0.2-0.5 cm ø) wetted with 30 ml distilled water. A mixture of 25 g of a soil sample (sieved at 0.6 mm) and ground perlite, supplemented with 30 ml of 0.1% $(NH_4)_2HPO_4$ solution is placed above the bottom layer. The test samples (approximately 500 mg) either as powder or film are allocated inside the soil-ground perlite mixture. Finally other 10 g of perlite wetted with other 30 ml distilled water are positioned in the vessel as top layer.

For measuring CO_2 evolved from test samples and blanks each vessel is equipped with a beker containing 40 ml of 0.05 N KOH solution placed on the top of the incubation medium (Fig. 6.7). At intervals (2-7 days of incubation), the CO_2 absorbing solutions are back titrated with 0.1 N HCl and replaced with fresh alkaline solution. Culture vessels can be incubated at a definite temperature (25 ºC) in the dark.

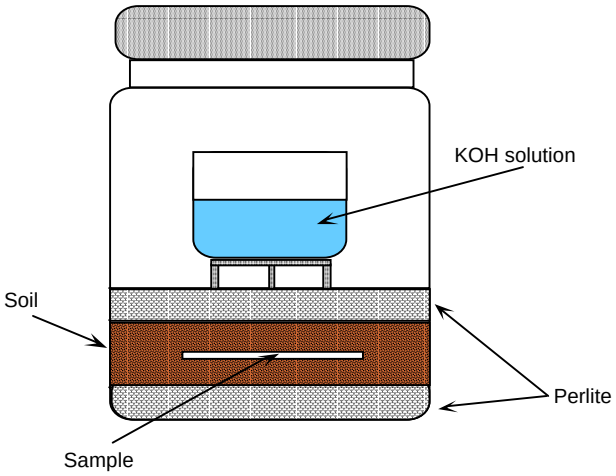


Fig. 6.7 Biometer Flask apparatus utilized in the simulating soil burial respirometric test

The biodegradability of a filter paper sample, normally utilized as positive reference material in most respirometric experiments, have been monitored within time by the same procedure. A thermoplastic, partially acetylated starch sample has been analyzed during the test. On the basis of theoretical CO_2 amounts of test samples, the relevant mineralization time profiles recorded during 160 days of incubation are reported in Figure 6.8.

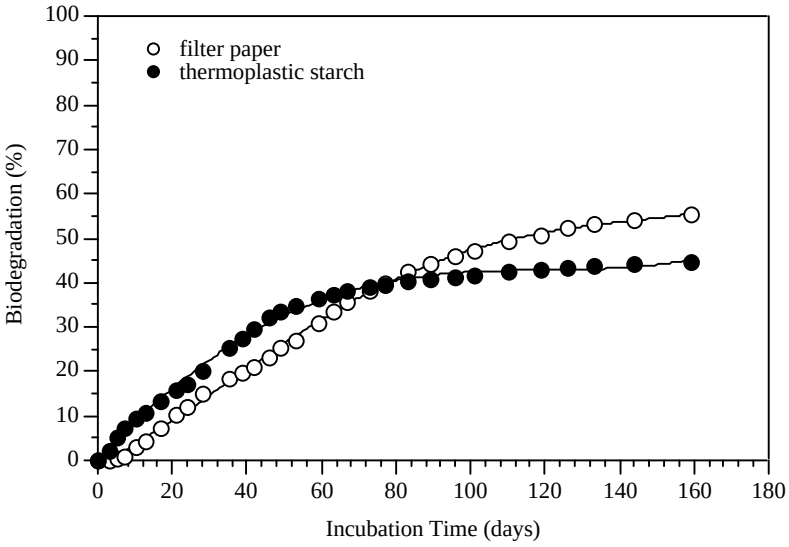


Fig. 6.8 Biodegradation profiles of filter paper and thermoplastic starch samples recorded in simulated soil burial test according to the Biometer Flask procedure

The reliability of the proposed experimental set-up is witnessed by the high extent of biodegradation reached by the filter paper sample, that is approaching 60% after 120 days. It is worth noticeable that the recorded value corresponds to the limit established in many official standard tests for the acceptance of the test results.

Simulated soil burial - air flow condition

The use of a mineral bed for reducing the amounts of soil as incubation media can be utilized also for the set-up of biodegradation experiments under CO_2-free air flow conditions. A typical experiment set-up is thus described.

The test is carried out in 1000 ml Erlenmeyer flasks containing a multi-layer substrate assembled as following. The bottom of each flask is filled with 20 g perlite wetted with 50 ml distilled water, the second (middle layer) is constituted by a mixture of 15 g of a garden soil sample (sieved at 0.6 mm) and 15 g ground perlite supplemented with 30 ml of 0.1% (NH4)2HPO4 solution. Test samples (500 mg approximately) are allocated inside this mixture; finally a third layer consisting of 20 g perlite hydrated with 50 ml distilled water is set on top. Culture flasks are placed in a water bath at 25˚`C and connected with silicone tubes with a high purity CO2-free air cylinder. The air flow is divided into the test and blank flasks and regulated with brass needle valves at approximately 30 ml/min (Figure 6.9)

The amount of carbon dioxide evolved from culture flasks is measured directly every two-four days by bubbling the exhaust air of each flask in a series of three cylindrical absorbers filled with 40 ml 0.025 N Ba(OH)2 solution and back-titration with 0.05N HCl of the solution contained into the proximal absorber connected to each flask. At the same time the remaining two absorbers are shifted by one place and the distal ones replaced with fresh alkaline solution containing absorbers.

The biodegradability of a gelatin cast film (GEL), poly(ε-caprolactone) (PCL) and poly(β-hydroxybutyrate-co-valerate) (PHB/HV) samples have been monitored within the time along with a 98 % hydrolyzed poly(vinyl alcohol) (PVA) cast film sample by using the described procedure (10, 42).
The readily biodegradable polyesters of natural (PHB/HV) and synthetic origin undergo significant mineralization as shown by the biodegradation reported in Figure 6.10.

It is worth noting that the extent of mineralization of the test and reference (cellulose) materials is still increasing after 160 days of incubation. This demonstrates that the soil microflora degrading ability is not substantially affected by the adopted incubation conditions. A very low tendency to biodegrade of PVA in soil is also ascertained.

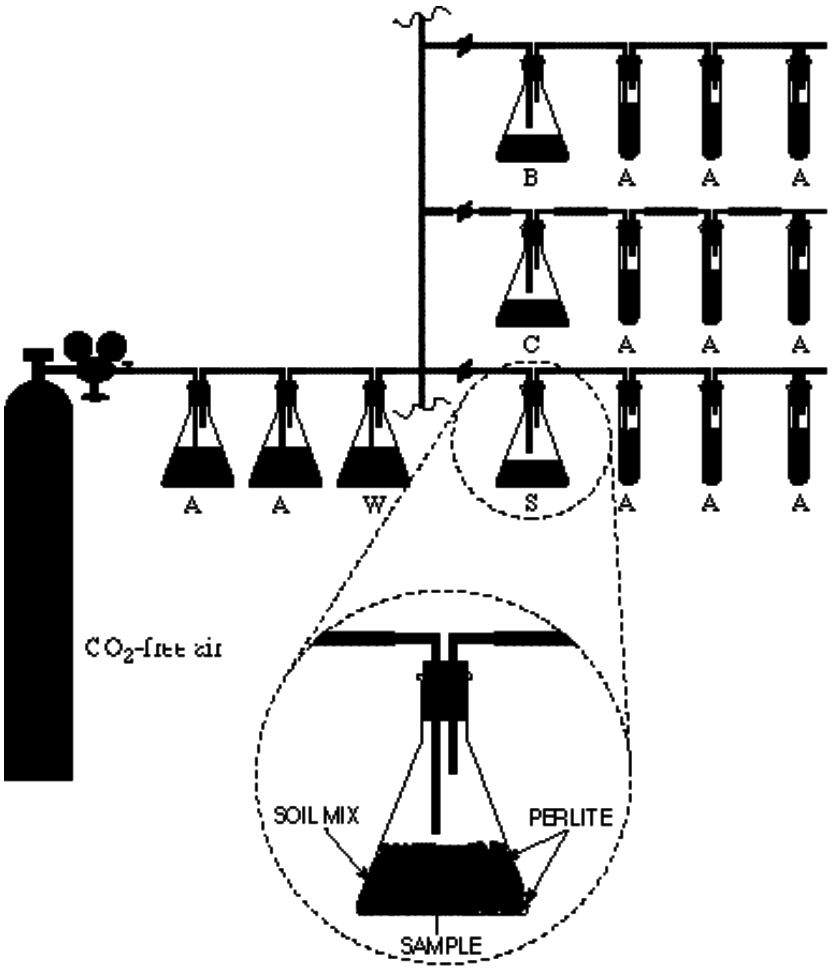


Fig. 6.9 The respirometric apparatus utilized in the simulated soil burial respirometric test under air flow conditions

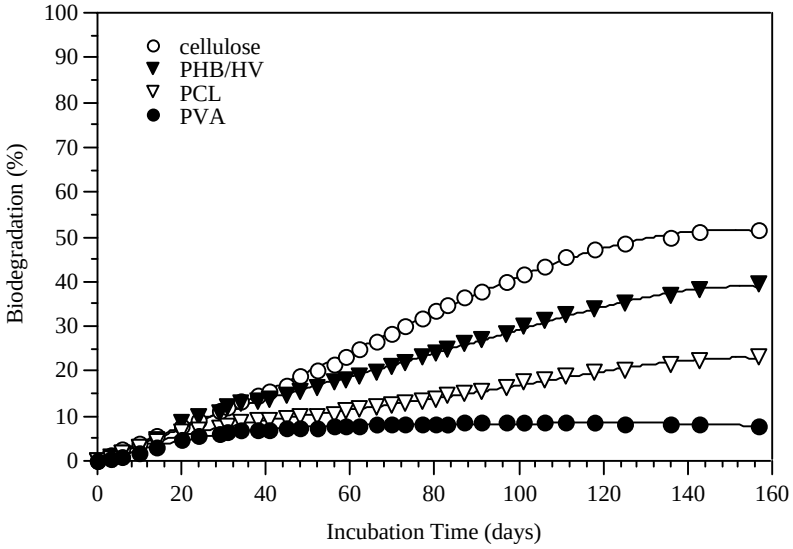


Fig. 6.10 Biodegradation profiles of filter paper, PCL and PHB/HV samples recorded during a simulated soil burial test under air flow conditions.

A very high extent of mineralization (80 %) of a gelatin based cast film is recorded under the adopted incubation conditions (Figure 6.11). This latter observation clearly evidence that the rate of microbial conversion to CO_2 is strictly dependent on the nature of the organic substrate to be tested. On the other hand, very limited degree of biodegradation can be also detected by the adopted procedure as revealed the moderate degrading material in soil such as PVA.

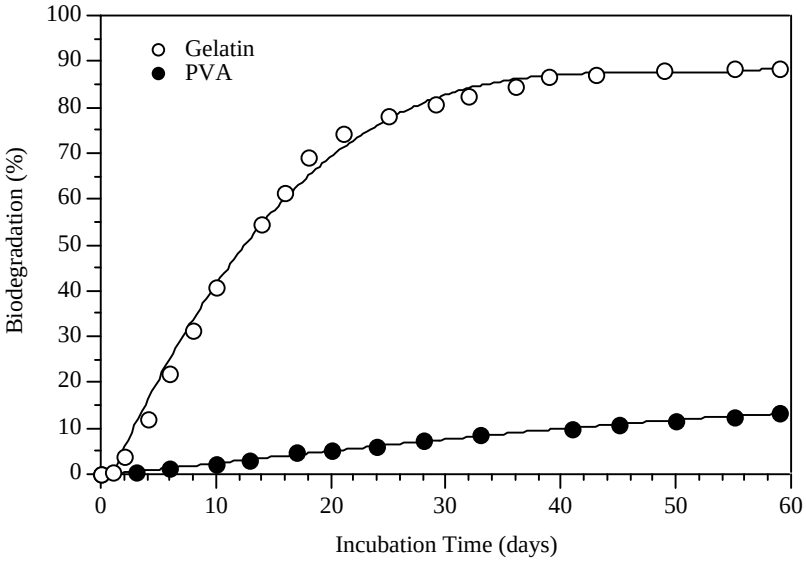


Fig. 6.11 Biodegradation profiles of gelatin and PVA based cast films recorded during a simulated soil burial respirometric test under air flow conditions

Aerobic biodegradation in an aqueous medium

In order to assess the potential biodegradation of polymeric materials in an aqueous media (e.g. liquid culture), the official tests procedures suggested in ASTM D5209-92 "Test method for determining aerobic biodegradation of plastic materials in the presence of municipal sewage sludge" (5), as well as in ISO 14852" "Evaluation of the ultimate aerobic biodegradability of plastic materials in an aqueous medium - method by analysis of released carbon dioxide" (6) can be utilized as fundamental guidelines to set up the experimental conditions.

A typical example of a respirometric biodegradation test in an aqueous medium using paper mill sewage sludge as inoculum is described below.

The test is carried out in 300 ml capacity Erlenmeyer flasks containing 100 ml of a mineral liquid medium having the following composition: K_2HPO_4 2.2 g, KH_2PO_4 0.8 g, Mg $SO_4 \cdot 7H_2O$ 0.2 g, NH_4NO_3 0.5 g, 1 ml per liter mineral medium of a trace elements solution having the following composition $MnSO_4 \cdot H_2O$ 0.15 g, $CaCl_2$ 1.3 g, H_3BO_3 0.06 g, $CoCl_2 \cdot 6H_2O$ 0.07 g, ZnCl 0.07 g, 0.1 N HCl 100 ml, is also added.

Inoculum preparation:

Paper mill sewage sludge inoculum has to be prepared at the same day of the test start by the following procedure: 30 ml of freshly collected sewage sludge are aerated for 30 min by bubbling air, then homogenized in a mixer for 2 min, leave to settling for other 30 min. Afterwards the supernatant can be directly used as microbial inoculum for test and blank cultures at 1% by volume ratio.

Test polymeric samples (100 mg approximately) are added to the cultures as sole carbon and energy sources either as water solution or milled (0.3 mm size) powder. Flasks are then incubated at room temperature on a rotatory shaker at 120 and connected with a high purity CO_2-free air cylinder. The air flow is divided into the different test and blank vessels and regulated with brass needle valves at approximately 30 ml/min.

The amounts of carbon dioxide evolved from each cultures is measured directly every two-five days by bubbling the relevant exhaust air in a series of three cylindical absorbers containing 40 ml of 0.025N $Ba(OH)_2$ solution and back titration with 0.05 N HCl of the alkaline solution contained in the proximal CO_2-absorbers. At the mean time the remaining two absorbers are shifted by one place, and the distal ones are replaced with an other containing alkaline fresh solutions (Figure 6.12).

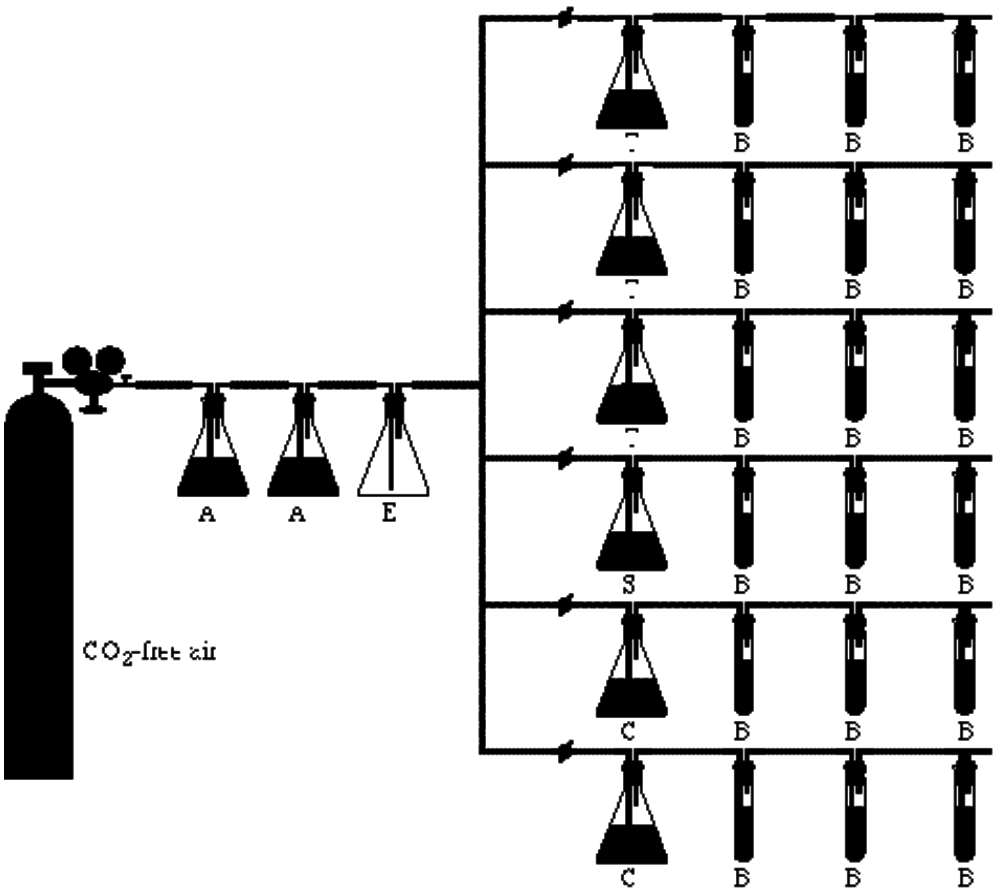


Fig. 6.12 The respirometric apparatus utilized in the respirometric tests in aqueous medium

By using the above described test procedure, the biodegradability of a PVA (98% hydrolyzed) blown film has been monitored along with a TLC grade cellulose powder sample utilized as positive reference material (40). Time profile of the mineralization degree of both PVA and cellulose sample are reported in Figure 6.13.

Under the adopted conditions the biodegradation extent of the PVA-based film reach values comparable to that of cellulose, even though after a larger incubation time (Figure 6.13). The biodegradation extent of the reference material (approximately 60%) after 50 days of incubation accounts for the test validity.

Aerobic biodegradation in an aqueous medium by using selected microbial inoculi

The test procedure suggested can be utilized in order to assess the biodegradability of polymeric samples in the presence of acclimated microbial populations. With selected microorganisms, the test duration could be usefully reduced, and detailed information on the biochemical routes can be more easily obtained by the analytical characterization of intermediates and end products without the hinder of several organic compounds usually associated to complex incubation media such as those deriving form sewage sludge.

An acclimated microbial inoculum can be obtained by an "enrichment" procedure as those suggested below for the selection of PVA-degrading microorganisms.

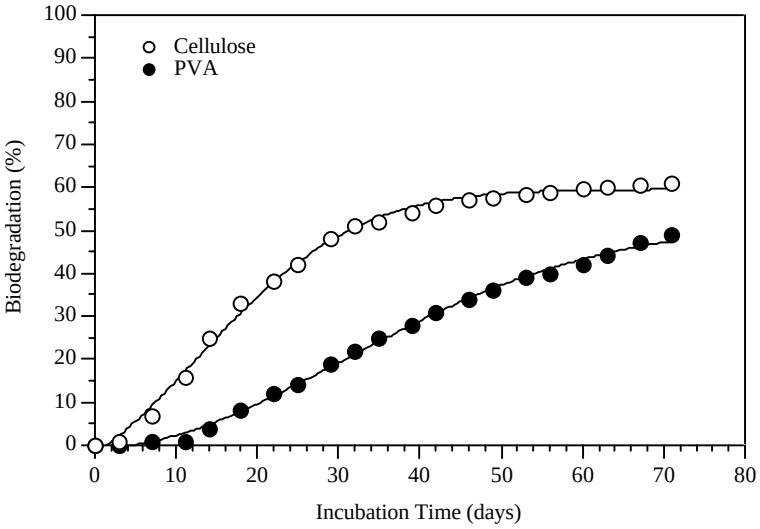


Fig. 6.13 Biodegradation profiles of PVA based cast film and cellulose samples recorded during a respirometric test in an aqueous medium

A paper mill sewage sludge sample is added to a mineral liquid medium in the presence of 0.1 % PVA solution as sole carbon and energy source. After 14 days of incubation, an aliquot of the first culture is used as 1% by volume inoculum for a new culture supplemented with the test material. The above procedure is repeated at least three times, and finally the last obtained PVA microbial culture can be directly utilized as microbial inoculum in a biodegradation respirometric experiment (40).

The time profiles of biodegradation degree of a PVA (98% hydrolyzed) sample and those of a TLC grade cellulose sample, recorded in a respirometric test carried out in an aqueous medium in the presence of PVA acclimated microorganisms are reported in Figure 6.14.

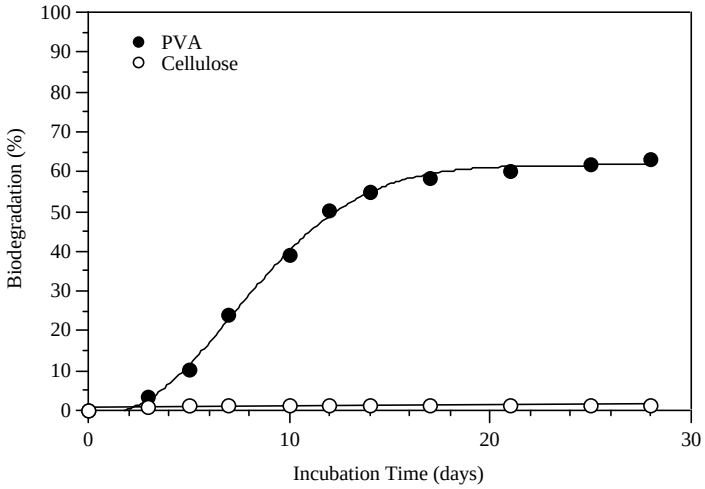


Figure 6.14 Biodegradation profiles of PVA based cast film and cellulose samples recorded during a respirometric test in an aqueous medium in the presence of an acclimated inoculum.

Under the adopted incubation conditions PVA based material undergo a fast bio-mineralization approaching 60% of net theoretical CO_2 after 15 days of incubation, whereas a substantial decrease of cellulose assimilation (1.5% at the 28^{th} day) is occurring as a consequence of the selection of specialized microbial species during the acclimation procedure. These results can be taken as a clear indication of the necessity of defining both the time frame and the environmental (e.g. incubation) conditions in assessing the biodegradability of plastic items.

References

1. M. S. Finstein, F. C. Miller, P. F. Strom, S. T. Mac Gregor, et al, Bio/Technol. **1**, 347 (1983)
2. J.-D. Gu, M. Gada, G. Kharas, D. Eberiel, S. P. McCarthy, R. A. Gross, Polym. Mater. Sci. Eng. **67**, 351 (1992)
3. J.-D. Gu, S. Yang, R. Welton, D. Eberiel, S. P. McCarthy, R. A. Gross, J. Environ. Polym. Degr. 2, 129 (1994)
4. K. E. Johnson, A. L. I. Pometto, Z. L. Nikolov, Appl. Environ. Microbiol. **59**, 1155 (1993)
5. ASTM, American Society for Testing and Materials, Environmentally Degradable Plastics, ASTM Philadelphia USA (1993)
6. ISO Internazional Organization for Standardization, http://www.iso.ch
7. CEN European Committee for Standardization, http://www.cenorm.be
8. A. Yabannavar, R. Bartha, Soil Biol. Biochem. **25**, 1469 (1993)
9. L. R. Krupp, W. J. Jewell, Environ. Sci. Technol. **26**, 193 (1992)
10. R. Solaro, A. Corti, E. Chiellini, J. Environ. Polym. Degr. **6**, 203 (1998)
11. J. D. Stahl, M. D. Cameron, J. Haselbach, S. D. Aust, Environ. Sci. Poll. Res. 7, 83 (2000)
12. J. A. Van Veen, J. N. Ladd, M. J. Frissel, Plant and Soil **76**, 257 (1984)
13. M. Alexander, Introduction of Soil Microbiology, Wiley, New York (1961)
14. T. D. Brock, M. D. Madigan, J. M. Martinko, J. Parker, Biology of Microorganisms 7th edition, Prentice Hall, Englewood Cliffs, New Jersey USA (1994)
15. W. Schönborn in H.-J. Rehm, et al., Biotechnology vol. 8, Verlag Chemie, Weinheim (1986).
16. ASTM, American Society for Testing and Materials, Annual Book of ASTM Standards vol. **8.03**, 1153 (1985)
17. A.-C. Albertsson, S.-O. Karlsson, In *Degradable Materials: Perspectives, Issues and Opportunities* S. A. Barenberg, J. L. Brash, R. Narayan, A. E. Redpath eds., CRC Press, Boca Raton (1990)
18. R. Y. Stanier, J.L. Ingraham, M. L. Wheelis, P. R. Pantier, The Microbial World fifth ed., Prentice Hall, Englewood Cliffs, New Jersey USA (1988)
19. H.-J. Rehm, G. Reed esd,. Biotechnology vol. 1, Verlag Chemie, Weinheim (1981)
20. O. W. Lowry, J. Biol. Chem. **193**, 265 (1951).
21. J. A. Donkersloot, S. A. Robrish, M. I. Krichewsky, Appl. Microbial. **24**, 179 (1972)
22. W. H. Holms, J. D. Hamilton, A. G. Robertson, Arch. Mikrobiol. 82, 95 (1972)
23. A. L. Andrady, J. M. S. Rev. Macromol. Chem. Phys. **C34**, 25 (1994)
24. J. H. Finley, Anal. Chem. **33**, 1925 (1961)
25. C. P. L. Grady Jr., Bitechnol. Bioeng. **27**, 660 (1985)
26. M. M. Berekaa, A. Linos, R. Reichelt, U. Keller, A. Steinbuchel, FEMS Microbiol. Lett. 184, 199 (2000)
27. R. N. Sturm, J. Am. Oil Chem. Soc. **50**, 159 (1973)
28. A. C. Marinucci, R. Bartha, Appl. Environ. Microbiol. **38**, 1020 (1979)
29. G. Colin, J. D. Cooney, D. J. Carlsson, D. M. Wiles, J. Appl. Polym. Sci. **26**, 509 (1981)
30. A.-C Albertsson, Z. G. Banhidi, L.-L. Beyer-Ericsson, J. Appl. Polym. Sci. **22**, 3435 (1978)
31. N. E.-D. Sharabi, R. Bartha, Appl. Environ. Microbiol. **59**, 1201 (1993)
32. A. Yabannavar, R. Bartha, Appl. Environ. Microbiol. **60**, 3608 (1994)
33. R. J. Larson, R. L. Perry, Water Res. **15**, 697 (1981)
34. U. J. Strotmann, H. Schwarz, U. Pagga, Chemosphere **30**, 525 (1995)
35. A. M. Buswell, H. F. Mueller, Eng. Chem. **44**, 550 (1952)
36. M. Hakkarainen, A.-C. Albertsson, S. Karlsson, J. Chromatogr. A **741**, 251 (1996)
37. A.-C. Albertsson, B. Erlandsson, M. Hakkarainen, S. Karlsson, J. Environ. Polym. Degr. **6**, 187 (1998)
38. G. Bellia, M. Tosin, G. Floridi, F. Degli Innocenti, Polym. Degrad. Stab. **66**, 65 (1999)
39. J. Shen, R. Bartha, Appl. Environ. Microbiol. **62**, 1428 (1996)
40. E. Chiellini, A. Corti, R. Solaro, Polym. Degrad. Stab. **64**, 305 (1999)
41. R. Bartha, D. Pramer, Soil Sci. **100**, 68 (1965)
42. E. Chiellini, P. Cinelli, A. Corti, E. R. Kenawy, R. Solaro, Macromol. Symp. **152**, 83 (2000)

CHAPTER 7

ENVIRONMENTALLY SOUND WASTE MANAGEMENT

Objectives

- Students will learn the principles, strategies and managing hierarchy of waste management;
- Students will understand the role of EDPs from the perspective of waste management;
- Students will study major waste management technologies and their relevance with EDPs;
- Students will study the legal framework governing waste management, and thus be prepared to develop, update and integrate policies, regulations and standards relevant to wastes and EDPs;
- Students will be able to develop, modify and/or apply economic and market oriented instruments to deal with waste management with respect to plastic and EDPs.

Summary

The general concepts and practice of environmentally sound waste management, with emphasis on plastics and linkages with EDPs, will be addressed in this lesson. Target group will be technical staff, R&D people and decision-makers, as prescribed by the LDV program.

7.1 Overview

Environmentally sound solid waste management (or generally shortened as SWM), as defined by UNEP, means taking all practical steps to ensure that wastes are managed in a manner which will protect human health and the environment against the adverse effects which may result from such wastes. Principles to be considered in such a waste management are recognized by the international community as followings:

(1) *The Source Reduction Principle* – means to minimize the generation of waste in quantity and its potential to cause pollution by appropriate plant and process designs and cleaner production;

(2) *The Integrated Life-cycle Principle* - by which substances and products should be designed and managed such that minimum environmental impact is caused during their generation, storage, transport, use, reuse, recycling, recovery and final disposal;

(3) *The Integrated Pollution Control Principle* - requires that the waste management should be based on a strategy which takes into account the potential for cross media and multi-media synergistic effects;

(4*) The Polluter Pays Principle* - by which the potential polluter must act to prevent pollution and those who cause pollution pay for remedying the consequences of that pollution;

(5) *The Standardization Principle* - which requires the provision of standards for the environmentally sound management of (hazardous) wastes at all stages of their processing, treatment, disposal and recovery;

(6) *Proximity of Waste Disposal Principle* – requires disposing of wastes in the nearest appropriate installations, aiming to minimize adverse impacts associated with the trans-boundary movement of wastes.

(7) *The Principle of Public Participation* - waste management options are considered in consultation with the public, and that the public has access to information concerning wastes management.

The logical starting point for solid waste management is to reduce the amounts of waste that must be managed at source. For the wastes that nevertheless are generated, a strong control should be involved over the life cycle from design, production, and use to after-use stages. Waste management should

follow the hierarchy of Four-R, namely Reduce, Reuse, Recycle (or recovery of materials) if can not be reused, and Recovery the energy content if not recyclable (or energy from wastes, EFW), before final disposal. It is worth noticing, however, recycling requires energy and generates products inferior in quality to virgin products, which can only be used in low-grade application with relatively limited market. A number of legal, institutional and technical conditions need to be met, particularly that:

- A regulatory and enforcement infrastructure, which will be discussed in Section 7.3;
- Authorized facilities have adequate technology and means of pollution control and effective monitoring, see section 7.2;
- More sophisticated yet efficient environmental measures, such as economic and market based instruments, to encourage more responsibility among producers and consumers, see section 7.4;
- Capacity building through training of the employees; Public participation and information through awareness raising campaign and consumer education.

Why EDPs are needed

Environmentally sound management of increasing amounts of difficult-to-treat or organic wastes is among the major concerns today. Some products are more difficult to recycle than others, for instance, foodservice disposables and egg cartons, and thus easy to be littered, because:

- These products contain high degree of contamination from food residues difficult to remove, resulting in very labor- and energy-intensive recycling;
- Also, because these products are lightweight and of high volume, high transport costs may be incurred in shipping the discarded products to the re-processors;

The principle of sustainable development requires the society to be based more and more on renewable resources. Furthermore, EDPs of renewable sources is neutral with respect to the carbon dioxide cycle.

Therefore, EDPs may serve as a promising solution to visual and sanitary problems caused by litters of disposable plastic products, to over-filled landfills by diverting part of bulky volume plastic packaging to other means of waste management. It also facilitates organic waste management by eliminating the cost involved in removing the collection bags before entering compost facilities. In addition, it helps to preserve the natural resources and contribute to sustainable development.

Self-check Questions

1. Following which hierarchy should the wastes be managed?
2. Why do we need EDPs?

Hints for Answers

1. See section 7.1;
2. See the text box in section 7.1.

Exercises

Group discussion:

"Municipal waste management in your country in general and how do you think EDPs can best fit in?"

Trainees are divided into several groups according to country or region. Each group will present the result of discussion and submit a short paper of maximum 3000 words or 3 pages.

Reading Materials

The need for an integrated approach

(Source: Waste generation and management, by European Environmental Agency (EEA)
http://themes.eea.eu.int/theme.php/issues/waste, July 26,2000)

The increasing waste quantities can not be solved in a sustainable way by waste management and recycling alone. There is an urgent need for integration of waste management into the strategy for sustainable development, where waste prevention, reduction, and minimization of emissions at source are given high priority. Waste must be handled as an integrated part of the total material flow through the society.

To stabilize or even reduce the waste amount, there is a need for many initiates besides cleaner technology, such as product development based on life cycle analysis; design for the disassembly; environmental management system in corporate, re-use of products and packages; improvement of product quality in terms of lifetime, better possibility for repair,; increased re-usable components of discarded products and increased consumer awareness of the need of changing lifestyles.

How to reduce wastes at the first place?

Source: The *International Source Book of Environmental Sound Technologies (ESTs) for SWM*, United Nations Environmental Program--Int'l Environmental Technology Center (UNEP-IETC)
(http://www.unep.or.jp/ietc/ESTdir/pub/MSW/index.html, June 26,2000)

There are four main ways that most city governments in developing countries can enhance waste reduction:

1. Inform citizens about source separation and recycling, and the needs of waste workers: extensive public education is needed to develop understanding of the need for further source separation to improve the potential for composting and to remove the stigma of association with waste materials.
2. Promote recycling industries and enterprises.
3. Divert organics. The greatest relief for the waste authority will come from reduction of organics, which implies, in the main, successful composting. Keeping organic pure for composting will require more thorough source separation than is done at present.
4. Advocate key areas for waste reduction at the manufacturing level (e.g., reduction of plastic packaging; coding of plastics to improve recycling).

Too much biodegradable waste ends up in landfills.

(Source: Waste — Environmental signals 2000 (Chapter 11), by EEA
http://themes.eea.eu.int/theme.php/issues/waste, July 24, 2000)

In EU, too much biodegradable waste was still going to landfill in many countries despite the fact that such waste could be recovered as compost or incinerated. Landfilling of biodegradable waste results in greenhouse gas emissions and is a loss of resources. An estimated 55 million tons of paper, paperboard, food and organic garden waste was landfilled in 1995 in EU Member States (less Portugal but including Iceland and Norway). If plastics are classed as biodegradable, this figure rises to 66 million tons. The EU Landfill Directive sets a target of reducing the amount of biodegradable municipal waste landfilled to 35 % by 2016, i.e. a maximum of 19 million tons. Minimizing waste to landfill is the core of the EU waste strategy and the Landfill Directive target for biodegradable wastes.

…Existing policies would not be sufficient to stabilize waste generation. Future product policies in EU will be of great importance as it calls for preventive strategy for the life cycle of products and services. Sufficient waste management must be supported by measures to reduce waste generation.

7.2 Technological Components of SWM

Environmentally sound management involves the use of appropriate state-of-the-art facilities operated under quality assured management regimes. Discussion about SWM technologies will follow the hierarchy indicated in section 7.1 when applicable. Since EDPs are created primarily to deal with the un-recyclable situation, recycling technologies of plastics will not be covered. Instead, more weight has been given to composting, a nature's way of recycling.

7.2.1 Collection and Separation

Source separation play a crucial role for the success of all follow-up SWM technologies, particularly for the proper operation of recycling and composting as well as their economic viability by affecting the quality of their products, as indicated by section 7.2.3 and 7.2.4. For the incineration, if the incoming waste is too wet or too dry, too rich in burnables or too high in non-burnables (glass and metals), there can be difficulties with the incinerator's functioning and emissions, as shown by section 7.2.5. Clear labeling is particularly important for effective sorting of plastics since their identification is difficult even for an expert.

Determining the "best" method requires tradeoffs between the conflicting objectives of low cost collection and low cost processing. For example, the use of plastic bags may lower collection costs, but may increase the cost of processing to remove unwanted plastic. Conversely, the use of curbside collection makes processing easier, but requires more capital expenditure for collection equipment.

7.2.2 Reuse

Reuse means that the after-service materials/products are used again, as a whole or partly, without any physical and chemical change being made. Reuse, for instance utilizing polystyrene products in the same form, is important not only because it delays the final disposal of a product, but also because it reduces the manufacture and purchase of new products. As a result, reuse prevents waste. In USA, nearly 30 percent polystyrene loose fill is used again, make it the most commonly reused plastics.

7.2.3 Recycling

Recycling, also termed as material recovery, involves physical and sometimes chemical change of recyclable products. Often the plants for virgin production perform recycling of same material. Though recyclables usually enter the process at a later stage than raw material for virgin production, they require extra pretreatment to remove the contaminants and properties possessed by recyclables when as a product. For example, plastics may be contaminated by dyes, pigments, non-plastics components or other types of polymers. The recycled material thus will not be suitable for the original application, leading to an inferior quality with a less demanded market.

The economics of recycling is not always favorable when hauling recyclables, especially lightweight products like plastics, long distances to recycling plant is needed. Cleaning cost of highly contaminated foodservice products is also significant. Above all, the ultimate success of recycling depends on whether there is a stable and reliable markets for the recycled materials.

7.2.4 Compost

Composting is a natural biological process, which converts organic material into a stable humus-like product called compost and generates CO2. During the composting process, various microorganisms, including bacteria and fungi, break down organic material into simpler substances. Majority of composting is aerobic process and mechanical mixing and aeration consume energy. Municipal composting can include a combination of backyard, on-site and centralized management approaches. Technology widely adopted are windrew and in vessel. Since approximately 50% of the waste stream are organic matter, composting can play an important role in the integrated waste management plans of any community.

A number of factors, including temperature, moisture, oxygen, particle size, and the carbon-to-nitrogen ratio of the waste and the degree of mixing involved, are important for the composting process and the

time it takes. Among which to control the quality of the material entering the process is the most important. This indicates the key role played by source separation.

Pros and cons of composting

An effective composting program can produce a high quality soil amendment with a variety of end uses. Diverting organic wastes from landfill sites helps to conserve landfill space and to reduce the production of leachate and methane gas (both of which add to the cost of operating a landfill). However, composting is much slower, subsequently requires more space, and needs more control than recycling or incineration. In general, the more actively it is controlled, the faster the process. The length of the process also depends on the degree of decomposition desired in the finished product. Most aerobic composting include a period of active composting, generally from 21 to 60 days, and a period of curing, generally from 6 months to a year. If not properly controlled, odors and pests may be generated.

Waste composting is still not viable as a large-scale waste management option because:

- The economics of this option usually does not compare favorably to landfill or incineration;
- Severe odor problems often associated with this technology that make siting very difficult;
- There are very limited viable, long-term markets for the compost produced.

7.2.5 Incineration

Waste-to-energy incineration is a substantial reduction of the weight (up to 75%) and volume (up to 90%) of solid waste, which can be valuable if landfill space is scarce, resulting heat to generate steam and electricity. Derived primarily from oil and natural gas, plastic produces significant amounts of heat energy when burned thus help to burn other waste more completely. For most plastics, thermal decomposition products are carbon dioxide, water vapors and a trace amount of non-toxic ash.

The high capital and operating costs, potential environmental impacts, technical difficulties of operating incinerators and the need for highly trained personnel make incineration a sound practice only when:

- Suitable landfill space is scarce, making incineration a cost-effective alternative;
- The necessary environmental controls of incinerator are properly installed and maintained;
- The facility is properly sized and sited to fit well with other components of the SWM system;
- Materials to be burned is combustible and has sufficient energy content, and near energy markets.

7.2.5 Landfill

Landfill is a vital component of any well-designed SWM system. They are the ultimate repositories of a city's wastes after all other SWM options have been exercised. While recycling and reuse grow in popularity, most of the waste still goes to landfills. Modern landfills are designed to protect the environment from the liquids and gases produced during the very slow breakdown by reducing the exposure of garbage to air, water and sunlight - conditions needed for degradation. By design, waste inside a modern landfill biodegrades very little - not paper, not plastics, not even food waste degrades. On the other hand, unwanted degradation of bio-wastes in many substandard landfills calls for diverting of bio-wastes from landfill by other means.

7.2.6 Integration of SWM

As more communities aim to recycle large portions of the waste stream, the need to comply with incinerator plant contracts for minimum tonnage and energy production may lessen incentives for materials recovery. Total energy production from incineration usually declines slightly due to the reduction in the amount of waste combusted. On the other hand, a successful materials recovery program can increase the energy content of waste because many recyclables (e.g., glass bottles, metal cans, and yard waste) are non-combustible or have low fuel value. Also, diverting glass and aluminum reduces boiler maintenance costs since melted glass can form slag on boiler walls, and aluminum can clog air circulation holes in furnace chambers. In addition, materials recovery programs (reuse and recycling) reduce the capital needed to build a new incineration and landfill. Therefore, proper planning for new facilities can ensure that materials recovery and landfill/ incineration are better integrated, possibly enhancing each other's operations.

Although incineration facilities are commonly sized to accommodate waste generated during peak times, introducing yard waste composting programs can eliminate much of the seasonal fluctuation in generation rates, and further reduce the need for excess capacity. Some materials (such as paper products) can be both recycled or composted. While clean paper is generally more valuable when recycled. Soiled paper or paper that cannot be recycled economically can be composted. If degradable materials were to be promoted as a viable remedy for dealing with solid waste, composting facilities to accept degradable /compostable materials should be widely available.

Self-check Questions

1. Why is source separation of wastes so crucial for the effective operation of SWM?
2. What limit the large-scale application of composting as a SWM component and what can be done to overcome such limitations?
3. What would be the influence of EDPs on various SWM components?

Hints for Answers

1. See section 7.2.1, 7.2.6 and Reading Materials;
2. See reading material on composting;
3. Refer to section 7.2.1, 7.2.4-7.2.6.

Exercise

On-site visits to:

1. A plastic recycling facility and a waste composting facility; or
2. Typical SWM facilities and environmentally sound managed SWM facilities as a comparison.

Pay attention to the advantages and disadvantages of each SWM technologies and the implication on EDPs. After the visit, everyone should prepare a brief summary and be cross-reviewed by each other.

Reading Materials

The composting methods

Source: The Composting Council of Canada **(** www.compost.org**,** June 20, 2000**)**

There are three basic types of centralized composting processes or methods:

1. In the *In-Vessel Method*, the organic material is composted inside a drum, silo, agitated bed, covered or open channel, batch container or other structure. The process conditions are closely monitored and controlled and the material is aerated and mechanically turned or agitated.
2. The *Aerated Static Pile Method* involves forming compostable materials into large piles, which are aerated by drawing air through the pile or forcing air out through the pile. The pile is not turned.
3. In the *Windrow Method*, compostable material is formed into elongated piles, known as windrows, which are turned mechanically on a regular basis.

In some cases, such as in the composting of grass clippings, the raw material may be too dense to allow for the proper flow of air or may be too moist. A common solution to this problem is to add a bulking agent, such as wood chips, to provide structure to material and to allow for proper airflow. The amount of bulking agent required is usually determined based on experience. Some facilities add commercial fertilizers to their composting process, but this can usually be avoided by combining different waste streams together in a specific "recipe". Inoculating the material with microbes is not normally required, since most wastes naturally contain the microbes needed for successful composting to occur.

Sound compost technologies and technical options

(Source: The *International Source Book of ESTs for SWM* , UNEP-IETC (http://www.unep.or.jp/ietc/ESTdir/pub/MSW/index.html, June 26,2000))

Volume reduction

All true composting processes result in volume reduction, since the action of the bacteria transforms materials into steam and gases, while insects and microorganisms also feed on the organics. Additional

volume reduction occurs due to removal of non-compostables during pre-processing or final screening, in addition to the moisture loss and volume reduction during composting itself. Thus a 100-ton-per-day facility will produce only 30 - 50 tons of compost per day. In places where land for siting is scarce, sound practice may entail selection of more intensive practices instead of more extensive land use (e.g. windrow facilities can range in size from about 1 to more than 20 acres.).

Critical lessons in sound composting practice

The analysis of compost failure yields the guidelines for sound composting practice discussed below.

- The material to be composted must be compostable in order to produce a marketable product: Both large- and small-scale systems can work well with highly compostable waste streams. In most cases (1) analyzing the waste stream and (2) if necessary, designing the separation protocol and separate collection system are as important to the success of composting as the selection of the technique itself.
- Manual pre-processing of mixed waste does work on a small to medium scale for the highly compostable waste streams in developing countries, but also in very small projects in industrialized countries; therefore: manual or manual-assisted processing is the soundest approach to bio-waste composting that can be sustainable over the long term in a technical sense; However, manual processing may not be either pleasant or safe for workers.
- Technical viability depends on three factors:
  1. There must not be excessive dependence on mechanical pre-processing.
  2. The scale of composting must not be too large. In general, the more complex the input stream, the smaller must be the scale to ensure proper composting process and a good product.
  3. The entire system from separation and collection to final screening must be designed together to deliver the appropriate input streams and to support the biological processes in composting.

Bacteria's central role in composting

The most common form of composting, aerobic composting, takes place in the presence of oxygen. Aerobic bacteria require a mix of approximately one part nitrogen to at least 30 and no more than 70 parts carbon in their food supply. Aerobic bacteria also require at least 40% but not more than 60% water in their environment, and a plentiful supply of oxygen. In the absence of any one of these four factors, the composting process will fail. The products of aerobic composting are steam, carbon dioxide, and decomposed organic material, called humus.

Anaerobic bacteria live in the absence of oxygen and can consume mixtures with a higher proportion of nitrogen and lower proportion of carbon. Anaerobic digestion can also occur at higher levels of moisture. The products of anaerobic digestion are methane gas and decomposed organic material. To recover the gas, anaerobic systems are enclosed in a pressurized environment.

Composting bacteria operate on the surfaces of compostable materials. That means that composting works well with small particles of waste and poorly with large pieces of organic material. For this reason, size reduction or shredding is frequently required prior to composting to allow for adequate bacterial decomposition. All solid waste composting is based on one or both of these biological processes. Differences in technology relate to input materials, pre-processing techniques, and the way in which the environment for bacterial action is created and maintained, but not to the composting process itself.

Monitoring and control of composting facility

Any waste management facility, including a composting site, has the potential to generate offensive odors or to attract pests. However, experience at hundreds of composting facilities has shown that proper design and operational procedures can prevent or control these problems. Excessive or offensive odors are generally a sign that the composting process is not proceeding properly, usually because of inadequate aeration or excessive moisture. Close monitoring of these factors can usually help to minimize odors.

Wherever decaying organic matter is present, certain microbes occur naturally. Spores of the fungus Aspergillus fumigatus are commonly cited as a source of concern. It is one of the most widely distributed microorganisms on earth known to exist in almost every interior and exterior environment. People are routinely exposed to low levels (and occasionally high levels) of this spores without consequence. The conclusion reached by health and environmental agencies in the US and Europe is

that normal, healthy individuals suffer no increased health risk by either working at, or living near, a compost facility.

An example of EDP composting in Switzerland

(Source: Int'l Center for agro-based materials, http://www.agriholland.nl/proterra/about.html, Dec. 2000)

State of the art in Switzerland

Various manufacturers of compostable, biodegradable bags (BAW bags) market their products in Switzerland. BAW bags for collecting organic waste are widely distributed by wholesalers. In addition, some municipalities are considering using it for the collection of organic waste, since there is a demand from consumers for clean and easy separation and collection of organic waste.

Composto tested and compared BAW bags made from Kraft paper, PolyCapro Lacton (PCL), PCL and cornstarch, and thermoplastic starch. The degradability of the bags was investigated in the laboratory (according to DIN draft 54900), on garden and communal compost heaps and in industrial composting facilities. Besides, the handling of biodegradable bags during use and organic-waste collection was studied from questionnaires and interviews. The impact on the environment was analyzed by performing life-cycle assessments of different collecting systems in order to answer the following key questions:

1. Which processing modes (private composting, industrial facilities) are compatible with biodegradable bags without involving any great disadvantages for organic-waste collection?
2. How quickly do the bags decompose at industrial and in private composting facilities?
3. What is the influence on compost quality?
4. Will the public's attitude to separate collection of wastes change?
5. Do the bags invite the inclusion of foreign matter in the organic-waste fraction?
6. How great is the environmental burden imposed by biodegradable bags compared with conventional collection methods (green buckets and containers with and without liners)?

Results of the degradability test

When questioned, one-half to two-thirds of households expressed a willingness to buy BAW bags for organic-waste collection. Positive adjectives used to describe them were 'clean', 'practical', 'hygienic' and 'compostable'. Negative adjectives were 'leaky' and 'costly'. Two-thirds of the community compost groups questioned would allow the use of BAW bags, while one-third would not. During the test period, organic-waste collection with BAW bags did not lead to an increase in foreign matter. Refuse collectors said that BAW bags made the collection of organic waste quicker, easier and more hygienic. For use in organic-waste collection the bags would have to be made more stable, recognizable and transparent.

The quality of the compost derived from the contents of BAW bags has been found to be up to the usual standard. Bags made out of PCL (Polycaprolacton) and TPS (Thermoplastic Starch) meet with the DIN draft 54900 decomposition specifications. Because of their longer decomposition period and their unsuitability for external assessment of foreign matter content (not transparent), bags made out of Kraft paper may be used on private compost heaps, but not in communal composting facilities and not for collection as organic waste. The heavy-metal analysis of the foils shows that no paints containing copper (blue and green) should be used; otherwise, the limit for heavy metals as set down in DIN draft 54900 will be exceeded.

7.3 SWM Policies and Regulations

The safe and effective operation of various SWM options (components) depends on the sound planning, administration, and management of the entire SWM system. This begins with an institutional and policy environment that views SWM as an important component in the sustainable development of a country. It continues with SWM regulations and standards that are designed to protect human health and the environment. It ends with the coordination of SWM programs, from waste reduction and resource recovery through collection and transfer to ultimate disposal.

7.3.1 Policies and Institutional Framework

Legal and institutional infrastructure, the foundation for the establishment and operation of SWM system, derive from the nations' strategy toward wastes. *Integrated waste management* is increasingly accepted as the SWM strategy worldwide. In line with the general framework, the waste managing hierarchy, discussed in section 7.1, constitutes the backbone of SWM in many countries. In order to implement the strategy and policies, a number of regulatory instruments are developed, as elaborated in section 7.3.2.

The challenge of increasing wastes calls for not only integrated SWM, but also the integration of waste policies with policies for products and consumption. This is particularly the case for the commercialization of EDPs, which depends on separated collection, the maturation of proper disposal (composting) both in technology and standards, and market development for the recycled products, the compost. Paying more attention on waste aspect while developing eco-label criteria may promote life cycle consideration of product aiming at waste minimization and stimulate market demand for the recycled. Following the same thought, public procurement should give preference to products with minimum wastes over their life span or contributing to waste minimization, such as EDPs and the compost, as discussed in section 7.4.3.

7.3.2 Regulations and Standards Regarding SWM

Waste related laws, regulations, ordinances, rules and/or bans, are legal actions, or direct instruments in contrast to economic instruments which are indirect, to support the strategy and policies, to address specific problems associated to certain type of wastes (packaging etc.) or waste management options. These so called command-and-control measures set up the bottom line for a society to regulate the behavior of its members with regard to wastes. Enforcement of regulations needs standards against which compliance with law can be judged and non-compliance can be punished. Economic instruments can not work either without regulations and standards, based on which a fee/tax can possibly be calculated and charged or a preference can be offered. Licensing and permitting are based on regulations and standards too. The voluntary agreement is actually an industry initiative to meet the environmental targets prescribed by the law and related standards. A complete legal system should also define the means of fine and sanction as a penalty for non-compliance and violation, and more important, as a way to prevent such behavior in future.

Packaging ordinance in Germany and other European countries are examples of regulations effective both in solving specific waste stream problems, e.g. diverting plastic wastes from landfill, and in promoting the waste strategy of material recovery superior to energy recovery and landfill by setting recycling targets for plastic and metal packaging. The section 8.2 of Chapter 8 will provide more insights into regulatory and standard issues.

The importance of separate collection is highlighted by the relevant regulations on products in many developed countries. In EU member states, it is mandatory for manufactures to clearly label the type of plastics on their products. As distinguishing different types of plastic, including EDPs, is difficult even for the experts, such simple measure will facilitate source separation by helping consumers in choosing the products and sorting them when discarding. Together with eco-labeling, clear and easy-understandable standards may regain trust in such products like EDPs and facilitate the administrative process involved.

Criteria selected as to whether it is environmentally sound disposal options

- Site selection and design standards for facilities
- Environmental assessment
- Operation/discharge standards and quality standards for the products, if any
- Training of operators of the facility
- Monitoring, control and documentation, emergency and contingency plans
- Treatment of residues, such as slag and dust from incineration plant.

7.3.3 Basel Convention and its Implication

The *Basel Convention on the Control of Trans-boundary Movements of Hazardous Wastes and their Disposal* entered into force on 5 May 1992. These wastes are hazardous to people or the environment because they are toxic, poisonous, explosive, corrosive, flammable, eco-toxic, or infectious. This global environmental treaty strictly regulates the trans-boundary movements of hazardous wastes and provides obligations to its Parties to ensure that such wastes are managed and disposed of in an environmentally sound manner. The Convention also aims to monitor and prevent illegal traffic, promote cooperation and provide assistance for the environmentally sound management of hazardous wastes, and develop Technical Guidelines (TG) for waste management among which TG for plastic wastes has been drafted. Classification of PVC wastes and PVC coated cables in the context of the Basel Convention, the costs involved in the disposal or recycling of plastic wastes and accessibility of appropriate technologies in developing countries were major issues in the debate for the draft.

In March 1994, Parties agreed to an immediate ban on the export from OECD to non-OECD countries of hazardous wastes intended for final disposal. They also agreed to ban, by 31 December 1997, the export of wastes intended for recovery and recycling from what are known as Annex VII countries (members of the EU, OECD, Liechtenstein) to non-Annex VII countries (all other Parties to the Convention). The Ban was formally incorporated in the Basel Convention as an amendment. However, the non-hazardous wastes, those can be safely recycled or reused including plastic wastes, would exempt from the Ban.

Self-check Questions

1. What does *integrated waste management* mean with regard to EDPs?
2. Why are the standards so important for the existence and effectiveness of SWM?
3. What is the implication of the Basel Convention and related provisions on EDPs?

Hints for Answers

1. See section 7.3.1 and Reading Materials;
2. See section 7.3.2;
3. The Technical Guideline under drafting, with its provisions on disposal and recycling of plastic wastes and accessibility of appropriate technologies in developing countries, may support the application of EDPs; Export of non-hazardous plastic wastes to which EDPs are belong is exempt from the Ban.

Exercise

Group discussion and reporting the outcome to the class:

1. Please draw a chart to visualize the legal infrastructure necessary for effective municipal SWM and interactions among different elements.
2. With the help of the chart, indicate what can be achieved by substituting EDPs for conventional plastics; what regulatory instruments should be added or integrated if EDPs were to be introduced successfully.

Reading Materials

Waste Production and Management within EU

(Source: Europe's Environment: The Dobris Assessment, Chapter 36, 1995. http://themes.eea.eu.int/showpage.php/issues/waste?pg=40434, July 24, 2000)

European countries have adopted various regulatory measures to minimize waste and ensure its safe management. Different regulatory frameworks have emerged which establish:

1. Programs to encourage waste reduction, re-use and recycling;
2. Standards and procedures to ensure safe storage, treatment and disposal; and
3. Programs to activate clean-up of contaminated sites.

Priority is given to five strategies summarized by the so-called 5R approach recommended by the Senior Advisers to ECE Governments on Environment and Water Problems (UNECE, 1992). These are based on: reduction, replacement, recovery, recycling and re-utilization of industrial products, residues or waste.

Prevention of waste movement

The importance of a coordinated action at the international level to control waste movements and reduce the potential threats of improper waste management has become clear with the discovery of the damage caused by transfrontier movements of hazardous waste. The Basle Convention on the Control of Trans-boundary Movement of Hazardous Waste and their Disposal (UNEP, 1989), signed by 116 countries in 1989, allocates to the exporter states the responsibility for ensuring that exported waste is managed in a safe manner. The convention does not forbid waste shipments, but establishes that shipments must receive the written informed consent of importing states before they can take place.

In EU countries, the export of waste is controlled under Council Regulation 93/259/EEC, which updates and extends the controls originally introduced in 1984 and implements the provisions of the Basle Convention. Under the Regulation, all exports of waste for final disposal outside the Union are banned. There are also specific controls on hazardous wastes being exported for recovery, and such exports from the EU, other than to OECD (Organization for Economic Cooperation and Development) countries, will be banned after 1997 as a consequence of Decision II/12 taken at the Second Conference of Parties at Geneva in March 1994. Transfrontier movements of wastes within Europe are influenced by a number of factors, including waste management capacity, regulatory standards and controls over transfrontier movements. Improvements in these respects across Europe, and particularly implementation of the Basle Convention, will help reduce these movements.

Privatization and support of the formal sector

(Source: The *International Source Book of ESTs for SWM*, UNEP-IETC (http://www.unep.or.jp/ietc/ESTdir/pub/MSW/index.html, July 18,2000)

Privatization involving contracting to formal-sector waste management companies often brings significant resources to the solid waste collection arena, and can represent an important element in sound practice. Privatization is sometimes mistakenly seen as a way to solve all of a government's waste management problems.

Privatization of waste collection generally involves the responsible government contracting out collection services to one or more private sector operators. There is competition at the point of securing the contract, but once a contract or a franchise is awarded, the contractor receives a managed monopoly from the government. When these arrangements are well managed and free of corruption, they can deliver a high level of cost-effective service often higher than the government could provide using its own workers.

By contrast, some privatization efforts have entailed the total retreat of the municipal government from the waste management business. In this circumstance, there is no management by government: private collection firms must go directly to generators and contract with individuals. This tends to create redundant systems, where multiple trucks roll down the same streets, with each picking up from only a few of the contiguous residents. The resulting scale effects are very unfavorable, which means that fees tend to be high, and smaller firms are likely to fail or become the target of corporate takeover. This can

lead rapidly to an unmanaged monopoly situation, and waste collection costs can become quite startlingly expensive.

Support to the informal sector

Local authorities can make good use of available resources by contracting to small-scale waste collection enterprises, and by providing support and recognition to waste pickers and itinerant collectors, effectively allowing their activities to be included in the overall MSWM system. This is particularly important when new waste services are being introduced, or where existing systems are being upgraded or modernized.

Sound practice in this arena is illustrated by the waste cooperatives for materials recovery and reuse in place in many regions of Asia and Latin America. These coops or associations employ workers (who might otherwise be waste picking without equipment or recognition) to separate wastes at sources, collect recyclable materials, and transport them to the collection centers for processing and sale.

7.4 Economic and Market Based Instruments

Sound practice virtually always requires a fiscal commitment from some level of government to design, finance and maintain the SWM system. The focus of economic instruments gradually shifts from financing the system and recovering costs directly from beneficiaries to encouraging waste reduction through cleaner production and sustainable consumption. Any such instruments will include these three components:

- A fiscal commitment to capitalize, maintain, and operate various components of SWM system;
- A way to recover all or part of the variable costs of SWM operation from its beneficiaries; and
- A monitoring and accounting system evaluating the efficiency and effectiveness of SWM system.

7.4.1 Charges and Taxes

The charges, fees and taxes are designed not only for cost recovery of SWM system, but also, and probably more important, to provide incentives for the change of behavior necessary for achieving certain environmental goal. If such economic instrument, functioning through the market mechanism, can be designed to reflect the environmental and social cost (or internalize the externalities as said by economists), of waste disposal, the environmentally optimum consumption and disposal level can be reached at the least cost of the society. A carefully designed and implemented economic instrument will be more efficient than direct regulation. Furthermore, it will promote a continual search for " cleaner" alternatives, such as EDP for plastic products, since it narrows down the price difference between conventional products and the substitutes.

How to calculate the taxes or charges and how to collect them are crucial to such schemes. Ideally the amount charged should include economic, environmental and social costs, while the way of collecting should incur least administrative cost. In reality, the costs mount up to such a level that deters most people from paying it. Therefore, SWM charges/taxes are usually only able to recover part of the economic cost and based either on volume ("pay per bag") or on weight, where generators pay for what they throw away. A too high fee has led to increased illegal dumping or burning of waste; When it is not noticeable, the expected reduction in consumption and subsequent waste generation may not be observed. In most countries, municipalities are still more likely to levy a flat fee included in a utility bill, or to simply pay for services out of taxes, though both have hardly any steering effect on the behavior.

Charges or taxes based on recycled content or degradability of plastic products are expected to have direct effect on consumption pattern, i.e., to reduce the consumption of plastic packaging and encourage the use of recycled products and EDPs. However, when the relevant standards are either not in place or not clearly defined, producers are inclined to seek higher recycled content and degradability at the expense of other less strictly regulated properties, for example durability. More likely they may simply declare so in order to get tax exemption and keep on selling at a low price. Clear and easy-to-access standards and product labeling will reduce such problems, in the meantime, help consumers in

choosing products and sorting them when discarding, re-gain trust in such products like EDPs and facilitate the administrative process.

As a summary, the success of an economic or market-oriented instrument depends on:

- The design, particularly the rate and collecting way with minimum administrative effort and cost;
- Clear and easily accessible standards;
- Adequate regulatory infrastructure, such as regulations and institutional capacity for enforcement and monitoring. This provides a platform only on which any market oriented instruments could work;
- Political acceptance since environmentally oriented taxes/charges would always be accused for giving foreign producer competitive advantages over domestic or local production.

7.4.2 Extended Producer Responsibility (EPR)

Extended producer responsibility (EPR) is an environmental strategy, which implies that the responsibility for a product over its life cycle lies with the producer. Before production is commenced, the producer should know how the waste, as a result of production, should be treated and how the product should be taken care of when discarded. Based on such rationale, producers, including manufactures and retailers in practice, are held responsible for particularly the take-back, recycling and disposal of the product. Originated from Sweden in early 1990s, EPR principles have achieved remarkable success in recovery of packaging and reduce the waste in several European countries, such as Sweden, Germany and Switzerland. Encouraged by these successes, more countries are interested or trying to incorporate EPR principles into their legislation and extend it to other waste streams, e.g. electronics.

The German EPR system, so-called Dual System, gained its name from the fact that this collecting, sorting and recycling system was established by industry, driven by the *Ordinance*, and operated in parallel with the municipal SWM system. It involves no deposit and is based on the voluntary source separation by consumers. The scraps are then re-processed by facilities set up by manufacturers of both packaging and soft drink participating in the system. In, Austria, a similar system called ARA is being implemented (see reading material). The Swedish EPR system is similar to Dual system except that the source separation is motivated by a deposit-refund mechanism. Though the recycling quota for aluminum can was pulled up from 75% to 90%, it was exceeded: 90.5% in 1997. However, the recycling requirement for PET bottles, 90%, has not been met, nor in Switzerland. This might be explained by the fact that metal scraps have a higher enough market value than PET and most of the plastic scraps. How to develop market for recyclables and “greener” products will be discussed in the next section.

7.4.3 Other Market Based Strategies Related to SWM

Eco-labelling

Eco-labeling programs are created primarily under the demand of consumers for simple and reliable information about the environmental performance of a product, particularly when it is claimed as environmental-friendly, when purchasing. Primary function of Eco-label is, on one hand, to stimulate industry to market greener and certified products, on the other hand, give the guarantee to the public that the products’ compliance with established ecological criteria has been tested by independent third parties.

Eco-label scheme facilitates individual consumers as well as public purchasers in selecting products with less environmental impacts. The successful acceptance of the scheme would also offer a considerable market incentive for manufacturers to switch to clean technology and to research in that direction. Eco-label is voluntary, meaning that it is not setting standards which all manufacturers must meet. Still it has been criticized as having the potential of being abused as a trade barrier for imports. Harmonization or mutual reorganization of various eco-labels therefore is being undertaken with limited result due to the large gaps in technology and environmental requirements in different countries. It might bring about similar dispute on trade and environment if eco-label were to be introduced to EDPs.

Public Procurement

Governments are the largest consumers in many countries. Their purchasing activities could generate significant market demand. Governments' commitment for sustainable development makes public procurement, traditionally following the economic principle, "greener", so does organization and corporate purchasing. Clear and enforceable quality standards for the recycled and "green" products, such as EDPs and compost from wastes, are essential to stimulate and sustain such green purchasing. The role that government can play in market development for the recycled and green substitutes include:

- Use them in public sector and public works projects, or give them away to businesses;
- Specify that government contractors use them in government-funded construction projects;
- Support price of recyclables and substitutes or remove/modify subsidies on conventional products;
- Provide technical assistance to composting facilities for quality control;
- Formulate standards and guidelines for the recycled and green substitutes and their application;

Self-check Questions

1. What are the advantages and challenges of economic instruments for SWM?
2. What do you think are the reasons that EPR principle is practiced only in limited countries?
3. Describe at least 3 major market development means for EDPs and justify.

Hints for Answers

1. See section 7.3.1;
2. Design of an effective EPR scheme, with minimum administrative effort and free-rider problem etc., is challenging for many countries, particularly when there is a large number of producers involved, a large population needs to be motivated and/or a large proportion of imports in the market. As a more sophisticated environmental measure, EPR imposes higher requirements on institutional capacity for enforcement and monitoring.
3. Public and organizational procurement, eco-label for EDPs, public education and campaign etc.

Exercise

A debate on pros and cons of application of three major economic and market oriented instruments, namely environmental charge/fee, EPR and Eco-label for EDPs. Trainees are divided accordingly into six groups by selecting either pro- or against one of the three topics.

Reading Materials

Environmental Economics - An Elementary Introduction

R. Kerry Turner, David Pearce, Ian Bateman; Publisher: Prentice Hall/Harvestester Wheatsheaf, 1994
ISBN: 0-7450-1083-0

A comprehensive yet concise and easily intelligible textbook, one of the most commonly used both at undergraduate and graduate level around the world.

EEA's report: Environmental taxes--Recent Development

(Source: http://org.eea.eu.int/documents/presentation.pdf)

Why environmental taxes

Environmental taxes are major tools to get price right based on polluter pay principle and to create market-based incentives for environmentally friendly behavior. It will internalize external costs, improve efficiency, raise revenue for environmental purpose, create environmental and economic benefits and integrate environmental requirements into sector policies. Taxes and charges are can play a crucial role in changing relative prices and giving clear signals.

Application in Europe

The use of environmental taxes is increasing in member states of EU. By 2001, eight member states will apply CO_2 taxes. The level of energy taxation is rising and there are more non-energy and non-

transportation taxes on products (batteries, packaging, etc.). For example, Denmark, Iceland and Italy taxed on plastic bags in 1996, Austria and Italy start to tax on packaging from 2000, and Denmark taxed on disposable tableware from 1996.

Does it work

There is increasing evidence of environmental effectiveness. Cases include CO2 tax, waste taxes, etc.. A study in UK claimed that the landfill taxes had impact and the tax should be tripled to achieve its objective. In Denmark, waste tax has had significant impact on reduction of taxable wastes (construction and demolition wastes, heavier fraction of household and other wastes)… A landfill tax is operational in a number of Member States. The aim is to improve the competitive position of recycling and incineration with energy recovery as treatment methods. The limited capacity of landfills is another factor that may motivate countries to impose a landfill tax.

In countries that have implemented landfill taxes, less biodegradable waste than the EU average goes to landfill. Finland is an exception; this can be partly explained in that the price difference between incineration and landfill still encourages disposal to landfill over incineration. The Finnish example shows that landfill taxes can be effective only as part of an integrated approach to waste management when economic instruments are used synergistically to promote the desired environmental outcome.

Economics of composting facility

(Source: Sound compost technologies and technical options, The *International Source Book of ESTs for SWM* , UNEP-IETC, http://www.unep.or.jp/ietc/ESTdir/pub/MSW/index.html, June 26,2000)

An accurate estimate of the cost of a composting facility requires detailed knowledge of project specific criteria such as location, site conditions, waste composition, facility size and level of technology. The cost of collecting and composting organic wastes should be evaluated as a component of an integrated system of waste management since increased diversion through composting will result in lower costs for collection and disposal of garbage.

The economic viability of composting depends on three factors; failure of any of the three can cause the system to fail:

- In the absence of a tradition of composting, landfill must be controlled and sufficiently expensive to make the moderate cost of composting (US$20 - 40 per ton) competitive with that of dumping.
- There must be a market or use for the compost at the quality it is produced. This market does not have to produce net income, but it has to be factored into the cost of composting as a positive or negative. The closer the market is, the more likely composting is to be sustainable.
- The waste streams composted have a large effect on compost quality and marketability. Enhancing removal of non-compostables is a necessary step and manual picking and final screening can help.

Germany: Annual report on consumer issues 1997

(Source: http://www.oecd.org//dsti/sti/it/consumer/prod/ger-97.htm, July 21,2000)

Eco-label and energy labeling

Through the energy consumption labeling law (EnVKG) and the ordinance (EnVKV), effective in July 1997, all relevant directives of the European Union on energy conservation in household appliances were transposed into German law (including compulsory labeling of refrigerators and freezers, washing machines, dryers and dishwashers).

It is worth mentioning that, compared with other countries, the proportion of foreign companies being awarded the German "blue angel" eco-label is comparatively large. In 1997, producers from the United States, Korea and the Czech Republic were awarded the eco-label for their products. In many industries the eco-label is becoming an ecological product standard that producers can no longer ignore.

Eco-label of European Union

(Source: http://europa.eu.int/comm/environment/ecolabel/program.htm, July 21, 2000)

EU Eco-label aims to set up a Europe-wide program that represents a much simpler and effective tool for producers and consumers in order to support the concept of the environmental labeling of products. It answers the need to halt the proliferation of national schemes, which are not in line with the creation of a single market in the EU.

The aim is to award a Community eco-label to products with a reduced environmental impact. It is voluntary. Criteria are being worked out to individual product groups. When consumers see products with the eco-label they will know that those products have been carefully assessed and have been found to make less of an environmental impact than competing products.

Ecological criteria for each product are defined on the basis of a "cradle-to-grave"(i.e. life cycle) assessment of the environmental impact of the product group, starting with extraction of the raw materials, and ending with disposal after use. The Commission makes proposals for the definition of product groups and ecological criteria that needs approval from member states.

Eco-label is selective

The label is awarded only to those products with the lowest environmental impact in a product range. Product categories are carefully defined so that all products that have direct "equivalence of use", as seen through the eyes of the consumer, are included in the same product group. For instance, a recent study on possible eco-label criteria for rubbish bags is examining the two main sub-categories: paper and plastic bags. However, the proposed criteria were suspended after the completion of the study.

Eco-label works with a multi-criteria approach

Ecological criteria are not based on a single parameter, but rather rest on a study which analyses the impact of the product on the environment throughout its entire life-cycle.

Packaging waste management (ARA system) in Austria

1. The ARA –SYSTEM (ARA = Altstoff Recycling Austria)

Founded in 1993, the ARA is comprised of privately operating non-profit organizations. They are independent of each other and organize the collection and/or recycling of the collected material abed on efficiency, cost-effectiveness, and ecology. It is devised after the success of German Green Dot system.

For plastic packaging, 3 responsible organizations involved:

- ARA: signs license contracts to all relevant companies, collects license-fees and transfers the money to ARGEV and ÖKK
- ARGEV: organization of collection and sorting of plastic packaging. Execution done by disposal companies that are contract-partners
- OKK: organizing, coordinating and controlling the recovery of all collected plastic packaging materials. Recovery is executed in 7 national and 2 international plants (CH, NL)

2. Requirements set by the AUSTRIAN PACKAGING ORDINANCE (October 1993)

Producers and importers of packaged goods, fillers and packers are RESPONSIBLE for collection and recovery of their packaging. They are allowed to transfer this responsibility to companies that have established an area collecting and recovery-system that has been approved by the Federal Ministry of Environment, Youth and Family Affairs. Reduction of the percentage of landfilled packages:

Targets for 1998: not more than 90000 t
Targets for 2001: not more than 60000 t

3. Fixed percentage of mechanical recycling for several packaging materials

Table 7.1 Target for mechanical recycling

Targeted packaging materials	Percentage related to total amount of transport and sales packaging
Paper, cardboard, corrugated board	90%
Glass	93%
Ceramics	95%
Metals	95%
Plastics	**40%**

Cardboard composite drinks packaging	40%
Other composites	40%

4. Costs and Financing of Plastic Packaging Recovery in Austria

The expenses for collection, recovery and organizational costs are subsequently allocated to the license–tariffs based on expected annually required financial resources. In 1997, the fees for Plastic Packaging was 1485 Million ATS (~ 125 Million $US). Total plastic packaging waste in Austria is 200000 – 220000 tons/year of which 83000 tons of plastic packages were collected in 1997.

Collection and Sorting (ARGEV): 70% of fees
Recovery (ÖKK): 25% of fees
Organization (ARA): 5% of fees

5. License Tariff for large volumes of commercial plastic packages in Austria

Table 7.2 License tariff for shrink-films (in USD)

	1995	1996	1997	1998	1999
ÖKK recovery	-----	0,26	0,26	0,22	0,15
ARGEV collection and sorting	-----	0,37	0,37	0,32	0,27
ARA licensing	-----	0,03	0,03	0,03	0,02
Total	-----	0,66	0,66	0,57	0,44

Table 7.3 License tariff for low volume of plastic packages (> 5 Litres or 1,5 m^2 , in USD)

	1993/94	1995	1996	1997	1998	1999
ÖKK recovery	0,59	0,40	0,36	0,36	0,32	0,24
ARGEV collection & sorting	0,69	1,18	1,21	1,21	1,23	1,22
ARA licensing	0,04	0,07	0,07	0,07	0,07	0,07
Total	1,32	1,65	1,64	1,64	1,62	1,53

Table 7.4 Tariff for large volumes of plastic packages in Austria (> 5 Litres or 1,5 m^2, USD)

	1993/94	1995	1996	1997	1998	1999
ÖKK recovery	0,27	0,40	0,42	0,42	0,36	0,21
ARGEV collection and sorting	0,69	0,61	0,50	0,50	0,50	0,47
ARA licensing	0,03	0,05	0,04	0,04	0,04	0,03
Total	0,99	1,06	0,96	0,96	0,90	0,71

Austria's EPR: ARA system for packaging wastes

(http://www.ara.at/ara_engl/, Dec., 2000)

The Austrian Packaging Ordinance (VerpackVO, Federal Law Gazette No. 645/1992) was put in effect on the basis of the Austrian Waste Management Act. It has been in force since October 1,1993, and requires producers, distributors, and importers that put packaging or packed goods on the Austrian market to take back their packaging free of charge and recycle it. In 1996, the ordinance was revised for the second time (Federal Law Gazette No. 648/1996)and was effective since December 1, 1996.

The Packaging Ordinance applies to companies that put packaging, products that are directly processed into packaging, or packed goods on the Austrian market. By signing a contract (License Agreement) with ARA and licensing (i.e. notification and payment) the packaging they distribute on the Austrian market, companies fulfill their legal obligations for the amount of packaging that has been notified and paid for. More than 12,000 companies – including more than 1,000 partners from neighboring countries – use the services provided by ARA.

Case Study

The energy module and the recycling module of SWM software are provided for examination. The modules are developed based on life cycle inventory. Suppose your organization is involved in the waste management, you are assigned to evaluate the usability and applicability of the modules to your work. Using the knowledge learnt and methodology supplemented in the Reading Materials, please justify your evaluation and conclusion and indicate what should be done to improve the modules in order to be used for the purpose of your organization.

There are several types of organizations from which all the trainees have to choose one:

- A municipality which is considering the introduction of EDPs in order to solve the littering problem caused by plastic food disposables; or
- A company investigating the feasibility of producing EDPs as food disposables and garbage bags for bio-waste, or
- A R&D institution which is developing EDPs used as food disposables and garbage bags.

Energy Module

The module estimates the environmental burdens associated with the production, delivery and use of different forms of energy, which is the major source of a number of pollutants considered. Fuel combustion emissions of CO_2 and CH_4 used in this study were obtained from the Canada (Jaques, 1992). NOx and SO_2 were derived from US (USEPA, 1993). Emission data for production and delivery of fuel and electricity were from UK Dept. of the Environment (Pira Int'l., 1996).

Emission factors for the electricity generation, obtained from various sources above, must reflect the different method and fuels used in power generation. The module gives the users the options of specifying a custom grid or selecting from the predefined grids of different combination of fuels.

Municipal Waste Recovery Facilities (MWRF) Module

This module calculates the environmental burden associated with MWRF activities. These are essentially a function of energy consumption, which is in turn decided by the extent of mechanization. Major energy consuming equipment in MWRF are ferrous magnets, eddy current separators and air classifiers. The user is required to input energy consumption per ton of materials processed at the MWRF and split between energy and natural gas. A default of 100 MJ/ton (Environment Canada, 1996) is also provided.

The quantities of air emission associated with the production and use of energy required by MWRF were calculated using emission factors of the energy module. As to liquid effluent from MWRF, only those associated with the production and use of energy consumed by MWRF are counted. The percentage of MWRF residues needs to be input by users, though default of 5% is provided when data is unavailable.

Plastic Recycling Module

The module estimates the environmental burden associated with the reprocessing of the material from the wastes. It also evaluates the burdens avoided as a result of displacing virgin material (offset burden or displacement credits).

The energy for the production of virgin resin was estimated based on the life cycle inventory conducted by American Plastic Council (APC) and the Environment and the Plastic Industry Council (EPIC). Their database provided estimates for energy and emissions from the production of all major plastics found in municipal solid wastes: PET, HDPE, LDPE, PP, PS and PVC.

The energy required to reprocess the recovered plastic materials is reported to be between 25.4-33.2 GJ/ton for LDPE, 7.6 GJ/ton for HDPE (White et al., 1995). Very limited published data exist on

process emissions from the production of recovered plastics. The emission data of HDPE used in the module.

Material losses during reprocessing have been assumed to be 15% of the material shipped from the MWRF to recycling facility. Based on a MWRF residue of 5%, this means that for every ton of plastic collected at curb, 807.5 kg of recycled plastic can be generated.

Composting Module

It calculates the environmental burdens associated with composting paper, food waste and yard waste. The user is required to input the composition of the waste collected for composting. Facility-specific energy consumption data can be input or the default values for windrew (21 kwh/ton) and in-vessel (30kwh/t) composting can be used.

Emissions of CO_2, particulate matter and VOCs are obtained mainly from UK Environmental Agency (ADAS Environmental, 1998). The average concentrations of selected heavy metals are measured in a variety of studies from both bio-waste, leaf and yard feed stock streams.

As the boundary for waste management processes used in the model ends at the point at which a useful product is recovered, the model does not evaluate the impacts during land application of the compost. The model does not consider the effluent to water from the composting operations. Emissions to water from composting therefore are limited to those associated with energy production.

Source of Information

1. Jaques, 1992. Canada's greenhouse gas emission. Environmental Protection and Conservation, EPS 5/AP/4. Environmental Canada, Ottawa, Ontario.

2. USEPA, 1993. Compilation of air pollutant emission factors, Volume 1: Stationary point and area sources. Draft: Municipal waste landfills.

3. Pira Int'l., 1996. Environmental benefit of offset energy, Prepared for ETSU, UK Dept. of the Environment.

4. Environment Canada, 1996. An assessment of the physical and economic dimensions of SWM in Canada. Perspective of SWM in Canada, Volume 1, prepared by Resource Integration System Ltd.

5. White et al., 1995. Integrated solid waste management: A life cycle inventory. Chapman & Hall.

6. ADAS Environmental, 1998. An assessment of the behavior of organic micro-pollutants I waste composting processes. Draft report, prepared by UK Environmental Agency.

Notes:

The case study can be carried out without reference to the source of information for detailed data. They are meant to provide extra information for interested trainees.

Website Directory

1. http://themes.eea.eu.int/theme.php/issues/waste

A homepage maintained by the European Environment Agency and EIONET partners, it is claimed to be Europe's gateway to environmental information in terms of data, maps, bio-diversity clearinghouse mechanism. Major environmental issues covered include:

Acidification, Air quality, Bio-diversity change, Chemicals, Climate change, Human health, Natural resources, Noise, Ozone depletion, Waste and other issues. Sectors and activities covered are agriculture, energy, fisheries, households, industry, population and economy, tourism and transport. The current page on waste includes 15 reports and 3 links:

1. Waste Annual topic update 1999
2. Waste — Environmental signals 2000 (Chapter 11)
3. Dispersion of hazardous substances -Environment in EU at the turn of the century (Chapter 3.3)
4. Waste generation and management - Environment in EU at the turn of the century (Chapter 3.7)
5. Waste - Europe's Environment: The Second Assessment (Chapter 7)
6. Sludge Treatment and Disposal: Environmental Issues Series No. 7
7. Waste - Europe's Environment: The Dobris Assessment (Chapter 15)
8. Waste Production and Management - Europe's Environment: The Dobris Assessment (Chapter 36)
9. Development and application of waste factors — an overview. Technical report No 37
10. Dangerous substances in waste: Technical report N° 38
11. Hazardous waste generation in selected European countries
12. Baseline projections of selected waste streams
13. Information on waste management practices: Technical Report No 24, Published 1999
14. Report on an overall data model for ETC/Waste: Technical Report No 23 , Published 1999
15. Annual topic update 1998: Topic Report 06/99 European Topic Center on Waste

Links

1. DG-Environment: Waste
2. European Topic Center on Waste
3. Waste Prevention Association

2. http://www.unep.or.jp/ietc/ESTdir/pub/MSW/index.html

The project "International Source Book on Environmentally Sound Technologies (ESTs) for Municipal Solid Waste Management (MSWM)" was initiated in response to the Rio Declaration and to the recommendations of Agenda 21, Chapters 21 and 34, specifically for the purpose of promoting the transfer and application of ESTs for improved management of municipal solid wastes.

This Source Book is directed toward MSWM decision-makers of developing countries and countries in transition, NGOs and community-based organizations involved in waste management. The Book also aims to serve as a general reference guide to researchers, scientists, science and technology institutions and private industries on a global state-of-the-art on ESTs for MSWM.

The list of information sources, containing information on nearly 300 organizations working on municipal solid waste management, is available by using our Searchable Information Directory on ESTs called "maESTro" within this web site. It provides various information such as a contact addresses, organizational profiles, specific fields covered, missions and mandates, and materials and services available from each organization.

3. http://www.agriholland.nl/proterra/about.html

Proterra - International Center for Agro-based Materials

Proterra is an International Center which develops the market for Agro-based Materials such as biodegradable polymers, natural cellulose fibers and composites. Proterra's main goal is to stimulate the application of renewable raw materials in durable goods, packaging and disposables. Proterra is active in market development and market exploitation programs.

- Current Activity: Renewable Materials in sustainable building
- Proterra's Activities in the Future
- Results of the project 'Packaging based on Biopolymers'

This website contains a lot of specific information regarding technical, standards and legal issues related to EDPs, as outlined below:

1. Biopolymers
- 1.1 What makes a polymer a bio-polymer?
- 1.2 Properties of biopolymers
- 1.3 Applications of biopolymers
- 1.4 Different types of biopolymers

2. Composting standards of ISO, ASTM etc.

3. Report on packaging based on biopolymers

4. http://www.oecd.org/

Homepage of Organization for Economic Cooperation and Development, which covers the following areas:

Aging Society; Agriculture, Food and Fisheries; Biotechnology; Competition; Regulatory Reform; Economics; Education and Skills; Electronic Commerce; Emerging and Transition economies; Employment ; Energy ; **Enterprise, Industry and Services ; Environment**; Finance and Investment; Food Safety; Growth; Health; Science and Innovation; Statistics; Sustainable Development ; Taxation Trade; Transport etc.. Among these, Enterprise, Industry and Services; Environment are most relevant.

ENVIRONMENT AT THE OECD

Welcome to the OECD Environment website, updated regularly. You will find information here about the work of the OECD Environment Program 1999-2000, other work on environmental issues undertaken in the OECD, and related news and events. There are links to the 12 main activities of the Environment Program, including economic and environmental policies, globalization, resource efficiency, sustainable consumption, health and safety, waste management, environmental data and information, environmental performance review and environmental indicators.

5.http://europa.eu.int/comm/environment/index_en.htm

The website of European Commission on the environment contains Policy areas, Legislation, Funding opportunities, Publications and Key speeches. A lot of useful information is available under policy areas that are grouped in the following environmental themes:

General policy and overviews, Air, Waste, Water, Urban environment, Nature protection and biodiversity, Industry (Eco-label, Eco-management and audit scheme (EMAS), integrated product policy, pollution etc.), Chemicals and Biotechnology (Dangerous substances, chemicals and genetically modified organisms, accident prevention, dioxin exposure and health, etc.), Environmental law and economics (environment and employment, economics, laws, financing, etc.)

CHAPTER 8

REGULATIONS AND STANDARDS

Objective

- Students will understand the context and major driving forces from regulators for the development of and demand for EDPs in the last decade in major countries and regions.
- Students will learn environmental as well as quality requirements related to EDPs and their variation in different countries and regions.
- Students will study the relevant standards, their structure and key elements and the method of how to obtain relevant regulation and standards to his/her project.
- Students will be able to search and apply relevant regulations and standards.

Summary

In this chapter, regulatory driving forces for EDPs in major countries and regions are discussed. Their implication on regulation, standardization and market are also analyzed.

8.1 Regulations in Major Countries and Regions

Laws and regulations are the major tools in protecting the environment. Under the framework of national policies, Parliament passes laws that govern the nation or community. To put those laws into effect, certain agencies are authorized to create and enforce regulations since laws often do not include all the details. Regulations set specific rules about what is legal and what the penalty will be for the violation.

A policy document should outline the rationale, national goals and key steps to achieve the goals. It should generally contain the followings:

1. Problems and risks of mis-management;
2. Reasons for and goals of management (of wastes , of EDPs etc);
3. Listing approved treatment methods for wastes (plastics);
4. Responsibilities inside and outside the management system;
5. Assessment of the costs of the management;
6. Key steps in achieving the goals with more details in a separate technical guidelines;
7. Documentation and reporting requirements;
8. Training requirements;
9. Rules governing health and safety issues.

A national law should include the followings:

- Clear and proper legal definitions;
- Detailed requirements for and responsibilities of all actors(authority, producers, users, etc)
- A methodology for record keeping and reporting;
- A regulatory system for enforcing the laws;
- The penalties to the offenders and designation of courts where cases can be tried.

Accountability is crucial to adequate SWM systems. Government has the ultimate responsibility for public health and welfare, and this makes governments ultimately accountable for the performance and adequacy of the MSWM system, which enforcement requires appropriate institutional structure and capacity. Though private service is considered more efficient, there is potential loss of control and tendency toward a minimum level of service. As a result, even though governments can choose to transfer operations to the private sector, performance must be inspected and ensured, a challenge for the institutional capacity too.

8.1.1 Relevant Environmental Laws and Regulations

In the past, legal actions supportive to strategy and hierarchy are underdeveloped, than those targeting specific waste problems, and less enforceable due to their general character. However, new efforts are underway, supporting the strategy in more concrete terms. For example, the EU Directive on the Landfilling of Waste set up targets for the reduction of biodegradable municipal wastes going to landfill. Consequently, new initiatives on composting are also being focused on.

In Europe, **European Parliament and Council Directive 94/62/EC of 20 December 1994 on packaging and packaging waste,** the so-called Packaging Directive proved to be the decisive law for EDPs. It strengthened clearly that the prevention of waste should be 'a first priority', while reuse, recycling and other forms of recovering are 'additional fundamental principles' for the 'reduction of the final disposal of such waste'. It covers all packaging in EU market and all packaging waste including plastics, metal, paper, glass and so on. Article 6, *Recovery and recycling*, was proved to be the most influential provision for it set quantitative targets and timetable for member states, exhibited in Table 8.1.

Table 8.1 Key requirements set by the EU Packaging Directive

Timetable	Recovery and recycling targets (by weight) for packaging wastes
By 2001	Recovery rate: 50%-65% should be recovered Recycling rate: 25-45%; Min. 15% for each material.
By 2006	Recovery and recycling rate: determined by the Council

The Directive specifies the requirement on *marking and identification* of packaging. This is particularly important for plastics since the identification of different plastic material is even difficult for a professional, which is proved to be a big obstacle for an economically viable sorting and thus recycling. It highlights the key role played by public participation for the success of (packaging) waste management. Therefore, it is important to let the public know what they are expected to do, why and how to do.

The Directive recognized that standardization is essential for the implementation of this Directive among member states. It encouraged the development of national standards in accordance with EU standards, i.e. CEN standards, under the framework of the Directive. As a comprehensive regulation including most aspect regarding packaging, it also promoted the use of *economic instruments*. This opened the way for economic and market based approaches which are discussed in Chapter 7.

European Parliament and Council Directive 1999/31/EC of 26 April 1999 on the landfill of waste, proved to be another regulatory driving force for EDPs in Europe. The core of this so called 'Landfill Directive' lies in the Article 5, *Waste and treatment not acceptable in landfills,* in which member states are required to take measures to reduce biodegradable wastes going to landfills. It too set up quantitative targets and timetable, see Table 8.2.

Table 8.2 Key requirements set by the EU Landfill Directive

Timetable	Reduction of bio-degradables in landfill (of total weight of biodegradable municipal wastes produced in 1995)
By 2004	Reduced to 75%
By 2007	Reduced to 50%
By 2014	Reduced to 35%

It is clear that the current recovery and recycling practice in Europe are not able to reach the targets set by Packaging Directives unless composting as a way of organic recycling would be introduce at a commercial scale. While the Landfill Directive imposes direct pressure on diverting organic wastes from landfill by ' organic recycling' approaches, both aerobic (composting) and anaerobic (biogas

production) methods as defined by the Directive. Both directives require, and generate the demand for, the development and large scale application of degradable materials of which EDPs can find its niche. A new EU legislation that can have an influence on EDPs is composting directive. As a step further from Packaging and Landfill Directives, it encourages source separation of wastes. The first draft was finished in Nov. 2000 and the directive is expected to be in effective in one to two years.

USA is among the earliest countries that ban or restrict the use of certain plastic products for environmental reasons (see Reading Materials) at state level, on the basis of Resource Conservation and Recovery Act (RCRA) issued in 1976. RCRA gave US EPA the authority to control hazardous waste from the generation, transportation, treatment, storage, to disposal of hazardous waste. RCRA also set forth a framework for the management of non-hazardous wastes. The Pollution Prevention Act issued in 1990 focused industry, government, and public attention on reducing the amount of pollution through cost-effective changes in production, operation, and raw materials use. The law recognized that source reduction is fundamentally different and more desirable than waste management or pollution control. In its definition for pollution prevention, it also includes practices in recycling, source reduction, and sustainable agriculture. Instead of nationwide regulations, each state developed their own regulations to enforce federal laws and acts.

In Asia, notably East Asia, different countries and regions took similar acts in the last decade dealing with waste and plastic waste management. In Japan, the law on plastic recycling was issued in 1991. Together with earlier *Law of cleaning and treatment of wastes*, it provides legal framework for the management of plastic and plastic wastes. In China, the *Law of Solid Waste Pollution Prevention and Control* came into force from April 1st of 1996. It defines the responsibility of producers, sellers and users in recovery and recycling of the recyclable packaging in a fashion conformed to relevant regulations. Producers and users should choose easily recyclable, disposable and environmentally assimilatable materials as packaging, or as product in the case of agricultural milch film. Similar law on wastes came into effect in 1991 in South Korea. It specified the adoption of economic instrument, such as deposit, for waste management. Further in 1993 came the regulation on packaging method and criteria for packaging material, aiming at reducing packaging waste. It required producers to reduce the plastic packaging for electronic appliance by 30% by weight of the amount used in 1992. Taiwan also set up quantitative objectives for waste management. For example, in its regulation on PET bottle effective from 1989, a recovery rate of 50% was prescribed and it was raised to 60% in 1992. The *General Methods for recycling and Cleaning of Containers* was issued in 1994 which specified the recycling targets for various packaging materials. For instance, the target for expanded polystyrene (EPS) was set as 50%. While the real recycling rate achieved in 1995 was 56.1%, indicating an effective implementation. This led to the cancel of ban on EPS as disposable food container.

8.1.2 EDPs Related Health and Hygiene Regulations

In many countries, regulations specify that packaging should fulfill quality requirements, regulations and standards on health, safety and hygiene in the meantime of meeting environmental requirement. Health and safety regulation related to the possible application of EDPs falls in several categories, namely, food contact, medical devices and synthetic implants.

Food Contact

In Europe, EU Directive 90/128/EEC of 1990 relates to plastic materials and articles intended for direct contact with food staffs. It was amended by Directive 92/39/EEC issued in 1992, 93/9EEC, 95/3/EC and 96/11/EC. The Directive specifies the migration limits for constituents of plastics to foodstuff since it can endanger human health when in increased concentration. Directive 82/711/EEC, 85/572/EEC and provisions in the annexes to 90/128/EEC are for verification of compliance. It requires that, as early as in marketing stage, a written declaration for compliance with 89/109/EEC must accompany the plastic materials and articles intended for contact with foodstuffs.

In US, products with food contact and all medical devices are subject to the regulations of Food and Drug Administration (FDA). Regulations for food contact are derived mainly from federal Food,Drug and Cosmetic Act, issued in 1938.

Medical Devices and Synthetic Implants

All medical devices are subject to FDA regulations and standards in US, including R&D and testing, manufacture, product effectiveness and safety, labeling, record keeping, approval, storage, advertising and promotion of products. Other relevant laws include Federal Food, Drug and Cosmetic Act, the Public Health Service Act, and the Controlled Substances Act, to name a few. Proof of safety and efficacy for medical applications must be submitted for FDA approval or clearance. However, those made from homo-polymers or copolymers of glycolide, lactide,caprolactone,p-dioxanone and trimethylene carbonate have been cleared for marketing by FDA. Other polymers are under investigation for use as materials in biodegradable medical applications.

Requirement for marketing of medical devices in EU is regulated by Directive 93/42/EEC of 14 June 1993 concerning medical devices and transplants of man-made origin. It requires the transition to national law of EU member states where all medical devices must have a Certification Equivalent (CE Mark) prior to marketing in the Community. Manufacturing and quality assurance documentation and inspection mandated by ISO 9000 standards are required to obtain the CE Mark. Products in compliance with all provisions of applicable directives must bear this mark. Medical devices directives (93/42/EEC, 93/68/EEC and 98/79EC) fall into this category, while Packaging Directive doesn't provide for the CE marking.

Self-check Questions

1. What is the major regulatory driving forces for EDPs in Europe, USA and Asia?
2. In your opinion, what are the major differences between regulation in Europe and Asia and what implication this difference may have on waste management as well as EDPs?
3. What laws and regulations apply to wastes management and EDPs in your country? List and briefly describe their contents.

Hints for Answers

1. See section 8.1.1.
2. Reference information provided in section 8.1.1 and Reading Materials.
3. The legal outline that provided in first part of Section 8.1 would help to organize your description.

Exercise

Based on the meditation on the questions above and with the help of lecturing and exercise of Chapter 7, each trainee prepares a short paper to identify the major problems and obstacles in the legal system in your country that affect sound management of wastes, for example, what is missing or unsufficient. Then, suggest on how to improve the effectiveness and enforceability of legislation regarding waste management and EDPs in your country. In the group discussion that follows, trainees will present their result and receive comments from their group members and the trainers.

Reading Materials

European Parliament and Council Directive 94/62/EC of 20 December 1994 on packaging and packaging waste

(http://europa.eu.int/eurlex/en/lif/dat/1994/en_394L0062.html, Oct., 2000)

There are 25 articles altogether. Article 6, is about *recovery and recycling*, and proved to be the most influential provision because it set quantitative targets and timetable for member states. It is necessary to copy it below in whole:

1. In order to comply with the objectives of this Directive, Member States shall take the necessary measures to attain the following targets covering the whole of their territory;

(a) no later than five years from the date by which this Directive must be implemented in national law, between 50 % as a minimum and 65 % as a maximum by weight of the packaging waste will be recovered;

(b) within this general target, and with the same time limit, between 25 % as a minimum and 45 % as a maximum by weight of the totality of packaging materials contained in packaging waste will be recycled with a minimum of 15 % by weight for each packaging material;

(c) no later than 10 years from the date by which this Directive must be implemented in national law, a percentage of packaging waste will be recovered and recycled, which will have to be determined by the Council in accordance with paragraph 3 (b) with a view to substantially increasing the targets mentioned in paragraphs (a) and (b).

2. Member States shall, where appropriate, encourage the use of materials obtained from recycled packaging waste for the manufacturing of packaging and other products.

3.
(a) The European Parliament and the Council shall, on the basis of an interim report by the Commission, and four years from the date referred to in paragraph 1 (a) on the basis of a final report, examine the practical experience gained in the Member States in the pursuance of the targets and objective laid down in paragraphs 1 (a) and (b) and 2 and the findings of scientific research and evaluation techniques such as eco-balances.
(b) No later than six months before the end of the first five-year phase referred to in paragraph 1 (a) the Council shall, acting by qualified majority and on a proposal from the Commission, fix targets for the second five-year phase referred to in paragraph 1 (c). This process shall be repeated every five years thereafter.

4. The measures and targets referred to in paragraph 1 (a) and (b) shall be published by the Member States and shall be the subject of an information campaign for the general public and economic operators.

5. Greece, Ireland and Portugal may, because of their specific situation, i. e. respectively the large number of small islands, the presence of rural and mountain areas and the current low level of packaging consumption, decide to:
(a) attain, no later than five years from the date of implementation of this Directive, lower targets than those fixed in paragraph 1 (a) and (b), but shall at least attain 25 % for recovery;
(b) postpone at the same time the attainment of the targets in paragraph 1 (a) and (b) to a later deadline which, however, shall not exceed 31 December 2005.

6. Member States which have, or will, set programs going beyond the targets of paragraph 1 (a) and (b) and which provide to this effect appropriate capacities for recycling and recovery, are permitted to pursue those targets in the interest of a high level of environmental protection, on condition that these measures avoid distortions of the internal market and do not hinder compliance by other Member States with the Directive. Member States shall inform the Commission thereof. The Commission shall confirm these measures, after having verified, in cooperation with the Member States, that they are consistent with the considerations above and do not constitute an arbitrary means of discrimination or a disguised restriction on trade between Member States.

Council Directive 1999/31/EC of 26 April 1999 on the landfill of waste

(http://europa.eu.int/eur-lex/en/lif/dat/1999/en_399L0031.html, Oct. 28, 2000)

There are 20 articles in this Directive, only the most important and relevant ones are quoted below:

Article 5. Waste and treatment not acceptable in landfills

1. Member States shall set up a national strategy for the implementation of the reduction of biodegradable waste going to landfills, not later than two years after the date laid down in Article 18(1) and notify the Commission of this strategy. This strategy should include measures to achieve the targets set out in paragraph 2 by means of in particular, recycling, composting, biogas production or materials/energy recovery. Within 30 months of the date laid down in Article 18(1) the Commission shall provide the European Parliament and the Council with a report drawing together the national strategies.

2. This strategy shall ensure that:
(a) not later than five years after the date laid down in Article 18(1), biodegradable municipal waste going to landfills must be reduced to 75 % of the total amount (by weight) of biodegradable

municipal waste produced in 1995 or the latest year before 1995 for which standardised Eurostat data is available

(b) not later than eight years afte the date laid down in Article 18(1), biodegradable municipal waste going to landfills must be reduced to 50 % of the total amount (by weight) of biodegradable municipal waste produced in 1995 or the latest year before 1995 for which stadardised Eurostat data is available;

(c) not later than 15 years after the date laid down in Article 18(1), biodegradable municipal waste going to landfills must be reduced to 35 % of the total amount (by weight) of biodegradable municipal waste produced in 1995 or the lates year before 1995 for which standardised Eurostat data is available.

Two years before the date referred to in paragraph (c) the Council shall reexamine the above target, on the basis of a report from the Commission on the practical experience gained by Member States in the pursuance of the targets laid down in paragraphs (a) and (b) accompanied, if appropriate, by a proposal with a view to confirming or amending this target in order to ensure a high level of environmental protection. Member States which in 1995 or the latest year before 1995 for which standardised EUROSTAT data is available put more than 80 % of their collected municipal waste to landfill may postpone the attainment of the targets set out in paragraphs (a), (b), or (c) by a period not exceeding four years. Member States intending to make use of this provision shall inform in advance the Commission of their decision. The Commission shall inform other Member States and the European Parliament of these decisions. The implementation of the provisions set out in the preceding subparagraph may in no circumstances lead to the attainment of the target set out in paragraph (c) at a date later than four years after the date set out in paragraph(c).

3. Member States shall take measures in order that the following wastes are not accepted in a landfill:

(a) liquid waste;

(b) waste which, in the conditions of landfill, is explosive, corrosive, oxidising, highly flammable or flammable, as defined in Annex III to Directive 91/689/EEC;

(c) hospital and other clinical wastes arising from medical or veterinary establishments, which are infectious as defined (property H9 in Annex III) by Directive 91/689/EEC and waste falling within category 14(Annex I.A) of that Directive.

(d) whole used tyres from two years from the date laid down in Article 18(1), excluding tyres used as engineering material, and shredded used tyres five years from the date laid down in Article 18(1) (excluding in both instances bicylce tyres and tyres with an outside diameter above 1 400 mm);

(e) any other type of waste which does not fulfil the acceptance criteria determined in accordance with Annex II.

4. The dilution of mixture of waste solely in order to meet the waste acceptance criteria is prohibited.

Article 6. Waste to be accepted in the different classes of landfill

Member States shall take measures in order that:

(a) only waste that has been subject to treatment is landfilled. This provision may not apply to inert waste for which treatment is not technically feasible, nor to any other waste for which such treatment does not contribute to the objectives of this Directive, as set out in Article 1, by reducing the quantity of the waste or the hazards to human health or the environment;

(b) only hazardous waste that fulfils the criteria set out in accordance with Annex II is assigned to a hazardous landfill;

(c) landfill for non-hazardous waste may be used for:

- (i) municipal waste;
- (ii) non-hazardous waste of any other origin, which fulfil the criteria for the acceptance of waste at landfill for non-hazardous waste set out in accordance with Annex II;
- (iii) stable, non-reactive hazardous wastes (e.g. solidified, vitrified), with leaching behavior equivalent to those of the non-hazardous wastes referred to in point (ii), which fulfil the relevant acceptance criteria set out in accordance with Annex II. These hazards wastes shall not be deposited in cells destined for biodegradable non-hazardous waste,

(d) inert waste landfill sites shall be used only for inert waste.

Article 7. Application for a permit

Member States shall take measures in order that the application for a landfill permit must contain at least particulars of the following:

(a) the identity of the applicant and of the operator when they are different entities;
(b) the description of the types and total quantity of waste to be deposited;
(c) the proposed capacity of the disposal site;
(d) the description of the site, including its hydrogeological and geological characteristics;
(e) the proposed methods for pollution prevention and abatement;
(f) the proposed operation, monitoring and control plan;
(g) the proposed plan for the closure and after-care procedures;
(h) where an impact assessment is required under Council Directive 85/337/EEC of 27 June 1985 on the assessment of the effects of certain public and private projects on the environment (8), the information provided by the developer in accordance with Article 5 of that Directive;
(i) the financial security by the applicant, or any other equivalent provision, as required under Article 8(a)(iv) of this Directive. Following a successful application for a permit, this information shall be made available to the competent national and Community statistical authorities when requested for statistical purposes.

Regulations in North America

In US, state governments promulgated their own regulation on plastic waste management under the auspices of federal law, presented in Table 8-3.

Table 8.3 Some states' regulation on plastics, USA

States	*Items regulated*	*Requirements*	*Implementation*	*Date*
California	Trash bag	By 1993: contain 10% (by weight) recyclable in material; by 1995: 30%.	Not available	1993
Wisconsin	All hard containers	Material should contain 10% recyclable by weight	Not available	1995
Florida	Bottles and cans with volume up to 1 gallon	0.01 USD/unit was charged as waste management fee in sale unless 25% recycling be achieved or recyclable in product material reach 25% by weight.	Material recovery reached 50% in Florida	1994

8.2 Relevant Standards

Guidelines and standards dealing with waste management facilities, such as incineration and composting, as well as the associated requirements on source separation lag far behind in many countries, hindering the proper operation of the facilities and the economic viability as well. Take the example of composting, Europe and North America are the only areas of the world with clear compost quality standards. Though a few other countries have developed standards and criteria for EDPs, lack of regulations and standards for composting and compost obstacle the large-scale application of EDPs. The European emphasis on compost marketability has led its development from mixed waste composting to composting of source-separated bio-waste, which greatly improved the compost quality.

8.2.1 Principles and Methods of Standardization

Enforcement of law and regulation can not be possible without standards. Only against the precise provisions of standards will it be possible to check the behavior of the regulated, and thus the compliance of the law. Standards also provide a consistent and reliable signal for consumer about the certain facet of the products, for instance eco-profile in the case of EDPs, which has a significant influence on its market demand. Consensus has been reached that the standards for definitions, tests and acceptance criteria are necessary for the success of EDPs.

Once public authority agreed on a mandate, in principle it is the interested parties who search for technical solutions. However, in the areas of environment and health and safety, participation of public authority on a technical level is important in the standardization process. The new procedure in standardization followed by EU is exhibited below in Fig 8.1.

1. A mandate is drawn up, following consultation with member states.
2.The mandate is transmitted to European standards organizations (e.g. CEN).
3. European standards organizations accept and elaborate a joint program.
4. Technical committee elaborates a draft standard.
5. European standards organizations and national standards bodies organize a public enquiry.
6. The technical committee considers comments.
7. National standards bodies vote/ European standards organizations ratify.
8. European standards organizations transmit references to the Commission.
9. The Commission publishes the references.
10. National standards bodies transpose the European standards.
11. National authorities publish references of national standards.

Fig. 8.1 New standardization procedure followed by EU

Standards should have a sound scientific basis and be practically feasible. Clear definition is essential for any standards. Test procedure should be written and followed as a general procedure, which provides basic and minimum requirements. The next level would be acceptance criteria and certification, to which the quality control and quality assurance is crucial. This is ensured by the impartiality and independence of testing and certification institutions from industry.

8.2.2 Compostability and Biodegradability Standards

Up to date, the majority of the relevant standards address the composting disposal environment, given the importance of composting as an ecologically sound disposal method that generates useful soil amendment product. All available compostability standards are constructed around 4 basic characteristics: (1) material characteristics, (2) biodegradation, (3) disintegration and (4) compost quality.

- Concerning *material characteristics* the general requirements are clear and agreed upon. Some certain vagueness exists regarding the absence of other noxious components but this can probably be clarified further in future updates of the standard.
- Regarding *biodegradability*, more agreement than difference is reaching with the time passing by among major standardization organizations, from ISO, CEN/DIN to ASTM. Some main difference however can be identified as followings:
  - Regarding the *pilot-scale composting test* the same standard has been proposed by CEN and ISO but although the proposal at ISO is introduced by DIN, the procedure described in the DIN V 54900 is for some aspects significantly different (e.g. controlled temperature).
  - For the *full-scale composting test* a different approach can be noticed between ASTM and DIN, respectively with an 'active processing' and a 'passive embedding'.
- *Disintegration* or physical and visual disappearance of a specific form of packaging can be evaluated in a pilot-scale or a full scale composting test. Disintegration must be >90% over a sieve of 2 mm.
- The *quality of compost* may not be negatively influenced by the addition of compostable products to the compost feedstock. Yet, more R&D are still needed in order to establish precise testing procedures and criteria of (mainly animal) eco-toxicity tests.

Challenges for Future

The compatibility with *anaerobic bio-gasification* plants so far has been more or less neglected in compostability standards. If compostable products are envisaged to be applied on a large scale, they will need to be treatable in bio-gasification systems also since the total capacity of this type of plants has increased dramatically. It has been demonstrated that certain polymers (e.g. lignin) have a completely different biodegradation pattern under anaerobic conditions than under aerobic conditions. On the other hand, all industrial bio-gasification processes contain an aerobic stabilization phase as an integral part and the anaerobic compost ultimately produced will be used for the same purposes (agriculture, horticulture, etc.) as aerobic compost. The standards will need to specify precisely the particularities needed for treatability of biopolymers in anaerobic systems.

The limitations of compostability must be understood. A positive evaluation of compostability of a given material may not be extrapolated automatically to claims on *biodegradability in specific environments* such as soil or marine conditions. In these environments lower temperatures and eventually less aggressive microbial life prevail which can reduce degradation percentages significantly. On the other hand disintegration within a short time frame is probably less important. For these reasons it is necessary to develop specific standards and acceptance criteria for environments other than compost where biopolymers are used and are claiming biodegradability.

So far standards are developed for industrial composting. Home composting is regarded as waste reduction at source therefore should be encouraged. However, its small-scale, variation and complexity in comparison with industrial composting make standardization effort difficult.

As principle, EDPs standards should cover:
- The exposed environment (simulating the real disposal system or environment)
- The test method to measure degradability (mechanical and chemical property loss) and biodegradability (microbial assimilation/degradation)
- The fate and effects of the degraded products
- Classification based on intended application

8.2.3 Relevant Standards on Health and Safety

The end use of biodegradable materials for medical use comprises the direct interaction of these materials and their degradation products with patients. The safety of the patient is of course of great

importance. In recent years the safety of medical devices are in Europe guided by the International Organization for Standardization (ISO).

ISO provides a series of standards on the "biological evaluation of medical devices". The first edition of 1999 gives the following parts:

1. Evaluation and testing
2. Animal welfare requirements
3. Tests for genotoxicity, carcinogenicity, and reproductive toxicity.
4. Selection of tests for interactions with blood
5. Tests for in vitro cytotoxicity
6. Tests for local effects after implantation
7. Ethylene oxide sterilization residuals
8. Framework for the identification and quantification of potential degradation products
9. Tests for irritation and sensitization
10. Tests for systemic toxicity
11. Sample preparation and reference materials
12. Identification and quantification of degradation products from polymers
13. Identification and quantification of degradation products from ceramics
14. Identification and quantification of degradation products from metals and alloys
15. Toxicokinetic study for degradation products and leachables
16. Chemical characterization

With regard to biodegradable materials the ISO 10993-9 (Biological evaluation of medical devices – part 9: Framework for identification and quantification of potential degradation products) has been filed in 1999. This ISO standard does not give a detailed description of experiments for testing biodegradation, but offers a guideline for evaluation.

Three American Society for Testing and Materials (ASTM) standards are nowadays available for Biomaterials testing of (specific) materials:

- F1635-95 Standard test method for in-vitro degradation testing of poly(L-lactic acid) resin and fabricated form for surgical implants
- F1925-99 Standard specification for virgin poly(L-lactic acid) resin for surgical implants.
- F1983-99 Standard practice for assessment of compatibility of absorbable/resorbable biomaterials for implant applications

Self-check Questions

1. What are the basic elements for standardization and development of standards?
2. What are the main contents of currently developed standards regarding compostability and biodegradability in your country, if any, in comparison with international standards?
3. List all standards that you think may apply to EDPs in your country, including those concerning health, hygiene and safety.

Hints for Answers

1. See section 8.2.1and Reading Materials.
2. See section 8.2.2 and Reading Materials.
3. Inspired by text and Reading Materials, may need to search information about your country.

Exercise

1. You have made a polymeric device composed of a copolymer of lactic acid and glycolic acid. In what way and by what methods would you evaluate the biocompatibility of this material?

2. "An important issue in standards development is balancing shelf life with rapid degradability. A product must be able to remain intact in inventory until purchased and on a shelf until used. After use, it must degrade quickly." (Taken from a consultancy's report on EDPs). Please comment on the statement, from both theoretical and practical or enforcement point of view, then suggest how to deal with similar problems. Put your results in a paper of 1-2 pages.

Reading Materials

How to search regulations, standards and relevant documents?

The European Union website (http://europa.eu.int/eur-lex/en/) includes Official Journal, treaties, community legislation in force and case laws. Clicking through Legislation in force, two searching channels are provided, by alphabet or by category. The environmental and health legislation can be found under the 15th category titled *Environment, consumers and health protection* which is divided further into environment, consumer, health protection and animal health.

The search functions provided enable to search certain legislation by keyword or by document number. Take the Directive on food contact plastic as an example. Chose *Plain Search,* select *Legislation in force* and type "90/128/EEC" in, nine documents including the Directive and others amended to it will come up. Alternatively, you can search by document number and publishing year combined. In the case of the above Directive, it is 1990 in year, then it prompts document number as 390L. After 390L you should add 0128 which is the last digits of the Directive number.

The European Committee for Standardization (CEN) has website (http://www.cenorm.be/) containing links to all national standardization bodies in member states. Abstract of national standards transformed from EU standards can be viewed, while the full text can be purchased on-line. CEN has initiated environmental helpdesk to guide standard developers to take into account the environmental aspect when formulating standards. An environmental guideline and a checklist for possible environmental aspects for developers of standards were provided.

You can purchase the texts of European Standards, transposed as national standards, from CEN national members and affiliates listed below: (The website provides links to all the following organizations. http://www.cenorm.be/aboutcen/products/standards.htm)

National Members and the representative expertise they assemble from each country

Austria (ON), Belgium (IBN/BIN), Czech Republic (CSNI), Denmark (DS), Finland (SFS), France (AFNOR), Germany (DIN), Greece (ELOT), Iceland (STRÍ), Ireland (NSAI), Italy (UNI), Luxembourg (SEE), Netherlands (NEN), Norway (NSF), Portugal (IPQ), Spain (AENOR), Sweden (SIS), Switzerland (SNV), United Kingdom (BSI).

Associates

ANEC,	European Association for the co-operation of consumer representation in standardization;
CEFIC,	European Chemical Industry Council;
EUCOMED,	European Confederation of Medical Devices Associations;
FIEC,	European Construction Industry Federation;
NORMAPME,	European Office of Crafts, Trades and Small and Medium-sized Enterprises for standardization;
TUTB,	European Trade Union Technical Bureau for Health and Safety

Counsellors (European Institutions)

EC,	the European Commission
EFTA	European Free Trade Association

Affiliates

Albania (DPS); Bulgaria (SASM); Croatia (DZNM); Cyprus (CYS); Estonia (ESK); Hungary (MSZT); Latvia (LVS); Lithuania (LST); Malta (MSA); Poland (PKN); Romania (ASRO); Slovakia (SUTN); Slovenia (SMIS); Turkey (TSE)

ASTM --standard organisation in US

The equivalent of CEN in US is American Society for Testing and Materials (ASTM), one of the largest voluntary standards development organizations in the world. ASTM is a not-for-profit organization that provides a forum for the development and publication of voluntary consensus standards for materials, products, systems and services. The ASTM standards become legally binding only when agovernment body makes them so, or when they are cited in a contract.

ASTM standards are written by ASTM's 32,000 volunteer members, from more than 100 countries around the world, who are producers, users, ultimate consumers, and general interest parties, such as academia and government representatives. These members serve on ASTM's 129 technical committees that are devoted to specific areas of interest. .ASTM Standards can be purchased in the Store area of the ASTM web site (http://www.astm.org/). Using a credit card, you can download standards, receive standards by fax, or by mail. Standards vary in cost, based on their length. Average cost for an ASTM standard is about $25.

ISO Standards on medical applications

ISO 10993-9 Biological evaluation of medical devices – part 9: Framework for identification and quatification of potential degradation products.

The latest European Standards available from the National Members of CEN from October 2000 (http://www.cenorm.be/news/press_notices/waste.htm, Oct., 2000)

EN 13427	Requirements for the use of European Standards in the field of packaging and packaging waste (the 'umbrella' or guidance document)
EN 13428	Requirements specific to manufacturing and composition - Prevention by source reduction
EN 13429	Packaging - Re-use
EN 13430	Requirements for packaging recoverable by material recycling
EN 13431	Requirements for packaging recoverable in the form of energy recovery, including specification of minimum interior calorific value
EN 13432	Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging

These standards are the first in the field of the environment to follow the principles of the 'new approach', by which legislation is limited to 'essential requirements' and the detailed technical specifications are drafted by competent standardization bodies. In brief, the manufacturer has the choice of not using these harmonized standards but in that event has an obligation to prove by other methods that he meets the legal requirements.

The directive itself (94/62/EC) aims at a trade-off between increased recycling and guaranteed free movement of goods. CEN's standards give practical advice to manufacturers to sustain policies of continuous improvement to minimize the quantity of packaging used, bearing in mind that food packaging, for example, has to fulfil criteria for strength and insulation to prevent contamination or loss. Furthermore, the packaged product must remain acceptable to the consumer and this fact is recognized in the statement of the essential requirements. Given the difficult balance to maintain and owing to the complexity of the production, packaging and transport train CEN decided on a management systems approach rather than writing quasi-legislation - which is not within its power - that would resul in 'pass/fail' criteria and so ban certain types of packaging.

Prevention by source reduction will make the packer/filler primarily responsible and solely responsible when re-use is claimed; for organic recovery the convertors will have to verify the calorific value from incineration or compostibility. To claim re-use the packer/filler, in addition, must intend the package for re-use. It must be possible to clean, wash or repair the packaging before refilling, and a logistic system that supports re-use must demonstrably be in place.

CEN's environmental checklist when developing standards

(http://www.cenorm.be/sectors/ehd.htm , Nov., 2000)

CEN recognizes that every product has some impact on the environment during its life cycle. Provisions of standards may have a significant influence on the extent of these impacts. Therefore, the Environmental Helpdesk of CEN provided a Guideline, with basically four steps and a checklist summarized below, for standard developers to consider the environmental aspects. The matrix provided in this checklist suits particularly product standards. For standards other than product standards, it is recommended to use it as much as possible.

Matrix—Environmental checklist

Environmental aspects		Product life-cycle			
		Production and Pre-production	Distribution(includ. packaging)	Use	End of life
		A	B	C	D
1	Resource use				
2	Energy consumption				
3	Emission to air				
4	Emission to water				
5	Waste				
6	Noise				
7	Migration of hazardous substances				
8	Impacts on soil				
9	Risks to the environment from accidents or misuse				

Comments

With the help of the matrix, the process can be completed in the following 4 steps:

1. Identify each environmental aspect relevant to the product without assessing its relationship to the draft standard. Fill each box with "yes" (if there is an environmental aspect) or "no" (if there is no significant environmental aspect or if the box is not relevant).
2. Indicate whether this environmental aspect can be realistically influenced and addressed by the provisions in the standard. Mark these boxes with three asterisks (***). Write the number of the standard clauses where the environmental aspects are addressed, in the appropriate boxes.
3. Make proposals on how each aspect can be addressed in the draft standard: Use the box "Comments" for providing any additional information. A short description of each environmental aspect (boxes filled with "yes") and how they are addressed (or why they are not) can be given here.
4. Document the results of the assessment by using the checklist (matrix). Please note that when assessing various environmental aspects during the life cycle of a product, it is essential to avoid shifting of environmental burden from one life cycle phase to another or from one medium to another.

5. Application of life cycle assessment in developing standards for eco-label

(Source: http://europa.eu.int/comm/environment/ecolabel/guidel.htm, Feb., 2001)

A guideline for application of life cycle assessment, it is recommended by EU Eco-label award Scheme. Policy-makers, competent bodies and practitioners must remain aware of the current capabilities and limitations of life cycle assessment and should support its continuous development. It should be clear, however, that life cycle assessment is only a decision-support tool; it cannot replace decision-making.

Life cycle assessment and other environmental decision-support systems

Two major approaches are available for environmental analysis: environmental impact assessment (EIA) and life cycle assessment (LCA). The EIA approach concentrates on localized activities. Generally, the environmental impacts are studied as site-specific effects. This precludes much attention to life cycle aspects of the activity in question. The LCA approach is a cradle-to-grave analysis of products and services and, possibly, of policies and strategies. However, products are currently the

main area of application. Generally, environmental impacts are studied as non-site specific effects. This is due to the fact that many processes cannot be bound to specific locations and that the inclusion of too much local detail would render the analysis impracticable.

The Eco-label and related labels on environment, health and safety

In the fields of environment, health and safety, single-issue mandatory labels, single-issue voluntary labels and multi-issue voluntary labels can be distinguished. This distinction is in line with the research being carried out within the OECD and ISO. Multi-issue mandatory labels do not exist. At present eco-labeling is the only environmental multi-issue approach.

Data quality

Problems regarding the availability and quality of data are at the core of all LCA studies. The more sophisticated the study, the greater the data problem. Identification of key issues should direct the data gathering process. An important issue is how to deal with the conflicting interests of credibility and confidentiality. Here, the distinction between background and foreground data is relevant. Background data should always be public. To maintain credibility, foreground data used for criteria setting should also be public, if necessary, in an anonymous form.

Case Study

Two groups are established for this case study. One is comprised mainly of policy makers and regulators, the other R&D people and technical staff. Mixture to some extent is however accepted as it may produce inspiring and enlightening results. The tasks are designed for both groups.

Task A

Draft an outline of a regulatory package for national legislation in your country on dissemination of EDPs and integrated waste management with EDPs as one element.

- Outline the structure of the package (what policies, laws, guidelines etc. are needed.)
- Draft the main elements to be included in each regulatory document. For example, the key contents of the national law governing municipal wastes should be listed.

Task B

- Outline a package of standards required to fulfill the goals and requirements set by the legal package drafted in Task A.
- Make suggestions on what should be done in order to make the regulatory system including standards work more effectively toward environmentally sound waste management with EDPs as an element.

Your answers to the questions and exercises of this chapter and part of Chapter 7 are already partly solving the two tasks. Final result of the group should be written in a paper and presented to the whole class. A discussion in plenary will be carried out on whether the two results are compatible and what can be learned and supplemented from each other.

CHAPTER 9

KINETIC MODELLING OF POLYPROPYLENE OXIDATION

Objectives

- Students will learn basic concepts of free radical degradation mechanisms of the polyolefins via thermal or photo-oxidation.
- Students will learn and understand the principles of homogeneous oxidation of polypropylene.
- Students will learn the basic principles and methods of kinetic modeling of heterogeneous polypropylene oxidation.

Summary

Polypropylene is today one of the most widely used of all commodity polymers. This success occurred in spite of the polymer being one of most oxidatively unstable and susceptible to environmental degradation. Understanding of the mechanism and kinetics of oxidative degradation may lead to the design of new strategies for stabilization or enhanced environmental degradability, depending on the polymer exploitation. This chapter highlights key features of the recent paper of G. M. George and M. Celina, who analyzed the kinetic modeling of homogeneous and heterogeneous oxidation of polypropylene.

9.1 Background

For polyolefins the chemical changes to the polymer due to environmental degradation processes are the result of a free radical oxidative chain reaction [1]. Evidence for this process has been gained from measurements such as oxygen consumption by the heated polymer and accumulation of hydro-peroxide and carbonyl species as measured by infrared spectrophotometry [2,3]. These products are a consequence of the oxidative scission reactions that lower the polymer molecular weight and produce density changes and shrinkage owing to the changes in the intermolecular forces.

The earliest work related to the free radical oxidation model was that by Backström on the radical chain theory of auto-oxidation [4]. This was followed by the detailed studies of Bolland and Gee [5], which resulted in the current model of radical initiation, propagation and termination being applied to polymer oxidation [6,7]. The radical processes during the thermal or photo-oxidation of polyolefins are, in principle, identical, with only minor differences owing to variations in initiation or secondary photo-chemistry [6-8]. Evidence for the reaction scheme and products that may be formed has been obtained from analysis of polyolefins at high extent of oxidation. As the sensitivity of analytical techniques is improved, possible reaction steps may increase in both number and complexity. In spite of this, the free radical oxidation mechanism has generally been believed to consist of the following steps [6-9]:

Initiation:	Polymer PP	$\longrightarrow$	$P^{\bullet} + P^{\bullet}$	(1)
	2 POOH	$\longrightarrow$	$POO^{\bullet} + P^{\bullet} + H_2O$	(2)
Propagation:	$P^{\bullet} + O_2$	$\longrightarrow$	$POO^{\bullet}$	(3)
	$POO^{\bullet} + PH$	$\longrightarrow$	$POOH + P^{\bullet}$	(4)
Chain-branching:	POOH	$\longrightarrow$	$PO^{\bullet} + {}^{\bullet}OH$	(5)
	$PH + {}^{\bullet}OH$	$\longrightarrow$	$P^{\bullet} + H_2O$	(6)
	$PH + PO^{\bullet}$	$\longrightarrow$	$P^{\bullet} + POH$	(7)
	$PO^{\bullet}$	$\longrightarrow$	various chain-scission reactions	(8)
Termination:	$POO^{\bullet} + POO^{\bullet}$	$\longrightarrow$	POOOOOP ($\rightarrow$ POOP + O_2)	(9)
	$POO^{\bullet} + P^{\bullet}$	$\longrightarrow$	POOP	(10)
	$P^{\bullet} + P^{\bullet}$	$\longrightarrow$	Polymer PP	(11)

In thermal oxidation and photo-oxidation, the initiation reaction, eq. (1), results from the thermal or photo-dissociation of chemical bonds. The light quanta in solar radiation are energetically sufficient to

cleave PO-OH (176 kJ/mol) and P-OOH (293 kJ/mol), but hardly POO-H (377 kJ/mol) bonds. The large difference in the bond dissociation energy between PO-OH and P-OOH means that the formation of $PO^{\bullet}$ and $^{\bullet}OH$ radicals will be the predominant reaction of photo-cleavage during irradiation [8]. Hydro-peroxide groups have a very low molar absorptivity at a wavelength of 340 nm. The O-O bond has no low-lying stable excitation state, and the potential energy surfaces of the first excited state are dissociative. The quantum yield in the near ultraviolet (UV) is close to 1.0, however, the photolysis of hydro-peroxides under solar irradiation is a slow process due to average lifetime of hydro-peroxide group of 4-5 days [9,10].

Prediction of useful lifetime of a polymer at high temperature and in the presence of UV radiation is of great importance for technological exploitation of plastics as well as for assessment of their environmental degradability. From a detailed understanding of the processes involved in polymer degradation, it should be possible to develop a quantitative kinetic model for the oxidation of the polymer. The tool that has been employed to approach this goal has been chemical kinetics. The theory of free radical chain reaction kinetics in the gas phase or well-mixed liquid state has been long established [4,5]. Although molten polymers differ from low molar mass compounds in solution because of entanglements and viscosity the theory for oxidation during melt processing is consistent with homogeneous chemical kinetics. In this system, the concentration of reactants may be averaged over the volume to produce a representative value. However, polyolefines in service is used well below melting temperature (T_m), but above glass transition temperature (T_g) and thus the segmental motion of the polymer chains on which the free radicals are formed will be restricted. In addition, the oxidation reactions will be occurring only in the amorphous phase where the concentration of dissolved oxygen is sufficient [1]. Therefore, at one extreme, homogeneous chemical kinetics may be employed, at the other extreme, evidence exists for highly localized oxidation as a prelude to cracks formation in the solid polymer, so that a model for heterogeneous oxidation is considered appropriate [11,12].

In this chapter, we survey the paper by George and Celina [9] in which the authors review the kinetic modeling of homogeneous and heterogeneous oxidation of polypropylene.

9.2 Kinetic Models for Polymer Oxidation

9.2.1 Homogeneous Oxidation Kinetics

The starting point in the kinetic analysis of the oxidation of polypropylene is measurement of the extent of oxidation of the polymer as a function of time. The most common measurement is the uptake of oxygen by the polymer. The reaction mechanism for free radical oxidation, eqs. (1-11), was used to relate the consumption of oxygen to the formation of oxidation products in polypropylene. A kinetic interpretation was based on the steady-state approximation equating the rates of the initiation and termination reactions. With this approach it was possible to derive mathematical equations describing the consumption of oxygen and the formation of oxidation products. Simplify the oxidation of polypropylene to the reaction sequence of eqs. (2,3,4,9,10,11) the consumption of oxygen could be related to the formation of hydroperoxides at high oxygen pressure:

$$-d[O_2]/dt = k_4(k_3/k_9)^{1/2} [POOH][PH] \quad (12)$$

and at low oxygen pressure:

$$-d[O_2]/dt = k_3(k_2/k_{11})^{1/2} [POOH][O_2] \quad (13)$$

Eqs. (10,11) were neglected at high oxygen pressure and eqs. (9,10) were redundant at low oxygen pressure. A more complex relation was presented [13] obtained with the assumption that the kinetic chain is not too short and that $k_{10}^2 = k_9 k_{11}$:

$$-d[O_2]/dt = (k_3k_4 [PP][O_2] r_i^{1/2})/(k_3k_9^{1/2} [O_2] + k_4k_{11}^{1/2} [PP]) \quad (14)$$

where r_i means initiation rate constant. One of the obvious feature of the oxidation of polypropylene is the formation of hydro-peroxides as a product. The kinetics of oxidation becomes that of a branched-

chain reaction as the number of free radicals in the system increases with the time in which the steady state approximation may not be valid. The oxidation may oscillate between the branched-chain and linear-chain regimens, obviously, under the conditions of service, the oxidation of the polymeric hydrocarbons cannot reach the critical conditions and homogeneous kinetic treatments of polypropylene oxidation involve perturbations of the steady-state approximation.

The application of models involving degenerate branching has been a particular feature of kinetic treatments by several authors [14,15] who expressed the maximum rate of oxygen consumption at high oxygen pressure assuming the oxidation chain is sufficiently long:

$$-d[O_2]/dt^{\bullet}_{max} = \sigma\, k_4\, [PH]^2/(2k_9)^{1/2} \qquad (15)$$

by neglecting the reactions in eqs. (10,11), where σ means probability of degenerate chain branching. In many kinetic studies, the basic information is obtained from the slope of the oxygen-uptake curve in the linear region of a "steady-state" rate of O_2 consumption. Any attempt to establish precise rate constants for the initiation, propagation and termination in the auto-oxidation of polypropylene is complicated because intermediate oxidation products of a saturated hydrocarbon are 10-100 times more susceptible to a complex co-oxidation of highly degraded polymer and its oxidation products [16].

Although the analysis and evaluation of relative oxidation rates and rate coefficients from oxygen uptake and related methods may sometimes be useful, those measurements are fundamentally related to a homogeneous process and, therefore, can yield only average information about the polymer oxidation behavior. The limited mobility of radicals and oxidation products of a non-uniform degradation are not taken into consideration when applying models originally derived to describe the reactions and kinetics occurring in the liquid state or in solution.

9.2.2 Heterogeneous Oxidation of Polypropylene

The free radical oxidation scheme of hydrocarbons was primarily aimed at explaining the chemical changes in the material during oxidation. The chemical oxidation in the solid polymer, however, can be complicated by physical features of the material and the oxidative process. The physical aging of a polymer (physical embattlement) can occur despite overall low extents of oxidation. mechanical properties, such as, impact resistance, can be reduced without changes in the visual appearance of the polymer.

The localization of oxidation in polypropylene has been repeatedly investigated using specific-staining techniques in combination with UV microscopy. Carbonyl groups in oxidized polyolefins were stained to visualize localized oxidation at the surface of the polymeric material [17,18]. Various staining techniques were used and involved SO_2, Sudan III and methylene blue [19]. The UV microphotographs from staining experiments on oxidized solid polypropylene showed the preferential oxidation of micro-sized spots, which were attributed to the activity of catalyst residues.

The limited mobility of radicals in the solid polymer matrix is considered to be one of the main reasons for heterogeneous polymer degradation. Cage recombination of the polymer peroxy radicals is considered as the main difference between liquid and solid-state photo-oxidation processes [8,16]. In liquid-state photo-oxidation diffusion will quickly randomize radical populations, whereas in the solid state the polymer peroxy radical will separate only by slow segmental diffusion. Sufficient evidence has been obtained in the past that the mobility of radicals varies considerably between the crystalline and amorphous phase.

Polymer morphology plays an integral part in the course of degradation. The oxidation of semi-crystalline polymers, such as polyolefines, is generally considered to occur within the amorphous region, which can be treated as a boundary phase of the neighboring crystalline regions [8]. Buchachenko [20] described the material as a micro-heterogeneous system, with an imperfect sub-molecular structure in which the crystalline regions alternate with the amorphous ones. This feature leads a non-homogeneous distribution of reagents, such as oxygen, oxidation products, and stabilizers, that are concentrated in the amorphous and defective parts of the polymers. These regions also contain the most reactive groups of the macromolecules, such as peroxidized groups and unsaturated bonds.

Local concentrations of reagents, and, therefore, local rates of chemical reactions, should differ strongly from average ones.

The oxidation of polyolefines below their melting point is a reaction between a gas and solid, and similar to all such reactions, may be susceptible to rate control by the diffusion of oxygen into the sample, rather than the rate of reaction with the sample. To oxidize the material, oxygen has to adsorb on the surface and diffuse into the material. In most polyolefins, then amorphous region is more susceptible to oxygen diffusion, and the solubility of oxygen within this region is much higher than in the crystalline fraction. Polypropylene is expected to show diffusion-controlled kinetic behavior similar to that of other polyolefins [21].

Evidence that the thermal oxidation of polypropylene may be initiated by catalyst residues was obtained from degradation studies of slightly compressed polypropylene films using UV microscopy [18]. Common polypropylene powder is manufactured by a slurry process that uses second-generation catalyst system suspended in hydrocarbon solvents. The morphological features of the produced polypropylene particles are extremely complex, with different levels of order [22]. Highly efficient and improved catalytic systems have resulted in optimized polymerization process, reduced operating costs and increased polymer yields per employed catalyst, but have also made catalyst deactivation steps unnecessary. Highly reactive catalyst residues can, therefore, remain in the polymer. Catalyst residues of currently employed catalytic systems can be of wide chemical variety, composition, and activity, and they are able to interfere with the thermooxidative mechanism of the polymer, leading to oxidative initiation centers and reduced stabilities of the polymer [18]. In particular, it has been suggested that the residual catalyst particles provide the sites of initiation of the heterogeneous oxidation, with the number of kinetic chains depending on the concentration of the catalyst [23].

One of the manifestations of the oxidation of polypropylene, as either a liquid or as a solid, is the emission of weak visible light - chemiluminiscence - from the onset of the oxidation [24]. The particular features of this chemiluminiscence have been interpreted as presenting direct evidence for the heterogeneous oxidation of polypropylene. Mechanistic studies of chemiluminiscence during the oxidation of hydrocarbons and polymers have been advanced by studies of model compounds, and the spectral analysis of the emission suggests the emitting chromophore may be the triplet state of a carbonyl compound [25]. Several possible reactions have been considered to be energetically feasible for the formation of an excited triplet state of carbonyl oxidation product:

$$R_2CHOOH \longrightarrow R_2HCO^{\bullet} + {}^{\bullet}OH \qquad \Delta H = 147 \text{ kJ/mol} \qquad (16)$$

$$R_2HCO^{\bullet} + {}^{\bullet}OH \longrightarrow R_2C{=}O^{*} + H_2O \qquad \underline{\Delta H = -471 \text{ kJ/mol}} \qquad (17)$$

$$\Delta H_{total} = -314 \text{ kJ/mol}$$

$$R_2CHO^{\bullet} + {}^{\bullet}R \longrightarrow R_2C{=}O^{*} + RH \qquad \Delta H = -323 \text{ kJ/mol} \qquad (18)$$

$$R_2CHO^{\bullet} + {}^{\bullet}OOR \longrightarrow R_2C{=}O^{*} + O_2 + ROH \qquad \Delta H = -460 \text{ kJ/mol} \qquad (19)$$

In the preceding sections there have been successively finer levels of heterogeneity introduced to help explain the experimental observations of the oxidation of solid polypropylene. The challenge in the study of the heterogeneous oxidation is to develop a model that encompasses the foregoing observations and allows parameters to be determined that are consistent with the experimental oxidation kinetics. Modeling of a system that incorporates an inert crystalline component and an oxidizable amorphous component has been straightforward and it is possible to rationalize the oxidation profiles as a function of sample depth using known solubility and diffusion coefficient data for oxygen in polyolefines [26]. Any model that is developed for the heterogeneous oxidation of the amorphous region must be consistent with the experimental observations made of the oxidation-time profile, as given by oxygen uptake infrared spectroscopy, or chemiluminiscence.

The purpose of a kinetic model of heterogeneous oxidation of the amorphous region is to explain the observations from a wide range of experimental studies of the oxidation of polypropylene as a powder, film, or bulk samples, and , from this, gain insight into the reaction mechanism. It has been argued that the homogeneous kinetic model may be applied to a non-homogeneous polymer by considering the parameters as average values [27]. However, it has been noted [28] that there is a fundamental problem between microscopic and macroscopic kinetics owing to the measurement of the appropriate concentration terms to appear in the kinetic equations. If oxidation is occurring non-uniformly in the amorphous domains, then these are the kinetic terms that must be evaluated in the rate law using

domain concentrations that will be much higher than the mean concentration averaged over the whole polymer.

In the following it is intended to summarize those approaches that have been made to a heterogeneous model for the oxidation of polyolefines [9,11,12,28]. These have common starting points in which it is considered that oxidation initiates non-uniformly at a few sites in the amorphous region of the polymer. From these sites, oxidation is able to spread so that progressively larger fraction of the total amorphous region is oxidizing as a function of time. Within one of these reactive zones, a free radical chain reaction will take place at a high local rate, but because there are few such zones initially, the average rate of oxidation is negligible (i.e. there is an apparent induction period on the macroscopic scale).

A highly illustrative approach to heterogeneous oxidation has been provided by using a random-walk model [29] for the spatial propagation of oxidation initiated at catalyst residues. Because diffusion of macroradicals in the solid state is highly restricted, low molecular weight "jogger" radicals are considered the spreading agents. This spreading of the zones by jogger radicals micro-diffusion at the same time as the extent of oxidation within the original zone also increases. the jogger radical $r^{\bullet}$ is formed from a macroradical with a rate constant k_d and will migrate a distance:

$$R \cong k_d \, (D/k_r \, [PH])^{1/2} \qquad (20)$$

with a characteristic diffusion coefficient D, until it is immobilized by reaction with the polymer [PH] with a rate constant k_r to form a macro-radical $P^{\bullet}$ (or $PO_2^{\bullet}$), which again, may then produce oxidation products.

$$r^{\bullet} + PH\,(+O_2) \longrightarrow rH + P^{\bullet}\,(PO_2^{\bullet}) \qquad (21)$$

The diffusion process has been simulated by Monte Carlo method, with the following points concluded from the study:

- a high initiation rate of 10^{-6} to 10^{-5} mol/kg was used at N initiation sites (ranging from 5 to 100),
- the diffusion coefficient D is taken as that for gases in polypropylene (10^{-5} cm^2/s at 130 °C),
- within a lifetime t_r of 10^{-2} s, the jogger radical $r^{\bullet}$ moved an average distance of 3 μm or ten lattice sites before becoming immobilized by the reaction in eq. (21),
- at the immobilization site, damage could accumulate and accumulation for a time t_d was considered sufficient to form a micro-crack. When the micro-crack merged into a percolation cluster, the sample failed by fracture.

This model, while requiring values for several radical lifetimes and related parameters, provided an interesting approach to heterogeneous modeling. It was also possible to examine the role of hat inhibitors play in such a system. For example, the effectiveness of the inhibitor decreased if there was a substantial concentration of initiating impurities (catalyst residues), and not all of the inhibitor was consumed before the sample failed. Another interesting conclusion was that the durability increased dramatically if the number of initiation centers were decreased rather than the rate of radical generation being decreased to the new average value for the same number of initiation centers. This is a consequence of the bimolecular recombination of radicals produced at higher rate in an initiation zone. These results have been interpreted as indicating that the greatest increase in the durability of polypropylene will be achieved by eliminating the initiation centers by deactivating the catalytic impurities.

The epidemic model draws on the observation that the oxidation profile from polypropylene shows features characteristic of the infectious spreading of a disease through a population [30]. In simple epidemic model, a small number of infected individuals are introduced into a large, fixed population, and the aim is to determine the spread of the infection as a function of time. The population, after a given time period, may be divided into three classes: (i) those that have become infected, (ii) those that have either recovered from the disease (and are immune) or are dead, and (iii) those that are still susceptible to infection.

In applying this epidemic model to the heterogeneous oxidation of a solid polymer the same procedure may be followed. In this case a number of infectious zones are randomly distributed within the polymer. Within each zone are impurities or catalyst residues that result in a chain reaction producing

macromolecule scission, formation of volatile oxidation products, and a high local concentration of free radicals. These free radicals are than able to spread from the initial zone by infecting the adjacent polymer. The termination reaction of these radicals serve to reduce the infectious population, replacing it with dead or oxidized material. In the epidemic model three distinct populations in the amorphous region of the polymer may be assigned after a short time of oxidation:

- the remaining or un-oxidized fraction p_r
- the infectious or oxidizing fraction p_i
- the dead or oxidized fraction p_d

These populations may be described by a series of coupled differential equations, which were developed by noting that oxidation can spread only if an infectious zone has uninfected material available within a contact distance, and this will be proportional to p_r:

$$- dp_r/dt = bp_rp_i = bp_r (1 - p_r) \quad (22)$$

where b [s^{-1}] is the rate coefficient for spreading and p_d is small compared with p_r at short times of oxidation, such that $p_i = 1 - (p_r + p_d) \cong 1 - p_r$, and

$$- dp_d/dt = \alpha p_i \quad (23)$$

where α [s^{-1}] is the rate coefficient for formation of oxidized material from the infectious (free radical) fraction, and this encompasses a range of elementary rate processes for both propagation and termination steps in the oxidation.

$$- dp_i/dt = bp_rp_i - \alpha p_i \quad (24)$$

These equations may be solved [31] if the initial fraction p_o is small to give the time dependence of the infectious fraction p_i as:

$$p_i = p_rp_o \exp[b - \alpha]t \quad (25)$$

Thus, in this simple model, the spreading of the oxidation through the amorphous region of polypropylene should depend on the initial infectious fraction (i.e. number of catalytic initiation centers) and the difference between the spreading rate coefficient b and removal coefficient α. This model can be explored by using parameters explicitly determined from the analysis of chemiluminiscence profile [31]. The only assumption required is that the intensity of chemiluminiscence, I, at any time is given by:

$$I = \varphi r p_i \quad (26)$$

where φ is the chemiluminiscence quantum efficiency and r is an average rate for termination of peroxy radicals within an infectious zone under the conditions of the oxidation. This relating of chemiluminiscence to the infectious fraction enables a linear function to be obtained from which p_o, α, and b may be calculated:

$$\ln(I_{max}/I) = \ln(\alpha/b) + (b - \alpha) (t_{max} - t) \quad (27)$$

$$\ln[(1 - p_o)/p_o] = bt_{max} + \ln(\alpha/b - \alpha) \quad (28)$$

where I_{max} is the maximum chemiluminiscence intensity after oxidation time t_{max}. To derive eqs. (27,28) it was required that φr should not change up to the maximum chemiluminiscence intensity. This is supported by the constancy of activation energies, which is one of the earlier experimental observations. The relations of eqs. (27,28) hold only in the early stages of oxidation (when p_r is very much greater than $p_i + p_d$), but this is sufficient to enable a linear plot to be obtained and values of α, b, and p_o determined for single particles of polypropylene of different origin [31].
The values determined in the first 15% of the oxidation were used to simulate the chemiluminiscence curve as representing the change in the infectious fraction over the entire oxidation. The following

points may be noted from the infectious parameters p_o, b, and α for three separate single particles of polypropylene reactor powder:

- there is wide variation in the value of p_o, the initial infectious fraction, both between and within sample types. This probably reflects the statistical nature of the distribution of residual catalyst and its activity from particle to particle. The values of p_o indicate that between 0.02 and 2% of the sample is oxidizing at the start of the induction period.
- the spreading rate coefficient b is approximately constant within a polypropylene type, and the difference between the values for two different polymer types is sufficiently small to suggest that it is a fundamental property of the polymer.
- The removal rate coefficient α is significantly different between the polymer types and may be more sensitive to the morphology of the particle.

The simulated profiles for the three separate fractions as a function of oxidation time for different polymer types are of some interest. The following pints may be noted from the curves profiles:

- the difference between two infectious distributions (p_i) is linked to the difference between the initial fraction oxidizing p_o. This is consistent to the importance of the number of active sites, as noted from different modeling studies of Rapoport [29].
- for both polymer types, the peak in p_i occurs when $p_d \sim 0.3$ (i.e. approximately 30% extent of oxidation).
- the oxidation does not become homogeneous (i.e. $p_r \rightarrow 0$) until well past the maximum in p_i and at least 50% extent of oxidation.
- the removal parameter α controls the rate of formation of the oxidized fraction p_d compared with the infectious fraction and accounts for the more rapid buildup of p_d in the polymer samples.

When the oxidation kinetics of a heterogeneous solid polymer are measured, it is necessary to consider that a significant fraction of the polymer will not be reacting, hence the concentration terms that are required for the kinetics equations are those for the amorphous region that are oxidizing at any point in time. If a polymer is oxidizing heterogeneously with only a fraction of the available number of the amorphous domains oxidizing, then to determine the true concentration of reactants and oxidation products it is necessary to probe a sample with a thickness of one amorphous domain using a technique with a spatial resolution of less than one amorphous domain. Because is considered that these domains may be 10-30 nm [29] this is then beyond the capabilities of current techniques.

A generalization of the concentration problem in heterogeneous oxidation may be obtained by considering that, in a chemical kinetic treatment of oxidized product formation in a heterogeneous system, it is necessary to obtain the actual local rate of reaction of a microscopic amorphous domain, dC_a/dt, but in practice, it is only possible to obtain a measured rate of a macroscopic volume dC_m/dt. The relation linking these two rates is the volume fraction V_d of the oxidizing polymer in the total volume such that the concentration terms are linked by: $C_a = C_m/V_d$, that is because V_d is very much less than 1 especially early in the oxidation, then $C_a >> C_m$. The oxidation rate in the amorphous domains is given as the derivative:

$$dC_a/dt = d(C_m/V_d)dt = 1/V_d\, dC_m/dt - C_m/V_d^2\, dV_d/dt \qquad (29)$$

That is, the true amorphous region oxidation rate will depend also on how V_d changes with the time. The principal way that this occurs in the heterogeneous model is by infectious spreading and eq. (25) shows how the oxidizing fraction changes with time. In the homogeneous model it is assumed that V_d does not change throughout the oxidation so the measured oxidation rate is related directly to the microscopic oxidation rate:

$$dC_m/dt = V_d\, dC_a/dt \qquad (30)$$

Another extreme example would be if the oxidation rate within an amorphous domain was a constant, r, such as that the change in the measured reaction rate depends only on the rate of spreading of the oxidation through the polymer:

$$dC_m/dt = Cm/Vd\, dC_a/dt + rV_d \qquad (31)$$

It is unlikely that the conditions described by the eq. (31) would prevail over the full extent of the oxidation, although it was reported that the infectious spreading model, with a constant rate r of free

radical termination in the oxidizing domain, was able to describe the chemiluminiscence profile from oxidizing polypropylene.

To summarize the kinetic models for homogeneous and heterogeneous oxidation of polypropylene analyzed in the paper of George and Celina [9], it is not generally possible to translate data from macroscopic measurements of oxidation product formation (or oxygen uptake) with time of reaction onto a kinetic equation that may be used to predict the useful lifetime of the polymer, owing to the heterogeneous oxidative behavior of polypropylene. However, considerable insight may be gained into the process that ultimately lead to micro-crack formation and polymer embrittlement by considering the spatial development of the oxidation that initiates from catalyst particles and related impurity centers.

Self-check questions

1. What is the molecular mechanism of photo-oxidative polyolefine degradation?
2. Under what condition the homogeneous reaction kinetics formalism can be applied to study radical oxidation of polyolefins?
3. What is the meaning of "local concentration" in solid polypropylene oxidation reaction kinetics?
4. What are the main features of the "epidemic model"?

Reading Materials

1. Hawkins, W. *Polymer Degradation and Stabilization*, Springer Verlag, Berlin (1984)
2. Iring, M. and Tudos, F. (1990) *Prog. Polym. Sci.* **15**, 217
3. Knight, J.B., Calvert, P.D. and Billingham, N.C. (1985) *Polymer* **26**, 1713
4. Backström, H.L. (1929) *J. Am. Chem. Soc.* **51**, 90
5. Bolland, J.L and Gee, G. (1946) *Trans. Faraday Soc*. **42**, 236
6. Al-Malaika, S., In: *Comprehensive Polymer Science*, G. Allen and J. Bevington (eds.), Pergamon Press, Oxford (1989), p. 539
7. Grassie, N.,Scott, G. Polymer Degradation and Stabilization, Cambridge Univ. Press, (1985)
8. Rabek, J.F. *Photostabilization of Polymers – Principles and Applications*, Elsevier Applied Science, London (1990)
9. George, G. and Celina, M., In: *Handbook of Polymer Degradation*, S.H. Hamid (ed.), 2nd ed., M. Dekker, New York (2000), p. 277
10. Carlson, D.J., Garton, A. and Wiles, D.M., In: *Developments in Polymer Degradation - 1.*, N. Grassie (ed.), Aplied Science, London (1979), p. 219
11. Celina, M. and George, G.A. (1995) *Polym. Degrad. Stabil.* **50**, 89
12. Gugumus, F. (1996) *Polym. Degrad. Stabil.* **52**, 159
13. Vink, P. (1979) *J. Appl. Polym. Sci. Appl. Polym. Symp.* **35**, 265
14. Emanuel, N.M. and Buchachenko, A.L., *Chemical Physics of Polymer degradation and Stabilization*, VNU Science, Utrecht (1987)
15. Kyriushkin, S.G. and Schlyapnikov, Y.A. (1989) *Polym. Degrad. Stabil.* **23**, 185
16. Mayo, F.R. (1972) *J. Polym. Sci. Plym. Lett. Ed.* **10**, 921
17. Johnson M. and Williams, M.E. (1976) *Eur. Polym. J.* **12**, 843
18. Billingham, N.C. and Calvert, P.D. (1985) *Pure Appl. Chem.* **57**, 1727
19. da Costa, R.A., Coltro, L. and Galembeck, F. (1990) *Angew. Makromol. Chem.* **180**, 85
20. Buchachenko, A.L. (1976) *J. Polym. Sci. Symp.* **57**, 299
21. Langlois, V., Meyer, M., Audouin, L. and Verdu, J. (1992) *Polym. Degrad. Stabil.* **36**, 207
22. Wristers J. (1973) *J. Polym. Sci. Polym. Phys. Ed.* **11**, 1601
23. Livanova, L.M. and Zaikov, G.E. (1997) *Polym. Degrad. Stabil.* **57**, 1
24. George, G.A., In: *Luminiscence Techniques in Solid State polymer Research*, L. Zlatkevich (ed.), M. Dekker, New York (1989)
25. Kellogg, R.E. (1969) *J. Am. Chem. Soc.* **91**, 5433
26. Clough, R.L. and Gillen, K.T. (1992) *Polym. Degrad. Stabil.* **38**, 47
27. Zlatkevich, L. (1995) *Polym. Degrad. Stabil.* **50**, 83
28. Gugumus, F. (1996) *Polym. Degrad. Stabil.* **53**, 161
29. Rapoport, N. Proceedings 17th International Conference on Advances in Stability and Degradation of Polymers, Lucerne (1995), p. 245
30. Murray, J.D., *Mathematical Biology*. Springer Verlag, Berlin (1989), p. 610
31. George, G.A., Celina, M., Lerf, C., Cash, G. ,Wenddell, D. (1997) *Macromol. Symp.* **115**, 69

CHAPTER 10 LIFE CYCLE ASSESSMENT

Objectives

1. Students will develop an understanding of the rationale for design of environmentally friendly products – specifically biodegradable or bio-based plastics
2. Students will have an overview of LCA, the background and the different elements of an LCA – the principles and framework
3. Students will learn how to conduct Life cycle inventory analysis (LCI), i.e. from defining product system, function unit, system boundaries, data selection and their quality, to criteria for initial inclusion of inputs and outputs.
4. Student will understand the procedure of impact assessment (LCIA) – mandatory elements for conducting an LCIA.
5. Students will learn the methods for interpretation of LCA study and critical review, and be aware of the limitation and applicable areas of LCA.

Introduction

EDPs are developed as ecologically alternatives to conventional plastics. Many tools have been developed to measure and thus to compare the environmental impacts associated with the manufacturing and consuming and disposal of products. One of these techniques is life cycle assessment (LCA). LCA has been increasingly adopted since 1990s by decision makers to assess the environmental performance and potential impacts associated with a product or a service. The chapter will introduce basic concepts, principles and methodology for conducting and reviewing LCA studies, particularly those for EDPs and bio-based plastic products.

10.1 Facts about LCA

ISO (International Standards Organization) defines LCA as a technique for assessing the potential environmental aspects associated with a product or service by:

- compiling an inventory of relevant inputs and outputs of a product system
- evaluating the potential environmental impacts associated with those inputs and outputs
- interpreting the results of the inventory and impact in relation to the objectives of the study.

LCA studies the impact on the environment throughout the life cycle of a product from raw material acquisition to production, use, and ultimate disposal. Thus LCA is holistic environmental and energy audit that focuses on the entire life cycle of the product, not a single step of manufacturing or emission.

ISO 14040 (Environmental management – Life cycle assessment – Principles and framework), ISO 14041(Environmental management – Goal & scope definition and inventory analysis), ISO 14042 (selection of impact categories, category indicators, and characterization models, assignment of LCI results, normalization and weighting.) and ISO 14043 (Life cycle interpretation) gave more description of the key elements and procedures of LCA. However, compared with other guidelines, it is still difficult for beginners to acquire more concretely the LCA techniques from ISO standards. The chapter and the case study are devised to facilitate understanding of ISO 14040 serial standards as well as of the LCA methodology.

10.1.1 Application of LCA

LCA can be used internally:

- ❖ Identifying opportunities to improve the environmental aspects of products or processes at various points in their life cycle (e.g., strategic planning, priority setting etc.)
- ❖ LCA is regarded as an excellent process development tool to provide an insight about production, to set process improvements goals and to provide eco-profile data of processes.

As it shows the environmental impacts separately for every production step in a processing plant, it is easy to find the most expensive step from the ecological (e.g. CO2-emissions) and, to a less extent, the economical point of view (e.g. energy demand) as well. Improving the most relevant steps is most advantageous for the environmental management of an enterprise and might bring in money too, especially in the long term.

For example in the same case study of this chapter, the biodegradable Mater-Bi loose fills can be obtained by using a conventional extruder. Novamont SpA evaluated their LCA to find the most ecologically favorable way for their production. LCA showed that the production of loose fills from granules directly at the customer's site, turned out to be most convenient as it minimized the transportation costs.

LCA can be used externally:

- Decision-making in industry, government or non-governmental organizations for strategic planning purposes, and improving the overall environmental and economic performance
- Selection of relevant indicators of environmental performance, including measurement techniques
- Marketing (for example an environmental claims, eco-labeling scheme or environmental product declaration)

For example, the German agency of environment insists on LCA studies of biodegradable mayerials including comparative assertions as a base for the political strategy for waste management (disposal of biodegradable materials in the organic waste or not) and packaging taxes.

The LCA study of the biodegradable Mater-Bi bags shown in the case study of this chapter serves for decision making, i.e. in waste management to choose the bags, which are allowed in the organic waste collection and as a selling argument for potential customers. An increasing number of customer cares about the environmental engagement of the bidders and therefore a good documentation of the products' life cycle might be advantageous.

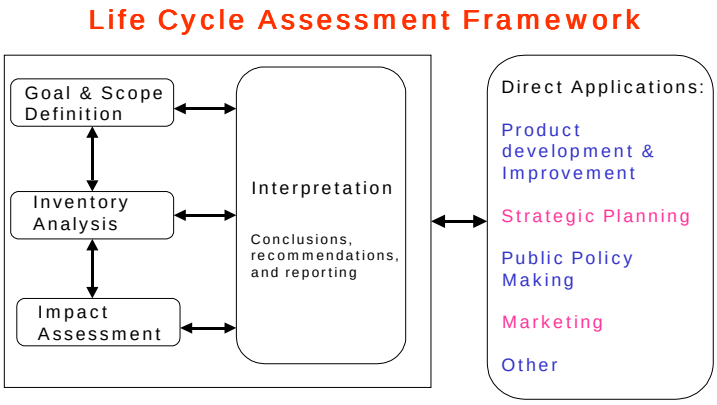


Fig. 10.1 Life cycle assessment phases and its application

10.1.2 Limitation of LCA

LCA is not an exact calculation because many of the parameters can not be measured exactly and have to resort to assumptions. Data from literature (e.g. for energy or transports) are average values. Some LCA-software tools consider this inaccuracy and allow a special error of calculation. The scope, assumptions, data quality, methodologies and output of LCA studies have to be transparent. The quality of the database determines the quality of the LCA.

The depth of detail and time frame of an LCA study may vary to a large extent, depending on the definition of goal and scope. Therefore results of different studies can hardly be compared to each other.

The general categories of environmental impacts need consideration are resource use, human health, and ecological consequences. While economics generally do not figure in LCA, it should be a part of any LCA study because ultimately costs will be an important deciding factor in the change to an environmentally preferable option or to choose between two options. Thus, ecology + economics = eco-efficiency is the key driver for widespread acceptance of environmentally preferable products.

There is subjectivity in LCA, particularly in impact assessment, such as the value choice, modeling and evaluation of impact categories. Therefore, transparency is critical for LCA to minimize the danger of being manipulated. For this purpose, ISO standardize the procedure of critical review.

Critical Review

The use of LCA results to support comparative assertions raises special concerns and requires critical review since this application is likely to affect interested parties that are external to the LCA study. A critical review may facilitate understanding and enhance the credibility of LCA studies, for example, by involving interested parties. Different kinds of critical review are possible: internal or external expert review and third party review by interested parties.

The critical review process has to ensure that:

- the methods used to carry out the LCA are consistent with the International Standard ISO 14040.
- the methods used to carry out the LCA are scientifically and technically valid.
- the data used are appropriate and reasonable in relation to the goal of the study.
- the interpretations reflect the limitations identified and the goal of the study.
- the study report is transparent and consistent.

10.2 Methodology

LCA can be carried out according to ISO 14040 procedures and several other widely recognized guidelines on LCA, such as European Environmental Agency (EEA) guidelines and that of Nordic Council, will guarantee a high quality and acceptance. There are four fundamental phases for conducting an LCA, as defined by ISO 14040.

10.2.1 Goal & Scope Definition

Goal shall unambiguously state the intended application, the reasons for carrying out the study and the intended audience. The scope of the study should be sufficiently well defined to ensure that the breath, depth, and detail of the study are compatible and sufficient to address the stated goal.

The most crucial points in defining the scope of an LCA study are the functional unit, the system boundaries, the types of impact, the methodology of impact assessment and subsequent interpretation to be used, and the data requirements.

Take the case study of this chapter as an example. Functional unit can be based on the material that constitutes the EDPs bag, i.e. 1 kg of the Mater-Bi trash bag, since it is the amount of EDP material that counts in waste facility (composting). It is also justifiable to base on the function it will fulfill, i.e. as a package for organic wastes. Therefore the functional unit could be 1kg or 1cubic meter of organic waste the Mater-Bi trash bag and the reference bag can properly handle.

Determining system boundary depends heavily on relevance and data availability, as indicated by the case study. The decision should be convincingly justified.

10.2.2 Inventory Analysis (LCI)

LCI involves identification and quantification of inputs and outputs of the entire product system. The attached figure 10.2 shows the components of product system. Both qualitative and quantitative data for inclusion in the inventory have to be collected for each unit process within the system boundaries. The data constitute the input to the life cycle assessment.

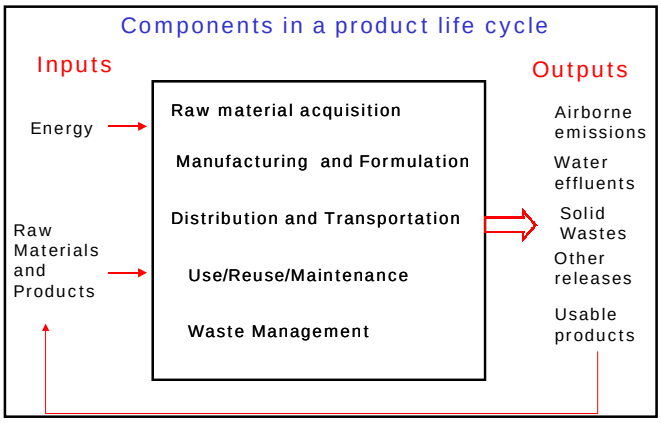


Fig. 10.2 Diagram of the life cycle inventory phase

10.2.3 Life-cycle Impact assessment (LCIA)

The impact assessment phase of LCA aims to evaluate the significance of potential environmental impacts by using the results of the life cycle inventory analysis. In general, this process involves association of inventory data with specific environmental impacts. The phase mostly includes steps such as: classification (assigning of inventory data to impact categories), characterization (modeling of the inventory data within impact categories) and evaluation (aggregating and weighting the results). Evaluation is a very sensitive process and should only be used in special cases provided that the data prior to evaluation remain available.

An impact assessment consists of the following basic steps:

A. Selection of impact categories, category indicators, and characterization models

1. resource depletion,	Indicators: fossil fuel, ores etc. (tons/kg) per functional unit
2. global warming,	Indicators: CO_2 equivalents per functional unit
3. ozone depletion,	Indicators: CFC-11(kg) per functional unit
4. human health	Indicators: acute toxicity LC50/LD50 per functional unit
5. eco-toxicity,	Indicators: acute toxicity, or acceptable concentration
6. photochemical smog	Indicators: ethylene (g) per functional unit
7. acidification,	Indicators: SO_2 -equivalent or H+ per functional unit
8. eutrophication/nitrification,	Indicators: PO_4- equivalents per functional unit
9. Photochem. ozone formation,	Indicators: ethane (g) per functional unit

B. Classification – Assignment of LCI results to each impact category.
For example, SO_2 emission is allocated among the impact category of human health, winter smog and acidification. NOx may be assigned to both acidification and ground-level ozone smog.

C. Characterization – calculation of category indicator results

Calculate category indicator: $EF_{ij} * Load_{ij}$ where:
EF_{ij} is the Equivalency factor of the i^{th} impact category for j^{th} environmental loading, $load_{ij}$ is the j^{th} environmental loading contributing to the I^{th}impact category

For example, CH_4 is another significant greenhouse gases contributing to the global warming. Its emission then be converted to a common unit (CO_2 equivalent) by multiplying certain Equivalency factor, proved scientifically and accepted internationally. The converted results will then be aggregated to a numerical single indicator.

There are other steps defined by ISO as optional steps to obtain further information, i.e. normalization, grouping and weighting. Normalization could provide a better understanding of the relative magnitude of each impact indicator of a product. Grouping and weighting will involve ranking, which could be subjective, as different individuals, organizations or societies may have different priority, depending on physical capacity for environmental impact assimilation and social preference etc. As a result, it is possible to reach different conclusion based on same indicator results. That might be one of the reasons that ISO put these steps aside as optional.

10.2.4 Interpretation and Report

Interpretation is the phase of LCA in which the findings from the inventory analysis and the impact assessment are combined to reach conclusions and make recommendations. The findings of this interpretation may take the form of conclusions and recommendations to decision-makers, consisting of the goal and scope of the study.

The results of the LCA have to be reported fairly, completely and accurately to the intended audience. Which means that the results, data, methods, assumptions and limitations have to be transparent and presented in sufficient detail to allow the reader to understand the complexities and trade-off inherent in the LCA study.

ISO 14047-14049 – Case Studies Guidance for application, will provides useful help for understanding how LCA is actually carried out. The next revision may see integration of the LCA documents.

Exercise

The class will be divided into groups of 2-3 persons. Each group will select a product system of their interest/choice. The groups will then break up to work on developing a goal and scope definition for their selected product system (1 hr). They will reconvene and each group will present their findings. The groups will then break up to work on the inventory analysis element of the LCA. The groups will set up the problem, using a, b, c, x, y etc numbers for values they don't have. An excel spreadsheet program will be used by the groups (requires each group to have a computer). Groups will reconvene to present their work. The other elements of the LCA will be worked on in a similar fashion.

Reading Materials

European Environmental Agency (1998), Copenhagen
Life Cycle Assessment (LCA) - A Guide to Approaches, Experiences and Information Sources,

The Nordic Council of Ministers (1995), Copenhagen
Nordic Guideline on Life cycle assessment,

ISO 14000 serial standards:

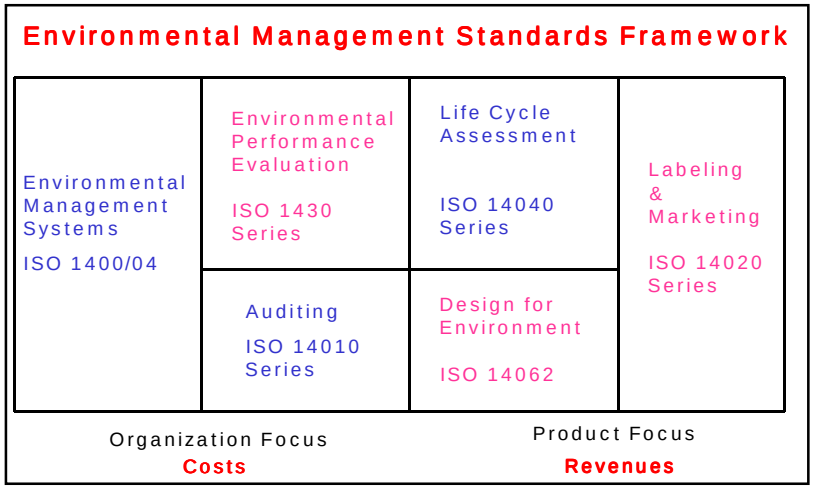
ISO was established in 1945 and has 135 member bodies. Each country's National Standards Body represents the country at ISO. There are 187 Technical committees, and ISO develops full consensus International Standards for products (plastics, paper, etc), processes, and services. There are more than 12, 500 Standards and documents.

Technical Committee 207 on Environmental Management Standards was formed to develop "Standardization in the Field of Environmental Management" – the ISO 14000 series Standards. Sixty-

one countries are participating in TC207, 15 have observation status, and there are 42 liaison organizations. It is ISO's largest committee.

The following figure shows the key subcommittees of TC 207. LCA Standards development activities are conducted as a subcommittee of TC207. The Environmental Management Standards framework and the relationship to the various subcommittees is depicted in the figure above. It should be noted that the Management systems, auditing, and performance evaluation are focused on the organization, whereas LCA, labeling, design for the environment are focused on the product.

ISO 14001 and 14004 were published in 1996 and are the approved Standards for Environmental Management Systems (EMS). ISO 14015 is the new auditing standard.



Some useful websites:

http://www.trentu.ca/faculty/lca/

Society of Environ. Toxicology and Chemistry(one of LCA pioneers) site: http://www.setac.org/

The Worldwide resources for LCA: http://www.ecosite.co.uk/

Case Study

Students are required to make a critical review of the following LCA on biodegradable bag for organic waste collection from the perspective of either internal, external or interested party, following the procedure in the corresponding text of ISO 14040.

The students should evaluate the validity of each key elements and steps, in the meantime, suggest alternatives. For example, the case didn't give the information on what kind of functional unit it adopted. It can be 1kg of different bags, or 1kg or 1cubic meter of organic waste that different bags can handle, or other options. Students are encouraged to propose and then comment on different alternatives and justify their choice.

Mater-Bi Bags for Organic Waste Collection

Life cycle assessments were applied in 1997/98 to analyze the degree of ecological damage caused by the production and disposal of Mater-Bi bags[1] used in households to collect organic waste. Paper bags which can be composted, and PE multipurpose bags which cannot be composted, were used as points of reference.

The life cycles included raw material acquisition, the production and processing and/or disposal of the bags as well as routes of transport. Packaging, distribution, utilization and collection as well as transport to the wholesalers could not be considered due to the dependency of these processes on the respective bulk buyers and retailers.

Life cycle profiles were drawn up using the modified impact-oriented model [3,5] and the impact categories of Eco-Indicator '95[4]. All in all the degree of ecological damage could be identified in thirteen different impact categories. The calculations were obtained by application of the life cycle assessment software EMIS (Environmental Management and Information System, Version 2.2). Most data were taken either from internationally recognized literature (energy supply[6], production and processing of paper, PE [polyethylene][7,8], disposal processes[9, 10], transport[2]) or they were supplied by the manufacturers. In order to analyze sensitivity, new unit processes for the agricultural production of maize in France and for organic waste incineration were created. Assessments were carried out separately for each impact category because of previously specified boundaries of reliability.
The production and disposal of Mater-Bi bags (Table 1) causes less environmental damage than that of paper bags in eleven out of thirteen impact categories. In the two remaining categories the Mater-Bi bag causes the same or greater degree of ecological damage.

The Mater-Bi bag and the multipurpose PE bag are equivalent in 7 impact categories; the Mater-Bi bag achieves better results in four categories, but worse results in the two remaining categories. However, Mater-Bi bags generate less environmental damage than PE multipurpose bags in 10 categories if one considers the waste adhering to the bags and being incinerated together with them. In 2 categories both bags obtain the same results, while in one category the production of Mater-Bi bags generates more environmental damage. It does not prove relevant for the overall results whether maize produced in Switzerland or respectively in France, is used. Mater-Bi bags made of French maize were selected for the overall assessment as maize on the European market is mainly produced in France.

Table 1 Mater-Bi bag in comparison with other products.

Mater-Bi bag compared with	paper bag	PE bag	PE bag [a)]
much better	5	2	6
better	6	2	4
comparable	1	7	2
worse	1	0	1
much worse	0	2	0
total result	**better**	**comparable**	**better**

a) Including organic waste incineration

Assessments of the Mater-Bi bag and the multipurpose PE bag show that both can be regarded as equivalent, as long as the focus remains on production and disposal (disregarding compostable waste

incineration). If the compostable waste that is incinerated with the PE bags is taken into account, the Mater-Bi bag offers a better ecological value.

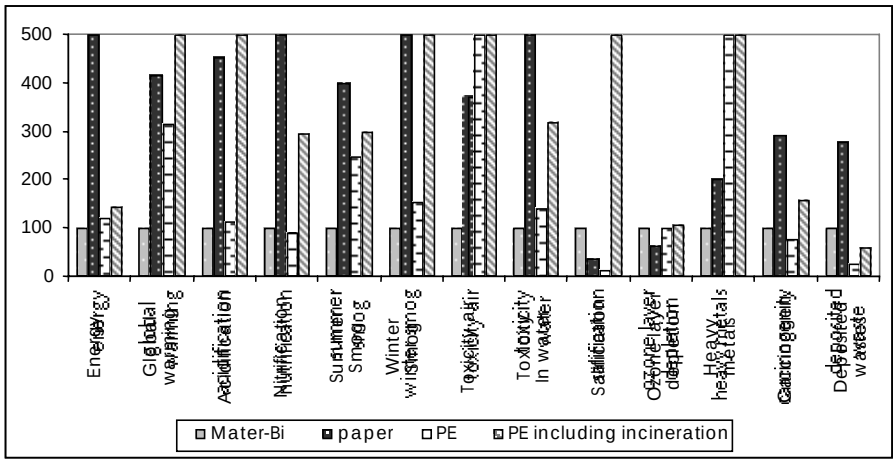


Figure 1. Impact assessment of the three products (Mater-Bi, paper and PE bag)

There is no doubt that for the municipal collection of organic waste biodegradable bags should be recommended. Short routes of transport and minimal use of packaging material should be weighty criteria as for the choice of product.

Conclusion

- LCA studies are of increasing importance for biodegradable products: to improve the production process, for external communication, for politics.
- International Standards such as ISO 14040, available database and special software tools guarantee a high quality and decreasing costs of the calculations.
- The change of agriculture to a more sustainable farming would amplify the ecological advantage of bio-polymers made out of renewable resources. This aspect becomes more and more relevant regarding the increasing excess of farmland.
- LCA is a necessity for products that are intended to be sold using ecological arguments.

Reference

1. The case study is extracted from a LCA study conducted by Dr. Gérard Gaillard from the Eidgenössische Forschungsanstalt für Agrarwirtschaft und Landtechnik, "Federal Research Centre for Agriculture and Cultivation Methods", Switzerland.
2. SSP Umwelt, 1995, Ökoinventar Transportarten, Modul 5, Verlag Infras.
3. Heijungs, R., Guinee, J.B., Huppes, G., Lankreijer, R.M., Udo De Haes, H.A. & A. Wegener Sleeswijk 1992, *Environmental Live Cycle Assessment of Products, Guide and Backgrounds*, (R. Heijungs Ed), CML Leiden.
4. Goedkoop, M. 1995, *The Eco-indicator 95*, Amersfoort 1995.
5. Buwal, 1996, Ökobilanz stärkehaltiger Kunststoffe. SRU 271, Bundesamt für Umwelt, Wald und Landschaft, Bern
6. ETH, 1996: Ökoinventare von Energiesystemen, ENET Bern.
7. Buwal, 1995, Vergleichende ökologische Bewertung von Anstrichstoffen im Baubereich, Band 2. SRU 232, Bundesamt für Umwelt, Wald und Landschaft, Bern
8. Buwal, 1996, Ökoinventare für Verpackungen. SRU Nr. 250 (2 Bände), Bundesamt für Umwelt, Wald und Landschaft, Bern
9. ETH, 1996: Ökoinventare von Entsorgungsmodulen, ENET Bern.
10. Aebersold, A., Eichenberger, S., Künzli Hauenstein, M., Schmid, H. And Schmidweber, A., 1993, Vergären oder Kompostieren? Entscheidungshilfen für die Systemwahl. NDS Umweltlehre 1993, Universität Zürich, Zürich.

CHAPTER 11 MARKET ANALYSIS

Objective

- Students will have the basic concepts about market analysis.
- Students will understand the importance of market analysis for EDPs application.
- Students will study the major methods of evaluating market demand for EDPs and of conducting a market analysis.
- Students will learn marketing skill for new products, particularly EDPs products.

11.1 Introduction to the Market Analysis

The industrial market research can be defined as a collection, recording and systematic analysis of data related to problems about goods marketing and industrial services.

There are no general rules since there are different markets; in any case all market researches need the definition of their aim and a data collection. They can face different activities: advertising researches, macro-economical researches, and product analysis.

The American Marketing Association underlines that the most common market researches required by companies are forecasts of requirements, competition analysis, market analysis, sale analysis. A frequent market research about products will allow products to fit users' needs. In such a way companies are able to check the product trend on the market.

A market research can be considered as the application of scientific methods to industrial problems. The first step of a market research is to define the problem that must be solved. The objective should be established clearly and with accuracy in order to evaluate timing, operative conditions and all the necessary information sources. The following two phases consist of a **deskwork** and a **fieldwork**.

11.1.1 Desk Work

The items under the heading of deskwork comprise a list of secondary sources, which are divided as of *internal* and *external* nature. The *Company invoices, general correspondence, budgets, and balance sheets form the internal sources*. A large quantity of these data is not often used but it is a mistake since substantial information are thus disregarded with distortion of the resulting ultimate market analysis vision. In particular it is extremely recommended to check the original documents, in order to obtain a wider range of information elements.

The collection of internal data does not require particular methods and techniques but it is essential to know the company organization and to establish an adequate system framed to the specific market analysis and company profile management. For instance the analysis of sales should be arranged in order to supply information about market structure, products, geographic areas where business relationships have been established, customers' characteristics, customers needs and market infrastructures.

The *external sources* include statistical data, public bodies' reports, and Chamber of Commerce reports. The difficulty for an adequate weighing of the external data is that there are many sources and a lot of data to be taken into account for evaluation. Moreover it is necessary to pay a great deal of attention to the accuracy of these data and what period they do refer to, since they could not be up-to-date.

If the market research is addressed to a new product it is obviously difficult to find statistics and specific information about. So in this case it is more convenient and effective to start from the scratch and set on an accurate fieldwork.

11.1.2 Field Work

A fieldwork is the collection of main data, focusing on
- Definition of “industrial universe and industrial framework”
- Execution of a range of interviews
- Set up of an inquiry method
- Preparation of a scheme of questionnaire
- Assessment and selection of an interview method

The “industrial universe” is the whole number of Companies that use or could be considered as potential users of a specific product. Moreover it is quite important to identify all the sectors and merceological segments involved in order to make a complete search. The collection of specific data can be made by a questionnaire whose preparation is quite delicate to avoid any prejudice or misunderstanding in the potential interviewees.

For the questionnaire, it is necessary to establish the items to be discussed and submitted to the interviewees according to the profile of the committed market research. Some information categories are listed below:

- *Facts and knowledge*. Opinions about particular products, services, industrial fields or organizations; the way they are gathered and how much they are known.
- *Opinions*. Identification of attitudes towards products, services or organizations and how much they are rooted.
- *Reasons*. The reasons of some market attitudes that are the needs and wish which stimulate buyers towards well-defined products or services.
- *Purchase attitude*. Definition of consumption models in a well-defined period of time. Suggestions for future attitudes could be collected even directly through the quantification of different satisfaction levels for existing products, expectation nature.
- *Purchase process and organization*. The components of the “purchase center”, basis of its power, their priorities and supplier evaluation.
- *Statistics information*. Data concerning companies such as their business fields, number of employees, invoicing volume, their markets.

11.1.3 Final Stage of the Undertaken Research

Once the market research is completed all the material must be organized through the following steps.

- Completeness, accuracy and consistency in the check of questionnaires .
- Coding of answers, that is a technical procedure classifying, elaborating and analyzing the collected data.
- Collected data processing in order to elaborate data for an immediate comprehension and analysis.
- Data analysis.
- Report draft.
- Final Report

11.1.4 Evaluate the Market Demand for a New Product

It is quite important to evaluate the potential demand for a new product with well identified performances and characteristics and thus to decide marketing strategies. Market requirements are the total quantity of products that could be bought in a period of time and in a particular geographic area.
It is well understandable that there is a big quantity of factors influencing the demand of a product. These factors can be both external and internal in character.

The maximum number of users favorably incline to buy is identified as “potential market”. This last one is very dynamic since it can change according to the general economical situation. In the economical world any company is interested in acquiring a wide share of the potential market. The overall view of the trend of sales vs. industrial marketing effort can be represented in two different

modes as sketched in the following Fig.11.1 and Fig. 11.2. The following items are evidenced in the market share of a company, the feasible quantity of a product and the level of the potential demand.

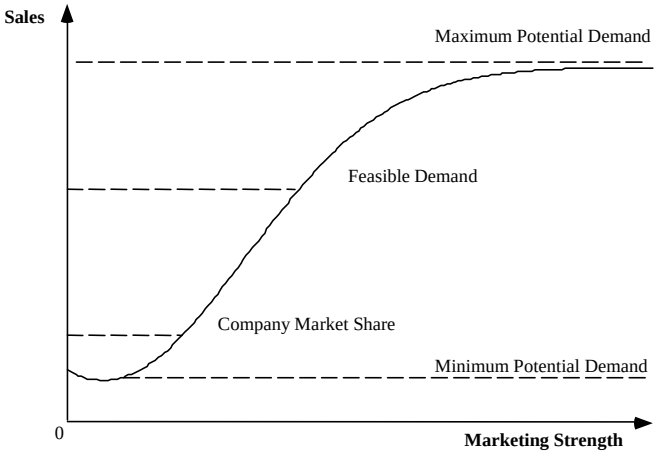


Fig. 11.1 Sales trend *vs* marketing strength

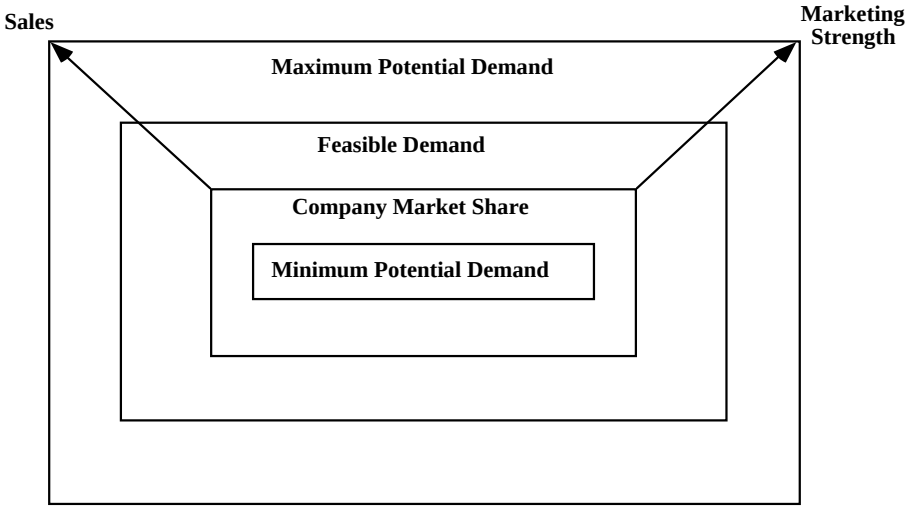


Fig. 11.2 Sales trend *vs* marketing strength

The maximum level of the potential market/demand is often a holistic one as the potential market may evolve in almost continuous manner within the time depending on very many external factors independent of the product cost-performance profile. For instance the potential market during a period of economical recession can be very much affected with a heavy penalization with respect to the virtual situation in a different economical boom (Fig.11. 3).

In general a forecast on product sales is based on three elements:

- Connection among different variables
- Stability of the market and economical situation
- Management

The most important element concerns the existence of a relationship between variables, which must be evaluated (*dependent variables*), usually sales, and a second variable *(independent variables*), usually time. The second element is that the relationship between variables is considered steady or changeable in a foreseeable way. In any case this is a rare event if not impossible since future is always uncertain and circumstances can change suddenly. A third element finally considers that the variables can be

summarized into mathematical models easy to be used and understood. However data for these models should be available at a reasonable cost, that is not often the case.

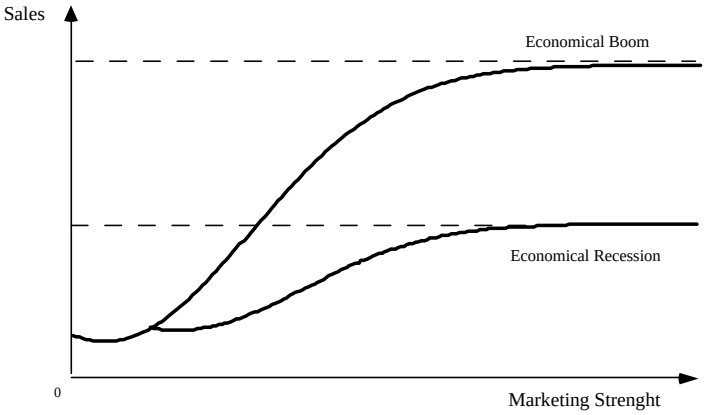


Fig. 11.3 Effect of the general economic situation on sales trend *vs* marketing strength

In order to forecast the market demand there are three different techniques based on

- Recording of opinions
- History of products
- Market research

The first method is based on opinions of people who usually work inside the companies but being very subjective, results can be affected by a broad level of uncertainty. The method based on the product history is an analysis of past sales with their trend. Market researches get their information from salesmen, experts, effective and potential customers. Salesmen can supply useful information since they are in touch with customers and are able to verify future purchases. Customers can reveal what kind of products they are interested in and be helpful in understanding the future demand.

11.1.5 Conclusive Remarks on Market Research

Creativity and innovation have an important role in the industrial evolution but it must be taken into account that products should satisfy potential users by meeting their needs.

The success of a new product depends on the market approval and following requests. It is important to know all the needs of users in order to create a successful product. This is the aim of market researches that should be made frequently to assess what the market is requiring. Moreover the market research individualizes the characteristics and the advantages which make a product different from the others. The identification of these factors also allows for the management to develop a new product effectively and in accordance with users' expectations. Unfortunately most companies are used to make market researches in a rudimentary and not systematic way and often under the pressure of a decrease in sales volume.

These days this is not the case holding time for the environmentally degradable polymeric materials and plastics for which companies with consolidate experience in the production of polymeric materials and in converting them in commodity or specialty plastic items, have already faced the scenario for the future entering on the market of EDPs. The requirements for a sustainable industrial development can act as powerful key to provide the access to EDPs in merceological segments for which the most convenient way to a recovery of the post-consume items is represented by bio-recycling under controlled conditions.

11.2 Marketing of Environmentally Degradable Plastics

EDP materials including the biodegradable ones used for biomedical and pharmaceutical applications, are meeting an ever increasing interest especially at industrial level as shown by the fairly high number of patents application and assignment.

The target markets for biodegradable plastics are:

- Packaging materials: single or limited use disposable packaging and film applications
- Disposable non-woven and hygiene products (diapers, personal care, medical plastics),
- Consumer goods -- cups, plates, cutlery, containers, egg cartons, razor handles, toys etc.
- Coatings for paper and film.
- Marine plastics -- fishing lines, nets, pots etc., plastics used in ships (Marpol treaty)
- Agricultural mulch film, and other agricultural related plastic products
- Loose-fill and rigid foam packaging products

Specialty markets will be established first before large-scale usage of biodegradable resin occurs. This will consist of toys, pens, planters or other products where biodegradability is a novelty. In three to five years the most promising application is the use of biodegradable plastics for organic trash bags. Biodegradable plastic bags have better strength and water resistance than paper. In the future, it is likely that other waste streams, such as food waste will be required to be composted. This would increase the size of the market for biodegradable bags. Cost is one of the major barriers to successful market penetration. It has been found that the customer/end-user may pay a 10 to 20% premium on biodegradable or environmentally friendly products. However, most of today's biodegradable resins are much more expensive, in part due to limited production.

Composting is an environmentally sound approach to transfer biodegradable waste, including new biodegradable plastics, into useful soil amendment products. Composting plastic and paper waste, along with other biodegradable waste, can generate much needed carbon-rich soil (humic material). When the economic costs of soil loss and degradation and off-site effects are included in the cost/benefit analyses of agriculture, it makes sound economic sense to invest in composting programs to reduce widespread erosion. Composting bio-wastes not only provides ecologically sound waste disposal but also provides much needed compost to maintain the productivity of soil and sustainable agriculture.

A number of factors have contributed to the growth of the composting infrastructure:

- Legislative mandates have been the biggest factor. Typical provisions include separation or ban of yard waste from landfills. Some are outright bans, others offer incentives and endorsements.
- Established or raised recycling goals. Depending upon the composition of MSW, the only way to reach goals of 30, 40, or 50% recycling is to compost. Many countries consider it a way of recycling.
- Relative costs for disposal are much higher. The unpopularity of landfills, and strict regulations governing them, have pushed landfill costs sharply upward. In many countries composting is becoming competitive with other waste management approaches.
- Separation technologies have improved and contaminants can be effectively separated. In addition, community separation programs have had excellent participation.
- Public procurement policies. Markets for the finished compost have been created by Government fiat. Government agencies and local governments are required to procure compost product for land maintenance activities, highway construction, landscaping, re-cultivation, and soil erosion control.

Organic recycling through composting is a major initiative throughout Europe. An EC Packaging Directive requires 25-45% recycling rates with organic recycling being the major component. Germany and the Netherlands require their bio-wastes (organic wastes) to be collected for "organics recovery" (Composting, bio-gasification) programs. The Scandinavian countries also have major composting infrastructures in place.

Conclusion

The development of EDPs has increased rapidly over the last decade though the commercial application is still in an early stage. Consensus has been reached that standard for definitions and tests as well as precise acceptance criteria are an absolute necessity for this industry to be successful. Worldwide harmonious Standards have been developed and certification/logo schemes are being put in place based on these National & International Standards. Government needs to provide a market-pull for EDPs by purchasing requirements, incentives, and/or regulations. Organic recovery through composting or anaerobic digestion (bio-gasification) is creating the pull for EDPs products.

Exercise

Students will be divided into two groups and given certain EDPs new product. Using the methods learned, both are required to work out a marketing plan of this product based on a market analysis. Then each group will present and justify their results. They will make comments on other's result and suggestion on how to improve.

CHAPTER 12 INDUSTRIAL CASES

Objectives

EDPs have been developed and used since the problems of conventional plastics were exposed to human being. But due to the obstacles of the high costs, the undeveloped compost infrastructure, the low level of public awareness and the half-fledged stages of legislation, mass production as well as public consumption of EDPs is yet far from the goal where EDPs are indispensable for our lives. From the industrial cases of this chapter regarding the major EDP products and their producers, we can have a perspective about the real world of EDPs.

Summary

In 1998, total demand for EDPs in the United States, Western Europe and Japan reached 18,000MT valued at over U$95million. Total consumption of EDPs in these three regions will increase to over 91,000MT in 2003, representing an average annual growth rate of over 37% over the 5 year period from 1998 to 2003. This growth projection assumes that approximately 140,000MT of new production capacity are brought on stream prior to 2003, allowing producers to achieve dramatic price reductions.

Supply/Demand for EDPs by Major Regions - 1998 (1,000MT)

	US	Europe	Japan
Annual Capacity	11	29	6
Production	10	8	1.5
Consumption	9	7	2

In 1998 the United States was the dominant market for EDPs, accounting for about half of world consumption; Western Europe accounted for about 40% and Japan accounted for about 10%. However, a large proportion (over 60%) of the world's 1998 production capacity was located in Western Europe.

Major EDP producers by Major Regions - 1998

United States

Company/location	*Annual Capacity (million p. in 1999)*	*Remarks*
Bioplastics/Lansing, MI	<0.5	**Envar**, reactive blend of starch, polycaprolactone
Cargill Dow Polymers/ Savage, MN	10 (a)	**Eco-PLA,** poly lactic acid Started production in 1994
Dupont/ Old Hickory, TN	Unknown(b)	**Biomax,** PET based
Eastman Chemical Company/	3	**Easter,** Bio copolyester Started production in 1994
Kingsport Novon International	Unknown(c)	**Novon,** plasticized starch
Planet Polymer Technology	1	**EnviroPlastic,** custom engineered alloys
Union Carbide Corporation	10	**TONE,** polycaprolactone
Uni-Star Industries, Ltd.	<1	**Star Kore,** starch-based polymers
Total	25	

Notes:

(a) Cargill Dow is expecting the capacity of the Savage, Minnesota plant 20million pounds per year as demand increases.

(b) Dupont has modified its facility in Old Hickory, Tennessee to provide capacity to manufacture Biomax. The total plant capacity(for both PET and Biomax) is 200 million pounds per year.

(c) ECOssais purchased the assets of Novon out of Chapter 11 bankruptcy in 1998 related to themanufacture of Degra Novon. Novamont purchased all rights related to Poly Novon technology

Europe

Company/location	*Annual Capacity (1,000MT in 1999)*	*Remarks*
Bayer Antwerpen nv Antwerpen, Belgium	10	**BAK**
Neste Chemistry Porvoo, Finland	Unknown(a)	Poly lactic acid
BASF Aktiengesellschaft/ Ludwigshafen, Germany	10(b)	**Ecoflex**, Copolyester
Biotech GmbH Enmerich, Germany	1(c)	Starch-based
Novamont S.p.A Terni, Italy	8(d)	**Mater-Bi**, Starch-based
Solvay Interox/ UK	Unknown(e)	**CAPA**, polycaprolactone

Notes:
(a) Neste Oy produces poly lactic acid in pilot quantities.
(b) BASF utilizes an existing shared polyester facility at Ludwigshafen to produce Ecoflex at capacity up to 10,000MT per year.
(c) E. Khashoggi Industries acquired 100% of Biotec shares from Militta in April 1999.
(d) In 1990, Novamont started up its production line with a capacity of 4,000MT per year. A second line was completed in 1997, bringing total Mater-Bi capacity to 8,000MT per year. Maize/Corn is the primary source of starch.
(e) Solvay produces both low-molecular-weight polycaprolactone oligomers for use in the polyurethane industry and high-molecular-weight polymer (CAPA 600series) at its Warrigton facility. It also operates a 15,000-ton per year caprolactone monomer facility at the site.

Japan

Company/location	Annual Capacity (1,000MT in 1999)	Remarks
Chisso Petrochemical Corp./ Chiba	2.0(a)	Novon, Starch alloy
Daicel Chemical Industries/ Hiroshima	0.3(b)	Celgreen, PCL
Mitsui Chemical, Inc./ Fukuoka	0.5(c)	Lacea, PLA
Shimadzu Corporation	0.1(d)	Lacty, PLA
Showa High Polymer	0.01(e)	Bionelle, Copolyester

Notes:
(a) Chisso has licensed Wamer-Lambert's Novon technology for starch based polymers. The company expanded its compounding capacity to 2,000MT per year in 1995.
(b) The total capacity for Celgreen is 300MT per year. Much of the product from this facility is used in other than biodegradable application.
(c) Mitsui Toatsu Chemical Inc. and Mitsui Petrochemical Industries Ltd. merged into MitsuiChemical, Inc. as of October 1997. Mitsui Chemical's semicommercial facility started up in Jan. 1996.
(d) Shimadzu's semicomercial plant has a capacity of several hundred MT per year and started up in April 1997.
(e) Showa High Polymer's Takasaki plant is a pilot plant for special grades.
(f) Showa High Polymer completed its Tatsuno plant in October 1993.

Overall Industry Trends

1. Nearly all EDPs are products that are in the early stages of market development. Producers have developed second-generation products that demonstrate good biodegradability characteristics, and they are in various stages of commercializing these products.

2. Between 1998 and 2003, worldwide growth in EDP consumption will be significantly higher than GDP growth. This growth will be possible due to new capacity coming on stream during the period, which should allow producers to dramatically drop prices.

3. The industry is establishing a reputation with consumers for having products that are good forthe environment. An important part of this effort is establishing biodegradability/compostability tests and standards that are well grounded scientifically.

4. One of the main obstacles to widespread use of EDPs is cost. Although prices are expected todecline as more capacity comes on-line, EDPs will remain more expensive than commodity polymers through the year 2001 and for the foreseeable future.

5. A second major obstacle is lack of composting infrastructure. Large-scale composting would provide the ideal disposal environment for spent biodegradables. Western Europe has made progress toward developing a composting infrastructure, but infrastructure is lacking in the U.S.

6. Legislation in Western Europe, and to a lesser extent in the U.S. and Japan, has helped to spur demand. Future legislation will be depend not only the environmental awareness of politicians but also on their perceptions of how these polymers fit into plastic recycling strategies. The European Union Packaging Waste Directive accepts composting as a form of recycling.

7. Due to the high cost of EDPs, applications are in special niches with unique environmental considerations. Loose-fill packaging and compost bags are the two major end uses, constituting nearly 90% of demand in 1998. Food service items are also an important application.

Case 1. Cargill-Dow

Background

In late November 1997, The Dow Chemical Company, the fifth largest chemical company in the world and Cargill, an international marketer and processor of agricultural, food, financial and industrial commodities formed Cargill Dow Polymers LLC, a 50/50 joint venture company, to develop and market polylactic acid (PLA) polymers derived from renewable agricultural resources, such as corn.

Cargill's EcoPLA team was established in 1989 as part of Cargill's corn Milling Division, in an effort to develop a large-scale, cost-effective approach to PLA production and during a successful 15-month joint development program, Dow made intensive investments. Cargill and Dow combined efforts to evaluate the potential for PLA polymers as well as the benefits of joint PLA product development and marketing.

The combination of these two global companies has a synergy effect in that Cargill provides process technology and a low cost manufacturing position for lactic acid and PLA resins, as well as a strong patent portfolio of critical technology. In the meantime, Dow has world class polymer science, applications technology and access to a global customer base that accelerate the commercialization of PLA.

The Joint venture has offices in Midland, Michigan; Minnesota; and Freeport, Texas. In addition, there are sales personnel in Europe and Asia. Key application areas targeted for PLA polymers include cast films, fibers and non-wovens, blown films, rigid containers and paper/board coatings.

NatureWorks and Application

Cargill Dow LLC has invented a new technology to produce performance polymers entirely from annually renewable resources. Using their patented technology to produce NatureWorks, they start with natural sugars (derived from plants such as corn, wheat, beets and rice) and use fermentation to create lactic acid (a food additive) and some simple refining steps to create polylactide polymers (PLA). The result is the only commercially viable polymer to combine performance and cost competitiveness with outstanding environmental benefits.

The application is mainly as packaging in the following fields:

1. Films
   - ♦ Confectionery twist wrap to premium wrapping for flowers, toiletries and prestige gifts
   - ♦ Bags for compost and garden refuse as well as agricultural mulch films
   - ♦ Flavored cereals, coffee packs and pet foods
   - ♦ Window films for envelopes, cartons and other packages
2. Containers
3. Coated Papers and Boards

Manufacturing Process

Lactic Acid is difficult to polymerize directly to high-molecular weight (50,000-110,000) polymers. In current commercial practice, lactic acid is first oligomerized to a linear chain with a molecular weight of 300-3,000 by removing water. The oligomer is then depolymerized to lactide, a cyclic dimer. This six-member ring is purified and subjected to ring-opening polymerization to produce poly (lactic acid) with a molecular weight 50,000, see Fig. 12-1.

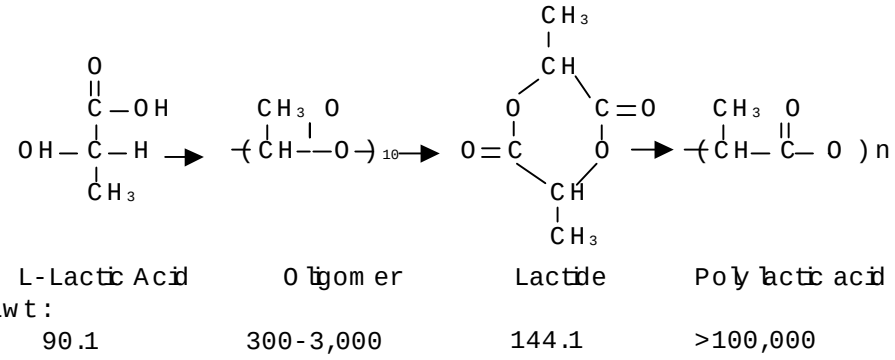


Fig. 12.1 Chemistry of PLA

Renewable Resource

A renewable resource such as corn is milled, separating starch from the raw material. Unrefined dextrose, in turn, is processed from the starch. Future technology enhancements may eliminate the milling step and allow for utilization of even more abundant agricultural by-products.

Fermentation

Cargill Dow LLC turns dextrose into lactic acid using a fermentation process similar to that used by beer and wine producers. This is the same lactic acid that's used as a food additive and is found in muscle tissue in the human body.

Intermediate Production

Through a special condensation process, a cyclic intermediate dimer, referred to as a lactide, is formed.

Polymer Production

This monomer lactide is purified through vacuum distillation. Ring opening polymerization of the lactide is accomplished with a solvent-free melt process.

Modification for Customers

A wide range of products that vary in molecular weight and crystallinity can be produced, allowing Cargill Dow to modify PLA for a wide range of applications.

Cost Structure

Production from L-Lactic Acid, 1998(a)(Cents/pound)

Net Variable Costs	
Raw Material	122
Utilities	1
Labor and Supplies	22
Other Production Costs	
Depreciation(b)	18
Others(c)	73
Total Production Cost	236(U$5.20/kg)

(a) Cost are for a 10 million pound-per-year capacity plant, U.S. Gulf Coast, Assumed raw material costs include U$0.79 per pound for L-lactic acid.
(b) Depreciation is based on a total investment of U$21million.

(c) Other costs include plant overhead, taxes and insurance, general and administrative expenses, sales and research.

Case 2. Mater-bi

Novamont

Novamont is recognized internationally as a leader in EDP technologies. Originally a subsidiary of Montedison (an unit of The Ferruzzi Group), Novamont was acquired by a group of commercial banks, including Banka Commercial Italiana and Investori Associate II, in 1996.Novamont developed and is making Mater-Bi, a family of EDPs based on materials derived from corn, wheat, and potato starch. Mater-Bi materials consist of starch blended with PCL. Novamont made a successful progress in commercialization of Mater-Bi in Australia during 2000 Sydney Olympics.

The result of a strong collaboration bringing together National Starch's unique expertise in natural polymer chemistry and Novamont's experience in material science and technology, the cutlery has made a significant contribution to the target of 80% organic waste from the Olympics. Further, the Mater-Bi cutlery has already been used in McDonalds restaurants in Austria and Sweden for 3 years.

Mater - Bi

Mater-Bi, a family of EDPs consists of starch blended with PCL. Mater-Bi is trademarked by Nonamont and was first marketed in 1992. The initial Mater-Bi contained only 10% starch, but later up to 50-70%, with an eventual target of 90%.

Like conventional plastics, Mater-Bi scrap can be recycled. When disposed of, Mater-Bi acts the same as organic domestic waste such as food. It can be also incinerated with non-toxic or metallic residue. Landfilling is not recommended because other recycling options are more favorable.

Mater-Bi material has mechanical and physical properties similar to PE, with tensile strength decreasing inversely to starch content. The hydrophilic nature of the polymer provides Mater-Bi films with advantages such as supple feel and better printability and compatibility with water-based inks. But due to Mater-Bi's hydrophilic nature, end products cannot contain water and have reduced mechanical properties when exposed to relative humidity of over 80%.

Application

1. Personal care
Diapers sanitary napkins, cotton swabs, and soap holders, containers for cosmetics.

2. Catering
Plates, cutlery, cups, straws, cup lids.

3. Packing
Films for dry food, thermoformed trays, packing systems, foamed items, yogurt cups, compostable shopping bags.

4. Small commodities
toys, pet products, pens, cartridges, pencil sharpeners, rulers.

5. Agriculture & waste
Mulch films, nursery pots, twines, Bags and liners for organic waste

Manufacturing Process

Mater- Bi is prepared by blending a starch-based component with other components in an extruder in the presence of water or plasticizer. The temperature and pressure conditions are such that the starch is destructurized, and the composition forms a thermoplastic melt. Three main classes of Mater-Bi is commercially available:

- Class Z - starch and polycaprolactone

- Class V - starch content greater than 85%
- Class Y - starch and cellulose derivatives

Prices

German list Prices for Mater-bi as of 1999:

	DM/kg	U$/kg
Class Y	8	4.6
Class Z	9	5.15
Class V	3.2	1.85

Case 3. Kuraray-PVA

Kuraray is one of the leading chemical companies in Japan with its 75- year- history covering manufacture of functional resins, fine chemicals, man-made leather, medical products, synthetic fibers. Kuraray was the world's first company to industrially produce PVA resin under the brand name “EVAL”, which is the raw material for the synthetic fiber vinylon.

EVAL-Application

- Textile sizing and finishing.
- Laminating adhesive in solid fiberboard and spiral wound tubes and cores, and a Component in industrial adhesives.
- Sizer in paper and paperboard manufacture.
- Water-soluble films for packaging and release applications.
- Protective colloid in emulsion polymerization processes.
- Photosensitive coatings.
- Binders for building products such as ceramics, ceiling tiles, floor coatings and particle board.
- Binders for pigmented paper coatings, ceramic materials, and nonwoven fabrics.

PVA was commercialized as a water-soluble synthetic polymer for use as an ingredient of the synthetic resin, vinylon. Since then, its applications expanded dramatically for textile sizing agents, paper processing agents, emulsification dispersants, as well as in the fields of films and general industrial use.

In the paper production field, PVA has become an essential product as a dispersant, binder; and surface coating agent for heat- and pressure-sensitive paper, and a surface coating agent of ink-jet paper. It is also used for boosting the strength of information recording paper, lightweight newspaper and newspaper for color printing, preventing the penetration of printing inks and fluffing in order to improve the recycling rate of liner white cardboard. It is also used to improve surface and printing luster.

In the adhesive field, cold-curing, water-resistant woodwork adhesives instant and high-speed curing and two-pack type with main ingredient and primer; high-performance water-based vinyl urethane adhesives; and water-soluble hot-melt adhesives are used in a broad range of fields.

As emulsification-suspension agents, PVA is mainly used for water-resistant emulsifiers as a water-resistance-boosting agent of polyvinyl acetate EVA emulsion. For PVC polymerization suspension agents, PVA is mainly used as the main and secondary dispersion auxiliary agents.

Manufacture Process

PVA is the largest volume water-soluble polymer produced in the world. It can be commercially obtained by the hydrolysis of poly(vinyl acetate)PVAc by batch and continuous processes. The latter is

wider utilized in the large-scale productions. In the industrial continuous process a typical free radical polymerization of vinyl acetate is followed by the alkaline alcolysis of PVAc, see Fig. 12.2. PVA molecular weight control is usually accomplished by choosing appropriate residence time in the polymerization reactor, vinyl acetate feed rate, solvent (methanol) concentration, radical initiator concentration, and polymerization temperature.

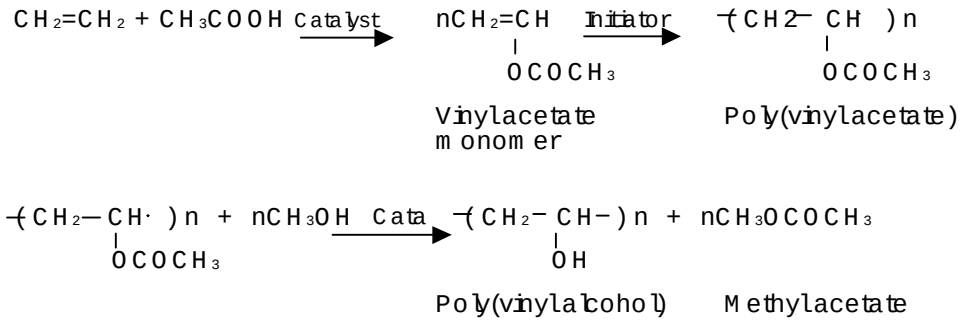


Fig. 12.2 Chemistry of PVA

Case 4. Bionelle

Showa-Denko

Showa is a major Japanese producer of unsaturated polyesters, emulsions, and phenolics, with sales in 1993 exceeding $261 million. The company is a member of the Showa Denko group, which also owns Showa Denko, K.K., a multiline chemical company. It is producing Bionolle (R) polyesters chains extended with disocyanate

Bionelle

Showa High Polymer began production of Bionelle at its 3,000MT per year plant at Tatsuno, Hyogo Prefecture in October 1993. It had been operating a Bionelle bench-scale plant since 1991 to evaluate the product's processibility and other characteristics.

Bionelle is thermoplastic polyester produced from glycols and aliphatic dicarboxylic acids such as succinic acid or modified acids see Fig. 12.3. Polyethylene succinate and polybutylene succinate/adipate are major Bionelle polyesters with useful physical properties. The two major grades are Bionelle #1000, polyethylene succinate (PBSU), and Bionelle #3000, polybutylene succinate adipate (PBSA). Bionelle #3000 biodegrades more quickly than Bionelle #1000 in compost, moist soil and water. In 1997 Bionelle acquired the European compost certification, OK Compost, and then acquired OK Biodegradable certification.

Application

- Injection Molding : Cutlery, brush
- Multi-filament : Conjugated fiber, knotless net, Raschel woven net, Non woven fabric for diaper, disposable medical supplies, sanitary napkin
- Mono-filament : Fishing net, rope
- Film : paper lamination, packaging, shopping bag
- Sheet extrusion : Food tray
- Blow molding : Sampoo bottle, drug bottle, cosmetic bottle, beverage bottle

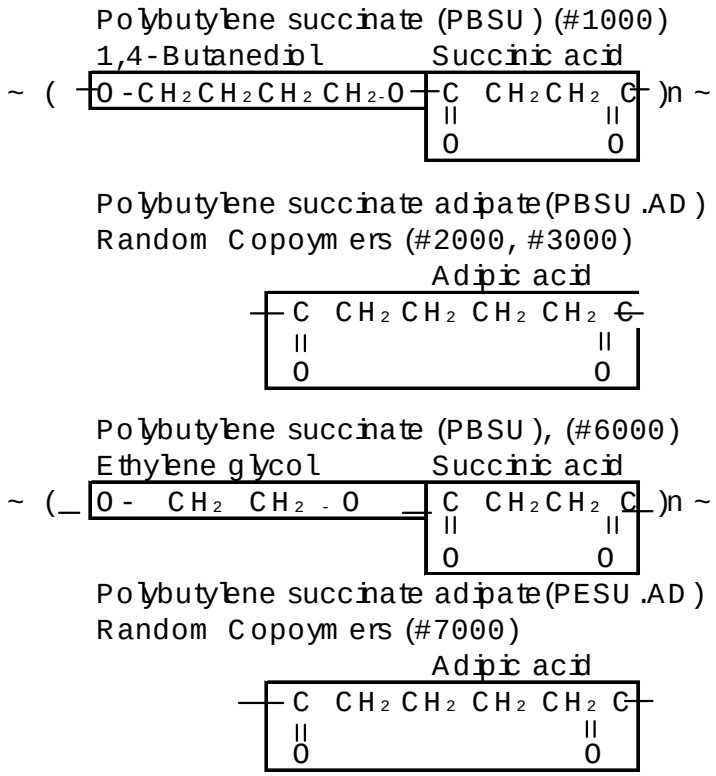


Fig. 12.3 Chemistry of Bionelle

Case 5. Polyester Amides

Bayer Corp.

Bayer Group is an international chemical and healthcare company that operates in nearly every country worldwide. In 1997, annual sales totaled DM 55 billion and production, DM5.4 billion. Bayer Corp is a public company within the Bayer Group that operates businesses in the field of healthcare, life sciences, chemicals, and imaging technologies.

BAK

Bayer manufactures BAK 1095 and BAK 2195 biodegradable thermoplastic polyester amides. BAK 1095 resin is a semicrystaline, yet largely transparent thermoplastic, that breaks down into water, carbon dioxide and biomass under aerobic conditions. The degradation rate is comparable to that of other organic materials that are composted. In addition to its compostability, BAK 1095 is soluble in ethyl alcohol. The resin will not degrade in a landfill or under other sealed conditions.

The property profile of BAK 1095 resin has many similarities to low-density polyethylene (LDPE). In addition to being fully degradable under composting conditions, the resin is also noted for its high toughness and tensile strain at break. BAK 1095 resin can be processed into film and also into extruded or blow-molded parts on conventional machinery used for processing thermoplastics. It is suitable for thermoforming and can be colored, printed, hot-sealed and welded. Bayer has developed BAK 2195, an injection-molding grade of this biodegradable thermoplastic that exhibits greater stiffness.

Application

Potential applications for BAK 1095 resin include uses in the horticulture, agriculture and food sectors where highly soiled plastics could be disposed of in conjunction with compostable waste.
Price of BAK 1095 is U$2.60/pound.

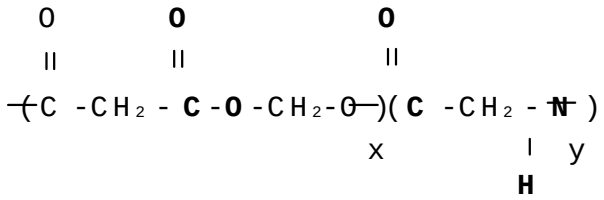


Fig. 12.4 Chemistry of BAK 1095

Case 6. Polyesters

BASF

BASF AG, founded in 1865, is the headquarters for the global BASF Group of chemical businesses. BASF offers a full range of chemical and chemical-related products in Europe, North America, Asia, South America, and other growing markets. In 1997, BASF had consolidated sales of $32billion and over 100,000 employees worldwide.

ECOFLEX

BASF has developed ECOFLEX, a biodegradable copolyester. Biodegradable and ecotoxicologically safe monomers, such as adipic acid, dimethylterephthalate, and 1,4-butanediol, were used to create the biodegradable copolyester, which consists of aliphatic diols and aliphatic and aromatic dicarboxylic acids. Hydrophilic components, monomers with branching effects, and chain lengthening compounds are also incorporated to produce products with different characteristics. For example, BASF has developed a version of this biodegradable copolyeter with high tensile strength and high percentage elongation to break for film application. BASF has also been developing biodegradable copolyester-starch blends, for application such as flexible films.

Application

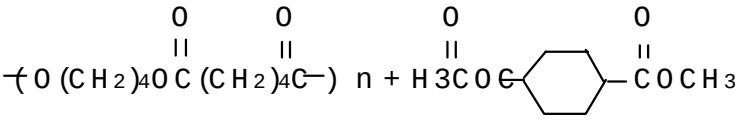
- Disposal goods : cup, knife, fork, razor, straw, diaper
- Paper coating : disposable bowl, cup, pot, tray
- Agriculture and Horticulture : mulch film, plant pot, rope or string, clip, matrix for controlled release of fertilizer/pesticides
- Bottle : shampoo, detergent, medicine, cosmetic and beverage
- Packaging : loose-fill packaging, shrinkable film
- Medical : injection syringe, mouthpiece for endoscope, casting tape

Manufacturing Process

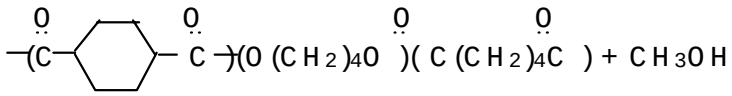
Aliphatic/aromatic copolyester is some of the newest biodegradable polymers commercially available. They are prepared as high-molecular-weight polymers in a bulk three-step polymerization. First hydroxyl-terminated aliphatic polyester prepolymer is made by dehydration condensation:

1,4-butanediol Adipic Acid

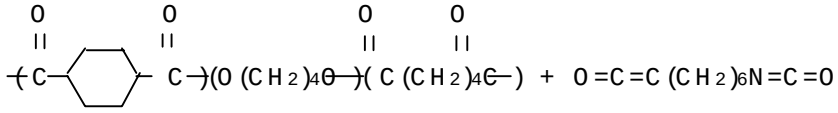
The prepolymer next undergoes transesterification polycondensation with DMT:

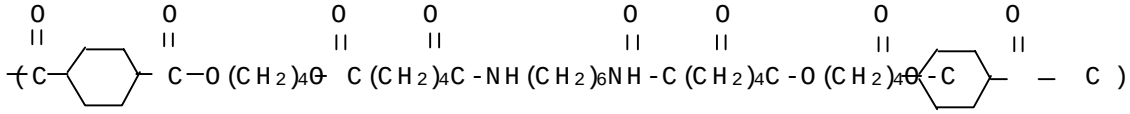


Dimethyl Terephthalate



The aliphatic/aromatic copolyester is formed into a high-molecular weight polymer by chain extension, for example by reacting with hexamethylene:





Cost Structure

Aromatic Copolyester Production, 1998(a)(Cents per pound)

Net Variable Costs	
Raw Material	84
Utilities	1
Labor and Supplies	9
Other Production Costs	
Depreciation	10
Others(b)	**22**
Total Production Cost	**126(U$2.78/kg)**

(a) Cost are for a 22 million pound-per-year capacity plant, U.S. Gulf Coast, Assumed raw material costs include U$0.99 per pound for 1,4-butanediol.
(b) Other costs include plant overhead, taxes and insurance, general and administrative expenses, sales and research.

Case 7. Polyester

Eastman

Eastman Chemical manufactures a wide variety of chemicals, fibers and plastics. Eastman has produced polyesters for 30 years and is the world's leading producer of thermoplastic polyesters, such as PET. It plays a major role in polyester recycling efforts.

Eastar

Based on its expertise in cellulosics and polyester core technologies, Eastman has developed several environmentally responsible, high quality specialty polymers. It manufactures Eastar; a biodegradable thermoplastic copolyester made from conventional diacids and glycols.

Eastar A150 Copolyester is a poly (1,4-cyclohexylene-dimethylene terephthalate/isophthalate). It is produced by reacting terephthalic acid and isophthalic acid with the glycol 1,4-cyclohexanedimethanol. Eastar A150 is intended primarily for extrusion into film and sheeting for packaging applications. It has excellent hydrolytic stability and good heat stability. Eastar A150 copolyester can be used in food contact applications as well as in packaging material for meat or poultry foods prepared

Application

- Blister packaging
- Food packaging
- Food-contact applications
- Rigid medical

APPENDIX 1.

DEFINITIONS RELATED TO WASTE MANAGEMENT AND EDPS

Activated sludge Biomass produced in the aerobic treatment of wastewater by the growth of bacteria and other micro-organisms in the presence of dissolved oxygen. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Activated sludge bio-mass produced in the aerobic treatment of waste water by the growth of bacteria and other micro-organisms in the presence of dissolved oxygen.(ISO/FDIS14851)

Activated sludge bio-mass produced in the aerobic treatment of waste water by the growth of bacteria and other micro-organisms in the presence of dissolved oxygen.(ISO/FDIS14852)

Aqueous waste liquid waste that predominantly consists of water.(CEN TC 292 N329)

Asbestos waste waste that contains significant amounts of asbestos. NOTE - The value of "significant" may be defined in legislation. (CEN TC 292 N329)

Battery waste waste that has been batteries or components of batteries. NOTE - See waste Directive (and Battery waste Directive, in preparation). (CEN TC 292 N329)

Biochemical oxygen demand (BOD) The mass concentration of dissolved oxygen consumed under specified conditions by the aerobic biological oxidation of a chemical compound or organic matter in water; expressed in this case as mg oxygen uptake per mg or g test compound. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Biochemical oxygen demand (BOD) the mass concentration of the dissolved oxygen consumed under specified conditions by the aerobic biological oxidation of a chemical compound or organic matter in water, expressed as milligrams of oxygen uptake per milligram or gram of test compound.(ISO/FDIS14851)

Biodegradability Potential of a material to be biodegraded. (CEN 261069:1996)

Biodegradable A material is called biodegradable with respect to specific environmental conditions if it undergoes a biodegradation to a specified extent within a given time measured by standard test methods. (CEN 261069:1996)

Biodegradable waste See Landfill Directive. (CEN TC 292 N329)

Biodegradation Degradation caused by Biological activity especially by enzymatic action leading to a significant change in the chemical structure of a material. (ISO/CD 16929)

Biodegradation Degradation caused by biological activity especially by enzymatic action leading to a significant change of the chemical structure of a material. (CEN 261069:1996)

Biodegradation Degradation caused by biological activity especially by enzymatic action leading to a significant change of the chemical structure of a material. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Biodegradation Degradation caused by biological activity especially by enzymatic action leading to significant change of the chemical structure of a material. (ISO/CD15986.2)

Biological treatability The potential of a material to be aerobically composted or anaerobically bio-gasified.(Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Biological treatability The potential of a material to be aerobically composted or anaerobically biogasified. (ISO/CD 16929)

Biological treatability The potential of a material to be aerobically composted or anaerobically biogasified. (ISO/CD15986.2)

Biological waste waste that has been living organisms.(CEN TC 292 N329)

Bio-sludge waste sludgy waste arising from biological treatment of sewage or sewerage sludge.(CEN TC 292 N329)

Bottom ash combustion residue arising at the bottom of combustion furnaces.(CEN TC 292 N329)

Bulky waste waste that due to its bulky character needs special considerations for its management.(CEN TC 292 N329)

Chemical degradation Degradation caused by chemical agents including catalysts leading to a significant change of the chemical structure of a material. (CEN 261069:1996)

Chemical oxygen demand (COD) The mass concentration of oxygen equivalent to the amount of a specified oxidant consumed by a chemical compound or organic matter when a water sample is treated with that oxidant under defined conditions; expressed in this case as mg oxygen consumed per mg or g test compound. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Chemodegradability Potential of a material to be chemical degraded. (CEN 261069:1996)

Chemodegradable A material is called chemodegradable with respect to specific chemical agents if it undergoes a chemical degradation to a specified extent within a given time measured by standard test methods. (CEN 261069:1996)

Claim verification confirmation of the validity of an environmental claim using specific pre-determined criteria and procedures with assurance of data reliability. (ISO/CD 16929)

Co-combustion Combustion of a mixture of fuels. (CEN 261069:1996)

Combustible material Any material, capable of releasing energy by burning. (CEN 261069:1996)

Combustion Process of burning. Chemical conversion by means of an oxidant, usually oxygen, accompanied by the release of heat. (CEN 261069:1996)

Combustion residue waste that remains after combustion. NOTE - See Waste Directive. (CEN TC 292 N329)

Compost Organic soil conditioner obtained by biodegradation of a mixture principally consisting of various vegetable residues, occasionally with other organic material and having a limited mineral content. (CEN 261069:1996)

Compost Organic soil conditioner obtained by biodegradation of a mixture principally consisting of various vegetable residues, occasionally with other organic material, and having a limited mineral content. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Compost Organic soil conditioner obtained by biodegradation of a mixture principally consisting of various vegetable residues, occasionally with other organic material and having a limited mineral content. (ISO/CD 16929)

Compost Organic soil conditioner obtained by biodegradation of a mixture principally consisting of various vegetable residues, occasionally with other organic material, and having a limited mineral content. (ISO/CD15986.2)

Compost quality The compost quality is defined by the relevant national or EC standards.(CEN 261069:1996)

Compostability Property of a material to be biodegraded in a composting process. To claim compostability it must have been demonstrated that a material can be biodegraded and disintegrated in a composting system (as can be shown by standard test methods) and completes its biodegradation during the end use of the compost. The compost must meet the relevant quality criteria. Quality criteria are e.g.: heavy metal content. no eco-toxicity, no obviously distinguishable residues. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Compostability Property of a material to be biodegraded in a composting process. To claim compostability it must have been demonstrated that a material can be biodegraded and disintegrated in a composting system (as can be shown by standard test methods) and completes its biodegradation during the end-use of the compost. The compost must meet the relevant quality criteria. Quality criteria are e.g.: heavy metal content, no eco-toxicity, no obviously distinguishable residues. (ISO/CD 16929)

Compostability Property of a material to be biodegraded in a composting process. To claim compostability it must have been demonstrated that a material can be biodegraded and disintegrated in a composting system (as can be shown by standard test methods) and completes its biodegradation during the end-use of the compost. The compost must meet the relevant quality criteria. Quality criteria are e.g.: heavy metal content no eco-toxicity, no obviously distinguishable residues. (ISO/CD15986.2)

Compostability Property of a packaging to be biodegraded in a composting process. To claim compostability, it must have been demonstrated that a packaging can be biodegraded and disintegrated in a composting system (as can be shown by standard test methods) and completes its biodegradation during the end-use of the compost. The compost must meet the relevant quality criteria. Quality criteria are e.g. :- heavy metal content;- no eco-toxicity ;- no obviously distinguishable residues. (CEN 261069:1996)

Compostable waste waste that is suitable for treatment mainly by aerobic biodegradation under controlled conditions and using micro-organisms. NOTE - Biodegradable organic material is normally compostable, but also other aspects such as toxicity shall be considered. (CEN TC 292 N329)

Composting an aerobic process designed to produce compost. NOTE. Compost is an organic soil conditioner obtained by biodegradation of a mixture consisting principally of vegetable residues, occasionally with other organic material, and having a limited mineral content. (ISO FDIS 14855)

Composting Composting is an aerobic process to produce compost. Compost is an organic soil conditioner obtained by biodegradation of a mixture principally consisting of various vegetable residues, occasionally with other organic material, and having a limited mineral content. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Composting Composting is an aerobic process to produce compost. Compost is an organic soil conditioner obtained by biodegradation of a mixture consisting principally of various vegetable residues, occasionally with other organic material and having a limited mineral content. (ISO/CD 16929)

Concentration of suspended solids of an activated sludge The amount of solids obtained by filtration or centrifugation of a known volume of activated sludge and drying at about 105° C to constant weight. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Concentration of suspended solids of an activated sludge the amount of solids obtained by filtration or centrifugation of a known volume of activated sludge and drying at about 105°C to constant mass.(ISO/FDIS14851)

Concentration of suspended solids of an activated sludge the amount of solids obtained by filtration or centrifugation of a known volume of activated sludge and drying at about 105° C to constant mass.(ISO/FDIS14852)

Constituent (of a packaging material) pure chemical material or substance helping to make up the whole packaging material. (CEN Draft EN 13432)

Construction waste waste arising from construction of buildings.(CEN TC 292 N329)

Degradability Potential of a material to be degraded. Degradability of a material shall be specified and measured by standard test methods in order to determine its classification with respect to waste management. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Degradability Potential of a material to be degraded. Degradability of a material shall be specified and measured by standard test methods in order to determine its classification with respect to waste management. (ISO/CD15986.2)

Degradability Potential of a material to be degraded. Degradability of a material shall be specified and measured by standard test methods in order to determine its classification with respect to waste management. (CEN 261069:1996)

Degradable A material is called degradable with respect to specific environmental conditions if it undergoes a degradation to a specific extent within a given time measured by specific standard test methods. (CEN 261069:1996)

Degradable A material is called degradable with respect to specific environmental conditions if it undergoes a degradation to a specific extent within a given time measured by specific standard test methods. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Degradable A material is called degradable with respect to specific environmental conditions if it undergoes a degradation to a specific extent within a given time measured by specific standard test methods. (ISO/CD15986.2)

Degradable Plastic A plastic designed to undergo a significant change in its chemical structure under specific environmental conditions, resulting in a loss of some properties that may very as measured by standard test methods, appropriate to the plastic and the application in a period of time that determines its classification. (Draft ISO/CD 15315)

Degradable Polymer A polymer which will degrade in specific circumstances. (Draft ISO/CD 15315)

Degradable waste waste that predominantly consists of easily biologically, chemically or physically degradable organic matter.(CEN TC 292 N329)

Degradation An irreversible process leading to a significant change of the structure of a material, typically characterised by a loss of properties (e.g. integrity, mechanical strength, change of molecular weight or structure) and/or fragmentation. Degradation is affected by environmental conditions and proceeds over a period of time comprising one or more steps. (CEN 261069:1996)

Degradation An irreversible process leading to a significant change of the structure of a material., typically characterised by a loss of properties (e.g. integrity, molecular weight or structure, mechanical strength) and/or fragmentation. Degradation is affected by environmental conditions and proceeds over a period of time comprising one or more steps. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Degradation An irreversible process leading to a significant change in the structure of a material typically characterised by a loss of properties (e.g. integrity, molecular weight or structure mechanical strength) and/or fragmentation. Degradation is affected by environmental conditions and proceeds over a period of time comprising one or more steps. (ISO/CD 16929)

Degradation An irreversible process leading to a significant change of the structure of a material, typically characterised by a loss of properties (e.g. integrity, molecular weight or structure, mechanical strength) and/or fragmentation. Degradation is affected by environmental conditions and proceeds over a period of time comprising one or more steps. (ISO/CD15986.2)

Degradation phase The time from the end of the lag phase of a test until about 90 % of the maximum level of biodegradation has been reached, recorded in days. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Degradation phase The time from the end of the lag-phase of a test until about 90% of the maximum level of biodegradation has been reached, noted in days. (ISO/CD 701.2)

Degradation phase The time of maximum degradation activity from the end of the lag-phase up to the beginning of the plateau. .(ISO/DIS 14021.2)

Degradation phase the time, measured in days, from the end of the lag phase of a test until about 90 % of the maximum level of biodegradation has been reached. (ISO FDIS 14855)

Degradation phase the time, measured in days, from the end of the lag phase of a test until about 90 % of the maximum level of biodegradation has been reached.(ISO/FDIS 14851)

Degradation phase the time, measured in days, from the end of the lag phase of a test until about 90 % of the maximum level of biodegradation has been reached.(ISO/FDIS 14852)

Demolition waste waste arising from demolition of buildings.(CEN TC 292 N329)

Digested sludge A mixture of the settled phases of sewage and activated sludge, which have been incubated in an anaerobic digester at about 35'C to reduce bio-mass and odour problems and to improve the de-waterability of the sludge. Digested sludge consists of an association of anaerobic fermentative and methanogenic bacteria producing carbon dioxide and methane. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Disintegration the physical breakdown of a material] into very small fragments. (ISO FDIS 14855)

Disintegration of a test material The obvious decay of a compact test material at the end of a digestion process qualitatively or quantitatively determined by suitable methods. (ISO/CD 701.2)

Disintegration of a test material The obvious decay of a compact test material at the end of a composting process qualitatively or quantitatively determined by suitable methods.(ISO/DIS 14021.2)

Disintegration of a test material The physical falling apart of a material into very small fragments. (ISO/CD 16929)

Disintegration The decay of a compact test item at the end of a composting process qualitatively or quantitatively determined by suitable methods. (ISO/CD15986.2)

Disintegration The obvious decay of a compact test material at the end of a composting process qualitatively or quantitatively determined by suitable methods. (ISO/DIS 14021.2)

Disintegration The physical falling apart of a material into very small fragments. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Disposal (Definition given by Directive on Packaging and Packaging waste: Article 3).Any of the applicable operations provided for in Annex II.A to Directive 75/442/EEC. NOTE: Within the context of the life cycle of packaging and packaging waste, disposal can be considered as the ultimate operation on packaging waste which is not recovered. (CEN 261069:1996)

Dissolved inorganic carbon (DIC) That part of the inorganic carbon in water which cannot be removed by specified phase separation, for example by centrifugation at 40000 ms^{-2} for 15 min or by membrane filtration using membranes with pores of 0.2 - 0.45µm diameter. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Dissolved inorganic carbon (DIC) that part of the inorganic carbon in water which cannot be removed by specified phase separation, for example by centrifugation at 40 000 $m*s^{-2}$ for 15 min or by membrane filtration using membranes with pores of 0,2 µm to 0,45µm diameter.(ISO/FDIS 14852)

Dissolved organic carbon (DOC) That part of the organic carbon in the water which cannot be removed by specified phase separation, for example by centrifugation at 40000 ms^{-2} for 15 min or by membrane filtration using membranes with pores of 0.2 - 0.45µm diameter. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Dissolved organic carbon (DOC) that part of the organic carbon in water which cannot be removed by specified phase separation, for example by centrifugation at 40 000 $m*s^{-2}$ for 15 min or by membrane filtration using membranes with pores of 0,2 µm to 0,45 µm diameter.(ISO/FDIS 14851)

Dissolved organic carbon (DOC) that part of the organic carbon in water which cannot be removed by specified phase separation, for example by centrifugation at 40 000 m-s-2 for 15 min or by membrane filtration using membranes with pores of 0,2µm to 0,45 µm diameter.(ISO/FDIS 14852)

Electronic waste waste that has been electric or electronic equipment. NOTE - This concept embraces a variety of waste streams. (CEN TC 292 N329)

End of life vehicle waste that has been a vehicle.(CEN TC 292 N329)

Energy recovery Use of combustible packaging waste as a means to generate energy through direct incineration with or without other waste but with recovery of the heat. NOTE : From a technical point of view, any process where the calorific value or the sensible heat of a material is converted into useful heat or electricity. (CEN 261069:1996)

Environmental aspect element of an organisation's activities or products that can interact with the environment. (ISO/CD 16929)

Environmental claim statement, or symbol that indicates the environmental aspects of a product. NOTE An environmental claim may be made on product or packaging labels, through product literature, technical bulletins, advertising, publicity or similar applications. (Draft ISO/DIS 14021.2)

Environmental impact any change to the environment, whether adverse or beneficial, wholly or partially resulting from an organisation's activities, or products. (ISO/CD 16929)

Excavation residues waste arising from excavation.(CEN TC 292 N329)

Explanatory statement any explanation which is needed so that an environmental claim can be properly understood by a purchaser, potential purchaser or user of the product. (ISO/CD 16929)

Filter-cake waste formed when the liquid phase of a liquid or sludgy waste is completely or partly removed.(CEN TC 292 N329)

Flue-gas cleaning residues waste arising from cleaning of flue gases . NOTE - See Waste Directive. (CEN TC 292 N329)

Fly ash waste that is entrained in a flue gas stream. NOTE - See Waste Directive. (CEN TC 292 N329)

Fuel Any material used as a source of energy. (CEN 261069:1996)

Garden and park waste waste arising from garden or park management.(CEN TC 292 N329)

Gasification Transformation of organic material by partial oxidation into a gaseous fuel or a synthesis gas. (CEN 261069:1996)

Hazardous biological health care waste hazardous biological waste that arises from medical activities such as diagnosis, monitoring, treatment, prevention of disease or alleviation of handicap in humans or animals and related research, performed under the supervision of person(s) authorised by virtue of their qualifications to do so.(CEN TC 292 N329)

Hazardous biological waste hazardous waste that has been living organisms.(CEN TC 292 N329)

Hazardous health care waste hazardous waste that arises from medical activities such as diagnosis, monitoring, treatment, prevention of disease or alleviation of handicap in humans or animals, including related research, performed under the supervision of person(s) authorised by virtue of their qualifications to do so. NOTE - The hazard can be of the following natures: - Biological (recognisable anatomical waste) - Infectious- Chemical, toxic or pharmaceutical, including cytotoxins-- Radioactive Sharps (e.g. needles, scalpels) (CENTC 292)

Hazardous waste See Hazardous waste Directive and Hazardous waste List. (CEN TC 292 N329)

Health-care waste waste arising from medical activities such as diagnosis, monitoring, treatment, prevention of disease or alleviation of handicap in humans or animals and related research, performed under the supervision of person(s) authorised by virtue of their qualifications to do so.(CEN TC 292 N329)

Household hazardous waste See Hazardous waste Directive, when amended. (CEN TC 292 N329)

Household waste waste arising in households.(CEN TC 292 N329)

Incineration Combustion of waste as a means of disposal, with or without energy recovery. (CEN 261069:1996)

Inert waste See Landfill Directive. (CEN TC 292 N329)

Infectious health care waste infectious waste that arises from medical activities such as diagnosis, monitoring, treatment, prevention of disease or alleviation of handicap in humans or animals, including related research, performed under the supervision of person(s) authorised by virtue of their qualifications to do so. NOTE - This category includes e.g. waste that is known or assessed to be contaminated with a)organic material from treatment or diagnosis of humans or animals with known or clinically suspected disease caused by biological agents belonging to groups 3 and 4 or identified through the procedure set out in Article 3 of the Council Directive 90/679/EEC of 26 November 1990 (amended by Directive 93188 EEC) on the protection of workers from risks related to exposure of biological agents of Article 16 (1) of Directive 89/391/EEC or b) biological agents belonging to groups 2, 3 and 4 of Council Directive 93188/EEC, having been cultivated to significantly elevated numbers. (CEN TC 292 N329)

Infectious waste see hazardous waste Directive.(CEN TC 292 N329)

Inherent biodegradability The potential of a Material to be biodegraded established under laboratory conditions, (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Inherent biodegradability The potential of a material to be biodegraded established under laboratory conditions. (ISO/CD15986.2)

Kitchen waste degradable waste arising from households.(CEN TC 292 N329)

Lag-phase The time from the start of a test until adaptation and selection of the degrading micro-organisms is achieved and the biodegradation degree of a chemical compound or organic matter has increased to 10% of the theoretical maximum degradation, noted in days. (ISO/CD 701.2)

Lag-phase The time from the start of a test until adaptation and selection of the degrading micro-organism are achieved and the biodegradation degree of a chemical compound or organic matter bas increased to about 1 0 % of the maximum level of biodegradation, recorded in days. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Lag-phase The time from the start of a test until the degradation of the test material significantly starts. (ISO/DIS 14021.2)

Lag-phase the time, measured in days, from the start of a test until adaptation and/or selection of the degrading micro-organisms is achieved and the degree of biodegradation of a chemical compound or organic matter has increased to about 10 % of the maximum level of biodegradation. (ISO FDIS 14855)

Lag-phase the time, measured in days, from the start of a test until adaptation and/or selection of the degrading micro-organisms is achieved and the degree of biodegradation of a chemical compound or organic matter has increased to about 10 % of the maximum level of biodegradation.(ISO/FDIS14851)

Lag-phase the time, measured in days, from the start of a test until adaptation and/or selection of the degrading micro-organisms is achieved and the degree of biodegradation of a chemical compound or organic matter has increased to about 10 % of the maximum level of biodegradation.(ISO/FDIS14852)

Landfilled waste See Landfill Directive. (CEN TC 292 N329)

Leachate See Landfill Directive.(CEN TC 292 N329).

Life cycle consecutive and inter-linked stages of a product system, from raw material acquisition or generation of natural resources to the final disposal. (ISO/CD 16929)

Liquid waste See Landfill Directive.(CEN TC 292 N329).

Material identification words, numbers or symbols used to designate composition of components of a product or packaging. NOTE l. A material identification symbol is not considered to be an environmental claim. NOTE 2. Examples of national standards and industry publications dealing with material identification symbols are given in Annex C. (ISO/CD 16929)

Maturity of compost (Rottegrad) Assignment of the maturity of a compost based on the measurement of the maximum temperature in a self-heating test using Dewar vessels. (ISO/CD 16929)

Maximum level of biodegradation the degree of biodegradation, measured in per cent, of a chemical compound or organic matter in a test, above which no further biodegradation takes place during the test. (ISO FDIS 14855)

Maximum level of biodegradation the degree of biodegradation, measured in per cent, of a chemical compound or organic matter in a test above which no further biodegradation takes place during the test.(ISO/FDIS14851)

Maximum level of biodegradation the degree of biodegradation, measured in per cent, of a chemical compound organic matter in a test, above which no further biodegradation takes place during the test.(ISO/FDIS14852)

Maximum level of biodegradation The maximum biodegradation degree of a chemical compound or organic matter in a test noted in percent above which no further biodegradation takes place during the test. (ISO/CD 701.2)

Maximum level of biodegradation The maximum biodegradation degree of a chemical compound or organic matter in a test recorded in per cent, above which no further biodegradation takes place during the test. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Mechanical degradation Disintegration caused by mechanical influences i.e. forces to be vibration and shock, shear stress, abrasion, pressure, rupture leading to a significant change of the physical structure of a material. (CEN 261069:1996)

Mechanically degradability Potential of a material to be mechanically degraded. (CEN 261069:1996)

Mechanically degradable A material is called mechanically degradable with respect to specific mechanical loads if it undergoes a mechanical degradation to a specified extend within a given time measured by standard test methods. (CEN 261069:1996)

Metal finishing waste waste arising from metal finishing operations. NOTE - Metal finishing operations includes chemical, mechanical or physical treatment. (CEN TC 292 N329)

Metal waste waste that predominantly consists of metal.(CEN TC 292 N329)

Mining waste waste arising from prospecting, extraction, treatment and storage of mineral resources and the working of quarries.(CEN TC 292 N329)

Mono-combustion Combustion of a single fuel. (CEN 261069:1996)

Night soil waste that consists of human excreta accumulated in a container.(CEN TC 292 N329)

Oily waste waste whose oil content dictates its subsequent management.(CEN TC 292 N329)

One-way packaging Packaging which is designed to be used only once. (CEN 261069:1996)

Organic recovery. Biological treatment of plastic waste in aerobic composting or anaerobic biogasification facilities with following aerobic stabilisation with the aim to reduce the waste and obtain compost. (ISO/CD15986.2)

Packaging derived fuel (PDF) Fuel derived by separate collection of combustibles, mainly consisting of used packaging. (CEN 261069:1996)

Packaging litter. Used packaging which has been left in the environment as a result of uncontrolled disposal. (CEN 261069:1996)

Packaging material that is used to protect or contain a product during transportation, storage, marketing or use. NOTE Packaging also includes any item that is physically attached to, or included with, a product or its container for the purpose of marketing the product or communicating information about product. (ISO/CD 16929)

Packaging waste See Packaging waste Directive. (CEN TC 292 N329)

Packaging waste(Definition given by Directive on Packaging and Packaging waste: Article 3). Any packaging or packaging material covered by the definition of waste Directive 75/442/EEC, excluding production residues. NOTE : packaging or packaging materials which fall out of the commercial cycle or out of the chain of utility. Such items of material way be subject to recovery processes or may be sent for final disposal. (CEN 261069:1996)

Pesticide waste waste that contains significant amounts of pesticides. NOTE -the value of "significant" may be defined in legislation. (CEN TC 292 N329)

Pharmaceutical waste waste that contains significant amounts of medicine residues arising from pharmaceutical manufacturing or being waste prescription medicine. NOTE - The value of "significant" may be defined in legislation. (CEN TC 292 N329)

Photodegradability. Potential of a material to be photodegrated. (CEN 261069:1996)

Photodegradable Plastic: ISO CD 472 Plastics - vocabulary A degradable plastic in which the degradation results from the action of natural daylight.(Draft ISO/CD 15315)

Photodegradable. A material is called photodegradable with respect to specific environmental conditions if it undergoes a photodegradation to a specified extent within a given time measured by standard test methods. (CEN 261069:1996)

Photodegradation. Degradation caused by absorption of visible and UV light. (CEN 261069:1996)

Plateau phase The time from the end of the biodegradation phase until the end of the test, recorded in days. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Plateau phase The time from the end of the biodegradation phase when the maximum level of biodegradation is reached until the end of the test. (ISO/CD 701.2)

Plateau phase The time from the end of the degradation phase when the maximum degradation degree is reached and no further significant degradation takes place during the test until the end of the test. .(ISO/DIS 14021.2)

Plateau phase the time, measured in days, from the end of the biodegradation phase until the end of a test. (ISO FDIS 14855)

Plateau phase the time, measured in days, from the end of the biodegradation phase until the end of a test.(ISO/FDIS14851)

Plateau phase the time, measured in days, from the end of the biodegradation phase until the end of a test.(ISO/FDIS14852)

Pre-conditioning The pre-incubation of an inoculum under the conditions of the subsequent test in the absence of a chemical compound or organic matter, with the aim of improving the performance of the test by acclimatisation of the micro-organisms to the test conditions(Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Pre-conditioning the pre-incubation of an inoculum under the conditions of the subsequent test in the absence of the chemical compound or organic matter under test, with the aim of improving the test by acclimatisation of the micro-organisms to the test conditions.(ISO/FDIS14851)

Pre-conditioning the pre-incubation of an inoculum under the conditions of the subsequent test in the absence of the chemical compound or organic matter under test, with the aim of improving the test by acclimatisation of the micro-organisms to the test conditions.(ISO/FDIS14852)

Pre-exposure The pre-incubation of an inoculum in the presence of a chemical compound or organic matter, with the aim of enhancing the ability of this inoculum to biodegrade the test material by adaptation and selection of the micro-organism. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Pre-exposure the pre-incubation of an inoculum in the presence of the chemical compound or organic matter under test, with the aim of enhancing the ability of the inoculum to biodegrade the test material by adaptation and/or selection of the micro-organisms.(ISO/FDIS14851)

Pre-exposure the pre-incubation of an inoculum in the presence of the chemical compound or organic matter under test, with the aim of enhancing the ability of the inoculum to biodegrade the test material by adaptation and/or selection of the micro-organisms.(ISO/FDIS14852)

Primary biodegradation The structural change (transformation) of a chemical compound by micro-organisms resulting in the loss of a specific property, (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Primary fuel Principal fuel(s) of an energy conversion plant. (CEN 261069:1996)

Product any goods or service. (ISO/CD 16929)

Qualified environmental claim environmental claim which is accompanied by an explanatory statement that describes the limit of the claim. (ISO/CD 16929)

Recoverable packaging Packaging which is capable of undergoing the process of recovery". (CEN 261069:1996)

Recovery (Definition given by Directive on Packaging and Packaging waste: Article 3). Any of the applicable operations provided for in Annex II.B to Directive 75/442/EEC. NOTE : An operation which diverts waste from final disposal. The principal operations are recycling and energy recovery. (CEN 261069:1996)

Recovery process waste See Waste Directive.(CEN TC 292 N329).

Recyclable packaging Packaging which is capable of undergoing the process of recycling. (CEN 261069:1996)

Recycling (Definition given by Directive on Packaging and Packaging waste: Article 3). Reprocessing in a production process of the waste materials for the original purpose or for other purposes including comporting but excluding energy recovery. (CEN 261069:1996)

Refuse derived fuel (RDF) Waste treated to make it more suitable as a fuel. (CEN 261069:1996)

Returnable Packaging Packaging for which there is a specific collection system. (CEN 261069:1996)

Reusable packaging Packaging which is capable of reuse. (CEN 261069:1996)

Reuse (Definition given by Directive on Packaging and Packaging waste: Article 3)Any operation by which packaging, which has been conceived and designed to accomplish within its life cycle a certain number of trips or rotations, is refilled or used for the same purpose for which it was conceived ; such re-used packaging will become packaging waste when no longer subject to re-use. . (CEN 261069:1996)

Road sweepings waste that has been collected from streets and public areas. NOTE - Road sweepings includes natural waste like leaves and sand. (CEN TC 292 N329)

Secondary fuel Fuel used in addition to or instead of the primary fuel. (CEN 261069:1996)

Self-declared environmental claim environmental claim that is made, without independent third-party certification by manufacturers, importers, distributors. retailers or anyone else likely to benefit from such a claim. (ISO/CD 16929)

Sewerage sludge sludgy waste separated in sewerage systems.(CEN TC 292 N329)

Shredder residues waste arising from shredding after removal of components for recovery.(CEN TC 292 N329)

Slop aqueous waste that contains oil from machinery or tanks in ships.(CEN TC 292 N329)

Sludgy waste waste that is semisolid, but pourable because of its content of liquid.(CEN TC 292 N329)

Solid household waste solid waste arising from households.(CEN TC 292 N329)

Solid waste waste that predominantly consists of material that has the properties of a solid. NOTE - Cf. sludgy waste. (CEN TC 292 N329)

Solidified waste waste that has been physically or chemically stabilised.(CEN TC 292 N329)

Support fuel Fuel used to maintain combustion. (CEN 261069:1996)

Test material Packagings or packaging materials made from organic compounds normally tested in compact forms at a suitable size.(CEN/TC 261/SC4 N 42)

Themodegradable A material is called thermo-degradable if it undergoes a thermal degradation to a specified extent within a given time measured by standard test methods, excluding any combustion process. (CEN 261069:1996)

Theoretical amount of evolved carbon dioxide (ThCO_2) the maximum theoretical amount of carbon dioxide evolved after completely oxidising a chemical compound, calculated from the molecular formula and expressed as milligrams of carbon dioxide evolved per milligram or gram of test compound. (ISO FDIS 14855)

Theoretical amount of evolved carbon dioxide (ThCO_2) the maximum theoretical amount of carbon dioxide evolved after completely oxidising a chemical compound, calculated from the molecular formula and expressed as milligrams of carbon dioxide evolved per milligram or gram of test compound.(ISO/FDIS14852)

Theoretical amount of released carbon dioxide (ThCO_2) The theoretical maximum amount of carbon dioxide released after oxidising a chemical compound completely, calculated from the molecular formula; expressed in this case as mg carbon dioxide released per mg or g test compound. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Theoretícal oxygen demand (THOD) The theoretical maximum amount of oxygen required to oxidise a chemical compound completely, calculated from the molecular formula expressed in this case as mg oxygen required per mg or g test compound. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Theoretícal oxygen demand (THOD) the theoretical maximum amount of oxygen required to oxidise a chemical compound completely, calculated from the molecular formula, expressed as milligrams of oxygen uptake per milligram or gram of test compound.(ISO/FDIS14851)

Thermal degradability Potential of a material to be thermal degraded. (CEN 261069:1996)

Thermal degradation Degradation caused by heat leading to a significant chang**e** of the physical and/or chemical structure of a material. Combustion is not part of this definition. (CEN 261069:1996)

Total dry solids amount of solids obtained by taking a known volume of test material or compost and drying at about 105°C to constant weight. (CEN Draft prEN 13432)

Total dry solids the amount of solids obtained by taking a known volume of test material or inoculum and drying at about 105° C to constant weight. (ISO/CD 701.2)

Total dry solids The amount of solids obtained by taking a known volume of test material or compost and drying about 105°C to constant weight. .(ISO/FDIS14851)

Total dry solids The amount of solids obtained by taking a known volume of test material or compost and drying at about 105°C to constant weight. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Total dry solids the amount of solids obtained by taking a known volume of test material or compost and drying at about 105°C to constant mass. (ISO FDIS 14855)

Total dry solids The amount of solids obtained by taking a known mass of test material or compost and drying at 105°C to constant weight. (ISO/CD 16929)

Total dry solids The amount of solids obtained by taking a known volume of test item or compost and drying at about 105°C to constant weight. (ISO/CD15986.2)

Total dry solids The amount of solids obtained by taking a known volume of test material or compost and drying about 105°C to constant weight. .(ISO/DIS 14021.2)

Total inorganic carbon (TIC) All that inorganic carbon in the water deriving from carbon dioxide and carbonate. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Total organic carbon (TOC) all the carbon present in organic matter which is dissolved or suspended in water.(ISO/FDIS14852)

Total organic carbon (TOC) All that carbon present in organic matter which is dissolved and suspended in the water. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Total organic carbon (TOC) all the carbon present in organic matter which is dissolved or suspended in water.(ISO/FDIS14851)

Treated waste Waste that has been treated to make it more suitable for recovery or disposal. (CEN 261069:1996)

Ultimate aerobic biodegradation The breakdown of a chemical compound or organic matter by micro-organisms in the presence of oxygen into carbon dioxide, water and mineral salts of any other elements present (mineralization) and the production of new bio-mass. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Ultimate aerobic biodegradation the breakdown of an organic compound by micro-organisms in the presence of oxygen into carbon dioxide, water and mineral salts of any other elements present (mineralization) plus new bio-mass.(ISO FDIS 14855)

Ultimate aerobic biodegradation the breakdown of an organic compound by micro-organisms in the presence of oxygen into carbon dioxide, water and mineral salts of any other elements present (mineralization) plus new bio-mass.(ISO/FDIS14851)

Ultimate aerobic biodegradation the breakdown of an organic compound by micro-organisms in the presence of oxygen into carbon dioxide, water and mineral salts of any other elements present (mineralization) plus new bio-mass.(ISO/FDIS14852)

Ultimate anaerobic biodegradation The breakdown of a chemical compound or organic matter by micro-organisms in the absence of oxygen to carbon dioxide, methane, water and mineral salts of any other elements present (mineralization) and the production of new bio-mass. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Ultimate biodegradability breakdown of an organic chemical compound by micro-organisms in the, presence of oxygen to carbon dioxide, water and mineral salts of any other elements present (mineralization) and new bio-mass or in the absence of oxygen to carbon dioxide, methane, mineral salts and new bio-mass. (CEN Draft prEN13432)

Ultimate biodegradability The breakdown of a chemical compound or organic matter by micro-organisms in the presence of oxygen to carbon dioxide, water and mineral salts of any other elements present (mineralization) or in the absence of oxygen to carbon dioxide, methane, water **,** mineral salts and the production of new bio-mass. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Ultimate biodegradability The breakdown of an organic chemical compound by micro-organisms in the presence of oxygen to carbon dioxide, water and mineral salts of any other elements present (mineralization) and new bio-mass or in the absence of oxygen to carbon dioxide, methane, mineral salts and new bio-mass. (ISO/CD15986.2)

Ultimate biodegradation of a test material The breakdown of an organic by micro-organisms resulting to methane, carbon dioxide, water and mineral salts of any other elements present (mineralization) and new bio-mass.(ISO/CD 701.2)

Ultimate biodegradation of a test material The level of biodegradation achieved when the test material is utilised by micro-organisms resulting in the production of carbon dioxide, water, mineral salts and new microbial cellular constituents (bio-mass). (CENTC 261/SC4)

Used packaging Packaging or packaging material remaining after the removal of the product it contained. (CEN 261069:1996)

Used packaging with hazardous residues Used packaging with residues of hazardous substances or products. NOTE: Hazardous substances are defined by International and National regulations (91/689/EEC of 12 December 1 991). (CEN 261069:1996)

Vitrified waste waste that has been stabilised by vitrification.(CEN TC 292 N329)

Volatile solids The amount of solids obtained by subtracting the residues of a known volume of test material or compost after incineration at about 550°C from the total dry solids content of the same sample. The volatile solids content is an indication of the amount of organic matter. .(ISO/FDIS14851)

Volatile solids amount of solids obtained by subtracting the residues of a known volume of test material or compost after incineration at about 550°'C from the total dry solids content of the same sample. The volatile solids content is an indication of the amount of organic matter. (CEN Draft prEN13432)

Volatile solids The amount of solids obtained by subtracting the residues of a known volume of test Material or compost after incineration at about 550°C from the total dry solids content of the same sample. The volatile solids content is an indication of the amount of organic matter. °C to constant weight. (Proposal of Pagga to ISO TC 61 of 03 sept.1997)

Volatile solids The amount of solids obtained by subtracting the residues of a known volume of test material or inoculum after incineration at about 550° C from the total dry solids content of the same sample. The volatile solids content is an indication of the amount of organic matter. (ISO/CD 701.2)

Volatile solids the amount of solids obtained by subtracting the residue of a known volume of test material or compost after incineration at about 550°C from the total dry solids of the same sample. NOTE The volatile-solids content is an indication of the amount of organic matter present. (ISO FDIS 14855)

Volatile solids The amount of solids obtained by subtracting the residues of a known mass of test material or compost after incineration at about 550°C from the total dry solids content of the same sample. (ISO/CD 16929)

Volatile solids The amount of solids obtained by subtracting the residues of a known volume of test item or compost after incineration at about 550°C from the total dry solids content of the same sample. The volatile solids content is an indication of the amount of organic matter. (ISO/CD15986.2)

Volatile solids The amount of solids obtained by subtracting the residues of a known volume of test material or compost after incineration at about 550°C from the total dry solids content of the same sample. The volatile solids content is an indication of the amount of organic matter. .(ISO/DIS 14021.2)

Waste anything for which the generator or holder has no further use and which is disposed of or released to the environment. (ISO/CD 16929)

Waste oil waste that predominantly consists of non-food oil.(CEN TC 292 N329)

Waste See Waste Directive .(CEN TC 292 N329)

Waste-to-energy process Combustion of waste, with the primary goal of energy recovery. (CEN 261069:1996)

Wastewater sludge sludgy waste, produced during wastewater treatment.(CEN TC 292 N329)

REFERENCES

ISO/DIS 14021.2 :1988 (E)	Environmental labels and declarations - Self-Declared environmental claims
ISO/CD 701.2 1998	Plastics - Evaluation of the ultimate anaerobic biodegradability and disintegration under high solids anaerobic digestion conditions - Method by analysis of released bio-gas
ISO/CD 15315 1999	Ageing, chemical and environmental resistance
draft prEN 13432	Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging
ISO/FDIS 14855 1999	Determination of the ultimate aerobic biodegradability and disintegration of plastic materials under controlled composting conditions - Method by analysis of evolved carbon dioxide
CEN/TC 292 N329 1998	Characterization of waste - Terminology, Part I: material related terms and definitions
ISO/CD 16929 1998	Plastics - Evaluation of the disintegration of plastic materials under defined composting conditions in a pilot scale test
CEN 261069:1996	Packaging - Packaging and the environment - Terminology
ISO/CD 15986.2 1999	Plastics - Evaluation of compostability - Test scheme for final acceptance
ISO/FDIS 14851 1999	Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium - Method by measuring the oxygen demand in a closed respirometer
ISO/FDIS 14852 1999	Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium - Method by analysis of evolved carbon dioxide
Proposal of Pagga to ISO TC 61	Definitions for biodegradation tests of ISO TC 61