Article

Not peer-reviewed version

Thermophilic Composting as a Means to Evaluate the Biodegradability of Polymers used in Cosmetic Formulations

---

Timothy W. Gillece * , Helen K. Gerardi , Roger L. McMullen , William T. Thompson

Posted Date: 29 March 2024

doi: 10.20944/preprints202403.1813.v1

Keywords: biodegradation; ASTM D5338; ASTM D6400; polysaccharides; sustainability; respirometry; microplastics; polymer; composting; OECD

doi:10.20944/preprints202403.1813.v1

Article

Thermophilic Composting as a Means to Evaluate the Biodegradability of Polymers used in Cosmetic Formulations

Timothy W. Gillece *, Helen K. Gerardi, Roger L. McMullen and William T. Thompson

Ashland Inc., 1005 US HWY 202/206, Bridgewater, NJ 08807, USA; tgillece@gmail.com (T.G.); hgerardi@.ashland.com (H.G.); bjcycling@gmail.com (W.T.); rmcmullen@ashland.com (R.M.)
* Correspondence: tgillece@ashland.com

Abstract: In the past decade, a growing demand for sustainable cosmetic ingredients has yielded numerous biodegradation protocols. While OECD (Organization for Economic Co-operation and Development) aquatic assays are suitable for water-borne chemicals, it is crucial for the personal care industry to consider the persistence of plastics in soil, compost, and municipal sludge. Adopting this cradle-to-grave holistic approach would strengthen product appeal, while increasing the accuracy and ethical integrity of green-product labeling. Our study employs quantitative CO_2 detection and thermophilic composting protocols specified in ASTM D5338, along with pass level criteria outlined in ASTM D6400, to assess the mineralization of plastics commonly formulated into personal care products. The results indicate that many cellulose ethers, cationic guars, starches, and labile polyesters demonstrate satisfactory disintegration, biodegradation, and seed germination rates to secure an ASTM D6400 compostability claim. In contrast, macromolecules designed with carbon-carbon backbones resisted acceptable mineralization in composting experiments, suggesting that unadulterated municipal compost lacks the microbial diversity to enzymatically digest many synthetically derived resins. Additionally, polymers that demonstrated acceptable biodegradability in internal and published OECD aquatic studies—including chitosan and polyvinyl alcohol—exhibited limited fragmentation in local municipal compost; hence, untested correlations between aquatic, soil, and compost testing outcomes should never be assumed.

Keywords: biodegradation; ASTM D5338; ASTM D6400; polysaccharides; sustainability; respirometry; microplastics; polymer; composting; OECD

---

1. Introduction

The successful commercialization of plastics began in the 1950s, and global production has gradually increased to more than 330 million metric tons (MMT) per annum [1]. Consequently, plastics pollution has paralleled global production. According to a 2018 EPA report, 32 MMT of discarded plastic was generated in the United States. Of this total, about 75% was disposed of in regulated dumpsites and landfills—which underscores the pressing need for sustainable waste reduction and disposal methods. In nearly every region of the world, successful cosmetic and personal care products are packaged in single-use rigid plastic containers—including vessels composed of polyethylene terephthalate (PET), polyethylene, polyvinyl chloride (PVC), polystyrene (PS), and polylactic acid (PLA). In addition, each year approximately 0.7 MMT of plastics is blended into personal care formulations, where dilute resins function as rheology modifiers, emulsifiers, film formers, opacifiers, and cleansing aids [3]. Although a few macromolecules in finished chassis are discernible with the naked eye, most polymers in liquid formulations are too soluble or minuscule to be visibly perceived. When rinsed from the body, these overlooked plastics, including polyacrylates, cross-linked thickeners, and polyethylene, flow down drains and enter cesspools, septic tanks, and public sewer systems. In septic systems, a fraction of the soluble or dispersed plastics may circumvent residential containment tanks and percolate through leach fields and into the water table; or, in towns with public sewer systems, micronized debris may elude sewerage remediation and enter

doi:10.20944/preprints202403.1813.v1

neighboring rivers and oceans [4,5]. Consequently, when recalcitrant plastics reach local springs and waterways, microparticles may be inadvertently consumed by zooplankton, insects, amphibians, bivalves, and fish, and accumulate in our food chain [6,7]. In response to heightened environmental concerns, the North American Oceanic and Atmospheric Administration (NOAA) and European Chemicals Agency (ECHA) have led efforts to rigorously investigate the ecological impact of carbon-based synthetic microplastics, which are defined as water-insoluble plastic particulates less than 5 mm in length [8].

Polymers used in personal care formulations are synthetically or naturally derived. Synthetic polymers, such as polyacrylates, polyethylene glycol (PEG), and polycaprolactone (PCL), are routinely polymerized using non-renewable petrochemicals. In contrast, higher molecular weight (MW) natural polymers (bioplastics) are typically produced by purifying and chemically modifying extracts sourced from renewable resources, including terrestrial florae (e.g., starch, cellulose, zein, guar, PLA, and pectin), seaweed (e.g., alginates and carrageenan), shellfish (e.g., chitin and chitosan), and faunae (e.g., collagen, keratin, and gelatin) [9]. Synthetic and natural polymers may be further divided into degradable and non-degradable plastics. Degradable plastics deteriorate in the environment by mechanical, hydrolytic, thermal, photooxidative, and/or enzymatic mechanisms, whereas non-degradable resins are designated as persistent, with half-lives measured in years. Many synthetic personal care polymers are designed with thermodynamically stable -C—C- backbones and resist ambient photooxidative and hydrolytic degradation processes. Notable exceptions include specific grades of polyvinyl alcohol (PVA) and a select group of polyesters and polyurethanes, which have hydrolytically labile backbones [10]. In the absence of sunlight, however, aquatic or landfill temperatures must exceed 200 °C to thermo-oxidatively degrade these plastics in a timely manner—which is highly unlikely [11-13]. In contrast, most bioplastics resist abiotic hydrolysis, but are susceptible to abiotic fragmentation via photooxidation and enzymatic degradation by adapted aquatic and soil microbes.

Biodegradation is the breakdown of organic substances by microorganisms, and occurs in well characterized stages, including biodeterioration, biofragmentation, bio-assimilation, and mineralization [14]. Biodeterioration is a quasi-abiotic step, where tandem weathering processes, including extreme temperatures, mechanical work, abiotic hydrolysis, and photooxidation cause surface imperfections in the plastic, resulting in an increase in bioavailable surface area. Once the polymer matrix is compromised, biofragmentation ensues as macro-organisms (e.g., nematodes, earthworms, and snails) and microorganisms (e.g., bacteria, fungi, algae, and protozoa) colonize the polymer surface and employ microbial gut symbionts or secreted enzymes to cleave accessible macromolecular chains into shorter oligomers and monomers [15]. These smaller molecules are then digested by macro-organisms or are assimilated and/or mineralized by cells. The assimilation and mineralization steps terminate when microbiota chemically transform all bioavailable polymer fragments into usable biomass, biogas, inorganic salts, or waste. Enzymatic carbon mineralization processes occur aerobically or anaerobically, whereupon aerobic mineralization routes produce CO_2 and water, and slower anaerobic digestion yields methane, CO_2, water, and small amounts of H_2S and ammonia. Each stage of biodegradation is influenced by the properties of the ecosystem, including the biodiversity of its indigenous microbes. Hence, biodegradability assays and relevant claims should be tailored to the elements of the targeted local environment (e.g., fresh water, raw sewage, seawater, marine sediment, compost, landfill) [16]. Although native microorganisms cannot produce enzymes to biodegrade many synthetic polymers, they have suitably developed enzymes to biofragment and mineralize natural polymeric materials. For example, cellulose is a linear semi-crystalline biopolymer composed of D-anhydroglucose units that are covalently joined with β-1,4 glycosidic linkages; and, due to numerous intra- and intermolecular hydrogen bonds, cellulose forms insoluble and impenetrable fibrils that are difficult to enzymatically hydrolyze [17]. Nevertheless, over millions of years of co-evolution with woody plants, microbes have adapted collections of lytic enzymes to productively degrade lignocellulose [17,18]. Similarly, enzymes have been naturally adapted by microorganisms to mineralize other agro-polymers, including guar gum, chitosan, xanthan gum, and starch [19-22].

Consumer demand for environmentally friendly goods has sparked interest in biodegradation testing and innovative strategies for developing sustainable raw materials for the personal care industry. Current aquatic biodegradability testing protocols are set forth by international standards organizations such as ASTM International, International Organization for Standardization (ISO), and OECD and are routinely used to establish ecolabeling claims. Arguments for using aquatic OECD testing standards to solely establish biodegradability claims include the understanding that *ca.* 99% of treated municipal wastewater is discharged to local waterways as rejuvenated water; however, one could dispute that the fate of the accumulated 1% (w/w) municipal sludge (biosolids) should be considered as well [23-29]. Approximately three quarters of US households use municipal wastewater treatment facilities to treat sewage [30]. In these treatment facilities, wastewater influent receives primary, secondary, activated sludge, disinfection, anaerobic digestion, and filtration treatments [31]. After releasing the rejuvenated water—which may contain numerous soluble and dispersed polymer fragments—the residual biosolids are condensed and subsequently: a) incinerated (16%); b) sent to landfills (27%); c) deposited as fertilizer in home gardens and US farms (55%); or d) used in land reclamation or other projects (2%) [31-33]. In land use, sludge treatments are typically added to the soil surface as top dressing, or are blended with water, air, wood chips, straw, and decaying plant matter to produce enriched *compost*—in which composting is defined as the conversion of mixed organic waste into materials that are beneficial to plant life [34]. The good news: composting effectually recycles biowaste and amends agricultural soil by improving its aeration, water holding capacity, pH, and soil structure, as well as its bioavailable carbon, nitrogen, and micronutrient composition. Furthermore, composting is an important solution for communities looking to economically manage large volumes of organic refuse—including kitchen, agricultural, industrial, and municipal waste [34]. The potentially concerning news: each batch of biosolids added to farmland likely introduces enduring oligomers and microplastics [35-42].

To model the municipal composting of plastics in a laboratory setting, respirometry studies are accomplished in an aerobic milieu using municipal compost and standard biodegradability testing protocols such as ASTM D5338, ISO 14855-1, and ISO 14855-2 [43-48]. For each of these standard methods, ASTM D6400 and EN 13432 define the required disintegration, mineralization, and terrestrial safety pass level criteria to label a plastic as compostable in municipal or industrial facilities [46,47,49]. In this study, compost testing protocols outlined in ASTM D5338, and pass level criteria defined in ASTM D6400, are used to evaluate the biodegradability of several biopolymers and synthetic plastics in an inoculum of local municipal compost.

2. Materials and Methods

2.1. Source of Municipal Compost

Municipal compost was provided by NaturCycle LLC (Plainville, NY, USA), and sampled from the Morris County Municipal Utilities Authority (MCMUA) facility in Parsippany, NJ, USA. The compost was analyzed externally by MCMUA and met the seal of approval of the US Composting Council—by which Test Methods for the Examination of Composting and Compost (TMECC) standard testing assays are used to assess trace heavy metals, percent seedling emergence, compost maturity, pH, electrical conductivity, select pathogens, particle size, moisture, organic matter, and ash content. As per specifications in ASTM D5338, the compost was sifted at the collection site to a particle size of less than 10 mm using a USA Standard 10 mm brass test sieve (Dual Manufacturing Co., Franklin Park IL, USA), and residual inert materials—including small sticks and stones, plastic particles, and glass—were manually removed.

2.2. List of Tested Polymers

A description of the commercial polymers used in our study is specified in Table 1. In addition, Table 2 contains a cross-reference of the polymer identification used in the text against its marketed trade name.

Table 1. Various commercial grades of polysaccharides, proteins, and synthetic resins were used in the study. The chemical or trade name, common/INCI name, moisture level, ash content, and maximum theoretical CO_2 produced by complete carbon mineralization of each tested material are provided in the table.

Chemical / Trade Name	INCI/Common Name	Moisture	Ash	CO_2 (g)[*]
Aqualon 7L2F[a]	Cellulose Gum[^]	7.5	36.4	139
Aqualon 7H4F [a]	Cellulose Gum[^]	6.9	36.0	144
Aquasorb A380[a]	Cellulose Gum[^]	5.1	35.3	142
Aquasorb A500[a]	Cellulose Gum[^]	6.0	35.5	142
Avicel PH101[b]	Microcrystalline Cellulose	5.4	0.2	154
Avicel PH102[b]	Microcrystalline Cellulose	6.0	0.1	165
Bovine gelatin[c]	Gelatin	11.0	1.0	164
κ-Carrageenan[c]	Carrageenan	6.9	33.9	84
Cassia gum[a]	Cassia Gum	10.8	2.0	145
Chitin[c]	Chitin	5.4	1.1	164
Chitosan[c]	Chitosan, 50-190 KDa, 76%	7.1	0.3	153
Citrus peel pectin[c]	Pectin	7.2	15.1	146
Crosslinked pAA[d]	Carbomer	8.6	0.1	174
Guar gum[c]	Guar Gum	11.3	1.1	171
Keratin (bleached hair)[e]	Bleached European dark brown hair	12.7	6.5	153
Keratin (virgin hair)[e]	Virgin European dark brown hair	11.7	1.7	163
Locust bean gum[c]	Ceratonia Siliqua Gum	12.5	1.0	147
Maize amylopectin[c]	Amylopectin	11.9	0.6	147
N-Hance 3196[a]	Guar Hydroxypropyltrimonium	9.5	1.0	151
poly(OAA/Acrylates/BAEM)[f]	OAA/Acrylates/BAEM Copolymer	3.6	2.4	226
Polycaprolactam[c]	Nylon 6	0.5	0.1	212
Polycaprolactone (80 kDa)[c]	Polycaprolactone	1.2	0.1	234
Polyethylene[c]	Polyethylene	0.0	0.3	313
Polylactic acid (60 kDa)[c]	Polylactic Acid	0.6	0.1	186
Polystyrene[c]	Polystyrene	0.1	0.1	335
Potato starch[c]	Potato Starch	8.1	0.6	151
PVA, ≥ 85% hyd.[c]	Polyvinyl Alcohol, 30 kDa, ≥ 85%	3.4	0.8	197
PVA, ≥ 98% hyd.[c]	Polyvinyl Alcohol, <50 kDa, ≥ 98%	3.4	0.2	194
SigmaCell Type 20 cellulose[c]	Cellulose (cotton linters)	5.0	0.1	156
Sodium alginic acid[c]	Alginic Acid, Sodium Salt	13.2	23.2	114
Soluble starch (potato)[c]	Soluble Starch	17.0	0.9	138
Soy protein acid hydrolysate[c]	Hydrolyzed Soy Protein	11.7	18.1	146
Styleze ES-1[a]	Guar Hydroxypropyltrimonium	9.3	2.8	165
Styleze ES-Dura[a]	Guar Hydroxypropyltrimonium	7.7	3.0	153
Xanthan gum[c]	Xanthan Gum	15.0	0.6	131
Zein protein[c]	Zein	6.8	1.8	220
α-Cellulose[c]	Microcrystalline Cellulose	7.5	0.4	153

[a]Ashland Inc. (Wilmington, DE, USA); [b]IFF Inc. (New York, NY, USA); [c]Merck KGaA (Darmstadt, Germany); [d]Lubrizol (Wickliffe, OH, USA); [e]European dark brown hair (1-2 mm fiber snippets) from International Hair Importers and Products, Inc. (Glendale, NY, USA); [f]Nouryon (Amsterdam, Netherlands); pAA=polyacrylic acid; OAA=*tert*-octylacrylamide; BAEM=*tert*-butylaminoethyl methacrylate; Acrylates=methyl methacrylate, acrylic acid, hydroxypropyl methacrylate; [^]sodium carboxymethyl cellulose; [*]based on 100 g of polymer as received.

Table 2. Cross reference of Polymer ID used in the text against its commercial trade name.

Polymer ID	Commercial Trade Name
CMC-1	Aqualon 7L2F
CMC-2	Aqualon 7H4F
CMC-3	Aquasorb A380
CMC-4	Aquasorb A500
MCC-1	Avicel PH101
MCC-2	Avicel PH102
CGG-1	N-Hance 3196
CGG-2	Styleze ES-1
CGG-3	Styleze ES-Dura

2.3. *Seed Types used in Germination Studies*

To appraise the terrestrial safety of successfully biodegraded plastics, Champion radish (JD Hardware LLC, Wellsville, UT, USA), Purple Top White Globe turnip (Burpee, Warminster, PA, USA), and Heirloom Robust Barley (The Plant Good Seed Company LLC, Ojai, CA, USA) seeds were chosen for the germination studies.

2.4. *Polymer Ash Content*

Ash content (± 0.2%) was determined using a TA Instruments (New Castle, DE, USA) Q5000 thermogravimetric analyzer (TGA) and the following protocol: switch purge gas to dry air (25 mL/min); isothermal 40 °C for 5 min; ramp 10 °C/min to 600 °C; isothermal 30 min (n=3). TA Instruments Universal Analysis v4.5A software was used to quantify residual ash.

2.5. *Polymer Dry Solids Measurements*

Prior to commencing respirometry studies, ASTM D5338 indicates that the dry solids of compost-polymer admixtures be adjusted to 50-55% (w/w), and loss on drying (LOD) protocols defined in Standard Method 2540D be applied for adjusting moisture content [43,49]. In Standard Method 2540D, the sample is dehydrated to constant weight in a drying oven set to 103-105 °C for no less than 60 min, and the weight difference is used to calculate the initial dry solids. To expedite moisture analyses, we instead developed a 15 min higher-temperature (140 °C) evaporation method using a Sartorius (Göttingen, Germany) MA-30 moisture analyzer. To validate the protocol, Table 3 contrasts neat compost dry solids results using Standard Method 2540D and findings from other commonly employed LOD procedures.

Table 3. Compost solids determination using various standard LOD techniques.

Drying Method	Sample Size (g)	Temperature (°C)	Time (h)	Dry Solids (%)
Sartorius MA 30	4-6	140	0.25	47.9 ± 1.1 (n=10)
TGA Q5000 isotherm	0.02	175	1.0	46.2 ± 1.3 (n=3)
Forced-air oven (2540D)	25-35	105	24	48.3 ± 1.1 (n=5)
Vacuum oven (2540D)	25-35	105	24	48.1 ± 0.3 (n=5)
DVS isotherm*	0.02	25	24	48.8 ± 1.5 (n=4)

*TA Instruments Q5000 SA (25 °C; 0% RH using dry $N_{2,g}$; 24 h isotherm).

2.6. *CHN Analysis of Tested Polymers*

Using carbon-hydrogen-nitrogen (CHN) analysis and Equation 2, average measurements (n≥2) were used to assess the maximum theoretical CO_2 produced in the aerobic biofragmentation of the as-received polymers. The % carbon results were subsequently converted to the maximum theoretical CO_2 produced (i.e., assuming 100% mineralization) and are presented in Table 1. For each replicate, *ca.* 1.5 mg of sample was weighed into a tin sample capsule and analyzed directly with a Thermo Fisher Scientific Flash 2000 CHNS/O combustion analyzer (Waltham, MA, USA) using the following instrumental parameters: helium carrier = 140 mL/min; oxygen = 250 mL/min; helium reference = 100 mL/min; sample run time = 720 sec with 12 sec sampling delay; oxygen injection end = 5 sec; oven temperature = 65 °C; furnace temperature = 950 °C. The measurement was calibrated linearly using a 2,5-bis(5-*tert*-butyl-benzoxazol-2-yl)thiophene standard. In addition, CHN analyses estimated the C/N ratio of the compost as *ca.* 19:1 (w/w), which is acceptable for composting carbon-rich polymers [43].

2.7. ASTM D5338 and ASTM D6400: Biodegradability Controls, Calculations, and Compostability Labeling Claims

The composting apparatus described in ASTM D5338 incorporates a minimum of twelve vessels, in which the test material, blank (compost only), negative control, and positive control are run in triplicate at 58 ± 2 °C in diffuse light. The blank vessels are used for zeroing CO_2 contributions provided by the indigenous carbonaceous matter in the compost (Eq. 1). Since it takes 10-600 years for polyethylene to naturally biodegrade [41], ASTM D5338 specifies polyethylene as the negative control, wherein the physical form of the negative control should match that of the test sample—powders, films, and cylindrical slugs were used in our studies. The universal positive control is defined as ≤ 20 µm high purity microcrystalline cellulose (MCC) powder. ASTM D5338 requires that the positive control biodegrades ≥ 70% in ≤ 45 days or the composting assay is judged as invalid.

The % biodegradation of the test polymer is defined by Equation 1,

$$\%\ biodegradation = \frac{CO_2\ polymer\ (g) - CO_2\ blank\ (g)}{CO_2\ theoretical\ (g)} \times 100, (1)$$

in which *CO_2 polymer* is the detected CO_2 mass (44 g/mol) produced by the mineralized test polymer in compost; and *CO_2 blank* is the detected mass of CO_2 produced by the compost. In Equation 2,

$$CO_2\ theoretical\ (g) = polymeric\ carbon\ (g) \times \frac{44}{12}, (2)$$

CO_2 theoretical is the maximum theoretical CO_2 evolved by the test polymer—which assumes that all polymeric carbon (12 g/mol) is catabolized to gaseous CO_2 [43]. For our calculations, the polymeric carbon was determined empirically using the CHN analysis method detailed in the "Polymers: CHN Analysis" section. The *CO_2 theoretical* values for each tested polymer are itemized in Table 1. According to ASTM D5338, the % biodegradation may be reported in absolute form (i.e., Eq. 1) or defined relative to the performance of the positive control.

ASTM D6400 defines criteria to meet requirements for labeling materials as biodegradable and/or compostable in aerobic municipal and industrial composting facilities: 1) In tandem with environmental conditions outlined in ASTM D5338, materials must naturally degrade ≥ 90% in ≤ 6 months; 2) After 84 d, no more than 10% of its dry weight is retained after passing the composted plastic through a 2-mm sieve; and 3) Composted plastics must successfully demonstrate acceptable terrestrial safety [43-47].

2.8. Composting of Polymers: Thermophilic Respirometry

A Columbus Instruments 24-vessel Micro-Oxymax respirometer (Columbus, OH, USA), equipped with a computer and software to archive and display biodegradation results, was used for the composting studies. The instrumentation includes a nondispersive mid-IR (NDIR) 0-10% CO_2 sensor, expansion interfaces to direct sampled gas through a common CO_2 sensor, fresh air pump, water vapor condensers, and an environmental enclosure for monitoring and controlling respirometry studies. The 4.26 µm NDIR sensor was calibrated using a certified 9.02 ± 0.02% CO_2 (with N_2 gas diluent) primary standard (Praxair, Morrisville, PA, USA). Fresh ambient air was continuously introduced to the vessels using a linear diaphragm pump with a calibrated flow rate of 250 mL/min. Other timed instrumental settings include sensor purge = 240 sec; purge measurement = 60 sec; sample circulation = 240 sec; sample measurement = 60 sec.

Polymer samples were added to the compost as powders, cylindrical slugs, or films, in which the dimensions of the resin particles were always less than 2 cm. After adjusting the dry solids of the compost to *ca.* 50% solids, exactly 25, 30, or 100 g of polymer was mixed with 600 g of compost (dry basis) and added to a 5 L Pyrex glass respirometry vessel. Twenty-four sample vessels were then placed in a heated environmental enclosure (58 ± 0.5 °C) and incubated for ≥ 45 d (positive control) or ≤ 180 d. Every seventh day the vessels were removed from the chamber and manually shaken to inhibit air and water vapor channeling. In our investigation, the Micro-Oxymax was operated in open circuit mode at ambient atmospheric pressure, wherein the system purges measured CO_2 from the system after successively logging the differential CO_2 concentration between the atmosphere and each composting vessel. The production of CO_2 was automatically logged every 4 h.

2.9. Differential Scanning Calorimetry (DSC)

In the wet DSC methodology for analyzing the thermal stability of hair keratin, snippets of hair are soaked in water and then heated through the denaturation temperature (T_D) in sealed pressurized stainless-steel crucibles. By heating the fiber cortex in the wet state rather than the dry state, water plasticization of the cortical matrix separates denaturation and pyrolysis events. The T_D probes the plasticity and viscosity of the amorphous fiber matrix, whereas the denaturation enthalpy (ΔH_D) represents the energy required to decompose the ordered α-helical keratin and interfacial bonding between the α-helical and matrix proteins. A TA Instruments Q2000 DSC with Perkin-Elmer (Waltham, MA, USA) high-volume pressure pans were used for the studies. Approximately 10 ± 0.5 mg of 2–3 mm length fiber snippets were loaded into high-volume steel crucibles and 50 µL of distilled water was added using a micropipette. The pans were crimped with a Perkin-Elmer Quick Press and then loaded into an indium-calibrated DSC cell using an automated sample cassette and robot. The T_D and ΔH_D of the fibers in excess water were evaluated using the following protocol: 1) Equilibrate at 20°C; 2) Isothermal for 2 min; 3) Ramp 2 °C/min to 160°C.

In other standard DSC experiments, a TA Instruments Q2000 was used to determine the glass transition (T_g) and melting temperatures (T_m) of PCL, PLA, and Nylon 6 by evaluating the second heats, wherein the polyesters were heated in perforated aluminum DSC hermetic pans from -100 °C to 200 or 250 °C at 10 °C/min under a dry nitrogen purge.

TA Instruments Universal Analysis v4.5A software was used to evaluate T_D, ΔH_D, T_g, and T_m.

2.10. Scanning Electron Microscopy (SEM)

Composted materials were imaged using SEM. Samples were fixed to aluminum Pelco pin stubs using double-sided 25-mm conductive carbon tabs (Ted Pella, Redding, CA, USA) and then coated with Au/Pd using a high-vacuum Leica EM ACE600 sputter coater (Leica Mikrosysteme GmbH, Wetzlar, Germany). A Hitachi SU-5000 FESEM (Hitachi High Technologies, Schaumburg, IL, USA) was used to image the samples at several magnifications.

2.11. Compost Viability: Seed Germination Studies

ASTM D6400 and OECD Guideline 208 specify that materials may be designated as compostable if the compost with degraded plastic passes terrestrial safety testing protocols [47,50]. One directive involves monitoring seed germination, where trends in compost viability factors, including the visual assessment of germination, fresh shoot weight or height, and plant health, are contrasted with seedling studies implemented with untreated compost [47]. ASTM D6400 indicates that the germination rate of the composted mixture shall be no less than 90% of the germination rate observed in blank compost. ASTM D6400 adds modifications to OECD Guideline 208, which are found in Annex E of EN 13432. One such modification states that 25-50% of the potting soil mixture should include compost containing the disintegrated polymers [46].

In the assays, 300 g of compost with degraded polymer was thoroughly blended with 600 g fresh compost. Radish, turnip, and barley seeds were then placed in contact with the mixture, and compost viability factors were documented after *ca.* 50 % of the seedlings emerged in the vessel containing only untreated compost (control). Each viability test was performed in triplicate, where 6 seeds were added to three 4-inch diameter plastic gardening pots (n=18) that had been filled with blended compost. In each pot, the six seeds were evenly dispersed and covered with approximately ¼ inch of compost. After watering generously, a transparent humidity dome was placed on each pot to minimize moisture evaporation. After germination, the humidity domes were removed from the gardening pots to boost light exposure for the developing florae (Figure 1). A broad spectrum (400-840 nm) 3 x 30 W full-spectrum LED grow light with a 12 h on/off schedule setting (Jinhongtu, Zhongshan City, China) was used to simulate sunlight. The ambient conditions in the laboratory were 24 ± 2 °C and 33-46% RH. Compost viability factors were documented for ≥ 15 d.

Figure 1. Radish (left), turnip (center), and barley (right) plants grown in neat compost for 15 days.

3. Results and Discussion

Throughout aerobic biofragmentation, assimilation, and carbon mineralization, microbes use enzymes and processes such as the electron transport cycle to systematically convert polymeric intramolecular bonding energy into fuel—which is subsequently used for cellular activities including reproduction, cell growth, ion transport, and chemical synthesis. The net catabolic activity of cellular respiration includes the incorporation of nutrients and energy into cells (i.e., assimilation) and the sequential oxidation (i.e., mineralization) of oligomeric carbon into CO_2 gas or carbonate salts. In the present work, we used respirometry and guidelines in ASTM D5338 and ASTM D6400 to examine the carbon mineralization rates of several common polymers using municipal compost and quantitative CO_2 gas detection.

3.1. Biodegradation of Common Personal Care Polymers

Tables 4, 5, and 6 summarize the 90-days composting results, in which the data describes the relative biodegradability, as well as the absolute rate and extent of mineralization for the composted polysaccharides, proteins, and synthetic polymers.

Table 4. Thermophilic biodegradation of polysaccharides using municipal compost (± 4%).

Polymer ID	Relative Biodegradability (%)	Absolute Biodegradability (%)					
		5	10	20	30	60	90 d
Sodium alginic acid	100	50	96	122	175	177	**179**
Soluble starch (potato)	100	57	89	109	119	134	**144**
Potato starch	100	87	96	99	101	128	**140**
Xanthan gum	100	39	67	98	102	121	**122**
Citrus peel pectin	100	51	63	74	78	100	**120**
Guar gum	100	35	60	89	100	101	**102**
MCC-2	100	16	33	57	83	94	**99**
MCC-1	100	19	32	58	81	90	**97**
Cassia gum	100	77	89	92	95	95	**97**
Locust bean gum	100	71	80	84	86	89	**94**

Amylopectin (maize)	100	69	80	84	86	92	**93**
α-cellulose	100	6	13	28	48	82	**88**
CMC-4 (powder)	100	5	10	38	65	85	**87**
Chitin	100	17	39	55	63	75	**85**
CMC-1	100	5	12	33	55	72	**84**
SigmaCell Type 20 cellulose	100	58	73	79	**81**	**81**	**81**
CGG-2	98	12	43	58	66	74	**79**
CGG-1	95	19	33	51	63	70	**77**
CMC-4 (film shards)	93	14	21	46	57	64	**75**
κ-Carrageenan	90	3	13	36	49	67	**75**
CMC-3	90	39	59	68	**73**	**73**	**73**
CGG-3	90	37	42	49	56	63	**72**
Chitosan (pH = 6.0)	22	2	4	8	10	12	**18**
Chitosan (as received)	0	**0**	**0**	**0**	**0**	**0**	**0**

Table 5. Thermophilic biodegradation of proteins using municipal compost (± 4%).

Polymer ID	Relative Biodegradability (%)	Absolute Biodegradability (%)					
		5	10	20	30	60	90 d
Soy acid hydrolysate	100	78	99	118	135	147	**151**
Zein	100	78	85	95	**98**	**98**	**98**
Bovine gelatin	100	82	90	**91**	**91**	**91**	**91**
Keratin (bleached hair)	100	45	76	**87**	**87**	**87**	**87**
Keratin (virgin hair)	11	1	5	8	**9**	**9**	**9**

Table 6. Thermophilic biodegradability of synthetic polymers using municipal compost (± 4%).

Polymer ID	Relative Biodegradability (%)	Absolute Biodegradability (%)					
		5	10	20	30	60	90 d
PCL (80 kDa), 30g	100	5	13	39	70	99	**102**
PLA (60 kDa)	100	10	11	31	35	61	**90**
PCL (80 kDa), 100g	100	6	13	27	30	**32**	**32**
poly(OAA/Acrylates/BAEM)	15	10	**12**	**12**	**12**	**12**	**12**
Polycaprolactam	12	8	**10**	**10**	**10**	**10**	**10**
Polystyrene	6	**5**	**5**	**5**	**5**	**5**	**5**
PVA, ≥ 31kDa, ≥ 98% hyd.	4	**3**	**3**	**3**	**3**	**3**	**3**
Crosslinked pAA	3	**2**	**2**	**2**	**2**	**2**	**2**
PVA, 30 kDa, ≥ 85% hyd.	1	**1**	**1**	**1**	**1**	**1**	**1**
Polyethylene	0	**0**	**0**	**0**	**0**	**0**	**0**

3.2. Biodegradation of Natural Polymers

Table 4 presents biodegradation data for several classes of natural and modified carbohydrates, including starches, marine carbohydrates, natural galactomannans, cationic guar gum (CGG), MCCs, and sodium carboxymethyl cellulose (CMC). The examined starches comprise natural potato, soluble potato, and maize amylopectin, whereas the composted marine carbohydrates include chitin, chitosan, carrageenan, and sodium alginate. In addition, the biodegradability trends of natural galactomannans, including guar gum, locust bean gum, and cassia gum, are compared to the those of CGG (i.e., CGG-1, CGG-2, and CGG-3). Lastly, Table 4 contrasts the composting results of several MCC powders against the biodegradation rates of various CMC grades (i.e., CMC-1, CMC-3, and CMC-4).

At first glance, Table 4 suggests that several polysaccharides biodegraded more than 100% on an absolute basis; however, the phenomenon is rationalized by considering the *priming effect*, which expedites the decomposition of indigenous organic matter upon adding simple polysugars, including mono- and polymeric glucose, to neat compost. Although the mechanism is not completely understood, it is known that priming with simple carbohydrates introduces surplus chemical energy that stimulates microbial activity [51-53]. Our results suggest that complex carbohydrates such as starch, sodium alginate, xanthan gum, citrus peel pectin, and hydrolyzed soy protein also induce positive priming (see Tables 4 and 5).

To elucidate priming contributions, cylindrical slugs of sodium alginate, xanthan gum, potato starch, soluble starch, citrus peel pectin, and hydrolyzed soy protein were produced and composted, whereby slug disintegration was used as a visual cue for monitoring the disintegration rates of each bioplastic. In contrast to the attrition rate of SigmaCell Type 20 slugs, in which erosion in compost was slow and biodegradation plateaued at << 100%, thermophilic respirometry of sodium alginate, natural potato starch, xanthan gum, soluble potato starch, pectin, and hydrolyzed soy protein slugs induced rapid slug disintegration and >> 100% absolute biodegradation. Indirectly, the results suggest that the disintegrated bioplastics wholly mineralized; however, it is also possible that priming preferentially mineralized compost organic matter. To discriminate between the CO_2 released from catabolized bioplastics and the CO_2 liberated from biodegraded compost carbon, sodium alginate, xanthan gum, natural potato starch, citrus peel pectin, and hydrolyzed soy protein were composted in carbon-free vermiculite using guidelines in ISO 14855-2 [45]. As an example, Figure 2 presents the biodegradation rate data for xanthan gum, which is a high MW biopolymer of glucose, mannose, and glucuronic acid. The overlay demonstrates that xanthan gum powder and xanthan gum slugs degraded > 120% when mineralized in municipal compost, whereas the biodegradation of xanthan gum powder composted in activated vermiculite plateaued at *ca.* 100%. The results indicate that xanthan gum introduced positive priming, in which xanthan gum likely mineralized, while surplus CO_2 biogas was generated by augmented mineralization of indigenous carbon in the neat compost. Like xanthan gum, other tested bioplastics exhibited positive priming in composting studies and demonstrated 45-days biodegradation plateaus at ~ 100% mineralization in activated vermiculite: sodium alginate = 100.4%; potato starch = 100.7%; citrus peel pectin = 94.6%; and hydrolyzed soy protein = 93.8%.

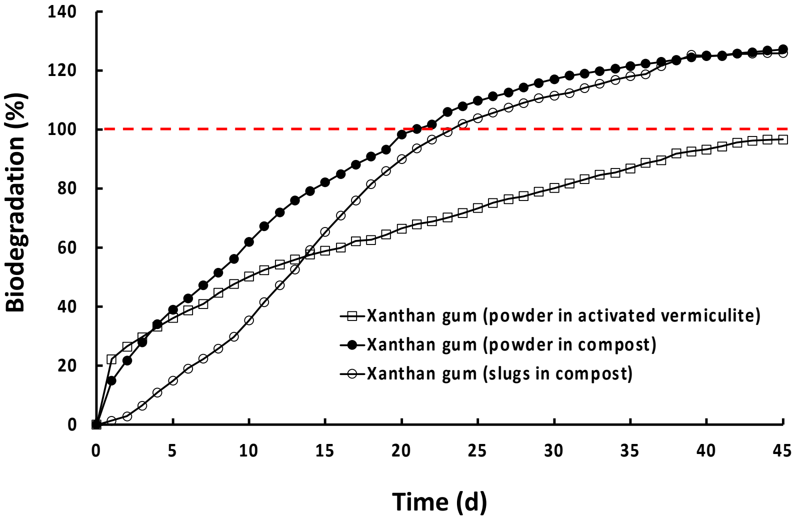


Figure 2. Effect of intrinsic compost carbon content on the measured biodegradability rates of a) xanthan gum powder and b) xanthan gum slugs in mature municipal compost; and c) xanthan gum powder in activated vermiculite. The red dashed line demarcates 100% absolute biodegradability, in which biodegradation >> 100% suggests contributions from positive priming.

Not surprisingly, Table 4 indicates that starch exhibited positive priming in municipal compost. Natural potato and corn starches are polymers of glucose and contain *ca.* 25% amylose and 75%

amylopectin. Amylose is the linear fraction, and is polymerized with α-1,4 glycosidic linkages. Similarly, amylopectin contains glucosyl units joined with α-1,4 glycosidic bonds but has a much higher degree of polymerization (DP) than amylose, including intermittent branching with α-1,6 glycosidic side chains. In our composting studies, soluble potato and natural potato starches demonstrated similar rates and extents of hydrolysis, suggesting that enzymatic digestion may be linked to amylopectin content [54]. However, although the initial fragmentation rates of maize amylopectin and natural potato starch were similar, the mineralization rate of maize amylopectin decreased markedly after 7-10 days, indicating that higher-DP chains in amylopectin likely influenced the rate of hydrolysis at > 80% biodegradability. Moreover, the immediate hydrolysis of soluble potato and natural potato starches (50-90% in ≤ 5 days) suggests the presence of thermally stable exocellular amylases in the compost at the start of testing, where the rapid enzymatic production of glucose monomers and oligomers in the compost likely activated positive priming.

Table 4 also details the mineralization rates of several marine carbohydrates, including sodium alginate, chitin, carrageenan, and chitosan. Sodium alginate is a linear polysaccharide derived from brown seaweed and is chemically the biopolymer of the sodium salts of mannuronic and guluronic acids. Like starch, sodium alginate demonstrated significant priming effects, including rapid initial mineralization, as well as 179% absolute biodegradability after 90 days of thermophilic composting. The rapid initial biodegradation of sodium alginate suggests that active exocellular hydrolytic enzymes may be present in the compost, demonstrating that previous secretions from the compost inocula “recognized” the added carbohydrate. Moreover, the microorganisms appear to possess the enzymatic machinery to mineralize mannuronic and guluronic acids, while simultaneously increasing the biofragmentation of indigenous carbon in the compost. Although compositionally different, like sodium alginate, carrageenan is another high MW extract derived from (red) seaweed. As shown in Figure 3, the *κ*-isomer of carrageenan may be described as a sulfated (one ester sulfate per repeating unit) anionic gum composed of alternating units of β-d-galactose and 3,6-anhydro-*α*-d-galactose, which are linked by *α*-(1,3) and β-(1,4) glycosidic bonds. Although *κ*-carrageenan is ultimately consumed by composting microbes, Table 4 shows that the initial biodegradability rate is slow and there is a lack of priming compared to sodium alginate, indicating that the indigenous compost microbiome initially lacked the ability to rapidly mineralize *κ*-carrageenan.

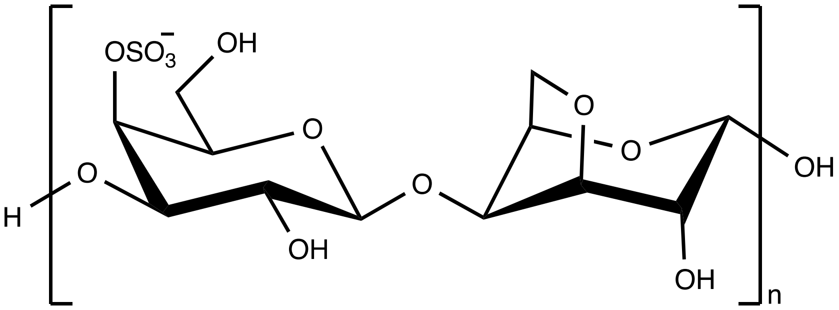
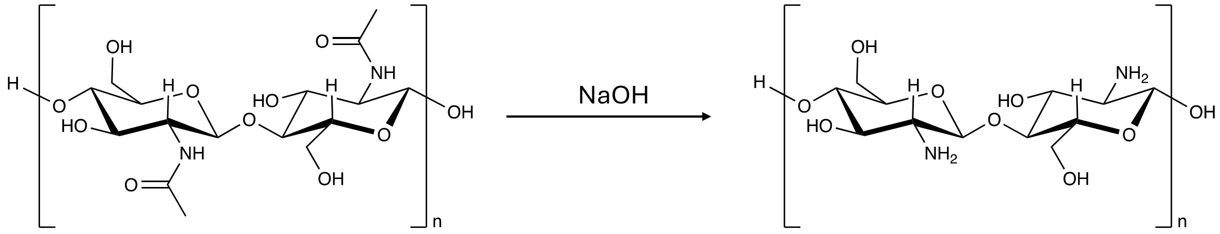


Figure 3. Molecular structure of *κ*-carrageenan.

Table 4 also summarizes the biodegradation results for shrimp chitin and two composting experiments using chitosan. Chitin is the second most abundant polysaccharide found in nature—cellulose is the first. It plays an important role in the structural integrity of the cell walls of fungi and is also a key element of the exoskeleton of crustaceans (e.g., crab, lobster, and shrimp shells) and insects (e.g., beetles and cockroaches). The chitin used in our study is a water-insoluble carbohydrate isolated from the exoskeleton of marine shrimp and is chemically the polymer of N-acetylglucosamine (Figure 4a). Like chitin, our chitosan is sourced from shrimp shells and is compositionally the deacylated form of chitin (Figure 4b). In our studies, chitin steadily biodegraded > 95%, revealing no priming effects. Curiously, in opposition to published results, low MW linear chitosan with 76% deacetylation exhibited limited mineralization in municipal compost [55]. Instead, chitosan remained persistent until the powder was slurried in an aqueous HCl-adjusted pH 6 solution, dried, and then introduced to the compost. Ultimately, the acid-treated chitosan slowly biodegraded *ca.* 40% in 180 days, clearly substantiating that deacetylating chitin decelerates mineralization kinetics.



(a) (b)

Figure 4. Chemical conversion of (a) chitin to (b) chitosan.

Galactomannans are polysaccharides with β-(1,4)-mannopyranose (M) backbones and *α*-(1,6)-galactopyranose (Ga) linked side groups, whereupon the M/Ga ratio of the seed extract varies depending on the plant source from which it is derived. Table 4 documents the biodegradability results for guar, locust bean, and cassia gums, which have M/Ga ratios of 2:1, 4:1, and 5:1, respectively. The data suggests that higher mannose content leads to greater initial biodegradation (absolute), in which cassia gum (89%) > locust bean gum (80%) > guar gum (60%) after 10 days of composting. However, after 90 days of thermophilic composting, each polysaccharide similarly mineralized *ca.* 100% (relative). Interestingly, the initial biodegradability of galactomannans in compost contrasts with trends in water solubility, wherein the solubility of galactomannans increases with increasing galactose content. CGG is a derivatized form of guar gum which includes a hydroxypropyl group on the pendant D-galactose unit (Figure 5). Table 4 also documents mineralization data for chemically modified guar gum polymers, including CGG-1, CGG-2, and CGG-3, and demonstrates that CGGs were degraded by exocellular gumases and/or galactomannanases *ca.* 60-70% (absolute) in less than 30 days, while mineralizing > 85% (relative) in ≤ 90 days. Comparing the mineralization rates of natural guar gum to the examined CGG grades, quaternization of guar gum appears to slow but not obstruct the extent of biodegradation.

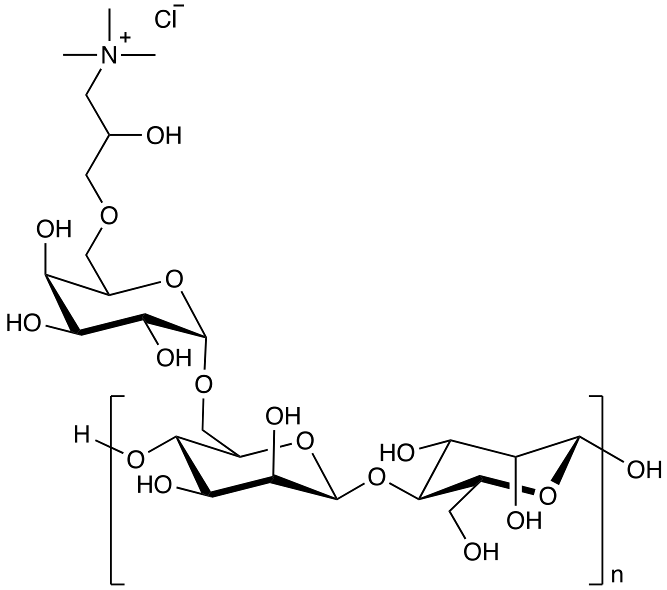


Figure 5. Molecular structure of CGG.

Additionally, Table 4 contrasts the biodegradability of MCC against commercial grades of CMC. MCC-1 (DP = 309), MCC-2 (DP = 309), and SigmaCell Type 20 (DP = 321) MCC grades have much lower DP values than *α*-cellulose (DP = 4,389); furthermore, average particle sizes for the powders vary between D_{v50} = 20 and 120 µm, with MCC-2 and α-cellulose having the largest particles [56,57]. Consequently, trends in Table 4 demonstrate that higher MCC powder mineralization rates are more associated with lower MW cellulose grades rather than a specific particle size distribution. CMC is derived from cellulose, a polysaccharide that is the most abundant biopolymer in nature and found in the cell walls of plants as well as in wood and cotton. CMC is used extensively as a rheology modifier, film former, and water retention agent in cosmetic preparations. The MW trends are as follows: CMC-4 (10^6 Da) > CMC-3 >> CMC-1 (10^5 Da). As indicated by the structural formula in Figure 6, there are several possible chemical structures that may evolve during the carboxymethyl substitution of cellulose. For example, carboxymethylation can take place at any of the three hydroxyl substituents on each anhydrous cellulose ring, wherein the degree of substitution (DS) is the average number of hydroxyl groups on the ring that have been substituted with carboxymethyl moieties. The examined CMC lots possess statistically similar DS. In distinction, there are significant particle size differences between the CMC samples: CMC-3 powder (*ca.* D_{v50} = 500 µm) and CMC-4 film shards (*ca.* 1 cm x 1 cm x 2 mm) have > 10-fold larger particle sizes than CMC-1 and CMC-4 powders. Comparisons of mineralization rates between the CMC grades demonstrate that vessels containing larger CMC particles showed higher initial biodegradation rates: in 10 days, CMC-3 (59%) and CMC-4 film shards (21%) mineralized polymeric carbon more rapidly than CMC-1 (12%) and CMC-4 powders (10%). One likely reason for the initial trend is that larger CMC particles less intimately complex compost particles, wherein rapid CMC dissolution can raise the bulk compost viscosity and deleteriously affect air and moisture diffusion in the composting vessels. Consequently, higher initial mineralization rates for the CMC grades are associated with particle morphology rather than DP, MW, or chemistry. However, CMC-1 and CMC-4 powders ultimately mineralized carbon more completely, indicating that slower erosion of larger particles in CMC-3 powder and CMC-4 film shards appears to limit biofragmentation. In summary, the biodegradation results demonstrate that MCC mineralized more rapidly than the CMC lots, suggesting that our municipal compost microorganisms are more suitably adapted for depolymerizing unmodified cellulose; however, CMC-1 powder, CMC-3 powder, CMC-4 powder, and CMC-4 film shards ultimately mineralized ≥ 90% on relative basis in < 90 days.

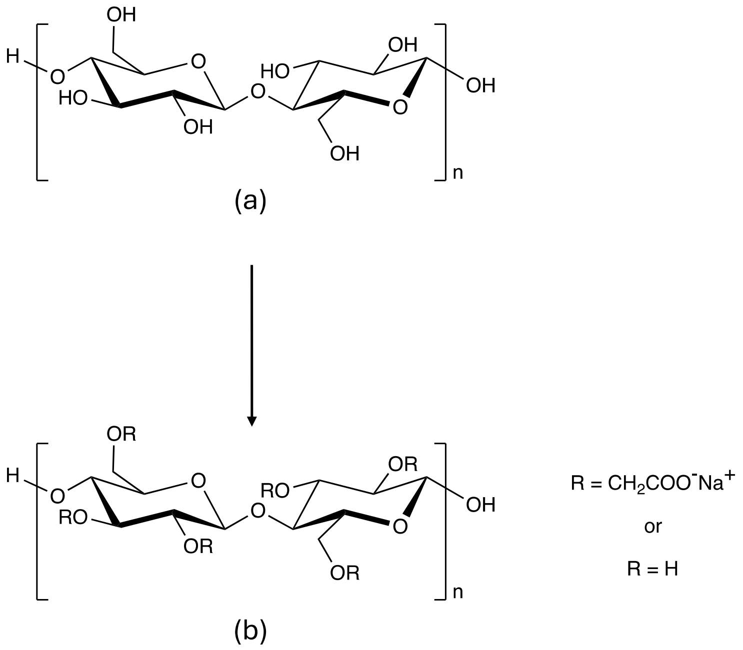


Figure 6. Conversion of (a) cellulose into the sodium salt of (b) CMC.

The remaining carbohydrate in Table 4 is citrus peel pectin. Citrus peel pectin is a structural heteropolysaccharide, and is used as a gelling agent, especially in the food industry. Pectins consist of linear backbones of α-(1,4)-D-galacturonic acid residues partially esterified with methanol. In

doi:10.20944/preprints202403.1813.v1

addition, neutral sugar side chains and periodic L-rhamnose residues introduce irregularities in the backbone [58]. Citrus peel pectin demonstrated positive priming by mineralizing 120% in 90 days, suggesting that pectin increased the microbial degradation of compost carbon while lytic enzymes such as exocellular esterases, depolymerases, and pectinases successfully mineralized pectin chains [59].

3.3. Biodegradation of Proteins

Table 5 summarizes the biodegradability of several proteins in municipal compost. Proteins with predominantly linear polyamide backbones, including zein, bovine gelatin, and hydrolyzed soy protein degraded ≥ 90% in less than 20 days. In addition, hydrolyzed soy protein demonstrated positive priming, in which > 115% of theoretical CO_2 was measured at 15 days. Interestingly, bleached European dark brown keratin fiber snippets (Figure 7A) fully mineralized, on a relative basis, whereas virgin snippets only decomposed *ca.* 11% after 90 days. The result suggests that the municipal compost microbiome lacked specialized enzymes such as disulfide reductase for initiating cystine breakage but possessed effective proteases for subsequent catabolysis of linear polypeptides. Evidence of structural differences between the virgin and oxidized fibers was provided by wet DSC [60]. Prior to composting, most disulfide bonds in the bleached keratin fibers snippets were proven chemically oxidized to cysteic acid, wherein analysis of the endotherm presented $T_D = 117.4 \pm 1.2$ °C and $\Delta H_D = 5.8 \pm 0.6$ J/g. In contrast, the amorphous matrix viscosity, intermediate filaments, and interphase domains of the virgin fibers were predominantly intact, demonstrating moisture-corrected wet DSC values of $T_D = 142.0 \pm 0.1$ °C and $\Delta H_D = 20.0 \pm 0.9$ J/g. At the 7-days mark (*ca.* 62% biodegradation), SEM was used to image the biofragmentation of bleached keratin. Figures 7B-F clearly illustrate evidence of microbial attack on the chemically oxidized hair snippets, indicating that bleached fibers are susceptible to cross-cuticle and cortical fragmentation by various keratinolytic and/or generalized proteolytic enzymes.

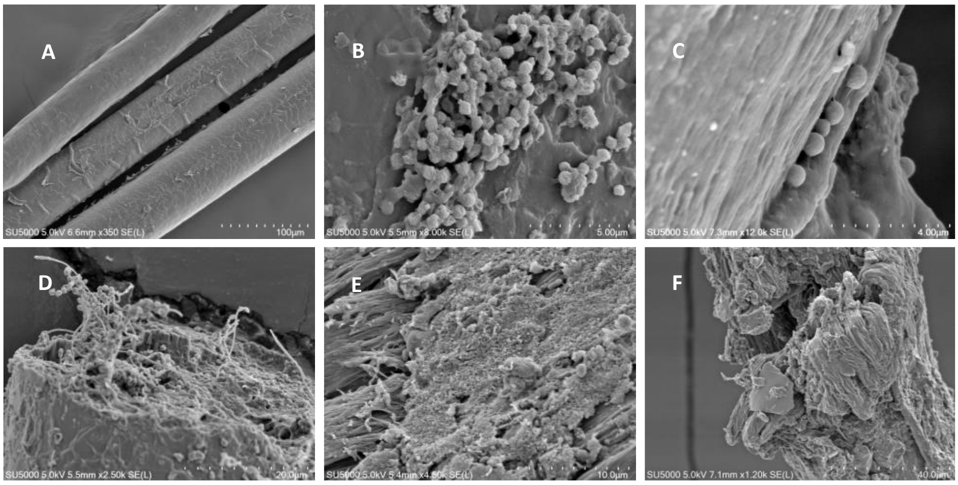


Figure 7. Effect of microbial activity on the composition and microstructure of bleached European dark brown hair fibers: A) Bleached fiber snippets prior to thermophilic composting (day 0); B-E) evidence of microbial growth (likely *M. brevicaulis*) on the surface of composted fiber snippets (day 7); E-F) enzymatically compromised ultrafine structure of composted bleached hair fiber snippets (day 7).

3.4. Biodegradation of Synthetic Polymers

Conventionally, synthetic polymers have been used in personal care products due to their purity, performance benefits, and acceptable toxicological profiles. Table 6 details composting results for the synthetic polymers. As expected, polyethylene and PS powders showed negligible biodegradability in municipal compost. Interestingly, the two grades of 30 kDa PVA (*ca.* 85 and 98% hydrolyzed) demonstrated insignificant biodegradability (*ca.* 1-3%) as well. The low biodegradability of PVA in compost differs from outcomes reported in published enhanced OECD 301B aquatic studies, which report > 60% degradation in less than 60 days [61]. In OECD biodegradation studies, PVA is studied in dilute aqueous solution (100-400 ppm), and the PVA chains are less likely to aggregate into crystal structures; however, in composting PVA is 3-8% (w/w) of the composting mixture; and, during testing, we noted that PVA particles remained intact, suggesting that the water in the compost had little effect on disrupting interchain hydrogen bonding and the inherent crystallinity of PVA. Hence, it is probable that successful biodegradation of PVA in OECD aquatic studies is linked to particle morphology and acclimated aquatic sludge microbiota and implies that our local municipal compost lacks the compulsory microbiome and adapted exocellular enzymes to hydrolyze 1,3-diol repeating units in PVA. Like polyethylene, PS, and PVA synthetics, crosslinked pAA and poly(OAA/Acrylates/BAEM) contain persistent -C—C- backbones and are common polyacrylates that are routinely formulated into commercial hair care styling gels and sprays. Although the crosslinked anionic thickener showed negligible biodegradability, poly(OAA/Acrylates/BAEM) demonstrated 15% biodegradability in municipal compost; however, increasing mineralization ceased after *ca.* ten days.

The remaining synthetics, including PCL, PLA, and polycaprolactam, contain hydrolyzable ester and amide chain backbones. PCL (30 g) showed the swiftest biodegradability in municipal compost, fully degrading in < 60 days. PCL is a thermoplastic polyester derived from petroleum and, due to its low glass transition (T_g = -60 °C) and melting temperatures (T_m = 60 °C), the polymer softens at the ASTM D5338 thermophilic testing temperature. Although PCL (30 g) mineralized, it should be noted that PCL charged at 100 g solids did not fully degrade—instead plateauing at 32% mineralization. One plausible explanation for poorer mineralization at higher polymer solids may be souring of the compost, where the lower pH of the composted plastic matrix catalyzed excessive hydrolysis of PCL (100 g), which in turn lowered the pH of the composted mixture from pH 7.4 to pH 5.7. Compared to PCL, PLA (30 g) also fully degraded, but the rate of enzymatic hydrolysis was much slower than PCL (30 g), requiring > 90 days to fully mineralize. The slower mineralization rate may be partially linked to differences in thermal properties, wherein PLA is a bio-based thermoplastic with higher T_g (60 °C) and T_m (170 °C) values.

Polycaprolactam is a tough, hydrolytically stable polyamide (Nylon 6) with T_g = 50 °C and T_m = 210-220 °C. In contrast to the polyesters, polycaprolactam degraded only 10%, indicating that unfragmented polycaprolactam remained solid and semicrystalline during thermophilic composting. This result advocates that exocellular enzymes such as lipases, esterases, and proteases may selectively degrade proteins, but do not necessarily catalyze the chain scission of all polyamides.

3.5. Seed Germination Studies

We conducted phytotoxicity assessments of the biodegradable bioplastics and synthetic polymers by determining the seed germination and initial growth behavior of radish, turnip, and barley seeds in a mixture containing the composted plastics. Table 7 provides the seed germination success results for all biodegradable polymers, revealing that each plastic mineralized and produced compostable mixtures with suitable seed germination properties. Specifically, ASTM D6400 and OECD Guideline 208 require that ≥ 78% of radish seeds, ≥ 77% of turnip, and ≥ 82% barley seeds must germinate in a timely manner to pass terrestrial safety standards. In addition, for polymers demonstrating successful biodegradability and germination, Figure 8 illustrates the requisite average growth rates for radish, turnip, and barley seedlings in neat compost (n=18), wherein each curve is bounded by ± 10% pass criteria limits defined by ASTM D6400 and OECD Guideline 208. In our studies, all polymers that germinated also demonstrated successful 15-days shoot growth rates [47,50].

It should be noted that although PCL (30 g) was deemed compostable, the polyester showed unacceptable biodegradation and germination performance when the mass of PCL in the compost was increased to the ASTM D5338 recommended 100 g. Akin to ineffective biodegradation, seed germination failure was likely related to increased hydronium concentration and souring of the bulk compost matrix, whereupon the composted PCL matrix was evaluated as weakly acidic (pH = 5.7).

Table 7. Compost terrestrial safety of biodegradable polymers: seed germination success summary (15 days growth).

Polymer ID	% Germinated (Radish)	% Germinated (Turnip)	% Germinated (Barley)	Pass/Fail
Amylopectin	94	89	94	Pass
CMC-1	83	83	89	Pass
CMC-3	89	89	100	Pass
CMC-4	83	83	83	Pass
MCC-1	94	89	89	Pass
MCC-2	89	89	89	Pass
κ-Carrageenan	89	89	94	Pass
Cassia gum	89	89	94	Pass
Chitin	94	94	83	Pass
Compost	89	83	89	Pass
Gelatin	94	89	89	Pass
Guar gum	100	89	94	Pass
Keratin (bleached hair)	94	94	100	Pass
Locust bean gum	94	94	89	Pass
CGG-1	100	89	94	Pass
PCL (30 g)	94	89	89	Pass
Pectin	89	89	94	Pass
PLA (30 g)	94	94	94	Pass
Potato starch	94	100	100	Pass
SigmaCell Type 20	94	94	89	Pass
Sodium alginate	89	83	94	Pass
Soluble starch	83	89	83	Pass
Soy protein acid hydrolysate	94	94	89	Pass
CGG-2	94	100	89	Pass
CGG-3	94	89	100	Pass
Xanthan gum	94	83	83	Pass
Zein	94	94	83	Pass
α-cellulose	100	94	94	Pass

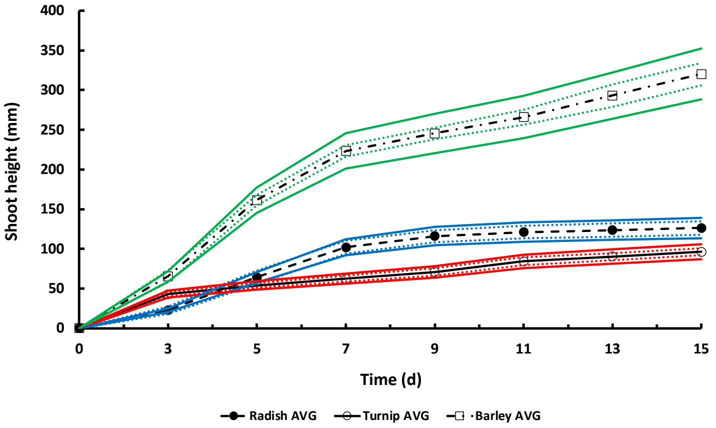


Figure 8. Growth rate of seedlings in neat compost. Terrestrial safety studies indicate that barley grows more rapidly in neat compost than radish and turnip plants. The 95% confidence intervals (dotted lines) and mean ± 10% bands (solid lines) bracket the mean shoot height values as a function

of time for each seed type. As per guidelines in ASTM D6400, the seedlings were germinated in a 1:2 (w/w) mixture of composted plastics and neat compost.

3.5. Comparing Aquatic and Composting Degradation Rates

Unlike OECD aquatic biodegradability testing, ASTM D6400 does not incorporate acceptability criteria for labeling a system as inherently, readily, or ultimately biodegradable by assessing short-term mineralization rates. Instead, a system simply passes or fails ASTM D5338, and ASTM D6400 subsequently labels the plastic as biodegradable if the polymeric carbon is mineralized ≥ 90% in ≤ 6 months. Nevertheless, for research purposes, we informally contrasted internal 28-days OECD aquatic degradation rate data (unpublished) against 28-days composting results.

Figure 9 compares select polymer mineralization outcomes from aquatic testing using OECD 301D [23], OECD 306 [24], and OECD 302B [26] against respective composting results from ASTM D5338 composting studies. Table 4 and Figure 9 indicate that CGG, cellulose, and CMC demonstrated steady biodegradation in municipal compost. Additionally, keeping in mind that OECD testing is typically restricted to 28 days at 20-28 °C, it is fair to conclude that CGG, cellulose, and CMC likewise demonstrated significant biodegradability in aqueous media treated with activated sludge and OECD 301D methodology. Furthermore, the results reveal that compost inocula mineralized water-insoluble cellulose more rapidly than sludge microbiota; however, the data in Figure 9 also indicates that higher MW chains (DP=4,389) inhibited the biofragmentation rate of cellulose in both municipal compost and aqueous media inoculated with activated sludge. In comparison, the aqueous mineralization of CGG in compost was significantly slower than fragmentation rates measured in water that had been inoculated with activated sludge using the more aggressive OECD 302B biodegradation testing protocol. Finally, composting and aquatic biodegradability rates were contrasted for PCL and PVA, in which OECD 306 and OECD 301D were used, respectively, to assess aquatic biodegradability. Interestingly, PCL displayed inherent biodegradability in seawater, while also degrading quickly in compost (> 60% in 28 days using 30 g PCL). Conversely, PVA yielded mixed results, in which PVA exhibited persistence in municipal compost, while demonstrating inherent biodegradability in an aquatic environment using activated sludge and OECD 301D.

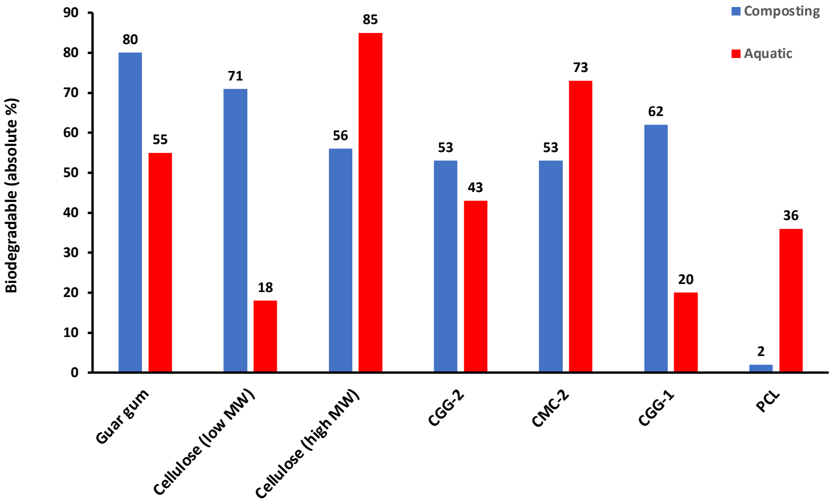


Figure 9. Comparison of 28-days biodegradation rates using aquatic and ASTM D-5338 composting protocols. The following OECD aquatic methods were applied: 301D (low MW cellulose = SigmaCell Type 20; high MW cellulose = α-cellulose; CMC-2; PVA); 302B (CGG-1; CGG-2); 306 (guar gum; PCL). See References 23, 24, and 26 for method details for the OECD protocols.

OECD aquatic biodegradation protocols are performed mesophically (10-42 °C), whereas ASTM D5338 testing is accomplished thermophically (> 42 °C); hence, we expected notable differences in biodegradation that could be linked to the 58 °C testing temperature, physical phase of the tested polymers, active microbiome diversity, and the distribution of lytic enzymes. Nevertheless, the results generally suggest that materials which degrade in aqueous media also degrade in municipal compost; however, the biodegradability data for PVA, PCL, and chitosan (discussed in Section 3.2) suggests that positive correlations between OECD aquatic and ASTM D5338 outcomes should be measured and not assumed (see Figure 9). Furthermore, standard OECD aquatic protocols use ≤ 400 ppm of polymer, whereas our composting studies included *ca.* 5-15% (w/w) resin; consequently, polymer degradation rates in water and compost should not be expected to correlate linearly, with CMC-2 being a notable exception ($R^2=0.99$). That is—in most cases—we observed that the initial mineralization rates of dilute and biodegradable polymer chains in aqueous media superseded the biodegradation rates of solid polymer aggregates dispersed in a solid matrix of damp municipal compost particles.

4. Conclusions

Although aquatic testing biodegradation protocols are primarily used to assess the enzymatic degradation of polymeric waste from rinsed off personal care formulations, we believe it is worthwhile to consider the fate of municipal sludge and its potential use in downstream home garden and agricultural applications. For example, in research performed in Spain, the number of microplastics isolated from sludge-treated crop fields increased by *ca.* 700 microplastic particles per kilogram of soil with each additional batch of sludge—and the distribution of microplastic contaminants in the soil was ubiquitous, where non-degrading plastic particles were isolated from 97% of analyzed soil samples (35). In another study, biodegradable films comprising polybutylene adipate terephthalate (PBAT) and PLA/polyhydroxyalkanoates (PLA/PHA) were found to disintegrate > 99%; however, after the study, significant numbers of other nano- and microplastics were isolated from the compost (36). Results from a third study suggest that microplastics may indeed influence the vigor of beneficial soil macro- and microorganisms—in the analysis, polyethylene particles were added to sludge and were found to negatively affect the health and production of vermicomposting earthworms, as well as the intrinsic biodiversity of bacteria in the biosolids (37). Other studies examined the sluggish mineralization kinetics of modern synthetic plastics: using carbon isotope assays and personal care polymers, Gaytán and Wilske review the fate of recalcitrant polyacrylates and demonstrate that mineralization rates in cultured aqueous media (0.200 %/day) are much different than decomposition rates observed in soil (0.001 %/day) (38-40). Additionally, according to a recent review, digestive enzymes that degrade persistent plastics have been isolated from bacteria, fungi, and the gut bacteria of insects—including enzymes that fragment polyethylene, PS, polypropylene, polyurethanes, PVC, and PET; however, enzymatic biodegradation of these recalcitrant polymers is quite slow, amounting to the conversion of merely 0.1% (w/w) polymeric carbon to CO_2 each year (41).

In our studies, the majority of examined bioplastics demonstrated satisfactory disintegration, biodegradation, seed germination, and shoot growth rates, which are traits of compostable materials using ASTM D5338 methodology and ASTM D6400 pass level criteria. Contrary to the performance of most carbohydrates, proteins, and labile polyesters, macromolecules designed with -C—C- backbones resisted carbon mineralization in composting experiments, suggesting that our municipal compost lacks the thermophilic microbiome and associated enzymatic activity to successfully hydrolyze carbon-carbon chain backbones. Regarding the applicability of ASTM D5338 for modeling the deposition and mineralization of water-soluble personal care polymers in compost-amended soil—dissolution, dilution, and diffusion of resin chains in municipal waste streams likely diminish the influence of bulk particle properties on polymer biodegradation rates. In addition, ASTM 5338 was designed to examine the biodegradation of plastics in municipal compost at 58 °C, whereas the authentic range of bioactivity in soil is *ca.* 10-30 °C (i.e., mesophilic temperature range). Hence, to appropriately research the biodegradability of very dilute polymer concentrations in sludge, compost, or soil, the standard biodegradation protocol, test sample, and respirometry instrumentation should be adapted to minimize polymer chain aggregation, while simultaneously targeting the expected biodegradation environment and optimizing the CO_2 detection limit of the respirometer.

Author Contributions: Conceptualization, T.G.; methodology, T.G. and W.T.; software, T.G. and W.T.; validation, T.G., H.G. and R.M.; formal analysis, T.G., R.M. and H.G.; investigation, T.G, W.T. and H.G.; resources, R.M. and H.G.; data curation, W.T.; writing—original draft preparation, T.G., R.M. and H.G.; writing—review and editing, R.M., H.G. and T.G.; visualization, T.G., H.G. and R.M.; supervision, T.G., R.M. and H.G.; project administration, T.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgements: Special thanks to Charles Duprey and NaturCycle LLC for generously supplying the municipal compost. The authors further recognize Waldo Alvarado, Charles Leiba, Marie Lougheed, Russell Meade, John Sorce, Paul Suszczynski, and Dave Toeltl of Ashland Inc., and Tim Hans of Columbus Instruments LLC, for their dedicated efforts in flawlessly assembling the Bridgewater composting laboratory; and to Dan Brown, Larry Feeley, Joe Sosnowik, Wayne Xu, and Drs. Nigel Crabtree, Carrie Jantzen, Osama Musa, Sangrama Sahoo, and Fan Wu of Ashland Inc. for supporting analytical measurements, technical guidance, and/or project funding.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lebreton, L., Andrady, A. Future scenarios of global plastic waste generation and disposal. *Palgrave Commun.*, **2019**, *5*. Available online: doi: 10.1057/s41599-018-0212-7, accessed April 11, 2022.
2. U.S. Environmental Protection Agency (EPA) (2020) Advancing sustainable materials management: 2018 fact sheet. Available online: https://www.epa.gov/sites/default/files/2021-01/documents/2018_ff_fact_sheet_dec_2020_fnl_508.pdf, accessed April 11, 2022.
3. Polymers in liquid formulations—technical report: a landscape view of the global plfs market, Royal Society of Chemistry. Available online: https://www.rsc.org/globalassets/22-new-perspectives/sustainability/liquid-polymers/rsc-polymer-liquid-formulations-technical-report.pdf, accessed October 17, 2022.
4. Bashir, S., Kimiko, S., Mak, C., Fang, J., Gonçalves, D. Personal care and cosmetic products as a potential source of environmental contamination by microplastics in a densely populated Asian city. *Front. Mar. Sci.* **2021**, *8*. Available online: doi: 10.3389/fmars.2021.683482.
5. Cousins, I.T., Ng, C.A., Wang, Z., Scheringer, M., Why is high persistence alone a major cause of concern? *Environ. Sci.: Processes Impacts* **2019**, *21*, 781-792.
6. Rozman, U., Kalčíková, G. The first comprehensive study evaluating the ecotoxicity and biodegradability of water-soluble polymers used in personal care products and cosmetics. *Ecotoxicol. Environ. Saf.* ***2021***, *228*. Available online: doi: 10.1016/j.ecoenv.2021.113016.
7. Miller, M.E., Hamann, M., Kroon, F.J. Bioaccumulation and biomagnification of microplastics in marine organisms: a review and meta-analysis of current data. *PloS one* **2020**, *15*. Available online: doi: 10.1371/journal.pone.0240792.
8. ECHA Annex XV Restriction Report - Proposal for a Restriction:Intentionally added microplastics, Version 1.2. Available online: https://echa.europa.eu/documents/10162/05bd96e3-b969-0a7c-c6d0-441182893720, accessed November 7, 2019.
9. Senak, L., McMullen, R.L. Dilute Solution Properties and Characterization of N-Vinyl Pyrrolidone Based Polymers. In *Handbook of Pyrrolidone and Caprolactam Based Materials*, Musa, O.M., Ed.; John Wiley & Sons: New York, USA, 2022; pp. 1313–1364.
10. Halima, N.B. Poly(vinyl alcohol): review of its promising applications and insights into biodegradation. *RSC Adv.* **2016**, *6*, 39823-39832.
11. Chamas, A., Moon, H., Zheng, J., Qiu, Y., Tabassum, T., Jang, J., Abu-Omar, M., Scott, S., Suh, S. Degradation rates of plastics in the environment. *ACS Sustain. Chem. Eng.* **2020**, *8*, 3494-3511.
12. Zeenat, Elahi, A., Bukhari, D., Shamim, S., Rehman, A. Plastics degradation by microbes: a sustainable approach. *J. King Saud Univ. Sci.* **2021**, *33*. Available online: doi: 10.1016/j.jksus.2021.101538.
13. Yousif E., Haddad, R. Photodegradation and photostabilization of polymers, especially polystyrene: review. *Springerplus* **2013**, *2*. Available online: doi: 10.1186/2193-1801-2-398.
14. Lucas, N., Bienaime, C., Belloy, C., Queneudec, M., Silvestre, F., Saucedo, J. Polymer biodegradation: mechanisms and estimation techniques—a review. *Chemosphere*, **2008**, *73*, 429-442.
15. Bilal, H., Raza, H., Bibi, H., Bibi, T. Plastic biodegradation through insects and microbes. *J Biores. Manag.* **2021**, *8*, 95-103.

16. De Wilde, B., Mortier, N., Verstichel, S., Briassoulis, D., Babou, M., Mistriotis, A., Hiskakis M. Report on current relevant biodegradation and ecotoxicity standards, kbbpps work package 6: biodegradability, deliverable 6.1 (2013). Available online: http://www.biobasedeconomy.eu/research-knowledge/kbbpps-knowledge-based-bio-based-products-pre-standardization, accessed April 8, 2022.
17. Béguin, P. Aubert, J-P. The biological degradation of cellulose, *Microbiol. Rev.* **1994**, *13*, 25-58.
18. Dimarogona, M., Topakas, E., Christakopoulos, P. Cellulose degradation by oxidative enzymes, *Comput. Struct. Biotechnol. J.* **2012**. Available online: doi: 10.5936/csbj.201209015.
19. Kaur, A., Rishi, V., Soni, S.K., Rishi, P. A novel multi-enzyme preparation produced from *Aspergillus niger* using biodegradable waste: a possible option to combat heterogeneous biofilms, *AMB Express* **2020**, *10*. Available online: doi: 10.1186/s13568-020-00970-3.
20. Giri, T.K. Chapter 15: Chitosan Based Nanoparticulate System for Controlled Delivery of Biological Macromolecules. In *Nanomaterials for Drug Delivery and Therapy*, Grumezescu, A.M. Ed.; William Andrew Publishing: Norwich, NY, USA, 2019; pp. 435-459.
21. Huang, C. Li, X., Du, Y. Biodegradation of xanthan by newly isolated *Cellulomonas sp.* lx, releasing elicitor-active xantho-oligosaccharides-induced phytoalexin synthesis in soybean cotyledons. *Process Biochem.* **2005**, *40*, 3701-3706.
22. Sarian, F.D. van der Kaaij, R.M., Kralj, S., Wijbenga, D.J., Binnema, D.J., van der Maarel, M.J., Dijkhuizen, L. Enzymatic degradation of granular potato starch by *Microbacterium aurum* strain B8.A. *Appl. Microbiol. Biotechnol.* 2012, **93**, 645–654.
23. *OECD (1992)*; Test No. 301: Ready Biodegradability, OECD Guidelines for the Testing of Chemicals, Section 3. OECD Publishing: Paris, France. Available online: doi: 10.1787/9789264070349-en, accessed April 2, 2022.
24. *OECD (1992)*; Test No. 306: Biodegradability in Seawater, OECD Guidelines for the Testing of Chemicals, Section 3. OECD Publishing: Paris, France. Available online: doi: 10.1787/9789264070486-en, accessed April 2, 2022.
25. *OECD (1981)*; Test No. 302A: Inherent Biodegradability: Modified SCAS Test, OECD Guidelines for the Testing of Chemicals, Section 3. OECD Publishing: Paris, France. Available online: doi: 10.1787/9789264070363-en, accessed April 2, 2022.
26. *OECD (1992)*; Test No. 302B: Inherent Biodegradability: Zahn-Wellens/ EVPA Test, OECD Guidelines for the Testing of Chemicals, Section 3. OECD Publishing: Paris, France. Available online: doi: 10.1787/9789264070387-en, accessed April 2, 2022.
27. *OECD (2009)*; Test No. 302C: Inherent Biodegradability: Modified MITI Test (II), OECD Guidelines for the Testing of Chemicals, Section 3. OECD Publishing: Paris, France. Available online: doi: 10.1787/9789264070400-en, accessed April 2, 2022.
28. OECD (2014); Test No. 310: Ready Biodegradability—CO_2 in sealed vessels (Headspace Test), OECD Guidelines for the Testing of Chemicals, Section 3. OECD Publishing: Paris, France. Available online: doi: 10.1787/9789264224506-en, accessed April 2, 2022.
29. Shchegolkova, N.M, Krasnov, G.S., Belova, A.A., Dmitriev, A.A., Kharitonov, S.L., Klimina, K.M., Melnikova, N.V., Kudryavtseva, A.V. Microbial community structure of activated sludge in treatment plants with different wastewater compositions, *Front. Microbiol*. **2016**, *7*. Available online: doi: 10.3389/fmicb.2016.00090.
30. U.S. Census Bureau (1990); Detailed Housing Characteristics, Structural Characteristics: 1990 (Table 12). Available online: https://www2.census.gov/library/publications/dicennial/1990/ch-2/ch-2-1.pdf, accessed April 2, 2022.
31. Rolsky C., Kelkar, V. Degradation of polyvinyl alcohol in us wastewater treatment plants and subsequent nationwide emission estimate. *Int. J. Environ. Res. Public Health*. **2021**, *18*. Available online: doi: 10.3390/ijerph18116027.
32. Venkatesan, A.K., Done, H.Y., Halden, R.U. United states national sewage sludge repository at Arizona State University—a new resource and research tool for environmental scientists, engineers, and epidemiologists. *Environ. Sci. Pollut. Res. Int.* **2015**, *22*, 1577-1586. Available online: doi: 10.1007/s11356-014-2961-1, accessed April 13, 2022.
33. Environmental Protection Agency, Basic information about biosolids. Available online: https://www.epa.gov/biosolids/basic-information-about-biosolids, accessed April 18, 2022.
34. Hubbe, M.A., Nousa, M. Sánchez, C. Composting as a way to convert cellulosic biomass and organic waste into high value soil amendments: a review. *Bioresources*, **2010**, 5, 2808-2854.
35. P. van den Berg, E. Huerta-Lwanga, F. Corradini, V. Geissen, Sewage sludge application as a vehicle for microplastics in Eastern Spanish agricultural soils, *Environ. Pollut.* **2020**, *261*. Available online: doi: 10.1016/j.envpol.2020.114198.
36. Sintim, H.Y., Bary, A.I., Hayes, D.G., English, M.E., Schaeffer, S.M., Miles, C.A., Zelenyuk, A., Suski, K., Flury, M. Release of micro- and nanoparticles from biodegradable plastic during in situ composting. *Sci. Total Environ.* **2019**, *675*, 686-693.

37. Zhong, H., Yang, S., Zhu, L., Liu, C., Zhang, Y., Zhang, Y. Effect of microplastics in sludge impacts on the vermicomposting. *Bioresour. Technol.* **2021**, *326*. Available online: doi: 10.1016/j.biortech.2021.124777.
38. Wilske, B., Bai, M., Lindenstruth, B., Bach, M., Rezaie, Z., Frede, H.G., Breuer, L. Biodegradability of a polyacrylate superabsorbent in agricultural soil. *Environ. Sci. Pollut.* **2014**, *21*, 9453-9460. Available online: doi: 10. 1007/s11356-013-2103-1.
39. Gaytán, I., Burelo, M., Loza-Tavera, H. Current status on the biodegradability of acrylic polymers: microorganisms, enzymes and metabolic pathways involved, *Appl. Microbiol. Biotechnol.* **2021**, *105*, 991-1006. Available online: doi: 10.1007/s00253-020-11073-1.
40. Diaz, L., Savage, G.M., Golueke, C.G. Ch. 12: Composting of Municipal Solid Wastes. In *Integrated Solid Waste Management: Engineering Principles and Management Issues*, 2nd ed., Tchobanoglous, G., Kreith., F., Eds.; McGraw-Hill: New York, USA, 2002; pp. 1-70.
41. Mohanan, N., Montazer, Z., Sharma, P.K., Levin, D.B. Microbial and enzymatic degradation of synthetic plastics. *Front. Microbiol.* **2020**, *11*. Available online: doi: 10.3389/fmicb.2020.580709.
42. Grima, S., Bellon-Maurel, V., Feuilloley, P., Silvestre, F. Aerobic biodegradation of polymers in solid-state conditions: a review of physiochemical parameter settings in laboratory simulations. *J. Polym. Environ.* **2000**, *8*, 183-195.
43. ASTM Standard D5338-15 (2015); Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials Under Controlled Composting Conditions, Incorporating Thermophilic Temperatures. ASTM International: West Conshohocken, PA, USA.
44. ISO 14855-1 (2012); "Determination of the Ultimate Aerobic Biodegradability and Disintegration of Plastic Materials under Controlled Composting Conditions—Method by Analysis of Evolved Carbon Dioxide," Organization for Standardization.
45. ISO 14855-2 (2018); "Determination of the Ultimate Aerobic Biodegradability of Plastic Materials under Controlled Composting Conditions—Method by Analysis of Evolved Carbon Dioxide, Part 2: Gravimetric Measurement of Carbon Dioxide Evolved in a Laboratory-Scale Test," Organization for Standardization.
46. EN 13432 (2012); "Packaging—Requirements for Packaging Recoverable Through Composting and Biodegradation—Test Scheme and Evaluation Criteria for the Final Acceptance of Packaging," European Committee for Standardization.
47. ASTM Standard D6400-12 (2015); Standard Specification for Labeling Plastic Designed to be Aerobically Composted in Municipal or Industrial Facilities. ASTM International: West Conshohocken, PA, USA.
48. ASTM D5988-03 (2003); Standard Test Method for Determining Aerobic Biodegradation in Soil of Plastic Materials or Residual Plastic Materials after Composting. ASTM International: Philadelphia, PA, USA.
49. Standard Method 2540 D (2017); Total Suspended Solids Dried at 103–105°C, in Standard Methods for the Examination of Water and Wastewater, 23rd ed., American Public Health Association: New York, USA.
50. OECD (2006); Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, in OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing: Paris, France.
51. Bellia, G., Tosin, M., Degli-Innocenti, F. Test method of composting in vermiculite is unaffected by the priming effect. *Polym. Degrad. Stabil.* **2000**, *69*, 113-120.
52. Fontaine, S., Mariotti, A., Abbadie, L. The priming effect of organic matter: a question of microbial competition? *Soil Biol. and Biochem.* **2003**, *35*, 837-843.
53. Bernard, L., Basile-Doelsch, I., Derrien, D., Fanin, N., Fontaine, S., Guenet, B., Karimi, B., Marsden, C., Maron, P-A. Advancing the mechanistic understanding of the priming effect on soil matter mineralization. *Funct. Ecol.* **2022**, *36*, 1355-1377.
54. Gaenssle, A., Satyawan, C.A., Xiang, G., van der Maarel, M, Jurak, E. Long chains and crystallinity govern the enzymatic degradability of gelatinized starches from conventional and new sources. *Carbohydr. Polym.* ***2021***, *260*. Available online: doi: 10.1016/jcarbpol.2021.117801.
55. Ju, S., Shin, G., Lee, M., Koo, J.M., Jeon, H., Ok, Y.S., Hwang, D.S., Hwang, S.Y., Oh, D.X., and Park, J. Biodegradable chito-beads replacing non-biodegradable microplastics for cosmetics. *Green Chem.* **2021**, *23*, 6953-6965.
56. Kothari, N., Bhagia, S. Zaher, M., Pu, Y., Mittal, A., Yoo, C.G., Himmel, M., Ragauskas, A.J., Kumar, R., Wyman, C.E. Cellulose hydrolysis by *Clostridium thermocellulum* is agnostic to substrate structural properties in contrast to fungal cellulases. *Green Chem.* **2019**, *21*, 2810-2822.
57. Mattonai, M., Pawcenis, D., del Seppia, S., Łojewska, J., Ribechini, E. Effect of ball-milling on crystallinity index, degree of polymerization and thermal stability of cellulose. *Bioresour. Technol.* **2018**, *270*, 270-277.
58. Kanmani, P., Dhivya, E., Aravind, J., and Kumaresan, K. Extraction and analysis of pectin from citrus peels: augmenting the yield from *Citrus limon* using statistical experimental design. *Iranica J. Energy Environ.* **2014**, *5*, 303-312.
59. Khattab, A.M. The Microbial Degradation for Pectin, Pectins—The New-Old Polysaccharides. *IntechOpen*. **2022**. Available online: doi.10.5772/intechopen.100247.
60. Gillece, T., Senak, L., McMullen, R.L. Characterization of bleached hair: vibrational spectroscopy, thermal analysis, and determination of equivalent damage factor. *J. Cosmet. Sci.* **2021**, *72*, 519-546.

61. Byrne, D., Boeije, G., Croft, I., Hüttmann, G., Luijkx, G., Meier, F., Parulekar, Y., Stijntjes, G. Biodegradability of polyvinyl alcohol based film used for liquid detergent capsules. *Tenside Surfactants Deterg.* **2021**, *58*, 88-96.