Screening of Enzyme-Catalyzed Polyester Synthesis

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Introduction

Polyesters are synthesized by polycondensation via direct esterification or transesterification of aliphatic or aromatic acid derivatives and diols. Conventionally, synthesis is performed at high temperatures of 140 °C to 280 °C using metal catalysts such as zinc acetate or titanium oxides. As an alternative, enzyme catalyzed synthesis of polyesters using lipases has sparked scientific interest in recent years due to numerous benefits like biocompatibility and significant chemo-, regio- and stereoselectivity [1]. One such enzyme is lipase B of *Candida antarctica* (CALB) whose performance is dependent on reaction conditions such as pH and temperature. An additional parameter is the reduced pressure which helps to remove the water formed in the polycondensation to shift the reaction equilibrium to the side of the products [2]. Tailoring the reaction parameters for different enzymes presents a major goal to promote bio-catalyzed processes on an industrial level.

Polycondensation with CALB-IB150

The mass polycondensation of adipic acid and 1,4-butanediol (ratio 0.95:1, Fig. 1a) was chosen to identify favorable reaction conditions for CALB, immobilized on Immobead-150 (IB150). The products were analyzed using size exclusion chromatography (SEC) and ^{1}H-NMR spectroscopy (Fig. 1b,c). Using the latter method, the degree of esterification (d_E) was determined (Tab. 1). Multiple experiments revealed that 40 °C and atmospheric pressure were favorable at the beginning of the reaction while an increased temperature and 20 mbar promoted further condensation to high molecular weights.

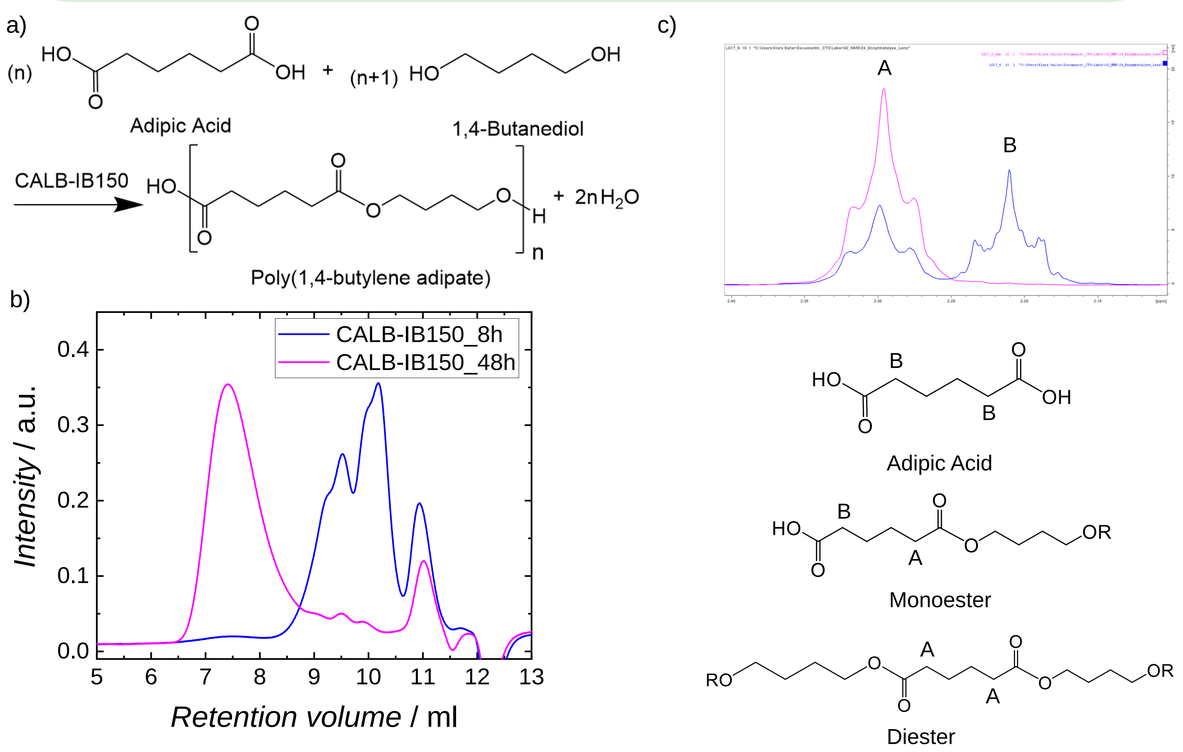

Figure 1: a) Reaction scheme of the enzyme-catalyzed polycondensation using CALB-IB150 (enhanced temperature regime: 8 h 40 °C, 40 h 60 °C; enhanced pressure regime: 8 h 1 bar, 40 h 20 mbar, b) results from the SEC measurements after 8 h and 48 h of the polycondensation using CALB-IB150 and c) ^{1}H NMR peaks used for the determination of the degree of esterification (d_E).

Esterification activity

To elevate enzyme-catalyzed polyester synthesis to an industrial level, low-cost alternatives to immobilized CALB are of significant relevance. For the identification of promising candidates, a range of non-immobilized enzymes, together with CALB-IB150, were tested for their esterification activity (*EA*). For this purpose, butyric acid and 1-butanol in *n*-heptane were left to react with 2-10 mg catalyst. The amount of butyl butyrate was determined by titration with ethanolic NaOH, and the results are given in *U* (µmol min^{-1} mg^{-1} catalyst). As seen in Fig. 2, CALB showed a significant increase of esterification activity upon immobilization (CALB-IB150). As lipase from porcine pancreas and lipase from *Candida rugosa* are accessible and have shown similar *EA* to free CALB, they were selected to be examined further in polycondensation reactions.

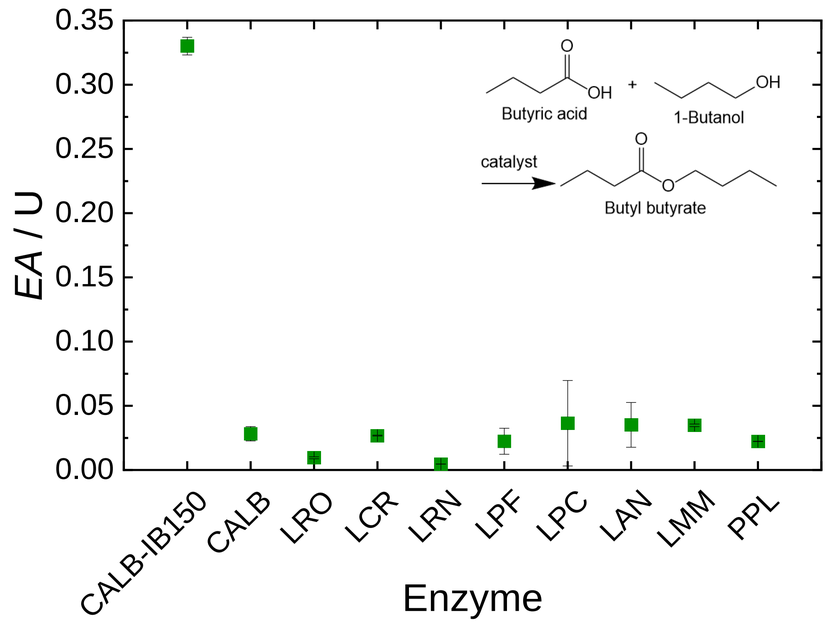

Figure 2: Results for the esterification activity (*EA*) of different enzymes. Immobilized lipase B from *Candida antarctica* (CALB-IB150), lipase B from *Candida antarctica* (CALB), lipase from *Rhizopus oryzae* (LRO), *Candida rugosa* (LCR), *Rhizopus niveus* (LRN), *Pseudomonas fluorescens* (LPF), *Pseudomonas cepacia* (LPC), *Aspergillus niger* (LAN), *Mucor miehei* (LMM) and porcine pancreas (PPL).

Enzyme screening

Lipase from porcine pancreas and lipase from *Candida rugosa* were investigated in the polyester synthesis of adipic acid and 1,4-butanediol described above. Due to low activities compared to immobilized CALB, the catalyst amount was increased from 1 to 5 wt.%. Using higher temperatures than in the enhanced polycondensation reactions with CALB-IB150, oligomeric condensation products were formed using both non-immobilized lipases (Fig. 3, Tab. 1). Further enhancement of polyester formation and increased molecular weight is expected for both enzymes upon immobilization on suitable carrier materials.

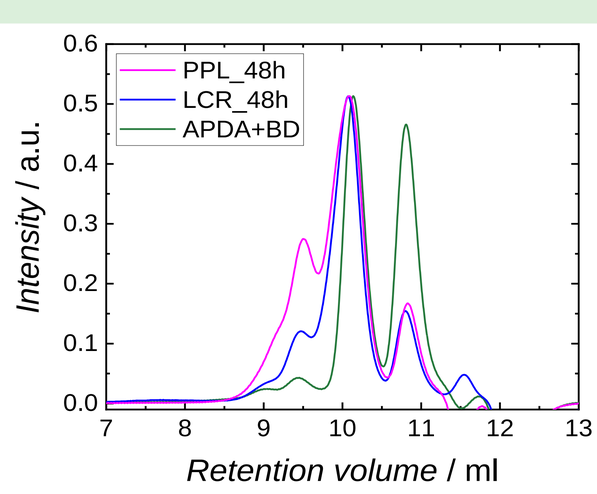

Figure 3: Results of the SEC measurements showing the presence of oligomeric condensation products after 48 h. *Candida rugosa* lipase (LCR, temperature regime: 8 h 40 °C, 21 h 60 °C, 19 h 80 °C; pressure regime: 8 h 1 bar, 40 h 20 mbar; enzyme amount: 1 wt.%) and porcine pancreas lipase (PPL, temperature regime: 8 h 60 °C, 40 h 80 °C; pressure regime: 8 h 1 bar, 40 h 20 mbar; enzyme amount: 5 wt.%).

Table 1: Molar masses, dispersity index (*Đ*) and degree of esterification (d_E) of the polycondensation reactions after 24 h and 48 h using CALB-IB150, porcine pancreas lipase (PPL) and *Candida rugosa* lipase (LCR).

	M_n / g mol^{-1}	M_w / g mol^{-1}	*Đ*	d_E / %
CALB-IB150_8h	140	740	5.3	47
CALB-IB150_48h	1 880	17 560	9.3	100
PPL_48h	230	440	1.9	41
LCR_48h	150	735	4.9	26

Conclusion

- Enhanced parameter selection for the polyester synthesis using adipic acid, 1,4-butanediol and CALB-IB150 as catalyst resulted in polyesters with high molecular weights at only low temperatures, which is not achievable with conventional metal catalysis.
- Significant activity in esterification and polyesterification reactions was found for the low-cost lipases from porcine pancreas as well as *Candida rugosa* which should further be improvable upon immobilization of the enzymes.

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References

[1] Nikulin M., Švedas V., Molecules (Basel, Switzerland), (**2021**), *26*(9).
[2] Dourado Fernandes C., Francisco Oechsler B., Sayer C., Oliveira D. de, Hermes de Araújo P. H., European Polymer Journal, (**2022**), *169*.