Feruloyl esterases immobilization in mesoporous silica particles

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Feruloyl esterases (FAEs) can be used to hydrolyze ferulic acid (FA) from lignocellulosic biomass but also, when put in different reaction systems, in modifying FA and other hydroxycinnamic acids to produce bioactive compounds through transesterification and esterification reactions. FAEs are immobilized on mesoporous silica materials (MPS) and studied.

Background

Immobilization of enzymes is an advantageous technique for increasing the reusability and stability of enzymes. It also eases the downstream processes. Moreover, it has been shown that immobilization can alter the selectivity of enzymes [1] [2].

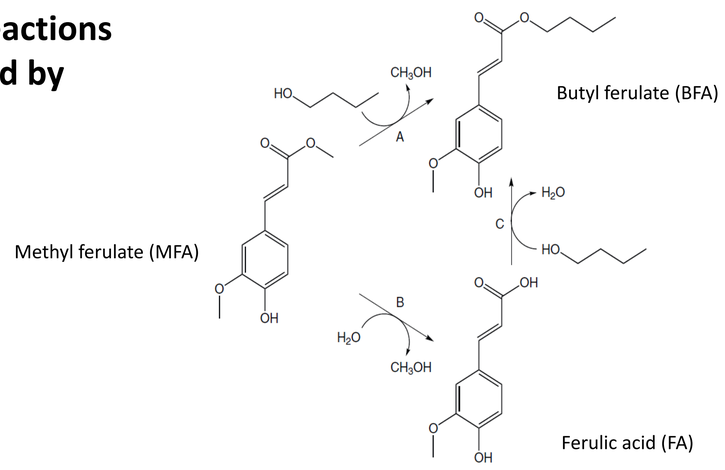
MPS of the SBA-15 type possess properties such as large surface area, defined pore geometry, mechanical and thermal stability and they have tunable pore sizes.

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.

Transesterification reactions happen at reduced water content in the reaction system. This can be achieved by using solvents and/or ionic liquids. The composition of the reaction system also influences the selectivity of enzymes towards the hydrolytic and/or synthetic reactions.

Esterified hydroxycinnamic acids, such as FA, are bioactive compounds recognized for their antioxidant, tumor suppressing and antibacterial properties[3].

Some reactions catalyzed by FAEs

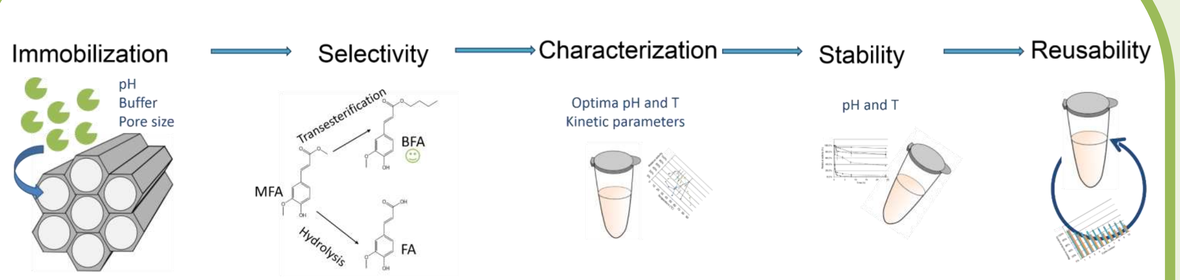


FAEs can catalyze different reactions: (A) Transesterification of MFA with 1-butanol generating BFA and methanol. (B) Hydrolysis of MFA generating ferulic acid and methanol (natural reaction at high water contents). (C) Esterification of FA with 1-butanol generating BFA and water [2].

Aims of the project

- ✓ Evaluate how immobilization and reaction condition affects the selectivity of the FAE using organic solvents and ionic liquids
- ✓ Evaluate the impact of immobilization on the enzyme kinetic parameters under optimized immobilization and reaction conditions in organic solvents
- ✓ Assess the industrial potential of one immobilized FAE for transesterification in organic solvents
- ✓ Assess the impact of some ionic liquids on FAEs
- ✓ Evaluate the influence of enzyme glycosylation

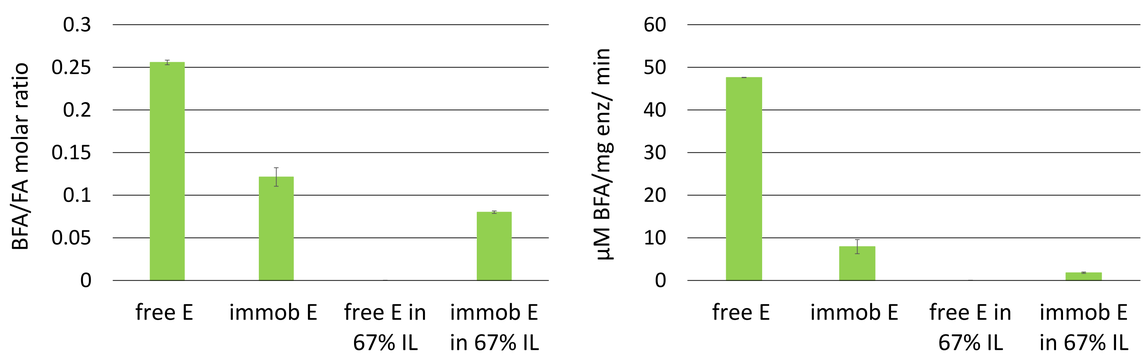
Characterization of an immobilized FAE [3]



	Km (mM)	kcat (s^{-1})	kcat/Km ($s^{-1}M^{-1}$)	Topt (°C)	pH opt
free enzyme – hydrolysis	0.43	31.4	73000	50	7.5
free enzyme – transesterification	36.0	0.14	3.79	25	7.0
immobilized enzyme – hydrolysis	0.43	6.47	15000	40	7.0
immobilized enzyme – transesterification	30.9	0.01	0.35	30	7.0

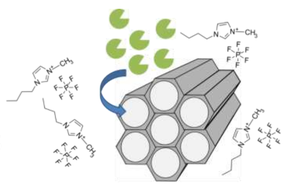
Optimum conditions differed mainly in temperature. Km was not affected upon immobilization. However kcat was much lower, resulting in a lower catalytic efficiency. Reusability of the immobilized enzyme during 10 cycles of 48h showed a good behavior: more than 20% of activity remained after 20 days.

Effect of ionic liquids



Enzymes were put into a mixture of 7.5% buffer plus either only butanol (92.5%) or butanol (25.5%) and [BMIM] [PF_6] (67%).

The immobilized enzyme activity in ionic liquids was decreased compared to the activity observed in the buffer-butanol mixture. However, immobilization has a sheltering effect on the enzyme since the free enzyme in ionic liquid was completely inactive.



Future perspectives

- ✓ Immobilization behavior is highly enzyme dependant, therefore different feruloyl esterases will be investigated. Focus will be put on their original host organism: fungal versus bacterial enzymes.
- ✓ Only one ionic liquid was tested, therefore different ones will be evaluated in order to assess if the immobilization sheltering effect observed for [BMIM][PF_6] is observed for other ionic liquids as well.
- ✓ When enzymes are produced in different host organisms, the glycosylation extent and pattern can differ. Therefore, the effect of glycosylation on immobilization and transesterification capacity of enzymes will be investigated.

[1] Thörn, C., Gustafsson, H., and Olsson, L. (2011). Immobilization of feruloyl esterases in mesoporous materials leads to improved transesterification yield. J. Mol. Catal. B Enzym. *72*, 57–64.
[2] Bonzom,C., Schild, L., Gustafsson, H., and Olsson, L. *Manuscript in preparation*.Feruloyl esterase immobilization in mesoporous silica particles and characterization in hydrolysis and transesterification.
[3] De Vries, R.P., and Visser, J. (2001). Aspergillus Enzymes Involved in Degradation of Plant Cell Wall Polysaccharides. Microbiol. Mol. Biol. Rev. *65*, 497–522.