

Feruloyl esterases, effects of glycosylation on activity, stability and immobilization

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BACKGROUND

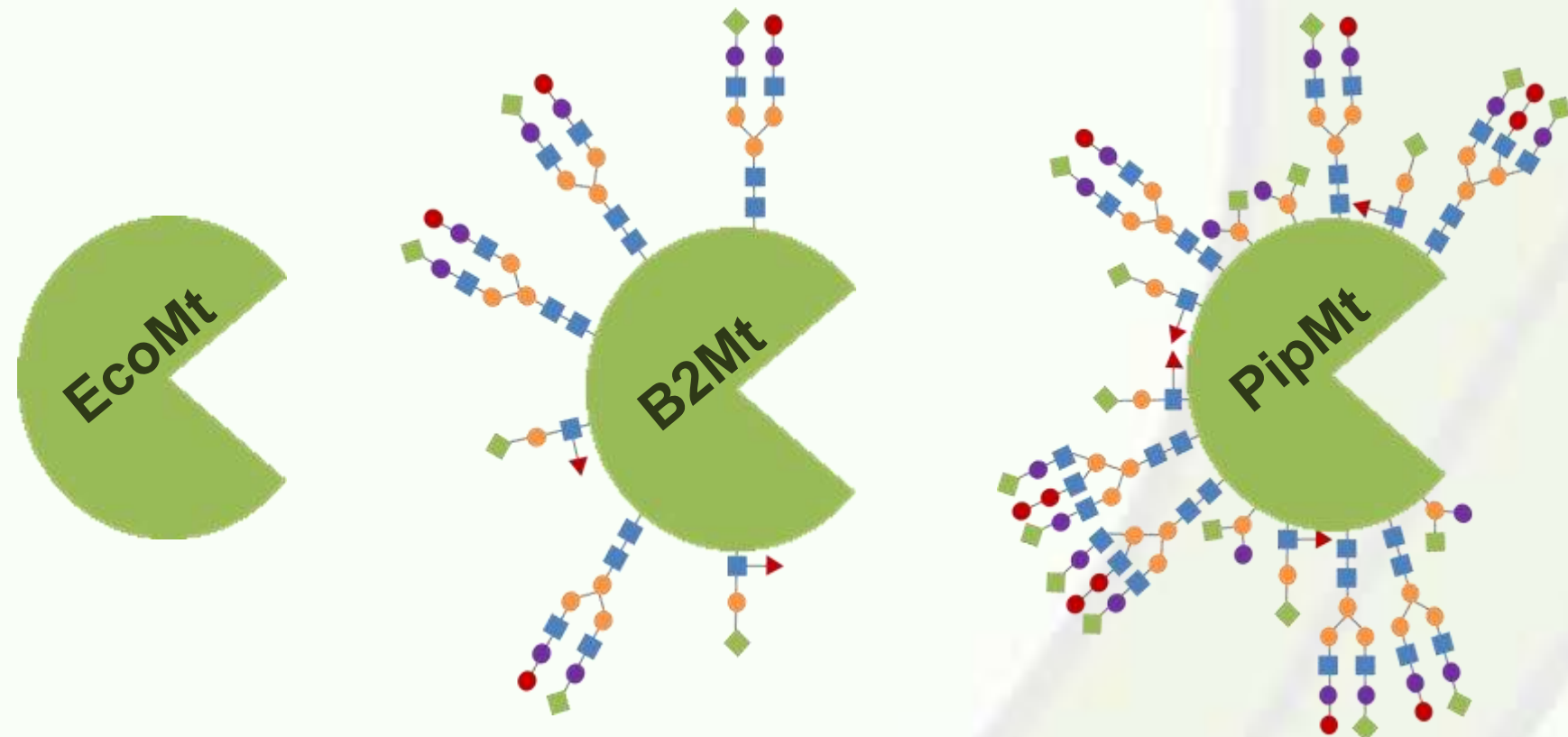
Feruloyl esterases (FAE; EC 3.1.1.73) are a subclass of carboxylic ester hydrolases. They catalyze the hydrolysis of ester linkages in plant cell walls materials releasing ferulic acid and other hydroxycinnamic acids and therefore have been classified in the CAZy database [1] in the carbohydrate esterase (CE) family. Currently, all FAEs in the CAZY database belong to the CE1 family.

We studied the Fae1a from *Miceliophthora thermophila* expressed in different host organisms and therefore having different glycosylation extent/patterns.

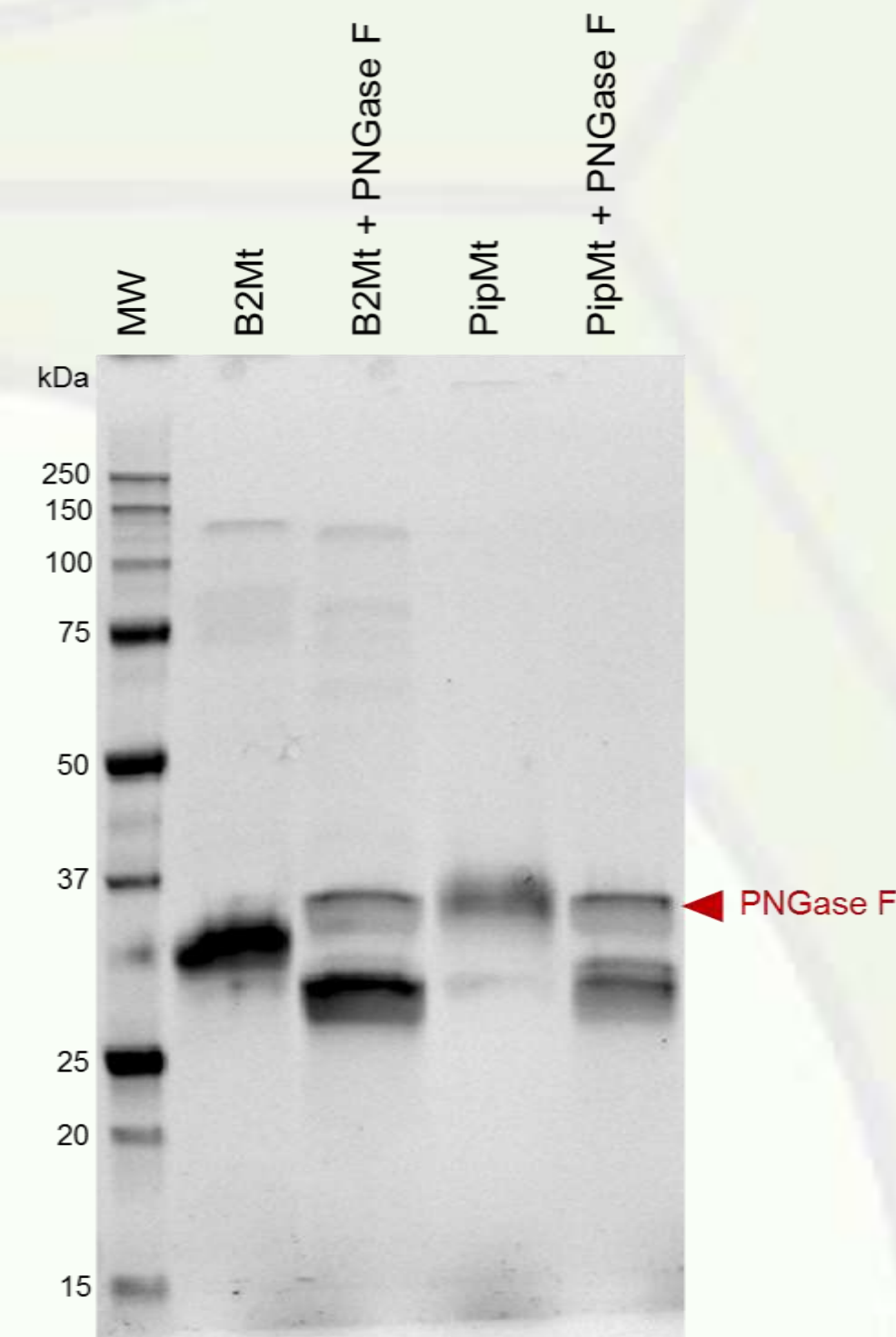
Our hypothesis was that glycosylation would influence the immobilization behavior of the studied FAE in mesoporous silica particles as well as its activity.

GLYCOSYLATION

Depending on the production host of the enzyme, the glycosylation extent and/or pattern can vary from none in *Escherichia coli* to hyper-glycosylation in *Pichia pastoris*. *M.thermophila* was expected to have an intermediate glycosylation level.



The different glycosylation extents were confirmed by PNGase F digestion and SDS-Page gel analysis.

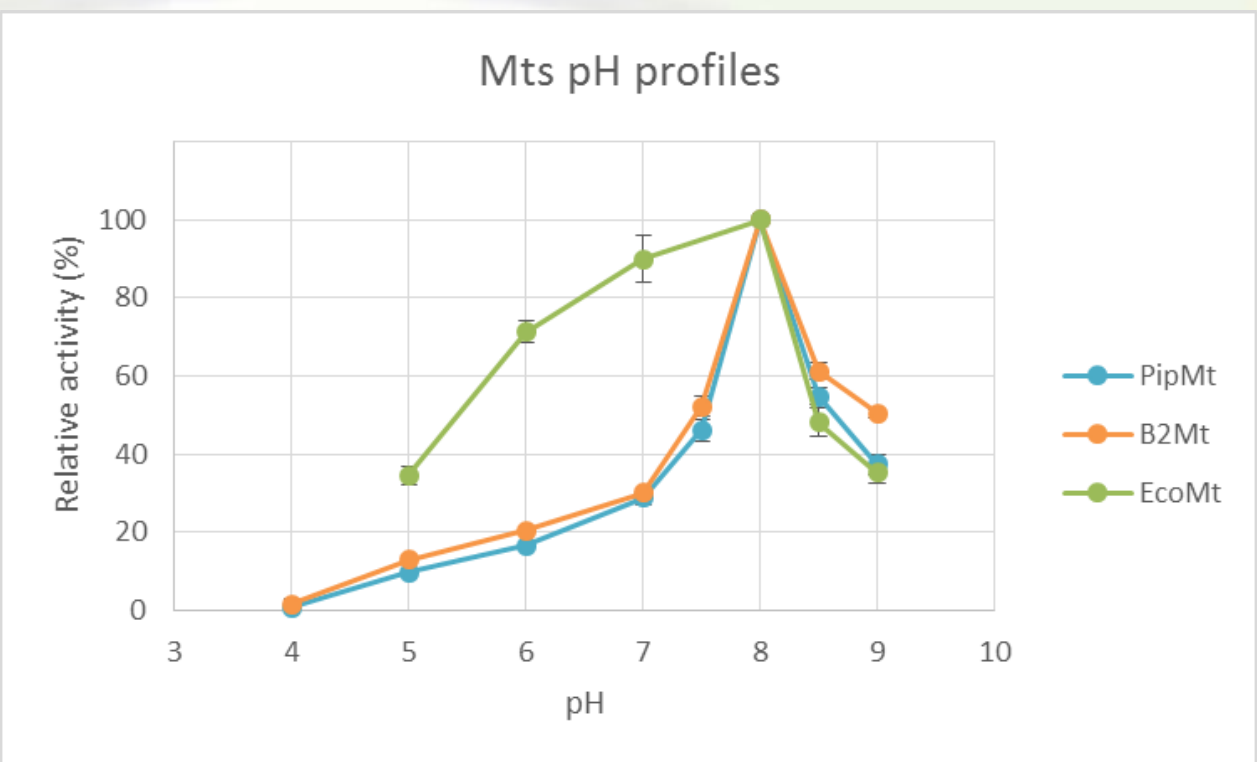


Protein	Before (kDa)	After (kDa)	Δ (kDa)
PipMt	33	26	7
B2Mt	27	24	3

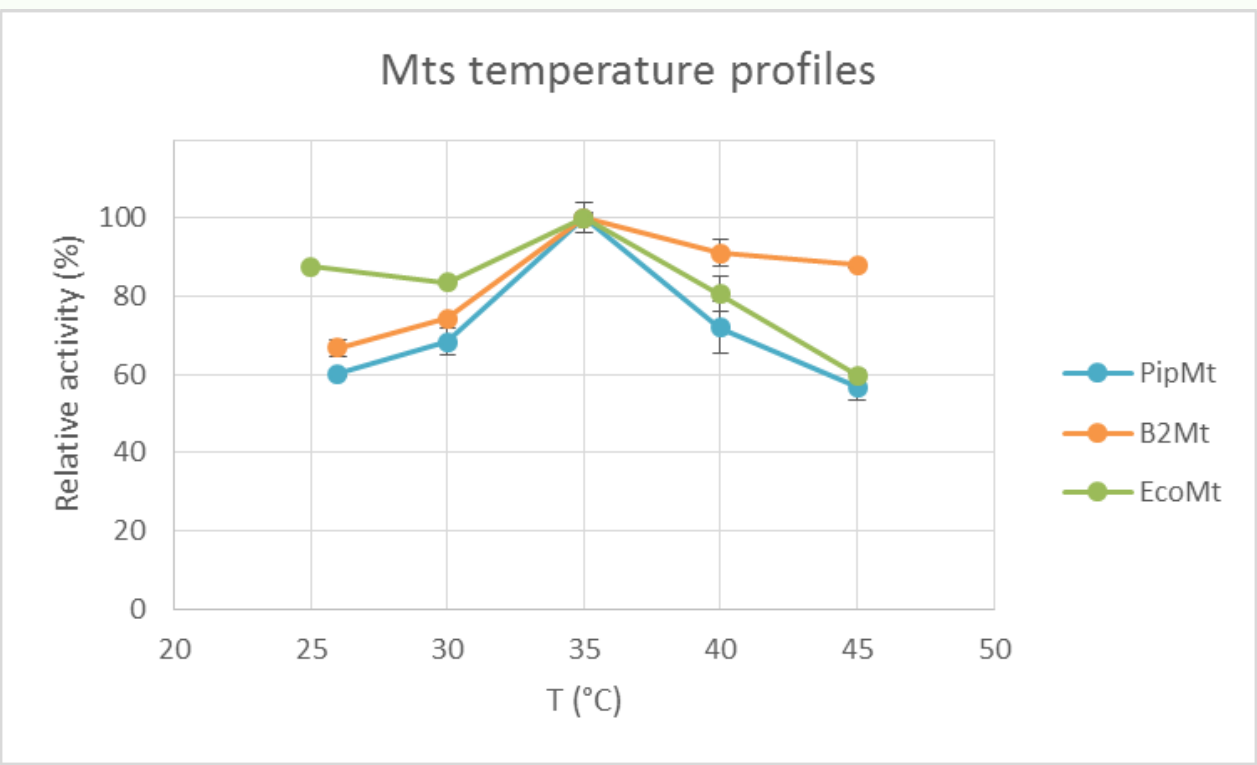
Glycosylation has been shown to alter activity and stability of enzymes [3]. It was therefore decided to investigate the behavior of the different forms of the same enzyme.

PROFILES

The three forms of the enzyme were tested for pH and temperature profiles.



Both glycosylated forms displayed similar behaviors with pH. The non-glycosylated enzyme was more tolerant to low pHs.



The original enzyme (B2Mt), having the intermediate glycosylation level, was the most tolerant to high temperatures. The non-glycosylated one (EcoMt) was the most robust at low temperatures.

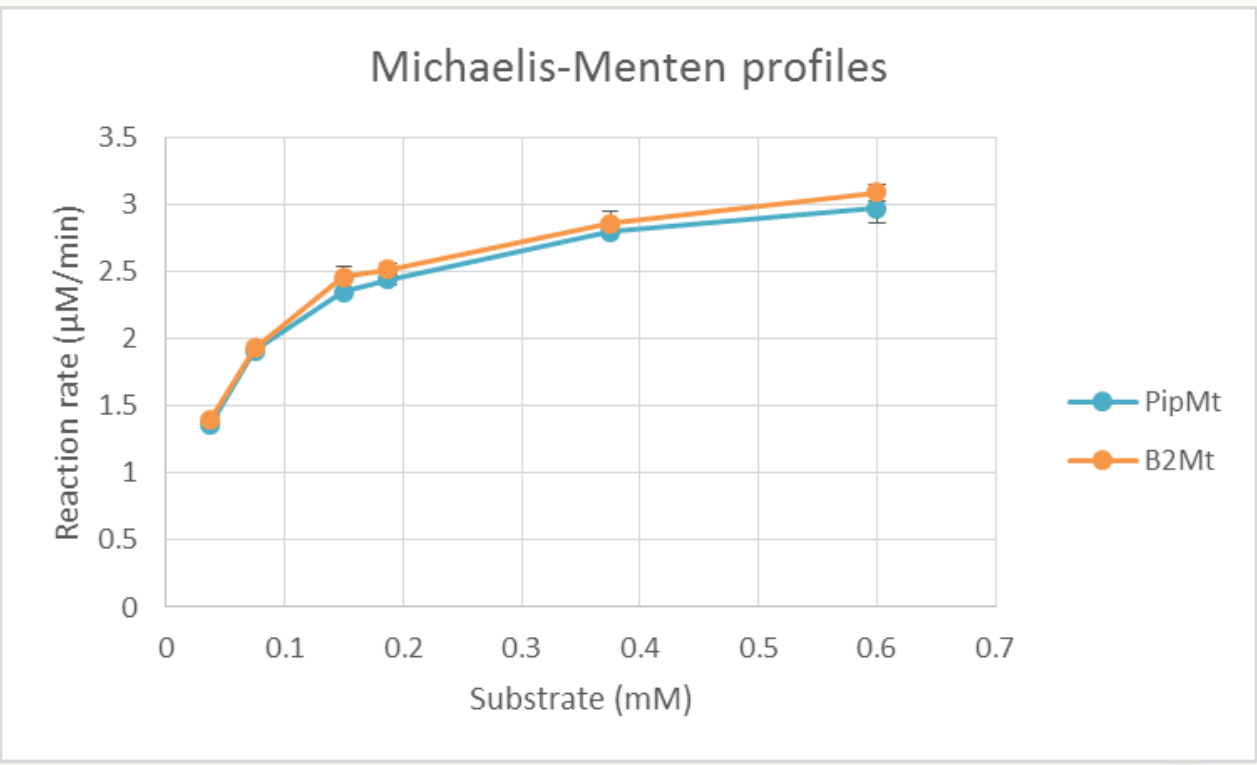
Activity retained after 24h at 20°C		
pH	PipMt	B2Mt
5.0	64%	85%
6.0	79%	84%
7.0	66%	49%

Both glycosylated enzymes were relatively stable at 20°C for 24h.

At the determined preferred pH and temperature, the kinetic constants of the enzymes were evaluated.

KINETIC CONSTANTS

K_m, V_{max} and k_{cat} of the two glycosylated enzymes were evaluated.



	PipMt	B2Mt
K _m (μM)	51.3 ± 2.1	52.9 ± 2.5
V _{max} (μM/min)	3.2 ± 3.2 10 ⁻²	3.3 ± 3.9 10 ⁻²
k _{cat} (s ⁻¹)	42.8 ± 0.4	31.8 ± 0.4

K_m was the same for both glycosylated enzymes. Small differences were observed on k_{cat}.

IMMOBILIZATION BACKGROUND

Immobilization is a powerful tool to allow enzyme reuse and therefore decrease their cost in the overall process.

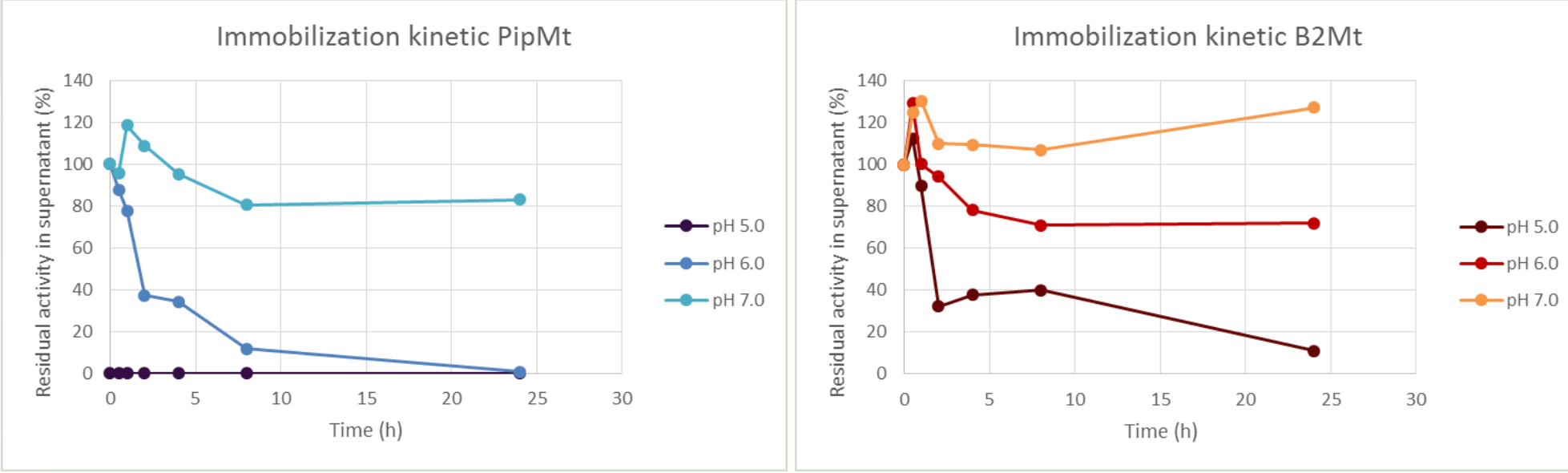
Mesoporous silica particles (MPS) are porous supports made of silica which pore size can be tuned from 2 to 50 nm depending on the synthesis conditions [2]. A pore size matching the enzyme size can greatly reduce leaching after immobilization. The pore system also provides a sheltering and more packed environment, mimicking the inside of a cell.

IMMOBILIZATION KINETIC

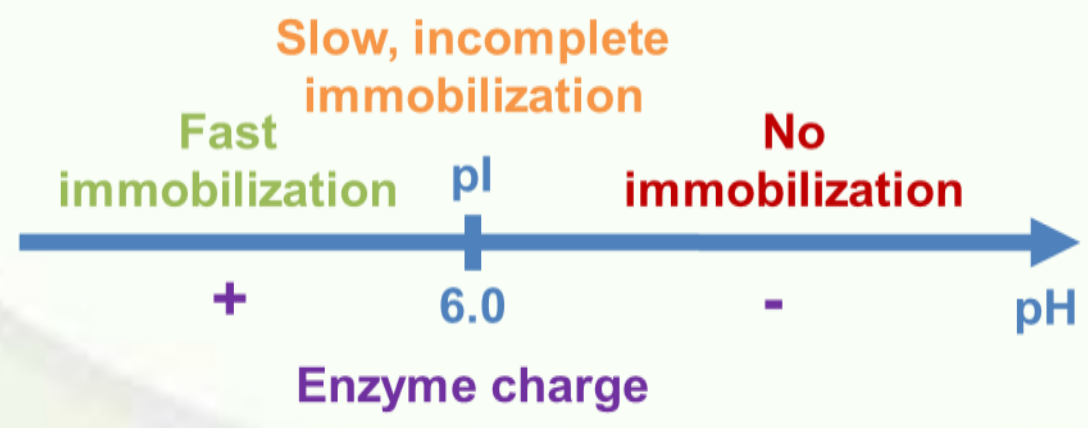
Immobilization on solid carriers can be achieved by different approaches, one of them is by physical adsorption. This technique relies mainly on the surface charges of the support and enzyme.

Because they are negatively charged, mesoporous silica particles allow for rapid enzyme immobilization by physical adsorption.

MPS of 10 nm pore size were used to immobilize enzymes estimated to be 4-5 nm in diameter.



For B2 Mt, at different pHs different behaviors were observed which can be linked to the enzyme pI, determined experimentally to be at 6.0 [4].



Based on the obtained profiles, PipMt seems to have a higher pI. This is in agreement with the fact that glycosylation are known to alkify the pI.

A different behavior is expected for the non glycosylated enzyme which pI should be closer to the theoretical one calculated by expasy to be at 4.4.

CONCLUSIONS

- ✓ The different glycosylation extents were confirmed.
- ✓ All three forms of Fae1a display the same pH and temperature optima with some differences in the profiles
- ✓ Both glycosylated forms have the same kinetic parameters K_m and k_{cat}.
- ✓ Different immobilization behaviors were observed which we hypothesized are linked to the enzyme's pI

Future work:

- Stability of the enzymes
- The non-glycosylated form: EcoMt