Lipase from Candida antarctica supported on 3-D printed structured resin packings for reactive distillation

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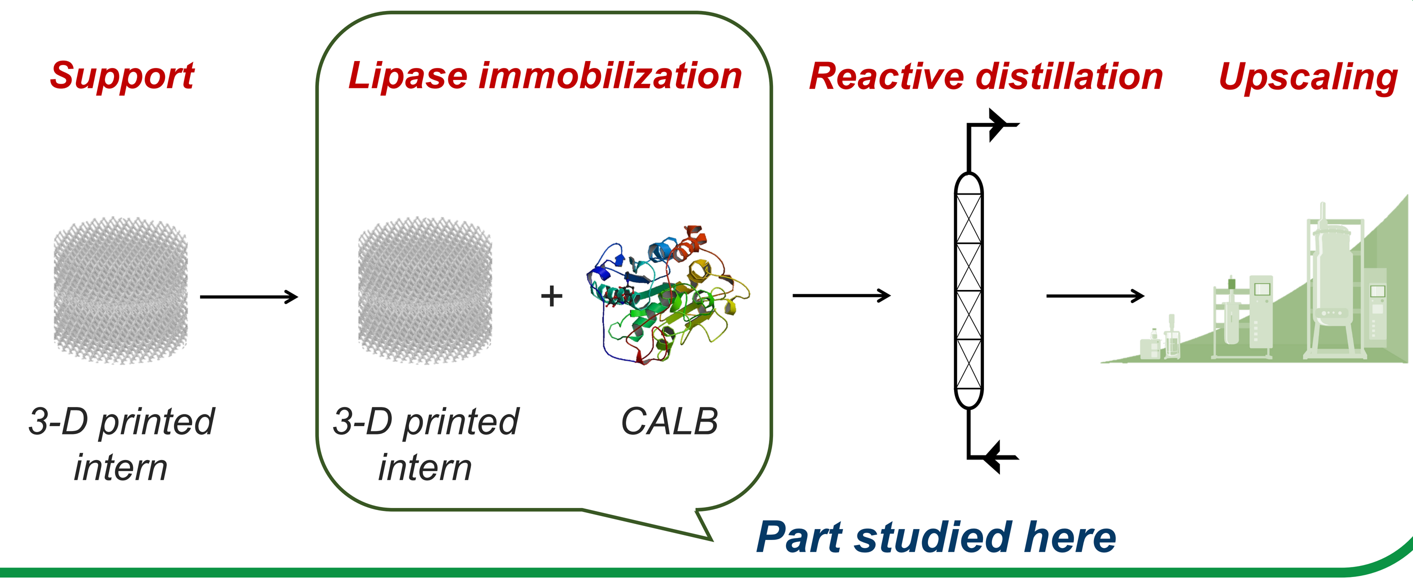
Introduction

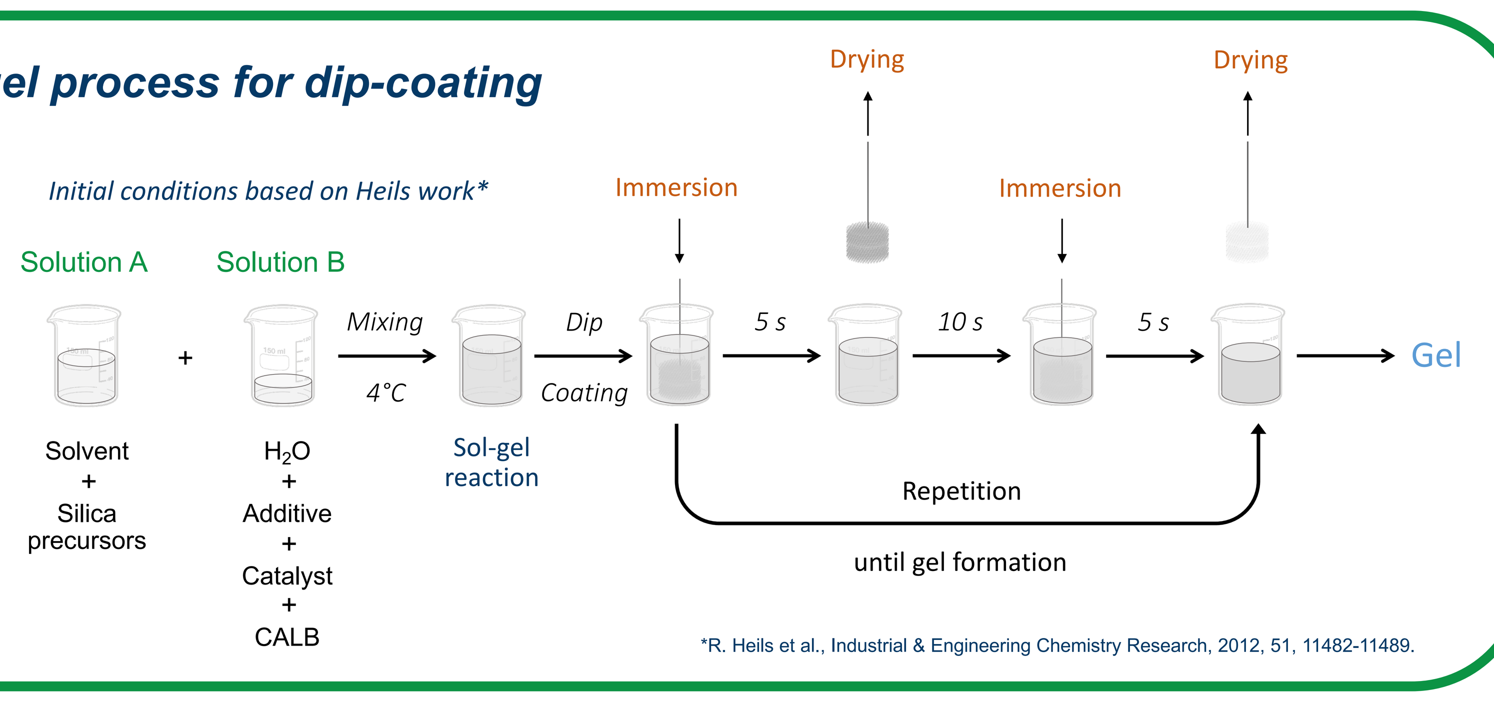
Reactive distillation was carried out for the enantioselective transesterification of racemic 2-pentanol with ethylbutyrate using a supported enzymatic catalyst. The reaction was designed to achieve the reaction-separation coupling by using **structured resin packings** coated with a **sol-gel** containing the **lipase from *Candida antarctica* (CALB)**. This project includes three main studies, the immobilization of lipase, reactive distillation, and upscaling. We here focus on the immobilization of CALB by improving the gel properties for the coating process and lipase retention. Parameters such as catalyst loading, silica precursors, and alcohol were considered to improve the process of **dip-coating** in terms of gelation time, gel quality, and the resistance and stability of the gel coated on the structured resin packings.



Sol-gel process, immobilization of CALB, and coating on structured resin packings

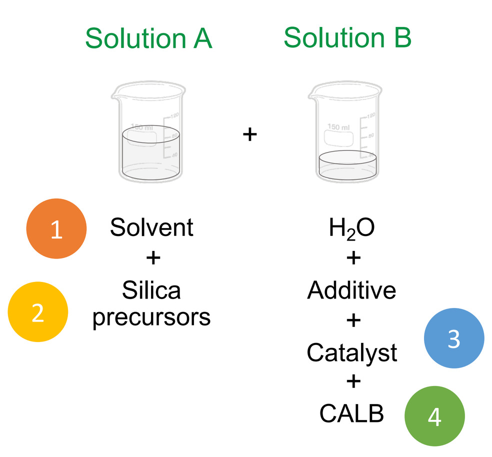
Sol-gel process for dip-coating

The sol-gel process for dip-coating starts with two solutions, one with the silica precursors in a solvent and another one with water, a catalyst, an additive, and the enzyme immobilized. The mixture of these two solutions allows the hydrolysis reaction, then, condensation producing gel. Dip-coating happens during sol-gel reaction until gel formation.



*R. Heils et al., Industrial & Engineering Chemistry Research, 2012, 51, 11482-11489.

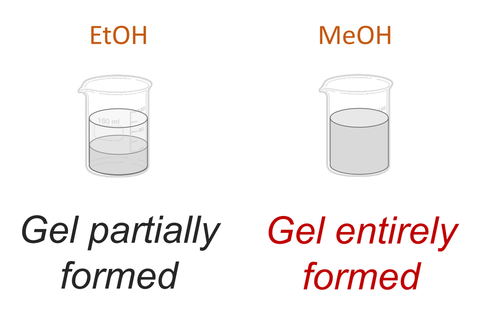
Studied parameters

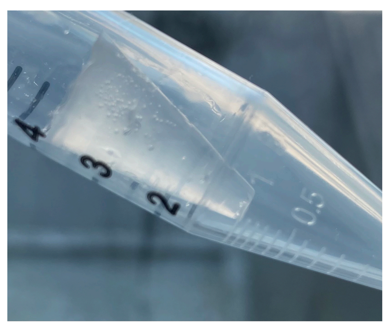


Water and additive were not studied here

1 Solvent

Alcohols are used for the miscibility of alkoxides. The length of the carbon chain plays a role in the reaction. Methanol and ethanol were tested.





Picture of an entirely formed gel, it has the texture of glass

2 Silica precursors

Two alkoxides were used, TMOS and MTMS, MTMS brings hydrophobia to the gel and makes it water-repellent. Gel quality and TMOS/MTMS ratio were studied.

3 Catalyst

Three catalysts were tested, NaOH, NH_4OH, and Na_2CO_3, the best one was chosen to belong to gelation time, TMOS/MTMS ratio, and CALB activity.

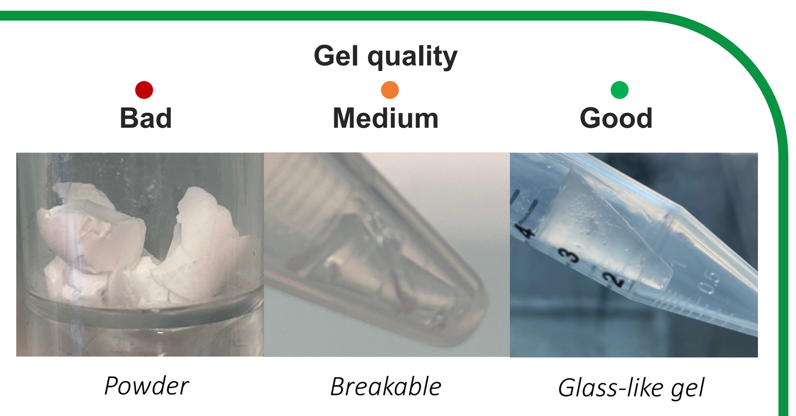
Four TMOS/MTMS ratios per catalyst were tested

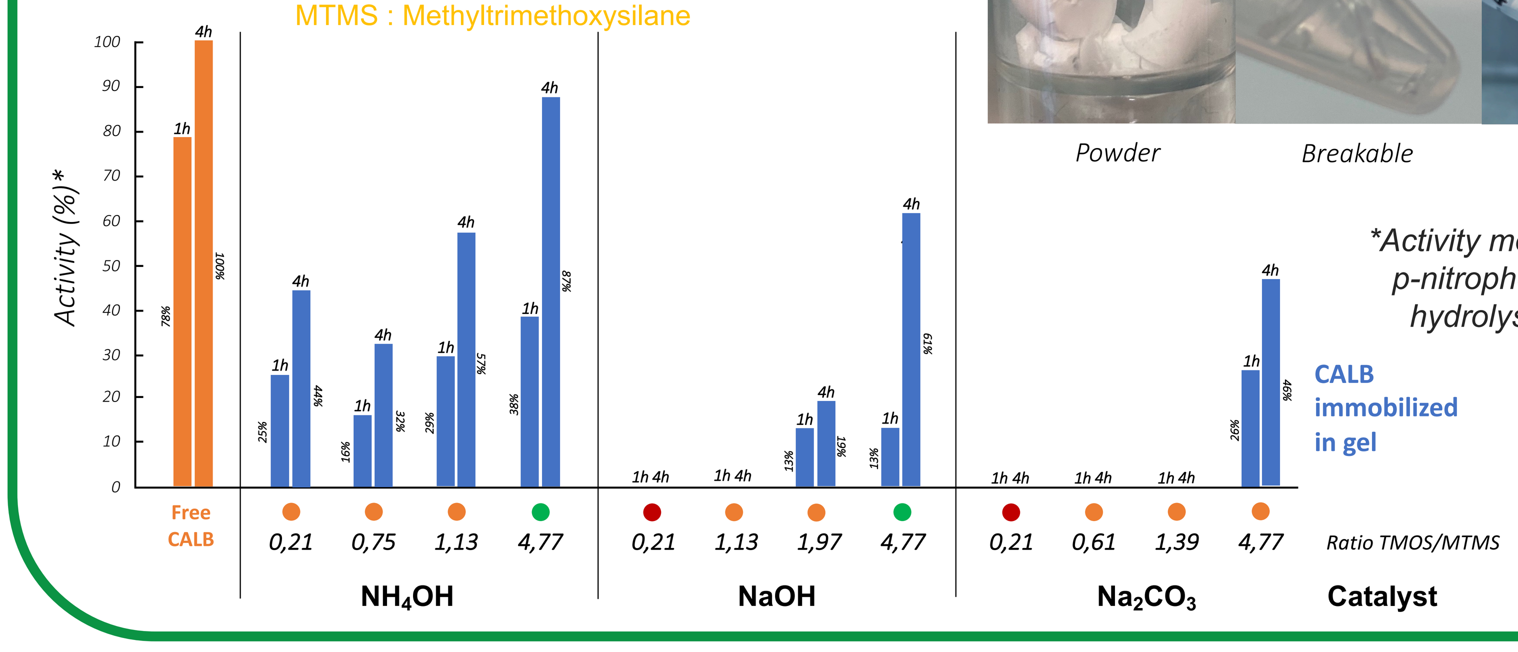
Catalyst was chosen on the best CALB activity

TMOS/MTMS ratio was chosen based on gel quality

TMOS : tetramethylorthosilicate

MTMS : Methyltrimethoxysilane

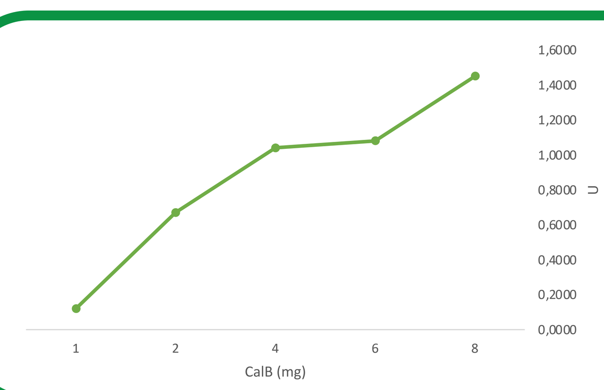




*Activity measured using p-nitrophenylbutyrate hydrolysis reaction

4 CALB

Different concentrations were applied on the optimized gel, 1, 2, 4, 6, and 8 mg of lipase. CALB activity and gel modifications were observed.



As expected, the activity of lipase increased with its quantity rising. We decided to choose **6 mg per 100 mg of support** as an optimum concentration of CALB because, from 8 mg, the homogeneity of the gel has gone bad. The gelation time was really quick and the coating process became complicated. This study also shows that **the gel has a high capacity to contain an important quantity of lipase**.

Results

Solution A

Solvent: **MeOH** allowed better gel quality

Silica precursors: Despite the importance of MTMS, a **higher TMOS/MTMS** ratio makes a better quality gel and improves lipase activity

Solution B

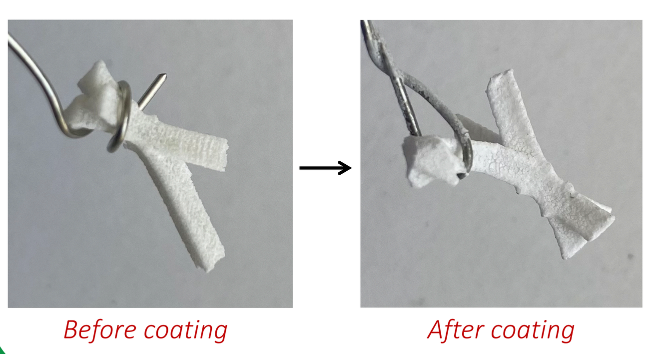
Catalyst: With the help of the TMOS/MTMS ratios study, the measurement of CALB activity and the gel quality allows **NH_4OH** to be the best catalyst

CALB: Several concentrations of CALB were tested with an optimized gel, and an optimum of **6 mg** of CALB per 100 mg of the structured resin packings was chosen.

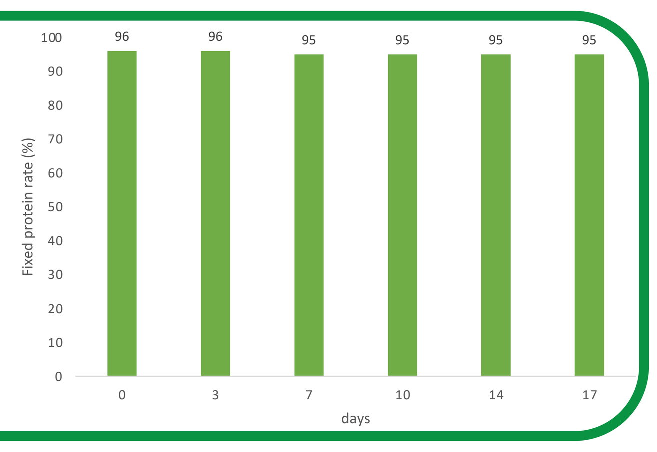
Final conditions

Solution A		Solution B	
MeOH	37.6%	H_2O	18%
TMOS	34.4%	PEG 400	1%
MTMS	8.3%	NH_4OH	0.4%
		CALB	0.3%

Coating on structured resin packings



Multi-thin layers are deposited on the structured resin packings, the force of the packings to keep the gel on and the resistance of the gel to sustain the lipase are tested by putting the packings in water for several days and see if the gel remain on the support. The rate of fixed protein is measured to determine the gel retention capacity. **We observed that the gel remains on the support and the following graph prove that the gel has a high capacity to retain the protein.**



Conclusions

- Successfully **optimized parameters** of the gelation
- The efficiency of the **3-D resin** to keep the gel on
- **High retention capacity** of the gel for the protein
- CALB remains **highly active** in the gel despite the reagents diffusion issue

Contact for more informations