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### Introduction

Immobilized lipases are excellent biocatalysts for the enzymatic synthesis of short- and medium-chain fatty esters used as food flavour compounds. [1-3] Lipase from Candida antarctica B (GenoFocus, South Korea) was immobilized by entrapment in sol-gel hybrid matrices obtained with epoxy functionalized silane precursors. The catalytic efficiency of the immobilized lipase was investigated in the synthesis of the flavour ester pentyl hexanoate (apple, pineapple aroma) in organic solvent/solvent free media, by esterification of pentyl alcohol and hexanoic acid. The esterification reaction was performed at 36°C and

# **Methods**

### **ENZYME IMMOBILIZATION BY SOL-GEL ENTRAPMENT**





equimolar ratio of substrates for 16 hours. High reaction yields (>90%) were obtained, the immobilized lipase retaining an activity yield of 96% compared to the free enzyme. The immobilized lipase maintained constant activity for more than 10 reaction cycles. Continuous production of the flavour ester pentyl hexanoate in a packed-bed reactor was developed using the immobilized lipase as a catalyst in a hexane solvent system. Continuous esterification at fixed temperature and substrate ratio was performed and the effect of flow rate on the molar conversion of alcohol was evaluated. Product formation was determined by GC-FID analysis of samples collected within a set time frame, following the stabilization of the system.

### Results

**BATCH MODE** 



 $R = C_6 H_{11}$  $R' = C_5 H_{11}$ R'-OH =

#### **ENZYMATIC SYNTHESIS OF FLAVOR ESTER**

**FLOW MODE** 

5.0



Substrate molar ratio: 1/1 Substrate concentration: 0.5 M Biocatalyst amount: ~ 0.25 g Temperature: 36 °C **Reaction time: 5 h** 

Temperature: 36 °C Stirring speed: 1000 rpm Reaction time: 16 h

Substrate molar ratio: 1/1

**Biocatalyst loading: 20% (w/v)** 

#### Ester yield of enzymatic synthesis with native and immobilized lipase from Candida antarctica B

 Native enzyme Sol-gel entrapped enzyme Adsorption immobilized enzyme -Flow 0,2 ml/min -Flow 1,0 ml/min -Flow 0,6 ml/min 12500 100 Productivity (µmole/h/g) 10000 80 7500 60 5000 40 2500 20 2.5 3.5 4.0 4.5 1.5 2.0 3.0 1.0 0.0 0.5 16 12 13 15 Reuse cycles Time (h)

Productivity of enzymatic synthesis in a packed-bed reactor with sol-gel entrapped Candida antarctica lipase B

Relative yield (%)

### Conclusion

- Batch mode ester synthesis: Using a new sol-gel entrapped biocatalyst with Candida antarctica lipase B, the flavor ester pentyl hexanoate was successfully synthesized. The entrapped biocatalyst showed high operational stability for the synthesis of natural aroma esters in solvent-free system, maintaining its activity even after 17 consecutive reaction cycles. This could be considered as an opportunity for further scaling-up and application for the efficient synthesis of other similar products.
- Flow mode ester synthesis: The results showed that the flow rate has a significant effect on the conversion of substrates, higher rates leading to productivities up to 10000 µmole/h/g and shorter retention times. This study demonstrates the applicability of sol-gel entrapped lipases in a packed-bed reactor for continuous aroma ester synthesis.

### References

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