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Targeting microplastic particles in the void of diluted suspensions

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ABSTRACT

Accumulation of microplastic in the environment and food chain will be a grand challenge for our society. Polyurethanes are widely used synthetic polymers in medical (e.g. catheters) and industrial products (especially as foams). Polyurethane is not abundant in nature and only a few microbial strains (fungi and bacteria) and enzymes (polyurethaneases and cutinases) have been reported to efficiently degrade polyurethane. Notably, in nature a long period of time (from 50 to > 100 years depending on the literature) is required for degradation of plastics. Material binding peptides (e.g. anchor peptides) bind strongly to polymers such as polypropylene, polyethylene terephthalate, and polyurethane and can target specifically polymers. In this study we report the fusion of the anchor peptide Tachystatin A2 to the bacterial cutinase Tcur1278 which accelerated the degradation of polyester-polyurethane nanoparticles by a factor of 6.6 in comparison to wild-type Tcur1278. Additionally, degradation half-lives of polyester-polyurethane nanoparticles were reduced from 41.8 h to 6.2 h (6.7-fold) in a diluted polyester-polyurethane suspension (0.04% w/v).

1. Introduction

Worldwide plastic production increased dramatically in the 20th century with the development of synthetic polymers due to their durability, lightweight, and cost effectiveness (Barnes et al., 2009; Hidalgo-Ruz et al., 2012; Andrady, 2011). About 335 million tons of plastics were produced worldwide in 2016 (PlasticsEurope, 2017). Synthetic plastics such as polyethylene (PE), polyvinyl chloride (PVC), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and polyurethane (PUR) are used in numerous applications (e.g. packaging, textiles, pipes, tyres) (Shah et al., 2008). Plastic waste in the environment is degraded in nature by e.g. photo-, bio-, thermooxidative depolymerization, and friction (Barnes et al., 2009; Browne et al., 2011). Environmental pollution by plastic debris was first mentioned in the 1970s (Carpenter et al., 1972). Extensive degradation of plastic waste generates microplastic (mostly defined as particles with a size smaller than 5 mm) (Shah et al., 2008; Zheng et al., 2005; do Sul and Costa, 2014). Microplastics pose a threat to human health since the particles are reported to attract and store toxic compounds such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDs), and bisphenols (Bouwmeester et al., 2015). Microplastic particles have been found in small fishes (Boerger et al., 2010) and larger mammals (e.g. sperm whales) (de Stephanis et al., 2013). Main polluter are PP particles, PE particles, and PUR foams that are commonly used as microplastics in the pharmaceutical and cosmetic industries for production of domestic consumables, which finally end up as waste in the water cycle (PlasticsEurope, 2017; Sharma and Chatterjee, 2017). Due to their physicochemical properties, the degradation of plastics is very slow (half-life of PE debris in sea water is estimated to be ~50 years) (Kim et al., 2015) and thereby polluting coastal and marine habitats (Sharma and Chatterjee, 2017). Management strategies to avoid microplastics contaminations are of high importance to ensure a supply of healthy food and water (Eerkes-Medrano et al., 2015).

Biodegradation might be the preferred strategy to decompose microplastics (Shah et al., 2008). PET and PUR are biodegradable through hydrolytic cleavage of ester or urethane bonds (Nakajima-Kambe et al., 1999). Polyurethanes find versatile applications in tires, sponges, insulation, coats, paints, adhesives, and fibers (Shah et al., 2008; Zheng et al., 2005; Howard, 2002). Fungi and bacteria are reported to degrade polyester-polyurethane through enzymatic hydrolysis of ester linkages (Nakajima-Kambe et al., 1999; Howard, 2002). Fungal biodegradation of polyester-polyurethane was for instance reported for four species of fungi isolated from soil (e.g. *Curvularia senegalensis*, *Fusarium solani*, *Aureobasidium pullulans*, and *Cladosporium* sp.) (Crabbe et al., 1994). A

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variety of bacterial strains such as *Corynebacterium* sp. (Kay et al., 1991), *Pseudomonas aeruginosa* (Kay et al., 1991), *Comamonas acidovorans* (Nakajima-Kambe et al., 1995), *Acinetobacter calcoaceticus* (El-Sayed et al., 1996), and *Bacillus subtilis* (Rowe and Howard, 2002) are reported to use polyester-polyurethane polymers as carbon, nitrogen and energy source for growth. Several studies refer to esterase-activities in the microbial degradation of polyester-polyurethane (Rowe and Howard, 2002; Akutsu et al., 1998). Membrane associated (e.g. PudA) (Akutsu et al., 1998) and extracellular (e.g. PueA, PueB) (Allen et al., 1999; Ruiz et al., 1999; Vega et al., 1999) polyurethanases (PUases from *Comamonas acidovorans* TB-35, *Pseudomonas chlororaphis*, and *Pseudomonas fluorescens*) were purified and investigated for polyurethane degradation in a two-step process with PUR esterase (PudA). In the first step, a surface binding domain promotes hydrophobic adsorption of PudA to the PUR surface and enhances in the second step, the hydrolysis of ester bonds (Akutsu et al., 1998).

Cutinases (EC 3.1.1.74) caught attention due to their ability to hydrolyze polyesters with a high molecular weight (Chen et al., 2013). Cutinases are α/β hydrolases originally derived from plant pathogenic fungi (e.g. *Fusarium solani pisi*) (Purdy and Kolattukudy, 1975). Apart from plant fungi, a variety of cutinases were isolated and characterized from bacterial strains (thermophilic actinomycetes) (Chen et al., 2013; Wei et al., 2014a; Zimmermann and Billig, 2011), for instance *Thermobifida fusca* (Kleeberg et al., 2005), *Thermobifida alba* (Hu et al., 2010), *Thermobifida cellulosilytica* (Acero et al., 2013), *Thermomonospora fusca* (Fett et al., 1999), and *Thermomonospora curvata* (Wei et al., 2014b). Hydrolysis of aliphatic polyesters e.g. polycaprolactone (PCL) (Kleeberg et al., 2005; Baker et al., 2012) as well as aliphatic-aromatic co-polyesters such as PET (Wei et al., 2014b; Acero et al., 2011; Gamerith et al., 2017; Ribitsch et al., 2012; Roth et al., 2014) and polytrimethylene terephthalate (PTT) (Eberl et al., 2008) by cutinases have also been reported. Engineering studies on cutinases (Acero et al., 2013; Silva et al., 2011) and fusion to binding domains (Ribitsch et al., 2015; Ribitsch et al., 2013; Zhang et al., 2013) have revealed enhanced polymer degradation. In latter approach, cutinases (from *T. cellulosilytica* and *T. fusca*) were fused to binding domains like hydrophobins (Ribitsch et al., 2015), carbohydrate binding modules (CBM) (Ribitsch et al., 2013; Zhang et al., 2013), and polyhydroxyalkanoate binding modules (PBM) (Ribitsch et al., 2013). In all three cases, the activity toward PET and thus the hydrolysis of PET was enhanced compared to cutinases without a binding domain.

Immobilization of proteins to polymer surfaces such as PP and PS has also been reported at ambient temperature in water through adhesion promoters that were termed as anchor peptides (Rübsam et al., 2017, 2018a). The anchor peptide LCI (47 aa) (Gong et al., 2011) was reported to form a dense monolayer on PP surfaces (Rübsam et al., 2017). LCI served as adhesion promotor to PP and enabled semi-purification of enzymes in 96-well microtiter plates (Zou et al., 2018). Tachystatin A2 (TA2, 44 aa) (Osaki et al., 1999) and LCI were evolved in directed evolution campaigns using the PePevo method and yielded TA2 and LCI variants with increased binding strength to PS and PP in presence of surfactants (LAS and Triton X-100) (Rübsam et al., 2018a). In a KnowVolution campaign LCI's binding strength was improved for stronger PP binding in presence of a non-ionic surfactant (Triton X-100) (Rübsam et al., 2018b). Additionally, short synthetic material binding peptides (7-12mers) were identified from phage libraries and used for directed enzyme immobilization (Zernia et al., 2018), biomining of molybdenite (MoS2) (Cetinel et al., 2018), and a targeted surface binding on polystyrene (PS) (Qiang et al., 2017), polycarbonate (PC), and poly(methylmethacrylate) (PMMA) (Kumada et al., 2012).

Here we report an enhanced degradation of anionic and aliphatic polyester-polyurethane nanoparticles (Impranil® DLN-SD) in diluted suspensions using the anchor peptides TA2 and LCI for targeting and the cutinase Tcur1278 for degradation. Anchor peptides fused to the cutinase Tcur1278 (from *Thermomonospora curvata*) promoted binding and depolymerization of polyester-polyurethane nanoparticles. Degradation of polyester-polyurethane nanoparticles or films was monitored by a MTP-plate based turbidity assay (OD_{600}), DLS, or FE-SEM analysis.

2. Experimental

All chemicals used in this study were of analytical grade or higher quality, and were purchased from AppliChem (Darmstadt, Germany), Sigma Aldrich Chemie (Taufkirchen, Germany) or VWR International (Darmstadt, Germany) unless specified. DNA polymerases were prepared in-house. All oligonucleotides were purchased from Eurofins MWG Operon (Ebersberg, Germany). Polyester-polyurethane dispersion (Impranil® DLN-SD) was purchased from Covestro AG (Leverkusen, Germany). The expression strain Pichia pastoris (*Komagataella phaffii*) BSYBG11 and plasmid pBSYA1S1Z were purchased from bisy e.U. (Hofstätten/Raab, Austria). The *E. coli* strains DH5α and BL21 Gold (DE3) were obtained from Agilent Technologies (Santa Clara, USA).

2.1. Cloning of eGFP-anchor fusion constructs

The reporter protein eGFP was used to determine anchor peptide binding to polyester-polyurethane surfaces. The *egfp* gene was genetically fused to the N-terminus of the anchor peptides (LCI or TA2) using a stiff spacer helix (17 amino acids: AEAAAKEAAAKEAAAKA) (Arai et al., 2001) and a TEV protease cleavage site (7 amino acids: ENLYFQG) (Kapust et al., 2001) as functional separators of both units (for all gene sequence see Table S1). The constructs were generated by phosphorothioate-based ligase-independent gene cloning (PLICing) (Blanusa et al., 2010) and produced as published before (Primer: see Table S2) (Rübsam et al., 2018a).

2.2. Binding analysis of eGFP-anchor to polyester-polyurethane

eGFP-17aa Helix-TEV (negative control), eGFP-17aa Helix-TEV-LCI, and eGFP-17aa Helix-TEV-TA2 were produced in *E. coli* BL21 Gold (DE3) as reported before (Rübsam et al., 2017, 2018a, 2018b). The binding of eGFP as well as eGFP-anchors to polyester-polyurethane was investigated using cell-free extract (CFE) of eGFP fusion peptides (1.5 mg/mL lysozyme in 50 mM Tris-HCl buffer, pH 8.0, 1 h, 37 °C, and 900 rpm). The fluorescence of all CFEs was normalized to 100,000 RFU using a 96-well MTP reader (FLUOstar Omega, BMG LABTECH GmbH, Ortenberg, Germany; excitation (Ex) 485 nm, emission (Em) 520 nm, gain 1000, 35 reads/well). Glass slides were coated with polyester-polyurethane (Impranil® DLN-SD) and air dried. eGFP and eGFP-anchor binding (50 µL) to polyester-polyurethane was performed at room temperature (10 min). All non-bound peptides and the negative control eGFP were washed off in three successive washing steps (3 × 1 mL 50 mM Tris-HCl buffer, pH 8.0). Unspecific binding of eGFP was minimized as previously reported by washing the polyester-polyurethane coated slides with sodium dodecyl benzenesulfonate (LAS) (1 mL, 0.5 mM in 50 mM Tris-HCl buffer, pH 8.0) (Rübsam et al., 2018a). Binding of eGFP-anchors to polyester-polyurethane was confirmed by detection of the fusion partner eGFP (Leica TCS SP8 microscope, Leica Microsystems GmbH, Wetzlar, Germany; Ex: 485 nm, Em: 520 nm, gain 800).

2.3. Generation of Tcur1278 and Tcur1278-anchor fusion constructs

The wild type (WT) *tcur1278* (GenBank accession: CDN67545.1) derived from *Thermomonospora curvata* DSM4318335 was ordered from Eurofins MWG Operons (Ebersberg, Germany) as *Pichia pastoris* codon optimized synthetic gene (see sequence in Table S1). The gene was introduced into pBSYA1S1Z vector by PLICing. The PCR protocols and used primers are listed in supplementary materials (Tables S2–S4). The PLICing product was transformed into electro-competent *E. coli* DH5α. The resulting plasmid, pBSYA1S1Z-Tcur1278 was isolated and

transformed into freshly prepared electro-competent *Pichia pastoris* BSYBG11 for expression. The plasmid, pBSYA1S1Z-Tcur1278 served as the vector backbone for the fusion construct pBSYA1S1Z-Tcur1278-17aa Helix-TEV-TA2, which was generated by PLICing. The final proteins of target were named as Tcur1278-WT und Tcur1278-TA2.

2.4. Production of Tcur1278-WT and Tcur1278-TA2

Tcur1278-WT and Tcur1278-TA2 were overexpressed in *Pichia pastoris* BSYBG11. The cultivation of pre-cultures was performed in 10 mL YPD medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L D-glucose, 100 µg/mL zeocin) using a 100 mL Erlenmeyer flask (30 °C, 200 rpm, 70% humidity, 48 h; Multitron II, Infors GmbH, Einsbach, Germany). The main cultures (200 mL YPD medium supplemented with 100 µg/mL zeocin in 1 L Erlenmeyer flask) were inoculated using the pre-cultures as inoculum to an OD_{600} nm of 0.5. After cultivation (30 °C, 200 rpm, 70% humidity, 72 h; Multitron II, Infors GmbH), the culture supernatant was separated from the cell broth by centrifuging (Sorvall, ThermoFischer Scientific, Darmstadt, Germany; 4 °C, 11,279 × *g*, 15 min). Culture supernatant (200–400 mL) was concentrated using an Amicon Ultra-15 Centrifugal Filter Unit (Merck Millipore Ltd., Tullagreen, Ireland; Cut off: 10 kDa) to 30–40 mL. Tcur1278-WT and Tcur1278-TA2 were purified via a cation exchange column (SOURCE™ 30S, GE Healthcare, Darmstadt, Germany) according to manufacturer's protocol (Running buffer: 50 mM HEPES buffer, pH 7.6, elution buffer: 2 M NaCl).

2.5. Polyester-polyurethane degradation in MTP (turbidity)

Continuous turbidimetric measurements were performed in 96-well MTP (PS-F-bottom, Greiner Bio-One, Frickenhausen, Germany). MTPs were prepared with 130 µL polyester-polyurethane dispersion diluted in buffer (1:100–1:1200 in 100 mM Tris-HCl, pH 8.0). Reactions were started by supplementing culture supernatant (20 µL) or purified protein (0.6 nM). The degradation of polyester-polyurethane was detected over time at 600 nm in a microtiter plate reader (TECAN Sunrise, Tecan Group AG, Männedorf, Switzerland; cycles: 700, kinetic interval: 120 s, RT, duration: 23 h).

2.6. Dynamic light scattering

Purified Tcur1278-WT (0.6 nM), Tcur1278-TA2 (0.6 nM), and buffer control were supplemented to a polyester-polyurethane dilution of 1:900 (in 100 mM Tris-HCl, pH 8.0) and incubated by stirring (ambient temperature, 600 rpm, 72 h). Dynamic light scattering (DLS) measurements were accomplished to determine the hydrodynamic radius (R_h) of the degraded polyester-polyurethane particles with an ALV/CGS-3 goniometer, an ALV/LSE 5004 tau digital correlator, and a JDS uniphase laser operating at 632.8 nm. All measurements were taken in triplicates at an angle of 90 °C and at 20 °C after equilibrating the samples for at least 3 min.

2.7. Field emission scanning electron microscopy

The degradation of polyester-polyurethane by Tcur1278 and Tcur1278-TA2 was visualized with field emission scanning electron microscopy (FE-SEM). A stainless steel support was used for coating of polyester-polyurethane. The stainless steel support (1 cm^2) was cleaned by sonication in NaOH (Bandelin Sonorex Digitec, Berlin, Germany; 1 M, 50 mL, 30 min) and in ddH_2O (500 mL, 30 min). Afterwards, the stainless steel was washed with absolute ethanol (50 mL) and air-dried. The polyester-polyurethane dispersion (10 µL) was coated on the steel support and air-dried as well. Negative control (Tris-HCl buffer, pH 8.0), Tcur1278 and Tcur1278-TA2 were added to the polyester-polyurethane film (10 µL of a 20 µM stock solution) and incubated (ambient temperature, 24 h and 40 h, respectively). Incubation was performed in parafilm (IDL GmbH & Co. KG, Nidderau, Germany) sealed petri dishes (PS, 94/16 MM, Greiner Bio-One) to avoid evaporation. The samples were rinsed with ddH_2O (3 × 1 mL) and air-dried for the subsequent FE-SEM analysis (S-4800 FE-SEM, Hitachi, Schaumburg, USA; accelerating voltage: 2 kV, working distance: 3.8–4.4 mm, magnification: 25080 ×).

3. Results

The result section is divided into four parts. The first part shows the proof of binding of the selected anchor peptides on polyester-polyurethane films. The second section describes the polyester-polyurethane degradation performance by cutinase wild type and cutinase fusion protein with one anchor peptide. In the third part, polyester-polyurethane nanoparticles were degraded and changes in size were determined by DLS. The fourth part highlights a FE-SEM analysis of degraded polyester-polyurethane films on a stainless steel support.

3.1. Binding analysis of eGFP-LCI and eGFP-TA2 to polyester-polyurethane

The anchor peptides LCI and TA2 are reported to bind to the synthetic polymers PP and PS (Rübsam et al., 2018a) and were therefore selected for polyester-polyurethane binding studies. Binding performance of anchor peptide fusion proteins with eGFP can easily be determined on targeted polymer surfaces (PE, PS, PP) by fluorescence microscopy (Rübsam et al., 2018a). Glass slides were coated with eGFP (negative control; Fig. 1A), eGFP-LCI (Fig. 1B), and eGFP-TA2 (Fig. 1C) (normalized fluorescence to 100,000 RFU), washed to remove unspecifically bound proteins, dried and subsequently analyzed by detecting the fusion partner eGFP on the polyester-polyurethane surface via confocal microscopy.

eGFP-TA2 was able to bind to polyester-polyurethane whereas neither the negative control eGFP nor eGFP-LCI showed a detectable fluorescence on the targeted surface after washing (Fig. 1). Accordingly, TA2 was selected as the anchor of choice to generate the fusion protein Tcur1278-TA2.

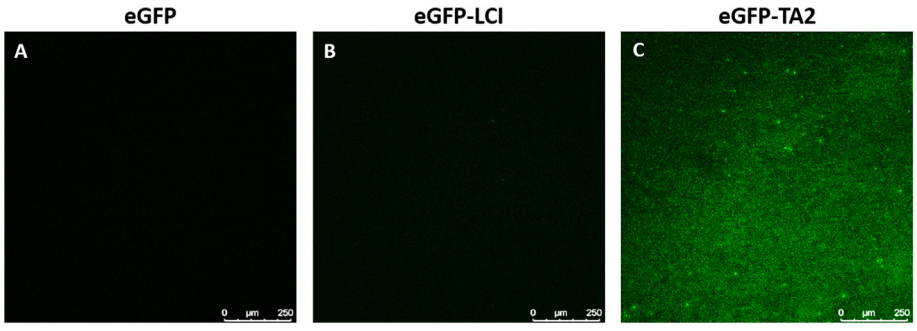

Fig. 1. Binding of eGFP-anchor peptides to Impranil® DLN-SD. Binding of negative control eGFP, eGFP-LCI, and eGFP-TA2 (CFE, 100,000 RFU) was observed by incubation (10 min, ambient temperature) on Impranil® DLN-SD coated glass slides. All unbound proteins were washed off with ddH20 (3 × 1 mL), followed by a washing step with LAS (0.5 mM, 5 min) to prevent non-specific binding of eGFP. Binding of eGFP anchor-peptides was analyzed using confocal microscopy (Leica TCS SP8 microscope; Ex: 485 nm, Em: 520 nm, gain 1000, Leica Microsystems GmbH (Wetzlar, Germany).

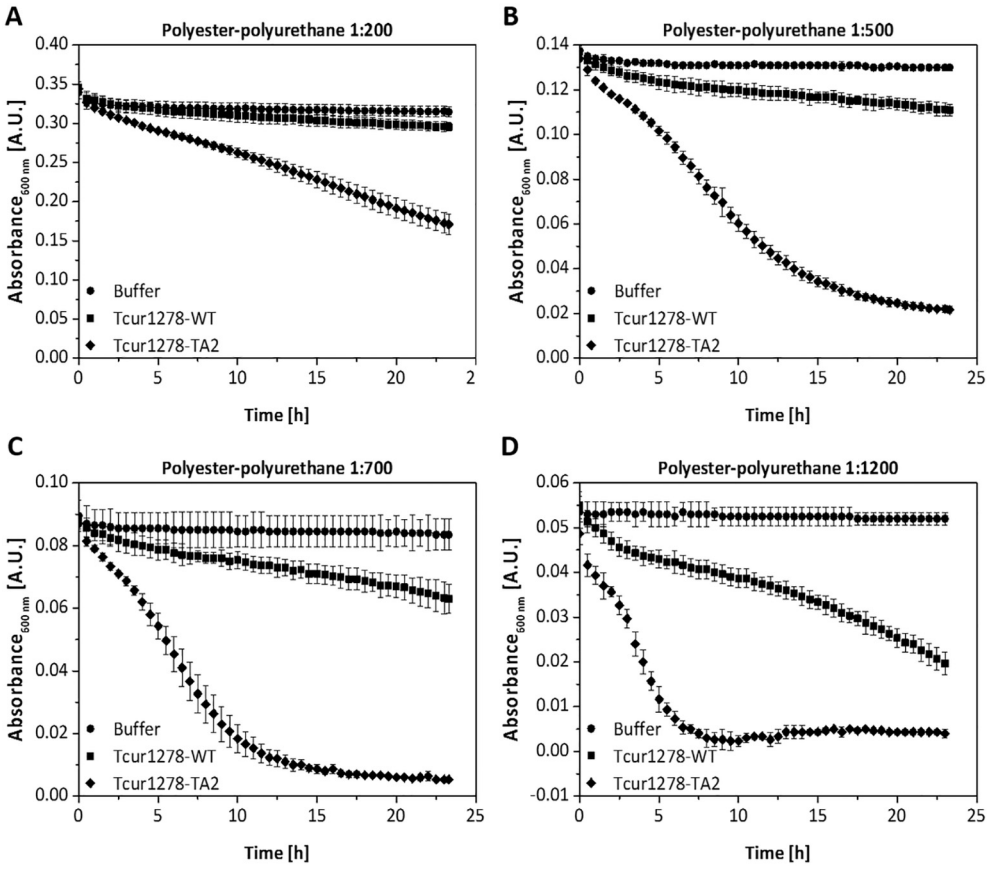

Fig. 2. Continuous turbidity measurements of polyester-polyurethane particle degradation at 600 nm. Enzymatic degradation of polyester-polyurethane dilutions of 1:200 (A), 1:500 (B), 1:700 (C), and 1:1200 (D) for about 24 h. Measurements of buffer control (circle), Tcur1278-WT (square), and Tcur1278-TA2 (diamond) were performed in triplicates.

3.2. Polyester-polyurethane degradation by Tcur1278-anchor fusion proteins

The degradation of polyester-polyurethane nanoparticles is based on the hydrolytic cleavage of ester bonds by Tcur1278 and can be monitored by the decrease of turbidity at 600 nm over time (Alvarez-Barragan et al., 2016). Tcur1278-TA2 and Tcur1278-WT (0.6 nM; equimolar concentration) depolymerization was determined in series of diluted suspensions of polyester-polyurethane nanoparticles (1:100: 0.4% w/v to 1:1200: 0.033% w/v). The degradation of the particles at 1:200 (0.2% w/v), 1:500 (0.08% w/v), 1:700 (0.057% w/v), and 1:1200 (0.033% w/v) polyester-polyurethane dilution are represented in Fig. 2.

In all measurements, no turbidity loss was observed for the negative control (100 mM Tris-HCl, pH 8.0) during the whole reactions time (~one day). The latter suggests that polyester-polyurethane nanoparticles do not self-degrade. Reduced turbidity due to polyester-polyurethane degradation was observed in presence of Tcur1278-WT and Tcur1278-TA2. Kinetic measurements suggest that Tcur1278-TA2 degrades polyester-polyurethane significantly faster than Tcur1278-WT (up to 6.6-fold; Table 1). Two performance indicators were defined to compare degradation kinetics of polyester-polyurethane by Tcur1278-WT and Tcur1278-TA2. The first indicator is the degradation kinetics, which represents the reciprocal slope of the turbidity measurements. The second performance indicator is the half-life of polyester-polyurethane nanoparticles ($t_{1/2}$), at which half of the initial particles are degraded. Table 1 shows the absolute values of degradation kinetics of Tcur1278-WT and Tcur1278-TA2 at varied polyester-polyurethane dilutions. Comparison between Tcur1278-WT and Tcur1278-TA2 discloses that at every dilution of polyester-polyurethane, the adhesive fusion enzyme Tcur1278-TA2 was capable to achieve a more rapid degradation of polyester-polyurethane than Tcur1278-WT (respective improvements are given in fold in Table 1). A factor of 6.6-fold

Table 1
Degradation kinetics of polyester-polyurethane nanoparticles by Tcur1278-WT and Tcur1278-TA2 at constant protein concentration (0.6 nM) over one day. The measured values and their respective standard deviations (±) were obtained from four technical replicates.

Polyester-polyurethane[a]	Tcur1278-WT [(−1) ∗ 10–3 A.U./h]	Tcur1278-TA2 [(−1) ∗ 10–3 A.U./h]	Improvement [fold]
1:100 (0.4%)	3.31 ± 0.09	8.58 ± 0.51	2.59 ± 0.17
1:200 (0.2%)	2.01 ± 0.10	6.50 ± 0.37	3.24 ± 0.24
1:300 (0.133%)	1.30 ± 0.13	5.85 ± 0.44	4.49 ± 0.56
1:400 (0.1%)	1.12 ± 0.11	6.17 ± 0.57	5.52 ± 0.77
1:500 (0.08%)	1.14 ± 0.03	7.06 ± 0.33	6.19 ± 0.34
1:600 (0.067%)	1.18 ± 0.01	7.79 ± 0.23	6.62 ± 0.20
1:700 (0.057%)	1.25 ± 0.12	7.12 ± 0.72	5.70 ± 0.78
1:1200 (0.033%)	1.22 ± 0.03	6.63 ± 0.49	5.42 ± 0.43

[a] Polyester-polyurethane dilution with nanoparticle content in w/v%.

improvement in degradation kinetics of polyester-polyurethane was achieved by Tcur1278-TA2 at a dilution of 1:600 (0.05% w/v nanoparticles). The statistical significance of the regression coefficients was determined by the Fischer's test for analysis of variance (ANOVA; OriginPro 9.1). Tcur1278-TA2 degraded polyester-polyurethane nanoparticles significantly better at all tested dilutions than Tcur1278-WT ($p \leq 0.01$).

Furthermore, Fig. 3 illustrates the half-lives ($t_{1/2}$ in hours) of polyester-polyurethane degradation for Tcur1278-WT and Tcur1278-TA2. The higher the dilution, the lower is the half-life of enzymatically degraded polyester-polyurethane nanoparticles. Notably, a reduced half-life is observed in all cases with Tcur1278-TA2 fusion protein compared to Tcur1278-WT. The half-life of polyester-polyurethane was reduced by a factor of 6.7-fold at a dilution of 1:600 (0.067% w/v nanoparticles) by Tcur1278-TA2 (Tcur1278-WT: 41.8 h and Tcur1278-

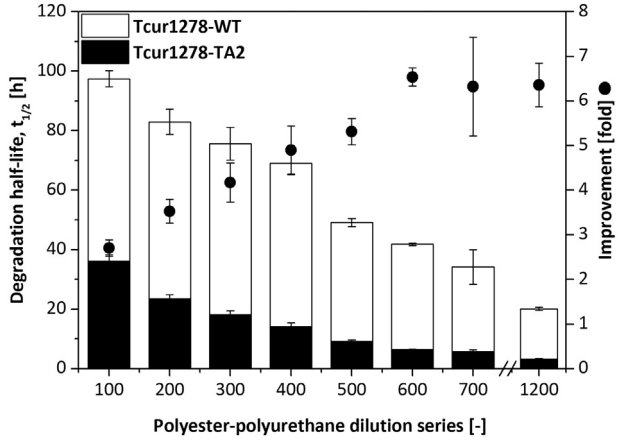

Fig. 3. Degradation half-life of polyester-polyurethane particles. The half-life ($t_{1/2}$) of the polyester-polyurethane particles for each investigated dilution is constituted for Tcur1278-WT (white) and Tcur1278-TA2 (black). $t_{1/2}$ is defined as the time point, at which half of the initial particles are degraded. The circles represent the improvement for each dilution series achieved by Tcur1278-TA2 compared to Tcur1278-WT. All values and the standard deviations were determined in triplicates for each data set.

TA2: 6.2 h). Interestingly, Tcur1278-TA2 degradation kinetics and half-lives ($t_{1/2}$) are in good correspondence with each other at every polyester-polyurethane dilution (Fig. S2).

3.3. Dynamic light scattering of polyester-polyurethane nanoparticles

Size of polyester-polyurethane particle was determined by DLS measurements after three days of cutinase treatment. The overall hydrodynamic radius of the analyzed nanoparticles was 95.3 nm. On average, the hydrodynamic radius of the particles was reduced from 95.3 nm to 66.3–67.7 nm by Tcur1278-WT and Tcur1278–TA2 treatment (Fig. 4). Notably, the degradation of polyester-polyurethane by Tcur1278-TA2 resulted in smaller particles (down to 0.08 nm) when compared to Tcur1278-WT (down to 0.33 nm; see extracted diagram in Fig. 4). Polydispersity index (PDI) of non-enzymatically treated polyester-polyurethane was determined to be 0.102 ± 0.013 and PDI values of Tcur1278-WT and Tcur1278-TA2 were 0.093 ± 0.019 and

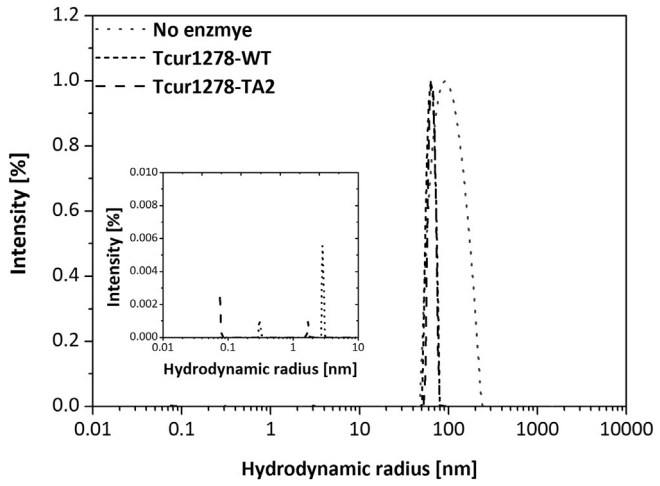

Fig. 4. Hydrodynamic radii (Rh) of degraded polyester-polyurethane particles measured by DLS. The nanoparticle size distribution is represented in samples with no enzymatic treatment, Tcur1278-WT, and Tcur1278-TA2. The extracted diagram (bottom left) shows degraded polyester-polyurethane particles between 0.01 and 10 nm. All measurements were taken in triplicates at an angle of 90 °C and at 20 °C after equilibrating the samples for at least 3 min.

0.099 ± 0.012, respectively.

3.4. FE-SEM analysis of polyester-polyurethane degradation by Tcur1278-WT and Tcur1278-TA2

Degradation of a polyester-polyurethane film at ambient temperature was investigated by FE-SEM for up to 40 h of cutinase treatment. For the latter investigation, a stainless steel support was coated with Impranil® DLN-SD. No degradation of polyester-polyurethane films occurred in the reaction buffer (100 mM Tris-HCL, pH 8.0) when applied onto the film (Fig. 5A and B). Degradation of the polyester-polyurethane film by Tcur1278-WT was not observed after 24 h (Fig. 5C) and 40 h (Fig. 5D) of treatment. Only the Tcur1278-TA2 fusion protein was able to significantly degrade the polymer film within 24 h and 40 h of incubation. After 24 h, a degradation of polyester-polyurethane film by Tcur1278-TA2 was visible, indicated by cracks on the polyester-polyurethane film surface (Fig. 5E; darker color). The degradation was more prominent after 40 h, since most of polyester-polyurethane film was degraded and the stainless steel support underneath became visible (Fig. 5F).

4. Discussion

Microplastics belong to the most severe pollutants worldwide and are of serious concern for human health and from an economic prospective (Bouwmeester et al., 2015; Galloway, 2015). In order to minimize existing environmental pollution, sustainable management strategies are especially needed for microplastics (Sherman and Van Sebille, 2016). To prevent the excessive generation of new microplastics, zero waste strategies and waste management are discussed (Eriksen et al., 2014). The improvement of waste recovery is a crucial point for successful waste management; only 9% of all plastics have been recycled in 2015 (Geyer et al., 2017). Even though wastewater treatment plants have a plastic removal rate of 98%, the effluent wastewater is considered as a major pathway for microplastics to enter into the environment (Murphy et al., 2016; Ziajahromi et al., 2017). The analysis of microplastics in wastewater has been in the focus of many recent reports (Shim et al., 2017; Löder et al., 2015; Kedzierski et al., 2016). Degradation of microplastic particles by microbial and enzymatic means is a promising strategy to convert plastic waste into carbon dioxide, monomers, new biomass and valuable compounds (Grima et al., 2000).

Nature developed a very successful concept for the degradation of natural occurring polymers such as cellulose; specialized enzymes (e.g. cellulases) bind via an adhesion promotor (the cellulose binding domain CBD) to cellulose, which enhances hydrolysis by keeping the hydrolysis domain on the cellulose surface (Linder and Teeri, 1997; Bolam et al., 1998). Similar domains can be found on enzymes depolymerizing chitin (Beintema, 1994) and polyhydroxyalkanoate (Kasuya et al., 1999).

Reported polyester-polyurethane degrading enzymes (e.g. esterases or cutinases) do not possess a hydrophobic binding domain for a targeted polymer degradation. Mimicking nature's CBD concept, the cellobiohydrolase I binding domain was fused to a carboxymethyl cellulase (CSCMCase) which enhanced the catalytic activity toward cellulose from no detectable degradation activity to 2.66 U/mg (Thongekkaew et al., 2013). Fusion of binding modules such as hydrophobins, CBMs, and PBMs to hydrolases has been reported before for PET-degradation (Ribitsch et al., 2015; Ribitsch et al., 2013). Hydrophobins (HBF4 or HBF7) were fused to a PET-degrading cutinase (Thc_Cut1) enhancing the degradation up to 16 times (24 h at 50 °C; 5 µM enzyme) (Ribitsch et al., 2015). The fusion of a PBM to the enzyme Thc_Cut1 enhanced hydrolysis activity for 11-fold compared to the wild type enzyme (72 h at 50 °C; 25 mM enzyme) (Ribitsch et al., 2013). In one study, PBM was utilized as well to degrade polyurethane polyester co-polymers (Gamerith et al., 2016). The fusion of polyamidase and PBM (PA-PBM)

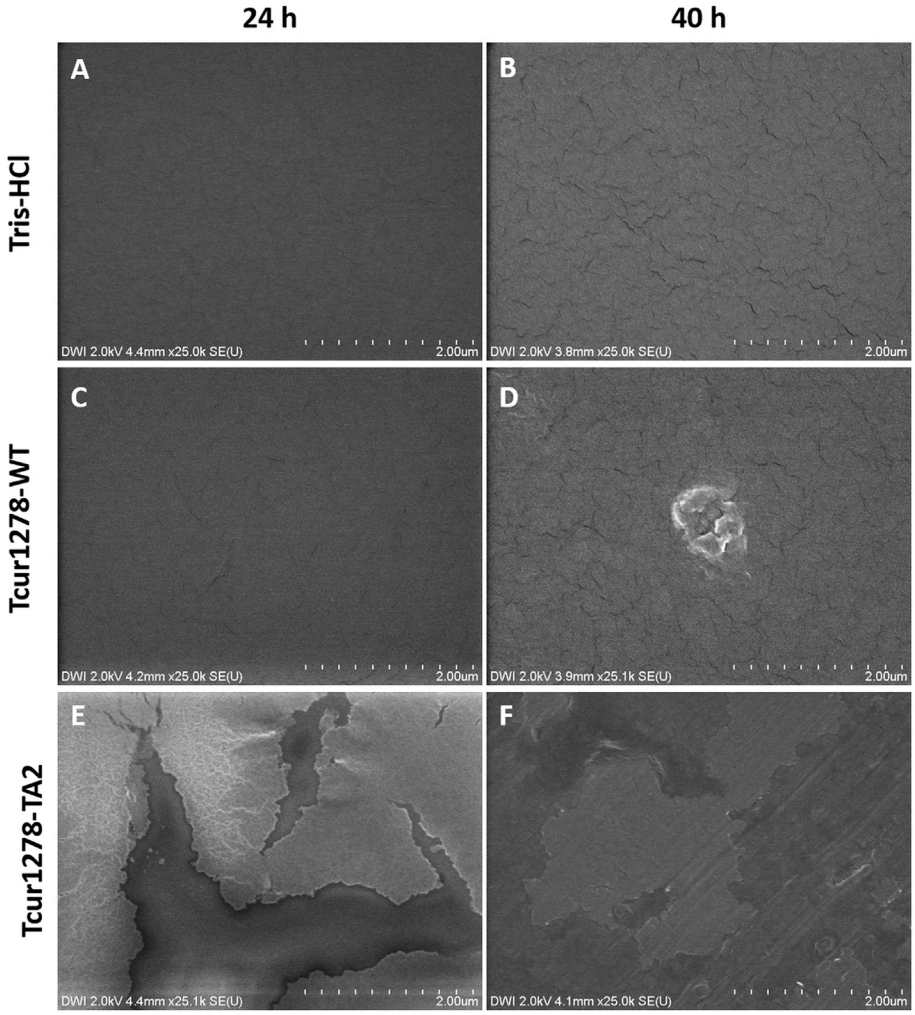

Fig. 5. FE-SEM analysis of polyester-polyurethane degradation. A stainless steel support was coated with Impranil® DLN-SD. Negative control Tris-HCL buffer (100 mM, pH 8.0), Tcur1278-WT, and Tcur1278-TA2 (10 µL and 20 µM each) were incubated on the polyester-polyurethane film for 24 and 40 h, respectively. A) Buffer control (24 h), B) buffer control (40 h), C) Tcur1278-WT (24 h), D) Tcur1278-WT (40 h), E) Tcur1278-TA2 (24 h), and F) Tcur1278-TA2 (40 h) (S-4800 FE-SEM; accelerating voltage: 2 kV, working distance: 3.8–4.4 mm, magnification: 25080×, Hitachi, (Schaumburg, USA)).

was reported to hydrolyze the employed polyurethane polymer four times more efficiently than the native enzyme (7 days at 50 °C; 2.5 µM enzyme) (Gamerith et al., 2016).

We fused the polyester-polyurethane binding anchor peptide TA2 to the cutinase Tcur1278 to achieve a targeted degradation of polyester-polyurethane nanoparticles and investigated in contrast to most studies the degradation kinetics at ambient temperatures to be close to environmentally relevant conditions (e.g. wastewater treatment plants with low microplastic concentration). Degradation kinetics of Tcur1278-TA2 was generally improved for all investigated polyester-polyurethane dilutions compared to Tcur1278-WT. The degradation kinetics of Tcur1278-WT decreased from $(-1)*3.3*10^{-3}$ A.U./h at a dilution of 1:100 to an average of $(-1)*1.18*10^{-3}$ A.U./h (final dilution 1:1200) retaining 35.6% of initial degradation kinetics. In comparison, the degradation kinetics of Tcur1278-TA2 decreased only from $(-1)*8.6*10^{-3}$ A.U./h to an average of $(-1)*6.7*10^{-3}$ A.U./h retaining 78.4% of initial degradation kinetics. In essence, the degradation kinetics of Tcur1278-TA2 was improved by 6.6 fold at a dilution of 1:600 in comparison to the Tcur1278-WT and the $t_{1/2}$ was reduced by 6.7-fold at a dilution of 1:700. At high particle dilutions, the degradation improvement of Tcur1278-TA2 over Tcur1278-WT becomes more prominent due to an unfavorable enzyme-particle ratio. Increasing the particle concentration is expected to minimize the obtained degradation improvement of Tcur1278-TA2 over Tcur1278-WT.

A deeper look at the degraded nanoparticles (before and after enzyme treatment) revealed a smaller hydrodynamic radius of the polyester-polyurethane nanoparticle which shifted from ~95 to ~66 nm (Fig. 4). An enhanced degradation activity of Tcur1278-TA2 was proven by identifying smaller nanoparticles (0.08 nm vs 0.33 nm for Tcur1278-WT) after treatment. FE-SEM results show that polyester-polyurethane films on a stainless steel support are significantly faster degraded by Tcur1278-TA2 at room temperature (24 and 40 h; Fig. 5) compared to Tcur1278-WT. Interestingly, there is a strong difference in the degradation performance of Tcur1278-WT (nanoparticle dispersion are in contrast to films rapidly degraded).

To the best of our knowledge, our report represents the first systematic study on the degradation of polyester-polyurethane nanoparticles in highly diluted suspensions at ambient temperature. Degradation was achieved with an enzyme concentration down to 0.6 nM (Tcur1278-TA2). Low enzyme concentrations and applications at ambient temperature are key parameters for the development of sustainable microplastic degradation processes. Notably, there is a high variation in chemical properties and compositions of synthetic polymers/polymer blends that can affect the performance of hydrolyzing enzymes (e.g. adhesion promotors might be affected by additional coatings or plasticizers). Recently, the reengineering of anchor peptides was reported via a directed evolution campaign (following the KnowVolution strategy) (Rübsam et al., 2018b) and employing a

specialized diversity generation protocol for short peptides (termed PePevo) (Rübsam et al., 2018a). Thereby the anchor peptides TA2 and LCI were improved toward stronger polymer binding (PS and PP, respectively) in the presence of surfactants.

5. Conclusions

Anchor peptides provide a versatile tool for a targeted degradation of microplastics at ambient temperature in highly diluted suspensions. The anchor peptide (TA2) significantly enhanced the Tcur1278 degradation kinetics toward polyester-polyurethane nanoparticles and films on a stainless steel support. Peptide engineering methods and strategies will very likely enable to immobilize polymer degrading enzymes in a specific manner (even on polymer blends) to achieve an effective and controlled depolymerization and thereby enable on the long run the development of sustainable biotechnological processes for conversion of plastics into valuable compounds. Optimized enzyme-anchor peptide fusion proteins might find applications in municipal wastewater treatment plants, for instance, in the third or fourth treatment step to degrade the remaining micro- and nanoparticles.

Conflicts of interest

There are no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.12.029.

References

Acero, E.H., Ribitsch, D., Steinkellner, G., Gruber, K., Greimel, K., Eiteljoerg, I., Trotscha, E., Wei, R., Zimmermann, W., Zinn, M., Cavaco-Paulo, A., Freddi, G., Schwab, H., Guebitz, G., 2011. Enzymatic surface hydrolysis of PET: effect of structural diversity on kinetic properties of cutinases from thermobifida. Macromolecules 44 (12), 4632–4640.

Acero, E.H., Ribitsch, D., Dellacher, A., Zitzenbacher, S., Marold, A., Steinkellner, G., Gruber, K., Schwab, H., Guebitz, G.M., 2013. Surface engineering of a cutinase from *Thermobifida cellulosilytica* for improved polyester hydrolysis. Biotechnol. Bioeng. 110 (10), 2581–2590.

Akutsu, Y., Nakajima-Kambe, T., Nomura, N., Nakahara, T., 1998. Purification and properties of a polyester polyurethane-degrading enzyme from *Comamonas acidovorans* TB-35. Appl. Environ. Microbiol. 64 (1), 62–67.

Allen, A.B., Hilliard, N.P., Howard, G.T., 1999. Purification and characterization of a solublepolyurethane degrading enzyme from *Comamonas acidovorans*. Int. Biodeterior. Biodegrad. 43 (1), 37–41.

Alvarez-Barragan, J., Dominguez-Malfavon, L., Vargas-Suarez, M., Gonzalez-Hernandez, R., Aguilar-Osorio, G., Loza-Tavera, H., 2016. Biodegradative activities of selected environmental fungi on a polyester polyurethane varnish and polyether polyurethane foams. Appl. Environ. Microbiol. 82 (17), 5225–5235.

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62 (8), 1596–1605.

Arai, R., Ueda, H., Kitayama, A., Kamiya, N., Nagamune, T., 2001. Design of the linkers which effectively separate domains of a bifunctional fusion protein. Protein Eng. 14 (8), 529–532.

Baker, P.J., Poultney, C., Liu, Z.Q., Gross, R., Montclare, J.K., 2012. Identification and comparison of cutinases for synthetic polyester degradation. Appl. Microbiol. Biotechnol. 93 (1), 229–240.

Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. B Biol. Sci. 364 (1526), 1985–1998.

Beintema, J.J., 1994. Structural features of plant chitinases and chitin-binding proteins. FEBS Lett. 350 (2–3), 159–163.

Blanusa, M., Schenk, A., Sadeghi, H., Marienhagen, J., Schwaneberg, U., 2010. Phosphorothioate-based ligase-independent gene cloning (PLICing): an enzyme-free and sequence-independent cloning method. Anal. Biochem. 406 (2), 141–146.

Boerger, C.M., Lattin, G.L., Moore, S.L., Moore, C.J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Mar. Pollut. Bull. 60 (12), 2275–2278.

Bolam, D.N., Ciruela, A., McQueen-Mason, S., Simpson, P., Williamson, M.P., Rixon, J.E., Boraston, A., Hazlewood, G.P., Gilbert, H.J., 1998. Pseudomonas cellulose-binding domains mediate their effects by increasing enzyme substrate proximity. Biochem. J. 331 (Pt 3), 775.

Bouwmeester, H., Hollman, P.C., Peters, R.J., 2015. Potential health impact of environmentally released micro-and nanoplastics in the human food production chain: Experiences from nanotoxicology. Environ. Sci. Technol. 49, 8932–8947.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines worldwide: sources and sinks. Environ. Sci. Technol. 45 (21), 9175–9179.

Carpenter, E.J., Anderson, S.J., Harvey, G.R., Miklas, H.P., Peck, B.B., 1972. Polystyrene spherules in coastal waters. Science 178 (4062), 749–750.

Cetinel, S., Shen, W.-Z., Aminpour, M., Bhomkar, P., Wang, F., Borujeny, E.R., Sharma, K., Nayebi, N., Montemagno, C., 2018. Biomining of MoS 2 with peptide-based smart biomaterials. Sci. Rep. 8 (1), 3374.

Chen, S., Su, L.Q., Chen, J., Wu, J., 2013. Cutinase: characteristics, preparation, and application. Biotechnol. Adv. 31 (8), 1754–1767.

Crabbe, J.R., Campbell, J.R., Thompson, L., Walz, S.L., Schultz, W.W., 1994. Biodegradation of a colloidal ester-based polyurethane by soil fungi. Int. Biodeterior. Biodegrad. 33 (2), 103–113.

do Sul, J.A.I., Costa, M.F., 2014. The present and future of microplastic pollution in the marine environment. Environ. Pollut. 185, 352–364.

Eberl, A., Heumann, S., Kotek, R., Kaufmann, F., Mitsche, S., Cavaco-Paulo, A., Gubitz, G.M., 2008. Enzymatic hydrolysis of PTT polymers and oligomers. J. Biotechnol. 135 (1), 45–51.

Eerkes-Medrano, D., Thompson, R.C., Aldridge, D.C., 2015. Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res. 75, 63–82.

El-Sayed, A.H.M.M., Mahmoud, W.M., Davis, E.M., Coughlin, R.W., 1996. Biodegradation of polyurethane coatings by hydrocarbon-degrading bacteria. Int. Biodeterior. Biodegrad. 37 (1), 69–79.

Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS One 9 (12).

Fett, W.F., Wijey, C., Moreau, R.A., Osman, S.F., 1999. Production of cutinase by *Thermomonospora fusca* ATCC 27730. J. Appl. Microbiol. 86 (4), 561–568.

Galloway, T.S., 2015. Micro-and nano-plastics and human health. Marine anthropogenic litter Springer, pp. 343–366.

Gamerith, C., Herrero Acero, E., Pellis, A., Ortner, A., Vielnascher, R., Luschnig, D., Zartl, B., Haernvall, K., Zitzenbacher, S., Strohmeier, G., Hoff, O., Steinkellner, G., Gruber, K., Ribitsch, D., Guebitz, G.M., 2016. Improving enzymatic polyurethane hydrolysis by tuning enzyme sorption. Polym. Degrad. Stab. 132, 69–77.

Gamerith, C., Vastano, M., Ghorbanpour, S.M., Zitzenbacher, S., Ribitsch, D., Zumstein, M.T., Sander, M., Acero, E.H., Pellis, A., Guebitz, G.M., 2017. Enzymatic degradation of aromatic and aliphatic polyesters by *P. pastoris* expressed cutinase 1 from Thermobifida cellulosilytica. Front. Microbiol. 8.

Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3 (7).

Gong, W., Wang, J., Chen, Z., Xia, B., Lu, G., 2011. Solution structure of LCI, a novel antimicrobial peptide from *Bacillus subtilis*. Biochemistry 50 (18), 3621–3627.

Grima, S., Bellon-Maurel, V., Feuilloley, P., Silvestre, F., 2000. Aerobic biodegradation of polymers in solid-state conditions: a review of environmental and physicochemical parameter settings in laboratory simulations. J. Polym. Environ. 8 (4), 183–195.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ. Sci. Technol. 46 (6), 3060–3075.

Howard, G.T., 2002. Biodegradation of polyurethane: a review. Int. Biodeterior. Biodegrad. 49 (4), 245–252.

Hu, X., Thumarat, U., Zhang, X., Tang, M., Kawai, F., 2010. Diversity of polyester-degrading bacteria in compost and molecular analysis of a thermoactive esterase from *Thermobifida alba* AHK119. Appl. Microbiol. Biotechnol. 87 (2), 771–779.

Kapust, R.B., Tözsér, J., Fox, J.D., Anderson, D.E., Cherry, S., Copeland, T.D., Waugh, D.S., 2001. Tobacco etch virus protease: mechanism of autolysis and rational design of stable mutants with wild-type catalytic proficiency. Protein Eng. 14 (12), 993–1000.

Kasuya, K.-i., Ohura, T., Masuda, K., Doi, Y., 1999. Substrate and binding specificities of bacterial polyhydroxybutyrate depolymerases. Int. J. Biol. Macromol. 24 (4), 329–336.

Kay, M.J., Morton, L.H.G., Prince, E.L., 1991. Bacterial degradation of polyester polyurethane. Int. Biodeterior. 27 (2), 205–222.

Kedzierski, M., Le Tilly, V., Bourseau, P., Bellegou, H., César, G., Sire, O., Bruzaud, S., 2016. Microplastics elutriation from sandy sediments: a granulometric approach. Mar. Pollut. Bull. 107 (1), 315–323.

Kim, M., Hyun, S., Kwon, J.-H., 2015. Estimation of the environmental load of high- and low-density polyethylene from South Korea using a mass balance approach. Arch. Environ. Contam. Toxicol. 69 (3), 367–373.

Kleeberg, I., Welzel, K., Vandenheuvel, J., Muller, R.J., Deckwer, W.D., 2005.

Characterization of a new extracellular hydrolase from *Thermobifida fusca* degrading aliphatic-aromatic copolyesters. Biomacromolecules 6 (1), 262–270.
Kumada, Y., Murata, S., Ishikawa, Y., Nakatsuka, K., Kishimoto, M., 2012. Screening of PC and PMMA-binding peptides for site-specific immobilization of proteins. J. Biotechnol. 160 (3–4), 222–228.
Linder, M., Teeri, T.T., 1997. The roles and function of cellulose-binding domains. J. Biotechnol. 57 (1), 15–28.
Löder, M.G.J., Kuczera, M., Mintenig, S., Lorenz, C., Gerdts, G., 2015. Focal plane array detector-based micro-Fourier-transform infrared imaging for the analysis of microplastics in environmental samples. Environ. Chem. 12 (5), 563–581.
Murphy, F., Ewins, C., Carbonnier, F., Quinn, B., 2016. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. Environ. Sci. Technol. 50 (11), 5800–5808.
Nakajima-Kambe, T., Onuma, F., Kimpara, N., Nakahara, T., 1995. Isolation and characterization of a bacterium which utilizes polyester polyurethane as a sole carbon and nitrogen source. FEMS Microbiol. Lett. 129 (1), 39–42.
Nakajima-Kambe, T., Shigeno-Akutsu, Y., Nomura, N., Onuma, F., Nakahara, T., 1999. Microbial degradation of polyurethane, polyester polyurethanes and polyether polyurethanes. Appl. Microbiol. Biotechnol. 51 (2), 134–140.
Osaki, T., Omotezako, M., Nagayama, R., Hirata, M., Iwanaga, S., Kasahara, J., Hattori, J., Ito, I., Sugiyama, H., Kawabata, S.-i., 1999. Horseshoe crab hemocyte-derived antimicrobial polypeptides, tachystatins, with sequence similarity to spider neurotoxins. J. Biol. Chem. 274 (37), 26172–26178.
PlasticsEurope, 2017. Plastics - The Facts 2017: An Analysis of European Plastics Production, Demand and Waste Data.
Purdy, R.E., Kolattukudy, P.E., 1975. Hydrolysis of plant cuticle by plant pathogens. Properties of cutinase I, cutinase II, and a nonspecific esterase isolated from *Fusarium solani pisi*. Biochemistry 14 (13), 2832–2840.
Qiang, X., Sun, K., Xing, L., Xu, Y., Wang, H., Zhou, Z., Zhang, J., Zhang, F., Caliskan, B., Wang, M., 2017. Discovery of a polystyrene binding peptide isolated from phage display library and its application in peptide immobilization. Sci. Rep. 7 (1), 2673.
Ribitsch, D., Acero, E.H., Greimel, K., Eiteljoerg, I., Trotscha, E., Freddi, G., Schwab, H., Guebitz, G.M., 2012. Characterization of a new cutinase from *Thermobifida alba* for PET-surface hydrolysis. Biocatalysis Biotransformation 30 (1), 2–9.
Ribitsch, D., Yebra, A.O., Zitzenbacher, S., Wu, J., Nowitsch, S., Steinkellner, G., Greimel, K., Doliska, A., Oberdorfer, G., Gruber, C.C., Gruber, K., Schwab, H., Stana-Kleinschek, K., Acero, E.H., Guebitz, G.M., 2013. Fusion of binding domains to *Thermobifida cellulosilytica* cutinase to tune sorption characteristics and enhancing PET hydrolysis. Biomacromolecules 14 (6), 1769–1776.
Ribitsch, D., Acero, E.H., Przylucka, A., Zitzenbacher, S., Marold, A., Gamerith, C., Tscheliessnig, R., Jungbauer, A., Rennhofer, H., Lichtenegger, H., Amenitsch, H., Bonazza, K., Kubicek, C.P., Druzhinina, I.S., Guebitz, G.M., 2015. Enhanced cutinase-catalyzed hydrolysis of polyethylene terephthalate by covalent fusion to hydrophobins. Appl. Environ. Microbiol. 81 (11), 3586–3592.
Roth, C., Wei, R., Oeser, T., Then, J., Follner, C., Zimmermann, W., Strater, N., 2014. Structural and functional studies on a thermostable polyethylene terephthalate degrading hydrolase from *Thermobifida fusca*. Appl. Microbiol. Biotechnol. 98 (18), 7815–7823.
Rowe, L., Howard, G.T., 2002. Growth of Bacillus subtilis on polyurethane and the purification and characterization of a polyurethanase-lipase enzyme. Int. Biodeterior. Biodegrad. 50 (1), 33–40.
Rübsam, K., Stomps, B., Böker, A., Jakob, F., Schwaneberg, U., 2017. Anchor peptides: a green and versatile method for polypropylene functionalization. Polymer 116, 124–132.
Rübsam, K., Weber, L., Jakob, F., Schwaneberg, U., 2018a. Directed evolution of polypropylene and polystyrene binding peptides. Biotechnol. Bioeng. 115 (2), 321–330.
Rübsam, K., Davari, M.D., Jakob, F., Schwaneberg, U., 2018b. KnowVolution of the polymer-binding peptide LCI for improved polypropylene binding. Polymers 10 (4), 423.
Ruiz, C., Main, T., Hilliard, N.P., Howard, G.T., 1999. Purification and characterization of two polyurethanase enzymes from *Pseudomonas chlororaphis*. Int. Biodeterior. Biodegrad. 43 (1–2), 43–47.
Shah, A.A., Hasan, F., Hameed, A., Ahmed, S., 2008. Biological degradation of plastics: a comprehensive review. Biotechnol. Adv. 26 (3), 246–265.
Sharma, S., Chatterjee, S., 2017. Microplastic pollution, a threat to marine ecosystem and human health: a short review. Environ. Sci. Pollut. Res. 24 (27), 21530–21547.
Sherman, P., Van Sebille, E., 2016. Modeling marine surface microplastic transport to assess optimal removal locations. Environ. Res. Lett. 11 (1), 014006.
Shim, W.J., Hong, S.H., Eo, S.E., 2017. Identification methods in microplastic analysis: a review. Anal. Methods 9 (9), 1384–1391.
Silva, C., Da, S., Silva, N., Matama, T., Araujo, R., Martins, M., Chen, S., Chen, J., Wu, J., Casal, M., Cavaco-Paulo, A., 2011. Engineered *Thermobifida fusca* cutinase with increased activity on polyester substrates. Biotechnol. J. 6 (10), 1230–1239.
de Stephanis, R., Giménez, J., Carpinelli, E., Gutierrez-Exposito, C., Cañadas, A., 2013. As main meal for sperm whales: plastics debris. Mar. Pollut. Bull. 69 (1), 206–214.
Thongekkaew, J., Ikeda, H., Masaki, K., Iefuji, H., 2013. Fusion of cellulose binding domain from *Trichoderma reesei* CBHI to *Cryptococcus* sp. S-2 cellulase enhances its binding affinity and its cellulolytic activity to insoluble cellulosic substrates. Enzym. Microb. Technol. 52 (4), 241–246.
Vega, R.E., Main, T., Howard, G.T., 1999. Cloning and expression in *Escherichia coli* of apolyurethane-degrading enzyme from *Pseudomonas fluorescens*. Int. Biodeterior. Biodegrad. 43 (1–2), 49–55.
Wei, R., Oeser, T., Zimmermann, W., 2014a. Synthetic polyester-hydrolyzing enzymes from thermophilic actinomycetes. In: Sariaslani, S., Gadd, G.M. (Eds.), Advances in Applied Microbiology. vol 89. pp. 267–305 Vol. 89.
Wei, R., Oeser, T., Then, J., Kuhn, N., Barth, M., Schmidt, J., Zimmermann, W., 2014b. Functional characterization and structural modeling of synthetic polyester-degrading hydrolases from *Thermomonospora curvata*. AMB Express 4.
Zernia, S., Frank, R., Weiße, R.H.J., Jahnke, H.G., Bellmann-Sickert, K., Prager, A., Abel, B., Sträter, N., Robitzki, A., Beck-Sickinger, A.G., 2018. Surface-binding peptide facilitates electricity-driven NADPH-free cytochrome P450 catalysis. ChemCatChem 10 (3), 525–530.
Zhang, Y., Wang, L., Chen, J., Wu, J., 2013. Enhanced activity toward PET by site-directed mutagenesis of *Thermobifida fusca* cutinase-CBM fusion protein. Carbohydr. Polym. 97 (1), 124–129.
Zheng, Y., Yanful, E.K., Bassi, A.S., 2005. A review of plastic waste biodegradation. Crit. Rev. Biotechnol. 25 (4), 243–250.
Ziajahromi, S., Neale, P.A., Rintoul, L., Leusch, F.D., 2017. Wastewater treatment plants as a pathway for microplastics: development of a new approach to sample wastewater-based microplastics. Water Res. 112, 93–99.
Zimmermann, W., Billig, S., 2011. Enzymes for the biofunctionalization of poly(ethylene terephthalate). In: Nyanhongo, G.S., Steiner, W., Gubitz, G.M. (Eds.), Biofunctionalization of Polymers and Their Applications. 125. pp. 97–120.
Zou, Z., Mate, D.M., Rübsam, K., Jakob, F., Schwaneberg, U., 2018. Sortase-mediated high-throughput screening platform for directed enzyme evolution. ACS Comb. Sci. 20 (4), 203–211.