OXIPRO

CBM3-tagged N-acetylglucosamine oxidase: An Eco-friendly approach for hydrogen peroxide generation in cotton textile manufacturing

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INTRODUCTION

The growing demand for better biocatalytic processes requires enhancing enzyme reusability and robustness. Innovations in enzyme immobilization explore new supports and methods for easier recovery while improving stability and activity, boosting enzyme efficiency, and reducing downstream costs significantly. The present work evaluates the use of carbohydrate binding modules (CBMs), a new technology based on biomolecular scaffolds, as a potential tool for the immobilization of enzymes, such as N-acetylglucasamine oxidase (NagOX), in cellulose-rich materials of biological origin and low value (**Fig.1**).
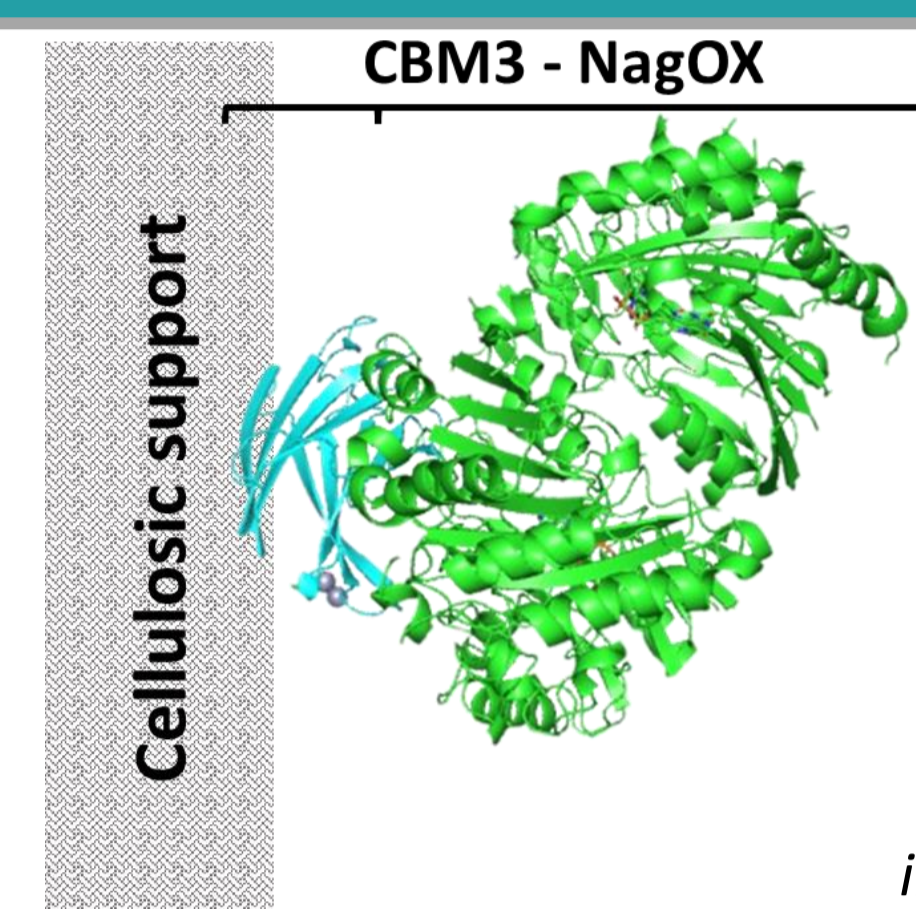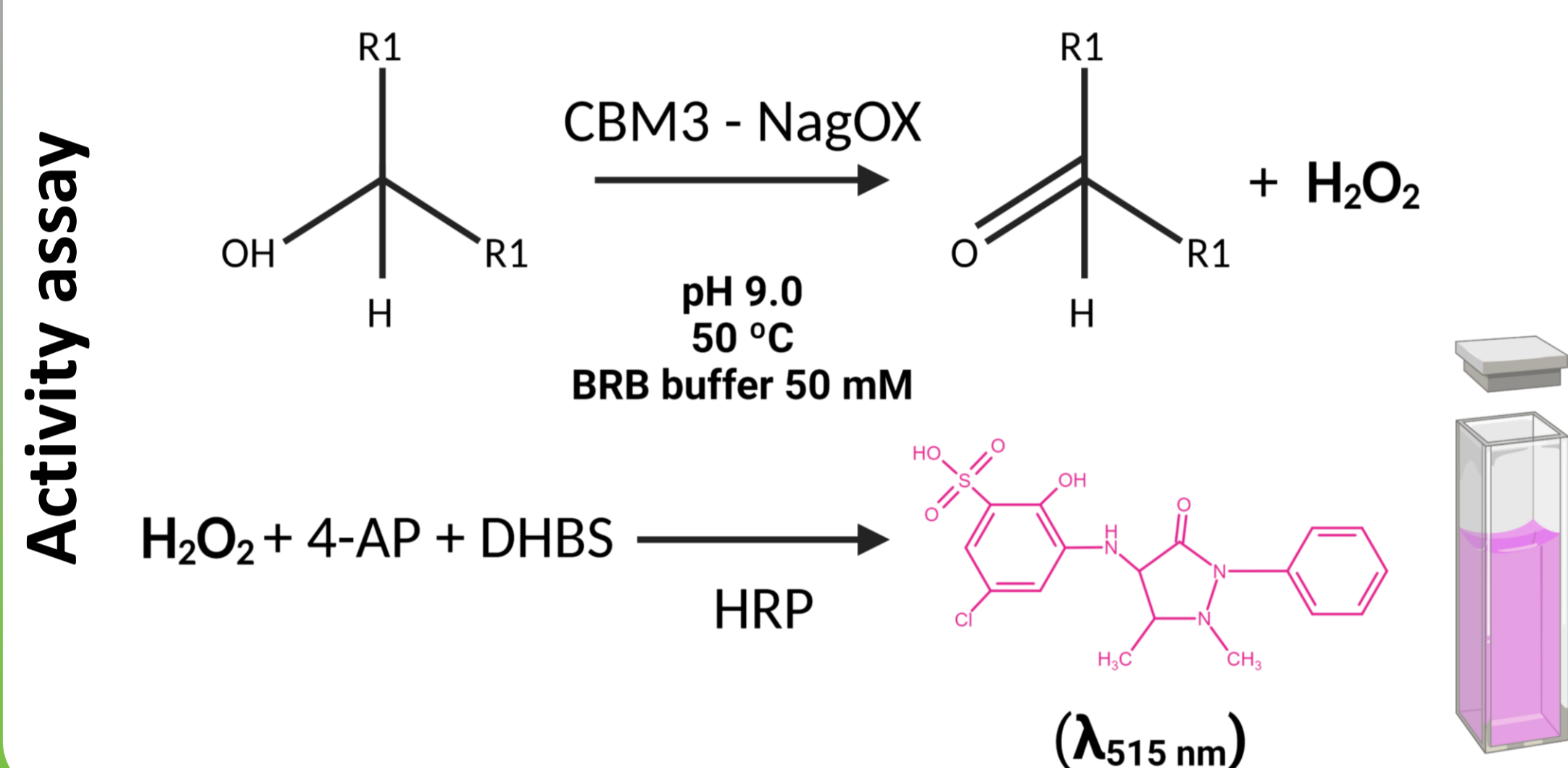

Fig.1. CBM3-NagOX immobilization scheme

METHODS

Activity assay

$$CBM3 - NagOX,\ pH\ 9.0,\ 50\ ^\circ C,\ BRB\ buffer\ 50\ mM$$

$$\text{(alcohol)} \xrightarrow{\text{CBM3 - NagOX}} \text{(ketone)} + H_2O_2$$

$$H_2O_2 + 4\text{-}AP + DHBS \xrightarrow{HRP} \text{(pink product)}\ (\lambda_{515\ nm})$$

Supports assessment

Pressure-flow experiments
- Commercial supports
- 20 mL FPLC column
- MiliQ water at RT
- ÄKTA Pure FPLC

Enzyme immobilization
- Ratio 1:10 support - cell lysate
- Activity offered in a range of 6 – 5 UI/g supp
- 25 ºC
- pH 7.0

Loading studies
- Ratio 1:10 support - cell lysate
- Activity offered in the range of 6 - 450 UI/g supp
- Support saturation achieved upon reducing the IY below 90%.

RESULTS

① Assessment of cellulosic supports

A variety of commercial cellulose resins are packed and tested using FPLC in order to evaluate the increase in column internal pressure when applying different water flows (**Fig.2**). The objective was to determine the most suitable resin candidates for future process scaling.
MT100 and **MT500 (Medium)** are the supports that yield the best results and are therefore selected for studying enzymatic immobilization on them.
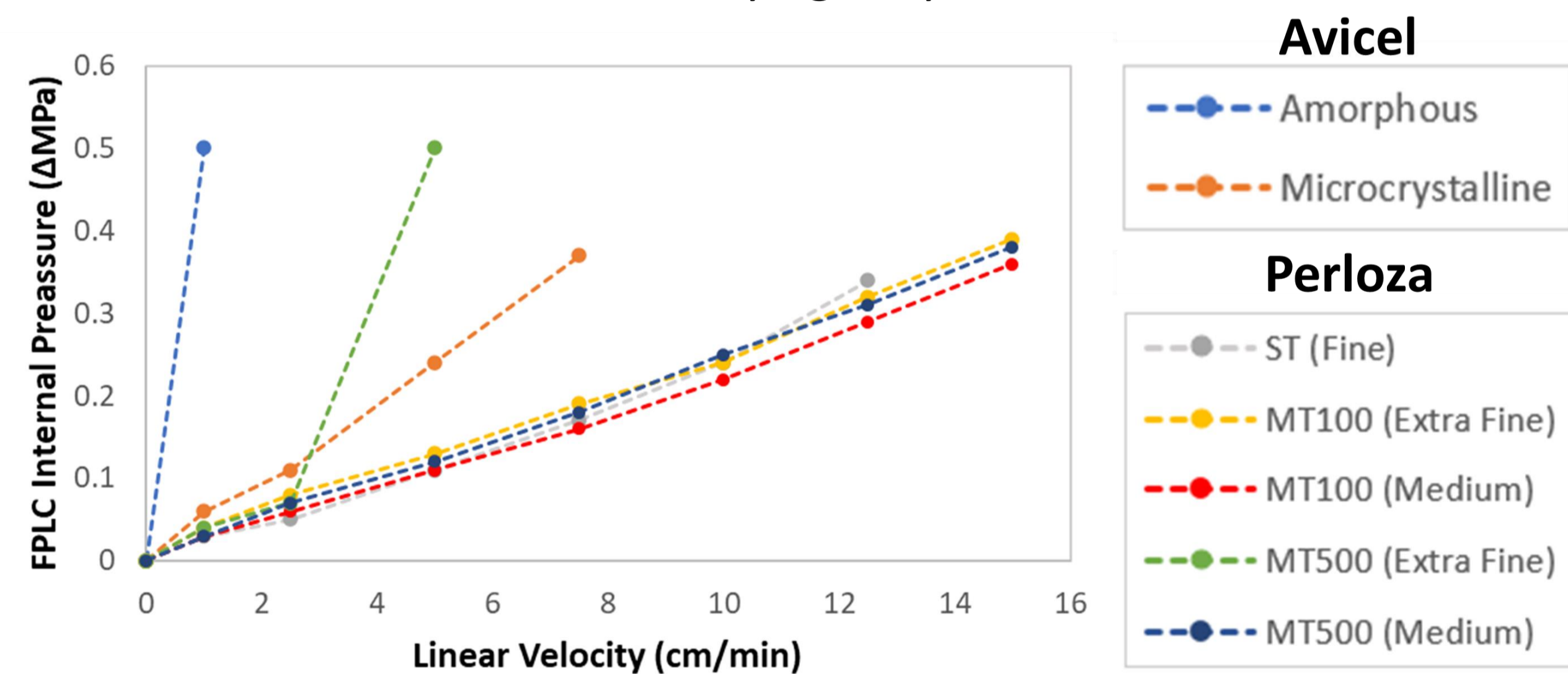

Fig.2. Pressure-flow experiments of the different cellulosic supports

② Immobilization of CBM3-NagOX

Cellulose-based supports with superior performance in FPLC were chosen to immobilize the CBM3-NagOX enzyme (**Fig.3 – 4**). Results indicated efficient enzyme retention, with immobilization yields of **95%** and **99%** on MT100 and MT500, respectively, and a recovered activity of **90%** on both supports. MT100 demonstrated a higher maximum enzymatic load (**97.08 UI/g support**) compared to MT500 (**49.60 UI/g support**).
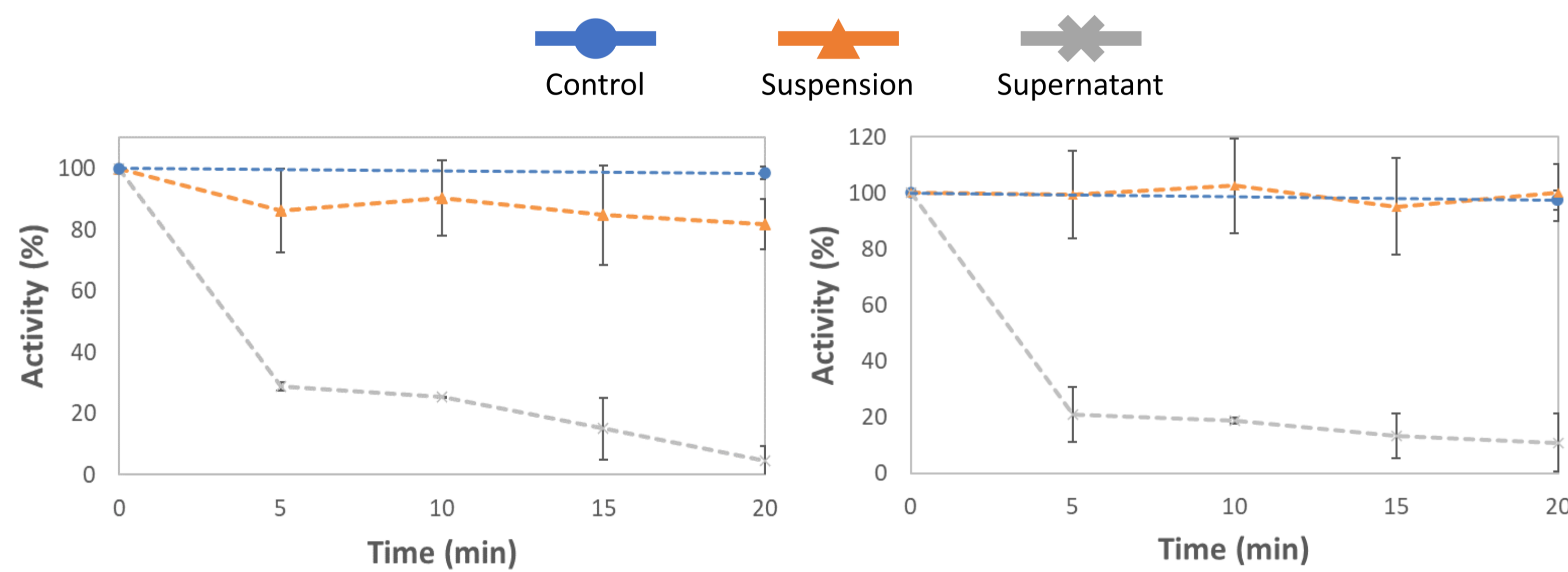

Fig.3. Immobilization kinetic of CBM3-NagOX in Perloza MT100 (Medium) support.

Fig.4. Immobilization kinetic of CBM3-NagOX in Perloza MT500 (Medium) support.

③ Study of alternative supports

Other cellulosic materials, like **cellulose-acetate nanofiber membranes** and **delignified coffee grounds**, were also tested for immobilizing the CBM-tagged enzyme (**Fig.5 – 6**). While successful, their immobilization parameters yielded notably lower results compared to Perloza supports, with activity recovery percentages of **25%** in coffee and **10%** in membranes.
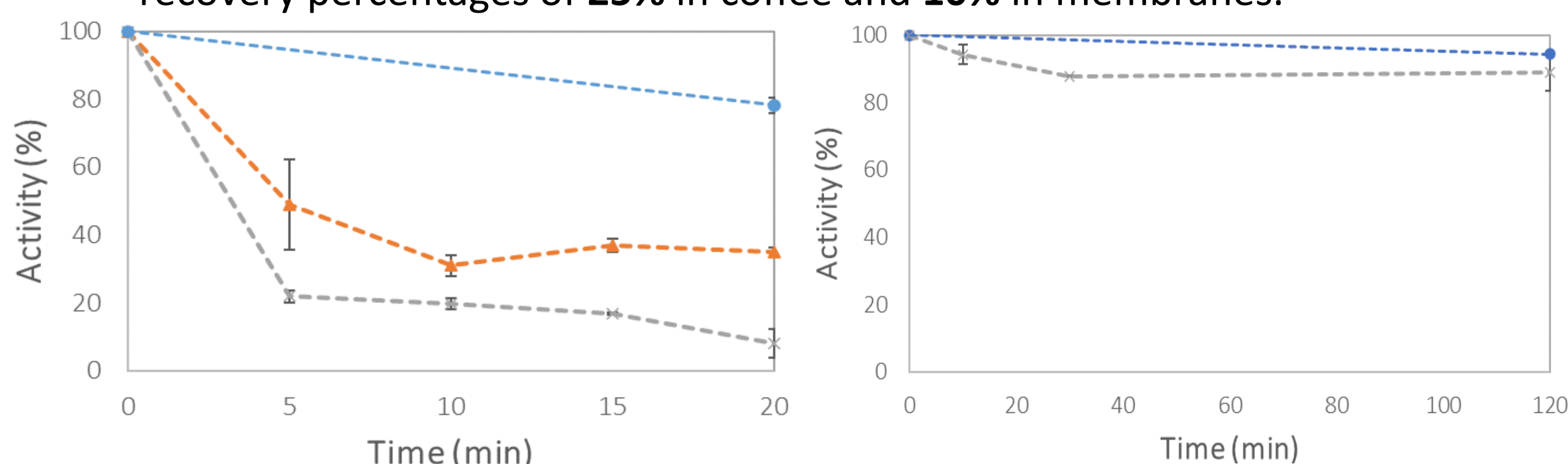

Fig.5. Immobilization kinetic of CBM3-NagOX in delignified coffee grounds

Fig.6. Immobilization kinetic of CBM3-NagOX in cellulose-acetate nanofiber membranes

CONCLUSIONS

- The MT100 and MT500 (Medium) supports by Perloza exhibit optimal mechanical properties for FPLC applications, with MT100 chosen for future studies due to its superior enzymatic loading capacity.
- CBM tags offer an efficient method for enzymatic immobilization without support functionalization.
- Immobilizing CBM3-NagOX on various cellulose supports highlights the versatile adherence capabilities of these biomolecular scaffolds to cellulose matrices.
- These findings hold promise for economically and environmentally friendly biocatalyst design and preparation.