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COUPLED TWO REACTOR-CONCEPT FOR AN *IN SITU* - PRODUCT CRYSTALLIZATION IN TRANSAMINASE-CATALYZED REACTIONS

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Introduction

The unfavorable reaction equilibria of many amine transaminases-catalyzed reaction systems require additional methods to shift the biocatalytic reaction to the product side.[1] Here we recently presented an alternative, crystallization-based approach to selectively remove the product amine from solution as a sparingly soluble product salt of 3,3-diphenylpropionic acid (3DPPA) (Fig. 1).[2]

In this concept study we plan to enhance this approach by using a two coupled reactors in a semi-continuous approach for the continuous synthesis of the desired amine.

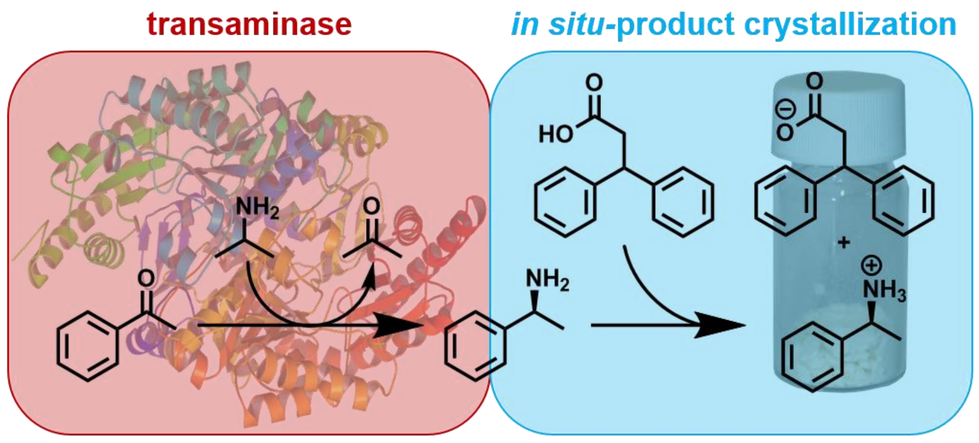


Fig. 1: crystallization-based in situ product removal in an amine transaminase-catalyzed reaction

Results and Discussion

Solubility differences

The main driving force of the *in situ*-product crystallization is the low solubility of the product amine salt (in this case (*S*)-1-phenylethylamine; PEA-3DPPA) and the higher solubility of the amine donor salt (isopropyl amine; IPA-3DPPA). The corresponding solubilities are pH-dependent, but fortunately overlap with the activity maximum of the investigated amine transaminase from *S. pomeroyi* (Tab. 1).

Tab. 1: pH-dependent solubility in 100 mM phosphate buffer at 30 °C.

pH	IPA-3DPPA	PEA-3DPPA
6	8.57 mM	6.60 mM
7	51.57 mM	6.81 mM
8	61.73 mM	6.29 mM
9	60.45 mM	8.25 mM

Substrate variation

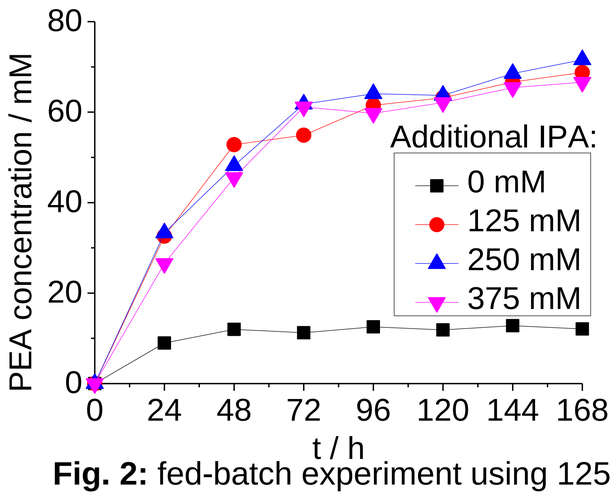
Tab. 2: substrate variation ISPC-concept

substrate	without ISPC	with ISPC
acetophenone	19 %	75 %
3-F-acetophenone	21 %	69 %
4-F-acetophenone	11 %	61 %
3-Cl-acetophenone	8 %	46 %
4-Cl-acetophenone	8 %	65 %
3-MeO-acetophenone	10 %	37 %
4-MeO-acetophenone	4 %	8 %
1-cyclohexylethanone	0 %	8 %
2-pentanone	61 %	53 %
2-hexanone	37 %	72 %
2-heptanone	20 %	78 %
methyl isobutyl ketone	36 %	96 %

100 mM substrate, 250 mM isopropyl amine, 3 U·mL^{-1} lyophilized whole cells, 30 °C; 125 mM 3DPPA for ISPC; 200 mM phosphate buffer pH 7.5

The presented ISPC-concept was also successfully applied for selected acetophenone derivatives and other non-aromatic substrates (**Tab. 2**). As expected, low conversions were obtained without ISPC due to the low, but still over-stoichiometric use of 250 mM IPA. A simple addition of 3DPPA increases product formation significantly for almost all investigated substrates. The enantioselectivity of the catalyst was not changed.

Use of donor amine salt



Alternatively 3DPPA and amine donor IPA can be applied directly as a combined salt. However, additional IPA is required to achieve sufficient conversion during *in situ*-crystallization in a fed-batch reaction (Fig. 2).

Fig. 2: fed-batch experiment using 125 mM IPA-3DPPA and 100 mM acetophenone (+ 10 mM every 24 h), pH 7.5, 30 °C, 1.5 U ·mL^{-1}

Two reactor-concept

Full utilization of the applied substrates can only be acquired by a continuously-operated process concept, which includes a crystallization of the product salt from reaction solution. The reactor is herein coupled to a secondary reactor, which re-adjust the concentrations of the substrates within the mother liquor after conversion (Fig. 3).

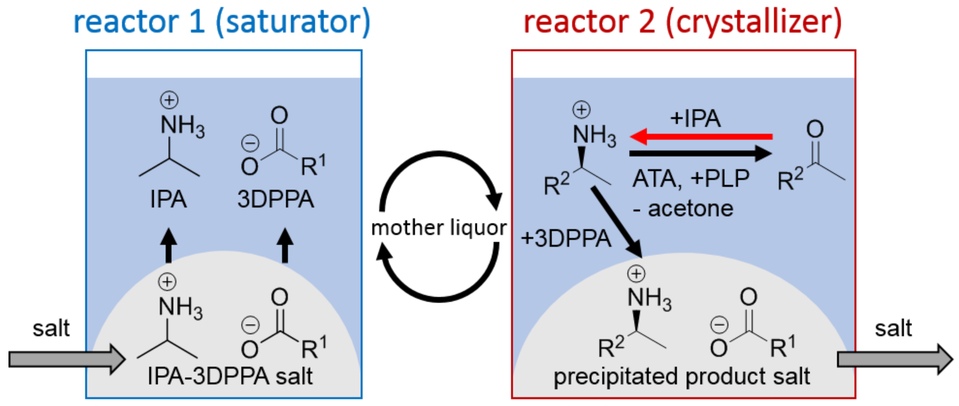


Fig. 3: process concept for a coupled two reactor system

Summary

- ISPC facilitates higher conversion for various substrates
- two reactor-system is currently developed for a continuous reaction system

References

[1] a) F. Guo, P. Berglund, *Green Chem* **2017**, *19*, 333.; b) M. T. Gundersen, R. Abu, M. Schürmann, J. M. Woodley, *Tetrahedron: Asymmetry* **2015**, *26*, 567.

[2] a) D. Hülsewede et al., European Patent Application 17202282.4, filed on November 17th, **2017.**; b) D. Hülsewede et al., *Eur. J. Org. Chem.*, accepted. DOI: https://doi.org/10.1002/ejoc.201800323

[3] a) S. A. Kelly et al., *Chem. Rev*. **2018**, *118*, 349.; b) I. Slabu, J. L. Galman, R. C. Lloyd, N. J. Turner, *ACS Catal*. **2017**, *7*, 8263.

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