



# Biomass Hydrolysis: Enzyme Synergies

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## Introduction

Cellulose is the principle component of plant biomass that is currently used for fuels and productions. Cellulose (Figure 1) is a polymer that must be transformed into its individual glucose molecules for subsequent fermentation to ethanol or other biofuels.

This transformation is typically performed using cellulase enzymes and is termed hydrolysis.

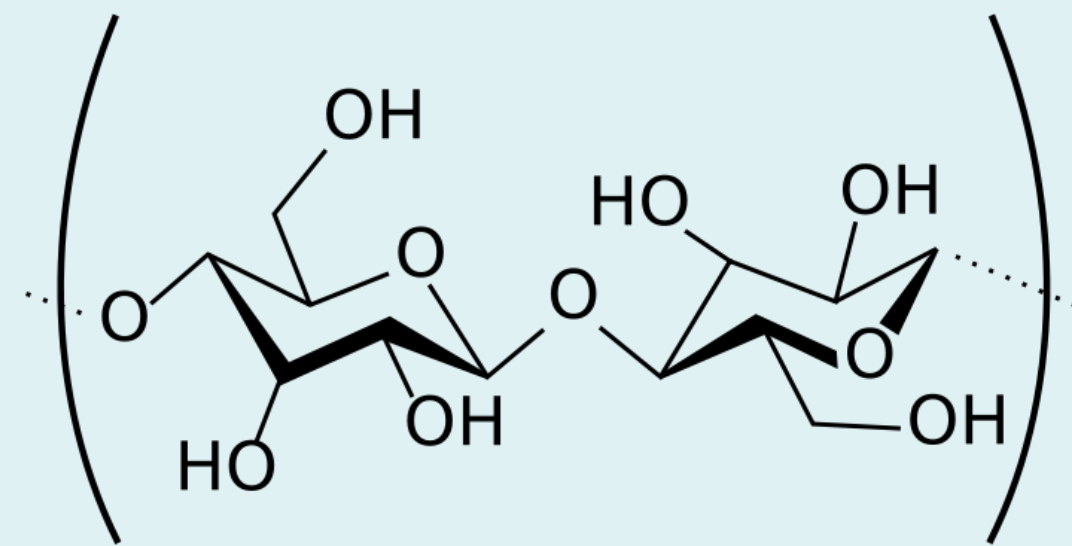


Figure 1. Cellulose

One of the major operating costs of the biological conversion of plant biomass into ethanol is cellulase enzymes. The role of the ligninolytic enzyme manganese peroxidase (Figure 2) in reducing cellulase use was investigated.

MnP is able to degrade lignin and may be able to reduce cellulase poisoning by lignin or increase cellulose accessibility to cellulases. The catalytic cycle of MnP includes oxidizing a Mn<sup>2+</sup> to Mn<sup>3+</sup>, which diffuses to the lignin substrate and then induces a degradation reaction. The Mn<sup>3+</sup> is stabilized by organic acids. This unique mode of action will allow MnP to be immobilized and thereby reused in a processing strategy.

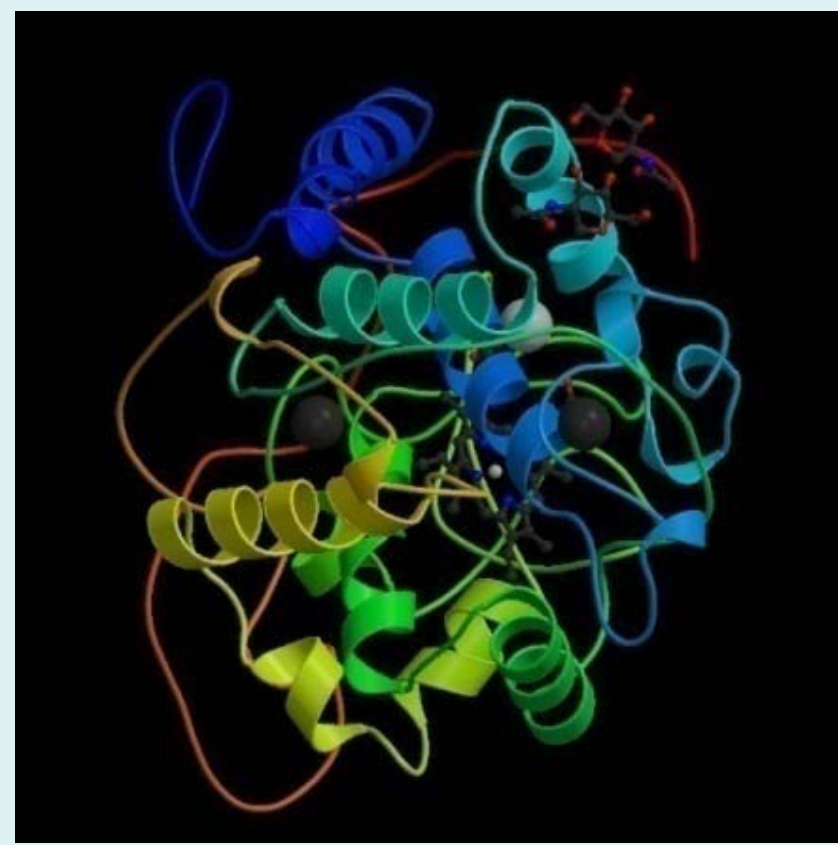
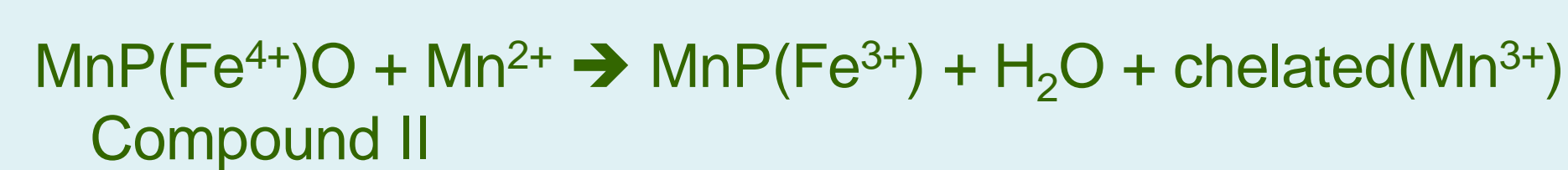
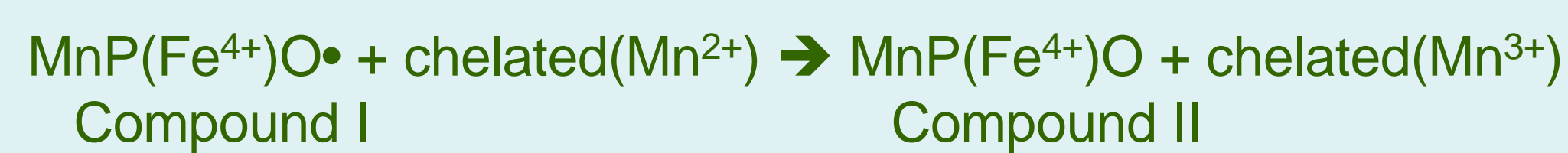
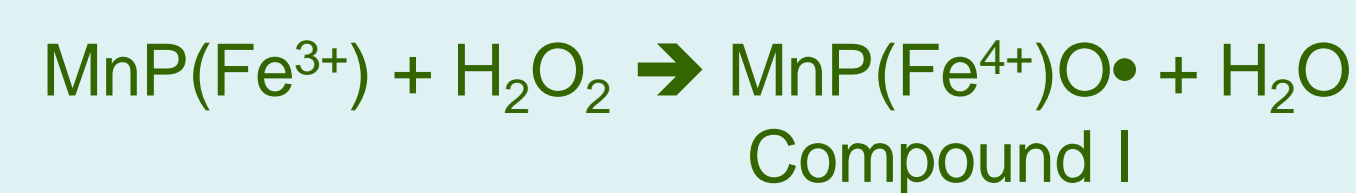


Figure 2. Crystal structure of MnP

### MnP Radical Cycle



### Lignin Radical Initiation



Figure 3. Lignin degradation catalyzed by MnP



## Methodology

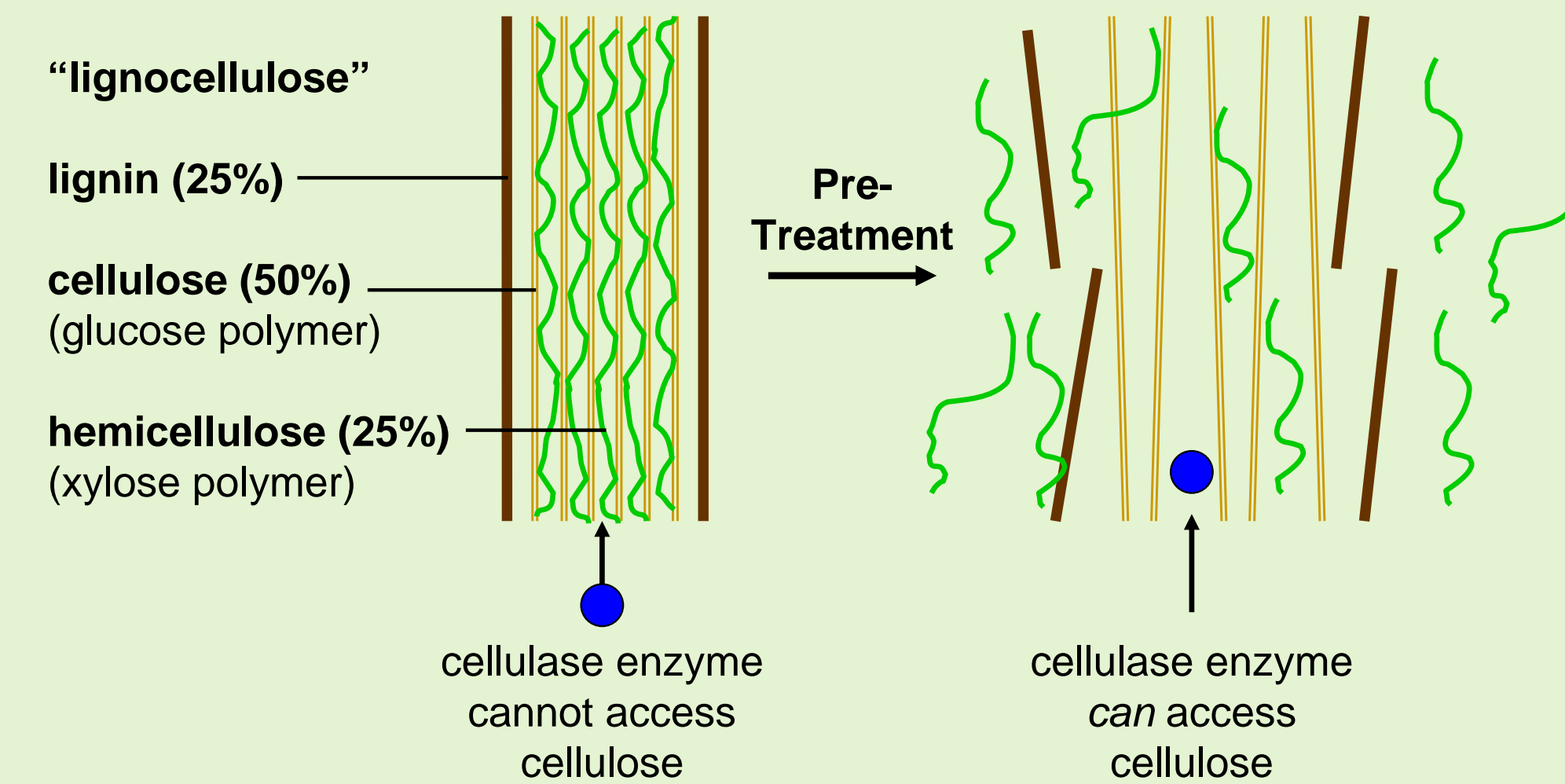


Figure 4. Wood or corn stover was pretreated using acid hydrolysis.



Figure 5. Wood chip and corn stover.

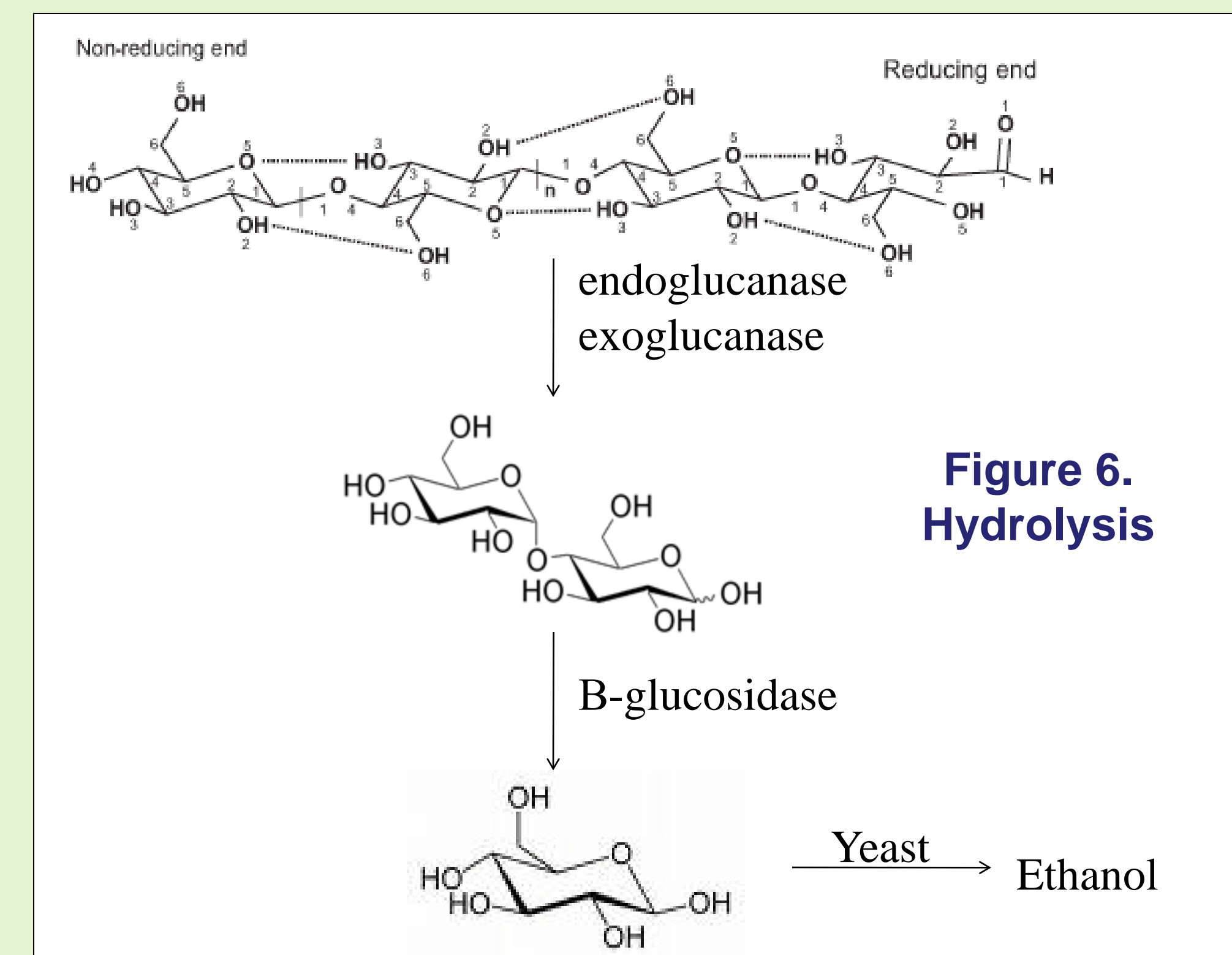
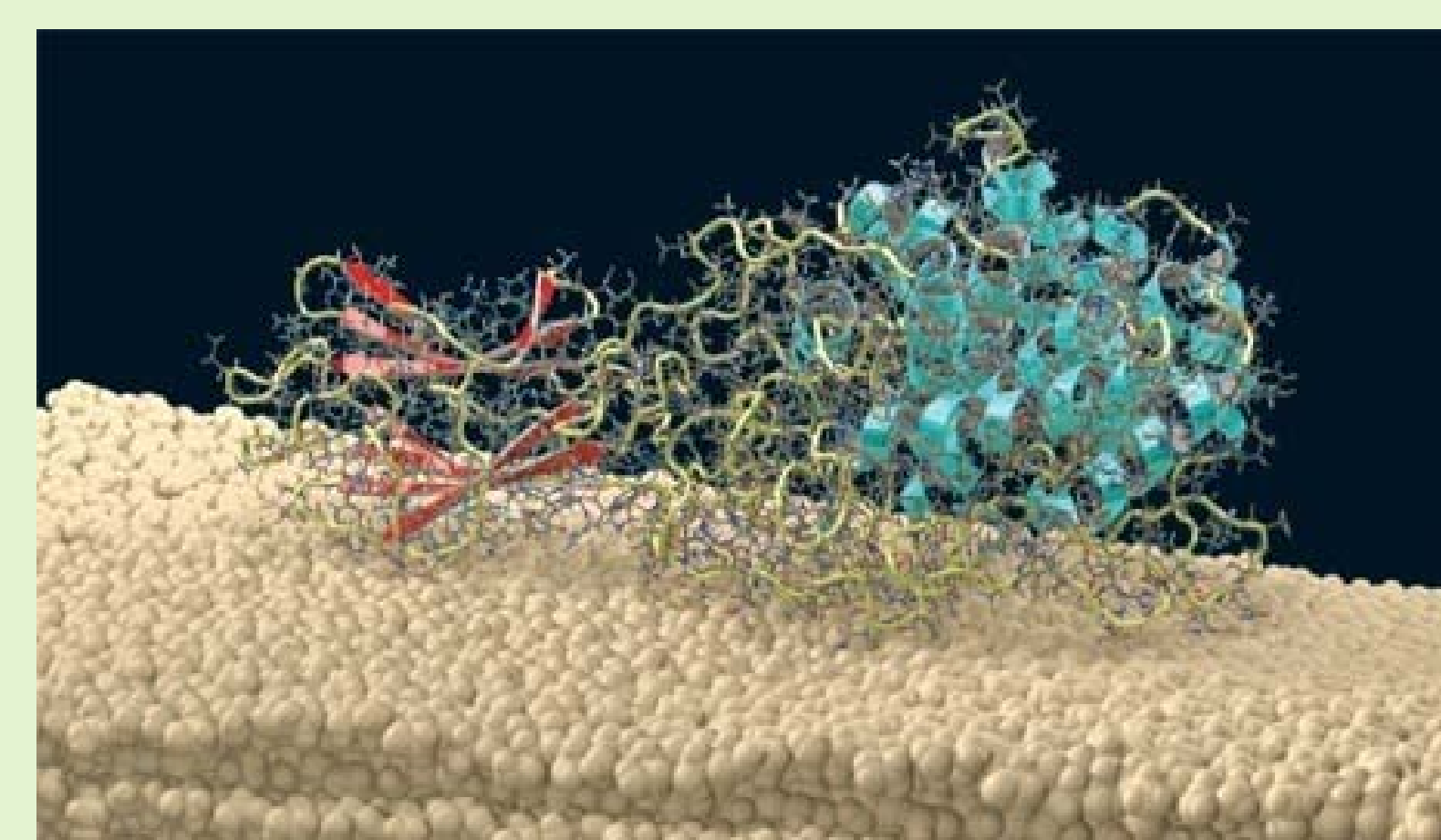


Figure 6. Hydrolysis

Figure 7. Cellulase Enzyme



The effect of rMnP addition to the cellulase hydrolysis of cellulose was investigated. The reaction was carried out in vials with filter paper or pretreated wood biomass as a substrate (Figure 5).

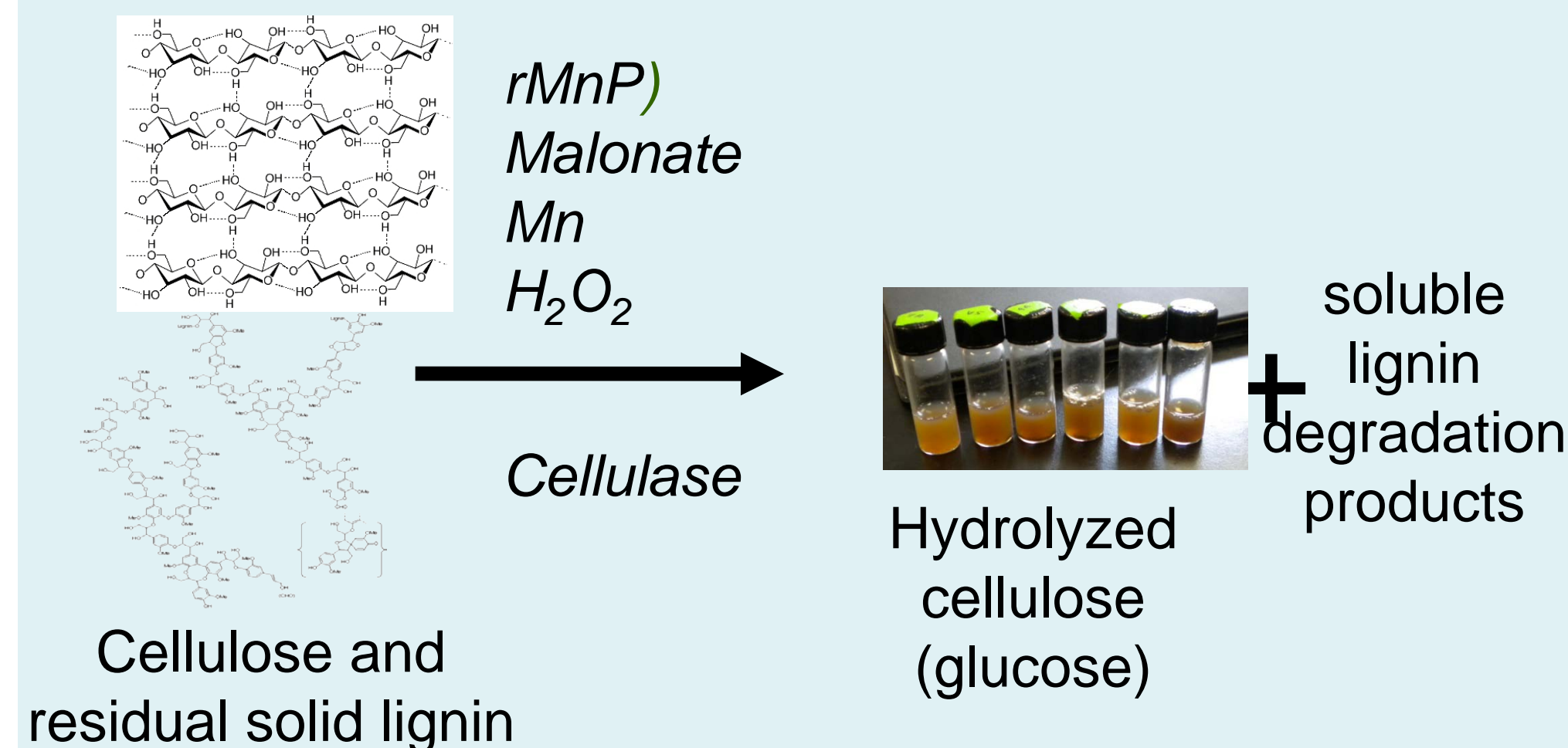


Figure 8. Enzymatic hydrolysis

rMnP was produced in bioreactor cultivation (Figure 9) and concentrated with acetone precipitation, filtration and lyophilization.



Figure 9. Bioreactor Production of rMnP

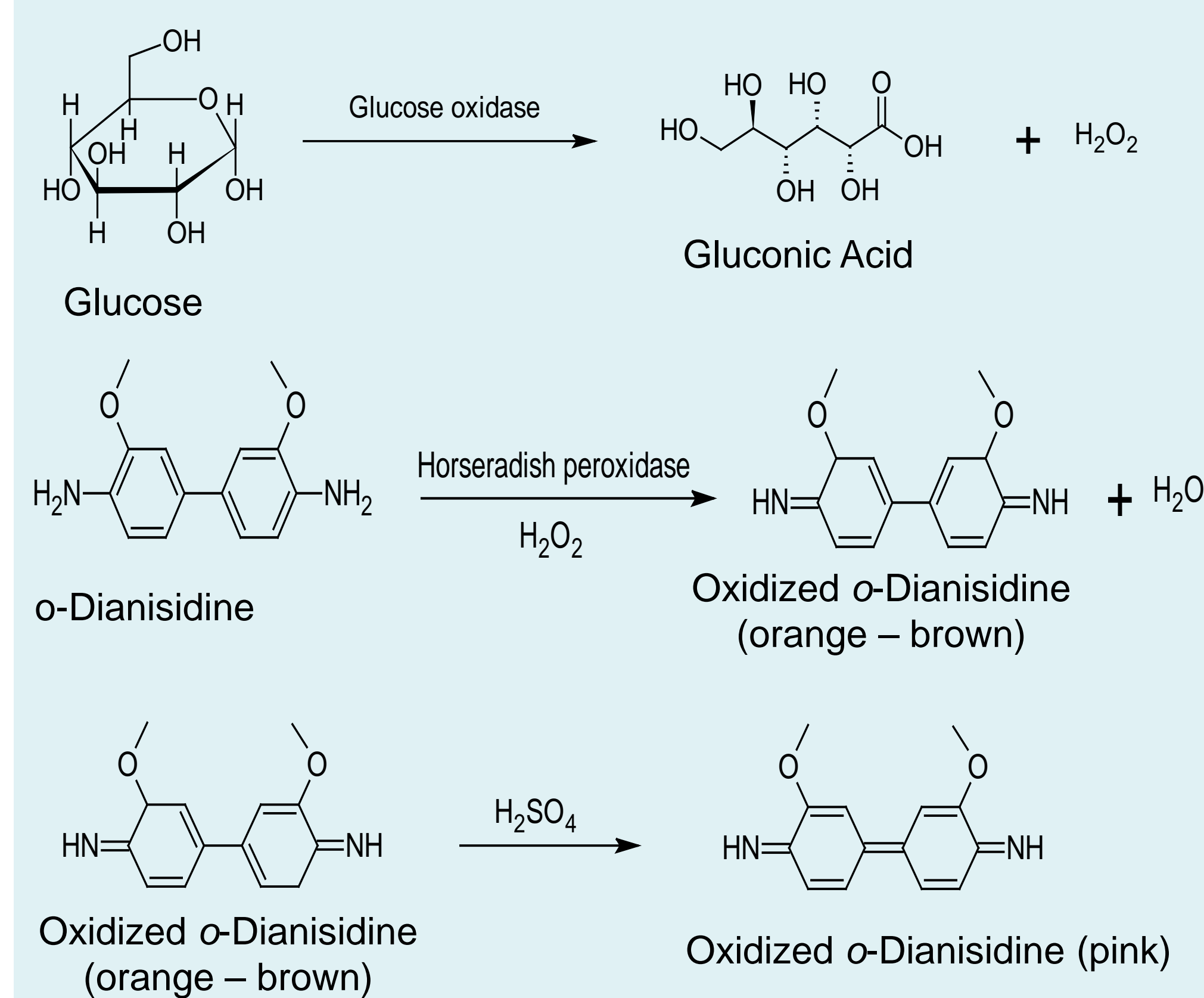


Figure 10. Reaction scheme and plate for the experimental determination of glucose in from hydrolyzed biomass



## Results

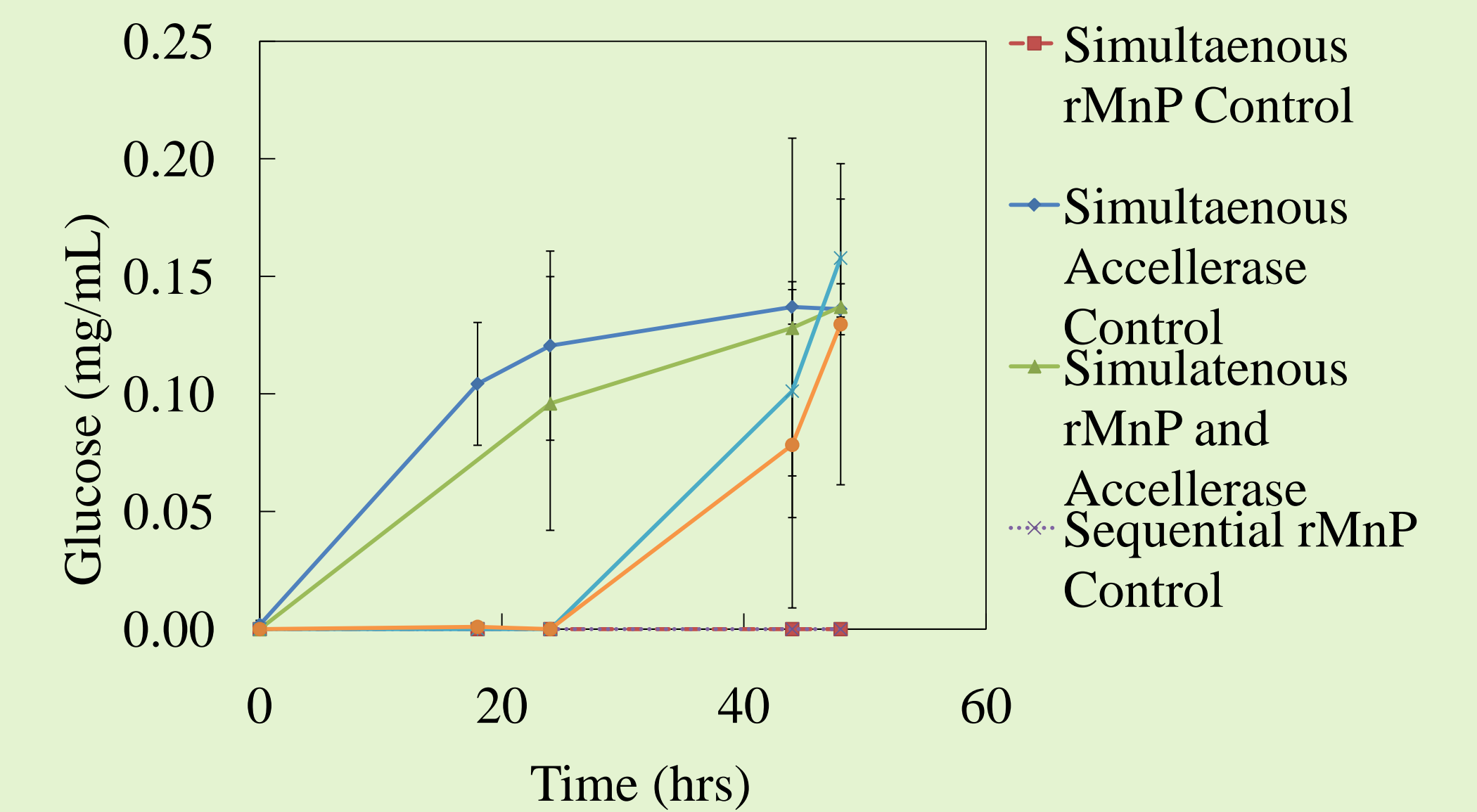


Figure 11. Sequential and simultaneous rMnP and cellulase hydrolysis of pretreated Ponderosa Pine

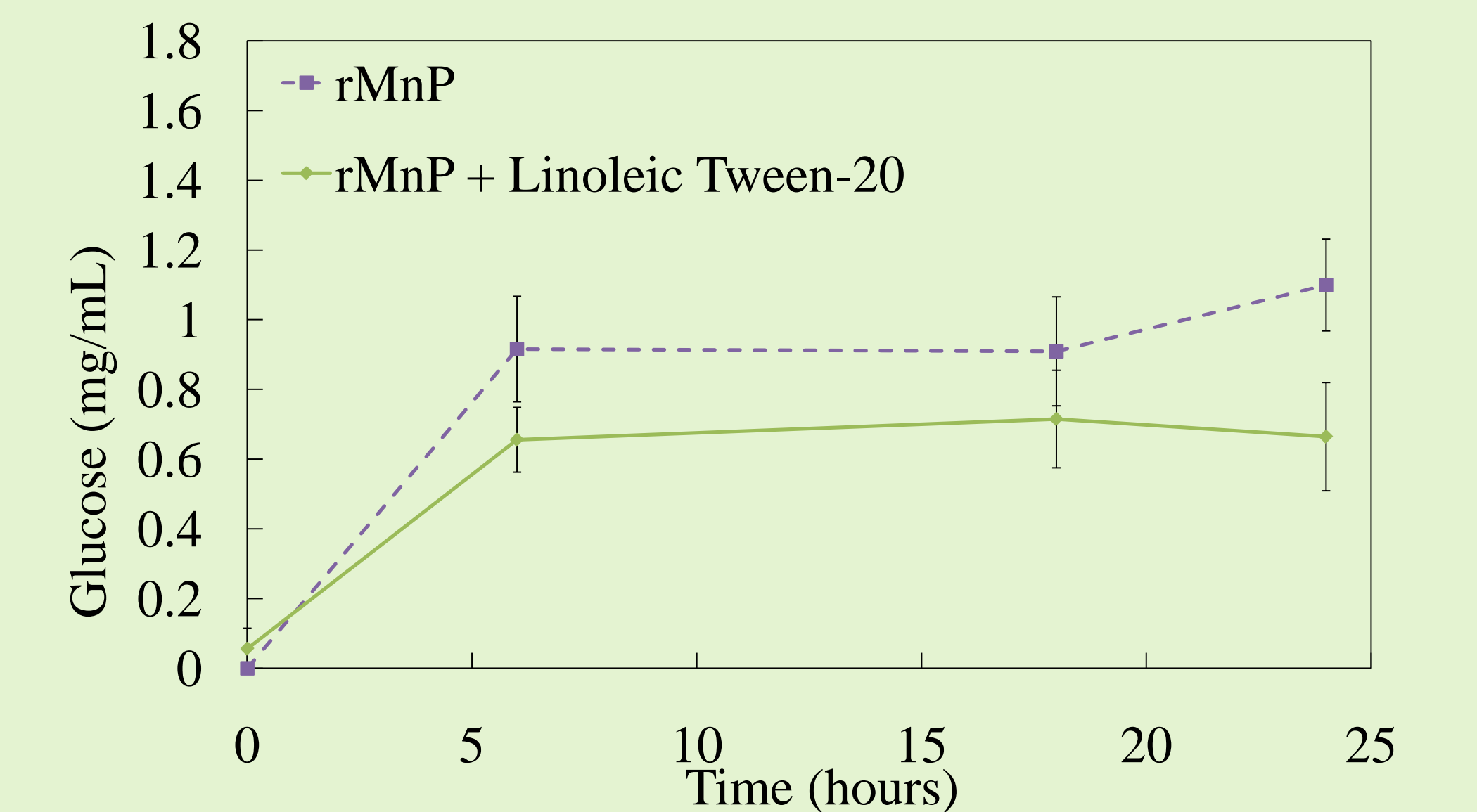


Figure 12. Sequential rMnP and cellulase hydrolysis of pretreated corn stover, with and without the redox mediator linoleic acid

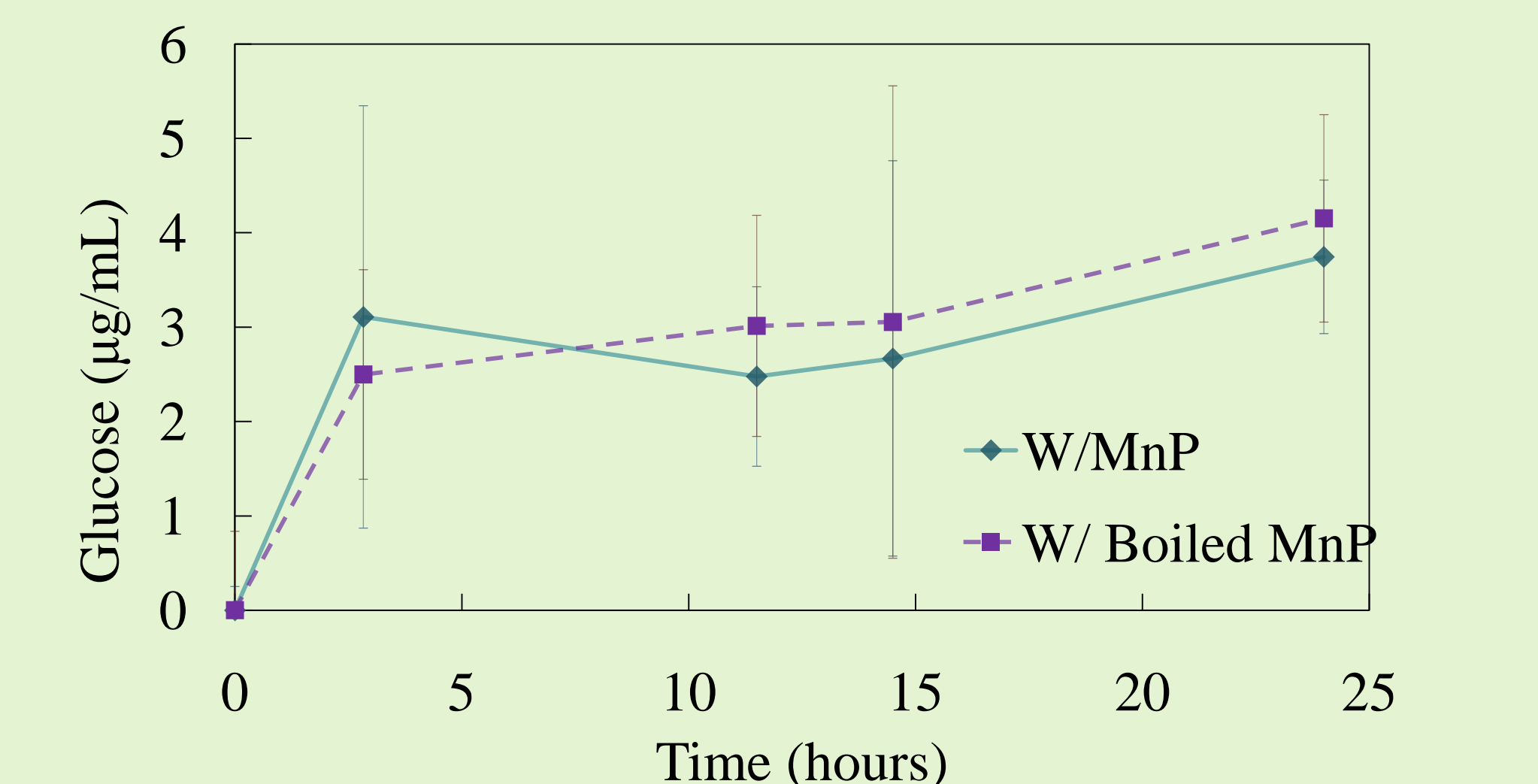


Figure 13. Sequential rMnP and cellulase hydrolysis of pretreated corn stover, with active and inactive rMnP

## Acknowledgements



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