Immobilization of *Tth*LPMO9G on Carbon Felt for potential Electrochemical Applications

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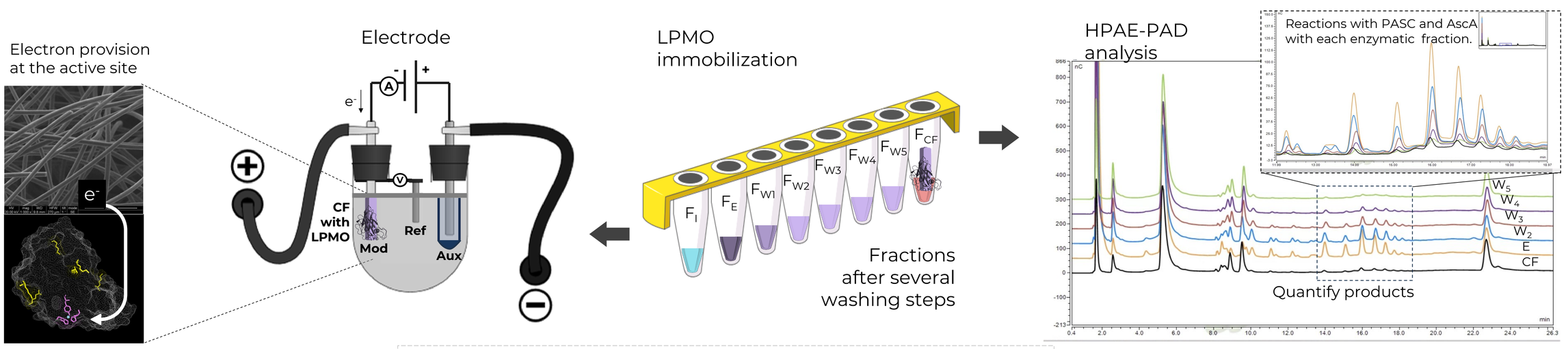
Introduction

This study investigates the immobilization of lytic polysaccharide monooxygenases (LPMOs) on conductive carbon material to eventually facilitate electron transfer directly to the enzyme's active site. LPMOs are challenging to work with due to their specific features and fragile active site, making optimization essential in the immobilization process. By establishing methods for stable LPMO attachment and controlled electron provision, this project lays the groundwork for advancing electrochemical applications in lignocellulose degradation, potentially marking the first instance of external electron provision to an immobilized LPMO.

Methods



1. *Yield (%) = 100 × (immobilised activity/starting activity)*
2. *Efficiency (%) = 100 × (observed activity/immobilised activity)*
3. *Activity recovery (%) = 100 × (observed activity/starting activity)*

- *Immobilised activity = I - (E+W1+W2+W3+W4+W5)*
- *Starting activity = I*
- *Observed activity = CF*

LPMO Immobilization on Carbon Felt:

- Oxidize the carbon felt to introduce carboxyl groups **(-COOH)**.
- Activate carboxyl groups using EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide).
- Stabilize with Sulfo-NHS (N-hydroxysulfosuccinimide) to form amide bonds for covalent enzyme attachment.

Procedure Optimization:

- Assess the carboxyl (COOH) content of the carbon felt after oxidation.
- Test different buffers for optimal immobilization conditions.
- Optimize reaction times to enhance measurable PASC oxidation activity.

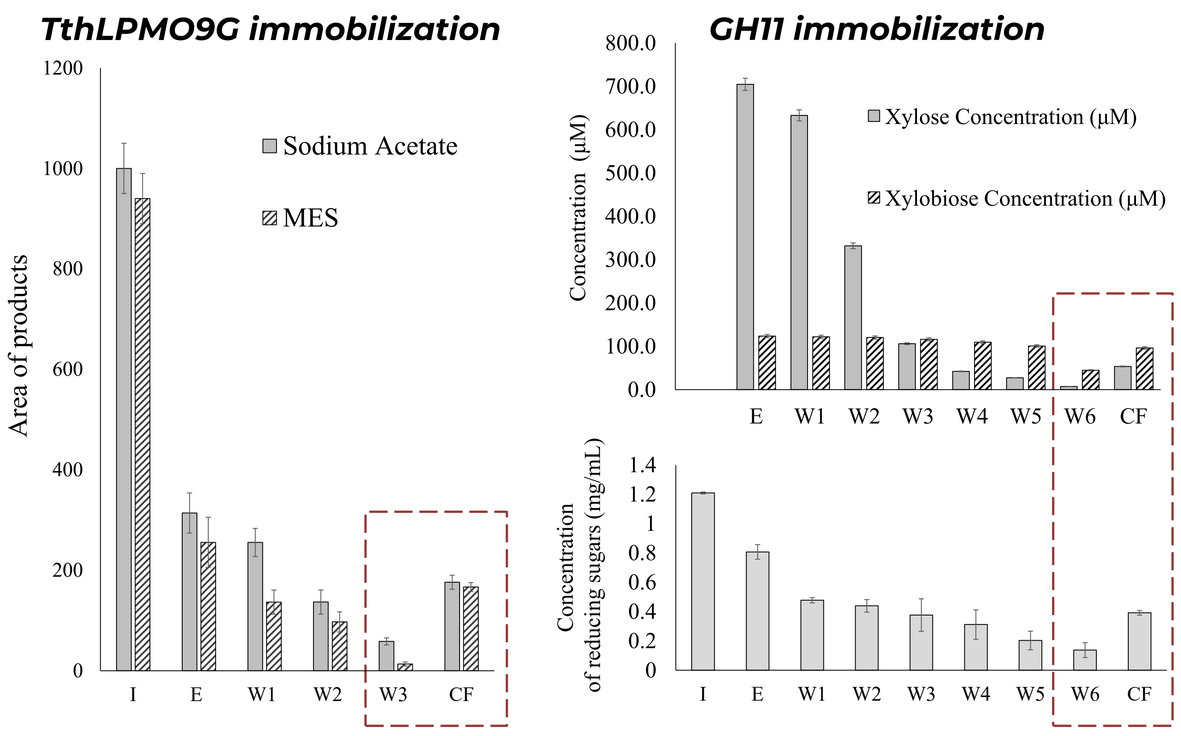
Determining Immobilization Yield, Efficiency and Recovery:

- Conduct reactions using cellulose and a reducing agent with the immobilized enzyme on carbon felt.
- Measure the activity after washing steps to confirm how much enzyme remained immobilized.

Electron Provision via Electrode:

After successful immobilization, test the LPMO activity by oxidizing PASC without reducing agent and assess electron transfer through the electrode.

Results



Immobilization optimization parameters		Reaction optimization parameters			
Enzyme Immobilized	**Content of COOH on CF (mol -COOH / g CF)**	**Hours of Fractions incubated with PASC**	**Yield (%)**	**Efficiency (%)**	**Activity recovery (%)**
Xylanase GH11	0.0206	24	37.69	21.62	8.15
*Tth*LPMO9G → *optimization of immobilization steps*	0.0005	24	49.77	6.73	3.35
*Tth*LPMO9G → *evaluation of stability of immobilized enzyme (second reaction cycle)*	0.0018	24	63.27	6.06	3.83
*Tth*LPMO9G	0.0018	4	51.55	15.01	7.74
*Tth*LPMO9G	0.0018	24	75.16	1.71	1.28
*Tth*LPMO9G	0.0018	72	76.40	0.76	0.58

Note: Highlighted indicates the optimized parameter for each immobilization batch

Bottlenecks for LPMO immobilization

- LPMO experiences inactivation during enzyme reactions aimed at oxidizing PASC, which complicates accurate activity determination.
- The oxidized carbon felt control generates a high background signal, interfering with activity measurements using the 2,6-DMP assay.
- High background signals from oxidized carbon felt also impact protein concentration measurements at 280 nm, making it difficult to accurately quantify enzyme loading.
- Direct electron provision to the enzyme was not achieved, as the cellulose substrate was attached to the cellulose fibers.
- When measured with voltammetry (FCV) in the absence of substrate, the carbon felt with immobilized enzyme displayed an oxidation and reduction pattern similar to that of carbon felt alone, indicating no direct electron transfer to the enzyme.

Conclusions

1. Parameter Optimization: MES buffer provided better stability than sodium acetate buffer. Additionally, a higher carboxyl content on oxidized carbon felt, measured by conductometric titration, resulted in improved enzyme immobilization. Final reactions with PASC oxidation indicated that a 4-hour reaction time produced better results than 24- or 72-hour reactions.
2. Procedure Standardization: Xylanase GH11 immobilization was effective in releasing reducing sugars (DNS method) and xylose, xylobiose (HPAEC-PAD), establishing a standardized protocol baseline for LPMOs.
3. Electron Transfer: Electrode-based electron transfer was not achieved, pointing to an area for future work with immobilized enzymes.

Future work for LPMO immobilization

- Optimize Immobilization Conditions: Further refine buffer selection, carbon felt oxidation levels, linker chemistry, and reaction times to enhance enzyme stability and activity.
- Enhance Electron Transfer Mechanisms: Develop methods to achieve consistent electron transfer via electrodes, potentially using conductive coatings, electron mediators, or linkers between the enzyme and carbon felt.

Acknowledgments

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