ACCELERATING THE BIODEGRADATION OF POLY(LACTIC ACID) AT MESOPHILIC TEMPERATURES

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ABSTRACT

As plastic production and consumption increase globally, so does the amount of plastic waste to be disposed of and managed. Most worldwide plastic waste ends in landfills, open dumps, or leaks into the environment. Hence, new alternatives, such as developing biobased and biodegradable polymers, have been proposed to tackle the ever-growing plastic waste problem and help reduce the amount of plastic coupled with organic waste reaching landfills or incineration facilities. It is essential to understand the degradation behavior of these novel polymers to guarantee their ultimate biodegradation together with organic waste. Among them, poly(lactic acid) (PLA), a popular biobased and compostable polymer produced from renewable sources has garnered much interest due to its low environmental footprint and ability to replace conventional polymers and be disposed of in industrial compost environments. Although PLA is industrially compostable when subjected to a suitable set of conditions (i.e., aerobic thermophilic conditions for an extended period), its acceptance in industrial composting facilities is affected adversely due to longer timeframes to degrade than the readily biodegradable organic fraction of waste. So, PLA's requirement to be fully exposed to thermophilic conditions for prolonged periods to biodegrade has restricted its adoption and hindered its acceptance in industrial composting facilities. This dissertation proposes three different approaches to improve PLA biodegradation under mesophilic conditions to open new avenues of biodegradation, such as home/backyard composting and guaranteeing industrial composting biodegradation at similar times as that of readily biodegradable materials.

For the first approach, a reactive blend of PLA with thermoplastic starch (TPS) was produced and evaluated for biodegradation under mesophilic (37°C) and thermophilic (58°C) conditions. Films were tested for biodegradation by analysis of evolved CO_2 for 180 days in simulated composting conditions in an in-house built direct measurement respirometer (DMR). The films' average molecular weight (M_n) and crystallinity (X_c) were tracked throughout the test

duration, and the kinetic degradation rate was calculated. The introduction of TPS positively affected accelerating PLA hydrolysis during the lag phase in both mesophilic and thermophilic conditions due to increased chain mobility, resulting in faster degradation of PLA at both biodegradation conditions.

The second approach involved the introduction of biostimulants in compost to target different stages of biodegradation and enhance the enzymatic activity of microorganisms. PLA and biostimulants, Fe_3O_4 nano-powder, skim milk, gelatin, and ethyl lactate were introduced into the compost media at 37°C. The CO_2 evolution, M_n, and X_c of PLA, PLA added with single biostimulants, and PLA added with a combination of biostimulants were evaluated to investigate the degradation of PLA. To attain M_n values of ≲10 kDa for PLA, PLA added with skim milk experienced a biodegradation acceleration of 15%, 25% with gelatin, and 22% with ethyl lactate. Fe_3O_4 enhanced the biodegradation rate by 17% whereas the combination of gelatin and Fe_3O_4 resulted in a substantial increase of biodegradation rate of around 30%.

The last approach explores the use of enzymatic pretreatments. PLA films were pretreated with proteinase K enzyme at 37°C for 7 and 10 days and at 58°C for 2 and 5 days. These films were later introduced in inoculated vermiculite at 37°C and 58°C in the DMR to investigate the effect of pretreatment by simulating home and industrial composting settings. The results showed a higher CO_2 evolution and visible degradation for PLA films pretreated with proteinase K compared to the untreated control PLA films.

This dissertation presented three innovative methods to speed up PLA film biodegradation in composting. It provides potential solutions to remove the barrier for degrading PLA in home and industrial composting conditions and to help address the plastic pollution challenge by effectively degrading biodegradable polymers with organic waste.

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LIST OF ABBREVIATIONS

BA	bonds of 1,4-butanediol of adipic acid
BOD	biochemical oxygen demand
BT	bonds of 1,4-butanediol and terephthalic acid
CO_2	carbon dioxide
EFP	environmental footprint
EPS	extracellular polymeric substances
GHG	greenhouse gas
MSW	municipal solid waste
X_c	degree of crystallinity
M_{no}	initial number-average molecular weight
M_n	number-average molecular weight
M_w	weight-average molecular weight
MWD	molecular weight distribution
OH	hydroxyl group
PBAT	poly(butylene adipate-*co*-terephthalate)
PBS	poly(butylene succinate)
PBSA	poly(butylene succinate-*co*-adipate)
PBSe	poly(butylene sebacate)
PBSeT	poly(butylene sebacate terephthalate)
PBST	poly(butylene succinate terephthalate)
PBT	poly(butylene terephthalate)
PDLA	poly(D-lactide)
PDLLA	poly(D, L-lactide)
PE	poly(ethylene)

PEA	poly(ethylene adipate)
PES	poly(ethylene succinate)
PET	poly(ethylene terephthalate)
PGA	poly(glycolic acid)
PHA	poly(hydroxyalkanoates)
PHB	poly(hydroxybutyrate)
PHU	polyhydroxyurethane
PLA	poly(lactic acid)
PLLA	poly(L, L-lactide)
PS	polystyrene
PU	polyurethane
PVOH	poly(vinyl alcohol)
SEC	size exclusion chromatography
SUP	single-use plastic
TCA	tricarboxylic acid
THF	tetrahydrofuran
TPS	thermoplastic starch
UV	ultraviolet radiation
VOC	volatile organic compounds

CHAPTER 1: INTRODUCTION

1.1 Background and motivation

The last two decades have seen a significant revolution in the plastic industry concerning the fate of polymers in the environment post-consumer use [1]. As the global production of plastic increases, so does the attention towards its disposal and waste management scenario [2]. The current problem of plastic waste finding its way to oceans and remote locations, such as the alps, arctic has revealed the lack of waste management systems and policies targeting their responsible use and consumption [3–5]. The growing concern and environmental awareness of the problem of white pollution have been a driving force to develop green, biodegradable, recyclable, or compostable polymers with a low environmental footprint [6,7]. Replacing the fossil-based polymers with biodegradable polymers such as poly(lactic acid) (PLA), thermoplastic starch (TPS), polycaprolactone (PCL), and polyhydroxyalkanoate (PHA) is perceived as one of the many solutions to tackle the plastic pollution problem [8,9]. Implementing these polymers can expand their commercial market and help divert organic waste from reaching landfills.

PLA is a bio-based polymer that can be synthesized from the fermentation of dextrose obtained from crops such as corn, sugarcane, and cassava [10,11]. PLA is compostable in nature and can be biodegraded under the right conditions to produce carbon dioxide, water, and biomass, which is cycled back and non-toxic to the environment. In addition, PLA displays a moderate barrier to gas and flavor, is easily processed using extrusion, has high clarity and good stiffness equivalent to traditional polymers such as polystyrene [12].

PLA has found its applications in food packaging such as containers and trays [13–16]. It can be collected with the organic fraction of municipal solid waste (MSW). The organic collection of MSW can be treated in industrial composting treatment facilities. These organic composting treatment facilities were created keeping in mind the considerable volume of

organic waste that comes from food waste streams. However, they were not designed to process bioplastics such as PLA, whose degradation rate is lower than readily biodegradable feedstocks such as food waste.

Since PLA undergoes chemical hydrolytic degradation at temperatures higher than its glass transition temperature (T_g ~ 58°C), which has been reported as the primary controlling degradation mechanism, this brings a considerable change in the mechanical properties and reduction in molecular weight to a point where it can be easily assimilated by microorganisms present in the given environment [13]. PLA shows a more extended biodegradation lag phase at lower temperatures and degrades at a slower rate than other readily biodegradable materials such as starch and cellulose. This dependence of PLA on degrading at elevated temperatures reduces its disposal scenario to industrial composting and limits degradation on home or backyard composting [14,15].

Furthermore, since industrial composting facilities handle large volumes and the turnaround time can be faster than the current standard requirements, PLA may not be wholly degraded at the end of the composting process [16]. Any variation in the process related to the frequency of turning the compost pile, uniform mixing to aerate the piles, maintaining optimal temperature range, and avoiding the formation of pockets of anaerobic conditions through the compost heaps can easily derail the biodegradation of PLA, thereby leaving half degraded or at times whole packages of the same by the end of the composting cycle [17]. This defeats the purpose of compostable polymers and produces low-quality, contaminated, and unmarketable compost (soil conditioner and fertilizer). Overall, this negative effect has discouraged composting facilities from accepting biodegradable polymers like PLA [15,16,18].

Since biodegradation of PLA is a complex phenomenon and involves the action of extracellular enzymes released by the microbes at the same time, researchers have studied

and advocated that apart from chemical hydrolysis, the action of the hydrolases class of enzymes plays an essential role in the breakdown of the high molecular weight PLA by severing the backbone chain [19–23]. Since PLA is marketed as a compostable polymer, inspecting different paths for improving PLA degradation in composting and ambient conditions is essential. So, enhancing the degradation of PLA under lower temperature conditions such as mesophilic range can open new avenues to dispose of PLA in-home/backyard composting and provide some assistance towards guaranteeing the degradation at industrial composting facilities.

Biostimulation, which is the addition of selective compounds in the degradation environment, is one way of inducing enzymatic degradation and accelerating the degradation of PLA. Researchers have studied the prospective of biostimulation by introducing different groups of nutrients and enhancing the degradation activity. Modifying the PLA structure through a process such as blending PLA with other biodegradable polymers to tailor its properties such as hydrophobicity, tensile strength, and elongation at the break while still retaining/ improving the biodegradability can be another option to advance its slow degradation rate. Finally, bioaugmentation is another favorable option that involves introducing specific microbial strains known to degrade PLA in the current environment and boosting its degradation rate. These three methods, modification of PLA structure (blending), biostimulation, and bioaugmentation, can open the venue to make PLA easily compostable in industrial facilities and provide an opportunity for home composting.

1.2 Overall goal and objectives

This dissertation aims to elucidate the degradation process of PLA at mesophilic temperatures and investigate different pathways to accelerate the aerobic biodegradation of PLA in simulated composting conditions. Three objectives have been outlined to accomplish this goal:

1) To modify PLA structure by blending PLA with other biobased biodegradable polymers to increase its biodegradation.

2) To assess and understand the effect of adding biostimulants to enhance the biodegradation of PLA by altering different steps involved in the biodegradation process.

3) To investigate the effect of pretreating PLA with proteinase K enzyme prior to introducing the films in a compost environment.

1.3 Dissertation overview

This dissertation is organized as follows.

The current chapter, Chapter 1, details the background and motivation for this dissertation study, including the main goal and the specific objectives outlined to accomplish this goal.

Chapter 2 lays out an extensive literature review regarding the main polymer classification, abiotic and biotic polymer degradation mechanism, mesophilic environments, polymer properties affecting the biodegradation rate, different assessment techniques to measure biodegradation, and polymers that can be blended with PLA to improve its properties and biodegradation. The chapter also covers the main class of enzymes active in PLA biodegradation and concludes by highlighting the biodegradation pathway of PLA and other biodegradable polymers.

Chapter 3 investigates the effect of reactively blending thermoplastic starch with PLA and compares biodegradation at two different simulated composting settings: industrial (thermophilic temperature range ~ 58°C) and home/backyard (mesophilic temperature range ~ 37°C). The study explores how the addition of thermoplastic starch improves PLA biodegradation.

Chapter 4 investigates the biostimulation technique to improve PLA biodegradation at mesophilic conditions. Different biodegradable biostimulants were added to the compost

environment to stimulate the native microbial population and target different steps involved in the biodegradation process.

Chapter 5 explores the effect of a combination of biostimulants on PLA biodegradation when introduced in the compost environment. The combination of biostimulants targets two different steps (i.e., adding compounds to trigger enzyme generation and electron donor/acceptor compounds) in the PLA biodegradation process.

Chapter 6 presents the results of the bioaugmentation technique wherein PLA films are pretreated with proteinase K enzyme and subjected to industrial and home composting simulated degradation setting. A comparative analysis of PLA degradation at two different temperatures, 37°C and 58°C, for two different pretreatment time intervals was investigated.

Chapter 7 summarizes all the works in this dissertation and concludes with future work recommendations.

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CHAPTER 2: LITERATURE REVIEW

2.1 Abstract

Finding alternatives to diminish plastic pollution has become one of the main challenges of modern life. A few alternatives have gained potential for a shift towards a more circular and sustainable relationship with plastics. Biodegradable polymers, derived from both bio- and fossil-based sources, have emerged as one feasible alternative to overcome inconveniences associated with the use and disposal of non-biodegradable polymers. Biodegradation of biodegradable polymers depends on the environments, abiotic and biotic factors, and the polymer bulk and surface properties, resulting in a plethora of parameters that create a complex process whereby biodegradation times and rates can vary immensely. The intent of this review is to provide background and a comprehensive, systematic, and critical assessment of the factors affecting the biodegradation of biodegradable polymers, with special focus on the mesophilic range. To accomplish this goal, the literature on biodegradable polymers since 1990 was assessed to create a holistic understanding of the main proxies responsible for biodegradation such as abiotic and biotic mechanisms of degradation, environments, factors, microbial populations, and polymer properties. Insights gained and remarks for potential future research are provided with focus on mesophilic aerobic environments and the main biodegradable polymers produced.

2.2 Introduction

Plastics are pervasive and have become an indispensable part of our everyday life. The nature of plastics and their easy processability, durability, low cost, and availability favor their use, opening up an array of opportunities in market segments such as consumer goods, food and medical packaging, plastic films and pots for the agriculture sector, construction, and automotive parts [1,2]. Between 1950 and 2020, global plastic production

reached an accumulated amount of c. 9,500[1] million metric tons [1,3]. With annual production of c. 370 million metric tons in 2020, estimates for 2030 are c. 600[2] million metric tons. The global value of the plastic industry in 2020 was around USD 580 billion [4,5], and is expected to reach USD 800[3] billion by 2030. Flexible and rigid single-use plastic (SUP) represents c. 40% of the total global plastic production [1].

The benefits of plastics and SUPs are numerous, and include lower water and energy consumption during production and use than glass and metal, which in turn reduces the cost of resources used [6]. However, the ability of plastics to persist, even in harsh environments, has led to white pollution (i.e., leakage and accumulation of plastics in the environment). SUPs have been blamed as one of the main offenders for white pollution and are a growing concern for our modern society since increasing amounts end up in landfills as a portion of municipal solid waste (MSW), as litter on land and in drainage systems worldwide, and ultimately leaking into rivers and oceans [7–9]. At present, c. 8 million metric tons of plastic end up in our oceans annually, in addition to the 150 million metric tons that are already circulating in marine environments since the dawn of the plastic era [10,11]. A recent prediction, if business continues as usual without mitigation measures, is that c. 90 million metric tons of plastic waste will reach the world's aquatic environments by 2030 [12].Plastics ending up in the environment mostly start as macromolecular structures formed as films, bottles, trays, etc. (macroplastics) and then break down into smaller fragments called microplastics, which are formed due to mechanical abrasion, radiation light, and heat, and

[1] Estimations were obtained from references [1,3]. Results are based on production estimated from reference [3] until 2015 and the addition of production for the 2016–2020 period from reference [1].

[2] Estimation was obtained based on a linear projection growth rate from 2006–2018 from each global region from references [1,3] and extrapolated to 2030.

[3] Estimation obtained based on a linear global projection growth rate from 2016–2020 from references [4,5] and extrapolated to 2030.

can even be reduced to nanoplastics. Microplastics are a concern due to the ability to concentrate pollutants and become a channel for bioaccumulation, while nanoplastics are also a health concern since they can potentially translocate in cell membranes of living organisms and become a source of transporting toxic chemicals [13–15].

Most of the plastic waste in the ocean comes from land-based sources, such as agricultural soils, open dumps and industries, or mismanaged plastic waste from land litter and incomplete collection, and then ending up in sludge, sewage, and polluted streams, finding its way through river pathways and leading to global marine pollution [11,16,17]. Apart from rivers [16], wind and snow have also been identified as responsible for transporting airborne plastic debris to locations perceived uninhabitable and remote such as the polar regions and the French and Swiss Alps [18,19]. So, plastic pollution has called attention worldwide in the form of a global crisis leading to ecological imbalance [7,20].

A consumer paradigm shift about plastics has occurred due to the growing amount of unmanaged disposal of flexible SUPs, pushing industries to embrace the long-term circular economy of plastics [21–23]. As part of this circular economy, new challenges have been highlighted such as *novel policies* targeting responsible consumption, a push for *worldwide waste management infrastructure* creation to recover plastics, and the development, production and use of *highly recyclable or biodegradable* plastics with lower environmental footprint (EFP) [24–26]. Novel policies targeting responsible consumption have been developed, such as the 2030 Agenda for Sustainable Development by the United Nations establishing the seventeen Sustainable Development Goals (SDGs) to achieve a better and more sustainable future for all [27]. Specifically, Goal 12 stipulates sustainable consumption and production, which has been adopted by countries around the world to create novel policies about use of materials such as plastics [28]. For example, various U.S. states have established "extended producer responsibility" for packaging and have banned plastics bags [29–31].

Furthermore, bans or extra fees for some SUPs are already effective in the European Union and countries in Asia such as China and Indonesia [32–34], and are in development in New Zealand and Australia [35].

The need for worldwide waste management infrastructure has been noted: in 2016, the world generated c. 2 billion metric tons of MSW, and is expected to generate c. 2.6 billion metric tons of waste by 2030 if no measures are taken to curb the growing generation of waste [36]. High and upper-middle income economies generated 66% of the global MSW in 2016, representing c. 1.34 billion metric tons, and are expected to generate c. 1.6 billion metric tons by 2030; lower-middle and low-income economies generated 34% of global MSW in 2016, representing c. 0.7 billion metric tons, and are expected to generate 1.0 billion metric tons by 2030 [36]. Inadequate or scarce waste management solutions are prevalent in lower-middle and low-income economies. In 2016, high and upper-middle income economies had MSW collection rates of 96% and 82%, respectively, in urban areas; whereas lower-middle and low-income economies had collection rates of 51% and 39%, respectively, in urban areas [36]. Therefore, as economies move from low and lower-middle to upper-middle and high-income, MSW generation will increase, and waste management systems and collection must improve accordingly. Concentrated efforts are being directed to improve material recovery facilities around the world, with special emphasis on the lower-middle and low-income economies [37,38]. However, without MSW collection, white pollution will not be deterred.

To tackle the problem of plastic pollution, cradle approaches to deal with the production of highly recyclable and biodegradable polymers with lower EFP are increasingly being considered [39,40]. Replacing fossil-based plastics with bio-based plastics is one strategy shown to reduce the greenhouse gas (GHG) emissions produced by plastics [41–43]. For example, substitution of c. 66% of the world's fossil-based plastics with bio-based plastics has been estimated to reduce GHG emissions by 241 to 316 Mt CO_2-eq. per year [44]. To date,

mechanical recycling has been the main recovery method for polymers such as poly(ethylene terephthalate) (PET) and poly(ethylene) (PE). However, novel chemical upcycling methods for polymers are being explored, which entail the use of rejected plastic (waste) and converting it to a high-value resource in the form of fuels, chemicals and novel polymers, thus avoiding the buildup of plastic and reducing the use of fossil fuels [45]. Most of these new recovery methods are also being explored for flexible SUPs [46].

Among the cradle approaches to deal with littering and recovery of plastics, the production of biodegradable polymers is a promising solution, primarily since they can be recovered by traditional waste management options, including mechanical and chemical recycling and energy recovery, and the additional recovery route through aerobic industrial and home composting or anaerobic digestion. If enough volume of isotropic biodegradable polymers is collected and treated, they can be recycled. However, if volumes collected are low and/or the materials are contaminated, they can be routed towards biodegradation recovery scenarios such as industrial and home composting, with soil biodegradation being a special route for agriculture films.

The efficacy of biodegradation is conditioned by drastically different environmental conditions, such as heat, humidity, and acidic or alkaline media, and by the polymer characteristics such as chemical structure and physical properties.

Previous reviews on the biodegradation of polymers in some of these environments and conditions have focused on biodegradable polymers in general [47–49], biodegradable polyesters [50,51], and mechanisms of degradation [52,53]. Furthermore, recently works have reviewed and identified gaps and research needs in this area [54,55]. This comprehensive review expands on these previous works, and aims to provide an overview of the mechanisms, environments, and factors affecting the biodegradation of biodegradable polymers, giving special attention to the mesophilic degradation range (20 - 45°C). The specific goals are:

1) to provide a transdisciplinary background on the aspects affecting the biodegradation of biodegradable polymers,

2) to describe the different methods used for assessing biodegradation at mesophilic temperatures, and

3) to provide insights on the degradation pathway followed by polymers susceptible to biodegradation.

2.3 Bio- and fossil-based biodegradable polymer classification

Figure 2.1 provides a general classification of polymers according to their feedstock source and their ability to experience biodegradation. The first group of polymers are bio-based in nature and non-biodegradable, such as bio-based poly(ethylene) (Bio-PE) and bio-based poly(ethylene terephthalate) (Bio-PET). The second group of polymers are bio-based and biodegradable, such as poly(lactic acid) (PLA), poly(hydroxyalkanoate)s (PHAs), cellulose, and starch. The third group includes polymers that are derived from fossil-based sources, but also present biodegradable characteristics, such as poly(butylene adipate-*co*-terephthalate) (PBAT) and poly(caprolactone) (PCL). The fourth and final group corresponds to the conventional group of polymers that are derived from fossil-based sources and are non-biodegradable, such as low- and high-density PE (LDPE, and HDPE) and PET. This classification is very general since the characteristics of the material, the environment, and the rate of biodegradation for polymers vary widely among these groups.

As shown in **Figure 2.1**, biodegradability of a polymer in regular environmental conditions is not strictly related to the source of the polymer, and factors such as its chemical structure and physical properties are essential [56]. Some bio-based polymers, such as bio-PE and bio-PET, are more difficult to degrade than their fossil-based counterparts (i.e., PE and PET); whereas some fossil-based polymers, such as fossil-poly(butylene succinate) (PBS)

and PBAT, are as easily biodegradable as some bio-based polymers, such as bio-PBS and PHAs, when assessed under standard conditions [57,58].

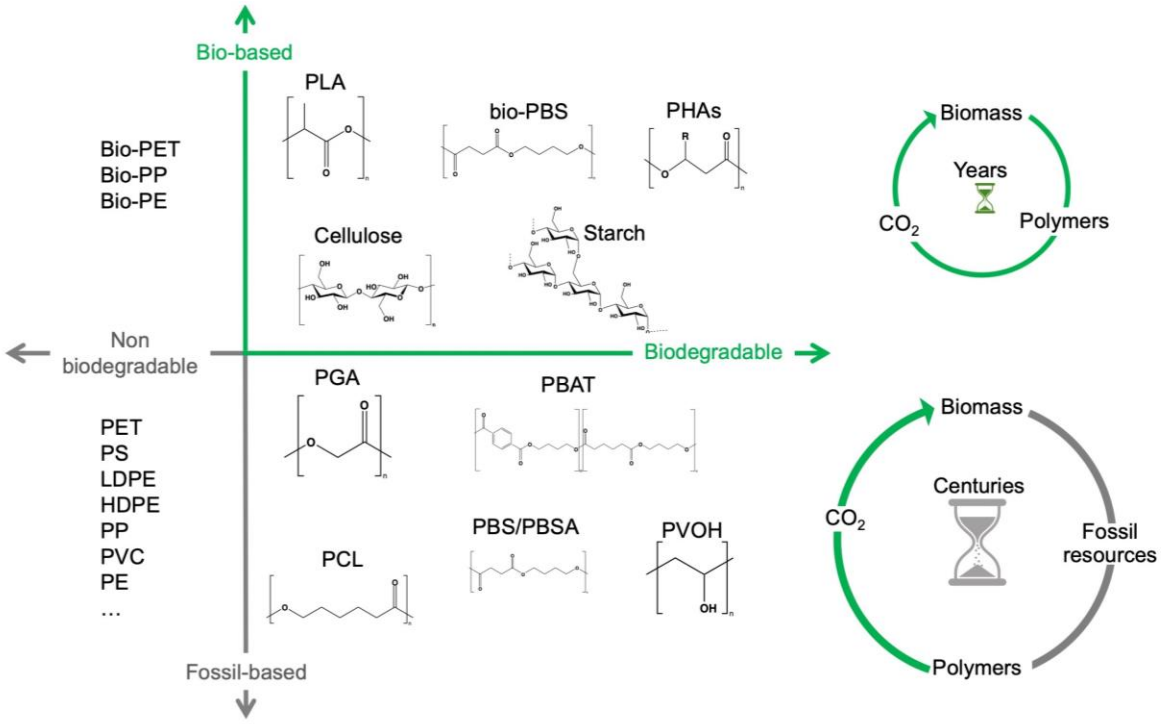


Figure 2.1 Classification of polymers considering their bio-based or fossil-based feedstock and ability to be biodegradable or non-biodegradable in environments as compost, soil, and aquatic; and carbon cycle of bio-based and fossil-based polymers; adapted from [59,60].

Considering the carbon used to produce polymers, the main benefits of biodegradable polymers can be obtained when the polymers are produced from renewable resources since they can replenish the carbon cycle (i.e., the times needed to create them and to convert them to biomass are equivalent) (**Figure 2.1**). Fossil-based polymers can also be considered renewable like the bio-based polymers, but the main difference between both is the amount of time needed to convert to biomass and then back to their original form. Biodegradable polymers produced from bio-based resources take far less time to be converted to biomass whereas the fossil-based polymers take millions of years to achieve the same. The longer time frames are due to the imbalance between the rate of consumption and the replenishment

rate, which further leads to mass imbalance in the carbon cycle. There is no additional carbon footprint associated with renewable-carbon feedstock used to produce biodegradable polymers, such as starch-heavy crops not intended for human consumption (e.g., starches from field/feed corn), due to quite similar time frames for consumption and conversion to biomass [43,60,61].

2.4 Abiotic and biotic polymer degradation mechanisms

Polymer degradation is defined as an irreversible change of the chemical structure, physical properties, and visual appearance due to the chemical cleavage of the polymer's constitutive macromolecules by one or more mechanisms acting concurrently [49]. More than one mechanism can simultaneously take place due to the action of external factors, and one mechanism can be more dominant than others at any time [48]. External factors associated with the environment, such as heat, humidity, radiation, and acidic or alkaline conditions, could modify the degradation process and its rate. The degradation process can alter polymer properties such as mechanical, optical, electrical, discoloration, phase separation or delamination, erosion, cracking and crazing [49]. The four main abiotic mechanisms associated with polymer degradation are mechanical, thermal (or thermo-oxidative), photo (photo-oxidative), and hydrolytic (chemical) degradation, some of which can be assisted by catalysis. In addition, ozone degradation (chemical) is considered a mechanism of degradation for polymers but is less common (**Figure 2.2**). Mechanical and thermal degradation can occur at the early stage of polymer processing. Photodegradation is observed when polymers are exposed to radiation like ultraviolet (UV) light or gamma rays. Abiotic hydrolytic (chemical) degradation is considered the most significant mechanism for biodegradable polymers such as polyesters. These abiotic mechanisms of degradation can be combined, accelerating the final biotic enzymatic process, for example, increasing the

exposed surface area for interaction with the microbial population [48]. We will be discussing hydrolytic and biotic degradation in detail below.

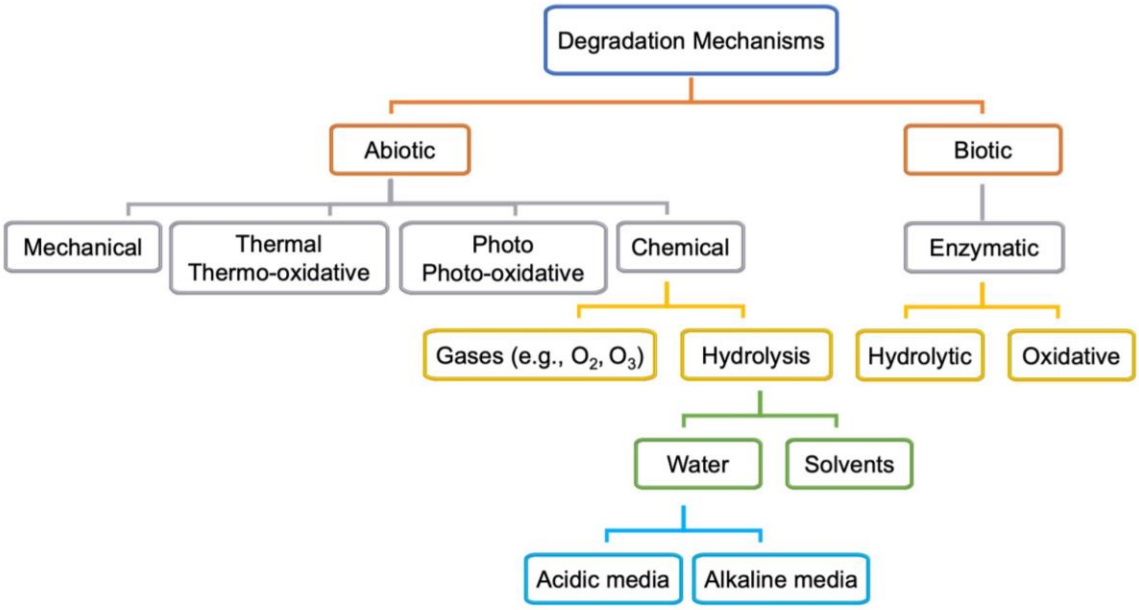


Figure 2.2 Main abiotic and biotic mechanisms of polymer degradation.

2.4.1 Hydrolytic degradation

Chemical hydrolytic degradation or abiotic hydrolysis is one of the main abiotic degradation mechanisms for biodegradable polymers, especially for aliphatic and aliphatic/aromatic polyesters. In this review, we refer to this mechanism also as chemical hydrolysis. With the uptake of water, susceptible chemical bonds in polymers can undergo chain scission, resulting in a reduction of M_w, loss of mass and mechanical properties, and increased surface area of the polymer, thereby increasing the available sites for attack by enzymatic activity, the biotic step initiated by microorganisms [48,52,62].

Chemical hydrolysis proceeds via two mechanisms when considering the macro structure: bulk or surface erosion. Depending on the conditions, these mechanisms can occur independently or combined. Bulk erosion is the dominant mechanism when the water diffusion is higher than the hydrolysis reaction rate, and surface erosion is dominant when

the water diffusion into the polymer matrix or bulk is lower than the hydrolysis reaction rate [48,50,63].

When bulk erosion is the dominant mechanism, the M_w of the polymer bulk is reduced so that the polymer loses its mechanical properties in a short period of time. Due to the M_w reduction and higher mobility of shorter polymeric chain segments, crystallinity may change. Loss of mass and change/s in geometric shape take more time. The byproducts of bulk erosion are first accumulated; when the polymer chains are short enough, and reaching n-mers size, they can start to diffuse out. When the polymer undergoes surface erosion, the loss of mass is mostly from the surface while the bulk remains intact. As degradation advances, the loss of mass happens faster at the surface and the polymer gets smaller in size. When compared with bulk erosion, the mechanical properties and M_w are preserved for an extended period, and release of byproducts from the surface occurs from the beginning [63].

The kinetic rate of the chemical hydrolysis – surface or bulk dominant – depends on and can be affected by several factors associated with the polymer itself and the environment. The roles of some factors are discussed in the next sections and additional information can be found elsewhere [48,50,63,64].

In terms of environmental factors, increases in temperature and moisture intensify the rate of chemical hydrolysis [65]. Polymer chain mobility increases as the temperature increases. Hence, the susceptibility of hydrolysable bonds to undergo chain scission increases. The chemical potential of water on the surrounding media plays a significant role in hydrolysis of polymers [66]. Hydrolysis in acidic or alkaline conditions can occur through different mechanisms so the byproducts of the reactions can differ [67]. Finally, catalysts can modify the rate of the hydrolytic process [68,69].

In terms of polymer factors, hydrophilic polymers are more susceptible to hydrolytic degradation than are hydrophobic polymers [48]. Hydrolysis depends on the presence of

hydrolysable covalent bonds, such as esters, ethers, anhydrides, carbamide (urea), and ester amide (urethane), which increase the rate of chemical hydrolysis [52,70]. **Table 2.1** compares the half-lives of hydrolysable bonds in various polymers and shows that poly(anhydride)s are subjected to rapid hydrolysis due to the presence of hydrolysable bonds of very low half-life. By contrast, polyamides are very resistant to hydrolysis due to the resistance of the amide bonds to hydrolysis. The kinetics of the hydrolysable bond half-life presented in **Table 2.1** can increase or decrease due to the influence of neighboring groups.

The presence of amorphous regions increases the chemical hydrolysis rate due to easy diffusion of water into the polymer matrix, compared to semi- and crystalline polymers showing well-organized structure where diffusion is limited, even at temperature higher than T_g [71–73]. So, for polymers with lower or similar values of T_g than the mesophilic range, the diffusion likely is mostly controlled by the amorphous region where chemical hydrolysis is dominant.

Table 2.1 Half-lives of hydrolysable bonds (in water at pH 7 and 25 ºC). Adapted from [74].

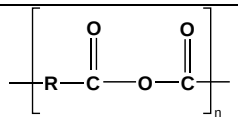
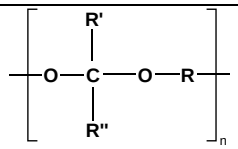
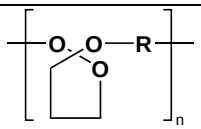
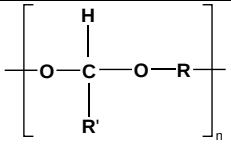
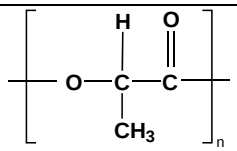
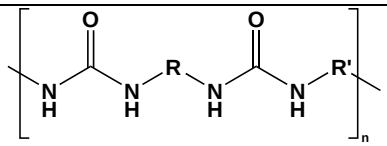
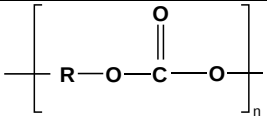
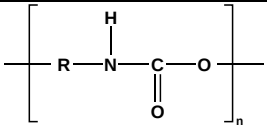
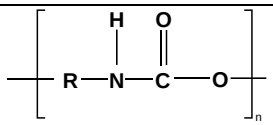
Polymer	Chemical structure	Half-life*
Poly(anhydrides)		0.1 hours
Poly(ketal)		3 hours
Poly(ortho esters)		4 hours

Table 2.1 (cont'd)

Poly(acetal)		0.8 years
Poly(ester)		3.3 years
Poly(urea)		33 years
Polycarbonate		42,000 years
Polyurethane		42,000 years
Polyamides		83,000 years

**Half-life: time required for 50% hydrolysis in water at pH = 7 and 25 °C for the low M_w (methyl, ethyl) model compounds.*

The macro structure properties, such as size and shape of the polymer, are factors that condition whether the dominant mechanism will be either surface or bulk erosion. In this way, a material can go from surface to bulk erosion when its thickness is reduced to a value lower than a critical value, called critical sample thickness (L_{crit}) [64,75].

Polyesters that contain ester groups are degraded mainly by chemical hydrolysis. Bulk degradation is predominant for aliphatic polyesters, such as poly(glycolic acid) (PGA), PLA, PCL, and PBS. The main stages of the hydrolytic degradation of polyesters undergoing bulk erosion can be summarized as 1) diffusion of water in the polymer matrix (amorphous regions); 2) water reacting with random ester linkage to produce shorter chains; 3)

autocatalysis due to the presence of acid chain ends in the medium; and 4) release of water-soluble oligomers and monomers creating a void core and subsequent reduction in M_w [48,76]. The duration of the chemical hydrolysis process depends mainly on the initial M_w, crystallinity, temperature, and pH [53].

PLA is an example for chemical hydrolysable polymer degradation that does not require enzymes to catalyze the hydrolytic degradation. In this sense, the media to which the material is exposed and factors such as temperature, pH, and moisture play major roles in delaying or speeding up the hydrolytic degradation rate. In an industrial composting process (~58 ℃ and ~60% RH), PLA can absorb water and undergo chemical hydrolytic degradation. However, at lower temperatures, such as in agricultural soil environments (~25 ℃ and ~45% RH), the rate of chemical hydrolysis is low, increasing the time for the enzymatic hydrolysis process to start. One of the main differences between bulk and surface erosion mechanisms can be recognized in the diffusion of the degradation byproducts. During bulk degradation of polyesters, these hydrolysis-formed oligomers and monomer byproducts, such as carboxylic acid and hydroxyl groups, are trapped and accumulated inside the bulk, leading to an autocatalytic degradation that tends to accelerate the degradation kinetics [50,77]. Burkersroda et al. [75] reported that hydrolytic degradation of PLA, evaluated at 37 °C, follows a bulk erosion mechanism for thicknesses between 0.5 and 2 mm, a core-accelerated erosion for thicknesses between 2 mm and 74 mm, and surface erosion for thicknesses greater than 74 mm. Hoüglund et al. [78] reported that the hydrolysis of 100% PLLA increased upon the addition of a low percentage of d-Lactide units, due to reduction of the polymer order structure, showing the effect of tacticity and optical purity on the hydrolytic degradation of PLA. PLA hydrolysis is delayed in comparison to PGA hydrolysis due to the presence of the methyl group in PLA that blocks the attack of water to interact with the hydrolysable bonds

[48,63]. A review of the hydrolysis of PLA at mesophilic and thermophilic conditions has been reported by Tsuji [64].

PBAT, due to the presence of an aromatic group in its polyester chain experiences a lower hydrolytic degradation rate than polyester with only aliphatic units as PLA and PGA [79]. The presence of the aromatic group reduces chain flexibility, provides less susceptible bonds, and creates an steric interference effect to the access of the susceptible ester bonds [80]. The soft aliphatic domain bonds consisting of 1,4-butanediol and adipic acid monomers (BA) are more susceptible to hydrolysis than the hard aromatic bonds of 1,4-butanediol and terephthalic acid monomers (BT). In this sense, PBAT displays good biodegradability when the aromatic moiety concentration is kept below 55 mol% [81]. Kijchavengkul et al. [82] also demonstrated that the increase of crosslinking on PBAT has a detrimental effect, not only on chemical hydrolysis but also in enzymatic hydrolysis.

Polymers that undergo surface erosion are desirable when designing medical devices and for drug release, since the retention of mechanical properties and capacity for a controlled release of drugs can be achieved by mass loss without compromising the M_w. Polymers that can mostly undergo surface erosion are polyanhydrides, some poly(ortho esters), and some polycarbonates [83–85].

2.4.2 Biotic enzymatic degradation

Biotic enzymatic degradation is the mechanism of degradation where microorganisms break down organic substances through an enzymatic process. The four main stages of biotic degradation are shown in **Figure 2.3**. The main outcome of biotic degradation is reduction of the polymer to small molecules that are utilized by the microorganisms as a source of carbon and energy, resulting in final products like CO_2 and H_2O in aerobic conditions or CO_2, H_2O and CH_4 in anaerobic conditions. Microorganisms like bacteria and fungi are actively involved in the biodegradation process. These microorganisms have their own optimal growth

conditions; for this reason, biotic degradation is a complex process where several factors associated with the polymer, microorganisms, and the environment come into play [65].

Abiotic chemical hydrolysis degradation, and biotic enzymatic degradation are the two main processes for the cleavage of polymer bonds and degradation [63]. The biodegradation process involves several steps, some of them are abiotic, and some are biotic and act synergistically to decompose the organic matter. Some of the abiotic mechanisms described above, such as photo, hydrolytic, or even mechanical degradation, can enhance the biotic degradation process by increasing the surface area for biofilm formation or by reducing the M_w [53]. However, the dominant mechanism in the biotic degradation process is related to biotic agents.

The first stage of the biodegradation process, as shown in **Figure 2.3**, is biofilm formation. In the second stage, depolymerization, microorganisms secrete extracellular enzymes. These agents can cleave the molecules by random or end chain scission, reducing the M_w and resulting in the generation of oligomers, dimers, and monomers. The susceptibility of a polymer to microbial attack depends on enzyme availability, availability of sites in the polymer for enzyme attack, enzyme specificity for the polymer, and the presence of cofactors [53]. In the third stage, bioassimilation, the molecules transported from the depolymerization stage are assimilated into the microorganism's metabolism and are used to produce energy, new biomass, and primary and secondary metabolites. In the final stage, mineralization, simple molecules such as CO_2, CH_4, H_2O, N_2, and different salts from intracellular metabolites, which are completely oxidized, are released to the environment [86]. These non-toxic components are redistributed through the carbon, nitrogen, and sulfur cycles in nature. Some simple and complex metabolites, such as organic acids, terpenes, aldehydes, and antibiotics, can reach the extracellular surroundings by excretion [52].

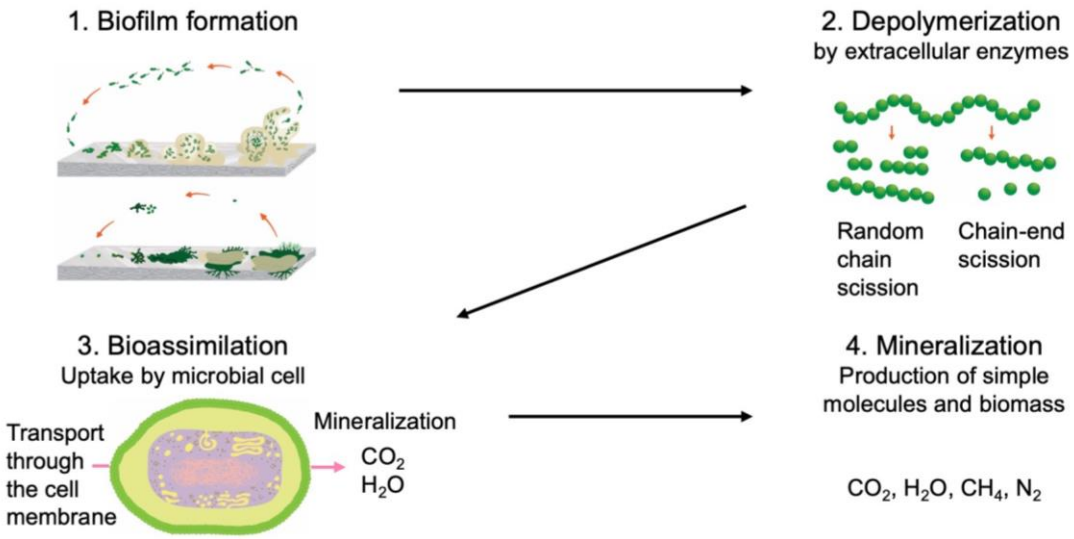


Figure 2.3 Main stages of the biotic degradation process: (1) biofilm formation, (2) depolymerization, (3) bioassimilation, and (4) mineralization. Adapted from [87].

The literature has also described these processes as biodeterioration. Biodeterioration is commonly described as the undesirable degradation of materials by microorganisms, and it is considered the mechanism responsible for causing several irreparable damages. In this review, to avoid any confusion regarding the stages of the biodegradation, we will not use this term to describe any particular process. The term biodeterioration is mostly used to describe a combined mechanism when microorganisms are in contact with a polymer surface and the polymer experiences fouling, degradation of leaching components (as additives), biocorrosion (enzymes and radicals attack additives and polymer backbone), hydration and penetration in the bulk polymer, and changes in color of the polymer matrix [88,89].

2.4.3 Biofilm formation

Biofilm structure and formation have been identified as the dominant phase of life for microorganisms on Earth. Studies have shown that microorganisms, in general, live in aggregates or mixed species rather than as single cells in pure cultures [90,91]. When a biodegradation process occurs, biofilm formation is considered the first step and a necessary

step in the process. However, the formation of a biofilm on a surface does not necessarily imply that biodegradation will occur [89].

In biofilm formation, a microbial community is established on a surface. These surfaces, such as metals, sediments, or polymers, can exist in different forms, have different properties, and different compositions. Biofilms are considered highly sophisticated and complex synergistic structures originated by the selective attachment of phylogenetically and functionally diverse communities of bacteria, fungi, protozoans, or algae [92]. The organization of microorganisms on a surface is specific for the material and dependent on that material's surface properties and the environmental conditions. Biofilms can be developed in solid/liquid and solid/air interfaces [89]. The first step of biofilm formation for bacteria is the microorganism's initial attachment to the surface via the cell pole or the flagellum (within minutes after the first contact with the substrate) (**Figure 2.4a**). The initial attachment is a reversible step. The second step of biofilm formation is the microorganisms' irreversible attachment to the surface using a glue-like substance and tail-like structures. The attached microorganisms start producing slimy extracellular polymeric substances (EPS), formed by proteins, polysaccharides, nucleic acids, lipids, and humic substances, and develop clusters of cells in contact with each other and with the substrate. EPS production allows the microbial community to develop a complex structure highly influenced by the environmental factors, and is the main factor responsible for the adhesion to surfaces and for the integrity of the biofilm [93]. During this second step, the growth of microbial communities can occur in a matter of hours. Biofilm maturation occurs in the third step, when cell clusters embedded in the EPS become mature and layered. A high level of biofilm maturation is achieved as cell clusters and microcolonies reach their maximum average thickness in the fourth step. In the final step, as the maturation of the colonies progresses, the complex structures weaken, detach from the substrate, release, and

propagate. This variable sized group of cells can now attach to a different zone of the surface, or another previously optimally developed biofilm. The detachment step is characterized by cells evacuating from the interior of the clusters, forming void spaces [90,94].

In the case of fungi population, the development of filamentous fungi biofilm has been proposed (Figure 2.4b) [95]. The first step, similar for bacteria biofilm, implies the deposition and adsorption of spores and/or hyphal fragments. The second step implies the development of a fungal EPS for active attachment to the surface. In the third step a microcolony is formed with branching of a monolayer hyphal and extension of the EPS for better adherence of the microcolony to the surface of the substrate. In the fourth step a colony is formed, a hyphal compacted network is developed, and the maturation of the colony occurs. Finally, in the fifth step the dispersal or release of new cells takes place. These new cells can start a new cycle. A detailed discussion of the biofilm formation mechanism can be found elsewhere [96–98].

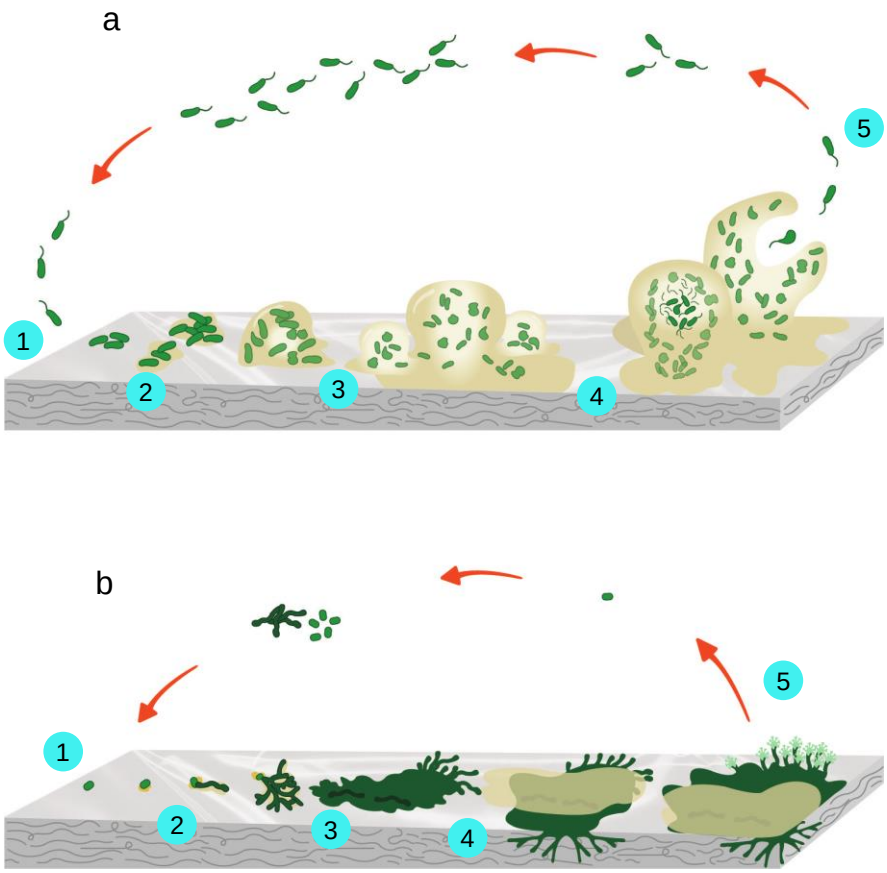


Figure 2.4 a) bacteria biofilm formation, steps: (1) attachment of microorganisms to the surface using a specialized glue-like substance and tail-like structures, (2) colonization, (3) growth, (4) maturation, and (5) detachment; b) Fungi biofilm formation, steps: (1) attachment of microorganisms to the surface using a specialized glue-like substance and tail-like structures, (2) colonization, (3) growth, (4) maturation, and (5) detachment. Adapted from [91,95,99].

Table 2.2 Functions of extracellular polymeric substances (EPS) in biofilm formation. Reproduced with permission from [93].

Function	Relevance for biofilms
Adhesion	Allows the initial steps in the colonization of abiotic and biotic surfaces by planktonic cells, and the long-term attachment of whole biofilms to surfaces
Aggregation of bacterial cells	Enables bridging between cells, the temporary immobilization of bacterial populations, the development of high cell densities and cell-cell recognition

Table 2.2 (cont'd)

Cohesion of biofilms	Forms a hydrated polymer network, mediating the mechanical stability of biofilms, determines biofilm architecture, and allows cell-cell communication
Retention of water	Maintains a highly hydrated microenvironment for biofilm microorganisms, leading to their tolerance of desiccation in water-deficient environments
Sorption of organic compounds	Allows the accumulation of nutrients from the environment and the sorption of xenobiotics
Sorption of inorganic ions	Promotes polysaccharides gel formation, ion exchange, mineral formation, and the accumulation of toxic metal ions
Enzymatic activity	Enables the digestion of exogenous macromolecules for nutrient acquisition and the degradation of structural EPS, allowing the release of cells from biofilms
Nutrient source	Provides a source of carbon, nitrogen, and phosphorus containing compounds for utilization by the biofilm community
Electron donor or acceptor	Allows redox activity in the biofilm matrix
Export of cell components	Releases cellular material as a result of metabolic turnover
Sink for excess energy	Stores excess carbon under unbalanced carbon-to-nitrogen ratios
Binding of enzymes	Results in the accumulation, retention, and stabilization of enzymes through their interaction with polysaccharides

Many of the identified extracellular enzymes, such as oxidoreductases and hydrolases, are responsible for the degradation of biopolymer substrates. Extracellular enzymes can break down substrates available on the EPS or from the surface to which the biofilm is attached; the resulting low molecular mass products are used as a source of carbon and energy by the microorganisms. Hence, enzymes capable of degrading substrates turn the EPS into an external digestive system for the microorganism [93]. Extracellular enzymes are key for the breakdown of water-soluble substrates (e.g., polysaccharides, proteins, and nucleic acids) or water-insoluble substrates (e.g., cellulose, lipids, and bio- or fossil-based polymers chains), leading to depolymerization [93,100].

2.4.4 Depolymerization

The enzymatic activity that occurs after biofilm formation is the main contributor to the depolymerization step. Enzymatic activity can occur via a hydrolytic or oxidative route (**Figure 2.5**), involving either random or end chain scission [53,65].

The oxidative mechanism is called enzymatic oxidation. In the case of non-hydrolysable polymers, due to the absence of hydrolysable groups, redox reactions are the most effective way to break the backbone made of C-C bonds. However, extracellular enzymes must have redox potentials high enough to allow the electron extraction from non-reactive C-H or C-C bonds. A high redox potential requirement could be an important obstacle for ultimate polymer biodegradation [101].

Enzymatic hydrolysis mirrors abiotic chemical hydrolysis. As chemical hydrolysis progresses, the M_w is reduced and consequently the polymer becomes available for enzymatic hydrolysis, which starts dominating the depolymerization step. For hydrolysable polymers, with ester, carbonate or amide groups, the hydrolytic enzymatic degradation by extracellular hydrolases has been reported and is presented and discussed in further section in detail.

Within the major enzyme classes (**Table 2.3**), hydrolases (EC 3) and oxidoreductases (EC 1) are the main groups of enzymes linked to depolymerization.

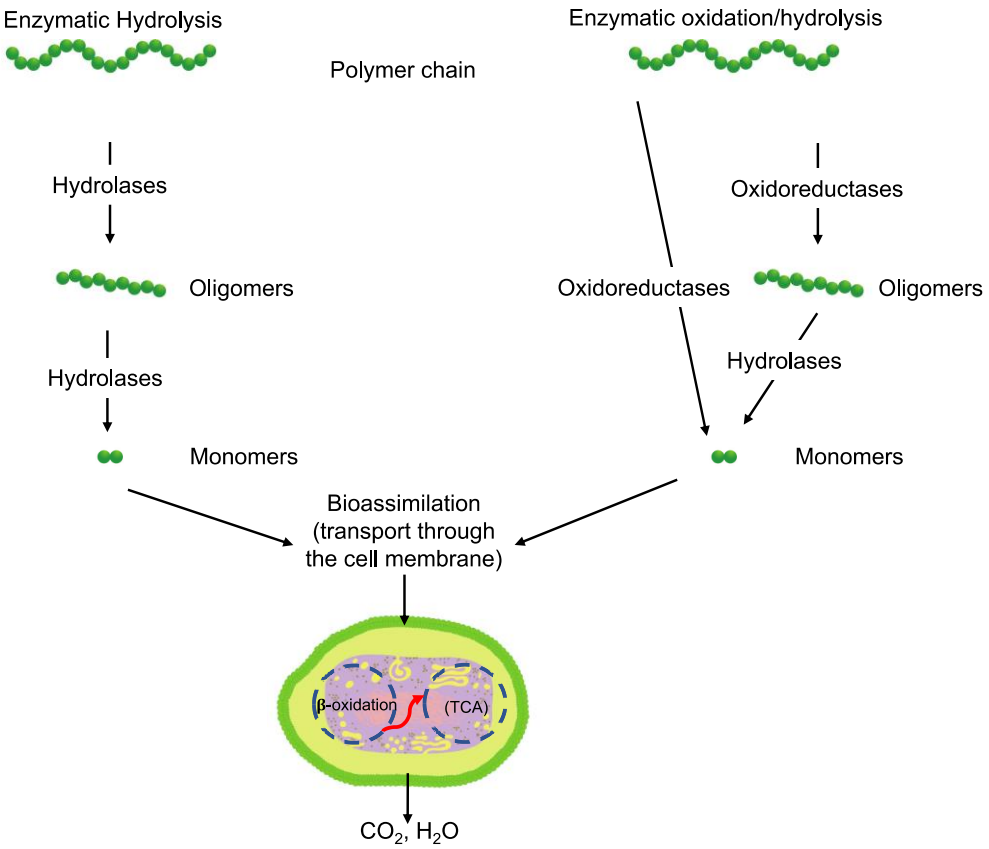


Figure 2.5 Enzymatic hydrolysis or oxidation routes for depolymerization. Adapted from [101].

Table 2.3 International Union of Biochemistry and Molecular Biology (IUBMB) classification of enzymes by function of the reactions they catalyze. Adapted from [102].

EC Number	Enzyme class	Reaction
1	Oxidoreductases	Oxidation-reduction
2	Transferases	Chemical group transfers
3	Hydrolases	Hydrolytic bond cleavages
4	Lyases	Nonhydrolytic bond cleavages
5	Isomerases	Changes in arrangements of atoms in molecules
6	Ligases	Joining together of two or more molecules

The lower activation energy needed for enzymatic hydrolysis of ester linkages, such as those in aliphatic and semi-aromatic polyesters, appears to facilitate the depolymerization

of polyesters in comparison to polyolefins, where non-hydrolysable linkages are present. However, large differences have been reported in the rates of biodegradation for polyesters as a function of their morphology and chemical structure. For example, the aromatic polyester PBT is considered non-biodegradable; however, the copolymer obtained from terephthalic acid and adipic acid, PBAT, is biodegradable. Besides the presence of the aromatic ring in both structures as well as hydrolysable bonds, the presence of the adipic acid component in PBAT improves the flexibility of the polymer structure, making it more susceptible to attack by extracellular enzymes [51].

Enzymes are macromolecules made up mostly of proteins, which are complex chemical structures, with high M_w and hydrophilic groups acting as biocatalysts that accelerate the depolymerization reaction rates by lowering the activation energy of the reaction [103]. The simplest enzymes consist entirely of amino acids while conjugated enzymes contain a non-protein component, a cofactor (or co-enzyme) along with a protein component.

Extracellular enzymes are released when optimal conditions are present between the polymer surface and the attached biofilm. Enzymes bind to a substrate by their active site and transform the substrate into a product. **Figure 2.6** shows the steps of this process. First, an enzyme binds to its substrate and positions it properly in its active site to catalyze the reaction. In the second step, the enzyme-substrate complex is formed. In the third step, the enzyme-substrate complex aligns reactive groups in the substrate and places strain on specific bonds, reducing the activation energy required for making the reaction to occur. In the fourth step, the cleaved products are released. Finally, in the fifth step, the enzyme is ready to begin the catalytic cycle again.

The main factors influencing the susceptibility of a polymer towards microbial attack by extracellular enzymes are:

Enzyme availability. Availability is determined by the type of microorganisms and the environment.

Available sites on the polymer for enzyme attack. Extracellular enzymes are classified as exo- and endo-enzymes. Exo-enzymes are responsible for chain end scission, while endo-enzymes are responsible for random chain scission [104].

Enzyme specificity. Enzymes are known as catalysts of biochemical reactions with high substrate specificity. This means that an enzyme catalyzes a special reaction with high efficiency. Therefore, many different reactions catalyzed by different enzymes can run in parallel simultaneously. The specificity is a function of the three-dimensional structure of the enzyme [104].

Presence of cofactors. Cofactors are additional chemical groups incorporated to the structure of the active site of the enzyme to facilitate a biochemical reaction. Cofactors can be metal ions (e.g., calcium, magnesium, potassium, sodium, or zinc) or co-enzymes (organic cofactors). A common function of cofactors is to provide a geometric place for the substrate to bound to the enzyme by maintaining the stability and activity of the enzyme at the active site [105].

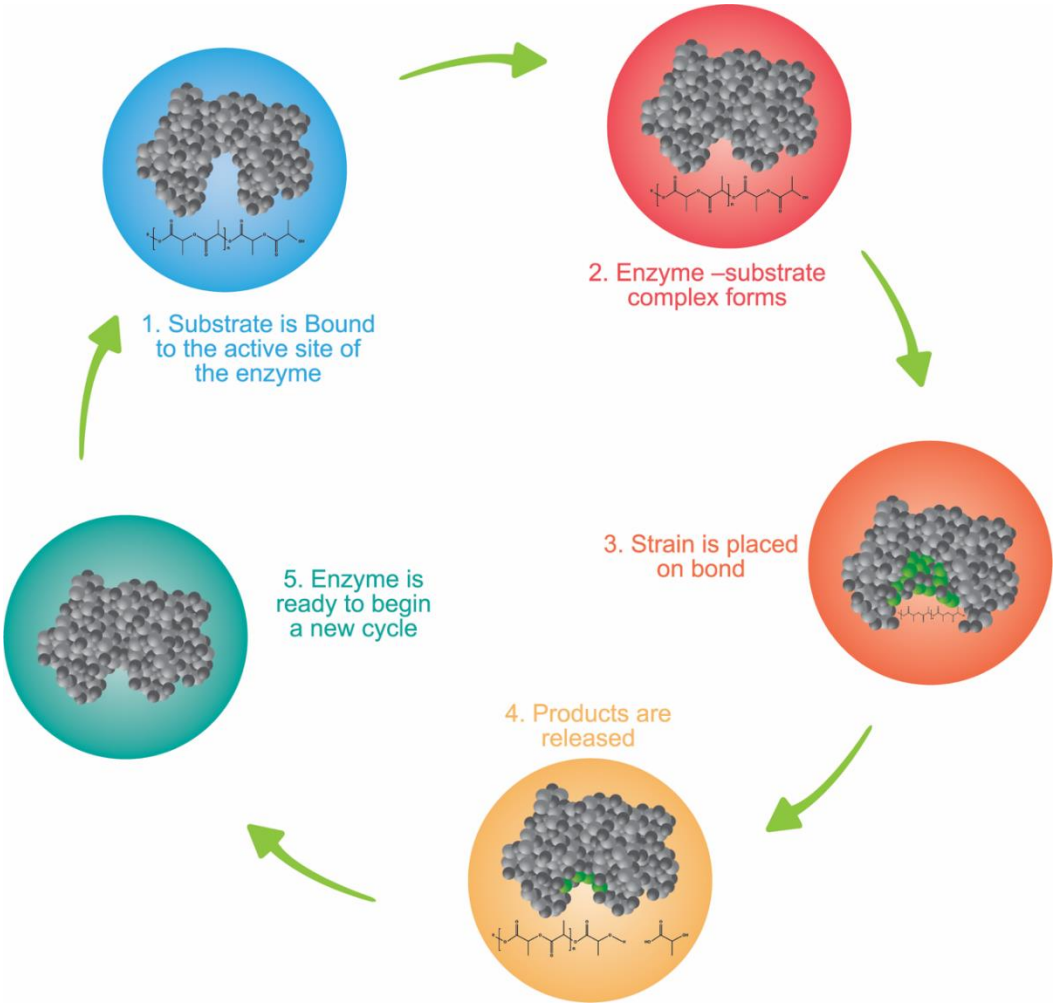


Figure 2.6 Catalytic cycle of an enzyme. Adapted from [86].

The priority of extracellular enzymes is obtaining carbon to ensure a supply of resources. Additionally, the microbial community is able to shift enzyme production between groups of substrate-specific enzymes and non-specific enzymes, to match substrate requirements. In other words, enzymes are selectively produced to increase the supply of the most limiting element and to target the most available substrates [106]. From an energy point of view, enzyme production is energy intensive. For this reason, microorganisms produce enzymes at the expense of growth and metabolism when nutrients are scarce. Furthermore, when available nutrients are scarce, microorganisms can produce adaptive

enzymes to obtain resources from complex sources [107]. On the other hand, when assimilable nutrients are available and abundant, the production of constitutive enzymes may be decreased [108].

For polymer degradation, depolymerases are the extracellular enzymes secreted by microorganisms that cleave complex polymeric substrates into oligomers, dimers, and monomers. The hydrolytic cleavage can be by exo-attack or endo-attack. Exo-attacks occur at the end of the polymer chain, and the byproducts are oligomers or monomers that can be assimilated by the cell. Endo-attacks occur randomly along the polymer chain, reducing the M_w; hence, products are not assimilable without further depolymerization [109,110]. An important characteristic of extracellular enzymes is that they are too large to penetrate deeper into the polymer material. For this reason, enzymes can only act on the polymer surface, making depolymerization by enzymatic activity a surface erosion process [104]. Increasing the surface area can increase the rate of depolymerization by extracellular enzymes [111]. Fragments small enough to go through the membrane cells as monomers are transported inside the cell and transformed to obtain energy for the growth process by the action of intracellular enzymes. Usually these are oxidative enzymes, and the process is called bioassimilation or assimilation.

2.4.5 Bioassimilation

Bioassimilation is related to the acquisition or uptake of substances for the microbial metabolic process. Compounds small enough to pass the semi-permeable membrane after the depolymerization step can be potentially processed by the metabolism of the microorganism and finally mineralized to CO_2 (dissimilation) or be used for biosynthesis of new products through metabolic pathways (assimilation) (**Figure 2.7**). In general, the periplasmic space – the cell membrane – is where the cleavage takes place, and from where oligomers can be

transported across the cytoplasmic membrane for further oxidation in the β-oxidation cycle. Oligomers can be internalized with the aid of surfactants produced by microorganisms during biofilm formation and be used as carbon and energy sources by the action of intracellular enzymes. The presence of water for the transport of components is a critical factor during the bioassimilation step [86].

2.4.6 Mineralization

Mineralization, or ultimate biodegradation, refers to the degradation of polymer fragments to the mineralized components and biomass, plus CO_2 and H_2O in aerobic conditions or CO_2, H_2O, and CH_4 in anaerobic conditions (**Figure 2.7**). Depending on the polymer composition, other compounds also can be released, including sulphide, sulphate or sulphite, ammonia, nitrite or nitrate, phosphate or phosphite, chloride, and fluoride. By measuring the mineralization levels (*i.e.*, CO_2 released or evolved), biodegradation rates and % mineralization can be quantified. Bioassimilated monomers are part of the catabolism cycle. During this step, organic compounds, such as carbohydrates and proteins, are used as metabolites of the tricarboxylic (TCA) cycle or Krebs cycle by aerobic and anaerobic respiring microorganisms to produce energy [93,112]. Insights on external factors affecting mineralization of biodegradable polymers can be found elsewhere [113].

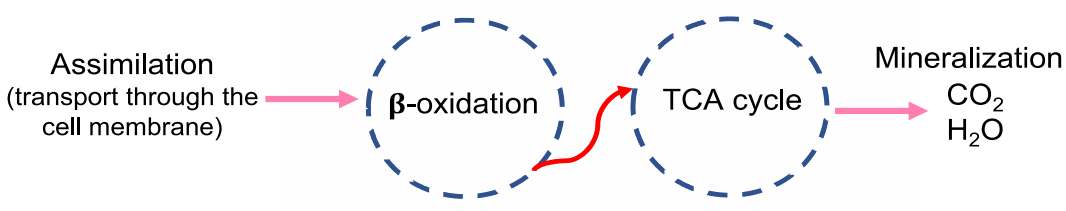


Figure 2.7 Microbial bioassimilation and mineralization during the polymer biodegradation process. Adapted from [114].

2.5 Biodegradation environments

There are many feasible waste management recovery processes for polymers, ranging from recycling, biodegradation included as defined by the U.S. Environmental Protection Agency (EPA), waste-to-energy conversion, and landfill, each with trade-off environmental impacts. Littering or leakage to the environment must not be considered waste management processes. Each of these waste management environments present specific conditions that can tailor the degradation rate of polymers. So, evaluation of degradation of a polymer in different environments may reveal different rates due to the influence of external abiotic and biotic factors [115–117].

Heat (temperature) and other factors in the environment, such as aeration, acidic/alkaline media, and water content, play crucial roles in how degradation of polymers takes place. Temperature is one fundamental parameter affecting the rate of degradation and has been studied in detail [76,118–120]. Besides affecting the abiotic mechanisms of degradation, temperature drives chemical and microbial changes through the different phases of biodegradation. Microbial activity is temperature dependent since each microorganism's optimal population and growth is driven by different temperature regimes. Marine environments and some lakes and rivers are associated with temperatures in the psychrophilic range (0–20 °C); however, depending on the geographical region, rivers and lakes can also reach the mesophilic range (20–45 °C). Soil and home composting environments are mostly in the mesophilic range, whereas composting under industrial conditions is mostly conducted in the thermophilic range (45–60 °C). **Table 2.4** presents a summary of the temperature ranges and the main environments where biodegradation occurs.

Table 2.4 Typical temperature ranges and conditions for different environments where polymers can be subjected to aerobic biodegradation.

Temperature range, °C	Environment	General description	Management
20–30	Soil/Agricultural soils	Large scale. Soil structure (texture, porosity), moisture, aeration, radiation	Uncontrolled
20–45	Home composting	Small scale. C/N ratio, moisture, aeration, heat, pH	Controlled
45–60	Industrial composting	Medium scale. C/N ratio, moisture, aeration, pH	Controlled

2.5.1 Soil environments

Soils provide diverse environments where the biodegradation of polymers can take place. Soil is a typical disposal scenario for biodegradable and non-biodegradable polymers employed as agricultural mulch films [121,122]. For several decades, non-biodegradable fossil-based polymers, such as PE, have been employed as mulch films for crops. However, in the last 15 years, bio-based and fossil-based biodegradable polymers have gained market momentum since their use can avoid the removal of the plastic film after harvest and reduces the leakage of plastic debris [123,124]. The study of plastic use in agriculture is known as "plasticulture" and includes products such as drip irrigation tape, greenhouse covers, hoop-house covers, silage bags, row covers, hay bale wraps, plastic trays and pots, and mulch films [125,126].

Soil is a diverse and typical habitat for microorganisms [127,128], and biodegradation usually takes place in the mesophilic range of temperature. Biodegradation of polymers in soil is affected by both biotic and abiotic components such as microorganisms, solid particles (i.e., organic matter and inorganic minerals), water, and air. Solid, liquid, and gaseous phases make up the soil environment, along with different organisms. The liquid and gaseous

phases, water and air, vary with the climatic conditions and human activity, whereas the solid phase generally is resistant to these activities [127]. The main parameters used to classify a soil are based on its granularity and porosity due to the amount of clay, sand, and silt (**Figure 2.8**). The texture and structure of soils is determined by the relative proportions of clay, sand, and silt and their relative sizes [129,130]. Silty soils can possess high water retention capacity, but clay soils possess the highest water retention capacity. Hence, fungal populations can be supported by a dry, sandy, well-ventilated soil, whereas an insufficiently aerated clay soil provides habitat for facultative bacteria [131].

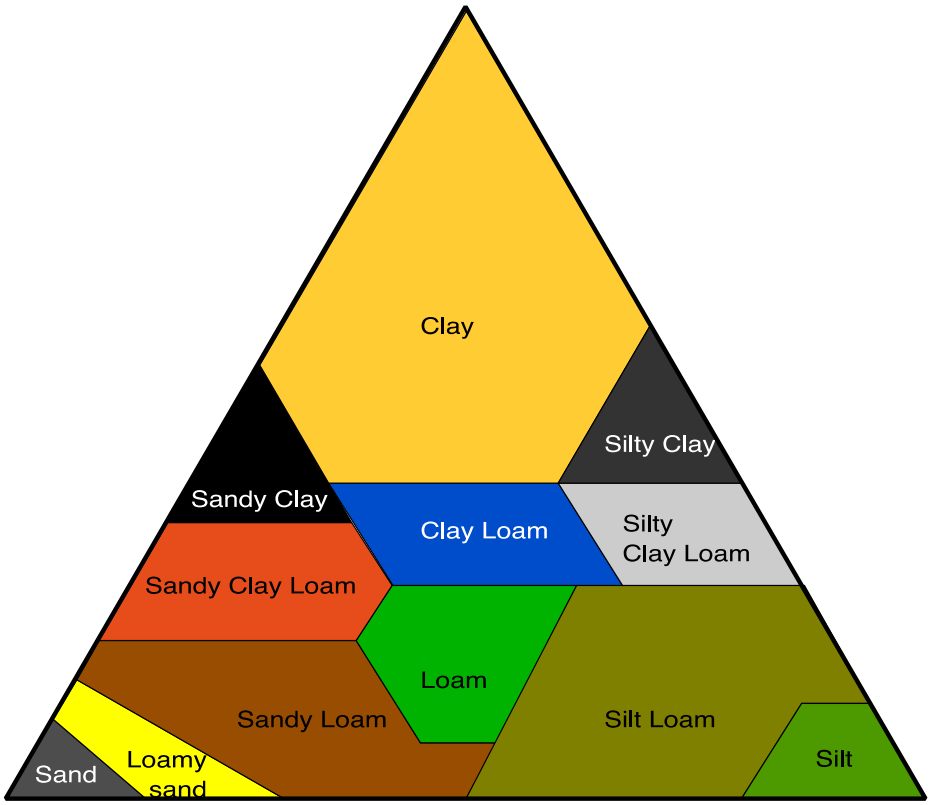


Figure 2.8 USDA soil texture classes determined according to the relative proportions of sand, clay, and silt. Adapted from [129,132].

The chemical and biological properties of soils are characterized by acidic/alkaline media, cation exchange capacity, organic carbon concentration, and soil respiration [133]. These properties control the formation and activity of the microbial diversity, and the

combination of the mentioned factors create habitats where only certain microorganisms can grow [70]. The distribution of the different particles creates pores of different sizes that can retain water or surrounding living organic material. The soil connectivity determines the circulation of nutrients, soluble organic compounds, and water and is ultimately tied to the pore geometry and network [134]. Thus, the size of the pores is a factor that determines and helps to explain the spatial separation of living organisms [127].

The biodegradation process occurring in soil environments should consider the surface layer and underground matrix [135]. The surface layer of the soil is highly affected by abiotic factors. On the other hand, the underground matrix is associated with the microbial population and factors for its optimal activity [70]. The factors playing major roles in the biodegradation process in soil are soil texture and structure, water content, organic matter, pH, temperature, O_2, and sunlight [131].

Water content and retention is a function of the texture and structure of the soil, as discussed above. While a dry soil encourages the formation of fungal populations, a wet soil promotes the genesis of bacterial populations [131]. Fungi spread through the soil using hyphae, which are thin filaments forming the mycorrhizal network. Under dry conditions, while in search of water and nutrients, the hyphae spread and take different routes. The fungi continue enlarging this network and bridge the gaps between different small pockets of water and nutrients, thus enabling survival and growth in soil, where the moisture content may be low [136].

Microorganisms can adapt to specific ranges of pH values. Thus, the soil pH is a factor that can limit the growth of microorganisms. Alkaline to neutral pH favors bacterial growth whereas acid pH favors fungal development [131]. The pH influences the availability of nutrients and concentrations of trace metals such as zinc, iron, calcium, magnesium, and phosphorus. Fungi take in these molecules across their membranes by creating a proton

gradient; this proton gradient affects the ability to take up the nutrients when exposed to extreme pH conditions [137]. In acidic media, certain nutrients, such as phosphorus, become less available and other nutrients like magnesium and aluminum can become more toxic, thus creating a hostile environment for helpful soil bacteria.

The O_2 content of the medium determines whether the microbial population expressed is aerobic or anaerobic. Soil temperature governs the physical, chemical, and biological processes in the soil. Changes in soil respiration rate, due to the fluctuation in temperatures also affect the bioactivity. Microbial activity is inhibited or reduced drastically with lowering temperatures [125]. Radiation, mostly from UV light, can inhibit the growth of microbial populations, depending on the intensity of the radiation. The optimal conditions of temperature, organic matter, aeration and O_2, and water content are in the first 30 cm of the soil layer [129,135].

Agricultural soils can be considered as a particular type of soil environment, and have been extensively studied in the plasticulture field [123,126]. One of the most studied applications has been polymeric mulch films, which undergo several steps in biodegradation. This process involves a period of intense photodegradation when the mulch film starts crosslinking and eroding, followed by an intensive period of biodegradation [125,138–140]. **Figure 2.9** shows a typical life cycle of polymeric mulch films in agriculture soils.

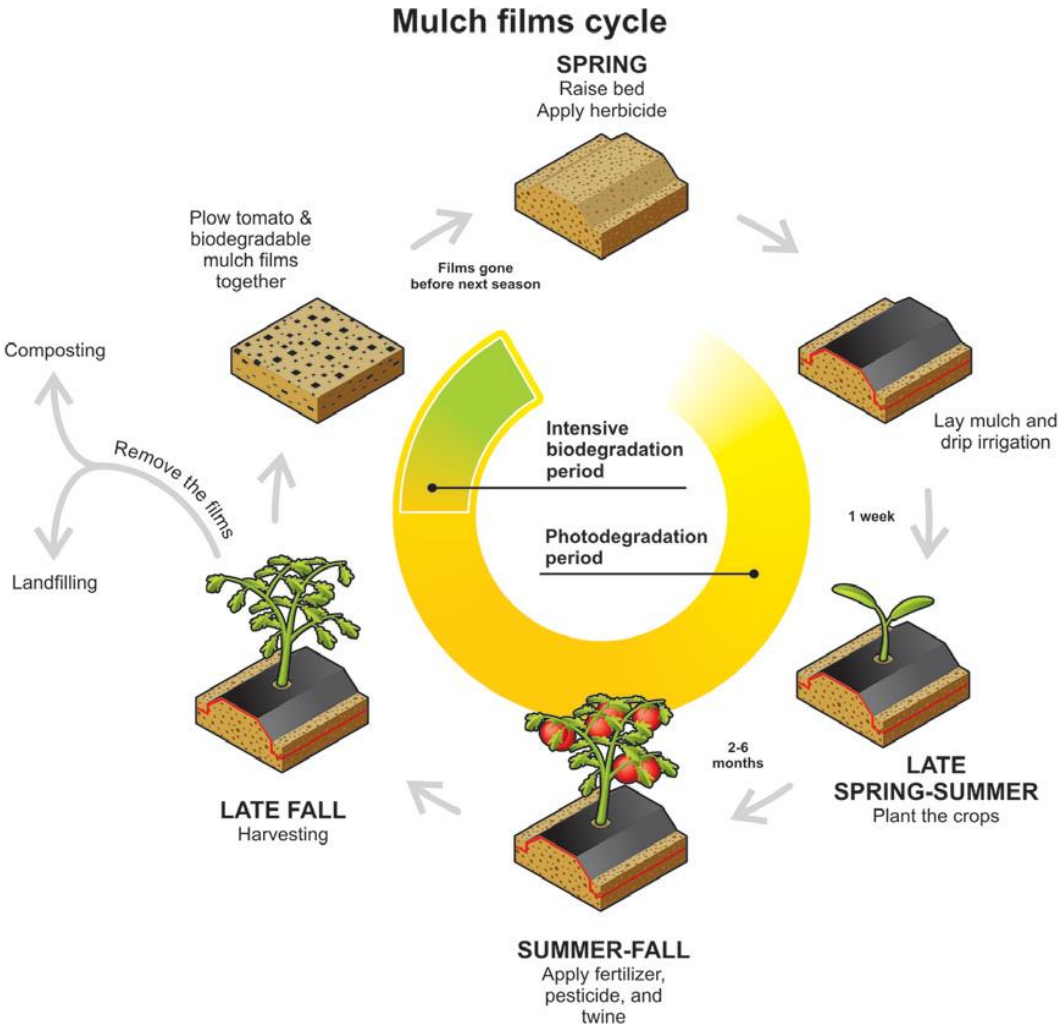


Figure 2.9 Biodegradable mulch film cycle, starting from raising the bed and applying herbicide in spring, to harvesting and the disposal of the films in late fall, and the associated degradation processes. [141] (Copyright 2008. Reproduced with permission from Elsevier Science Ltd.).

2.5.2 Home and industrial composting

Home composting could become a common disposal scenario for polymers and polymer blends used for packaging (food and beverages) [142]. Home composting is garnering interest since it can be very instrumental in diverting the household organic fraction from going to landfill [143]. Additionally, as consumers are becoming more aware of plastic pollution, home composting has also become important as a potential methodology to reduce organic waste and contaminated packages that cannot be efficiently recovered or diverted through the MSW

management system. The US Composting Council describes home composting as the natural aerobic decomposition of organic wastes or materials, usually in small-scale composters by "slow-stack" treatment methods where temperatures are in the psychrophilic (0–20 °C) to mesophilic (20–45 °C) range [144]. As per the US Composting Council, home composting can also be labeled as "backyard" or "composting at home". However, the terminology varies in different geographical regions worldwide since "composting at home" may imply composting in designed vessels inside the apartment or house [145,146], and "backyard" composting may refer to uncontrolled composting units outside the house subject to the environmental conditions.

The typical matrix for home composting includes biowastes, which are generated in the kitchen, and garden waste such as weeds and leaves (**Figure 2.10**). Many key factors, such as temperature, pH, moisture, composter efficiency, substrate, C/N ratio, and microbial populations, affect the home and industrial composting process [147,148]. Home composting is a far less controlled process in comparison to industrial composting, since the process takes place in the backyard and hence on a smaller scale. Usually, it never reaches the high temperatures in the thermophilic range for long periods of time, as seen in industrial composting. The small size of the installation, accompanied with difficulties in reaching an optimum control of factors, result in home composting requiring a longer time to achieve a mature compost [131]. The material volumes that can be handled and the abundance of microorganisms are lower for home composting settings. In addition, seasonal changes can influence "backyard composting" depending on the geographical location, and hence lower and more variable temperatures are inevitable.

Regardless of whether the composting is done at home, in a community backyard, or in an industrial facility, the composting process must ensure a succession of microbial communities (i.e., mesophiles–thermophiles–mesophiles) and the corresponding temperature

regimes to operate. These factors are necessary to guarantee the safety and quality of the compost process and the final product.

One advantage of home composting is that it can be helpful in rural and suburban areas where collection of organics is limited or there is no infrastructure for industrial composting [143,149,150].

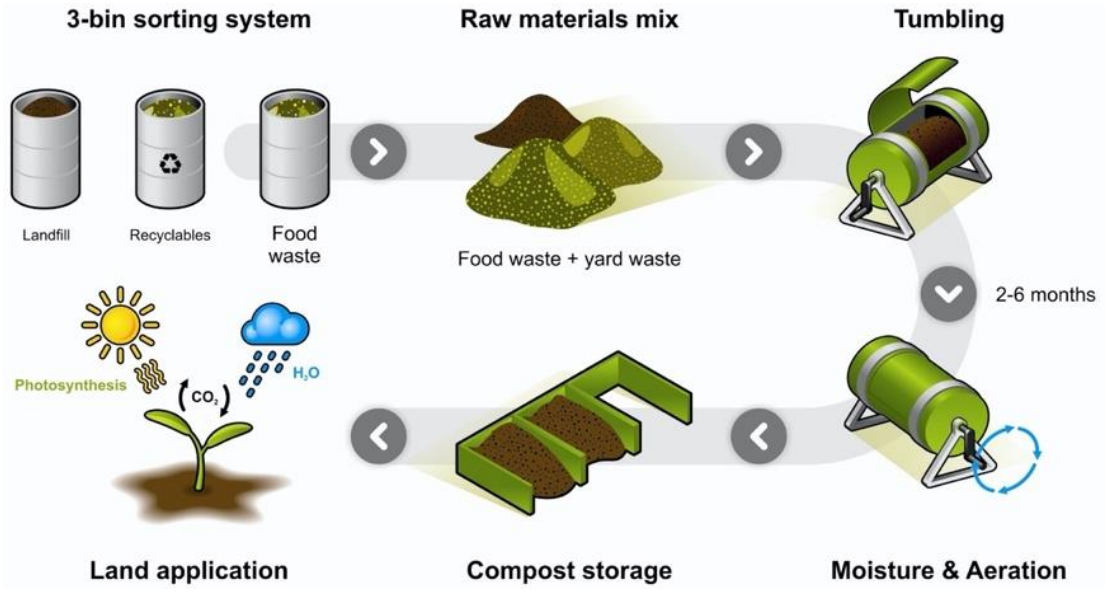


Figure 2.10 Home composting representation. [60] (Copyright 2008. Reproduced/adapted with permission from Wiley & Sons, Ltd.).

Industrial composting is a process designed to handle large volumes of yard, food, and manure waste [60,148,151]. By employing better aeration, moisture control, and higher temperatures the biodegradation in industrial composting is accelerated significantly in comparison to natural and home composting processes. The industrial composting process requires a proper system in place for collection of wastes and a good infrastructure (e.g., windrow, aerated static piles, and in-vessel composting) [152]. Biodegradation in industrial composting takes place mostly in the thermophilic temperature range. **Figure 2.11** shows a representation of an industrial composting process.

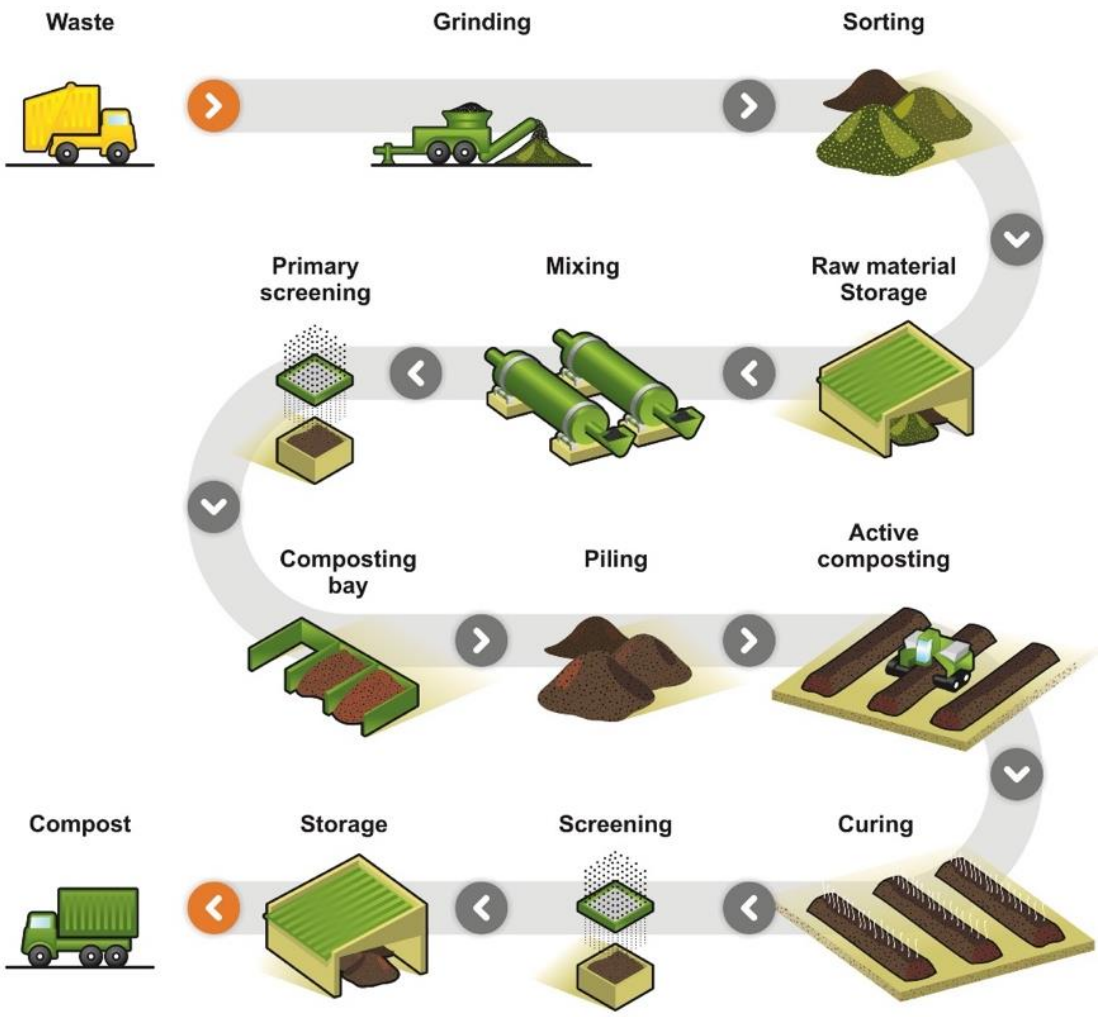


Figure 2.11 Industrial composting process. [60] (Copyright 2008. Reproduced with permission from Wiley & Sons, Ltd.).

The industrial composting process follows four main stages. The first stage is the mesophilic stage (20–45°C), where microorganisms decompose the simplest organic, degradable substances into CO_2 and water in an exothermic reaction. The high amount of substrate ensures high microbial activity, which leads to the generation of large quantities of metabolic heat energy that causes the temperature to rise swiftly. The second stage is the thermophilic stage (45–60 °C) where bacteria and fungi mesophiles become less active and are replaced by thermophiles. As the temperature rises above 55 °C, microorganisms such as pathogens are destroyed. For safety reasons, several certifying entities require that the

temperature must reach above a certain temperature, such as 55 °C, and remain at that level for a set period of time, such as 15 days, to ensure that the resulting compost is pathogen free [153]. Temperatures in some industrial compost facilities during the early stages commonly reach values of c. 70 °C [154]. Such high temperatures expedite the disintegration process of high energy carbohydrates and structurally complex molecules. As the disintegration process comes to an end, there is no longer any supply of these high energy compounds, and the third stage kicks into action where the mesophiles take over once again. The third stage is a transition stage from high to low temperature. The final stage, also called "curing" or maturation, can take several months to result in stabilized compost [131]. The total composting time varies in systems used worldwide, from two to more than six months; thus, certified compostable packages can encounter difficulties to fully disintegrate in some operations [155].

2.5.3 Aquatic environment

Natural aquatic environments (i.e., oceans, rivers, and lakes) unfortunately are environments where discarded polymers from activities such as fishing and shoreline recreation are commonly found [11,16,156]; however, these are not formal waste management scenarios and must not be considered as such. The natural aquatic environment is a non-desired end-of-life scenario due to the creation of white pollution and a lack of proper conditions for biodegradation and control of the process due to its complexity [157]. Biodegradation in the aquatic environment can happen in lakes, rivers, and oceans as well as in reservoirs, and wastewater facility treatments (aerobic or anaerobic); however, our discussion is focused on the natural aquatic environments.

Geographical considerations of the aquatic environment play an important role in understanding the presence and flow of plastics. Lakes are generally low-flow environments and act as a point for accumulation for plastics and microplastics [158]. Rivers are considered

the essential route for transporting plastics to the ocean. Considering their proximity to urban and industrial areas, rivers become an easy access point to the marine environment with respect to plastic pollution. Plastics are extensively carried out during floods in cities with poor waste management systems [12]. For the marine environment (seawater), three main habitats can be considered when addressing the degradation of plastics (**Figure 2.12**): the pelagic zone, an illuminated and aerated column of water; the littoral zone, which is the beach sediment periodically covered by water due to waves or tide; and the sublittoral zone, which is the seabed interface up to 200 meters in depth that is aerated and photosynthetically active. The physical and chemical properties of seawater, including the essential nutrients for living organisms, vary with the depth, latitude, and proximity to land. Because of this variation, the microbial populations within seawater also vary. Furthermore, the degradation process of the plastics entering this environment can be altered by agitation and turbulence caused by ocean currents, salinity, temperature gradients, and solar radiation among others [159,160]. Biodegradation of polymers in aquatic environments is described in terms of scarce evolution for synthetic biodegradable polymers. However, high efficiency has been reported for natural polymers as cellulose, starch, and PHAs regardless of the low temperatures reached (**Table 2.9**). The "plastisphere," the development of biofilms on the surface of polymers present in water, has been extensively studied to elucidate the main components and behavior of microorganisms during the colonization and depolymerization of polymers in aquatic environments [161–163].

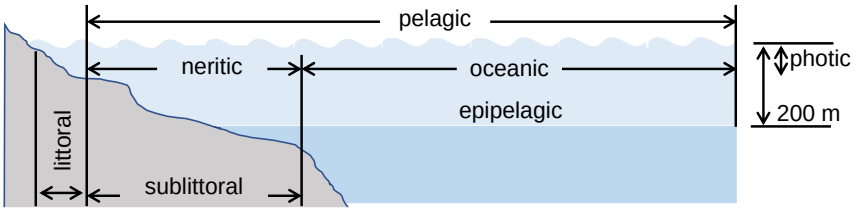


Figure 2.12 Main habitats to consider when addressing the degradation of polymers in the marine environment. The pelagic zone, an illuminated and aerated column of water; the littoral zone, which is the beach sediment periodically covered by water due to waves or tide; and the sublittoral zone, which is the seabed interface up to 200 meters in depth that is aerated and photosynthetically active. Adapted from [164].

2.6 Factors and properties that affect degradation rate

The rate of polymer degradation is affected by the degradation mechanisms, the environments, and the polymer properties. This framework creates a complex interplay governing what is reflected in the rate and efficiency of the whole degradation process.

In the next subsections, we selectively provide a discussion of factors important for mesophilic biodegradation and correlate these factors to the information already provided about mechanisms and environments. Detailed discussions of these factors are also provided in selected reviews [48,50,53].

2.7 Environmental factors

Factors that can affect the degradation rate of a polymer are related to the environment where the degradation process takes place, and include thermal energy (heat), acidic/alkaline media, moisture, aeration, and microbial populations. Some of these factors are more relevant or critical than others and are important during the abiotic and biotic degradation stages affecting both the polymer's properties and the microbial activity.

2.7.1 Heat

The amount of thermal energy, identified as the temperature of the system, is one of the main factors affecting the rate of abiotic and biotic degradation mechanisms and varies

with the environment (**Table 2.4**). In this section, we are not expanding on the thermal degradation mechanism; instead, we briefly discuss temperature as a factor that can modify the rate of other mechanisms such as chemical hydrolysis and microbial activity. At an early stage in the degradation process, mechanisms such as chemical hydrolysis can be dominant and the temperature plays a crucial role on the rate [64,165]. For example, for PLA, the chemical hydrolytic degradation is dependent on the temperature since a large initial reduction of the M_w is needed before microorganisms can assimilate the byproducts [64]. Higher temperatures activate chain mobility, increasing free volume and polymer rearrangements. If the temperature is higher than the T_g of the polymer, mobility and reaction are accelerated, increasing the rate of polymer degradation (**Table 2.5**). Furthermore, the presence and potential growth of different microorganisms depends on the environment temperature, and a change in temperature regulates both presence and activity [86,131]. Biodegradation rate is a function of temperature, mostly described by the Arrhenius equation above and below T_g [120].

2.7.2 Moisture

The presence of water plays a crucial role in the degradation of hydrolysable chemical bonds, such as in polyesters, since they are susceptible to chain scission reactions [166,167]. Furthermore, microorganisms need water for transport of nutrients through the cell membrane and for growth. The amount of water in the different environments, such as soil and home or industrial composting, can create different surroundings for the microorganisms. Low levels of moisture can lead to dry environments with low biological activity [168]. High values of moisture will lead to loss of the porosity of the matrix (soil or compost), turning the process into one with anaerobic conditions [169]. Pore spaces are essential for the normal air flow and aerobic regimen; the optimal humidity range for microbial activity is a function of the percentage of pore space needed that does not obstruct

the air flow required for the microbial activity [170]. For example, for the composting process, an optimal range of moisture content is 45 to 65% [148].

2.7.3 Acidic and alkaline media

Acidic or alkaline media can modify the rate of reactions and the mechanism of hydrolytic degradation [64]. For example, for PLA in acidic conditions the hydrolysis proceeds via a chain-end scission, while in alkaline solution the hydrolysis takes place via backbiting [64]. In the case of PCL films evaluated at extreme pH values (1 and 13) at 37 °C, different behavior was observed for reduction of M_w and crystallinity, suggesting a surface erosion process in alkaline media and bulk erosion in acidic media [171]. During the biodegradation process, pH values close to neutral are highly favorable for the growth of microbial populations. In soil environments, a pH range close to alkaline-neutral values is favorable for bacteria populations, whereas fungi are more tolerant to acidic and alkaline media; fluctuations of pH are considered a harmful situation for living organisms [131,172].

2.7.4 Light and UV radiation

If sufficient energy is absorbed by light and UV radiation, polymers can be subjected to photodegradation, experiencing changes in their chemical structure and physical properties. Light and UV radiation is important in agricultural soils and aquatic environments. So, photodegradation can be the precursor of the degradation process before microorganisms can use byproducts [82,140].

2.7.5 C/N ratio

Microorganisms need carbon as a source of energy and nitrogen for synthesis of amino acids, proteins and nucleic acids [148]. The C/N ratio is a key parameter in environments such as compost and soil. Optimal values for the C/N ratio in compost and soil are in the range of 15:1 to 30:1. During the active aerobic phase of breakdown, microorganisms use around 30 parts of carbon for each part of nitrogen, due to the high energy requirement. If

carbon levels are higher, microorganisms need to undergo several life cycles to oxidize the excess carbon, slowing down the biodegradation process. If carbon levels are low, microorganisms do not have sufficient energy source to survive [148].

2.7.6 Oxygen flow and porosity

Aeration and porosity are key factors for the normal activity of the microbial population in soil and compost environments. To maintain aerobic conditions the porosity should allow O_2 concentrations of around 5%. Porosity is highly correlated with the air flow within a matrix. Low porosity hinders air flow, whereas high porosity can lead to excessive aeration and low water retention capacity. The shape, size, and structure of particles of the matrix (soil or compost) affects its texture. Therefore, a tight packing arrangement reduces the porosity and the compressed matrix impacts the air flow [148].

2.8 Polymer properties

The factors affecting degradation associated with the bulk polymer matrix can be categorized as chemical structure and physical properties such as morphology, crystallinity, constitutional unit, flexibility, crosslinking, M_w, tacticity, density, shape, and polarity. The surface properties affecting degradation are related mostly to hydrophobic/hydrophilic ratio, roughness, surface energy, and available surface area.

2.8.1 Bulk properties

Chain flexibility. A polymer chain that is highly flexible is more accessible to attack by microorganisms. Longer aliphatic chains can exhibit high biodegradation rates. However, aromatic rings can act as obstacles, providing steric hindrance to the enzyme attacking the ester bonds, thereby lowering the rate of biodegradation [70]. During the depolymerization step, enzyme binding is favored by high flexibility of the polymer chains. In this sense, it is aptly recognized that microorganisms are more likely to start the biodegradation process in the amorphous region of the polymer [48,49]. Polymers with T_g values in or below the

mesophilic range, such as PCL, PBS, PBAT, PHAs, and PGA, will be more flexible in favoring chemical and enzymatic hydrolysis in the mesophilic range (see **Table 2.6**). Flexibility and mobility are enhanced by copolymerization, blending, or by increasing the temperature, and are reduced by crystalline domains [80,173].

Table 2.6 Polymer structure and thermal properties (T_g,T_m) of the biodegradable polymers discussed in this work.

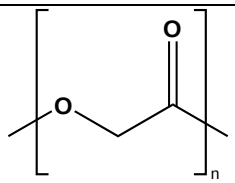
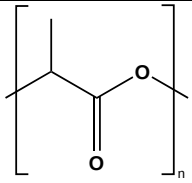
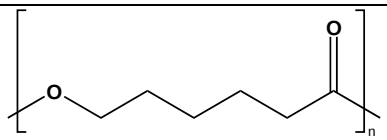
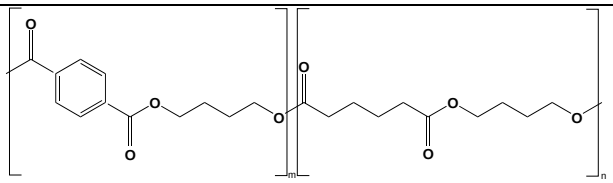
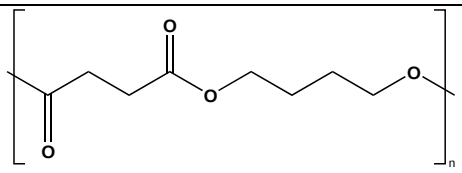
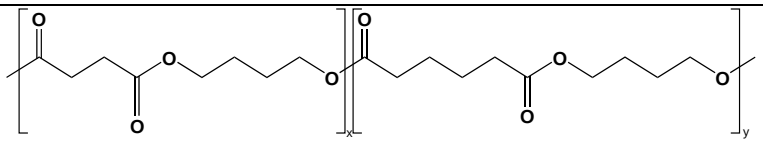
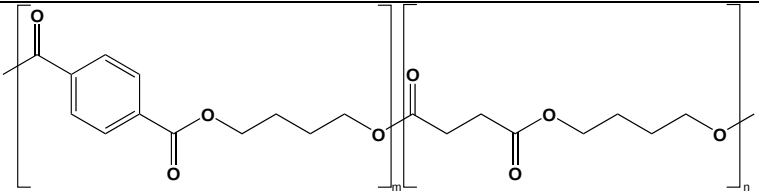
Polymer	Structure	T_g (°C)	T_m (°C)
PGA		35–40	220–230
PLA		55–65	170–200
PCL		-60	58–63
PBAT		-30	106
PBS		-28 to -32	112–114
PBSA		-43 to -45	95
PBST		-20 to -30	~179

Table 2.6 (cont'd)

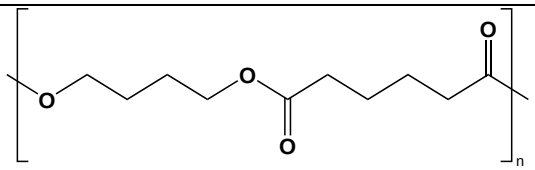
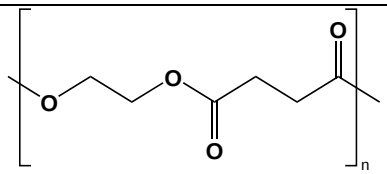
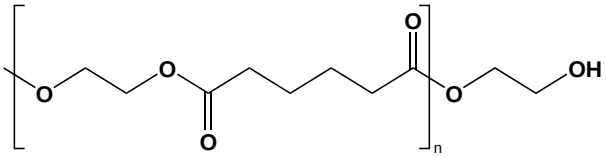
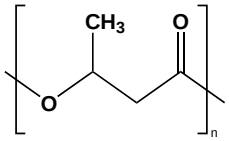
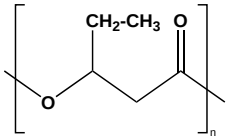
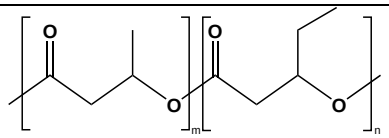
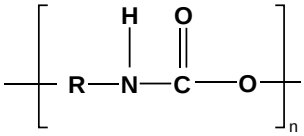
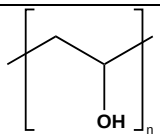
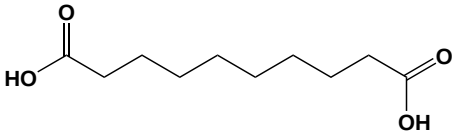
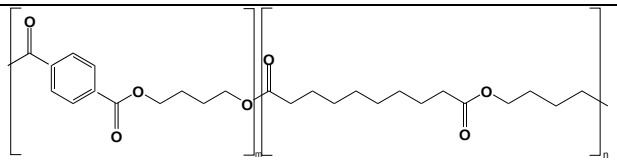
PBA		-61 to -64	41–61
PES		-9 to -17	96–105
PEA		-46 to -50	48
PHB		4	180
PHV		-10	100–200
PHBV		-8 to -1	180
PU		-63	-
PVOH		-	-
PBSe		-62	65

Table 2.6 (cont'd)

PBSeT		~ -43	25 to 91

Chemical structure (functional units and functional groups). Chemical structure is an inherent property of a material and determines whether the polymer is prone to undergo biodegradation. The chemical structure depicts the spatial arrangement of chemical bonds and atoms in the molecule influencing the molecular geometry and governs how the molecules are packed together allowing the formation of crystalline or amorphous regions. The presence of bulky groups in the main chain, such as aromatic rings, restricts the free movement of the polymer molecule, reducing chain flexibility such as in PBT. However, when the linear copolymer of adipic acid and 1,4-butanediol is added to the main chain of PBT to obtain PBAT, polymer flexibility improves and susceptible hydrolysable bonds are introduced, so the polymer is more flexible and prone to biodegradation [81]. Modifications such as inclusion of functional groups by copolymerization in the main chain of initially non-biodegradable chemical structures can make a polymer more prone to biodegradation [56]. The addition of functional groups also can impart a hydrophilic nature to a hydrophobic polymer thus improving its likelihood of undergoing biodegradation [109].

Chain structure configuration (side chains and crosslinking). The length of side chains influences the degradation process. For example, Li et al. [174] concluded that the enzymatic degradation of PHA was dependent on the length of side chain in the PHA structure. Crosslinking can occur and play a significant role in polymer mass transfer properties and chain flexibility hindering biodegradation. Kijchavengkul et al. demonstrated that increasing the amount of crosslinking reduces the biodegradation of PBAT [175].

Crystallinity. Crystallinity can increase the stiffness and density of a polymer [48]. A high crystalline fraction decreases the abiotic and biotic degradation rates. The amorphous region is more susceptible to chemical hydrolysis due to the ease of water diffusion. A characteristic of the crystalline region is its low mass transfer to gases and vapors, decreasing the rate of the hydrolytic degradation [176,177]. Extracellular enzymes mainly attack the amorphous region of the polymer structure [104,178]. Biodegradable polymers are, in general, semicrystalline polymers with a crystalline and amorphous region.

Molecular weight (M_w). To obtain polymers with usable thermal, mechanical, and barrier properties a high M_w is required. However, microorganisms assimilate polymers when selected thresholds of low M_w fractions of the polymer are reached. The higher the M_w value of the polymer residue, the harder it is for microorganisms to assimilate the chain segments and assimilate that to their cell, which reduces the rate of the biodegradation. So, a critical threshold low M_w value must be reached to kick off the degradation by enzymatic attack [70]. Generally, this M_w is attainable by a precursor degradation mechanism such as photodegradation or chemical hydrolysis, as with polyesters. In the case of PLA, the polymer first undergoes primarily chemical hydrolysis, accelerated under industrial composting conditions, until reaching a $M_w \leq 10$ kDa, and then enzymatic activity becomes the dominant degradation mechanism, with a high mineralization rate [113].

Density and porosity. Denser and more compact polymers have lower chances to experience water diffusion. For polyesters, chemical hydrolysis is generally the initial trigger mechanism of degradation, mostly through a bulk erosion process, so water diffusivity of the polymer plays a crucial role. One way to modify the diffusion or the hydrophilicity of a polymer matrix is by blending different polymers. So, biodegradable blends and copolymers

can be used to tailor some of these bulk properties. Blends of PLA and TPS have shown higher biodegradation rates [179].

2.8.2 Surface properties

Hydrophobic/hydrophilic ratio, surface roughness, surface energy, and surface/volume ratio are the more relevant factors during the degradation process. Chemical hydrolysis is highly affected by the hydrophobic/hydrophilic ratio of the polymer surface. Furthermore, enzyme activity, biofilm formation, and colonization are also linked to surface properties.

Hydrophobic/hydrophilic ratio. In the case of isotropic polymers, surface and bulk water sensitivity plays a major role in the degradation process. Hydrophobic surfaces will not allow water to be adsorbed and will delay water uptake, so that any degradation mechanism triggered by water diffusion will be delayed. **Table 2.7** shows that polymers with hydrophobic surface and high-water diffusion, such as the polyester PLA, mostly degrade under a bulk degradation process [63]. So, by tailoring the surface and bulk hydrophobicity and the water diffusion of the polymer matrix, the overall chemical hydrolysis can be controlled, as shown for PLA [166]. In terms of enzymatic activity, a hydrophobic/hydrophilic balance allows the presence of necessary water for optimal microbial activity [162]. Some studies have demonstrated that biofilms develop faster on hydrophobic nonpolar surfaces [180]. However, Tsuji et al. reported an alkaline treatment to increase the hydrophilicity of PLLA and PCL to improve enzymatic attack. The effect was important for PLLA films, where enzymatic attack by Proteinase K was higher on hydrophilic surfaces [181,182]; however, the attack by lipases on PCL films remained unchanged [182]. The fact that lipases need a hydrophobic surface to be active could be an important conditioning of the scarce activity on PCL films. Furthermore, the exposure to hydrophobic surfaces has been reported to be a relevant signal for the production of extracellular enzyme cutinases by fungi to act on the surface of polyesters such as PCL, PBS, and PBSA, among others [183]. Tribedi et al. reported the effect

of cell hydrophobicity when comparing enzymatic esterase activity of two strains of *Pseudomonas* on the surface of PES. The strain with higher hydrophobicity also showed higher microbial activity, which is indicative that the interaction and hydrophobic balance between the microorganism and polymer surface is also relevant for microbial and enzymatic activity [184].

Table 2.7 Water diffusion and surface property as related to the main degradation process.

Water diffusion	Surface	Degradation process	Example
Low	Hydrophilic	Surface	PHAs
High	Hydrophilic	Bulk/surface	Starch, TPS, Cellulose
High	Hydrophobic	Bulk	PLA, PCL, PBS, PCL
Low	Hydrophobic	Surface (depending on the ratio of hydrophobic depletion and water diffusion)	PLA with chain extender

Surface roughness. Surface roughness is a measure of the finely spaced micro-irregularities on the surface texture and depicts the irregularities on the polymer surface. Some researchers have used surface roughness as an indicator of surface biodegradation [125,185]. The types of microbes able to colonize a surface and the formation of biofilms depend on the surface roughness. Increased roughness favors bacterial adhesion because of the greater area of contact between the polymeric material and the bacterial cells [186]. A rough surface offers micro- and nano-irregularities in the range of 0.5 to 2 μm, which appear as voids and can provide sites for microorganisms to attach and eventually access the polymer chains, increasing the rate of biodegradation [187,188].

Surface area. The shape (e.g., film, pellet, powder, and fiber) and size (macro, micro, and nano) of the polymer play important roles during the degradation process [53]. For example, thicker polyester samples take more time to biodegrade [75]. The surface area

available has a high effect on the rate of biodegradation: as the surface to volume ratio increases with time, so does the speed at which biodegradation occurs. Pits and cracks continue to increase as time proceeds, and gradually the sample shape and size change, enabling access to the inside of the matrix [189]. Extracellular enzymes are highly active on the surface of a polymer since they are relatively large to penetrate the bulk. Hence, increasing the surface area available for enzymatic attack translates into an increase in the kinetics of the biodegradation process. Herzog et al. [111] showed that the enzymatic degradation of a polyester by *Candida cylindracea* at 40 °C was more effective on nanoparticles (100 nm diameter) than on thick films (110 µm thickness) of the same polyester. The effect of morphology on water biodegradation of PHBV was evaluated by Komiyama et al. [190]. Samples evaluated in powder form showed the faster biodegradation due to the larger surface area available for biofilm formation in comparison to film, undrawn fiber, and fivefold drawn fiber.

2.9 Biodegradation assessment

The misuse of the terms "biodegradable" or "biodegradation" has given rise to inflated and unsubstantiated claims. Claims about general biodegradable products that are used to deceive consumers into believing that products are environmentally friendly have been coined as "greenwashing." It is essential to avoid such false claims, guarantee transparency to consumers, and stop the unqualified use of vague terms. Certification for biodegradation, per se, does not exist worldwide. Some polymer and paper materials are certified for biodegrading in specific environments such as home and industrial composting, soil, and water [142]. Standards and methods have been developed to aid certification, to avoid confusion, and to define the environment and conditions in which the samples can be biodegraded [191].

2.10 Standards for evaluation of biodegradation at mesophilic conditions

Several organizations are associated with developing the standards for biodegradability of materials in different environments for different countries and world regions [191]. Various reviews and reports have provided the standards available for biodegradation in soils [192], aquatic environments [193], or home and industrial composting [194,195]. In this review we specifically summarize, in **Table 2.8**, the different standards used to assess biodegradability under aerobic conditions for mesophilic temperatures and tracking evolution of CO_2 and O_2 demand and cite published works that reported the use of these standards. Furthermore, standards with specifications of materials to be evaluated and for certification are described in ASTM 6400, ISO 17088, and EN13432. The environmental conditions in which the biodegradation takes place are an important aspect since the biodegradability of a material differs from one environment to another. Development of standards for assessing biodegradation in different environments is essential [196,197].

Table 2.8 Standards for assessing aerobic biodegradation of polymers at mesophilic conditions in different environments, and selected studies that used the standards to conduct their biodegradation tests.

Standard	Name	Parameter evaluated	Biodegradation requirement	Environment	Temperature range	Time frame	Selected published works
ISO 14852:2018	Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by analysis of evolved carbon dioxide	Measure CO_2 evolved	> 60% for reference material (end of test)	Natural aqueous medium (inoculum from activated sludge, compost, or soil)	20–25 °C (± 1 °C)	6 months	[198–200]
ISO 14851:2019	Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium — Method by measuring the oxygen demand in a closed respirometer	Measure O_2 demand	> 60% for reference material (end of test)	Natural aqueous medium (inoculum from activated sludge, compost, or soil)	20–25 °C (± 1 °C)	6 months	[115,201–207]

Table 2.8 (cont'd)

ISO 17556:2019	Plastics — Determination of the ultimate aerobic biodegradability of plastic materials in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved	Measure O_2 demand, CO_2 evolved	> 60% for reference material (plateau phase or end of test)	Soil	20–28 °C (preferably 25 °C, ± 2 °C)	6 months	[115,208–210]
ISO 19679:2019	Plastics — Determination of aerobic biodegradation of non-floating plastic materials in a seawater/sediment interface — Method by analysis of evolved carbon dioxide	Measure CO_2 evolved	> 60% for reference material after 180 days	Seawater / sandy sediment interface	15–25 °C (don't exceed 28 °C, ± 2 °C)	≤ 24 months.	[211]

Table 2.8 (cont'd)

ISO 18830:2016	Plastics — Determination of aerobic biodegradation of non-floating plastic materials in a seawater/sandy sediment interface — Method by measuring the oxygen demand in closed respirometer	Measure O_2 demand	> 60% for reference material (after 180 days)	Seawater / sandy sediment interface	15–25 °C (don't exceed 28 °C, ± 2 °C)	≤ 24 months.
ISO 22403:2020	Plastics — Assessment of the intrinsic biodegradability of materials exposed to marine inocula under mesophilic aerobic laboratory conditions — Test methods and requirements	Measure CO_2 evolved	≥ 90% for reference material (within 2 years)	Marine	15–25 °C (don't exceed 28 °C, ± 2 °C)	24 months.

Table 2.8 (cont'd)

ISO 22404:2019	Plastics — Determination of the aerobic biodegradation of non-floating materials exposed to marine sediment — Method by analysis of evolved carbon dioxide	Measure CO_2 evolved	> 60% for reference material (after 180 days)	Marine sediment	15–25 °C (don't exceed 28 °C, ± 2 °C)	≤ 24 months.
ISO 23977-1:2020	Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater — Part 1: Method by analysis of evolved carbon dioxide	Measure CO_2 evolved		Sea water	15–25 °C	≤ 24 months

Table 2.8 (cont'd)

ISO 23977-2:2020	Plastics — Determination of the aerobic biodegradation of plastic materials exposed to seawater — Part 2: Method by measuring the oxygen demand in closed respirometer	Measure O_2 demand		Sea water	15–25 °C	≤ 24 months	
EN 17033:2018	Plastics – Biodegradable mulch films for use in agriculture and horticulture – Requirements and test methods	Measure CO_2 evolved	> 90% conversion	Agriculture soil	20–28 °C (25 °C preferred, ± 2 °C)	24 months	[120]
ASTM D5988-18	Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in Soil	Measure CO_2 evolved	> 70% for reference material after 180 days (starch or cellulose)	Soil and mature compost	25 ± 2 °C	6 months	[120,189,218–227,208,228,229,209,212–217]

Table 2.8 (cont'd)

ASTM D6691-17	Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in the Marine Environment by a Defined Microbial Consortium or Natural Sea Water Inoculum	Measure CO_2 evolved	> 70% for reference material	Marine (seashore and open ocean). Synthetic seawater with pre-grown population of at least 10 aerobic marine micro-organisms. Natural seawater with inorganic nutrients	30 ± 2 °C	10–90 days	[115,230–232]
ASTM D7991-15	Standard Test Method for Determining Aerobic Biodegradation of Plastics Buried in Sandy Marine Sediment under Controlled Laboratory Conditions	Measure CO_2 evolved	> 60% for reference material (after 180 days)	Marine (tidal zone, sandy sediment + seawater)	15–25 °C (do not exceed 28 °C, ± 2 °C)	24 months	[230,233]

Table 2.8 (cont'd)

ASTM D5929-18	Standard Test Method for Determining Biodegradability of Materials Exposed to Source-Separated Organic Municipal Solid Waste Mesophilic Composting Conditions by Respirometry	Measure O_2 uptake, Measure CO_2 evolved	Total O_2 uptake > 80g Volatile fatty acids > 2g/kg (invalid test)	Municipal solid waste inoculated with compost	40 ± 2 °C	45 days	
AS 5810-2010	Biodegradable plastics—Biodegradable plastics suitable for home composting	Measure CO_2 evolved	≥ 90% (dry weight) degradation of test sample.	Organic waste, kitchen waste	25 ± 5 °C (< 30 °C)	12 months	[234]
NF U52-001:2005	Biodegradable materials for use in agriculture and horticulture – Mulching products – Requirements and test methods	Measure CO_2 evolved	60% for reference (cellulose) in soil, 90% for cellulose in compost or water media	Soil, compost, and water	28 ± 5 °C	12 months in soil, 6 months in compost, 6 months in water	

2.11 Methods for biodegradation assessment

Different methodologies, both quantitative and qualitative, are used to determine the biodegradation process. When used in combination, the different methodologies help to recognize if there is any disagreement among the achieved results. Also, supporting quantitative methodologies, such as CO_2 evolution and M_w reduction, with qualitative methodologies, such as scanning electron microscopy (SEM), visual observation, and spectroscopy, is helpful in corroborating the biodegradation of the material under study. The main methodologies to assess and report the degree of biodegradation in aerobic conditions have been summarized in several reviews [49,195,235–237]. The oldest and most common methodology is the gravimetric reduction in weight or mass loss of the material under biodegradation. Significant deterioration in mechanical properties has also been reported as a degree of biodegradation. Macro visualization, mass loss, and deterioration of mechanical properties are methods for the approximate assessment of biodegradation. These methods are more related to physical degradation of the material and not to the biological process conducted by a population of microorganisms. In general, are more useful for gaining insights during the early step of polymer biodegradation as during abiotic degradation or during biofilm formation on the surface of the polymer.

For enzymatic activity clear zone formation, turbidimetric assays, and techniques that monitor the release of soluble products into the supernatant solution as Total Organic Carbon (TOC), and spectroscopy combined with chromatography have been reported. Nowadays the use of microbalance with dissipation monitoring measurements constitutes an additional analytical technique to evaluate the evolution of the enzymatic hydrolysis of hydrolysable polymers.

For tracking CO_2 evolution and mineralization, respirometric methods has been developed and are supported by standards for assessing the conversion of the C present in

the polymer to CO_2. Furthermore, standards also describe for specific environments the measure of biochemical oxygen demand (BOD) instead of CO_2. Radio labeling and tracking of C has been reported as an adequately technique to complement with respirometric methods.

Associated with each of the main evaluation methodologies are several techniques used to quantify the degree of biodegradation. **Table 2.9** lists published studies conducted to measure biodegradation using techniques to measure CO_2 and/or O_2 under aerobic conditions at mesophilic temperatures. This section provides a brief description of the methodologies used and the published studies using those methodologies in the mesophilic range.

Table 2.9 Biotic degradation of polymers at mesophilic conditions measuring CO_2 evolution or O_2 demand. Polymer details as shape, initial molecular weight (M_w), and initial crystallinity (X_c); environment in which the biodegradation study is conducted, testing temperature, the extent of biodegradation with the time frame, and the corresponding selected studies are mentioned.

Parameter	Polymer (shape, initial M_w, initial X_c)	Environment	Temperature, °C	Main result (test duration)	Published studies
CO_2	Cellulose (powder)	Soil	15, 20, 28	-	[219]
CO_2	Cellulose (paper mulch)	Soil in laboratory conditions	27	-	[237]
CO_2	PBS (dumbbell, 21.2 kDa, 57.6%)	Soil compost in laboratory conditions	25 ± 2	65% CO_2 evolution (180 days)	[226]
CO_2	PCL (powder, 100 kDa)	Compost in laboratory composting conditions	40	20% mineralization (180 days)	[246]
CO_2	PLA (films, 100–200 kDa), starch (powder)	Soil in laboratory conditions	28, 40	PLA (100kDa): 10–40% mineralization (28 ºC, 180 days), PLA (200 kDa): 30–95% mineralization (40 ºC, 180 days)	[247]
CO_2	PLA (sheets, 170 and 180 kDa)	Soil inoculated in laboratory conditions	30	5–40% mineralization (60 days)	[222]
CO_2	PLLA (film, 100 kDa, 30–35%)	Aquatic laboratory conditions	25, 37	PLA (25 ºC): 10% mineralization (180 days), PLA (37 ºC): 12% mineralization (180 days)	[248]
CO_2	PLA (films, 163 kDa)	Soil in laboratory conditions	30	10–25% mineralization (150 days)	[236]

Table 2.9 (cont'd)

CO_2	PHB (powder and film), PCL (powder), starch (powder)	Soil in laboratory conditions	22 ± 3	PHB powder: 91% mineralization (90 days), PCL powder: 102% mineralization (270 days), PHB films: 26% mineralization (210 days)	[225]
CO_2	PHBV (powder, -, 68.9%), cellulose (powder)	Marine in laboratory conditions	25	PHBV: 90% mineralization (450 days)	[241]
CO_2	PHBV (film), cellulose (powder)	Soil in laboratory conditions	28	PHBV: 90% mineralization (120 days)	[224]
CO_2	PHB (film), PBSe (film), PBSeT (film)	Marine in laboratory conditions	25	PHB: 70% mineralization (360 days) and 95% mineralization (200 days), PBSe: 95% mineralization (365 and 200 days), PBSeT: 85% mineralization (360 days) and 90% mineralization (200 days)	[218]
CO_2	PLLA (powder and film, 5, 11, 34, 256 kDa, 0, 18, 42%)	Compost	30, 37	PLA (5 kDa): 70% mineralization (40 days), PLA (11 kDa): 55% mineralization (40 days), PLA (34 kDa): 35% mineralization (40 days), PLA (256 kDa): 20% mineralization (40 days)	[249]

Table 2.9 (cont'd)

CO_2	PHA, PBS, cellulose (powder)	Soil in laboratory conditions	25, 37	PHA (25 °C): 95% mineralization (150 days), PHA (37 °C): 90% mineralization (180 days), PBS (25 °C): 90% mineralization (200 days), PBS (37 °C): 75% mineralization (180 days)	[217]
CO_2	PU (films)	Soil/Sturm test	30	10 g CO_2 evolution (30 days)	[250]
CO_2	PBAT (films, -, 9%)	Soil	25	5% mineralization (100 days)	[251]
CO_2	PBSe (powder), cellulose (powder)	Soil	28	55–90% mineralization (140 days)	[195]
CO_2	PHB (film), PBSe (film), PBSeT (film), cellulose (powder)	Soil	25	PHB: 95% mineralization (360 days), PBSe: 90% mineralization (360 days), PBSeT: 90% mineralization (360 days)	[216]
CO_2	Cellulose (powder)	Soil	25 ± 2	-	[234]
CO_2	UV irradiated PLA (powder, 198 kDa)	Inoculated sterilized compost, Sturm test	37	PLA (compost): 35–45% mineralization (40 days), PLA (Sturm test): 10–20% mineralization (40 days)	[73]
CO_2	PHB (powder, 470 kDa))	Sturm test	27	10–80% mineralization (28 days)	[252]
CO_2	PLA (film, -, 20.8), PHBV (film, -, 72.6), cellulose	Soil	23–25	PLA: 5% mineralization (190 days), PHBV: 25% mineralization (190 days)	[227]
CO_2	PHA (films), PHB (films)	Soil	23 ± 4	PHA: 0.2 mM/mg CO_2 (90 days), PHB: 0.3 mM/mg CO_2 (90 days)	[221]

Table 2.9 (cont'd)

CO_2	PHB (film, 175–225 kDa, 48–52%) PHBV (films, 400–300 kDa, 48–52%) with 1% nucleating agent	Microorganisms from marine environment in simulated laboratory conditions	30	PHB: 80–95% mineralization (115 days), PHBV: 90–100% mineralization (115 days)	[239]
CO_2	PHBV (films, 455 kDa, 47%), cellulose (powder)	Marine (foreshore sand, sand & seawater, seawater) in laboratory conditions	25	PHBV (foreshore sand): 90% mineralization (250 days)	[253]
CO_2	PHA (film), PLA (bag, bottle)	Marine	30	PHA: 38–45% mineralization (180 days), PLA (bag): 4.5% mineralization (180 days), PLA (bottle): 3.1% mineralization (180 days)	[254]
CO_2	PHBV (film, 500–600 kDa, 14–58%), cellulose, starch	Soil	25	PHBV: 90% mineralization (250 weeks)	[223]
CO_2	PHA (film), cellulose (paper)	Soil	20 ± 2	PHA: 70% mineralization (660 days)	[235]
CO_2	PLA with chain extender (films sheets, 449 kDa, 0.9%), PBAT (films sheet, 44 kDa, 15.2%), cellulose (powder)	Soil in laboratory conditions	28	PLA: 10% mineralization (180 days), PBAT: 20% mineralization (180 days)	[220]
CO_2	PLA (sheets), PHB (sheets), PBS (sheets), TPS (sheets), PCL (sheets), cellulose (powder)	Soil, home composting*, marine pelagic, and fresh water	25 ± 2, 28 ± 2, 30 ± 1, and 21 ± 1	PLA (soil): negligible (141 days), PLA (home composting): <20% mineralization (365 days), PLA (marine water): <10% relative biodegradation	[128]

Table 2.9 (cont'd)

CO_2	PU (films, 48.7 kDa)	Sturm test	35, 30	7.6–8.6 g/l CO_2	[255–257]
CO_2	PBAT (films)	Soil	30	15% mineralization (120 days)	[258]
CO_2	PU (films)	Sturm test	35	4.46 g/l CO_2	[259]
CO_2	PBSA (films)	Sturm test	37	78% mineralization (40 days)	[260]
CO_2	PLA (sheets)	Sterilized soil, non-sterilized soil, non-sterilized inoculated soil in laboratory conditions	30	PLA inoculated: 20% mineralization (60 days)	[228]
CO_2	Cellulose (foil)	Respirometer	20	-	[215]
CO_2	PBS (sheets, 90 kDa, 58.9%), PEA (sheets, 88 kDa, 40.6%)	Sturm test (activated sludge)	25	PBS: 18% mineralization (40 days), PEA: 12% mineralization (50 days)	[261]
CO_2	PBSA (films), cellulose (powder)	Compost	25	70% mineralization (55 days)	[242]
CO_2	PHA (films), PVOH (films)	Sea water	30	PHA: 100% mineralization (100 days), PVOH: 85% mineralization (100 days)	[240]
CO_2	PCL, PHBV, PBSA, PVOH, PEA, starch, cellulose	Aqueous solution	30	PCL: 26% mineralization, PHBV: 53% mineralization, PBSA: 3% mineralization, PVOH: 5% mineralization, PEA: 36% mineralization (2 weeks)	[205,206]
CO_2	PLA 3001D (films, -, 7.7%), cellulose (powder)	Aqueous mineral solution (including wastewater)	30	5% mineralization (115 days)	[207]
CO_2	PBAT (films, 56–38 kDa)	Soil incubation	25	7–15% mineralization (6 weeks)	[149]

Table 2.9 (cont'd)

CO_2	PU (foam)	Soil	21 ± 2	43% mineralization (192 days)	[229]
CO_2	PU (foam), cellulose (paper)	Soil	27 ± 1	10% mineralization (320 days)	[230]
CO_2	PU (foam)	Sewage water/modified Sturm test	22 ± 2	32–45.6% mineralization (60 days)	[262]
CO_2	Non-isocyanate polyurethane (NIPU) polyhydroxyurethane (PHU) (film)	Soil	20–28	40% mineralization (120 days)	[231]
O_2	PCL (powder), cellulose (powder)	Aqueous environment	25	30–35% BOD (150 days)	[208]
O_2	PHB (film, 735 kDa, 65%), PHBV (film 484 kDa, 46%), PCL (films, 187 kDa, 63%), PES (film, 87 kDa, 61%), PEA (film, 144 kDa, 74%), PBS (film, 79 kDa, 63%), PBA (film, 81 kDa, 70%), PBSe (films, 31.5 kDa, 68%)	Freshwater (river)	25	PHB: 75 ± 16% BOD, PHBV: 76 ± 2% BOD, PCL: 75 ± 8% BOD, PES: 83 ± 2% BOD, PEA: 70 ± 3% BOD, PBS: 3 ± 1% BOD, PBA: 20 ± 4% BOD, PBSe: 6 ± 3% BOD (28 days)	[263,264]
O_2	PHB (film, 735 kDa, 65%), PHBV (film 484 kDa, 46%), PCL (films, 187 kDa, 63%), PES (film, 87 kDa, 61%), PEA (film, 144 kDa, 74%), PBS (film, 79 kDa, 63%), PBA (film, 81 kDa, 70%)	Freshwater (lake)	25	PHB: 52 ± 7% BOD, PHBV: 71 ± 1% BOD, PCL: 77 ± 1% BOD, PES: 77 ± 1% BOD, PEA: 68 ± 8% BOD, PBS: 12 ± 8% BOD, PBA: 80 ± 13% BOD (28 days)	[263]

Table 2.9 (cont'd)

O_2	PHB, PHBV, PCL, PES, PEA, PBS, PBA	Seawater (bay)	25	PHB: 27 ± 10% BOD, PHBV: 84 ± 2% BOD, PCL: 79 ± 2% BOD, PES: 1 ± 1% BOD, PEA: 65 ± 3% BOD, PBS: 1 ± 1% BOD, PBA: 20 ± 2% BOD (28 days)	[263]
O_2	PHB, PHBV, PCL, PES, PEA, PBS, PBA	Seawater (ocean)	25	PHB: 14 ± 10% BOD, PHBV: 78 ± 5% BOD, PCL: 43 ± 14% BOD, PES: 3 ± 2% BOD, PEA: 46 ± 13% BOD, PBS: 2 ± 0% BOD, PBA: 10 ± 5% BOD (28 days)	[263]
O_2	Cellulose (filter paper)	Seawater (pelagic, eulittoral, sublittoral, supralittoral, deep sea, buried under sediments)	11–26	-	[213]
O_2	PLA (film), PBAT (film), PCL (film and powder), cellulose (powder)	Inoculum from activated sludge	30 ± 2	PLA: 3.7% BOD, PBAT: 15.1% BOD, PCL (film): 34.8% BOD, PCL (powder): 37.7% BOD (28 days)	[209]
O_2	PLA (films, fibers), PHA (films)	Soil	30, 40	PLA (films, 30 °C, 20 days): 9.8–10.3% BOD, PLA (films, 40 °C, 10 days): 11.8–17.9% BOD, PLA (fiber, 30 °C, 20 days): 9% BOD, PLA (fiber, 40 °C, 10 days): 16% BOD, PHA (films, 30 °C, 20 days): 26.3% BOD, PHA (films, 40 °C, 12 days): 49.5% BOD	[265]

Table 2.9 (cont'd)

O_2	PBS (sheets), cellulose (powder)	Inoculum from activated sludge	25	PBS: 31% BOD (80 days)	[212]
O_2	PHBV (powder, 376 kDa, 58.5%), cellulose (powder)	Aqueous conditions	20	PHBV: 80 % BOD (28 days)	[214]
O_2	PLA (film)	Lake water, compost, soil in laboratory conditions	20	PLA (lake water): ~5 mgO_2/dm^3 water, PLA (compost): ~25 mgO_2/kg compost, PLA (soil): ~100 mgO_2/kg soil (28 days)	[266]
O_2	PCL (film), PLA (film)	Compost, activated sludge, river water, sea water	20	PCL (compost): 140 mgO_2/dm^3, PLA (compost): 125 mgO_2/dm^3, PCL (activated sludge): 120 mgO_2/dm^3, PLA (activated sludge): 115 mgO_2/dm^3, PCL (river water): 10 mgO_2/dm^3, PLA (river water): 8 mgO_2/dm^3, PCL (sea water): 5 mgO_2/dm^3, PLA (sea water): 5 mgO_2/dm^3 (7 days)	[267]
O_2	PBAT (film, 16 kDa)	Mineral medium	25	10% BOD (22 days), 45% BOD (45 days)	[268,269]
O_2	PHBV (powder, film, undrawn fiber, fivefold-drawn fiber, 250 kDa)	Freshwater, seawater	25	Powder: 18% BOD, film: 18% BOD, undrawn fiber: 18% BOD, fivefold-drawn fiber: 8% BOD (28 days)	[196]
O_2	PCL (powder), cellulose (powder)	Activated sludge	25	PCL: 20–100% (100 days)	[210]
O_2	PLA (powder), PCL (powder)	Aqueous conditions	30	PLA: 35% (40 days), PCL: 100% (days)	[211]

Table 2.9 (cont'd)

O_2	PLA (film, particle), PBAT (film, particle), PBS (film, particle), PBSA (film, particle), PCL (film, particle), PHB (particle)	Seawater in laboratory conditions	27	PLA: 0.3 % BOD, PBAT: 1–1.4% BOD, PBS: 0.1–1.3% BOD, PBSA: 0.4–29.2 % BOD, PCL: 14.5–40.9% BOD, PHB: 44–60.4% BOD (4 weeks)	[270]

BOD: biochemical oxygen demand; *using ISO 14855; **relative to the reference material

2.12 Mass loss and mechanical properties deterioration

Measurement of mass loss is the most commonly used method to indicate the extent of degradation and is indicated as mass loss measured from the samples retrieved during the degradation test [195]. Mass loss is used mostly to designate the degradation occurring on the polymer surface and is contingent on the disintegration phenomena. Many researchers have reported the use of mass loss determination to indicate that the material has undergone degradation. Furthermore, deterioration of mechanical properties (assessed on films, sheets, or dumbbell specimens) indicative of degradation by the action of abiotic mechanisms has been reported along with mass loss.

Quartz crystal microbalance with dissipation (QCM-D). In terms of enzymatic degradation, microbalance weight loss technique in the nanogram scale has been reported during enzymatic hydrolysis of aliphatic and aromatic polyesters as PCL and PBAT. This is a unique approach to monitor the dynamic of the enzymatic hydrolysis and has showed high sensitivity [262–266].

2.13 Macro and micro visual analysis of the polymer surface

Macro visual analysis is the second most commonly used technique after mass loss. Macro visual changes of the polymer do not necessarily indicate biodegradation, but these changes are usually the first evidence of microbial colonization and biofilm formation.

Micro visual inspection using microscopic techniques like SEM, transmission electron microscopy (TEM) or atomic force microscopy (AFM) can impart more knowledge regarding the biodegradation process at the early stage, specifically biofilm formation and the structure of the sample [49]. The topographical changes occurring in the polymer are usually seen as the formation of holes, cracks, cavities (material erosion), discoloration, or surface roughness [267]. Kijchavengkul et al. studied the surface evolution during

biodegradation of PBAT films and demonstrated the consequences of the degradation by using SEM methodology among

other techniques [268]. For PBAT samples with c. 30% or less crosslinking, biofilm formation was observed. Large number of microbes consumed PBAT samples, creating pits in the film surface. For samples with more than 30% crosslinking, no cavities were observed on the PBAT film surface, indicating that increased crosslinking results in reduced biodegradation [268]. Shah et al. reported changes in surface morphology, such as pit formation and erosion, due to the biodegradation of PHBV films in a basal salt medium after two weeks of immersion [269]. Techniques as TEM and AFM were extensively used for identification of chemical and enzymatic degradation of polyesters [270–273].

2.13.1 Chromatography

Size exclusion chromatography (or gel permeation chromatography). It is used to study the reduction of M_w. Reduction and distribution in M_w are a preferred parameter that provides evidence of the biodegradation process. When accompanied with mineralization, the M_w reduction can provide more insights into understanding the process.

High performance liquid chromatography is widely used for qualitative and quantitative analysis of soluble compounds derived from enzymatic activity that are released into solution.

Lu et al. examined biodegradability of PPC/starch composites in soil at room temperature; the study of M_w change for unburied, 40 and 180 days along with weight loss and other qualitative techniques like FTIR, SEM, and photographs helped the researchers conclude that PPC was the last component to biodegrade post microbial colonization and starch degradation [274]. Reduction of *Mw* by chemical hydrolysis has been reported for aliphatic and aromatic polyesters as PLA, PCL, PHB, and PBAT, among others [64,275,276].

2.13.2 Spectroscopy

A qualitative way to assert biodegradation is by identifying the chemical changes in the polymer structure [195]. These changes could translate into the formation of low M_w compounds resulting from the polymer degradation. Changes in the molecular structure can be identified by various spectroscopic analysis methods.

Nuclear magnetic resonance (NMR): The nuclei of any given type (C, H, N, P, or O) resonate at different energies. The information from the NMR signal (position and pattern) gives critical information about the nuclei environment and presence [79,277]. The use of NMR has been reported for the degradation of different polymers in different environments. Kijchavengkul et al. studied the biodegradation of PBAT in compost and tracked the evolution of the BT and BA dimers using ^{1}H NMR and showed that the soft aliphatic portion and the amorphous region are more susceptible to hydrolysis and biodegradation than the rigid aromatic portion and the crystalline region [79].

Fourier-transform infrared spectroscopy (FTIR): FTIR analysis of any given material provides a specific fingerprint spectrum for that material, and the appearance and disappearance of peaks associated with the functional groups can help explain the changes happening in the material structure [79,195]. *Mass spectroscopy* is an analytical technique widely used for identification of products during enzymatic degradation of polymers. In general, it is used along with techniques as liquid chromatography.

Weng et al. studied the biodegradation of PHB/PLA blends buried in soil at different depths at c. 20 °C; the FTIR spectra showed that the peaks in the 4000 to 3000 cm^{-1} region were broad in nature due to the formation of -OH and -COOH groups after degradation [278]. Furthermore, Mbarki et al., conducted both the FTIR and NMR analysis on PDLA samples immersed in the soil/liquid culture at 37 °C and found no significant difference in the chemical structure before and after immersion (45 days for FTIR and 28 days NMR); the

conclusion derived was that the biodegradation phenomena was only surface and not bulk [279].

2.13.3 Plate (clear zone formation) and turbidimetry assays

Plate tests were originally designed to gauge the resistance of plastics to degradation via microorganisms. However, in addition to testing resistance they are now used to see if the polymer can support the growth of microorganisms through biofilm formation. The polymeric material is dispersed in a petri dish containing a mineral salts agar medium that serves as the sole carbon source. The polymer in the surface, suspended in the medium, is then inoculated with microorganisms and held for a predetermined amount of time at a constant temperature to allow the microorganisms to grow. The formation of a halo or clear zone around the microorganism colony marks an end for this test, since the clear zone indicates that the microorganism can at least depolymerize the polymeric material. The test is also used in screening, isolating, and identifying the potential degrading microorganisms for any given polymer [280,281]. Urbanek et al. isolated, screened, and assessed the degrading capability of Antarctic soil microorganisms on PCL, PBS, and PBSA at low temperature with the help of the clear zone formation technique [282].

2.13.4 Respirometric tests for CO_2 evolution and biochemical O_2 demand

These tests involve measuring the consumption of O_2 or formation of CO_2 under aerobic conditions. The CO_2 evolved can be measured by three different techniques [60]: in cumulative measurement respirometry (CMR) the evolved CO_2 (trapped in basic solution such sodium hydroxide, barium hydroxide) is quantified by titration method [283]; in gravimetric measurement respirometry (GMR) the evolved CO_2 is trapped in absorption columns and the weight increase is used to quantify the amount of CO_2 [283]; and in direct measurement respirometry (DMR) the evolved CO_2 is quantified by means of an inline non-dispersive infrared gas analyzer or gas chromatograph [113]. Kale et al. compared the use

of CMR, GMR, and DMR to assess the biodegradation of PLA under simulated composting conditions, and found similar evolution of biodegradation [283]. They concluded that the biodegradation process is further dependent on various factors, including shape, size, thickness, and sample/compost ratio, among others. The advantages and disadvantages associated with these techniques are explained in detail elsewhere [268].

Techniques measuring O_2 consumption, reported as BOD, are assessed in specific aquatic environments as sewage sludge and wastewater. However, standards have been also developed for assessing O_2 consumption in soil environments (**Table 2.8**).

2.13.5 Radiolabeling

An understudied approach for assessing the degree of biodegradation is the use of radiolabeled carbon. This is one of the absolute tests to determine biodegradation and involves tracking carbon from biodegradable polymers into CO_2 and biomass. The approach is based on labeling the carbon atoms in the polymer backbone with carbon isotopes: ^{13}C (stable in nature) and ^{14}C (radioactive) [284,285].

Early works conducted by Albertsson et al. showed that the technique of radiolabeling polymers using ^{14}C were useful not only for detecting the biotic stage of the biodegradation process but also the abiotic stage [286–289]. In this sense, PE films produced using a ^{14}C marker showed $^{14}CO_2$ evolution of the carbonyl oxidized byproducts when the films were exposed to soil [286].

Studies in the area of biodegradable polymers addressed in this review are insufficient. Zumstein et al. employed the use of ^{13}C labeled polymer along with isotope-specific analytical methods (i.e., cavity ring-down spectroscopy) to track the biodegradation of PBAT in soil [140]. This technique allowed for tracking of the basic biodegradation steps by distinguishing the labelled PBAT CO_2 from the CO_2 evolved due to the mineralization of organic matter in the soil.

In summary, two or more methods are commonly employed together to determine biodegradation. The change in M_w, weight loss, and surface analysis are used widely but these alone do not guarantee biodegradation, and at most hint towards disintegration of the material under study. The evolution of CO_2 and radiolabeling represents the complete assessment of the breakdown of the material into biomass and need to be employed on a more regular basis for biodegradation studies. Though the respirometry method gives the mineralization value, radiolabeling is far more advanced by showing the actual integration of polymer carbon into the microbial biomass.

With respect to standards, there is no international standard specifying how home composting should be conducted for effective biodegradation of biodegradable polymers. Also, many standards for determining polymer biodegradation in aquatic environments, as listed in **Table 2.8**, mention temperatures (laboratory simulated settings conditions) that are much higher than the actual conditions encountered in real-world environments. In general, adaptations of the international standards to specific conditions are implemented to assess and report, for example, biodegradation of polymers in home composting or at mesophilic temperatures.

2.14 Microorganisms and enzymes able to biodegrade polymers

The ability to degrade biodegradable polymers is widely distributed among bacteria, fungi, and actinomycetes, and there is much variation in ability. **Table 2.10** lists extracellular enzymes and/or microorganisms able to biodegrade polymers in different mesophilic environments, as reported in the published literature.

2.14.1 Microbial Population

Some microorganisms can digest several polymer structures in different environments, and degradation rate efficiency can differ. A high portion of the published works have reported the digestion activity of a specific microorganism in the highly

controlled conditions of incubated or culture media; under these conditions, the polymer substrate is mostly the only source of nutrient for the microorganism. In contrast, in less restricted environments, such as soil, home composting, industrial composting, or aquatic environments, the complexity of the biological activity process increases, and several sources of substrates and microorganisms may be available. In natural soil or aquatic environments, an active population of microorganisms with different requirements, in terms of nutrients and optimal growth conditions, are competing or working cooperatively for the resources available.

The presence of microorganisms and the formation of a biofilm, due to the colonization

of the polymer surface, creates an effect that sometimes can alter the abiotic degradation of the polymer. For example, when PCL biodegradation was evaluated under low stirring, the impediment of biofilm formation resulted in a higher weight loss [298].

Table 2.10 Enzymes and/or microorganisms with activity for degrading biodegradable polymers when tested in mesophilic conditions. Different parameters such as the enzymes released, microbial species used, the environment from which the microorganisms were isolated / testing media, polymer studied, the temperature and pH for conducting the biodegradation study, optimal conditions for the microorganisms, and studies reporting them are mentioned.

Enzymes*	Microorganism*	Environment	Polymer	T (°C), pH	Optimal conditions of T (°C) and pH	Reference
Alcalase (3.4.21.62)	*Bacillus licheniformis* (B)	Buffer solution	PLA	40, 8.0	60, 9.5	[290]
Amidase (3.5.14)/esterase (55 kDa)	*Rhodococcus equi* strain TB-60	Soil/culture	PU	30, 7	45, 5.5	[291]
Carboxyl esterase (3.1.1.1)	*Alcanivorax borkumensis* (B), *Rhodopseudomonas palustris* (B)	Culture	PCL, PDLLA, PBSA	30, 8.0	30–37, 9.5–10	[292]
Carboxyl esterase	*Alcanivorax borkumensis* (B)	Culture	PES, PHBV, PDLLA	30, 8.0	55–60, 9.5–10	[292]
Chymotrypsin (3.4.21.1)	-	Culture	PLLA, PEA	37, 7.0	-, -	[293]
Cutinase (3.1.1.74) (21.6 kDa)	*Aspergillus oryzae RIB40 (F)*	Culture	PBS, PBSA, PLA	37, 8.0	35–55, 9.0	[183]
Cutinase	*Alternaria brassicicola (F), Aspergillus fumigatus (F), Aspergillus oryzae (F), Humicola insolens (F), Fusarium solani (F)*	Culture	PCL	40, 3, 5, 8	-, -	[294]
Cutinase	*Fusarium solani* (F)	Buffer solution	PBAT	30, -	-, -	[140]

Table 2.10 (cont'd)

Cutinase (21 kDa)	*Cryptococcus magnus* (F)	Larval midgut of stag beetle (Aegus laevicollis)/culture	PBS, PBSA, PCL, PDLLA, PLLA	30, 7.4	40, 7.5	[295]
Cutinase	*Fusarium solani* (F)	Buffer solution	PCL	37, 7.2	-, -	[296]
Cutinase (20 kDa)	*Fusarium sp. FS1301 (F)*	Soil/liquid culture	PBS, PCL	30, -	50, 8.0	[297]
Cutinase (19.7 kDa)	*Paraphoma*-related fungal strain B47-9 (F)	Barely phyllophane/liquid culture	PBAT, PBS, PBSA, PCL, PDLLA	30, 7.2	45, 7.2	[298]
Cutinase	*Pichia pastoris* (F)	Buffer solution	PBS	37, 7.4	-, -	[299]
Cutinase	-	Culture	PBS, PBA	37, 7.4	-, -	[300]
Cutinase (20.3 kDa)	*Pseudozyma antarctica* JCM 10317 (Y)	Culture	PBS, PBSA, PCL, PLLA, PDLLA	30	40, 9.5	[301,302]
Cutinase	*Fusarium solani* (F), *Fusarium moniliforme* (F)	Culture	PCL	22	9–10	[303]
Cutinase	*Bacillus* sp. KY0701	Culture	PCL	30, 7	50, 7	[304]
Cutinase	*Aspergillus oryzae* (F)	Buffer solution	PCL	40, 8	-, -	[305]
Cutinase	*Pseudozyma jejuensis* OL71 (F)	Leaves of Citrus unshiu/culture	PCL	30, -	-, -	[306]
Cutinase-like enzyme (22 kDa)	*Cryptococcus flavus* GB-1 (Y)	Culture	PBSA	30, 6.8	45, 7.8	[307]

Table 2.10 (cont'd)

Cutinase-like enzyme	*Cryptococcus* sp. Strain S-2 (F)	Liquid culture	PBS, PLA, PCL	30, -	37, 7.0	[308]
Close related to Cutinase	*Pseudomonas pachastrellae* JCM12285^T (B)	Marine, coastal seawater/culture	PCL	30, -	-, -	[309]
Elastase	-	Culture	PLA	37, 7.0	-, -	[293]
Esterase (3.1.1.1)	*Aspergillus* sp. strain S45 (F)	Solid waste dumpsite/liquid culture	PU	30, 7.0	-, -	[240]
Esterase	*Bacillus* sp. AF8 (B), *Pseudomonas* sp. AF9 (B), *Micrococcus* sp. 10 (B), *Arthrobacter* sp. AF11 (B), *Corynebacterium* sp. AF12 (B)	Soil/culture	PU	30–35	-, -	[250]
Esterase	Hog liver	Buffer solution	PGA	37, 7.5	-, -	[310]
Esterase	*Bacillus subtilis* (B)	Buffer solution	PCL, PLA	37, -	-, -	[258]
Esterase	*Aspergillus tubingensis* (F)	Soil/solid and liquid culture	PU	(30, 37, 40), (5 – 9)	37, 7.0	[311]
Esterase	*Bacillus licheniformis* (B)	Compost/liquid culture	PLLA	32, 7.4	-, -	[312]
Esterase	*Alicycliphilus* sp. (B)	Culture	PU	37, 7	-, -	[313]
Esterase	*Leptothrix* sp. TB-71 (B)	Soil, fresh water/culture	PBSA, PES, PCL	30, -	-, -	[314]

Table 2.10 (cont'd)

Esterase (62 kDa)	*Comamonas acidovorans* strain TB-35 (B)	Soil/liquid culture	PU	30, 7.2	45, 6.5	[315–317]
Esterase (28 kDa)	*Curvularia senegalensis* (F)	Soil/liquid culture	PU	(21–25), 30, 35, 45, (4.0–8.0)	-, 7–8	[318]
Esterase (42 kDa)	*Comamonas acidovorans* (B)	Culture	PU	30, 5–8	-, -	[319]
Esterase	*Penicillium verrucosum* (F), *Aspergillus ustus* (F)	Compost soil/culture	PLA	30, 5.6	-, -	[320]
Esterase	*Pseudomonas aeruginosa* MZA-85 (B), *Bacillus subtilis* MZA-75 (B)	Soil/liquid culture	PU	37, 7.0	-, -	[246–248]
Esterase	*Pseudomonas aeruginosa* strain S3 (B)	Culture	PLA	30–37, 8	37, 8	[321]
Esterase	*Pseudomonas* (B)	Soil/Culture	PES	30, -	-, -	[184]
Esterase	Porcine liver	Buffer solution	PLA	40, 8.0	40, 8.0	[290]
Close related to esterase	*Bacillus pumilus* strain KT1012 (B)	Soil, water/culture	PES, PCL	30, 7.0	40–45, -	[322]
Lipase (3.1.1.3)	*Rhizopus delemar* (F)	Buffer solution	PLA	37, 7.2	- ,-	[323]
Lipase	*Acidovorax delafieldii Strain BS-3 (B)*	Soil/solid and emulsified substrate	PBS, PBSA	30, 7.0	-, -	[324]
Lipase	*Rhizopus oryzae* (F), *Burkholderia* sp. (B)	Liquid culture	PCL	30, -	-, -	[308]

Table 2.10 (cont'd)

Lipase	*Candida rugosa (F)*	Buffer solution	PCL, PLA	37, -	-, -	[258]
Lipase (36 kDa)	*Aspergillus niger* MTCC 2594 (F)	Liquid culture	PCL, PLA	30, 7	37, 7.0	[325]
Lipase	*Aspergillus oryzae* (F)	Buffer solution	PCL	37, 7.0	-, -	[326]
Lipase	*Aspergillus tubingensis* (F)	Soil/solid and liquid culture	PU	(30, 37, 40), (5–9)	37, 5.0	[311]
Lipase	*Burkholderia cepacia* PBSA-1 (B), *Pseudomonas aeruginosa* PBSA-2 (B)	Soil/culture	PBSA	27, 37	-,	[251]
Lipase	*Candida cylindracea* (F)	Buffer solution	PLA	40, 8.0	40, 8.0	[290]
Lipase	*Candida antarctica* (F)	Buffer solution	PCL, PBS	45, 7.2	-, -	[296,327,328]
Lipase	*Candida rugosa* (F)	Liquid culture	PU	(20–50), (4–9)	35, 7.0	[329]
Lipase	*Chromobacterium viscosum* (B), *Rhizopus orizae* (F), *Rhizopus niveus* (F)	Culture	PCL, PBS, PBSA	37, 7.0	-, -	[330]
Lipase (23 kDa)	*Cryptococcus* sp. MTCC 5455 (F)	Liquid culture	PBAT	25, -	-, -	[331]
Lipase	*Cryptococcus* sp. MTCC 5455 (F)	Buffer solution	PU	30, 7.0	37, (7.0–8.0)	[332]

Table 2.10 (cont'd)

Lipase	*Lactobacillus plantarum* (B)	Culture	PCL	37, 8.0	-, -	[333]
Lipase (25 kDa)	*Penicillium* sp. strain 14-3 (F)	Soil/liquid culture	PEA	30, 6.0	45, 4.5	[334]
Lipase	*Pseudomonas* (B)	Buffer solution	PLLA, PCL, PDLLA	37, 7.0	-, -	[335,336]
Lipase	*Pseudomonas cepacia* (B)	Buffer solution	PCL	37, 7,0	-, -	[201]
Lipase	*Pseudomonas cepacia* (B), *Rhizopus delemar* (F)	Buffer solution	PCL, PPS	30, 7.2	-, -	[337]
Lipase	*Pseudomonas fluorescens* (B)	Buffer solution	PCL	37, 7.4	-, -	[338]
Lipase (22 kDa)	*Cryptococcus* sp. (Y)	Buffer solution	PBS, PBSA	30, 7	-, -	[339]
Lipase	*Fusarium solani* (F)	Culture	PCL	22, 6.8	-, -	[340]
Lipase (34 kDa)	*Pseudomonas* sp. strain DS04-T (B)	Activated Sludge/liquid medium	PLLA, PCL, PHB	37, 8	50, 8.5	[341]
Lipase	*Rhizopus oryzae* (F)	Solution	PBS, PLLA, PBA	40, 5	40, 7	[263]
Lipase	*Rhizopus arrhizus* (F)	Buffer solution	PCL	30, 7	-, -	[182]
Lipase	*Pseudomonas* (B)	Buffer solution	PCL	25, 37, 7	-, -	[342]
Lipase	*Rhizopus oryzae* (F)	Buffer solution	PBAT	30, -	-, -	[140]
Lipase	*Rhizopus delemar* (F)	Buffer solution	PU	37, -	-, -	[343]

Table 2.10 (cont'd)

Lipase	*Pseudomonas* (B)	Buffer solution	PCL	37, 7	-, -	[344]
Lipase	*Achromobacter* sp (B), *Candida cylindracea* (F), *Rhizopus arrhizus* (F), *Rhizopus delemar* (F), *Geotrichum candidum* (F)	Buffer solution	PEA, PCL	37, 7.0	-, -	[345]
Lipase	*Bacillus* sp. (B)	Soil/culture buffer solution	PBAT	30–37, 7.4	-, -	[346]
Lipase	*Pseudomonas* sp. (B)	Buffer solution	PEA	37, 7.0	-, -	[347]
Lipase	*Stenotrophomonas* sp. YCJ1	Soil/culture	PBAT	30, 7.2	37, 7.5	[348]
Lipase	*Candida Antarctica* (F)	Buffer solution	PBAT	45, 7.2	-, -	[349]
PBAT hydrolase (close related to lipase)	*Rhodococcus fascians* NKCM 2511 (B)	Soil/liquid culture	PBAT, PCL, PBSA, PES, PBS (low activity)	25, -	-, -	[259]
PBAT hydrolase (close related to cutinase) (18.9 kDa)	*Rhodococcus fascians* (B)	Liquid culture	PBAT, PCL, PBSA, PES, PBS	30, 7	-, -	[350]
PBAT hydrolase (close related to Lipase)	*Bacillus pumilus* (B) (NKCM3101, NCKM3201, NCKM3202, KT1012), *Brevibacillus choshinensis* PBATH (B)	Soil/liquid culture	PBAT (low activity), PBSA, PBS, PES, PCL	30, 7.0	-, -	[81]
PLA depolymerase (related to lipase)	*Paenibacillus amylolyticus* Strain TB-13 (B)	Soil/culture	PBS, PBSA, PDLLA, PCL, PES	37, 8	45–55, 10.0	[351]

Table 2.10 (cont'd)

PBAT hydrolase	*Isaria fumosorosea* strain NKCM1712 (F)	Soil/culture	PBAT, PBA, PBS, PBSA, PES, PHB, PCL	25–45, 7.0	-, -	[260]
PBS-degrading enzyme (44.7 kDa)	*Aspergillus* sp. XH0501-a (F)	Soil/culture	PBSA	30	40, 8.6	[352]
PCL depolymerase (63.5 kDa) (esterase)	*Brevundimonas* sp. strain MRL-AN1 (B)	Liquid culture	PCL, data not shown for PLA, PES, PHB, and PHBV	37, 7	30, 6–8	[353]
PCL depolymerase	*Penicillium oxalicum* strain DSYD05-1 (F)	Soil/liquid culture	PCL, PHB, PBS	30, 6.8	-, -	[354]
PCL depolymerase	*Alcaligenes faecalis* TS22 (B)	Culture	PCL	30, -	-, -	[355]
PCL depolymerase	*Paecilomyces lilacinus* strain D218 (F)	Soil/solid culture	PCL	30, 5.2	30, 3.5–4.5	[356]
PLA depolymerase (58 kDa)	*Pseudomonas tamsuii* TKU015 (B)	Soil/culture	PLLA	30, 7.0	60, 10	[357]
PLLA degrading enzyme	*Actinomadura keratinilytica* T16-1 (B)	Culture	PLLA	45, 7	45, 6–8	[358]
PHA depolymerase (3.1.1.76)	*Alcaligenes faecalis* (B)	Buffer solution	PHB, PHBV, PHA	37, 7.4	-, -	[359]
PHA depolymerase (48 kDa)	*Pseudomonas stutzeri* YM1414 (B)	Fresh water/buffer solution	PHB	37, 7.4	55, 9.5	[360]
PHA depolymerase	*Ralstonia pickettii* T1 (B)	Buffer solution	PHB, PHBV	37, 7.5	-, -	[174]

Table 2.10 (cont'd)

PHA depolymerase	*Ralstonia pikettii* T1 (B), *Acidovorax* sp. TP4 (B)	Buffer solution	PHA	37, 38, 7.5, 8.0	-, -	[361]
PHA depolymerase	*Comamonas* sp. DSM 6781 (B), *Pseudomonas lemoignei* LMG 2207 (B), *Pseudomonas fluorescens* GK13 DSM 7139 (B)	Liquid culture	PHB, PHV, PHBV	30, 7.2	-, -	[362]
PHA depolymerase (50 kDa)	*Comamonas testosteroni* (B)	Buffer solution	PHB, PHBV	37, 7.4	-, 9.5–10	[363]
PHA depolymerases (33.8 and 59.4 kDa)	*Pseudomona mendocina* DS04-T (B)	Mineral medium	PHB, PHBV	37, -	50, 8 and 8.5	[364]
PHA depolymerase (intracellular)	*Pseudomonas putida* LS46 (B)	Culture	PHB, PCL, PES	30, 7	-, -	[365]
PHB depolymerase (3.1.1.75)	*Alcaligenes faecalis* (B)	Culture	PHB	37, 7.4	-, -	[366]
PHB depolymerase	*Alcaligenes faecalis (B), Pseudomonas stutzeri (B), Comamonas acidovorans (B)*	Buffer solution	PHB, PEA, PES	37, 7.4	-, -	[367]
PHB depolymerase (57 kDa)	*Aspergillus fumigatus* (F)	Buffer solution	PHB, PHBV, PEA, PES	45, 8.0	70, 8	[368,369]
PHB depolymerase (49 kDa)	*Comamonas testosteroni* strain ATSU (B)	Soil/culture	PHB, PHBV	37, 7.4	70, 8.5	[370]
PHB depolymerase (42.7)	*Aureobacterium saperdae* (B)	Buffer solution	PHB	37, 7	45, 8	[371]

Table 2.10 (cont'd)

PHB depolymerase (57 kDa)	*Aspergillus fumigatus* 76T-3		PHB, PES, PBS	45, -	55, 6.4	[372]
PHB depolymerase (50–48 kDa)	*Emericellopsis minima* W2 (F)	Wastewater/liquid culture	PHB, PHBV	30, 8.0	55, 9.0	[373]
PHB depolymerase (40 kDa)	*Microbacterium paraoxydans* RZS6 (B)	Dumping yard/culture	PHB	30, -	30, 7	[374]
PHB depolymerase (46.8 kDa)	*Penicillium* sp. DS9701-D2 (F)	Activated sludge/culture	PHB	28–30, 6.8	30, 5	[375]
PHB depolymerase	*Streptoverticillium kashmirense* AF1 (A)	Sewage sludge/culture	PHBV	30, 8	-, -	[376]
PHB depolymerase (50 kDa)	*Acidovorax* sp. strain TP4 (B)	Pond water, river water, farm soil/culture	PHB	30, 8.5	-, -	[377]
PHB depolymerase (47 kDa)	*Arthrobacter* sp. strain W6 (B)	Soil/culture broth	PHB, PHBV	30, 7	50, 8.5	[378]
PHB depolymerase (85 kDa)	*Fusarium solani* Thom (F)	Wastewater/culture	PHB	25, 8	55, 7	[379]
PHB depolymerase (62.3 kDa)	*Bacillus megaterium* N-18-25-9 (B)	Culture	PHB	30–37, 9	65, 9	[380]
PHB depolymerase (44.8 kDa)	*Penicillium* sp. (F)	Culture	PHB	40, 4–6	50, 5	[381]
PHB depolymerase (61.8–70 kDa)	*Marinobacter* sp. NK-1 (B)	Culture	PHB	37, 7.4	-, 8	[382,383]
PHB depolymerase	*Nocardiopsis aegyptia* sp. nov. DSM 44442^T (B)	Marine seashore sediments/culture	PHB, PHBV	30, 7	-, -	[384]

Table 2.10 (cont'd)

PHB depolymerase (33 kDa)	*Penicillium funiculosum* (F)	Culture	PHB	30, 7.5	-, 6.5	[385]
PHB depolymerase (36 kDa)	*Penicillium simplicissimum* LAR13 (F)	Soil/culture	PHB	25, 30, 37, -	45, 5.0	[386]
PHB depolymerase	*Paecilomyces lilacinus* D218 (F)	Soil/liquid culture	PHB, PCL	30, 6.0	50, 6.5–7.5	[356]
PHB depolymerase	*Pseudomonas fluorescens* (B), *Pseudomonas aeruginosa* (B), *Pseudomonas putida* (B)	Contaminated soil/culture	PHB, PHBV	30, 7.9	-, -	[105]
PHB depolymerase (48 kDa)	*Comamonas acidovorans* YM1609 (B)	Freshwater/culture	PHB, PHBV	37, 7.4	-, -	[387]
PHB depolymerase	*Pseudomonas stutzeri* (B)	Sea water/Buffer solution	PHB	30–45, 7.4	-, 7–7.5	[388]
PHB depolymerases (44, 46 kDa)	*Agrobacterium* sp. K-03 (B)	Culture	PHB, PHBV	30, 8	45, 7,9 and 8.1	[389]
PHB depolymerase (49 kDa)	*Streptomyces exfoliatus* K10 (B)	Culture	PHB	25–37, 8	40, 8.5–9	[390]
PHB depolymerase (40 kDa)	*Pseudomonas pickettii* (B)	Culture	PHB	37, 7.4	40, 5.5	[391]
PHB depolymerase (53 kDa)	*Comamonas* sp. (B)	Solid culture	PHB	37, 8	-, -	[392]

Table 2.10 (cont'd)

PHB depolymerase (65 kDa)	*Alcaligenes faecalis* AE122 (B)	Seawater/culture	PHB	37,	-, -	[393]
PHB depolymerase (95.5 kDa)	*Alcaligenes faecalis* AE122 (B)	Seawater/culture	PHB	30, 6.8–7.5	55, 9	[394]
PHB depolymerase (40 kDa)	*Aspergillus fumigatus* (F)	Culture	PHB	30–32, 8	-, -	[395]
PHB depolymerase (48 kDa)	*Alcaligenes faecalis* T_1 (B)	Activated sludge/culture	PHB	30, 7.5	-, 7.5	[396]
PHB depolymerase	*Ralstonia pikettii* (B)	Culture	PHB, PHBV	20, 7.5	-, -	[270]
PHB depolymerase (45 kDa)	*Paecilomyces lilacinus* F4-5 (F)	Soil/culture	PHB, PHBV	27–37, 7	50, 7	[397]
PHB depolymerase (52.2 kDa)	*Diaphorobacter* sp. PCA039 (B)	Culture	PHB, PHBV	30, -	45, 8	[398]
PHB depolymerase (63.7 kDa)	*Aspergillus fumigatus* 202 (F)	Soil/culture	PHB	30, 37, 45, 7	45, 7	[399]
PHB depolymerase (20 kDa)	*Penicillium expansum* (F)	Wastewater/culture	PHB	30, 5	50, 5	[400]
PHB depolymerase	*Streptomyces* sp. SNG9 (B)	Marine/liquid culture	PHB, PHBV	30, 7	-, -	[401]

Table 2.10 (cont'd)

PHB depolymerase (45 kDa)	*Bacillus* (B), *Clostridium* (B), *Streptomyces* (B), *Alcaligenes* (B), *Comamonas* (B), *Pseudomonas* (B), *Zoogloea* (B)	Soil, lake water, activated sludge, air/liquid culture	PHB, PHV, PHBV	4–58, 4.8–10.6	29–35, 9.4	[402]
PHB depolymerase (37 kDa)	*Penicillium funiculosum* (F)	Culture	PHB	30, 5	-, 6	[403]
PHB depolymerase (48 kDa)	*Paecilomyces lilacinus* D218	Buffer solution	PHB, PHBV	30, 6.8	45, 7	[404]
PHB depolymerase	*Aspergillus clavatus* strain NKCM1003 (F)	Soil/culture	PES, PHB, PCL, PBS	30, -	-, -	[405]
PHBV depolymerase (36, 68, 72, 90 kDa)	*Aspergillus* sp. NA-25 (F)	Soil/solid culture	PHBV	30, 7.0	45, 7.0	[406]
PHBV depolymerase (43.4 kDa)	*Acidovorax* sp. HB01	Activated sludge/	PHBV, PHB, PCL	37, 6.8	50, 7	[407]
PHBV depolymerase (51 kDa)	*Streptomyces* sp. strain AF-111 (B)	Sewage sludge/culture	PHBV	30–37,	35–55, 7–8	[408]
PHV depolymerase (43.6 kDa)	*Pseudomonas lemoignei* (B)	Liquid culture	PHB, PHV	37, 8	-, -	[409,410]
Polyurethanase – lipase (28 kDa)	*Bacillus subtilis (B)*	Soil/liquid culture	PU	30, 7	-, -	[411]
Polyurethanase esterase (27 kDa)	*Pseudomonas chlororaphis* (B)	Liquid culture	PU	30, 7.2	-, 7–8	[412]

Table 2.10 (cont'd)

Polyurethanase esterase/protease (63 kDa), Polyurethanase esterase (31 kDa)	*Pseudomonas chlororaphis* (B)	Yeast extract salts medium	PU	30, -	-, 8.5 and 7	[413]
Polyurethanase protease (29 kDa)	*Pseudomonas fluorescens* (B)	Liquid culture	PU	30, 7.2	25, 5.0	[414]
Polyurethanase lipase	*Pseudomonas protegens* strain Pf-5 (B)	Liquid culture	PU	27, 7.4	-, -	[415]
Polyurethanase (66 kDa)	*Acinetobacter gerneri* P7 (B)	Liquid culture	PU	30, 7.0	37, 8.0	[416]
Polyurethanase – protease	*Alternaria solani* Ss1-3 (F)	Soil/liquid culture	PU	(20–35), (4.0–8.0)	30, 7.0	[417]
Polyurethanase – esterase and amidase	*Alicycliphilus* sp. BQ8 (B)	Liquid culture	PU	37, 7.0	-, -	[418]
Polyurethanase serine hydrolase family (21 kDa)	*Pseudomonas chlororaphis* (B), *Pestalotiopsis microspora* (E2712A, 3317B) (F), *Lasiodiplodia* sp. E2611A (F), *Bionectria* sp. strain E2910B (F), *Aspergillus niger* (F), *Pleosporales* sp. E2812A (F)	Soil/liquid culture	PU	30, -	-, -	[419]
Protease (3.4.21)	*Amycolatopsis orientalis* (A)	Liquid culture	PLLA	30–40, 7.0	-, -	[420]
Protease	*Bacillus licheniformis* (B)	Buffer solution	PLA	37, -	-, -	[258]

Table 2.10 (cont'd)

Protease	*Tritirachium album* (F), *Lentzea waywayandensis* (A), *Amycolatopsis orientalis* (A)	Culture	PLLA	30, 7	-, -	[421]
PLA degrading enzyme close related to Protease (40–42 kDa)	*Amycolatopsis* sp. strain 41 (A)	Soil/liquid culture	PLLA	37, 7.0	37–45, 6.0	[422]
Protease, esterase, and lipase	*Amycolatopsis* sp. strain SCM_MK2-4 (A)	Soil/liquid, solid culture	PLA, PCL	30, 7.0	-, -	[423]
Protease, PLA degrading enzyme	*Stenotrophomonas pavanii* CH1 (B), *Pseudomonas geniculata* WS3 (B)	Soil, wastewater sludge/liquid culture	PLA	30, -	30, 7.5 30, 8.0	[424]
Proteinase K (3.4.21.64)	-	Buffer solution	PLLA	37, 8.6	-, -	[182]
Proteinase K	-	Buffer solution	Amorphous PLLA (not crystalline PLLA)	37, 8.6	-, -	[335]
Proteinase K	*Tritirachium album*	Liquid culture	PLA	30, -	-, -	[308]
Proteinase K	-	Culture	PLLA, PES, PEA, PBS, PBSA, PCL	37, 7.0	-, -	[293]
Proteinase K	-	Culture	PLLA	37, 8.6.	-, -	[181,425]

Table 2.10 (cont'd)

Proteinase K	*Tritirachium album*	Buffer solution	PLA	37, -	-, -	[258]
(PVAase)-$Cu_3(PO_4)_2$	*Bacillus niacini* (B)	Culture	PVOH	30, 8.0	30, 7	[426]
PVOH oxidase (1.1.3.30)	*Sphingomonas* sp. (B)	Activated sludge/culture	PVOH	25, 7.5	-, -	[427]
PVOH oxidase	*Sphingopyxis* sp. PVA3 (B)	Activated sludge/culture	PVOH	30, 7.2	-, -	[428]
PVOH degrading enzyme (30 kDa)	*Pseudomonas* (B)	Buffer solution	PVOH	27, 7.3	40, 7–9	[429]
PVOH degrading enzyme	*Streptomyces venezuelae* GY1	Culture	PVOH	30, 8	-, -	[430]
PVOH degrading enzyme	*Penicillium* sp. WSH0-21 (F)	Activated sludge/culture	PVOH	30, 7	-, -	[431]
PVOH degrading enzyme (67 kDa)	*Alcaligenes faecalis* KK314	River water/culture	PVOH	30, 7.2	-, -	[432]
Serine enzyme (3.4.21) (24 kDa)	*Amycolatopsis* sp. strain K104-1 (A)	Soil/liquid medium	PLLA	37, 7.0	55–60, 9.5	[433]
Subtilisin (3.4.21.62)	-	Culture	PLA, PEA, PBS, PBSA, PCL	37, 7.0	-, -	[293]

Table 2.10 (cont'd)

Trypsin (3.4.21.4)	-	Culture	PLA, PEA	37, 7.0	-, -	[293]
Aliphatic-aromatic co-polyester degrading enzyme (27–31 kDa)	*Roseateles depolymerans* TB-87 (B)	Soil, fresh water/culture	PBS, PBSA, PCL, PBST, PES	20–40, 6–11	35, 7	[434,435]
Esterase and protease activity	*Paenibacillus amylolyticus* TB-13 (B)	Soil/culture	PLA, PBSA, PBS, PCL, PES	30, -	-, -	[436]
Esterase and amidase	-	Buffer solution	PU	37, 7	-, -	[437]
PU esterase (48 kDa)	*Pseudomonas fluorescens* (B)	Culture	PU	37, -	-, -	[438]
Lipase, manganese peroxidase, laccase	*Penicillium brevicompactum* OVR-5 (F)	Liquid medium	PVOH	28, -	30, 7	[439]
Fungal peroxidase (1.11.1.7), Laccase (1.10.3.2)	*Aspergillus* sp. (F)	Buffer solution	PU	30, 7	-, -	[440]
Esterase deacetylase (3.5.1.)	*Comamonas* sp. strain NyZ500	Activated sludge/culture	PVOH	37, -	-, -	[441]
-	*Pseudomonas aeruginosa* (B)	Culture	PU	37, -	-, -	[442]

Table 2.10 (cont'd)

-	*Nocardioides* OK12	Culture	PHB, PHBV	30, -	-, -	[443]
-	*Aspergillus flavus* (F)	Culture	PU	28, 6–6.5	-, -	[444]
-	*Aspergillus versicolor* (F)	Culture	PBSA	30, 7.2	-, -	[445]
-	*Pseudomonas chlororaphis* ATCC 55729 (B)	Culture	PU (foam)	29, -	-, -	[446]
-	*Aspergillus fumigatus* (F), *Paecilomyces farinosus* (F), *Fusarium solani* (F), *Penicillium simplicissimum* (F), *Penicillium minioluteum* (F), *Penicillium pinophilum* (F), *Penicillium funiculosum* (F)	Activated sludge soil/farm soil	PHB	28, 37, -	-, -	[243]
-	*Pseudonocardia* sp. RM423 (A)	Culture	PLA	30, 7	-, -	[226]
-	*Fusarium solani* (F), *Candida ethanolica* (F)	Compost, Soil	PU	25, 45	-, -	[447]
-	*Enterobacter* sp. IBP-VN1 (B), *Bacillus* sp. IBP-VN2 (B), *Gracilibacillus* sp. IBP-VN3 (B), *Enterobacter* sp. IBP-VN4 (B), *Enterobacter* sp. IBP-VN5 (B), *Enterobacter* sp. IBP-VN6 (B)	Seawater/culture	PHB, PHBV	27.1–30.4, 7.0–7.5	-, -	[448]
-	*Acidovorax delafieldii* (B7-7, B7-21, B7-28) (B), *Streptomyces acidiscabies* A2–21 (A), *Streptomyces griseus* A2–10 (A), *Fusarium oxysporium* F1–3 (F), *Paecilomyces lilacinus* F4–5 (F), *Paecilomyces farinosus* F4–7 (F)	Natural Soil/incubated artificial soil	PHBV	30, -	-, -	[449]

Table 2.10 (cont'd)

-	*Pseudomonas aeruginosa* (B)	Soil/liquid culture	PDLA	37, -	-, -	[279]
-	*Fusarium solani* WF-6 (F)	Soil/culture	PBS	30, -	-, -	[450]
-	*Flammulina velutipes* (F)	Culture	PVOH	28, -	-, -	[451]
-	*Aspergillus flavus* (F), *Aspergillus oryzae* (F), *Aspergillus parasiticus* (F), *Aspergillus racemosus* spp. (F)	Soil/culture	PHB, PHBV	28 – 30, 6 – 7	-, -	[452]
-	*Azospirillum brasilense* BCRC 12270 (B)	Liquid culture	PBSA	30, 7.0	-, -	[453]
-	*Aspergillus fumigatus* (F)	Compost/culture media	PCL	23, 25, 30, 37, 5.5	-, -	[178,454]
-	*Aspergillus fumigatus* (F) strain NKCM1706	Soil/culture	PBS, PBSA, PES, PHB, PCL	30, 7	30, -	[455]
-	*Leptothrix* sp. TB-71 (B)	Culture nutrient broth	PBST, PBAT	30, -	-, -	[456]
-	*Burkholderia cepacia* (B)	Culture	PLLA	35, 7	-, -	[457]
-	*Bacillus pumilus* strain 1-A (B)	Soil/Culture	PBSA, PBS, PCL	30, 7.0	-, -	[458]
-	*Bacillus* sp. JY14 (B)	Marine/culture	PHB, PHBV	30, -	-, -	[459]
-	*Pseudomonas* sp. (B)	Marine water/culture	PCL	25, -	-, -	[460]
-	*Actinomadura* AF-555 (A)	Soil/culture	PHBV	37, -	-, -	[269]

Table 2.10 (cont'd)

-	*Trichoderma viride* (F)	Soil/liquid culture	PLA	28, -	-, -	[461]
-	*Chryseobacterium* S1 (B), *Sphingobacterium* S2 (B), *Pseudomonas aeruginosa* (S3, S4) (B)	Compost/liquid culture	PLA	30, 7.2	-, -	[462]
-	*Amycolatopsis* sp. (SST, SNC, SO1.2, SO1.1) (A)	Soil/basal medium	PLLA	30, 7	-, -	[463]
-	*Amycolatopsis* sp. (A)	Culture	PLLA, PCL, PHB	30, 7.3	-, -	[464]
-	*Amycolatopsis* sp strain 3118 (A)	Soil/liquid medium	PLLA	(30, 37, 43, 48), 7.0	43, 7.0	[465]
-	*Amycolatopsis* sp. strain HT-32 (A)	Soil/liquid culture	PLLA	30, 7.0	-, -	[466]
-	*Amycolatopsis* sp. strain KT-s-9 (A)	Soil/liquid medium	PLLA	30, -	-, -	[467]
-	*Acidovorax facilis* (B), *Varivorax paradoxus* (B), *Pseudomonas syringae* (B), *Comamonas testosteroni* (B), *Cytophaga jhonsonae* (B), *Bacillus megaterium* (B), *Bacillus polymyxia* (B), Streptomyces spp. (B), *Aspergillus fumigatus* (F), *Paecilomyces marquandii* (F), *Penicillium daleae* (F), *Penicillium simplicissimum* (F), *Penicillium ochrochloron* (F), *Penicillium adametzii* (F), *Penicillium chermisimun* (F), *Penicillium restrictum* (F), *Acremonium* sp. (F)	Soil/incubated	PHB, PHBV	(15, 28, 40), (3.5, 3.9, 6.3, 6.5, 7.1)	-, -	[172]

Table 2.10 (cont'd)

-	*Acinetobacter calcoaceticus*, *Arthrobacter artocyaneus*, *Bacillus aerophilus*, *Bacillus megaterium*, *Bacillus* sp., *Brevibacillus agri*, *Brevibacillus invocatus*, *Chromobacterium violaceum*, *Cupriavidus gilardii*, *Mycobacterium fortuitum*, *Ochrobactrum anthropi*, *Staphylococcus arlettae*, *Staphylococcus haemoliticus*, *Staphylococcus pasteuri*, *Pseudomonas acephalitica*, *Rodococcus equi*, *Bacillus cereus*, *Bacillus megaterium*, *Bacillus mycoides*, *B. agri*, *Gordonia terrari*, *Microbacterium paraoxydans*, *Burkholderia* sp, *Streptomyces*, *Mycobacterium* spp, *Nocardiopsis*, *Gongronella butleri*, *Penicillium*, *Acremonium recifei*, *Paecilomyces lilacinus*, *Trichoderma pseudokoningii*,	Soil	PHB, PHBV	(26–31), -	-, -	[468]
-	*Amycolatopsis thailandensis* strain CMU-PLA07^T (A)	Soil/liquid culture	PLLA	30, -	-, -	[469]
-	*Bacillus pumilus* B12 (B)	Soil/minimal salt medium agar	PLA	30, -	-, -	[470]
-	*Kibdelosporangium aridum* (B)	Solid/liquid culture	PLLA	30, 6.6–7.8	-, -	[471]
-	*Lentzea* (B), *Saccharothrix* (A), *Amycolaptosis* (B), *Kibdelosporangium* (B), *Streptoalloteichus* (B)	Culture	PLLA	30, 7	-, -	[472]
-	*Pseudonocardia alni* AS4.1531^T (A)	Soil	PLA	30, -	-, -	[473]
-	*Saccharothrix waywayandensis* (A)	Culture	PLLA	30, 7	-, -	[474]
-	*Tritirachium album* ATCC 22563 (F)	Liquid culture with gelatin	PLLA	30, -	-, -	[475]

Table 2.10 (cont'd)

-	*Parengyodontium* (F), *Aspergillus* (F), *Penicillium* (F), *Fusarium* (F)	Soil/agar medium	PLLA, PCL	25, 7.0, 6.0	-, -	[476]
-	*Stenotrophomonas maltophilia* LB 2-3 (B)	Compost/sturm test	PLLA exposed to UV irradiation	37, 7	-, -	[242]
-	*Mortierella* sp. (F), *Doratomyces microsporus* (F), *Fusarium solani* (F), *Fennellomyces* sp. (F), *Aspergillus fumigatus* (F), *Verticillium* sp. (F), *Lecanicillium saksenae* (F), *Cladosporium* sp. (F), *Trichoderma* sp. (F)	Compost, soil	PLLA	25, 7.2	-, -	[477]
-	*Bordetella petrii* PLA-3 (B)	Compost	PLLA	30, 37, 7.0	-, -	[239]
-	*Flammulina velutipes* (F)	Quartz sand/culture	PVOH	28, -	-, -	[451]
-	*Bacillus cereus* RA 23 (B)	Oil sludge/culture	PVOH	30, 7.0	28, 7	[478]
-	*Bacillus* sp. (B), *Curtobacterium* sp. (B)	Sewage sludge/culture	PVOH	35, 8.0	-, -	[479]
-	*Eutypella* sp. BJ (F)	Soil compost/culture	PVOH	30, -	-, -	[480]
-	*Geomyces pannorum* (F), *Phoma* sp. (F)	Soil/solid culture	PU	< 25, 5.5, 6.7	-, -	[481]
-	*Geomyces* sp. B10I (F), *Fusarium* sp. B3'M (F), *Sclerotinia* sp. B11IV (F)	Antarctic soil/liquid culture	PCL, PBS	(14, 20, 28), -	-, -	[282]

2.15 Extracellular enzymes

Figure 2.13 depicts the main extracellular enzymes reported for depolymerization of aliphatic and aliphatic/aromatic polyesters, PUs derived from ester, where the ester bond cleavage is considered as the rate-determining step, [104]; and PVOH. These enzymes belong to the esterase (EC: 3.1) and peptidase (EC: 3.4) groups of the main group hydrolases (EC: 3); and oxidoreductases (EC 1).

Enzymes like cutinases, esterases, lipases, and PHA/PHB depolymerases are the main extracellular enzymes for enzymatic hydrolysis of the ester group and belong to the α/β hydrolase family that are structurally similar but with diverse functionality [173,482,483]. The natural activity of the esterase group of enzymes is the hydrolysis of lipids. Proteases are the main group for enzymatic degradation of the peptidase group. For polyurethanes, various esterases, proteases, amidases (EC: 3.5.1.4), and ureases (EC: 3.5.1.5) also have been reported to induce enzymatic degradation. In this case, esterases are involved in ester scission, and ureases are more inclined to scission of urethane bonds and are more resistant to chemical and enzymatic hydrolysis [101]. In the case of PVOH, and also some PU, an oxidative pathway prior to the hydrolytic enzymatic degradation has been reported, and the main extracellular enzymes are the oxidoreductases (EC: 1).

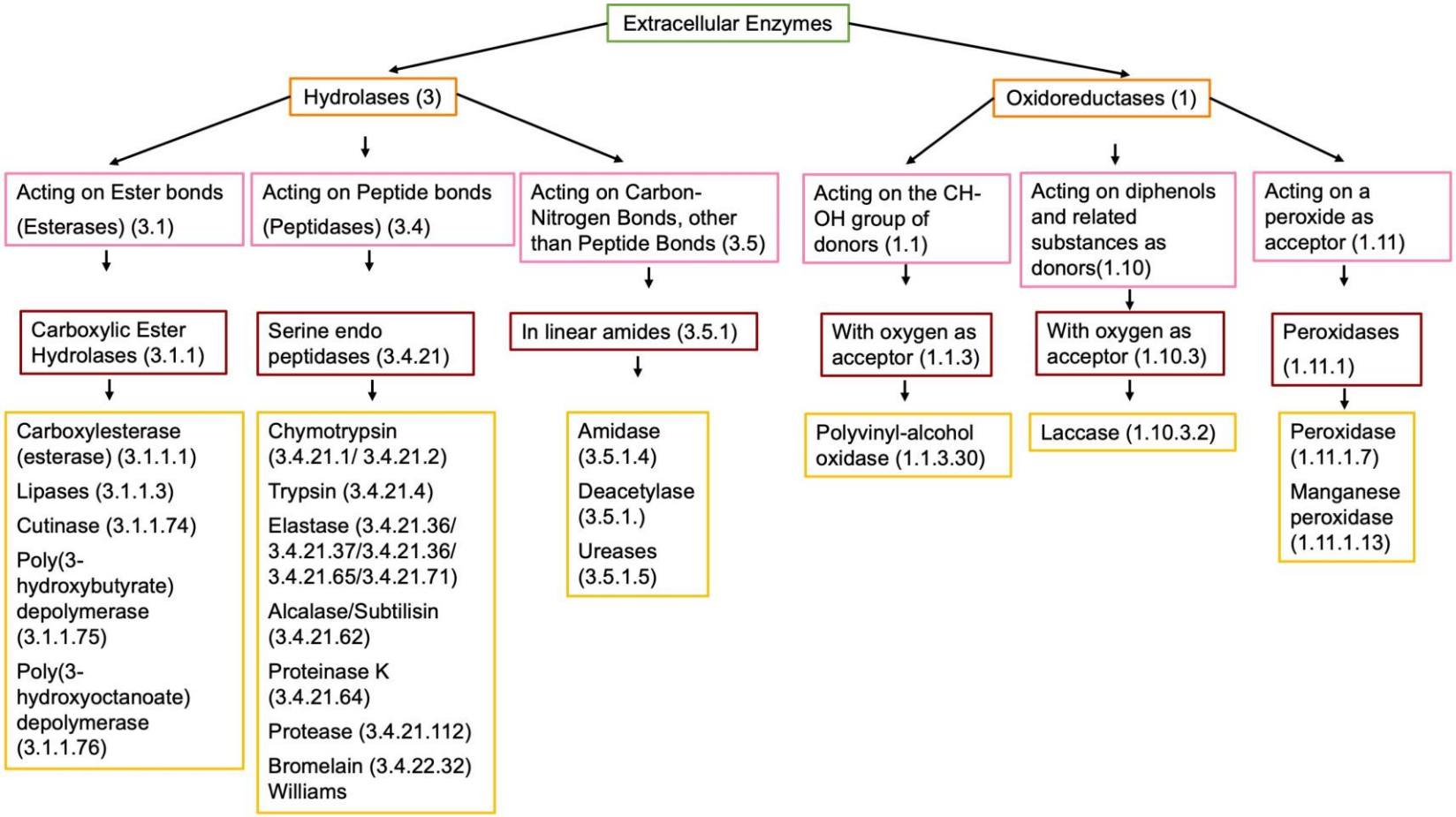


Figure 2.13 Classification of the main extracellular enzymes reported for enzymatic activity of aliphatic and aliphatic/aromatic polyesters, PUs derived from esters, and PVOH. The numbers in parentheses are the enzyme codes according to the Enzyme Commission (EC) nomenclature [102].

2.15.1 Carboxylesterases

In general, carboxylesterases (3.1.1.1) are reported as esterases, creating some confusion in the literature since the main group classification (3.1) is esterases. Since the natural function of esterases is the hydrolysis of lipids, for polymer attack they need a hydrophobic surface to be activated for scission of ester bonds. Carboxylesterases, in general, act hydrolyzing short-chains (C < 10) and present a lid domain that covers the active site. The lid domain (present also in lipases), when binding to the hydrophobic substrate, opens the active site to promote the catalysis. The lid domain structure is important since some differences can determine the specificity of the enzymes toward some substrates. Disulfide bonds is not present in carboxylesterases [484]. Hajighasemi et al. reported the action of carboxylesterases from *Alcanivorax borkumensis* and *Rhodopseudomonas palustris* on PLA and other polyesters; the enzymatic endo and exo activity resulted in the production of oligomers, dimers, and monomers of PDLLA but did not show activity for PDLA or PLLA [292].

2.15.2 Lipases

Lipases (3.1.1.3) are water-soluble extracellular enzymes reported to show enzymatic hydrolysis activity for several biodegradable polymers such as PLA (PLLA and PDLA), PCL, PBS, PBSA, PBAT, PBA, PEA, and PU esters. The typical structure of lipases is a protein structure covered by a lid-like structure. Similar to carboxylesterases, lipases need a hydrophobic surface to be activated since its natural function is the hydrolysis of lipids; increased lipase activity is observed when a hydrophobic substrate starts to form an emulsion due to its contact with a hydrophilic aqueous medium [482,485]. However, the difference respect to carboxylesterases is that lipases prefer to break down long chains (C > 10). Lipases are unable to hydrolyze ester bonds in intermediates that become water soluble [111,173]. However, Rizzarelli and Impallomeni reported some ability of lipases to hydrolyze

dissolved esters in water solution [486]. Such findings indicate that the nature of the polymer could be more important than the stereo chemistry in the vicinity of the ester bond for substrate preference by enzymes with lid-like structures, such as lipases. Also, some works have reported that lipases act preferentially by random chain scission, showing an endo-type behavior where M_w reduction is highly affected in comparison to end chain scission [487].

In the case of lipases, the active site is found in a deep cavity of the protein structure. This is shielded by a lid-like α-helical structure that is reoriented when in contact with the substrate. The degree of freedom of polymer chains to move is a key factor in controlling the hydrolytic depolymerization of polyesters. This mobility ensures that the polymer chain can fold itself and fit in the active site of the lipase enzyme to carry out the depolymerization [482]. Hence, polyesters must be mobile enough to reach the active site of the lipase, making thermal and conformational properties other key factors for depolymerization since exposure temperature controls the mobility of polymer chains [173]. In general, lipases require a hydrophobic surface to reach full hydrolytic activity. For this reason, lipases are not likely to be observed developing high enzymatic activity for the PHAs family of aliphatic polyesters. The molecular weight of lipases from bacteria has been reported in the range of 20 to 77 kDa [482], thus their size allows activity on the surface of polymers.

2.15.3 Cutinases

Cutinases (3.1.1.74) are hydrolytic enzymes considered the smallest members of the α/β hydrolase superfamily (20–25 kDa) [488,489]. Enzymatic activity for cutinases has been reported for several biodegradable polymers. Cutinases are mainly produced and released by fungal pathogens and are able to degrade the polyester cutin, a natural crosslinked lipid polymer composed of n-C_{17} and n-C_{18} hydroxy and epoxy fatty acids, present in plant cell

walls and insoluble in water. However, some bacteria can also produce cutinases [490]. Cutinases are able to show enzymatic activity without needing interfacial activation like lipases and are capable of being active in both soluble and emulsified substrates [490], primarily due to the absence of the hydrophobic lid that covers the active site. Furthermore, the active site of cutinases is considered large enough to locate and catalyze even high M_w polyesters [491]. Cutinases act mostly against aliphatic polyesters; however, results for aliphatic-aromatic polyesters are scarce. A comparative study of five extracellular cutinases released by five species of microorganisms found that some cutinases are more stable and have higher activity towards polymer substrates than others; the higher stability and efficiency were related to additional disulfide bond formation [294]. In general, the presence of covalent disulfide bonds and neutral charge in the crowning area of the active site provides extra stability to the tertiary structure by linking regions of proteins. The presence of disulfide bonds in a cutinase was also reported by Liu et al. [305], and together with a favored catalytic triad resulted in improved activity, enhanced thermostability, and higher activity towards PCL. A cutinase (21.6 kDa) from the fungus *Aspergillus oryzae* was able to degrade PBS and PBSA and also showed low activity for PLA [183]. Furthermore, PCL was reported to be an optimal substrate for cutinases [303].

A study on the effect of pH on the surface charge of the area around the active site of cutinases reported that the active site becomes more positive as pH decreases from alkaline to acidic values, resulting in lower activity towards polymers such as PCL [294]. Electrostatic surface potentials generated by charged residues affect the enzyme/substrate interaction, transition stage stabilization, and efficiency during the product release stage [294]. Similar results were reported from the interaction of cutinase and PBS; the release of acidic monomers from PBS affected the pH and the activity of the cutinase, lowering the degradation rate of the PBS films [296,299]. The presence of the cofactors Ca^{2+}, Na^{+}, and K^{+}

increased the activity of cutinases towards polymers such as PCL, PBS, and PBSA; however, the cofactors Mg^{2+} or Zn^{2+} did not show a significant effect or significantly inhibited the activity of the enzymes [295,297,298].

2.15.4 PHA, PHB depolymerases

PHA and PHB depolymerases (3.1.1.75 and 3.1.1.76) are produced by microorganisms and accumulate within the cells as an intracellular carbon and energy storage. Thus, they can undergo enzymatic degradation by functioning intra or/and extracellular. PHB depolymerases (3.1.1.75) show activity against short-chain length PHAs as PHB, PHV, and PHBV, while others (3.1.1.76) show more depolymerization of medium-chain length PHAs [489]. The primary structure of PHA depolymerases is formed by two functionally domains, a catalytic domain and a substrate binding domain, and is activated by the presence of Ca^{+2} and Mg^{+2} or inhibited by Cu^{+2}, Fe^{+2}, Mn^{+2}, and Hg^{+2} [489]. Inhibition of enzymatic hydrolysis due to the presence of detergents highlights the likely presence of a hydrophobic region near the active site of PHA depolymerases [489]. Furthermore, PHA depolymerases are reported to have exo and endo behavior since they were able to release monomers and oligomers [489]. The extended presence of hyphae due to the colonization of fungi on PHBV surface has been reported as evidence of enzymatic degradation by extracellular PHAs depolymerases released by fungus [449]. In terms of the structure, some PHA depolymerases are reported to belong to the serine esterases group due to the presence of lipase boxes [386]. Even though PHA depolymerases are specific for PHAs, enzymatic activity has been reported also for other polyesters.

The presence of additional carbon sources may reduce the enzymatic activity against polymers. For example, the reduction of PHA depolymerase produced by *Aspergillus* sp. showed a repression apparently influenced by the type of carbon source added to the media, which was indicative of a regulated behavior as a function of the available carbon source

[452]. This finding is in accordance with the hypothesis that when abundant labile nutrients are present, the decomposition of more recalcitrant compounds is inhibited [107]

2.15.5 Peptidases (Proteinase K and Protease)

Peptidases (3.4), a group of enzymes acting on peptide bonds, are also commonly called proteases, generating some confusion in the literature. Peptidases or proteases hydrolyze peptide bonds that link amino acids in a protein. For example, Proteinase K (3.4.21.64) and proteases (3.4.21.112) belongs to the serine endo peptidases (3.4.21), enzymes that preferentially catalyze bond scission in the middle of the substrate chain. Also, it has been reported that enzymes belonging to the serine endo peptidases are able to hydrolyze polyesters like PLA. Proteinase K and proteases have been identified for major enzymatic activity on PLA. Lim et al. [293] reported the ability of Proteinase K to depolymerize PES, PEA, PBS, PBSA, and PCL but at lower levels of enzymatic activity than on PLA. More specifically, Proteinase K showed a higher enzymatic activity for PLLA (amorphous preferentially) than for PDLA and PDLLA.

The activity of these classes of enzymes towards PLA is still not fully understood in the sense that these enzymes are more prone to attack the scission of peptide bonds. Tokiwa and Jarerat [492] concluded that enzymes showing activity on PLA belong to the peptidases or protease-type group, and these enzymes are able to recognize the repeated L-lactic acid unit of PLA as the natural homologue L-alanine unit of silk fibroin, a natural protein present in silk. Later work by Lim et al. [293] reported the enzymatic ability of serine proteases on PLA, PHB, PES, PEA, PBS, PBSA, and PCL; in particular, alpha-chymotrypsin, a mammalian enzyme, showed preferential activity for PLA. In studies on PLA biodegradability, the incorporation of agents to the media, such as silk fibroin or gelatin, as a nitrogen source to induce production of protease, has resulted in increased enzymatic activity for PLA since proteases are more prone to interact with peptide bonds [424].

2.15.6 Amidases and ureases

In addition to esterases and proteases, PUs derived from esters can be enzymatically degraded by amidases (3.5.1.4) and ureases (3.5.1.5). Amidases attack the amide groups, proteases can attack amide and urethane bonds, esterases attack the ester bonds, and ureases catalyze the hydrolysis attack of the urea groups. However, the information in terms of amidases and ureases showing enzymatic activity towards PUs derived from esters in mesophilic environments is scarce [493–496].

2.15.7 Oxidoreductases PU and PVOH-oxidases

Oxidoreductases (1) have shown activity for PVOH and PU within the groups EC 1.1, EC 1.10, and EC 1.11. More specifically 1.1.3 (with O_2 as electron acceptor), polyvinyl-alcohol oxidase (1.1.3.30) or dehydrogenase have shown enzymatic activity on PVOH; these are enzymes that can act extra or intracellular. Laccase (EC 1.10.3.2) has been reported to show enzymatic activity against both PVOH and PU.

2.16 Biosurfactants and synthetic surfactants

Biosurfactants are amphipathic molecules with the capacity of reducing surface and interfacial tension between liquids, solids, and gases. They contain both hydrophilic and hydrophobic moieties that can improve the interaction between phases of different degree of polarity and hydrogen bonding [497,498]. Microorganisms are capable of synthesize and release biosurfactants such as glycolipids and phospholipids to emulsify the substrate and stimulate other functions as extracellular enzymatic activity [499].

Hydrophobins are a type of amphipathic surfactant secreted by fungi microorganisms, that besides other functions, they attach to the surface of biodegradable polymers and stimulate the hydrolysis by extracellular enzymes. They present a dual behavior with hydrophobic and hydrophilic parts (amphipathic proteins), and are adsorbed to the surface of the polymer, condensing, and stimulating its enzymatic hydrolysis by

recruiting extracellular enzymes [500,501]. Hydrophobins are important to support the growth of fungal aerial structures (hyphae) and conidiospores by playing an important role for fungal adhesion to hydrophobic surfaces, development of a protective surface coating, and reduction of water tension [500,501].

Synthetic commercial surfactants are widely used during studies of extracellular enzymatic activity on polymers and are classified in function of the nature of their polar grouping. It has been reported the use of ionic and nonionic surfactants. Some common synthetic nonionic surfactants are added to culture studies for emulsification, like commercials ones as polysorbate 80 and polyoxyethylene type, to increase microbial activity on the polymer surface by increasing the hydrophilicity of the surface [109].

Interaction between surfactants and enzymes is still a subject of exploration. Holmberg mentioned that probably nonionic surfactant are more benign than ionic surfactants. The way in what ionic surfactants interact with enzymes can introduce significant changes in the conformational structure of the enzyme [502]. Detailed discussion about bio and commercial surfactants can be found elsewhere [497,498,502,503].

2.17 Polymers susceptible to biodegradation

The main group of biodegradable polymers susceptible to biodegradation in the mesophilic range are the aliphatic and aliphatic-aromatic polyesters; besides that, the soft segment of PUs derived from esters and PVOH are also considered biodegradable to some extent. Commercialized cellulose and starch-derived polymers, which are bio-based and naturally biodegradable, are also important to consider when discussing biodegradable polymers. **Figure 2.14** shows the tentative pathways of the most common bio- or fossil-based polymers reported to undergo depolymerization by specific microorganisms. These polymers are reviewed individually in this section.

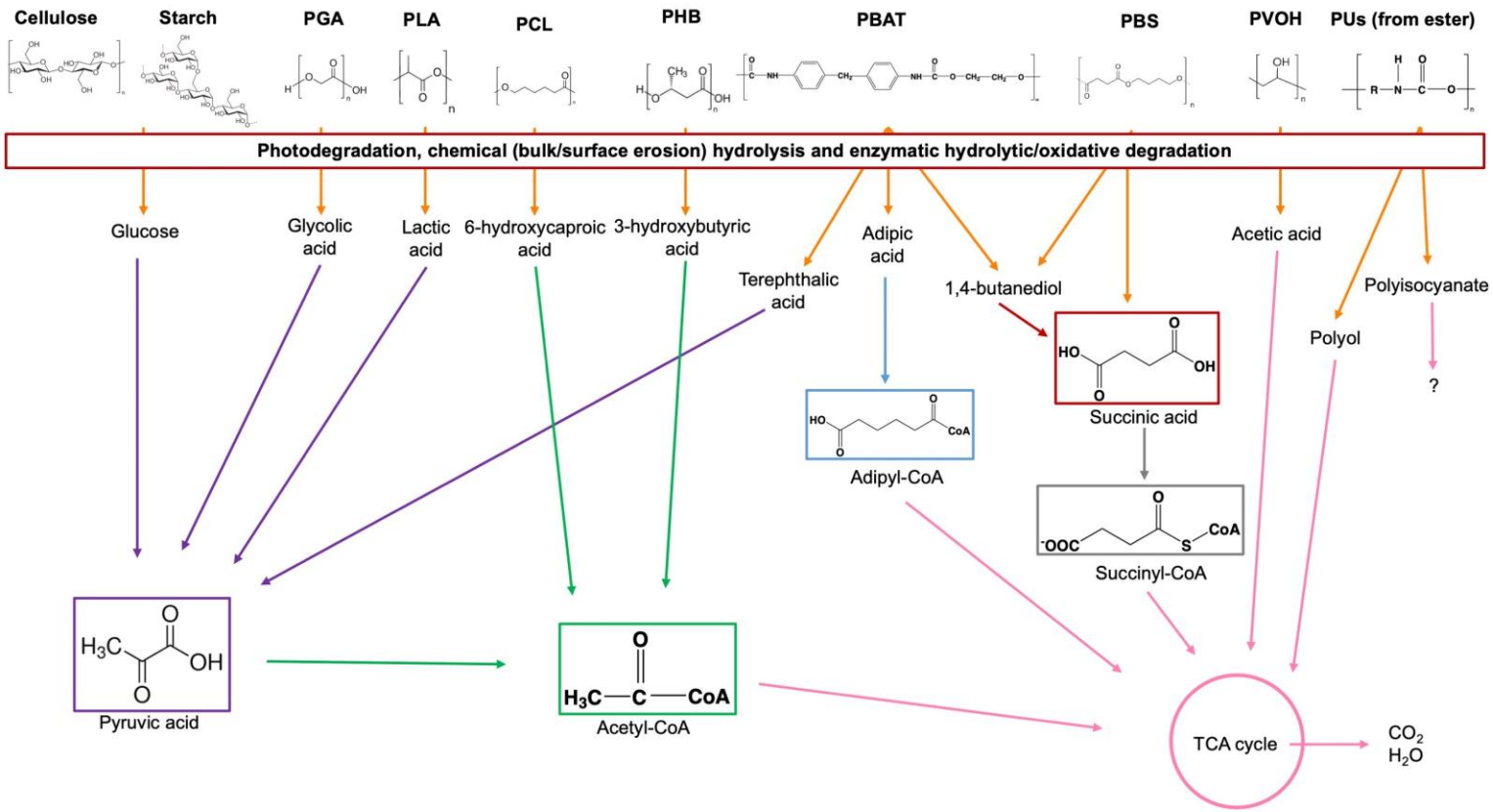


Figure 2.14 Main tentative biodegradation pathways for biodegradable polymers in aerobic conditions.

2.17.1 Cellulose

Cellulose is a linear homopolymer of D-glucose units joined by β–1,4 glycosidic linkages, with a degree of polymerization ranging from several hundreds to over 10,000 [504]. Each glucose molecule is upside down in relation to the neighboring glucose molecule so that the repeating unit is cellobiose, consisting of two glucose molecules linked by a β–1,4 glycosidic bond. The fibrils of cellulose can have crystalline and amorphous regions. Depending on the origin and treatment, the crystallinity of cellulose can vary from fully amorphous to fully crystalline. Higher crystallinity makes cellulose resistant to chemical attacks. In the secondary wall of plant cells, cellulose forms several sheets organized as parallel microfibrils. These microfibrils are embedded in the matrix of hemicellulose and lignin. Pure cellulose is available in several forms, such as cotton and filter paper. Before 1950, cellulose-based polymers were one of the most important groups of polymers. Cellulose nitrate, the oldest plastic, was produced by replacing nitrates on all three hydroxyl groups of the cellulose glucose units. Several other cellulose ether and ester thermoplastics, such as cellulose acetate and cellulose butyrate, have been produced through the years [505]. The main cellulose ethers are: methylcellulose (MC) – non-thermoplastic, water-soluble with high O_2 barrier, generally used as filler and thickener agent; carboxymethyl cellulose (CMC) – hydrophilic, non-thermoplastic, generally used as a viscosity modifier and thickener; hydroxypropyl cellulose (HPC) – thermoplastic, with water barrier and grease resistance, generally used for coatings, and as binder and thickener; and hydroxypropyl methyl cellulose (HPMC) – non-thermoplastic, non-heat-sealable, generally used for coating purposes. Cellulose esters are thermoplastic, and they are produced by the reaction of organic or inorganic acid substituting the hydroxyls of the glucose unit. The prominent cellulose esters include cellulose acetate (CA), which is thermoplastic, used for molding and extrusion, and

can exist in several forms such as cellulose acetate butyrate (CAB), cellulose acetate propionate (CAP), and cellulose triacetate (CTA) [505].

Cellulolytic and non-cellulolytic mixed populations of microorganisms are present where cellulosic waste is present. These microorganisms interact synergistically to complete the biodegradation of cellulose, which is ultimately converted to CO_2 and H_2O in aerobic environments through the pathways shown in **Figure 2.14**, and to CO_2, CH_4, and H_2O in anaerobic environments.

Cellulose, as well as starch, is enzymatically hydrolyzed to glucose by extracellular enzymes, which are produced by bacteria and fungi (**Figure 2.15**). Natural polymers, such as cellulose and starch, are mostly attacked for enzymatic hydrolysis by cellulases and α/β amylases. In addition, oxidoreductases have been identified that can act prior to hydrolytic enzymes on cellulose [506].

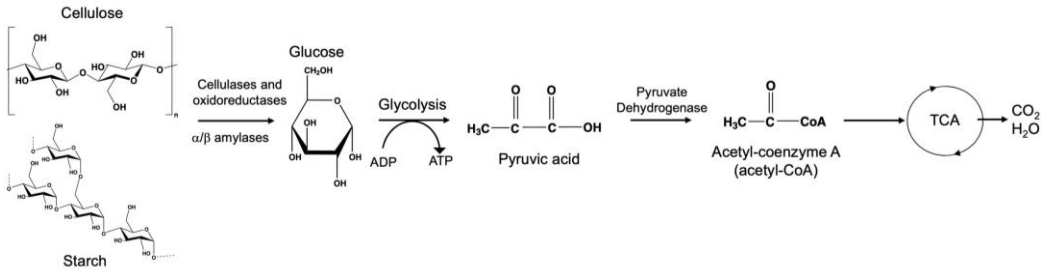


Figure 2.15 Pathway for enzymatic degradation, bioassimilation and mineralization of the natural polymers, cellulose and starch.

After glucose is produced, glycolysis converts the glucose to pyruvic acid, which acts as the precursor for the TCA cycle. Glucose, together with the adenosine triphosphate (ATP), which is the molecule providing the energy source in the cell plus NAD^+, and inorganic phosphate, breaks down into two pyruvates. In the pyruvic acid cycle, **Figure 2.16**, three main steps take place. First, a carbonyl group is removed from pyruvic acid, releasing CO_2 to the surrounding media and resulting in a two-carbon hydroxyethyl group bound to the

enzyme pyruvate dehydrogenase. Second, the hydroxyethyl group is oxidized to an acetyl group and the electrons are picked up by the NAD^+ (nicotinamide adenine dinucleotide), forming NADH. This electron will later be used by the cell to create energy through the ATP process. And third, the enzyme bound to the acetyl group is transferred to CoA, producing a molecule of acetyl CoA. This molecule is then further converted through the TCA cycle [86].

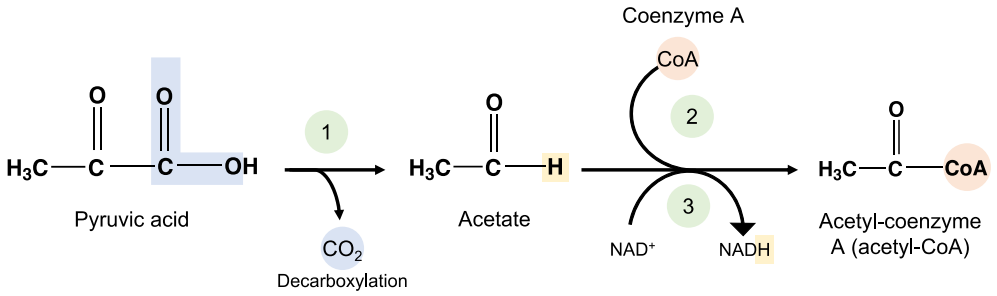


Figure 2.16 Pyruvic acid to Acetyl-CoA reaction pathway. Adapted from [86].

Cellulose biodegradation occurs primarily by cellulolytic microorganisms belonging to the bacteria and fungi. Aerobic biodegradation of cellulose occurs mostly by cellulolytic bacteria; several species identified in the genera *Cellulomona*, *Pseudomona*, *Thermomonospora*, and *Microbispora* have been shown to biodegrade cellulose [507].

Cellulose undergoes biodegradation in several environments. Amorphous forms of cellulose are used as positive controls for biodegradation studies due to their negligible chemical hydrolysis and rapid enzymatic hydrolysis rates and assimilation by microorganisms. In thermophilic and mesophilic environments, such as industrial composting or soil biodegradation, cellulose is widely used as a positive control, as stated in ASTM and ISO standards (**Table 2.8**). In marine environments, mineralization of cellulose powder was reported to reach ~95% after 450 days of testing at 25 °C [233], indicative of its high biodegradability in aquatic environments. Anunciado et al. [217] used cellulose in the form of a mulch paper, instead of powder, as a positive control in soil and composting

conditions; after 365 days of testing mineralization values were in the range of 50 to 80% for samples in soil at 27 °C.

2.17.2 Starch

Low-cost starch, mainly obtained from crops not intended for human consumption, is a bio-based material that can be blended with other polymers to produce novel bio-based and biodegradable blends. Starch consists of two main molecules making up the constitutional unit: amylose (linear) and amylopectin (branched). Starches with high amylose content have been used to produce suitable blends and to improve the thermal, mechanical, and gas barrier properties of the resulting blends [508–512]. The T_g of pure starch is above its decomposition temperature, meaning the material does not soften and flow. To make it processable, starch needs to be combined with plasticizers such as glycerol, poly(ethylene glycol) (PEG), or sorbitol to obtain thermoplastic starch (TPS). The starch granules are plasticized by using plasticizers under heating, which provides a viscous melt that can then be processed using traditional methods such as extrusion foaming and injection molding [513]. TPS is highly hydrophilic, resulting in leaching of plasticizer during storage and poor dimensional stability and mechanical properties with time [514]. However, TPS can be used to blend with other bio-based polymers, improving O_2 barrier and elongation at break due to the presence of glycerol [515–522]. Since the properties of TPS by itself are not sufficient for producing polymeric structures for some applications, the possibility of blending TPS with other polymers to improve its mechanical and water barrier properties has opened a wide field for the development of novel TPS blends, with reactive functionalization as one of the suitable methods to enhance the compatibilization of TPS [512].

Since starch is sensitive to water, starch or the portion of blends containing TPS will mostly hydrolyze by enzymatic hydrolysis to glucose. The main extracellular enzymes

involved during enzymatic degradation of starches are α/β-amylases (**Figure 2.14**). The general pathways for biodegradation and bioassimilation/mineralization of starch are shown in Figure 2.15 and Figure 2.16, respectively.

Starch, TPS, or TPS blends with other biodegradable polymers have shown high production of CO_2, which is indicative of the high biodegradability of TPS even in mesophilic environments. Ho and Pometto [118] reported values of mineralization of ~70% for starch at 28 at 40 °C in a soil environment under laboratory conditions after 180 days of testing. The main characteristic was rapid initial degradation at 40 °C, with a negligible abiotic phase of degradation, reaching the plateau stage at around day 60; lower activity was observed at 28 °C, reaching the plateau stage at around day 100.

2.17.3 Poly(glycolic acid) – PGA

PGA, the simplest aliphatic polyester, is a biodegradable and biocompatible thermoplastic, extensively used for many decades in the medical field for implants [523]. PGA can be synthesized using many mechanisms. Direct polycondensation polymerization of glycolic acid results in low M_w PGA (M_w < 50 kDa). Ring opening polymerization of glycolic acid results in high M_w PGA (M_w >50 kDa). Solid-state polycondensation is used to increase the M_w by increasing the polymer chain lengths in the absence of heat and O_2, by constant removal of byproducts using inert gas or under vacuum [524,525].

PGA has a T_g in the range of 35 to 40 °C and T_m between 220 to 230 °C (**Table 2.5**) [526]. PGA displays good gas barrier properties due to its crystalline and stereochemistry structure [523]. PGA is also resistant to most organic solvents. In addition, the high density of c. 1.53 g/cm^3 awards PGA good mechanical properties compared with other biodegradable polymers; however, the high cost associated with the PGA production process has hampered its entry into the consumer market as compared to other biodegradable polymers [523]. In

general, PGA is blended with other polymers to improve their properties. For example, when PGA is blended with PLA, the result is better mechanical properties and improved flexural modulus of the PLA/PGA blend [527]. Due to its high O_2 and H_2O barrier properties, PGA can be used in packaging of products sensitive to O_2 [528]. PGA is widely used in biomedical applications such as sutures, drug delivery, and tissue engineering [529].

PGA degradation starts by abiotic degradation, and chemical hydrolysis is by a non-specific chain scission of the ester backbone, with bulk erosion as the dominant mechanism (**Figure 2.14**) [50,523]. Therefore, water diffusion activated by temperature plays a crucial role in the initial hydrolysis of the ester backbone. The absence of asymmetrical methyl groups turns PGA more hydrophilic than PLA, increasing its bulk chemical hydrolysis rate. Currently, there is limited published information on PGA depolymerization by enzymatic activity in the mesophilic range in open environments. Since PGA has been used mainly for biomedical applications, most of the biodegradation data is from *in vivo* studies at 37 °C. Extracellular enzymes like esterases have been reported to have enzymatic activity on PGA sutures [310]. After the initial chemical and enzymatic hydrolysis, PGA is degraded into small oligomers and glycolic acid, which can be bioassimilated and oxidized to become a substrate for the TCA cycle, as shown in **Figure 2.17**.

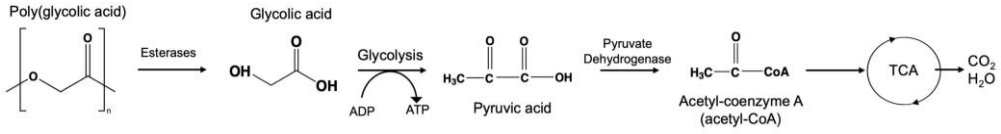


Figure 2.17 Biodegradation pathway for PGA in aerobic conditions.

In terms of CO_2 evolution and mineralization studies, biodegradation of PGA in a marine environment at around 30 °C, which is high for marine environments, showed a longer lag phase than for cellulose, but 75% mineralization was reached at 28 days for both PGA and cellulose [523]. At thermophilic conditions in a simulated industrial composting

environment at 58 °C, PGA showed lower mineralization than cellulose; 70% mineralization was reached at around day 40 for cellulose and at around day 70 for PGA [523].

2.17.4 Poly(lactic acid) – PLA

PLA, a biodegradable aliphatic polyester, is a widely used alternative for conventional fossil-based plastics. In addition to PLA being biocompatible, biodegradable (compostable), its production from renewable resources results in energy savings and lower greenhouse gas (GHG) emissions [530]. The building block for PLA is lactic acid or lactide, which is derived from the fermentation of glucose obtained from varied sources such as corn and sugar cane. Lactic acid has two enantiomers: L-lactic and D-lactic acid [531]. Lactide can be produced in three stereochemical configurations: L, L-lactide; L, D-lactide, and D, D-lactide. High M_w PLA is obtained by ring opening polymerization of the different lactides and polycondensation of low M_w lactic acid [532,533]. PLA presents acceptable thermal, mechanical and barrier properties and its main applications include food and medical product packaging, medical devices, fibers, textiles, plasticulture, and automotive parts [532]. The ratio of L-lactic and D-lactic acid in a final PLA formulation plays a crucial role in its final properties and degradation rate [532–534].

The hydrolysable ester bonds in the backbone of PLA structure (**Table 2.5**) makes it susceptible to chemical hydrolysis. Several mechanistic, phenomenological, and probabilistic models have been developed for PLA and can be extended to other aliphatic polyesters, explaining how diffusion and geometric properties can modify the pathways and incentivize one or the other mechanism [75]. The mechanism proceeds in different stages, starting with water diffusion into the material, followed by the degradation of amorphous regions. After degradation of the amorphous regions, the random chain scission and cleavage of ester bonds results in the release of soluble oligomers and monomers [62], which can be used as substrates for bioassimilation (**Figure 2.18**). The hydrolysis rate of PLA, as well as other

polyesters, is highly dependent on temperature (below or above T_g), pH, and several other properties of the polymer such as M_w and crystallinity, as reviewed elsewhere [64]. In the absence of other factors accelerating other mechanisms, chemical hydrolysis is the most important mechanism in the mesophilic range for the abiotic process.

The degradation activity of PLA by microorganisms has been monitored by different methods and correlated to different biodegradation stages. The crystal structure change or biofilm formation for PLA degraded in a compost environment was observed using SEM [239]. Weight loss indicating depolymerization of PLA was measured by size exclusion chromatography [424], the degree of biofragmentation of PLA fibers was monitored by X-ray diffraction (XRD) [290], and the generation of lactic acid was detected using an enzymatic bioanalysis kit [475].

The biotic degradation stage implies enzymatic activity and microbial assimilation. The enzymatic degradation of PLA involves interaction of the polymer with a reagent, such as water, in the hydrolysis reaction. Hydrolases, such as proteases and esterases, catalyze the hydrolysis reactions. When lactic acid becomes available for bioassimilation, it is transported through the semipermeable membrane and is oxidized to pyruvic acid through a dehydrogenization reaction, which then follows the pyruvic acid pathway (**Figure 2.18**), as previously described.

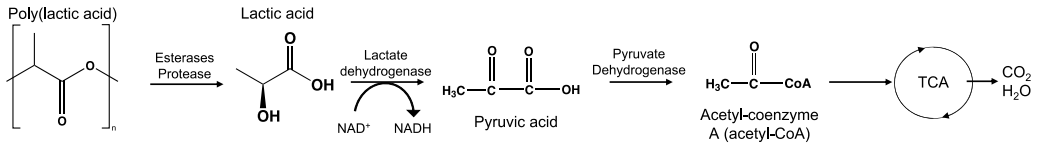


Figure 2.18 Biodegradation pathway for PLA in aerobic conditions.

Various bacteria, fungi and actinomycetes strains have been identified as having some ability to degrade PLA in different forms such as pellet, film, powder, and sheet. These

microorganisms were isolated from different environments, such as soil, compost, wastewater sludge, by enrichment culture media, while some were procured from research facilities. The extracellular enzymes secreted by these microorganisms have been reported to preferentially degrade the amorphous regions of PLA, since the backbone chains are highly disordered and have higher mobility as compared to the crystalline region. This flexibility and mobility aids in the binding of the backbone chain to ensure a fit into the active site of the enzyme [323]. The extracellular enzymatic activity efficiency is dependent on the type of PLA (PLLA, PDLA, or PDLLA) as well as the temperature, crystallinity, and M_w of the PLA [239,535].

The enzymatic degradation of PLA involves the hydrolases, with esterases (3.1) and peptidases (3.4) as the main groups of enzymes. Carboxyl esterases ABO2449 and RPA1511 (3.1.1.1) have been reported to hydrolyze PLLA and PDLA with the highest activity for ABO2449 at 30 to 37 °C and for RPA1511 at 55 to 60 °C [292]. The analysis of the hydrolysis suggested that, like other hydrolases (e.g., nucleases and proteases) that are active in depolymerizing polymeric substrates, these enzymes can exhibit both exo and endo-esterase types of cleavage [292]. Several esterases (3.1) able to degrade PLA such as lipases, cutinases, and carboxyl esterases. Peptidases (3.4) have been reported to be able to degrade PLA in culture media. For example, Proteinase K (3.4.21.64) has been shown to be efficient during scission of polymer chains, favoring the hydrolysis of the amorphous region of PLLA [536]. The enzymatic degradation of PLA revealed the preferential activity of proteases for PLLA and for PDLA of lipase/cutinase/esterase type. The enzymatic activity of lipase on PLLA was affected by the addition of Na^{+} and K^{+} that increased the activity. However, Zn^{+2}, Mg^{+2}, Cu^{+2}, and Fe^{+2} showed inhibition of the enzymatic activity [341]. Furthermore, the presence of anionic surfactant showed a significant inhibition of Proteinase K activity towards PLLA [293]. However, the presence of the same anionic surfactant showed a dual behavior during

enzymatic activity of α/β hydrolases on PDLLA, facilitating the binding of carboxylesterases on PDLLA and also reducing the hydrolytic activity by lipase-like esterases [292,537]. Nonionic surfactant also reduced the enzymatic activity towards PDLLA by lipase-like esterases [537]. Several studies have reported the importance of factors as stereochemistry, crystallinity, and hydrophilicity on the enzymatic degradation of PLLA, PDLA, and PDLLA mostly by action of Proteinase K [538–543].

Some biodegradation studies for PLA in the mesophilic range and different environments have reported high values of mineralization while other studies have reported low values of CO_2 evolution or mineralization. Ho and Pometto reported values of mineralization for three types of PLA films in soil environments under laboratory conditions; after 180 days of testing, mineralization values ranged from 10 to 45% at 28 °C and from 30 to 90% at 40 °C, depending on the film [118]. The effect of temperature also can be observed in the work of Muniyasamy et al., where mineralization values for PLA films in soil environment at c. 25 °C were a negligible 5% after 190 days of testing [225]. Biodegradation studies by Kim et al. in compost showed different mineralization values for PLA with different M_w and crystallinities after 40 days of testing [239]. The dependence of enzymatic activity on the initial M_w of PLA was evident, with mineralization values of c. 70% for low M_w PLA (5 kDa) and c. 30% for higher M_w PLA (34 kDa). The same study reported a similar trend for different levels of crystallinities at 30 °C, with reduced biodegradation rates for PLA with high crystallinity [239].

In aquatic environments, biodegradation of PLLA granules resulted in mineralization value of c. 10% after 180 days of testing at 25 and 37 °C [238]. After 50 days of testing, mineralization values had reached a similar plateau at both temperatures. Lower

mineralization values are indicative of limited chemical and enzymatic hydrolysis in aquatic conditions for PLA.

When PLA (initial M_w c. 188 kDa) powder was exposed to UV irradiation and studied by using the Sturm test and in compost for 40 days, the highest mineralization values were found in both the Sturm test (c. 20%) and compost (c. 45%) for samples treated for 8 hours; longer UV irradiation treatment times resulted in decreased mineralization values after 40 days of testing [242]. The authors stated that a Norrish reaction was not identified as the main effect for reduced biodegradation with longer UV irradiation time, leaving the presence of crosslinking as the most probable one.

Biodegradation of PLA sheets after 180 days of testing in soil at 28 °C resulted in c. 10% of mineralization [218]. Lower values obtained for PLA sheets in comparison to powder samples indicates the effect of shape and size as important factors decreasing the chemical hydrolysis and the mineralization rate.

Biostimulation and bioaugmentation of soil environments to improve PLA biodegradation under mesophilic conditions was studied by Satti et al. [216]. After 150 days of testing, improved results, with respect to natural biodegradation of PLA, were obtained for biostimulated soil with lactate and bioaugmented soil with previously isolated PLA-degrading bacteria strains. Techniques such as biostimulation and bioaugmentation to improve biotic conditions are increasingly considered as feasible alternatives to increase the biodegradation rate of polymers. In this sense, UV irradiated PLA sheets in soil, inoculated with *Pseudomonas geniculata* WS3 at 30 °C, showed maximum biodegradation values of c. 35% after 60 days of testing. However, in the case of soil non inoculated, the biodegradation was just about 15% after 60 days [220].

2.17.5 Polycaprolactone – PCL

PCL is a synthetic, aliphatic biodegradable polymer, semicrystalline in nature, and is obtained by the ring opening polymerization of caprolactone [544]. PCL has a T_g of around −60 °C and a T_m of 60 °C. Since the T_g is so low, PCL shows high molecular chain mobility due to its rubbery state [545]. Also, this low T_g provides good flexibility and malleability to PCL [544]. PCL is non-hazardous and biocompatible, so the polymer is often used in biomedical applications such as tissue engineering, drug delivery, and in the construction of scaffolds and sutures [546]. PCL displays excellent rheological and viscoelastic properties. Aside from the many listed advantages, the mechanical properties are less suited for rigid applications. The inferior mechanical advantage coupled with improved degradation rate warrants the use of fillers and incorporation of different polymers to attain the necessary mechanical properties. PCL is usually a raw material for production of polyurethanes, as polyol polyester-type [495,547].

The main abiotic degradation mechanism for PCL in the mesophilic range is chemical hydrolytic degradation through bulk erosion [548]. Furthermore, PCL can photodegrade when exposed to radiation via Norrish II reactions [549]. UV treatment also has been effective in increasing the degradation rate of PCL films, making it easier for microorganisms to attack during the biodegradation phase [550]. Due to its relatively low T_m (60 °C), PCL can undergo thermal degradation at conditions similar to the thermophilic conditions of the industrial composting process. A short abiotic lag phase was reported for PCL in home composting conditions, showing a biodegradation trend similar to readily biodegradable materials like cellulose or starch [115]. However, the initial M_w of the used PCL was low (M_w c. 50 kDa) in comparison to others polyesters evaluated such as PLA and PHAs [551].

A comparison of the hydrolysis mechanisms for PCL in water and phosphate buffer solutions revealed that, in general, enzymatic hydrolysis was faster than abiotic chemical

hydrolysis in terms of mass loss, and that enzymatic hydrolysis is a surface erosion process whereas abiotic chemical hydrolysis is a bulk erosion process [338]. However, in more real-world conditions the enzymatic hydrolysis also could be affecting the chemical hydrolysis. For example, a comparison test for PCL abiotic degradation at 30 °C by *Aspergillus fumigatus* showed a different pattern; samples studied for chemical hydrolysis remained without surface changes, while samples in culture media showed an erosion pattern indicative of surface enzymatic degradation [178].

Carboxyl esterase (3.1.1.1), lipases (3.1.1.3), and cutinases (3.1.1.74) are able to degrade PCL. Also, low enzymatic activity by peptidases such as Proteinase K (3.4.21.64) has been observed. Cutinases from fungal phytopathogens are indicated as PCL depolymerases showing enzymatic activity [100]. Based on earlier works studying and identifying aerobic microorganisms able to biodegrade PCL, it has been reported that the natural polymers cutin and suberin are enzymatically degraded by lipases; since these materials are considered as natural analogous to PCL, enzymatic activity of PCL by lipases was potentially considered. Nishida et al. demonstrated that lipases are highly active in the degradation of PCL [552]. This finding was also indicative of potential microbial populations for PCL biodegradation being extensive in natural environments such as soil, home composting, and water. When enzymatic degradation of PCL by lipases and Proteinase K available in those environments was studied, lipase activity was reported but none for Proteinase K; the authors associated this result to the preferential specificity of lipases for ester bonds on hydrophobic substrates as in PCL [335].

Temperature and pH are key factors also identified in playing a main role in the degradation of PCL. The high stability of cutinases able to degrade PCL was associated with stabilization of the enzymes by neutral surfaces and additional disulfide bond formation [294]. Baker et al. compared cutinases for PCL degradation and showed that enzyme activity,

stability, and efficiency was affected by the type of microorganism that releases the extracellular enzyme and by temperature; the authors reported a similar residual activity for the enzymes at 25 °C, but reduced residual activity at 45 °C for some of the cutinases [294].

Li et al. reported the presence of 6-hydroxy-hexanoic acid instead of PCL oligomers during the enzymatic degradation of PCL by *Penicillium oxalicum*, indicative of an exo-type chain-end scission by the enzyme [354].

PCL degrading microorganisms and extracellular enzymes have been reported also in marine environments, showing relatively good activity in comparison to other aliphatic polyesters as PLA [553].

When 6-hydroxycaproic acid becomes available for bioassimilation after chemical and enzymatic depolymerization of PCL (**Figure 2.14**), it is transported through the semipermeable membrane, and then is converted to acetyl-CoA by β-oxidation of fatty acids, becoming available for the TCA cycle (**Figure 2.19**).

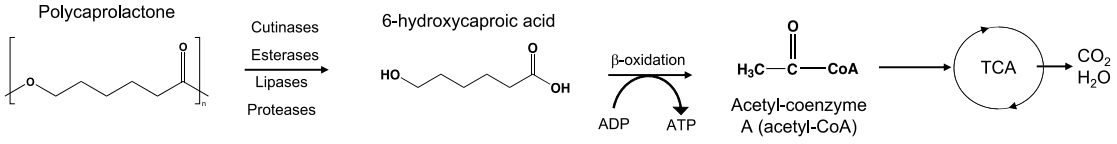


Figure 2.19 Tentative biodegradation pathway for PCL in aerobic conditions. Adapted from [544].

A few studies have demonstrated PCL biodegradation with production of CO_2 and mineralization at mesophilic conditions like soil or home composting. Ohtaki et al. reported a low mineralization value of c. 15% for PCL powder (M_w c. 100 kDa) in compost after 8 days of testing [138]. Modelli et al. reported ~102% mineralization for PCL in a soil environment in laboratory conditions at 22 °C after 270 days of testing [223]. Narancic et al. tested the biodegradation of PCL sheets in home composting and in marine (30 °C) and fresh water (21°C) environments [115]. In home composting, the PCL reached mineralization values of c.

90% after 180 days of testing, with a negligible abiotic degradation stage; however, PCL failed the marine (56 days) and freshwater tests, with mineralization values of c. 80 and 50%, respectively.

2.17.6 Poly(alkylene dicarboxylate)s

Poly(alkylene dicarboxylate)s are a family of biodegradable aliphatic polyesters derived from dicarboxylic acids and dihydroxy compounds [50]. This family includes PBA, PBS, PBSA, PBST, PBSe, PBSeT, PEA, PES, among others. Their general structures are presented in **Table 2.5**, and a general description of the main polymers of the family is provided here.

PBA is a biodegradable polyester that can be synthesized via polycondensation of adipic acid with 1,4-butanediol in the presence of a catalyst. Due to its low T_m (41–61 °C), PBA is generally copolymerized to obtain polyesters with improved mechanical properties. Potential applications for PBA are mainly in the medical area [50].

PBS, a biodegradable, linear, semi-crystalline, thermoplastic aliphatic polyester, is a result of the condensation polymerization of succinic acid (SA) and 1,4-butanediol (BDO). PBS can be 100% bio-based (bio-based SA and BDO), partially bio-based (bio-based SA and petrochemical BDO) or fossil-based (petrochemical SA and BDO), depending on the production route used [554]. SA is derived from maleic anhydride, which can be produced by the oxidation of butane or benzene or from the fermentation of carbohydrate sources such as glucose and starch [555]. BDO, on the other hand, can be derived via three routes: using petrochemicals, hydrogenation of SA, or fermentation of sugars [556]. PBS provides easy processability and mechanical properties comparable to LDPE and PP. The fact that PBS is flexible and not rigid and brittle like other biodegradable polymers (e.g., PLA, PBAT, and PHB) makes it a more viable and a cost-effective option for common applications [557]. The properties of PBS can be further fine-tuned for designated applications by blending with

other polymers. For example, PBS/PLA blends have improved toughness and elongation at break with the help of random copolymers of poly(butylene succinate-*co*-lactic acid) as compared with neat blends [558]. PBS applications vary from food packaging, agriculture mulch films, hygiene products, fishing nets, plant pots, and coffee capsules, among others [559].

PBSA is obtained when adipic acid is added when synthesizing PBS. The addition of adipic acid decreases the crystallinity and increases the degradation rate [50]. In comparison to PBS, PBSA has a lower T_m of c. 95 °C (**Table 2.5**) and higher flexibility in terms of mechanical properties [489].

PBST is an aliphatic/aromatic polyester synthesized by direct esterification and polycondensation using titanium tetraisopropoxide as a catalyst. PBST has a potential development, and works about PBST biodegradation are limited [50].

PEA, an aliphatic polyester, is produced by the polycondensation of ethylene glycol and adipic acid or by the polycondensation of dimethyl adipate and ethylene glycol [560]. PEA has a T_g of c. −50 °C and T_m of c. 48 °C (**Table 2.4**). The polymer displays good flexibility due to the low T_g, but at the same time demonstrates low mechanical strength [561]. PEA is usually blended with other polymers. When blended with PLA, PEA helps in reducing the brittleness, improving the thermal stability, and has also been shown to increase the elongation at break compared with neat PLA [562]. PEA has application as a plasticizer, when low migration, good plasticity and better mechanical properties are desired for the copolymer blend [563].

PES is synthesized by ring opening polymerization of succinic anhydride with ethylene oxide or by polycondensation of succinic acid and ethylene glycol (**Table 2.5**). PES is highly permeable to O_2 [50].

In terms of abiotic degradation, the density of the ester bond is a factor affecting the chemical hydrolysis rate of poly(alkylene dicarboxylates). As reported for PES, a smaller ester bond reduces the hydrophilicity, affecting the overall process of hydrolysis [50]. For PBS, abiotic degradation generally occurs through chemical hydrolysis, with bulk erosion as the predominant mechanism [48]. In general, PBS is copolymerized with the aim of increasing the hydrolysis rate. For example, the addition of more hydrophilic components, such as PEG, has been reported to increase abiotic hydrolysis; however, adipic acid is the most common component added to obtain PBSA [50]. Hayase et al. reported a higher degradation of PBSA than PBS in the presence of *Bacillus pumilus*, which was attributed to the faster degradation of the adipate units [458].

Extracellular enzymes with activity for poly(alkylene dicarboxylate)s have been reported, mostly for PBA, PBS, PBSA, PEA, and PES (**Table 2.10**). In general, lipase activity data is scarce for PBA, PBS, and PBSA; however, the activity of cutinases is well reported for PBS and PBSA (**Table 2.10**). Cutinases are indicated as being more active for polyesters with chain lengths less than 10 C [489].

Fungi and bacteria have been shown to depolymerize PBS. For example, Ishii et al. [455] reported succinic acid and 1,4-butanediol as hydrolysis products due to the action of *Aspergillus fumigatus* strain NKCM1706. Li et al. [352] reported the action of an exo extracellular enzyme on PBS as an exo attack, since products identified by mass spectrometry were succinic acid and butylene succinate monomers rather than PBS oligomers. Furthermore, the presence of butylene succinate monomers and not 1,4-butanediol showed that the enzyme cut the polymer chain from the carboxyl end [352]. In the case of PBSA, 1,4-butanediol, succinic acid, and adipic acid were detected by HPLC during depolymerization by *Leptothrix* sp. TB-71 [314]. Enzymatic activity of *Rhizopus delemar* lipases against PEA

produced, besides ethylene glycol and adipic acid, PEA oligomers; indicative of an endo attack of the lipase extracellular enzyme [345].

When SA and BDO become available for bioassimilation (**Figure 2.20**) after chemical and enzymatic hydrolysis, they are transported through the semipermeable membrane and converted to succinyl-CoA, becoming available for the TCA cycle (**Figure 2.20**).

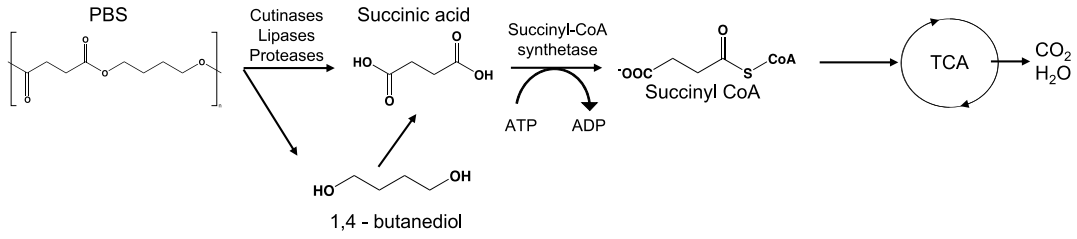


Figure 2.20 Tentative biodegradation pathway for PBS in aerobic conditions. Adapted from [564].

Some studies reported mineralization (or CO_2 production) for PBS, showing limited biodegradation in the mesophilic range, including home composting, soil, marine and freshwater environments. Narancic et al. tested the biodegradation of PBS sheets in soil, home composting, and in marine and freshwater environments, and found mineralization values lower than 20% after 365 days of testing in soil and home composting, whereas values were c. 20 and 5% in marine and freshwater after 56 days of testing, respectively [115]. However, other group evaluated PBS in powder form in soil environments at 25 and 37 °C, and mineralization values reached c. 85 and 80%, respectively, after 180 days of testing [210].

PBSe and PBSeT (films) were assessed in a marine environment at 25 °C under laboratory conditions, and similar mineralization values of c. 90% were obtained after 360 days of testing with stirring and without stirring the media containing the samples [211]. PBSe and PBSeT (films) in soil at 25 °C reached mineralization values of c. 90% after 360 days of testing [209]. Furthermore, when PBSe (powder) was evaluated in soil at 28 °C,

mineralization improved for samples with higher available surface area; at day 140, the mineralization values were c. 55% for samples with 33 cm^2 surface area, and 80 to 95% for samples with 89 to 1650 cm^2 surface area [189].

PBSA films was evaluated in compost at 25 ºC with values of mineralization of ~70% after 55 days of testing, with an abiotic phase duration of ~1 week [234].

2.17.7 Polyhydroxyalkanoates – PHAs

PHAs comprise a family of naturally occurring biodegradable aliphatic polyesters produced due to the fermentation of carbohydrate sources, such as sugar and lipids, by the action of a broad range of microorganisms [531,565]. PHAs are synthesized and stored as an intracellular energy resource for later metabolism under conditions of scarcity. PHAs can be classified according to the length of the side chain. The most common are short-chain-length PHAs, with 3 to 5 carbon atoms in their monomeric structure [50]. Poly(3-hydroxybutyrate) (PHB), poly(hydroxyvalerate) (PHV), and the copolymer poly(hydroxy-butyrate-*co*-valerate) (PHBV) are the most common, and PHB is abundantly manufactured. PHAs can be derived from renewable and non-renewable sources [566], and have excellent barrier and good thermo-mechanical properties [567]. However, drawbacks for PHAs in conventional thermal processing include a narrow processing window and high production costs. To improve the processability and ensure large-scale production, PHAs are often blended with other polymers. PHAs have been commonly used for cutlery, trays, food packaging, and cosmetics, and in the development of medical devices, surgical sutures, implants and tissue engineering, among others [568].

The most common PHAs undergo abiotic degradation by chemical hydrolysis scission of the ester bonds (**Figure 2.14**). A discussion is still open in the field whether PHAs go through a bulk or surface erosion process regardless of the thickness [48,50]. However, some studies have reported reduction of M_w, mass loss, and mechanical properties deterioration

during degradation, indicative of surface erosion as the dominant mechanism [569,570]. Specifically, for the copolymer PHBV, a surface erosion process for both enzymatic and chemical hydrolysis was reported [571].

After depolymerization of PHB (as an example PHA), 3-hydroxybutyric acid is bioassimilated and, through a redox reaction, converted to acetyl-CoA, which feeds the TCA cycle (Figure 2.21).

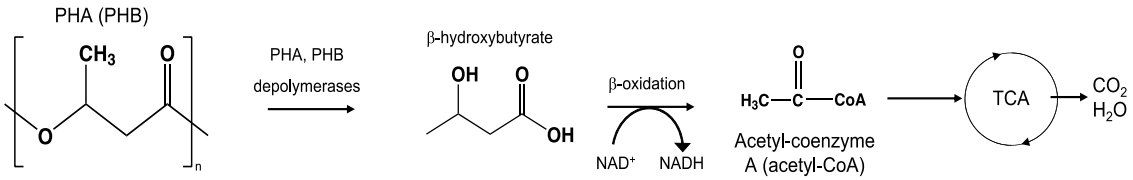


Figure 2.21 Tentative biodegradation pathway for PHB in aerobic conditions.

Degradation of short-chain-length PHAs by enzymatic activity from bacteria and fungi has been extensively reported [48,50,489,572]. In general, an increase in side-chain length decreases the hydrolytic rate of the PHAs [359].

PHAs can be metabolized by intra or extracellular depolymerases depending on its location. In this sense, *in vivo* granules are amorphous PHAs that can be metabolized by intracellular enzymes. Denatured PHAs, after cell lysis, becomes semi-crystalline and can be depolymerized by microorganisms that release extracellular depolymerases [489,573].

PHB depolymerases (3.1.1.75) and PHA depolymerases (3.1.1.76) are the main enzymes reported as able to degrade PHB and other PHAs. A bacterial PHB depolymerase has been shown to have two functions during the hydrolysis of PHB films, which takes place via adsorption and hydrolysis, binding, and catalytic domains [366]. Investigations revealed that the binding domain of the enzyme is non-specific for binding to the surface of PHA films; however, the active site in a catalytic domain is specific for the hydrolysis of the PHB molecule [366].

The activity of extracellular PHB depolymerases in enzymatic depolymerization occurs initially on the surfaces of the polymer after biofilm formation, and the rate is dependent on the M_w, crystallinity, and microbial community [574]. PHA depolymerases can be described as serine hydrolases with protein progression formed by four regions. First is the signal sequence, second is a catalytic area which contains the lipase box, third is a substrate-binding domain where the adsorption of the polymer substrate takes place, and eventually a domain which connects the catalytic area to the substrate securing areas [100].

Stimulation activity for PHB depolymerase was observed in presence of Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+} [105,380]. However, Fe^{+2}, Hg^{+2}, Mn^{+2}, and Cu^{+2} were reported as inhibitory of enzymatic PHB activity [399,489].

Nishida et al. reported the effect of crystallinity and amorphous fraction on microbial degradation [575]; showing that increased crystallinity repressed microbial activity.

PHB, PHV, and PHBV have been reported to be biodegradable in several mesophilic environments such as soils, composts, and natural waters (**Table 2.9**). A large microbial population has been identified as associated with biodegradation of PHB and the copolymer PHBV in mesophilic conditions [50]. Biodegradation in soil of PHBV films was reported as the combined action of fungi, bacteria and actinomycetes; however, the fungi population was identified as the dominant one due to the ability to increase the surface growth of hyphae [48,449]. Copolymer composition, crystallinity, microstructure, and surface morphology are factors reported to play an important role during biodegradation of PHBV in soil [221]. During degradation of PHBV in seawater, an increase in surface roughness was observed, which was reported as both surface erosion by chemical hydrolysis and enzymatic activity [48,576].

During biodegradation of PHBV (films) evaluated in soil at 25 °C, mineralization values of c. 25% were achieved after 190 days of testing [225]. Higher mineralization values

were reported for PHBV in marine environments. Thellen et al. [231] reported high mineralization for high M_w PHBV films with different contents of valerate in a marine environment at 30 °C; after 100 days of testing, mineralization values reached c. 100% for PHBV with 5 and 8% of valerate, and c. 90% for PHBV with 12% valerate. A recent study by Meereboer et al. [233] evaluated PHBV in powder form in a simulated marine environment at 25 °C; mineralization values were higher than 50% at day 190, and values reached c. 90% at the end of the test (450 days).

Mineralization of PHB in a soil environment was reported after 360 days of testing, with a degree of biodegradation of c. 95% at 25 °C [209]. High mineralization values were also reported by Narancic et al. [115]. After 136 days of testing in soil at 25 °C, mineralization values higher than 100%, showing priming effect, were reported; however, when evaluated in home composting, low mineralization values (less than 20%) were reported for PHB at 28 °C [115].

On the other hand, PHB in marine environment showed c. 70% of mineralization after 360 days of testing and c. 95% in the same test after 200 days, but with a stirring system; the difference in CO_2 evolution was attributed to the shortage of O_2 in the system without stirring [211]. Thellen et al. also reported high mineralization values (in the range of 80 to 90%) for high M_w and high crystallinity content PHB films in a simulated marine environment at 30 °C; this work was indicative of the high degradability of polymers from the PHA family even though the % crystallization and initial M_w was high for both PHB and PHBV [231]. Narancic et al. reported mineralization values of c. 90% for PHB sheets at day 56 of testing in a marine environment at 30 °C, whereas in a freshwater environment at 21 °C the values were c. 90% for PHB [115].

PHA biodegradation has been evaluated in soil. Gómez et al. reported mineralization values of c. 70% for PHA (injection molding samples) in soil after 650 days at 20 °C [215]. A

more recent study reported mineralization values of c. 90% for PHA (powder) in soil after 150 days at both 25 and 37 °C [210].

2.17.8 Poly(butylene adipate-*co*-terephthalate) – PBAT

PBAT is a co-polyester synthesized from 1,4-butanediol (BDO), adipic acid (AA), and dimethyl terephthalate by a polycondensation reaction. Adipic acid and BDO polymerize to produce their own polyester and water. Dimethyl terephthalate and BDO react to form their own polyester and methanol. The resulting polyester then reacts with the polyester of AA and BDO using tetrabutoxytitanium as a catalyst for the transesterification. The reactions are carried out at temperatures higher than 190 °C, under high vacuum, and usually require long times [577]. PBAT has a T_g of c. 30 °C and T_m of c. 106 °C. PBAT has good toughness and ductility, biodegradability, and is flexible. However, higher production costs, coupled with lower mechanical and heat resistance in comparison to common fossil-based plastics, has hindered PBAT development and acceptance in the consumer market [577]. These shortcomings can be overcome by blending PBAT with other biodegradable polymers. For example, blends of PBAT and PLA demonstrated higher yield stress, modulus, and rheological properties than those of neat PBAT [578]. PBAT is widely used for agricultural mulch films, and also for packaging applications including trash bags, shopping bags, wrapping films, and disposable food containers [579].

The reported abiotic mechanisms of degradation associated with PBAT are primarily mechanical, photodegradation, thermal, and hydrolysis. Mechanical degradation is, in general, associated with the entire spectra of biodegradable polymers; in the case of PBAT, erosion is a common situation due to its main application in agricultural mulch films. Photodegradation has been reported as the main abiotic mechanism of degradation for PBAT agricultural mulch films. A crosslinking effect as a result of exposure to sunlight has been

reported; the effect is known to delay the biodegradation rate of PBAT by decreasing the chain mobility of the polymer [141,175,276].

The addition of aliphatic acids to aromatic polyesters improves the water uptake and hydrolysis of these polymers. For example, by adding adipic acid to poly(butylene terephthalate), PBAT is obtained and it can undergo faster hydrolytic degradation. However, PBAT still offers more resistance to chemical hydrolysis than aliphatic polyesters, such as PLA, due to the steric hindrance of the large aromatic ring repeating units.

PBAT is depolymerized through chemical and enzymatic hydrolysis into adipic acid, 1,4-butanediol, and terephthalic acid (**Figure 2.14**). Then each compound is bioassimilated or undergoes a redox reaction to feed the TCA cycle, as shown in **Figure 2.22**. The adipic acid pathway is through adipyl-CoA, and 1,4-butanediol is converted to succinic acid and to succinyl-CoA. Several bioassimilation pathways have been reported for terephthalic acid. The most probable, in the case of PBAT, seems to be the transport of terephthalic acid through the cell membrane, followed by degradation to protocatechuic acid and then through the pyruvic acid pathway to Acetyl-CoA to enter the TCA cycle [564].

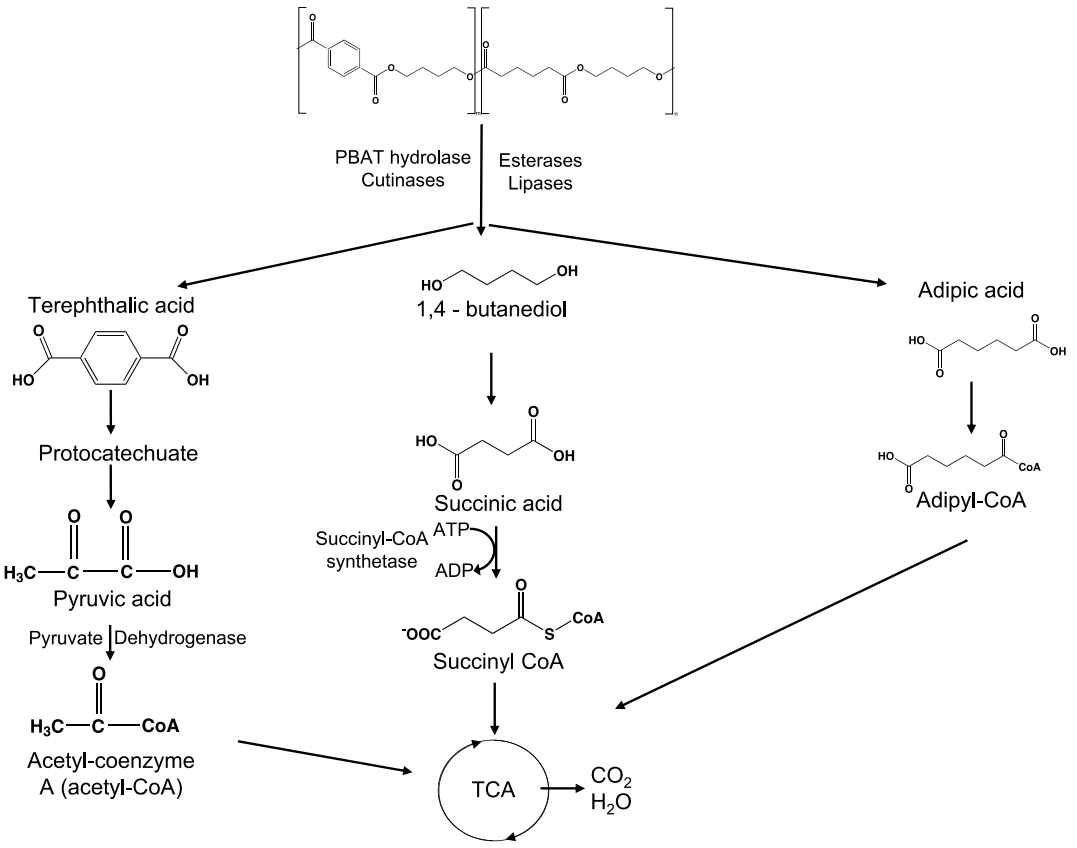


Figure 2.22 Tentative biodegradation pathway for PBAT in aerobic conditions. Adapted from [564,580].

In terms of biodegradation and enzymatic activity at mesophilic conditions, PBAT has been reported to be degraded by cutinases [298], lipases [331], and PBAT hydrolases [260]. As for chemical hydrolysis, enzymatic activity is affected due to the presence of aromatic groups that make enzyme accessibility more difficult for scission of the ester bonds that are close to these groups [50,51]. The presence of the aromatic ring has been associated with a decrease of the enzymatic activity by creating a steric impediment to access the active site of the enzymes. Butanediol-terephthalate bonds have been reported to be hydrolyzed at a lower rate in comparison to adipate-butanediol bonds [81].

Low values of enzymatic activity reported for the actinobacteria *Rhodococcus fascians* in comparison to a mesophilic PBAT-degrading fungus show that both the type of enzyme

and the microorganism producing the enzyme play major main roles in activity [259,260]. These results could be associated with the favorable conditions offered to microorganism populations in soil environments in the mesophilic range. The enzymatic hydrolysis of PBAT by a fungal strain generated terephthalic acid, adipic acid, and 1,4-butanediol, as identified by mass spectroscopy [260]. Furthermore, enzymatic activity of PBAT hydrolase by *Bacillus pumilus* on PBAT showed degradation products as adipate, 1,4-butanediol, and terephthalate [81].

Crosslinking due to exposure to UV-radiation treatment has also been shown to decrease the enzymatic activity against PBAT, due to the reduced flexibility of the polymer chains after crosslinking [581].

PBAT has been reported to be degraded in soil environments or soil in laboratory conditions (Table 2.9). In general, rates of biodegradation at mesophilic conditions are low. Biodegradation studies of PBAT showing CO_2 and mineralization in simulated and controlled media in the mesophilic range are limited [140]. Studies in more controlled environments like culture and/or buffer media are more commonly focused on identification of microbial activity and/or enzymatic activity towards PBAT. However, identification of extracellular enzymes able to degrade PBAT is relatively limited in comparison to those for common aliphatic polyesters. Most of the environments assessed for PBAT degradation are agricultural soils. CO_2 production from PBAT in soil environment media has been reported, with mineralization values of c. 10% after six weeks [140].

A novel approach by Zumstein et al. [140] demonstrated the mineralization of PBAT ^{13}C to $^{13}CO_2$, with higher values of mineralization for ^{13}C derived from depolymerization of the adipate structure, and lower values of mineralization associated with depolymerization of the aromatic terephthalate fraction. This finding is indicative of the increased complexity of aromatic polyesters towards depolymerization and assimilation. On the other hand, the

presence of the aromatic component in the co-polyester was shown to improve the overall rate of biodegradation, even in the mesophilic range like in the soil environment evaluated [140].

PBAT films with 1% of a chain extender had low mineralization values of c. 20% after 180 days in soil at 28°C [218]. The effect of the chain extender on delaying M_w reduction and biodegradation was evident.

An interesting outcome of biodegradation studies for PBAT in a soil environment is that the degradation products have been shown to be harmless to the microbial population [582]. Although biodegradation can be a longer process in the mesophilic environment, and the formation of PBAT degradation products does not affect the quality and health of the soil and its microbial population, development of some microorganisms over others can be modified [583].

2.17.9 Poly(urethanes)s – PUs

PUs are synthetic plastics, insoluble in water, and produced by the condensation reaction of polyols and polyisocyanate having urethane bonds [49,584]. Polyisocyanates and polyols react with a chain extender to give polyurethane polymers with alternate soft and rigid segments. Polyol forms the soft segment and can be obtained from polyester or polyether polyols; whereas the rigid segment is derived from the isocyanate and chain extender, and has restricted mobility compared with the soft polyol segment (**Figure 2.23**) [495]. The rigid segment is considered the crystalline region and the soft segment the non-crystalline or amorphous region of PUs [493,585]. Depending on the polyol used, the resulting PU can be identified as polyester PU or polyether PU. The resulting properties and degradation behavior are dependent upon the selection and chemistry of the soft segment [586].

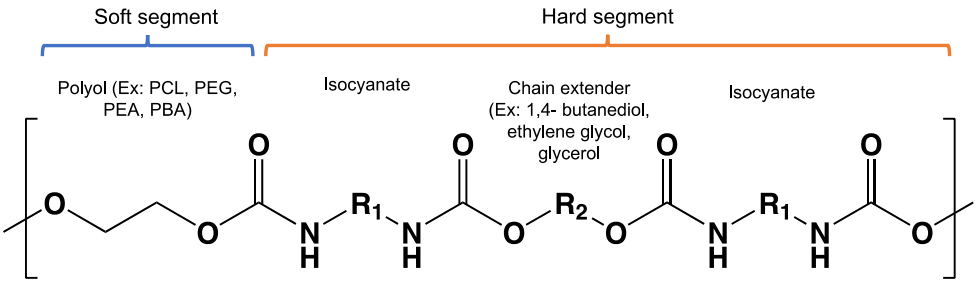


Figure 2.23 Soft and hard segment of the poly(urethane)s structure. Adapted from [495].

Poly(urethane)s are used in the medical, construction, and automotive fields, among others. Typically, products that contain PUs include furniture, paints, fibers, flexible foams, rigid foams, coatings, adhesives, synthetic skins, sutures, and tissue scaffolds [494,587,588]. Poly(urethane) elastomers (thermoplastic) are used in the medical field due to their high elasticity and toughness compared with other elastomers [584,587]. The good mechanical, thermal, and electrical properties of PUs allow these polymers to offer good adhesion for coatings, tensile strength, and abrasion resistance for several uses [587]. Poly(urethane) foam are a typically example of thermoset PUs [494].

Early studies demonstrated that PUs with long repeating units and hydrolytic groups were susceptible to biodegradation [589]. This review concentrates on polyester PUs. The ester bond of polyester PUs is susceptible to hydrolytic degradation and can be catalyzed with the help of extracellular enzymes. The extracellular enzymes for PU degradation have a hydrophobic area, which assists in attaching onto the polymer surface [585,587]. Microbial attack of PUs can occur by action of extracellular hydrolases such as ureases (3.5.1.5), amidases (3.5.1.4), proteases, and esterases (**Figure 2.14**). The cleavage site and the product of the breakdown is dependent on the type of the enzyme acting during depolymerization (**Figure 2.24**). Adipic acid and diethylene glycol were reported as degradation products by the action of extracellular enzymes on polyester PUs; however, no identification of the

isocyanate hard segment byproducts was reported [315,316]. Later work by Shah et al. reported the probable presence of a hydrolyzed portion of the hard segment, detected by FTIR spectrum, when polyester PU was attacked by both *Bacillus subtilis* MZA-75 and *Pseudomonas aeruginosa* MZA-85 [246]. Furthermore, the mixing of esterase and amidase has been reported to hydrolyze the hard segment via the urethane bonds [437].

A bacterial esterase was identified to degrade ester PUs by acting in a two-step reaction: first, a hydrophobic adsorption of the enzyme on the surface of the PU; and second, the hydrolysis of the ester bonds of the PU [316]. Studies of enzymatic activity have shown that the rate of biodegradation decreases with decreasing ester content, indicating the impact of the esterase activity as relevant for PU depolymerization [584]. Fungal communities have been identified to degrade PU to some extent [481].

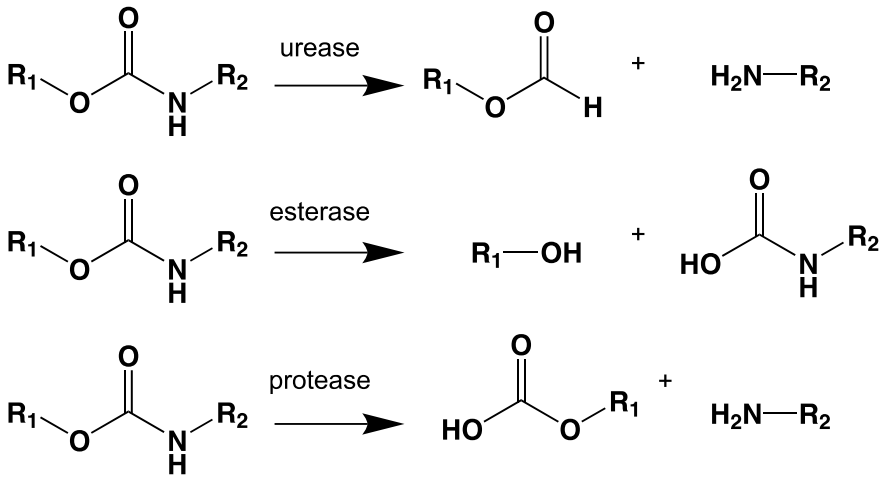


Figure 2.24 Sites of scission for urethane bonds in extracellular enzyme function. Adapted from [585].

A tentative route for metabolism of the soft segment (PEG) is presented in **Figure 2.25** for PU derived from ester.

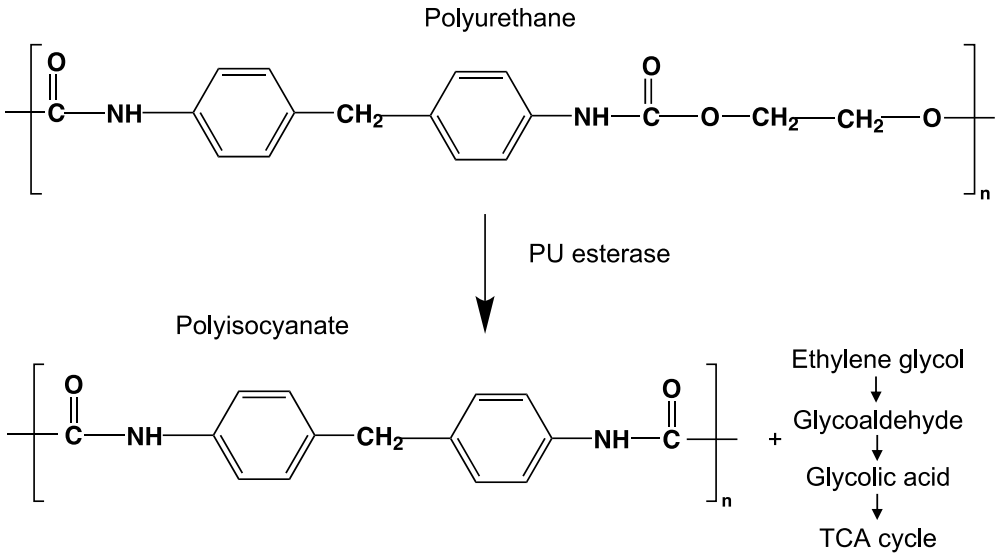


Figure 2.25 Tentative metabolism pathway for poly(urethane)s derived from esters. Adapted from [590].

Biodegradation studies showing CO_2 evolution or mineralization in mesophilic environments for PUs are limited. Most of the reported studies on PUs are for enzymatic activity of both fungi and bacteria. More investigations of the abiotic degradation process of PUs prior to the biotic degradation stage, such as hydrolysis or photodegradation, in the mesophilic range would help determine whether PUs derived from esters are fully biodegradable in soil, home composting, industrial composting, or water environments. The biodegradation of polyester-PUs studied under mesophilic composting conditions resulted in mineralization between 5 and 43% after 45 days of testing, and this wide range was attributed to the different chemical structures of the PUs [591]. A high content of the hard segment led to decreased biodegradation rates and mineralization, whereas biodegradation increased as the amount of diol carbon chains of the polyol (soft segment) increased. The hard segment composition in PUs was presented as a more dominant effect than the crystallinity or surface properties during PU biodegradation in composting [591]. The presence of aromatic

diisocyanates decreased the rate of biodegradation in comparison to PUs with aliphatic diisocyanates [591].

Biodegradation of PU films during the Sturm test showed high CO_2 evolution at 30 °C for 28 days in comparison to the control [240]. Also, a Sturm test revealed the production of CO_2 during the enzymatic hydrolysis of PU films by *Bacillus subtilis* MZA-75 and *Pseudomonas aeruginosa* MZA-85, hydrolyzing the ester portion in 1,4-butanediol and adipic acid products [246–248]. This result indicated that *Bacillus subtilis* was able to hydrolyze and assimilate the intermediates as carbon sources with final mineralization. An interesting outcome of the reported enzymes attacking ester PUs is the evidence of the presence of membrane-bound enzymes, besides extracellular enzymes. For an esterase not secreted to the culture medium, its high hydrophobicity was reported as the most probably cause for its membrane-bound characteristic [547].

A new approach is the development of non-isocyanate PUs (NIPU). NIPU are a promising and more sustainable alternative for traditional PUs [592,593]. However, studies in this area looking at degradation and biodegradation are still limited. Production and biodegradation assessment of polyhydroxyurethane, a NIUP based on cyclic carbonate and polyamine, was reported by Ghasemlou et al. [229]. Mineralization values for film samples reached c. 40% after 120 days of testing in soil conditions.

2.17.10 Poly(vinyl alcohol) – PVOH

PVOH is a synthetic, water-soluble polymer produced by partial or complete hydrolysis of polyvinyl acetate. Unlike other polymers, PVOH is not synthesized from the polymerization of its monomer (vinyl alcohol), due to the unstable nature of the high density of hydroxyl groups in the monomer. Polyvinyl acetate is first synthesized by the polymerization of vinyl acetate and then subjected to saponification wherein the ester groups of vinyl acetate are replaced by hydroxyl groups in the presence of caustic soda [594].

Different grades and properties of PVOH are available, depending on the degree of hydrolysis and the variation in initial length of the vinyl acetate polymer. PVOH is odorless and non-toxic in nature, has excellent resistance to aroma and gases, is resistant to solvents and oil, has good optical and adhesive properties, and film forming capacity [595]. In terms of disadvantages, PVOH is expensive, and mechanical properties are highly conditioned by the presence of water or humidity so it needs to be blended with other polymers to achieve more desirable properties [596]. Due to its good adhesion to other hydrophilic surfaces, PVOH is used widely in emulsifiers, binders, and hydrogels for a broad range of industries, including textile, paper sizing, fabrics, and packaging films as a protective film for laundry and dish detergents. The applications are not limited and extend to the biomedical, cosmetic, and food packaging industries [597,598].

The degree of solubility of PVOH in water can be tailored, depending on the amount of OH groups and remaining acetate bonds.

Besides the abiotic mechanism of biodegradation, PVOH could be considered as partially biodegradable since the number of microorganisms and enzymes identified to biodegrade it is rather scarce in comparison to polyesters.

Biodegradation of PVOH has been reported to start from random chain scission where the action of oxidative enzymes catalyzes the break of the carbon backbone. Mostly dehydrogenases or oxidases are responsible for the carbon-carbon bond scission. Hydrolases or aldolases have been reported as responsible for the chain scission of the hydroxyl group (**Figure 2.14**). Furthermore, a two-step process has been proposed for the enzymatic degradation of PVOH: the first step, by action of PVOH oxidases, involves the oxidation of hydroxyl groups to form diketone or monoketone structures; and the second step involves hydrolysis of the carbonyl structure formed by oxidized PVOH hydrolases [599].

Since PVOH is a water-soluble polymer, its biodegradation has been studied mostly in aqueous media. The identified microorganisms and enzymes able to biodegrade PVOH are associated mainly with contaminated environments, such as waste sludge, which are common end-of-life scenarios for PVOH.

Abiotic degradation of PVOH by UV/chlorine oxidation via generation of active free radicals has been investigated; in acidic media the efficiency was higher due to the higher ratio of [HOCl]/[OCl^-] [600]. The abiotic degradation of PVOH by photocatalytic oxidation or radiation and ozone also has been reported [478,601].

Published works have identified that microorganisms able to biodegrade PVOH are mostly from the genus *Pseudomonas* [602]. Also, many PVOH degradation pathways have been proposed for different bacteria such as *Alcaligenes* and *Pseudomonas* species [603]. These routes include scission of the polymer chain by an extracellular oxidase (dehydrogenase), followed by aldolase and hydrolase reactions, releasing compounds such as acetic acid and hydroxyl fatty acids that can be incorporated into the β-oxidation and TCA cycle, respectively [100]. In **Figure 2.26** is presented a tentative metabolization route for PVOH [439].

As stated, scarce mineralization was reported for PVOH films in water conditions, with ~ 10% after 100 days of testing at c. 30 ºC [232].

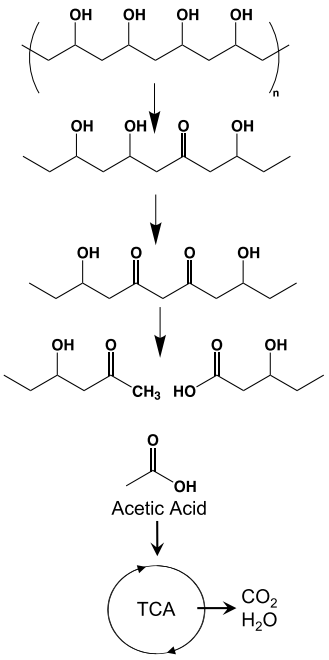


Figure 2.26 Proposed pathway for metabolism of PVOH. Adapted from [109,603].

2.18 Final insights and remarks

Addressing plastic pollution has become one of the main concerns of our modern society. The impacts of plastic waste in terms of global climate change, health, and social effects, circular economy, sustainable use of resources and production, and improved waste management systems have garnered the attention of industry, government, and NGO stakeholders and the society in general. The development of new plastic waste pacts and commitments to curb the use of virgin plastic are ongoing globally, with targets for 2025 [21]. However, the damage to ecosystems has already created deleterious impacts, which will require forward-thinking actions to remediate, to mitigate, and to avoid permanent damage [9].

The development of biodegradable polymers derived from both bio- and fossil-based resources has transcended from the lab scale to commercial applications in the last two decades, and these polymers have become an option for packaging and consumer goods

applications to mitigate the impact of plastic waste. However, as with any material created by society, biodegradable polymers also must reach a waste management end-of-life to avoid a rebound effect on creating additional pollution.

The degradation process for biodegradable polymers starts by the action of external abiotic and biotic factors. The main abiotic mechanisms of degradation associated with mesophilic environments are photodegradation, mechanical degradation, and chemical hydrolysis. Photodegradation in the presence of O_2 introduces modifications during the degradation of biodegradable polymers in specific environments such as agriculture soils, inducing a dual effect: chain scission that contributes to the degradation and also crosslinking that act to delay the process. The initial deterioration of the polymer structure enhances the mechanical degradation, generating micro and nano plastics but not guaranteeing biodegradation. Chemical hydrolysis is the crucial mechanism for the large majority of biodegradable polymers since most of them contain ester bonds that are prone to water attack.

The formation of biofilms, the stage prior to the release of extracellular enzymes, affects the whole dynamic of the degradation process, as discussed. Since biofilms create an extra layer on the polymer surface and potentially affect water diffusion during chemical hydrolysis, a better understanding of biofilm formation and its effect on water diffusion and bulk erosion are needed.

Extracellular enzymes act at the surface level of polymers, making enzymatic activity a surface erosion process. As presented in this review, the main groups of enzymes reportedly able to break chemical bonds in polymers belong to the esterase group (amidases, cutinases, esterases, lipases, and PHA depolymerases), proteases (specific for PLLA), and oxidoreductases (for PVOH and PU). Recent advances in the identification of protein sequences and residues, structural domains, mechanisms of substrate binding, kinetic

analysis, and the presence and effects of cofactors have provided a better understanding of enzymatic activity on biodegradable polymers. However, a better understanding of bioassimilation and mineralization is still needed at the biochemical level of monomer compounds produced from the chemical and enzymatic hydrolysis of biodegradable polymers.

In terms of polymer properties, the key bulk properties affecting biodegradation in the mesophilic range are stereochemistry, crystallinity, and M_w, which are tailored for each application. The amorphous region offers the optimal conditions for chemical hydrolysis due to the easy diffusion of water and also for exo and endo enzymatic attack by extracellular enzymes. Microorganisms start the assimilation process when low M_w compounds such as dimers and monomers are released. Since biofilm formation, microbial colonization, and enzymatic activity are surface related processes, the key surface properties of polymers impacting biofilm formation and colonization are hydrophobic/hydrophilic balance, roughness, and surface energy parameters.

This review has summarized the enzymes and microorganisms (e.g., bacteria, fungi, and actinomycetes) isolated from several environments and showing activity towards aliphatic, aliphatic-aromatic polyesters, PUs derived from esters, and PVOH. Usually the identification of microorganisms and/or enzymes involves techniques, such as culturing, where the polymer is the solely source of carbon for the biotic process. These studies provide unique insights on enzymatic activity and pathways of degradation. However, natural environments introduce far more complexities to the degradation process, creating a dynamic that undoubtedly affects the rate of degradation. Microbial consortia have demonstrated an increased efficiency for elimination of toxic metabolites in comparison to pure cultures. Studies showed that some microorganisms are directly involved in the degradation process, while other microorganisms showed activity towards eliminating toxic metabolites excreted by the first ones. However, besides symbiotic, mutual, and synergistic interactions, efficiency

differs among microbial consortia. Complex tracking of microorganism population dynamics during biodegradation should provide better insights on the real pathways of degradation and assimilation of these polymers in actual environments. Research in the areas of biostimulation (addition of specific nutrients to the soil, compost, or water environments to stimulate the activity of naturally occurring microorganism populations), bioaugmentation (addition of specific microorganisms to increase the biodegradation rate of an indigenous microbial population), and engineering of enzymes (modification of enzymes to reach specific reactions) are needed to address the complexities associated with microbial consortia involved in the biodegradation process, extracellular enzymes, and biocatalytic cascades of enzymes.

Standards, methodologies, and techniques have been developed to assess the degradation of polymers in the environments as discussed. Some are more focused on evaluating the degradation of mechanical properties and the mass loss due to various factors of abiotic mechanisms. However, the assessment of CO_2 or O_2 and ultimate mineralization must be the definitive assessment to determine the extent of biodegradability in a specific environment. The use of complementary techniques, such as carbon tracking and M_w reduction, constitutes important tracking parameters that must be incorporated when evaluating biodegradation to the mineralization level.

Many works reported the CO_2 or mineralization for the biodegradable polymers available in the market in soil, home composting, and aquatic environments at mesophilic conditions using several standards. From the aliphatic polyesters group (e.g., PGA, PEA, PLA, PCL, and PBS), chemical hydrolysis has been reported to be the main controlling step. Other aliphatic polyesters, such as PCL, PBS, and PBSA, were consistently reported to biodegrade in soil conditions. For the aliphatic-aromatic polyesters (PBAT and PBST), reports of PBAT biodegradation in soil and marine environments are limited. The

degradation of the natural polyesters PHB and PHBV in aquatic environments was reported extensively, showing the high level of biodegradability at mild conditions. For the PUs, the presence of the soft segment offers availability for enzymatic attack and biodegradation with mineralization at some extent; however, the bioassimilation pathway of the hard segment has not been well identified and/or described.

New, innovative methods to tailor the biodegradation of biodegradable polymers through creation of novel polymers with tailored biodegradation [604], biostimulation, bioaugmentation, and addition of natural enzymes [605–607] or modified enzymes [608] are opening new routes to accelerate the biodegradation process. However, these new methods must be connected to standards to fully track the biodegradation process and the end products in the environment, so that further insights on biodegradation pathways of polymers can be elucidated.

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CHAPTER 3: BREAKING IT DOWN: HOW THERMOPLASTIC STARCH ENHANCES POLY(LACTIC ACID) BIODEGRADATION IN COMPOST - A COMPARATIVE ANALYSIS OF REACTIVE BLENDS

3.1 Abstract

Poly(lactic acid) (PLA) is a sustainable, bio-based, and industrial compostable polymer with a recalcitrant abiotic degradation phase limiting its organic recovery to well-managed industrial composting facilities. We present a methodology to biodegrade PLA in industrial and home composting settings fully. Thermoplastic starch (TPS) and PLA were reactively blended by adding a chemical modifier and peroxide radicals to obtain a PLA-*g*-TPS blend by twin screw extrusion and later processed into films by cast extrusion. Biodegradation of the films was investigated using a direct measurement respirometer (DMR) for 180 days by tracking the CO_2 evolution in compost media at 58 and 37 °C, and the molecular weight (M_n) reduction was measured by size exclusion chromatography. The hydrophilic nature of TPS and its role as a nutrient source accelerated the degradation of PLA in both abiotic and biotic phases of the composting process. The kinetic curve of M_n reduction showed the positive effect of TPS on accelerating PLA hydrolysis during the lag phase in both mesophilic and thermophilic conditions due to increased chain mobility. This work unlocks the capability of PLA-based films to be successfully composted in industrial and home composting without compromising its desired properties for applications in everyday life.

3.2 Introduction

Poly(lactic acid) (PLA), a bio-based polymer derived from renewable resources with end-of-life scenarios such as recyclability and industrial compostability, is considered a green and sustainable polymer and an alternative polymer to displace the ever-growing fossil-based plastic pollution [1]. PLA has been used in medical applications, but its use has mainly grown

in food packaging applications [2,3]. PLA and its products are labeled and marketed as industrial compostable [4,5], so it can be collected with the organic fraction of municipal solid waste (OFMSW) routed to industrial composting. Marketing bio-based and compostable plastics, such as PLA, fuels the assumption that they can be discarded with the organic fraction and treated and recovered in the same manner [6,7]. However, some challenges arise when these polymers cannot fully disintegrate in the same time frame as readily biodegradable organic fraction wastes, such as food, starch, and cellulose, leaving fragments at the end of the compost cycle. Mostly, this situation occurs due to the slow abiotic degradation controlling phase, such as in the case of PLA, associated with inherent properties, such as high initial molecular weight (M_n) and crystallinity (X_c). Although composting of the organic fraction is accomplished in industrial composting facilities, the infrastructures have not been precisely designed to keep bio-based and compostable plastics in mind. Therefore, some composters are not keen on accepting these products [8–10].

Standards are available to delineate what conditions polymers should meet to be certified as compostable in industrial composting operations [11]. Conditions for home and industrial composting are different since temperatures and humidities encountered in these two environments differ drastically. Since the material volume, reached temperature, and abundance of microorganisms are lower in home composting, it is very challenging to maintain the necessary conditions for several days.

Regardless of whether the composting is done at home, in a community backyard, or in an industrial facility, the process must ensure a succession of microbial communities (i.e., mesophiles–thermophiles–mesophiles) and the corresponding temperature regimes to operate. These factors ensure and guarantee the safety and quality of the composting process and the final product. Furthermore, this process and high temperatures are crucial to degrade a polymer, such as PLA with a high glass transition temperature (T_g *c.* 58 °C) and

whose initial breakdown is based on being able to traverse a rapid abiotic stage to reduce the weight average molecular weight number (M_n) low enough ($\lesssim$10 kDa) so that the microorganisms can use PLA oligomers as a food source [12]. Furthermore, the industrial composting process is highly controlled. Efficient control of temperature, moisture, and airflow is exercised to achieve complete waste biodegradation. Any deviation from the required conditions in terms of turning frequency of the piles, consistent mixing to maintain adequate aeration and pH, and reduction of the composting process can lead to the formation of anaerobic pockets or increase the time needed for PLA to initially degrade through the abiotic reduction process and delay the complete breakdown of PLA, thereby leaving behind partially degraded or whole packages at the end of the composting cycle. Additionally, polymer fragments at the compost pile surface could take longer to degrade since they are not exposed to the same conditions. This is especially relevant for plastics that can constantly resurface during pile turning due to their lower density. So, incomplete biodegradation of PLA as well as other compostable plastics can impact the sale of compost by failing to abide by the agricultural compost requirements. To avoid this problem, some composting facilities are apprehensive about incorporating PLA with organic wastes [9]. Methods able to accelerate the biodegradation of PLA in industrial composting facilities and possibly expand its biodegradation efficiency in home/backyard composting are highly sought.

One potential way to improve PLA properties and its degradation rate in ambient conditions is by blending it with different biodegradable polymers to improve specific properties such as toughness, flexibility, and ductility and promote its complete biodegradation post-use [1,13–15]. One such polymer that can be used to blend with PLA to improve its biodegradability is starch, which is low-cost, renewable, non-toxic, readily available, and 100% bio-based. Thermoplastic starch (TPS) is a plasticized polymer used to

produce blends with different polymers. Starch undergoes rapid biodegradation by enzymatic hydrolysis to produce glucose, which is further assimilated to produce CO_2 and H_2O in compost or soil environments [16]. To process TPS efficiently and to blend and derive good interfacial adhesion between hydrophilic starch and hydrophobic PLA, compatibilizers are generally used [17–19]. Blending PLA and TPS helps improve the oxygen and water vapor barrier properties [20] and the elongation at break of the resulting matrix [21]. Furthermore, the hydrophilic feature of TPS can enhance PLA's sensitivity to humid environments and act as an excellent initial nutrient source for the microbes when introduced into the compost environment [22–24].

PLA is environmentally benign, to begin with, and the addition of starch derived from non-human crop consumption creates a safe polymer. Biodegradation of PLA-*g*-TPS ultimately will not leave behind traces of microplastics since degradation of the PLA evolves to lactic acid traces and degradation of the starch evolves to glucose, two naturally occurring and benign monomers that can be acted upon and utilized by the microorganisms. Adding TPS can further help accelerate the low abiotic degradation rate of PLA at mesophilic conditions, which otherwise takes longer. PLA-starch blends can open avenues for home/backyard composting and address the growing concern about microplastics [25–28] and their exo-toxicological effects [29,30] since their formation can be avoided.

In recent years, several studies have been conducted focusing on the biodegradation of PLA in soil environments [31–34] by introducing starch in the PLA matrix, where the biodegradation was determined by visual analysis, weight loss, or change in mechanical properties but not CO_2 evolution or M_n reduction. Biodegradation studies have also been conducted where additives such as wood flour [24], coir [35], and montmorillonite [36] have been blended with PLA, in addition to starch. Still, the additives failed to enhance properties, and the resulting biodegradation was attributed to the presence of starch. However, a

detailed assessment of PLA and TPS mineralization and M_n evolution is still lacking, and there is a need to understand the role of starch in accelerating the biodegradation of PLA in compost. In this work, we investigated the role of starch in expediting the biodegradation of PLA under thermophilic and mesophilic conditions in a compost environment, and we monitored CO_2 evolution and quantified the changes in M_n throughout the test duration so as to replicate real-life scenarios encountered under home composting and industrial composting conditions.

3.3 Materials and methods

3.3.1 Materials

PLA resin, Ingeo™ 2003D with L-lactic content of 96%, was provided by NatureWorks LLC (Minnetonka, MN, USA). Cassava starch was procured from Aldema LLC (Cooperativa Agricola e Industrial San Alberto Ltda., Puerto Rico, Misiones, Argentina), with an amylose content of 25 ± 6% wt/wt and moisture content of approximately 12%. Glycerol (>99.5%), maleic anhydride (MA), dicumyl peroxide (DCP), and cellulose (~20 μm particle size) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Except for the PLA resin, all materials were processed in the condition they were received in. PLA pellets were dried at 50 °C overnight at 67 kPa to avoid hydrolytic degradation while processing.

3.3.2 Preparation of PLA and thermoplastic starch (TPS) masterbatches and films

Cassava starch and glycerol (70/30% wt.) were mixed and held for 12 h before processing into the TPS masterbatch. The temperature profile for the TPS masterbatch was set at 25/100/105/110/115/120/120/120/115/115 °C for each zone from feed to the die, with a screw speed of 120 rpm. A ZSK-30 co-rotating twin screw extruder (Century, Traverse City, MI, USA) [37] was used to process the various masterbatches; the screw length was 1,260 mm and the diameter was 30 mm, with an L/D ratio of 42:1. PLA, MA, and DCP were mixed

prior to processing; PLA-*g*-MA (PLA with 2% wt. MA and 0.65% wt. DCP, based on PLA weight) was processed in the twin screw extruder. The temperature profile for the PLA-*g*-MA masterbatch was set at 140/150/160/160/160/170/170/170/170/160 °C for each zone from feed to the die, with a screw speed of 120 rpm, feed rate of 70 g/min, and residence time of approximately 3 min. The extrudate from the die was fed through a water bath and pelletized using a BT 25 pelletizer (Scheer Bay Co., Bay City, MI, USA). The PLA-*g*-MA masterbatch was kept in an oven at 50 °C for 3 h to remove any traces of moisture and was later transferred to the freezer and stored at −15 °C until processed into films. The same procedure was followed to produce a PLA control masterbatch. To obtain PLA-*g*-TPS, PLA (56% wt.), TPS (30% wt.), and PLA-*g*-MA (14% wt.) were processed together to produce the final reactive blend. The temperature profile for the PLA-*g*-TPS masterbatch was set at 140/150/160/160/160/170/170/170/170/160 °C for each zone from feed to the die, with a screw speed of 120 rpm, feed rate of 70 g/min, and residence time of approximately 3 min, which was dried in a vacuum oven at 50 °C for 3 h to remove any residual traces of moisture. Figure S1 of the supporting information depicts the processing of PLA-*g*-MA, PLA and TPS masterbatches and the temperature profile for the twin screw extruder.

The masterbatch was introduced in a RCP-0625 microextruder (Randcastle Extrusion Systems, Inc., Cedar Grove, NJ, USA) to produce cast films; the screw diameter was 1.5875 cm, with an L/D ratio of 24/1 and volume of 34 cm^3, and the temperature profile was set at 140/150/160/160/160/170/168 °C from feed to die. The screw speed, nip roller, and winding roller were set at 30 rpm, 50 rpm, and 12 rpm, respectively [38]. The same procedure was used to process PLA films. Figure S2 shows the processing of PLA-*g*-MA, PLA and TPS masterbatches into PLA-*g*-TPS film using the cast film extruder.

3.3.3 Biodegradation test in simulated compost conditions

The biodegradability of PLA and PLA-*g*-TPS films was investigated by analysis of evolved CO_2 in a simulated composting environment using an in-house built direct measurement respirometer (DMR) system [11,39]. The biodegradation tests were run at 37 ± 2 °C and 58 ± 2 °C to simulate mesophilic and thermophilic environmental conditions, respectively. The DMR chamber was maintained at specific temperatures, and a Li-COR® LI-820 non-dispersive infrared gas analyzer (Lincoln, NE, USA) was used to measure the evolving CO_2 concentration from each DMR bioreactor. The relative humidity was maintained at 50% ± 5% and the optimal airflow rate was set at 40 ± 2 sccm [40,41].

Compost was procured from the Michigan State University composting facility (East Lansing, MI, USA). The compost was filtered using a 10-mm screen to remove any larger lumps of material and then was conditioned at 37 ± 2 °C and 58 ± 2 °C for one week before the respective test use. The procured compost was characterized for its physicochemical parameters (presented in **Table A3.1**, Appendix 3B). When running the biodegradation test, deionized water was added to the compost to regulate and sustain the moisture content at around 50%. Vermiculite, an inert, inorganic hydrous phyllosilicate matrix, obtained from Sun Gro® Horticulture Distribution Inc. (Bellevue, WA, USA), was mixed 1:4 (wt/wt) with compost. Vermiculite does not affect the biodegradation test and helps provide better aeration, improving the accessible space, thus improving the microbial activity [42].

Each bioreactor was loaded with 400 g of compost which was then mixed with 8 g of film samples (cut into 1-cm^2 pieces) or 8 g of cellulose. Experiments with film samples in compost media were run in triplicate. Cellulose, a positive control reference, was used in the test due to its high biodegradation nature and as requested by ASTM D5338-15(2021) [11]. Three bioreactors were run with compost only (blank) and without any film samples or

cellulose to identify the background CO_2 evolution. CO_2-free air (≲30 ppm CO_2) was passed through each bioreactor, and the evolved CO_2 was measured for a set period. After completing measurements for each bioreactor, the whole system was purged using CO_2-free air to ensure no residual CO_2 was disturbing the baseline test [41]. The mineralization formula shown in equation (3.1) was used to calculate the amount of carbon that was converted to CO_2:

$$Mineralization\ \% = \frac{(CO_2)_t - (CO_2)_b}{M_t\ x\ C_t\ x\ \frac{44}{12}}\ x\ 100 \tag{3.1}$$

where $(CO_2)_t$ is the average cumulative mass of CO_2 evolved from the bioreactor containing the sample, $(CO_2)_b$ is the average cumulative mass of CO_2 evolved from the blank, M_t represents the total mass of the sample in the bioreactor, C_t is the total carbon content of the sample and is derived from CHN analysis, 44 is the molecular weight of CO_2, and 12 is the atomic weight of carbon. The equation numerator represents the actual CO_2 evolved from the sample after accounting for the background compost activity, and the denominator depicts the maximum theoretical CO_2 the sample produces, i.e., when 100% of the sample carbon is converted to CO_2.

3.3.4 Hydrolysis experiment

A hydrolysis test method adapted from ASTM D4754-18 [43] was run for PLA films at 58 ± 2 °C and 37 ± 2 °C to understand the hydrolytic degradation. The hydrolysis cell consisted of a stainless-steel wire, glass beads, and a glass vial with cap. PLA films were cut into small discs of 2-cm diameter, and ten such discs were strung into a stainless-steel wire and separated by glass beads. The vial was filled with 35 mL of HPLC-grade water (J.T. Baker, Center Valley, PA, USA). The water was preconditioned, and the hydrolysis cell was stored at the same temperature. Triplicates of the PLA films were retrieved at predetermined time intervals and dried before running size exclusion chromatography to assess the M_n reduction.

3.3.5 Size exclusion chromatography

The film samples were collected at specific intervals to determine the Mn, weight average molecular weight (M_w), and molecular weight distribution (MWD). Samples were weighed and dissolved in tetrahydrofuran (THF) solvent in a ratio of 2:1. THF solvent was used as a mobile phase. A size exclusion chromatography (SEC) unit from Waters Corp. (Milford, MA, USA) was supplied with an isocratic pump (Waters® 1515), an autosampler (Waters® 717), a series of Styragel® columns (HR4, HR3, HR2), and a refractive index detector (Waters® 2414). The detector was maintained at 35 °C, and a 1 mL/min flow rate was applied for the mobile THF solvent. The Mark-Houwink constants of K = 0.000174 mL/g and α = 0.736 were used to determine the M_n and M_w of the PLA fraction in the samples. A detailed description of the technique can be found elsewhere [44]. The data was analyzed using the Waters Breeze™ 2 software.

3.3.6 Elemental analysis

The carbon, hydrogen, and nitrogen content of the PLA and PLA-*g*-TPS films was calculated using a PerkinElmer Series II CHNS/O Elemental Analyzer (PerkinElmer Inc., Shelton, CT, USA). Approximately 2 mg of sample was weighed in a small tin capsule and tested. Samples were tested in triplicates. The values are presented in **Table 3.1**.

Table 3.1 Percent of carbon, hydrogen, and nitrogen content by weight of cellulose and film samples.

Material	% Carbon	% Hydrogen	% Nitrogen
Cellulose	42.50 ± 0.34	6.53 ± 0.05	0.04 ± 0.01
PLA	49.72 ± 0.19	5.72 ± 0.04	0.11 ± 0.07
PLA-*g*-TPS	48.20 ± 0.05	6.00 ± 0.04	0.05 ± 0.01

3.3.7 Statistical analysis

The statistical analysis was conducted using MINITAB™ software (Minitab Inc., State College Park, PA, USA). The statistical significance at $p < 0.05$ was evaluated using one-way ANOVA and Tukey-Kramer test. Data is reported as means ± standard deviation.

3.4 Results and discussion

PLA films have a recalcitrant abiotic degradation stage, limiting its deployment to well-managed industrial composting facilities. Facilities that rotate compost in a very short period (*c.* ≲2 months) and do not follow a practice of allowing a full cycle of mesophilic, thermophilic, mesophilic, and mature phases are not able to fully biodegrade PLA, leaving residual at the end of the period [8]. Previously, we developed PLA-*g*-TPS reactive blend films with suitable thermal, mechanical, and barrier properties for several applications, including packaging and agriculture, to be recovered through composting [37,45]. **Figure A3.3 b**, Appendix 3C shows the SEM images for PLA-*g*-TPS film showing uniform dispersion of the TPS phase in the PLA matrix by reactive blending as opposed to physical blending. Here, we present the biodegradation of these PLA-*g*-TPS films in simulated composting at thermophilic and mesophilic conditions using a DMR unit and report the M_w reduction and thermal properties.

3.4.1 CO_2 evolution and mineralization of PLA and PLA-*g*-TPS films

PLA-*g*-TPS films were evaluated alongside PLA in simulated composting conditions at 58 ± 2 °C and 37 ± 2 °C to understand the effect of reactive blending TPS on PLA degradation. **Figure 3.1a and b** present the CO_2 evolution and % mineralization of the blank (compost only), cellulose, PLA films, and PLA-*g*-TPS films in compost at 58 ± 2 °C. The blank showed maximum CO_2 evolution of 28.3 g at day 180. The positive control cellulose achieved a 40.8 g for CO_2 evolution and showed maximum mineralization of *c.* 100%. No lag phase

was observed for cellulose, as it is a readily available food source for microorganisms and easily biodegradable. Cellulose is degraded by the action of a battery of enzymes that work simultaneously and synergistically. Cellulases catalyze the hydrolysis of β-1,4-linkages in the cellulose [46]. The action of exoglucanases and endoglucanases on the ends and at random internal sections of cellulose's amorphous region produces varying lengths of cello-oligosaccharides, which are then hydrolyzed by glucosidases to produce glucose [47]. Glucose is finally converted to CO_2 through a series of further cycles. Previous research has shown fungi, a few bacteria species, and actinomycetes in compost and the soil environment produce cellulase and are completely involved in the degradation of cellulose [48–52].

The biodegradation curve for PLA showed a lag phase of around 20 days, attributed to the initial abiotic hydrolysis phase. Ester bonds of PLA are cleaved during the hydrolytic abiotic degradation phase due to its susceptibility to water. As hydrolytic degradation proceeds, PLA chains are broken into smaller fragments, releasing low M_w oligomer populations. These lactic acid oligomers are available for microbial assimilation, releasing CO_2 and water, which can be observed during the biotic degradation phase. A maximum CO_2 evolution of 39 g and mineralization of *c.* 77% was observed for PLA over the test duration of 180 days at 58 °C.

PLA-*g*-TPS films showed a CO_2 evolution of 42.8 g and a maximum mineralization of 100% over the test duration of 180 days. PLA-*g*-TPS did not show any lag phase compared to the 20-day phase for PLA. This finding can be ascribed to the presence of TPS acting as an initial food supply for the microorganisms until the PLA undergoes hydrolysis, fragmentation, and is depolymerized into small fragments available for further assimilation. Starch, like cellulose, is a natural hydrophilic, biodegradable polymer and can be used up almost immediately as the microorganisms get acclimatized to the compost environment. The presence of hydroxyl groups in TPS contributes to and enhances the biodegradation of PLA

by further enabling the disintegration process of PLA-*g*-TPS films. Several research studies have shown that introduction of starch increases the water absorption characteristics of a matrix. Maran et al. [53] demonstrated improved water absorption due to the presence of starch in specimens buried in soil for degradation studies. Also, the presence of starch increases the hygroscopic characteristics of films, which favors water absorption, thereby providing suitable conditions for fast hydrolysis and microbial invasion and colonization [34,54,55]. The presence of amylopectin branched hydroxylated chains improves the water penetration in PLA-*g*-TPS film matrices [33,56]. This sensitivity of starch to water helps in the enzymatic hydrolysis to glucose.

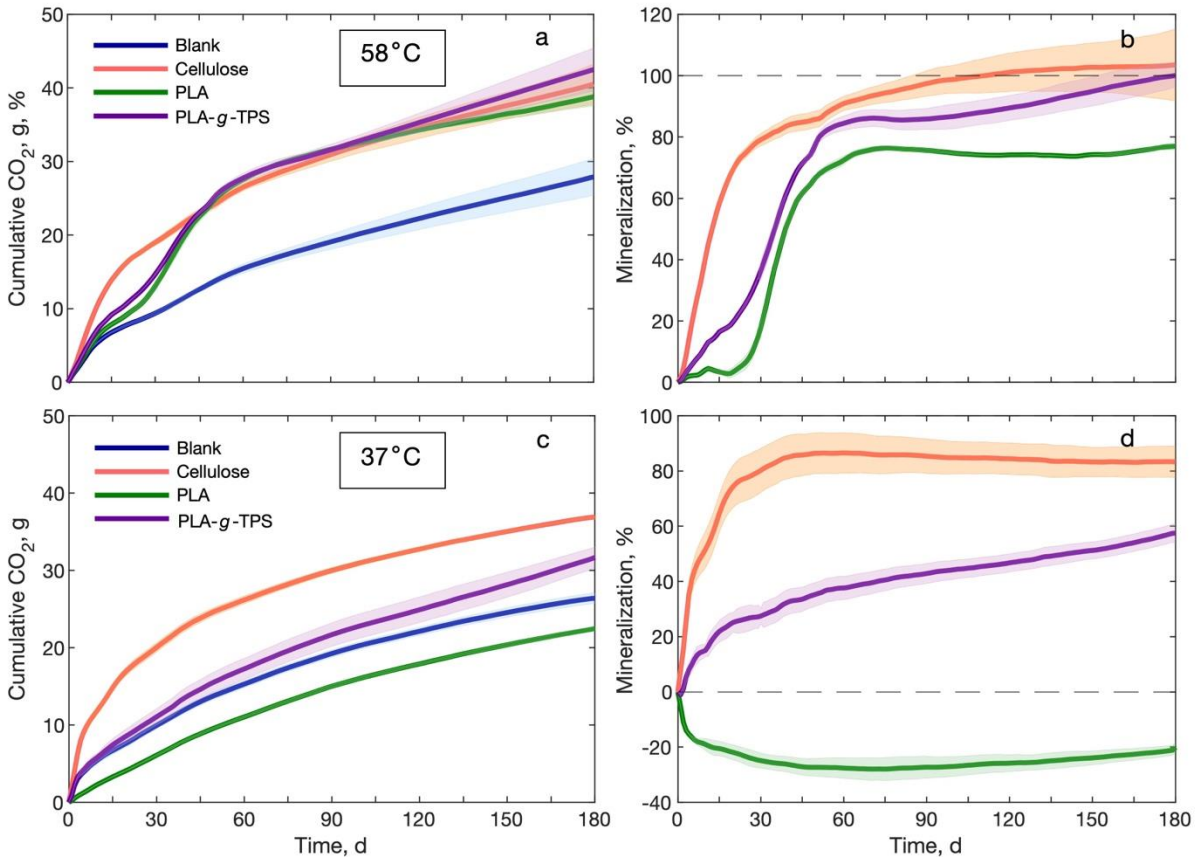

Figure 3.1 CO_2 evolution and % mineralization of PLA and PLA-*g*-TPS films in compost at 58 °C (a & b) and 37 °C (c & d). The shade in the background for each material represents the standard error between replicates.

Figure 3.1c and d depict the cumulative CO_2 evolution and % mineralization for cellulose, PLA films, and PLA-*g*-TPS films at 37 °C to evaluate the biodegradation behavior in mesophilic conditions. Cellulose achieved mineralization of *c*. 86% in 50 days and evolved around 37 g of CO_2 by the end of 180 days. There was no lag phase for cellulose, as also observed at 58 °C.

Bioreactors containing compost and PLA produced *c*. 22 g of CO_2 in compost around 180 days. In contrast, blank bioreactors evolved *c*. 26 g of CO_2, which is translated to negative mineralization in the case of PLA. In a blank bioreactor, the microbes can easily utilize the organic matter in the compost, whereas in a bioreactor containing PLA, the presence of PLA leads to a reduction in the working efficiency, which translates to reduced carbon conversion as a collective. The negative mineralization values are generated as an artifact and indicate that the blank bioreactors produce more CO_2 than the bioreactors containing PLA samples. This could mean that PLA offers a physical hydrophobic barrier to water and air, and the microbes have difficulty accessing the carbon source as nutrients. It could also indicate that there is non-uniform mixing of the compost and PLA samples in the bioreactors, which could additionally hinder the microbes. Any change in the optimal conditions for the biological activity, either due to an insufficient supply of water (dryness of the compost), excess of water (agglomeration), or obstruction in the airflow passage, can create an unfavorable environment for microbial development. The negative mineralization values in the case of PLA do not necessarily imply the absence of hydrolytic degradation or enzymatic activity due to the action of extracellular enzymes secreted by the microbes but more like inhibition of the microbial activity due to the hydrophobic layer barrier created by the presence of the sample. The lower values of CO_2 evolution in the case of PLA indicate that the samples are still undergoing hydrolysis and are yet to be reduced to a point ($\lesssim$10 kDa) needed to activate the

biodegradation stage where the microorganisms can start assimilating low M_w PLA, such as oligomers, dimers, and monomers for their biochemical processes. The hydrolytic degradation progresses very slowly when PLA is exposed to temperatures lower than T_g (*c.* 60 °C) due to scarce chain mobility of the amorphous fraction and low water diffusion in the crystalline fraction for effective bulk erosion [12,57,58]. PLA segments have little to no mobility and are not flexible below T_g, preventing diffusion or attack by water. Since the initial and rate-limiting step in PLA degradation is chemical hydrolysis, the lack of chain scissions, perhaps accompanied by simultaneous low surface colonization by microorganisms due to the hydrophobic surface [56], could have further prevented penetration by water molecules and slowed down the hydrolysis rate. Further attestation to this rationale is the lower CO_2 evolution values obtained for PLA (22 g) compared to the blank (26 g). The above reasons, as well as the lower chemical hydrolysis rate of PLA, can account for lower CO_2 evolution as compared to the blank.

In the case of PLA-*g*-TPS, *c.* 58% mineralization was observed by the end of the test (180 d) with an upward mineralization trend, since starch, like cellulose, is a readily available nutrient for the microbial species in the compost [59]. No lag phase was seen in the case of PLA-*g*-TPS films, which evolved 34.2 g of CO_2 by the end of the test. Ho and Pometto [22] documented mineralization of *c.* 70% for starch alone at 28 °C after 98 days and a higher mineralization rate with increasing temperature. The mineralization for PLA-*g*-TPS films at mesophilic conditions corroborates the research presented earlier [23,60,61]. The hydroxylated chains in TPS impart hydrophilicity to PLA, pave the way for water dispersion and hydrolysis in the blend matrix and support microbial development and growth [33,62]. The hydrophilic characteristics further stimulate microbial colonization and biofilm formation on the surface of PLA-*g*-TPS films. This is further corroborated by changes in the

roughness and contact angle for PLA and PLA-*g*-TPS film due to the addition of TPS, as seen in **Figure A3.4** and **Figure A3.5**, Appendix 3D.

Starch is enzymatically hydrolyzed by the action of extracellular enzymes, namely α/β-amylase belonging to the glycoside hydrolase family. Glucose produced as a result is then converted to pyruvic acid by glycolysis. Pyruvic acid is a precursor for the tricarboxylic acid cycle for energy production [63]. Once the microorganisms use starch as their food source, fragmented PLA films (holes and cracks) are left behind. These structural discrepancies in the form of macroscopic fractures aid in the biodegradation process. Starch biodegradation and PLA chemical hydrolysis processes complement each other, resulting in improved biodegradation of a starch-PLA mixture compared to PLA alone. Usually, at higher temperatures, such as industrial composting conditions, these complementary processes happen concurrently, and hence an improved degradation is seen for PLA. **Figure 3.1b and d** show that adding TPS to PLA not only accelerates the disintegration of PLA-*g*-TPS films in industrial composting but also opens a venue to make PLA blends biodegradable in home composting conditions if key parameters such as aeration and moisture are controlled.

3.4.2 Molecular weight reduction rate

The M_n was measured, and the reduction rate calculated during the abiotic hydrolysis stage was used to elucidate if the TPS fraction accelerated the hydrolysis of PLA besides also enhancing the biotic degradation. PLA and PLA-*g*-TPS films were retrieved at specific time intervals from the sampling bioreactors to track the reduction of M_w.

Figure 3.2 presents the MWD over time for PLA films and the PLA fraction in PLA-*g*-TPS films in thermophilic and mesophilic conditions. For 58 ± 2 °C (**Figure 3.2a & b**), both films show shifting of the peaks depicting a decrease in M_n and broadening of the peaks, indicating an increase in dispersity ($Đ$) associated with hydrolytic degradation of the PLA

chains. The shifting of peaks in the abiotic phase reveals that the chemical hydrolysis is due to random chain scission occurring in the bulk of the polymer and is not restricted to the surface [64,65]. The change in the peaks from monomodal to multimodal after day 15 is a characteristic feature of reconfiguration and crystallization of the newly crystalline region resulting from the regrouping of newly formed short polymer chains formed at $M_n \lesssim$10kDa. The amorphous region is subjected to hydrolysis, while the crystalline region remains stable [65]. Slight differences can be observed at day 20 on the MWD but may not be sufficient to impact the samples. Although the portion of PLA in PLA-*g*-TPS overall MWD follows the same path, no lag phase was observed in **Figure 3.1b**, supporting a more rapid disintegration of the samples.

For 37 ± 2 °C (**Figure 3.2c & d**), the PLA MWD shift occurred very slowly, as shown in **Figure 3.2c,** especially in the case of PLA. The peak amplitude remained the same, even at day 180, and a minimal shift of M_n is observed, indicating that chemical hydrolysis happened very slowly. Whereas for PLA-*g*-TPS films (**Figure 3.2d**), shifting and significant peak reduction are observed after day 90, indicating the reduction of the M_n and initial change in crystallinity (**Figure A3.6 and Table A3.2**, Appendix 3F). As biodegradation of the TPS component of the PLA-*g*-TPS film proceeded, so did hydrolysis of the PLA matrix, shown by the broadening of the peaks after day 60 and the peak reduction. This was accompanied by a stable increase in % mineralization, as shown in **Figure 3.1d**.

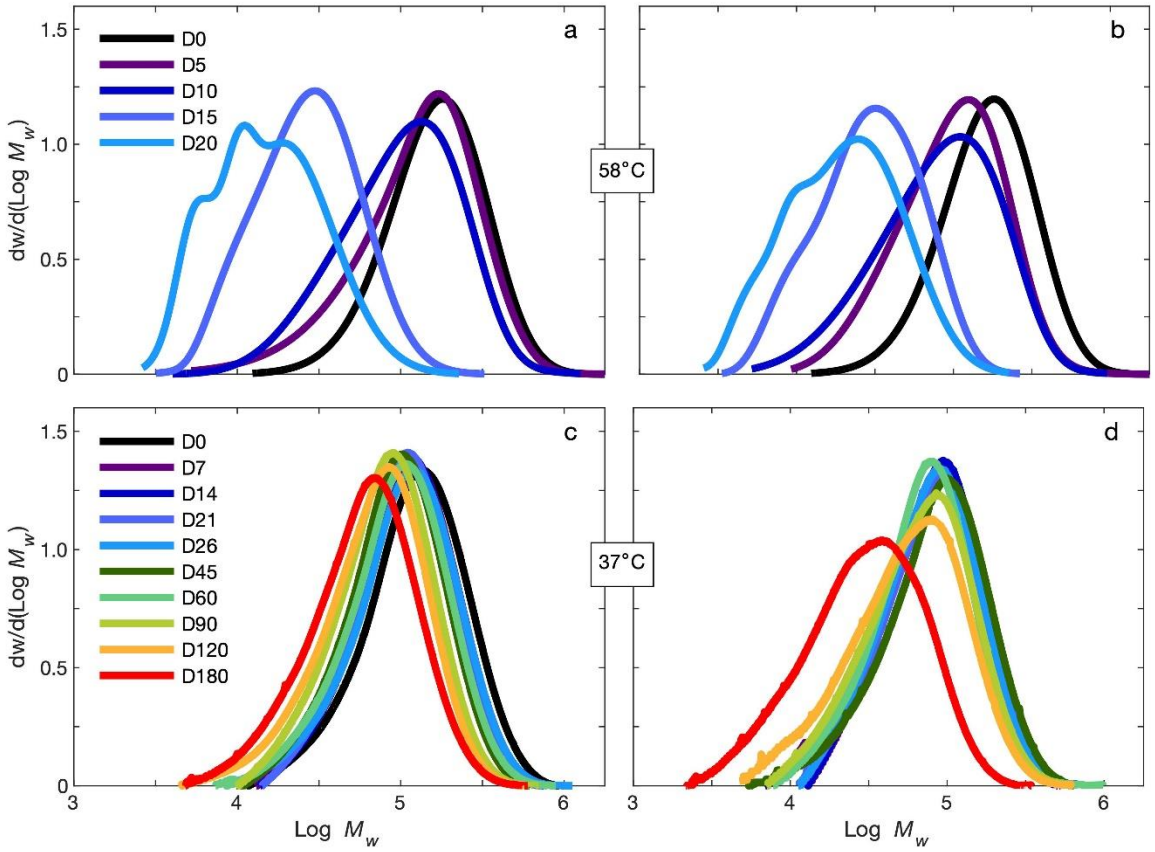

Figure 3.2 Molecular weight distribution of PLA and PLA-*g*-TPS films in compost at 58 °C (a & b) and 37 °C (c & d), respectively.

Figure 3.3 shows the reduction in M_n of PLA and PLA-*g*-TPS films in compost at 58 ± 2 °C and 37 ± 2 °C. In the thermophilic test, the samples could be retrieved only until day 20. For the mesophilic test, the samples were collected until day 180. **Figure 3.3** shows a more rapid reduction of the M_n at 58 ± 2 °C than at 37 ± 2 °C due to the higher hydrolysis rate at elevated temperatures. Furthermore, a larger deviation of the M_n of PLA-*g*-TPS seemed to occur at 180 days.

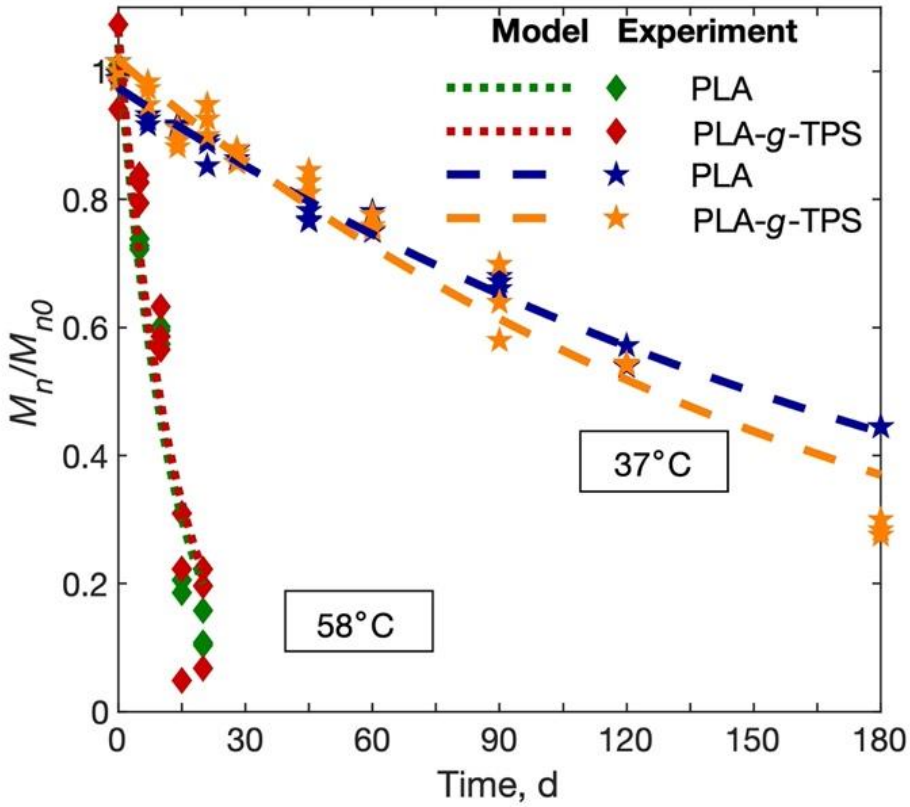

Figure 3.3 Normalized M_n reduction as a function of time for PLA and PLA-*g*-TPS films in compost at 58 °C and 37 °C. The experimental data were fitted using a first-order reaction of the form $M_n / M_{no} = e^{(-kt)}$, where M_{no} is the initial M_n, k is the rate constant, and t is the time. The data points indicate the original experimental triplicate values at specific times.

Hydrolysis experiments for PLA films were concurrently performed at 58 ± 2 °C and 37 ± 2 °C to decouple, simulate, and understand the abiotic phase (lag phase of biodegradation curve) dominated by chemical hydrolysis, as also reported elsewhere [44]. Hydrolysis in solid state (compost) and water are not exactly the same. We have previously conducted these experiments in sterilized vermiculite to show the difference; however, in this case, hydrolysis in water should be a sufficient proxy to model the abiotic stage. Additionally, the hydrolysis data obtained at 58 ± 2 °C was also used to predict the lifetime of PLA at 37 ± 2 °C using the master curve technique reported by Limsukon et al., who studied the hydrolytic degradation of PLA films over a series of temperatures (40 to 95 °C) and constructed a master curve to

predict the lifetime of PLA films at any temperature [57]. More details about this methodology can be found in section S5 of the Supporting Information. For this work, the fit obtained from the accelerated degradation testing at 58 ± 2 °C was used to predict the hydrolytic degradation at 37 ± 2 °C, as seen in **Figure 3.4**. To achieve the same reduction in M_n, *c.* 10 kDa or similar disintegration values for PLA, approximately 730 days were required at 37 ± 2 °C as compared to 25 days at 58 ± 2 °C.

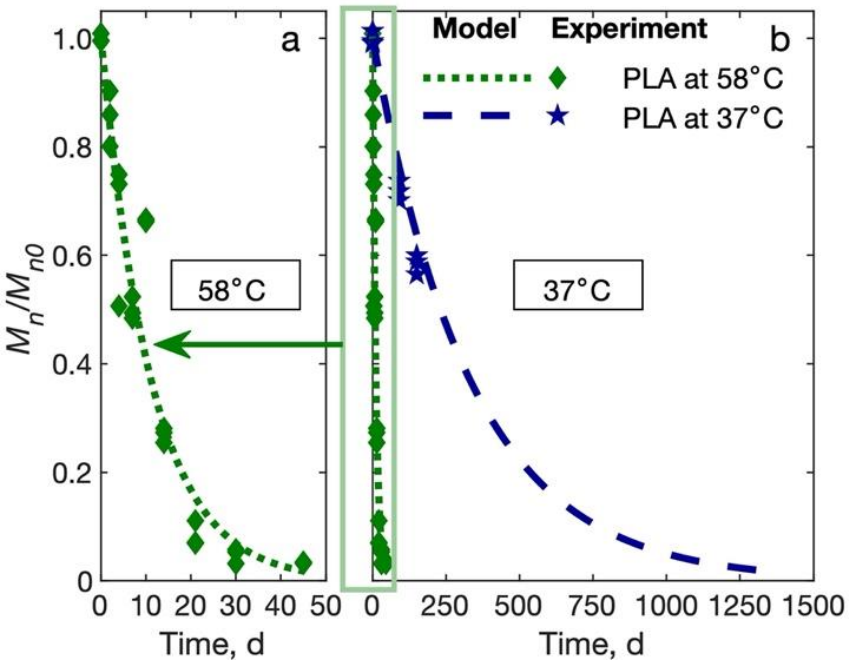

Figure 3.4 Hydrolysis of PLA at 58 ± 2 °C (a) and master curve prediction for hydrolytic degradation at 37 ± 2 °C (b). a) represents the actual experimental data obtained at 58 ± 2 °C. The data points indicate the original experimental triplicate values at specific times.

As shown in **Figure 3.3** and **Figure 3.4**, PLA degradation follows a first-order reaction, where chemical hydrolysis is the dominant phase at high and lower temperatures and controls the degradation rate. So, the degradation rate of PLA and PLA in PLA-*g*-TPS in compost at 37 ± 2 °C was modeled to a first-order reaction. **Table 3.2** details the rate constant k (d^{-1}) for M_n reduction of PLA and PLA-*g*-TPS films for both conditions. No significant difference in M_n reduction among PLA and PLA-*g*-TPS films was observed in thermophilic

conditions at 58 ± 2 °C. At the higher temperature, PLA is in the rubbery state, enabling easy diffusion of water and, thereby, more rapid hydrolysis. The presence of starch definitely aids in the initial disintegration and biodegradation of PLA [23], but at the higher temperature, the process of chemical hydrolysis is dominant and governs the reduction in M_n to differentiate any advantage associated with the presence of starch on the abiotic degradation of PLA.

Table 3.2 Rate constant (k) for PLA and PLA-*g*-TPS films evaluated in compost media at 58 ± 2 °C and 37 ± 2 °C.

Film	k (d^{-1}) at 58 ± 2 °C	k (d^{-1}) at 37 ± 2 °C
PLA	0.0885 ± 0.0010[a]	0.0045 ± 0.0001[a]
PLA-*g*-TPS	0.0900 ± 0.0167[a]	0.0056 ± 0.0002[b]

Values with different letters in a column are statistically different (α = 0.05 Tukey-Kramer Test).

The chemical hydrolysis process proceeds at a lower rate at 37 ± 2 °C than at 58 ± 2 °C. Conversely, there is a significant effect on the reduction in M_n of the PLA-*g*-TPS films due to the presence of starch in the PLA matrix at 37 ± 2 °C, suggesting that the addition of starch leads to enhanced degradation at mesophilic conditions. The presence of starch increases the hydrophilicity of the PLA-*g*-TPS films, further promoting water absorption and microbial colonization and leading to changes in the structural integrity. This results in the accelerated degradation of PLA in PLA-*g*-TPS films as compared to neat PLA with a different (k) rate constant at 37 ± 2 °C.

Lv et al. conducted a biodegradation study in outdoor soil conditions to evaluate and account for the role starch plays in PLA degradation, and found higher degradation of PLA in PLA/starch composites as compared to pure PLA; the authors reported the theoretical and experimental values of PLA degraded in the presence of starch through weight loss and component content analysis determination [31,66]. Yu et al. also demonstrated higher biodegradability, using the mass loss technique, for PLA/starch blends as compared to PLA

in soil conditions of 44 °C and high humidity due to the presence of acetylated starch [67]. Palai et al. recorded M_w reduction, depletion in mechanical properties, along with scanning electron microscopy morphological analysis, and reported higher degradation for PLA/starch blown films as compared to PLA and PLA/PBSA (poly butylene succinate-*co*-adipate) blend in a soil environment at 30 ± 2 °C due to the presence of TPS [68].

In this work, we demonstrated that reactive blending of starch with PLA presents an array of options for PLA to be easily accessed by water and microorganisms, thereby creating avenues for accelerated disintegration and rapid mineralization of PLA-*g*-TPS films at thermophilic conditions, which can increase the acceptance of PLA by industrial composting facilities. The blending of TPS and PLA also improves PLA biodegradation in mesophilic environments, thereby opening an opportunity for creation of PLA-based films that can be home composted. Overall, these findings can also help tackle the ever-growing problem of microplastics left behind after disintegration and incomplete biodegradation of plastics.

3.5 Conclusion

The biodegradation performance of a polyester-based reactive blend was evaluated in simulated composting conditions in both mesophilic and thermophilic conditions. PLA-*g*-TPS films showed the highest mineralization trend at 58 ± 2 °C by day 180, indicating that the presence of starch significantly affects the final biodegradation of PLA by reducing the initial lag phase and accelerating M_n reduction. Under mesophilic conditions (37 ± 2 °C), PLA-*g*-TPS showed improved degradation compared to PLA due to plasticized starch, achieving more than 57% mineralization. The fact that the amount of starch was around 30% of the blend indicates that PLA is being degraded and TPS influences M_n reduction of PLA as well. The presence of starch imparts hydrophilicity and surface roughness to PLA-*g*-TPS composites and creates favorable conditions for enhanced microbial activity. Furthermore, starch

accelerates the biodegradation of pure PLA by acting as a food reservoir for the growth of microorganisms while PLA undergoes chemical and enzymatic hydrolysis. Starch can be used to improve PLA degradation in industrial composting conditions by reducing the lag phase of disintegration of PLA and, even at mild conditions as in home composting settings, by inducing biodegradation during the initial days.

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APPENDIX 3A: MATERIAL PROCESSING

Maleic anhydride (MA) and dicumyl peroxide (DCP) along with PLA was used to produce PLA-*g*-MA masterbatch. Similarly, glycerol and starch were processed together to produce a thermoplastic starch (TPS) masterbatch. PLA pellets were processed to produce a PLA masterbatch. The different zones in the co-rotating twin screw extruder namely the feeding-melting zone, large kneading/mixing zone, conveying zone, short kneading zone, and conveying zone provide the necessary shear in the form of mechanical and thermal energy to produce TPS [69]. **Figures A3.1** and **A3.2** show the processing of PLA, PLA-*g*-MA, and TPS masterbatches and PLA-g-TPS films respectively.

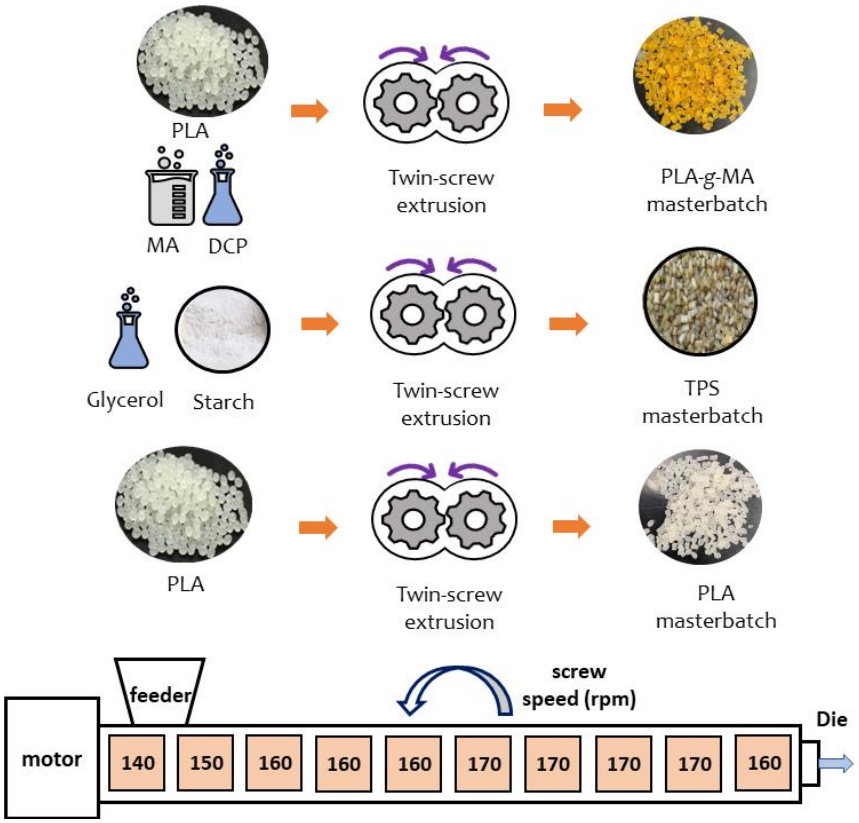


Figure A3.1 Processing of PLA-*g*-MA, TPS, and PLA masterbatches in a twin screw extruder and temperature profile from feeder to the die of the twin screw extruder.

The masterbatches produced from the twin screw extruder were blended in specific proportion (56% PLA, 14% PLA-g-MA, and 30% TPS) in a cast film extruder to produce PLA-*g*-TPS films.

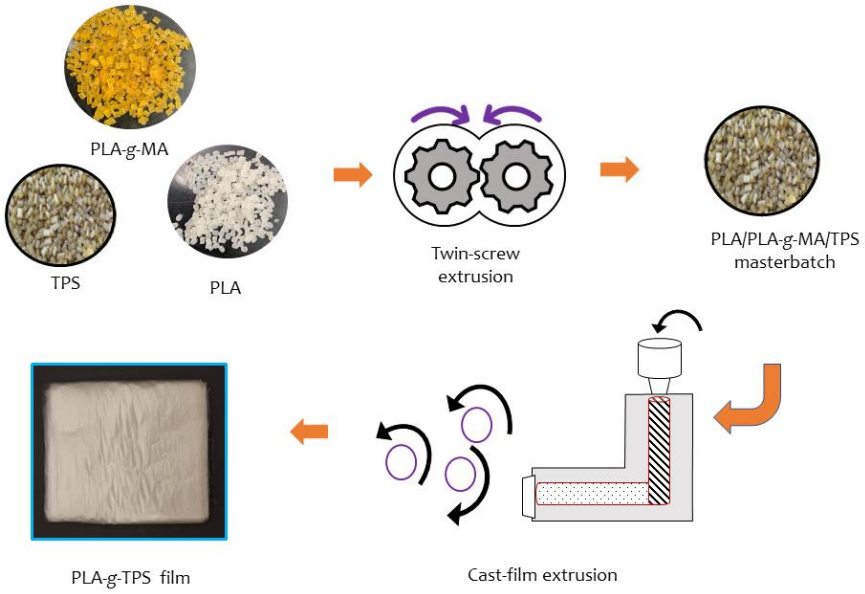


Figure A3.2 Processing of PLA-*g*-MA, TPS and PLA masterbatches into PLA-*g*-TPS film by cast film extrusion.

APPENDIX 3B: PHYSICOCHEMICAL CHARACTERISTICS

Some compost was collected and sent to the Soil and Plant Nutrient Laboratory at Michigan State University (East Lansing, MI, USA) to evaluate its physicochemical parameters (dry solids, volatile solids, and C/N ratio) as previously described elsewhere [12]. The physicochemical parameters are reported below in **Table A3.1**.

Table A3.1 Physicochemical parameters and total nutrient analysis of compost used in the biodegradation test.

Parameter	Compost
Dry solids, %	42.5
Volatile solids, %	41.7
pH	8.0
C/N ratio	10.1
Carbon, %	24.2
Nitrogen, %	2.42
Phosphorus, %	1.21
Potassium, %	3.15
Calcium, %	5.07
Magnesium, %	2.82
Sodium, %	0.58
Sulfur, %	0.58
Iron, ppm	9878
Zinc, ppm	480
Manganese, ppm	413
Copper, ppm	107
Boron, ppm	41
Aluminum, ppm	6751

APPENDIX 3C: MORPHOLOGICAL CHARACTERIZATION OF PLA-*g*-TPS REACTIVE BLEND

The PLA-TPS physical blend and PLA-g-TPS reactive blend were characterized by SEM to observe the morphological changes [70]. **Figure A3.3 a** shows the non-uniform dispersion of the TPS phase in the PLA matrix for PLA-TPS physical blend while **Figure A3.3 b** shows good compatibilization and distribution of TPS domain in the PLA matrix.

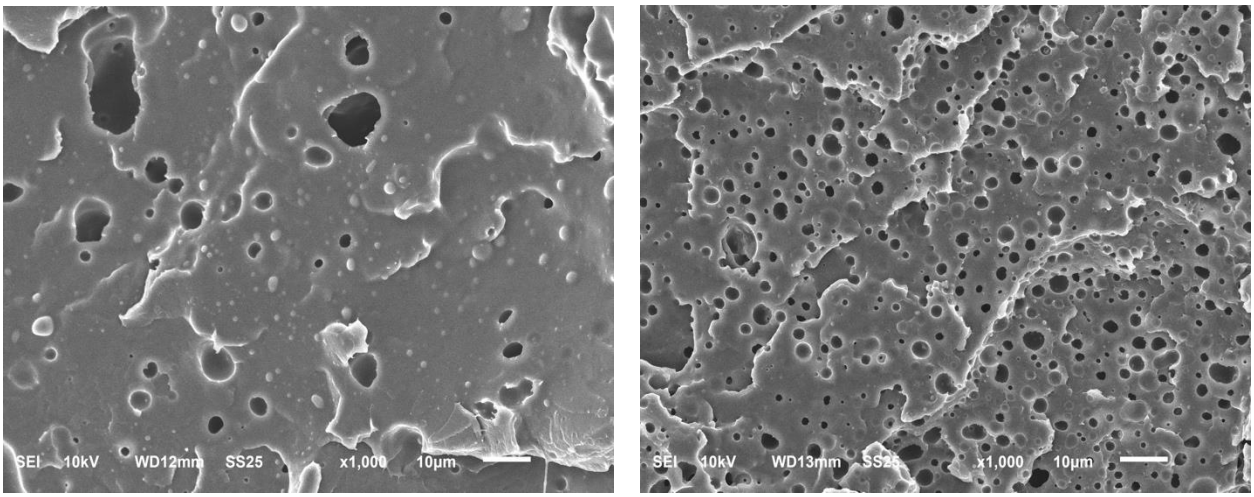
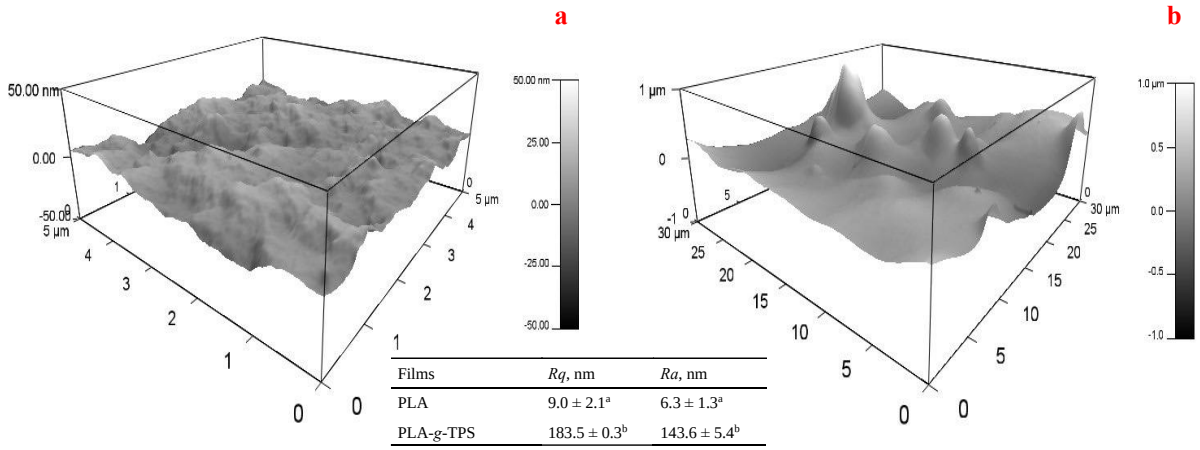


Figure A3.3 SEM images for (a) PLA-TPS physical blend and (b) PLA-*g*-TPS reactive blend.

APPENDIX 3D: ROUGHNESS AND CONTACT ANGLE MEASUREMENTS

The roughness of the PLA film and PLA-*g*-TPS films in **Figure A3.4 a** and **b** respectively was conducted using a CypherTM atomic force microscope (Oxford Instruments Asylum Research, Inc., Santa Barbara, CA, USA) in the contact mode. Roughness parameters, calculated as the root mean square (*Rq*) and average roughness (*Ra*), were determined for each type of film and were calculated from the Htr mode image. Images were obtained in the Dfr mode [71]. The film area for the determination of roughness was 900 µm2.



Films	*Rq*, nm	*Ra*, nm
PLA	9.0 ± 2.1[a]	6.3 ± 1.3[a]
PLA-*g*-TPS	183.5 ± 0.3[b]	143.6 ± 5.4[b]

Figure A3.4 Surface roughness of (a) PLA and (b) PLA-g-TPS films as measured by atomic force microscopy. Roughness parameters, calculated as the root mean square (Rq) and average roughness (Ra), were determined for each type of film and were calculated from the Htr mode image. Within columns, values followed by a different letter are significantly different at $p \leq 0.05$ (Tukey's test).

The contact angle of the films was measured by the sessile drop technique using an in-house built goniometer equipped with a diffuse light source and a digital camera. Film samples of 7 cm length and 2 cm wide were attached to microscope slides. Next, a drop of HPLC-grade water (3 µL) was deposited on each film surface, and the contact angle was determined by using the tangent method as seen in **Figure A3.5 a** and **b** [72].

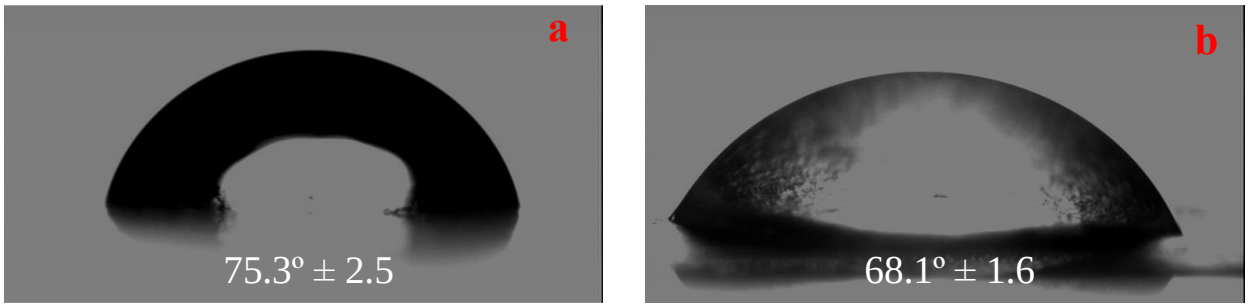


Figure A3.5 Water contact angle measurement on (a) PLA and (b) PLA-*g*-TPS films.

APPENDIX 3E: APPLICATION OF THE TIME-TEMPERATURE SUPERPOSITION PRINCIPLE FOR PREDICTING HYDROLYTIC DEGRADATION IN MESOPHILIC CONDITIONS

In a previous work, Limsukon et al. [73] studied the hydrolytic degradation of PLA film at temperatures from 40 to 95 °C and presented the lifetime prediction methods at the lower temperature of interest using the time-temperature superposition principle. The Williams-Landel-Ferry (WLF) equation was used to fit the hydrolysis of PLA film over the wide range of experiment temperatures crossing the T_g. The WLF equation is given as follows:

$$\log\alpha_T = -\frac{C_1(T - T_i)}{C_2 + (T - T_i)} \quad \text{(A3.1)}$$

C_1 and C_2 can be expressed as:

$$C_1 = \frac{E/R}{2.303\,(T_i - T_s)} \text{ and } C_2 = (T_i - T_s) \quad \text{(A3.2)}$$

where T and T_i are experimental temperature and the temperature of interest. T_s is the temperature at which the conformation entropy induced by segmental motion approaches zero (is about 50 K below the T_g of PLA immersed in water which is 51.6 °C or 324.75K [74]). E is the pseudo activation energy over a temperature range from below to above T_g. R is the gas constant (8.314 J/mol·K). a_T is the factor in shifting the data obtained at temperature T to overlap one at T_i.

To predict the hydrolysis of PLA in the mesophilic condition, a_T value was calculated by substituting C_1 and C_2 into S1 to shift the experimental data curves of 58 °C and construct the master curve at 37 °C using E of the hydrolysis of neat PLA film measured within the temperature range crossing the T_g reported as 2720 J/mol ; a_T can be determined as 0.03205.

Finally, the master curve of the hydrolysis at 37 °C can be constructed by multiplying the hydrolysis time range by $1/a_T$ and plotting as a function of the prediction line of hydrolysis at 58 °C. A detailed description of the calculation can be found elsewhere [73,75].

APPENDIX 3F: CRYSTALLINITY MEASUREMENTS

The films retrieved from the sampling bioreactors were analyzed for the evolution of crystallinity throughout the test for PLA and PLA-*g*-TPS films at both 37 °C and 58 °C, respectively. The crystallinity (X_c) of the samples was tracked using a DSC Q100 (TA Instruments, New Castle, DE, USA). Samples weighing between 5 and 10 mg were sealed in aluminum pans and subjected to a cycle ranging from −5 °C to 210 °C. The samples were cooled to −5 °C and then heated to 210 °C at a ramp rate of 10 °C/min under a nitrogen atmosphere where the purge flow was maintained at 70 mL/min. The data obtained was analyzed using the software Thermal Universal Analysis 2000, V4.5 (TA Instruments). The % X_c was calculated using the equation S3:

$$X_c(\%) = \frac{(\Delta H_m - \Delta H_c)}{\Delta H_m^{\circ}\left(1 - \frac{\%wt_{filler}}{100}\right)} \times 100 \qquad \text{(A3.3)}$$

where ΔH_m is the heat of fusion, ΔH_c is the enthalpy of cold crystallization, ΔH_m° is the enthalpy fusion for 100% pure crystalline PLA (93 J/g), and wt_{filler} is the weight fraction of TPS (30%).

Table A3.2 Crystallinity evolution for PLA and PLA-*g*-TPS films at 58°C and 37°C.

Material	58 ± 2 °C		37 ± 2 °C	
	Day 0	Day 15	Day 0	Day 90
PLA	3.1 ± 0.6	35.5 ± 0.2	28.2 ± 3.5	32.3 ± 1.8
PLA-*g*-TPS	7.0 ± 0.6	39.8*	7.0 ± 0.6	18.5 ± 2.2

***** Only one measurement is noted for the sample due to the fragile state of the film.

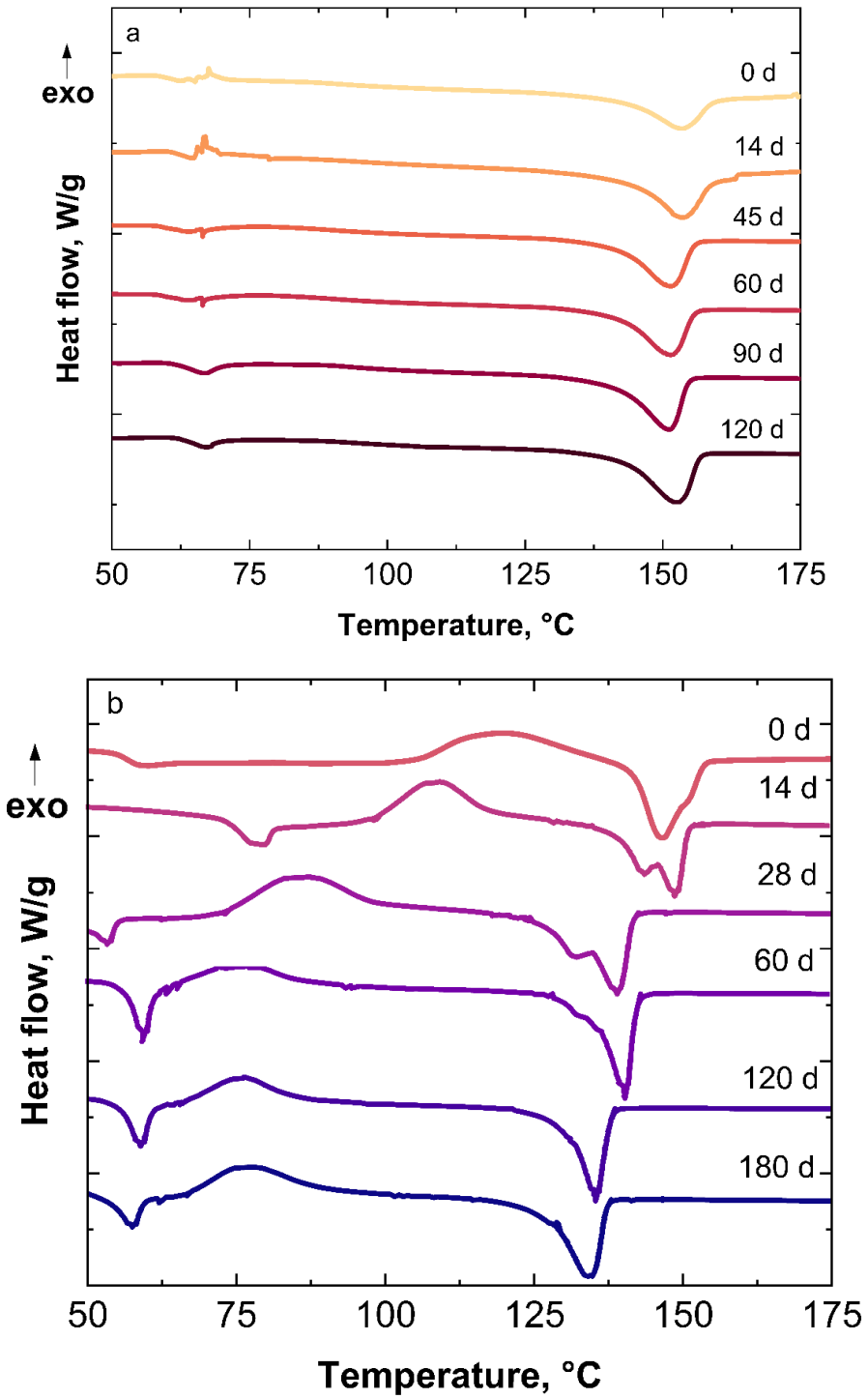


Figure A3.6 DSC thermograms showing the evolution of crystallinity of a) PLA and b) PLA-g-TPS films in compost at 37 °C.

CHAPTER 4: ACCELERATING BIODEGRADATION: ENHANCING POLY(LACTIC ACID) BREAKDOWN AT MESOPHILIC ENVIRONMENTAL CONDITIONS WITH BIOSTIMULANTS

4.1 Abstract

Poly(lactic acid) – PLA – has garnered interest due to its low environmental footprint and ability to replace conventional polymers and be disposed of in industrial composting environments. Although PLA is compostable when subjected to a suitable set of conditions (i.e., aerobic thermophilic conditions for an extended period), its broader acceptance in industrial composting facilities has been affected adversely due to longer degradation timeframes than the readily biodegradable organic waste fraction. PLA must be fully exposed to thermophilic conditions for prolonged periods to biodegrade, which has restricted its adoption and hindered its acceptance in industrial composting facilities, also negating its home composting potential. Thus, enhancing PLA biodegradation is crucial to expand its acceptance. PLA's biodegradability was investigated in a compost matrix under mesophilic conditions at 37°C for 180 days by biostimulating the compost environment with skim milk, gelatin, and ethyl lactate to enhance the different stages of PLA biodegradation. The evolved CO_2, number average molecular weight, and crystallinity evolution were tracked. To achieve Mn ≲10 kDa for PLA, biodegradation rate was accelerated by 15% by adding skim milk, 25% by adding gelatin, and 22% by adding ethyl lactate. This work shows potential techniques to help biodegrade PLA in compost by adding biostimulants.

4.2 Introduction

Poly(lactic acid) (PLA) is a well-known biodegradable thermoplastic polymer and has garnered a lot of interest due to its ability to exhibit reasonable properties as compared to fossil-based conventional polymers [1]. In addition, PLA has turned out to be a key player

leading the global bioplastic segment as per the market research conducted by nova-Institute which estimates an increase in bioplastics production from 2.18 million tonnes in 2023 to 7.43 million tonnes in 2028 [2]. Due to its biodegradable nature instead of contributing to plastic waste in landfills, PLA can be rerouted to composting facilities [3]. PLA is known to undergo chemical hydrolytic degradation during composting, significantly reducing its molecular weight [4]. This reduction in molecular weight precedes the assimilation by microorganisms and final mineralization to CO_2. However, it is difficult to degrade PLA at lower temperatures (e.g., backyard or home composting environments – mostly mesophilic conditions) because of its dependence on high temperatures (≳60°C) to undergo chemical hydrolysis. Additionally, PLA degradation in natural environments (usually associated with lower temperatures) is slow due to the sparse distribution of PLA-degrading microbes compared with other more aggressive thermophilic degradable environments such as industrial composting [5,6]. A detailed discussion about the compostability and biodegradation of PLA can be found elsewhere [7,8].

Biostimulation and bioaugmentation techniques are included under the bioremediation domain and are used to eradicate hazardous pollutants/waste materials that may be toxic to the environment. These techniques use enhanced settings or biological workers/microorganisms to eliminate pollutants. They also are designed to make up for the lack of factors that can speed up the removal process of pollutants in an eco-friendly approach. Biostimulation is an approach that addresses limiting factors such as nutrients (in the form of enzyme inducers),[9,10] electron donor or acceptor compounds,[11,12] nitrogen-supplying compounds,[13,14] and compounds that can activate the biochemical processes by providing necessary resources or chemicals to stimulate the environment [15,16]. The biostimulation technique has been used extensively to revitalize the native microbial

communities to boost the degradation of heavy and toxic metals,[11,17,18] diesel oil,[19,20] chlorinated hydrocarbons,[21] and petroleum hydrocarbons,[22,23] since these compounds are hard to biodegrade by native microorganism populations.

PLA biodegradation proceeds much faster at temperatures higher than its glass transition temperature (T_g) ~ 58°C, commonly reached in industrial composting conditions. Chemical hydrolysis, the dominant mechanism responsible for the significant reduction in molecular weight of PLA, takes place quickly at higher temperatures [24]. The resulting low molecular weight PLA is then available for further assimilation by the microorganisms.

Under thermophilic conditions (i.e., 45–60°C), the chemical hydrolysis of PLA proceeds quickly, efficiently, and in a suitable time frame compared to mesophilic conditions (i.e., 20–45°C). Accelerating PLA degradation, particularly hydrolytic degradation in compost, is exceptionally challenging because of the limitation of the solid complex matrix to be at high temperatures to activate the biodegradation process. Several research studies have focused on including metal compounds in PLA to catalyze its degradation [25–29]. But most of these metal compounds are included in PLA as blends/nanocomposites at lower concentrations, significantly altering physical properties such as number and weight average molecular weight, melting temperature, and T_g [30–33]. These final properties do not accurately represent the post-consumer PLA discarded packages ending up in compost, soil, or landfill scenarios.

Apart from the chemical hydrolysis, the biodegradation of PLA also entails enzymatic degradation, the release of extracellular enzymes by the microbes, which simultaneously break down PLA into low molecular weight compounds. The extracellular depolymerases released by the microorganisms in the case of PLA degradation belong mainly to the hydrolase class of enzymes, primarily proteases, and some also belong to lipase and cutinase [34–36]. The serine proteases produced by microbes disintegrate the ester bonds of PLA [37–

39]. Several studies have used the biostimulation technique to expedite the biodegradation of bio-based polymers. But most of these studies were conducted with PLA as the sole carbon source, in liquid media with specific microbial strains, and usually at higher temperatures [40–43]. So, there is a gap between these experiments conducted in highly controlled incubated culture media and the real-world unchecked environment in solid media such as soil, industrial composting, and home composting. The biological activity in such natural environments is highly complex due to the diversity of microorganisms competing or working together to consume organic resources.

This study aimed to evaluate the addition of different biostimulant compounds, which can be added externally in the compost media (solid) to accelerate the biodegradation of PLA in mesophilic conditions. The compounds selected as biostimulants were screened through multiple factors and were expected to meet various criteria: not toxic towards the microorganisms present, not antibacterial, able to disintegrate, consumable by the microorganisms, biodegradable in a suitable timeframe, cost-effective, and readily available or accessible.

Hydrolases (EC 3) are the hydrolytic enzymes responsible for degrading hydrolyzable polymers such as esters, carbonate, and amide groups. The ester bonds in the backbone of polyesters act as hydrolyzable linkages and are acted upon by esterases (EC 3.1) and proteases (EC 3.4), which are released by the PLA-degrading microbes. To induce the activity of these enzymes by microbes, skim milk and gelatin have been reported as suitable compounds [44–47]. So, skim milk and gelatin can incite proteolytic activity for enzymatic degradation, resulting in the cleavage of PLA polymer chains.

To stimulate the lactate-utilizing microbial species in compost, ethyl lactate can be used. Ethyl lactate belongs to the lactate esters family. It has been used as a food source for dechlorinators to remediate heavy metals in soil under anaerobic conditions [48–50]. It is

considered a green solvent with numerous advantages such as favorable low toxicity and low cost, and is readily available. It is environmentally benign, highly biodegradable, and has a strong solvency power [51,52]. Ethyl lactate undergoes hydrolysis to produce ethanol and lactate, where both can act as electron donor compounds for reductive degradation. Lactate has been shown to act as an electron donor compound in the case of anaerobic degradation [53,54]. Hydrolysis of ethyl lactate is a slow process ensuring a constant, long-term supply of hydrogen as the electron donor for the microbial redox process. The use of ethyl lactate as a biostimulant for polymer degradation has not yet been reported.

Thus, this study aimed to investigate the effectiveness of biostimulating the compost environment with compounds that may enhance enzymatic degradation (skim milk and gelatin), and with an electron donor compound (ethyl lactate) to improve the aerobic biodegradation of PLA in compost at mesophilic conditions under which it is more difficult to degrade PLA. The difference in the CO_2 evolution and changes in the number average molecular weight (M_n) and crystallinity (X_c) of PLA degradation with and without biostimulants was tracked to account for the activity of biostimulants in compost.

4.3 Materials and methods

4.3.1 Materials

Commercial PLA resin, Ingeo™ 2003D, with l-lactic acid content of 96%, with weight average molecular weight (M_w of $2.23 \pm 0.04 \times 10^5$ Da) and number average molecular weight (M_n of $1.14 \pm 0.07 \times 10^5$ Da) was obtained from NatureWorks LLC (Minnetonka, MN, USA). Skim milk powder was procured from the local grocery store. Gelatin of the brand McCormick & Co. (Hunt Valley, MD, USA) was purchased online. Ethyl lactate was procured from Sigma Aldrich (St. Louis, MO, USA). Cellulose of ~20 µm particle size was procured from from Sigma-Aldrich (Milwaukee, WI, USA).

4.3.2 Characterization of PLA and the biostimulants

The carbon, hydrogen, and nitrogen contents of PLA resin, skim milk, gelatin, and ethyl lactate were determined using a CHNS/O Elemental Analyzer, PerkinElmer 2400 Series II (Shelton, CT, USA), and are presented in **Table 4.1**. The methodology was provided in our previous work [55].

Table 4.1 Percent of carbon, hydrogen, and nitrogen content by weight of cellulose and film samples.

Material	% Carbon[a]	% Hydrogen[a]	% Nitrogen[a]
PLA	49.72 ± 0.19	5.72 ± 0.04	0.11 ± 0.07
Skim milk	41.28 ± 0.13	6.33 ± 0.03	5.75 ± 0.06
Gelatin	44.80 ± 0.30	7.00 ± 0.10	16.4 ± 0.10
Ethyl lactate	31.93 ± 0.98	5.46 ± 0.34	0.05 ± 0.03

a: Percentage by weight.

4.3.3 Biodegradation in compost

The biodegradation of PLA and the effect of biostimulant addition on the degradation of PLA were evaluated under aerobic mesophilic conditions using an in-house direct measurement respirometric (DMR) system for analysis of evolved CO_2 under simulated composting conditions. The test was adapted to ASTM D53338-15 and ISO 14855-12, as currently there are no standards in place for evaluating the aerobic biodegradation of plastic materials under controlled composting conditions at mesophilic temperatures [56–58]. The system was equipped with a non-dispersive infrared gas analyzer (NDIR) Li-COR® LI-820 (Lincoln, NE, USA), which measures the CO_2 concentration. The temperature and relative humidity (RH) of the chamber was maintained at 37 ± 2°C and 50% ± 5% RH, and the airflow

rate was controlled at 40 ± 2 cm^3/min. Detailed information about the DMR chamber can be found elsewhere [24].

Manure compost was obtained from the Michigan State University (MSU) Composting Facility (East Lansing, MI, USA). The compost was sieved using a 10-mm screen to remove any large debris or chunks that might be present, and post screening was conditioned at 37 ± 2°C. Deionized water was added to adjust the moisture content to 50%. Vermiculite, a hydrous phyllosilicate inorganic material of premium grade, was obtained from Sun Gro Horticulture Distribution Inc. (Bellevue, WA, USA). The vermiculite was mixed 1:4 with compost (dry weight basis). Samples of the compost were analyzed by the Soil and Plant Laboratory at MSU (East Lansing, MI, USA) for physicochemical parameters such as dry solids, volatile solids, pH, and C/N ratio, as presented in **Table 4.2**.

The bioreactors were packed with 400 g of compost, and then 8 g of PLA sample and 8 g of the selected biostimulant were added, and all the samples were tested in triplicate.

Table 4.2 Physicochemical parameters and total nutrient analysis of compost used in the biodegradation test.

Parameter	Compost	Parameter	Compost
Dry solids, %	42.5	Magnesium, %	2.82
Volatile solids, %	41.7	Sodium, %	0.58
pH	8.0	Sulfur, %	0.58
C/N ratio	10.1	Iron, ppm	9878
Carbon, %	24.2	Zinc, ppm	480
Nitrogen, %	2.42	Manganese, ppm	413
Phosphorus, %	1.21	Copper, ppm	107
Potassium, %	3.15	Boron, ppm	41
Calcium, %	5.07	Aluminum, ppm	6751

4.3.4 Biostimulation

Ethyl lactate, skim milk, or gelatin were added at 8 g to separate bioreactors and mixed thoroughly with the compost matrix containing the 8 g of PLA pellets to ensure uniform distribution. The bioreactor used to study the biodegradation of PLA samples is shown in **Figure 4.1**.

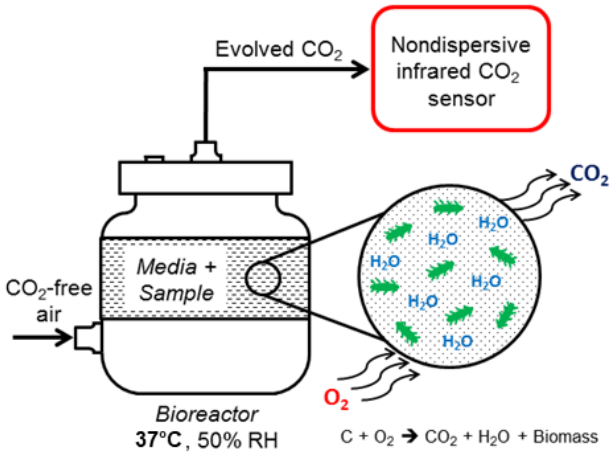


Figure 4.1 Bioreactor used to study the biodegradation of samples.

Moisture content was adjusted, and the optimal conditions were maintained by injecting the required amount of deionized water weekly into all bioreactors. CO_2-free air (<30 ppm) was supplied to each bioreactor and the CO_2 evolved was measured for a specific time. The measurement system was purged after every measurement to get rid of any traces of CO_2 from the previous bioreactor and to ensure that the baseline was achieved. Mineralization, which is defined as the total amount of carbon converted to CO_2 molecules, was calculated according to equation (4.1)

$$Mineralization\ \% = \frac{(CO_2)_t - (CO_2)_b}{M_t\ x\ C_t\ x\ \frac{44}{12}}\ x\ 100 \qquad (4.1)$$

The numerator expresses the CO_2 evolved from the sample, which is calculated by subtracting the average cumulative mass of CO_2 evolved from the blank $(CO_2)_b$ from the average cumulative mass of CO_2 evolved from the bioreactor containing the sample $(CO_2)_t$. The denominator represents the theoretical amount of CO_2 that can be produced by the sample and is calculated as a product of the total mass of the sample in the bioreactor (M_t), and the total carbon content of the sample as derived from CHN analysis (C_t). 44 is the molecular weight of CO_2, and 12 is the atomic weight of carbon.

4.3.5 Hydrolysis experiment

A hydrolysis test method adapted from ASTM D4754-18 [59] was run for PLA films at 58 ± 2 °C and 37 ± 2 °C to understand the hydrolytic degradation. The hydrolysis cell consisted of a stainless-steel wire, glass beads, and a glass vial with cap. PLA films were cut into small discs of 2-cm diameter, and ten such discs were strung into a stainless-steel wire and separated by glass beads. The vial was filled with 35 mL of HPLC-grade water (J.T. Baker, Center Valley, PA, USA). The water was preconditioned, and the hydrolysis cell was stored at the same temperature. Triplicates of the PLA films were retrieved at predetermined time intervals and dried before running size exclusion chromatography to assess the M_n reduction.

4.3.6 Size exclusion chromatography

A size exclusion chromatography (SEC) instrument was used to quantify the weight average (M_w), number average (M_n) molecular weight and molecular weight distribution (MWD) of the PLA treated with and without biostimulants. SEC system from Waters Corp. (Milford, MA, USA) was furnished with an autosampler (Waters® 717), a refractive index detector (Waters® 2414), an isocratic pump (Waters® 1515), and a series of Styragel® columns (HR4, HR3, HR2). The flow rate and temperature was maintained at 1mL/min and 35°C respectively. The Mark-Houwink constants of K = 0.000174 dL/g and α = 0.736 were

used for PLA dissolved in tetrahydrofuran solvent. Waters BreezeTM2 software was used to examine the data obtained.

4.3.7 Differential scanning calorimetry

A differential scanning calorimeter (DSC), model Q100 (TA Instruments, New Castle, DE, USA), was used to determine the glass transition temperature (T_g), melting temperature (T_m), crystallization temperature (T_c), and crystallinity (X_c) for the PLA samples retrieved from biostimulated compost. Samples weighing between 5–10 mg were sealed in aluminum pans and subjected to a heating cycle to understand the evolution of crystallinity. The samples were cooled down to −5°C, and then heated to 210°C at a ramp rate of 10°C/min. The cooling was achieved using a nitrogen cooling system that maintained the purge flow rate at 70 mL/min. Thermograms were analyzed using the Thermal Universal Analysis 2000 software, V4.5 (TA Instruments). The degree of crystallinity was calculated using equation (4.2)

$$\chi_c\ \% = \frac{\Delta H_m - \Delta H_c}{\Delta H_m^0}\ x\ 100 \tag{4.2}$$

where ΔH_m is the heat of fusion, ΔH_c is cold crystallization enthalpy, and ΔH_m^0 is the heat of fusion for 100% crystalline pure PLA (93 J/g).

4.3.8 Statistical analysis

All the statistical analyses was conducted using MINITAB™ 18 software (Minitab Inc., State College Park, PA, USA). One-way ANOVA and Tukey's test were used to evaluate statistical significance at $p < 0.05$. All the values are reported as mean ± standard deviation.

4.4 Results and discussion

The biodegradation of PLA in biostimulated compost with skim milk, gelatin, or ethyl lactate under mesophilic conditions (37°C) was evaluated for 180 days to understand the effect of these compounds on stimulating home composting operations and understanding the

potential effects in industrial composting. To decouple the effects of these compounds, the CO_2 evolution, M_n, and X_c of the samples were tracked.

4.4.1 Effect of skim milk on cellulose and PLA degradation

Figure 4.2a and **b** present the CO_2 evolution and % mineralization of the blank (compost only), cellulose, skim milk, PLA, cellulose+skim milk (Cell+skm), and PLA+skim milk (PLA+skm) in compost at 37°C. Cellulose evolved around 36.8 g of CO_2 and reached a mineralization of 87.7%, whereas skim milk showed around 39.2 g of CO_2 evolution and attained over 100% of its carbon conversion over 180 days. The priming effect is observed in the case of skim milk, due to over-degradation of the indigenous carbon present in the compost. Since skim milk and cellulose are readily biodegradable and can be easily utilized as a carbon source by the microorganisms present in the compost, no lag phase was observed. Skim milk was added to the compost to induce the protease activity of the microbes present, since serine protease (3.4.21.112), belonging to the peptidases, is in the class of extracellular enzymes able to hydrolyze the peptide bonds linking to the amino acids in the protein structure.

Skim milk comprises lactose, casein, and whey protein, making it a good precursor for the protease enzymatic activity by the microorganisms in the compost. Since cellulose and skim milk constitute excellent carbon sources when both are present in the compost, they are expected to show a much higher CO_2 evolution. However, the CO_2 evolution of the bioreactor containing both cellulose and skim milk was around 49.1 g, which is close to the CO_2 evolution of cellulose and skim milk separately and shows 96.6% biodegradation.

To better understand the interaction and isolate the degradation behavior of cellulose in the presence of skim milk ($Cell_{skm}$), we estimated the mineralization value wherein the background signal from the bioreactor containing skim milk is subtracted. As seen in **Figure 4.2b**, the mineralization % depicting the degradation behavior of cellulose reaches a

maximum value of 95.9%. The value is higher than for cellulose alone, as skim milk and cellulose are easily accessible to microorganisms.

PLA pellets in compost showed around 22.5 g of CO_2 evolution, whereas the blank produced around 26.1 g of CO_2, implying that no carbon from PLA was degraded. The negative mineralization values indicate that the blank bioreactors produced more CO_2 than the bioreactors containing PLA samples. In a blank bioreactor, the microbes are easily able to assimilate the organic matter in the compost, whereas the presence of PLA in a bioreactor reduces the working efficiency, which in turns shows diminished CO_2 production. PLA offers an initial physical hydrophobic barrier to water, making it difficult for the microorganisms to utilize it as a carbon source and establish a macro colony able to break down PLA. We did not see any mineralization in PLA due to the low temperature of 37°C, which is opposite to the degradation of PLA in thermophilic temperatures [1,24].

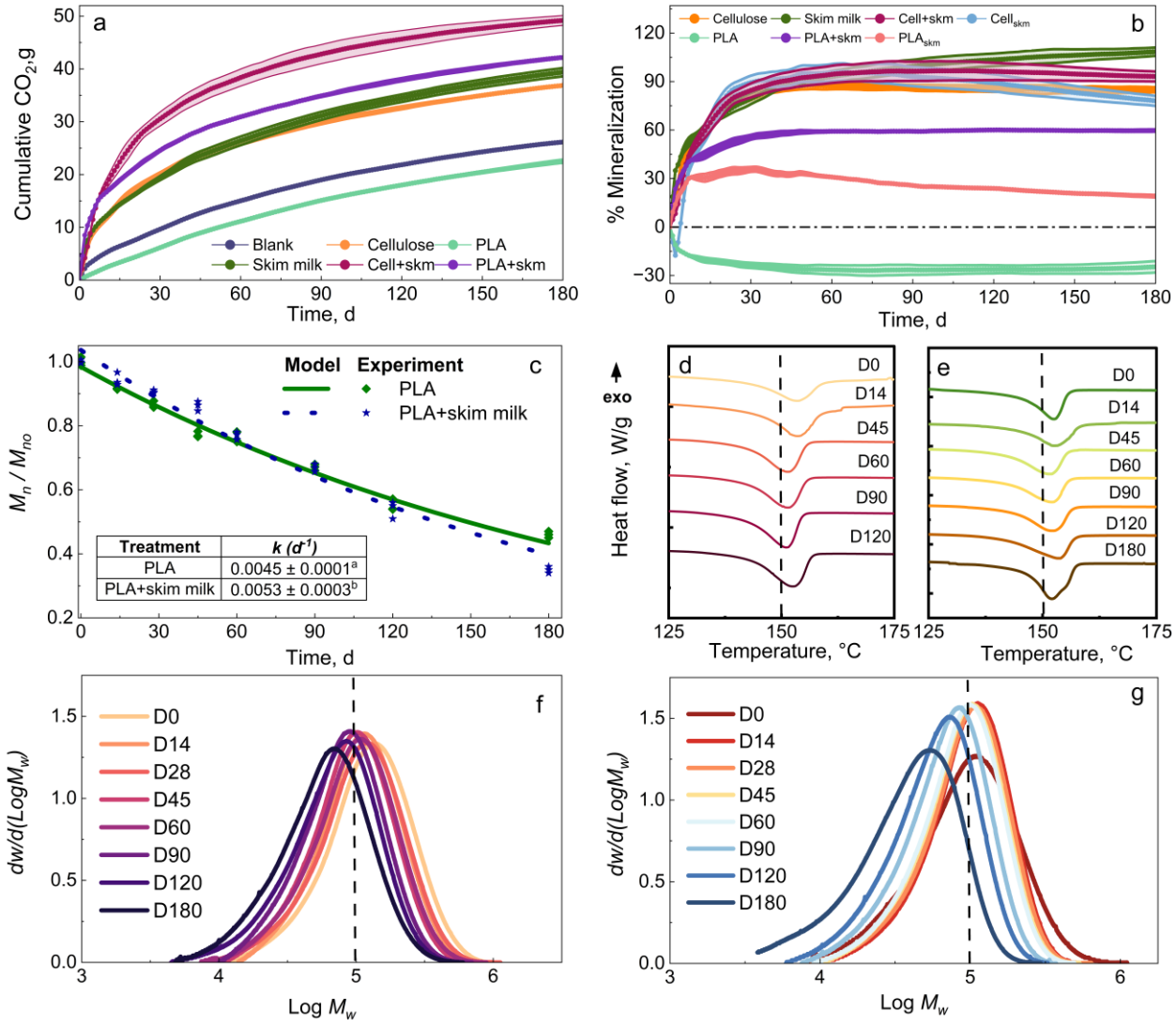


Figure 4.2 Cumulative CO_2 evolution (a) and mineralization (b) of blank, cellulose, PLA, skim milk, cellulose + skim milk (Cell+skm), PLA + skim milk (PLA+skm) in compost at 37°C. (c) represents the normalized M_n reduction as a function of time for PLA in control compost and compost biostimulated by skim milk. The experimental data was fitted using a first-order reaction of the form $M_n / M_{no} = e^{(-kt)}$, where M_{no} is the initial M_n, k is the rate constant, and t is the time. The inset shows the k-fitted values; values with different lowercase letters are statistically different ($\alpha = 0.05$ Tukey-Kramer Test). (d) and (e) depict DSC thermograms for PLA and PLA in compost biostimulated by skim milk, respectively. (f) and (g) show the MWD of PLA in compost and compost biostimulated with skim milk, respectively.

Chemical hydrolysis is the initial and primary driving mechanism in the biodegradation of PLA. It dramatically reduces molecular weight and aids in PLA assimilation by microorganisms [60–62]. The T_g of PLA is much closer to the industrial

composting conditions, which reduces the rigidity of its polymer chains, creating more free volume and rearranging the polymer chains for easy diffusion of water into the matrix.[63,64] The polymer hydrophilicity is also elevated at higher temperatures, further promoting microbial attachment besides the chemical hydrolysis [65,66]. Apart from this, the initial M_n of PLA plays a crucial role in the biodegradation of PLA [67]. The bioreactor containing PLA and skim milk (PLA+skm) shows CO_2 evolution of 42.1 g and maximum mineralization of 60.1% by the end of the test duration. This result indicates improved mineralization compared to PLA alone, where no mineralization was observed. To isolate the degradation behavior of PLA in the presence of skim milk, mineralization of PLA_{skm} (subtracting blank + skim milk) was calculated, as described earlier, and is shown in **Figure 4.2b**. We observed positive mineralization, indicating PLA's enzymatic degradation due to the presence of skim milk. To further corroborate the biostimulation activity of skim milk, samples of PLA were retrieved from the bioreactor at specific intervals, and their M_n was evaluated. The reduction in M_n of PLA was tracked until the end of the test, as seen in **Figure 4.2c**. For the skim milk treatment, the kinetic reduction rate of biostimulated PLA was higher than for PLA. There was a significant difference in the degradation rates for PLA alone and PLA treated with skim milk, as indicated by the k values shown in **Figure 4.2c** inset (PLA $k = 0.0045 \pm 0.0001$ and PLA+skim milk $k = 0.0053 \pm 0.0003$). This increase in k can be interpreted as a final reduction of around 75 days when PLA biostimulated with skim milk reaches $M_n \lesssim 10$kDa at 420 days, where microorganism assimilation of PLA n-mers accelerates biodegradation [24]. On the other hand, PLA needs at least 494 days to reach the same M_n conditions –an effective 15% reduction of time.

The PLA samples were also evaluated to understand crystallinity degradation and evolution at 37°C. The X_c increased from 28.3% to 31.4% for PLA and from 28.3% to 39.5%

for PLA samples biostimulated with skim milk, as seen in **Figure4.2d** and **e**, and the values are tabulated in **Table 4.3** respectively. The significant difference between the two indicates the more rapid degradation of PLA samples occurring in the presence of skim milk since the lowest M_n can reconfigure and recrystallize even though the test was conducted below but close to T_g. **Figure 4.2f** and **g** present the MWD for PLA in compost and compost biostimulated with skim milk, respectively. The peak amplitude for PLA in **Figure 4.2f** remains approximately the same throughout the test duration. No broadening of the peak and the negligible shift indicate that the chemical hydrolysis proceeded slowly at a mesophilic temperature of 37°C. Whereas for PLA biostimulated with skim milk, a significant shift and broadening of the peak are observed in **Figure 4.2g**, depicting the reduction in M_n, rearrangement of the PLA molecular chains and change in X_c. The presence of skim milk initiates protease activity and concurrent chemical hydrolysis, produces a significant peak shift.

Table 4.3 Crystallinity evolution for PLA and PLA biostimulated with skim milk.

Material	Day 0	Day 180
PLA	28.2 ± 3.5^{a}	31.4 ± 1.8^{a}
PLA + skim milk	28.2 ± 3.5^{a}	39.5 ± 1.5^{b}

Values with different letters within columns are statistically different (α = 0.05 Tukey-Kramer test).

4.4.2 Effect of gelatin on cellulose and PLA degradation

Gelatin was used as another candidate to stimulate the protease activity of microbial strains in compost. Gelatin has been reported as a precursor for protease activity [10,34,40,43]. **Figure 4.3a** and **b** show the CO_2 evolved and % mineralization of the blank, cellulose, gelatin, PLA, cellulose+gelatin (Cell+gel), and PLA+gelatin (PLA+gel) in compost at 37°C. Gelatin shows around 41.5 g of CO_2 evolution and mineralization of 116% in 180 days. To further understand the biostimulation of compost by gelatin, it was combined with

cellulose. The CO_2 evolution in this case (Cell+gel) was 58.5 g, which is as expected and higher compared to the individual values for cellulose and gelatin, and mineralization of 130.4%. To better understand the interaction and account for the degradation behavior of cellulose in the presence of gelatin ($Cell_{gel}$), the mineralization value was estimated at 180.5% by subtracting the background signal from the gelatin bioreactor. This higher value depicts that gelatin does not affect cellulose degradation, and both are used up as carbon sources by the microorganisms. Additionally, a significant priming effect is observed in the case of bioreactor containing cellulose biostimulated with gelatin. To understand the influence of gelatin on PLA degradation, PLA was introduced in the gelatin-amended compost. The bioreactor containing both PLA and gelatin (PLA+gel) shows CO_2 evolution of around 41.8 g and maximum mineralization of 56.4% by the end of the test (**Figure 4.3a**). Improved mineralization is observed as opposed to no CO_2 evolution for PLA alone without any biostimulation of compost. The effect of gelatin on PLA degradation is calculated and subtracted by plotting the mineralization of PLA_{gel} (subtracting blank + gelatin), as mentioned earlier. The positive mineralization indicates that gelatin's protease activity helps in PLA's enzymatic degradation (**Figure 4.3b**). This is validated by the significant difference observed in the kinetic rate of degradation for PLA in compost, with and without any biostimulation by gelatin, as seen in **Figure 4.3c** inset (PLA k = 0.0045 ± 0.0001 and PLA+gelatin k = 0.0060 ± 0.0002). This change in k indicates a final reduction of around 124 days when PLA biostimulated with gelatin reaches an $M_n \lesssim$10kDa at 371 days compared to PLA, which needs at least 494 days to reach the same M_n conditions – an effective 25% reduction of time. The significant difference in the evolution of X_c from 28.3% to 31.4% for PLA, and from 28.3% to 41.9% for PLA samples biostimulated with gelatin, as seen in **Figure**

4.3d and **e**, and as seen in **Table 4.4** respectively, further shows the improvement in the enzymatic degradation of PLA due to the presence of gelatin.

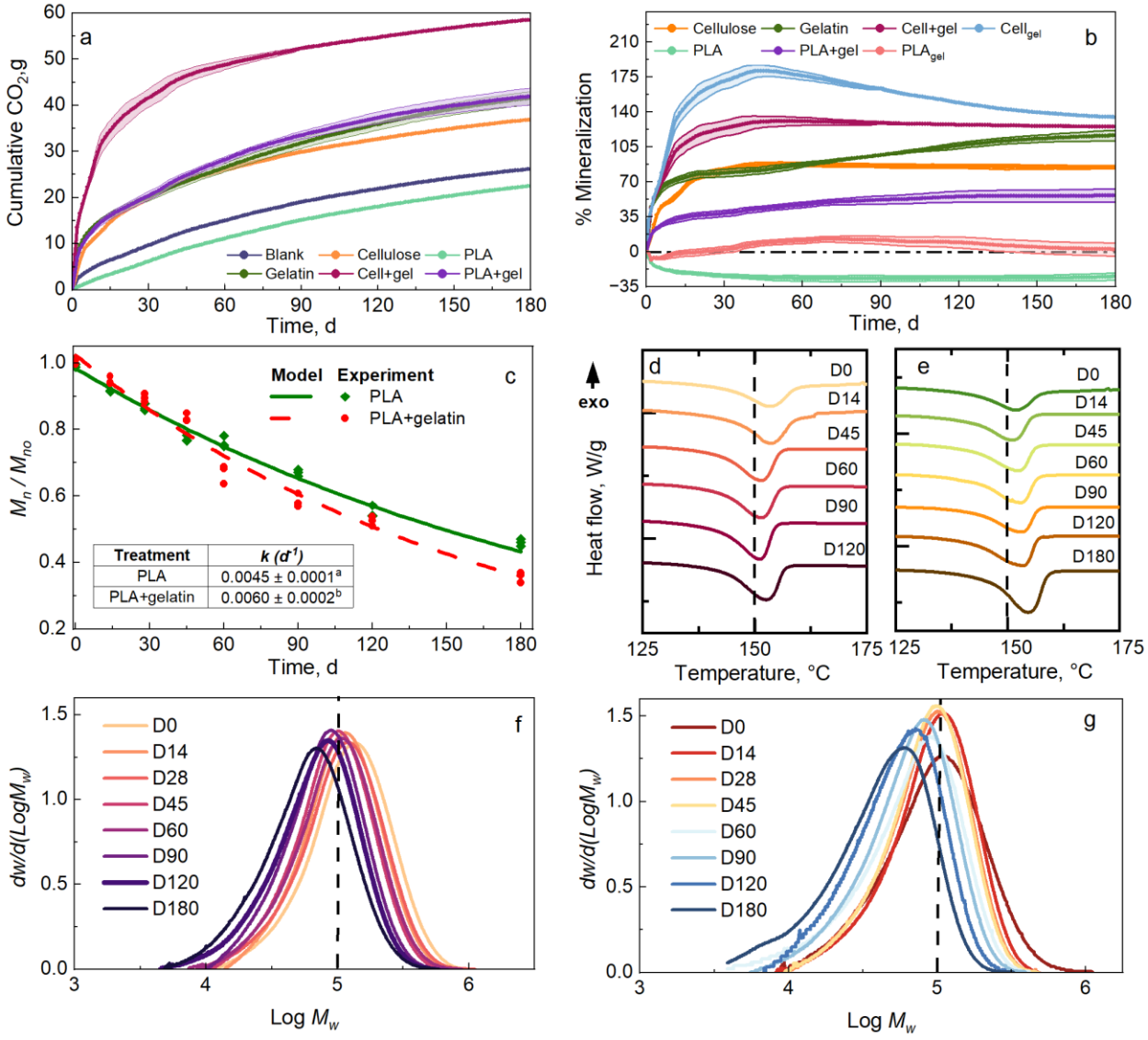


Figure 4.3 Cumulative CO_2 evolution (a) and mineralization (b) of blank, cellulose, PLA, gelatin, cellulose + gelatin (Cell+gel), PLA + gelatin (PLA+gel) in compost at 37°C. (c) represents the normalized M_n reduction as a function of time for PLA in control compost and compost biostimulated by gelatin. The experimental data was fitted using a first-order reaction of the form $M_n / M_{no} = e^{(-kt)}$, where M_{no} is the initial M_n, k is the rate constant, and t is the time. The inset shows the k-fitted values; values with different lowercase letters are statistically different ($\alpha = 0.05$ Tukey-Kramer Test). (d) and (e) depict DSC thermograms for PLA and PLA in compost biostimulated by gelatin, respectively. (f) and (g) show the MWD of PLA in compost and compost biostimulated with gelatin, respectively.

Table 4.4 Crystallinity evolution for PLA and PLA biostimulated with gelatin.

Material	Day 0	Day 180
PLA	28.2 ± 3.5[a]	31.4 ± 1.8[a]
PLA + gelatin	28.2 ± 3.5[a]	41.9 ± 1.9[b]

Note: Values with different letters within columns are statistically different (α = 0.05 Tukey-Kramer test).

The broadening and change in the amplitude of the MWD peaks through the test duration of 180 days, as seen in **Figure 4.3g** for PLA in compost stimulated with gelatin compared to **Figure 4.3f** for PLA alone, provide compelling evidence and confirm enhanced degradation for PLA with gelatin.

4.4.3 Effect of ethyl lactate on cellulose and PLA degradation

Ethyl lactate was used to stimulate the lactate-utilizing microbial species in the compost to improve PLA degradation at 37°C. **Figure 4.4a** and **b** show the CO_2 evolved and % mineralization of the blank, cellulose, ethyl lactate, PLA, cellulose+ ethyl lactate (Cell+el), and PLA+ethyl lactate (PLA+el) in compost at 37°C. Ethyl lactate evolves around 51.5 g of CO_2, the corresponding mineralization reaches around 270%, and no lag phase is observed. As noted earlier with skim milk and gelatin, a priming effect is observed with ethyl lactate. The CO_2 evolution of the bioreactor containing both cellulose and ethyl lactate (Cell+el) was only around 66.2 g and there was a corresponding mineralization of 182.4%, as both are readily available to be used as carbon sources by the microbial population. The higher value can be attributed to the lactate-utilizing microbial community, increasing the microbial population. The cellulose degradation in the presence of ethyl lactate ($Cell_{el}$) was decoupled by subtracting the CO_2 evolution from ethyl lactate – maximum mineralization of 116.7% was observed.

To understand how ethyl lactate affects the degradation of PLA, PLA was introduced into the bioreactor biostimulated by ethyl lactate. PLA in the presence of ethyl lactate (PLA+el) evolved around 51.6 g of CO_2, followed a similar trend as ethyl lactate over 180 days,

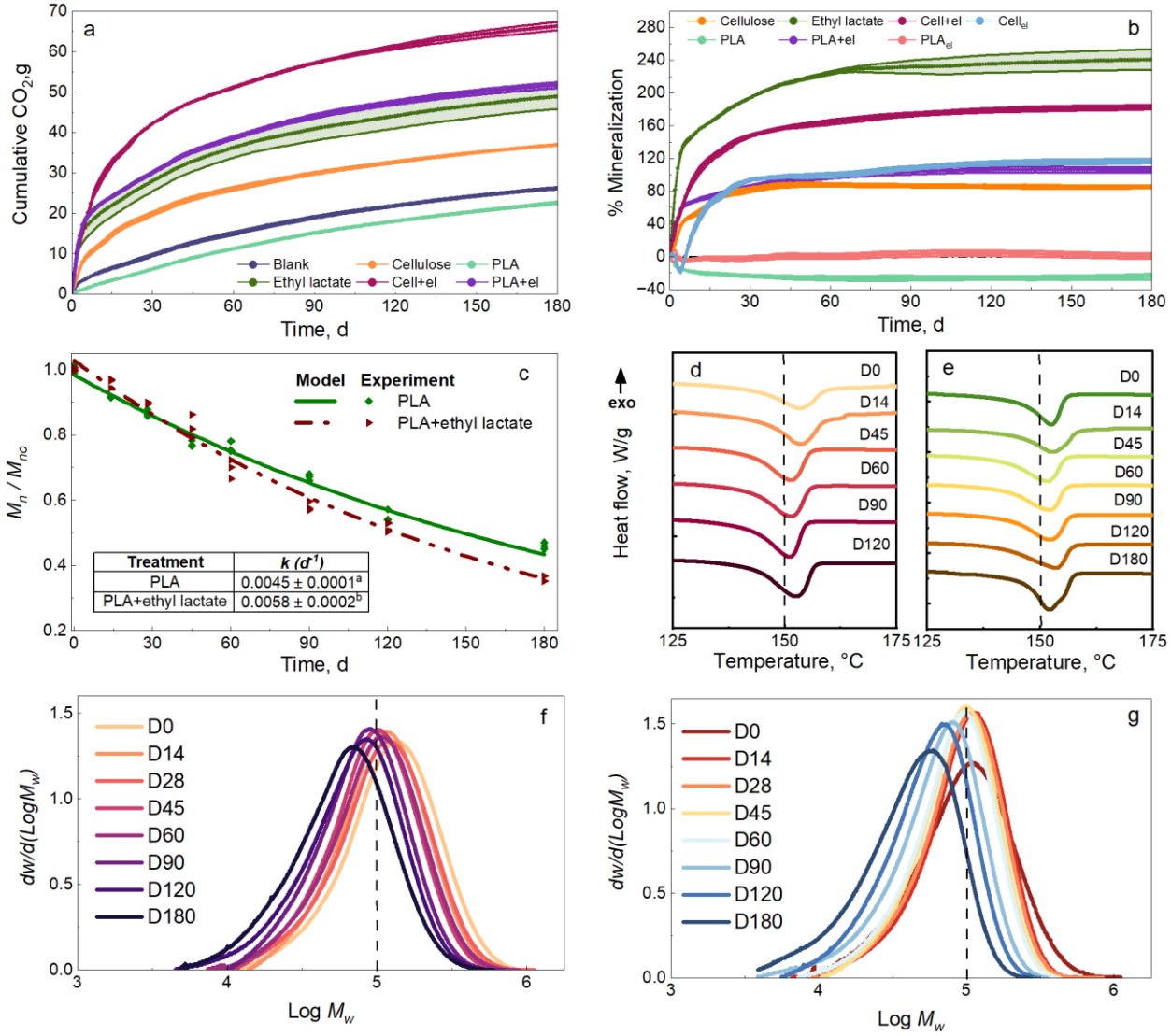


Figure 4.4 Cumulative CO_2 evolution (a) and mineralization (b) of blank, cellulose, PLA, ethyl lactate, cellulose + ethyl lactate (Cell-gel), PLA + ethyl lactate (PLA-gel) in compost at 37°C. (c) represents the normalized M_n reduction as a function of time for PLA in control compost and compost biostimulated by ethyl lactate. The experimental data was fitted using a first-order reaction of the form $M_n / M_{no} = e^{(-kt)}$, where M_{no} is the initial M_n, k is the rate constant, and t is the time. The inset shows the k-fitted values; values with different lowercase letters are statistically different ($\alpha = 0.05$ Tukey-Kramer Test). (d) and (e) depict DSC thermograms for PLA and PLA in compost biostimulated by ethyl lactate, respectively. (f) and (g) show the MWD of PLA in compost and compost biostimulated with ethyl lactate, respectively.

and showed maximum mineralization of 105%. A positive mineralization behavior is seen for PLA when the effect of ethyl lactate is accounted for (PLA_{el}), indicating the lactate-stimulating activity of ethyl lactate, but the biostimulation is lower than with skim milk and gelatin.

This finding is substantiated by a significant difference in the kinetic rate of degradation for PLA with and without the biostimulation effect of ethyl lactate, as indicated in **Figure 4.4c** inset (PLA k = 0.0045 ± 0.0001 and PLA-ethyl lactate k = 0.0058 ± 0.0002). This change in k indicates a final reduction of around 111 days when PLA biostimulated with ethyl lactate reaches an $M_n \lesssim$10kDa at 384 days compared to PLA, which needs at least 494 days to reach the same M_n conditions – an effective 22% reduction of time. The PLA samples were also evaluated to understand crystallinity degradation and evolution at 37°C. The X_c increased from 28.3% to 31.4% for PLA, and from 28.3% to 38.4% for PLA samples biostimulated with ethyl lactate, as seen in **Figure 4.4d** and **e**, respectively. The significant difference between the two indicates the degradation of samples as seen from **Table 4.5** occurring in the presence of ethyl lactate. **Figure 4.4f** and **g** present the MWD for PLA in compost and compost biostimulated with ethyl lactate, respectively. The peak amplitude for PLA in **Figure 4.4f** remains approximately the same throughout the test duration. No broadening of the peak, accompanied by a negligible shift, indicates that the chemical hydrolysis proceeded slowly at a mesophilic temperature of 37°C. Whereas for PLA biostimulated with ethyl lactate, a significant shift and broadening of the peak are observed in **Figure 4.4g**, depicting the reduction in M_n as previously shown for skim milk and gelatin.

Table 4.5 Crystallinity evolution for PLA and PLA biostimulated with ethyl lactate.

Material	Day 0	Day 180
PLA	28.2 ± 3.5^{a}	31.4 ± 1.8^{a}
PLA + ethyl lactate	28.2 ± 3.5^{a}	38.4 ± 2.1^{b}

Values with different letters within columns are statistically different (α = 0.05 Tukey-Kramer test).

The addition of biostimulants in the compost media accelerated the enzymatic degradation of PLA in mesophilic environments, which could be scaled to home composting settings. The biostimulants tested in the study are easily available and can be sourced locally, thus encouraging the practice of home composting, especially for contaminated PLA-based food packages post use. These practices can be applied and carried out by consumers in the backyard, and by university campuses diverting cafeteria and dining hall food waste from ending up in landfill. Additionally, the improvement in PLA degradation because of the presence of skim milk, gelatin, and ethyl lactate can further improve acceptance of PLA in thermophilic industrial composting environments, as these biostimulants can help improve the turnover time as compared to organic fraction such as food waste. In future work, we will be focusing on analyzing the effect of adding biostimulants periodically on accelerating PLA degradation. Understanding how the periodic addition of biostimulant impacts the mechanism of PLA's enzymatic degradation can further open avenues to investigate incorporation of enzymes directly to effectively biodegrade PLA.

4.5 Conclusion

The study investigated the biodegradation behavior of PLA using the biostimulation treatment under an aerobic simulated mesophilic setting. Different compounds – skim milk, gelatin, and ethyl lactate – were used to study the biostimulation effect by targeting different steps in PLA biodegradation. As expected, without any treatment, a very long abiotic lag phase was observed for PLA at mesophilic conditions (37°C), indicating and reaffirming a

very slow hydrolysis phase. This is depicted as negative mineralization over the test duration of 180 days. Improved CO_2 evolution was observed for PLA when the compost was biostimulated with the addition of skim milk, gelatin, or ethyl lactate. This finding was corroborated by tracking crystallinity evolution and molecular weight changes. PLA biodegradation was accelerated to reach $M_n \lesssim 10$kDa (where the biotic phase is taken over to consume the PLA n-mers) by 15% by adding skim milk, 25% by adding gelatin, and 22% by adding ethyl lactate. This work opens the door to help biodegrade PLA in compost conditions by adding selected biostimulants.

4.6 Acknowledgements

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CHAPTER 5: SPEEDING IT UP: DUAL EFFECTS OF BIOSTIMULANTS AND IRON ON BIODEGRADATION OF POLY(LACTIC ACID) AT MESOPHILIC CONDITIONS

5.1 Abstract

Plastic pollution presents a growing concern, and various solutions have been proposed to address it. One such solution involves the development of new plastics that match the properties of traditional polymers while exhibiting enhanced biodegradability when disposed of in a suitable environment. Poly(lactic acid) (PLA) is a biobased, compostable polymer known for its low environmental impact and ability to break down into harmless components within a specified timeframe. However, its degradation in industrial composting facilities poses challenges, and it cannot degrade in home composting. In this study, we investigated the biodegradability of PLA within a biostimulated compost matrix at mesophilic conditions (37°C) over 180 days. The compost environment was enhanced with Fe_3O_4 nanopowder, skim milk, gelatin, and ethyl lactate, individually and in combination, to target different stages of the PLA biodegradation process. We monitored key indicators, CO_2 evolution, number average molecular weight, and crystallinity, to assess the impact of the various biostimulants and iron. The results demonstrated that the most effective treatment for degrading PLA at mesophilic conditions was adding gelatin and Fe_3O_4. Gelatin accelerated PLA biodegradation by 25%, Fe_3O_4 by 17%, and a combination of gelatin and Fe_3O_4 by *c.* 30%. The effect of skim milk and ethyl lactate is also reported. This research introduces novel pathways to enhance PLA biodegradation in home composting scenarios, offering promising solutions to address the plastic pollution challenge.

5.2 Introduction

Poly(lactic acid) – PLA – is a biobased, biodegradable polymer that is an eco-friendly substitute for fossil-based polymers for a circular and sustainable economy. PLA is derived from natural resources and quickly degrades and breaks down in suitable waste management environments, such as industrial composting. PLA is a versatile biobased polymer because it has properties comparable to conventional polymers, is cost-effective, and can provide an additional disposal scenario, namely composting, at the end of a contaminated package life cycle [1]. These benefits, combined with the growing consumer awareness of plastic littering and white pollution, have propelled PLA to the forefront as the face of the green, biobased plastic movement [2].

Although PLA is industrially compostable, its practical and rapid biodegradation depends on reaching temperatures in the thermophilic range, 45 to 60°C, to undergo chemical hydrolysis and significantly reduce its molecular weight in a shorter period so that microorganisms present in the compost can use PLA oligomers as a food source. This constraint makes it difficult to degrade PLA at lower and ambient temperatures (i.e., mesophilic range, 20 to 45°C) [3]. Chemical hydrolysis is a crucial step in the PLA degradation mechanism, involving the breakdown of high into low molecular-weight polymer chains, such as oligomers, dimers, and monomers, which are easily assimilated by microorganisms [4]. However, at the lower temperatures commonly encountered in the home or backyard composting environments (herein referred to as backyard composting), chemical hydrolysis proceeds at a prolonged pace [5]. Boosting the hydrolytic degradation of PLA, particularly in backyard composting, is extremely difficult due to the limitation of high temperature essential to activate the biodegradation process [3].

Several research studies have included nanocomposites and metal compounds within the PLA matrix at lower concentrations to enhance its depolymerization and degradation [6–

8]. Including these metal oxides and nanoparticles significantly alters physical properties, such as number average molecular weight (M_n), melting temperature (T_m), and glass transition temperature (T_g) [9–11] of the resulting PLA or PLA blends, and may not be practical for food contact or single-use PLA discarded packages.

Enzymatic hydrolysis involving the release of extracellular enzymes is also essential for PLA degradation when low molecular weight PLA chains are available. PLA's degrading enzymes include the hydrolase class of enzymes, primarily proteases [3]. The serine proteases released by the microorganisms in response to the presence of amino acid compounds in compost have also been shown to cleave the ester bonds in PLA [12,13]. So, the introduction of enzymes to compost may assist in the biodegradation of PLA.

Adding various components besides enzymes, such as nutrients, electron donor/acceptor compounds, or compounds essential to trigger the biochemical processes of microorganisms in the given environment, is termed biostimulation [14–16]. Biostimulation techniques to enhance PLA biodegradation have been reported [17]. However, most of these studies were conducted in restricted settings where PLA was the only carbon source, in liquid media with specific microbial strains, and usually at higher temperatures, which do not replicate the conditions encountered during backyard composting [18,19].

In a previous study, we assessed the change in PLA biodegradation at a mesophilic temperature of 37°C in a solid composting matrix by adding biostimulants to the compost. The compounds selected as biostimulants in that study were screened through multiple criteria: anticipated to have no toxicity towards the microorganisms present, be able to degrade, be biodegradable in a suitable timeframe, be consumed, be cost-effective, and be readily available. The main goal was to introduce compounds enhancing the biotic degradation stage. Skim milk and gelatin were selected to trigger proteolytic activity. Ethyl lactate, belonging to the lactate esters family, was used to stimulate the lactate-utilizing

microbial species in backyard compost. All these compounds effectively enhanced the biodegradation of PLA in simulated backyard composting by at least 15%, as determined by the accelerated reduction of M_n [20].

In this study, we focused on evaluating the effect of adding a metal compound to catalyze the chemical hydrolysis of PLA and combine that with the previous demonstrated biotic enhancement. Metals can be added externally to the compost media rather than within PLA for humification purposes, as previously reported [21,22] and screening through the earlier criteria. **Table 5.1** presents the permissible limits of metal compounds as derived from regulatory standards for heavy metals in agricultural soils (mg/kg) [23], since the resulting amended compost may be applied to agricultural land, lawns, or home gardens. The primary standards include EEA 2007 [24], TMS 2007 [25], BPI 2021 [26], GB 15168-2018 [27], OMOE 2011 [28], and NZME 2012 [29].

Table 5.1 Critical limits of heavy metals in agricultural soils (mg/kg), adapted from [23].

Country	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
Australia	20	3	50	100	1	60	300	200
Canada	20	3	250	150	0.8	100	200	500
China	20 - 40	0.3 - 0.6	150-300	50-200	0.5-3.4	60-190	70-240	200-300
Germany	50	5	500	200	5	200	1000	600
Tanzania	1	1	100	200	2	100	200	150
Netherlands	76	13	180	190	36	100	530	720
New Zealand	17	3	290	$> 10^4$	200	N/A	160	N/A
UK	43	1.8	N/A	N/A	26	230	N/A	N/A
US	6.5	1.5	105	50	0.4	31	75	250

Considering the criteria mentioned before and the information from Table 5.1 only a few metals could be selected for further consideration due to limitations placed by permissible limits. The selected compounds were further scrutinized (Table 5.2) for their antibacterial properties, as reported in the literature, to evaluate their use directly in compost.

Table 5.2 List of elements screened for their antimicrobial properties.

Element	Antibacterial nature	References
Zn	Yes	[30,31]
Fe	No	[30,32,33]
Cu	Yes	[30]
Ni	Yes	[30]
Ti	Yes	[34,35]
Cl	Yes	[30,36]
Ag	Yes	[36,37]

Table 5.2 indicates that iron was an acceptable compound that could be used to target chemical hydrolysis. It was demonstrated that introduction of a Lewis acid, $FeCl_3$, can speed up the hydrolysis of PLA in an alkali solution [38]. However, due to chlorine's antimicrobial behavior, it was impossible to introduce $FeCl_3$ into compost. Alternatively, iron oxides, such as FeO, FeO_2, Fe_3O_4, or Fe_2O_3, could be the primary option and are in a form that is not toxic to the microbes in the compost [32,33]. Several of these iron forms are present in soil worldwide [39].

Thus, this study aimed to investigate the effectiveness of biostimulating the compost environment with compounds that may be able to enhance the chemical hydrolysis (Fe_3O_4), the enzymatic degradation (skim milk and gelatin), and electroconductivity (ethyl lactate as electron donor compound) during the aerobic biodegradation of PLA in compost at mesophilic conditions. Differences in CO_2 evolution, changes in M_n, and the crystallinity (X_c) of PLA

degradation with and without biostimulants were monitored to account for the activity of biostimulants in compost.

5.3 Materials and methods

5.3.1 Materials

PLA Ingeo™ 2003D resin, with L-lactic acid content of 96%, was obtained from NatureWorks LLC (Minnetonka, MN, USA). Iron oxide nano-powder (Fe_3O_4) was obtained from US Research Nanomaterials, Inc. (Houston, TX, USA). Skim milk powder was procured from a local store (Walmart, Lansing, MI, USA). Gelatin of the brand McCormick & Co. (Hunt Valley, MD, USA) was purchased on Amazon LLC. Ethyl lactate was procured from Sigma Aldrich™ (St. Louis, MO, USA).

5.3.2 Characterization of PLA and the biostimulants

The carbon, hydrogen, and nitrogen compositions of PLA resin, skim milk, gelatin, and ethyl lactate were determined using elemental analysis, (CHNS/O Elemental Analyzer, PerkinElmer 2400 Series II) (Shelton, CT, USA), and are presented in Table 5.3.

Table 5.3 Carbon, hydrogen, and nitrogen content (percentage by weight) of the tested materials.

Material	% Carbon[a]	% Hydrogen[a]	% Nitrogen[a]
PLA	49.72 ± 0.19	5.72 ± 0.04	0.11 ± 0.07
Skim milk	41.28 ± 0.13	6.33 ± 0.03	5.75 ± 0.06
Gelatin	44.80 ± 0.30	7.00 ± 0.10	16.4 ± 0.10
Ethyl lactate	31.93 ± 0.98	5.46 ± 0.34	0.05 ± 0.03

[a] Percentage by weight.

5.3.3 Biodegradation in compost

The biodegradation of PLA and the effectiveness of introducing biostimulants in compost on the degradation of PLA were evaluated under aerobic mesophilic conditions using a direct measurement respirometric (DMR) system [40–42]. The system included a non-dispersive infrared gas analyzer (NDIR) (Li-COR® LI-820, Lincoln, NE, USA) which measures the CO_2 concentration. The DMR system chamber was maintained at temperature of 37 ± 2 °C and relative humidity (RH) of 50% ± 5%. A flow rate of CO_2-free air (concentration <30 ppm to establish a low baseline) was controlled at 40 ± 2 cm^3/min. Detailed information about the DMR equipment can be found in other source [43].

The mature compost obtained from MSU composting facility was sifted using a 10-mm screen to get rid of any huge debris or chunks present, and then conditioned at 37°C until use. Deionized water was used to adjust the moisture content of compost to 50%. Saturated vermiculite (Sun Gro Horticulture Distribution Inc., Bellevue, WA, USA), was mixed with compost in 1:4 parts (dry weight). Samples of the resulting compost-vermiculite mixture were later sent to the Soil and Plant Laboratory at MSU for determining the physicochemical parameters. Data regarding the nutrient analysis is presented in **Table A5.1** (Appendix 5A) [44]. The bioreactors were packed with 400 g of compost, 8 g of PLA sample and the selected biostimulant, and all the samples were tested in triplicate. Blank (only compost) and positive control (cellulose) were also tested.

Biostimulation: Fe_3O_4 nanopowder at 17 g was mixed with the compost matrix in the bioreactor containing PLA to target the hydrolysis step. Ethyl lactate, skim milk, and gelatin were added at 8 g individually per bioreactor and mixed with the compost matrix thoroughly with the PLA pellets in it to ensure uniform distribution.

5.3.4 Size exclusion chromatography

The M_n and molecular weight distribution (*MWD*) of PLA for the control and each treatment with the biostimulants were measured using SEC (Waters Corp., Milford, MA, USA) as described elsewhere [4]. PLA samples weighing approximately 10 mg were retrieved at predetermined time intervals and dissolved in 5 mL of tetrahydrofuran (THF) solvent. A temperature of 35°C and a 1 mL/min flow rate were maintained during testing. The Mark-Houwink constants of K = 0.000174 dL/g and α = 0.736 were used to determine M_n ,M_w and MWD of the PLA samples. Data analysis was carried out using Waters Breeze™2 software.

5.3.5 Differential scanning calorimetry

A DSC model Q100 (TA Instruments, New Castle, DE, USA), was used to determine the T_g, T_m, crystallization temperature (T_c), and crystallinity (X_c) for the PLA samples retrieved from the regular and biostimulated compost. PLA samples weighing between 5 - 10 mg were packed in aluminum pans and cooled down to −5°C and then subjected to a heating cycle to reach 210°C at a ramp rate of 10°C/min . This helped to evaluate the evolution of X_c. The cooling was achieved using a nitrogen cooling system that maintained the purge flow rate at 70 mL/min. The degree of crystallinity was estimated using equation (5.1):

$$\chi_c\ \% = \frac{\Delta H_m - \Delta H_c}{\Delta H_m^0}\ x\ 100 \tag{5.1}$$

where ΔH_m is the heat of fusion, ΔH_c is cold crystallization enthalpy, and ΔH_m^0 is the heat of fusion for 100% crystalline pure PLA (93 J/g) [45].

5.4 Results and discussion

The CO_2 evolution of PLA samples in compost and biostimulated with Fe_3O_4 nanopowder and the combination of gelatin, skim milk, and ethyl lactate without and with Fe_3O_4 nanopowder was tracked over a test duration of 180 days at mesophilic conditions

(37°C). Samples were retrieved at specific times to evaluate the M_n and X_c evolution and determine the kinetic degradation rate.

5.4.1 Effect of Fe_3O_4 on cellulose and PLA degradation

Figure 5.1a and b shows the CO_2 evolution and mineralization, respectively, of cellulose, cellulose in compost biostimulated with Fe_3O_4 nanopowder (hereafter referred to as cellulose + Fe), PLA, and PLA in compost biostimulated with Fe_3O_4 nanopowder (hereafter referred to as PLA + Fe) at 37°C. Control compost (blank) evolved around 26.1 g of CO_2, and compost biostimulated with Fe_3O_4 nanopowder (hereafter referred to as blank + Fe) evolved around 27.2 g of CO_2. The minor difference can be attributed to the difference in weight of the compost introduced in the bioreactor, and the levels are not significantly different ($p > 0.05$). Cellulose in compost evolved around 36.8 g of CO_2, and reached a mineralization of 87.7%, whereas cellulose + Fe evolved around 44.6 g of CO_2, depicting a mineralization of 137.7%. The primary reason for the priming effect (>100% mineralization) observed in the case of cellulose + Fe may be attributed to the over-deterioration of the endemic carbon present in the compost [4]. PLA in compost showed around 22.5 g of CO_2 evolution, whereas blank produced around 26.1 g of CO_2, implying that no carbon from PLA was degraded. The negative mineralization values indicate more CO_2 production in the blank bioreactors than in the PLA bioreactors. PLA offers a physical hydrophobic barrier to water, making it difficult for the microorganisms to utilize it as a carbon source at the beginning of the test and until day 180 due to the low contribution of chemical hydrolysis at mesophilic temperatures. Overall, we did not see any mineralization in PLA due to the low temperature of 37°C, which is insufficient to activate the biotic stage. These values are very low compared to the degradation of PLA at thermophilic temperatures [4,44] but are similar to earlier reported values [5,46].

In the case of PLA and PLA + Fe, we observed the difference in the CO_2 evolution right from the start of the test. PLA + Fe evolved around 25.9 g of CO_2 compared with 22.5 g of CO_2 evolution for PLA. Similar to the case of cellulose + Fe, the reason for the difference in the CO_2 evolution for PLA can be attributed to the presence of Fe_3O_4. Fe_3O_4 promotes microbial activity in soil and enhances the nitrification potential [47]. Fe_3O_4 is also known to induce changes by enhancing enzymatic activity and microbial growth [32]. This characteristic can be corroborated by the kinetic degradation rate (k), as seen in **Figure 5.1c** inset (PLA k = 0.0045 ± 0.0001 d^{-1} and PLA+ Fe k = 0.0052 ± 0.0002 d^{-1}). The significant difference can be credited to the presence of Fe_3O_4. **Figure 5.1d and e** present the molecular weight distribution (MWD) for PLA in compost and compost biostimulated with Fe_3O_4. The peak amplitude for PLA in Figure 5.1dremained approximately the same throughout the test duration. No broadening of the peak and negligible shift indicates that the chemical hydrolysis proceeded slowly at a mesophilic temperature of 37°C. In contrast, for PLA + Fe, a significant shift to low M_w and broadening of the peak are observed in Figure 5.1e, depicting the reduction in M_n as shown in **Figure 5.1c**.

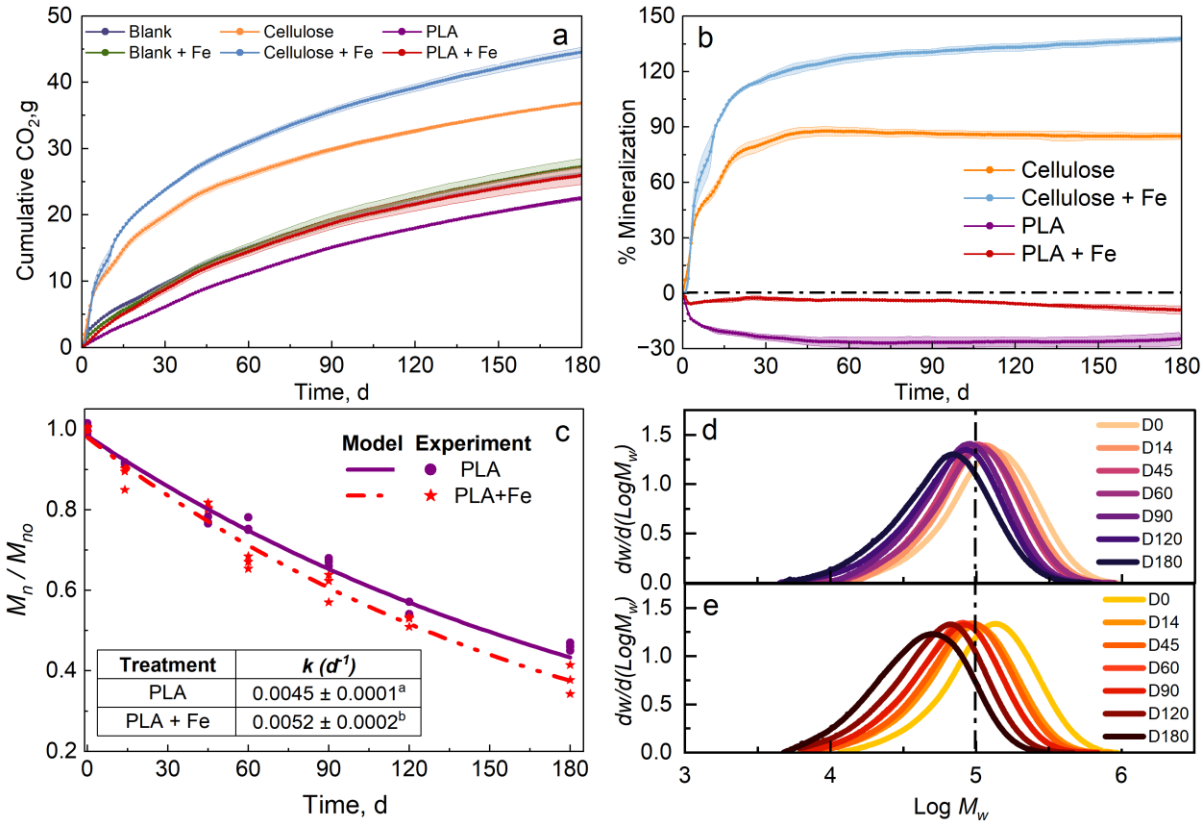

Figure 5.1 Cumulative CO_2 evolution (a) and mineralization (b) of blank, cellulose, PLA, blank + Fe_3O_4 (Blank + Fe), cellulose + Fe_3O_4 (Cellulose + Fe), PLA + Fe_3O_4 (PLA + Fe) in compost at 37°C. (c) represents the normalized M_n reduction as a function of time for PLA in control compost and compost biostimulated with Fe_3O_4. The experimental data was fitted using a first-order reaction of the form $M_n / M_{no} = e^{(-kt)}$, where M_{no} is the initial M_n, k is the rate constant, and t is the time. The inset shows the k-fitted values; values with different lowercase letters are statistically different (α = 0.05, Tukey-Kramer test). (d) and (e) show the MWD of PLA in compost and compost biostimulated with Fe_3O_4, respectively.

5.4.2 M_n reduction for PLA with biostimulants in compost

PLA samples were retrieved separately from the control compost and compost biostimulated with skim milk, gelatin, and ethyl lactate. **Figure 5.2** shows the reduction in M_n of PLA and biostimulated PLA tracked until the end of the test (180 days). A significant difference was observed in the kinetic reduction rates of PLA with biostimulant treatment compared with no biostimulation treatment. The CO_2 evolution values for PLA, PLA + skim milk, PLA + gelatin, and PLA + ethyl lactate are provided in Appendix 5B.

Skim milk was added to the compost to induce protease activity by the microbes [48–50]. Serine protease (3.4.21.112) belongs to the peptidases and is the class of extracellular enzymes able to hydrolyze the peptide bonds linked to amino acids in the protein structure. Skim milk is composed of different proteins, such as lactose, casein, and whey protein, making it a good precursor for enzymatic activity, as mentioned earlier. Other researchers have previously used skim milk to demonstrate extracellular protease synthesis. The microorganisms present in compost secrete protease to hydrolyze the milk protein. This protease is used by microorganisms capable of PLA degradation to depolymerize PLA [51]. This increase in k can be deduced as a final reduction of around 75 days on the biodegradation time when PLA in compost biostimulated with skim milk reaches $M_n \lesssim 10$kDa at 420 days (**Figure 5.2**). Microorganisms assimilate the PLA n-mers at this stage, accelerating the biodegradation stage [44]. On the other hand, PLA needs at least 494 days to reach the same M_n – an effective 15% reduction of time.

Gelatin is composed of protein and amino acids, and is a precursor for protease activity [19,52–54]. The addition of gelatin to compost produced an acceleration of PLA with an enhancement of k translated to a final reduction of around 124 days when PLA is biostimulated with gelatin, reaching an $M_n \lesssim 10$kDa at 371 days compared with at least 494 days for PLA alone – an effective 25% reduction of time (Figure 5.2).

Ethyl lactate, on the other hand, was used to stimulate the lactate utilizing microbes in the compost. Ethyl lactate undergoes hydrolysis to produce ethanol and lactate, where both can act as a constant long-term supply of hydrogen sources as electron donor compounds for reductive degradation and microbial redox process. Lactate has been shown to act as an electron donor compound in the case of anaerobic degradation [55,56]. Lactate has previously been used for anaerobic degradation, trichloroethane dichlorination, and sulfate reduction

[57,58]. PLA + ethyl lactate resulted in a change in k, which can be translated to around 111 days when PLA is biostimulated with ethyl lactate, reaching an $M_n \lesssim 10$kDa at 384 days compared to PLA alone – an effective 22% reduction of time (**Figure 5.2**).

A detailed discussion of the effect of gelatin, skim milk, and ethyl lactate on the biodegradation of PLA is provided in our previous work [20].

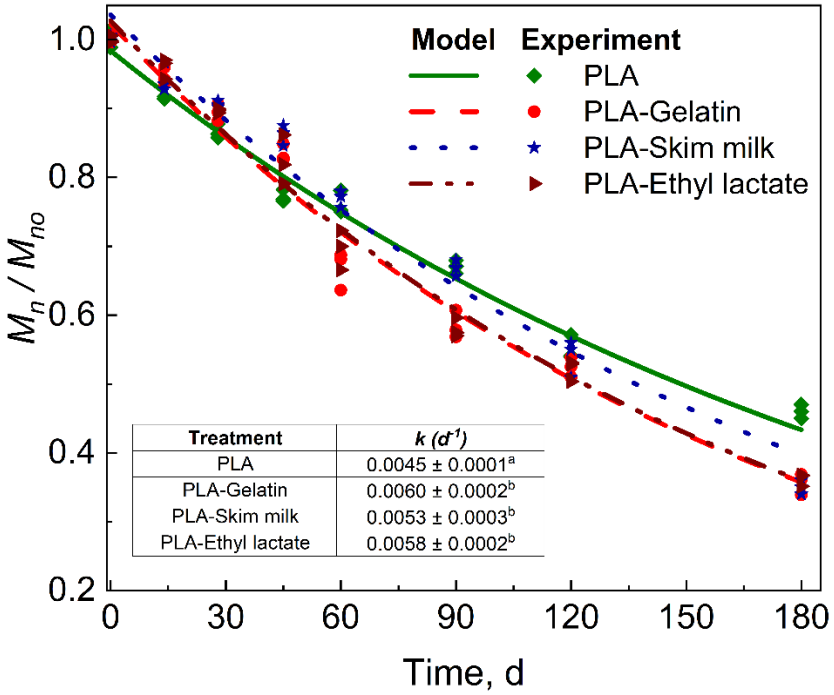

Figure 5.2 Normalized M_n reduction as a function of time for PLA in control compost and compost biostimulated with skim milk, gelatin, and ethyl lactate. The experimental data was fitted using a first-order reaction of the form $M_n / M_{no} = e^{(-kt)}$, where M_{no} is the initial M_n, k is the rate constant, and t is the time. The inset shows the k-fitted values; values with different lowercase letters are statistically different ($\alpha = 0.05$, Tukey-Kramer test).

5.4.3 Effect of Fe_3O_4 on cellulose and PLA degradation with gelatin as a biostimulant

Since gelatin resulted in the most significant M_n reduction for PLA, it was selected to discuss the effect of a combination of biostimulants (i.e., Fe_3O_4 nanopowder and gelatin). The data for Fe_3O_4 nanopowder, skim milk, and ethyl lactate are provided in Appendix 5B. When

Fe_3O_4 nanopowder was introduced in compost amended with gelatin, the gelatin acted as a precursor for the protease enzyme secretion by the microbes present in the compost and the Fe_3O_4 nanopowder provided the metal to catalyze the hydrolysis.

Figure 5.3a and b show the CO_2 evolution and mineralization of cellulose + Fe, gelatin + Fe, PLA + Fe, cellulose + gelatin + Fe (hereafter referred to as cell + gel + Fe), and PLA + gel + Fe in compost at 37°C. Gelatin + Fe resulted in around 60.1 g of CO_2 evolution and mineralization of 247.8% in 180 days. Gelatin was combined with Fe to target the chemical hydrolysis and enzymatic degradation steps. The CO_2 evolution in this case (Cell+gel+Fe 1) was 64.6 g, which is as expected and higher compared with the individual values for cellulose + Fe and gelatin + Fe and a mineralization of 97.7%. To better understand the interaction and to account for the degradation behavior of cellulose in the presence of gelatin (Cell+gel+Fe 2), the mineralization value was estimated at 80.5% by subtracting the background signal from the bioreactor containing gelatin + Fe. This higher value indicates that cellulose degradation was not affected by gelatin, and both are used up by the microorganisms as carbon sources.

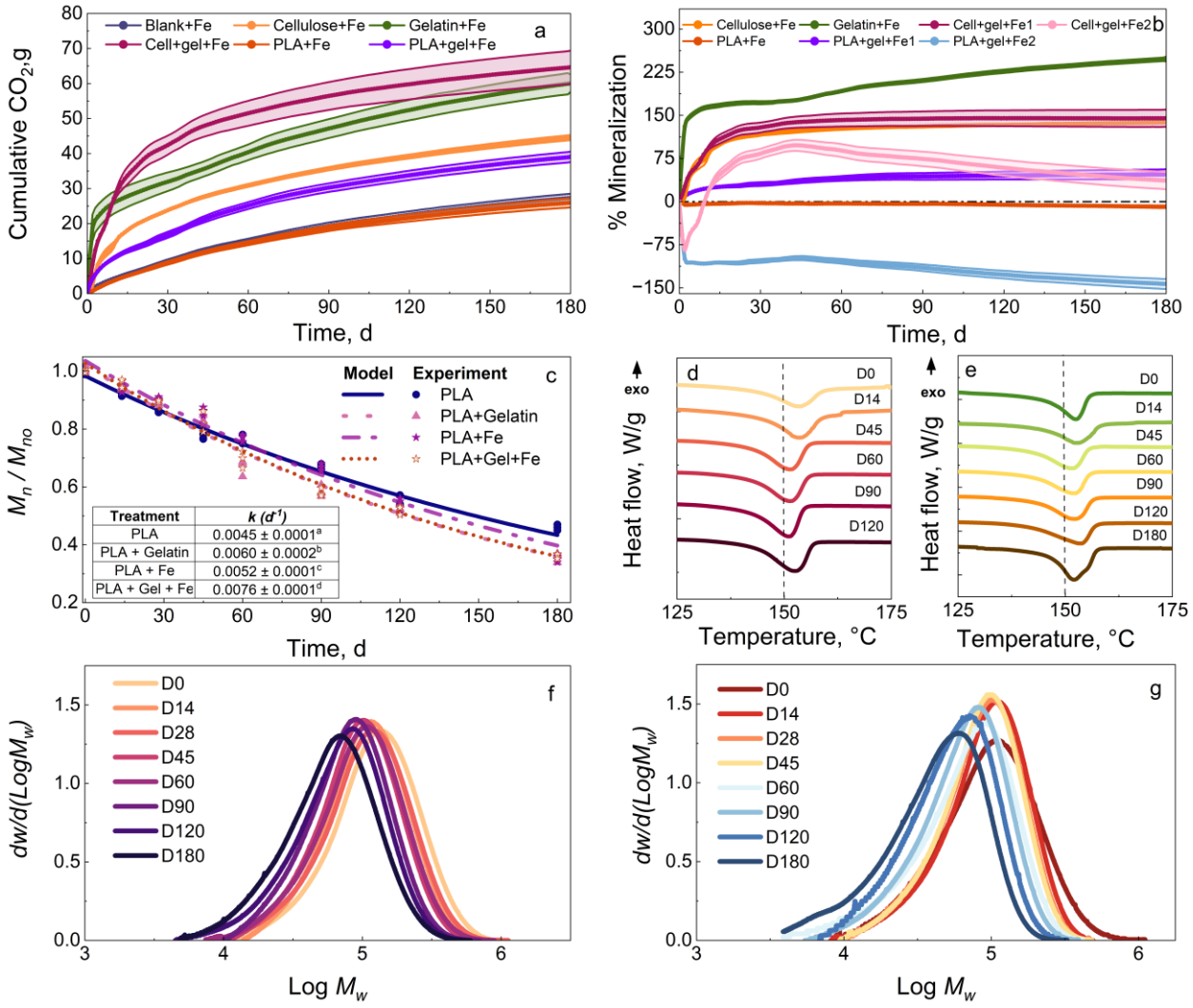

Figure 5.3 Cumulative CO_2 evolution (a) and mineralization (b) of blank + Fe, cellulose + Fe, PLA + Fe, gelatin + Fe, cellulose + gelatin + Fe (Cell+gel+Fe), PLA + gelatin + Fe (PLA+gel+Fe) in compost at 37°C. (c) represents the normalized M_n reduction as a function of time for PLA in control compost and compost biostimulated with gelatin, and gelatin + Fe. The experimental data was fitted using a first-order reaction of the form $M_n / M_{no} = e^{(-kt)}$, where M_{no} is the initial M_n, k is the rate constant, and t is the time. The inset shows the k-fitted values; values with different lowercase letters are statistically different (α = 0.05, Tukey-Kramer test). (d) and (e) depict DSC thermograms for PLA + Fe and PLA + Fe in compost biostimulated with gelatin. (f) and (g) show the MWD of PLA + Fe in compost and compost biostimulated with gelatin.

To understand the influence of gelatin + Fe on PLA degradation, PLA was introduced in the compost amended with gelatin + Fe. The bioreactor containing both PLA and gelatin + Fe (PLA+gel+Fe 1) generated CO_2 evolution of around 40.5 g and maximum mineralization

of 56.5% by the end of the test. Improved mineralization was observed as opposed to no CO_2 evolution for PLA alone without any biostimulation of the compost. The effect of gelatin + Fe on PLA degradation is calculated by plotting the mineralization of PLA+gel+Fe 2 (subtracting gelatin + Fe), as mentioned earlier. Negative mineralization does not necessarily indicate the absence of gelatin's protease activity in PLA's enzymatic degradation. This finding is validated by the significant difference observed in the kinetic rate of degradation for PLA in compost, with and without any biostimulation with gelatin, as seen in Figure 5.3c inset (PLA+Fe k = 0.0052 ± 0.0001 and PLA+Gel+Fe k = 0.0076 ± 0.0002). The significant difference in the evolution of X_c from 28.3% to 31.4% for PLA, and from 28.3% to 40.9% for PLA biostimulated with gelatin + Fe, as seen in Figure 5.3d and e **,** respectively, further shows the improvement in the enzymatic degradation of PLA due to the presence of gelatin + Fe. Gelatin acts as a precursor for the microbes to release protease enzyme, aiding in the enzymatic degradation of PLA. The broadening and change in the amplitude of the MWD peaks through the test duration of 180 days, as seen in Figure 5.3g for PLA in compost biostimulated with gelatin + Fe compared to PLA + Fe alone in Figure 5.3g , show compelling evidence for enhanced degradation for PLA in the presence of gelatin and Fe. Iron is an essential micronutrient, necessary for life-sustaining processes, and plays a critical role in cell growth of microbes [59]. Iron also functions as a cofactor, promoting and increasing enzymatic activity. Iron plays important roles in various biological processes such as respiration, oxido-reduction mechanism, nitrogen fixation, tricarboxylic acid cycle, and electron transport [33]. S. He et al. demonstrated that a soil matrix amended by Fe nanoparticles shifted the microbial community composition and stimulated the metabolic activity of the bacterial community present by enhancing their growth rate [33]. The soil nitrification potential of the Fe-amended soil was improved by 10% to 19% compared to

control soil, indicating that adding Fe aided in the increase of biomass capacity and eventually enhanced and boosted carbon cycling.

Zhang et al. further showed that the addition of Fe nanoparticles promoted the degradation of organic matter and amplified the dehydrogenase and urease activities, significantly improving the overall microbial activity and nitrogen mineralization [47]. Thus, adding Fe_3O_4 nanopowder improves the microbial metabolic activity, nitrification potential, and microbial population. When supplemented with the enzymatic activity associated with gelatin, these changes improve the degradation of PLA in compost compared with that of control PLA with no biostimulants present. Y. He et al. showed enhanced enzymatic and nitrification activity for organic matter degradation in a food-waste composting system due to compost amendment with Fe-carbon particles [60]. In addition, the bacterial and fungal communities exhibited significant improvement in the composting process due to the presence of iron, which can explain the improved PLA degradation found in the presence of Fe_3O_4 nanopowder.

Overall, the changes obtained in k indicate a final reduction of around 148 days when PLA stimulated with gelatin reaches an $M_n \lesssim 10$kDa at 346 days compared to at last 494 days for PLA – an effective 25% reduction of time. Similarly, time reductions of 17% or 30% were observed when PLA + Fe_3O_4 or gelatin and Fe_3O_4 nanopowder, respectively, were included in the compost.

As organic waste disposal is becoming more stringent worldwide and landfill disposal bans are increasing, several food industries are being impacted and need to find alternative end-of-life scenarios. The gelatin industry produces a large amount of sludge, resulting in a tremendous amount of waste generated, which includes collagen fibers, bone residues, and other inorganic materials. This gelatin sludge usually ends up in landfill or waste

management treatment without any pretreatment and creates several problems such as water contamination, greenhouse gas emission, and health risks for local habitats [61]. So, this waste could be diverted from landfills and used to produce mature compost along with the organic fraction of municipal solid waste. Gelatin sludge is high in nitrogen and organic matter content and can act as a valuable plant nutrient [61]. The nutritional value of the compost generated from treating gelatin waste and Fe_3O_4 nanopowder, considering the benefits mentioned earlier, complement each other to improve soil fertility once the compost is applied to agricultural land. The selection and combination of specific compounds can open a new route to accelerate the degradation of compostable polymers in industrial and home composting operations.

5.5 Conclusion

We investigated the role of different compounds—skim milk, gelatin, and ethyl lactate in combination with Fe_3O_4 nanopowder—on PLA degradation at 37°C by biostimulating the compost media. The different compounds were selected to target different stages of the biodegradation process. Without any biostimulant compounds, PLA continued to undergo a long abiotic lag phase affirming a slow hydrolysis phase, which was seen as a negative mineralization for the test duration of 180 days, whereas a boost in CO_2 evolution for PLA was observed in compost amended by Fe_3O_4 nanopowder in combination with gelatin, skim milk, or ethyl lactate. This observation was verified by the molecular weight change and crystallinity evolution. PLA biodegradation was accelerated by 30% to reach the biotic phase of $M_n \lesssim 10$kDa by the addition of gelatin and Fe_3O_4 nanopowder. The addition of biostimulants opens new avenues to improve PLA biodegradation in home composting conditions.

5.6 Acknowledgments

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APPENDIX 5A: PHYSICOCHEMICAL CHARACTERISTICS

Some compost was collected and sent to the Soil and Plant Nutrient Laboratory at Michigan State University (East Lansing, MI, USA) to evaluate its physicochemical parameters (dry solids, volatile solids, and C/N ratio) as previously described elsewhere [44]. The physicochemical parameters are reported below in **Table A5.1**.

Table A5.1 Physicochemical parameters and total nutrient analysis of compost used in the biodegradation test.

Parameter	Compost
Dry solids, %	42.5
Volatile solids, %	41.7
pH	8.0
C/N ratio	10.1
Carbon, %	24.2
Nitrogen, %	2.42
Phosphorus, %	1.21
Potassium, %	3.15
Calcium, %	5.07
Magnesium, %	2.82
Sodium, %	0.58
Sulfur, %	0.58
Iron, ppm	9878
Zinc, ppm	480
Manganese, ppm	413
Copper, ppm	107
Boron, ppm	41
Aluminum, ppm	6751

APPENDIX 5B: CO_2 EVOLUTION AND MINERALIZATION OF PLA IN THE PRESENCE OF SKIM MILK, GELATIN, AND ETHYL LACTATE

Figure A5.1 a and b shows the CO_2 evolution and % mineralization of cellulose, skim milk, PLA, cellulose + skim milk, and PLA + skim milk. Cellulose reached mineralization of 87.7% whereas skim milk attained over 100 % of its carbon conversion over a period of 180 days. Since skim milk and cellulose are readily biodegradable and can be easily utilized as a carbon source by the microorganisms present in the compost, no lag phase was observed. Skim milk was added to the compost with the goal of inducing the protease activity of the microbes present. PLA shows similar CO_2 evolution when compared to blank (compost only). This indicates that PLA is still undergoing chemical hydrolysis and is yet to breakdown to M_n of 10k Da, where it can be assimilated by microorganisms.

Due to the compost amendment with skim milk, PLA (PLA + skm1) shows a mineralization of approximately 60%. In order to account for the effect of skim milk, a separate mineralization plot (PLA + skm2) is derived. Around 35% mineralization is observed for the same depicting that the enzymatic degradation of PLA is enhanced due to the presence of skim milk. This is corroborated by the molecular weight analysis (PLA $k = 0.0045 \pm 0.0001$ and PLA-skim milk $k = 0.0053 \pm 0.0003$).

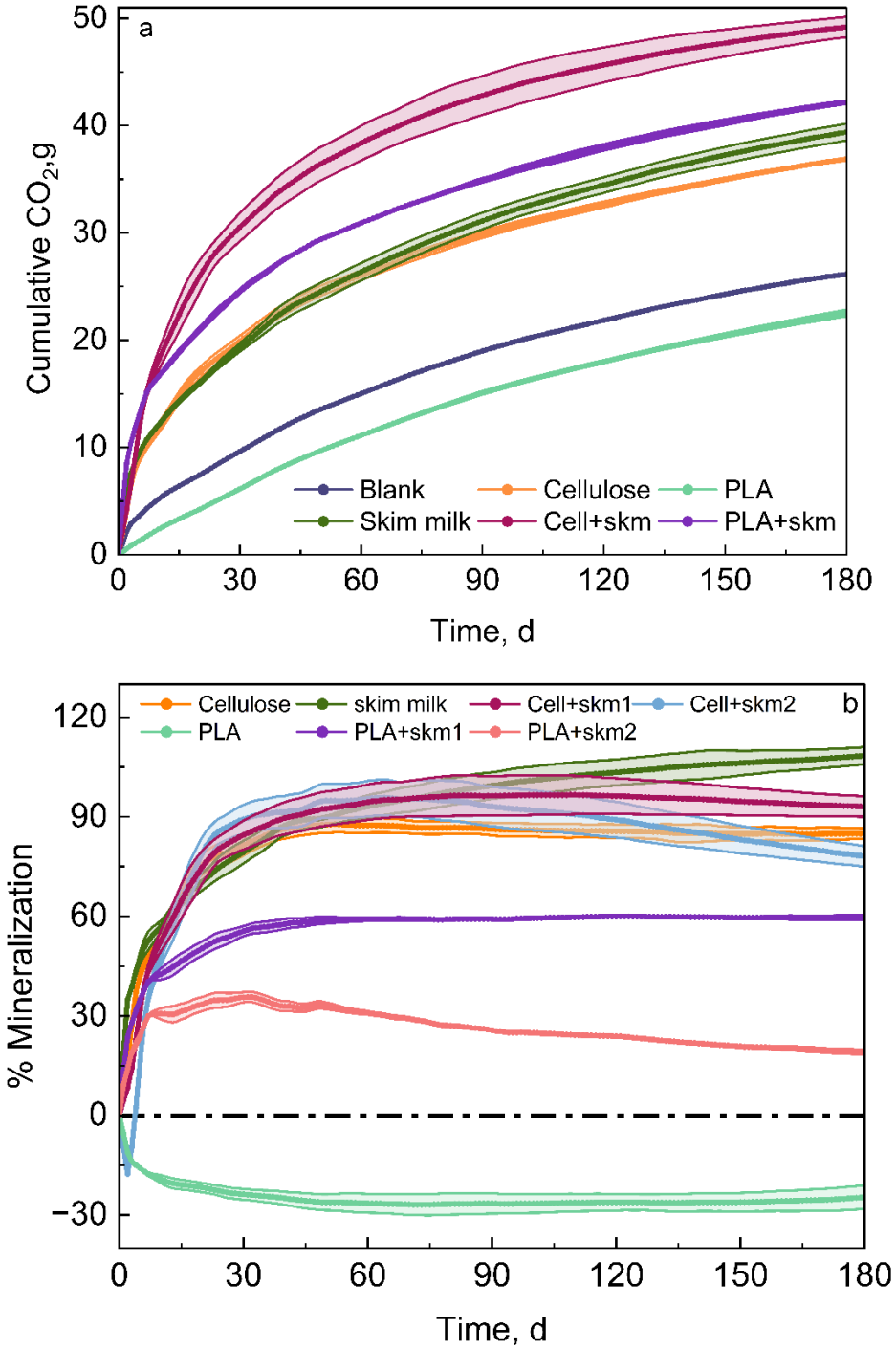

Figure A5.1 Cumulative CO_2 evolution (a) and mineralization (b) of blank, cellulose, PLA, skim milk, cellulose + skim milk (Cell+skm), PLA + skim milk (PLA+skm) in compost at 37°C.

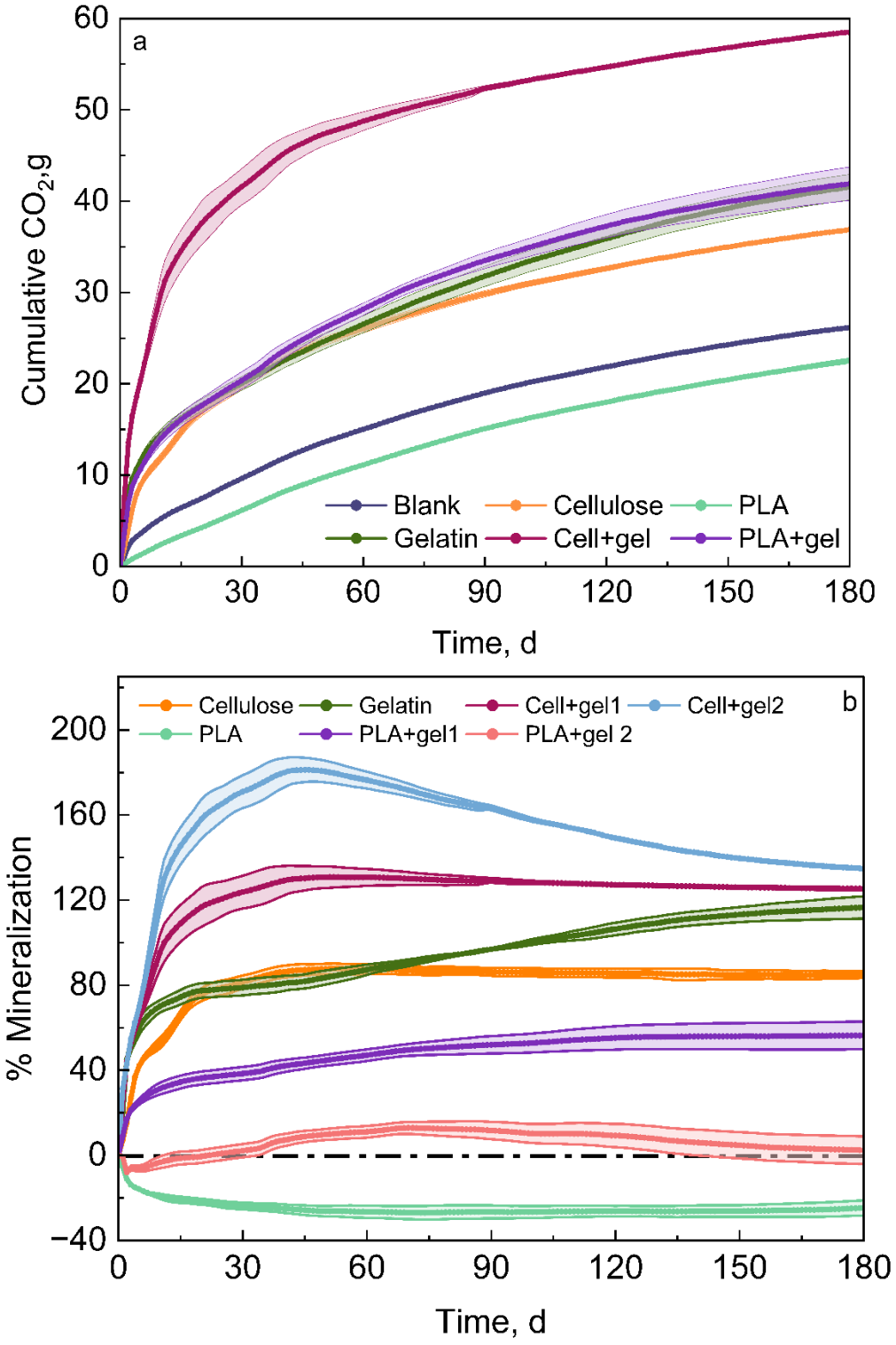

Figure A5.2 Cumulative CO_2 evolution (a) and mineralization (b) of blank, cellulose, PLA, gelatin, cellulose + gelatin (Cell+gel), PLA + gelatin (PLA+gel) in compost at 37°C.

Figure A5.2 a and b shows the CO_2 evolution and % mineralization of cellulose, gelatin, PLA, cellulose + gelatin, and PLA + gelatin. Cellulose reached mineralization of 87.7% whereas gelatin attained over 100 % of its carbon conversion over a period of 180 days. Since gelatin and cellulose are readily biodegradable and can be easily utilized as a carbon source by the microorganisms present in the compost, no lag phase was observed.

Gelatin is composed of protein which the microorganisms in compost use for their biochemical process. The microorganisms secrete protease enzyme to digest gelatin which is the same mechanism when PLA is introduced in gelatin amended compost. Due to the compost amendment with skim milk, PLA (PLA + gel1) shows a mineralization of approximately 60%. In order to account for the effect of gelatin, a separate mineralization plot (PLA + gel2) is derived. Though there seems to be negative mineralization, the molecular weight analysis [62] shows that gelatin helps in the enzymatic degradation of PLA. This coupled with the chemical hydrolysis of PLA [5], produces a significant difference with respect to the kinetic rate of degradation (PLA $k = 0.0045 \pm 0.0001$ and PLA-gelatin $k = 0.0060 \pm 0.0002$) [62].

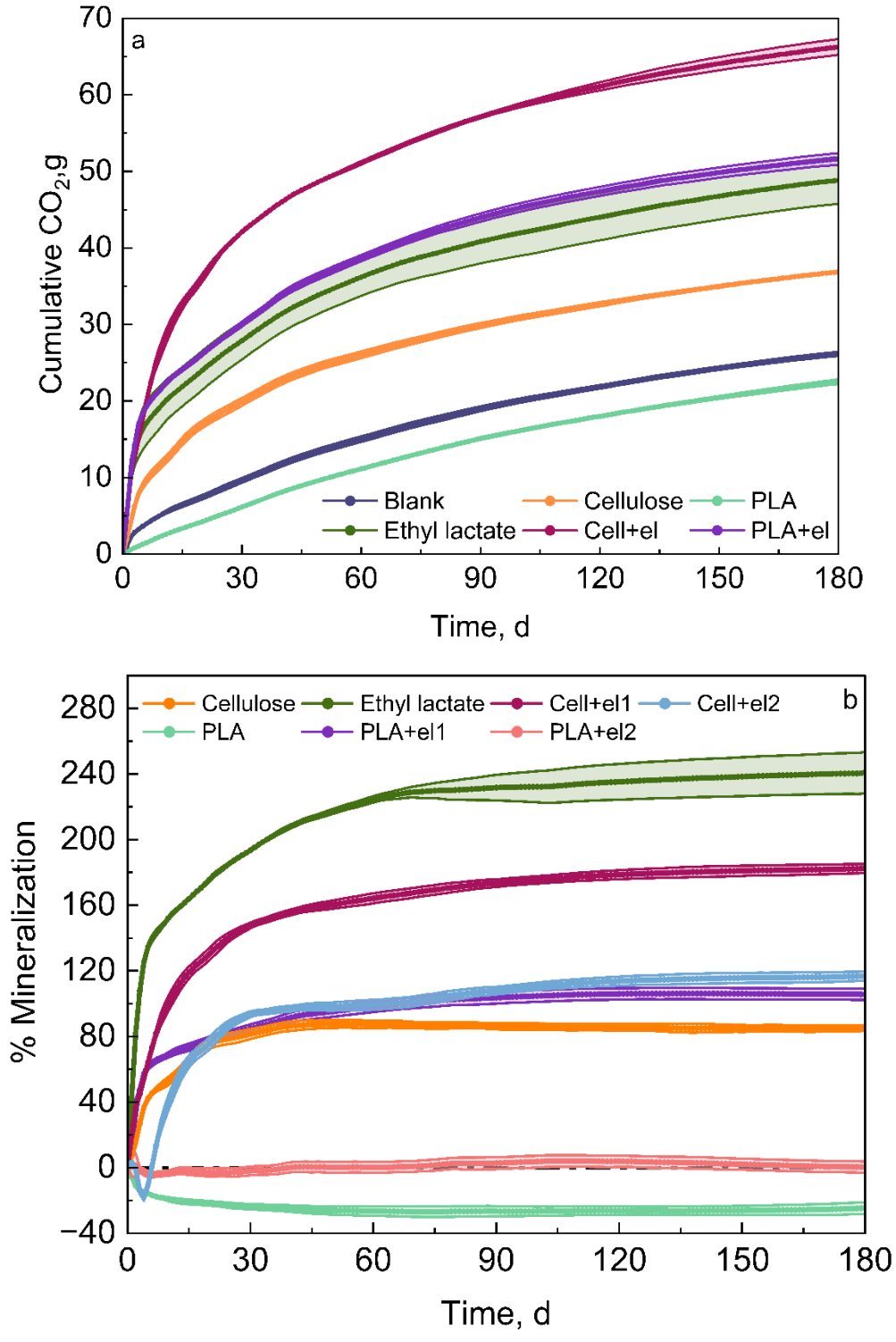

Figure A5.3 Cumulative CO_2 evolution (a) and mineralization (b) of blank, cellulose, PLA, ethyl lactate, cellulose + ethyl lactate (Cell+el), PLA + ethyl lactate (PLA+el) in compost at 37°C.

Figure A5.3 a and b shows the CO_2 evolution and % mineralization of cellulose, ethyl lactate, PLA, cellulose + ethyl lactate, and PLA + ethyl lactate. Cellulose reached mineralization of 87.7% whereas ethyl lactate attained over 100 % of its carbon conversion over a period of 180 days. Since ethyl lactate and cellulose are readily biodegradable and can be easily utilized as a carbon source by the microorganisms present in the compost, no lag phase was observed. Ethyl lactate was used to stimulate the lactate utilizing microbial community present in the compost.

Ethyl lactate evolves around 51.5 g of CO_2, and the corresponding mineralization reaches around 270%. The CO2 evolution of the amended compost containing both cellulose and ethyl lactate (Cell-el 1) was only around 66.2 g and there was a corresponding mineralization of 182.4%. PLA in the presence of ethyl lactate (PLA-el 1) evolved around 51.6 g of CO2, followed a similar trend as ethyl lactate over 180 days, and showed maximum mineralization of 105%. A positive mineralization behavior is seen for PLA when the effect of ethyl lactate is accounted for (PLA-el 2), indicating the lactate-stimulating activity of ethyl lactate. This is further confirmed by kinetic rates for PLA alone and PLA in compost amended with ethyl lactate (PLA $k = 0.0045 \pm 0.0001$ and PLA-ethyl lactate $k = 0.0058 \pm 0.0002$) [62].

APPENDIX 5C: CO_2 EVOLUTION AND MINERALIZATION OF PLA IN THE PRESENCE OF FE_3O_4 NANOPOWDER, SKIM MILK + FE, AND ETHYL LACTATE + FE

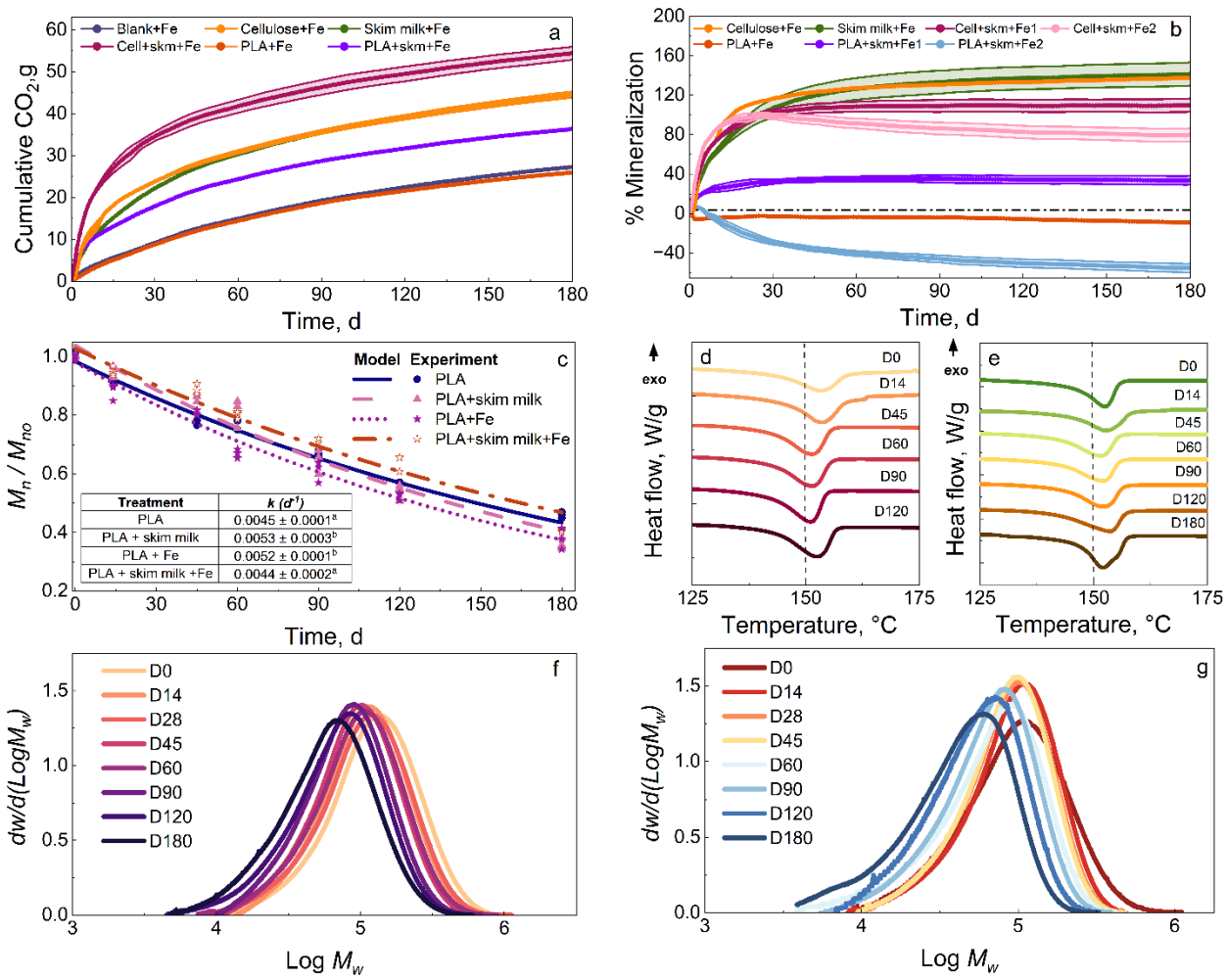

Figure A5.4 Cumulative CO2 evolution (a) and Mineralization (b) of blank + Fe, cellulose + Fe, PLA + Fe, skim milk + Fe , cellulose + skim milk + Fe (Cell+skm+Fe), PLA + skim milk + Fe (PLA+skm+Fe) in compost at 37°C. (c) represents the normalized Mn reduction as a function of time for PLA in control compost and compost biostimulated by gelatin, and gelatin + Fe. The experimental data was fitted using a first-order reaction of the form Mn /Mno = e(−kt), where Mno is the initial Mn, k is the rate constant, and t is the time. The inset shows the k-fitted values. Values in the column with different lowercase letters are statistically different (α = 0.05 Tukey-Kramer Test). (d) and (e) depict DSC thermograms for PLA + Fe and PLA + Fe in compost biostimulated by skim milk. (f) and (g) shows the MWD of PLA + Fe in compost and compost biostimulated with skim milk.

Figure A5.4 a and b shows the CO_2 evolution and mineralization of cellulose + Fe, skim milk + Fe, PLA + Fe, cell + skm + Fe, and PLA + skm + Fe in compost at 37°C. Skm +

Fe shows around 60.1 g of CO_2 evolution and mineralization of 247.8 % in 180 days. Skim milk was combined with Fe to target chemical hydrolysis and enzymatic degradation steps. The CO_2 evolution in this case (Cell+skm+Fe) sees a higher production of 54.5 g, which is as expected and higher compared to the individual values for cellulose + Fe (44.5 g). Cell+skm+Fe 1 shows a mineralization of 121.6% whereas Cell+skm+Fe 2 after accounting for skm+Fe shows a mineralization of 99.4% indicating that the presence of skim milk in no way affects the degradation of cellulose.

To understand the influence of skim milk + Fe on PLA degradation, PLA was introduced in the compost amended with skim milk and Fe. The bioreactor containing both PLA and skim milk + Fe (PLA+skm+Fe 1) shows CO_2 evolution of around 36.4 g and maximum mineralization of 35.3 % by the end of the test. Improved mineralization is observed as opposed to no CO_2 evolution for PLA alone without any biostimulation of compost. The effect of skim milk + Fe on PLA degradation is calculated by plotting the mineralization of PLA+skm+Fe 2 (subtracting skim milk + Fe). The negative mineralization does not necessarily indicate the absence of skim milk's protease activity in PLA's enzymatic degradation. The significant difference in the evolution of X_c from 28.3% to 31.4% for PLA, and from 28.3% to 37.9% for PLA samples biostimulated with skim milk + Fe, as seen in Figure S4 d and e**,** respectively further shows the improvement in the enzymatic degradation of PLA due to the presence of skim milk + Fe. Skim milk acts as a precursor for protease activity and the broadening and change in the intensity of peaks as seen in **Figure A5.4** f and g enforces that the addition of skim milk and Fe does enhance PLA degradation.

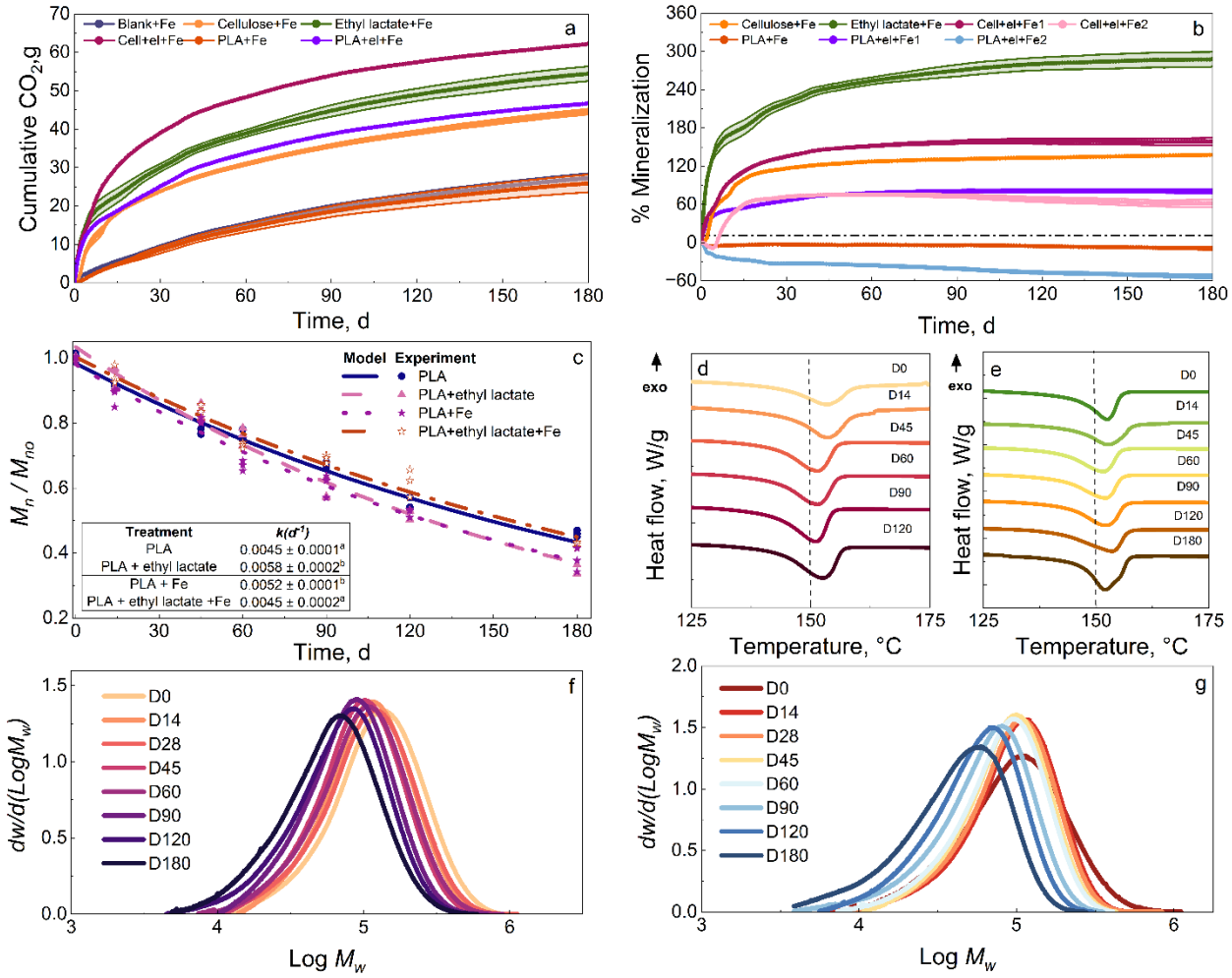

Figure A5.5 Cumulative CO_2 evolution (a) and Mineralization (b) of blank + Fe, cellulose + Fe, PLA + Fe, ethyl lactate + Fe , cellulose + ethyl lactate + Fe (Cell+el+Fe), PLA + ethyl lactate + Fe (PLA+el+Fe) in compost at 37°C. (c) represents the normalized M_n reduction as a function of time for PLA in control compost and compost biostimulated by gelatin, and gelatin + Fe. The experimental data was fitted using a first-order reaction of the form $M_n / M_{no} = e^{(-kt)}$, where M_{no} is the initial M_n, k is the rate constant, and t is the time. The inset shows the k-fitted values. Values in the column with different lowercase letters are statistically different (α = 0.05 Tukey-Kramer Test). (d) and (e) depict DSC thermograms for PLA + Fe and PLA + Fe in compost biostimulated by ethyl lactate. (f) and (g) shows the MWD of PLA + Fe in compost and compost biostimulated with ethyl lactate.

Figure A5.5 a and b shows the CO_2 evolution and mineralization of cellulose + Fe, ethyl lactate + Fe, PLA + Fe, cell + el + Fe, and PLA + el + Fe in compost at 37°C. El + Fe shows around 54.5 g of CO_2 evolution and mineralization of 288.1 % in 180 days. Ethyl lactate was combined with Fe to target chemical hydrolysis and lactate utilizing microbes in compost. The CO_2 evolution in this case (Cell+el+Fe) sees a higher production of 62.2 g, which is as

expected and higher compared to the individual values for cellulose + Fe (44.5 g). Cell+el+Fe 1 shows a mineralization of 156.7% whereas Cell+el+Fe 2 after accounting for el+Fe shows a mineralization of 75.1% indicating that the presence of ethyl lactate does not affect the degradation of cellulose.

To understand the influence of ethyl lactate + Fe on PLA degradation, PLA was introduced in the compost amended with ethyl lactate and Fe. The bioreactor containing both PLA and ethyl lactate + Fe (PLA+el+Fe 1) shows CO_2 evolution of around 46.5 g and maximum mineralization of 88.8 % by the end of the test. Enhanced mineralization is observed as opposed to no CO_2 evolution for PLA alone without any biostimulation of compost. The effect of ethyl lactate + Fe on PLA degradation is calculated by plotting the mineralization of PLA+el+Fe 2 (subtracting ethyl lactate + Fe). The negative mineralization does not necessarily indicate the absence of ethyl lactate's lactate stimulating microbial activity in PLA's enzymatic degradation. The significant difference in the evolution of X_c from 28.3% to 31.4% for PLA, and from 28.3% to 39.9% for PLA samples biostimulated with ethyl lactate + Fe, as seen in Figure S5 d and e**,** respectively further shows the improvement in the enzymatic degradation of PLA due to the presence of ethyl lactate + Fe. As seen in Figure S5 f and g ethyl lactate stimulates the lactate utilizing microbial community in the compost which aids in the degradation of PLA.

CHAPTER 6: ENHANCING BIODEGRADATION OF POLY(LACTIC ACID) IN MESOPHILIC AND THERMOPHILIC ENVIRONMENTAL CONDITIONS: THE ROLE OF PROTEINASE K AS A PRETREATMENT

6.1 Abstract

Plastic pollution poses a significant environmental challenge, prompting a shift towards biodegradable and biobased polymers such as poly(lactic acid) (PLA) as sustainable alternatives. Despite PLA's potential, a slow abiotic process hinders its degradation in composting environments, influenced by factors like high number molecular weight and crystallinity. Enzymatic degradation, particularly by PLA depolymerase like proteinase *K*, offers a promising solution to accelerate the degradation of PLA during composting if used as pretreatment. This study aimed to investigate the enzymatic degradation of PLA films pretreated with proteinase K and degraded in simulated home and industrial composting conditions. PLA films were pretreated with proteinase K at 37°C and 58°C at different times. PLA films treated in underwater conditions at 37°C were used as control. The accelerated CO_2 evolution and mineralization of the pretreated PLA films highlight the effectiveness of proteinase K pretreatment. This study shows the potential of enzymatic pretreatment to enhance PLA biodegradability at mesophilic and thermophilic temperatures, offering insights into sustainable waste management strategies to reduce the biodegradation rate of PLA films and packaging.

6.2 Introduction

Plastic pollution in the environment is a looming crisis. Given the crucial role that plastics play in creating single-use plastic packaging and the generation of white pollution, using biodegradable polymers in day-to-day life to combat mismanaged waste is seen as a welcoming solution. So, the commercial plastic industry is adopting biobased and

biodegradable plastics as an environmentally friendly alternative to fossil-based conventional polymers. Poly(lactic acid) - PLA - is one such polymer and is considered a major substitute for conventional fossil polymers because of its designed biodegradable nature when exposed to the proper set of composting conditions. So, the use of PLA has increased rapidly to support the growth of the circular sustainable bioeconomy, accompanied by the ongoing development in its production process.

Although PLA and its products are labeled as industrial compostable, it is essential to note that the time frame for the degradation process is longer as compared to the readily biodegradable organic waste fraction such as food, starch, and cellulose when PLA is collected together as a part of municipal solid waste and directed to an industrial composting facility [1]. This scenario arises because of the slow abiotic phase, which determines its degradation rate and is further influenced by molecular weight (M_n) and crystallinity (X_c). PLA is subjected to higher temperatures of 58°C-70°C in industrial composting facilities, which makes it able to degrade. However, its degradation is recalcitrant in soil and home composting environments where temperatures are much lower in the mesophilic range.

In environments such as soil and home composting, chemical hydrolysis proceeds at a prolonged rate due to the lower temperatures encountered, and biodegradation is mainly governed by biotic enzymatic degradation [2]. Abiotic degradation proceeds by reducing the M_n to values lower enough that the biotic enzymatic degradation can take over and accelerate the breakdown process. Biotic enzymatic degradation involves the breakdown of polymer chains into small molecules that the microorganisms can quickly assimilate. The biodegradation at such mesophilic conditions relies heavily on the depolymerization stage, wherein the microorganisms release the enzymes. For hydrolyzable polymers such as PLA, the main enzymes belong to the hydrolases (EC 3) class of extracellular enzymes reported for depolymerizing aliphatic polyesters. Depending on the substrate specificity, the PLA

depolymerases are categorized as protease and lipase types. Both classes of PLA depolymerases employ the serine hydrolase catalytic mechanisms, yet their different stereochemistry at the catalytic sites provides a structural basis for different specificities for substrates [3].

Proteinase K (3.4.21.64) and proteases (3.4.21.112) belong to the serine endo peptidases (3.4.21), which catalyze bond scission in the middle of the substrate chain and are also known to hydrolyze polyesters such as PLA. Proteinase K and proteases show enzymatic activity specifically for PLA containing L-lactate compared to PDLA and PDLLA. These enzymes can recognize PLA's repeating L-lactic acid unit as structurally homologous to the proteins composed of L-aminoacids [4]. Williams [5] first reported the hydrolysis of PLA by proteinase K sourced from *Tritirachium album*. Since then, the degradation of PLA by different enzymes has been studied substantially [2]. The commercially available proteinase K has been used extensively to study PLA enzymatic degradation.

Unlike mechanical and chemical recycling, enzymatic degradation consumes less energy, needs fewer chemicals, or generates less harmful compounds threatening the environment. So, enzymatic degradation can assist in further developing the degradation of PLA at lower temperatures. This study aimed to evaluate the enzymatic degradation of PLA by pretreating PLA with proteinase K enzyme in a buffer solution at 37°C and 58°C for different time intervals before introducing it into a simulated compost environment at 37°C to replicate home composting and at 58°C to simulate industrial composting. The CO_2 evolution of the samples was recorded, and the changes in M_n and X_c throughout the test duration were measured to understand how the pretreatment with proteinase K modified the biodegradation of PLA in the different composting conditions.

6.3 Materials and methods

6.3.1 Materials

Crystalline PLA films ranging 25 microns in thickness were obtained from EarthFirst (Columbus, OH, USA). The crystalline PLA films were heated at 180°C, for 10 mins and fast quenched in dry ice for 10 minutes to remove any residual crystallinity. Proteinase K from *Tritirachium album* (29.3 kDa) from Syd labs (Hopkinton, MA) was procured and used as received for enzymatic degradation. HPLC grade Tetrahydrofuran (THF) was obtained from (Sigma-Aldrich, St. Louis, MO, USA). All the chemicals were used as received.

6.3.2 Preparation of buffer and enzyme solution

Buffer powder (MSU, East Lansing, MI) was mixed in distilled water to prepare the buffer solution. NaOH pellets were mixed in the distilled water and added to the buffer solution until the pH reached 8.5. The proteinase K enzyme was added to the distilled water to get an aqueous solution of proteinase K (500 μg/mL). Based on the previous reports, the concentration was calculated and set to 500 µg/mL [6,7].

6.3.3 Enzymatic degradation by externally adding Proteinase K

The enzymatic degradation of PLA films was performed by externally adding the proteinase K solution and buffer solution to the PLA films (1 cm x 1 cm squares). The reaction solution was incubated at 37°C for 7 and 10 days and at 58°C for 2 and 5 days with constant stirring to determine the effect of exposing PLA films to Proteinase K at different intervals.

6.3.4 Hydrolysis experiment

A hydrolysis test was also run as a control to understand the hydrolytic degradation of the PLA films. The test procedure was adapted from ASTM D4754-18 [8]. The PLA films were cut into 1 cm x 1 cm and introduced into the beaker with HPLC-grade water (VWR, Radnor, PA, USA) at 37°C for 10 days. At the end of the test, the samples were dried and stored for further analysis.

6.3.5 Biodegradation in vermiculite

The biodegradation of PLA and the effectiveness of pretreating PLA films with proteinase K in inoculated vermiculite on the degradation of PLA were evaluated under aerobic simulated mesophilic and thermophilic conditions using two direct measurement respirometric (DMR) systems [9–11]. Shortly, the system included a non-dispersive infrared gas analyzer (NDIR) (Li-COR® LI-820, Lincoln, NE, USA) which measures the CO_2 concentration. The DMR system chamber was maintained at a temperature of 37 ± 2 °C and 58 ± 2 °C and provided with air at a relative humidity (RH) of 50% ± 5%. A flow rate of CO_2-free air (concentration <30 ppm to establish a low baseline) was controlled at 40 ± 2 cm^3/min. Additional detailed information about the DMR equipment can be found in another source [12].

Mature compost obtained from the MSU composting facility was sifted using a 10-mm screen to get rid of any considerable debris or chunks present and then conditioned at 37 ± 2 °C and 58 ± 2 °C until use. This compost extract was then amalgamated with a mineral solution in a 1:1 ratio, resulting in the inoculum solution. Detailed information regarding the preparation of the mineral solution can be found elsewhere [13]. Inoculated vermiculite provides the benefit of very low CO_2 evolution from the blank used as the baseline. Deionized water was used to adjust the moisture content of the vermiculite during testing to 50%. The resulting inoculated vermiculite mixture was sent to the Soil and Plant Testing Laboratory at the University of Missouri (Columbia, MO, USA) to determine the physicochemical parameters of the media. Data regarding the solid analysis is presented in **Table A6.1**, Appendix 6A. The bioreactors were packed with 400 g of inoculated vermiculite, and 8 g of all the samples were tested in triplicate and positive control (cellulose). Blank (only compost) was used as the baseline.

6.3.6 Size exclusion chromatography (SEC)

As described elsewhere, the M_n and molecular weight distribution (MWD) of PLA for the control and each biostimulant treatment were measured using SEC (Waters Corp., Milford, MA, USA) [14]. Shortly, PLA samples weighing approximately 10 mg were retrieved at predetermined intervals and dissolved in 5 mL of THF. A temperature of 35°C and a 1 mL/min flow rate were maintained during testing. The Mark-Houwink constants of K = 0.000174 dL/g and α = 0.736 were used to determine the absolute M_n, M_w, and MWD of the PLA samples. Data analysis was carried out using Waters Breeze™2 software from Waters.

6.3.7 Differential scanning calorimetry

A DSC model Q100 (TA Instruments, New Castle, DE, USA), was used to determine the glass transition temperature (T_g), (T_m), crystallization temperature (T_c), and crystallinity (X_c) for the PLA samples retrieved from the regular and biostimulated compost. PLA samples weighing 5 - 10 mg were packed in aluminum pans, cooled to −5°C, and then subjected to a heating cycle to reach 210°C at a ramp rate of 10°C/min. This information was used to evaluate the evolution of X_c. The cooling was achieved using a nitrogen cooling system that maintained the purge flow rate at 70 mL/min. The degree of crystallinity was estimated using equation (6.1):

$$\chi_c\ \% = \frac{\Delta H_m - \Delta H_c}{\Delta H_m^0}\ x\ 100 \tag{6.1}$$

where ΔH_m is the heat of fusion, ΔH_c is cold crystallization enthalpy and ΔH_m^0 is the heat of fusion for 100% crystalline pure PLA (93 J/g) [15].

6.3.8 Statistical analysis

The statistical analysis was conducted using MINITAB™ software (Minitab Inc., State College Park, PA, USA). The statistical significance at $p < 0.05$ was evaluated using one-way ANOVA and Tukey-Kramer test. Data is reported as means ± standard deviation.

6.4 Results and discussion

The CO_2 evolution of PLA control (PLA), PLA hydrolyzed films at 37°C for 10 days (PLA 37 hydro 10 D), and enzymatically pretreated PLA samples over 7 and 10 days at 37°C (PLA-37 $_{\text{proteinase K 7D}}$ and PLA-37 $_{\text{proteinase K 10D}}$) and PLA samples over 2 and 5 days at 58°C (PLA-58 $_{\text{proteinase K 2D}}$ and PLA-58 $_{\text{proteinase K 5D}}$) were tracked at simulated mesophilic conditions (37°C) and thermophilic (58°C) conditions using inoculated vermiculite as media, respectively.

6.4.1 M_n reduction and crystallinity evolution for PLA and PLA pretreated films

The M_n and X_c of PLA control, PLA hydrolyzed films at 37°C for 10 days, PLA pretreated with proteinase K at 37°C for 7 and 10 days, and PLA pretreated with proteinase K at 58°C for 2 and 5 days was determined to understand the effect of proteinase K pretreatment. **Table 6.1** presents the values for the same before introducing the samples in the DMR chamber for further biodegradation testing.

Table 6.1 Initial characterization of PLA, PLA hydrolyzed films, and PLA films treated with proteinase K at 37°C and 58°C.

Films	Treatment time (days)	37 ± 2 °C		58 ± 2 °C	
		Molecular weight (M_n, kDa)	Crystallinity (Xc)	Molecular weight (M_n, kDa)	Crystallinity (Xc)
PLA Control	0	79 ± 3.0[a]	0	79 ± 3.0[a]	0
PLA hydrolysis	10	75 ± 2.8[a]	2.5± 2.0[a]	75 ± 2.8[a]	2.5± 2.0[a]
PLA proteinase K	7	38 ± 1.0[b]	7.1 ± 1.5[b]	-	-
	10	17 ± 0.9[c]	12 ± 1.3[c]	-	-
	2	-	-	61 ± 3.0[b]	23 ± 3.5[b]
	5	-	-	47 ± 2.9[c]	43 ± 3.0[c]

A significant difference was seen for M_n for PLA control, PLA hydrolyzed films, and PLA films pretreated with proteinase K at both 37°C and 58°C. Apart from PLA and PLA

hydrolyzed films, again, a significant difference was seen for X_c for PLA proteinase K pretreated films. This indicates that proteinase K enzymatically hydrolyzes PLA and reduces it to a lower M_n. The values differ for the different pretreatment time intervals, as seen in **Table 6.1** for 37°C and 58°C. The difference can be associated with the optimal activity of proteinase K at 37°C. The PLA hydrolyzed films do not show any difference in M_n and X_c $_{compared}$ to PLA control films because of the slower chemical hydrolysis at 37°C.

6.4.2 CO_2 evolution and mineralization of PLA and PLA pretreated films in inoculated vermiculite

Figure 6.1 a and b present the CO_2 evolution and % mineralization of the blank (vermiculite only), cellulose, PLA control films, PLA hydrolyzed films at 37°C for 10 days, and PLA pretreated with proteinase K enzyme for 7 days and 10 days, respectively in inoculated vermiculite at 37 ± 2 °C. Blank showed CO_2 evolution of 2.4 g by the end of day 100.

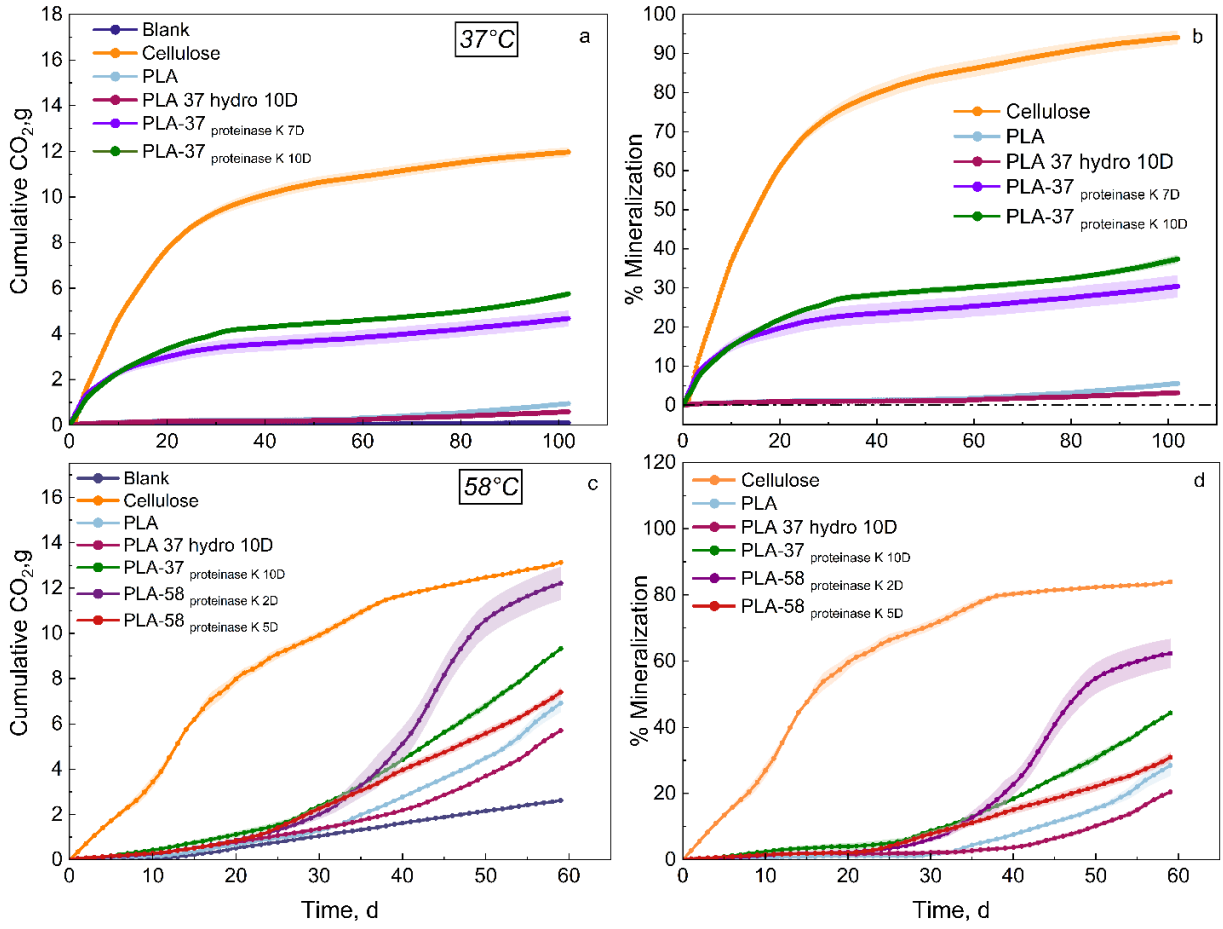

Figure 6.1 CO_2 evolution and % mineralization of PLA, PLA 37hydro 10D, PLA-37 $_{\text{proteinase K 7D}}$,and PLA-37 $_{\text{proteinase K 10D}}$ films in compost 37 °C (a & b) and PLA, PLA 37hydro 10D, PLA-58 $_{\text{proteinase K 2D}}$, PLA-58 $_{\text{proteinase K 5D}}$ films in compost at 58 °C (c & d). The shade in the background for each material represents the standard error between replicates.

Cellulose showed CO_2 evolution of 14.3 g and mineralization of *c.* 94% by day 100. PLA films subjected to hydrolysis at 37°C for 10 days showed a very low CO_2 evolution of 2.5 g, whereas PLA control films showed only 2.7 g. A lag phase is observed for both PLA control and PLA hydrolyzed films, which could mean that PLA offers a physical hydrophobic barrier to water and air before hydrolysis, and the microbes have difficulty accessing the carbon source as nutrients. The absence of mineralization does not necessarily imply the absence of hydrolytic degradation or enzymatic activity due to the action of extracellular enzymes secreted by the microbes but more like inhibition of the microbial activity due to the

hydrophobic layer barrier created by the presence of the high M_w PLA. The lower values for both indicate that PLA is still undergoing hydrolysis since PLA is exposed to temperatures lower than its T_g (*c.* 60 °C). PLA segments have little to no mobility and are not flexible below T_g, preventing diffusion or attack by water. Since the initial and rate-limiting step in PLA degradation is chemical hydrolysis, the lack of chain scission accompanied by the hydrophobic barrier provided by PLA can explain the lack of mineralization both for PLA control and PLA hydrolyzed films.

For PLA films pretreated with proteinase K enzyme for 7 days, CO_2 evolution of 6.6 g and mineralization of *c.* 28% was seen. In contrast, for PLA films pretreated with proteinase K enzyme for 10 days, 7.7 g of CO_2 was evolved, and *c.* 35% mineralization was observed by day 100. No lag phase was observed for proteinase K pretreated films, indicating that the pretreatment with proteinase K reduces PLA to M_n of ~ 10,000 Da, wherein the biotic degradation stage kicks in, and the microorganisms can utilize PLA for their biochemical processes. **Figure 6.1c and d** present the CO_2 evolution and % mineralization of the blank (vermiculite only), cellulose, PLA control films, PLA hydrolyzed films at 37°C for 10 days, and PLA pretreated with proteinase K enzyme for 2 days and 5 days, respectively in inoculated vermiculite at 58 ± 2 °C. The blank showed a maximum CO_2 evolution of 2.6 g at day 60. The positive control, cellulose showed CO_2 evolution of 13.1 g and mineralization of *c.* 84% at day 60. No lag phase is observed since cellulose is readily biodegradable and a food source for microorganisms. Cellulose is degraded by the action of a battery of enzymes that work simultaneously and synergistically. Cellulases catalyze the hydrolysis of ß-1,4-linkages in the cellulose [1,16]. The action of exoglucanases and endoglucanases on the ends and at random internal sections of cellulose's amorphous region produces varying lengths of cello-oligosaccharides, which are then hydrolyzed by glucosidases to produce glucose [17]. Glucose

is finally converted to CO_2 through a series of further cycles. Previous research has shown fungi, a few bacteria species, and actinomycetes in compost and the soil environment produce cellulase, which are involved in the degradation of cellulose [18–22].

PLA control films show a lag phase of around 35 days because of the initial abiotic hydrolysis phase, wherein PLA ester bonds are broken down mainly due to the hydrolysis by water. The high M_n PLA chains are cleaved to produce low M_n oligomers used by the microorganisms in the inoculated vermiculite, releasing CO_2 and water, which can be observed during the biotic degradation phase. A CO_2 evolution of 7.0 g and mineralization of *c*. 28.5% was observed for PLA at 58 °C. PLA films subjected to hydrolysis at 37°C for 10 days (**Figure 6.1b**) showed a CO_2 evolution of 5.7 g and mineralization of *c*. 20.5%. PLA hydrolyzed films followed a similar trend to PLA control films; however, the mineralization is slightly lower, maybe due to the higher initial crystallinity of PLA 37 hydro 10D films. PLA films pretreated with proteinase K enzyme at 37°C for 10 days (PLA-37 $_{\text{proteinase K 10D}}$) evolved 9.3 g of CO_2 and showed a mineralization of *c*. 44.3% at 58°C. The improved degradation can be attributed to the pretreatment with proteinase K at 37°C for 10 days. Proteinase K belongs to serine endo peptidases that preferentially catalyze bond scission in the middle of the substrate chain. The enzymes showing activity towards PLA belong to the protease type group or peptidases. They can recognize the repeating L -lactic acid unit of PLA as the natural homolog L -alanine unit of silk fibroin [2,4]. Though proteinase K is primarily known to cleave peptide bonds, it can exhibit a certain level of non-specific hydrolytic activity towards PLA.

The PLA films were also pretreated with proteinase K enzyme at 58°C for 2 and 5 days, respectively (PLA-58 $_{\text{proteinase K 2D}}$ and PLA-58 $_{\text{proteinase K 5D}}$) to evaluate the effect of temperature and time on the efficiency of proteinase K's enzymatic activity. PLA films pretreated for 2 days showed CO_2 evolution of 12.2 g and mineralization of *c*. 62.3%, whereas PLA films pretreated for 5 days evolved 7.4 g of CO_2 and exhibited a mineralization of *c*. 31%.

All the films, when pretreated with proteinase K, followed similar trends and showed considerable mineralization in comparison to the PLA control and PLA hydrolyzed films.

6.5 Conclusion

PLA films were pretreated with proteinase K to evaluate the effect of pretreatment on the overall biodegradation of PLA films in simulated compost conditions (inoculated vermiculite.) Subjecting PLA films to proteinase K pretreatment showed significant improvements in CO_2 evolution and mineralization rates, indicating accelerated degradation compared to untreated PLA films. These findings suggest that enzymatic pretreatment offers a promising approach to overcoming the challenges faced with PLA degradation, particularly in composting facilities operating at lower temperatures. Furthermore, the study highlights the potential of enzymatic hydrolysis as a sustainable and environmentally friendly method for managing PLA waste. Future research efforts could optimize pretreatment conditions and explore the scalability of enzymatic degradation processes for large-scale PLA waste management applications.

6.6 Acknowledgments

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APPENDIX 6A: PHYSICOCHEMICAL CHARACTERISTICS

Some inoculated vermiculite was collected and sent to the Soil and Plant Testing Laboratory at the University of Missouri (Columbia, MO, USA) to evaluate its physicochemical parameters (dry solids, volatile solids, and C/N ratio) as described elsewhere [23]. The physicochemical parameters are reported below in **Table A6.1**.

Table A6.1 Physicochemical parameters and total nutrient analysis of compost used in the biodegradation test.

Parameter	Compost
Dry solids, %	20.0
Volatile solids, %	4.53
pH	6.0-8.0
C/N ratio	3.57
Carbon, %	0.18
Nitrogen, %	0.05
Phosphorus, %	0.06
Potassium, %	3.15
Calcium, %	0.24
Magnesium, %	2.44
Sodium, %	0.15
Sulfur, %	0.07
Iron, ppm	6811
Zinc, ppm	17
Manganese, ppm	60
Copper, ppm	59
Boron, ppm	24
Aluminum, ppm	4.05

CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

7.1 Overall conclusion

Biobased, biodegradable polymers have emerged as a sustainable alternative to the ever-growing escalating waste management and disposal problems due to the increased fossil-based plastic production. Poly(lactic acid) - PLA, has garnered significant attention due to its biobased renewable origin, low environmental footprint, compostable nature, designable biodegradability, and because of its competitive price and expansion in different sectors such as packaging, plasticulture, and medical industries. Despite being biodegradable and compostable, the longer biodegradation timeframes associated with its degradation compared to the readily degradable organic fractions such as starch and cellulose have hindered PLA acceptance widely.

This dissertation addresses this issue to enhance PLA biodegradation under mesophilic and thermophilic conditions, to expand its application to different environments such as soil, and home/backyard composting, and to improve/ achieve a similar degradation timeframe with that of organic matter encountered in industrial composting settings.

Chapter 2 of this dissertation provides an in-depth literature review of the biodegradation of biodegradable polymers under mesophilic conditions, the different steps involved in the process, the role of enzymes, the different test methods used to determine degradation, key factors affecting the kinetic degradation rate. It also summarizes the different approaches and combines various factors to provide a holistic understanding of the complex biodegradation phenomena of biodegradable polymers, including PLA.

Chapter 3 of this dissertation investigated the reactive blending of PLA with thermoplastic starch (TPS) as one of the methods to accelerate biodegradation under mesophilic conditions, thereby simulating the home composting setting. The results revealed that reactive blending ensured uniform dispersion of TPS into the PLA matrix. The presence

of TPS as a food source for the microorganisms in the compost eliminated the extended lag phase caused by the chemical hydrolysis process wherein the breakdown of PLA chains to oligomers, dimers, and monomers, and a major reduction in the molecular weight is achieved. Since TPS could be used immediately, structural imperfections were created in PLA, further enhancing its biotic degradation rate. Without any blending, PLA underwent a long lag phase, which lasted until the end of the test, which was 180 days. The test data from 58°C biodegradation was also compared to illustrate and highlight the role of TPS in improving the overall degradation of PLA at thermophilic conditions.

Chapters 4 and 5 explain the use of a biostimulation technique to enhance the enzymatic biodegradation of PLA at 37°C. Different biostimulants, Fe_3O_4 nano-powder, skim milk, gelatin, and ethyl lactate, were identified to target the chemical hydrolysis and the biotic enzymatic degradation steps to facilitate and accelerate PLA degradation. Fe_3O_4 nano-powder, skim milk, gelatin, and ethyl lactate were introduced into the compost media at 37°C, and CO_2 evolution, M_n, and X_c of PLA were monitored through 180 days test duration. The introduction of individual biostimulants and a combination showed improved enzymatic degradation of PLA compared to no using biostimulants. Adding biostimulants mainly enhanced the enzymatic biotic phase, reflected in the improved kinetic degradation rate.

Chapter 6 focused on a pretreatment approach that involved pretreating PLA with proteinase K enzyme to investigate the effect of enzymes in accelerating enzymatic degradation. For the most part, in the reported research, enzymatic degradation has been conducted for a minimal amount of PLA in a liquid media, wherein PLA was the only isolated carbon source. In this chapter, PLA films were first enzymatically treated with proteinase K at 37°C and 58°C for different intervals to achieve different M_n and X_c. After pretreatment, the PLA films were introduced in an inoculated vermiculite solid matrix at 37° and 58°C to replicate the home and industrial composting environment. The results depicted improved CO_2

evolution and a shortened lag phase for the treated samples compared to the untreated PLA films.

The different approaches mentioned above offered practical strategies to accelerate PLA biodegradation and advance the understanding of the complex biodegradation process at lower temperatures, which are encountered in real end-of-life scenarios. The approaches utilized above also provide a safe way of degrading PLA, unlike incineration and landfilling, which bring many problems, including generating toxic gases and using higher temperatures, thereby increasing pollution, energy consumption, and the leaching of microplastics into the environment.

7.2 Recommendations for future work

The biodegradation of biodegradable polymers in different environments is a complex phenomenon that requires an array of expertise to investigate, understand, and advance the complex involved processes.

Starch has been extensively researched and studied in terms of polymer. Though adding starch enhances the biodegradation of PLA, several setbacks are encountered while processing starch into the PLA matrix on a larger scale. The current study only focused on including starch as one of the additives and focused more on the biodegradation aspect of the resulting blend. But overall, the blend should be able to provide the necessary properties expected of a package or film (e.g., food packaging), until the end-of-life cycle. Different additives and properties enhancement should be further considered and studied to provide a holistic approach.

One of the main uncontrollable parameters encountered in the home composting process is temperature due to the unreliable process control. The change from higher to lower temperatures as we move from industrial to home composting hugely influences the biodegradation phenomena. The dominant and rate-determining chemical hydrolysis phase

for PLA proceeds very slowly. Different biostimulants could be explored and studied to improve the hydrolytic phase without altering or modifying the PLA structure. In the case of the biostimulation approach, further delving and fine-tuning the optimal concentrations of biostimulants is essential to maximize their effectiveness while minimizing the associated costs and environmental impacts of accelerating PLA biodegradation. Systematic studies must be conducted by varying the concentrations of biostimulants to identify the optimal concentration and maximize the biodegradation efficiency. To provide a realistic and economic standpoint, laboratory-level studies should be scaled up to assess the feasibility and compatibility of the novel methodologies with the existing home infrastructure in place, specifically when compared to the industrial composting plants. Further testing of the phylogenetic makeup and microbial interactions due to adding biostimulants during the composting process should be conducted. This will help better understand the changes happening in the compost since compostability is a desired end-of-life scenario for contaminated PLA.

This dissertation used CO_2 evolution tracking and molecular weight determination to determine the acceleration of the biodegradation process, but further tests need to be conducted to understand the changes taking place at the molecular level and to comprehend better the process governing the interaction between the biostimulants, native microbial population in the compost, and PLA as a polymer model system. Carbon 13 label of polymer could also be a more expanded methodology to evaluate the biodegradation of PLA. Different scientific methods, such as enzymatic activity assays, spectroscopy analysis, and scanning electron microscopy imaging, can be complemented with computational modeling techniques to examine further and unravel complex pathways involved in PLA biodegradation. Machine learning can also be used to extract and analyze large existing microorganism datasets,

identify patterns, and develop prediction models for optimizing PLA biodegradation before running compostability tests.

The enzymatic pretreatment of PLA with proteinase K shows promising results, but different enzymes, pretreatment conditions, and durations should be further explored. This should be primarily conducted in a solid matrix such as compost as the current studies are limited to liquid media, a small amount of PLA tested, and isolated with specific microbial strains that do not replicate real-life disposal compost scenarios.

This dissertation has provided three different approaches that can be used to enhance the biodegradation rate of PLA. From a broader perspective, future efforts should focus on using these novel methodologies in collaboration with industries, governments, and composting facilities to test the implications and improve PLA recovery.