Feruloyl esterase immobilization in mesoporous silica: hydrolysis and transesterification reactions.

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A Feruloyl esterase (FAE) was used as the model enzyme to be immobilized in mesoporous silica particles (MPS). The aim of this study was to understand the influence of immobilization conditions on the enzyme activity and the impact of immobilization in MPS on the kinetics of enzymatic reactions.

Background

Mesoporous silica materials of the SBA-15 type possess properties such as large surface area, defined pore geometry, mechanical and thermal stability and they have tunable pore sizes.

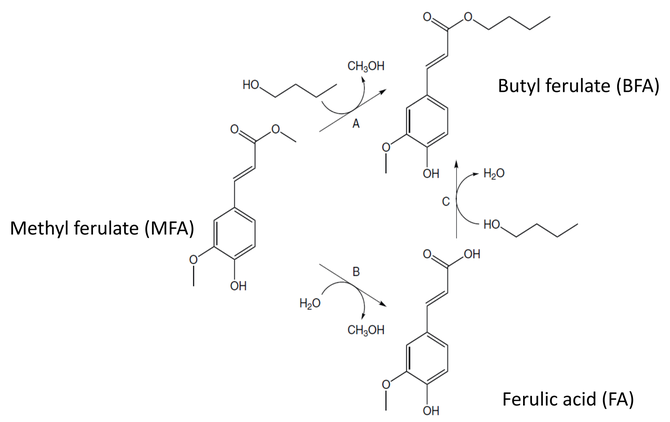
FAEs are of utmost interest in degrading lignocellulosic biomass: they catalyze the hydrolysis of ester linkages releasing ferulic acid and other hydroxycinnamic acids from hemicellulose. They can also catalyze the reverse reaction: transesterification.

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

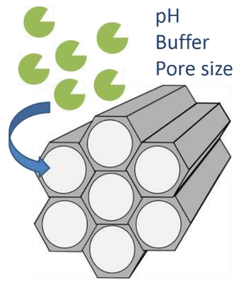
Some reactions catalyzed by FAEs

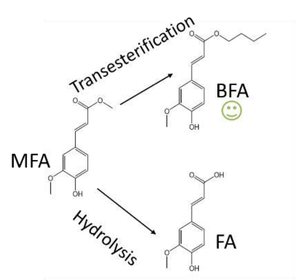


FAEs can catalyse different reactions: (A) Transesterification of MFA with 1-butanol generating BFA and methanol. (B) Hydrolysis of MFA generating ferulic acid and methanol (natural reaction at high water contents). (C) Esterification of FA with 1-butanol generating BFA and water [1].

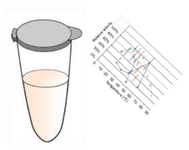
Experimental flowchart

Immobilization → Selectivity → Characterization → Stability → Reusability

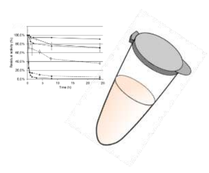


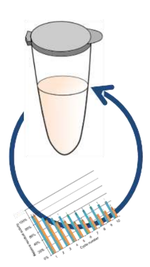


Optima pH and T
Kinetic parameters



pH and T

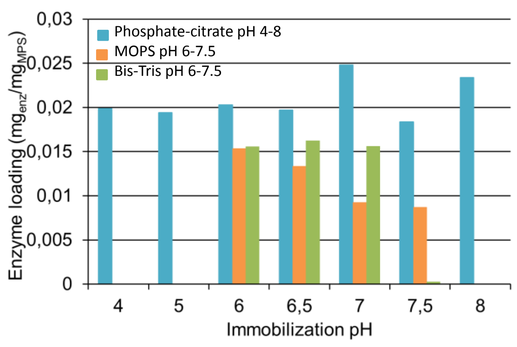




The used enzyme: E-FAERU is a commercially available FAE (Megazyme) coming from a rumen microorganism.

Immobilization parameters

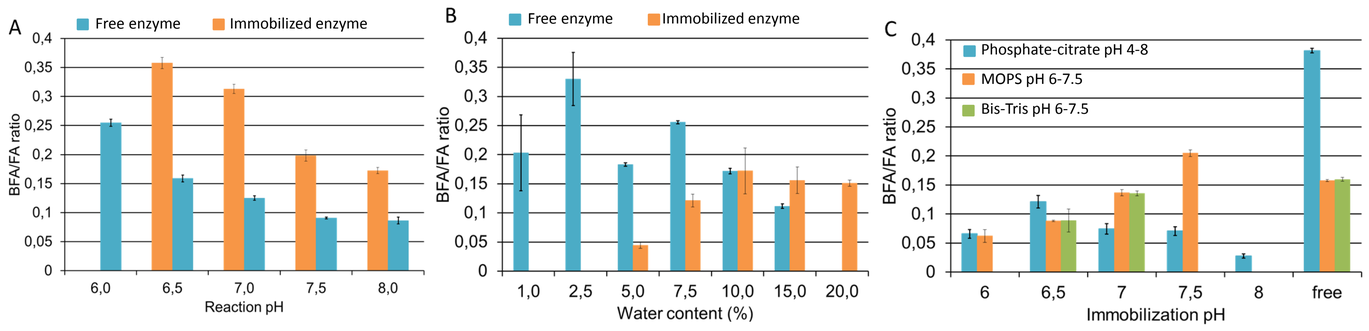
The influence of immobilization pH, buffer and pore size on the immobilization process were tested.



Pore size of the particles used (5 nm to 8.9 nm) did not have any significant impact on enzyme loading (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 found to be the best buffer.

Effects on selectivity

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



Selectivity of the enzyme was influenced by many parameters. Among them reaction pH and water content were the most influential. pH increase induced a decrease in the BFA/FA ratio. An optimum water content could be found for maximum transesterification reaction, it differed between free and immobilized enzyme. Immobilization pH had also some effect on selectivity.

Optimal conditions and kinetic parameters

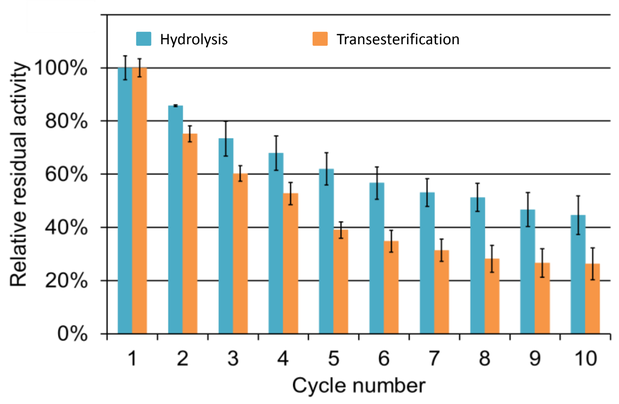
The FAE was characterized in terms of pH and T optimums in four different conditions: free/immobilized and in hydrolysis/transesterification. The kinetic parameters were then studied at the defined optimum conditions.

	Km (mM)	kcat (s^{-1})	kcat/Km ($s^{-1}M^{-1}$)	Topt (°C)	pH opt
free enzyme – hydrolysis	0.43	31.4	72928	50	7.5
free enzyme – transesterification	36.0	0.14	3.79	25	7.0
immobilized enzyme – hydrolysis	0.43	6.47	15005	40	7.0
immobilized enzyme – transesterification	30.9	0.01	0.35	30	7.0

Optimum conditions differed mainly in temperature. Km was not affected upon immobilization. However kcat was much lower, resulting in a lower catalytic efficiency.

Stability and reusability

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



Stability of the immobilized enzyme was not higher than the one of the free enzyme (data not shown). The immobilized FAE retained more than 20% of its activity after 10 cycles of 48h. Interestingly its selectivity shifted toward hydrolysis during the firsts cycles.

[1] Christian Thörn, 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.
[2] Cyrielle Bonzom, Laura Schild and Lisbeth Olsson. *Manuscript in preparation*.