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Fusarium solani pisi cutinase-catalyzed synthesis of polyamides

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ABSTRACT

Polyamides, or nylon, are widely used in fiber and engineering plastic materials, due to their good mechanical and thermal properties. Synthesis of oligomers from nylon-4,10, nylon-6,10, and nylon-8,10 were performed via polycondensation of diamines (1,4-butanediamine, 1,6-hexanediamine, and 1,8-diaminooctane) and diester (diethyl sebacate). These reactions were catalyzed by immobilized cutinase from *Fusarium solani pisi* on Lewatit beads, cutinase in the form of cross-linked enzyme aggregates (CLEA), or by immobilized Lipase B from *Candida antarctica* (N435). The highest maximal degree of polymerization (DP_{max}), up to 16, can be achieved in the synthesis of nylon-8,10 catalyzed by CLEA cutinase in diphenyl ether at 70 °C. By performing a reaction at cutinase optimal temperature (70 °C), CLEA cutinase in the synthesis of nylons shows good catalytic activity, like N435.

1. Introduction

Polyamides, also known as nylon, are widely used in fiber and engineering plastic materials, due to their good mechanical and thermal properties [1]. Synthesis of nylons can be accomplished via ring opening polymerization [2] and polycondensation [3]. Industrial synthesis of nylon is conducted at high temperatures, which leads to thermal degradation and results in undesired polymer products [4]. On the other hand, alternative options for synthesizing polymers at relative low temperatures can be achieved via enzymatic polymerization. Enzymatic polymerizations are known as environmentally friendly reactions, as they can be carried out in relatively low temperatures, and they use enzymes as catalysts [5–10].

Candida antarctica lipase B CALB is a highly used enzyme in biocatalysis. CALB is commercially available as N435, which is physically immobilized CALB within macroporous poly(methyl methacrylate) resin (known as Lewatit OC VOC 1600) [11]. N435 is known to have high catalytic activity over a broad range of esters, amides, and thiols [12]. CALB has been used in esterification and transesterification [13], aminolysis [14,15], and synthesis of polyester [5,7,16], polyamides [17–19] and poly(amide-co-ester) [20].

Here, we present, for the first time, synthesis of polyamides catalyzed by *Fusarium solani pisi* cutinase. Cutinases are hydrolytic enzymes that degrade cutin, which contains polyester and epoxy fatty acids (C_{16} and n-C_{18}). This enzyme is known as a small carboxylic ester hydrolase (M_w 22 kD) that bridges functional properties between lipases and esterases [21]. Like CALB, *F. solani pisi* cutinase belongs to the α/β hydrolase family, which has a catalytic triad in Ser120, His188, and Asp175. It is known, that the active site of this enzyme is not covered by a lid [21,22] and therefore *F. solani pisi* cutinases do not exhibit interfacial activation, just as CALB [23].

In order to increase temperature resistance and tolerance toward organic solvents [24] *F. solani pisi* cutinase was immobilized on Lewatit beads or used as cross-linked enzyme aggregates (CLEA). Using an immobilized enzyme allows it to be separated from the product more easily than a free enzyme, thereby minimizing protein contamination of the product [25,26].

The enzymatic polymerizations of diethyl sebacate and aliphatic diamines (C_4, C_6, and C_8) catalyzed by immobilized *F. solani pisi* cutinase on Lewatit beads or as CLEA cutinase are presented. The general reaction scheme is shown in Scheme 1. The same reaction was

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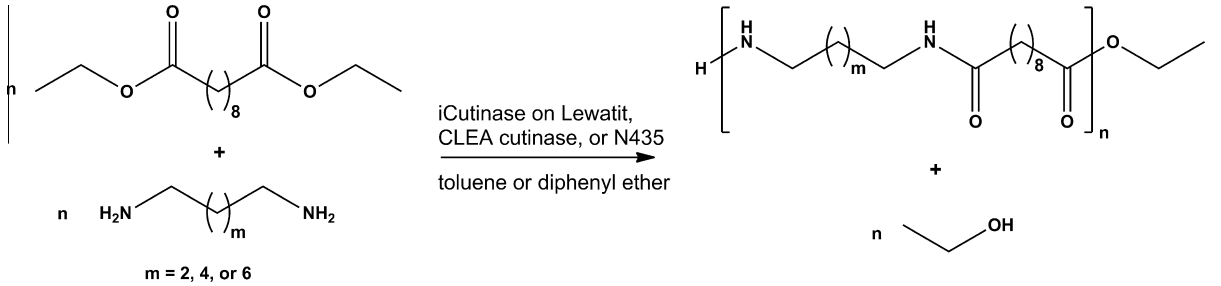


Scheme 1. Polycondensation of diethyl sebacate and diamines catalyzed by icutinase on Lewatit, CLEA cutinase, or N435.

also performed using N435 to determine whether cutinase displays catalytic activity in the synthesis of polyamides, as N435 does.

2. Experimental

2.1. Materials

F. solani pisi cutinase (Novozym 51032) was a gift from Novozymes, Denmark. Lewatit OC VOC 1600 was donated by Lanxess, Belgium. Ethanol, formic acid, and calcium hydride were purchased from Merck. 1,2-Dimethoxyethane, sodium phosphate dibasic, sodium phosphate monobasic, 4-nitrophenol butyrate, sodium cholate hydrate, ε-caprolactone, chloroform-*d*, diethyl sebacate, hexafluoroisopropanol, sodium trifluoroacetate, Lipase acrylic resin from *C. antarctica* (N435), molecular sieves 4 Å, and trifluoroacetic acid-*d* (TFA-*d*) were purchased from Sigma–Aldrich. A BCA Protein assay kit was purchased from Thermo Scientific. 1,8-Diaminooctane, 4-nitrophenol, and 2-(4-hydroxy-phenylazo)benzoic acid (HABA) were purchased from Fluka. 1,4-Butanediamine, 1,6-hexanediamine, tetrahydrofuran, and glutaraldehyde were obtained from Acros. Except for toluene, diethyl sebacate, and diamines, all chemicals were used without further purification. Toluene was dried by a solvent purification system (SPS). Diethyl sebacate (DES) was dried using CaH_2 and vacuum distilled. 1,4-Butanediamine (BD), 1,6-hexanediamine (HD), and 1,8-diaminooctane (DAO) were purified by sublimation.

2.2. Immobilization of F. solani pisi on Lewatit

The Lewatit beads were first activated with ethanol and dried under vacuum for 60 min to remove traces of ethanol [27]. Then, beads (0.2 g) were added to 3 mL cutinase (used as received with concentration of 20 mg/mL). The samples were incubated in a shaker at 100 rpm at 4 °C for 48 h. Subsequently, the supernatant was removed, and the remaining beads were washed with sodium buffer phosphate (0.1 M, pH 7.8). The concentration of cutinase that remained in the supernatant and washing solutions was determined using the BCA assay kit. The amount of immobilized cutinase on the Lewatit was determined as enzyme loading. The immobilized cutinase on Lewatit was freeze-dried for 48 h.

2.3. Preparation of CLEA F. solani pisi cutinase

Three milliliters of cutinase stock solution (20 mg/mL) were dissolved in 6 mL sodium phosphate buffer (100 mM, pH 7) [28]. Subsequently, 18 mL of 1,2-dimethoxylethane, and 480 μL glutaraldehyde (25% w/v in water) were added. The mixture was stirred at 4 °C for 17 h. After 17 h, 6 mL of 1,2-dimethoxyethane was added. Then, the mixture was centrifuged for 20 min at 7000 rpm. After centrifugation, the supernatant was separated from the residue. Later, the residue was washed with 30 mL of 1,2-dimethoxyethane, centrifuged, and decanted. The washing step was repeated three times. CLEA was collected and dried in a vacuum oven at 25 °C for 1 h.

2.4. Cutinase hydrolysis assay

The cutinase hydrolysis activity was determined by hydrolysis of 4-nitrophenyl butyrate (p-NPB) to 4-nitrophenol (p-NP). iCutinase on Lewatit or CLEA cutinase (10 mg) were added to 1 mL of sodium phosphate (11.3 mM), tetrahydrofuran (0.43 M), and pNPB (0.55 mM) in sodium phosphate buffer (50 mM, pH 7.0). The hydrolytic activities of cutinase were measured by UV–Vis spectrophotometer at 399 nm in a time range of 1 min against the blank solution [29]. Molar absorptivity of p-NP was considered to be 7919.2 M^{-1} cm^{-1} from the calibration curve. Specific activity of cutinase was defined as nmol of p-nitrophenol for 1 min per gram solid support.

2.5. Cutinase synthetic assay

The cutinase synthetic activities were determined by ring opening polymerization of ε-caprolactone. One milliliter of ε-caprolactone was added to a 25 mL two-neck flask containing of immobilized cutinase (10 mg or 10 μL for free cutinase). The reactions were carried out at 70 °C for 24 h at 100 rpm, under N_2 atmosphere. The monomer conversion and the degree of polymerization were determined by ^1H NMR measurements using $CDCl_3$ as solvent.

2.6. General procedures of enzymatic synthesis of nylon-4,10, nylon-6,10, and nylon-8,10

Nylons were synthesized via two different reaction conditions—one-step and two-step reactions.

2.6.1. One-step enzymatic synthesis of nylon-4,10, nylon-6,10, and nylon-8,10

Five milliliters of dried toluene was added to a mixture of 0.1 g of immobilized enzymes (icutinase on Lewatit, CLEA cutinase, or N435), 0.675 mL of diethyl sebacate (2.5 mmol), diamines (2.5 mmol), and 0.5 g of dried molecular sieves. The mixture was stirred at 70 °C, 100 rpm for 96 h, under N_2 atmosphere.

2.6.2. Two-step enzymatic synthesis of nylon-4,10, nylon-6,10, and nylon-8,10

Five milliliters of dried diphenyl ether was added to a mixture of 0.1 g of immobilized enzymes (icutinase on Lewatit, CLEA cutinase, or N435), 0.675 mL of diethyl sebacate (2.5 mmol), diamines (2.5 mmol), and 0.5 g of dried molecular sieves. The first step of the reaction was carried out at 70 °C for 20 h at 500 mmHg pressure, and the following step was carried out by decreasing the pressure to 100 mmHg for 24 h.

2.6.3. Control reactions for enzymatic synthesis of nylon-4,10, nylon-6,10, and nylon-8,10

As control reactions, one-step and two-step synthesis of nylon-4,10, nylon-6,10, and nylon-8,10 were performed without the addition of enzymes. The control reactions were carried out using the same equimolar amounts of diethyl sebacate and diamines as in the one-step and two-step reactions.

2.7. Kinetics of the one-step enzymatic synthesis of nylon-6,10 and nylon-8,10 catalyzed by CLEA cutinase

Reactions were carried out using the same equimolar amounts of diethyl sebacate and diamines as in the one-step reaction. The mixture was stirred at 70 °C, 100 rpm under N_2 atmosphere. The reaction was stopped at certain reaction times (0.25, 0.5, 1, 3, 6, 12, and 18 h). Small aliquots of the reaction mixture were withdrawn and analyzed by ^{1}H NMR spectroscopy using TFA-*d* as solvent. Reactants concentration [A] at certain reaction times were calculated from ^{1}H NMR analysis [20].

2.8. Instrumental methods

- Attenuated Total Reflection-Fourier Transform Infrared (ATR FT-IR) measurements were carried out on a Bruker IFS88 FT-IR spectrometer.
- UV–Vis measurements were performed on a Spextra Max M2 spectrophotometer.
- ^{1}H NMR measurements were performed on a 400 MHz Varian VXR apparatus, with $CDCl_3$ or TFA-*d* as solvent. The signals were referenced to tetramethylsilane (δ = 0.00 ppm).
- Maldi-ToF MS measurements were performed on a Biosystems Voyager-DE PRO spectrometer, in positive and linear mode, by accelerating the voltage to 20 kV. In a sample preparation, 20 mg/mL of 2-(4-hydroxy-phenylazo) benzoic acid (HABA) were used as a matrix, and 1 mg/mL of sodium trifluoroacetate as the salt for cationization was mixed with 6–7 mg/mL of the respective polymer sample in 1,1,1,3,3,3-hexafluoro-2-isopropanol (HFIP) solution [20].
- The Melting points of the nylons were measured by differential scanning calorimetry (DSC) Q1000.

3. Results and discussion

3.1. Immobilization of cutinase

Immobilization of *F. solani pisi* was successfully accomplished by physical adsorption on the Lewatit beads or by cross-linked enzyme aggregates (CLEA). In immobilization on Lewatit, cutinase is attached to the beads by pure physical adsorption/non-covalent linkages. From enzyme loading determination, the amount of cutinase attached varied between 127 and 143 mg cutinase per gram Lewatit beads. Fig. 1 shows the ATR-FTIR spectrum of the Lewatit beads before and after immobilization. After immobilization, there are three additional peaks at 1653, 1541, and 3286 cm^{-1}, which belong to amide I (C═O), amide II (N–H), and intermolecular hydrogen bonding, respectively, which confirms successful immobilization of the cutinase.

Immobilization of *F. solani pisi* via CLEA method was performed successfully via a simple crosslinking process using glutaraldehyde. Around 0.1 g CLEA was isolated from 3 mL free cutinase solution (20 mg/mL).

The immobilized cutinase on Lewatit and in the form of CLEA was examined for hydrolytic and synthetic assay; the results are summarized in Table 1. The hydrolysis assay revealed similar activity between free cutinase and cutinase after immobilization. In contrast to this, in the synthesis of polycaprolactone, free cutinase showed better catalytic activity than immobilized cutinase. This is probably because, after immobilization, some of the active sites are no longer accessible to solvent or monomer, as the active site is blocked by the Lewatit beads or cross-linked with glutaraldehyde. However, immobilized cutinase was used in the following synthesis reactions, due to easier separation from the reaction mixture, as well as minimizing protein contamination of the product [25].

From the synthetic activity assay (see Table 1) it becomes obvious that CLEA seems to be the better immobilization method for cutinase than the immobilization on Lewatit. This is according to literature as enzymes in CLEA preparations are usually more highly concentrated. Furthermore, immobilization on macroporous beads such as Lewatit beads leads to dilution of activity, due to the large amount of non-catalytic solid support [25].

3.2. Enzymatic synthesis of nylons

3.2.1. One-step reaction in toluene

Synthesis of polymers catalyzed by cutinase is still not widely explored; the enzymatic polymerization of polyesters catalyzed by *Humicola insolens* cutinase has been reported previously [27,30]. To the best of our knowledge, there are no previous reports on polymer synthesis catalyzed by *F. solani pisi*. Here, we reported polycondensation

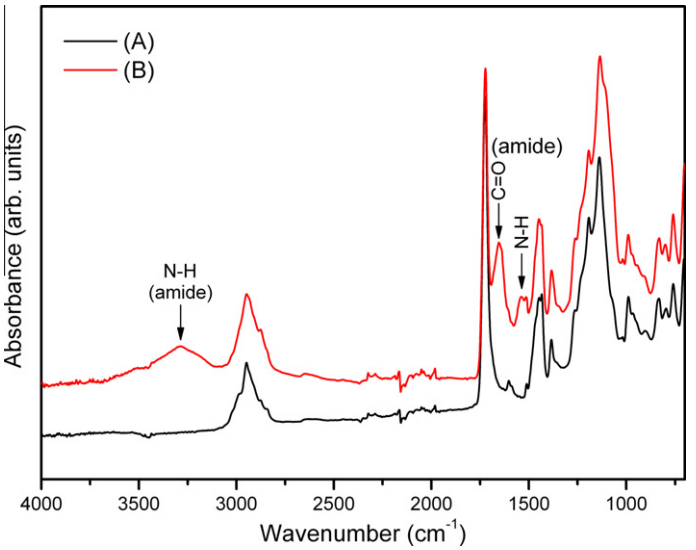


Fig. 1. ATR-FTIR spectra of (A) Lewatit beads and (B) immobilized cutinase on Lewatit beads.

Table 1
Enzyme loading and activity: free and immobilized cutinase.

Enzyme	Hydrolytic activity (nmol of p-NP/min)	Amount of enzyme in synthetic activity	Polycaprolactone	
			DPn	Monomer conversion (%)
iCutinase on Lewatit (enzyme loading: 135 ± 8 mg/g)	108 ± 19	10 mg	1	4
Cutinase as CLEA	124 ± 12	10 mg	3	19
Free cutinase (20 mg/mL)	124 ± 25[a]	10 μL	3	34

[a] Hydrolysis assay was carried out using 20 μL of free cutinase.

of diethyl sebacate and diamines (1,4-butanediamine, 1,6-hexanediamine, 1,8-diaminooctane) catalyzed by *F. solani pisi* cutinase. Polycondensation of diethyl sebacate and 1,8-diaminooctane catalyzed by N435 has been accomplished previously [20]. Therefore, we compared the catalytic activity of cutinase (immobilized on Lewatit beads or CLEA cutinase) to N435 in the polycondensation of diethyl sebacate and diamines, as well.

The polycondensation was conducted at 70 °C, as it previously was shown to be the optimal temperature for synthesis catalyzed by cutinase [27]. The reaction was performed for 4 d, because reactions catalyzed by cutinase – immobilized on Lewatit – resulted in no nylon-4,10 or nylon-6,10 when the reaction was performed for less than 4 d. Moreover, icutinase on Lewatit still maintains its catalytic activity after 4 d under stirring in toluene (see Table S1 in Supplementary data). In addition, the reaction was performed by adding molecular sieves and flowing N_2 to decrease the hydrolysis, due to the presence of water in the reaction.

In the control reaction without enzyme, no nylon was formed after 4 d at 70 °C, which proves that the formation of nylons is due to the catalytic activity of enzymes (cutinase or CALB). Monomer conversion was calculated from ^{1}H NMR (formation of amide bonds) of the reaction mixture after 4 d reaction; the results are summarized in Fig. 2. The highest monomer conversion was achieved for polycondensation catalyzed by CLEA cutinase, up to 56% and 53% in the synthesis of nylon-8,10 and nylon-6,10, respectively. The lowest monomer conversion was obtained in polycondensation catalyzed by icutinase on Lewatit. iCutinase on Lewatit, CLEA cutinase, and N435 used in this synthesis showed better catalytic activity toward long chain diamines ($C_8 > C_6 > C_4$). This result is in agreement with previous reports [30,31], which showed that cutinase and CALB possess higher catalytic activity towards long chains of diols.

^{1}H NMR spectra of the reaction mixtures after 4 d reaction time revealed a rise in the acid peak at 2.45 ppm, as shown in Fig. 3. The acid product was observed in all one-step reactions between diethyl sebacate and different diamines and is probably formed due to presence of water, which leads to hydrolysis during reactions. Kinetic studies of the polycondensation between DES and DAO, and polycondensations of DES and HD clearly show that the acid product was formed during the reaction and not by work-up procedure (e.g. work-up solvents, air, etc.). As toluene and monomers were dried before being used, the water was probably derived from the enzyme that was used in the reaction. Analysis of ^{1}H NMR spectra of the

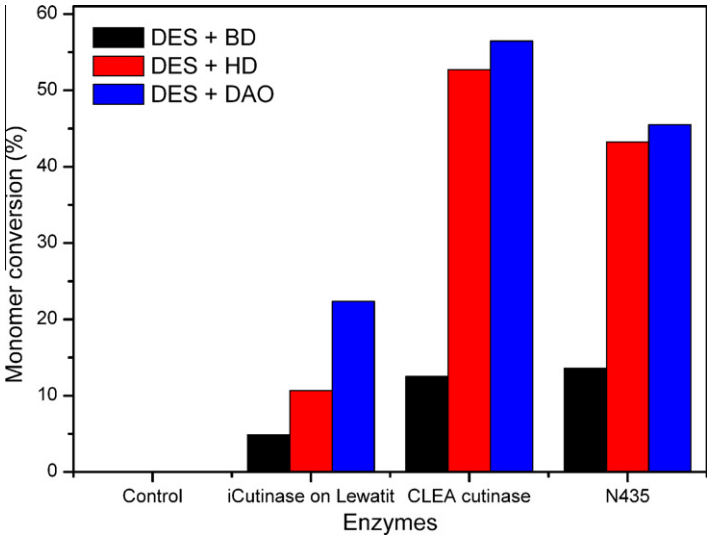


Fig. 2. Monomer conversion in the one-step polycondensation of diethyl sebacate (DES) and 1,8-diaminooctane (DAO), DES and 1,6-hexanediamine (HD), and DES and 1,4-butanediamine (BD).

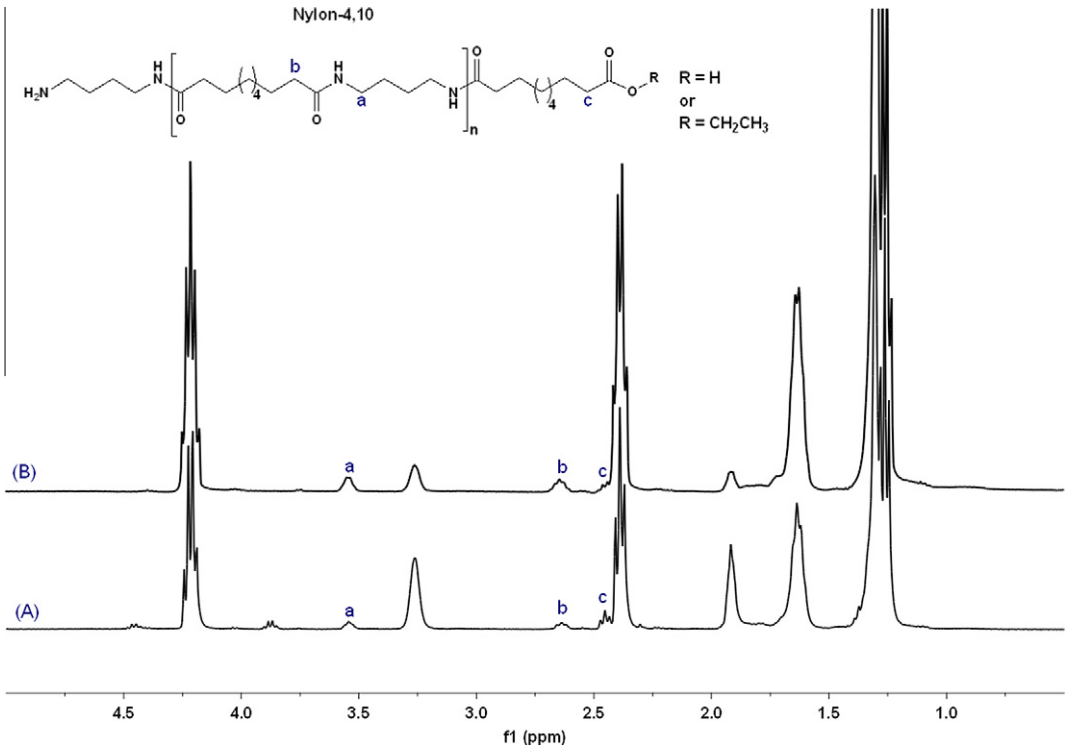
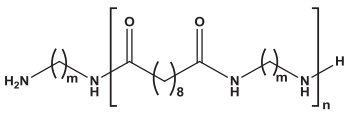
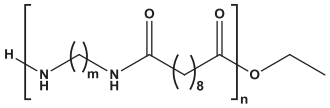
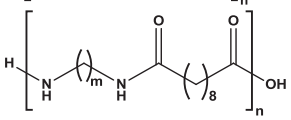


Fig. 3. ^{1}H NMR spectra of reaction mixtures of polycondensation of DES with BD using: (A) CLEA cutinase and (B) N435 as catalyst.

products after purification clearly shows that the nylons contained at least two different end groups—amine-ester and amine-acid end groups—as shown in Figs. S1–S3 in the Supplementary data.

Analysis of end-groups and the maximal degree of polymerization (DP_{max}) of the nylons was performed by MALDI-ToF measurements as well. Three different end groups were detected by MALDI-ToF: amine–amine, amine-ester, and amine-acid (Table 2). Ester–ester and cyclic end groups were not observed in the MALDI-ToF spectra of nylon-4,10, nylon-6,10, or nylon-8,10 catalyzed by icutinase on Lewatit, CLEA cutinase, or N435. Synthesis of nylon-8,10 catalyzed by N435 had been performed previously; the author showed five different end groups of nylon-

Table 2
Different microstructures and end groups of nylons.

Symbol adduct	Microstructures	Remaining mass for different nylons (amu)		
		$m = 4$	$m = 6$	$m = 8$
Na^+: ▢ H^+: □		88.2	116.2	144.2
Na^+: ▽ H^+: ▽		46	46	46
Na^+: ◯ H^+: ◯		18	18	18

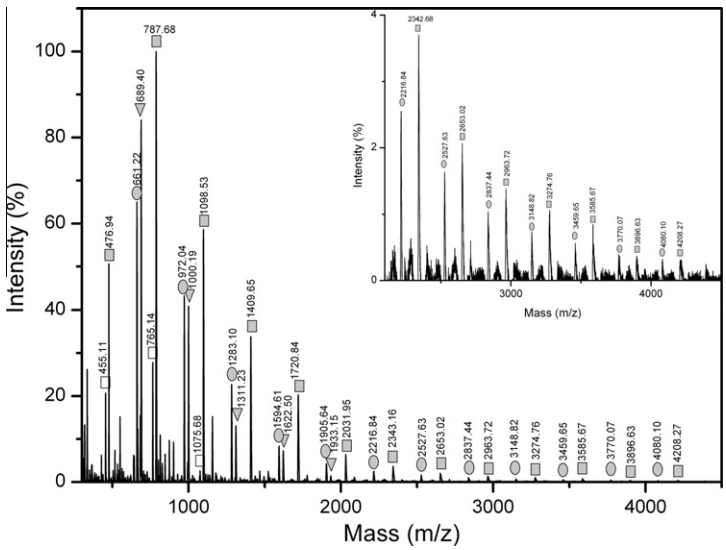


Fig. 4. MALDI-ToF spectrum of nylon-8,10 catalyzed by CLEA cutinase in one-step reaction.

8,10 including ester–ester and cyclic end groups [20]. In our results, the ester–ester and cyclic end groups of the nylons might have been lost during purification, and therefore, could not be detected by MALDI-ToF.

In MALDI-ToF MS spectra, the end group of the nylons can be determined as corresponding to $M = x + (n * \text{mass of repeated unit}) + Na^+$ [20], where n is the degree of polymerization and x is the mass of end groups, as shown in Table 2, which can be amine-ester, amine–amine, and amine-acid. For nylon-8,10, the mass of the repeating unit is 310.26 Da, $x = 144.16$ Da for amine–amine, $x = 46$ Da for amine-ester, and $x = 18$ Da for amine-acid. For example, the mass for $n = 2$, amine–amine end group is 787.68 Da.

MALDI-ToF spectra showed that the most abundant adduct is the sodium product. A trace amount of hydrogen adduct was also detected. Amine–amine and amine-acid are the dominant end groups that were formed, as shown in Fig. 4. The same results were observed in MALDI-ToF spectra of nylon-4,10 and nylon-6,10 (see Figs. S4 and S5 in Supplementary data). The one-step synthesis of nylon-8,10 catalyzed by CLEA cutinase resulted in the highest DP_{max}, up to 13, compared to the other nylons. This result indicates that cutinase possesses higher catalytic activity toward long-chain diamines C_8 and C_6.

This trend was also supported by a detailed kinetics study. Polycondensation of DES with different diamines follows a second order kinetics. The polycondensation of DES and DAO showed a higher reaction constant than the polycondensation between DES and HD, $0.0853\ M^{-1}\text{-}h^{-1} > 0.0628\ M^{-1}\ h^{-1}$, as shown in Fig. 5. Therefore, it can be concluded that cutinase prefer long chain diamines ($C_8 > C_6$).

The melting temperatures of nylon-8,10 and nylon-6,10 catalyzed by CLEA cutinase were measured, and the results were 138.58 and 132.16 °C, respectively. No melting temperature was observed for nylon-4,10 catalyzed by CLEA cutinase, as it resulted in a very short oligomer chain.

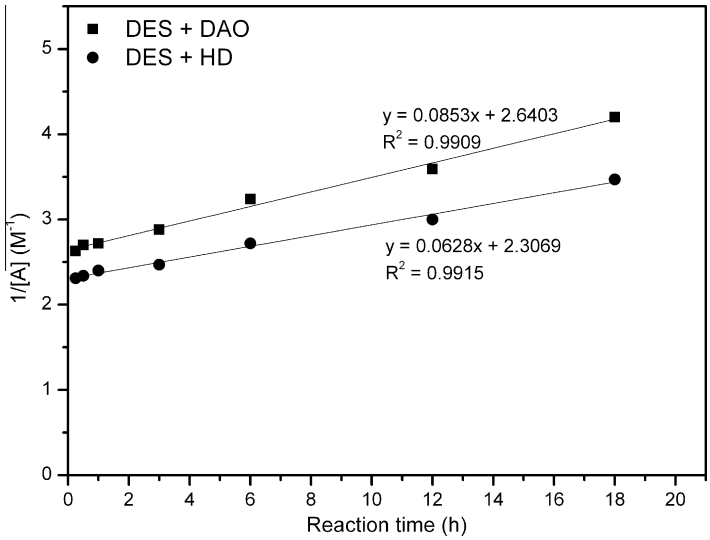


Fig. 5. Kinetic studies of polycondensation between DES and DAO, and DES and HD catalyzed by CLEA cutinase at 70 °C in toluene.

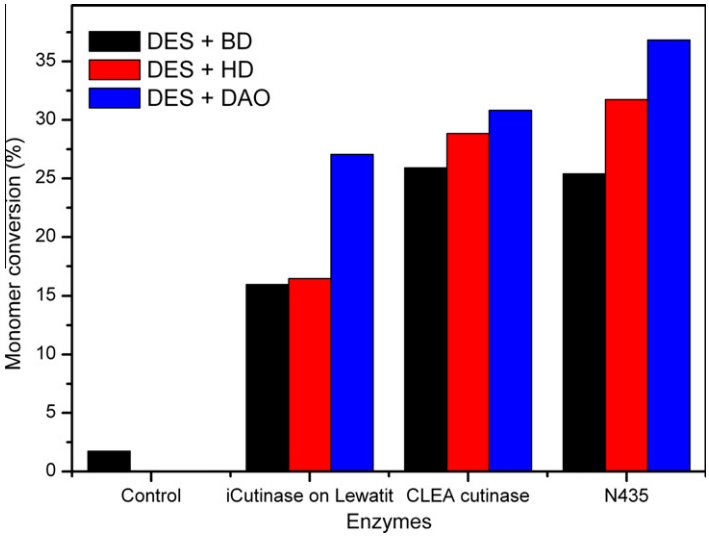


Fig. 6. Monomer conversion in the two-step polycondensation reaction of diethyl sebacate (DES) and 1,8-diaminooctane (DAO), DES and 1,6-hexanediamine (HD), and DES and 1,4-butanediamine (BD).

3.2.2. Two-step reaction in diphenyl ether

A two-step reaction was performed at 70 °C for 2 d under reduced pressure. Subsequently the pressure was reduced to 500 mmHg, and after 24 h it was decreased further, to 100 mmHg, while the temperature remained the same. An increase in temperature to 100 °C after the first 24 h did not improve monomer conversion in the reaction catalyzed by icutinase on Lewatit or CLEA cutinase.

By reducing the pressure, amide bond formation was observed in the control reaction between DES and BD, with low monomer conversion (~2%). When enzymes were added, monomer conversions were much higher than 15%. Thus; the observed polymerization is only due to enzyme activity. Moreover, control reactions between DES and HD, and between DES and DAO resulted in no nylons, as shown in Fig. 6. The highest monomer conversion (~37%) was obtained in the synthesis catalyzed by N435. When CLEA cutinase was used, the monomer conversion of the reaction between DES and DAO was 30%. Reaction under reduced pressure for 2 d resulted in lower monomer conversion than in the reaction under normal pressure (one-step reaction). This is because the reaction was performed in 4 d in the one-step reaction, while the two-step reaction was performed only for 2 d. On the other hand, monomer conversion increases for the synthesis of nylons catalyzed by icutinase on Lewatit.

Acid products were observed in all ^{1}H NMR spectra of reaction mixtures of the synthesis of nylons catalyzed by

Table 3
DP_{max} of nylon-4,10, nylon-6,10, and nylon-8,10.

Nylons	Catalysts	One-step reaction DP_{max} (MALDI)	Two-step reaction DP_{max} (MALDI)
Nylon-4,10	iCutinase on Lewatit	–[a]	4
	CLEA cutinase	4	8
	N435	5	8
Nylon-6,10	iCutinase on Lewatit	–[a]	5
	CLEA cutinase	8	11
	N435	7	10
Nylon-8,10	iCutinase on Lewatit	5	5
	CLEA cutinase	13	16
	N435	12	12

[a] Sample was not measured.

icutinase on Lewatit, CLEA cutinase, or N435. This is the same result as for the synthesis in a one-step reaction. It can be concluded, that reducing the pressure cannot remove water from the enzyme completely; thus, hydrolysis still occurred during the reaction and resulted in an amine-acid end group product.

DP_{max} of nylons synthesized in both one-step and two-step reactions are summarized in Table 3. Reduced pressure in the two-step reaction led to an increase of DP_{max} of nylons compared to DP_{max} nylons synthesized in the one-step reaction. It becomes obvious, that higher DP_{max} can be achieved due to the removal of ethanol as byproducts by reducing the pressure in the reaction. Under reduced pressure, CLEA cutinase exhibited slightly better catalytic activity than N435. Especially in the synthesis of nylon-8,10, the highest DP_{max}, up to 16, was achieved using CLEA cutinase as catalyst.

4. Conclusion

Immobilization of *F. solani pisi* cutinase by adsorption on Lewatit beads or cross-linked enzyme aggregates (CLEA) was performed successfully. Immobilization of cutinase using the CLEA method resulted in better catalytic activity than cutinase immobilized on Lewatit beads.

The enzymatic polymerizations of nylon-4,10, nylon-6,10, and nylon-8,10 oligomers were successfully performed via polycondensation of diamines (BD, HD, and DAO) and diester (DES) using immobilized cutinase on Lewatit, CLEA cutinase, or by immobilized Lipase B from *C. antarctica* (N435) in one-step or two-step reactions. In the control reaction of the one-step reaction, no oligoamides were formed. In the control reaction of the two-step reaction, only the reaction between BD and DES showed a low (2%) monomer conversion and no oligoamides were formed in the reaction between HD and DES, and between DAO and DES, which proves that the formation of oligoamides is due to the catalytic activity of enzymes.

Three different end groups were detected by MALDI-ToF in the oligomers of nylon-4,10, nylon-6,10, and nylon-8,10: amine–amine, amine-ester, and amine-acid.

By performing the reactions at cutinase optimal temperature (70 °C), CLEA cutinase shows good catalytic activity in the synthesis of nylons. Moreover, the highest DP_{max}, up to16, of nylon-8,10 can be achieved in the enzymatic polymerization catalyzed by CLEA cutinase in a two-step reaction. Cutinase and CALB exhibit high selectivity and catalytic activity toward diamines with longer chains ($C_8 > C_6 > C_4$).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eurpolymj.2012.12.010.

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