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

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INTRODUCTION

The cotton textile industry faces significant challenges due to the large amounts of contaminated effluent produced during cotton fiber processing, especially during desizing and pre-washing. To address this, new biotechnological tools are being developed to reduce water and chemical usage, minimizing pollution and using pollutants as feedstock to create valuable molecules.

This work presents the production of a genetically modified bacterial carbohydrate oxidase, N-acetyl glucosamine oxidase (NagOX), to enhance glucose acceptance as a substrate [1]. A key focus is the downstream process, where the enzyme is immobilized in a low-cost cellulosic matrix from various sources via the fusion of a Carbohydrate Binding Module type 3 (CBM3) to the protein sequence (**Figure 1**) [2]. The immobilized enzyme can be used in a continuous process to generate hydrogen peroxide from cotton textile process wastewater, which can then serve as a bleaching agent for cotton goods.

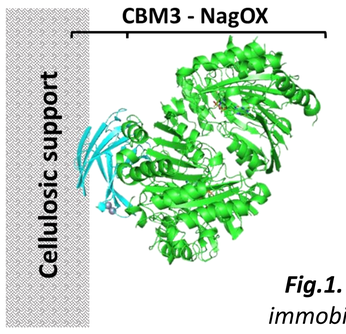
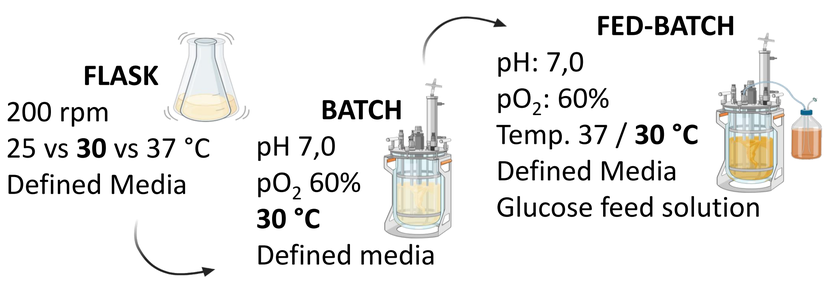


Fig.1. *CBM3-NagOX immobilization scheme*

METHODS

Enzyme production



Purification & immobilization

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 AU/g support
- 25 ºC
- pH 7.0

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

RESULTS

1 NagOX-CBM3-SUMO-His production

NagOX fused to His-SUMO-CBM3 at the N-terminus is produced in an antibiotic-free system with the auxotrophic *E.coli* strain M15ΔglyA [3]. The optimal induction temperature for enzyme synthesis is evaluated in flasks. Subsequently, batch production and culture intensification are carried out through a fed-batch strategy in 2 L bioreactor (**Figure 2**). Intensification allows reaching biomass concentration of **56.6 gDCW/L** compared to the **6.9 gDCW/L** achieved in batch. Similar maximum specific activities are observed in the three expression systems. Fed-batch production system augments volumetric activity 12-fold and enhances productivity 5-fold compared to the results obtained in batch (**Table 1**).

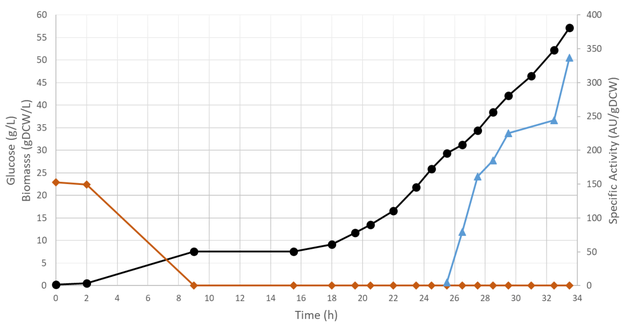


Fig.2. *Production profile of the His-SUMO-CBM3 fused NagOxL251R in a fed-batch strategy with E. coli M15ΔglyA pVEF. Culture conditions are 37 °C (batch), 30 °C (fed-batch), 60% pO2 and pH 7.0.* The data shown in the graph are about: (●) Biomass, (⧫) Glucose and (▲) Specific Activity.

Strategy	Specific activity (AU/gDCW)	Specific activity (AU/L)	Productivity (AU/L·h)
Flask	322.10	753.40	68.50
Batch	296.50	1594.20	107.10
Fed-Batch	336.90	19250.00	574.60

Tab.1. *Specific activity, volumetric activity and productivity results in different expression systems used for the expression of NagOX fused with His-SUMO-CBM3*

2 Immobilization of NagOX-CBM3-SUMO-His

The Perloza MT100 (Medium) cellulosic support was selected as the one with superior performance in FPLC (**Figure 3**), so it was chosen to immobilize the CBM3-NagOX enzyme (**Figure 4**). Results indicated efficient enzyme retention, with immobilization yields of **95%**, a recovered activity of **90%** and a maximum enzymatic load of **97.08 AU/g support**.

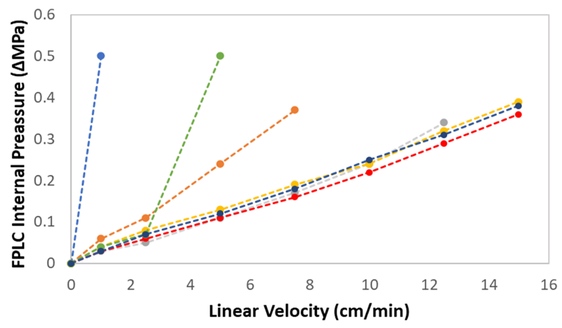


Fig.3. *Pressure-flow experiments of the different cellulosic supports. The supports studied are: (●) amorphous cellulose, (●) MT500 (Extra Fine), (●) microcrystalline cellulose, (●) MT500 (Medium), (●) ST (Fine), (●) MT100 (Medium), (●) MT100 (Extra Fine).*

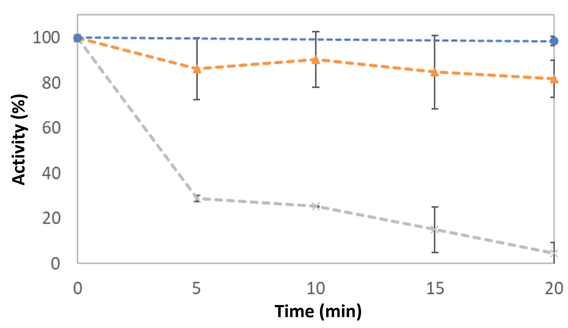


Fig.4. *Immobilization kinetic of CBM3-NagOX in Perloza MT100 (Medium) support. The immobilization samples are: (●) Control, (▲) Suspension and (✕) Supernatant.*

3 Oxidative and Catalase Activities

Using glucose as the main carbon source may promote the expression of endogenous *E. coli* catalases, HPI and HPII, which could mask the oxidative activity and underestimate NagOX activity. Previously, switching from glucose to glycerol was considered, but no improvement was observed. Therefore, the reduction of catalase activity by one-step purification and immobilization processes was assessed. With the CBM3 and x6 His tags present in the construct, enzyme purification/immobilization using microcrystalline cellulose and Sepharose-Ni^{+2} was compared (**Table 2**). Results confirmed that immobilizing NagOX-CBM3-SUMO-His tag on cellulosic supports like Perloza® MT100 is a better strategy for enhancing specificity and oxidative activity compared to Sepharose-Ni^{+2}.

Support	Catalytic activity ($AU/g_{support}$) Oxidative activity	Catalase activity
Sepharose - Ni^{+2}	5.40	51.30
Perloza® MT100 Medium	19.70	0

Tab.2. *Comparative Analysis of Oxidative and Catalase Activities between NagOX-CBM3-Perloza and NagOX-His Tag-Sepharose-Ni^{+2}*

CONCLUSIONS

- **Increased Biomass Concentration**
  Fed-batch strategy reaches 56.6 gDCW/L versus 6.9 gDCW/L in batch.
- **Higher Volumetric Activity**
  Fed-batch increases volumetric activity 12-fold compared to batch.
- **Improved Productivity**
  Productivity in fed-batch was increased 5-fold compared to batch.
- **Efficient Enzyme Immobilization**
  Immobilization with CBM3 achieves 95% immobilization yield and 90% recovered activity.
- **Superior Immobilization for Oxidative Activity**
  Immobilization with CBM3 in Perloza MT100 (Medium) enhances specificity and oxidative activity better than with His tag in Sepharose-Ni^{+2}.

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