Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Biodegradable High-Density Polyethylene-like Material

*M. Eck, S. T. Schwab, T. F. Nelson, K. Wurst, S. Iberl, D. Schleheck, C. Link, G. Battagliarin, S. Mecking**

SUPPORTING INFORMATION

Table of Contents

SUPPORTING INFORMATION

General Materials and Methods

Materials

All chemicals were used as received without further purification. 1,18-octadecanedioic acid was purchased from Elevance Renewable Sciences Inc. Dibutyltin oxide (for synthesis), para-nitrophenyl butyrate (≥ 98 %), 1,4-butanediol (99 %) and methanol (≥ 99.8 %) were purchased from Sigma Aldrich. Xylene (isomeric mixture, ≥ 99 %), ethylene glycol (≥ 99.5 %), and di-sodium hydrogen phosphate dihydrate (≥ 98 %) were purchased from Carl Roth. 2-Propanol (≥ 99.7 %) was purchased from VWR. 1,3-Propanediol (≥ 99 %) was purchased from Fluka Analytical. For LC-MS measurements, 2-propanol (≥ 99.9 %) was purchased from Merck, formic acid (98-100 %) from Riedel-de Haën and acetonitrile (≥ 99.9 %) from VWR. Sulfuric acid (96 %), sodium dihydrogen phosphate monohydrate (≥ 99.99 %), and para-nitrophenol (≥ 97 %) were purchased from Merck. High-density polyethylene Purell GB 7250 from LyondellBasell was used as reference material. Deuterated solvents for NMR spectroscopy were purchased from Eurisotop and dried over molecular sieves from Riedel de-Haën (0.4 nm). C_{19} diacid was synthesized as reported previously.[1] For enzyme experiments, Milli-Q (Millipore) water (resistivity = 18.2 MW cm; TOC ≤ 5 ppb) was used to prepare all solutions. All manipulations involving air- and/or moisture-sensitive substances were carried out under inert atmosphere using standard Schlenk and glovebox techniques.

Characterization techniques

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III HD 400 spectrometer. Chemical shifts were referenced to the resonance of the solvent (residual proton resonances for ^{1}H spectra). Mestrenova software by Mestrelab Research S.L. (version 14.1.2) was used for data evaluation.

Molecular weights of polymers were determined by gel permeation chromatography (GPC) in chloroform at 35 °C on a PSS SECcurity^2 instrument, equipped with PSS SDV linear M columns (2 x 30 cm, additional guard column) and a refractive index detector (PSS SECcurity^2 RI). A standard flow rate of 1 mL min^{-1} was used. Molecular weights were determined versus narrow polystyrene standards (software: PSS WinGPC, version 8.32).

Differential scanning calorimetry (DSC) measurements of polymers were carried out on a Netzsch DSC 204 F1 instrument (software: Netzsch Proteus Thermal Analysis, version 6.1.0) with a heating/cooling rate of 10 K min^{-1}. Data reported are from second heating cycles.

Thermogravimetric analysis was performed on a Netzsch STA 429 F3 Jupiter. Measurements were performed with 80 mL/min flowrate of a synthetic 80:20 mixture of N_2:O_2 at a heating rate of 10 K/min from 30 to 1000 °C.

Tensile tests were performed on a Zwick Z005/1446 Retroline tC II instrument at a crosshead speed of 5 mm min^{-1} on injection-molded tensile testing specimens (ISO 527-2, type 5A). The determination of the Young's modulus was conducted at a crosshead speed of 0.5 mm min^{-1}. Prior to tensile testing, the samples were preconditioned at room temperature. The Zwick Roell testXpert software version 11.0 was used for data evaluation.

Wide angle x-ray scattering (WAXS) diffractograms were recorded on a D8 Discover instrument (Bruker) with a Vantec (Bruker) or Lynxeye (Bruker) detector. Polymer crystallinities (χ_{WAXS}) were determined from the WAXS patterns as: $\chi_{WAXS} = [A_c(110)+A_c(200)] / [A_c(110)+A_c(200)+A_a]$ where A_c refers to the integrated area of the Bragg reflections from the orthorhombic PE crystal and A_a to the integrated area of the amorphous halo. A Voigt fit was used.

The surface interfacial tension of PE-2,18 was determined on injection-molded specimens by the method of Fowkes on a drop shape analyzer DSA25 by KRÜSS.[2]

Elemental analyses were conducted by the analytical services laboratory of the University of Konstanz on a UNICUBE analyzer fom Elementar.

Polymerization experiments

In a typical polycondensation for PE-2,18, a Schlenk flask was charged with 1,18-dimethyl octadecanedioate (1.0 equiv., prepared as reported previously[3]), ethylene glycol (2.0 equiv.), dibutyltin oxide (0.5 mol%) and a stirring bar. A cooled condensor flask that allows for monitoring of the volatiles released from the polymerisation mixture was connected and the setup was evacuated and purged with nitrogen. The temperature was raised to 150 °C (heating block temperature) and the mixture was stirred at 500 rpm for 1 h. Oligomerization commenced, and *vacuum* was gradually applied (900 mbar to 10 mbar) over the course of 4 h. The polymerization step was conducted at 150 °C for typically 16 h and at 160 - 180 °C for typically further 5 h at 100 rpm stirring and 2 x 10^{-2} mbar *vacuum*. The resulting polymer was dissolved at 160 °C in xylene, precipitated in -30 °C 2-propanol, washed with acetone and dried in a *vacuum* oven at 50 °C. Note that comparable results could also be obtained by using Ti(O^{n}Bu)$_4$ (0.25 mol%) as a catalyst.

PE-3,18 and PE-4,18 were synthesised from 1,18-dimethyl octadecanedioate and 1,3-propanediol and 1,4-butanediol, respectively, according to the procedure outlined for PE-2,18.

PE-18,18 and 1,18-octadecanediol were prepared as reported previously.[3]

SUPPORTING INFORMATION

Compounding and injection molding of PE-2,18

PE-2,18 was compounded in a Xplore MC 15 micro compounder at 140 °C and 50 rpm for 5 min. Injection-molded test specimens were prepared using a Xplore IM 5.5 micro injection molder. The cylinder and mold temperature were set to 140 °C and 50 °C, respectively, and an injection pressure of 16 bar for 10 s and 12 bar for 15 s was applied.

Chemical recycling of PE-2,18 by methanolysis

In a preliminary chemical recycling experiment, 15.0 g of melt-processed PE-2,18 were subjected to methanolysis (150 ml methanol) by stirring (200 rpm) for 16 h at 120 °C in a glass pressure vessel. The C_{18} monomer was recovered in 91 % yield by recrystallization from the methanolic solution (cf. Figure S8). Ethylene glycol was obtained from the filtrate by removal of methanol under reduced pressure and extraction of the residue with water (cf. Figure S9).

Enzyme activity determination

Enzymatic degradation experiments were carried out with three different commercially available, naturally occurring enzymes, namely the *Humicola insolens* cutinase (ChiralVision, Lipase 51032, StickAway), *Aspergillus oryzae* cutinase (Novozyme, Resinase HT) and *Thermomyces lanuginosus* lipase (Novozyme, Lipozyme TL 100 L). The activities of the enzymes HiC and TlL were tested against the model substrate para-nitrophenyl butyrate (pNPB) using an approach adapted from Ribitsch et al.[4] To this end, phosphate buffered (50 mM, pH = 7.2) pNPB solutions in different concentrations (280 µL, 0.3 –10 mM) were mixed with enzyme solutions (20 µL) in wells of a polystyrene 96-microwell plate. The formation of the hydrolysis product para-nitrophenol (pNP) was followed using a multimode microplate reader (Tecan) by monitoring the absorbance at 405 nm over time at 37 °C. The concentration of the formed pNP was calculated using a standard calibration curve (0 – 40 µM pNP) on the same plate. The measurements resulted in enzyme activities for HiC and TlL of 106 and 87 kat/L of enzyme stock solution, respectively, yielding a relative activity of 0.82 for TlL compared to HiC. Here, an activity of 1 kat corresponds to the release of 1 µmol pNP per second. The activities of AoC and TlL were determined against the substrate glycerol tributyrate by the supplier, and reported as 50 and 100 KLU/g, yielding a relative activity of 0.5 for AoC compared to TlL, and therefore a relative activity of 0.41 for AoC to HiC. Enzyme degradation experiments were carried out based on these relative activities: HiC was added at 2.0 mL enzyme stock solution/L degradation medium, or 0.21 kat/L, TlL was added at 2.4 mL/L, and AoC was added at 4.8 mL/L.

Polymer sample preparation for enzymatic hydrolysis experiments

The polymers PE-2,18 and PE-18,18 (cf. Figure S14 and Figure S16) were embrittled with liquid nitrogen for five minutes and subsequently milled to a fine powder with a Retsch ZM 200 Ultra Centrifugal mill at 14,000 rpm, using a ring sieve with a mesh size of 0.12 mm. The size distribution of the resulting particles was determined *via* laser diffraction using a Mastersizer 3000 (Malvern). To this end, the particles were dispersed in ethanol and stirred at 1000 rpm throughout the measurement process. Assuming spherical particles, the milling lead to particles with a narrow size distribution with a D_{50} of 30.3 ± 0.1 µm and span of 2.57 for PE-2,18 and a D_{50} of 43.7 ± 0.2 µm and span of 1.79 for PE-18,18. The given values are averages and standard deviations originating from 20 consecutive measurements (cf. Figure S15 and Figure S17).

Enzymatic hydrolysis experiments and mononomer quantification

All enzymatic degradation experiments were conducted in triplicate. The enzymatic degradation experiments were carried out in two different set-ups depending on the detected monomer.

For the detection of the monomer ethylene glycol (EG) of PE-2,18, 50 mg of milled polymer particles and 50 mL of enzyme buffer medium were added into a glass bottle and shaken with 200 rpm at the given temperature (37 or 25°C). The medium contained a 50 mM phosphate buffer solution at pH = 7.2 and enzyme stock solution, to reach an activity of 0.21 kat/L in the degradation medium. Samples were taken from the degradation experiments using a plastic syringe, filtered with a PTFE filter with a mesh size of 0.45 µm and added to 0.5 M aq. H_2SO_4 (1:1 v:v). The EG was separated using HPLC with a Rezex RHM-monosaccharide H+ 300 9 7.80 mm 8 lm ion exchange column (Phenomenex). The column was operated at 40 °C and 30 mM of sulfuric acid was used as mobile phase at a flow rate of 0.6 mL/min. For quantification of EG, a refractive index detector RID-20A (Shimadzu) was used and the signals obtained were analyzed with the Shimadzu Lab Solutions software version 5.81. The retention time of EG in this setup was 17.3 minutes. Negative controls containing only phosphate buffer and polymer without added enzyme were carried out for the individual experiments, and showed no detectable EG formation. A calibration curve and an exemplary chromatogram for EG are shown in Figure S20 and Figure S21, respectively.

For experiments comparing the enzymatic degradation of PE-18,18 and PE-2,18, 10 mg of milled polymer particles were added together with 20 mL of the aforementioned enzymatic buffer medium and five glass spheres with a diameter of 1 mm (for better mixing) into a

50 mL centrifuge tube. The tubes were rotated with 20 rpm at 37 °C for one week. To fully dissolve the C_{18} diacid, 20 mL of a 0.2 mM solution of C_{19} diacid as internal standard in 2-propanol were added to the mixture containing degradation medium and polymer particles after completion of the experiment. The resulting mixture was mixed for 30 min at 37 °C, and subsequently filtered with a PTFE filter with a mesh size of 0.45 µm. From this mixture, C_{18} and C_{19} diacid were independently quantified using a LC-MS (LCMS-2020, Shimadzu) with a ODS Hypersil™ C18 column (ThermoFisher Scientific). As mobile phase, a gradient of two solvents (A: 10 vol% acetonitrile and 0.1 vol% formic acid, filled up with MilliQ water, B: 100 % acetonitrile) was used. The gradient started with a 50 % concentration of B raising up to 95 % with a flow rate of 0.3 mL/min. The retention times of C_{18} and C_{19} diacid in this setup were 6.90 and 8.13 minutes, respectively. Calibration curves for both analytes and an exemplary chromatogram can be found in Figure S18 and Figure S19, respectively. Negative controls containing only phosphate buffer and polymer without added enzyme were carried out for the individual experiments, only detecting a negligible amount of C_{18} diacid, corresponding to less than 0.01 % of the polymer repeat units (cf. Figure S22). This signal is observed after a few hours already and stays constant over time, and most likely originates from residual monomer from the polymer synthesis.
Error bars, if shown, originate from standard deviations of triplicates.

Sample preparation for biodegradation tests

The polymer samples were melted at 110 °C under vacuum and a mild nitrogen stream and then cooled to room temperature to obtain homogeneous polymer sheets. The sheets were cut in small pieces and then cryo-milled using a Retsch ZM 300 ultra-centrifugal mill with a sieving unit with a cut-off of 0.5 mm. The isolated fractions with size below the cut-off were collected and dried in a *vacuum* oven at 36 °C for 48 hours. Finally, the polymer particles with size between 100 µm to 300 µm were isolated using a Retsch AS 200 sieving machine.

Biodegradation test under industrial composting conditions

The biodegradation experiments under industrial composting conditions were performed using a down-scaled version of the standard *ISO 14855 – Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions - Method by analysis of evolved carbon dioxide*. 1 L pressure DURAN laboratory glass bottles with a cap with one air inlet, which ended 5 cm over the compost, and one air outlet were used as reactors for the experiments. For each reactor, 118.8 g of 20 week-old compost (OWS Belgium, sieve fraction below 0.5 cm, pH 8.1, water content 46.5 %, volatile solids 31.4 %) were mixed with 1.2 g of 2 week old compost (fraction below 0.5 cm) and 8 g test material. Blank, positive control, and each of the materials were tested in triplicate. The samples were incubated at 58 °C ± 2 °C for 66 days. During incubation, air saturated with 100 % moisture was flushed through the bottles and collected through a GC spectrometer after condensation of the water. During the experiment, the reactors were opened, and the content mixed (twice during the first week, afterwards once per week).

Additional characterization data for polyesters

Additional characterization data for PE-2,18

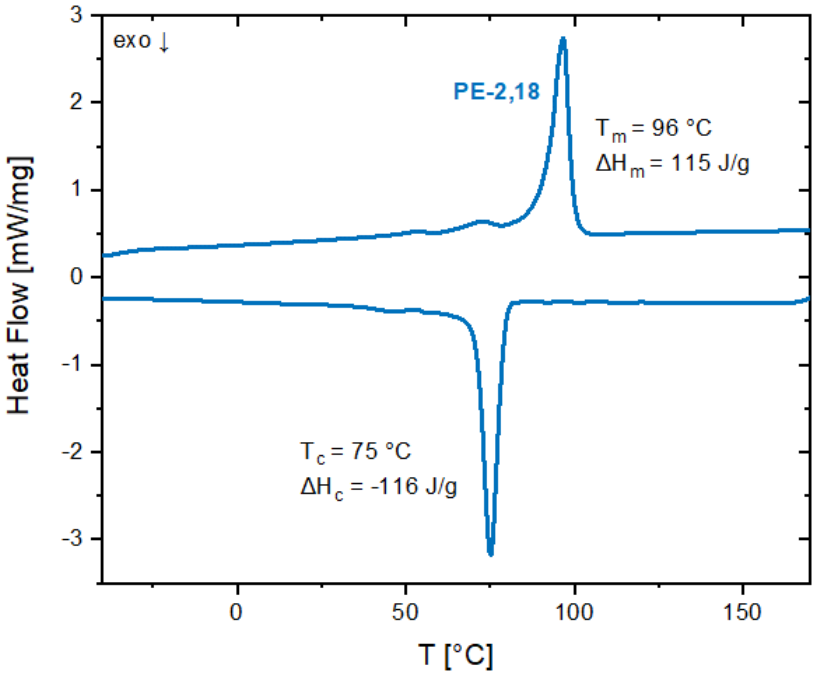

Figure S1. DSC trace of PE-2,18.

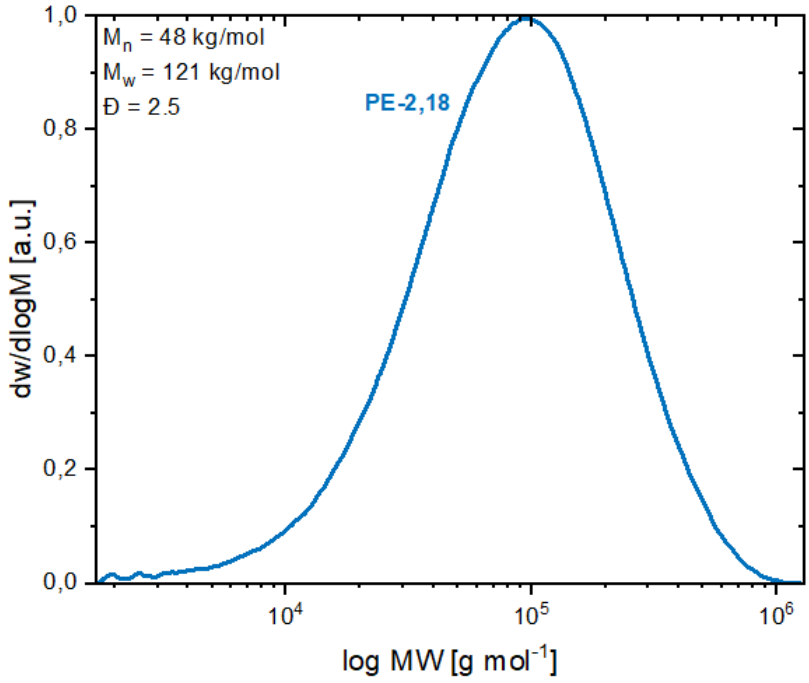

Figure S2. GPC trace of PE-2,18.

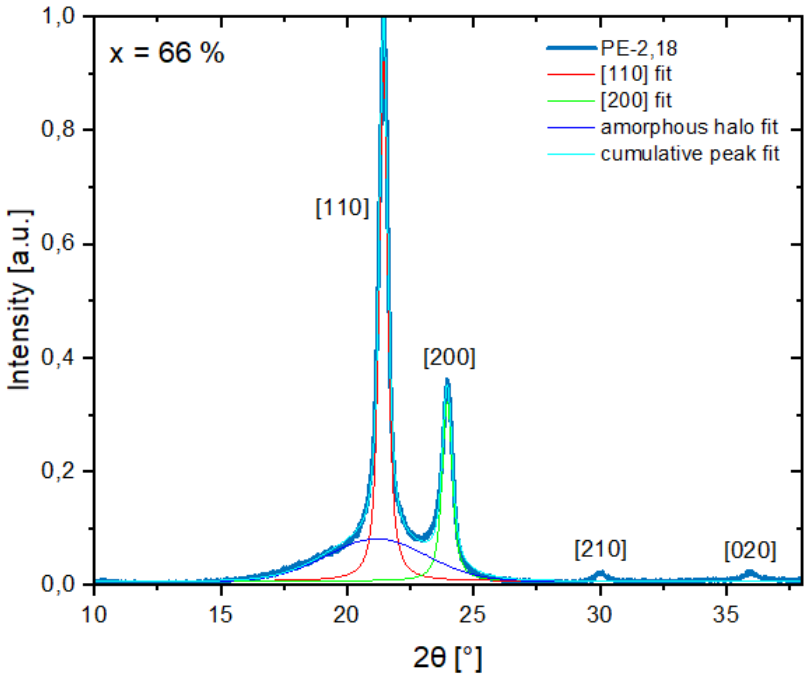

Figure S3. WAXS diffractogram of PE-2,18. The crystallinity determined by deconvolution of the crystalline reflexes and the amorphous halo (Voigt fit) amounts to 66 %.

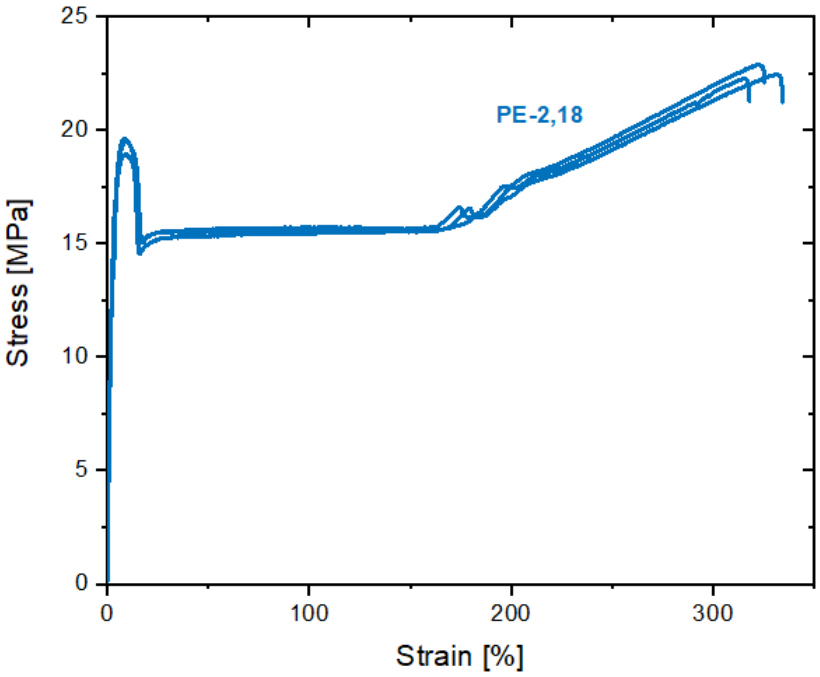

Figure S4. Stress-strain curves of PE-2,18.

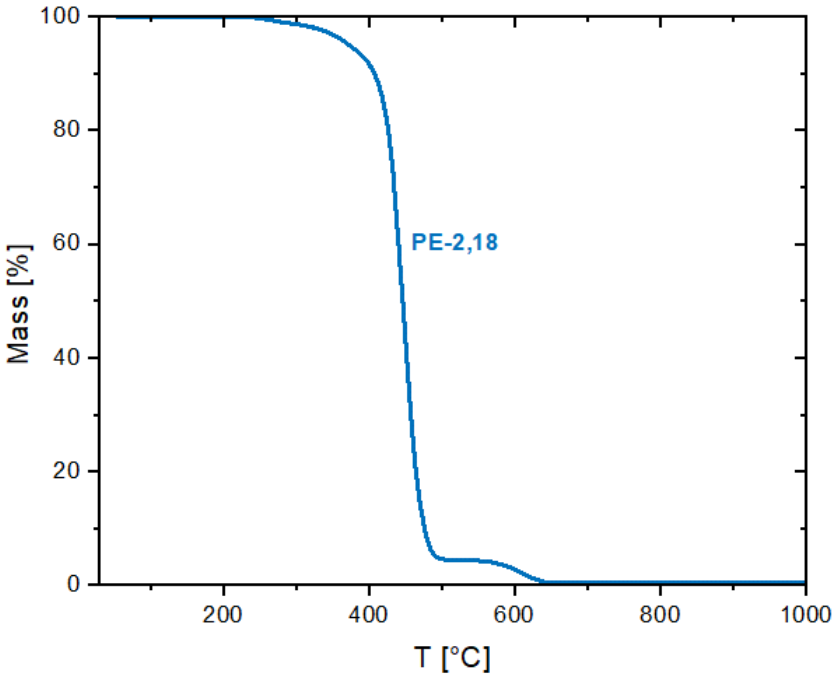

Figure S5. TGA trace of PE-2,18. 5 wt% weight loss was observed at T = 372 °C.

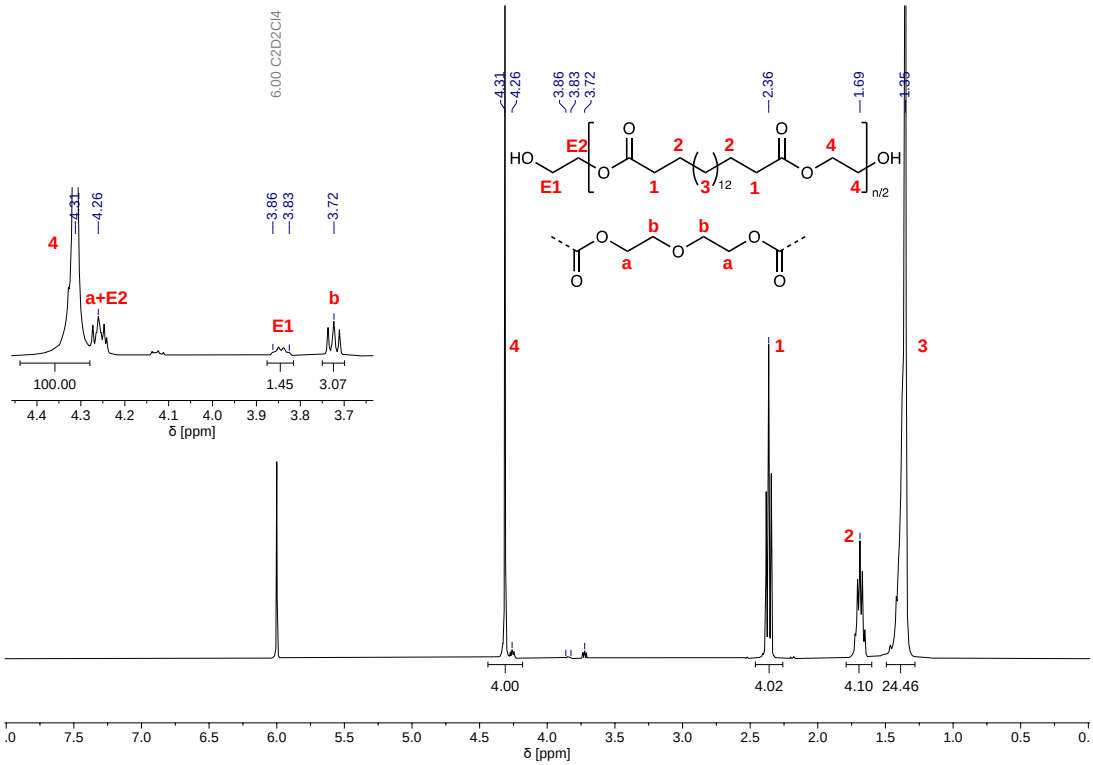

Figure S6. ^{1}H NMR spectrum (400 MHz, $C_2D_2Cl_4$, 383 K) of PE-2,18. The polymer contains negligible amounts of ether linkages (ca. 1.5 mol% diethylene glycol content), ether formation being a common side reaction in polyesterification reactions of the diols studied here (commercial PET: 2 – 4 mol% diethylene glycol content).[5] The resonances were assigned *via* 1D selective gradient TOCSY and 2D NMR experiments.

SUPPORTING INFORMATION

Table S1. Tensile properties of PE-2,18 in comparison to commercial HDPE.

	Number of specimens	Youngs Modulus E_t [MPa]	Stress at yield σ_y [MPa]	Elongation at break ε_{tb} [%]
PE-2,18	3	730 ± 40	19.3 ± 0.4	330 ± 10
HDPE	3	1130 ± 10	20.4 ± 2.7	460 ± 20

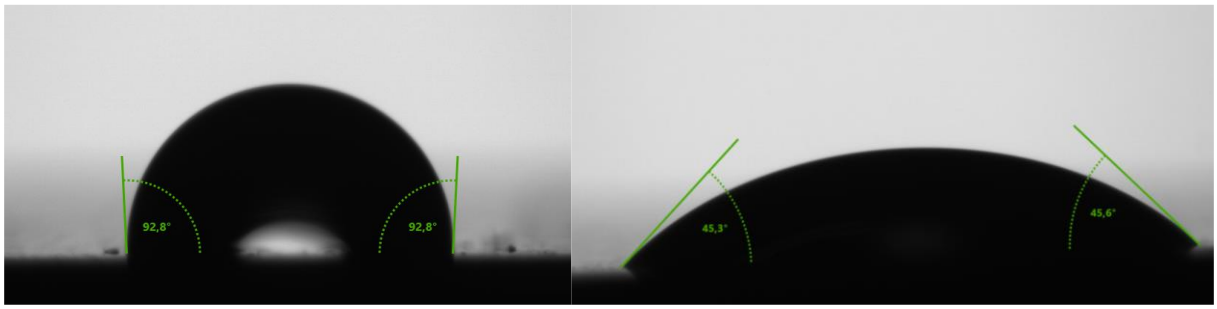

Figure S7. Drop of water (left) and diiodomethane (right) on PE-2,18.

Table S2. Contact angles of water and diiodomethane (DIM) on PE-2,18 and surface free energies in comparison to commercial HDPE.[6] Average of three drops on different sample positions with 30 measurements each are given (at 20 °C, Δt = 1 s between individual data points).

	Water Contact Angle [°]	DIM Contact Angle [°]	Total Surface Free Energy [mN/m]	Dispersive Surface Free Energy [mN/m]	Polar Surface Free Energy [mN/m]
PE-2,18	92.9 ± 1.0	45.6 ± 1.0	37.5 ± 0.7	36.7 ± 0.6	0.8 ± 0.2
HDPE	96.7 ± 1.4	54.6 ± 1.1	32.4 ± 0.9	31.7 ± 0.7	0.7 ± 0.2

Additional characterization data for chemical recycling experiments of PE-2,18

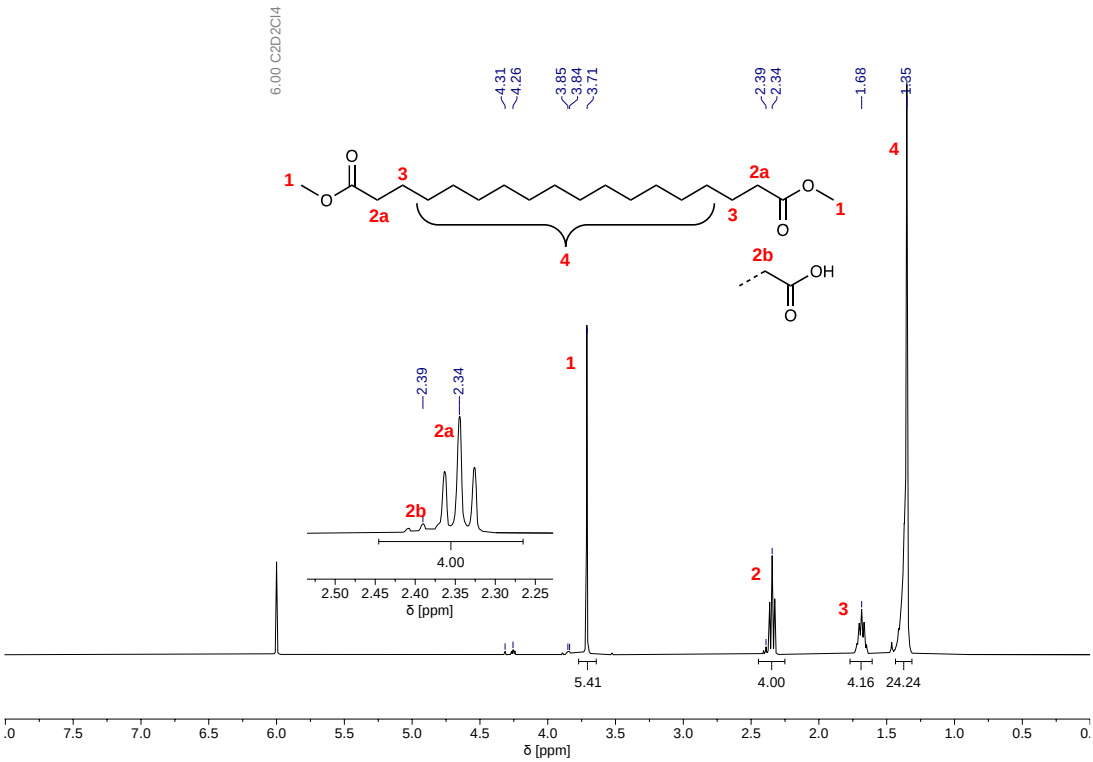

Figure S8. ^{1}H NMR spectrum (400 MHz, $C_2D_2Cl_4$, 383 K) of 1,18-dimethyl octadecanedioate recovered from the methanolysis product mixture of PE-2,18.

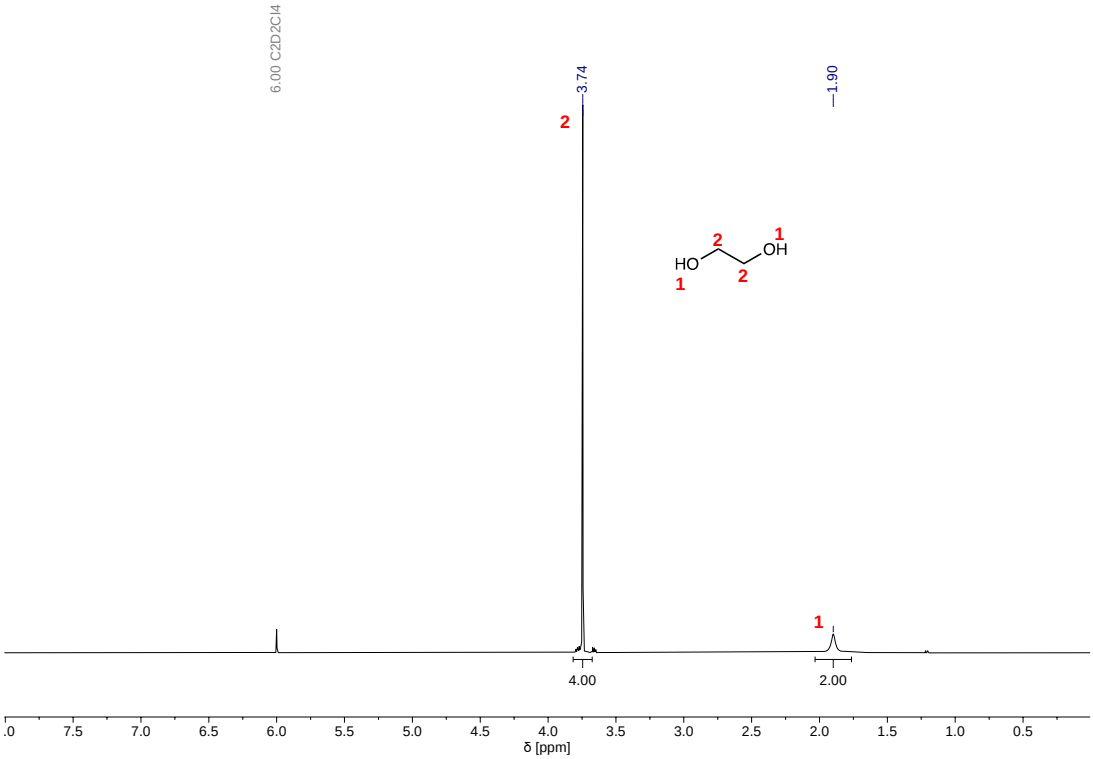

Figure S9. ^{1}H NMR spectrum (400 MHz, $C_2D_2Cl_4$, 383 K) of ethylene glycol recovered from the methanolysis product mixture of PE-2,18.

Additional characterization data for PE-3,18

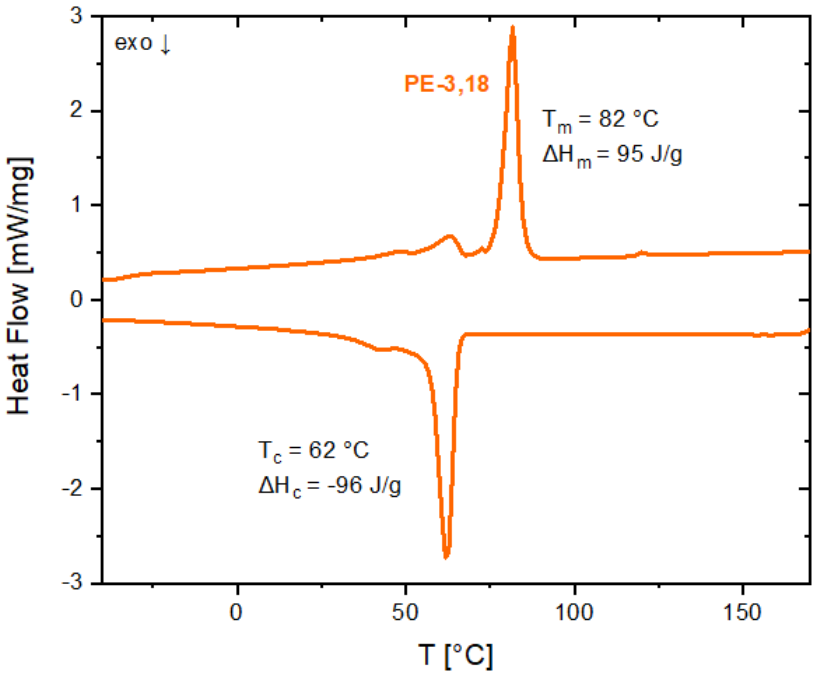

Figure S10. DSC trace of PE-3,18.

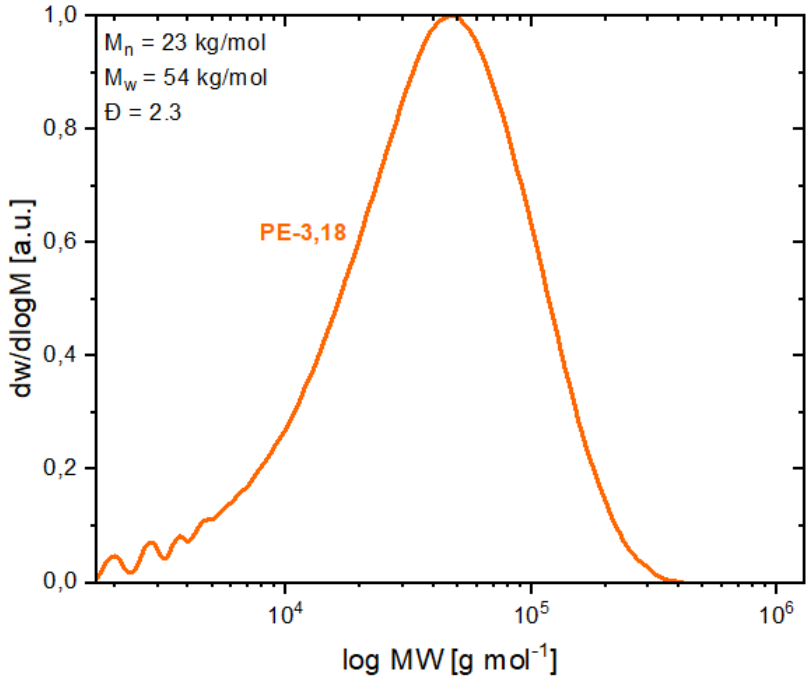

Figure S11. GPC trace of PE-3,18.

Additional characterization data for PE-4,18

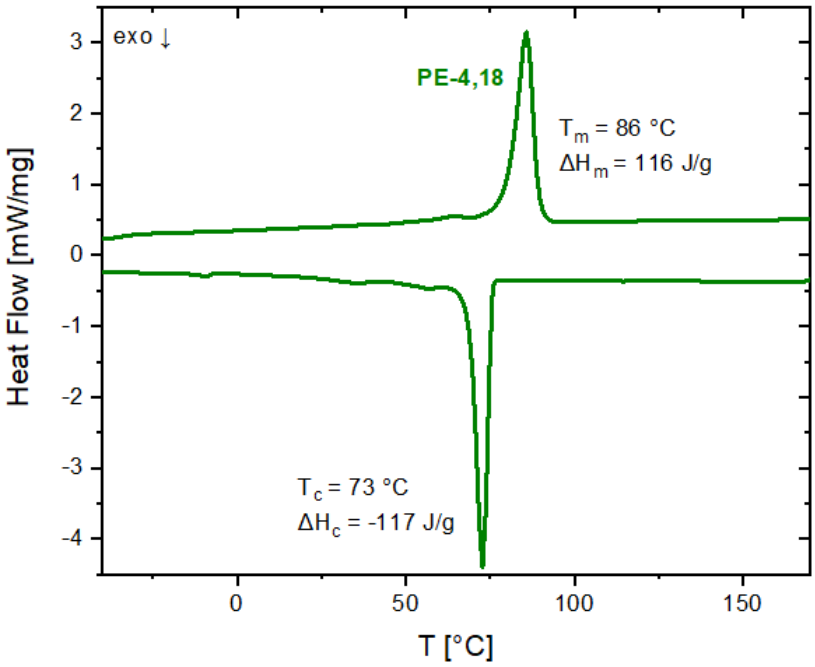

Figure S12. DSC trace of PE-4,18.

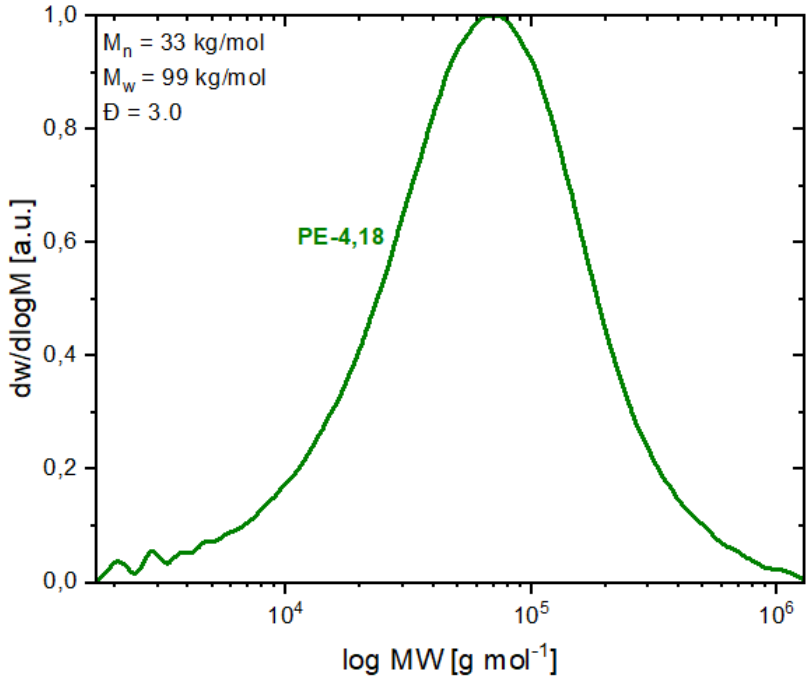

Figure S13. GPC trace of PE-4,18.

Additional data for enzymatic hydrolysis experiments

Additional data for polyesters used in enzymatic hydrolysis experiments

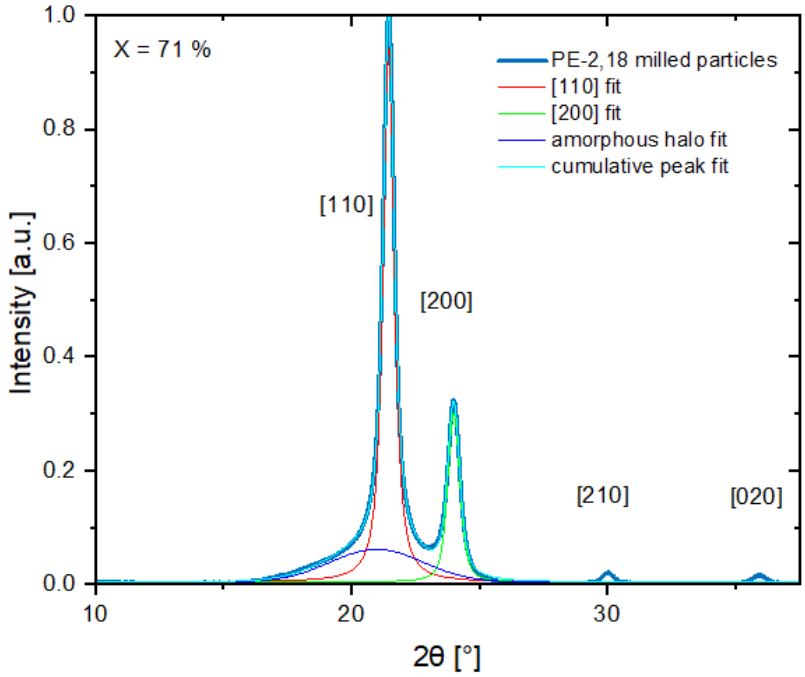

Figure S14. WAXS diffractogram of milled PE-2,18 used for enzymatic hydrolysis experiments.

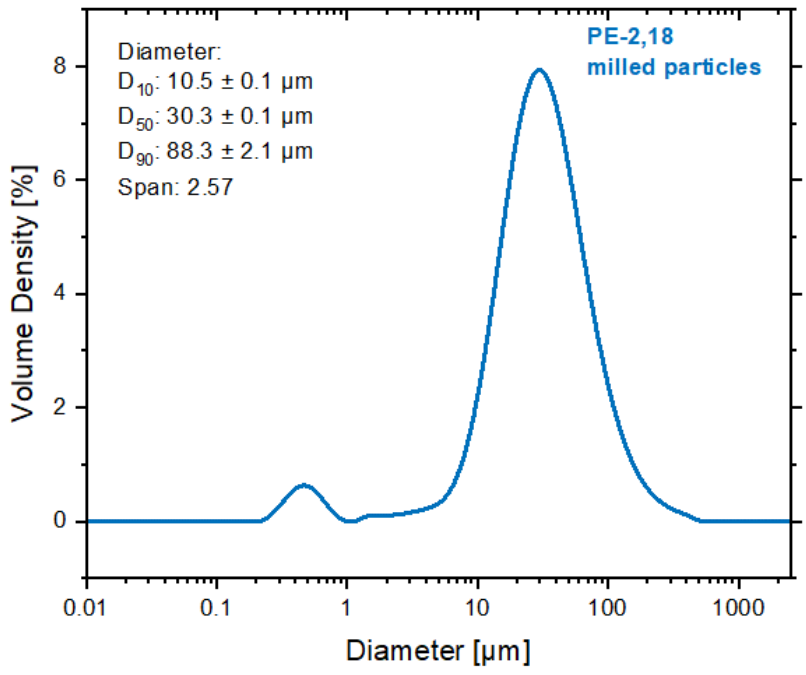

Figure S15. Particle size distribution of milled PE-2,18 particles used for enzymatic hydrolysis experiments measured *via* laser diffraction.

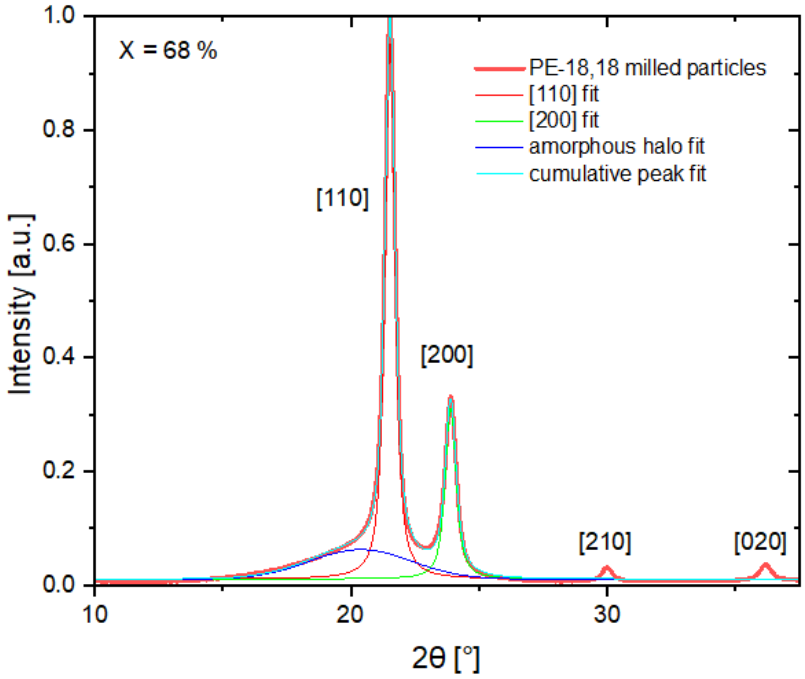

Figure S16. WAXS diffractogram of milled PE-18,18 used for enzymatic hydrolysis experiments.

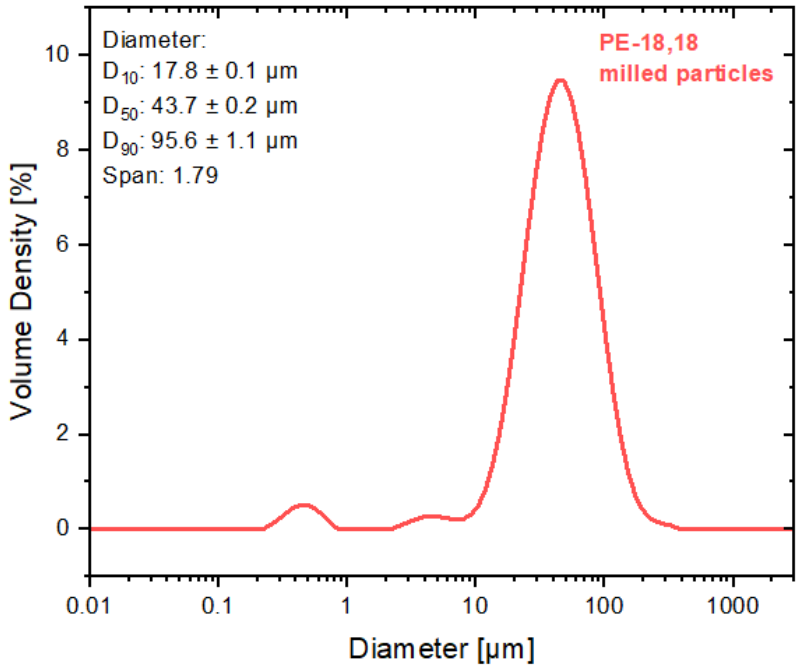

Figure S17. Particle size distribution of milled PE-18,18 particles used for enzymatic hydrolysis experiments measured *via* laser diffraction.

Additional data for HPLC and LC-MS measurements

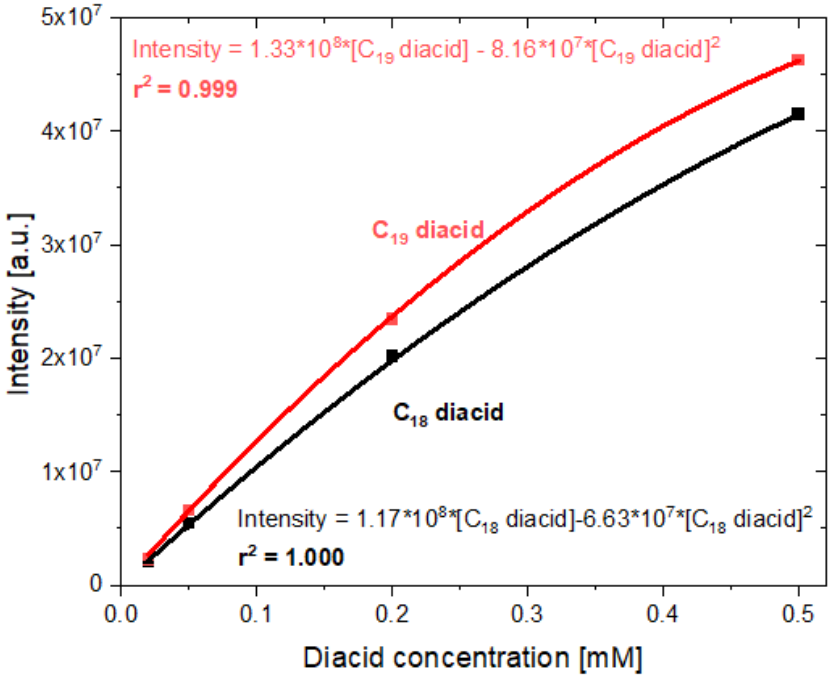

Figure S18. LC-MS calibration curve for different concentrations of C_{18} (black) and C_{19} diacid (red) with depicted quadratic fit.

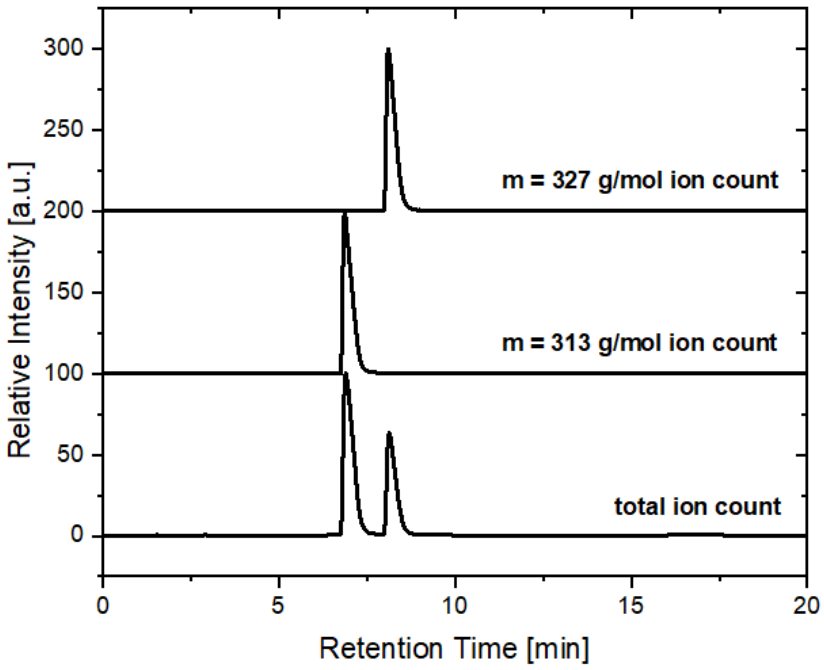

Figure S19. Exemplary LC-MS chromatograms for the internal standard C_{19} diacid (top, 327 g/mol) and the analyte C_{18} diacid (center, 313 g/mol) in the single ion mode, and the total ion count (bottom).

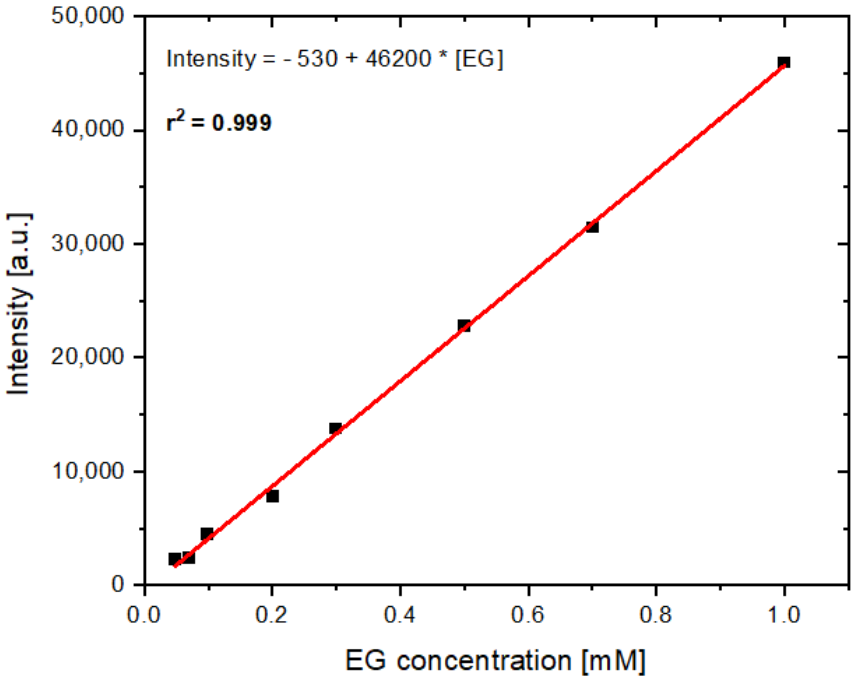

Figure S20. HPLC calibration curve of ethylene glycol (EG) with depicted linear fit.

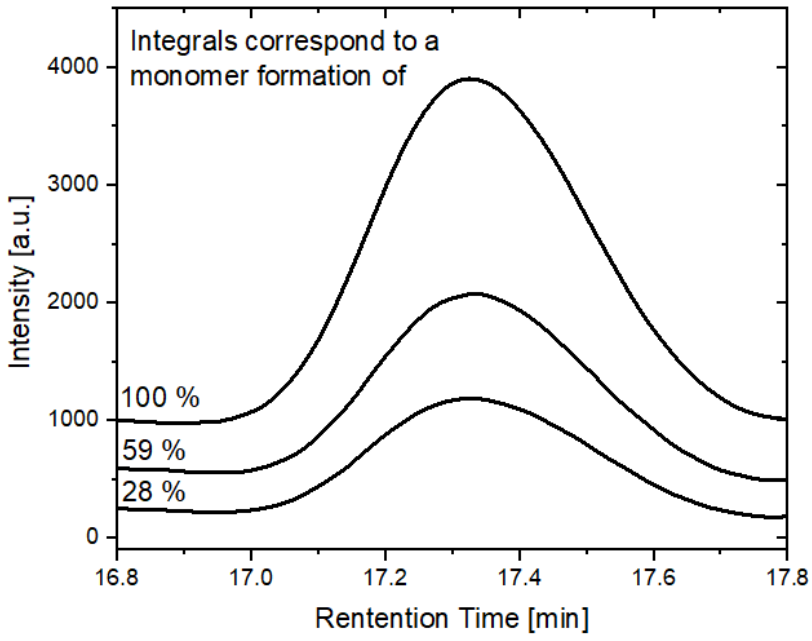

Figure S21. Three exemplary HPLC chromatograms of enzymatic degradation samples with variable amounts of ethylene glycol.

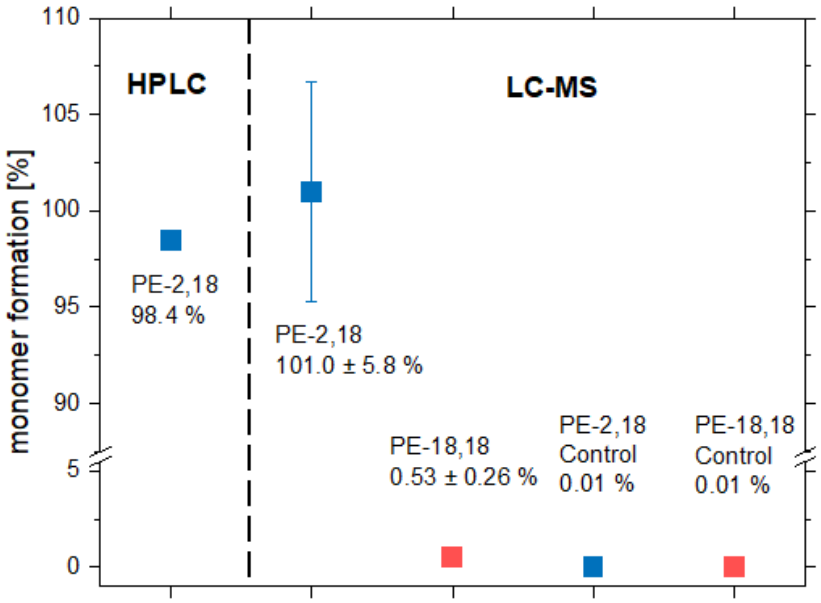

Figure S22. Hydrolysis of PE-2,18 and PE-18,18 by HiC at 37 °C and pH = 7.2 and control samples at 37 °C and pH = 7.2 after six days, monitored *via* HPLC (left) and LC-MS (right) quantification of EG and C_{18} diacid, respectively.

A quantitative comparison of the enzymatic hydrolysis of PE-2,18 and PE-18,18 was conducted as described in the method section. In short, 10 mg of the milled polymers PE-2,18 and PE-18,18 were enzymatically hydrolyzed with HiC in 20 mL buffer medium. The monomer formation for PE-2,18 in this setup was determined independently *via* HPLC (single measurement) and LC-MS (triplicate), to detect EG and C_{18} diacid, respectively. A complete hydrolysis was confirmed by both methods after six days. For PE-18,18 (triplicate), a monomer formation of only 0.53 ± 0.26 % could be detected under equal conditions. Control samples, treated equally as the hydrolysis samples but without addition of enzyme, accounted for less than 0.01 % of monomer formation, most likely due to residual monomer from the polycondensation synthesis.

Assessment of enzyme active site accessibility

The accessible surface area (ASA) of the active site of each enzyme tested here was assessed using a previously published protocol.[7] Published crystal structures were obtained from the RCSB Protein Data Bank (PDB) for *Humicola insolens* cutinase (HiC; PDB file '4OYY'),[8] *Aspergillus oryzae* cutinase (AoC; PDB file '3GBS'),[9] and *Thermomyces lanuginosus* lipase (TIL; PDB file '4ZGB')[10] and ASA values were calculated using the GETAREA algorithm[11] (accessed at http://curie.utmb.edu/getarea.html) as the surface area of the side chain of serine in the reported active site of each enzyme (HiC, serine 105; AoC, serine 126; TIL, serine 146). The values were determined for probes with radii ranging from 0.8 to 2.6 Å.
Figure S23 shows the determined ASA of active site serine vs. theoretical probe radius for the different enzymes. HiC and AoC both displayed high ASA (19.7 and 18.7 $Å^2$, respectively) for the smallest tested probe radius of 0.8 Å. The ASA of these enzymes steadily decreased with increasing probe radius, with HiC reaching an ASA of 0 at a probe radius of 2.6 Å, and AoC at a probe radius of 2.2 Å. In contrast, TIL showed a lower ASA at a radius of 0.8 Å (4.2 $Å^2$) and reached an ASA of 0 at a probe radius of only 1.4 Å. These trends in ASA imply that the active sites for HiC and AoC are more accessible than that of TIL. Furthermore, the stark difference in accessibility between HiC and AoC vs that of TIL agrees well with differences seen in the enzymatic hydrolysis rates of PE-2,18 by these enzymes (cf. Figure 3a); namely, that the enzymes which more readily hydrolyzed PE-2,18 (HiC and AoC) showed higher enzyme active site accessibility. These results indicate that, similarly to previous findings for enzymatic hydrolysis of aliphatic-aromatic polyesters PBAT[7] and PET[12], active site accessibility is a controlling parameter of the competency for enzymes to hydrolyze semi-crystalline long-chain aliphatic polyesters such as PE-2,18.

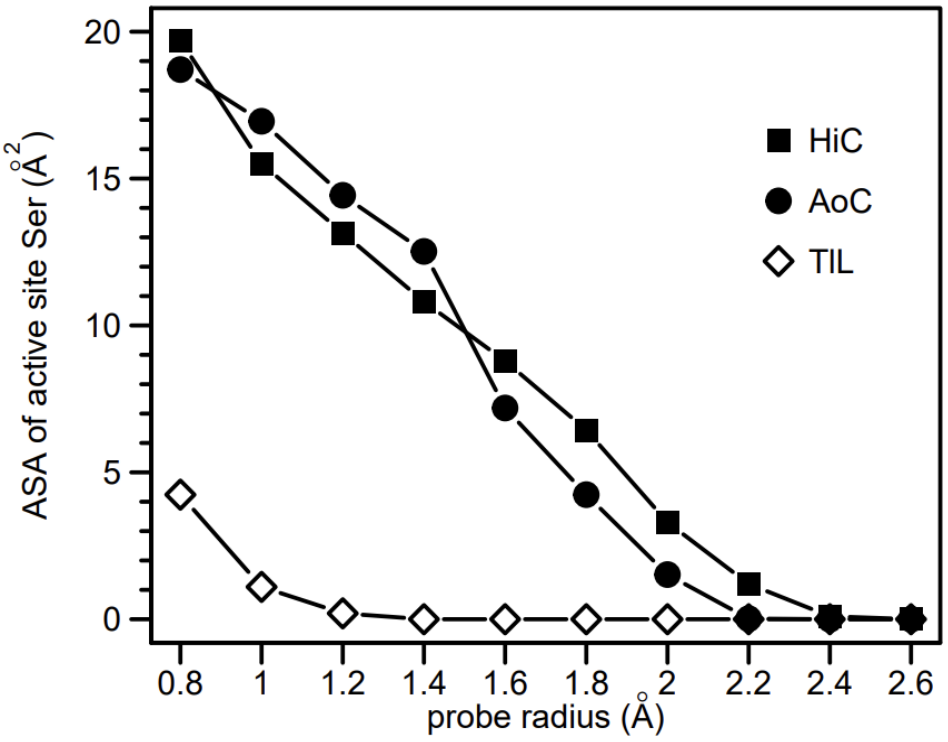

Figure S23. Available surface area (ASA) of enzyme active site serine (Ser) residues accessible to theoretical water probe molecules of varying radii. ASA values were calculated using published crystal structures and the GETAREA algorithm (see text for details).

Additional data for composting experiments according to ISO 14855-1

Additional characterization data for polyesters used in composting experiments

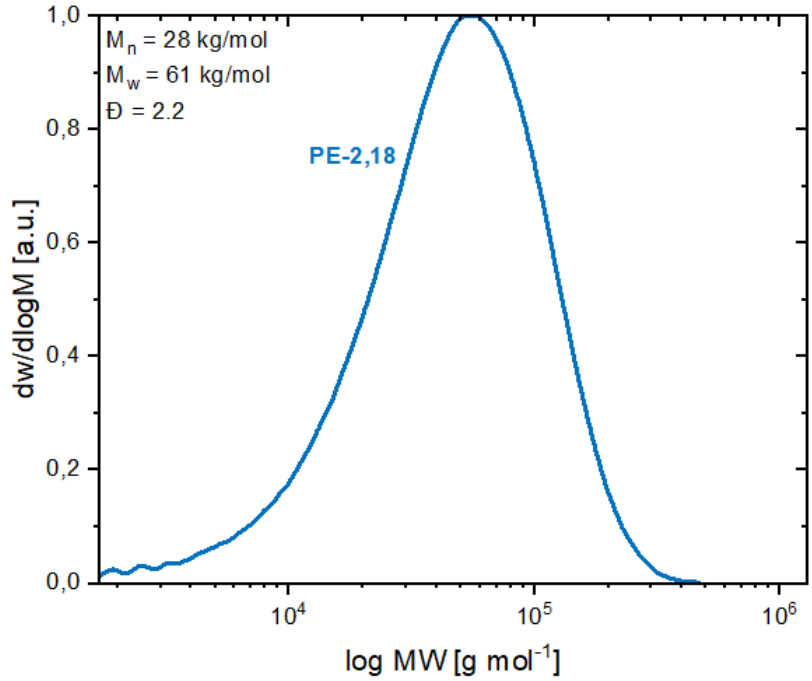

Figure S24. GPC trace of PE-2,18 used for composting experiments.

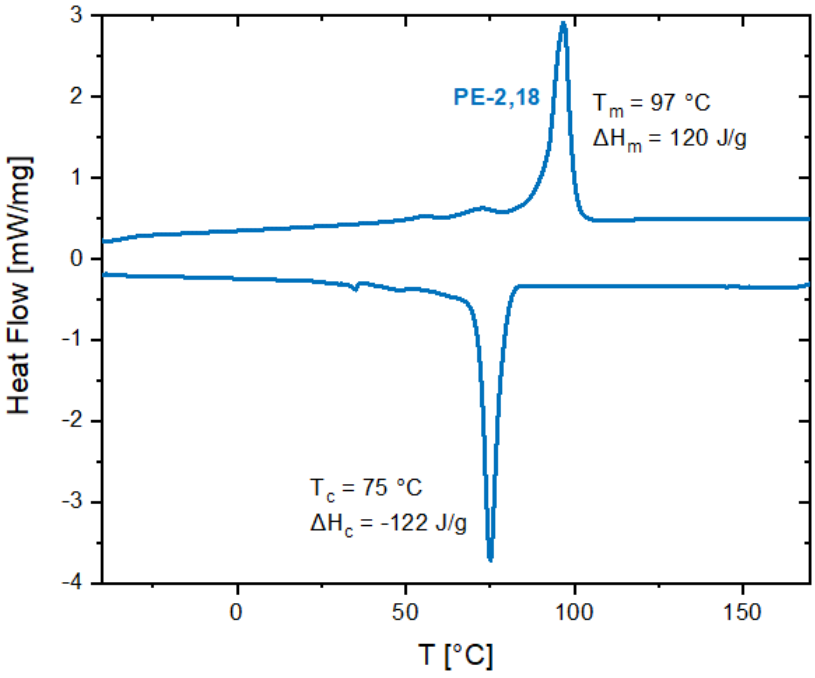

Figure S25. DSC trace of PE-2,18 used for composting experiments.

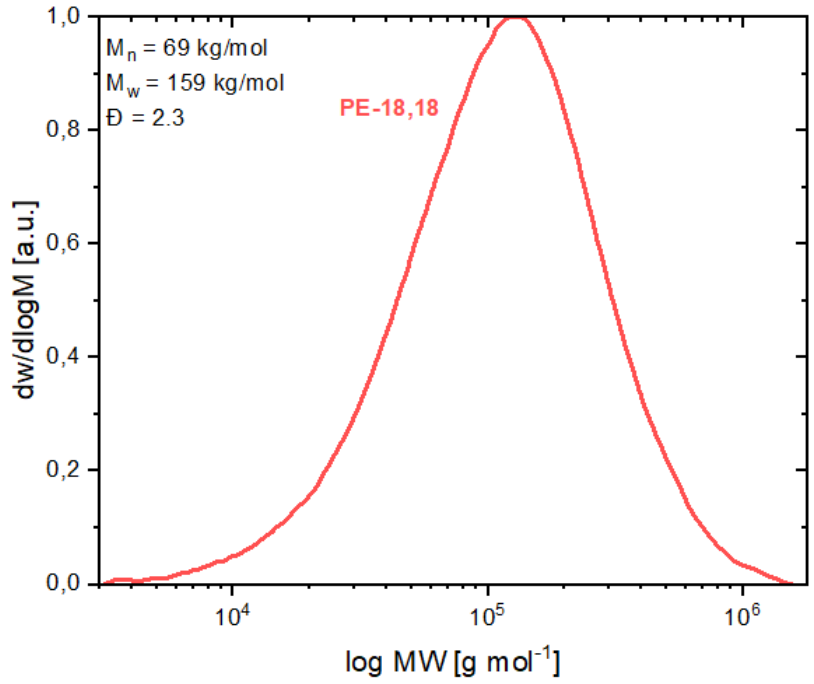

Figure S26. GPC trace of PE-18,18 used for composting experiments.

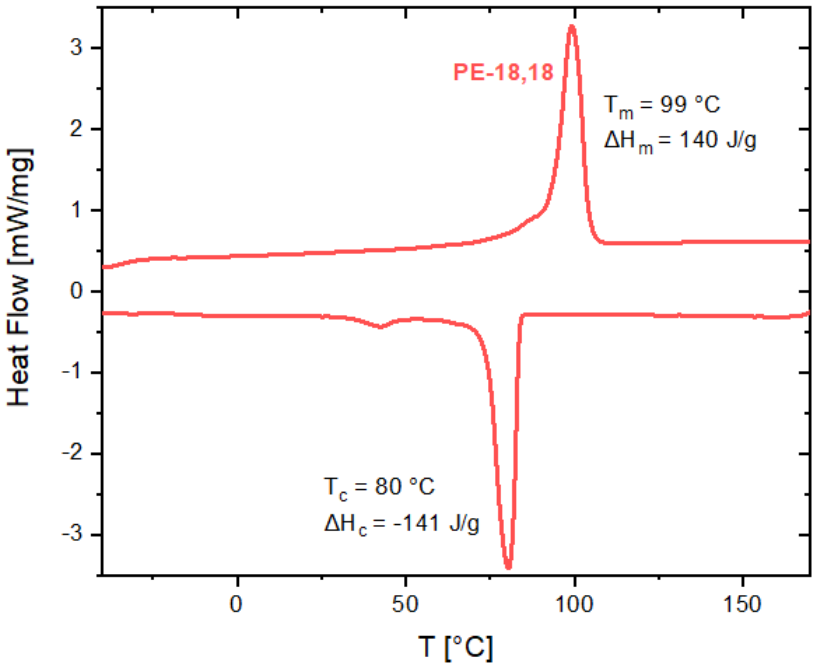

Figure S27. DSC trace of PE-18,18 used for composting experiments.

Table S3. Elemental analysis of polymers used for composting experiments. The average value of two measurements is given. Theoretical values are given in parentheses.

	C (%)	H (%)
PE-18,18	76.49 (76.54)	12.15 (12.13)
PE-2,18	70.18 (70.55)	10.69 (10.66)

Mineralization of PE-18,18 in compost

PE-18,18 mineralization in compost was measured in the same biodegradation test as PE-2,18 and cellulose (cf. Fig. 4), according to ISO 14855-1 (see Methods section for details on the testing setup and analysis). Figure S28 shows the mineralization curves of the three replicates of PE-18,18. Two of the replicates showed mineralization of PE-18,18 up to 23 % at 10 days, after which the mineralization plateaued, with cumulative amounts of 34 % at the end of the experiment (65 days). In contrast, the third replicate deviated after reaching 21 % mineralization and continued to biodegrade up to 92 %, when the experiment was stopped. While the reason for such a difference in mineralization extents for different replicates of PE-18,18 here is unclear, it is understood that microbial community composition and abundance are controlling factors for biodegradation.[13]

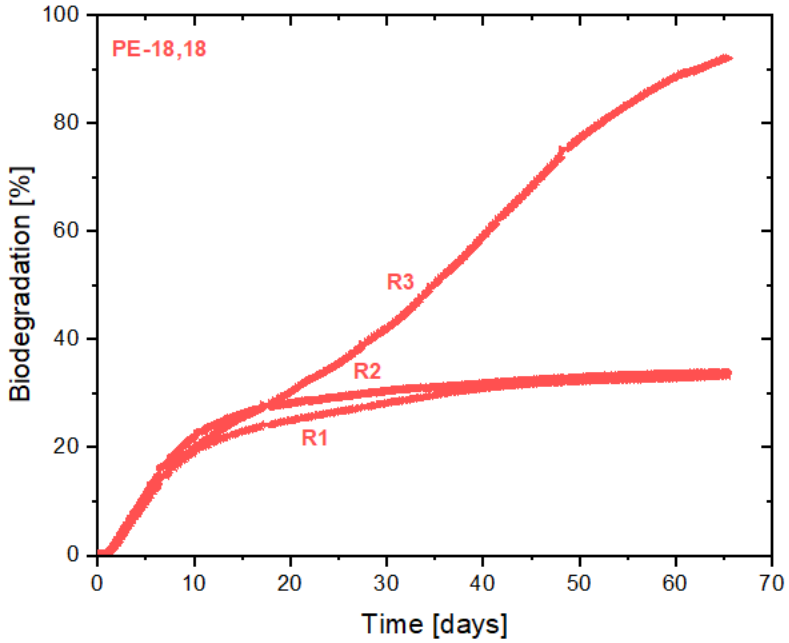

Figure S28. Mineralization curves based on CO_2 evolution measured under industrial composting conditions following the standard ISO 14855-1 for three replicates of PE-18,18.

SUPPORTING INFORMATION

References

[1] F. Stempfle, D. Quinzler, I. Heckler, S. Mecking, *Macromolecules* **2011**, *44*, 4159.
[2] F. M. Fowkes, *Ind. Eng. Chem.* **1964**, *56*, 40.
[3] M. Häußler, M. Eck, D. Rothauer, S. Mecking, *Nature* **2021**, *590*, 423.
[4] D. Ribitsch, E. Herrero Acero, K. Greimel, A. Dellacher, S. Zitzenbacher, A. Marold, R. D. Rodriguez, G. Steinkellner, K. Gruber, H. Schwab et al., *Polymers* **2012**, *4*, 617.
[5] M. Patkar, S. A. Jabarin, *J. Appl. Polym. Sci.* **1993**, *47*, 1749.
[6] M. Baur, F. Lin, T. O. Morgen, L. Odenwald, S. Mecking, *Science* **2021**, *374*, 604.
[7] M. T. Zumstein, D. Rechsteiner, N. Roduner, V. Perz, D. Ribitsch, G. M. Guebitz, H.-P. E. Kohler, K. McNeill, M. Sander, *Environ. Sci. Technol.* **2017**, *51*, 7476.
[8] D. Kold, Z. Dauter, A. K. Laustsen, A. M. Brzozowski, J. P. Turkenburg, A. D. Nielsen, H. Koldsø, E. Petersen, B. Schiøtt, L. de Maria et al., *Protein Sci.* **2014**, *23*, 1023.
[9] Z. Liu, Y. Gosser, P. J. Baker, Y. Ravee, Z. Lu, G. Alemu, H. Li, G. L. Butterfoss, X.-P. Kong, R. Gross et al., *J. Am. Chem. Soc.* **2009**, *131*, 15711.
[10] M. Kumar, J. Mukherjee, M. Sinha, P. Kaur, S. Sharma, M. N. Gupta, T. P. Singh, *Sustain. Chem. Process.* **2015**, *3*, 1.
[11] R. Fraczkiewicz, W. Braun, *J. Comput. Chem.* **1998**, *19*, 319.
[12] R. Araújo, C. Silva, A. O'Neill, N. Micaelo, G. Guebitz, C. M. Soares, M. Casal, A. Cavaco-Paulo, *J. Biotechnol.* **2007**, *128*, 849.
[13] a) M. T. Zumstein, R. Narayan, H.-P. E. Kohler, K. McNeill, M. Sander, *Environ. Sci. Technol.* **2019**, *53*, 9967; b) T. F. Nelson, S. C. Remke, H.-P. E. Kohler, K. McNeill, M. Sander, *Environ. Sci. Technol.* **2020**, *54*, 266; c) M. T. Zumstein, A. Schintlmeister, T. F. Nelson, R. Baumgartner, D. Woebken, M. Wagner, H.-P. E. Kohler, K. McNeill, M. Sander, *Sci. Adv.* **2018**, *4*, eaas9024.