Synthesis of Antioxidants with Free and Immobilised Fungal Feruloyl Esterases

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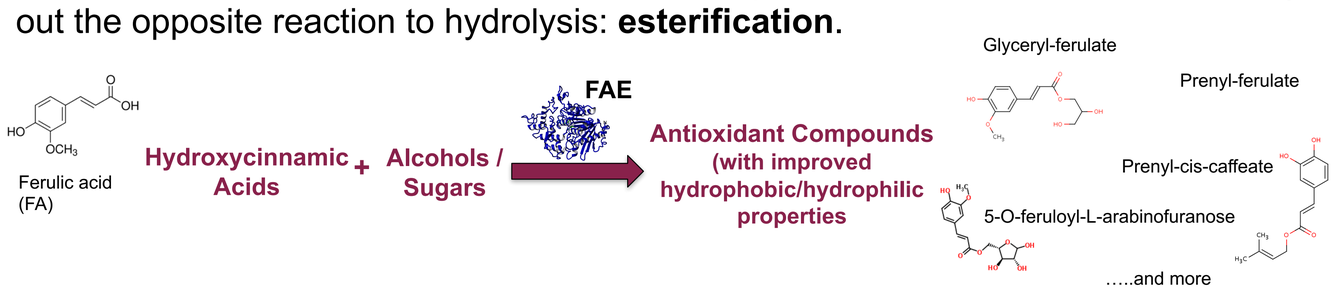
INDUSTRIAL BIOTECHNOLOGY

Introduction

Feruloyl esterases (**FAEs**, E.C. 3.1.1.73, CAZy family CE1) are enzymes that are secreted by a wide range of fungi and bacteria as part of the enzymatic system that hydrolyses plant biomass.
Under conditions of low water content, for example in solvents, FAEs can also carry out the opposite reaction to hydrolysis: **esterification**.



Hydroxycinnamic acids ester-bonded with various alcohols or sugars have promising applications in the food, cosmetics and pharmaceutical industry as **antioxidants**, thus the potential use of **FAEs as biocatalysts** for the synthesis of antioxidants has been investigated in recent years.

Material & Methods

Enzymes

Figure 1. A single colony of *Myceliophthora thermophila*. Image credit: www.gefor.4t.com

4 FAEs from fungus *Myceliophthora thermophila*: **A1, A2, B1, B2**

Produced in engineered overexpression strains by DuPont Industrial Biosciences

Mesoporous Silica (MPS)

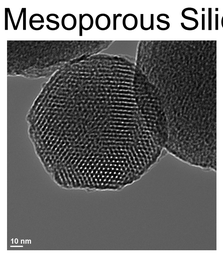


Figure 2. TEM image of SBA-15 mesoporous silica. Image credit: wikimedia

6 different MPS materials: SBA-15 with pore sizes of 6 nm, 7.8 nm, 9.3 nm and 10.2 nm. MCF-2 with pore size of 26 nm and KIT-6 with a pore size of 5 nm.

Produced by Milene Zezzi Do Valle Gomes and Hanna Gustafsson, Division of Applied Surface Chemistry, Department of Chemistry and Chemical Engineering, Chalmers.

Reactions

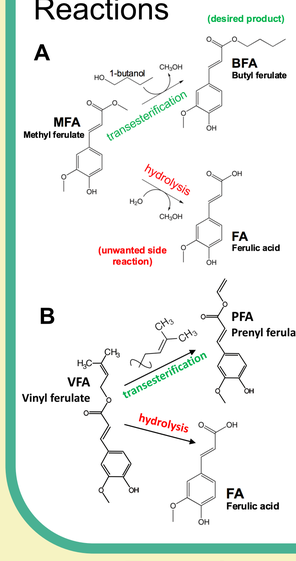


Figure 3. Reaction scheme of transesterification reactions. A) Synthesis of BFA and hydrolysis of MFA to FA. The reaction takes place in the solvent 1-butanol, which also acts as the alcohol donor. B) Synthesis of PFA and hydrolysiis of VFA to FA. The reaction takes place in different solvent-buffer systems.

Results

Immobilisation

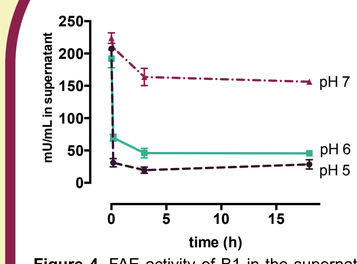


Figure 4. FAE activity of B1 in the supernatant during immobilisation on MPS SBA-15 with 9.3 nm pore size.

Immobilisation of enzymes is a very rapid process. The majority was adsorbed during the first 10 min. After about 3 h an equilibrium was reached. Enzyme-MPS can then be washed, dried and stored for later use.
Enzyme loading, i.e. how much enzyme can be immobilised, was very dependent on pH.

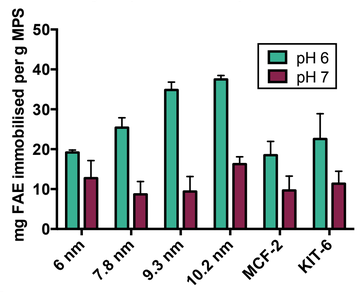


Figure 5. Amounts of B1 FAE immobilised on various MPS materials with different geometries and pore sizes.

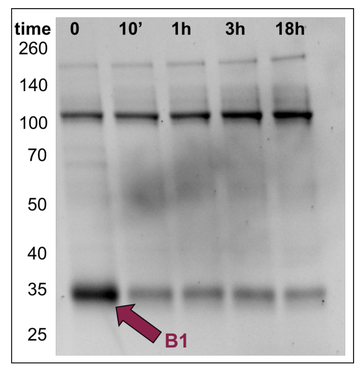


Figure 6. SDS-PAGE of supernatants during immobilisation of B1 on MPS SBA-15 with 9.3 nm pore size, pH 6.

The geometry and pore size of the MPS material, as well as the immobilisation pH, influence the enzyme loading. For B1, the material with 10.2 nm pore size at pH 6 resulted in the highest amount of immobilised enzyme.

When following the enzyme immobilisation with SDS-PAGE of the supernatant, it was clearly visible that the the relatively small B1 enzyme with a size of about 35 kDa was continually depleted in the supernatant, while the higher molecular proteins (>70 kDa) stayed in solution. Thus, an enrichment of enzyme on the MPS particles took place.

Reaction A: Methyl ferulate → butyl ferulate

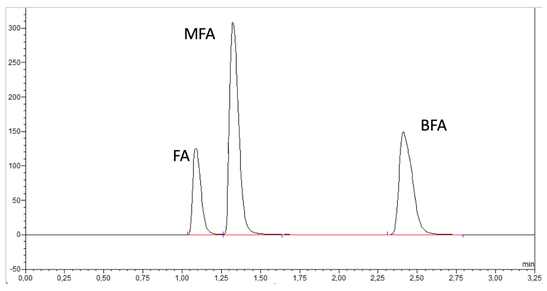


Figure 7. Chromatogram of a typical esterification reaction. Hydrolysis product FA, substrate MFA and desired product BFA appear as single peaks.

Reactions were run in 92.5 % 1-butanol and 7.5% buffer, pH 6.5, at 30°C for 24-48 h. Analysis was done on an HPLC instrument with PDA detector (300 nm), C18 column.

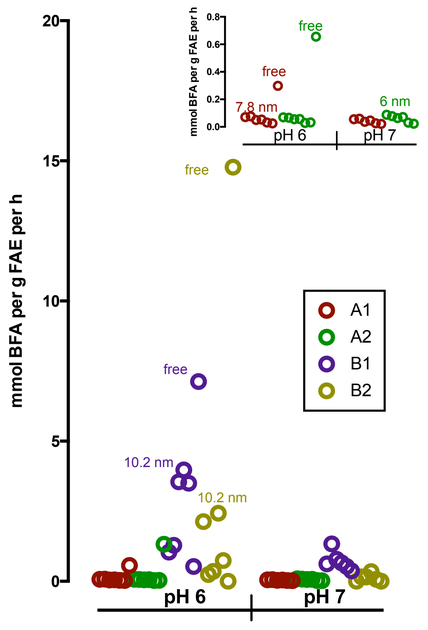


Figure 8. Overview of BFA production with A1, A2, B1 and B2, free or immobilised on different MPS materials. Each circle represents one condition, the best and second-best conditions for each sample are labelled. Inset in upper right corner shows zoomed in version of just samples A1 and A2.

The four enzymes showed differences in their ability to perform the esterification reaction from MFA to BFA. But not only the enzyme, also the kind of support material and the pH, at which the enzymes have been immobilised previously, influences product formation. Highest product formation was generally achieved with the free enzymes.

To determine the best reaction conditions, not only productivity, but also the ratio between esterification and hydrolysis reaction, expressed here as BFA:FA ratio, has to be considered. The higher the BFA:FA ratio, the better.
The B enzymes showed a much better BFA:FA ratio when immobilised, compared to the free enzymes

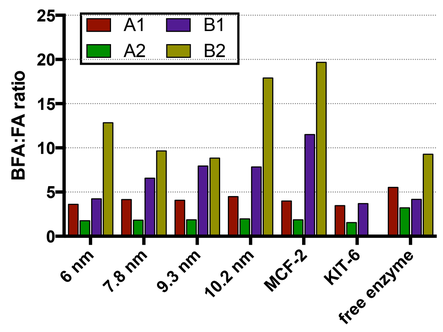


Figure 9. Product formation with free and immobilised A1, A2, B1, B2. Points for free enzymes, as well as for the best performing immobilised enzyme are labelled.

Re-usability

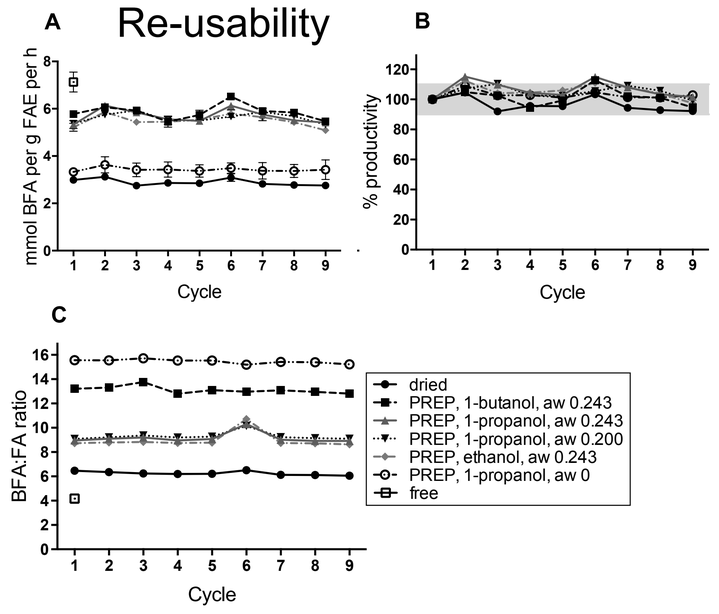


Figure 10. Re-usability of immobilised B1. A) Product formation per hour per g FAE. B1 enzyme was immobilised on SBA-15 10.2 nm and either dried conventionally, or rinsed with a solvent. Reaction was run over 9 reaction cycles. B) Loss of activity over 9 consecutive reaction cycles, expressed as percent of original activity. B) Loss of activity over 9 consecutive reaction cycles, expressed as percent of original activity.

In general, all MPS-enzyme preparations showed very high stability, with almost no loss of activity over 9 cycles. PREPs resulted in higher formation of product per hour than dried MPS-enzyme, especially when water was included in the PREPs media.
BFA:FA ratio was also higher in PREPs than in either free enzyme or dried MPS-enzyme.

Results

Reaction B: Vinyl ferulate → prenyl ferulate

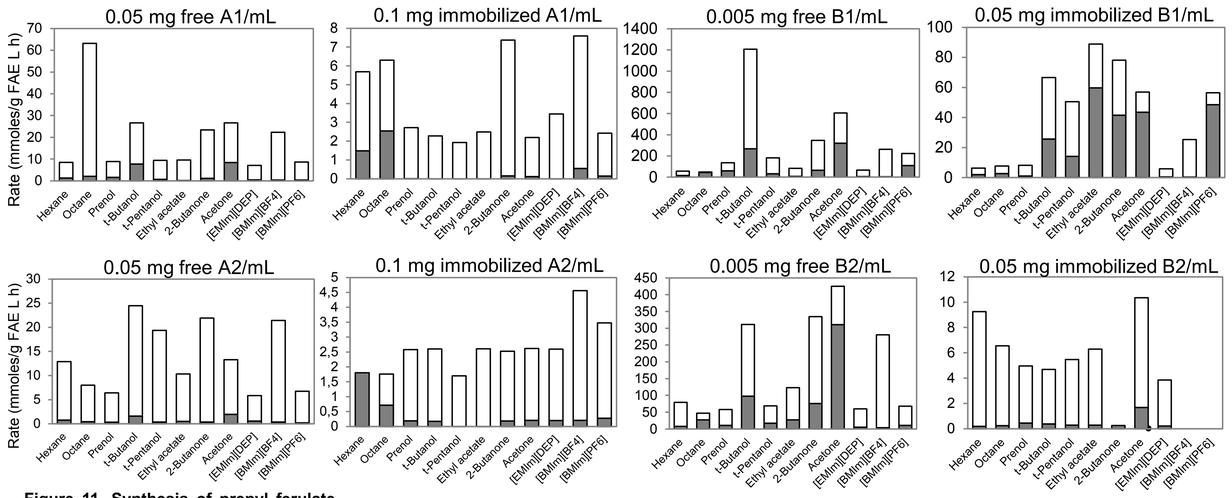


Figure 11. Synthesis of prenyl ferulate in reaction systems with different solvents. Transesterification product = grey, hydrolysis product = white.

Free enzymes show much higher production rate than immobilised enzymes. The PFA:FA ratio was best for immobilised B1. A big difference in rate and PFA:FA ratio could be observed between the different solvents used.

Conclusions

- By careful selection of pore size, enzymes can be enriched through immobilisation, making enzyme purification dispensable.
- Immobilisation increased synthesis:hydrolysis ratio of Reaction A favourably, but did not have such a clear effect in Reaction B.
- Free enzymes had a higher production rate than immobilised enzymes.
- Immobilisation stabilised enzymes and allowed re-use over >9 cycles (à 24h).
- Solvent rinsed MPS-enzyme (PREPs) showed higher activity than dried MPS-enzyme.
- Choice of solvents had a big influence of reaction rate.