Immobilization in MPS and characterization of a FAE for hydrolysis and transesterification reactions.

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The aim of this study was to understand the impact of immobilization in mesoporous silica particles (MPS) on the kinetics of enzymatic reactions. Feruloyl esterases (FAE) were used as the model enzymes to be immobilized in mesoporous silica particles.

Background

Mesoporous silica materials possess properties such as large surface area, defined pores geometry, mechanical and thermal stability and they are tunable.

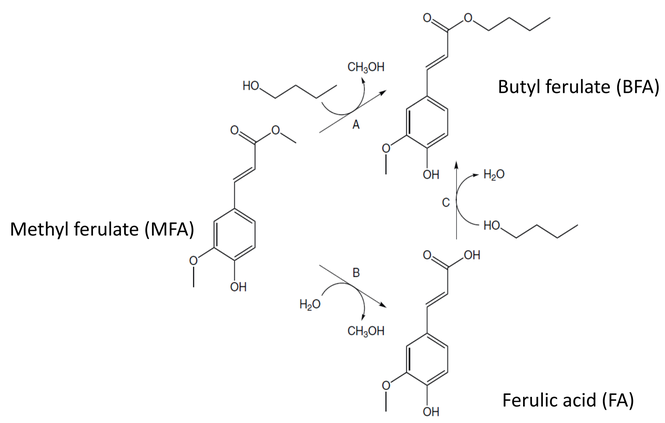
FAEs are of utmost interest in degrading lignocellulosic biomass: they catalyze the hydrolysis of ester linkages releasing ferulic acid and other hydroxycinnamic acids from hemicellulose. They can also catalyze the reverse reaction: transesterification.

Esterified hydroxycinnamic acids are bioactive compounds recognized for their antioxidant, tumor suppressing and antibacterial properties[1].

Aims of the study:

- ✓ Determine optimum immobilization conditions
- ✓ Determine optimum reaction conditions
- ✓ Evaluate how immobilization and reaction condition affects the selectivity of the FAE
- ✓ Evaluate the impact of immobilization on the enzyme kinetic parameters
- ✓ Assess the industrial potential of one immobilized FAE

Some reactions catalyzed by FAEs

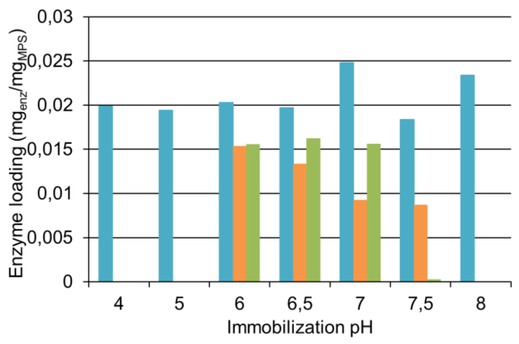


FAEs can catalyse different reactions: (A) Transesterification of MFA with 1-butanol generating BFA and methanol. (B) Hydrolysis of MFA generating ferulic acid and methanol (natural reaction at high water contents). (C) Esterification of FA with 1-butanol generating BFA and water [1].

E-FAERU is a commercially available FAE (Megazyme) coming from a rumen microorganism.

Immobilization parameters

Immobilization pH, buffer and pore size influence on the immobilization process were tested.



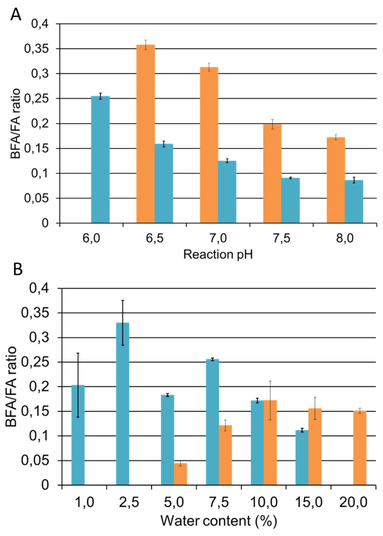
Three buffers were tested at different pH: phosphate-citrate (blue) pH 4-8, MOPS (orange) pH 6-7.5 and Bis-Tris (green) pH 6-7.5.
The effects on the enzyme loading were dependent on the used buffer. Phosphate-citrate was found to be the best buffer with an optimum at pH 7.0.

Pore size of the particles used (5nm to 8.9nm) did not have any significant impact on any of the two parameters studied (data not shown).

The buffer used for immobilizing the FAE on MPS and its pH were of utmost importance for good immobilization. Phosphate-citrate buffer was the best buffer both when looking at adsorption yield as well as at enzyme loading.

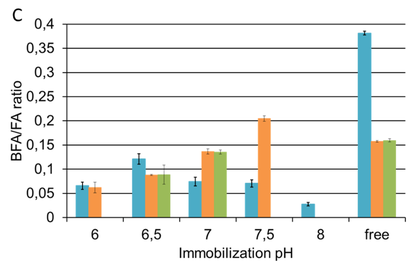
Effects on selectivity

pH and water content (in the solvent/buffer reaction mixture) effects on the transesterification vs hydrolysis ratio were investigated.



A The same trend was observed for the free (blue) and the immobilized (orange) enzyme with a decreasing BFA/FA ratio with increasing pH.

B For the free enzyme (blue) there was an optimum water content at 2.5%. Interestingly even at very low water content it was still active. For the immobilized enzyme (orange) at least 5% of water were needed to have activity.



C Good results were obtained with phosphate-citrate buffer (blue) for which the optimum was at pH 6.5. Other buffers: MOPS (orange) and Bis-Tris (green) gave good selectivity results also but were less good immobilization buffers.

Selectivity of the enzyme was influenced by many parameters. Among them reaction pH and water content were the most influential.

Optimal conditions and kinetic parameters

The FAE was characterized in terms of pH and T optimums in four different conditions: free/immobilized and in hydrolysis/transesterification. The kinetic parameters were then studied at the defined optimum conditions.

	Km (mM)	kcat (s^{-1})	kcat/Km ($s^{-1}M^{-1}$)	Topt (°C)	pH opt
free enzyme - hydrolysis	4,31E-01	1,13E+05	2,63E+02	50	7,5
free enzyme - transesterification	3,60E+01	4,92E+02	1,37E-02	25	7,0
immobilized enzyme - hydrolysis	4,31E-01	2,33E+04	5,40E+01	40	7,0
immobilized enzyme - transesterification	3,09E+01	5,20E+01	1,68E-03	30	7,0

Both for the free and immobilized enzyme, a decrease in the T_{opt} was observed for the transesterification Optimum pH were around 7.0 for all conditions.

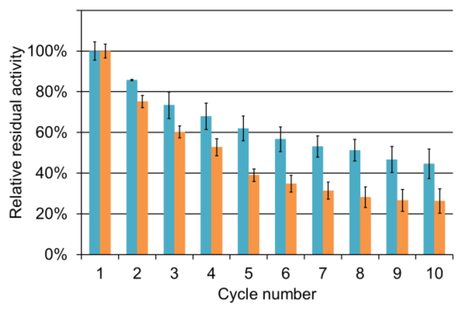
We observed a 100-fold increase in the affinity of the enzyme between hydrolysis and transesterification. Interestingly the affinity was not affected upon immobilization, however the turnover number was lowered resulting in a lower catalytic efficiency.

Optimum conditions differed mainly in temperature. Interestingly Km was not affected upon immobilization.

Stability and reusability

The stability and reusability of the biocatalyst immobilized on MPS was studied.

Stability at four pH conditions (pH 5-8) and at three temperatures (22-55°C) were tested over 24h (data not shown). At all pHs and the two lowest temperatures tested no significant stability improvement was observed. Only when going to higher temperature: 55°C the immobilized FAE had a higher residual activity after 24h.



Hydrolytic activity (blue) and transesterification activity (orange) After 10 cycles of 48h each the immobilized FAE retained more than 20% of its activity.
Surprisingly its selectivity changed over the course of the experiment because the transesterification activity of the enzyme decreased faster than its hydrolytic one.

Surprisingly, stability of the immobilized enzyme was not higher than the one of the free enzyme. The FAE retained more than 20% of its activity after 10 cycles of 48h. Interestingly its selectivity shifted toward hydrolysis during the firsts cycles.

Our results demonstrate that immobilization changes properties of the enzyme and is influenced by various parameters.

[1] Thörn, Christian, Hanna Gustafsson, and Lisbeth Olsson. *Journal of Molecular Catalysis B: Enzymatic* 72, no. 1–2 (October 2011): 57–64. doi:10.1016/j.molcatb.2011.05.002.