

# Inline Mid-Infrared Analytics of a Biocatalytic Kinetic Resolution in Reactive Distillation

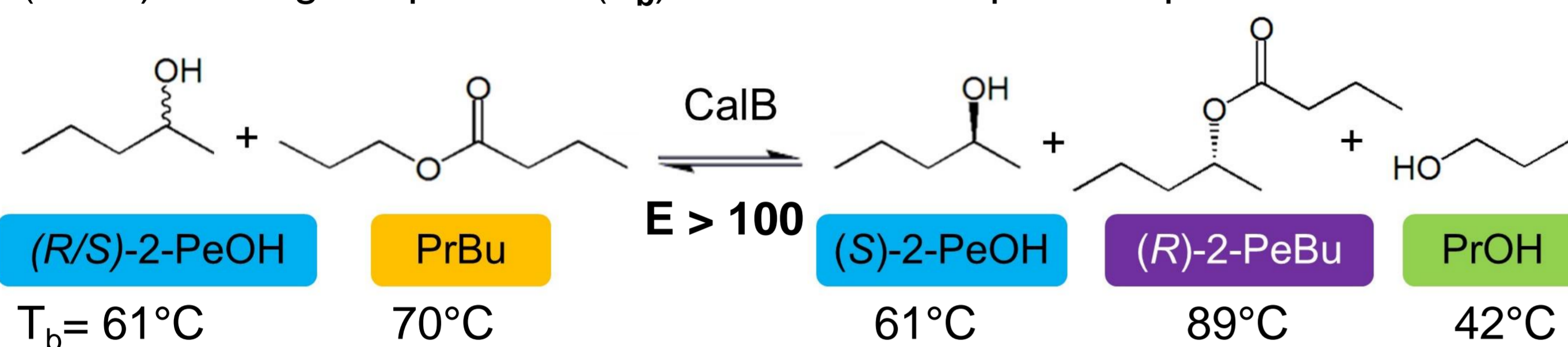
## Introduction & Substrate Combination

### Motivation:

- By substitution of chemical catalysts with **enantioselective biocatalysts** high valuable **chiral building blocks** are obtainable by **reactive distillation**
- Need for research for **simultaneous evaporation of the starting materials**, the **positioning of biocatalyst** and the **deactivation** of integrated **biocatalysts** at increased temperatures<sup>[1]</sup>
- Successful application of **enantiopure product formation and separation** by **in situ product removal (ISPR)** in a batch reactive distillation setup<sup>[2]</sup>

### Model Reaction for ISPR:

- The non-converted target compound (*(S)*-2-pentanol (**(S)-2-PeOH**) and (*(R)*-2-pentyl butyrate (**(R)-2-PeBu**) as well as propanol (**PrOH**) as byproducts are produced by the kinetic resolution (KR) of (*(R/S)*-2-pentanol (**(R/S)-2-PeOH**) and propyl butyrate (**PrBu**) catalyzed by *Candida antarctica* lipase B (**CalB**). Boiling temperature ( $T_b$ ) at the reduced process pressure of 80 mbar.



### Aims:

- ISPR and in situ separation** of target compound (**(S)-2-PeOH**)
- Inline process analytics** for the determination of concentration profiles of distillation column and analysis of the thermostability of the biocatalyst

## Experimental Setup - Reactive Distillation

### Column Specifications:

- 1.5 m height, 50 mm diameter** and **4 L bottom flask volume**
- Operating conditions  
Temperature **< 90 °C** and pressure **80 - 160 mbar**

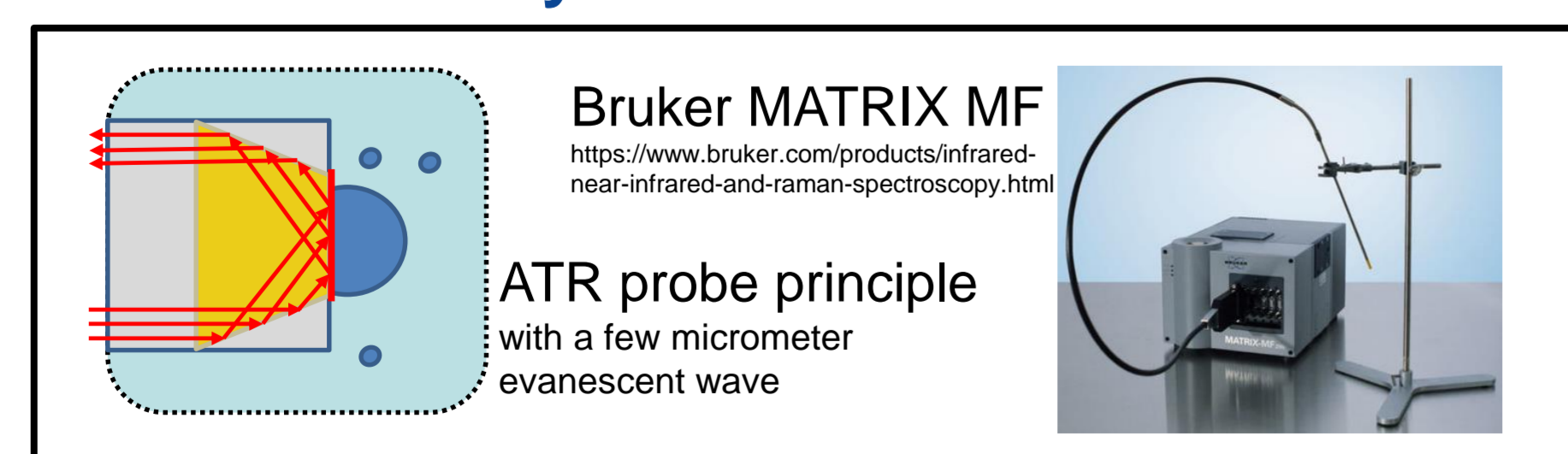
### Biocatalyst Preparation:

- Immobilized **CalB** (Novozym435@/CalBimmo®)

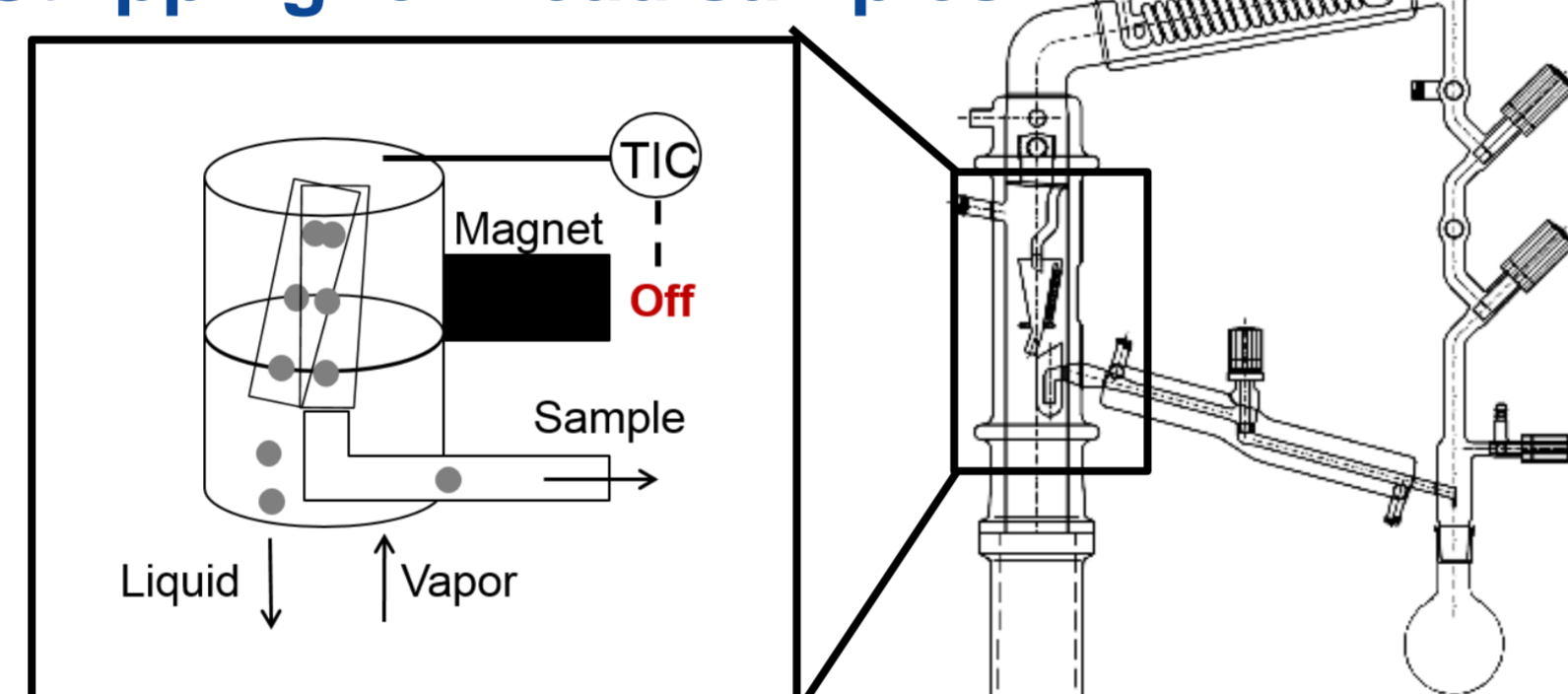
### Inline Analytics:

- FT Infrared spectrometer** Bruker Matrix MF
- 5 ATR optical fiber probes** IN350T
- Excitation of fundamental vibrations in mid infrared range** (fingerprint region)

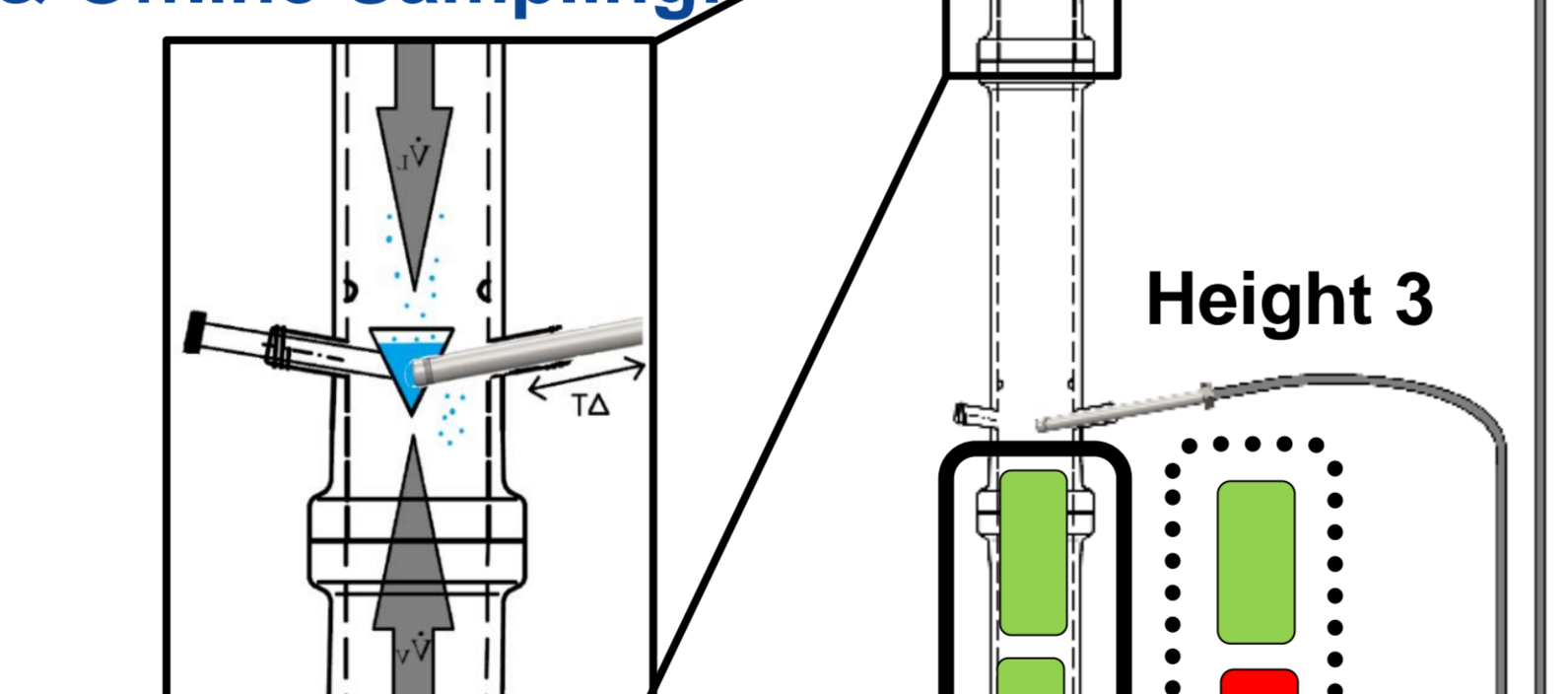
### FTIR Inline Analytics:



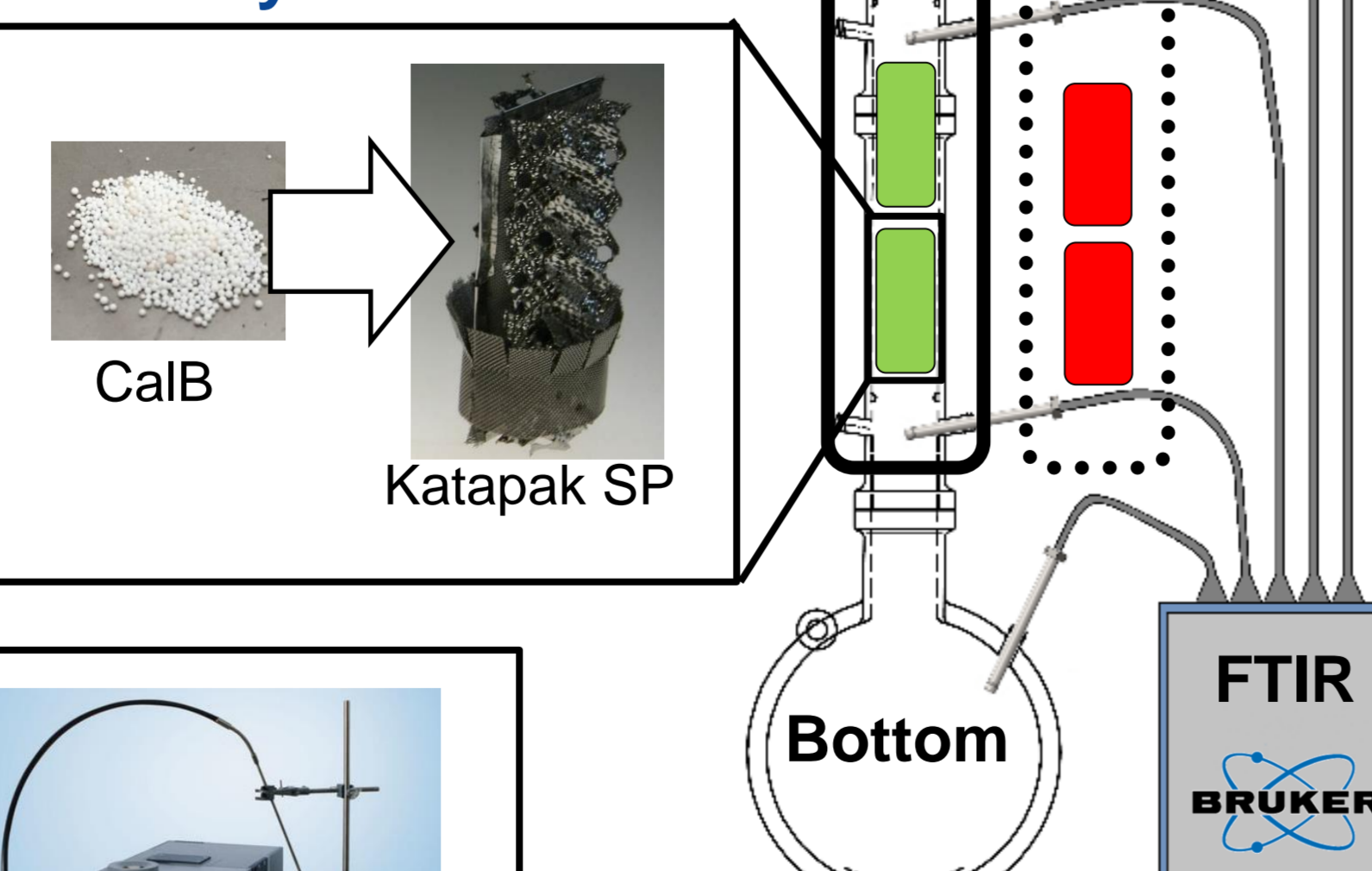
### Stripping for Head samples:



### ATR Probe Integration & Offline Sampling:



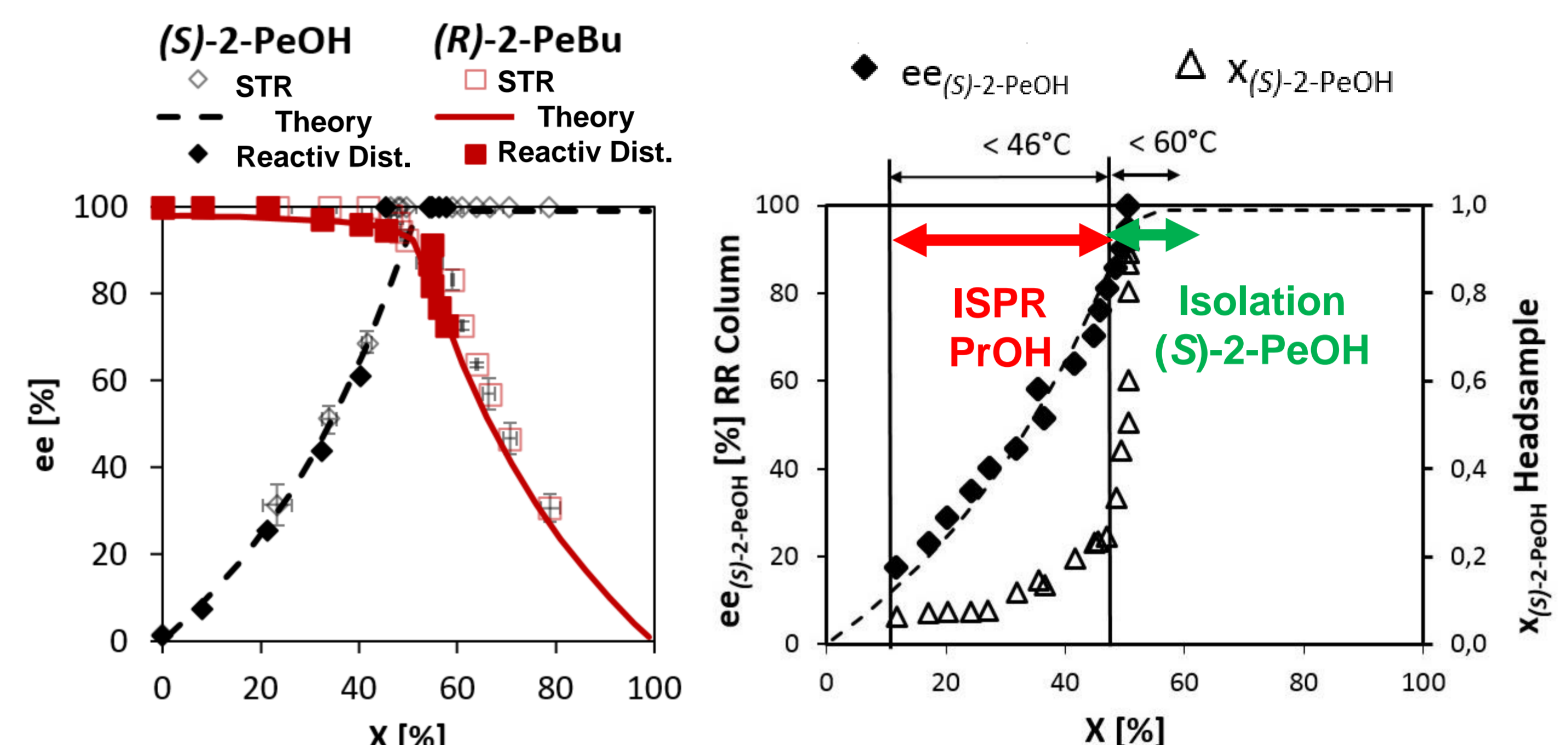
### Biocatalytic Zone:



Red Packings are filled with carrier without enzyme and green color indicates active Packings.

## ISPR Results & Target Compound Yield

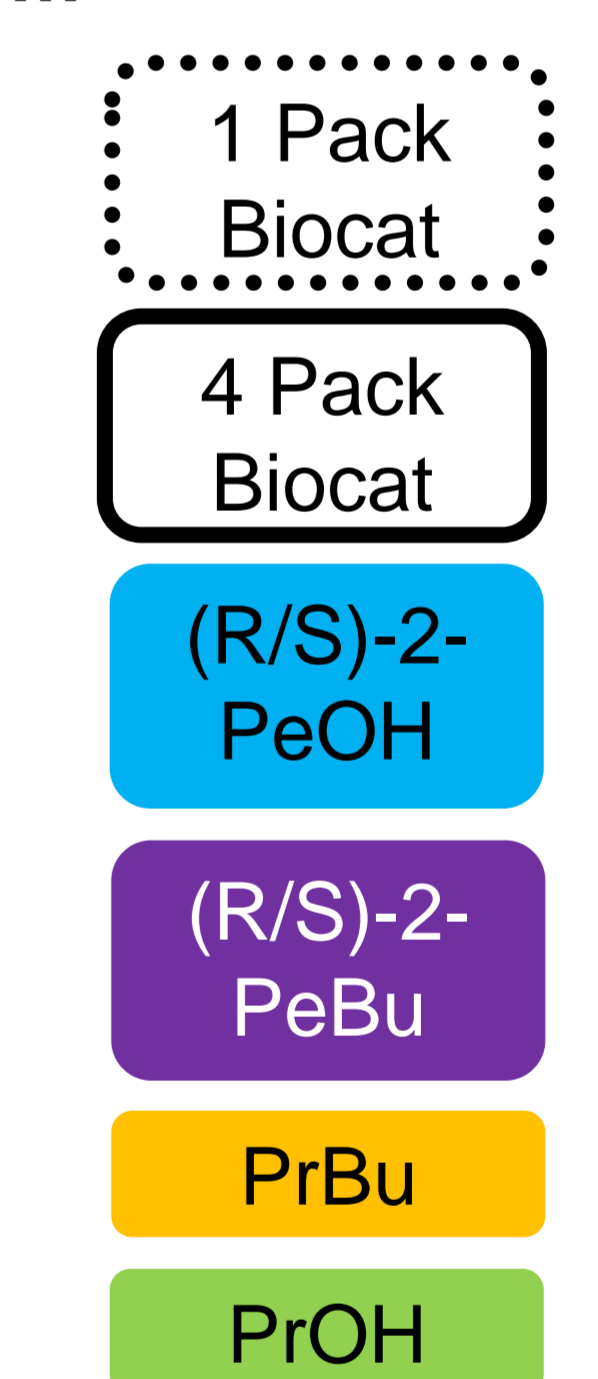
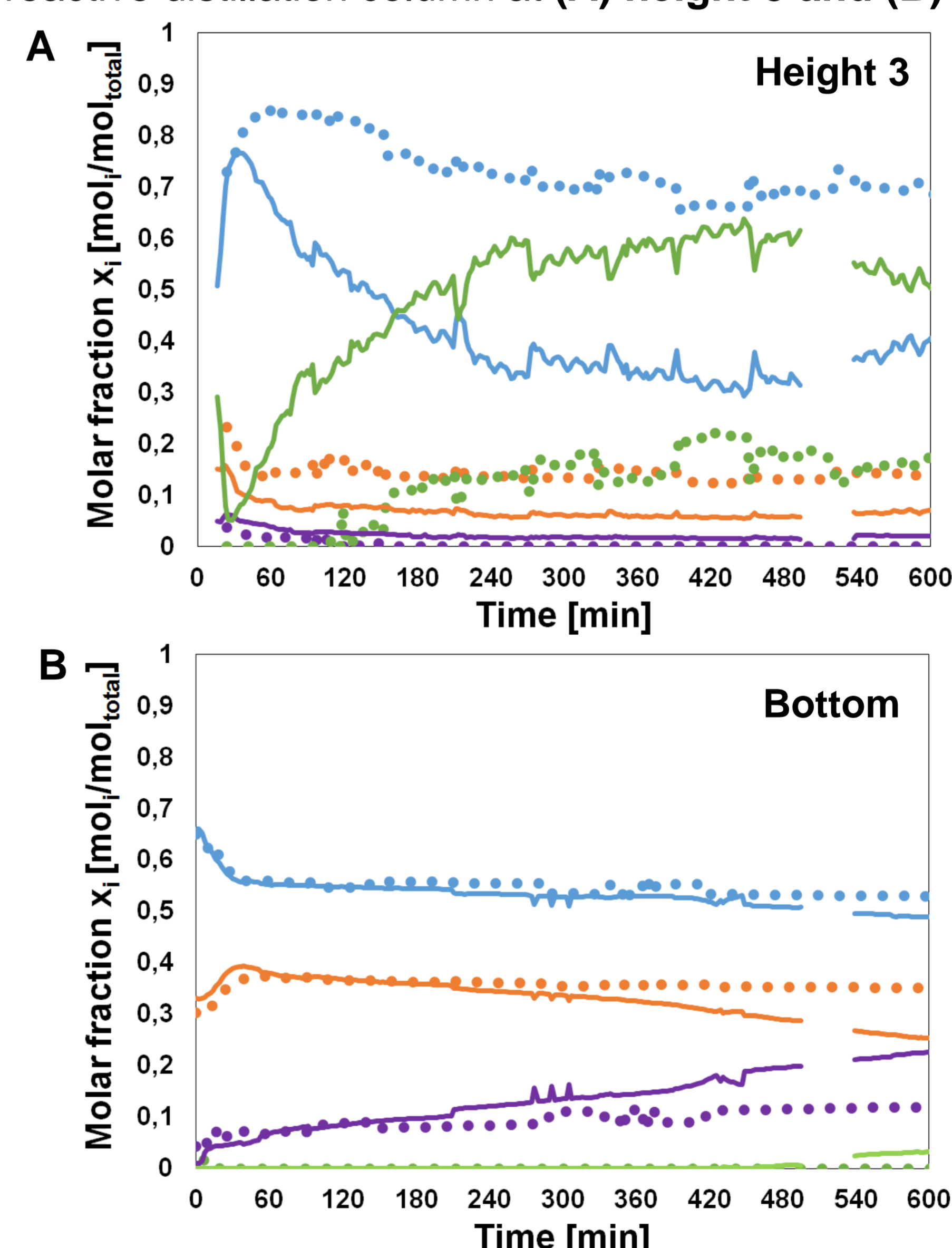
- An **equilibrium constant of 0.24** was determined by equilibrium reactions in a stirred tank reactor (STR) with initial molar substrate ratios of  $x_i = 0.1\text{--}0.9$  and corresponds to the **equilibrium conversion of 44%**
- ISPR of PrOH is required** to reach conversion for an ideal KR of  $X = 50\%$



- Best results** were achieved by starting concentration of  $x_{2\text{-PeOH}} = 0.64$  and  $x_{\text{PrBu}} = 0.36$  with head sample of  $Y_{\text{isolated}} = 13.4\%$  with  $x_{(S)\text{-}2\text{-PeOH}} = 0.95$  and enantiomeric excess  $ee_{(S)\text{-}2\text{-PeOH}} = 96 \pm 5\%$  (GC analysis)

## Inline MIR Process Analytic Results

- Calibration of a chemometric model (**Indirect Hard Modeling**) with the **concentration prediction with an error of < 3 mol-%**
- Best detectability** of the influence of the enzyme / biocatalyst loading in the reactive distillation column at **(A) height 3 and (B) bottom**



For better detectability and to reduce the complexity of the concentration time profiles these experiments were done without head stripping and ISPR.

- In-process determination of enzyme performance by MIR inline analytics**

## Conclusion

- Space-resolved **inline analytics** established with in-process **determination of enzyme performance**
- Full biocatalytic **KR** established in distillation column with high **ee & purity** was achieved by **in situ** isolation of **target compound (S)-2-PeOH** over head



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### References:

- R. Heils *et al.*, Ind. Eng. Chem. Res. 2012, 51, p. 11482–11489, DOI: 10.1021/ie300837v
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