Selective degradation of synthetic polymers through enzymes immobilized on nanocarriers

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Abstract

In order to develop new sustainable and reusable concepts for the degradation of omnipresent industrial plastics, immobilization of (bio)catalysts on nanocarriers offers unique opportunities for selective depolymerization and catalyst recovery. In this study, enzymes (lipase and cutinase) were covalently immobilized on carrier nanoparticles (SiO_2 and $\text{Fe}_3\text{O}_4@ \text{SiO}_2$) through 3-(aminopropyl)trimethoxysilane and glutaraldehyde linkers forming a stable bond to enzyme molecules. The presence of enzymes on the surface was confirmed by zeta potential and XPS measurements, while their degradation activity and long-term stability of up to 144 h was demonstrated by the conversion of 4-nitrophenyl acetate to 4-nitrophenol. Furthermore, enzymatic decomposition (hydrolysis/oxidation) of electrospun polycaprolactone fiber mats was verified through morphological (SEM) and weight loss studies, which evidently showed a change in the fiber morphology due to enzymatic degradation and accordingly a weight loss.

Introduction

Industrial plastics have become inevitable in every aspect of modern life and lifestyle due to their several desirable properties and cost-effective production in large volumes. For instance, 348 million metric tons of plastics were produced in 2017.^[1] However, due to lack of concepts for their recycling and their recalcitrant nature against natural biodegradation processes, millions of tons accumulate annually in terrestrial or marine ecosystems.^[2] There are several different research approaches for the degradation of varying plastic types, like blending conventional polyethylene (PE) with natural polymers such as starch or cellulose, but this can lead to an uncontrolled film degradation through lowered mechanical properties and reduces the lifetime of the modified film.^[3] Another approach of controlled PE degradation can be realized by the usage of pro-oxidants, typically metal catalysts like transition metal salts or oxides which are incorporated into the polymer for targeted implementation of hydroperoxides. UV-light or heat enables the polymer degradation to lower molecular weight hydrocarbons and oxidized products which will further be degraded by microorganisms.^[4] However, the European Commission published in 2018 a report on the risks of oxo-degradable plastics as it is not completely degraded leading to an accumulation of microplastic in the environment. Furthermore, there is no evidence that oxo-degradable plastic degrades in a reasonable time period in the open environment.^[5] In contrast, recycling is one of the most promising alternatives in reducing the environmental impact of industrial plastics. Interestingly, the number of studies including microorganisms and enzymes as capable reactants of plastic degradation has increased in recent

years.^[6] For example, some bacteria and fungi were identified for the partial degradation of plastics and enzymes such as PETases, which degrade polyethylene terephthalate (PET), are the best-explored and studied enzyme class with respect to the hydrolysis of synthetic polymers.^[6,7] Also serine hydrolases like lipases (EC 3.1.1.3) or cutinases (EC 3.1.1.74) are effective in the degradation of PET to terephthalic acid (TPA) and ethylene glycol^[6–8] with the salient advantage that no cofactor is needed when using lipases and cutinases.^[8] In this context, immobilization of reusable enzymes on magnetic nanoparticles could be a green pathway. Enzyme immobilization is already known since 1916, when Nelson et al. demonstrated the unhampered activity of invertase when it was adsorbed on a solid matrix.^[9] Since then, this concept has been significantly widened to immobilize enzymes on nanoparticles,^[10] fibers,^[11] or solid carriers.^[12] The advantages of nanoparticles over bulk materials are manifold, such as high surface area, ease of surface modification and size/shape dependent properties. To prevent the loss of enzyme activity due to change in the chemical nature or blocking of the catalytic triad, the immobilization procedure is critically important. The surface functionalization with enzymes can be performed via either covalent binding,^[13] adsorption,^[14] entrapment, or encapsulation.^[15] In comparison to the free enzymes, immobilized enzymes exhibit higher stability and higher activity compared to their unbound counterpart.^[16] Besides, enzymes immobilized on solid substrates are easier to handle as well as reusable.^[17] While the immobilization through adsorption is the simplest way using non-covalent interactions such as van der Waals, hydrogen, or ionic bonds,^[17] the covalent immobilization of

enzymes bestows higher stability and ease of separation, as shown for the functional biomolecules immobilized on magnetic nanoparticles.^[18]

In this work, two different enzymes, lipase and cutinase, were covalently attached on the surface of SiO₂ nanoparticles and core-shell Fe₃O₄@SiO₂ nanostructures. Both particle systems were surface modified with an inorganic linker, (3-aminopropyl)trimethoxy silane (H₂N(CH₂)₃Si(OCH₃)₃, APTMS), which was consequently activated with glutaraldehyde (GA) that was used for covalent attachment of enzymes through imine bond formation. For testing the efficiencies and long-term stabilities of enzyme-nanoparticle conjugates, degradation of 4-nitrophenyl acetate (4-NPA) as substrate to 4-nitrophenol (4-NP) was monitored. The efficacy of immobilized enzymes to decompose polymers was exemplarily tested for degrading polycaprolactone (PCL) fibers.

Materials and methods

Materials

All reagents and solvents were of analytical grade and used without further purification. 4-nitrophenyl acetate (4-NPA, ≥98%) and 4-nitrophenol (4-NP, >99,0%) were purchased from Sigma-Aldrich and TCI chemicals, respectively. Polycaprolactone (70,000–80,000 g/mol) was procured from Sigma-Aldrich. Acetonitrile (99.9%) was purchased from Fisher Chemicals, and the phosphate-buffered saline (PBS) buffer was purchased from Biowest. Enzymes (cutinase 2p from *Arxula adenivorans* and lipase FE-01 from *Thermomyces langinosus*) were obtained from ASA Spezialenzyme GmbH. Lipase was delivered as a solution (>18,000 U/mL, substrate: glycerintributyrate) while the 0.16 g cutinase (20,000 U/g, substrate: glycerintributyrate) was dissolved in 555 µl ultrapure H₂O.

Characterization

The Nova Nano 430 field-emission scanning electron microscope (SEM) operated at an acceleration voltage of 120 kV was used for analyzing the size and morphology of the as-prepared particles and electrospun fiber mats. For SEM sample preparation, particles were dispersed in ethanol, dropped onto a silicon wafer, and dried under ambient air. Fiber mats were put onto a carbon adhesive foil and were sputtered with gold before measurements. ZEISS Leo912 transmission electron microscope (TEM) operating at an acceleration voltage of 120 kV was used to analyze the core-shell particles. For TEM sample preparation, the particles were dispersed in ethanol and dropped onto a carbon-covered TEM grid, and dried under ambient air. X-ray diffractometry was performed for phase analysis using a STOE X-ray powder diffractometer equipped with Mo K α radiation ($\lambda=0.7093$ Å) and a Mythen 1 K detector. The zeta potential measurements were performed using a Zetasizer Nano ZS (Malvern Instruments) operating at a wavelength of 633 nm. Absorbances were measured using ELISA 96-well plate reader from BioTek, type ELx800. X-ray photoelectron spectroscopy

(XPS) was performed with an ESCA M-Probe spectrometer from SSI (Surface Science Instruments) equipped with a monochromatic Al K α (1486.6 eV) X-ray source. All binding energies were referenced to 284.8 eV based on the C 1s signal, and compositional calculations were performed using CasaXPS.^[19]

Synthesis of SiO₂ particles

SiO₂ particles were synthesized following a modified Stöber method.^[20] In a typical synthesis, 7.75 ml tetraethyl orthosilicate (Si(OEt)₄) was dropwise added to a solution of 90.5 ml EtOH, 32.5 ml H₂O, and 4.5 ml ammonium hydroxide (28–30%). Afterwards, the solution was stirred for two hours, and the particles were washed three times with ethanol (11,000 rpm, 15 min) and dried under ambient conditions.

Synthesis of Fe₃O₄@SiO₂ particles

This was a two-step synthesis in which first, α -Fe₂O₃@SiO₂ particles were synthesized as previously described by Gessner and Krakor et al.^[21] In a consequent step, the α -Fe₂O₃ core was reduced under H₂/Ar atmosphere at 400°C for 12 h with a heating rate of 10°C/min to obtain Fe₃O₄ nanoparticles, while preserving the shape.

Functionalization with cutinase and lipase

In a typical synthesis, 50 mg of Fe₃O₄@SiO₂ or SiO₂ particles were dispersed in 5 ml ethanol and heated up to 40 °C, and 1 ml APTMS was added.^[22] The dispersion was stirred for 48 h. Subsequently, the particles were washed three times with ethanol (11,000 rpm, 15 min) and dried in ambient air. 5 mg of APTMS-modified Fe₃O₄@SiO₂ or SiO₂ was dispersed in 400 µl PBS and 40 µl GA was added, followed by the addition of 50 µl cutinase or 50 µl lipase. The dispersion was stirred for 1 h and the particles were washed two times with PBS (11,000 rpm, 1.5 min). Additionally, the particles were modified with both enzymes. For this purpose, particles were dispersed in 400 µl PBS, 40 µl GA was added, and 50 µl cutinase as well as 50 µl of lipase were added. The dispersion was stirred for 1 h and washed two times with PBS (11,000 rpm, 1.5 min).

Protein quantification using Bradford assay

For determining the number of enzymes on the particle surface, Roti®-Nanoquant (*Carl Roth*) was used, which has been established as a modification of Bradford's protein assay.^[23,24] A calibration curve was prepared using bovine serum albumin (0–100 µg/ml) in a 96-microwell plate, and the absorbance was measured at 590 nm and 450 nm. A stock solution of SiO₂-lipase and SiO₂-cutinase conjugates (5 mg/ml) was prepared and diluted 1:5 with double-distilled water before measurements. To determine the enzyme concentration for Fe₃O₄@SiO₂ particles, the iron core was etched using HCl, as described in our previous publication.^[21] The hollow mesoporous silica capsules (HMSC) obtained were modified with enzymes as

described above, and the enzyme concentration was quantified using the Bradford assay.

Enzymatic degradation tests

The enzymatic degradation was monitored by degradation of 4-nitrophenyl acetate (4-NPA) to 4-nitrophenol (4-NP) followed by UV–Vis analysis. In a typical experiment, 9.15 mg (0.05 mmol) 4-NPA was dissolved in 1 ml of acetonitrile. 50 μ l of the 4-NPA solution was mixed with 4950 μ l PBS (pH 7.4) revealing a concentration of 0.5 μ mol/ml. Different concentrations of cutinase- or lipase-modified particles were prepared and 20 μ l of as-prepared dispersions were mixed with 1480 μ l of 4-NPA solution. After 10 min, 30 min, and 1 h incubation at ambient air, the UV–Vis absorption at 405 nm was measured using a 96-well plate microplate reader. The turnover (%) from 4-NPA to 4-NP was calculated through a calibration curve and was set in relation to the amount of 4-NPA used. In the case of SiO₂ particles modified with enzymes, pure particles were measured as a reference to subtract intrinsic absorption of the particles. In the case of Fe₃O₄@SiO₂ modified with enzymes, no reference was measured because the particles were separated magnetically before measuring the UV–Vis absorption.

Determination of a calibration curve

For recording a calibration curve, 6.94 mg 4-NP was dissolved in 1 ml acetonitrile. Afterwards, 50 μ l of the 4-NP solution was mixed with 1450 μ l of PBS, and a serial dilution in a ratio of 1:1 was performed. Following this, the UV–Vis absorption of as-prepared solutions was measured at 405 nm using a 96-well plate reader.

Electrospinning of the polycaprolactone (PCL) fibers mats

In a typical electrospinning experiment, 1 mg PCL was dissolved in 2550 μ l tetrahydrofuran (THF) and 2550 μ l dimethylformamide (DMF) and stirred overnight. The polymer solution was transferred into a syringe (5 ml) and pumped into a steel needle with a feed rate of 10 μ l/min. The steel needle was mounted 15 cm above the metal collector plate, and the electrical potential was adjusted to 13 kV for obtaining a homogeneous fiber morphology. Electrospinning was performed for 5 h. The as-fabricated fiber mat was dried overnight in ambient air.

Degradation of PCL fiber mats

For the degradation of PCL fibers, the fiber mats were cut into circular shape using a hole puncher. Afterwards, the mats were weighed, to analyze mass loss after the degradation process. In a consequent step, the mats were transferred into a 96-well plate and different amounts of enzyme functionalized particles or pure enzyme were added. Additionally, for reference purposes, particles modified with APTMS only and pure PBS were incubated with the fiber mats. The plates were incubated at ambient temperature, and after 24 h, the dispersion was mixed using a pipette. After 48 h, the mats were removed from the

solution and washed with water to remove enzyme-modified particles. Afterwards, fiber mats were dried under ambient conditions, and SEM analyses were performed. For mass loss measurements, the fiber mats were incubated for 96 h, washed with water to remove enzyme-modified particles, and dried under ambient conditions.

Results and discussion

X-ray diffraction measurements confirmed the formation of crystalline and phase pure α -Fe₂O₃ [Fig. S1(a), top], which are also in good agreement with the reference pattern of rhombohedral hematite (PDF no. 33-0664) with the space group $R\bar{3}c$ ($a = 5.03560(10)$ Å, $c = 13.4789(7)$ Å).^[25] Upon annealing, the XRD pattern showed the characteristic peaks (PDF no. 19-0629) corresponding to cubic Fe₃O₄ [Fig. S1(a), bottom]. In addition, peaks for FeO and Fe were visible, and a small amount of α -Fe₂O₃ as residual phase was also detected. This is possibly due to the reductive treatment of hematite in hydrogen atmosphere, which makes a precise control over the Fe:O stoichiometry in the reductive phase challenging due to the differential kinetics of the reduction of α -Fe₂O₃ that follows multiple steps (α -Fe₂O₃ $\rightarrow$ Fe₃O₄ $\rightarrow$ FeO $\rightarrow$ Fe) which leads to concomitant presence of minor amounts of subvalent (FeO) and metallic (Fe) iron phases.^[26] The successful reduction of α -Fe₂O₃ into Fe₃O₄ was also visible in the color change from red-brownish to black and an increase in the magnetization [Fig. S1(b)]. In addition, the TEM measurements [Fig. 1(b)] of the Fe₃O₄@SiO₂ particles confirmed the ellipsoidal form as well as the successful coating process. Figure 1(a) shows the SEM image of the SiO₂ particles revealing spherical particles of average size 392 nm $\pm$ 32 nm.

The successful attachment of the surface ligands on carrier particles was analyzed through zeta potential measurements [Fig. 1(d), Fig. S1(c) and (d)]. The pure Fe₃O₄@SiO₂ particles exhibited a zeta potential of – 11 mV, which changed towards a more positive value of + 28 mV after the functionalization with APTMS due to the surface-abundant amino groups.^[27] After the reaction of the terminal amino groups, present on the surface of Fe₃O₄@SiO₂-APTMS, with glutaraldehyde (GA, C₅H₈O₂), the zeta potential was found to decrease to 3 mV due to the presence of terminal aldehyde group. Upon addition of lipase or cutinase, the zeta potential values were found to be – 23 mV and – 20 mV, respectively. Zeta potential measurements of the SiO₂-modified particles showed a similar trend [Fig. 1(d)] with the zeta potential of pure SiO₂ particles (– 65 mV) changing to the positive range (42 mV) after their surface modification with APTMS. The addition of GA led to a zeta potential of 5 mV and the modification with cutinase resulted in more negative values (– 22 mV), which was also observed for the lipase-modified particles (– 30 mV). The quantification of immobilized enzymes using Bradford assay showed that ca. 23 μ g enzyme was present in 1 mg of SiO₂-lipase particles and 7.2 μ g was determined for 1 mg of SiO₂-cutinase particles. For the Fe₃O₄@SiO₂ particles, a quantification through Bradford

Figure 1. (a) SEM images of SiO₂ particles, (b) TEM images of Fe₃O₄@SiO₂ particles, (c) scheme for the functionalization of SiO₂ or Fe₃O₄@SiO₂ with cutinase or lipase, (d) table of the zeta potential changes for every functionalization step for Fe₃O₄@SiO₂ and SiO₂ particles with cutinase or lipase and (e) concentration (at.%) of carbon, nitrogen, oxygen, silicon, and iron for Fe₃O₄@SiO₂ after every functionalization step.

assay was not possible due to high absorption of Fe₃O₄ in the visible range of the electromagnetic spectrum, specifically at 590 and 450 nm.^[28] To minimize the self-absorption and to have an estimation of the enzyme concentration, the iron oxide core (α -Fe₂O₃) was etched using hydrochloric acid revealing hollow mesoporous silica capsules (HMSC).^[21] The HMSC was subsequently modified with both enzymes, and a Bradford assay was performed, which revealed a concentration of 57.6 μ g enzyme in 1 mg HMSC-cutinase and 49.8 μ g enzyme for the HMSC-lipase. However, given the higher density of magnetite, absolute numbers cannot be used for a quantitative comparison and are expected to be slightly lower for Fe₃O₄@SiO₂ particles.

The X-ray photoelectron spectra (XPS) of the samples were used to analyze the efficiency of the functionalization steps, the atomic concentrations (at.%) of Si, Fe, O, C (C 1s, 284.8 eV), and N (N 1s, ~399 eV) constituent on the surface of SiO₂ and Fe₃O₄@SiO₂ nanoparticles [Fig. 1(e), Figs. S2, S3, and S4]. For each modification step, the at.% of C, Si, O, (Fe), and N was found to vary with the attachment of functional groups to the particle surface resulting in thicker films.^[29] In general, the

carbon and nitrogen concentrations were found to increase with gradual decrease in the atomic concentrations corresponding to the compositions (Si, Fe) of nanoparticle core, which indicated the successful surface modification and its repeatability [Fig. 1(e), Figs. S2 and S3].^[30] Furthermore, XPS analysis showed higher concentrations of carbon and nitrogen for lipase than cutinase in both cases, SiO₂ and Fe₃O₄@SiO₂, which is in accordance with the quantification values determined by the Bradford assay. This showed a higher immobilization of lipase compared to cutinase for the SiO₂-modified particles.

The enzymatic activity of immobilized enzymes was analyzed through the gradual enzymatic degradation of 4-NPA in the presence of water to 4-NP and acetic acid [Fig. 2(a)]. Therefore, 4-NPA enters the active center, followed by the nucleophilic attack of serine to the carbonyl carbon, leading to the formation of a tetrahedral intermediate. This is stabilized by the amid groups of the oxyanion hole through hydrogen bonds. The formation of an acyl-enzyme intermediate leads to the release of the 4-NP. Subsequently, the acyl-enzyme intermediate passes through a nucleophilic attack with H₂O, leading to a second tetrahedral intermediate, stabilized by the oxyanion hole. In

Figure 2. (a) Reaction of the degradation of 4-NPA to 4-NP through the enzyme lipase or cutinase. Degradation of 4-NPA to 4-NP through enzyme-modified particles with (b) $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -cutinase, (c) $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -lipase, (d) SiO_2 -cutinase, and (e) SiO_2 -lipase. The particle concentrations range between 2 and 0.125 mg/ml.

the last step, the acetic acid is released whereby the catalytic triad turns into the stable original stage. The formation of 4-NP is analyzed through the absorption maximum at 405 nm.^[31] For this purpose, the particles were incubated with a known concentration of 4-NPA, and the absorption at 405 nm was measured at different time points.

Figure 2(b) and (c) shows the degradation of 4-NPA into 4-NP using $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -cutinase and $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -lipase particles after an incubation time of 10 min, 20 min, and 60 min, whereas the turnover (%) defines the amount of 4-NPA hydrolyzed to 4-NP. The concentration of 4-NP was determined using a calibration curve and was set in relation to the amount of 4-NPA used. After 10 min of incubation, 2 mg/ml $\text{Fe}_3\text{O}_4@$

SiO_2 -lipase degraded 4-NPA (79%) at a higher rate, when compared to $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -cutinase (43%). This trend is in accordance with the SiO_2 -modified particles (2 mg/ml), where SiO_2 -lipase degraded 76% of substrate and SiO_2 -cutinase only 19% 4-NPA after 10 min of incubation [Fig. 2(d) and (e)]. By using a longer incubation time of 60 min, the cutinase-modified particles degraded 4-NPA in the same range as described for lipase-modified particles. While $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -cutinase degraded 79%, $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -lipase showed a degradation rate of 88% and SiO_2 -cutinase degraded 61% while SiO_2 -lipase degraded 78%. These results are in accordance with the lower concentrations used in this study; however, in both cases, the lipase-modified particles were found to be more efficient in

comparison to the cutinase-modified particles. The different decomposition efficiencies correlate with the differential amounts of enzymes on the surface that showed a threefold increase in the surface-concentration of enzyme in SiO_2 -lipase conjugates (23 $\mu\text{g}/1\text{ mg}$ particles) when compared with SiO_2 -cutinase conjugates (7.2 $\mu\text{g}/1\text{ mg}$ particles).

To prove if the enzyme-modified particles showed long-term stability, degradation tests over a time period of 132 h with $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -lipase and $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -cutinase particles and over a time period of 144 h for SiO_2 -lipase and SiO_2 -cutinase were performed [Fig. S5(a)–(d)]. The modified particles were stored in between the measurements under ambient temperature in PBS. Our findings indicate that the immobilized lipase has a higher long-term stability in comparison to the cutinase. The highest stability was observed for $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -lipase particles. After 132 h of incubation, still sufficient enzymatic efficiency was present and 80% of 4-NPA was degraded using the highest concentration of $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -lipase particles (2 mg/ml). SiO_2 -lipase degraded 74% after 144 h. In the case of SiO_2 -cutinase particles, only 35% of 4-NPA was degraded while the lowest concentration of 0.125 $\mu\text{g}/\text{ml}$ showed no degradation efficiency. $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -cutinase particles showed limited long-term stability with only 12% 4-NPA degradation observed after 132 h with similar concentration of 2 mg/ml. A reason for the differences in long-term stability could be the differences in structure. It is known in literature that the lipase consists of a lid, made of a polypeptide chain,^[32] which secludes the active center from the medium. If a hydrophobic interface gets in contact with the lipase, the lid opens and permits the interaction.^[33] Apparently, this chemical characteristic enhances the long-term stability, if the active center is protected during storage.

The degradation of polycaprolactone (PCL) fibers using enzyme-modified $\text{Fe}_3\text{O}_4@\text{SiO}_2$ and SiO_2 particles was analyzed by cutting the electrospun PCL fiber mats into circular pieces of equal size (equivalent to the size of the well of the titer plate) that were incubated with a known amount of enzyme-modified particles. Additionally, APTMS-modified particles as well as pure PBS were incubated with the fiber mats as control, which were found to show no change in the morphology [Fig. S6 and Figs. 4(m), (n)]. However, the fiber mats incubated with 50 μl of pure enzymes (lipase and cutinase) showed complete degradation of the fiber mats as depicted in Fig. 3(o) and (p). Figure 3(a)–(h) shows the PCL fiber mats after the incubation with 2 mg/ml and 0.125 mg/ml of enzyme-modified particles. In all cases, a change in morphology is visible but the morphology differs depending on whether the fibers were subject to degradation by cutinase or lipase-modified particles. In the case of cutinase, the surface was found to have a porous (spongy) appearance with rough topography, which was more pronounced for the concentration of 0.125 mg/ml. This was not observed in the fibers degraded by lipase; however, the fibers appeared to be torn with fibrous morphology maintained. If particles, modified with both enzymes, are added, both surface

changes are visible in the same sample [Fig. 3(i)–(l)]. The different degradation mechanisms of lipases and cutinases were reported by Shi et al. in the degradation of poly(butylene succinate) films using cutinase of recombinant *Pichia pastoris* and the lipase Lipozyme CALB. The authors described the degradation through cutinase as surface erosion where the material is removed from the surface while the degradation through the lipase is attributed to bulk erosion where the film retains its shape and only some cavities are formed in the middle.^[34]

The degradation experiments with nanoparticle-enzyme conjugates were repeated to measure the mass loss of the fiber mats after an incubation time of 96 h (Table S1 and Fig. 4). The highest mass loss of 76.6% was measured for the PCL fibers incubated with $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -lipase [Fig. 4(e)], which is in accordance with previous results, where the fiber mat was completely degraded. This was followed by 59.9% mass loss for PCL fibers incubated with SiO_2 -lipase particles [Fig. 4(b)] that was significantly less when compared to PCL fiber mats incubated with SiO_2 -cutinase or $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -cutinase [Fig. 4(a) and (d)]. The combination of both enzymes led to 38% mass loss for $\text{Fe}_3\text{O}_4@\text{SiO}_2$ particles and 18% for SiO_2 particles [Fig. 4(c) and (f)].

Khan et al. analyzed the change of the molecular weight of PCL samples after the degradation through lipase using gel permeation chromatography revealing a decrease in molecular weight.^[35] This suggests that the PCL fibers are probably degraded to their monomers, caproic acid and caprolactone, and oligomers. Additionally, a set of particles was modified with both enzymes and degradation tests were performed to evaluate any possible cooperative effects. The $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -lipase/cutinase particles degraded much more fibers (38.4%) compared to SiO_2 -lipase/cutinase particles (1.8%) for the concentration of 2 mg/ml of modified particles. But for a lower concentration of 1 mg/ml, the difference in the degradation rates was smaller (11.2% for $\text{Fe}_3\text{O}_4@\text{SiO}_2$ -lipase/cutinase and 7.9% for SiO_2 -lipase/cutinase), which could be due to the varying ratio of lipase and cutinase on the surface. Shi et al. degraded PCL films using *Candida antarctica* lipase and *Fusarium solani* cutinase and observed that this lipase was also more suitable for the degradation of PCL films.^[36] An explanation could be that esterases preferentially degrade short-chain esters while classical lipases are more viable to degrade long-chain esters.^[37]

Conclusion

In this work, two different enzymes were immobilized on nanoparticles consisting of spherical SiO_2 and ellipsoidal $\text{Fe}_3\text{O}_4@\text{SiO}_2$ nanostructures. Through a surface modification strategy involving amino-silane chemistry, a bifunctional linker was covalently grafted onto the surface of the nanoparticles that was effective in the selective attachment of two different enzymes, lipase and cutinase. The conjugation of enzymes on nanoparticles provided high efficiency

Figure 3. SEM images of PCL fiber mats after the incubation with 2 mg/ml or 0.125 mg/ml of SiO_2 -cutinase ((a) and (b)), SiO_2 -lipase ((c) and (d)), Fe_3O_4 @ SiO_2 -cutinase ((e) and (f)), Fe_3O_4 @ SiO_2 -lipase ((g) and (h)), 2 mg/ml or 0.125 mg/ml SiO_2 -cutinase/lipase ((i) and (j)), 2 mg/ml or 0.125 mg/ml Fe_3O_4 @ SiO_2 -cutinase/lipase ((k) and (l)), 2 mg/ml SiO_2 -APTMS (m), 2 mg/ml Fe_3O_4 @ SiO_2 -APTMS (n), 50 μl cutinase (o), and 50 μl lipase (p) over a time period of 48 h.

to decompose polycaprolactone as a function of the activity and selectivity of lipase and cutinase. The efficacy of immobilized enzymes was demonstrated by time-dependent degradation of 4-NPA to 4-NP, which could be increased if the temperature optimum of the enzymes is used. The high stability (> 144 h) of as-prepared particles in conjunction with their repeatable use shows the promising potential of the demonstrated approach in controlled decomposition of polymers at room temperature. Since the immobilized

enzymes were stored at room temperature between the measurements, the long-term stability could be increased by using the recommended storage conditions of the manufacturer. In addition, various enzymes that have already been shown to be able to degrade PET, for example, could be immobilized to find sustainable repolymerization solutions for industrial plastics. The immobilization of the enzymes improves their stability and simplifies their handling such as magnetic-field assisted recovery, which can

Figure 4. Mass loss of PCL fiber mats after the incubation with enzyme-modified particles, (a) SiO₂-cutinase, (b) SiO₂-lipase, (c) SiO₂-lipase/cutinase, (d) Fe₃O₄@SiO₂-cutinase, (e) Fe₃O₄@SiO₂-lipase, and (f) Fe₃O₄@SiO₂-lipase/cutinase over a time period of 96 h.

be achieved by using magnetic carrier particles. Finally, the sustainability of this approach is given by the stability of immobilized enzymes without compromise of their activity that makes them available for multiple uses, which would also lead to a reduction in costs.

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Data availability

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Declarations

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Information

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