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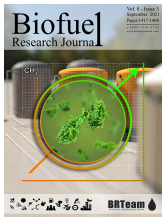

Original Research Paper

Combining pieces: a thorough analysis of light activation boosting power and co-substrate preferences for the catalytic efficiency of lytic polysaccharide monooxygenase *Mt*LPMO9A

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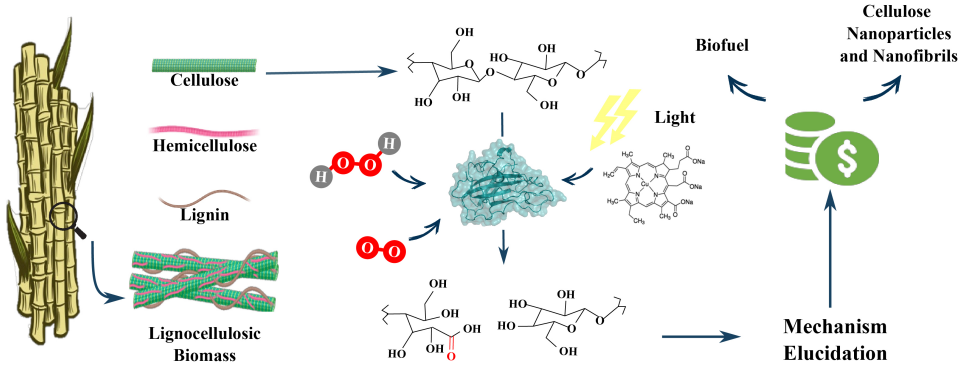
HIGHLIGHTS

- *Mt*LPMO9A can be efficiently activated by light at the presence of chlorophyllin.
- Photoactivation improves performance of *Mt*LPMO9A on crystalline cellulose.
- *Mt*LPMO9A can use both O_2 and H_2O_2 as co-substrates.
- For H_2O_2 reactions *Mt*LPMO9A requires chemical reductant.
- Photobiocatalysis mediated by LPMOs might play a role in plant biomass valorization.

GRAPHICAL ABSTRACT



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ABSTRACT

Cost-efficient plant biomass conversion using biochemical and/or chemical routes is essential for transitioning to sustainable chemical technologies and renewable biofuels. Lytic polysaccharide monooxygenases (LPMOs) are copper-dependent enzymes that make part of modern hydrolytic cocktails destined for plant biomass degradation. Here, we characterized *Mt*LPMO9A from *Thermothelomyces thermophilus* M77 (formerly *Myceliophthora thermophila*) and demonstrated that it could be efficiently driven by chlorophyllin excited by light in the presence of a reductant agent. However, in the absence of chemical reductant, chlorophyllin and light alone do not lead to a significant release of the reaction products by the LPMO, indicating a low capacity of *Mt*LPMO9A reduction (either *via* direct electron transfer or *via* superoxide ion, $O_2^{\bullet-}$). We showed that photocatalysis could significantly increase the LPMO activity against highly crystalline and recalcitrant cellulosic substrates, which are poorly degraded in the absence of chlorophyllin and light. We also evaluated the use of co-substrates by *Mt*LPMO9A, revealing that the enzyme can use both hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) as co-substrates for cellulose catalytic oxidation.

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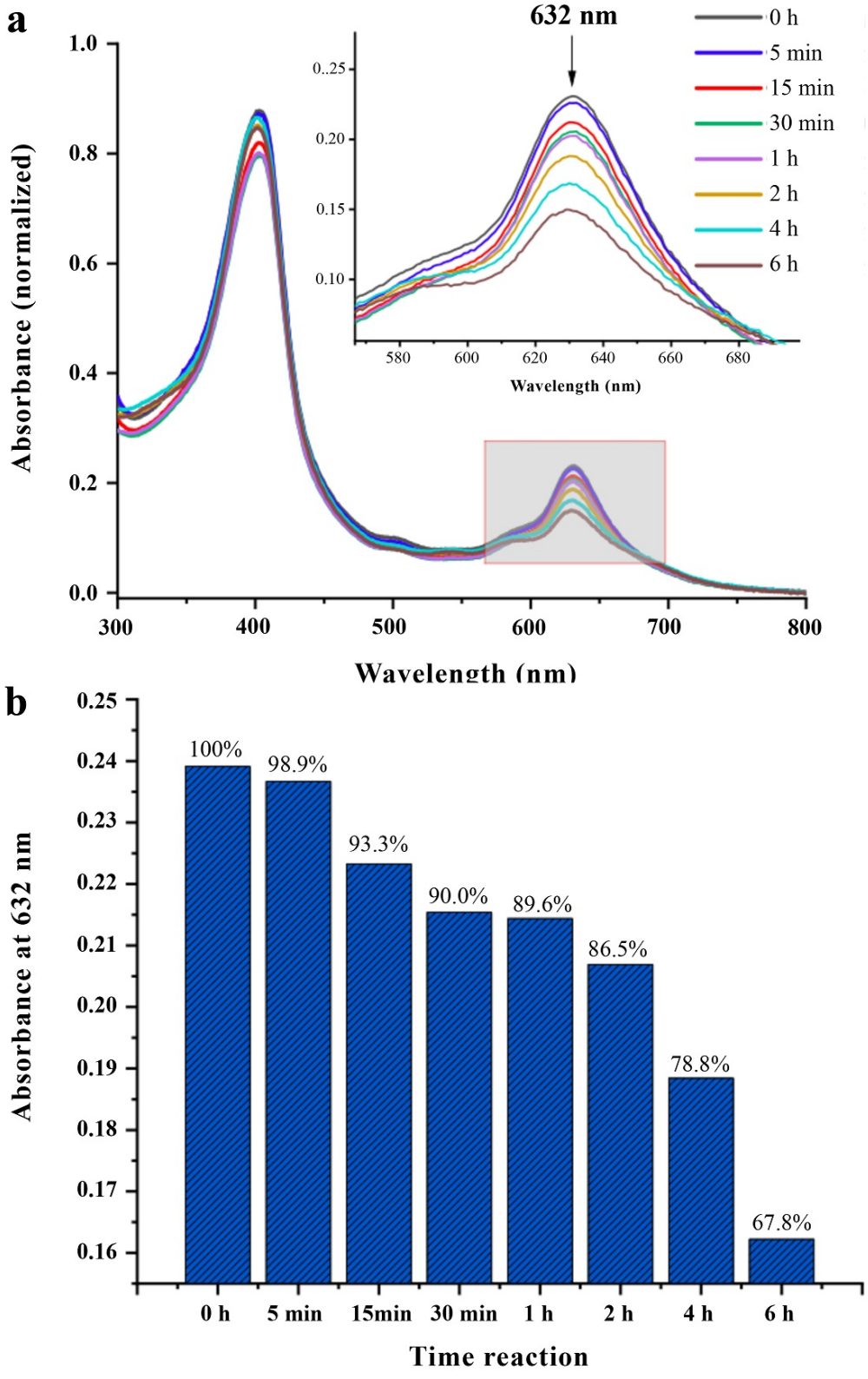

Fig. S1. Assessment of chlorophyllin degradation in reactions over time in the presence of AscA. **(a)** Evaluation of the behavior of chlorophyllin absorbance spectra over time (632 nm peak) and **(b)** Quantitative trend of chlorophyllin absorbance over time.

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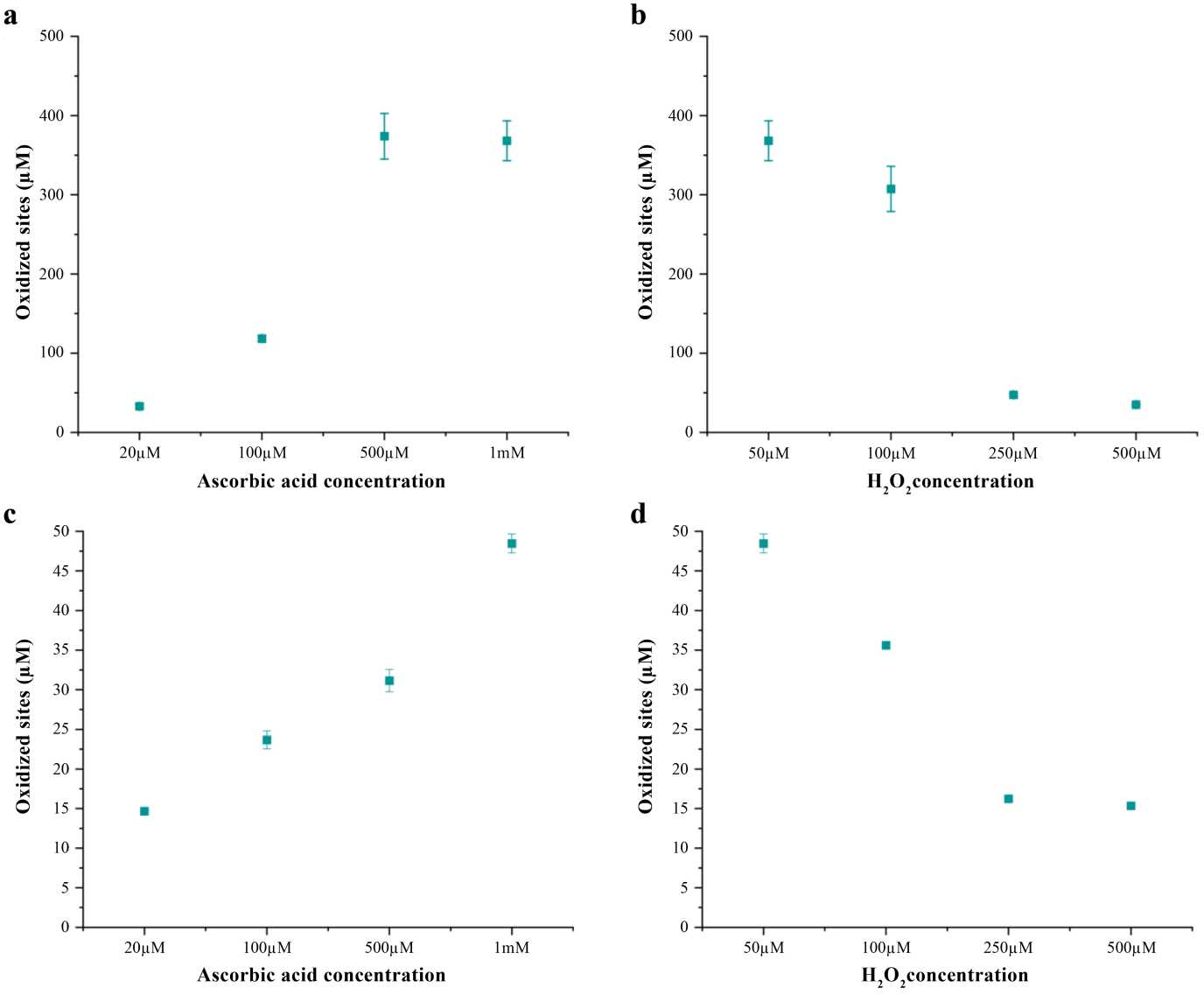

Fig. S2. Evaluation of the optimum conditions for peroxygenase reaction of *Mt*LPMO9A. Effects of ascorbic acid and H_2O_2 on *Mt*LPMO9A activity on PASC and Avicel were evaluated as follows: **(a)** reaction was performed with a fixed H_2O_2 concentration of 50 µM, varying the amount of ascorbic acid from 20 µM to 1mM on PASC as a substrate; **(b)** Using PASC as a substrate, ascorbic acid concentration was fixed at 1mM and the amount of H_2O_2 was varied from 50 µM to 500 µM. **(c)** Using Avicel as a substrate, reaction was performed with fixed H_2O_2 concentration of 50 µM and varying the concentration of ascorbic acid from 20 µM to 1mM. **(d)** Using Avicel as a substrate, ascorbic acid concentration was fixed at 1mM and the concentration of H_2O_2 was varied from 50 µM to 500 µM. Reactions were performed for 4 h at 50 ºC and under constant agitation of 1000 rpm.

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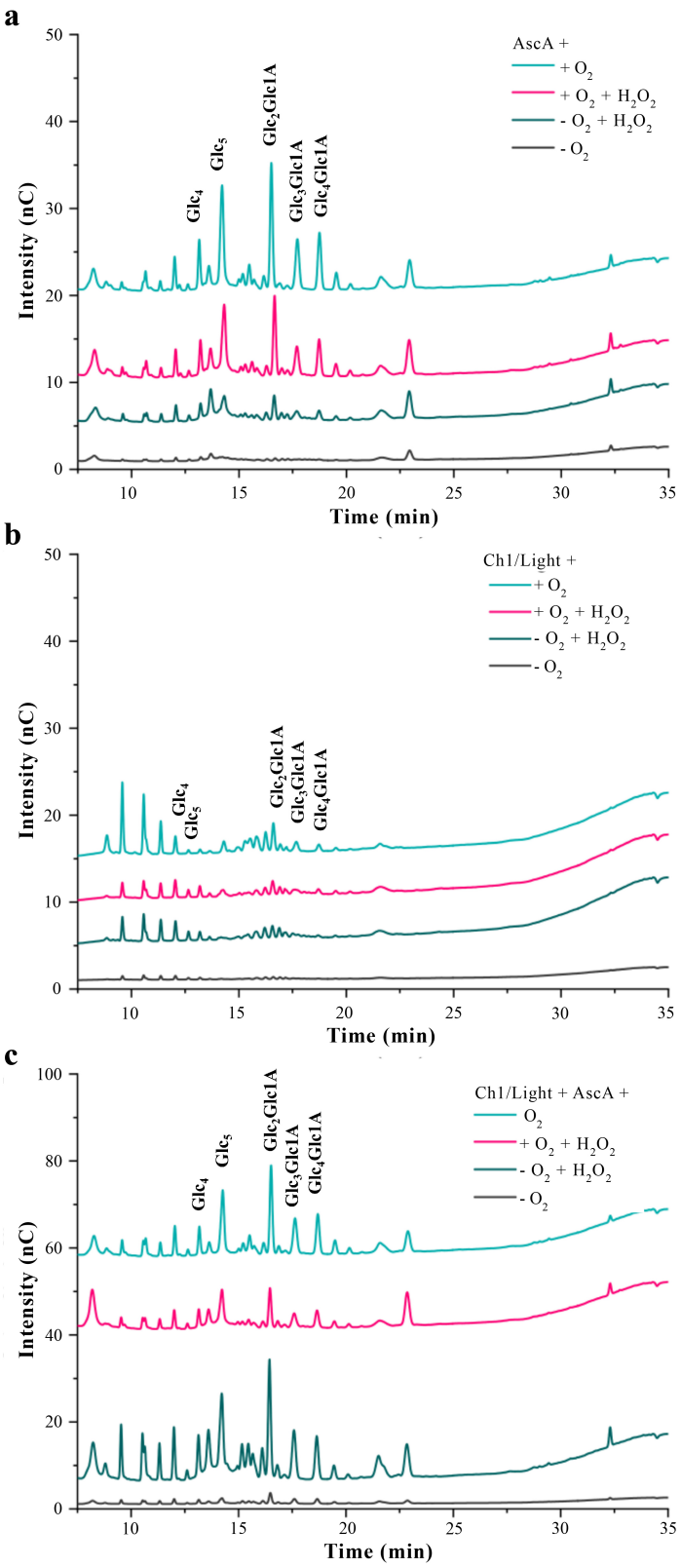

Fig. S3. Evaluation of *Mt*LPMO9A activity under different oxygen/hydrogen peroxide conditions on Avicel. *Mt*LPMO9A activity was evaluated on Avicel for 30 min using different combinations of co-substrates: **(a)** ascorbic acid as an electron donor ± O_2 ± H_2O_2, **(b)** photoactivated system with 500µM of chlorophyllin ± O_2 ± H_2O_2, and **(c)** coupled photoactivated system that combines 1mM of ascorbic acid with 500µM of chlorophyllin ± O_2 ± H_2O_2. The highlighted non-oxidized oligosaccharides are Glc4 (cellotetraose) and Glc5 (cellopentaose). C1-oxidized oligosaccharides are GlcGlc1A (cellobionic acid), Glc2Glc1A (cellotrionic acid), Glc3Glc1A (cellotetraonic acid) and Glc4Glc1A (cellopentaonic acid). Peak assignment was done using standards.

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