Universität Rostock Traditio et Innovatio

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

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Introduction

We have established an *in situ* – product crystallization (ISPC) concept to overcome the unfavorable reaction equilibria of amine transaminase-catalyzed reactions (**detailed information about this concept are shown at Poster P4-1 by Jan von Langermann**).[1] In this study we extend this work towards continuous and fed-batch processes to facilitate a full utilization of the applied amine donor during product formation. This is achieved by applying a donor amine salt consisting of isopropylamine (IPA) and 3,3-diphenylpropionic acid (3DPPA) to maintain a constant concentration throughout the reaction (Fig. 1).

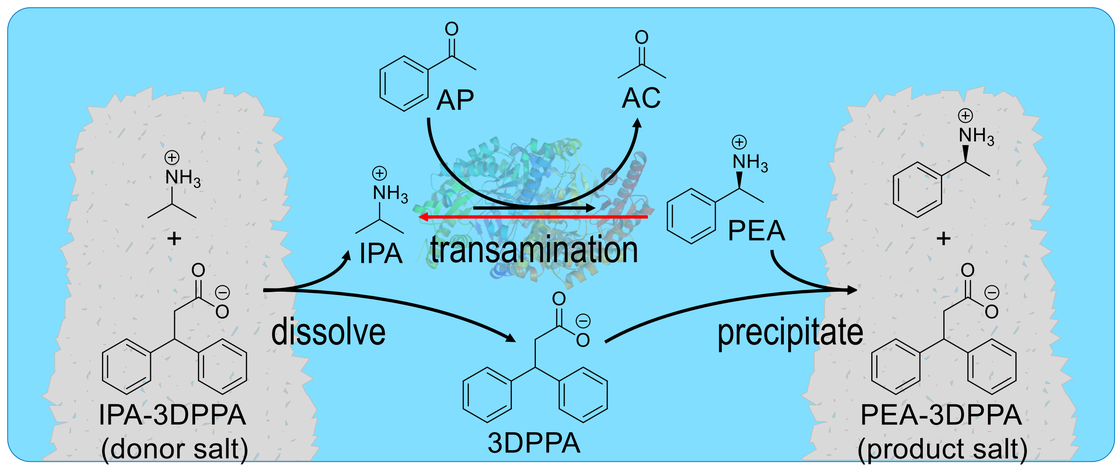

Fig. 1: reaction scheme for ISPC in transaminase-catalyzed reactions using donor salts

Results and Discussion

Driving force of ISPC

In order to use this ISPC system for a transamination reaction, only two conditions need to be met. The solubility of the product salt must be lower than the equilibrium position of the reaction (ISPC driving force) and significantly lower than the solubility of the donor salt (ΔL) (Fig. 2).

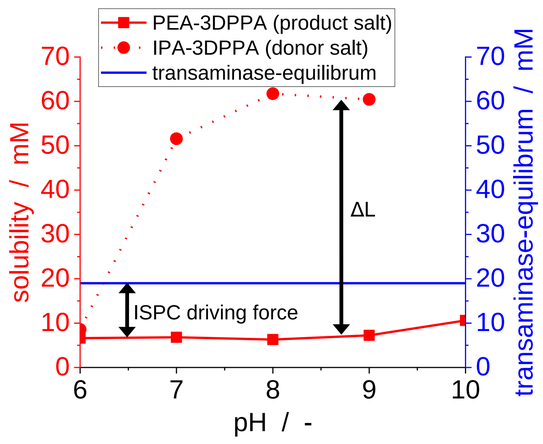

Fig. 2: solubility of IPA-3DPPA and PEA-3DPPA in H_2O

Productivity increase

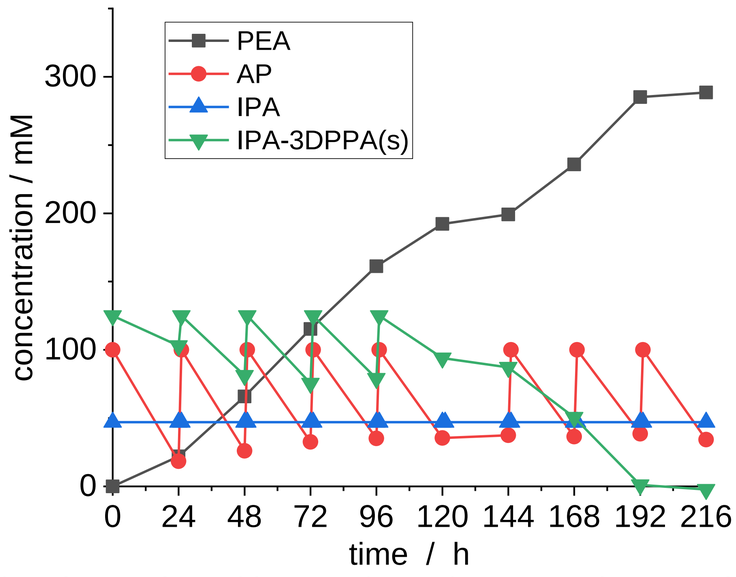

Fig. 4: fed-batch reaction with stepwise substrate & donor amine salt addition

Optimization of the reaction conditions

To determine the optimal conditions for ISPC, several parameters were varied. Here basically only two parameters are highly relevant (pH and IPA concentration; shown in Fig. 3). A pH of 7.5 represents the best compromise between product solubility and catalytic activitiy of the applied amine transaminases (Fig. 3A). Moreover, an additional use of 100 mM IPA further accelerates the ISPC-concept without interfering with crystallization (Fig. 3B).

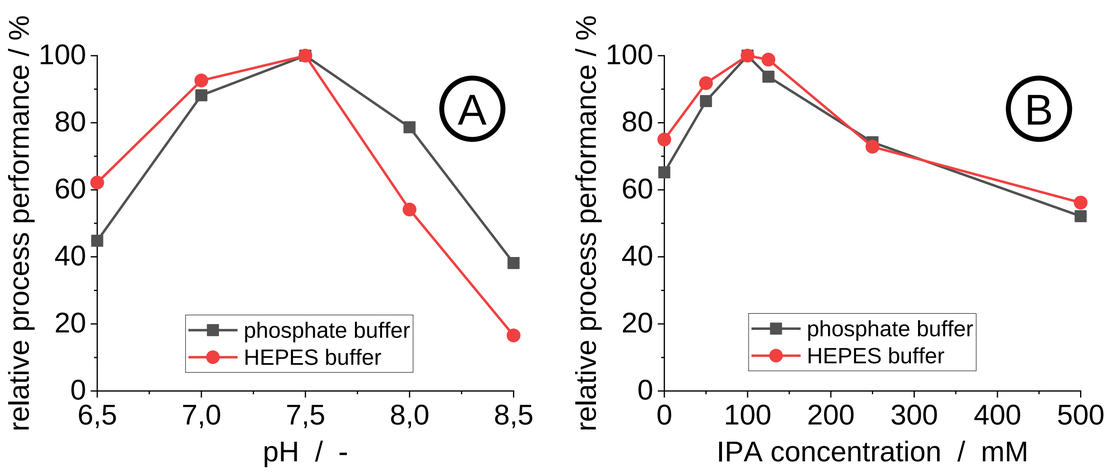

Fig. 3: (A) influence of the pH on the process performance; (B) influence of the IPA concentration on the process performance

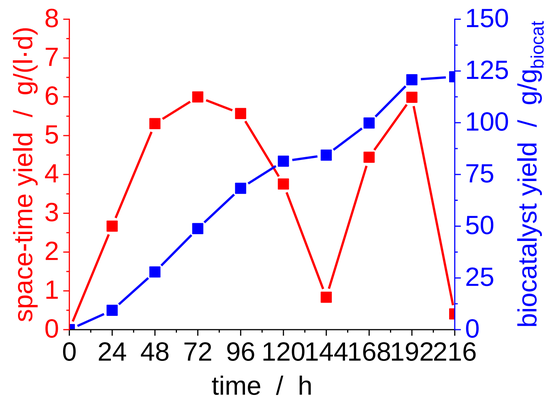

Fig. 5: space-time yield and bio-catalyst yield in the fed-batch reaction

The equilibrium shift can be exploited for high productivities by using a fed-batch process, which includes multiple addition of (solid) IPA-3DPPA (Fig. 4). Herein biocatalyst yield increases continuously to at least 125 $g_{product\ salt}/g_{biocatalyst}$ (Fig. 5).

Salt separation

In order to improve process productivity and the downstream processing even further, the salts and the biocatalyst have to be separated from each other in a continuous system (Fig. 6).

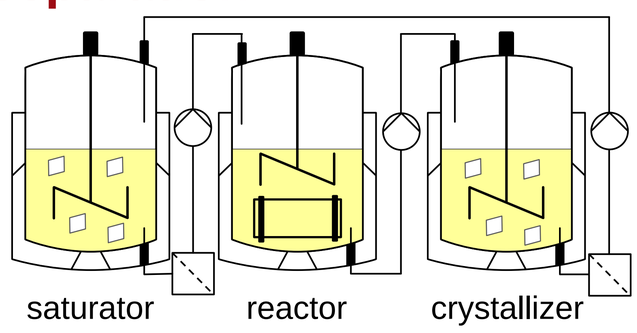

Fig. 6: multi reactor system for separation of saturation, crystallization and the biocatalytic reaction.

References

[1] a) D. Hülsewede et al., European Patent Application 17202282.4, filed on November 17th, **2017.**; b) D. Hülsewede et al., *Eur. J. Org. Chem.* **2018**, 2018, 2130.

Acknowledgements

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