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Insights on the aerobic biodegradation of polymers by analysis of evolved carbon dioxide in simulated composting conditions

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ABSTRACT

The development of novel biodegradable polymers as a way to create sustainable materials has required the development of methodologies to evaluate and understand their biodegradation. In this work, we first provide a critical summary of selected biodegradation tests performed in the last fifteen years for a number of biodegradable materials, providing relevant information about the materials tested, characteristics of the compost used and the method for testing. Then, we report a comparative analysis of the results obtained from eight different biodegradation tests performed in simulated composting conditions by analysis of evolved CO_2 and carried out in an in-house built direct measurement respirometer. The materials evaluated for biodegradation were cellulose, starch, glycerol, polyethylene, and poly(lactic acid). Our results along with the information provided in the literature allowed us to identify that one of the main issues of biodegradation testing is the low reproducibility due to the number of variables involved in the biodegradation process. It is difficult to provide fair comparisons of samples that are not within the same test. Therefore, we provide a critical overview of the different factors affecting the biodegradability, biodegradation rate, and biodegradation mechanisms of polymeric materials. Furthermore, we share the experiences and insights gained during the performance of the different biodegradation tests, and identify areas of opportunity for improving biodegradation testing through evolved CO_2. This information should create a common knowledge platform for people interested in studying the biodegradation of materials.

1. Introduction

Biodegradable polymers represent a promising way to reduce the amount of plastic waste disposed in landfills, with composting the preferred alternative for their disposal. Many biodegradable polymers have been developed in the last two decades with the desired performance properties [1–7] for replacing conventional polymers for applications where plastics are highly contaminated and are difficult to recover through recycling such as agricultural films and single-use products like packaging and disposable cutlery [8,9]. Thus, along with the development of these novel materials, evaluation and understanding of their biodegradation performance and their environmental impacts have become germane [8–11].

Different analytical techniques have been used to evaluate biodegradation of polymers in composting using a direct or an indirect approach. Even though techniques like visual observations, weight loss measurements, changes in mechanical properties, and changes in molecular weight, can provide insights into the degradation process of a polymer, they do not necessarily demonstrate biodegradation [12]. Therefore, respirometric methods, in which the consumption of oxygen and/or the evolution of carbon dioxide (CO_2) is measured, have become the preferred technique for such assessment.

During aerobic biodegradation, microorganisms use the polymer as a source of carbon for growth and their metabolic processes yield CO_2. The amount of CO_2 produced during metabolic reactions and the fraction of carbon that is incorporated into biomass is a function of the substrate type and concentration, physical attributes of the environment, species-specific characteristics of the degradative microbial populations, and population dynamics within a complex community of microbes [8,10,13,14]. In respirometric methods, the evolved CO_2 can be measured in either a discrete or a continuous way by using different techniques.

In cumulative measurement respirometry (CMR), the evolved

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CO_2 is trapped in a solution, *e.g.* sodium hydroxide (NaOH), throughout the test and then quantified by titration [8]. Similarly, in gravimetric measurement respirometry (GMR), CO_2 is captured in absorption columns filled with pellets of NaOH, and the amount of CO_2 is quantified by the weight increase in the columns [8]. When direct measurement respirometry (DMR) is used, the output air is directly analyzed using either a non-dispersive infrared (NDIR) sensor or a gas chromatograph (GC) coupled with a thermal conductivity (TCD) detector to quantify the amount of evolved CO_2 [8].

In this context, several respirometric systems have been designed and built by different research groups around the world [15–20] following international standards such as ASTM D5338 and ISO 14855 [21,22]. A detailed list and information about different available standards is provided elsewhere [23,24]. However, performing biodegradation tests is not an easy task; it is costly, time-consuming, and requires constant attention to the proper functioning of the equipment. Moreover, due to the biological nature of the process, there are many variables that must be properly controlled and/or monitored.

Table 1 shows the results of selected tests found in the literature using different methods for assessing biodegradation of materials in compost. The majority of these tests used CO_2 evolution to track the biodegradation of the materials, but some authors have used visual inspection and weight loss for estimating biodegradation. Table 1 also provides information relevant for biodegradation tests such as the material shape and thickness, molecular weight, and the physicochemical characteristics of the compost used for testing - whenever provided by the authors. However, when comparing the same materials, *e.g.* cellulose or PLA, there is large variation in biodegradation and the time to reach similar levels of biodegradation among tests. This variation makes it difficult to compare biodegradation values between and within tests. Therefore, further understanding and review of the different factors affecting biodegradation would be useful for conducting future biodegradation tests in which the key factors could be more strictly monitored and controlled, reducing such variability.

In this work, we report a comparative analysis of the results obtained from eight biodegradation tests of different materials (*i.e.,* cellulose powder (CP), glycerol (GC), cassava starch (CS), poly(lactic acid) (PLA), polyethylene powder (PE), and a blend of linear low density polyethylene (LLDPE) and low density polyethylene (LDPE)) performed in simulated composting conditions by the analysis of evolved CO_2 using the same DMR system. The data from published work is critically reviewed and compared with the data from our eight biodegradation tests performed by the evolved CO_2 approach. Finally, we share insights gained from the different biodegradation tests in an attempt to identify areas in need of improvement and to establish more standardized procedures for researchers interested in studying aerobic biodegradation of polymers by analysis of evolved CO_2.

2. Materials and methods

2.1. Materials

Cellulose powder (CP) with particle size ~20 μm and glycerol (GC) 99+% was purchased from Sigma-Aldrich (St. Louis, MO) and cassava starch (CS) containing 25± 6% amylose content from Erawan Marketing Co., LTD (Bangkok, Thailand). Polyethylene powder (PE), low density polyethylene resin (LDPE 501I) and linear low density polyethylene resin (DOWLEX 2045G) were obtained from Dow Chemical (Houston, TX), and poly(lactic acid) resin (Ingeo™ 2003D and 4032D) from NatureWorks LLC. (Minnetonka, MN). Materials were used as received unless specified and the same batch of a compound was used for all the tests.

2.1.1. Material processing and characterization

A 70% wt. LDPE- 30% wt. LLDPE blend film (hereafter referred to as LDPE) was produced by blown extrusion with an overall thickness of 0.023 ± 0.005 mm. The number average molecular weight (M_n), weight average molecular weight (M_w) and polydispersity (PI) of LDPE was 20.6 kDa, 92.6 kDa, and 4.5, respectively; and for PE 2.9 kDa, 22.0 kDa, and 7.6, respectively. Three Ingeo™ 2003D films with different molecular weights (PLA1 > PLA2 > PLA3) were obtained by cast extrusion, varying the temperature of processing (Table S1), with an overall thickness of 0.031 ± 0.006, 0.022 ± 0.003, and 0.034 ± 0.009 mm, respectively. Additionally, a PLA sheet (PLA4) was produced with Ingeo™ 4032D having an overall thickness of 0.255 ± 0.021 mm. The M_n, M_w, and PI of the different PLA samples are presented in Table S2. The carbon, hydrogen, and nitrogen content of the different test materials were determined by using a PerkinElmer 2400 Series II CHNS/O Elemental Analyzer (Shelton, CT, USA), and values are presented in Table 2. More details regarding the film processing, molecular weight determination and elemental analysis are provided in the supporting information, sections S1 to S3.

2.2. Biodegradation test

The aerobic biodegradation of the materials was evaluated under controlled composting conditions by analysis of evolved CO_2 using an in-house built DMR system, which uses a non-dispersive infrared gas analyzer (NDIR) for measuring the concentration of CO_2 evolved from the bioreactors. Detailed information about the equipment and the calculation method is provided in the supporting information, sections S5 and S6, and elsewhere [40]. Besides compost, CP and PLA pellets were also tested in inoculated vermiculite in the Jan14 test. Similarly, in the Nov15 test, CP, PLA1, PLA2, and PLA3 were evaluated in three different media: compost, inoculated vermiculite and uninoculated vermiculite. Additionally, analysis of the reduction of molecular weight of PLA was performed in these experiments. Table 3 shows a summary of the different tests performed, the materials that were evaluated in each test and the type of media used for testing.

2.2.1. Compost source

For the Sep12 test, Earthgro® organic humus and manure from Scotts Miracle-Gro (Marysville, OH) was used. For all the other tests, manure-straw compost prepared at the MSU Composting Facility (East Lansing, MI) was used. Detailed information about the preparation of this compost is provided in the supporting information, section S4.

In all cases, the compost was sieved on a 10 mm screen and preconditioned at 58 °C for a period of 3 days before use. Deionized water was added to increase the moisture content to about 50%. Saturated vermiculite premium grade (Sun Gro Horticulture Distribution Inc., Bellevue, WA) was added to the compost (1:4 parts, dry wt. compost) to provide better aeration.

2.2.2. Compost characterization

Samples of the compost from the different tests were sent to the Soil and Plant Nutrient Laboratory at Michigan State University (East Lansing, MI, USA) for determination of the physicochemical parameters. The dry solids (DS) were obtained after drying the compost sample at about 105 °C to constant mass. The volatile solids (VS) were obtained by the loss-on-ignition method, in which the residues after incineration at 550 °C are subtracted from the total DS. The pH was determined in a 1:5 compost-to-water suspension. The total organic carbon (TOC) was determined by calculation from the VS since carbon is typically considered to comprise about 58% of the VS [41]. The total nitrogen content was obtained

Table 1
Selected biodegradation tests in composting conditions reported in the literature and presented in reverse chronological order for 2015 through 1990, including information about the samples, compost and the main methods for assessing biodegradation.

Sample Materials	Form	Thickness, mm	M_n, kDa	M_w, kDa	PI	% Biodegradation	Time, d	Method for assessing biodegradation	Characterization of the compost					Ref.
									Dry solids, %	Volatile solids, %	pH	C/N	Temperature, °C	
PLA 4042 D	Film	0.04–0.06	150		1.7	CD	30	Visual inspection	45–60		4–8		45–70	[2]
CAB 500-5	Film	0.04–0.06	57			CD	>90							
PLA/CAB 80/20	Film	0.04–0.06				CD	9							
PLA/CAB 50/50	Film	0.04–0.06				CD	>90							
PLA/CAB/PEG 80/20/20	Film	0.04–0.06				CD	90							
PLA 4032 D	Film	0.2	217		2	100	28	Weight loss			6.5		58	[25]
PLA-PEG	Film	0.2				100	28							
PLA-ATBC	Film	0.2				100	28							
PLA-PHB-PEG	Film	0.2				100	35							
PLA-PHB-ATBC	Film	0.2				100	35							
Cellulose	Paper	0.35				78	115	CO_2 evolution (DMR-NDIR)	24.3	88.9	7.9	20	55	[26]
Plastarch	Sheet	0.48				51	115							
Paper pulp + soy wax	Sheet	2.14				12	115							
PET + additive	Sheet	0.36				1	115							
PLA				15		71	110	CO_2 evolution (CMR-Titration)	50.5	29	7.7	3.9	58	[27]
LA-EG-MA			10.3			53	110							
LA-EG-SA			10.8			51	110							
Cellulose	Powder					76	45							
PHBV-3	Film	0.01–0.08	404			80	110	CO_2 evolution (DMR-NDIR)	52.4	14.5	8.2	14.2	58	[3]
PHBV-20	Film	0.01–0.08	324			89	110							
PHBV-40	Film	0.01–0.08	324			91	110							
PHB	Film	0.01–0.09	240			80	110							
P(3HB, 4HB)	Powder		446			90	110							
Cellulose	Powder					83	110							
PBAT (Manure compost)	Film	0.04				67	45	CO_2 evolution (DMR-NDIR)				22.9	58	[28]
PBAT (Yard compost)	Film	0.04				34	45					47.1		
PBAT (Food waste compost)	Film	0.04				45	45					36		
PLA 7000 D	Sheet	3				60	80	CO_2 evolution (DMR-NDIR)	46.4		8.4		58	[29]
Cellulose	Powder					78	80							
PLA60/Starch40	Sheet	3				>80	80							
PLA90/Starch10	Sheet	3				~60	80							
PLA90/Wood-flour10	Sheet	3				~50	80							
Microcrystalline cellulose	Powder					>70	45	CO_2 evolution (CMR-Titration)	42–52	48	7.6	32		[30]
Industrial recycled cellulose	Particle size < 2.8 mm					>70	45							
PLA (Biomer L 9000)	Particle size < 2.8 mm			174.2	1.9	>60	80							
Wheat straw	Particle size < 2.8 mm					>70	45							
Soy straw	Particle size < 2.8 mm					>70	45							
PLA-Wheat straw (50:50)	Particle size < 2.8 mm			132.9	1.8	>60	60							
PLA-Soy straw (50:50)	Particle size < 2.8 mm			158.3	1.8	>60	60							
PCL	Particle size < 2.8 mm			171.7	1.6	>60	120							
Soy meal	Particle size < 2.8 mm					>70	45							
DDGS	Particle size < 2.8 mm					>70	45							
PCL-DDGS (70:30)	Particle size < 2.8 mm			162.3	1.6	>60	100							
PCL-Soy meal (70:30)	Particle size < 2.8 mm			168.2	1.6	>60	100							
Cellulose	Powder					72.4–82.5	45	CO_2 evolution (DMR-NDIR)	51	45	7.2		58	[17]
Potato starch-based tray						80		Weight loss (home composting)						[31]
Starch-based tray with a starch/PCL laminate						80								
Pressed wood pulp plate						40								
Pressed silvergrass pulp crate						80								
Molded coconut fiber tray						40								
Moulded recycled paper pulp tray						40								
PLA tray						<5								
Starch/PCL- extrudate sample						<5								
PP with biodegradability additive						<5								
						<5								

(continued on next page)

Table 1 (*continued*)

Sample Materials	Form	Thickness, mm	M_n, kDa	M_w, kDa	PI	% Biodegradation	Time, d	Method for assessing biodegradation	Characterization of the compost					Ref.
									Dry solids, %	Volatile solids, %	pH	C/N	Temperature, °C	
PP compounded with starch granules														
EPI						0	72	Weight loss	45	91.7	6.2	27.9	>50	[32]
Mater-Bi						27	72				−8.5			
Cellulose filter paper	Paper					100	72							
Microcrystalline cellulose	Powder					74	45	CO_2 evolution (CMR-Titration)	49	28.4	7.2	14.1	58	[4]
TPS	Powder					73	56							
TPDAS6	Powder					66	56							
TPDAS30	Powder					56	56							
TPDAS50	Powder					45	56							
TPDAS70	Powder					26	56							
TPDAS95	Powder					6	56							
PLA (2002 D)	Sheet	1				55	90	CO_2 evolution (CMR-Titration)	48	45.4	7.1	10.4	58	[5]
TPS	Sheet	1				87	90							
PLA/TPS 75/25	Sheet	1				61	90							
PLA/TPS/Coir 52/17/30	Sheet	1				59	90							
PLA/TPS/MA 75/25/1	Sheet	1				57	90							
PLA/TPS/Coir/MA 52/17/30/1	Sheet	1				54	90							
PBAT 25w (white)	Film	0.03	86.3			>60	120	CO_2 evolution (DMR-NDIR)	40–50				58	[6]
PBAT 35w (white)	Film	0.04	89.3			>60	120							
PBAT B (black)	Film	0.04	84.4			>60	120							
Corn starch	Powder					>70	120							
PLA	Sheet	0.3				86	120	CO_2 evolution (CMR-Titration)	52.5	28.2	8.5		58	[33]
Cellulose	Powder					87	120							
PLA bottle (96% L-lactide)				209.3	1.7	84	58	CO_2 evolution (CMR-Titration)					58	[34]
Cellulose	Powder					86	58							
PLA bottle (96% L-lactide)				209.3	1.7	81	58	CO_2 evolution (GMR-MODA)					58	[34]
Cellulose	Powder					70	55							
PLA bottle (96% L-lactide)				209.3	1.7	CD	30	Visual inspection	37		8.5		65	[35]
PLA tray (94% L-lactide)				222.7	1.7	CD	30							
Cellulose	Paper					72	45	CO_2 evolution (DMR-NDIR)	95	63	8.7	10	58	[36]
Kraft paper	Paper					62	45							
Mirel bag						64	45							
PLA straws						61	45							
Sugar cane plate						60	45							
Corn-based trash bag						60	45							
Ecoflex bag						60	45							
Polyethylene	Sheet					2	45							
Oxodegradable bag						2	45							
PCL	Particle size <10 mesh			50		52	45	CO_2 evolution (CMR-Titration)	52		7.4	43	58	[7]
CA	Particle size <10 mesh					22	45							
LDPE	Particle size <10 mesh			36.4		8	45							
Cellulose	Powder					70	45							
PCL/CA 60/40	Particle size <10 mesh					56	45							
PCL/CA 40/60	Particle size <10 mesh					65	45							
PLA bottle						64	63	CO_2 evolution (DMR-NDIR)	40–50				58	[18]
PET bottle						3	63							
Corn starch						72	63							
PLA bottle (96% L-lactide)				209.3	1.7	CD	<30	Visual inspection	37		8.5		65	[37]
PLA tray (94% L-lactide)				176.8	2	CD	<30							
PLA container (94% L-lactide)				215.5	1.7	CD	<30							
PLA/Starch/PLA	Sheet	2.19				78	45	CO_2 evolution (NS)	52.7	65.8	8	28.9	58	[38]
Microcrystalline cellulose	Powder					90	45	CO_2 evolution (DMR-NDIR)				10–40	52	[19]
Starch-polyester						87	45							
Starch-PVOH						72	45							
Biopol						88	45	CO_2 evolution (NS)	50–55	30	7–9	10–40	58	[39]
Kraft paper	Paper					80	45							
Microcrystalline cellulose	Powder					84	45							

Notes: Cells without values indicate that the authors did not report or calculate these values, films are samples with thickness ≤ 0.254 mm, sheets are samples with thickness > 0.254 mm. M_n: number average molecular weight, M_w: weight average molecular weight, CD: complete disintegration, NS: not specified, PLA: poly(lactic acid), CAB: cellulose acetate butyrate, PEG: poly(ethylene glycol), PHB: poly(hydroxybutyrate), ATBC: acetyl-*tri*-*n*-butyl citrate, LA: lactic acid, EG: ethylene glycol, SA: succinic acid, MA: malonic acid, PHBV: poly(hydroxybutyrate-*co*-hydroxyvalerate), PBAT: poly(butylene adipate-*co*-terephthalate), PCL: poly(caprolactone), DDGS: distillers dried grains with solubles, PP: poly(propylene), EPI: environmental product Inc. containing 3% of totally degradable plastic additive, Mater-Bi: starch/hydrophilic-biodegradable resin blend, TPDAS: thermoplastic dialdehyde starch, TPS: thermoplastic starch, MA: maleic anhydride, CA: cellulose acetate, LDPE: low-density polyethylene, PET: poly(ethylene terephthalate), Biopol: poly(hydroxy butyrate)/poly(hydroxy valerate) blend, PVOH: poly(vinyl alcohol).

Table 2
Carbon, hydrogen, and nitrogen content of the tested materials.

Material	ID	% Carbon[a]	% Hydrogen[a]	% Nitrogen[a]
Cellulose powder	CP	42.50 ± 0.34	6.53 ± 0.05	0.04 ± 0.01
Cassava starch	CS	41.75 ± 0.35	6.69 ± 0.08	0.01 ± 0.00
Glycerol[b]	GC	39.12	8.77	0.00
Polyethylene powder	PE	85.76 ± 0.29	15.13 ± 0.09	0.01 ± 0.01
LDPE/LLDPE film	LDPE	86.83 ± 0.10	14.84 ± 0.04	0.00 ± 0.00
Ingeo™ 2003D pellet	PLA pellet	50.40 ± 0.28	5.65 ± 0.05	0.00 ± 0.01
Ingeo™ 2003D film	PLA1	50.05 ± 0.05	5.65 ± 0.02	0.01 ± 0.01
	PLA2	49.93 ± 0.11	5.56 ± 0.02	0.01 ± 0.01
	PLA3	49.99 ± 0.05	5.60 ± 0.01	0.01 ± 0.01
Ingeo™ 4032D sheet	PLA4	50.00 ± 0.08	5.61 ± 0.01	0.04 ± 0.02

[a] Percentage by weight.
[b] Theoretical values based on chemical structure.

(with compost only) were evaluated. To simulate composting conditions, the bioreactors were placed in an environmental chamber set at a constant temperature of 58 ± 2 °C. Water-saturated CO_2-free air was provided to each bioreactor with a flow rate of 40 ± 2 sccm (cm^3/min at standard temperature and pressure). The bioreactors were incubated in the dark for at least 45 d or until the evolved CO_2 reached a plateau.

2.2.5. Biodegradation in vermiculite

Biodegradation tests were also carried out with inoculated and uninoculated vermiculite during the Nov15 test in an attempt to avoid the priming effect, which is discussed in Section 3.3, and to decouple biotic and abiotic degradation during the biodegradation test of PLA. In this case, vermiculite was mixed in a proportion of 1:4 (wt.) with the inoculum solution described in section 2.2.3, and with distilled water, respectively. The bioreactors were loaded with 400 g (wet wt.) of either inoculated or uninoculated vermiculite and mixed thoroughly with 8 g of the polymer. The bioreactors were then subjected to the testing conditions described in section 2.2.4.

Table 3
Biodegradation test, materials, and media used for testing.

Test ID[a]	Materials tested	Media for testing
Sep12	Blank, CP, LDPE	Commercial compost
Feb13	Blank, CP, LDPE	MSU compost (A)[b]
May13	Blank, CP, CS, LDPE	MSU compost (A)
Jul13	Blank, CP, LDPE	MSU compost (A)
Jan14	Blank, CP, CS, GC, PE, PLA1, PLA pellets	MSU compost (B), inoculated vermiculite
Jun14	Blank, CP, CS, PE, LDPE, PLA1	MSU compost (C)
Nov14	Blank, CP, PLA2, PLA4	MSU compost (C)
Nov15	Blank, CP, PLA1, PLA2, PLA3	MSU compost (C), inoculated vermiculite, uninoculated vermiculite

[a] Test ID refers to the month and year in which the test was performed.
[b] The compost ID (A, B, C) indicates that the initial compost was obtained from the same compost batch.

by the Dumas method [42], the ammonium (NH_4^-) concentration by the salicylate method [43], and the nitrate (NO_3^-) concentration by the cadmium reduction method [44]. Subsequent moisture content measurements were done in a moisture analyzer, model MX-50 from A&D Engineering, Inc. (San Jose, CA).

2.2.3. Preparation of inoculum solution

The solution used for inoculation of vermiculite was prepared by combining compost extract with a mineral solution (Table 4) at a 1:1 ratio [22]. Compost extract was prepared by mixing dry compost with deionized water (20% wt./vol.), stirring and letting sit for 30 min followed by filtration through a sieve with 1 mm mesh.

2.2.4. Biodegradation in compost

The bioreactors were loaded with either 500 g (wet wt.) of compost (first experiment) or 400 g of compost (subsequent experiments) and mixed thoroughly with 8 g of polymer sample. Film samples were cut to 1 cm^2 pieces and triplicates of each test material were analyzed. Additionally, triplicates of blank bioreactors

Table 4
Detailed composition of 1 L of mineral solution.

1 L of Mineral solution	
KH_2PO_4, g	1
$MgSO_4$, g	0.5
$CaCl_2$ (10% sol), mL	1
NaCl (10% sol), mL	1
Trace-element solution, mL	1
1 L of trace-element solution, mg	
H_3BO_3	500
KI	100
$FeCl_3$	200
$MnSO_4$	400
$(NH_4)_6Mo_7O_{24}$	200
$FeSO_4$	400

3. Results and discussion

This section first presents the physicochemical characteristics of the media (compost and vermiculite) that are relevant for the biodegradation test in composting conditions. Then, it provides a comparison of the results obtained by the analysis of evolved CO_2 approach in the eight tests with the different sample materials. To better understand and interpret the results of the biodegradation tests, a discussion of each of the factors affecting the biodegradation rate and the biodegradability of the materials is also presented. Likewise, some recommendations based on the literature and on our own experiences gained during the performance of these eight different biodegradation tests and more than 10 years of testing biodegradation of samples are provided to inform future testing. Finally, a case study is presented to gain additional understanding on the biodegradation mechanism of PLA, one of the most popular commercial compostable biobased polymers.

3.1. Biodegradation: CO_2 evolution and mineralization

An in-house built DMR system (as shown in Fig. S1) was used to perform the eight different biodegradation tests in which temperature, RH, air flow rate, CO_2 concentration, and time were monitored and measured (Fig. 1). Temperature and pH were stable at 58 °C and 7, respectively. The flow rate of air passing in each bioreactor was adjusted to 40 sccm throughout the testing period. Moisture content was measured periodically in a control bioreactor

to determine the amount of water required for adjustment. Detailed discussion of the effect of each physical parameter is presented in section 3.2.

3.1.1. Biodegradation in compost

The cumulative CO_2 and % mineralization curves obtained from the different biodegradation tests in compost are presented in Figs. 2–6. For the data analysis, the amount of CO_2 evolved from each bioreactor was calculated first (Eq. S(3)); subsequently, the average cumulative CO_2 (Eq. S(5)) and % mineralization (Eq. S(7)) of each test material was determined and plotted as a function of time. The % mineralization represents the relationship between the amount of CO_2 evolved from the test material and the theoretical amount of CO_2 that can be evolved from the same test material; *e.g.*, in the case of CP, with a 42.5% carbon content (Table S2) and the introduction of 8 g into a bioreactor, the theoretically possible CO_2 evolution from this material is 12.5 g (denominator of Eq. S(7)). Looking at the CO_2 evolution plots of the different materials, it seems that in general, the samples from the May13, Jul13, and Jun14 tests produced the highest amount of CO_2 and the samples from the Sep12 and Jan14 tests produced the least amount of CO_2 over time.

The blank bioreactors produced an amount of CO_2 ranging from 9.7 to 23.9 g after 60 days of testing, with the Jun14 test having the highest variability (Fig. 2). Even though all tests were performed under the same conditions, and in most of the cases using the same type of compost (except Sep12), there is significant difference in the production of CO_2 between some of the tests. The compost of the Sep12 test produced the lowest amount since it was a different kind of compost and due to the experienced drying conditions as explained later in Section 3.2.4.

The CO_2 evolved from the blank bioreactors represents the background, so their average is later subtracted from the amount of CO_2 produced by the test sample bioreactors to determine the mineralization, as shown in Eq. S(7) of the supporting information. The background and the variability value of evolved CO_2 between the blank replicates have a large influence on the final mineralization values.

For example, looking at the average values of the CO_2 evolution and % mineralization of cellulose (Fig. 3), the May13 and the Jul13 tests produced almost the same amount of CO_2; but the calculated mineralization in the Jul13 test was much higher than in the May13 test due to the CO_2 evolution from the blank. Similarly, the cellulose in the Jun14 test produced a much greater amounts of CO_2 than in the Jan14 test, but the average mineralization values were not very different from each other.

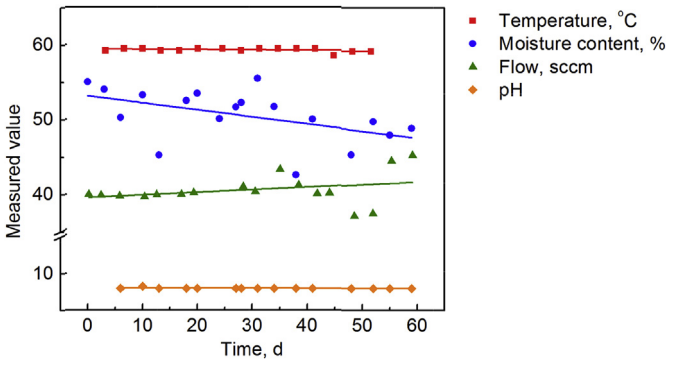

Fig. 1. Example of biodegradation test parameters as a function of time. Fitted lines ($y = \beta x + \alpha$) are included for visual guidance only. Air flow rate, temperature, moisture and pH monitored as a function of time in the May13 test during the first 60 days of testing.

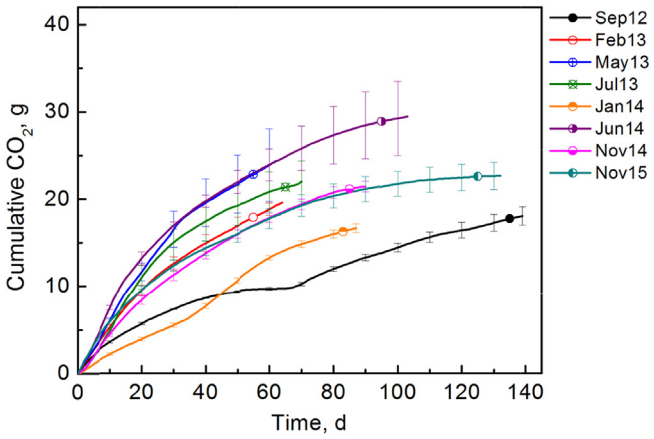

Fig. 2. Cumulative CO_2 evolution of blank bioreactors in the different biodegradation tests showing large variation of the CO_2 evolved although they were run under the same experimental conditions.

Overall, we can also state that except for the Sep12 and the Jan14 tests the behavior and amount of CO_2 evolved from most of the blank bioreactors is fairly similar (27.5–33.7 g at day 60). When accounting for the total background production, the % mineralization varied from 61.8 to 100.8%. These results are comparable to the ones reported in the literature (Table 1) with % mineralization of cellulose ranging 70–100% between 45 and 120 days.

The decrease in the mineralization curves of the Jan14 and the Jun14 tests indicates that the cellulose bioreactors were no longer producing more CO_2 than the blank bioreactors; a similar behavior was observed with the CS, being even more pronounced (Fig. 4).

A possible explanation of the behavior observed in the mineralization curve of starch (Fig. 4b) is that at the beginning of the test there is a rapid large increase in the microbial population since materials like starch are readily or easily available for microbial assimilation, but once these resources are depleted and/or limited, a decrease in the mineralization curve is observed. It should be considered that microorganisms do not only use carbon for generation of energy but also for growing [13]. Therefore, the maximum value of the curve can be reported as a result of the sample mineralization.

Fig. 4 shows that the CO_2 evolved from the CS bioreactors ranged from 19.1 to 26.5 g at day 60, while the % mineralization varied from 28.1 to 68.3. Table 1 shows results for two corn starch tests (70 and 72% mineralization after 120 and 63 days, respectively) [6,18], and two for TPS tests (73 and 87% mineralization after 56 and 90 days, respectively) [4,5]; however, those samples did not show the decline in the mineralization curve behavior, as reported in Fig. 4, based on the figures presented in the respective papers.

In some cases, especially in polymers that are not biodegradable like LDPE, negative mineralization values have been reported (Fig. 5). Physically these values make no sense, but they are possible since they are generated as an artifact when the blank bioreactors produce more CO_2 than the LDPE bioreactors [14]. These negative mineralization values could be attributed to a physical barrier offered by the polymer film, which limits the availability and/or the distribution of carbon and other nutrients for basic microorganism functions.

In the Jan14 and Jun14 tests, PE was evaluated to determine if this material, which is in the form of powder and with $M_n = 2.9$ kDa, is more susceptible to biodegradation than the LDPE film. Fig. 5 shows that the amount of CO_2 evolved from the LDPE bioreactors varied from 8.9 to 25.1 g at day 60, while the maximum mineralization was 6.8± 4.8%. The amount of CO_2 evolved from the

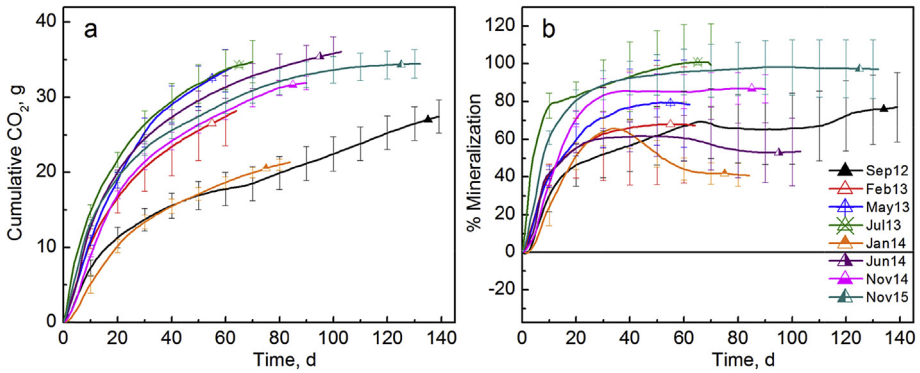

Fig. 3. Cumulative CO_2 evolution (a) and mineralization (b) of cellulose bioreactors in the different biodegradation tests. While similar or different CO_2 values were observed, the % mineralization is highly driven by the evolved CO_2 values for the blank test.

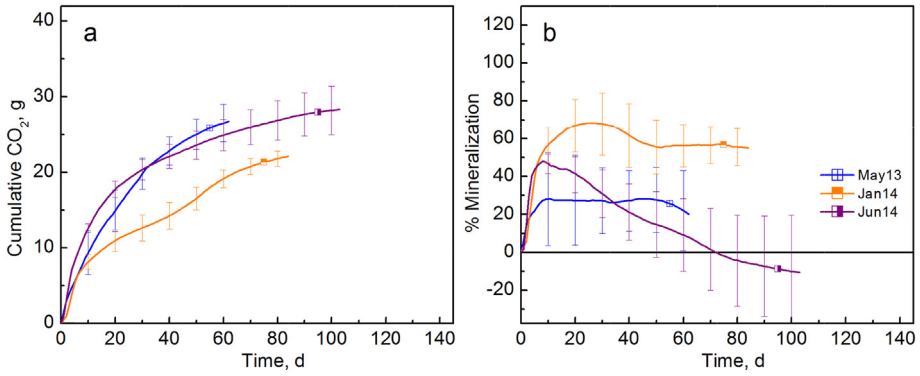

Fig. 4. Cumulative CO_2 evolution (a) and mineralization (b) of CS in the different biodegradation tests. Biodegradation tests show a fast increase in the mineralization during the first 10 days of testing.

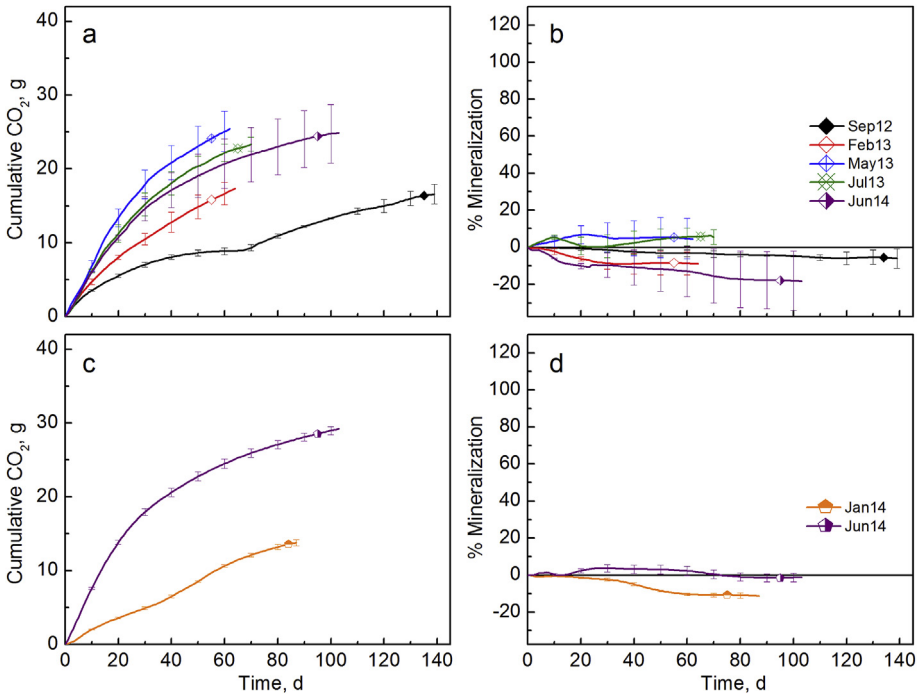

Fig. 5. Cumulative CO_2 evolution and mineralization of LDPE (a & b) and PE (c & d) bioreactors in different biodegradation tests. Negative values of mineralization are observed in many tests.

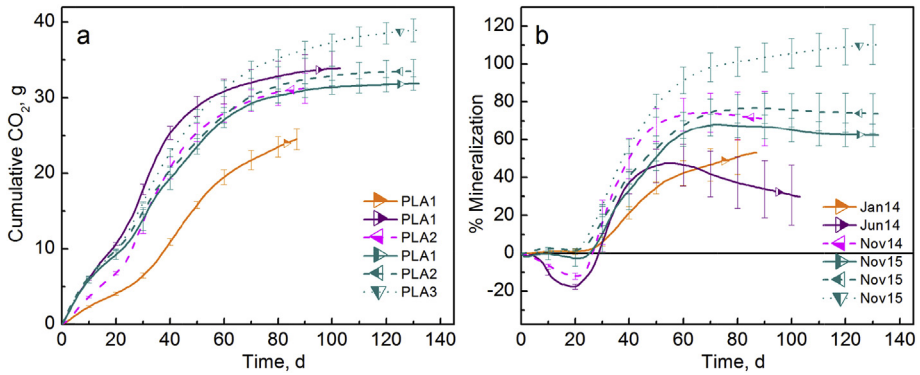

Fig. 6. Cumulative CO_2 evolution (a) and mineralization (b) of PLA bioreactors in the different biodegradation tests; solid, dashed and dotted lines represent PLA1 (93.5 kDa), PLA2 (82.9 kDa), and PLA3 (72.6 kDa), respectively.

PE bioreactors varied from 10.6 to 24.5 g at day 60, while the maximum mineralization was 3.7± 2.5%. Therefore, no significant increase in the biodegradability or mineralization of this material was found. The maximum mineralization of LDPE reported from the literature in Table 1 was 8% after 45 days. Similarly, Esmaeili et al. (2013) reported a mineralization of 7.6% after 126 days in soil and 15.8% in soil inoculated with a mixed culture of *Lysinibacillus xylanilyticus* and *Aspergillus niger* after the same period of time [45]. However, these mineralization values may be attributed to the microbial assimilation of organic carbon present in the samples used to modify the material or degradation products formed during oxidation reactions [14]. The influence of the chemical structure, form, and molecular weight of the materials on biodegradation is further discussed in Section 3.4.

Fig. 6 shows the biodegradation results of the PLA1, PLA2, and PLA3 films, indicating that the initial molecular weight of biodegradable polymers is highly influential on the biodegradation of PLA. The amount of CO_2 evolved from the PLA1 bioreactors varied from 19.5 to 30.8 g at day 60, while the mineralization varied from 47.4 to 68%. However, the production of CO_2 and mineralization increased as the molecular weight of PLA decreased, reaching a maximum mineralization of 109.1% with the lowest molecular weight. Mineralization over 100% is an indication of priming effect, which is attributed to the over-degradation of the indigenous organic carbon present in the compost when testing materials like glucose and its polymers [46], further discussed in Section 3.3.

The zero mineralization (or negative in some cases) at the early stage of the PLA test corresponds to the lag time, *i.e.,* the period in which the polymer chains are hydrolyzed -cleaved by the presence of water-until a certain degree of degradation has been reached and the degradation products become water soluble and available for microbial assimilation [47]. The specific biodegradation mechanism of PLA will be discussed in more detail in Section 3.5.

The results shown in this section indicate that the reproducibility between different tests is low, even if the tests were performed in the same equipment, using the same procedures, the same batches of materials, and excluding technical failures. The intrinsic variability in these biological tests makes it difficult to provide a fair comparison of samples that are not within the same test, and therefore to compare the results obtained between and within research groups. Hoshino et al. (2007) performed a round robin test for studying the aerobic biodegradation of PCL and PLA by the gravimetric method in seven countries, and they found that even though the method is effective for testing compostability of materials on a laboratory scale test, there is variation in the results which was mainly attributed to the compost [48].

Other researchers have reported that the inoculum quality is a source of variability that can affect the results of biodegradation tests [49]. Therefore, all the physicochemical characteristics of the compost must be reported since they may influence the efficiency and the rate of the biodegradation process. Based on the literature review and data provided in Table 1, we observed that in some papers, including previous papers from our research group, authors have reported only 3 or fewer parameters of the compost. Without a more extensive reporting of these parameters the final results and conclusions may be incomplete or misleading.

In this context, it would be relevant to further understand the different factors affecting the biodegradation rate and biodegradability of the sample materials, so in future biodegradation tests such factors can be strictly monitored and controlled in an attempt to improve the reproducibility of the test results. The physicochemical characteristics of the media used in the eight different biodegradation tests are shown and discussed in more detail in section 3.4.

3.1.2. *Biodegradation in vermiculite*

The biodegradation of CP and PLA samples was evaluated in inoculated vermiculite in the Jan14 and the Nov15 tests, and also uninoculated vermiculite in the Nov15 test. The results are shown in Fig. 7 and Fig. 8. The production of CO_2 from the blank bioreactors was very low, allowing better detection of the CO_2 signal from the sample bioreactors. During the Jan14 test (Fig. 7), the mineral solution described in Table 4 was not provided in the compost extract used for inoculation (Sections 2.2.3 and 2.2.5), and PLA was tested in the form of pellets as received from NatureWorks LLC. The PLA pellet bioreactors produced 5.6 ± 0.4 g of CO_2 and reached 34.5± 2.8% mineralization after 60 days of testing, while in compost they produced 19.0 ± 0.8 g of CO_2 and reached 39.2± 5.5% mineralization in the same period of time. Similarly, cellulose bioreactors produced 4.9 ± 0.5 g of CO_2 and reached a mineralization of 35.3± 3.9% after 60 days of testing, while in compost cellulose produced 18.7 ± 0.7 g of CO_2 and reached 44.3± 5.9% mineralization in the same period of time.

The PLA films with three different molecular weights (Table 2) were evaluated in the Nov15 test (Fig. 8) in which the inoculation of vermiculite was performed as described in Section 2.2.3 and 2.2.5. In this case, the cellulose bioreactors produced 7.3 ± 0.4 g of CO_2 and reached a mineralization of 60.2± 3.3% after 60 days of testing, while in compost they reached 95.7± 12.1% mineralization in the same period of time. PLA1, PLA2, and PLA3 produced 5.2 ± 0.6, 8.6 ± 0.9, and 7.2 ± 0.3 g of CO_2, and reached mineralization of 34.6 ± 4.4, 58.3 ± 5.8, and 48.5± 1.8%, respectively, after 60 days of testing. The mineralization in compost of these test materials was found to be 63.3 ± 6.7, 67.6 ± 7.1, and 91.5± 7.0% at day 60. No

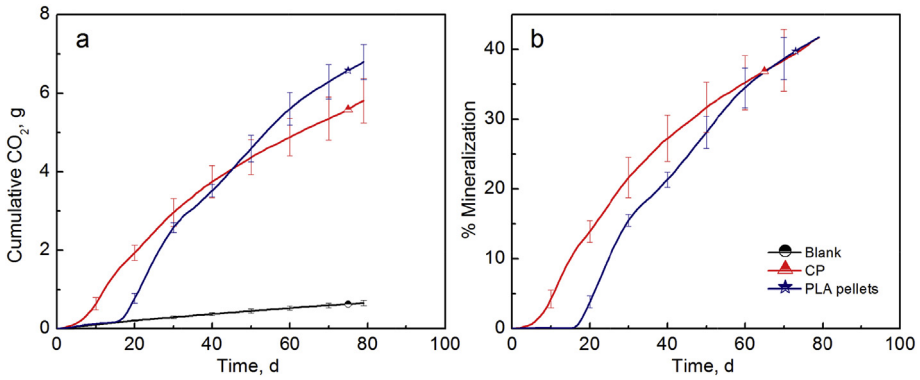

Fig. 7. Cumulative CO_2 evolution and mineralization of CP and PLA tested in inoculated vermiculite in the Jan14 test. Lower values of evolved CO_2 are seen when compared with compost tests, as expected.

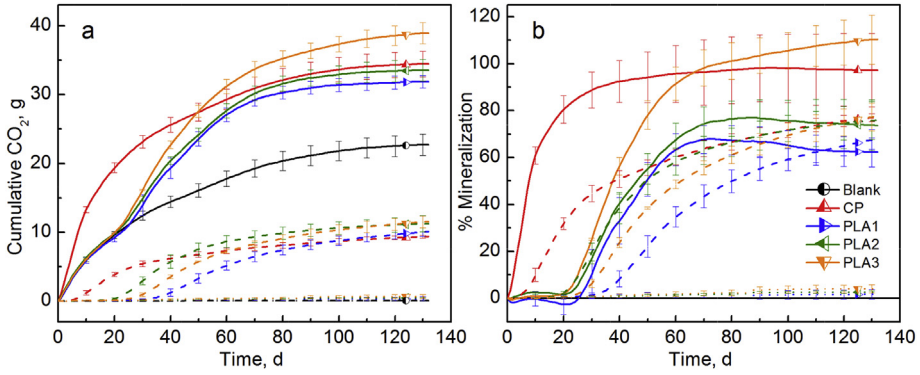

Fig. 8. Cumulative CO_2 (a) and mineralization (b) of CP, PLA1, PLA2, and PLA3 in the Nov15 test. Solid line, dashed line, and dotted line represent compost, inoculated vermiculite and uninoculated vermiculite, respectively. Large difference in CO_2 production can be observed between evolved CO_2 in inoculated and uninoculated vermiculite.

significant CO_2 evolution was found from the samples tested in uninoculated vermiculite, as expected.

Even though the biodegradation in inoculated vermiculite seems to be slower, the evolved CO_2 from the background is much lower and more stable. The use of inoculated vermiculite has proven to be an excellent way to test biodegradation although with unrealistic estimated biodegradation times. For example, the mineralization values of PLA1 and PLA2 (Fig. 8) are basically the same in either compost or vermiculite towards the end of the test (130 d). The % mineralization of PLA3 in vermiculite looks more similar to that of PLA1 and PLA2; while in compost it was believed to exhibit a priming effect since the mineralization was over 100%.

Furthermore, the higher mineralization reached by cellulose in the Nov15 test when compared with Jan14, could be due to additional supplementation of a mineral solution in the Nov15 test, which provides the basic nutrients required by the microorganisms to grow and multiply efficiently. In the case of PLA, other factors like molecular weight also play a role in the biodegradation process as discuss in Section 3.4.

3.2. Environment-related factors affecting biodegradation

The purpose of biodegradation as a disposal route of polymers is the total breakdown of their molecular structure and their complete assimilation back into the environment by the action of naturally occurring microorganisms like bacteria, fungi, and algae in a reasonable time frame (months to a few years) [14]. However, environmental conditions such as temperature, oxygen, and water availability play a crucial role in the biodegradation rate and biodegradation mechanism of a material.

3.2.1. Microorganisms

The amount and type of microorganisms present in the compost play a crucial role in the biodegradation of materials. As previously mentioned, environmental conditions like temperature, oxygen, water, pH, and nutrients can affect the kind of microorganisms present in the media and strongly influence their metabolic pathways, growth and survival [50]. In industrial composting, the microorganisms that predominate are mesophiles and thermophiles depending on the composting stage [51], while in laboratory controlled composting conditions the microorganisms that prevail are mostly thermophiles since the temperature is usually kept constant at 58 ± 2 °C. The microbial community in the compost is mainly formed by bacteria, fungi and possibly archaea and viruses. Bacteria are thought to be the major microbial domain responsible for the biodegradation process and bacteria belonging to the *Bacillus* species are more predominant in the thermophilic stage of composting [51]. A number of studies have been conducted to identify the microbial consortia present in the compost environment [52–54], and some have reported the isolation and identification of several species capable of the biodegradation of PLA [55–63], and other polymers [45,64–71]. The isolation of these bacteria has been done using selective enrichment and clear zone formation, in which the specific polymer was provided as the sole source of carbon. Further classification and identification of the isolated microbial strains has been performed by 16S rRNA sequence analysis [72,73]. However, few studies have use molecular ecological techniques and next generation sequencing which

allows the identification of the vast microbial diversity present in the compost including the uncultured microorganisms that may also play a crucial role in the biodegradation of polymers [72,74]. For example, terminal restriction fragment length polymorphism (T-RFLP), a cultivation independent technique used for comparative community analysis, can be used to monitor changes in complex microbial communities over time [75,76]. Recent studies have also shown the potential of metaproteomics to provide direct information about the microbial activity and the metabolic pathways occurring during the composting process [54]. Therefore, these novel techniques could be used along with biodegradation tests to gain insight into polymer biodegradation mechanisms and metabolic pathways.

The structure and diversity of microbial communities present in the soil are not likely to be the same in different regions of the world [50], which in turn may lead to different results when testing biodegradation of materials. Guo et al. (2010) have suggested the use of a specific microbial community to evaluate material biodegradability in a shorter period of time and improve the reproducibility of the results; such a community containing 20 selected microbial strains capable of degrading at least 14 types of biodegradable materials including among them starch, PLA, PCL, PHBV, and PVOH [73]. Thus, the use of synthetic microbial communities would allow the construction of defined systems with reduced complexity [74,77]. However, further studies with solid media, *e.g.* vermiculite, in composting conditions are required to prove the improved reproducibility of the results.

3.2.2. Temperature

Depending on the temperature, the microbial populations present in the media can be predominantly mesophilic or thermophilic. Usually, temperatures in the range of 54–60 °C are considered optimal for composting since this favors the thermophilic compost microorganisms. Moreover, elevated temperatures can accelerate reactions like hydrolysis. Temperatures above 60 °C would kill several microbial species and contribute to a faster drying of the compost, limiting the biodegradation rate [51,78]. Even though some authors have performed biodegradation studies using temperature profiles to simulate real composting, the recommendation is to keep it constant at 58 ± 2 °C if the purpose is to reduce the amount of time required for testing [39]. Fig. 1 shows that the temperature is one of the simplest parameters to control in simulated composting tests.

3.2.3. Oxygen availability

Depending on the oxygen availability, biodegradation can be aerobic or anaerobic [23,78]. Composting is a predominantly aerobic process in which microorganisms use oxygen to oxidize the carbon from the organic materials and produce CO_2, water, compost and heat [12,23]. Therefore, a continuous flow of air must be provided to ensure that aerobic conditions are maintained within the bioreactors [22,79].

It is recommended to set the air provided to each bioreactor to an optimal value; if the air flow rate is too low, oxygen becomes a limiting factor slowing down the biodegradation process. Conversely, high air flow rates can also be problematic in that it contributes to faster drying and cooling of the compost that also slows down the biodegradation process by decreasing water availability and temperature [78,79]. To determine the optimal air flow rate, it should also be considered that increasing the air flow rate decreases the concentration of CO_2 in the respired air stream and therefore the air flow rate for the test should be established as the one that allows the CO_2 concentration to be within the limits of the NDIR sensor [17]. In our system, the optimal air flow rate was found to be 40 sccm, and it was determined after a series of trial tests in which different known concentrations of CO_2 were injected into the bioreactors and different air flow rates were used for measurement of CO_2 with the NDIR sensor, as shown in the supporting information, section S7.

3.2.4. Water availability

Water availability is essential for the biodegradation process and usually moisture contents between 50 and 60% are preferable [51]. Water is a distribution medium for microorganisms and nutrients; it influences the microbial development and metabolic activity; and it is an important factor affecting the biodegradation rate [78]. For example in the Sep12 test (Fig. 9) cellulose produced ~16 g of CO_2 (Fig. 9a) and reached 59% mineralization (Fig. 9b) after 45 days of composting, and in the Nov15 test the same amount of cellulose produced about 26 g of CO_2 (Fig. 9c) and reached 93% mineralization (Fig. 9d). The difference in the biodegradation rate of the samples tested in the Sep12 and the Nov15 tests was mainly attributed to the water availability, assuming that the type of compost did not greatly influence this particular behavior. The effect of the compost characteristics is explained in Section 3.3.

In the case of the Sep12 test (Fig. 9a and b), water was not added at the beginning of the test, *i.e.* the moisture content depended only on the availability of water in the water-saturated air supplied to the bioreactors. After day 60, when the compost experienced considerable drying, distilled water was injected into each bioreactor every three days, clearly increasing the biodegradation rate and allowing the reestablishment of a healthy microbial population, as suggested by the Birch effect. Birch (1964) demonstrated that alternate drying and rewetting of soil results in stimulated mineralization of the soil organic matter (*i.e.*, higher release of CO_2) due to a rapid increase of the microbial activity in response to the water availability [80,81].

The addition of water is therefore necessary throughout the testing period; water-saturated air helps to prevent excessive drying of the compost, but it is in general not sufficient by itself to maintain the moisture content at the level required for the test. Thus, in the case of the Nov15 test (Fig. 9c and d), as well as all the other tests, water was added from the beginning of the test to each bioreactor every three days. The amount of water added was determined by first measuring the moisture content of the compost in the control bioreactors with a moisture analyzer and then calculating the amount of water required to increase the moisture content to 50%, based on the initial dry weight of the compost. Currently, a soil moisture sensor has been integrated into a bioreactor for constant monitoring and easier determination of compost moisture.

Other researchers have determined the amount of water required by weighing each bioreactor and then adding enough water to restore the initial weight [15]. Likewise, other researchers have collected the water condensate from each bioreactor and returned it to the bioreactor to keep the moisture levels constant [17]. However, these methods can be complicated for some equipment settings or when there is a large number of bioreactors.

Water is vital for the function of the composting process; however, excessive water leads to a reduction of the airspace within the compost matrix causing oxygen limitation or anaerobiosis [79]. In this context, it has been recommended that inorganic structural materials like vermiculite to be added to the compost, to provide increased porosity and help maintain aerobic conditions [22].

Furthermore, it is recommended that bioreactors are regularly shaken (*e.g.*, every three days) to homogenize the contents and to prevent the compost sticking together and clogging [17,19]. For example, if water is added to a bioreactor without mixing, then it is likely to have moisture variability throughout the compost that would result in zones with limited water for the biodegradation

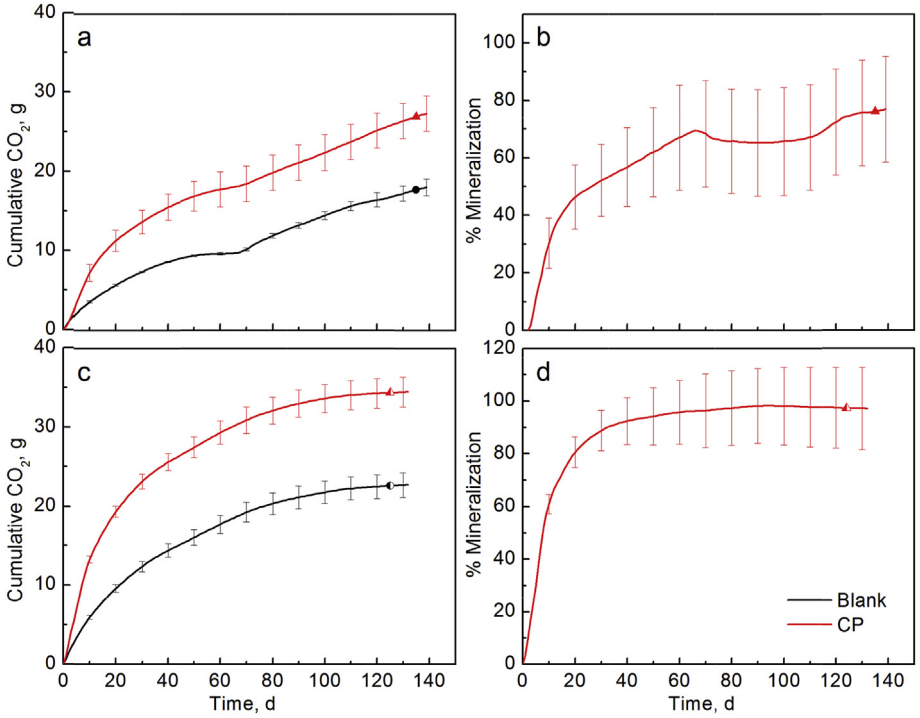

Fig. 9. Cumulative CO_2 of blank and cellulose and % mineralization of cellulose of two different tests Sep12 test (a & b) and Nov15 test (c & d), respectively. The biodegradation process of cellulose was more homogeneous and more efficient in the test in which water was added twice a week seeing as a high % mineralization in a short period of time.

process. Some authors have also found that the addition of water and shaking (material mixing) help restore favorable conditions for biodegradation increasing the biological activity of the compost [82].

3.3. Inoculum-related factors affecting biodegradation

The composition of the compost plays an important role in the biodegradation rate since, besides the microorganisms, it should provide the essential nutrients required for the microorganisms to grow and efficiently multiply. Previous researchers have shown that different raw materials such as manure, yard, and food waste have different physicochemical parameters and also different microbial activity, consequently producing different amounts of evolved CO_2 [28,49,83–85]. The media used in the Sep12 test was commercial compost that according to the manufacturer was made of 90% organic materials (humus) and 10% manure, while the media for the other tests was taken from different piles at the MSU Composting Facility, comprised of a 1:1 mixture of manure and straw. Therefore, the lower evolved CO_2 in the Sep12 test (Fig. 2) could also be attributed to the type of compost as previously demonstrated [28].

Table 5 shows the physicochemical characteristics of the several compost media used in the different biodegradation tests in comparison with the values recommended by the ISO 14855-1:2005 standard [22], which in turn are mostly based on quality standards and guidelines for compost maturity and stability found elsewhere [86,87].

3.3.1. Dry solids and volatile solids

The DS of the compost used in the different tests varied from 41.5 to 60.9%, which means that in most of the cases the initial moisture content was within a reasonable range [51,88]. Likewise, the VS of the compost used in the different tests varied from 23.1 to 44.6% of the DS, except in the Feb13 test in which the VS were particularly higher (67.6%) perhaps because the compost was not mature enough at the time or because in this particular test the analysis of the compost was done before mixing with vermiculite. From Table 1, considering the tests in which the biodegradation of cellulose was more efficient and in which the physicochemical parameters of the compost were provided, the DS and VS ranged from 49 to 52% and 28 to 48%, respectively.

The VS are an indication of the organic matter (OM) present in the compost, considering that other non-organic compounds (*e.g.*, carbonates and structural water) may be lost after ignition at 550 °C; the portion of organic carbon is typically considered to be 50–58% of the VS [51,89,90]. The usual recommendation is to keep the VS low since a high amount of OM may favor the priming effect, or the microorganisms may prefer it over the test material especially when testing more resistant materials like hydrophobic polyesters [49].

3.3.2. pH

In all of the tests, the pH was within the range 7–9 suggested by the ISO 14855 standard [22]. Other composting guidelines tolerate broader initial pH ranges (5.5–9) due to the natural buffering capacity of the compost and the wide range of microorganisms involved in the process [51,88]. However, a neutral pH is preferred for the survival and full activity of the microorganisms [78]. Lauber et al. (2009), showed that the microbial community diversity is highest in soils with neutral pH [50]. An acidic pH can cause inhibition while an alkaline pH is usually associated with loss of nitrogen as ammonia (NH_3) and odor problems [51,90]. From Table 1, considering the tests in which the biodegradation of cellulose was more efficient and in which the physicochemical parameters of the compost were provided, the pH ranged from 7.2 to 7.7, mostly neutral.

3.3.3. C/N

The C/N of the compost used in our different biodegradation

Table 5
Characteristics of the compost samples for each test and requirements according to ISO 14855 standard.

Parameters	ISO[b]	Sep12	Feb13	May13	Jul13	Jan14	Jun14	Nov14	Nov15
Dry solids, %	50–55	57.4	54.9	46.3	N/A	53.3	52.7	41.5	60.9
Volatile solids, %	<30	23.1	67.6	44.6	N/A	26.4	44.3	43.2	39.1
pH	7–9	7.6	8.9	9.1	N/A	7.8	7.9	8.5	7.4
Total Carbon, %	N/A[a]	13.4	39.2	25.9	N/A	15.3	25.7	25.1	22.7
Total Nitrogen, %	N/A[a]	1.1	2.3	1.1	N/A	0.9	2.4	2.4	2.1
C/N ratio	10–40	12.4	17.0	22.9	N/A	17.4	10.8	10.3	10.9
Compost activity[c]	50–150	49.6	35.7	73.2	N/A	39.0	81.1	63.0	62.5

[a] Not applicable or not available.
[b] Values based on ISO 14855-1:2005 standard.
[c] Average values measured in mg of CO_2 per g of VS in the first 10 days.

tests ranged from 10.3 to 22.9. The values reported in the literature (Table 1), considering also the tests in which the biodegradation of cellulose was more efficient and in which the physicochemical parameters of the compost were provided, varied between 14 and 43; which are basically within the wide range suggested by the ISO 14855 standard [22].

In our case, we obtained good results when using the compost piles with C/N of ~10 and ~23; however, other authors and composting guidelines have suggested different C/N, *e.g.,* Bernal et al. suggested the C/N to be below 12 [91], Daryl et al. below or equal to 25 [87], the Woods End Research Laboratory Incorporate below 17 [90], the Ontario Compost Quality Standard below 22 [88], the California Compost Quality Council below or equal to 25 but ideal of 10 [89], Stoffella et al. mentioned a reasonable range of 20–40 and a preferred range of 25–30 due to the large variation depending on the starting feedstock materials of the compost [51]. A list of the C/N of the different feedstock materials can be found elsewhere [51,84].

Despite the difference in C/N suggested by different sources, all agreed that high C/N slowed down the biodegradation rate since N was assumed to become a limiting factor for microbial growth while a low C/N caused excess N to be converted to NH_3 and to volatize, which is also not desirable as discussed earlier [51,90]. Huang et al. studied the effect of C/N on composting and found that a pile with an initial C/N of 30 had a more efficient composting process by achieving maturity faster than one with C/N of 15 [92]. Fig. 10 shows that the C/N of the compost *vs.* time in the blank bioreactors of the Nov14 test slightly decreased, though not significantly.

Besides the mineralization of carbon, one of the most important microbial processes is the mineralization of nitrogen [93]; microorganisms require nitrogen for their cell matter [54]. Under aerobic conditions, organic nitrogen is transformed into NH_3 or NH_4^+ during ammonification, subsequently into nitrites (NO_2^-) and finally into NO_3^- during nitrification [52,54,93,94]. In this context, nitrogen mineralization has been proposed to be used as a bio-indicator to evaluate the impact of biodegradable polymers in soil by measuring the concentrations of NH_4^+, NO_2^- and NO_3^- during the biodegradation process [93,94]. Mature compost is expected to have appreciable amounts of NO_3^- [90]. The concentrations of NH_4^+ and NO_3^- as a function of time during the Nov14 test are shown in the supporting information, section S9.

3.3.4. Compost activity

The ASTM D5338 and ISO 14855 standards recommend the compost to produce between 50 and 150 mg of CO_2 per gram of VS over the first 10 days as a measure of the compost microbial activity [21,22]. Fig. 11 shows the production of CO_2 per gram of VS of the compost media used in the different biodegradation tests.

From Fig. 11, the compost used in the Sep12 test was in the lower limit at 10 days, while the compost from the Feb13 and the Jan14

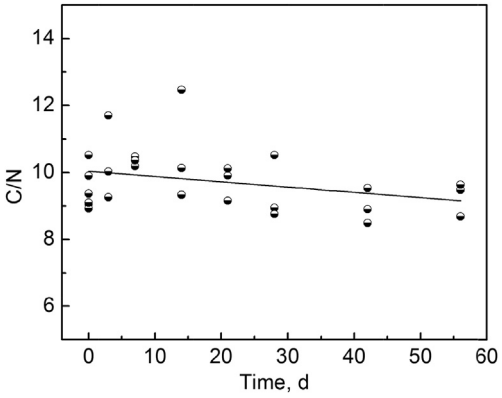

Fig. 10. C/N of the compost as a function of time during the Nov14 test. The fitted line ($y = \beta x + \alpha$) is included for visual guidance only.

tests did not produce the 50 mg minimum until about 13 days. The compost from all other tests (May13, Jun14, Nov14, and Nov15 tests) produced an amount of CO_2 within the suggested range; and based on Table 5, these active composts had similar amounts of VS (39.1–44.6%), amounts of carbon (22.7–25.9%), C/N (10.3–10.9), and pH (7.4–8.5), except that the May13 test compost had a higher C/N and pH (22.9 and 9.1, respectively). However, even though the Feb13 and the May13 tests belong to the same compost pile A, they display a different activity. A similar situation occurs with the Jun14, the Nov14, and the Nov15 tests that belong to compost pile C.

While the compost activity (CO_2 production in the first 10 days) is only a recommendation in the ASTM D5338-15 standard, it is required in the ISO 14855-1:2005 for the validity of the results. This criteria seems to be based on the composting standards and

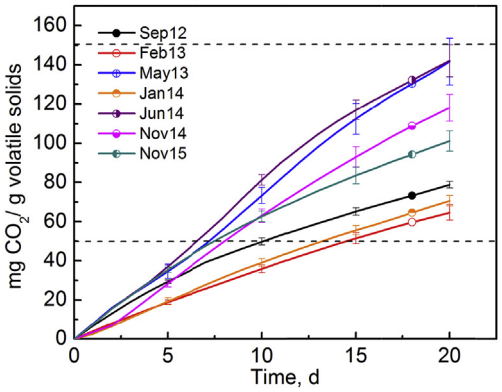

Fig. 11. Microbial activity of the compost measured as the production of CO_2 per gram of VS. Variation between 30 and 80 mg of CO_2 per gram of VS is seeing at 10 d.

guidelines for determination of compost stability, which is the rate or degree of OM decomposition [87]. Ge et al. (2006) state that for compost to be considered stable, it should have a CO_2 evolution rate less than or equal to 4 mg of carbon in the form of carbon dioxide per gram of VS per day (mg CO_2-C g^{-1} VS d^{-1}) [87]; the California Compost Quality Council requires the compost to produce 2–8 mg CO_2-C g^{-1} VS d^{-1} [89]; the Woods End Research Laboratory Incorporated classifies compost stability based on the mg of CO_2 per gram of VS per day produced as follows: high (<1), medium-high (1–4), medium (4–8), medium-low (8–13), and low (>13) [90].

In this context, it is important to mention that starting a biodegradation test with a large number of samples requires considerable resources and preparation time. Considerable loss is incurred if the experiment is discarded because the compost does not produce the 50–150 mg of CO_2 per gram of VS over the first 10 days as required by the ISO 14855-1:2005 standard. In this scenario, it is more important to consider if the compost is stable enough so the blank bioreactors, which are the background, do not produce large amounts of CO_2 that can hinder measurement of the CO_2 evolved from the bioreactors containing the test materials. In fact, a low production of CO_2 from the background is desired to improve the sensitivity of the measurement.

3.3.5. Other nutrients

In general, it is assumed that with a reasonable C/N, all other nutrients required by the microorganisms are available in sufficient quantities [51]. Table 6 shows the physicochemical characteristics and the total nutrient analysis of the three different media used in the Nov15 test: compost, inoculated vermiculite, and uninoculated vermiculite.

The physicochemical parameters of compost and vermiculite media are quite different. Vermiculite is a clay mineral with excellent water holding capacity, while compost is a more heterogeneous and complex matrix which contains additional organic compounds that can be assimilated by the microorganisms other than the test material [95,96].

According to Table 6, the amount of VS in vermiculite is very low as expected. The pH in inoculated vermiculite is lower due to the mineral solution used, while the C/N is higher in uninoculated vermiculite since no extra source of nitrogen was provided. In any case, the C/N is expected to increase when the test material is added to the media. Other element concentrations are similar between inoculated and uninoculated vermiculite, except sodium and sulfur due to the mineral solution used for inoculation. The high concentration of aluminum was expected in the vermiculite media.

3.3.6. Priming effect

The priming effect is the over-degradation of the indigenous organic carbon present in the compost when testing materials like glucose and its polymers [46]. Fig. 12 shows an excellent example of the priming effect. In the Jan14 test, the GC curve displayed an unusual high production of CO_2 in comparison with CP and CS (also readily biodegradable materials), and a mineralization near 200%, which physically makes no sense; the additional carbon converted to CO_2 is coming from the compost and not from the sample material.

It has been demonstrated that vermiculite is a good microbial carrier allowing the survival and full activity of the microorganisms, and it can be used as the solid media in biodegradation tests for avoiding the priming effect [46]. It has also been suggested that vermiculite increases reproducibility and aids in recovery of the by-products released during the degradation process, which is useful for determination of carbon balances [38,96].

3.4. Material-related factors affecting biodegradation

The physicochemical characteristics of the test materials such as chemical structure, hydrophilicity, crystallinity, molecular weight, shape, and surface area, among others, are also factors affecting the biodegradation rate and the biodegradability of the materials.

3.4.1. Chemical structure and properties

The intrinsic characteristics of the polymer such as mobility, tacticity, crystallinity, molecular weight, glass transition temperature (T_g), functional groups, plasticizers, and additives highly influence its biodegradability [12]. The unique chemistry of the polymer also dictates that the microorganisms should have metabolic pathways capable of targeting the polymer for biodegradation [97].

Fig. 13 shows the CO_2 evolution and mineralization of different materials from the Jun14 test, where there were two main groups of polymers tested. The first group, which included PE and LDPE polymers, did not show any meaningful mineralization (3.7± 1.6% for PE) while the second group, consisting of CP, CS, and PLA, reached a maximum mineralization of 61.7 ± 9.3, 48.0 ± 4.5, and 47.4± 9.8%, respectively.

Table 6
Physicochemical parameters and total nutrient analysis of different media used in the Nov15 test.

Parameter	Compost	Inoculated vermiculite	Uninoculated vermiculite
Dry solids, %	60.9	98	98.6
Volatile solids, %	39.1	2.0	1.4
pH	7.4	6.8	8.0
C/N ratio	10.9	7.2	27.1
Carbon, %	22.7	1.2	0.8
Nitrogen, %	2.08	0.16	0.03
Phosphorus, %	0.55	0.13	0.11
Potassium, %	2.48	4.32	4.26
Calcium, %	9.43	0.49	0.69
Magnesium, %	2.06	8.63	8.99
Sodium, %	0.40	0.15	0.03
Sulfur, %	0.42	0.05	0.01
Iron, ppm	15080	45330	47700
Zinc, ppm	163	80	86
Manganese, ppm	503	450	447
Copper, ppm	107	155	154
Boron, ppm	33	4	3
Aluminum, ppm	5955	42880	44200

Note: The total nutrient analysis was performed by inductively coupled plasma atomic emission spectroscopy (ICP-OES).

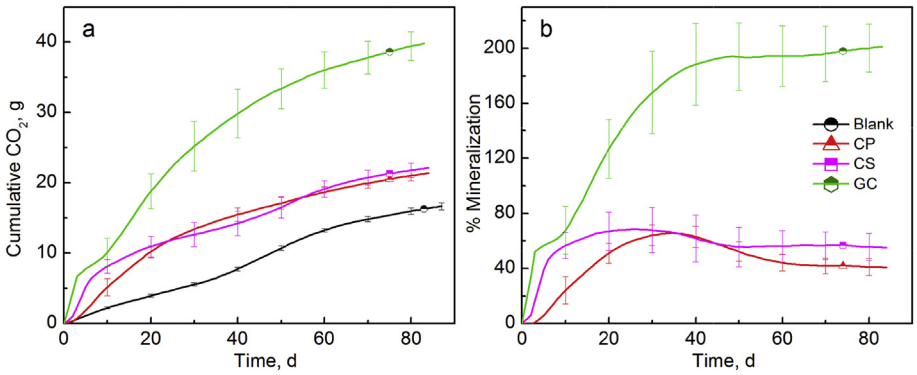

Fig. 12. Cumulative CO_2 evolution (a) and mineralization (b) of CP, CS, and GC in the Jan14 test. Mineralization values larger than 100% are observed for GC.

The different behavior between these two groups is due to the difference in the intrinsic characteristics of the polymer. On one hand, polymers like LDPE are not easily degradable due to their hydrophobic characteristics and relatively high stability [97], provided by the presence of single bonds between carbon atoms in the polymer chain that are especially difficult to break [98]. On the other hand, polymers like cellulose and starch tend to interact strongly with water due to their hydrophilic characteristics [98], and their biodegradation occurs relatively quickly. In the case of hydrolytically degradable polymers, the degradation rate is highly dependent on the nature of the functional groups comprising the polymer; some examples of functional groups contained in degradable polymers are: poly(α-hydroxy-esters), poly(β-hydroxy-esters), poly(ε-caprolactone), and poly(carbonates). A complete list of the different functional groups and their reactivity is provided elsewhere [47]. For example, polymers containing poly(α-hydroxy-esters), like PLA, tend to show lag periods due to the initial diffusion of water into the polymer matrix and the subsequent break down of the polymer into oligomers and monomers before actual biodegradation can take place. The biodegradation mechanism of PLA is discussed in more detail in Section 3.5.

In this context, Mezzanote et al. (2005) pointed out the question of whether it is correct or not to use cellulose as reference material since the microorganisms that are able to biodegrade materials like cellulose or starch are ubiquitous and perform cellulolytic activity, but it is not certain if they are equally able to perform esterase activity, which is required for the efficient and fast biodegradation of other materials like polyesters [49]. They have also suggested using biodegradable polyesters such as PCL as reference material besides cellulose. Similarly, other authors have proposed using PLA powder or PCL powder as reference materials for biodegradation tests [99,100].

Even if the test materials are the same, differences in composition and properties can highly influence their biodegradation rate. For example, Fig. 14 shows the biodegradation test results of two types of PLA evaluated in the Nov14 test. The PLA2 film is Ingeo™ 2003D while PLA4 film is Ingeo™ 4032D; the main difference between these two types of PLA is their composition in terms of the L-Lactide and D-Lactide content, which in turn affects the crystallinity of the material. PLA2 has a crystallinity of 6.14± 0.08% as measured by differential scanning calorimetry (DSC) (data not shown), while PLA4 was found to be completely amorphous due to its higher amount of D-Lactide. PLA4 produced more CO_2 than PLA2, especially at the early stage of the test. Likewise, the mineralization of PLA4 was higher than 100% towards the end of the test, indicating a priming effect.

These results are in agreement with the literature, in which other researchers have found that PLA with greater D-Lactide content presented higher and faster initial chemical hydrolytic degradation [101–103]. Besides, Tsuji and Miyauchi (2001) found that enzymatic hydrolysis, in which enzymes facilitate the cleavage of bonds, occurs mainly in the amorphous regions and on polymer chains with free ends [104]. However, these results may be also influenced by other factors such as molecular weight and thickness. PLA4 has lower M_n than PLA2 (M_n = 82.9 ± 6.7 and 75.0 ± 1.4 kDa, for PLA2 and PLA4 respectively); on the other hand, PLA4 is much thicker than PLA2 (0.022 ± 0.003 and 0.255 ± 0.021 mm, for PLA2 and PLA4, respectively).

3.4.2. Concentration

ASTM D5338-15 and ISO 14855-1:2005 recommend the ratio of the dry mass of the compost to the dry mass of the test material be

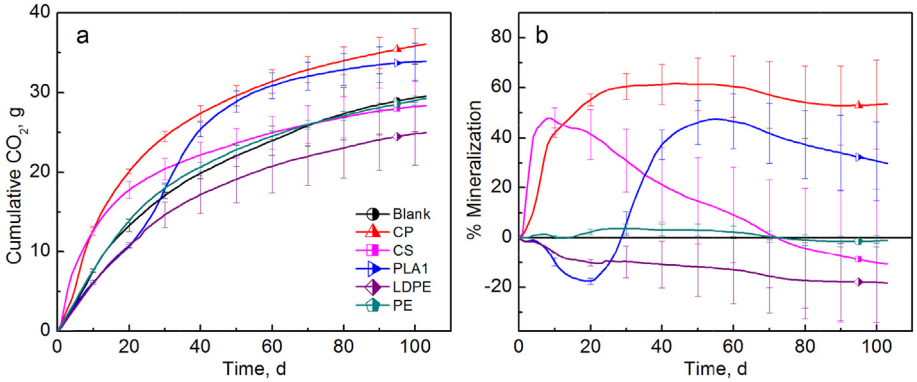

Fig. 13. CO_2 evolution (a) and mineralization (b) of different materials in the Jun14 test. Large difference of % Mineralization is observed for the different materials.

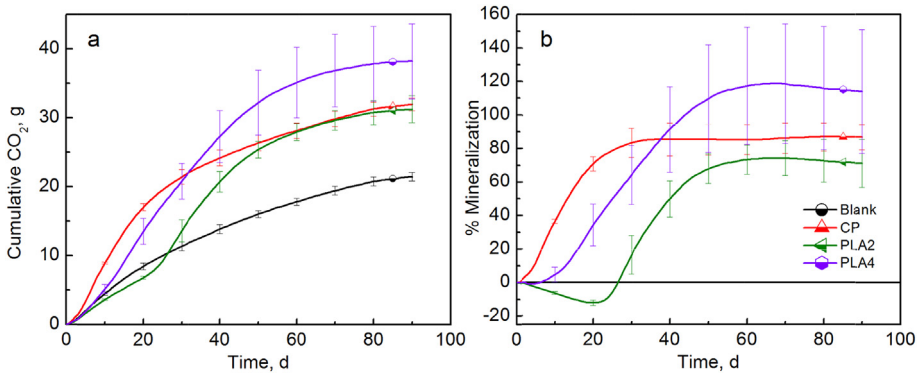

Fig. 14. Cumulative CO_2 (a) and mineralization (b) of CP, PLA2, and PLA4 in the Nov14 test. PLA 4032D shows faster and larger mineralization than PLA 2003D.

6:1; for example, 600 g of dry solids of the inoculum mixed with 100 g of dry solids of the sample. However, in our case we found this ratio not to be the most convenient because most of our samples are tested as films (1 cm × 1 cm squares) and not as powder, and the volume and area occupied by thin films is too large for good exposure when mixed with the compost. For example, in our bioreactors we can only fit 400 g wet weight of compost; considering that the initial moisture content was adjusted to 50% and that 20% of that weight was vermiculite, then each bioreactor contained 160 g dry weight of compost. If we followed the compost-to-material ratio recommended by the standards the weight of the sample should be 26 g; the inconvenience with this amount is that 26 g of films with a thickness of 0.00254 cm is too large (*i.e.,* density of PLA = 1.24 g/cm^3,V = 21 cm^3, A_{film} = 8252 cm^2) to be fit into the bioreactor and properly mixed with the compost. Moreover, if 26 g of cellulose were added to the bioreactor the production of CO_2 would be very high and fall outside the limits of the NDIR sensor. After many trial tests, we found that the optimal amount of material for our DMR system is 8 g or a 20:1 ratio (5% wt.). Again, similar to setting up the air flow rate mentioned earlier, the concentration of material should be adjusted in a way that the concentration of CO_2 is within the limits of the sensor and the production of CO_2 by the reference and the blank bioreactors are clearly differentiated.

Fig. 15 shows the cumulative CO_2 and % mineralization of CP (5%) and PLA pellets with two different concentrations (5% and 15%) where 15% is closer to the 6:1 ratio suggested by the standards. As expected, the production of CO_2 in the 15% was higher than the one in the 5% since the amount of available carbon is higher, but the % mineralization was not affected. The PLA pellets in both concentrations reach the same mineralization by the end of the test.

3.4.3. Shape

Biodegradation is usually, but not always, a surface erosion mechanism. Thus, materials in the form of powder usually degrade more easily since the area/volume ratio is maximized [8,78]. In this context, some researchers have suggested that the biodegradation test can be accelerated if the sample material is provided as a powder or small particles [105]. For example, the plastic materials can be converted into very thin films and then fragmented via cryogenic milling [106].

Fig. 16 shows the cumulative CO_2 evolution and % mineralization of CP and of PLA provided in different forms, *i.e.,* pellet and film, with surface area-to-volume ratio of ~12 and ~790, respectively. The production of CO_2, in this case, was not significantly different considering that the degradation of PLA via hydrolysis is a combination of both surface and bulk erosion [47]. However, Yang et al. (2005) found that PLA samples in the form of powder biodegraded faster than the film samples, and concluded that the biodegradability of slowly biodegrading polymers should be evaluated using samples with the same shape [105].

3.4.4. Comparison among different biodegradation tests

Even though it has been stated that comparing the results between different tests in a direct fashion would not be fair due to the many variables involved in the process, a possible way to perform such comparison would be to normalize against the mineralization of the positive reference, as suggested by ASTM D5338-15, in which the percentage of biodegradation relative to the positive reference (*e.g.,* cellulose) at the end of the test should be reported. In this context, if the t_i/t_T ratio, where t_i is the time at which the measurement was taken, and t_T the total time of the test, is plotted vs the ratio of material mineralization and cellulose mineralization, a possible way to compare the results could be envisioned (Fig. 17), assuming that the biodegradation behavior was similar in the tests being compared.

Fig. 17 shows that PLA1 reached a maximum mineralization ratio of 0.77 and 0.70 at a time ratio of 0.55 and 0.54 for the Jun14 test and the Nov15 test, respectively. Similarly, PLA2 reached a maximum mineralization ratio of 0.87 and 0.79 at a time ratio of 0.70 and 0.61 for the Nov14 test and the Nov15 test, respectively. This represents a difference of 9% between the mineralization ratio values in both cases. This approach to comparison should be further explored.

3.5. Study case: biodegradation of poly(lactic acid)

In the case of natural polymers like cellulose and starch, the biodegradation process is relatively fast and starts with the depolymerization of the material by the action of microbial extracellular enzymes that reduce the polymer to a size that is water soluble and able to be transported through the cell wall for subsequent assimilation by the microbial metabolic pathways [26,107]. Fig. 18 a shows the biodegradation of cellulose.

Microorganisms drive the biodegradation but other abiotic processes (oxidative, thermal, chemical or photodegradation) may also take place before or in parallel, such as in the case of PLA, where biodegradation is well known to involve abiotic hydrolysis (Fig. 18b) [74,108]. During the first step of PLA degradation, cleavage of ester linkages occurs due to their high susceptibility to water producing a significant reduction in the molecular weight of the polymer [107]. During the second step, the microorganisms are able to assimilate the low molecular weight lactic acid oligomers and monomers. We have found that the second step starts once the molecular weight is <10 k*Da*, as shown in Fig. 18b.

Fig. 18 shows that there are three phases in the biodegradation

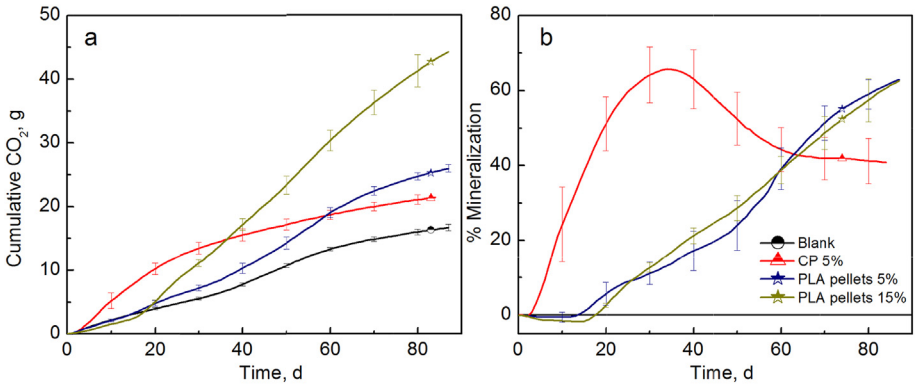

Fig. 15. Cumulative CO_2 and % mineralization of CP and PLA pellets with two different concentrations: 5% and 15%, in the Jan14 test. % Mineralization was not affected regardless of the initial amount of PLA.

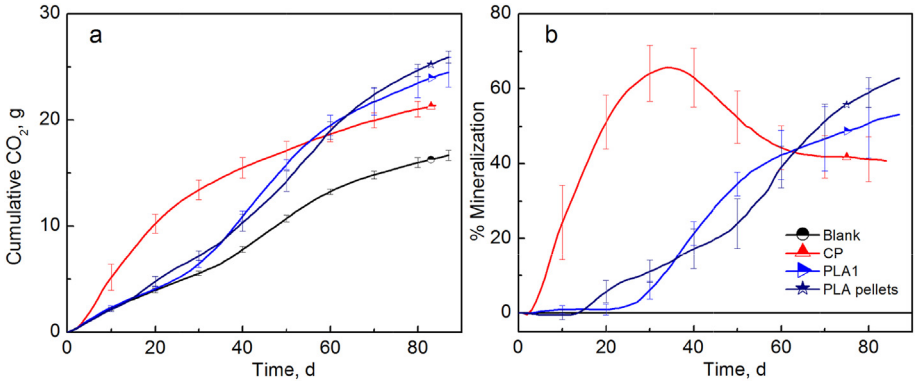

Fig. 16. Cumulative CO_2 evolution (a) and mineralization (b) of CP and of PLA provided in different forms: pellet and film, in the Jan14 test. % Mineralization was not extensively different regardless of the shape of the material.

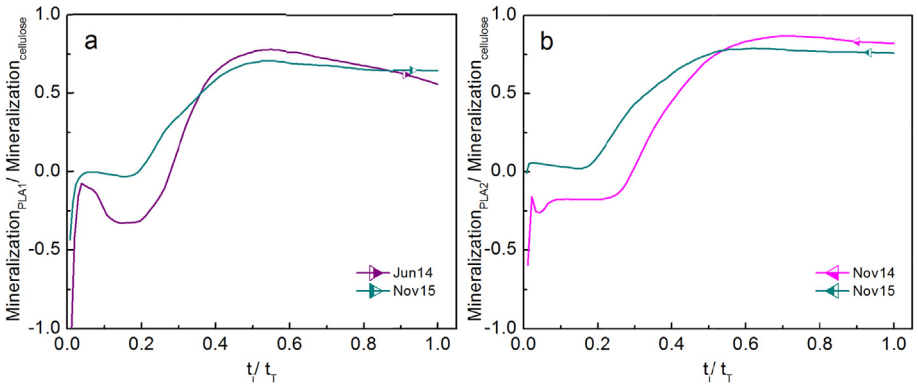

Fig. 17. Comparison of the mineralization values obtained for PLA1 in the Jun14 and the Nov15 tests (a), and the mineralization values obtained for PLA2 in the Nov14 and the Nov15 tests (b). The mineralization ratio when adjusting the time span of the test seems to be similar when comparing the same test material.

process: lag, biodegradation, and plateau phases. In the case of natural polymers like cellulose, the biodegradation phase starts almost immediately since fragmentation occurs quickly and the lag phase is assumed to occur due to the acclimatization of the microorganisms to the environment. In the case of polymers like PLA, there is an extended lag phase due to the relatively slow fragmentation of high molecular weight polymer chains. The biodegradation phase only occurs when enough low molecular weight oligomers and monomers become water soluble and are available for microbial assimilation [47]. According to Lyu et al. (2007), the molecular weight at which PLA becomes soluble in water is about 2–3 kDa [109].

The hydrolysis rate of the ester bonds in PLA can change depending on different factors such as water availability, pH, presence of ions, T_g, crystallinity, and molecular weight [47]. As previously mentioned, hydrolysis occurs mainly in the amorphous regions. As a result, during degradation, an increase in crystallinity can be observed. Furthermore, in the early stage of degradation, as T_g decreases from 64.0 ± 0.8 °C, as measured for PLA by DSC (data not shown), to temperatures below the test temperature

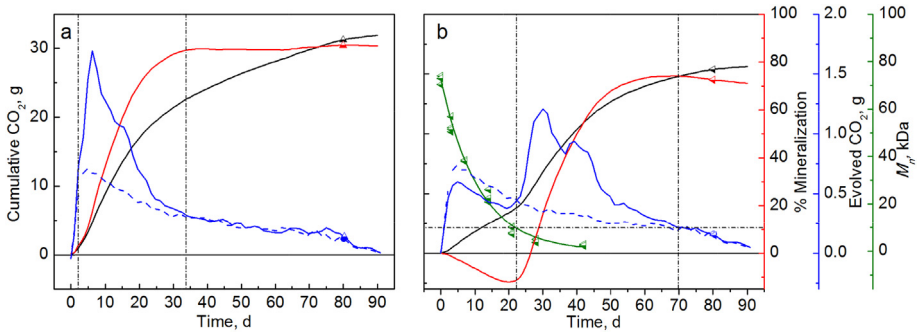

Fig. 18. Biodegradation of CP (a) and PLA2 (b) during the Nov14 test. The black, red, blue, and green lines represent cumulative CO_2, mineralization, evolved CO_2 per measurement, and M_n reduction, respectively. The dashed blue line represents the evolved CO_2 per measurement of the blank bioreactors. The green line indicates a fitting of an equation of the form $M_n = M_{n0}$ exp *(-kt)*, where M_{n0} is the initial M_n, k is the rate constant and t is the time. The black dash-dot lines are used as reference to indicate the beginning and end of the biodegradation phase, and the M_n at which the biodegradation phase gets started. Different lag phases and biodegradation phases were observed for CP and PLA2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(58 ± 2 °C), the oligomers and monomers have been reported to crystallize inside the PLA matrix since the polymer chains have sufficient mobility to rearrange into a more stable configuration [102,110]. Thus, temperature is a major factor affecting the biodegradation behavior of PLA. It has been reported that the biodegradation rate of PLA increases considerably at temperatures close to its T_g, while it does not significantly biodegrade at ambient temperature [109,111].

Some researchers have suggested that during the early hydrolysis step no microorganisms are involved [107,112,113], while others consider that enzymatic hydrolysis plays an important role along with abiotic hydrolysis [114]. Therefore, to decouple abiotic and biotic degradation, PLA films with three different molecular weights (Table 3) were evaluated in inoculated vermiculite and uninoculated vermiculite during the Nov15 test and the results are shown in Fig. 19 and Fig. 20. It is expected for polymers like PLA that both mechanisms compete against each other, with the fastest process the one that controls the initial degradation mechanism.

Fig. 19 shows that there was no significant production of CO_2 from the samples tested in uninoculated vermiculite. On the other hand, the PLA1, PLA2, and PLA3 produced 5.2 ± 0.6, 8.6 ± 0.9, and 7.2 ± 0.3 g of CO_2, respectively after 60 days of testing, and reached a mineralization of 34.6 ± 4.4, 58.3 ± 5.8, and 48.5± 1.8%, respectively, in the same period of time.

Fig. 20 shows the M_n as a function of time for the three PLA films tested in the three different media during the Nov15 test. The molecular weight decreased during the first three weeks and a first order reaction relationship was fitted to the experimental data. The

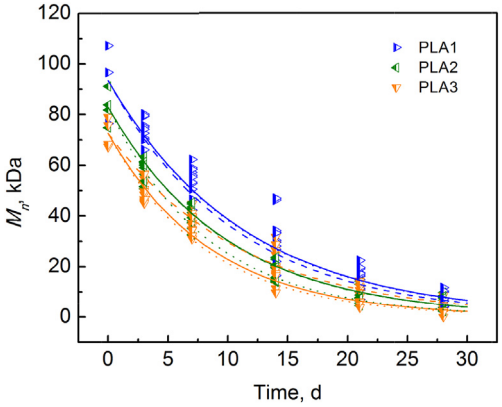

Fig. 20. Molecular weight reduction as a function of time for PLA1, PLA2, and PLA3 in compost (solid line), inoculated vermiculite (dashed line), and uninoculated vermiculite (dotted line), in the Nov15 test. Lines indicate fitting of a first order reaction of the form $M_n = M_{n0}$ exp *(-kt)*, where M_{n0} is the initial M_n, k is the rate constant and t is the time.

M_n reduction of each sample material was not significantly different regardless of the testing media (*i.e.*, compost, inoculated vermiculite, and uninoculated vermiculite), indicating that the abiotic step or hydrolysis is the main contribution to the degradation process of PLA in the early stage of degradation, and therefore it is a limiting factor for the subsequent biodegradation of PLA [106,107,113]. Fig. 20 shows that the initial molecular weight also

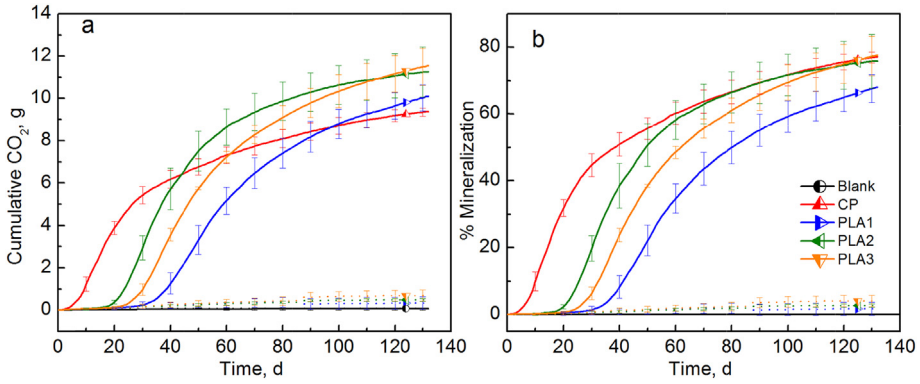

Fig. 19. Cumulative CO_2 (a) and mineralization (b) of CP, PLA1, PLA2, and PLA3 in the Nov15 test. Solid lines and dotted lines represent inoculated vermiculite and uninoculated vermiculite, respectively.

affects the hydrolysis rate and therefore the overall biodegradation. The PLA with higher M_n has longer polymer chains and more bonds to be cleaved, while the one with lower M_n has more polymer chains with free ends that can be cleaved producing more oligomers and monomers that are available for microbial assimilation. More detailed information about the biodegradation mechanism of PLA can be found elsewhere [74,109].

In our experiment, the rate constants (k) were not significantly different. However, it could be possible that the hydrolytic degradation of PLA occurs faster in an abiotic environment due to the accumulation of by-products, such as oligomers and monomers of lactic acid, which in turn reduce the pH of the media. This lower pH may cause an autocatalytic effect on the hydrolytic degradation [47]. On the other hand, in a biotic environment the microorganisms ideally assimilate those by-products continuously and the pH does not change significantly, perhaps assisted by the natural buffering capacity of the compost [51]. However, the effect of pH of the solid media like compost and vermiculite on the hydrolytic degradation rate of PLA needs further investigation.

4. Final remarks

We have provided a comparative analysis of the results obtained from eight different biodegradation tests for cellulose, starch, glycerol, polyethylene, and poly(lactic acid). These tests were carried out in the same in-house built DMR system following the analysis of evolved CO_2 approach. The results along with the critical analysis of the information provided in the literature allowed us to identify low reproducibility as one of the main issues for this kind of evaluation, caused mainly by the difficult to control variables in measurements of the biodegradation process.

In order to further understand such sources of variability a critical review of the literature regarding the different factors affecting biodegradation was provided. This analysis allowed us to identify some key parameters that can be more strictly monitored and controlled for an efficient biodegradation test, and therefore, to improve the current testing methodology.

Among the factors producing high variability is the quality and characteristics of the compost; therefore, a more strict control on moisture content, organic matter, and C/N should be required for the test, and all physicochemical parameters of the compost should be reported; otherwise, the interpretation of the results would not be complete. pH was found to be one of the easiest parameters to maintain due to the natural buffer capacity of the compost. If the test material is suspected to produce a priming effect, then biodegradation testing in vermiculite is recommended; also in cases where recovering of the by-products or determination of carbon balances is relevant for the study. Amendment of vermiculite with a mineral solution is recommended since it provides many of the nutrients required by the microorganisms.

Regarding environmental factors, temperature was the easiest parameter to control while water content was the most difficult and crucial. Maintaining the moisture content of the compost constant throughout the composting period is vital for the survival and reproduction of the microorganisms and other processes like hydrolysis. The optimal flow rate and optimal material concentrations should be found for each specific system in a way that allows proper measurement of CO_2 by the sensor and a clear differentiation between the background and the sample material.

If the test material is not expected to be readily biodegradable or to follow a similar behavior to cellulose, then the use of an additional positive control should be recommended (*e.g.,* a standardized PCL or PLA powder). In hydrolytically degradable materials like PLA, the shape did not show any significant difference since degradation occurs simultaneously in the surface and the bulk. For other types of materials, the usual recommendation is to use powder or small particles to increase the area/volume ratio and the biodegradation rate. Other polymer characteristics such as chemical structure, glass transition temperature, crystallinity, molecular weight, and functional groups highly influence the biodegradability and biodegradation rate of the material.

The biodegradation of PLA requires prior hydrolytic degradation, which breaks down the polymer chain into lactic acid oligomers or monomers that are easily assimilated by microorganisms. Thus, abiotic hydrolysis is the main contribution to the degradation process of PLA in the early stage of degradation and becomes a limiting factor for the subsequent biodegradation of this material. However, the hydrolysis rate is also dependent on the specific PLA properties like crystallinity and initial molecular weight.

The CO_2 evolution test can always provide valuable information about the biodegradability of a test material. However, if the purpose of the study is not only to evaluate and/or certify a material as biodegradable or compostable, but also to understand its biodegradation mechanism and environmental impacts, then additional tests are required such as determination of carbon balances, ecotoxicity tests, and molecular ecological techniques, among others.

Further biodegradation tests in composting conditions using different standardized reference materials and more strictly controlled inoculum (compost and/or vermiculite) characteristics and testing parameters could be performed in different labs around the world (*e.g.,* round robin test) by the analysis of evolved CO_2 in a DMR system in an attempt to unify and to improve this testing methodology.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.polymdegradstab.2017.01.017.

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