

ORIGINAL ARTICLE

Hydroxylation of polypropylene using the monooxygenase mutant 139-3 from *Bacillus megaterium* BM3

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Abstract

Enzymatic hydroxylation of polypropylene (PP) was investigated in order to increase hydrophilicity. A mutant (139-3) of the P450 monooxygenase from *Bacillus megaterium* expressed in *E. coli* DH5 α was purified using anion exchange chromatography. Hydroxylation of PP fabrics led to a dramatic increase of hydrophilicity as indicated by a water drop dissipation time of below 1 s compared to the hydrophobic reference material. Likewise, a 4.9 cm increase of rising height was measured which remained consistent after 144 h of storage. Similarly, enzymatic hydroxylation of PP films lead to a decrease of the WCA from 104.6° to 77.3° with no major change after exposure to air for 6 days. Using X-ray photoelectron spectroscopy, an increase in normalized atomic concentrations of oxygen from 1.40 to 4.98% for the CO-inhibited and enzyme treated sample, respectively, was measured confirming enzymatic hydroxylation.

Keywords: oxidoreductase, polypropylene, polymer, surface modification

Introduction

The properties of polyolefins regarding further functionalization can be considerably improved by the introduction of polar groups onto the surface (Naga et al. 2006). A number of chemical and plasma based methods have been developed for this purpose (Cui et al. 2002; Favaro et al. 2007). While with chemical methods such as the incorporation of hydroxylated cyclic molecules the polymer bulk properties can be tuned (e.g. glass transition temperature, T_g), plasma based processes target mainly surface properties such as wettability and adhesion (Cui et al. 2002; Favaro et al. 2007). Argon plasmas lead to bond scission creating active sites on the surface while oxygen plasmas additionally lead to surface oxidation (Beake et al. 1998; Han et al. 1999). Among plasma treatment techniques, air discharges are widely used. They are inexpensive since there is no need for a vacuum and, thus, also suitable for continuous processes treating large areas. In contrast, complex three dimensional

devices are more difficult to functionalize with such techniques. Enzymatic surface modification in aqueous solution could offer an environmentally friendly alternative working under mild conditions and without high energy consumption.

There are several successful reported examples of surface modification of synthetic polymers including polyalkyleneterephthalates, polyamides and polyacrylonitriles using mainly hydrolases (Guebitz & Cavaco-Paulo 2008). Polyethyleneterephthalate was hydrolyzed by enzymes, and half a dozen enzymes hydrolyzing PAT films, fibres and nano-particles were identified (Brueckner et al. 2008; Donelli et al. 2009, 2010; Eberl et al. 2008, 2009; Herrero-Acero et al. 2011; Ribitsch et al. 2011; Vertommen et al. 2005). Similarly, enzymatic hydrolysis of polyacrylonitrile either down to the corresponding acid or to the amide has been described (Fischer-Colbrie et al. 2006, 2007). Recently, a new enzyme hydrolyzing polyamide oligomers was crystallized (Negoro et al.

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2005) while cutinases and amidases have been described to hydrolyze various polyamides (Heumann et al. 2009). However, despite the potential for mild and specific enzymatic surface modification, enzymatic functionalization of polypropylene (PP) has not been exploited yet. Here, the potential of P450 monooxygenases from *Bacillus megaterium* BM3 for hydroxylation of PP was assessed.

Material and methods

Cultivation of recombinant E. coli DH5 α and expression of P450 BM-3 monooxygenases

Two recombinant (0821B; 139-3) and the wild-type P450 monooxygenase from *Bacillus megaterium* (BM-3) were expressed in *E. coli* DH5 α . The mutant monooxygenases were developed using a combination of directed evolution and site-directed mutagenesis in order to improve their ability to hydroxylate linear alkanes (Glieder et al. 2002). Recombinant *E. coli* DH5 α containing genes encoding different P450 BM-3 mutants were plated on LB agar plates containing 100 mg/L ampicillin, and were grown for 12 h at 37°C. Single colonies were picked for the inoculation of 5 mL TB-amp-medium. These pre-cultures were cultivated at 30°C and 130 rpm for 12 h, to reach a uniform cell density at the beginning of their stationary growth phase. Thereafter, 1000 mL Erlenmeyer flasks containing 250 mL TB-amp-medium (supplemented with 100 μ g ampicillin/mL and 1.5 μ L trace elements is oc) were inoculated with 1 mL of the overnight cultures of recombinant *E. coli* DH5 α . After 24 h of incubation, 125 μ L 5-aminolevulinic acid hydrochloride (1 M stock-solution) which acts as a heme precursor was added per 250 mL media. After 2 h, the expression was induced by adding 250 μ L IPTG (0.5 M stock-solution) and 125 μ L ampicillin. After 7 h, the medium was supplemented with 125 μ L 5-aminolevulinic acid hydrochloride, 250 μ L IPTG and 125 μ L ampicillin per 250 mL culture. After 15 h, cells were harvested by centrifugation at 4500 g for 20 min and washed with Tris/HCl buffer (30 mM, pH 7.5). Cells were stored until further use at -20°C in this buffer. All chemicals were purchased from Sigma.

Enzyme purification

Cells were suspended in 5 mL Tris/HCl buffer (30 mM, pH 7.5) and then sonicated for 3 min. The crude suspension was centrifuged for 60 min at 18 000 g. The resulting supernatant was diluted with buffer to a final volume of 25 mL and was then filtered through a sterile filter (0.5 μ m). Proteins (sample

volume of 10 mL) were separated using an anion-exchange column (HiPrep 16/10 QFF, 17 mL, from Pharmacia) with 30 mM Tris/HCl buffer (pH 7.5) as eluent at a flow rate of 5 mL/min. Proteins were eluted with increasing salt-concentration (1 M NaCl) simultaneously monitoring absorbance at 280 nm (protein) and 417 nm (heme). The most active fractions (brown coloured) were pooled together and frozen at -20°C.

Enzyme assays

The CO-binding-assay was used for quantification of functional P450-enzyme based on complex formation of reduced cytochrome P450 (Fe^{II}) with carbon-monoxide (CO) absorbing at 450 nm. For the measurement, 3 mL purified protein-solution (in Tris/HCl buffer, 30 mM, 1 M NaCl) were pipetted into a plastic tube and 10 μ L of methyl viologen dichloride hydrate (1% (w/v) in distilled water) were added as a redox indicator. Samples were reduced using sodium hydrosulfite with a final concentration of 20 mM. After mixing, the sample was separated into two portions. CO was bubbled through one of the samples for 1 min (approximately 1 bubble per second) while the second sample was used as a reference. A spectrum was run from 390 nm to 500 nm under aerobic conditions and the concentration of monooxygenases was calculated based on an extinction coefficient of 91 [mM⁻¹ cm⁻¹].

Monooxygenase activity was quantified based on the conversion of *p*-nitrophenoxystyrene (8-pNA) to *o*-xyloxyoctane and the chromophore *p*-nitrophenolate (Schwaneberg et al. 1999a); 50 μ L enzyme solution and 3 μ L of a 50 mM solution of 8-pNA in Dimethyl sulfoxide (DMSO) were added to 910 μ L KPi buffer (50 mM, pH 8.0). The reaction was started by the addition of 30 μ L of an aqueous solution of 6 mM Nicotinamide adenine dinucleotide phosphate (NADPH) and run for 100 s at 30°C. As a reference, 940 μ L buffer were used. Formation of *p*-nitrophenolate was monitored at 410 nm and enzyme activity was given in nkat representing the conversion of 1 nmol 8-pNA per second. For the synthesis of 8-pNA, 1-bromooctane (17 mmol, 2.96 mL), *p*-nitrophenolate (18.5 mmol, 2.98 g) and ethanol absolute (50 mL) were combined in a 100 mL round bottom flask fitted with a water-cooled reflux condenser. KI was added to act as catalyst and the reaction solution refluxed at 80°C for 16 h. The alcohol was removed with a vigreux-colonne. To the resulting residue, 15 mL of a 5%(w/v)-NaOH solution was added and the organic phase was re-dissolved in diethyl-ether, washed with water and dried over sodium sulphate. Afterwards, the solvent was removed by heating and the product recrystallized twice. The

structure was confirmed by Nuclear magnetic resonance spectroscopy, NMR (Varian Unity Inova 500 instrument).

Enzyme treatment of polypropylene materials

For a pre-screening, NADPH depletion as a measure of substrate hydroxylation was measured based on fluorescent emission at 445 nm when excited at 340 nm. Fabric samples of 2 × 2 mm were incubated in microtiter plates containing 1 nkat mL⁻¹ of monooxygenase and 0.5 mM NADPH in 300 μL of 100 mM phosphate buffer at 30°C.

For detailed analysis, PP fabrics and films (Ciba Inc.) were cut into pieces of 4 × 6 cm, and incubated in 100 mL Erlenmeyer flasks in 4.5 mL of 30 mM Tris/HCl buffer with different monooxygenase activities, incubation times and temperatures as indicated below. Erlenmeyer flasks were shaken at 250 rpm on an orbital shaker. To remove adsorbed enzymes or media impurities, the fabrics were washed with Na₂CO₃ (1 g/L, pH 9.5) for 2 h and with distilled water for 1 h. Afterwards, the samples were dried at 100°C overnight.

Inhibition of monooxygenase activity

For inhibition of monooxygenase activity, oxygen was removed from the above incubation mixture by flushing with nitrogen for 10 min. Subsequently, the mixture was flushed with CO for half an hour, and the PP sample was added and the vessel closed for incubation as described above.

Quantification of hydrophilicity increase

Hydrophilicity was quantified based on the rising height of water on fabric samples due to capillary forces (DIN 53924). Fabrics were fixed on a rod above a water bath immersed in the water for 1 cm, and after 10 min, the water level on the fabrics was measured in centimetres. Alternatively, the water drop dissipation test was used. A drop (20 μL distilled water) was placed on the surface of the fabric, and the time until dissipation was measured in seconds. For PP-films the contact angle, ϕ , between a water drop and the film surface was measured with the Drop Shape Analysis System DSA 100 (Krüss GmbH, Hamburg, Germany). Deionized water was used as test liquid with a drop size of 3 μL. The contact angle was measured after 3 s, and the data were obtained from the averages of the measurements taken from 10 different points of the sample surface. To estimate protein adsorption on samples, a 1% (w/v)-solution of ninhydrin in ethanol was

prepared. PP pieces were sprayed with this solution and then dried in a heating chamber at 100°C for half an hour.

XPS analysis

XPS analysis was carried out on a PHI-TFA spectrometer (Physical Electronic Inc.). PP samples were fixed in position and three different sample locations analyzed from two experimental series. The analyzed area was 0.4 mm in diameter, and the analyzed depth was about 3–5 nm. X-ray excitation was achieved with a monochromatic Al source at photon energy of 1486.6 eV. Wide-scan spectra were acquired at pass energy of 187 eV for identification and quantification of elements on the surfaces of the fabrics. The atomic concentrations of surface regions were calculated using relative sensitivity factors provided by the manufacturer. High-energy resolution spectra of C 1s, O 1s and S 2p were acquired with an energy resolution of about 0.6 eV with analyzer pass energy of 29 eV, to reveal binding energies of XPS peaks associated with different chemical states of elements.

Results and discussion

In recent years, the potential of hydrolases for surface modification of synthetic polymers including polyethylene terephthalate, polyamide and polyacrylonitriles has been demonstrated (Brueckner et al. 2008; Eberl et al. 2008, 2009; Fischer-Colbrie et al. 2006; Heumann et al. 2009).

For PP, which is not a substrate for hydrolases, oxidoreductases have been investigated for indirect enzymatic coating with phenolic molecules (Schroeder et al. 2008). In this study, direct enzymatic hydroxylation of PP was investigated.

For this purpose, monooxygenases from *Bacillus megaterium* BM3 were used which were previously designed by a combination of directed evolution and site-directed mutagenesis to hydroxylate linear alkanes (Glieder et al. 2002). The wild type enzyme and two mutants 139-3 and B were successfully expressed in *E. coli* DH5 α and monooxygenases purified from the cell lysates using anion exchange chromatography. In an initial screening with these enzymes increased depletion of the co-factor NADPH was seen in the presence of PP when compared to enzyme and PP controls. The most pronounced increases were seen for the mutant 139-3 (data not shown). These results can only be regarded as qualitative estimation since oxidation of NADPH can become uncoupled from substrate oxidation (Peters et al. 2003). However,

these findings were still encouraging for a detailed investigation of PP hydroxylation with P450_{BM3_139-3}.

P450_{BM3_139-3} was obtained in a one-step purification procedure using anion-exchange chromatography as previously described (Schwaneberg et al. 1999a, b) simultaneously monitoring protein and p450 concentration at 280 and 417 nm. The enzyme preparation had a p450 content of 16.3 μMol . A rate of 68.9 nkat ml^{-1} was measured at the temperature optimum, 44°C when using 8-pNA as substrate with 54.0 and 37.8 nkat ml^{-1} at 37 and 30°. However, at the optimum temperature, the enzyme showed a half-life time of only 210 min, while no significant activity loss was seen at 30°C after 12 h of incubation. Consequently, PP hydroxylation was carried out at 30°C.

P450_{BM3_139-3} treatment of PP fabrics lead to a dramatic increase in hydrophilicity. No water drop dissipation was seen at all with the very hydrophobic reference material while the dissipation time decreased to 4, 2 and below 1 s after incubation with 2 nkat mL^{-1} of P450_{BM3_139-3} for 2, 5 and 10 h, respectively. These results were confirmed using the rising height method (Table I).

The hydrophilicity increase measured after P450_{BM3_139-3} treatment was clearly primarily due to enzymatic hydroxylation, rather than due to protein adsorption, since carbon monoxide inhibited enzyme did not cause a very significant effect. Similarly, incubation of PP with heat inactivated enzyme preparations as well as with BSA (identical protein concentration) did not give any hydrophilicity increase over an incubation period of 24 h (data not shown). The hydrophilicity increase, depended on enzyme activity dosed, was quite permanent during exposure to air for 144 h, after an initial decrease of hydrophilicity between 24 h and 48 h exposure. A small decrease of hydrophilicity upon prolonged exposure to air has also been seen

Table I. Hydrophilicity of PP-fabrics (Rising height [cm]) incubated for 5 h with *Bacillus megaterium* BM3 (mutant 139-3) P450 monooxygenase and measured after different storage times.

Enzyme treatment [h]	Storage time [h]			
	24 CO ^a	24	48	144
	Rising height [cm]			
10	0.6	4.9	4.6	4.6
5	0.2	3.6	3.2	3.2
2	0.0	2.3	2.0	1.9
1	0.0	1.1	0.5	0.4

^aCO-inhibited enzyme.

Table II. XPS analysis of PP films treated with *Bacillus megaterium* BM3 (mutant 139-3) P450 monooxygenase.

Sample	C 1s	C 1s	C 1s
	aliphatic	CO/CN	COO/CON
	285 eV	286.6 eV	288.4 eV
Control	96.2	3.4	0.4
Enzyme	74.0	18.6	7.3
Enzyme + CO ^a	86.5	9.9	3.6

^aCO-inhibited enzyme.

for PP treated with dielectric barrier discharge plasma (Cui et al. 2002).

In addition to PP-fabrics, PP-films were treated with P450_{BM3_139-3}. In contrast to PP-fabrics, determination of hydrophilicity increases via changes in the contact angle to water was possible. Compared to a reference (104.6°), the contact angle decreased to 77.3° after 5 h incubation with 2 nkat ml^{-1} P450_{BM3_139-3}. After 6 days of exposure to air this value only changed to 75.7°. Relaxation and re-orientation of polar groups during exposure to air has been previously described as responsible for partial recovery (around 5° after 143 h) of the contact angle of dielectric barrier discharge plasma treated PP-films (Cui et al. 2002). Using this method, the contact angle of PP-films could be reduced to about 30° compared to 27° using the enzyme approach in this study.

X-ray photoelectron spectroscopy was used to quantify the chemical changes on the polymer surface. Upon treatment with P450_{BM3_139-3} normalized atomic concentrations of oxygen increased to 1.40 and 4.98% for the CO-inhibited and enzyme treated sample, respectively, when corrected for the amount of oxygen resulting from silicon oxide present as filler.

According to the C1s detail in the spectra for the three samples, clear differences were observed between enzyme treated samples and controls. The control sample contains mainly aliphatic hydrocarbon with a binding energy of 285 eV as expected for PP. After enzyme treatment, two more species appeared. Their higher binding energies are typical of binding states with higher oxidation, indeed the signal at around 286.6 eV can be assigned to species like $-\text{CH}_2-\text{O}(\text{C}-\text{O})$ or $-\text{CH}_2-\text{N}(\text{C}-\text{N})$ and the signal at 288.4 eV to carbon from carboxylic acids, esters or amides (COO/CON) (Table II).

The increase of $-\text{CH}_2-\text{O}$ species can be attributed to hydroxylation of PP by P450_{BM3_139-3}. However, the signal at 288.4 eV corresponding to carbon from carboxylic acids, esters or amides probably results from enzyme (= protein) adsorbed to PP. This is also evident from a nitrogen content of 5.4% indicating some irreversible enzyme adsorption.

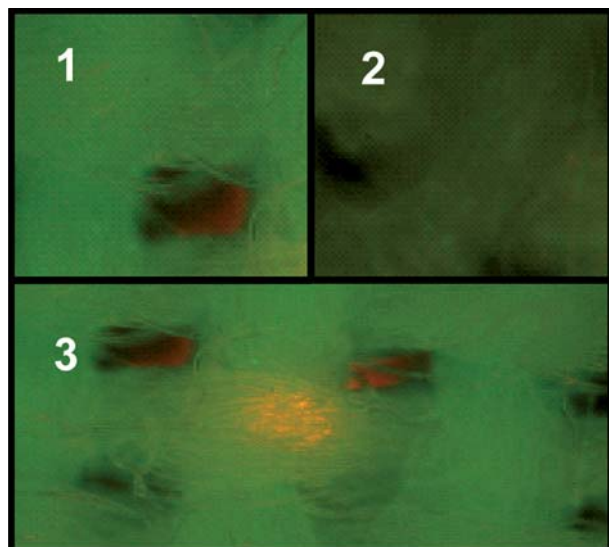


Figure 1. PP-fabrics incubated with FITC labelled BSA in the presence (1, 3) and absence (2) of *Bacillus megaterium* BM3 (mutant 139-3) P450 monooxygenase.

However, as noted there was neither an increase in wettability (see above) nor an increase in the nitrogen content when thermally inactivated P450_BM3_139-3 was incubated with PP. Thus, it seems that partial hydroxylation with concomitant hydrophilization promotes enzyme/protein adsorption. To confirm this effect, in addition to P450_BM3_139-3, fluorescently (FITC) labelled BSA was added to the incubation mixture. Interestingly enough, only in the presence of P450_BM3_139-3, FITC labelled BSA strongly adsorbed to the surface of PP and could not be removed with various washing procedures (Figure 1).

Irreversible adsorption of enzyme during treatment of synthetic materials has previously been reported for polyethyleneterephthalate. Using XPS analysis, an increase in the nitrogen content of up to 7.2% due to adsorption of lipase to PET was measured (Vertommen et al. 2005). Using angle resolved XPS, a protein layer thickness of 1.6–2.6 nm and 2.5–2.8 nm for cutinase and lipase, respectively, was measured. As in this study, washing even using ultrasound or organic solvents did not significantly decrease enzyme adsorption. In another study focusing on PET, it was demonstrated that severe washing procedures can remove adsorbed enzymes from PET (Brueckner et al. 2008), which, however, did not work for PP in this study.

Conclusions

Enzymatic hydroxylation of PP was demonstrated to dramatically enhance hydrophilicity. Introduction of

oxygen was confirmed by XPS, however, concomitant strong enzyme adsorption was observed. Future studies should focus on the effect of surface active molecules to decrease enzyme adsorption during hydroxylation. In addition, a wider range of hydroxylation enzymes should be investigated, for example, enzymes such as peroxygenases which do not depend on expensive co-factors.

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References

- Beake BD, Ling JSG, Leggett GJ. 1998. Correlation of friction, adhesion, wettability and surface chemistry after argon plasma treatment of poly(ethylene terephthalate). *J Mater Chem* 8:2845–2854.
- Brueckner T, Eberl A, Heumann S, Rabe M, Guebitz GM. 2008. Enzymatic and chemical hydrolysis of poly(ethylene terephthalate) fabrics. *J Polymer Sci Polymer Chem* 46:6435–6443.
- Cui NY, Brown NMD. 2002. Modification of the surface properties of a polypropylene (PP) film using an air dielectric barrier discharge plasma. *Appl Surf Sci* 189:31–38.
- Donelli I, Taddei P, Smet PF, Poelman D, Nierstrasz VA, Freddi G. 2009. Enzymatic surface modification and functionalization of PET: a water contact angle, FTIR, and fluorescence spectroscopy study. *Biotechnol Bioeng* 103:845–856.
- Donelli I, Freddi G, Nierstrasz VA, Taddei P. 2010. Surface structure and properties of poly-(ethylene terephthalate) hydrolyzed by alkali and cutinase. *Polymer Degrad Stabil* 95: 1542–1550.
- Eberl A, Heumann S, Kotek R, Kaufmann F, Mitsche S, Cavaco-Paulo A, Guebitz GM. 2008. Enzymatic hydrolysis of PTT polymers and oligomers. *J Biotechnol* 135:45–51.
- Eberl A, Heumann S, Brueckner T, Araujo R, Cavaco-Paulo A, Kaufmann F, Kroutil W, Guebitz GM. 2009. Enzymatic surface hydrolysis of poly(ethylene terephthalate) and bis (benzoyloxyethyl) terephthalate by lipase and cutinase in the presence of surface active molecules. *J Biotechnol* 143: 207–212.
- Favaro SL, Rubira AF, Muniz EC, Radovanovic E. 2007. Surface modification of HDPE, PP, and PET films with KMnO₄/HCl solutions. *Polymer Degrad Stabil* 92:1219–1226.
- Fischer-Colbrie G, Herrmann M, Heumann S, Puolakka A, Wirth A, Cavaco-Paulo A, Guebitz G. 2006. Surface modification of

- polyacrylonitrile with nitrile hydratase and amidase from *Agrobacterium tumefaciens*. *BioCat Biotrans* 24:419–425.
- Fischer-Colbrie G, Matama T, Heumann S, Martinkova L, Cavaco Paulo A, Guebitz G. 2007. Surface hydrolysis of polyacrylonitrile with nitrile hydrolysing enzymes from *Micrococcus luteus* BST20. *J Biotechnol* 129:62–68.
- Glieder A, Farinas ET, Arnold FH. 2002. Laboratory evolution of a soluble, self-sufficient, highly active alkane hydroxylase. *Nat Biotechnol* 20:1135–1139.
- Guebitz GM, Cavaco-Paulo A. 2008. Enzymes go big: surface hydrolysis and functionalisation of synthetic polymers. *Trends Biotechnol* 26:32–38.
- Han S, Koh SK, Yoon KH. 1999. Induced surface reactions and chemical states – A kiloelectronvolt ion irradiation on simple linear chain structure polymers in an O-2 environment. *J Electrochem Soc* 146:4327–4333.
- Herrero-Acero E, Ribitsch D, Steinkellner G, Gruber K, Greimel K, Eiteljoerg I, Trotscha E, Ren W, Zimmerman W, Zinn M, Cavaco-Paul A, Freddi G, Schwab H, Guebitz GM. 2011. Enzymatic surface hydrolysis of PET: effect of structural diversity on kinetic properties of cutinases from *Thermobifida*. *Macromol* in press.
- Heumann S, Eberl A, Fischer-Colbrie G, Pobeheim H, Kaufmann F, Ribitsch D, Cavaco-Paulo A, Guebitz GM. 2009. A novel aryl acylamidase from *Nocardia farcinica* hydrolyses polyamide. *Biotechnol Bioeng* 102:1003–1011.
- Naga N, Tsuchiya G, Toyota A. 2006. Synthesis and properties of polyethylene and polypropylene containing hydroxylated cyclic units in the main chain. *Polymer* 47:520–526.
- Negoro S, Ohki T, Shibata N, Mizuno N, Wakitani Y, Tsurukame J, Matsumoto K, Kawamoto I, Takeo M, Higuchi Y. 2005. X-ray crystallographic analysis of 6-aminohexanoate-dimer hydrolase - Molecular basis for the birth of a nylon oligomer-degrading enzyme. *J Bus Comm* 280:39644–39652.
- Peters MW, Meinhold P, Glieder A, Arnold FH. 2003. Regio- and enantioselective alkane hydroxylation with engineered cytochromes P450 BM-3. *JACS* 125:13442–13450.
- Ribitsch D, Heumann S, Trotscha E, Acero EH, Greimel K, Leber R, Birner-Gruenberger R, Deller S, Eiteljoerg I, Remler P, Weber T, Siegert P, Maurer KH, Donelli I, Freddi G, Schwab H, Guebitz GM. 2011. Hydrolysis of polyethyleneterephthalate by para-nitrobenzylesterase from *Bacillus subtilis*. *Biotechnol Progr* 27:951–960.
- Schroeder M, Fatarella E, Kovac J, Guebitz GM, Kokol V. 2008. Laccase-induced grafting on plasma-pretreated polypropylene. *Biomacromolecules* 9:2735–2741.
- Schwaneberg U, Schmidt-Dannert C, Schmitt J, Schmid RD. 1999a. A continuous spectrophotometric assay for P450 BM-3, a fatty acid hydroxylating enzyme, and its mutant F87A. *Anal Biochem* 269:359–366.
- Schwaneberg U, Sprauer A, Schmidt-Dannert C, Schmid RD. 1999b. P450 monooxygenase in biotechnology: I. Single-step, large-scale purification method for cytochrome P450 BM-3 by anion-exchange chromatography. *J Chromatogr A* 848: 149–159.
- Vertommen MAME, Nierstrasz VA, Veer Mvd, Warmoeskerken MMCG. 2005. Enzymatic surface modification of poly(ethylene terephthalate). *J Biotechnol* 120:376–386.