11
Analytical Methods for Monitoring Biodegradation Processes of Environmentally Degradable Polymers

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11.1
Introduction

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

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

11.2
Some Background

There is a worldwide research effort to develop biodegradable polymers for agricultural applications or as a waste management option for polymers in the environment. Until the end of the 20th century, most of the efforts were synthesis

Handbook of Biodegradable Polymers: Synthesis, Characterization and Applications, First Edition. Edited by Andreas Lendlein, Adam Sisson.

oriented, and not much attention was paid to the identification of environmental requirements for, and testing of, biodegradable polymers. Consequently, many unsubstantiated claims to biodegradability were made, and this has damaged the general acceptance.

An important factor is that the term biodegradation has not been applied consistently. In the medical field of sutures, bone reconstruction, and drug delivery, the term biodegradation has been used to indicate degradation into macromolecules that stay in the body but migrate (e.g., UHMW polyethylene from joint prostheses), or hydrolysis into low-molecular-weight molecules that are excreted from the body (bioresorption), or dissolving without modification of the molecular weight (bioabsorption) [4, 5]. On the other hand, for environmentally degradable plastics, the term biodegradation may mean fragmentation, loss of mechanical properties, or sometimes degradation through the action of living organisms [6]. Deterioration or loss in physical integrity is also often mistaken for biodegradation [7]. Nevertheless, it is essential to have a universally acceptable definition of biodegradability to avoid confusion as to where biodegradable polymers can be used in agriculture or fit into the overall plan of polymer waste management. Many groups and organizations have endeavored to clearly define the terms "degradation," "biodegradation," and "biodegradability." But there are several reasons why establishing a single definition among the international communities has not been straightforward, including:

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

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

Low-density polyethylene has been shown to biodegrade slowly to carbon dioxide (0.35% in 2.5 years) [8], and according to some definitions can thus be called a biodegradable polymer. However, the degradation process is so slow in comparison with the application rate that accumulation in the environment will occur. The same applies for polyolefin–starch blends which rapidly loose strength, disintegrate, and visually disappear if exposed to microorganisms [9–11]. This is due to

utilization of the starch component, but the polyolefin fraction will nevertheless persist in the environment. Can these materials be called "biodegradable"?

11.3 Defining Biodegradability

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

1) For all practical purposes of applying a definition, material manufactured to be biodegradable must relate to a specific disposal pathway such as composting, sewage treatment, denitrification, and anaerobic sludge treatment.

2) The rate of degradation of a material manufactured to be biodegradable has to be consistent with the disposal method and other components of the pathway into which it is introduced, such that accumulation is controlled.

3) The ultimate end products of aerobic biodegradation of a material manufactured to be biodegradable are CO_2, water, and minerals and that the intermediate products include biomass and humic materials. (Anaerobic biodegradation was discussed in less detail by the participants.)

4) Materials must biodegrade safely and not negatively impact the disposal process or the use of the end product of the disposal.

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

In the following sections, biodegradation of polymeric materials is looked upon form the chemical perspective. The chemistry of the key degradation process is represented by Eq. (11.1) and (11.2), where $C_{polymer}$ represents either a polymer or a fragment from any of the degradation processes defined earlier. For simplicity here, the polymer or fragment is considered to be composed only of carbon, hydrogen, and oxygen; other elements may, of course, be incorporated in the polymer, and these would appear in an oxidized or reduced form after biodegradation depending on whether the conditions are aerobic or anaerobic, respectively.

Aerobic biodegradation:

$$C_{polymer} + O_2 \rightarrow CO_2 + H_2O + C_{residue} + C_{biomass} \quad (11.1)$$

Anaerobic biodegradation:

$$C_{polymer} \rightarrow CO_2 + CH_4 + H_2O + C_{residue} + C_{biomass} \quad (11.2)$$

Complete biodegradation occurs when no residue remains, and complete mineralization is established when the original substrate, $C_{polymer}$ in this example, is completely converted into gaseous products and salts. However, mineralization is a very slow process under natural conditions because some of the polymer undergoing biodegradation will initially be turned into biomass [13, 14]. Therefore, complete biodegradation, and not mineralization, is the measurable goal when assessing removal from the environment.

11.4 Mechanisms of Polymer Degradation

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

11.4.1 Nonbiological Degradation of Polymers

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

Important features affecting chemical polymer degradation and erosion include (i) the type of chemical bond, (ii) the pH, (iii) the temperature, (iv) the copolymer

composition, and (v) water uptake (hydrophilicity). These features will not be discussed here, but have been covered in detail by Göpferich [4].

11.4.2
Biological Degradation of Polymers

Polymers represent major constituents of the living cells which are most important for the metabolism (enzyme proteins and storage compounds), the genetic information (nucleic acids), and the structure (cell wall constituents and proteins) of cells [18]. These polymers have to be degraded inside cells in order to be available for environmental changes and to other organisms upon cell lysis. It is therefore not surprising that organisms, during many millions of years of adaptation, have developed various mechanisms to degrade naturally occurring polymers. For the many different new synthetic polymers that have found their way into the environment only in the last 70 years, however, these mechanisms may not as yet have been developed.

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

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

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

11.5
Measuring Biodegradation of Polymers

As can be imagined from the various mechanisms described above, biodegradation does not only depend on the chemistry of the polymer but also on the presence of the biological systems involved in the process. When investigating the

		(1) ***aquatic***	(2) ***high solids***
a)	***aerobic***	aerobic wastewater treatment plants surface waters, e.g., lakes and rivers marine environments	surface soils organic waste composting plants littering
b)	***anaerobic***	anaerobic wastewater treatment plants rumen of herbivores	deep sea sediments anaerobic sludge anaerobic digestion/ biogasification landfill

Figure 11.1 Schematic classification of different biodegradation environments for polymers.

biodegradability of a material, the effect of the environment cannot be neglected. Microbial activity and hence biodegradation is influenced by

1) the presence of microorganisms
2) the availability of oxygen
3) the amount of available water
4) the temperature
5) the chemical environment (pH, electrolytes, etc.).

In order to simplify the overall picture, the environments in which biodegradation occurs are basically divided in two environments: (a) aerobic (with oxygen available) and (b) anaerobic (no oxygen present). These two in turn can be subdivided into (1) aquatic and (2) high-solids environments. Figure 11.1 schematically presents the different environments, with examples in which biodegradation may occur [21, 22].

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

Numerous ways for the experimental assessment of polymer biodegradability have been described in the scientific literature. Because of slightly different definitions or interpretations of the term "biodegradability," the different approaches are therefore not equivalent in terms of information they provide or the practical significance. Since the typical exposure to environment involves incubation of a polymer substrate with microorganisms or enzymes, only a limited number of

measurements are possible: those pertaining to the substrates, to the microorganisms, or to the reaction products. Four common approaches available for studying biodegradation processes have been reviewed in detail by Andrady [13, 14]:

1) monitoring accumulation of biomass
2) monitoring the depletion of substrates
3) monitoring reaction products
4) monitoring changes in substrate properties.

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

11.5.1 Enzyme Assays

11.5.1.1 Principle

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

11.5.1.2 Applications

The type of enzyme to be used, and quantification of degradation, will depend on the polymer being screened. For example, Mochizuki *et al.* [24] studied the effects of draw ratio of polycaprolactone fibers on enzymatic hydrolysis by lipase. Degradability of PCL fibers was monitored by dissolved organic carbon (DOC) formation and weight loss. Similar systems with lipases have been used for studying the hydrolysis of broad ranges of aliphatic polyesters [25–30], copolyesters with aromatic segments [26, 31–33], and copolyesteramides [34, 35]. Other enzymes such as α-chymotrypsin and α-trypsin have also been applied for these polymers [36, 37]. Biodegradability of poly(vinyl alcohol) segments with respect to block length and stereochemical configuration has been studied using isolated poly(vinyl alcohol)-dehydrogenase [38]. Cellulolytic enzymes have been used to study the biodegradability of cellulose ester derivatives as a function of degree of substitution and the substituent size [39]. Similar work has been performed with starch esters using amylolytic enzymes such as α-amylases, β-amylases, glucoamylases, and amyloglucosidases [40]. Enzymatic methods have also been used to study the biodegradability of starch plastics or packaging materials containing cellulose [41–46].

11.5.1.3 Drawbacks

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

11.5.2 Plate Tests

11.5.2.1 Principle

Plate tests have initially been developed in order to assess the resistance of plastics to microbial degradation. Several methods have been standardized by standardization organizations such as the ASTM and the ISO [47–49]. They are now also used to see if a polymeric material will support growth [23, 50]. The principle of the method involves placing the test material on the surface of a mineral salts agar in a petri dish containing no additional carbon source. The test material and agar surface are sprayed with a standardized mixed inoculum of known bacteria and/or fungi. The test material is examined after a predetermined incubation period at constant temperature for the amount of growth on its surface and the rating is given.

11.5.2.2 Applications

Potts [51] used the method in his screening of 31 commercially available polymers for biodegradability. Other studies where the growth of either mixed or pure cultures of microorganisms is taken to be indicative for biodegradation have been reported [6]. The validity of this type of test and the use of visual assessment alone have been questioned by Seal and Pantke [52] for all plastics. They recommended that mechanical properties should be assessed to support visual observations. Microscopic examination of the surface can also give additional information.

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

11.5.2.3 Drawbacks

A positive result in an agar plate test indicates that an organism can grow on the substrate, but does not mean that the polymer is biodegradable, since growth may appear on contaminants, plasticizers present, oligomeric fractions still present in

the polymer, and so on. Therefore, these tests should be treated with caution when extrapolating the data to field situations.

11.5.3 Respiration Tests

11.5.3.1 Principle

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

11.5.3.2 Applications

The closed bottle BOD tests were designed to determine the biodegradability of detergents [61, 64]. These have stringent conditions due to the low level of inoculum (in the order of 10^5 microorganisms/L) and the limited amount of test substance that can be added (normally between 2 and 4 mg/L). These limitations originate from the practical requirement that the oxygen demand should not be more than half the maximum dissolved oxygen level in water at the temperature of the test, to avoid the generation of anaerobic conditions during incubation.

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

11.5.3.3 Suitability

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

11.5.4 Gas (CO_2 or CH_4) Evolution Tests

11.5.4.1 Principle

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

11.5.4.2 Applications

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

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

The percentage of carbon converted to biomass instead of carbon dioxide depends on the type of polymer and the phase of degradation. Therefore, it has been suggested to regard the complete carbon balance to determine the degree of degradation [90]. This implies that besides the detection of gaseous carbon, also the amount of carbon in soluble and solid products needs to be determined. Soluble products, oligomers of different molecular size, intermediates, and proteins secreted from microbial cells can be measured as COD or as DOC. Solid

products, biomass, and polymer remnants require a combination of procedures to separate and detect different fractions. The protein content of the insoluble fraction is usually determined to estimate the amount of carbon converted to biomass, using the assumptions that dry biomass consists of 50% protein, and that the carbon content of dry biomass is 50% [90–92].

11.5.4.3 Suitability

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

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

11.5.5 Radioactively Labeled Polymers

11.5.5.1 Principle and Applications

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

11.5.5.2 Drawbacks

Problems with handling the radioactively labeled materials and their disposal are issues on the down side to this method. In addition, in some cases, it is difficult

to synthesize the target polymer with the radioactive labels in the appropriate locations, with representative molecular weights, or with representative morphological characteristics.

11.5.6 Laboratory-Scale Simulated Accelerating Environments

11.5.6.1 Principle

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

11.5.6.2 Applications

The OECD Coupled Unit test [106] simulates an activated sludge sewage treatment system, but its application for polymers would be difficult as DOC is the parameter used to assess biodegradability. Krupp and Jewell [107] described well-controlled anaerobic and aerobic aquatic bioreactors to study degradation of a range of commercially available polymer films. A relatively low loading rate of the semicontinuous reactors and a long retention time were maintained to maximize the efficiency of biodegradation. Experimental setups have also been designed to simulate marine environments [108], soil burial conditions [108–110], composting environments [111–114], and landfill conditions [115] at laboratory scale, with controlled parameters such as temperature and moisture level, and a synthetic waste, to provide a standardized basis for comparing the degradation kinetics of films.

A wide choice of material properties can be followed during the degradation process. However, it is important to select one which is relevant to the end-use of the polymer material or provides fundamental information about the degradation process. Weight loss is a parameter frequently followed because it clearly demonstrates the disintegration of a biodegradable product [116–118]. Tensile properties are also often monitored, due to the interest in the use of biodegradable plastics in packaging applications [54, 119, 120]. In those polymers where the biodegradation involves a random scission of the macromolecular chains, a decrease in the average molecular weight and a general broadening of the molecular weight distribution provide initial evidence of a breakdown process [86, 121, 122]. However, no significant changes in material characteristics may be observed in recovered

material if the mechanism of biodegradation involves bioerosion, that is, enzymatic or hydrolytic cleavage at the surface. Visual examination of the surface with various microscopic techniques can also give information on the biodegradation process [123–126]. Likewise, chemical and/or physical changes in the polymer may be followed by (combinations of) specific techniques such as infrared [10, 127] or UV spectroscopy [84, 128], nuclear magnetic resonance measurements [122–129], X-ray diffractometry [130, 131], and differential scanning calorimetry [132, 133].

11.5.6.3 **Drawbacks**

An inherent drawback in the use of mechanical properties, weight loss, molecular weights, or any other property which relies on the macromolecular nature of the substrate is that in spite of their sensitivity, these can only address the early stages of the biodegradation process. Furthermore, these parameters can give no information on the extent of mineralization. Especially in material blends or copolymers, the hydrolysis of one component can cause significant disintegration (and thus loss of weight and tensile properties), whereas other components may persist in the environment, even in disintegrated form [13]. Blends of starch, poly(3-hydroxybutyrate) or poly(ε-caprolactone) with polyolefins are examples of such systems [11, 43, 134].

11.5.7
Natural Environments, Field Trials

Exposures in natural environments provide the best true measure of the environmental fate of a polymer, because these tests include a diversity of organisms and achieve a desirable natural closeness of fit between the substrate, microbial agent, and the environment. However, the results of that exposure are only relevant to the specific environment studied, which is likely to differ substantially from many other environments. An additional problem is the timescale for this method, since the degradation process, depending on the environment, may be very slow (months to years) [23]. Moreover, little information on the degradation process can be gained other than the real time required for weight loss or total disintegration.

Nevertheless, field trials in natural environments are still used to extrapolate results acquired in laboratory tests to biodegradation behavior under realistic outdoor conditions [123, 135, 136].

11.6
Conclusions

The overview presented above makes clear that there is no such thing as a single optimal method for determining biodegradation of polymeric materials. First of all, biodegradation of a material is not only determined by the chemical composition and corresponding physical properties; the degradation environment in which the material is exposed also affects the rate and degree of biodegradation.

Furthermore, the method or test to be used depends on what information is requested.

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

In most cases, it will not be sufficient to ascertain macroscopic changes, such as weight loss and disintegration, or growth of microorganisms, because these observations may originate from biodegradation of just one of separate components. The ultimate fate of all individual components and degradation products must be included in the investigations. This implies that it is essential that both the polymeric materials and also intermediate degradation products have to be well characterized in order to understand the degradation process. For a good number of biodegradable materials, this means that a lot of work still needs to be done.

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12
Modeling and Simulation of Microbial Depolymerization Processes of Xenobiotic Polymers

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12.1
Introduction

Microbial depolymerization processes are classified into two categories, exogenous type and endogenous type. In an exogenous depolymerization process, molecules reduce their sizes by separation of monomer units from their terminals. Examples of polymers subject to exogenous depolymerization processes include polyethylene (PE). PE is structurally a long-chain alkane of normal type. The initial step of the oxidation of *n*-alkanes is hydroxylation to produce the corresponding primary (or secondary) alcohol, which is oxidized further to an aldehyde (or ketone) and then to an acid. Carboxylated *n*-alkanes are structurally analogous to fatty acids and subject to β-oxidation processes to produce depolymerized fatty acids by liberating two carbon units (acetic acid). It is also shown by gel permeation chromatography (GPC) analysis of PEwax before and after cultivation of a bacterial consortium KH-12 that small molecules are consumed faster than large ones [1].

As is seen in the previous discussion, the mechanism of PE biodegradation is based on two essential factors: the gradual weight loss of large molecules due to the β-oxidation and the direct consumption or absorption of small molecules by cells. A mathematical model based on those factors was proposed, and PE biodegradation was studied using the model [2–5]. The biodegradability of PE between the microbial consortium KH-12 and the fungus *Aspergillus* sp. AK-3 was compared [4]. The transition of weight distribution of PE over 5 weeks of cultivation was numerically simulated using the weight distribution before and after 3 weeks of cultivation, and a numerical result is compared with an experimental result [5].

Polyethylene glycol (PEG) is another example of polymer subject to exogenous depolymerization processes. PEG is depolymerized by liberating C_2 compounds, either aerobically or anaerobically [6, 7]. The mathematical techniques originally developed for the PE biodegradation was extended to cover the biodegradation of PEG. Problems were formulated to determine degradation rates based on the weight distribution of PEG with respect to molecular weight before and after the cultivation of the microbial consortium E-1 [8]. Those problems were solved

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numerically, and the transition of the weight distribution was simulated [9, 10]. Dependence of degradation rate on time was also considered in modeling and simulation of depolymerization processes of PEG [11–13].

Unlike exogenous type depolymerization processes in which monomer units are separated from terminals of molecules, molecules are separated internally in endogenous type depolymerization processes. Hydrolysis is often involved in endogenous type epolymerization processes, while oxidation plays an essential role in exogenous type depolymerization processes. One of the characteristics of endogenous type depolymerization processes is the rapid breakdown of large molecules to produce small molecules in an early stage of depolymerization, whereas molecules lose their weight gradually throughout these processes. Polyvinyl alcohol (PVA) is an example of polymer subject to endogenous type depolymerization. PVA is a carbon-chain polymer with a hydroxyl group attached to every other carbon unit. It is degraded by random oxidation of hydroxyl groups and hydrolysis of mono/diketones. A mathematical model for endogenous depolymerization process was proposed, and enzymatic depolymerization process of PVA was studied. [14–16]. Mathematical model originally proposed for the enzymatic degradation of PVA was applied to enzymatic degradation of polylactic acid (PLA), and the degradability of PVA and PLA was compared [17]. Dependence of degradation rate on time was considered in study of depolymerization processes of PLA [18].

In the following sections, the mathematical models for exogenous type and endogenous type depolymerization processes are described. Numerical techniques to determine degradation rates and to simulate transitions of weight distribution are illustrated. Some numerical results are also introduced.

12.2 Analysis of Exogenous Depolymerization

12.2.1 Modeling of Exogenous Depolymerization

Polyolefins are regarded as linear saturated hydrocarbons, and considered chemically inert in a natural setting. However, it has been shown that PE is slowly degraded and its degradation is promoted by irradiation or oxidation. Slow degradation of PE was shown by measurement of $^{14}CO_2$ generation [19]. Linear paraffin molecules of molecular weight up to approximately 500 were utilized by several microorganisms [20]. Oxidation of *n*-alkanes up to tetratetracontane ($C_{44}H_{90}$, mass of 618) in 20 days was reported [21]. Several experiments were performed to investigate the biodegradability of PE. Commercially available PEwax was used as a sole carbon source for soil microorganisms [1]. Microbial consortium KH-12 obtained from soil samples degraded PEwax, which was confirmed by significant weight loss (30–50%). GPC analysis of PEwax showed that small molecules were consumed faster than large ones in the depolymerization processes of PE.

While experiments revealed the nature of the microbial depolymerization process of PE, it was also viewed theoretically. PE is classified structurally as hydrocarbon, and it is subject to the following metabolic pathways [22]:

1) Terminal oxidation:

$$RCH_3 \rightarrow RCH_2OH \rightarrow RCHO \rightarrow RCOOH$$

2) Diterminal oxidation:

$$H_3CRCH_3 \rightarrow CH_3RCOOH \rightarrow HOH_2CRCOOH \rightarrow OHCRCOOH \rightarrow HOOCRCOOH$$

3) Subterminal oxidation:

$$RCH_2CH_2CH_3 \rightarrow RCH_2CH(OH)CH_3 \rightarrow RCH_2C(O)CH_3 \rightarrow RCH_2OC(O)CH_3 \rightarrow RCH_2OH + CH_3COOH$$

A PE molecule carboxylated by one of these oxidation processes is structurally analogous to the fatty acid, and becomes subject to β-oxidation. Then a series of terminal separation of monomer units follow.

In view of the foregoing theoretical and experimental aspects of PE biodegradation, the following assumptions were made:

1) Each molecule loses its weight by a fixed amount per unit time.
2) Some molecules are directly consumed by microorganisms.
3) The consumption rate per unit time depends on the sizes of molecules.

The mathematical model (12.1) based on these assumptions was proposed, and the biodegradability of PE was studied by analyzing the model [2–5, 14]

$$\frac{dx}{dt} = -\alpha(M)x + \beta(M+L)\frac{M}{M+L}y \quad (\alpha(M) = \rho(M) + \beta(M)) \tag{12.1}$$

where variables t and M represent the cultivation time and the molecular weight, respectively. The variable x equals $w(t,M)$ which denotes the total weight of M molecules (the PE molecules with molecular weight M) present at time t. The parameter L represents the amount of the weight loss due to the terminal separation, and the variable y is given by $y = w(t,M+L)$, that is, the total weight of $(M+L)$-molecules present at time t. The functions $\rho(M)$ and $\beta(M)$ represent the direct consumption rate and the weight conversion rate from the class of M-molecules to the class of $(M-L)$-molecules, respectively. The first term of the right-hand side of Eq. (12.1) is the total weight loss in the class of M-molecules due to the direct consumption and the β-oxidation, and the second term represents the weight conversion from the class of $(M+L)$-molecules to the class of M-molecules due to the β-oxidation.

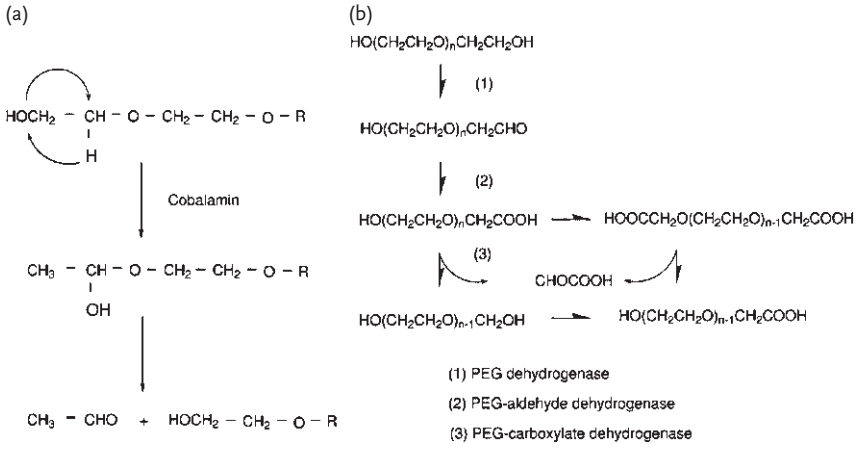

Figure 12.1 Anaerobic metabolism (a) and aerobic metabolism (b) of PEG.

The mathematical model (12.1) was originally proposed for the PE biodegradation. However, it can be viewed as a general biodegradation model for exogenous depolymerization processes, which covers not only the PE biodegradation but also other polymers such as PEG. A PEG molecule is first oxidized at its terminal, and then an ether bond is separated (Figure 12.1) [6, 7]. This process corresponds to β -oxidation for PE, and we call it oxidation because oxidation is involved throughout the depolymerization process [6, 7]. Note that $L = 44$ (CH_2CH_2O) in the exogenous depolymerization of PEG, whereas $L = 28$ (CH_2CH_2) in the β-oxidation of PE.

Equation (12.1) forms an initial value problem together with the initial condition

$$w(0,M) = f(M) \tag{12.2}$$

where $f(M)$ represents the initial weight distribution. Given the total consumption rate $\alpha(M)$ and the oxidation rate $\beta(M)$, the solution of the initial value problem is a function $w(t,M)$ that satisfies Eq. (12.1) and the initial condition (12.2). Given the initial condition (12.2) and an additional final condition at $t = T > 0$

$$w(T,M) = g(M) \tag{12.3}$$

Equation (12.1) forms an inverse problem together with the conditions (12.2) and (12.3). It is a problem to determine the degradation rates $\alpha(M)$ and $\beta(M)$ for which the solution $w(t,M)$ of the initial value problems (12.1) and (12.2) also satisfies the final condition (12.3). It has been shown that the following condition is a sufficient condition for a unique positive total degradation rate $\alpha(M)$ to exist, given the β -oxidation rate $\beta(M+L)$ and the weight distribution $w(M+L)$ [4, 5]:

$$0 < g(M) < f(M) + \frac{M\beta(M+L)}{M+L}\int_0^T w(s,M+L)\mathrm{d}s \tag{12.4}$$

Polymer molecules must penetrate through membranes into cells in order to become subject to direct consumption. The rate of the penetration decreases, as the molecular size increases. Therefore, the rate of direct consumption must also decrease as molecular size increases. In addition, there must be a limit of penetration with respect to molecular size. It follows that $M_\rho > 0$ such that $\rho(M) = 0$ for $M > M_\rho$. Note that

$$\alpha(M) = \beta(M) \quad \text{for} \quad M > M_\rho \tag{12.5}$$

since $\alpha(M) = \rho(M) + \beta(M)$. The weight distribution of PEG with respect to the molecular weight M introduced in the following sections is given in the range $3.1 \leq \log M \leq 4.2$. The molecular weight in this range should be greater than M_ρ.

12.2.2 Biodegradation of PEG

Polyethers are utilized for constituents in a number of products including lubricants, antifreeze agents, inks, cosmetics, etc. They are also used as raw materials to synthesize detergents or polyurethanes. Those polymers are either water soluble or oily liquid, and eventually discharged into the environment [6]. Since they are not tractable to incineration or recycling, their biodegradability is an important factor of environmental protection against their undesirable accumulation [7]. Polyethers include PEG, polypropylene glycol, and polytetramethylene glycol, and they are polymers whose chemical structures are represented by the expression $HO(R–O)_nH$, for example, PEG: R = CH_2CH_2, polypropylene glycol: R = CH_3CHCH_2, polytetramethylene glycol: R = $(CH_2)_4$ [23].

PEG is produced in the largest quantity among polyethers. Its major part is consumed in production of nonionic surfactants. Metabolism of PEG has been well documented. PEG is depolymerized by liberating C_2 compounds, either aerobically or anaerobically [6, 7] (Figure 12.1).

12.3 Materials and Methods

12.3.1 Chemicals

All reagents used were of reagent grade.

12.3.2 Microorganisms and Cultivation

Microbial consortium E-1 was used as a PEG degrader, which was cultivated as described previously. The culture was centrifuged to remove cells and the resultant supernatant was subjected for HPLC analysis.

12.3.3
HPLC analysis

Molecular weights of PEG before and after cultivation were measured by a Tosoh HPLC ccp&8020 equipped with Tosoh TSK-GEL G2500 PW (7.5 $\phi \times$ 300 mm) with 0.3 M sodium nitrate at 1.0 mL/min at room temperature. Detection was done with an RI detector (Tosoh RI-8020) (Figure 12.2). The molecular weights were calculated with authentic PEG standards (Figure 12.3). Figure 12.4 shows HPLC profiles of PEG before and after cultivation of microbial consortium E-1 based on the HPLC outputs and the PEG standards.

12.3.4
Numerical Study of Exogenous Depolymerization

Mathematical model (12.1) is appropriate for the depolymerization processes under a steady microbial population. However, the change of microbial population should be taken into account over a period in which a microbial population is still in a developing stage. In such cases, the degradation rate should be time dependent in the modeling of exogenous depolymerization processes:

$$\frac{dx}{dt} = -\beta(t,M)x + \beta(t,M+L)\frac{M}{M+L}y \tag{12.6}$$

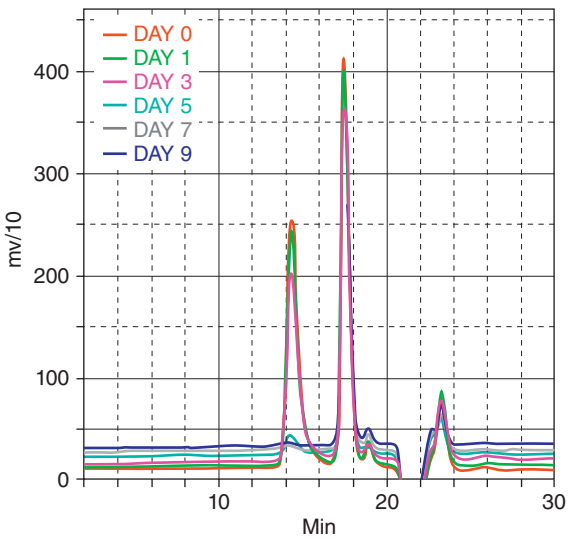

Figure 12.2 HPLC outputs of PEG before and after the cultivation of the microbial consortium E-1.

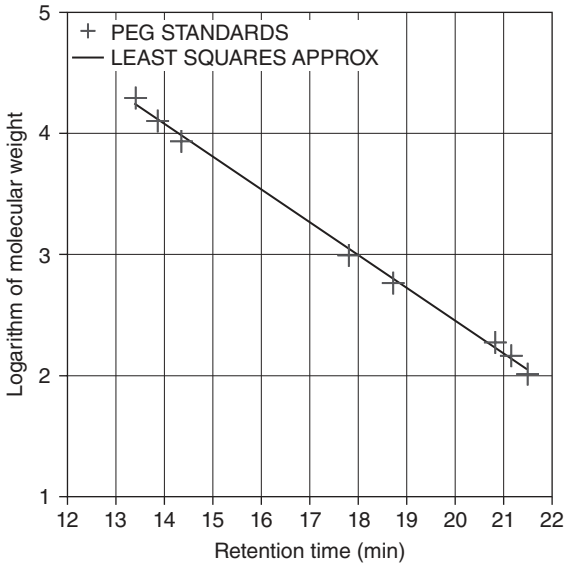

Figure 12.3 PEG standards.

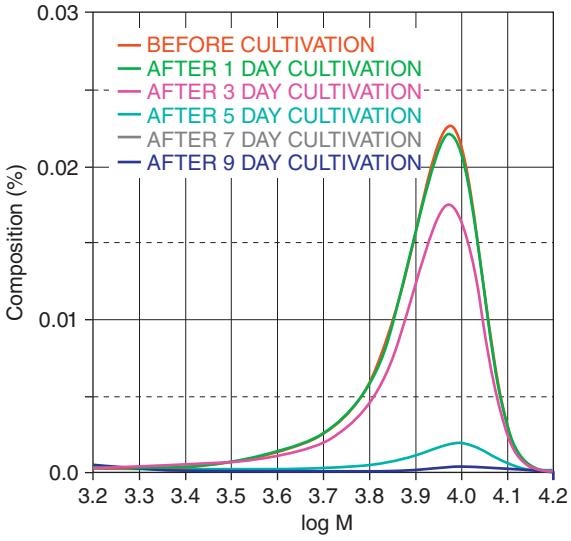

Figure 12.4 HPLC profiles of PEG before and after the cultivation of the microbial consortium E-1 [11, 12].

Solution $x = w(t,M)$ of (12.6) is associated with the initial condition (12.2). Given the degradation rate $\beta(t,M)$, Eq. (12.6) and the initial condition (12.2) form an initial value problem.

Time factors of the degradation rate such as microbial population, dissolved oxygen, or temperature affect molecules regardless of their sizes. The dependence of degradation rate on those factors is uniform over all molecules, and the degradation rate should be a product of a time-dependent part $\sigma(t)$ and a molecular dependent part $\lambda(M)$

$$\beta(t,M) = \sigma(t)\lambda(M) \tag{12.7}$$

Note that $\sigma(t)$ and $\lambda(M)$ represent the magnitude and the molecular dependence of degradability, respectively.

In order to simplify the model, let

$$\tau = \int_0^t \sigma(s)\mathrm{d}s \tag{12.8}$$

and

$$W(\tau,M) = w(t,M), \quad X = W(\tau,M), \quad Y = W(\tau,M+L)$$

Then

$$\frac{\mathrm{d}X}{\mathrm{d}\tau} = \frac{\mathrm{d}x}{\mathrm{d}t}\frac{\mathrm{d}t}{\mathrm{d}\tau} = \frac{1}{\sigma(t)}\frac{\mathrm{d}x}{\mathrm{d}t}$$

and the exogenous depolymerization model (12.6) is converted into the equation

$$\frac{dX}{d\tau} = -\lambda(M)x + \lambda(M+L)\frac{M}{M+L}Y \tag{12.9}$$

This equation governs the transition of weight distribution $w(\tau,M)$ under the time-independent or time-averaged degradation rate $\lambda(M)$. Given the initial weight distribution $f(M)$, Eq. (12.9) forms an initial value problem together with the initial condition

$$W(0,M) = f(M) \tag{12.10}$$

Given an additional condition at $\tau = \mathrm{T}$, Eq. (12.9) forms an inverse problem together with the initial condition (12.10) and the final condition (12.11), for which the solution of the initial value problems (12.9) and (12.10) also satisfies the final condition

$$W(\mathrm{T},M) = g(M). \tag{12.11}$$

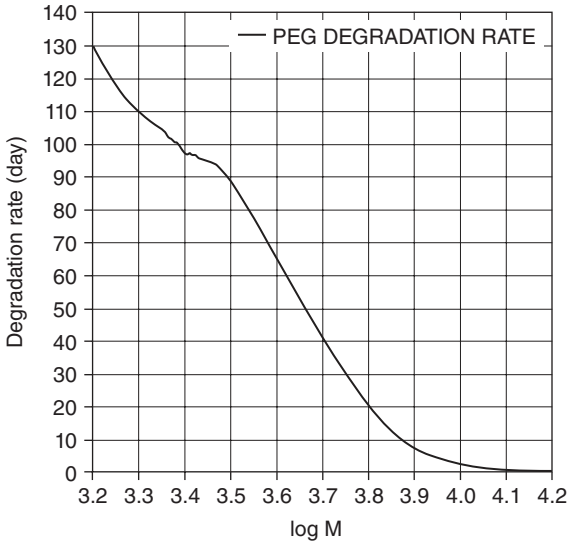

Figure 12.5 Degradation rate based on the weight distribution of PEG before and after the cultivation of the microbial consortium E-1 for 3 days [11, 12].

When the solution $W(\tau, M)$ of the initial value problem (12.9), (12.10) satisfies the condition (12.11), solution $w(t, M)$ of the initial value problems (12.6) and (12.2) satisfies the condition (12.3), where

$$T = \int_0^T \sigma(s)ds \tag{12.12}$$

Note that the inverse problem consisting of (12.9)–(12.11) is essentially identical to the inverse problems (12.1)–(12.3). Numerical techniques developed for the latter was applied to the former to find the degradation rate $\lambda(M)$ based on the weight distribution before and after cultivation for 3 days [12, 13] (Figure 12.5).

12.3.5 Time Factor of Degradation Rate

A microbial population grows exponentially in a developing stage, and the increase of biodegradability results from increase of microbial population. It is appropriate to assume that the time factor of the degradation rate $\sigma(t)$ is an exponential function of time

$$\sigma(t) = e^{at+b} \tag{12.13}$$

In view of Eq. (12.8)

$$\tau = \int_0^t \sigma(s)\mathrm{d}s = \int_0^t e^{as+b}\mathrm{d}s = \frac{e^b}{a}(e^{at} - 1) \tag{12.14}$$

It has been shown that the parameters a and b are uniquely determined provided the weight distribution is given at $t = T_1$ and $t = T_2$, where $0 < T_1 < T_2$, and let

$$\mathrm{T}_1 = \int_0^{T_1} \sigma(s)\mathrm{d}s \tag{12.15}$$

$$\mathrm{T}_2 = \int_0^{T_2} \sigma(s)\mathrm{d}s \tag{12.16}$$

The condition (12.15) leads to

$$\sigma(t) = e^b e^{at} = \frac{a\mathrm{T}_1 e^{at}}{e^{aT_1} - 1} \tag{12.17}$$

Now in view of (12.14),

$$\tau = \mathrm{T}_1 \frac{e^{at} - 1}{e^{aT_1} - 1} \tag{12.18}$$

Equation (12.16) leads to

$$\mathrm{T}_2 = \mathrm{T}_1 \frac{e^{aT_2} - 1}{e^{aT_1} - 1}$$

which is equivalent to the equation

$$h(a) = 0 \tag{12.19}$$

where

$$h(a) = \frac{e^{aT_2} - 1}{e^{aT_1} - 1} - \frac{\mathrm{T}_2}{\mathrm{T}_1}$$

It has been shown that the condition

$$\frac{T_2}{T_1} < \frac{\mathrm{T}_2}{\mathrm{T}_1} \tag{12.20}$$

is a necessary and sufficient condition for Eq. (12.19) to have a unique positive solution [11].

In order to determine a and b, let $T_1 = \mathrm{T}_1 = 3$. The initial value problems (12.9) and (12.10) were solved numerically with the degradation rate shown in Figure 12.5 to reach the weight distribution at $\tau = 30$ (Figure 12.6). Note that Figure 12.6

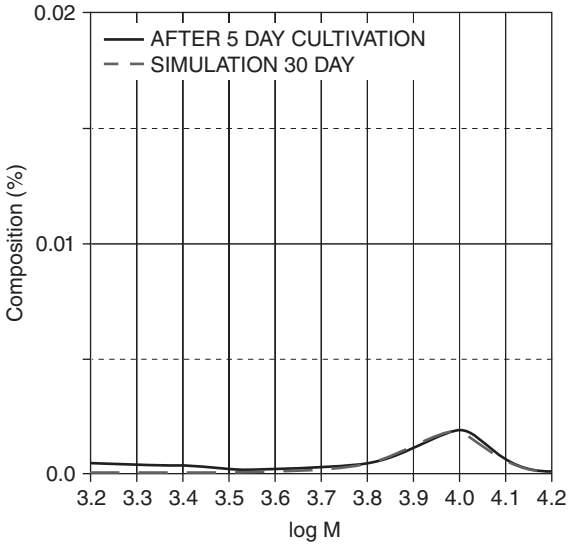

Figure 12.6 Weight distribution of PEG after cultivation for 30 days according to the time-independent model based on the initial value problems (12.9) and (12.10), and the degradation rate shown in Figure 12.5. The experimental result obtained after cultivation for 5 days is also shown [12, 13].

also shows the weight distribution after cultivation for 5 days. It is appropriate to set $T_2 = 5$ and $T_2 = 30$. Equation (12.19) was solved numerically with the Newton's method, and a numerical solution, which was approximately equal to 1.136176, was found [12].

12.3.6 Simulation with Time-Dependent Degradation Rate

Once the degradation rate $\beta(t,M) = \sigma(t)\lambda(M)$ is determined, the initial value problems (12.6) and (12.2) can be solved directly to see how the numerical results and the experimental results agree. The initial value problem was solved numerically with techniques based on previous results [3–5].

Given the initial weight distribution shown in Figure 12.4, the degradation rate $\lambda(M)$ shown in Figure 12.5, and the function $\sigma(t)$ given by Eq. (12.17) with the value of a obtained numerically, the initial value problems (12.6) and (12.2) was solved numerically with the Adams–Bashforth–Moulton predictor–corrector in PECE mode in conjunction with the Runge–Kutta method to generate approximate solutions in the first three steps [24]. Figure 12.7 shows the transition of the weight distribution for 5 days under cultivation of the microbial consortium E-1. Figure 12.8 shows the numerical result and the experimental results for the weight distribution after 1-day cultivation. Note that no information concerning the weight distribution after 1-day cultivation was used to determine the degradation

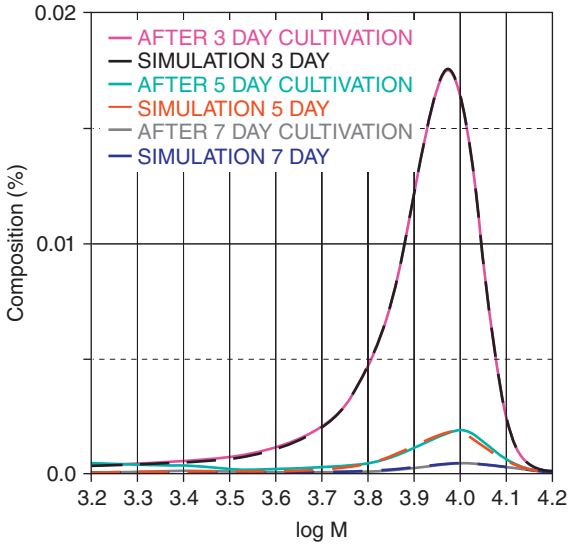

Figure 12.7 The weight distribution of PEG before and after 5-day cultivation, and the transition of the weight distribution based on the initial value problems (12.6) and (12.2) with $\sigma(t) = e^{at+b}$, $a \approx 1.136176$, $b = \ln\{aT_1/(e^{aT_1} - 1)\}$, and $T_1 = T_1 = 3$ [12].

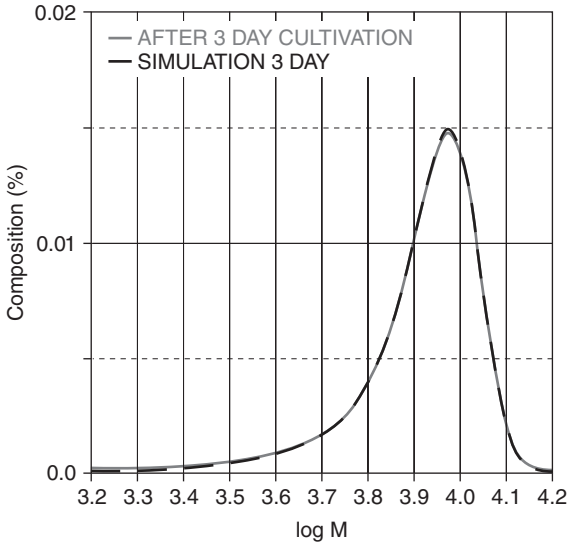

Figure 12.8 The weight distribution of PEG after 1-day cultivation and the weight distribution based on the initial value problems (12.6) and (12.2) with $\sigma(t) = e^{at+b}$, $a \approx 1.136176$, $b = \ln\{aT_1/(e^{aT_1} - 1)\}$, and $T_1 = T_1 = 3$ [12].

rate. Nevertheless, Figure 12.8 shows an acceptable agreement between the numerical result and the experimental result.

12.4
Analysis of Endogenous Depolymerization

12.4.1
Modeling of Endogenous Depolymerization

PVA is in general degraded in a succession of two processes: oxidation of a couple of pendant hydroxyl groups either by oxidase or by dehydrogenase followed by hydrolysis. The sequence of reactions results in a cleavage of carbon–carbon chain at a carbonyl group and an adjacent methyne group [25] (Figure 12.9). Matsumura *et al.* proposed a new metabolism of PVA by oxidation of a hydroxyl group and aldolase reaction of a monoketone structure, which results in a cleavage of carbon–carbon chain between a methyne group adjacent to a carbonyl group and an adjacent hydroxymethyne group [26] (Figure 12.9). Irrespective of metabolic pathways, PVA is in general depolymerized by oxidation and the resultant cleavage

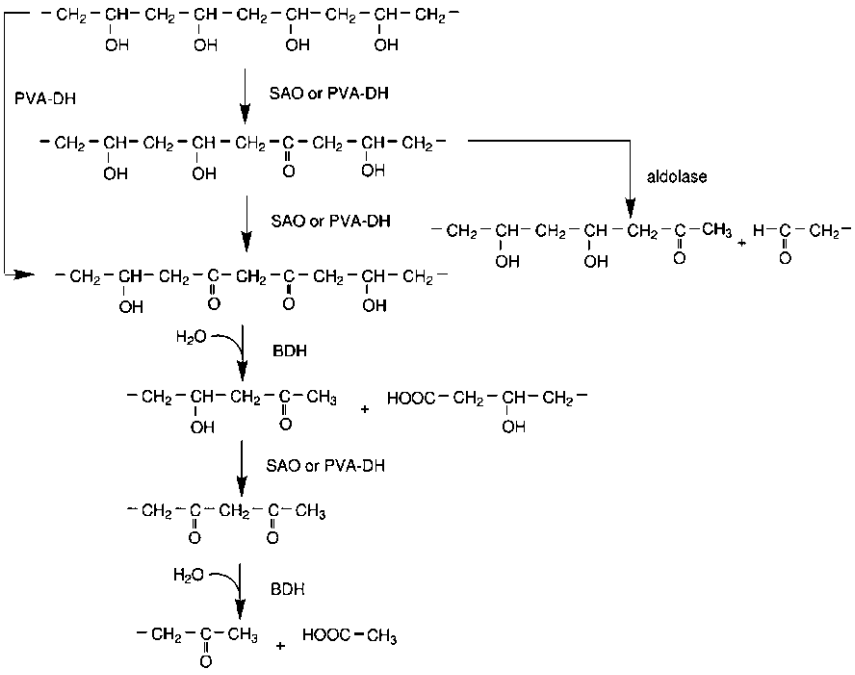

Figure 12.9 Metabolic pathways of PVA [25, 26]. SAO: secondary alcohol oxidase, PVA-DH: PVA dehydrogenase, BDH: β-diketone hydrolase.

of carbon–carbon chain between two carbonyl groups/a carbonyl group and an adjacent hydroxymethyne group, which produces smaller molecules of random sizes.

In order to mathematically model endogenous depolymerization processes of polymers such as PVA, let $w(t,M)$ be its weight distribution with respect to the molecular weight M at time t. Denote by $C(A,B)$ the class of all molecules whose molecular sizes lie between A and B. Then the total weight of $C(A,B)$ present at time t is the integral of $w(t,M)$ with respect to M over the interval $[A,B]$

$$\int_A^B w(t,M)\mathrm{d}M \tag{12.21}$$

For $K \le M$, let $p(t,K,M)$ denote the time rate of transition from $w(t,M)$ to $w(t,K)$ due to endogenous depolymerization. The transition of the weight from $C(A,B)$ to $C(D,E)$ per unit time is the integral of $p(t,K,M)$ with respect to (K,M) over the region R

$$\iint_R p(t,K,M)\mathrm{d}M\,\mathrm{d}K$$

where

$$R = \{(K,M) \mid K \le M,\ D \le K \le E,\ A \le M \le B\}$$

The total weight decrease in $C(A,B)$ per unit time is given by

$$\int_A^B \int_0^M p(t,K,M)\mathrm{d}K\,\mathrm{d}M \tag{12.22}$$

while the total weight increase per unit time is given by

$$\int_A^B \int_M^\infty p(t,M,K)\mathrm{d}K\,\mathrm{d}M \tag{12.23}$$

The equation

$$\frac{\mathrm{d}}{\mathrm{d}t}\int_A^B w(t,M)\mathrm{d}M = \int_A^B \frac{\partial w}{\partial t}(t,M)\mathrm{d}M$$

holds for the rate of change of the quantity (12.21), and it equals the difference between the quantities (12.22) and (12.23)

$$\int_A^B \frac{\partial w}{\partial t}(t,M)\mathrm{d}M = -\int_A^B \int_0^M p(t,K,M)\mathrm{d}K\,\mathrm{d}M + \int_A^B \int_M^\infty p(t,M,K)\mathrm{d}K\,\mathrm{d}M$$

which leads to

$$\int_A^B \left\{ \frac{\partial w}{\partial t}(t,M) + \int_0^M p(t,K,M)\mathrm{d}K - \int_M^\infty p(t,M,K)\mathrm{d}K \right\} \mathrm{d}M = 0$$

Since this equation holds for an arbitrary interval $[A,B]$

$$\frac{\partial w}{\partial t}(t,M)+\int_0^M p(t,K,M)\mathrm{d}K-\int_M^\infty p(t,M,K)\mathrm{d}K=0$$

It follows that $w=w(t,M)$ satisfies Eq. (12.24) [13–16]

$$\frac{\partial w}{\partial t}(t,M)=-\int_0^M p(t,K,M)\mathrm{d}K+\int_M^\infty p(t,M,K)\mathrm{d}K \tag{12.24}$$

Let $\gamma(t,M)$ be the amount which $w(t,M)$ loses per unit time and per unit weight. The amount, which $w(t,M)$ loses per unit time, is $\gamma(t,M)w(t,M)$, which is expressed in terms of $p(t,K,M)$

$$\gamma(t,M)w(t,M)=\int_0^M p(t,K,M)\mathrm{d}K$$

This amount is distributed over weight classes over the interval $[0,M]$. For $K\in[A,B]$, let $q(K,M)$ denote the increase in $w(t,K)$ per unit weight due to the weight loss in $w(t,M)$.

Then $p(t,K,M)=\gamma(t,M)q(K,M)w(t,M)$. It follows that

$$\begin{aligned}\int_A^B \gamma(t,M)w(t,M)\mathrm{d}M&=\int_A^B\int_0^M p(t,K,M)\mathrm{d}K\,\mathrm{d}M\\&=\int_A^B\int_0^M \gamma(t,M)q(K,M)w(t,M)\mathrm{d}K\,\mathrm{d}M\\&=\int_0^M q(K,M)\mathrm{d}K\int_A^B \gamma(t,M)w(t,M)\mathrm{d}M\end{aligned}$$

Since this equation must hold for an arbitrary interval $[A,B]$

$$\int_0^M q(K,M)\mathrm{d}K=1 \tag{12.25}$$

Equation (12.24) leads to

$$\frac{\partial w}{\partial t}(t,M)=-\gamma(t,M)w+\int_M^\infty \gamma(t,K)q(M,K)w(t,K)\mathrm{d}K \tag{12.26}$$

Given an initial weight distribution in terms of a prescribed function $f(M)$, Eq (12.26) forms an initial value problem together with the initial condition

$$w(0,M)=f(M) \tag{12.27}$$

Given an additional weight distribution at $t=T>0$ in terms of a prescribed function $g(M)$

$$w(T,M)=g(M) \tag{12.28}$$

Equation (12.26) and the conditions (12.27) and (12.28) form an inverse problem to find the degradation rate $\gamma(t,M)$, for which the solution of the initial value problems (12.26) and (12.27) also satisfies the condition (12.28).

A time factor of degradability such as enzyme concentration or temperature affects degradation regardless of molecular sizes. Then it can be assumed that the degradation rate $\gamma(t,M)$ is a product of a function of t and a function of M:

$$\gamma(t,M) = \sigma(t)\lambda(M)$$

Let

$$\tau = \int_0^t \sigma(s)\mathrm{d}s$$

and

$$W(\tau,M) = w(t,M)$$

Then

$$\frac{\partial W}{\partial \tau} = \frac{\partial w}{\partial t}\frac{\partial t}{\partial \tau} = \frac{1}{\sigma(t)}\frac{\partial w}{\partial t}$$

It follows from Eq. (12.26) that

$$\frac{\partial W}{\partial \tau}(t,M) = -\lambda(M)W + \int_M^\infty \lambda(K)q(M,K)W(\tau,K)\mathrm{d}K \tag{12.29}$$

Equation (12.29) is associated with initial condition

$$W(0,M) = f(M). \tag{12.30}$$

Let

$$\mathrm{T} = \int_0^T \sigma(s)\mathrm{d}s$$

Then the inverse problem is formulated to find the degradation rate $\lambda(M)$ for which the solution of the initial value problems (12.29) and (12.20) also satisfies the condition

$$W(\mathrm{T},M) = g(M) \tag{12.31}$$

The initial value problems (12.29) and (12.30) correspond to the initial value problems (12.26) and (12.27), and the inverse problems (12.29)–(12.31) correspond to the inverse problems (12.26)–(12.28).

Suppose that $q(K,M)$ is a product of a function of K and a function of M:

$$q(K,M) = c(K)d(M)$$

Then Eq. (12.29) becomes

$$\frac{\partial W}{\partial \tau}(t,M) = -\lambda(M)W + c(M)\int_M^\infty \lambda(K)d(K)W(\tau,K)\mathrm{d}K \tag{12.32}$$

The condition (12.25) leads to

$$\int_0^M q(K,M)\mathrm{d}K = \int_0^M c(K)d(M)\mathrm{d}K = d(M)\int_0^M c(K)\mathrm{d}K = 1$$

In case the number of degraded molecules are uniformly distributed over the interval [0, M] [15–17]

$$c(K) = 2K \quad d(M) = \frac{1}{M^2} \tag{12.33}$$

For these $c(K)$ and $d(M)$, Eq (12.32) is the model originally proposed for the enzymatic degradation of PVA. In case the weight of degraded molecules are uniformly distributed over the interval [0, M] [18]

$$c(K) = 1 \quad d(M) = \frac{1}{M} \tag{12.34}$$

Differentiating both sides of Eq. (12.32) with respect to M leads to

$$\begin{aligned}\frac{\partial^2 W}{\partial M \partial \tau} &= \frac{\partial}{\partial M}\{-\lambda(M)W\} + c'(M)\int_M^\infty \lambda(K)d(K)W(\tau,K)\mathrm{d}K - \lambda(M)c(M)d(M)W \\ &= \frac{\partial}{\partial M}\{-\lambda(M)W\} + \frac{c'(M)}{c(M)}\left\{\frac{\partial W}{\partial \tau} + \lambda(M)W\right\} - \lambda(M)c(M)d(M)W\end{aligned}$$

and

$$\frac{\partial}{\partial M}\left\{\frac{\partial W}{\partial \tau} + \lambda(M)W\right\} = \frac{c'(M)}{c(M)}\left\{\frac{\partial W}{\partial \tau} + \lambda(M)W\right\} - \lambda(M)c(M)d(M)W \tag{12.35}$$

Numerical techniques have been developed for the inverse problem to find the degradation rate $\lambda(M)$ for which the solution of the initial value problems (12.35) and (12.30) also satisfies the condition (12.31), in case $c(K)$ and $d(M)$ are given by (12.33). Those techniques can be extended to cover the general case.

12.4.2 Analysis of Enzymatic PLA Depolymerization

The experimental and analytical study of endogenous depolymerization is continued to cover degradation of PLA [17, 18]. PLA used for the experiment was poly(L-lactide). Figure 12.10 shows the GPC patterns of PLA given in terms of retention time versus voltage. The figure also shows the baselines of the GPC patterns. Figure 12.11 shows standards of molecular weight versus retention time. The figure also shows the least-squares approximation that fits the standards. Figure 12.12 shows the weight distribution of PLA before and after enzymatic degradation for 5 and 67 h based on the shift according to the baselines shown in Figure 12.10, the transformation according to the least-squares approximation shown in Figure 12.11, and scaling according to the residual amounts. An inverse problem was formulated to determine the degradation rate for which the solution of the initial value problem also satisfies the weight distribution after incubation for 5 h. PLA was solved in chloroform and emulsified by sonication for enzymatic degradation. As the time elapsed, chloroform was lost by evaporation, resulting in reduced degradation rates. In previous studies [18], the temporal change of degradability was considered, and a temporally dependent degradation rate was incorporated into the endogenous depolymerization model. The endogenous depolymerization is studied in this chapter. A mathematical model of endogenous depolymerization, which covers the previously proposed models as special cases, is introduced.

It is shown that a general model can be transformed to a form to which previously developed techniques are applicable. Techniques to solve an inverse problem

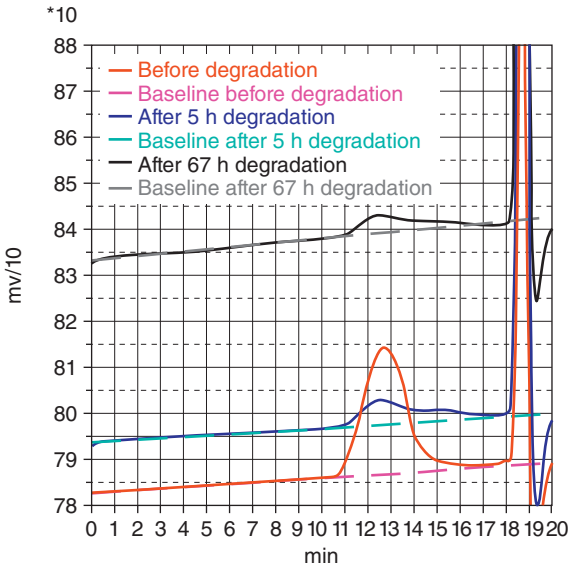

Figure 12.10 GPC patterns of PLA given in terms of retention time versus voltage [16, 17]. The baselines are also shown.

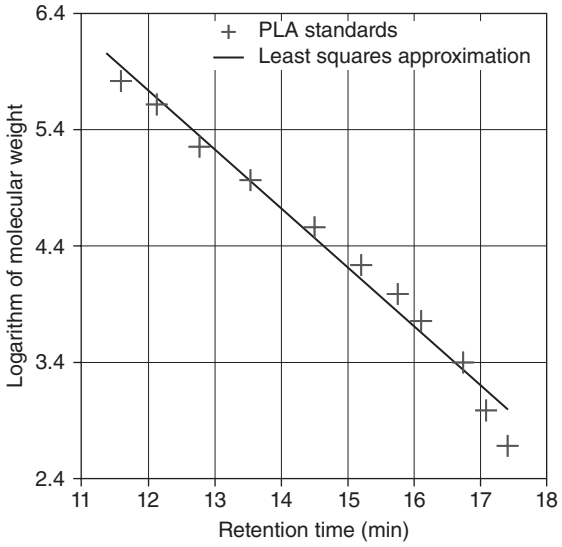

Figure 12.11 Standards of molecular weight versus retention time, and the least-squares approximation which fits the standards [17].

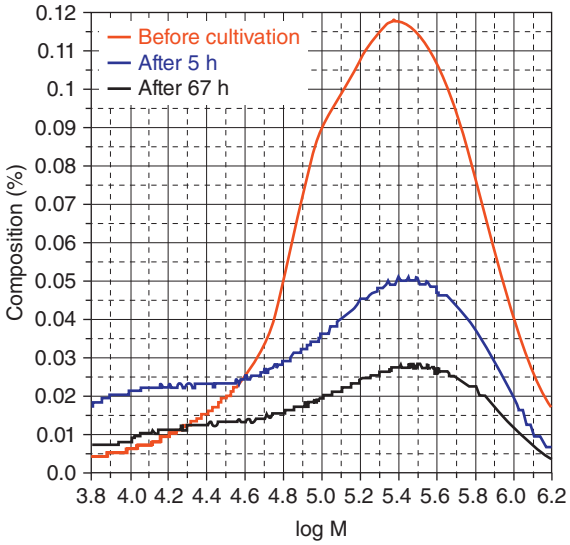

Figure 12.12 Weight distribution of PLA before and after enzymatic degradation. Residual amounts of PLA after incubation for 5 and 67 h were 40% and 27%, respectively [17, 18].

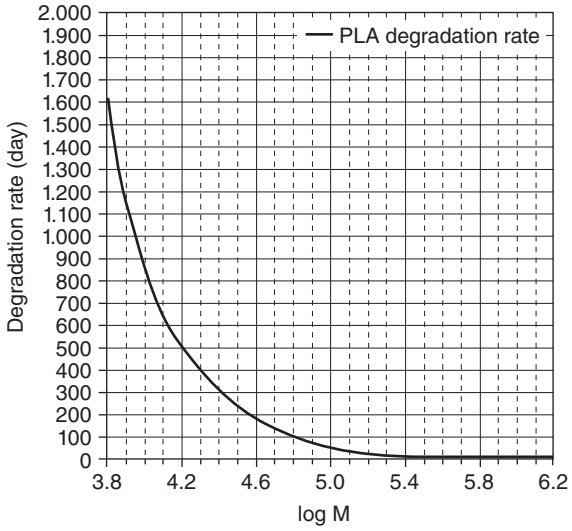

Figure 12.13 Degradation rate of PLA based on the GPC profiles obtained before and after incubation for 5 h shown in Figure 12.12. $c(K) = 1$ and $d(M) = 1/M$ [17].

to determine the degradation rate and to simulate the transition of weight distribution are described, and numerical results are introduced.

The weight distribution before incubation shown in Figure 12.12 was set as the initial condition (12.30), and the weight distribution after incubation for 5 h shown in Figure 12.12 was set as the final condition (12.30) to solve the inverse problem numerically for the function $c(K)$ and $d(M)$ given by the expressions (12.34). Figure 12.13 shows the graph of the degradation rate $\lambda(M)$. Figure 12.14 shows a result of numerical simulation for transition of weight distribution over incubation period for 10 h based on the degradation rate shown in Figure 12.13. Figure 12.15 shows the experimental result for weight distribution after incubation for 5 h and a numerical result to simulate the experimental result based on the degradation rate shown in Figure 12.13.

Chloroform used to dissolve PLA was lost by evaporation as the time elapsed. The loss of chloroform resulted in reduction of the degradation rate. Figure 12.16 shows the experimental result for weight distribution after incubation for 67 h and a numerical result for the weight distribution after incubation for 8.5 h based on the degradation rate. The figure shows that it takes only 8.5 h to reach the stage after incubation for 67 h with the average degradation rate over incubation period for the first five hours.

12.4.3
Simulation of an Endogenous Depolymerization Process of PLA

A technique to determine the time factor $\sigma(t)$ has been proposed [18]. Since the decrease of degradability was due to evaporation of chloroform, it is appropriate to assume that $\sigma(t)$ is an exponential function of time:

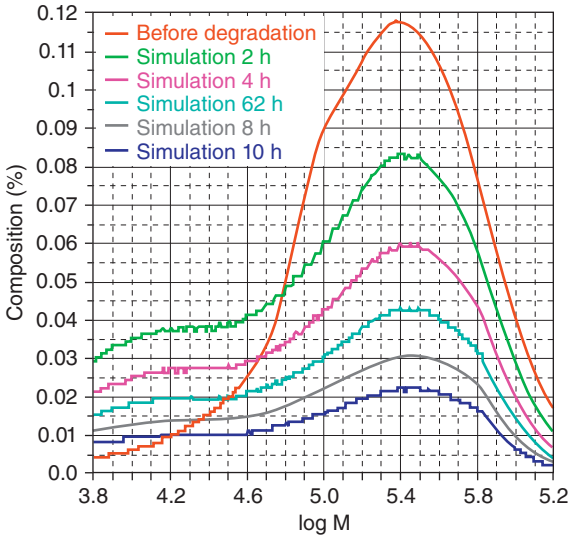

Figure 12.14 Transition of weight distribution for over incubation period for 10 h based on the degradation rate shown in Figure 12.13 [18].

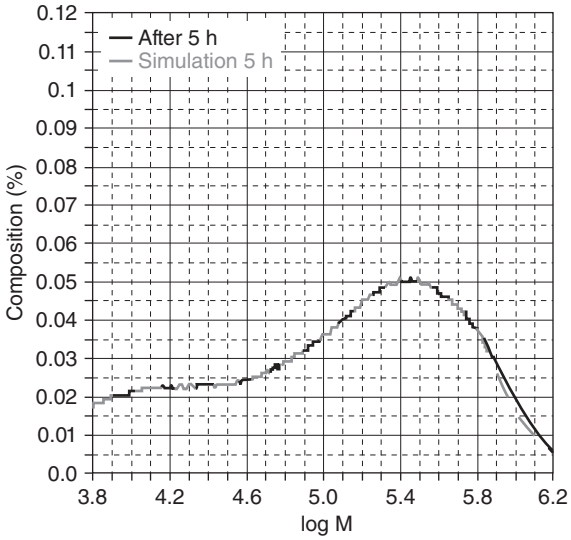

Figure 12.15 Weight distribution after incubation for 5 h and numerical result to simulate the experimental result [18].

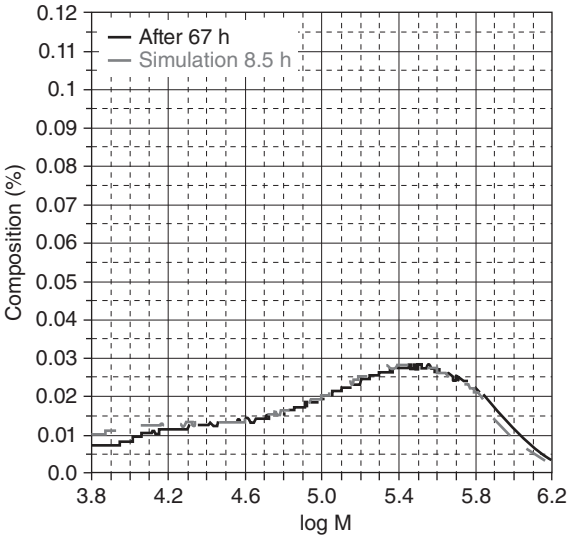

Figure 12.16 Weight distribution after 67 h of incubation and numerical result to simulate the weight distribution after incubation for 8.5 h [18].

$$\sigma(t) = e^{-at+b} \tag{12.36}$$

Then τ is given by

$$\tau = \int_0^t \sigma(s)\mathrm{d}s = \int_0^t e^{-as+b}\mathrm{d}s = \frac{e^b}{a}(1-e^{-at}) \tag{12.37}$$

Given T_1 and T_2 with $T_1 < T_2$, let

$$\mathrm{T}_1 = \int_0^{T_1} e^{-as+b}\mathrm{d}s \tag{12.38}$$

$$\mathrm{T}_2 = \int_0^{T_2} e^{-as+b}\mathrm{d}s \tag{12.39}$$

In particular, in view of the result shown in Figure 12.16, the values of parameters can be set as follows: $T_1 = \mathrm{T}_1 = 5/24$ (day), $T_2 = 67/24$ (day), and $\mathrm{T}_2 = 8.5/24$ (day).

Equations (12.38) and (12.39) lead to

$$\mathrm{T}_1 = \frac{e^b}{a}(1-e^{-aT_1})$$

It follows from Eq. (12.37) that

$$\tau = \mathrm{T}_1 \frac{1-e^{-at}}{1-e^{-a\mathrm{T}_1}}$$

Now Eq. (12.39) leads to

$$\mathrm{T}_2 = \mathrm{T}_1 \frac{1-e^{-a\mathrm{T}_2}}{1-e^{-a\mathrm{T}_1}} \tag{12.40}$$

Define $h(a)$ by

$$h(a) = \frac{1-e^{a\mathrm{T}_2}}{1-e^{a\mathrm{T}_1}} - \frac{\mathrm{T}_2}{\mathrm{T}_1}$$

Now Eq. (12.40) is equivalent to

$$h(a) = 0 \tag{12.41}$$

Equation (12.41) was solved numerically for the values of parameters: $T_1 = \mathrm{T}_1 = 5/24$, $T_2 = 67/24$, and $\mathrm{T}_2 = 8.5/24$, and an approximate value of the solution $a \approx 4.259$ was found. Figure 12.17 shows a result of numerical simulation for the transition of weight distribution of PLA over incubation period for 67 h.

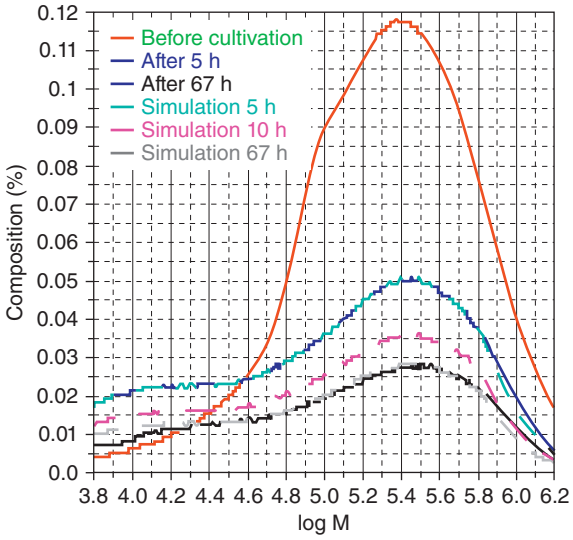

Figure 12.17 Transition of weight distribution over incubation period for 67 h [18].

12.5 Discussion

The degradation rate $\lambda(M)$ of the exogenous depolymerization model is the ratio of the total weight of M-molecules degraded per unit time. It also represents the ratio of the number of M-molecules that undergo exogenous depolymerization processes. It might be assumed to be independent of the molecular size M, for the exogenous depolymerization processes take place only at the terminals of the molecules. In practice, however, this is the case for molecules of moderate sizes. As metabolic enzymes are located in cell membranes, they take effect in periplasms, and at least one end of a molecule must penetrate through its outer membrane to become subject to an exogenous depolymerization process [7].

In the presence of sufficient number of microorganism, the number ratio of molecules in contact with microorganisms in a fixed period of time can be assumed to be independent of the molecular weight. When a molecule makes contact with a microorganism, a part of a fixed length should be taken into a cell in a fixed period. If that part happens to contain one of the terminals, the enzyme takes effect and an exogenous depolymerization process starts. The possibility for a part of a fixed length to contain a terminal becomes less when the molecule becomes large. This is the reason why the oxidation rate is a decreasing function of the molecular weight. The result shown in Figure 12.5. clearly indicates the dependence of the rate of membrane transport with respect to the molecular weight, and our mathematical analysis has revealed the role of membrane transport in exogenous depolymerization processes, which is unpredictable from experimental results alone.

The exogenous depolymerization model originally developed for PE biodegradation has been applied successfully to PEG biodegradation. The numerical results show how molecules are incorporated into cells in exogenous depolymerization processes. The validity of the result concerning the oxidation rate has been confirmed by the numerical simulation (Figures 12.7 and 12.8). This is a typical microbial depolymerization process of exogenous type, where monomer units are split from the terminals of molecules.

The only factor assumed in construction of the endogenous depolymerization model was random separation of molecules. There are other factors of weight changes in processes through the metabolic pathways of PVA as was described, but those changes should be negligible when compared to the weight shift due to random separation of molecules due to enzymatic degradation. As a mathematical model for enzymatic degradation of PVA, a linear second-order hyperbolic partial differential has been derived from the original model. Given a prescribed function that represents the degradation rate and an initial condition, it forms an initial value problem of a linear partial differential equation. On the other hand, given the initial condition and an additional final condition, it forms an inverse problem to determine the degradation rate for which the solution of the initial value problem also satisfies the final condition.

It is shown that the inverse problem can be reduced to a nonlinear ordinary differential equation whose unknown variable represents the weight flux into the

class of all molecules of a fixed size from the classes of all larger molecules due to random depolymerization. The necessary and sufficient condition, under which the initial value problem of the ordinary differential equation has a solution defined locally, has been established. The theory was described by introducing a numerical result based on the ordinary differential equation. Note that one can hardly predict the existence of its solution for a given range of the independent variable. On the contrary, the inverse problem has been solved successfully for the given range of the molecular weight applying the HPLC data obtained before and after enzymatic degradation of PLA. The result of numerical simulation shows that the technique to solve the inverse problem is practically acceptable.

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