Karlsruhe Institute of Technology

BIO Interfaces In Technology and Medicine

Institute for Biological Interfaces
IBG-1 | Biomolecular Micro- and Nanostructures

Characterization of Industrially Relevant Enzyme Systems for Flow-catalysis

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Biocatalysis in Industry

- Reduction of reaction steps
- No protection groups are needed
- Highly selective biocatalysts

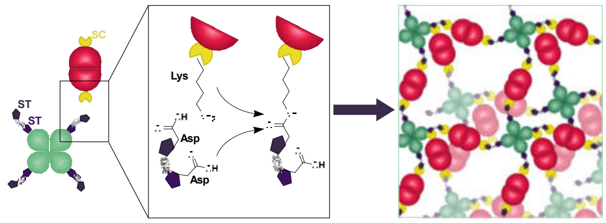
Motivation

- Identify and characterize biocatalytic systems for production of valuable molecules
- Efficiently immobilize enzymes in microreactors using the SpyCatcher/SpyTag system[1]
- Optimize synthesis through a combination of enzyme engineering and process optimization strategies

Immobilization Strategies

All-Enzyme Hydrogel (AEH)[2,3]

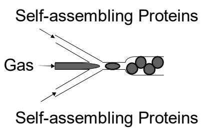
- Genetically encoded SpyCatcher (SC) and SpyTag (ST)
- Covalently crosslinked enzymes upon mixing
- Multiple binding sites allow the use of mono- and multimeric enzymes

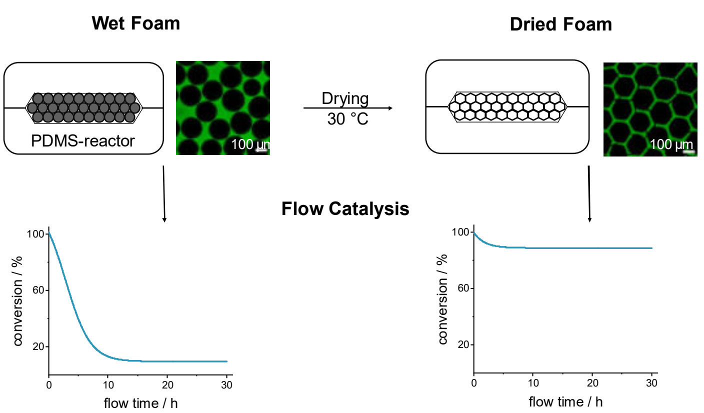


Enzyme Foam[4]

- Monovalent foam via microdroplet foaming of self-assembling enzymes
- High surface-to-volume ratio for efficient perfusion and mass transport
- Characteristic hexagonal structure forms after drying
- Can be directly integrated into PDMS reactor

Flow Focusing





Increased stability upon drying

Conclusion

It has been shown that the foam formulation is transferable to other enzyme systems. Nevertheless, the system under investigation requires further enzyme engineering and process optimization to enhance yield efficiency.

References

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[2] T. Peschke, P. Bitterwolf, S. Gallus, Y. Hu, C. Oelschlaeger, N. Willenbacher, K. S. Rabe, C. M. Niemeyer, *Angew. Chem.*, **2018**.
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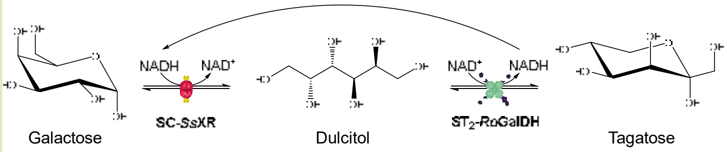
Application to a D-Tagatose Producing System[4,5]

Tagatose

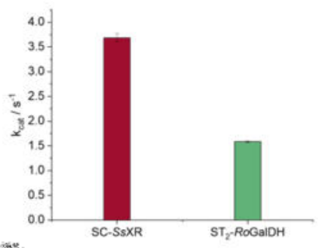
- Rare sugar suitable as a low-caloric sweetener
- Chemical synthesis complex and inefficient

Oxidoreductive 2-step biocatalytic reaction

- Xylose Reductase from *S. stipitis* (*Ss*XR)[5] converts Galactose to Dulcitol under the consumption of NADH
- Galactose Dehydrogenase from *R. oryzihabitans* (*Ro*GalDH)[6] oxidises Dulcitol to Tagatose while regenerating the cofactor

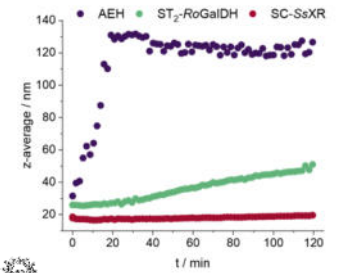


Activity of Fusion Enzymes



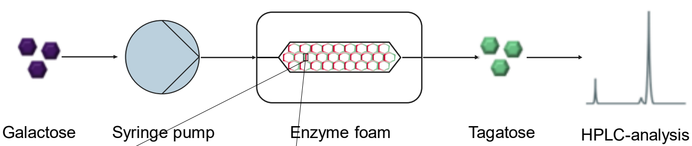
XR fused to an SC (SC-*Ss*XR) showed higher turnover numbers than GalDH fused to two ST (ST$_2$-*Ro*GalDH).

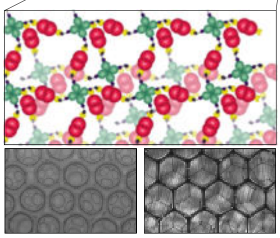
Stability of free Enzymes and as AEH



DLS measurements showed that formulation as AEH increases stability of ST$_2$-*Ro*GalDH in reaction buffer

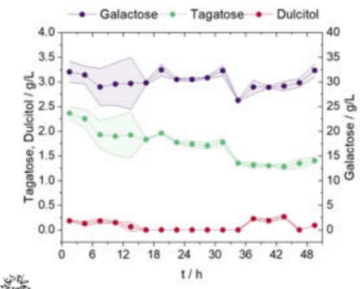
Foam Production and Use in Flow-catalysis





7 days of drying at 30 °C [4]

Successful enzyme-foam production with formation of characteristic honeycomb structure



- Tagatose production over a period of ~ 50 h
- Low, decreasing yields. Probably due to flushing out of enzyme-conjugates.