USING ENZYMES IN SOLVENTS: SIMPLE CONCEPTS AND AMBITIOUS SOLUTIONS FOR POLYESTER SYNTHESIS

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Status quo

Numerous factors are influencing the activity of the enzymatic catalyst:

- reaction temperature
- solubility of the substrates
- water content (side product)
- acidity of the reaction mixture [1]
- polarity [1,2]

Objective

- screening of temperature-dependent activity of the biocatalyst
- influence of the polarity of substrates using aliphatic diols
- effect of the addition of a solvent to the screened reaction systems

Experimental

- adipic acid, $HO(CH_2)_nOH$ with n = 2-10 (ratio 1:1.05)
- 1 *wt*% *Candida antarctica* lipase B on Immobead 150
- reaction temperatures: 40 °C, 60 °C, 80 °C

…in mass:

- water removal using 0.1 L min^{-1} N_2 flow
- size: 40 g polyester
- analysis: ^{1}H-NMR and size exclusion chromatography

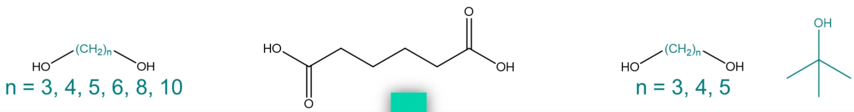
…in solution:

- *tert*-butanol as solvent (ratio 1:1 by weight of monomers)
- water not removed
- size: 0.5 g polyester
- analysis: ^{1}H-NMR

Results and Discussion

mass polymerizations

solution polymerizations



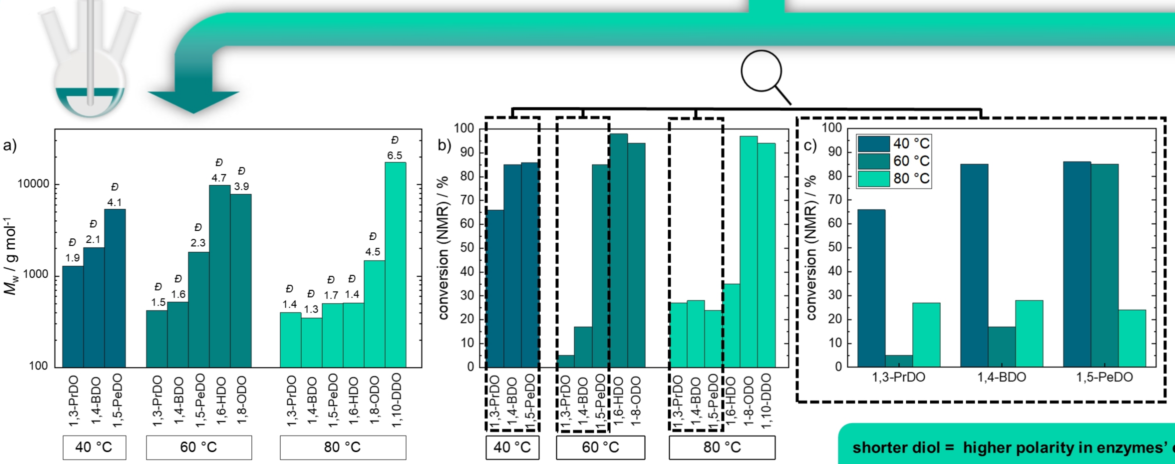


Figure 1: Results of mass polymerizations after 24 h reaction time using adipic acid and either 1,3-propanediol (1,3-PrDO), 1,4-butanediol (1,4-BDO), 1,5-pentanediol (1,5-PeDO), 1,6-hexanediol (1,6-HDO), 1,8-octanediol (1,8-ODO), or 1,10-decanediol (1,10-DDO) at 40 °C, 60 °C or 80 °C. a) Weight average molecular weights (M_w). b) Conversion according to ^{1}H-NMR. Conversions of 1,3-PrDO, 1,4-BDO and 1,5-PeDO at different temperatures for c) mass polymerizations and d) solution polymerizations in *tert*-butanol.

shorter diol = higher polarity in enzymes' environment

⟶ activity decreased with increasing temperature

addition of lower polarity solvent = lower polarity in enzymes' environment

⟶ activity increased with increasing temperature

The mass polymerizations (Fig. 1 a-c) show a clear dependence of the catalytic activity on diol length and temperature. While lower temperatures benefit the biocatalyst performance using more polar diols, less polar diols allow for higher reaction temperatures promoting significant conversions and molecular weights. With decreasing diol length, the polarity of the overall reaction mixture increases (Fig. 2), which also results in an increase in the acid's solubility. This solubility is further enhanced with rising temperature which highlights the correlation between some of the crucial parameters in enzyme-catalyzed polyester synthesis. When adding *tert*-butanol as a solvent to identical reaction systems, higher conversions in solution polymerizations are observed for short-chain diols at 60 °C compared to mass polymerizations (Fig. 1 c-d). The amount of solvent has a levelling effect on the overall polarity inside the reactions (Fig. 2) which is assumed to have a positive effect on the enzyme activity at higher temperatures. Water removal will be required to further enhance the performance in solution polymerizations.

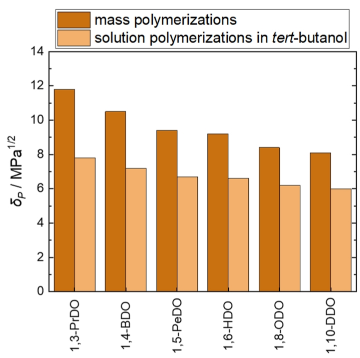


Figure 2: Polarity of the biocatalysts' environment including adipic acid and either 1,3-propanediol (1,3-PrDO), 1,4-butanediol (1,4-BDO), 1,5-pentanediol (1,5-PeDO), 1,6-hexanediol (1,6-HDO), 1,8-octanediol (1,8-ODO), or 1,10-decanediol (1,10-DDO) and/or *tert*-butanol based on Hansen parameters [3].

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Conclusion

- **Biocatalyst performance in the polycondensation reactions depend on the length of diols and temperature, both influencing polarity and acidity of the reaction medium.**
- **The addition of *tert*-butanol as solvent has a levelling effect.**
- **Screenings as presented are helpful for understanding the enzyme's activity and allow for selection of suitable parameters and reaction components.**

References

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[2] Laane, C.; Boeren, S.; Vos, K.; Veeger, C. Rules for optimization of biocatalysis in organic solvents. Biotechnol Bioeng 1987; 30: 81-7. *Biotechnology and bioengineering* **2009**, *102* (1), 2-8; discussion 1. DOI: 10.1002/bit.22209.

[3] Abbott, S.; Yamamoto, H. *Hansen Solubility Parameters in Practice (HSPiP) software*, 5th edition, version 5.4.08, **2015**. https://www.hansen-solubility.com/HSPiP/.