Available online at www.sciencedirect.com

ScienceDirect

J. Biochem. Biophys. Methods 39 (2006) 89–99

JOURNAL OF biochemical and biophysical methods

www.elsevier.com/locate/jbbm

New model substrates for enzymes hydrolysing polyethyleneterephthalate and polyamide fibres

Sonja Heumann [a,b], Anita Eberl [a], Herbert Pobeheim [a], Stefan Liebminger [a], Gudrun Fischer-Colbrie [b], Eva Almansa [a], Artur Cavaco-Paulo [c], Georg M. Gübitz [b,*]

[a] *Research Centre Applied Biocatalysis, Petersgasse 14, A-8010 Graz, Austria*
[b] *Graz University of Technology, Department of Environmental Biotechnology, Petersgasse 12, A-8010 Graz, Austria*
[c] *University of Minho, Textile Engineering Department, 4800 Guimarães, Portugal*

Received 4 November 2005; received in revised form 8 February 2006; accepted 9 February 2006

Abstract

Recently the potential of enzymes for surface hydrophilisation and/or functionalisation of polyethyleneterephthalate (PET) and polyamide (PA) has been discovered. However, there was no correlation between enzyme class/activity (e.g. esterase, lipase, cutinase) and surface hydrolysis of these polymers and consequently no simple assay to estimate this capability. Enzymes active on the model substrates bis (benzoyloxyethyl) terephthalate and adipic acid bishexyl-amide, were also capable of increasing the hydrophilicity of PET and PA. When dosed at the identical activity on 4-nitrophenyl butyrate, only enzymes from *Thermobifida fusca, Aspergillus* sp., *Beauveria* sp. and commercial enzymes (TEXAZYME PES sp5 and Lipase PS) increased the hydrophilicity of PET fibres while other esterases and lipases did not show any effect. Activity on PET correlated with the activity on the model substrate. Hydrophilicity of fibres was greatly improved based on increases in rising height of up to 4.3 cm and the relative decrease of water absorption time between control and sample of the water was up to 76%. Similarly, enzymes increasing the hydrophilicity of PA fibres such as from *Nocardia* sp., *Beauveria* sp. and *F. solani* hydrolysed the model substrate; however, there was no common enzyme activity (e.g. protease, esterase, amidase) which could be attributed to all these enzymes.

Keywords: Polyamide; Polyethyleneterphthalate; Enzyme; Model substrate

* Corresponding author. Tel.: +43 316 873 8314; fax: +43 316 873 8815.
E-mail address: guebitz@tugraz.at (G.M. Gübitz).

0165-022X/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jbbm.2006.02.005

1. Introduction

Synthetic polymers form the base for more than 55% of all textile materials with a world wide fibre production of 33.6 million tons [8]. Besides many beneficial properties, synthetic fibres show various disadvantages in processabilty and wearing comfort mostly due to low hydrophilicity and flexibility. Undesired characteristics of the most widely used synthetic fibres based on polyethyleneterephthalates (PET) include difficulties in finishing, build-up of electrostatic charge, the tendency to pilling, insufficient washability and wearing comfort due to low water absorbency [1,25]. Although alkaline treatment of PET can easily increase hydrophilicity, favourable properties of polyester such as strength are negatively influenced [25].

Recently, partial surface hydrolysis of PET using enzymes has been shown to increase hydrophilicity of PET fabrics without compromises in fibre strength [1,4,9,17,25,26]. Interestingly, only very few representatives among lipases, esterases, cutinases and other not further defined hydrolases have been shown by these authors to hydrolyze synthetic polyesters. Thus, there exists no correlation between standard enzyme assays e.g. for lipase activity and the ability to hydrolyse PET. Enzymatic surface hydrolysis of PET was usually monitored either by indirect methods such as quantification of increased hydrophilicity [9] or direct methods such as HPLC based detection of released substances [25]. All these methods are very useful for mechanistic studies but they are rather time-consuming due to the experimental set-up and low hydrolysis rates of the PET polymer.

Like PET fibres, polyamide based fibres are hydrophobic and show poor wettability. Although alkaline hydrolysis is an effective way to improve fibre wettability, this treatment is difficult to control and extensive damage of fibres can be obtained [22]. Recently we have reported that targeted surface modification can be achieved with hydrolytic enzymes [21,23] while other authors have reported enzymatic hydrolysis of nylon oligomers [11,12,20]. In addition, the potential of oxidative enzymes modification of polyamides has been reported [7,15].

For the monitoring of enzymatic hydrolysis of PA the increase of hydrophilicity of fabrics can be quantified like for PET [9]. Furthermore, a method has been established to determine the increase of amino groups formed during enzyme hydrolysis based on their derivatisation with TNBS (2,4,6-trinitrobenzenesulfonic acid) and subsequent spectrophotometric detection at 420 nm [22]. Additionally, the same authors have developed a method for the estimation of surface amino groups resulting from enzyme treatment based on dyeing with α-bromoacrylamido reactive dyes. All these methods give a good insight in enzymatic hydrolysis of PA, however, these techniques are rather time consuming. Thus, in order to allow further exploitation of biodiversity and/or enzyme engineering to obtain more efficient enzymes for modification of PET and PA materials, there is a strong demand for more efficient screening assays. In this study we have developed screening assays for PA and PET hydrolysing enzymes based on model substrates.

2. Materials and methods

2.1. Chemicals and enzymes

2.1.1. Chemicals

Acetonitrile and methanol used were HPLC quality and purchased by Roth (Carl Roth GmbH, Karlsruhe, Germany). Water was distilled twice. All other chemicals were analytical grade from SIGMA.

2.1.2. Enzymes

The hydrolase from *Thermobifida fusca* was kindly provided by Prof. Müller from GBF Braunschweig, Germany [16]. The esterase TEXAZYM PES sp5 was obtained from inoTEX Ltd., Dvur Kralove, Czech Republic while Lipase PS was obtained from Amano Enzyme Inc. and the neutral–alkaline protease Multiplus L was from Genencor Inc. Further enzyme preparations were obtained by shake flask cultivation: *Aspergillus* sp. St5 and *Nocardia* sp. PA1 were cultivated according to the conditions described previously [9]. Briefly, a mineral salt media with pH 5.5 with cutin as a carbon source and (pH 7.0) with PA model substrate as the only carbon source were used, respectively. In case of *Aspergillus* sp. extra cellular fraction was used while in case of *Nocardia* sp. the intracellular fraction was applied. *Beauveria* sp. was cultivated in a medium consisting of (g/L) 10 wheat bran, 4.0 $MgSO_4 \cdot 7H_2O$, 1.0 glucose, 1.5 yeast extract, 1.5 KH_2PO_4, 2.5 Na_2HPO_4 and 1.0 ammonium sulphate at pH 4.0 and the extra cellular enzymes were used for all further experiments [3]. The extracellular cutinase from *Fusarium solani pisi* was overproduced by *Saccharomyces cerevisiae* SU50 as described previously [21].

2.2. Model substrates

The synthesis of the PET model substrate bis (benzoyloxyethyl) terephthalate was carried out in two steps [2,9]. Briefly, benzoylchloride and a 1.2 fold excess of 2-chloroethanol were mixed and stirred at 110 °C for 24 h to form the benzoic acid 2-chloroethylester. After removal of excess of 2-chloroethanol with a rotavapor, terephthalic dissolved in dimethylformamide acid (1:2 mol/mol) and triethylamine (1:1 mol/mol) were added and esterification was carried out at 140 °C for 24 h. Further purification included solvent exchange to toluol, filtration, crystallization from methanol and silica gel chromatography (Fig. 1).

The model substrate of the PA adipic acid bishexyl-amide was synthesised in one step. 100 mL hexylamine (1.1 mol) and 50 mL dimethyl adipate (0.3 mol) were mixed and stirred at 85 °C for 72 h. After cooling down to room temperature, the product was washed with 100 ml of 50 mM sulphuric acid to remove excess hexylamine and then with 100 ml of water

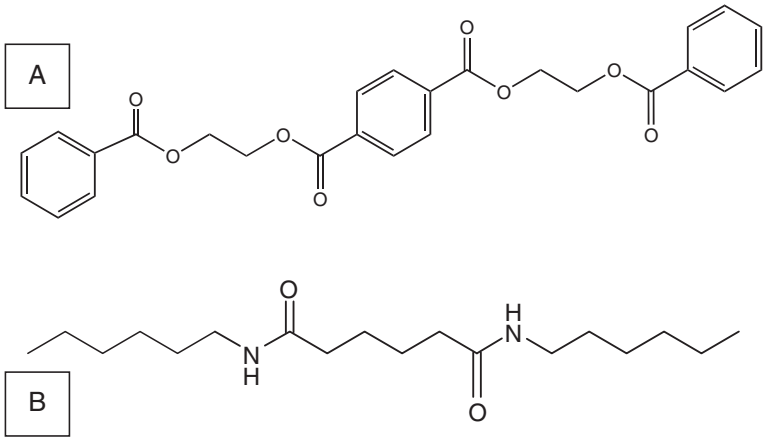

Fig. 1. A — Structure of PET model substrate (bis (benzoyloxyethyl) terephthalate), B — Structure of PA model substrate (adipic acid bishexyl-amide).

and 100 ml of acetone. The product was dried at 100 °C to constant weight giving a white powder (Fig. 1).

2.3. Enzyme activity

2.3.1. Esterase activity

Esterase activity was measured using 4-nitrophenyl butyrate as a substrate. 84 μL of 4-nitrophenyl butyrate were dissolved in 1000 μL DMSO and stored at −20 °C until use. The assay mixture consisted of 1000 μL of a potassium phosphate buffer (50 mM, pH 7.0), 100 μL of the enzyme solution and 10 μL of the substrate solution. The increase of the absorbance at 405 nm was measured at room temperature using a spectrophotometer type Hitachi U 2001. A blank was measured using 100 μL buffer instead of sample. The increase of the absorbance at 405 nm indicates an increase of 4-nitrophenolate due to hydrolysis of the substrate. The activity was calculated in units, where 1 unit is the amount of enzyme required to hydrolyze 1 μmol of substrate per minute under the given assay conditions.

2.3.2. Protease activity

Protease activity was determined using azocasein as substrate. 150 μL of the sample were mixed with 250 μL of a 2% azocasein solution in phosphate buffer (25 mM, pH 7.5). After an incubation time of 30 min at 37 °C, the reaction was stopped by adding 1.2 mL 10% trichloroacetic acid and then centrifuged (4 min, 3000 g). 600 μL of the supernatant were added to 700 μL of a 1 M NaOH solution and mixed. The absorbance at 440 nm was monitored using a Hitachi U 2001 spectrometer. The activity was calculated in units where 1 unit was defined as the amount of enzyme required to produce the absorbance change of 1.0 in 1 cm cuvette under the conditions of the assay per minute.

2.3.3. Amidase activity

The assay of amidase activity was carried out in a reaction mixture (320 μL) containing 220 μL potassium phosphate buffer (50 mM, pH 7.0), 20 μL of a 430 μM hexane amide solution and 100 μL enzyme solution. The reaction was carried out at 30 °C for 60 min and stopped with 32 μL 10% trichloroacetic acid. After centrifugation at 16,000 g for 5 min the released ammonium was determined colourimetrically by the phenol/hypochlorite method [24]. 100 μL reaction mixture were added to 350 μL hypochlorite solution together with 350 μL phenol reagent. The absorbance was read at 625 nm after 15 min incubation at 50 °C. The amount of ammonium released was determined from a standard curve. One unit of the amidase activity was defined as the amount of enzyme required for the hydrolysis of 1 μmol of amide (corresponding to the formation of 1 μmol ammonium) per minute in 1 mL enzyme solution under the conditions of the assay.

2.4. Monitoring hydrolysis of the PET model substrate

2.4.1. Sample preparation

20 mg model substrates were treated with 0.6 mL enzyme solution diluted to give the esterase activity on 4-nitrophenyl butyrate as indicated below. After 60 min of incubation the reaction was stopped by adding 10 μL conc. sulphuric acid. The samples were kept at room temperature for 15 min and then centrifuged at 16,000 g (Hereaus, Biofuge primo) and filtered with syringe filter (13 mm, with 0.2 μm pore size) for HPLC use.

2.4.2. HPLC

The HPLC equipment used was a DIONEX P 580 PUMP with an ASI-100 Automated Sample Injector and a PDA-100 Photodiode Array Detector. For analyses of the benzoic acid as a product of the PET model substrate a reversed phase column (Source 5RPC ST 4.6/150 amersham pharmacia biotech) was used. Separation was achieved by elution using a composition of 40% methanol, 10% 10 mM sulphuric acid and 50% water. A flux of 1 mL/min and a temperature of 40 °C were adjusted. The amount of benzoic acid released was determined from a standard curve. One unit of the enzyme is defined as the amount of enzyme that catalyzes the formation of benzoic acid at the rate of 1 μmol/min.

2.5. Monitoring hydrolysis of the PA model substrate

2.5.1. Sample preparation

20 mg model substrates were treated with 0.6 mL enzyme solution diluted to give the esterase activity on 4-nitrophenyl butyrate as indicated below and incubated as described for the PET model substrate.

2.5.2. HPLC

For analyses of adipic acid as a product of model substrate of PA the analytes were separated with a reversed-phase column Discovery 15 cm (Supelco) installed in the HPLC system described above. Separation was achieved by elution using 8% acetonitrile, 10% 10 mM sulphuric acid and 82% water. A flux of 1 mL/min and a temperature of 40 °C were adjusted. The amount of adipic acid released was determined from a standard curve. One unit of the enzyme is defined as the amount of enzyme that catalyzes the formation of adipic acid at the rate of 0.5 μmol/min.

2.6. Enzyme treatment of synthetic fabrics

2.6.1. Enzyme treatment

The synthetic fabrics were washed with sodium phosphate ($Na_2HPO_4 \cdot 2H_2O$, 5 mM) solution for 30 min as pre-treatment to remove the finishes on the surface; finally the fibres were rinsed with distilled water. The fabrics were cut into pieces of 3×18 cm and treated in 250 mL Erlenmeyer flasks with the enzyme solutions for 60 min. Respective controls with denatured enzymes 100 °C for 10 min or without enzyme were prepared accordingly. Afterwards fabrics were washed with sodium carbonate (9.4 mM, pH 9.5) and distilled water (4 times) to remove adsorbed enzymes and other media impurities. The fabrics were dried at room temperature overnight. The absence of protein impurities adsorbed on the fibre surfaces was confirmed using Coomassie blue staining method comparing with a reference.

2.6.2. Measurement of hydrophilicity

To measure the surface modification of synthetic polymers two different analytic methods indicating changes in hydrophilicity were used: Water absorption was determined using rising height and drop test.

Rising height was measured using a method slightly modified from DIN 53924. The method consisted in suspending the fabrics on a glass rod and bringing a water bath at the bottom of the fabrics, the fabrics dept at 1 cm in the distilled water for 10 min. After 10 min the water level on the fabric is measured immersed up to 1 cm.

Drop dissipation measurement (called *drop test*) was carried out according to AATC specification. A drop of distilled is suspended on the surface of the fabric. The time the drop takes to disappear in the fabric is measured (20 μL).

3. Results

The synthesis of the PET model substrate bis (benzoyloxyethyl) terephthalate was done in two steps in overall yields between 45% and 50%. The synthesis of the PA model substrate adipic acid bishexyl-amide gave a higher yield of 90%. The structures and purities of both model substrates were confirmed with NMR. NMR spectra were recorded on a Varian Unity Inova 500 instrument; ^{1}H and ^{13}C data were measured at 499.8 and 125.7 MHz, respectively (data not shown).

To find a possible correlation between enzyme activity on the model substrates and on the polymeric materials a total of 56 experimental and commercial enzymes were tested in a screening based on hydrophilicity increase of PET and PA (data not shown). Only few enzymes were found to be capable of attacking PET or PA which are all listed in Tables 1 and 2.

3.1. Polyester

Interestingly, all enzymes which were able to cleave the model substrate for polyethyleneterephthalate were able to modify the surface of this synthetic textile material based on substantial increases in hydrophilicity. The increase of hydrophilicity was quantified with the rising height and drop test (Table 1). The activity on the model substrate was measured based on the quantification of benzoic acid using HPLC. This method proved to be simple and reliable (Fig. 2).

In order to obtain comparable results the PET model substrate was incubated with different enzymes but with the same activity against p-nitrophenyl butyrate (10 U). Although activity on the model substrates indicates the capability of the enzymes to increase hydrophilicity there was no linear correlation. While the enzyme preparation of *Aspergillus* sp. caused a slightly higher hydrophilicity increase on PET (4.3. cm) than the hydrolase from *T. fusca*, the activity of the latter enzyme on the PET model substrate was more than two hundred fold higher. Moreover we have experienced even decreasing hydrophilicity after prolonged incubation or higher enzyme activities (data not shown). These findings are most likely due to different modes of action of the enzymes (endo/exo) and the release of smaller fragments at a certain stage of incubation which would again decrease hydrophilicity.

Table 1
Activity of different enzymes on a PET model substrate and increase of hydrophilicity of PET fabrics

Enzyme	Activity on PET model substrate [U]	Rising height [cm] [a]	Drop test [%] [b]
Thermobifida fusca	4.27	3.3	66
TEXAZYME PES sp5	0.06	2.1	48
Lipase PS	0.40	4.2	n.d.
Aspergillus sp. St 5	0.02	4.3	71
Beauveria sp.	5.46	1.2	76
F. solani	0.05	2.3	24

All enzymes were dosed at 10 U against *p*-nitrophenol butyrate.
n.d. not determined.
[a] Difference between control and sample.
[b] Relative decrease of water absorption time between control and sample.

Table 2
Activity of different enzymes on a PA model substrate and increase of hydrophilicity of PA fabrics

Enzyme	Protease activity [U]	Activity on PA model substrate [mU]	Rising height [cm][a]	Drop test [%][b]
Thermobifida fusca	0	0	0	0
Nocardia sp.	0	9.2	4.5	79
Beauveria sp.	40.0	40.6	2,5	83
Multiplus L	146	0	0	0
F. solani	0	4.1	n.d.	50

All enzymes were dosed at 10 U against *p*-nitrophenol butyrate.
n.d. not determined.
[a] Difference between control and sample.
[b] Relative decrease of water absorption time between control and sample.

3.2. Polyamide

For determination of enzyme activity on the PA model substrate adipic acid bishexylamide the release of adipic acid was measured. In Fig. 3 the chromatograms of a sample was shown.

Potential candidates among enzyme from different classes which could be able to cleave the amide bonds in polyamide could show protease, esterase and amidase activity. A screening of 56 experimental and commercial enzyme preparation yielded only three preparations increasing the hydrophilicity of PA fabrics. All used enzymes had esterase activity against *p*-nitrophenyl butyrate and therefore the enzymes were dosed based on the same esterase activity of 10 U. Two enzyme preparations active on PA showed protease activity while only one enzyme had amidase activity against hexanoamide (*Nocardia* sp.). For comparison the data of a commercial protease are also listed.

The enzyme preparations from *F. solani*, *Beauveria* sp. and *Nocardia* sp. increased the hydrophilicity of PA by 50% to 83% based on water absorption. However, the activity on the model substrate varied between 4 and 40 mU which is less than with PET but still

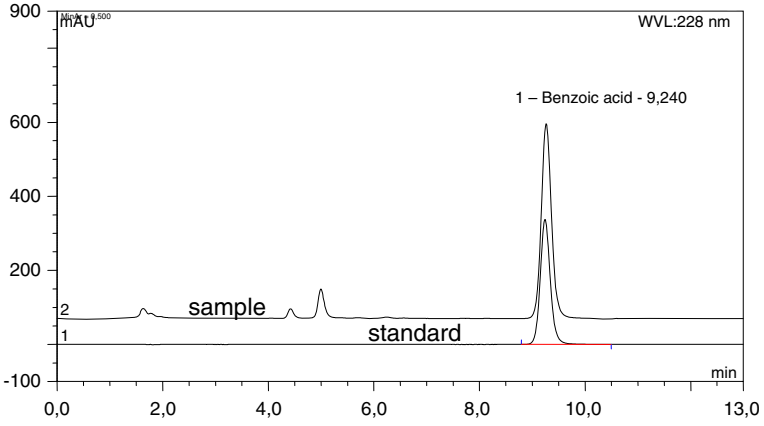

Fig. 2. Detection of benzoic acid after enzymatic hydrolysis of bis (benzoyloxyethyl) terephthalate.

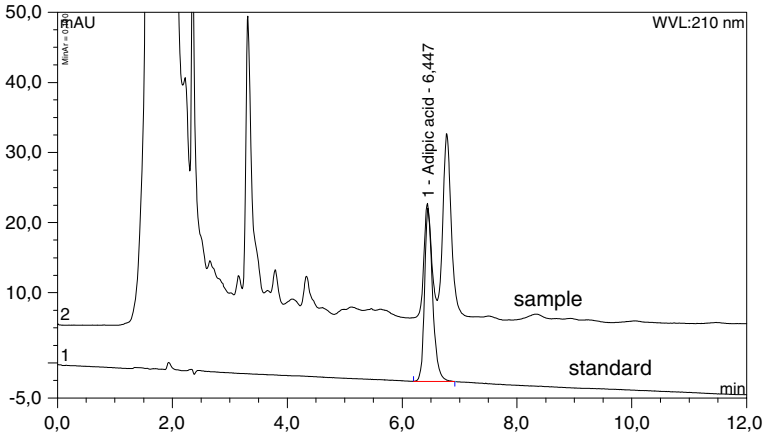

Fig. 3. Detection of adipic acid after enzymatic hydrolysis of adipic acid bishexyl-amide.

considerable high. Again, this behaviour could be explained with a different mode of action of the enzymes (endo/exo) on the fabrics.

4. Discussion

In the last few years the high potential of enzymes for surface modification of polyethyleneterephtalates and polymamides has been reported. However, no relationship between enzyme classification or origin and activity on these insoluble polymers has been reported. For example, although only few enzymes hydrolysing PET have been described so far in the literature, such enzymes have been found among lipases, esterases, cutinases, other hydrolases and were from both fungal and bacterial origin [1,9,16,21,26]. Consequently there was no correlation between standard enzyme activity (e.g. on *p*-nitrophenyl butyrate) and the capability of hydrolases to attack PET.

Similarly, we found in this study, that when dosed at the same activity on *p*-nitrophenyl butyrate some of these enzymes significantly increase the hydrophilicity of PET while others did not at all. Other authors have isolated 36 actinomycete strains from compost samples showing esterase activity on *p*-nitrophenyl butyrate. Cultivation of these isolates in media containing PET yarn and suberin, a plant polyester composed of aliphatic and aromatic moieties, should induce PET hydrolysing enzymes. Interestingly, only enzyme preparations from two of these isolates and from *T. fusca* caused an increase of the absorption at 240 nm of reaction mixtures containing PET fibres indicating the release of terephthalic acid or its esters [1]. Again these data show that there is only a marginal correlation between the capabilities of enzymes to hydrolyse *p*-nitrophenyl butyrate and PET fibres. Spectrophotometric monitoring of the release of terephthalic acid has also been used to monitor hydrolysis of PET with cutinase from *Pseudomonas mendocina*. These spectophotmetric results were confirmed by dyeing methods indicating an increase of surfacial hydroxyl groups [1,26].

The hydrophobic character of PET has previously prompted researchers to screen for PET hydrolysing enzymes among lipases. Interestingly, several PET hydrolysing enzymes with esterase activity on *p*-nitrophenyl-acetate including the *Aspergillus* sp. enzyme preparation tested here did neither hydrolyse *p*-nitrophenyl decanoate nor *p*-nitrophenyl palmitate which are typical

lipase substrates [9]. In contrast, other authors have selected several enzymes based on their natural role to hydrolyse ester bonds in lipids such as from *Humicola* sp., *Candida* sp. and *Pseudomonas* sp. and have indeed found activity on PET fibres [4]. Similarly, the LPS lipase and Texazyme sp5 assessed in this study were active on both lipase substrates and PET fibres.

More recently, the potential of cutinases to hydrolyse PET has been accessed. These enzymes are in nature responsible for the decomposition of the plant polyester cutin which is a major component of plant cuticles composed of inter-esterified C_{16} and C_{18}-hydroxy, and epoxy-hydroxy fatty acids. Indeed a hydrolase from *T. fusca,* which might be a cutinase, has been reported to hydrolyse dibutylterephtalate [14], to degrade PET materials [16] and also increased hydrophilicty of PET as shown here. Other authors have successfully used cutin to induce production of PET hydrolysing enzymes by various fungal species [1,9]. Similarly, a recombinant cutinase from *F. solani pisi* was able to hydrolyse PET fibres [21] which we confirmed here. However, the latter authors have routinely measured cutinase activity on a general lipase substrate *p*-nitrophenylpalmitate (pNPP) due to the lack of simple assays specific for cutinase only. Only recently a more specific substrate (4-nitrophenyl (16-methyl sulfone ester) hexadecanoate) has been described for the measurement of cutinase activity while labelled cutin is routinely used for this purpose [5].

Summarizing our experimental findings and the information available in the literature it is obvious that there is no correlation between enzyme activities on standard substrates and the ability to hydrolyse PET. However, we have found that activity on PET can be correlated with cleavage of the PET model substrate bis (benzoyloxyethyl) terephthalate. Although there is no direct relationship, all enzymes active on model substrate were capable of increasing hydrophilicity of fabrics. This oligomeric substrate is hydrolysed much faster than PET fibres and hydrolysis can be monitored more easily. Despite time consuming procedures based on dyeing or hydrophilicity measurement [1,9,26] more simple spectrophotometric quantification of terephthalic acid released from PET might lead to misleading results on enzyme activity [1,21,26]: Most applications related to enzymatic functionalisation of PET aim at the partial surface hydrolysis of PET leading e.g. to increased hydrophilicity. Thus, enzymes should obviously preferentially act “endo-wise” in the middle of the polymer chains and not successively cleave terephthalic acid and ethylene glycol from the end of the chains which would not lead to any beneficial effect but to not-desired weight loss. Since spectophotometric monitoring was never carried out until total hydrolysis of PET, this assay would preferentially detect “exo-acting” enzymes.

These substrate preferences might also be the reason for the fact that the correlation between activity on model substrate and hydrophilicity increase was not linear. Both endo and exo-acting enzymes might have similar activities on the oligomer while endo-acting enzymes would lead to much higher hydrophilicity increases of fabrics. Additionally, even prolonged action of endo-acting enzymes might lead to a situation where small fragments are released from the surface leading to constant or even decreasing hydrophilicity. Moreover, it has been shown for enzymes from *F. solani* and from *T. fusca* that crystallinity greatly affects the capability of the enzymes to hydrolyze PET [16,25]. Thus, it is obvious that there are sensitive balance points in terms of incubation time/enzyme activity which will lead to the maximum possible hydrophilicity increase.

The model substrate for PET developed here can also be used for the induction of PET hydrolysing enzymes. It has previously shown, that cutin or other polyester like materials can induce production of PET hydrolysing enzymes in actinomycetes and other microorganisms [1,9,10].

Lignin degrading fungi have been found to degrade nylon [6,7,13]. Oxidative enzymes (i.e. manganese peroxidase) were responsible for this capability. Further studies on nylon degrading peroxidases revealed a mechanisms involving hydrogen peroxide and superoxide anion radicals different from known Mn-peroxidase reactions [19]. Peroxidases seem to attack methylene groups adjacent to the nitrogen atoms while the reaction proceeds auto-oxidatively [7]. Despite these highly interesting mechanistic aspects and some potential of nylon degrading peroxidases in environmental biotechnology, such enzyme have less potential for surface modification due to difficulties in the control of the oxidative cleavage.

Already more than ten years ago enzymes capable to hydrolyse nylon oligomers have been isolated and characterised extensively by Negoro and coworkers [11,12,18,20]. Both in *Flavobacterium* spp. and *Pseudomonas* spp. these enzyme activities were coded on plasmids. However, the hydrolytic enzyme activity of these enzymes was restricted to substrates soluble in water and nylon was not attacked [19]. Searching for enzymes hydrolysing polyamides one would obviously start to screen among e.g. amidases or proteases. Interestingly enough, the nylon oligomer degrading enzymes described above did not hydrolase amide bonds in more the 100 natural substrates [18]. Similarly, only one of the enzyme preparation tested in this study showed amidase activity. In contrast, a cutinase from *F. solani* hydrolysed polyamide in this study which has also been recently reported to hydrolyse PET in addition to PAs [21].

From these facts it is obvious, that standard enzyme activities do not correlate to the ability to hydrolyse PA. On the other hand, quantification of enzymatic hydrolysis of PA such as via derivatisation of the formed amino groups with TNBS (2,4,6-trinitrobenzenesulfonic acid) and subsequent spectrophotometric detection at 420 nm or dying with α-bromoacrylamido reactive dyes is rather time consuming [22]. In contrast, hydrolysis of model substrate adipic acid bishexyl-amide developed in this study correlates with activity on PA. Furthermore, this substrate could be used for the screening of PA hydrolysing which have previously (for nylon oligomers) been shown to be inducible [18]. It has previously been shown that the activity of a nylon oligomer hydrolysing enzyme could be improved 200-fold by genetic engineering [18]. Using model substrates a similar strategy could be followed to improve PA hydrolysing enzymes. Moreover, reaction engineering seems to be an important issue and it has previously been shown that enzymatic hydrolysis of PA can be increased in the presents of solvents [23]. Again model substrate based assays could facilitate screening for enzymes with high stability under these conditions.

5. Simplified description and future applications

In this study we show that measurement of enzyme activity on the oligomeric model substrates bis (benzoyloxyethyl) terephthalate and adipic acid bishexyl-amide can be used to estimate enzymatic surface hydrolysis of polyethyleneterephthalate (PET) and polyamide fibres (PA). This assay avoids the use of time consuming methods e.g. for the determination of increases of hydrophilicity of fibre materials and has thus a potential for screening procedures for more efficient enzymes and functionalisation of synthetic materials.

Acknowledgement

We say thank you to Markus List for measuring the hydrophilicity of PET and PA fabrics. The research was financed by the Commission of the European Union (“BIOSNYTEX”), the SFG, the

FFG, the city of Graz, and the province of Styria. NMR spectra were kindly recorded by Carina Illaszewicz and Joerg Weber.

References

[1] Alisch M, Feuerhack A, Mueller H, Mensak B, Andreaus J, Zimmermann W. Biocatalytic modification of polyethylene terephthalate fibres by esterases from actinomycete isolates. Biocatal Biotransform 2004;22:347–51.
[2] Allan, et al. Studies in pyrolysis. Part 9. J Chem Soc 1957:2107–13.
[3] Almansa E, Fischer-Colbrie G, Heumann S, Cavaco-Paulo A, Gübitz GM. Screening of fungi for modification of synthetic fibres. 3rd Intl Conf Textile Biotechnol Graz, Austria June 13–16; 2005. p. 2.
[4] Andersen B, Borch K, Abo M, Damgaard B. Method of treating polyester fabrics. US Patent Nr. 5,997,584 (1999) 1–20.
[5] Degani O, Salman H, Gepstein S, Dosoretz CG. Synthesis and characterization of a new cutinase substrate, 4-nitrophenyl (16-methyl sulfone ester) hexadecanoate. J Biotechnol 2006;121:346–50.
[6] Deguchi T, Kakezawa M, Nishida T. Nylon biodegradation by lignin-degrading fungi. Appl Environ Microbiol 1997;63:329–31.
[7] Deguchi T, Kitaoka Y, Kakezawa M, Nishida T. Purification and characterization of a nylon-degrading enzyme. Appl Environ Microbiol 1998;64:1366–71.
[8] Engelhardt A. The fiber year 2002. Winterthur, Switzerland: The Saurer Group; 2003.
[9] Fischer-Colbrie G, Heumann S, Liebminger S, Almansa E, Cavaco-Paulo A, Gübitz GM. New enzymes with potential for PET surface modification. Biocatal Biotransform 2004;22:341–6.
[10] Gouda MK, Kleeberg I, Van den Heuvel J, Müller RJ, Deckwer WD. Production of a polyester degrading extracellular hydrolase from *Thermomonospora fusca*. Biotechnol Prog 2002;18:927–34.
[11] Kakudo S, Negoro S, Urabe I, Okada H. Nylon oligomer degradation gene, NylC, on plasmid pOAD2 from *Flavobacterium* strain encodes endo-type 6-aminohexanoate oligomer hydrolase: purification and characterization of the nylC gene product. Appl Environ Microbiol 1993;59:3978–80.
[12] Kakudo S, Negoro S, Urabe I, Okada H. Characterization of endo-type 6-aminohexanoate-oligomer hydrolase from *Flavobacterium* sp. J Ferment Bioeng 1995;80:12–7.
[13] Klun U, Friedrich J, Krzan A. Polyamide 6 fibre degradation by a lignolytic fungus. Polym Degrad Stab 2003;79:99–104.
[14] Marten E, Muller RJ, Deckwer WD. Studies on the enzymatic hydrolysis of polyesters. I. Low molecular mass model esters and aliphatic polyesters. Polym Degrad Stab 2003;80:485–501.
[15] Miettinen-Oinonen A, Silvennoinen M, Nousiainen P, Buchert J. Modification of synthetic fibres with laccase. Proceedings of The Second International Symposium on Biotechnology in Textile, April 3–6, 2002, Athens, USA; 2002. p. 13.
[16] Muller R-J, Schrader H, Profe J, Dresler K, Deckwer WD. Enzymatic degradation of poly(ethylene terephthalate): rapid hydrolyse using a hydrolase from *T. fusca*. Macromol Rapid Commun 2005;26:1400–5.
[17] Muller RJ, Kleeberg I, Deckwer WD. Biodegradation of polyesters containing aromatic constituents. J Biotechnol 2001;86:87–95.
[18] Negoro S. Biodegradation of nylon oligomers. Appl Microbiol Biotechnol 2000;54:461–6.
[19] Nomura N, Deguchi T, Shigeno-Akutsu Y, Nakajima-Kambe T, Nakahara T. Gene structures and catalytic mechanisms of microbial enzymes able to biodegrade the synthetic solid polymers nylon and polyester polyurethane. Biotechnol Genet Eng Rev 2001;18:125–47.
[20] Prijambada ID, Negoro S, Yomo T, Urabe I. Emergence of a nylon oligomer degradation enzymes in *Pseudomonas aeruginosa* PAO through experimental evolution. Appl Environ Microbiol 1995;61:2020–2.
[21] Silva C, Carneiro F, O'Neill A, Fonseca LP, Cabral JSM, Gübitz GM, et al. Cutinase — a new tool for biomodification of synthetic fibers. J Polym Sci 2005;43:2448–50.
[22] Silva C, Cavaco-Paulo A. Monitoring biotransformations in polyamide fibres. Biocatal Biotransform 2004;22:357–60.
[23] Silva C, Matama T, Cavaco-Paulo A. Influence of organic solvents on cutinase stability and accessibility to polyamide fibers. J Polym Sci A Polym Chem 2005;43:2749–53.
[24] Skouloubris S, Labigne A, Dereuse H. Identification and characterization of an aliphatic amidase in *Heliobacter pylori*. Mol Microbiol 1997;25:989–98.
[25] Vertommen MAME, Nierstrasz VA, Veer Mvd, Warmoeskerken MMCG. Enzymatic surface modification of poly (ethylene terephthalate). J Biotechnol 2005;120:376–86.
[26] Yoon MY, Kellis J, Poulouse AJ. Enzymatic modification of polyester. AATCC Rev 2002;2:33–6.