Polymer Biodegradability 2.0: A Holistic View on Polymer Biodegradation in Natural and Engineered Environments

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Polymer Biodegradability 2.0: A Holistic View on Polymer Biodegradation in Natural and Engineered Environments

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Abstract Biodegradable polymers are an important part of the solution toolbox to achieve circularity in the plastic economy and overcome negative impacts of a linear plastic economy. Biodegradable polymers need to excel not only on a mechanical performance level in the application to fulfill their function during the use phase but also on a biodegradation performance level after use. The biodegradation performance is tailored to the application and the receiving environment of the polymer product after use, which can be both engineered systems (e.g., compost, anaerobic digestors, wastewater treatment plants) and natural systems (e.g., soils, freshwater, or marine environments). This chapter addresses key aspects of polymer biodegradability and biodegradation in both natural and engineered systems with the goal to advance a more holistic view on the topic and, thereby, provide guidance for all stakeholders working on developing, testing, and regulating biodegradable polymers. These aspects include definitions of biodegradability and biodegradation, elucidating polymer- and environmental factors that control the biodegradation process, a discussion of the analytical chemistry of polymer biodegradation, polymer biodegradability testing and certification, as well as a brief overview of research needs. In accordance with the diverse backgrounds of the authors of the chapter, this chapter targets all stakeholder groups from academics to industry and regulators.

Keywords Polymer biodegradation · Biodegradability · Mineralization · Biodegradable plastics · Bioplastic · Compostability

1 Introduction

Polymers – including structural polymers, on which this chapter focuses, and water-soluble polymers – play essential roles in our modern life and fulfill many important functions in diverse applications. For instance, structural polymers are used in consumer goods such as shoes and garments, in the transportation industry, in electronic goods and for hygienic packaging of our food. Water-soluble polymers are used widely in home and personal care products and in agricultural formulations. In all the above-mentioned applications, the polymers deliver a desired functionality at high efficiency and thereby contribute to the sustainability of the application.

The many unique benefits offered by using polymers are undisputed. Yet, academic and industrial researchers working on developing and applying polymers (and plastics composed thereof) face two major challenges: to establish circularity in the use of polymers (including preventing the accumulation of persistent (micro- and nano) plastics in the environment) and to become carbon neutral. Addressing these challenges requires research and innovation on polymers as well as new approaches in their use, all as part of a multiple "solution toolbox" [1–3]. The use of biodegradable and biobased polymers (both being part of the larger class of the so-called biopolymers) in specific applications is considered an integral part of this overall "solution toolbox" [4] (see also introduction of book).

There are numerous applications in which the use of biodegradable instead of non-biodegradable polymers offers benefits toward attaining circularity and preventing environmental plastic pollution [5, 6]. These applications include, but are not limited to, compostable plastics (e.g., compostable bags for collection of domestic biowaste) and agricultural plastics (e.g., soil-biodegradable mulch films) [4]. In the first case, compostable (and possibly also biobased) plastics enable organic waste recycling and closing the nutrient loop [7]. Austria and Italy are good examples for countries which successfully implemented waste management structures that include compostable bags (e.g., "*Biosackerl*" in Austria) to collect organic waste. Using compostable plastic bags is essential to allow collecting biowaste separately from the normal household waste and, at the same time, to reduce or even avoid contamination of the compost by conventional plastics that persist in the compost. The use of compostable polymers (and plastics) thus is a prerequisite to ensure a high quality of the final compost [8]. Among the agricultural applications using biodegradable polymers are mulch films that are placed onto soils to increase crop yields [9, 10]. Conventional mulch films are composed of non-biodegradable polyethylene (PE) and require a minimum thickness of at least 25 μm to ensure that they can be completely recollectable from the field after use. Thinner films would suffice to fulfill the needed mechanical performance of the films during the use phase but would impair complete recollection of the film after its use phase, resulting in soil contamination by residual PE film fragments. Following use in the field, the recollected PE films often contain crop and soil attachments which render recycling and reuse of these films difficult if not impossible, leaving only undesirable incineration or landfilling as disposal options. As compared to conventional PE films, soil-biodegradable mulch films composed of biodegradable polymers can be thinner, such as 15 μm, as they do not need to be recollected after use but instead are plowed into the soil to then undergo biodegradation. This practice also substantially lowers the end-of-life costs because biodegradable films – as opposed to conventional PE-based films – do not need to be recollected from the field, transported, and disposed of. The possibility to use thin biodegradable films (instead of thicker conventional films) comes with the additional benefit that less polymer material is needed for the mulch film application.

In specific applications (such as thin mulch films), the use of polymers that are biodegradable in the open environment is beneficial over the use of conventional, non-biodegradable polymers in that it helps to overcome environmental plastic pollution [4, 6]. Biodegradation as an end-of-life treatment is particularly warranted for applications in which complete recollection from the environment after use and/or reuse and recycling of the collected polymer material are not feasible. It was recently proposed to delineate three categories for such applications: (1) applications in which polymers (and plastics) are intentionally left in the environment after the application (i.e., seed coatings), (2) applications in which polymers (and plastics) are lost to the open environment through abrasion (e.g., paints and geotextiles), and (3) applications which have a high potential that the deployed polymer (and plastic) items are lost during (or after) use (e.g., certain fishing gear) [6].

Compostable bags and soil-biodegradable mulch films are only two of a larger number of applications [4, 6] in which biodegradable polymers are recognized as an important component of the overall "solution toolbox" by academics, industry, NGOs, and decision makers. These applications have in common that biodegradation is the targeted end-of-life scenario or the preferred scenario for polymers that unintentionally leak into the environment from specific applications [11, 12]. Consequently, decision makers and regulators rightly demand that biodegradation standard- and legislative support frameworks are based on stringent scientific evidence demonstrating that the polymers indeed undergo biodegradation. In essence, biodegradable polymers therefore must perform on two levels: on a "*mechanical performance level*" during processing and during the application (e.g., processability and tensile strength of the polymer) and on a "*end-of-life biodegradation performance level*," following the use phase.

Providing scientific proof for "end-of-life biodegradation performance" – and, thereby, to ensure the acceptance of biodegradable polymers by different stakeholder groups – requires a revised and extended approach of understanding and assessing polymer biodegradability and biodegradation in receiving environments. The latter include both engineered systems (e.g., anaerobic digestors, industrial and home composting, wastewater treatment plants) and natural environments (i.e., soils, marine, and freshwater systems).

This revised and extended approach of polymer biodegradability and biodegradation is critical to achieve three specific targets.

Target 1: *Establish reliable and science-based laboratory testing systems to provide a clear framework for the development of biodegradable polymers.*

The first target is to demonstrate, by experimental incubation tests, that a specific polymer material (or item made thereof) indeed biodegrades in the specific targeted receiving environment to a defined extent over a given time. This target is challenging to achieve given that biodegradation in almost all cases needs to be followed over incubation periods of several months and up to a few years, thereby requiring both extensive testing time and robust testing systems. Ideally, these test systems are designed to have incubation conditions that are representative of the conditions in the actual targeted receiving environment of the polymer. At the same time, the handling and control of the test systems need to be feasible. Furthermore, the system needs to allow for a continuous and direct monitoring of polymer biodegradation during the test period, as discussed in more detail below. For the certification of biodegradable polymers (and items made thereof), biodegradation standards need to define sufficiently stringent criteria that polymers passing the tests indeed biodegrade at desired rates in the actual receiving environment [13]. These criteria are also critical to prevent misuse of labels by helping to identify polymers or polymer additive technologies (e.g., pro-oxidant additives for conventional, non-biodegradable polymers) that do not fulfill the biodegradation criteria and therefore falsely claim polymer biodegradability [14–16]. Finally, standards with clear criteria set the boundaries for research on and innovation of future biodegradable polymers and provide guidance and boundaries for academic laboratories and industrial research and development.

Target 2: *Align laboratory testing systems with real (*in situ*) environmental conditions.*

The design of laboratory testing methods to determine polymer biodegradability (see Target 1) must be well-aligned with the conditions that prevail in the targeted receiving environment (e.g., engineered systems such as industrial composting or natural systems such as soils). This alignment is critical to ensure that results from laboratory tests adequately capture the "biodegradation performance" of the polymer also under real in situ conditions. The transferability of biodegradability results obtained in laboratory incubations to real in situ conditions in the actual receiving environment needs to be fulfilled for laboratory testing systems to be acceptable in standards and for certification in the long-term. This second target requests establishing separate laboratory testing systems for the different targeted receiving environments. The tests need to be adaptable to the specifics of the receiving environment in terms of temperature, humidity, and anticipated residence time of the polymer in that system. Demonstrating transferability of biodegradation results from the laboratory to real in situ field systems currently is a central request by different stakeholders, including political decision makers.

Target 3: *Ensure complete biodegradation of the polymer in the receiving environment.*

Stringent and elaborate testing of polymer biodegradation in the laboratory needs to be complemented by efforts to demonstrate complete biodegradation of the polymer also in the receiving environment over a time frame that is acceptable by different stakeholders. This target is needed to ensure no formation of residual persistent polymer particles that accumulate in the receiving environment. Achieving this target includes the development of analytical methods to track biodegradation and the formation dynamics and continuous biodegradation of potentially forming micro- and nanometer-sized plastic fragments as intermediates in the biodegradation process.

To fulfill all the three above-defined targets, an extended approach (i.e., "Polymer biodegradability 2.0") is needed. This approach needs to be based on a fundamental understanding of polymer biodegradation in the targeted receiving environment.

In this chapter, we abide to the recently proposed concept that polymer biodegradability is a *system property* in that biodegradability depends on both the polymer material properties *and* the characteristics of the receiving environment [6]. This concept expands from the traditional view in which polymer biodegradability is considered primarily (and sometimes exclusively) a polymer material property. We also explicitly emphasize that the physicochemical properties of a biodegradable polymer that render it biodegradable are desired: intentional "designed to biodegrade" (i.e., biodegradation performance) fundamentally differs from "biodeterioration," a term that has often been used to describe undesired deterioration of a polymer by processes involving microorganisms. Because of the multifactorial material and environmental effects on polymer biodegradation rates, elucidating this process and developing new materials requires expertise from a multitude of research disciplines, including – but not limited to – polymer chemistry, environmental chemistry, and microbiology. This chapter summarizes fundamentals and

key concepts of polymer biodegradation in engineered and natural systems. This chapter also briefly highlights recent advances in analytical approaches, testing approaches, and certification standards for polymer biodegradation. The goal is to help advance a more holistic view on polymer biodegradability in both natural and engineered systems and, thereby, provide guidance for all stakeholders of developing, testing, and regulating biodegradable polymers.

2 Definition of Polymer Biodegradability and Biodegradation

Stringent and clear definitions of polymer biodegradability and biodegradation are critical to advance our understanding of polymer biodegradation, testing and certifying polymer biodegradability and to clearly communicate these aspects. Conversely, the absence of concise definitions will result in misunderstandings and, more problematically, can even lead to false claims of polymer biodegradability and biodegradation.

We herein define "*polymer biodegradation*" as follows [6]:

Polymer biodegradation is the process by which microorganisms completely metabolize the organic carbon in the polymer under formation of carbon dioxide and microbial biomass, under oxic conditions, or carbon dioxide, methane, and microbial biomass, under anoxic conditions.

This definition holds true for polymer biodegradation both in engineered and natural systems. In fact, it applies not only to structural polymers (which are used to make plastics and which are at the focus of the discussion in this chapter) but also to non-structural, water-soluble polymers. For polymers containing organically bound heteroatoms (i.e., N, P, and S), these heteroatoms need to be converted to the respective inorganic salts or also be incorporated into microbial biomass during the biodegradation process. However, the major biodegradable polymers currently marketed are composed solely of C, H, and O (see below).

Based on the above definition, we can express polymer biodegradation as a simple reaction [17, 18]:

$$C_{polymer} \rightarrow C_{CO2}\ (+C_{CH4}) + C_{microbial\ biomass} \quad (1)$$

where $C_{polymer}$ is the organic carbon of the polymer (or polymers, in case of plastics composed of more than one), C_{CO2} and C_{CH4} is the polymer-derived carbon that has been metabolically converted by microorganisms to carbon dioxide and methane, respectively, and $C_{microbial\ biomass}$ is the polymer-derived carbon incorporated into microbial biomass (i.e., intracellular bio(macro)molecules such as building blocks of cells).

Conversion of $C_{polymer}$ to C_{CO2} is commonly referred to as "mineralization." The evolved CO_2 may either be formed directly from polymer carbon by respiration of

polymer-derived substrates or it may form from the mineralization of polymer carbon first incorporated into microbial biomass. The latter formation pathway reflects that microbial biomass containing polymer-derived carbon is itself a transient carbon pool: $C_{microbial\ biomass}$ in both alive and dead microbial cells can be reworked to C_{CO2}. Microbial necromass may also be reworked into the (non-living) natural organic matter pool in the specific receiving environment (e.g., soil organic matter in soils), from which mineralization may be slow. Polymer carbon conversion to CO_2 (and CH_4) and to microbial biomass is a desirable and acceptable biodegradation endpoint.

While Eq. 1 describes the "educts" and "products" of polymer biodegradation, it does not capture that polymer biodegradation is a time-dependent process. The latter can be accounted for by balancing polymer carbon at any time during the biodegradation according to Eq. 2:

$$C_{polymer}\ (t) = C_{polymer}\ (t_0) - [C_{CO2}\ (t) + C_{microbial\ biomass}\ (t)] \tag{2}$$

where $C_{polymer}\ (t)$ and $C_{polymer}\ (t_0)$ are the amounts of polymer carbon at time point t during the biodegradation process and at the onset of the biodegradation process t_0, respectively, and $C_{CO2}\ (t)$ and $C_{microbial\ biomass}\ (t)$ are amounts of polymer-derived carbon mineralized to CO_2 and transferred into microbial biomass at time t, respectively. For simplicity, we here consider biodegradation only under oxic conditions and, therefore, omit CH_4 as a possible biodegradation product under anoxic conditions from the equations. Furthermore, we make the simplification in Eq. 2 that $C_{microbial\ biomass}$ does not undergo reprocessing over time (which is not the case).

Polymer biodegradation can be directly followed in laboratory incubations in which the conversion of $C_{polymer}$ to C_{CO2} is followed over time through respirometric analyses. Respirometric analyses are critical to verify biodegradation given that CO_2 is the final product of the biodegradation process under toxic conditions. We can therefore rearrange Eq. 2 for $C_{CO2}(t)$ to obtain Eq. 3:

$$C_{CO2}\ (t) = C_{polymer}\ (t_0) - C_{polymer}\ (t) - C_{microbial\ biomass}\ (t) \tag{3}$$

While respirometric analyses provide proof for polymer biodegradation, Eq. 3 highlights that these analyses provide no information on the relative importance of $C_{microbial\ biomass}\ (t)$ and residual $C_{polymer}\ (t)$ to the non-mineralized polymer carbon. The nature of the non-mineralized polymer carbon thus remains unknown when only using respirometric analyses. Significant formation of $C_{microbial\ biomass}$ would imply that respirometric analyses of C_{CO2} underestimate the actual extent of polymer biodegradation.

One common approach in standard test methods to account for the fact that polymer carbon may be incorporated into biomass (i.e., the formation of $C_{microbial\ biomass}$) is to express C_{CO2} formed in polymer biodegradation not in absolute terms (i.e., % of polymer carbon converted to CO_2) but instead relative to the C_{CO2} formed from a positive control – a known biodegradable reference (bio) polymer, such as

cellulose or polyhydroxyalkanoates (PHA) – run in parallel incubations in the same matrix (e.g., soil or sediment). Biodegradation of these positive control materials often shows plateauing mineralization extents at values smaller than 100%, with the "missing" non-mineralized carbon being ascribed to $C_{\text{microbial biomass}}$ formation.

Expressing the amounts of C_{CO2} formed from polymers relative to the amounts of C_{CO2} formed from positive control reference polymers during incubation assumes that microorganisms in the tested medium have a similar metabolic utilization pattern for substrates derived from the polymers of interest and from the positive control reference polymer. More specifically, it is assumed that microorganisms in the tested medium have comparable carbon use efficiencies (CUEs) (i.e., the ratio of substrate carbon incorporated into microbial biomass relative to the total substrate carbon taken up) for the substrate molecules from the polymer of interest and from the positive control. While this assumption may be valid, information on CUEs for polymer-derived substrates is currently missing. The CUEs of polymers are likely dependent not only on the chemistry of the polymer-derived molecules and their specific intracellular metabolic processing, but also on the rate at which polymer-derived substrates become available to microbial cells during the biodegradation process: low CUEs are expected when rates at which polymer-derived substrates become available to microbial cell are low given that the cell metabolism is then likely directed toward energy generation rather than biomass formation. Furthermore, extents of incorporation of polymer carbon into microbial biomass may strongly depend also on the availability of nitrogen and phosphorus as these are required for biomass buildup, as previously shown for natural substrates [19].

We note that new solvent extraction-based analytical approaches to quantify $C_{\text{polymer}}(t)$ over the course of incubations promise to help identify the nature of non-mineralized polymer carbon and, therefore, the extent to which polymer carbon is incorporated into microbial biomass (Sect. 4 below provides more information).

Equation 3 forms the basis for elucidating polymer biodegradation and testing polymer biodegradability, as detailed in Sects. 3–5 in this chapter. Biodegradation standards typically stipulate a high extent of mineralization of polymer carbon (commonly 90% of the carbon, either in absolute terms or relative to the extent of mineralization of a reference polymer, as discussed above) over a pre-defined incubation period. These incubation periods are specific to the product application of the tested biodegradable polymer and typically vary between weeks to months (e.g., for compostable polymers and plastics made thereof) and up to 2 years (e.g., for polymer biodegrading in natural systems, such as mulch films in agricultural soils).

Given the specific definition of "biodegradation," this term ought not to be used interchangeably with other terminology that describes alterations in the physicochemical properties of a polymer (or plastic) item, including "*degradation*," "*disintegration*," "*breakdown*," "*biodeterioration*," "*biotransformation,*" and "*fragmentation*." While the latter processes may (all) be involved in polymer biodegradation (e.g., a biodegradable polymer or plastic may also undergo fragmentation into smaller particles during its biodegradation process), the term

"biodegradation" is distinct from all others in that it stipulates that the entire polymer carbon is microbially converted to CO_2 (and CH_4) and microbial biomass.

In addition to the term "polymer *biodegradation*" that describes a process, the term "polymer *biodegradability*" is also commonly used. The latter term describes the propensity of a polymer (or, for a plastic, of the polymer(s) contained in the plastic) to undergo biodegradation in a specific receiving environment. As stated above, we herein adopt the view that biodegradability is a *system property* in that it depends both on the physicochemical properties of the polymer and on the abiotic and biotic conditions of the receiving environment in which polymer biodegradation is tested or occurs. Because conditions vary substantially between different engineered and natural systems (e.g., industrial compost vs. soils vs. freshwater systems vs. marine systems), certifications and labels of polymers being "*biodegradable*" ought to specify in and for which engineered or natural environment biodegradation was tested and certified (i.e., "*industrially compostable*," "*soil biodegradable*," "*freshwater biodegradable,*" or "*marine biodegradable*") (see also Sect. 5 of this chapter). Defining the receiving environment for which polymer biodegradation has been certified is critical to overcome the common misconception of biodegradable polymers being "universally" biodegradable across all receiving environments (which may, however, be indeed the case for a few biodegradable polymers). Finally, complementing certification labels with information on the receiving environment does not imply that this polymer shows the same biodegradation rates within different types (e.g., types of soils or types of marine sediments) and habitats of the same receiving environment (e.g., beach, seafloor, and water column habitats in marine environments). Instead, a certified biodegradable polymer may show variations in biodegradation rates between different types of the same receiving environment or between habitats of the same receiving environment for which the certificate was issued.

We conclude the discussion of terminology with the term "*intrinsically biodegradable*." This attribute has been proposed for polymers that have demonstrated extensive biodegradation in test systems that favor the biodegradation (e.g., elevated temperatures, controlled humidity, presence of nutrients). As such, "intrinsic biodegradability" may be viewed as a material attribute and as a proof that the polymer can, in principle, be metabolically utilized by microorganisms. However, the term "*intrinsically biodegradable*" should not be misinterpreted to imply that the polymer is universally biodegradable across receiving environments – in fact, it may not biodegrade in environments with conditions disfavoring its biodegradation. Section 3 will summarize key polymer properties and environmental conditions that govern polymer biodegradability.

3 Process Elucidation of Polymer Biodegradation

3.1 Steps in Polymer Biodegradation

As defined in the previous subsection, polymer biodegradation refers to the process in which polymer carbon is metabolized by microorganisms for energy production under the formation of CO_2 (and CH_4) (catabolism) and for the formation of microbial biomass (anabolism). Therefore, the presence of microbial degraders in the polymer-receiving environment is central to biodegradation. The uptake of polymer-derived molecules into microbial cells and the intracellular processing of these molecules may, however, not be the rate-limiting steps of the overall biodegradation. Instead, the biodegradation rate in many engineered and natural systems is controlled by the rate at which the polymer breaks down into molecules sufficiently small for microbes to take the molecules up into their cells and metabolically use them as substrates [20, 21]. Molecules that can readily be taken up into microbial cells are expected to have an upper size limit in the range of 1,000 Da, a molecular weight that is substantially smaller than the typical molecular weights of polymers. Efficient breakdown of the polymer into small, microbially utilizable molecules separates biodegradable polymers from conventional polymers: while the latter may fragment into small particles – micro- and nanoplastics – these fragments persist and are not readily converted to organic molecules sufficiently small to be taken up by microorganisms. Consequently, degradation of conventional plastics commonly results in the formation of micro- and nanoplastics that accumulate in the environment. While biodegradation of biodegradable polymers may also involve fragmentation of the polymer into small polymer particles, these particles are only intermediates and continue to undergo biodegradation (possibly even at increasing rates given that the small polymer particles have higher surface-to-volume ratios than the original polymer specimen).

We herein describe polymer biodegradation as a two-step process (Fig. 1). The first step is the extensive breakdown of the macromolecular chains of the polymer into small (i.e., low-molecular-weight) organic molecules. For most commercial biodegradable polymers, this breakdown occurs by hydrolysis of hydrolyzable bonds in the polymer backbone. For some polymers, such as polylactic acid (PLA), hydrolysis is primarily abiotic and increases in rate with increasing temperature. Abiotic hydrolysis may not only be constrained to the polymer surface but also occur in the bulk phase of the polymer if water molecules diffuse into the polymer matrix. These polymers then undergo "bulk erosion" [22, 23]. Yet, for many biodegradable polymers, abiotic breakdown is slow. Instead, breakdown of these polymers is catalyzed by extracellular enzymes that are secreted by microorganisms. Given that these enzymes have dimensions of a few nanometers, they cannot diffuse into the bulk phase of the polymers. Enzymatically mediated breakdown is therefore commonly restricted to the polymer surface, resulting in polymer "surface erosion" [22, 23].

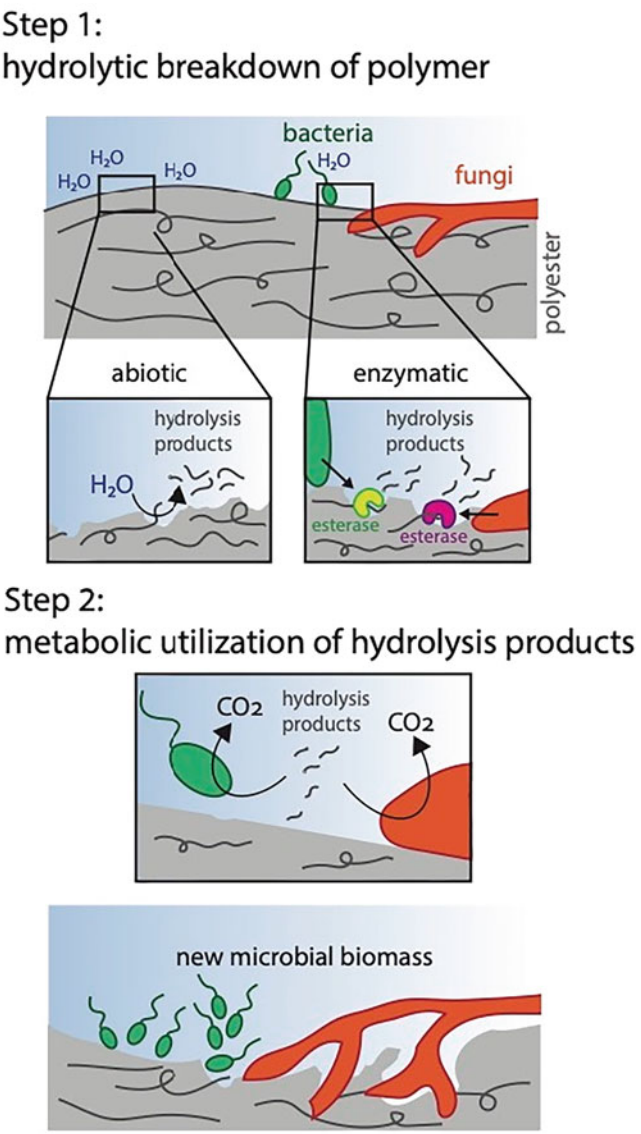

Fig. 1 Schematic for the two central steps in the process of polymer biodegradation: breakdown of the polymer into low molecular weight products (step 1) and subsequent uptake and metabolic utilization of these breakdown products by microorganisms (step 2). Polymer breakdown in step 1 may occur abiotically and/or be mediated by extracellular microbial enzymes. The breakdown

For polymers requiring enzymatic catalysis in the breakdown, biodegradation rates and extents are controlled by the amount and activity of the respective enzymes in the specific environment in which biodegradation occurs. These enzymes are present in the receiving environment not because of an evolutionary response to the presence of biodegradable polymers but instead because the enzymes have evolved to catalyze the breakdown of natural biopolymers that have functional groups which are present also in the biodegradable polymer. For instance, cutinases have evolved to hydrolyze ester bonds in cutin, a wax-like polyester that forms a protective cover on the surfaces of plant leaves. These cutinases also hydrolyze ester bonds in the backbone of many synthetic polyesters [24]. Because the polymer-degrading enzymes are of microbial origin, enzymatic breakdown is expected to increase with increasing colonization of the polymer surface by microorganisms that secrete the respective enzymes.

It is because of the importance of enzymatic breakdown and microbial colonization that polymer biodegradation is oftentimes also referred to as a three-step process: microbial surface colonization is viewed as an additional step that is followed by the breakdown of the polymer chains and microbial utilization of breakdown products. Herein, we instead advocate viewing biodegradation as a two-step process in which the first step, polymer breakdown, may (or may not) involve microbial colonization of the polymer and enzymatic polymer breakdown. We favor this view of a two-step process because it is more universal. For example, microbial colonization is not a necessity if breakdown occurs abiotically or if the enzymes actively breaking down the polymer are secreted by microorganisms that have not colonized the polymer surface. Furthermore, the conceptual framework of a two-step process also applies to water-soluble polymers in that they may undergo enzymatically mediated breakdown but lack a rigid surface that can be colonized by microbial cells.

Most commercially important biodegradable polymers contain hydrolyzable bonds in their backbone. Polyesters dominate among the synthetic polymers, but hydrolytic breakdown and biodegradation have also been described for other polymers, including polyurethanes [25–27], polyamides [28, 29], and polycarbonates [30]. The hydrolyzable bonds in the polymer backbone can be considered "intended breaking points" to allow for step 1 of biodegradation. However, the mere presence of hydrolyzable bonds (e.g., ester bonds) in a polymer does not imply that it is biodegradable. This is showcased by polyethylene terephthalate (PET), a synthetic polyester. Enzymes that hydrolyze ester bonds in amorphous regions of PET have recently been identified [31–33] and are currently investigated for their potential use

Fig. 1 (continued) needs to result in molecules of sufficiently small size to be taken up into microbial cells. Inside the microbial cell, these molecules are metabolically utilized resulting in the formation of CO_2 and microbial biomass under aerobic conditions or CO_2, CH_4 and microbial biomass under anaerobic, methanogenic conditions

in PET recycling [34]. Yet, enzymatic hydrolysis of crystalline domains in PET remains slow [35, 36]. Consequently, PET remains a non-biodegradable polymer.

Besides hydrolytic reactions, oxidations constitute a second class of (enzymatically mediated) reactions that may result in the breakdown of synthetic polymers. Oxidases occur naturally and are key in the breakdown of specific biopolymers in the natural environment: for instance, the breakdown of lignin is catalyzed by manganese peroxidases, hydroperoxidases, and laccases secreted by white-rot fungi [37–39]. However, compared to hydrolytic breakdown, oxidative breakdown of synthetic polymers is expected to have two principal limitations. The first limitation is that oxidases require dioxygen or activated oxygen species (such as hydrogen peroxide) as co-substrates. While these can be present under oxic conditions, they are absent in anoxic natural systems such as many aquatic sediments. Consequently, polymers relying on oxidative breakdown are expected to be stable in sub-oxic and anoxic systems. Such stability in anoxic systems is well known for the natural biopolymer lignin which undergoes oxidative breakdown: wooden Viking ships have been preserved for centuries in anoxic marine sediments. As compared to oxygen, water as the reaction agent in hydrolytic polymer breakdown is present in almost all natural environments (except for extremely dry (micro-) environments in which hydrolytic breakdown of a biodegradable polymer may be impaired). The second limitation of oxidative breakdown is that the oxidants in enzymatic oxidations, particularly highly reactive oxidants, react in a less directed manner as compared to water that selectively hydrolyzes specific bonds. Consequently, not all oxidations on a polymer structure are expected to result in backbone cleavage and polymer breakdown. Instead, additional follow-up reactions may be required to result in cleavage of the polymer backbone. These two principal challenges of oxidative breakdown likely explain why most synthetic biodegradable polymers rely on hydrolytic and not oxidative breakdown in step 1.

A critical assessment of oxidative polymer breakdown is particularly compulsory for the so-called pro-oxidant additive technologies (i.e., oxo-additives) that claim to render polyolefins biodegradable. Polyolefins persist in the environment because of the high stability of the carbon–carbon bonds in their backbone. The pro-oxidant additives contain complexed transition metals (i.e., typically stearates of Co, Fe, and Mn) [40, 41] and are added to polyolefins at a few weight percent. These transition metals are activated either thermally or photochemically by UV light and, in the presence of O_2, trigger the formation of reactive oxygen species that attack the backbone carbon in polyolefins, ultimately leading to polyolefin fragmentation, scissions of their C-C bonds, introduction of oxygen functionality, and a decrease in the polymer molecular weight. However, for several reasons, there is broad consensus that such pro-oxidant additives fail to render polyolefins biodegradable in natural systems. First, a fundamental shortcoming of this technology is its dependence on the presence of O_2, rendering this technology ineffective under anoxic conditions that are present or even prevail in many natural environments (e.g., soils, water bodies and sediments). Second, (UV) light is absent from many natural systems, implying that there is no UV light activation of pro-oxidant additives in such systems. Furthermore, temperatures in the environment are much

lower than those typically used in laboratory settings to demonstrate activation of the pro-oxidant additives. Therefore, pro-oxidant activation and polymer breakdown demonstrated under enhanced UV light irradiation and/or at elevated temperatures, as performed by companies marketing pro-oxidants, fall short of demonstrating that activation also adequately occurs in the natural environment. Activation under natural conditions has not been demonstrated, even if claimed in recent studies: activation of polyolefins containing metal additives under natural Florida irradiation conditions failed to address that these irradiance conditions are not globally representative nor disclosed that polymer specimen were mounted in a manner expected to result in artificially high weathering temperatures [42]. Thirdly, direct experimental evidence for biodegradation of pro-oxidant-activated polyolefins in any relevant environment and without artificial pre-treatment remains missing from the literature. In fact, several studies have shown that pro-oxidant containing polyolefins do not biodegrade even after extensive activation [15]. Based on these considerations, the scientific community and regulators in many countries agree that pro-oxidant additive technology do not render polyolefins biodegradable [16, 43]. This view has led to a ban of pro-oxidant technology in the European Union [44].

3.2 Factors Controlling Polymer Biodegradation

Polymer biodegradation is dependent on both the physicochemical properties of the biodegradable polymer (e.g., the presence of hydrolyzable bonds in the backbone of the polymer, the crystallinity of a polymer) and the abiotic and biotic conditions in the receiving environment (e.g., temperature, presence of specific microorganisms that secrete hydrolases that break down the polymer, presence of water, etc.) [6, 23]. These conditions are thus decisive in determining the extent to which the biodegradation potential of the polymer is leveraged. In the following, we will provide a brief overview of key polymer-dependent and environment-dependent factors that control polymer biodegradation in natural and engineered systems. These factors provide the basis on which biodegradation in different receiving environments can be compared and tested. We refer to reviews that also summarize the factors controlling polymer biodegradation [23, 45].

3.2.1 Polymer-Dependent Factors that Control Polymer Biodegradation

Polymer backbone chemistry. The backbone chemistry of the polymer is a key criterion determining its biodegradability. As alluded to above, most commercially important biodegradable polymers contain hydrolyzable bonds in their backbone. Figure 2 shows the structures of selected commercially important biodegradable polyesters as well as the hydrolytic reaction that leads to bond breaking in polyester backbones. These polymers include aliphatic and aliphatic-aromatic (co)polyesters (e.g., PLA, PBAT, PBA, PBS, PHA, PBSA, PCL) that undergo hydrolysis of the

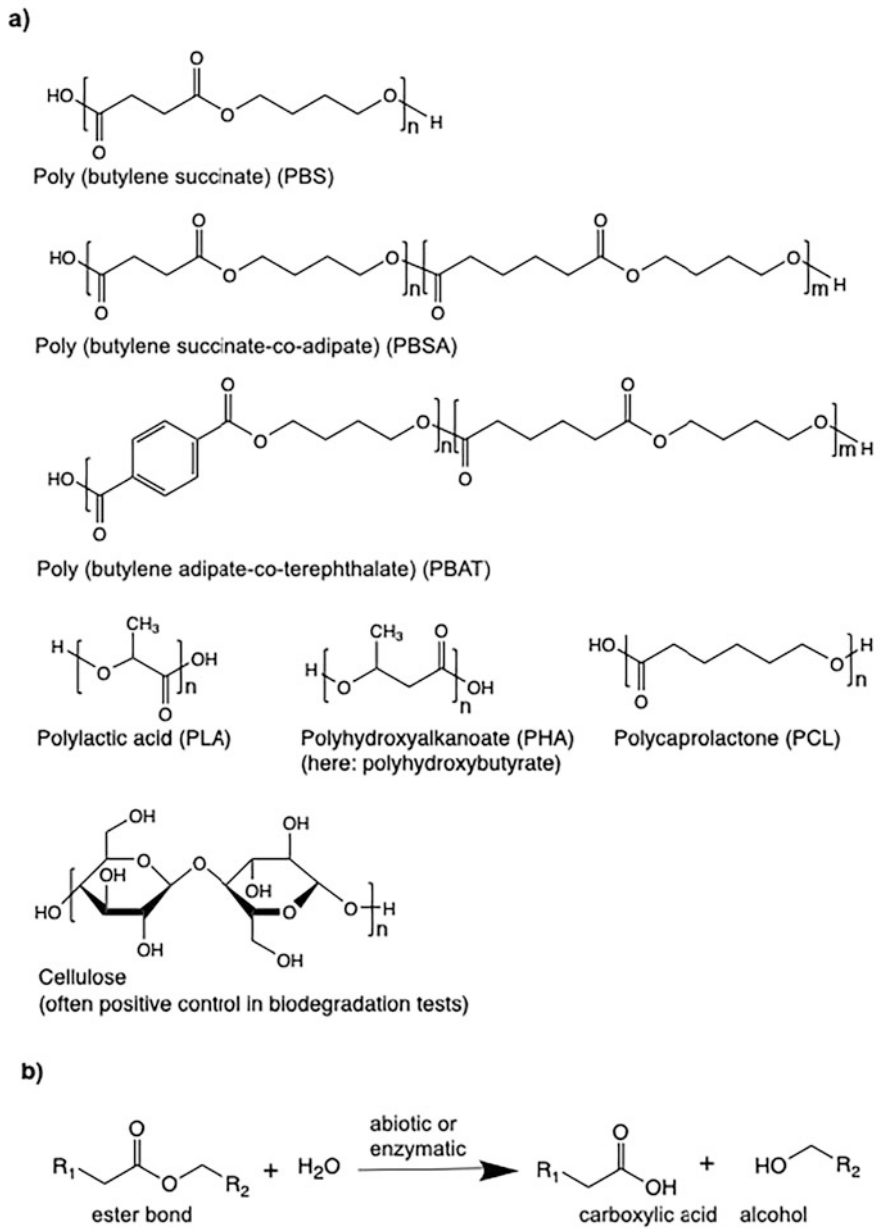

Fig. 2 (**a**) Chemical structures of commercially important biodegradable polyesters (including aliphatic and aliphatic-aromatic co-polyesters) and of cellulose, which often is used as a positive control in polymer biodegradation tests. (**b**) Hydrolysis of an ester bond into a carboxylic acid and an alcohol during the initial step (i.e., breakdown) of polyester biodegradation. Hydrolysis can occur abiotically but is enzymatically mediated for many polyesters

ester bonds to form a carboxylic acid and an alcohol. Figure 2 also shows the chemical structure of cellulose, a natural biopolymer with glycosidic bonds that is commonly used as positive control in biodegradation tests. While polyesters dominate the market of biodegradable polymers, biodegradation has also been demonstrated for other hydrolyzable polymers, such as polyamides (i.e., Nylons) [28, 29, 46].

Polymer form and morphology. The rate of polymer breakdown in step 1 of biodegradation increases as the surface-to-volume ratio of the polymer increases, particularly when the breakdown is enzymatically catalyzed and thus constrained to the polymer surface [45, 47]. As a consequence, larger plastic particles or items composed of one or more biodegradable polymers may require extensive time periods to biodegrade due to their small surface-to-volume ratio. Fragmentation of these items into micro-and nanometer-sized particles during biodegradation may largely enhance biodegradation rates as the surface-to-volume ratio increases. This point is noteworthy given that the formation of micrometer-sized polymers and plastics (i.e., microplastics) is often considered a concern per se without considering the chemistry of the polymer [48]. However, while conventional non-biodegradable polymers form nano- and microplastic particles that are persistent and accumulate in the environment, micrometer-sized particles composed of biodegradable polymers are only of transient nature as the polymers continue to biodegrade to CO_2 (and CH_4) and microbial biomass.

For non-crystalline and semicrystalline polyesters, enzymatically mediated ester hydrolysis was reported to increase with decreasing glass transition temperature, T_g [49, 50]. Similarly, for semicrystalline polyesters, enzymatic hydrolyzability was found to be inversely correlated to the melting temperature, T_m [21, 51, 52]. Both dependencies reflect constrained mobility of polyester chains in glassy domains and microcrystalline lamellae, respectively, which impairs the formation of enzyme-substrate complexes and hence hydrolytic cleavage of the ester bond. The volume occupied by glassy domains in amorphous polymers and of crystallites in semicrystalline polymers depends not only on the polymer chemistry, the tacticity, and the molecular weight distribution of polymer chains, but also on the processing history of the polymer. The latter implies that the morphology of a polymer in a plastic product may differ from the morphology of the same polymer before it was processed. In such cases, it is mandatory to test the biodegradation of the actual plastic product and not (only) of the pure polymer(s) that are present therein.

Testing the biodegradability and assessing the biodegradation rates of actual plastic products instead of only the constituting polymer(s) is also warranted for plastics that contain significant amounts of additives or that contain more than one polymer (i.e., polymer blends). Both the presence of additives and polymer blending have been reported to affect plastic biodegradability [53, 54]. Blending may lead to enhanced biodegradation of the polymers in the blend as compared to only the individual polymers [54].

3.2.2 Environment-Dependent Factors that Control Polymer Biodegradation

Several abiotic and biotic environmental factors affect polymer biodegradation, including temperature, pH, humidity, oxygen and nutrient availability, and UV light irradiance as well as the abundance and activity of specific microbial degraders that secrete extracellular enzymes that catalyze polymer breakdown. These factors are often interdependent (e.g., microbial activity depends on temperature, pH, and humidity), resulting in multifactorial effects on polymer biodegradation. This interplay challenges any assessment of the relative importance of individual factors for polymer biodegradation, particularly when polymer biodegradation is determined in natural environments characterized by simultaneous spatiotemporal variations of several factors. Laboratory incubations under defined and controllable conditions provide a viable means to separately assess the importance of individual environmental factors (in addition to assessing the dependence of biodegradability on material properties, which is of key interest to the development of novel biodegradable polymers).

Presence and activity of microbial degraders and their extracellular enzymes. Microorganisms are critical to polymer biodegradation as they take up the small organic molecules released during polymer breakdown and metabolically convert them to CO_2 (and CH_4) and microbial biomass. Furthermore, for biodegradable polymers that break down enzymatically, the presence of specific microorganisms is required that secrete these active enzymes.

The abundance of microorganisms expressing and secreting competent enzymes is strongly polymer-dependent. Microorganisms secreting extracellular enzymes to break down naturally occurring biopolymers, such as cellulose, are abundant across environments [55]. By comparison, microorganisms secreting enzymes active on synthetic biodegradable polymers may be much less abundant and typically require that the synthetic polymers have sufficient structural resemblance to the biopolymer that is the natural substrate for the secreted enzyme (see above example for cutinases). However, the environmental occurrence of microorganisms that secrete enzymes active on synthetic polymers does not imply that the polymer also biodegrades in the environment. An illustrative example is PET: while some microorganisms have been identified that secrete enzymes hydrolyzing the amorphous regions (but not the crystalline domains) in PET [31, 56], PET remains non-biodegradable in natural environments.

Temperature. Among the abiotic factors affecting polymer biodegradation, temperature is of key importance [57]. Over a wide range of temperatures in both natural and engineered system, increases in temperature will result in increase in the rates of biotic and abiotic chemical reactions that are involved in polymer biodegradation. However, polymer biodegradation rates are expected to increase only up to a temperature optimum in manipulated test systems. At temperatures above this optimum, biodegradation rates likely decrease, reflecting thermal inactivation of extracellular enzymes catalyzing polymer break down. This is because the microbial

community and their secreted enzymes in a given polymer-receiving environment are typically well adapted to the prevailing temperatures in that environment, including thermophiles (>45°C, e.g., found in industrial compost), mesophiles (i.e., 20–45°C; e.g., found in tropical soils and waters), and psychrotrophs and psychrophiles (<20°C; found in temperate and cold soil and water environments).

Temperature may affect polymer biodegradation rates also by altering the physical properties of the polymer. For instance, the enzymatic hydrolyzability of semicrystalline polyesters is expected to increase as the environmental temperature increases toward T_m [52, 58, 59]. Similarly, abiotic hydrolysis of specific polyesters is expected to increase with temperature due to increased water diffusion rates into the bulk polymer, as is well-established for polylactic acid, particularly at temperatures above its T_g of 60°C [60, 61].

For assessing polymer biodegradation rates, temperature therefore is a key parameter to be controlled. In laboratory incubation studies, the temperature is typically set to a constant value that is environmentally relevant, albeit in the upper end of the temperature range under real, in situ conditions (e.g., 20–25°C for biodegradation tests in soils). Incubations run at elevated temperatures have recently also been proposed as a viable means to determine biodegradation rates of polymers designed to undergo only slow biodegradation at ambient, environmentally relevant temperatures [62]. In the referred study, biodegradation rates increased with increasing incubation temperature according to the Arrhenius rate law, suggesting that rates measured at elevated incubation temperatures can be used to predict rates at lower temperature which may be challenging to determine experimentally [62]. However, because temperature affects polymer biodegradation rates in numerous ways (see above), more research is warranted to establish for which systems such rate predictions are applicable. Extrapolating rates measured from above certain temperature thresholds that lead to drastic changes in polymer morphology (e.g., from above the glass transition temperature that defines the transition from glassy to rubbery state of polymers) to environmental temperatures may lead to large errors in the predicted rates. Furthermore, care must be taken not to set precedence for studies that determine polymer biodegradation rates only at elevated temperatures without providing scientific data on how these rates can be extrapolated to lower, environmentally relevant temperatures. Non-linearity in the response of biodegradation rates with temperature also have implications for testing polymer biodegradability: it may not be advisable to use the mean annual temperature in incubations mimicking temperate climates if biodegradation rates scale non-linearly with temperature. In such cases, it is possible that biodegradation rates in incubations run at mean annual temperatures underestimate actual biodegradation rates in the open environment if biodegradation mainly occurs during the warm summer weeks to months.

Humidity, pH, and O_2 availability. The presence of water and dioxygen is critical for the biodegradation of polymers that undergo hydrolytic and oxidative breakdown, respectively. The activities of both microorganisms secreting these enzymes as well as the enzymes themselves have an optimal pH range above and below which their activities are expected to decrease. For polymers that release protons during

hydrolytic breakdown, as is the case for polyesters, the buffer capacity of the medium surrounding the polymer (or within a biofilm growing on the polymer surface) may be important to prevent local acidification that may lead to decreased biodegradation rates. Like temperature, the microbial community in each specific receiving environment is expected to be well adapted to – and shaped by – the humidity, pH, and oxygen availability in that system.

Photoirradiation. Sunlight exposure of polymers is often believed to facilitate its biodegradation by triggering photochemical oxidation that ultimately lead to chain scission and thereby polymer breakdown into smaller units. This view may originate from numerous studies on photo-induced chemical degradation of polymers which also may involve the embrittlement of polymeric items and their subsequent fragmentation into smaller particles. However, photochemical effects on degradation were mainly reported for conventional polymers which, in the absence of light, show only very slow breakdown due to their high backbone stability. By comparison, the effects of photoirradiation on the breakdown of biodegradable polymers are less studied and understood. In fact, besides triggering polymer chain scission reactions, photoirradiation can also cause crosslinking reactions between individual polymer chains. Photochemical crosslinking has been reported, for instance, for PBAT and lowered its enzymatic hydrolyzability [63]. Such photochemical crosslinking can be eliminated through the addition of photostabilizers to PBAT-containing plastics, thereby preserving enzymatic hydrolyzability – and thus likely also biodegradability – of the PBAT even after prolonged exposure to sunlight. An additional point to consider is that only a fraction of polymers that are released to natural and engineered systems are light-exposed. For this reason, photochemical breakdown unlikely is a viable reaction pathway to render polymers universally biodegradable in the environment.

Nutrient availability. The availability of carbon (C), nitrogen (N), and phosphorus (P) controls the activity and metabolic substrate utilization patterns of microorganisms in both natural and engineered systems. Polymer biodegradation may be suppressed in environments with a high availability of labile naturally occurring carbon substrates. Similarly, microorganisms isolated in growth media containing a specific biodegradable polymer as the sole carbon source may instead preferentially utilize natural substrates as carbon source under real, in situ conditions in the respective natural or engineered environment from which the microorganisms were isolated.

In systems with limited availability of N and P, unfavorable nutrient stoichiometry is expected to constrain polymer biodegradation: the synthesis of extracellular enzymes involved in polymer breakdown as well as the incorporation of polymer carbon into microbial biomass requires N and P [9]. In fact, carbon use efficiencies of natural substrates, including biopolymers, decrease with increasing substrate C to N ratio [19]. Most of the commercially relevant biodegradable polymers do not contain N and P. Microorganisms growing on the carbon of these polymers therefore need to source N and P from the environment surrounding the polymer [57]. Limited N and P availability may lead to microorganisms accumulating polymer-derived carbon in biomolecules in intracellular storage compartments, as was recently suggested by

NanoSIMS imaging of fungal cells growing on ^{13}C-labelled PBAT films incubated in soils [20].

3.3 Variation in Polymer Biodegradation Between Receiving Environments

The previous subsection highlighted biotic and abiotic factors of polymer biodegradation in natural and engineered systems. Because these factors largely differ between polymer-receiving environments, the biodegradation of a specific polymer may vary substantially between these environments. These variations may also occur among environmental systems of the same type (e.g., among agricultural soils), spatially within a given system (e.g., between the open water, the sediment surface or inside the sediment within a marine system), as well as temporarily in a system (e.g., seasonal temperature fluctuations in natural environments). The resulting differences in biodegradation rates of a specific polymer between different environments have been accounted for, in a first step, in certification schemes for biodegradable polymers (and plastics composed thereof) that specify the environment for which biodegradation is attested (i.e., "*soil biodegradable*," "*compostable*", or "*marine biodegradable*"). This system-specific labelling of polymer biodegradation promises to help overcome the misconception that "biodegradable polymers" universally biodegrade across all environmental systems. A systematic compilation of biodegradability data of polymers in different systems (e.g., in form of a catalog) would be helpful to guide product developments for specific applications [6].

The important notion that polymer biodegradation is environment-specific does not exclude the possibility that specific biodegradable polymers biodegrade across natural and engineered systems. For instance, the biopolymers cellulose and polyhydroxyalkanoates are reported to biodegrade across marine, freshwater, soil, and composting environments [64–66]. Conversely, other biodegradable polymers such as PBS, PLA, and PBAT have so far mainly been shown to biodegrade only in a subset of systems, including compost and soils [4, 5]. However, recent studies provided evidence for the biodegradation of some of these polymers in additional environments (e.g., PBAT in marine systems) [67]. Clearly, systematic studies assessing the biodegradation performance of commercially important biodegradable polymers across receiving environments are warranted.

Due to the absence of benchmarking studies systematically comparing biodegradation of a set of polymers across natural and engineered environments, it is currently impossible to quantitatively rank polymer biodegradability among the receiving systems. Yet, based on the existing data, polymer biodegradation potential is generally high under industrial composting conditions and, albeit to a lower extent, in home composting. Biodegradation is typically slower in soils, freshwater, and marine environments.

A clear communication is required in studies assessing the biodegradation of a certified biodegradable polymer in environments other than the one for which it was certified (e.g., biodegradation of a compostable polymer in a marine system) [68]. In such cases, the potential finding of insufficient biodegradation of the polymer in environments other than the ones for which it was certified biodegradable is ill-suited to call the certification (or the certified biodegradability of the tested product) into question. At the same time, there is significant scientific merit of assessing polymer biodegradation in systems other than the one for which the plastic was certified biodegradable. Such studies would provide valuable information on biodegradation rates and thus lifetimes of polymers that reside in environments other than the one for which they were certified biodegradable.

4 Setups and Analytical Methods to Study Biodegradation and to Test Biodegradability of Polymers

The analytical chemistry of polymer biodegradation is important for various reasons: it is critical for the understanding and interpretation of the polymer biodegradation process, it provides the data required for certifying a polymer product as biodegradable for specific receiving environments (see Sect. 5), and it aids in the development of future polymers with desired biodegradation performance for the use in specific applications and targeted receiving environments. Furthermore, consensus on analytical methods and testing setups is required as they provide the basis for quality control standards that protect against methods and test schemes that fall short of providing compelling evidence for polymer biodegradation. This subchapter will summarize general experimental approaches and analytics established to follow polymer biodegradation and also highlight novel analytical techniques that promise to give new insights into the biodegradation process. Most of the analytical methods presented herein are used in laboratory incubation studies under well-controlled conditions. However, the subchapter will also refer to analytical methods for the assessment of polymer biodegradation in mesocosm experiments, as well as in the actual natural or engineered receiving environment (i.e., under real, in situ conditions, such as in agricultural soils, aquaculture, or industrial composting facilities). Data collected on the latter levels are particularly helpful for predicting biodegradation rates of biodegradable polymers in their receiving systems.

4.1 Laboratory Incubations

Polymer biodegradability is most often tested in laboratory systems in which the polymer is incubated in the selected media (or inoculum) of interest (e.g., compost,

soil, or sediment) [69]. Over the course of the incubation, the headspace in each flask is either discontinuously or continuously analyzed for the formation of polymer-derived CO_2 (or depletion of O_2) under oxic incubation conditions (or CO_2 and CH_4 under anoxic incubation conditions). These respirometric analyses of formed CO_2 (and CH_4) are mandatory to demonstrate polymer biodegradation according to the above definition of biodegradation given that CO_2 (and CH_4) are the ultimate biodegradation endpoints (i.e., mineralization) (Eqs. 1–3). Other measurement endpoints besides respirometry – including visual disappearance, weight loss, decrease in surface area, and changes of material properties of a polymer – are indirect and thus provide no proof of biodegradation. Similarly, microscopy imaging of polymer surfaces showing colonization by microorganisms (i.e., the presence of a "plastisphere" [70]) provides no proof for polymer biodegradation because microbial colonization is well documented also for non-biodegradable, conventional polymers [71, 72]. Measurement endpoints that are complementary to respirometric analyses may, however, provide additional information and thereby help to attain a more detailed understanding of polymer biodegradation dynamics.

Laboratory incubation systems may either be *static* (i.e., the incubation flasks are closed and are opened only periodically for the analysis of formed CO_2 (and CH_4) and/or for the replenishment of consumed O_2) or *dynamic* (i.e., the headspace gas in the incubation flasks is continuously replaced by a gas stream through the flasks). Irrespective of the type of setup, one analytical challenge of measuring plastic mineralization is to distinguish the polymer-derived CO_2 (and CH_4) from CO_2 (and CH_4) formed by mineralization of naturally occurring organic material in the tested systems (e.g., soil organic matter in a soil). This challenge is typically addressed by running parallel control incubations containing the same environmental medium without added polymer (i.e., "blanks"). The amount of CO_2 (and CH_4) formed in these polymer-free controls is then subtracted from the amount formed in the actual incubation flasks containing both the polymer and the environmental medium. This subtraction relies on the assumption that the addition of polymer to the medium does not affect background mineralization of naturally occurring organic substrates. While this assumption likely is an acceptable approximation for most systems, changes in background mineralization of natural organic matter induced by the addition of a labile substrate are well documented as the so-called "priming effects" [73–75]. If significant priming occurs in response to polymer addition, it will result in uncertainties in the quantification of polymer carbon converted to CO_2 (and CH_4). These uncertainties can be overcome by extracting and quantifying residual polymer at the end of the incubation and/or by using polymers labelled with carbon stable (^{13}C) or radioactive (^{14}C) isotopes and by quantifying the formation of $^{13}CO_2$ or $^{14}CO_2$ (and $^{13/14}CH_4$), as further detailed below.

Static systems typically quantify polymer mineralization by monitoring the cumulative amount of polymer carbon converted to CO_2 (and CH_4) and/or of oxygen consumed over time. There are three common approaches. In the first approach, discrete air samples are repeatedly withdrawn from the headspace of the closed incubation bottles, followed by quantifying the CO_2 (and CH_4) concentration(s) in

the withdrawn sample (e.g., by gas chromatography coupled to mass spectrometric detection). The second and third approaches rely on deploying a CO_2 trap in the incubation flasks (e.g., alkaline solutions or NaOH pellets that trap CO_2 as carbonate salts). The trap continuously removes the formed CO_2 from the headspace. In the second approach, the amount of trapped carbonate is regularly quantified. Alternatively, in the third approach, the pressure drop in the incubation flask is monitored. This drop results from the consumption of oxygen through polymer mineralization and trapping of the formed CO_2. The use of CO_2 traps is, however, limited to incubations under oxic conditions as the traps do not capture CH_4 that may form in anoxic incubations. Furthermore, pressure drop measurements are incorrect if gases other than CO_2 form in the incubation flasks, such as N_2 through denitrification. Also, reactions that consume O_2 (e.g., nitrification reactions) can interfere with the measurements. The formation of any additional gases or the consumption of gases through reactions other than the mineralization would result in smaller or larger, respectively, net pressure drops and thus to underestimations or overestimations of the extent of polymer mineralization, respectively. In some cases, it may be advisable to use nitrification inhibitors. It is also important to keep in mind that mineralization in a static, closed system under oxic conditions results in continuous oxygen consumption. These systems may therefore need to be periodically opened to replenish O_2 in the headspace of the incubation flasks as oxygen depletion will influence microbial activity. Headspace O_2 concentrations can be independently monitored, e.g., by optodes, to identify the appropriate timepoint for O_2 replenishment.

In *dynamic systems*, the headspace in the incubation flasks is constantly exchanged with new gas and the concentration of CO_2 (and CH_4) in the effluent gas from the flasks is continuously quantified, typically by infra-red absorbance measurements [76] or gas chromatography coupled to a thermal conductivity detector (TCD). Therefore, in contrast to static systems, dynamic systems determine polymer mineralization *rates* which, upon integration over time and accounting for the volumetric gas flow rates through the system, yield cumulative amounts of polymer carbon mineralized. While dynamic systems require accurate control of volumetric gas flow rates through the bottles, they have the advantage over static systems that they minimize the risk of O_2 depletion in the incubation flasks. Dynamic systems may, however, encounter sensitivity problems for polymers with very slow mineralization rates given that the formed CO_2 (and CH_4) is continuously diluted in the gas stream that is delivered through the flasks. In such cases, the volumetric flow rates of the gases through the incubation flasks may need to be lowered to result in higher steady state CO_2 (and CH_4) concentration(s) (or static incubations become the preferred choice). Alternatively, it is possible to deliver CO_2-free air to the incubators to increase sensitivity to CO_2 formed in the incubators.

Laboratory incubations provide the unique advantage of allowing to control and, if desired, vary incubation conditions while directly following polymer mineralization into CO_2 (and CH_4). Factors controlling biodegradation, such as temperature or humidity, can thus be systematically tested. At the same time, laboratory incubations are reductionist representations of the actual natural or engineered systems in which

polymer biodegradation will occur. Incubations in the laboratory are typically run under constant incubation conditions (e.g., temperature and humidity) whereas in situ conditions in the actual receiving environment may show substantial spatiotemporal variations (e.g., daily or seasonal temperature fluctuations, micro-scale heterogeneities). These differences in incubation conditions emphasize the need to establish the extent to which results from laboratory polymer biodegradation studies are transferrable to biodegradation of the same polymer under real, in situ conditions in the actual natural or engineered receiving system. In this context, it has been proposed to complement laboratory biodegradation studies with mesocosm and field biodegradation studies [77].

4.2 Mesocosm and Field Studies

Mesocosm studies are considered to help link results from laboratory incubations under well-controlled conditions to results from biodegradation studies under real, in situ conditions at the field scale. Mesocosms are larger in size than laboratory incubations and, thereby, are likely more representative subunits of the actual polymer-receiving environment. For instance, the rates of polymer biodegradation at the freshwater-sediment interface can be determined in mesocosm tank tests in which the water is repeatedly or continuously replaced, mimicking continuous water exchange under real, in situ conditions in lake, river, or marine settings. Similarly, soil mesocosms can be set up containing significantly more soil (i.e., tens to several hundred kg of soil) than used in laboratory incubations (typically 100 g to 1 kg of soil) and thus may better address spatial variations in polymer biodegradation in soils. Soil mesocosms can be set up to also have a vegetation cover and to be periodically irrigated, two boundary conditions representative of in-field soil incubations that are not readily implementable in laboratory incubations in small flasks. By contrast to laboratory and mesocosm incubations, field studies typically allow for no or only minimal control of the incubation conditions (e.g., temperatures or, for soils, soil water contents). Consequently, monitoring of the environmental conditions during the incubations is of special importance. In field studies, variations in conditions can be pronounced (e.g., diurnal and seasonal fluctuations in temperature and precipitation). A major advantage of field studies is that they allow assessing polymer biodegradation under real, in situ conditions. At the same time, field studies have the disadvantage that the few selected field study sites may not be representative of other receiving environments of the same type (e.g., agricultural soils) and that they are susceptible to weather events that may largely affect polymer biodegradation (e.g., two-year soil incubations in the field with an exceptionally cold and long winter or a very warm and dry summer may not be representative of typical winters and summers). It is therefore important to closely monitor key abiotic factors (i.e., temperature, moisture) during field incubations to help rationalize observed biodegradation rates.

Mesocosm and field studies are significantly more demanding in terms of infrastructure, space, maintenance, and costs than most laboratory studies. Furthermore, from an analytical perspective, a central "drawback" of mesocosm and particularly field incubations is that they are not "enclosed" and therefore cannot readily be coupled to respirometric analyses of polymer carbon mineralization to CO_2 (and CH_4). As a result, polymer biodegradation is typically assessed by indirect measurement parameters only. These include changes in the physicochemical properties of the polymer (e.g., decrease in tensile strength), gravimetric weight loss of the polymer over time, and determining polymer physical disintegration or visual disappearance (e.g., photographic analysis of residual polymer film area relative to the initial film area) [78]. Because these latter measurement endpoints provide only indirect, circumstantial evidence for polymer biodegradation, mesocosm and field incubations cannot replace laboratory incubations in which the mineralization of polymer carbon to CO_2 (and CH_4) is directly demonstrated. A tiered approach has been proposed in which mesocosm and field biodegradation tests are conducted only for those polymers (and plastics composed thereof) that have demonstrated environmental biodegradability in laboratory incubations. Furthermore, novel solvent extraction methods (see next subsection) allow to accurately quantify the residual polymer in mesocosm and field incubations and thus to overcome the analytical deficiencies of the above-mentioned indirect methods.

4.3 Recent Analytical Advancements in the Assessment of Polymer Biodegradation

Laboratory incubations coupled to respirometric analyses are central to establishing polymer biodegradability. Yet, these traditional incubations have a major shortcoming: they do not allow to determine (and thus delineate) the amount of polymer-carbon incorporated into microbial biomass over the course of the incubations, nor the amount of residual non-mineralized polymer at the end of the incubations. Furthermore, the lack of analytical methods to quantify the total non-mineralized polymer-added carbon (i.e., which includes carbon incorporated into biomass (i.e., $C_{\text{microbial biomass}}$ (t) in Eq. 3) and residual polymer carbon (i.e., C_{polymer} (t) in Eq. 3)) implies that it was previously impossible to demonstrate closed mass balances on the added polymer carbon over the course of incubations [79]. Closing mass balances is, however, desirable particularly for long-term incubations over several months to years. A reliable and direct determination of the amount of biomass formed from polymer carbon remains a major challenge and likely is possible only when using isotopically labelled polymers. Conversely, solvent extractions to quantify C_{polymer} (t) do not require the use of labelled polymer [80, 81]. The possibility to quantify $C_{\text{microbial biomass}}$ (t) and/or C_{polymer} (t) is important to assess the true extent of biodegradation: while polymer carbon incorporated into microbial biomass is a desirable biodegradation outcome, the presence of residual polymer would imply

that polymer biodegradation was incomplete. As described above, mesocosm and field studies were missing analytical methods to accurately quantify the decrease in residual polymer concentrations over the course of the incubations but instead relied on measurement endpoints that provided only indirect evidence for biodegradation. The following subsections summarize recent analytical advancements that overcome these shortcomings and, thereby, will eliminate existing interpretational ambiguities on polymer biodegradation in laboratory, mesocosm, and field experiments.

4.3.1 Stable-Carbon Isotope Labelling of Polymers

Early work demonstrated that using radiocarbon ^{14}C-labelling allows to quantify the mineralization of minute amounts of compounds released from non-biodegradable polyethylene to $^{14}CO_2$ [82, 83]. The unique possibilities of using ^{13}C-labelled polymers in biodegradation studies have only recently been advocated and demonstrated [84–86]. As compared to radiocarbon ^{14}C-labelling, stable-isotope (^{13}C)--labelling does not require radioisotope-specific safety measures, implying that there are no restrictions to work in assigned laboratory space and that it is possible to analyze samples on all instruments (and not only those that are specifically dedicated to the analysis of radioactive substances). The challenges of using ^{13}C are the costs of labelling and the need for (costly) stable-carbon isotope-sensitive analytics. Consequently, we anticipate that the use of ^{13}C-labelled polymers (or plastics composed thereof) in biodegradation studies will be restricted to selected model polymers in laboratory incubations.

Using ^{13}C-labelled polymers has the unique advantage that polymer-derived ^{13}C can be selectively tracked through the biodegradation process. By quantifying both $^{13}CO_2$ and $^{12}CO_2$ in the headspace or effluent gas of incubation bottles, mineralization of the polymer can be discriminated from the background mineralization of natural organic matter, given the different isotopic compositions of the polymers and the natural substrates. The selective quantification of polymer-derived $^{13}CO_2$ implies that the measurements are not susceptible to priming effects. Furthermore, for polymers containing more than one monomer unit, it is possible to synthesize ^{13}C-labelled variants that carry the label in different monomer units. Separate incubations of these variants allow to demonstrate that carbon from all monomer units in the specific polymer undergoes microbial utilization (and thus that all building blocks biodegrade), as recently demonstrated in laboratory soil incubations for PBAT and PBS [20, 80].

Using ^{13}C-labelled polymers further allows to quantify the total residual, non-mineralized polymer-added carbon left in the incubation flasks at the end of the incubation and, thereby, to close the polymer carbon mass balance over the course of the incubations. Closed mass balances on PBS carbon over the course of 425 days of soil incubations in the laboratory were recently demonstrated: at the end of these incubations, the non-mineralized PBS-^{13}C remaining in the soil was quantified by combusting small soil aliquots in an elemental analyzer and quantifying the amount of formed $^{13}CO_2$ by isotope ratio mass spectrometric detection [80]. In

contrast, when incubating non-labelled polymers closing mass balances in this manner is impossible given that combustion-derived CO_2 from the polymers and soil organic matter cannot be differentiated.

The use of ^{13}C-labelled polymers also allows to demonstrate the incorporation of polymer-derived carbon into microbial biomass, as recently done for ^{13}C-labelled PBAT in soils [20]. Using nanoscale secondary ion mass spectrometry (Nano-SIMS), microbial cells growing on the surface of the PBAT films were shown to be enriched in PBAT-derived ^{13}C [20]. A recent study used ^{13}C-labelled PE to show that minute amounts of carbon from photo-irradiated PE were incorporated into the biomass of *Rhodococcus ruber*. This study demonstrated that ^{13}C labelling is sufficiently sensitive to even demonstrate microbial utilization of carbon released from non-biodegradable polymers [87]. This finding, however, does not imply that the microbes biodegraded PE. Instead, the irradiation likely resulted in the formation of a minute amount of low-molecular-weight breakdown products in PE which, upon release from the PE into solution, were utilized by *Rhodococcus ruber*. This interpretation is supported by earlier studies with ^{14}C-labelled PE [82, 83]. Future studies may use other isotope-sensitive spectromicroscopy techniques to image microorganisms on polymer surfaces, extract biomolecules to infer extent of ^{13}C-incorporation into biomass (e.g., through analysis of ^{13}C-labelled phospholipid fatty acids), and/or identify microbial degraders (e.g., through DNA-stable isotope probing) [88, 89].

Finally, using ^{13}C-labelled polymers in biodegradation studies offers new possibilities in validating newly developed incubation setups and analytical methods (e.g., by demonstrating closed mass balances on polymer ^{13}C) that may subsequently be used for incubations of non-labelled polymers. Method validation using ^{13}C-labelled polymers is expected to be particularly helpful for incubations in media rich in background organic carbon, including compost and anaerobic digestate, given that ^{13}C from polymers can be analytically differentiated from highly abundant ^{12}C in the organic carbon background.

4.3.2 Quantification of Residual Polymer

Many biodegradable polyesters are soluble in chloroform, which opens the possibility to solvent-extract these polyesters from incubation matrices (e.g., soils or sediments). Two recent studies reported the accurate quantification of PBAT, PLA, and PBS in soils by using Soxhlet or accelerated solvent extraction of the polyesters into methanol-chloroform solvent mixtures, followed by the quantification of the extracted polyester(s) by quantitative proton nuclear magnetic resonance spectroscopy (q-^{1}H-NMR) [80, 81]. This approach likely is extendable to other chloroform-soluble biodegradable polyesters – such as polyhydroxyalkanoates (PHA), polycaprolactones (PCL), and other synthetic aliphatic and aliphatic-aromatic polyesters (e.g., PBA or PBSA) – as well as to solid matrices from receiving environments other than soils (e.g., lake or marine sediments and compost).

Applied to laboratory incubations, these solvent extraction-based methods allow to quantify residual polyesters in the incubation matrix, $C_{polymer}$ (t), over the course and/or at the end of incubations. When combined with respirometric analyses to quantify polymer mineralization to C_{CO2} (t) and assuming closed mass balances on polymer carbon (which can be directly verified when using ^{13}C-isotope labelled polymers), it is possible to indirectly assess the amount of polymer carbon incorporated into microbial biomass, $C_{biomass}(t)$ (i.e., $C_{biomass}(t) = C_{polymer}(t_0) - C_{polymer}(t) - C_{CO2}(t)$, see Eqs. 2 and 3). Furthermore, a quantification of both $C_{biomass}(t)$ and $C_{CO2}(t)$ allows to calculate the carbon use efficiencies (CUEs) for the polymers.

In mesocosm and field studies, these solvent extractions would allow to monitor the decrease in the concentration of biodegradable polymer(s) over time. Given the extraction efficiencies of polymers are shown to be close to (or ideally at) 100% – as achieved for PBAT, PLA, and PBS by spike-recovery experiments in soils [80, 81] – these extractions provide an accurate and direct determination of residual polymer and thus can be used to follow polymer biodegradation. For mesocosm and field incubations, the experimental setup may require that the tested polymer is first placed into a mesh bag to prevent physical loss. This bag then is deployed in the mesocosm or in the field to ensure that the sample and its content can be recollected in its entirety. This approach is well established for plant leaf decomposition studies and has recently been extended to polymer biodegradation studies in field soils [90]. However, the latter study placed only the polymer in the mesh bag (in addition to using visual inspection to determine residual polymer) and did not solvent-extract the residual polymer. Going forward, it may be advisable to also add soil (or the corresponding environmental matrix of interest) into the mesh bag to ensure close polymer-soil contact and to minimize potential loss of fine polymer fragments through the mesh upon retrieval of the sample. The mesh size thus needs to be chosen carefully to be sufficiently small to ensure that the polymer is retained but sufficiently large to allow for the exchange of water, nutrients, air, and microbes between the in- and outside of the mesh bag.

5 Standard Tests and Certifications for Polymer Biodegradability and Biodegradation

5.1 Motivation for Testing

Norms (and synonymously *standards* or *guidelines*) define methodologies to be used and outcomes to be achieved to claim polymer biodegradability. These norms are issued by national and international standardization institutions such as the European Committee for Standardization (CEN), ASTM International (formerly American Society for Testing and Materials), the International Organization for Standardization (ISO), or the Organization for Economic Co-operation and Development (OECD). Norms serve to achieve comparability and harmonization on polymer

biodegradability testing on national and international levels and provide a basis for clear communication on experimental setups and methods used to test polymer biodegradability. *Standard test methods* aim to standardize test procedures, harmonize test conditions, and ensure reproducibility and comparability of test results between different testing laboratories. *Standard specifications* define methods and requirements that need to be fulfilled in order to comply with the standard (e.g., pass levels). Consequently, polymer biodegradability claims can be made only based on standard specifications and not solely on standard methods. We note that some countries' standardization institutes offer companies the option of paid-for (i.e. sponsored) standards. For instance, the British Standards Institution (BSI) specification PAS 9017:2020 is a sponsored specification which lists requirements that supposedly allow to claim biodegradability of pro-oxidant additive containing polyolefins in the terrestrial environment. This sponsored specification requests an artificial high-temperature or intense UV-light pre-treatment of the pro-oxidant-containing polyolefin under conditions that are not environmentally representative, before the thereby generated polyolefin degradation products are submitted to a respirometer test in soil. Thus, this specification falls short of meeting the more stringent and scientifically-accepted requirements put forward by below-listed standards (e.g., from ISO and ASTM), including that biodegradability cannot be tested on a polymer material after it has been pre-treated at environmentally unrealistic high temperatures or UV light exposures. Instead, a polymer (or plastic) material that is released into the environment must undergo biodegradation 'as is' without any environmentally-unrealistic pretreatment that results in artificial polymer breakdown.

Certifications are issued by independent third parties after the proof of compliance of a polymer's biodegradability with the standard specifications [91]. In the absence of standard specifications, certification bodies can also define own certification schemes. Certificates are often awarded in the form of a *label* that can serve as a transparent and reliable means of communication for polymer biodegradability between businesses (B2B) or from business to consumers (B2C). Labelling itself is regulated in some countries to prevent misuse and false claims on the biodegradability of a polymer or a product.

Standards and certification schemes for biodegradable polymers and plastics have been originally developed to fill a gap left by the OECD guidelines for the testing of chemicals. More specifically, specifications for biodegradable polymers and plastics address several important aspects. The most important aspects are the *chemical characteristics* related to the chemical composition of the material that is tested for biodegradability and regulating the use of specific chemicals in these materials, e.g. heavy metals and additives, *biodegradation testing* which defines test conditions and pass requirements, *disintegration testing* which defines conditions and outcomes to claim that the tested item or product on the market will disintegrate at the end-of-life in the targeted receiving environment, e.g., disintegration of a compostable bag in industrial compositing, and *ecotoxicity testing* which ensures that no toxic metabolites form during biodegradation. While all aspects are equally important for the

certification of biodegradable polymers and plastics, the next sections will focus solely on the aspects related to *biodegradation testing*.

5.2 Levels of Testing

Standardized laboratory biodegradation tests are used to demonstrate that the polymer (or a plastic item composed thereof) can be metabolized by microorganisms present in the sampled matrix used for the incubation. The matrix can be obtained from polymer-receiving engineered systems (e.g., compost or wastewater) or natural habitats (such as soils, freshwater, or the sea). The tests typically rely on the previously described respirometric methods and quantify the extent of mineralization of the test material to CO_2 (e.g., in ISO 14852) and/or the biological oxygen demand (BOD), i.e., the consumption of oxygen that results from aerobic respiration of the test specimen, such as in ISO 14851, both corrected for background CO_2 formation or BOD determined in blank incubations containing the sampled matrix but devoid of the polymer/plastic specimen. In standardized laboratory biodegradation test methods, incubation conditions often favor microbial activity. At the beginning of the test, sufficient nutrients may be added to the incubation to ensure that mineralization will not be impacted by nutrient limitations. Similarly, regular mixing of the matrix in the incubation flasks or the addition of coarser inert particles to the flask may be used to ensure that oxygen readily penetrates the matrix containing the test material. In addition, for technical reasons, concentrations of the test material in the incubations may exceed actual anticipated concentrations of the tested material in the field. Higher test material concentrations ensure an optimal signal-to-noise ratio in the quantification of mineralization, especially in tests with very active inoculums and thus high background mineralization/BOD, such as composting tests, e.g., ISO 14855.

Respirometry methods to assess the biodegradability of materials are preferred for technical and economic reasons. To claim biodegradability, standard requirements and certification schemes for biodegradable polymers typically request a high degree of mineralization (e.g. of 90%) of the polymer organic carbon to CO_2 (and CH_4) over time periods of several months (e.g., industrial compost) up to 2 years (e.g., soil). These extents of mineralization may be achieved either in absolute terms (i.e., $\geq$90% of the added polymer carbon is mineralized to CO_2) or relative to a known biodegradable reference material. The latter often is cellulose which may show incomplete mineralization, i.e., a percentage smaller than 100% of the cellulose carbon is converted to CO_2. It is commonly assumed that the non-mineralized cellulose carbon is not present as residual cellulose but rather has been incorporated into the microbial biomass pool (e.g., cellulose mineralizes to 70%, the remaining 30% of non-mineralized cellulose carbon is considered incorporated into microbial biomass). In this case, a polymer or plastic material incubated in the same environmental matrix meets the requirement for being "biodegradable" if 63% of its carbon is mineralized to CO_2 (i.e., 90% of 70% for cellulose). The underlying assumption is

that the CUEs of the polymer and the biodegradable reference material are comparable. This assumption, however, has not been experimentally verified. The determination of CUEs for different biodegradable polymers is a research need, as detailed in Sect. 5. Any polymer carbon incorporated into microbial biomass contributes to polymer biodegradation but is not captured by mineralization measurements.

While standardized testing based on radiocarbon ^{14}C-isotope labelling exists and would allow to close mass balance on polymer carbon (i.e., ASTM D6340 and D6692 for plastics – akin to other guidelines typically used for small molecules and not yet validated for polymers, such as OECD 307, 308, 309, and 314 for ^{14}C), this approach is not routinely applied because of safety regulations for working with the ^{14}C radioisotope as well as cost restrictions in labelling the polymer. As discussed above, ^{13}C labelling of polymers was recently shown to allow closing mass balances in incubation studies [80]. For practicability, however, routine tests are conducted with unlabelled polymers. Determining an extensive formation of CO_2 (and CH_4), the gaseous end product(s) of biodegradation, is taken as a valid proof of biodegradability even if mass balances cannot be closed. Future studies and tests may consider complementing respirometric analyses with solvent extraction and quantification of potentially present residual polymers from the incubation flasks at the end of incubations, as described above.

Small-scale laboratory tests only partially reflect real conditions encountered in situ in the field. In addition, small-scale testing setups often do not allow the proper assessment of final test items at the end of the incubations, due to the limited size of the incubation vessels and the optimized laboratory conditions. Therefore, laboratory tests primarily provide information on the *biodegradability* of a polymer but currently do not allow to predict the actual *biodegradation rates* and *extents* in engineered or natural environments. To achieve the latter and overcome inherent deficits of laboratory tests, field, mesocosm and pilot tests have been developed. *Field tests* allow to monitor polymer and plastic biodegradation under real, in situ conditions. Indirect measurements of biodegradation are commonly used in such field tests such as polymer or plastic disintegration (e.g., ISO 22766 for the marine environment or UNI/PdR 79:2020 for composting) and weight loss of the tested material (ASTM D7473). Because these measurement endpoints are only indirect, the results from these tests need to be interpreted with caution. Also, these tests should be conducted only in combination with laboratory tests that provide the direct proof of polymer biodegradability. Yet, when using such indirect methods, efforts should be taken in designing the experiment to minimize measurement artifacts. For instance, the test specimen could be enclosed in a mesh to minimize loss of fragments, such as in ISO 22766 or UNI/PdR 79:2020. Future studies may employ solvent extraction-quantification approaches (as described above) which provide a quantitative measure of the residual polymer remaining at a given time point [81].

Field tests can provide specific biodegradation *rates* which can be used to model the lifetime of the tested material under specific environmental conditions [77]. *Mesocosm tests* (e.g., ASTM D7473) or *tank tests* (e.g., ISO 23832) can be seen as a methodological link between well-controlled laboratory tests and field

tests. Similarly, *pilot-scale tests* are used to simulate engineered environments, e.g., industrial composting conditions (ISO 16929).

Based on standard test methods and specifications and considering a balance between effort, cost, efficiency, and feasibility, the SAPEA report [6] described a hierarchical series of multi-tier and interactive testing schemes [92, 93] to assess the environmental fate and potential impact of biodegradable polymers and plastic materials in natural systems. Yet, systematic studies that compare the biodegradation of a given polymer or plastic item across the different test levels (laboratory, mesocosm, field) and that establish correlations between these levels are missing.

5.3 Status of Standard Test Methods and Specifications

The development of methods and specifications has constantly progressed in recent years, in parallel to the increase in the development and commercialization of biodegradable polymers and products. While several methods are already available for determining the biodegradability of different materials and items, specifications have been mainly developed for categories of products already present on the market. Standard methods and specifications have been especially targeting *industrial composting* as the intended end-of-life. For example, ISO 14855 is applied to investigate the biodegradability of plastic materials by respirometric methods under laboratory composting conditions and it is typically complemented by disintegration tests, such as ISO 20200 or ISO 16929. Specifications for industrial composting conditions and different categories of products have been developed, such as for plastics (ISO 17088, EN 14995, ASTM D6400), carrier bags (ISO 5412), and packaging (ISO 18606, EN 13432, ASTM D6868). These standards have in common that they define basic requirements for packaging and packaging materials to be considered biodegradable and compostable in industrial composting facilities. More recently developed standard methods address *home composting* (EN 17427) as intended end-of-life. These methods are similar to the ones for industrial composting testing, but testing is performed at lower temperatures (e.g., 25°C ± 5°C).

While biodegradability in *anaerobic digestion* is partially covered, e.g., in EN 13432 (with organic waste as inoculum) and in ISO 14853 (with sludge as inoculum), further developments of methods and specifications are currently under discussion. These methods and specifications are needed given the increasing importance of anaerobic digestion as dedicated end-of-life treatment.

Biodegradation in natural environments has been addressed in recent years, but there are only few specifications due to the limited number of products targeting these environments as intended end-of-life. ASTM D5988 and ISO 17556 testing methods describe laboratory incubations to assess the biodegradability of plastic materials in *soil* as inoculum. Standard specifications have been developed for the certification of soil-biodegradable mulch films (EN 17033 and ISO 23517). *Fresh water* testing is mainly covered by the standards ISO 14851 and 14852, for which different inocula can be utilized. Conversely, no standardized methods for polymers

or plastic biodegradability are available for sediments from freshwater environments or the water-sediment interface, nor have specifications been developed so far for freshwater. More broadly covered is the *marine* environment with different zones, such as eulittoral, benthal and pelagic, and methods, ranging from lab testing to mesocosm and field tests (see references in Table 1). A standard specification for marine biodegradability testing in the laboratory has been published in 2020 at ISO level (ISO 22403).

For other relevant environmental systems into which biodegradable polymers and plastics may be transferred and which are not yet covered by standards, new biodegradation standards can be developed, or existing standards can be modified. One example for environments for which no standards exist are anoxic systems, i.e., with limited or no oxygen available such as muddy aquatic sediments. Ideally, appropriate standards for all relevant environmental compartments should be developed in a timely manner and be supported by collecting data on biodegradation performance in these compartments. This would aid in the development of novel polymers or blends with desirable biodegradation performances in these environmental compartments.

Substantial progress has been made in recent years on standard test methods and specifications. Yet, there are still gaps. For instance, numerous standards were developed for polymers and products that undergo comparatively fast biodegradation (weeks to months and up to 2 years). These standards are suitable for polymers and plastics in applications which require mechanical stability performance for rather short lifespans. There are, however, numerous applications in which the mechanical performance of a polymer is needed for a longer lifespan [94]. These applications include, for instance, polymeric geotextiles. Standard test methods and specifications need to be developed for these applications in which biodegradation needs to be slower to ensure that the product is stable for a sufficiently long time in the application e.g., up to 5 years.

5.4 Certifications

Registered biodegradable polymers are used in the production of biodegradable finished plastic products – e.g., bags and packaging – which then must be certified in the next step. Testing at an acknowledged test institute also serves to prevent that the plastic item contains non-conforming additives, such as problematic stabilizers, plasticizers, and inks. The certificate entitles its holder to use the regional label for biodegradable plastics on his packaging or article, in combination with a certification number. An overview of certifications and labels for industrially compostable products for different geographical regions is shown in Table 2. A description of the certification process and a list of certifiers is provided elsewhere [6, 91].

Table 1 Status of standards test methods and specifications for biodegradability and biodegradation in compost, soil, freshwater, and marine environments. Empty fields imply that an applicable standard is missing

		Standard test method			**Standard specifications related to the environment**
Compost	Laboratory tests (biodegradation)	ISO 14855-1			ISO 17088:2021 EN 13432:2000-12 ISO 5424:2022 ISO 5412:2022
		ISO 14855-2			
	Pilot-scale/field tests (disintegration)	ISO 16929:2021			
	Lab-scale tests (disintegration)	ISO 20200:2015			
Home compost	Laboratory tests (biodegradation)	ISO 14855-1			EN 17427:2022
		ISO 14855-2			
	Field tests (disintegration)				
	Pilot-scale tests (disintegration)	EN 17428:2022			
Soil	Laboratory tests (biodegradation)	ASTM D5988-2018			ISO 23517:2021 EN 17033:2018
		ISO 17556:2019			
	Field tests				
	Lab-scale tests (disintegration)				
		Standard test method: pelagic zone (water column scenario)	**Standard test method: benthic zone (seafloor scenario)**	**Standard test method: eulittoral zone (beach scenario)**	**Standard specification**
Freshwater	Laboratory tests (biodegradation)	ISO 14851:2019			
		ISO 14852:2021			
		ISO 14853:2016 (anaerobic)			
	Field tests				
	Tank tests				
Marine	Laboratory tests	ASTM D6991-2017 (Under revision by ASTM WK82370)		ASTM D7991-2022	ISO 22403: 2020 ASTM D7081:2015 (Under revision by ASTM WK75797)
		ASTM D6692-2001 (Withdrawn 2010)			

(continued)

Table 1 (continued)

		ISO 23977-1: 2020	ISO 18830: 2016	ISO 22404: 2019	
		ISO 23977-2: 2020	ISO 19679: 2020		
	Field tests	ISO 15314:2018	ISO 22766: 2020	ISO 22766: 2020	
		ISO/CD 16636			
	Tank tests		ASTM D7473-2012 (under revision by ASTM WK71923)		
		ISO 23832:2021	ISO 23832: 2021	ISO 23832: 2021	

6 Research Needs

This subchapter provides a perspective on research needs on specific aspects of polymer biodegradation and biodegradability in engineered and natural environments. The identified research needs to consider both fundamental and practical aspects of polymer biodegradability and biodegradation.

6.1 Variations in Polymer Biodegradation in and Across Different Receiving Environments

Most polymer and plastic biodegradation studies are conducted in laboratory incubations, including incubations in composts, soils, water, and sediments. However, laboratory incubation studies that assess the variations in the biodegradation rates and extents of a set of biodegradable polymers across members of the same receiving environment (e.g., different agricultural soils) as well as across different receiving environments (e.g., compost, soil, freshwater, and marine systems) remain scarce. Such systematic studies are warranted to provide information on the variability of biodegradation rates and extents within members of the same receiving environment (e.g., among soils) but also between different types of receiving environments (e.g., soils and lakes). This is particularly important for benchmarking biodegradability of a given polymer across potential receiving environments. While many applications of biodegradable polymers have targeted receiving environments, e.g., compostable bags in industrial compost or biodegradable mulch films in soils, it is of interest to test biodegradation in environments other than those targeted to provide information on biodegradation rates and hence residence times of these polymers or plastic items composed thereof in other environments. Two case examples for which this

Table 2 Labels issued by certification bodies for industrially compostable products and standards linked to the certification [95–99]

Organization	DIN-Certco	TÜV AUSTRIA	CIC	Jätelaito-syhdistys	BPI / USCC	BPS
Location	Germany	Belgium	Italy	Finland	USA	Japan
Label						
Standard	EN 13432 ASTM D 6400-04	EN 13432	EN 13432	EN 13432	ASTM D 6400-04	GreenPLA certification scheme

information can be relevant are, first, incompletely biodegraded compostable plastics that may be transferred with the compost to agricultural soils or, second, soil-biodegradable mulch films that may be transported by runoff from agricultural soils or wind erosion to an adjacent creek or lake. In fact, some recent studies addressed this research need by assessing biodegradation of plastics in freshwater and marine systems [67, 100]. Such tests on the biodegradability of polymer products in systems other than the primary receiving environment should be complemented by systematic studies on the probability of transport of the polymer products and its degradation products across boundaries of environmental compartments (e.g., from agricultural fields to adjacent lakes). Also, benchmarking biodegradability of existing biodegradable polymers across receiving environments will help in the design of novel polymer materials with desired biodegradation performances in specific receiving environments.

6.2 Assessing Uncertainties Associated with the Transferability of Results of Polymer Biodegradability from Laboratory Tests to the Real in-situ Biodegradation in the Receiving Environment

A central criticism of laboratory incubations is that their conditions only partly reflect conditions encountered in the actual natural or engineered receiving environment, raising concerns that laboratory biodegradation rates and extents have only limited predictive power for polymer biodegradation under real, in situ conditions in the field. To address this concern, studies are needed in which the biodegradation of selected polymers is quantified in the same receiving environment (i.e., an agricultural soil or at a water-sediment interface) but across incubation scales: in the laboratory, in mesocosms, and in the field. In such studies, polymer or plastic biodegradation may be followed using the same analytical methods on all three incubation scales, for instance by quantifying residual polymers over the course of the incubation using solvent extraction (see above). It is expected that polymer biodegradation rates are higher in laboratory tests that are run at incubation temperatures higher than those in the actual receiving environment. Conversely, biodegradation rates in laboratory incubations – particularly when run at small scales and for an extended time of months to a few years – may decrease if essential nutrients (e.g., N and P) become depleted. These limitations are less likely to arise in open, larger scale studies in mesocosms or in the field where nutrients and microorganisms can be replenished naturally. Determining uncertainties associated with extrapolating laboratory incubation results to biodegradation in the field is critical to accurately predict the lifetimes of biodegradable polymers and plastics under real in situ conditions in the actual receiving environment.

6.3 Identification of Key Degrading Enzymes and Microbial Degraders

Most commercially available biodegradable polymers undergo enzymatically mediated breakdown as the initial step of biodegradation. For synthetic polymers, it is likely that relatively few microbial degraders can secrete competent enzymes unlike enzymes that hydrolyze natural biopolymers which are likely to be expressed by a larger number of microorganisms. While extracellular enzymes hydrolyzing synthetic polyesters have been identified and purified, little is known on the number, activity, specificity, and identity of enzymes that are involved in the hydrolytic breakdown of polyesters in situ. The importance of enzymatic breakdown in the biodegradation of polymer and plastics is reflected in recent efforts to identify competent enzymes [101], develop high-throughput assays to screen for polyesterhydrolyzing enzymes [52, 102] as well as the use of metagenomics, metatranscriptomics, and metaproteomics to identify esterases and microorganisms involved in the biodegradation of synthetic polyesters [103].

Future work is needed using these approaches to characterize hydrolases involved in polymer breakdown in situ and identify potential restrictions on enzyme activities and expression and secretion levels (e.g., by limited N and P availability). Furthermore, the occurrence of specific enzymes ought to be linked to the organisms expressing these enzymes. Given that the enzymatic hydrolysis of polyesters often is the rate-determining step in polyester biodegradation, it is expected that rates of polymer biodegradation correlate with the abundance and activity of the enzyme-secreting microorganisms. In contrast, biodegradation rates do not necessarily correlate with the microorganisms that incorporate most of the polyester's breakdown products into their biomass – as could be determined by DNA stable isotope probing (DNA-SIP) – nor do they correlate with the most abundant microorganisms colonizing the surfaces of biodegradable polymers and plastics.

6.4 Microbial Metabolic Utilization of Plastic and Polymer Carbon

Polymer or plastic biodegradation may involve a significant incorporation of polymer carbon into microbial biomass, $C_{\text{microbial biomass}}$ (see Eq. 1). However, routine laboratory testing of the biodegradability of polymer and plastic uses only respirometric analyses of polymer carbon mineralization into CO_2. Future studies are warranted that determine the carbon use efficiencies (CUEs) of polymer-derived carbon in different receiving environments. Determining CUEs is, for instance, possible by combining respirometric analyses of formed CO_2 (i.e., C_{CO2} in Eqs. 1–3) with solvent extractions and quantifications of residual polymer(s) from the incubation medium. The incorporation of polymer carbon into microbial biomass can then be calculated assuming closed mass balance (i.e., $C_{\text{microbial biomass}}$

(t) = $C_{polymer}$ (t_0) − C_{CO2} (t) − $C_{polymer}$ (t); Eqs. 2 and 3). Under the assumption that C_{CO2} primarily stems from the catabolic utilization of polymer carbon (and not mineralization of $C_{microbial\ biomass}$), the carbon use efficiency can be estimated as CUE = ($C_{polymer}$ (t_0) − (C_{CO2} (t) + $C_{polymer}$ (t)))/($C_{polymer}$ (t_0) − $C_{polymer}$ (t)).

The CUE values will help to constrain the extent to which respirometric analyses underestimate true polymer biodegradation in laboratory incubations. The degree of underestimation increases with increasing CUE. Furthermore, the CUE of the polymers can be compared to the CUEs of reference (bio)polymers used as positive biodegradation controls. The polymers and the reference (bio)polymers ought to have similar CUEs to substantiate the approach of expressing the final extents of mineralization of polymers relative to the mineralization extent of such positive reference biomolecules. Finally, information on how CUE values vary with plastic composition and changes in abiotic factors (e.g., nutrient availability) will provide guidance both in the development of novel biodegradable polymers and the optimization of conditions in the incubations (e.g., nutrient addition) that lead to higher CUEs (and thereby faster biodegradation of the polymers). These insights from laboratory incubations can then be transferred to real in situ conditions in the actual receiving environment.

6.5 *Slowly Biodegrading Polymers*

A major challenge when developing novel biodegradable polymers is to balance the two desired polymer performance levels: the *mechanical performance level* during processing and during the use-life of the application – which typically requires stability of the polymer – and the *end-of-life biodegradation performance level*, which requires that the polymer readily undergoes biodegradation after use. These two performance levels may be mutually exclusive in cases where mechanical performance and stability demands for a specific application are high and, at the same time, requested biodegradation times after the use of the polymer are short. One application in which these performance levels can be balanced are biodegradable mulch films with above-ground stability for up to several months during use and subsequently extensive biodegradation in soil over a period of 2 years after use. However, there are also potential applications of biodegradable polymers which demand long stability of the material during use (i.e., product stability over several years). Such applications may include, but are not limited to, polymer components in fishing gear as well as geotextiles deployed in the environment, for instance to stabilize slopes and inhibit soil erosion. The latter are commonly composed of conventional, non-biodegradable polymers which are expected to persist in the environment for decades to centuries given that they are not meant to be collected after use.

Future research efforts are needed on the development and testing of polymer materials that can be used in such applications and with reliable biodegradation performance over a few years. The testing of such materials is challenging because

these polymers need to show slow, yet continuous biodegradation rates. Given the extended biodegradation times and hence increased lifetimes in the environment, molecular breakdown as the initial step of biodegradation needs to occur reliably and continuously to ensure that these materials indeed biodegrade and thus do not accumulate in the open environment.

The extended lifetimes are also a challenge for testing the biodegradation of these materials. The tests may be conducted under accelerated biodegradation conditions, such as slightly elevated temperatures (which is different from the above-discussed inappropriate thermal pre-conditioning of polymers at unrealistically high temperatures). In such cases, however, experimental evidence is required that the rates measured at higher temperatures can indeed be extrapolated to environmental conditions at lower temperatures (e.g., by establishing that the Arrhenius rate law applies to the biodegradation of these polymers). Extrapolations across threshold temperatures at which the polymers undergo significant changes (e.g., the glass transition temperature, T_g) need careful assessment. These biodegradation tests also need to be complemented by appropriate certification schemes. From the regulatory side, stringent criteria are needed to define how data on biodegradation rates can be extrapolated and how associated uncertainties are statistically evaluated and documented. Periodic in situ monitoring of the biodegradation of these items after application is advisable to ensure that actual biodegradation rates match predicted rates.

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