Electronic Supplementary Material (ESI) for Green Chemistry.

Supplementary Information

Enzymatic surface hydrolysis of poly(ethylene furanoate) thin films of various crystallinities

S. Weinberger,[a] K. Haernvall,[b] D. Scaini,[c] G. Ghazaryan,[d] M. T. Zumstein[e], M. Sander[e], A. Pellis,[a,†] and G. M. Guebitz,[a,b]

Materials & Methods

S1 Chemicals, reagents and enzymes

Hexafluoroisopropanol (supplier: TCI, article number: H0424), tris(hydrolymethyl)aminomethane (tris) (Sigma-Aldrich, T87602), potassium chloride (KCl) (Sigma-Aldrich, 60130). Furandicarboxylic acid (FDCA) and all other chemicals and solvents were purchased from Sigma-Aldrich at reagent grade, and used without further purification if not otherwise specified. Poly(ethylene furanoate) (PEF) pellets were provided by Synvina. Cutinase from *Humicola insolens* (HiC) was obtained from Novozymes and used as received without further purifications. Cutinase 1 from *Thermobifida cellulosilytica* (Thc_cut1) was produced and purified as previously described [1].

S2 Esterase activity and protein concentration assays

Enzymatic activity (PNPB assay) and protein concentration determination according to the BioRad protein assay (Bio-Rad Laboratories GmbH, Vienna, Cat.No: 500-0006) were carried out as previously described [2].

S3 Polyester films hydrolysis

0.5x1.0 cm of the PEF or PET films of various crystallinities (0, 10 and 20%) were incubated with 1.0 mL of 5 µM HiC of Thc_cut1 in 0.1 M TRIS/HCl buffer pH 7 for 24, 48, 72 or 96 h. Incubations were conducted in an orbital shaker set at 100 rpm and 65 °C since good biocatalyst stability and activity over time were previously reported for these conditions [3]. Blank reactions were carried out in buffer without addition of the biocatalyst. All reactions were performed in triplicates. For the films, the % weight loss after the enzymatic reaction was calculated after the washing of the films.

S4 Polyester hydrolysis measurements by quartz crystal microbalance (QCM).

We used a QCM E4 system (Q-Sense, Sweden) to monitor the changes in the adlayer mass of thin polyester films during their enzymatic hydrolysis, as previously described [4]. QCM monitors changes in the resonance frequency of a quartz crystal embedded in a QCM sensor. The measured changes in the resonance frequencies can be converted to changes in the adlayer mass using the Sauerbrey equation (Eq.1):

$$\Delta \mathrm{m} = \mathrm{C} \bullet -\frac{\Delta \mathrm{f_i}}{\mathrm{i}} \qquad \text{Eq. 1}$$

where Δm is the change in the adlayer mass (ng/cm^2), C is the sensor-specific mass sensitivity constant (= 17.7 $ng \bullet cm^{-2} \bullet Hz^{-1}$), and Δf_i is the change in the resonance frequency of the i^{th} overtone (Hz). We used the fifth overtone (i= 5) for plotting and calculations.

For the QCM-D measurements, we spin-coated polyester films onto gold-coated QCM sensors (QSX 301, Microvacuum) from polyester solutions in hexafluoroisopropanol (0.5 wt%). Spin coating was achieved by transferring 40 µL of polyester solution onto a QCM sensor that was mounted on a spin coater (WS-650MZ-23NPP, Laurell Technologies) and by subsequently spinning the sensor for 1 min (4000 rpm, acceleration: 1500 rpm•s^{-1}). After air-drying the films at room temperature, we pre-equilibrated the coated sensors to a pH-buffered solution (3 mM Tris, 10 mM KCl, pH 7) at room temperature for 14 h. We subsequently mounted the sensors into individual QCM flow cells and delivered a pH-buffered solution (same chemistry as used for pre-equilibration) over the sensors at 40°C and at a volumetric flow rate of 20 µL/min. When the resonance frequencies were constant, we switched from enzyme-free solutions to solutions containing the hydrolytic enzyme (70 nM for both HiC and Thc_Cut1). Polyester hydrolysis resulted in increasing resonance frequencies (i.e., decreasing adlayer masses) and was continued until these frequencies levelled off at final constant values.

We assessed the extent of polyester mass loss during the hydrolysis by determining the final adlayer mass after hydrolysis and sensor drying to mass of polyester that we originally spin-coated onto the sensor (i.e., mass loss as percent ratio of final adlayer mass to total spin coated film mass). These measurements were conducted on dry sensors in air. We cleaned the QCM instrument and sensors in between each set of experiments as previously described [5].

S5 Differential Scanning Calorimetry.

The thermal analysis of the neat and modified PET fabrics was performed using a PerkinElmer (DSC, Diamond) apparatus under a nitrogen flow (20 mL min^{-1}). In order to investigate the effect of treatment on the degree of crystallinity, PET samples of 3-5 mg were heated in aluminum pans from 25 to 270 °C at a heating rate of 10 K min^{-1}. The corresponding data and curves were recorded, and the degree of crystallinity (χc) was calculated by using the equation below:

$$\chi c = (\Delta H_m - \Delta H_{cc})/\Delta H_m^0$$

where ΔH_m is the enthalpy of melting, ΔHcc is the enthalpy of cold crystallization, and ΔH_m^0 is the enthalpy of fusion per gram of a perfect crystal of infinite size. The latter is 140 J/g for 100% crystalline PET [6].

S6 Estimated thicknesses of spin-coated polyester films.

The thicknesses of spin-coated polyester films for QCM-D measurements were estimated from the spin coated polyester masses (measured as mass difference of the sensor before and after spin coating in air) and densities for PEF and PET of 1.43 and 1.33 g/cm3 reported in the literature [7]. The calculated thicknesses were 80.8 ± 2.7 nm and 76.2 ± 1.6 nm for PEF and PET, respectively (mean ± standard deviation of four thin films).

S7 Imaging and Analysis of the Polymer Surface via Scanning Electron Microscopy and Atomic Force Microscopy.

Scanning electron microscopy (SEM) was used to investigate the morphology of the polyester samples treated with Thc_Cut1, HiC and only with buffer (100 mM, pH 7) as the control. Before analysis samples were washed and dried as previously described. Images were acquired collecting secondary electrons on a Gemini SUPRA 40 SEM (Carl Zeiss NTS GmbH, Oberkochen) working at an acceleration voltage of 5 keV. Prior to SEM imaging, samples were gold metalized in a metal sputter coater (Polaron SC7620).

Atomic force microscopy (AFM) was used to obtain high resolution 3D images of PET fabrics samples' surfaces. Samples were mounted on metallic plates using epoxy glue and subsequently characterized using an MFP-3D AFM (Asylum Research, Oxford Instruments, Santa Barbara, CA, U.S.). Measurements were carried out in the air at 21 ºC working in dynamic mode. Cantilevers, characterized by a resonant frequency of 320 kHz and force constant of 40 nN/nm (NSG30, NT-MDT, Moscow, RU), were used working at low oscillation amplitudes with a half free-amplitude set point. Images were acquired at 256 × 256 pixels at a one line/s scan speed. All AFM data were analyzed using Gwyddion open-source SPM analysis software. In particular three-dimensional reconstructions were used to evaluate surface roughness. Surface roughness was computed as the root-mean-square (RMS) value of the height irregularities of AFM images.

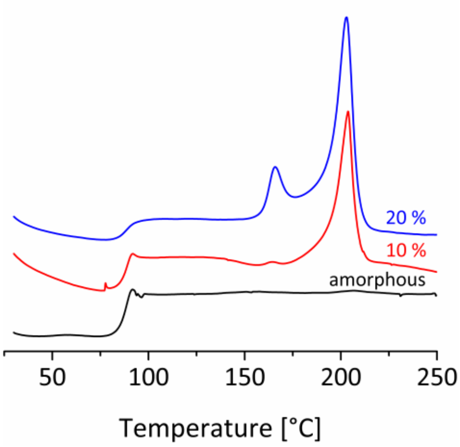

Fig. S1. Differential scanning calorimetry (DSC) analysis of PEF films having different crystallinities produced by compression molding and annealing.

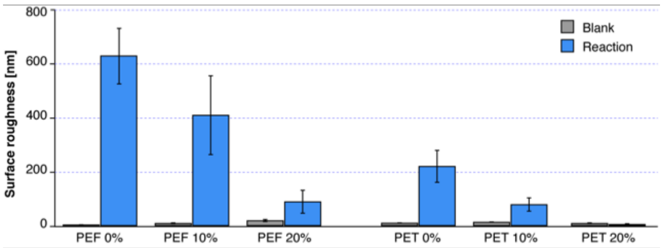

Fig. S2. Surface roughness of the hydrolyzed PEF and PET films vs blanks (i.e., enzyme-free controls) as assessed by atomic force microscopy (AFM) on the film surfaces.

Table 1. HFIP-GPC results of the PET and PEF samples before and after processing.

Sample name	M_n	M_w	PDI
PET pellets	40138	80359	2.002
PET film 0%	39493	81806	2.071
PET film 10%	38982	78151	2.005
PET film 20%	39564	81013	2.048
PEF pellets	51318	121444	2.367
PEF film 0%	49837	111880	2.245
PEF film 10%	53895	113377	2.104
PEF film 20%	52406	115597	2.206

References

[1] E. Herrero Acero, D. Ribitsch, G. Steinkellner, K. Gruber, K. Greimel, I. Eiteljoerg, E. Trotscha, R. Wei, W. Zimmermann, M. Zinn, A. Cavaco-Paulo, G. Freddi, H. Schwab, G. Guebitz, Enzymatic Surface Hydrolysis of PET: Effect of Structural Diversity on Kinetic Properties of Cutinases from Thermobifida, Macromolecules 44 (2011) 4632-4640

[2] A. Pellis, V. Ferrario, M. Cespugli, L. Corici, A. Guarneri, B. Zartl, E. Herrero Acero, C. Ebert, G.M. Guebitz, L. Gardossi, Fully renewable polyesters via polycondensation catalyzed by Thermobifida cellulosilytica cutinase 1: an integrated approach, Green Chemistry (2017)

[3] C. Gamerith, M. Vastano, S. M. Ghorbanpour, Sabine Zitzenbacher1†, Doris Ribitsch1,2, Michael T. Zumstein4, Michael Sander4, Enrique Herrero Acero1†, Alessandro Pellis2* and Georg M. Guebitz1,2, Enzymatic Degradation of Aromatic and Aliphatic Polyesters by P. pastoris Expressed Cutinase 1 from Thermobifida cellulosilytica, Frontiers Microbiol. https://doi.org/10.3389/fmicb.2017.00938 (2017).

[4] M. T. Zumstein, H.-P. E. Kohler, K. McNeill and M. Sander, Environ Sci Technol, 2016, 50, 197–206.

[5] A. Armanious, M. Aeppli and M. Sander, Environ Sci Technol, 2014, 48, 9420–9429.

[6] Wunderlich, B. Thermal Analysis; Academic Press, 1990, 417-431.

[7] S. K. Burgess, J. E. Leisen, B. E. Kraftschik, C. R. Mubarak, R. M. Kriegel, W. J. Koros, Chain Mobility, Thermal, and Mechanical Properties of Poly(ethylene furanoate) Compared to Poly(ethylene terephthalate). Macromolecules, 2014, 47, 1383-1391