

Effect of wood polymers degradation during heat treatment on extracellular enzymatic activities involved in beech degradation by *Trametes versicolor*

Serge Lekounougou^{a,b}, Gildas Nguila Inari^a, Mathieu Pétrissans^a, Stéphane Dumarçay^a, Jean Pierre Jacquot^b, Eric Gelhaye^b, Philippe Gérardin^a

^aLaboratoire d'Etudes et de Recherches sur le Matériau Bois, UMR_A 1093

^bIFR 110, UMR_A 1136, Interactions Arbres/Micro-organismes

Increasing interest for heat treatment considered as a safe alternative to wood preservation due to its “non biocidal” character

Development of different industrial processes in Europe and Canada

- improved decay resistance

- improved dimensional stability

- colour modification

- reduction of mechanical strength properties

Several reasons have been proposed to explain improved durability

- hydrophobic character of wood

- generation of biocidal components

- modification of wood polymers

- degradation of hemicelluloses

- reduction in cell wall porosity

Aims of this study is to investigate the effects wood polymers modification due to heat treatment on enzymes involved wood degradation

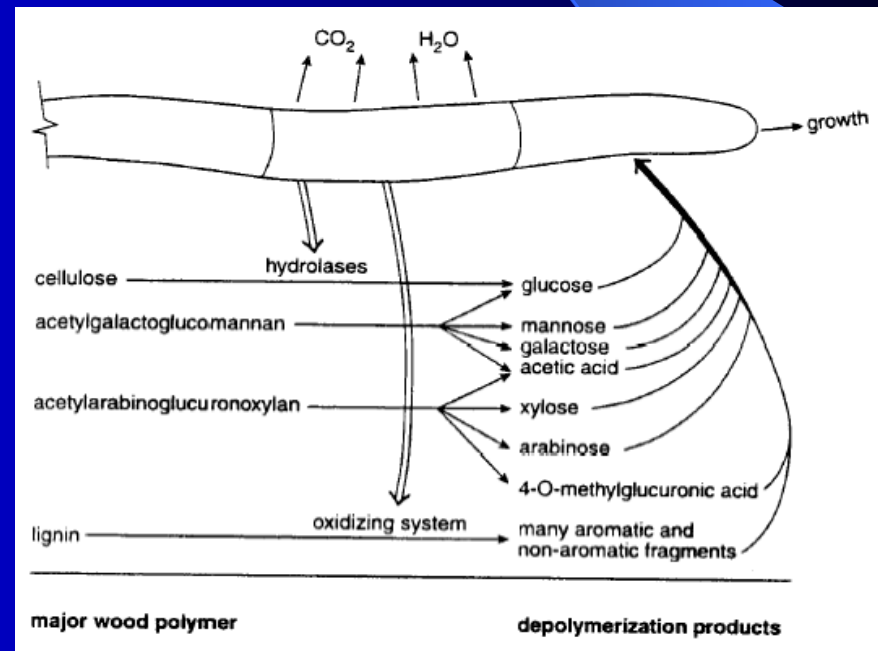
Extracellular enzymes involved in wood degradation by *Trametes versicolor*

◆ Ligninolytic enzymes involved in lignin degradation

- Laccases
- Peroxydases

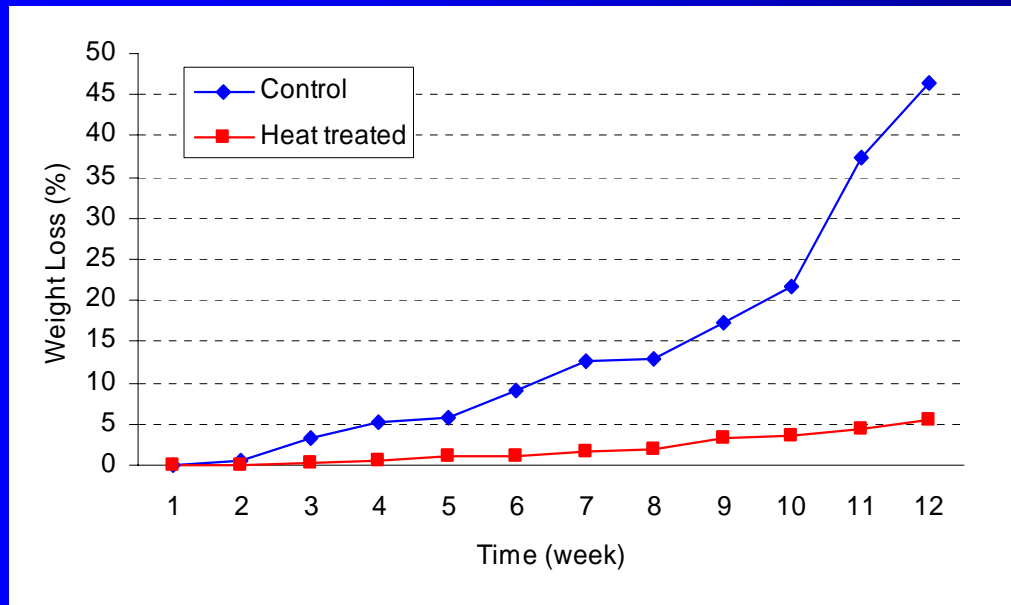
◆ Hydrolytic enzymes involved in polysaccharides degradation

- Cellobiohydrolases
- Endoglucanases
- β -Glucosidases
- Xylanases
- Xylosidases
- Glucuronidases
- Arabinosidases...



Heat treatment was performed of beech blocks under N₂ at 240°C during 8h.
Mass loss = 22% ± 0.5

Blocks were then exposed to *Trametes versicolor* on malt agar medium according to a procedure derived from EN-113 standard for different times, before mass loss and enzymatic activity evaluation

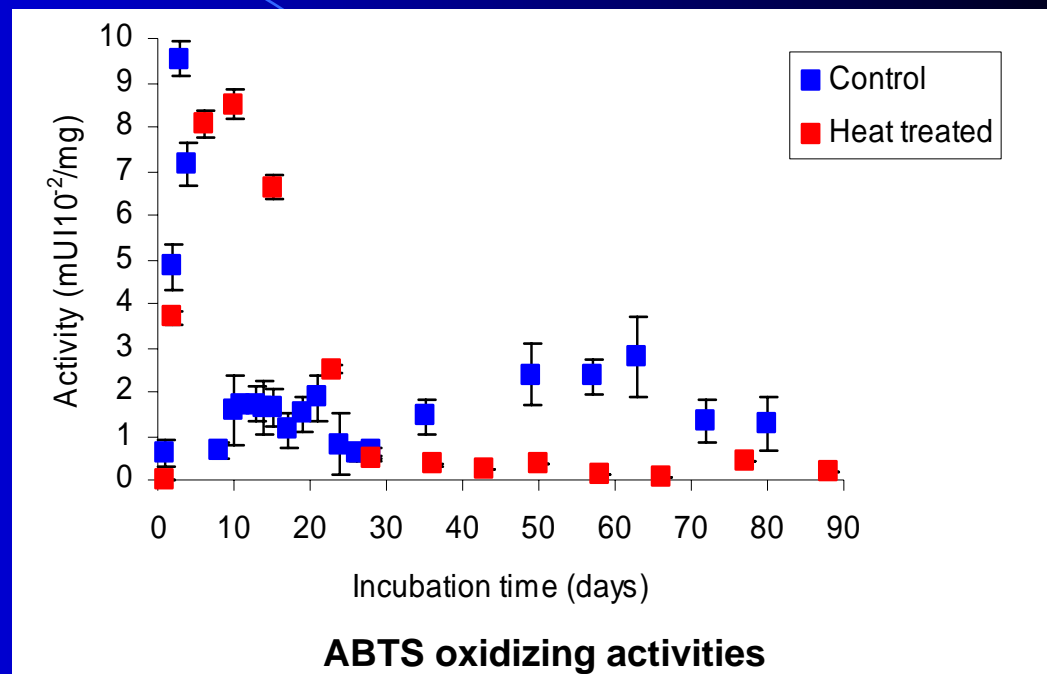
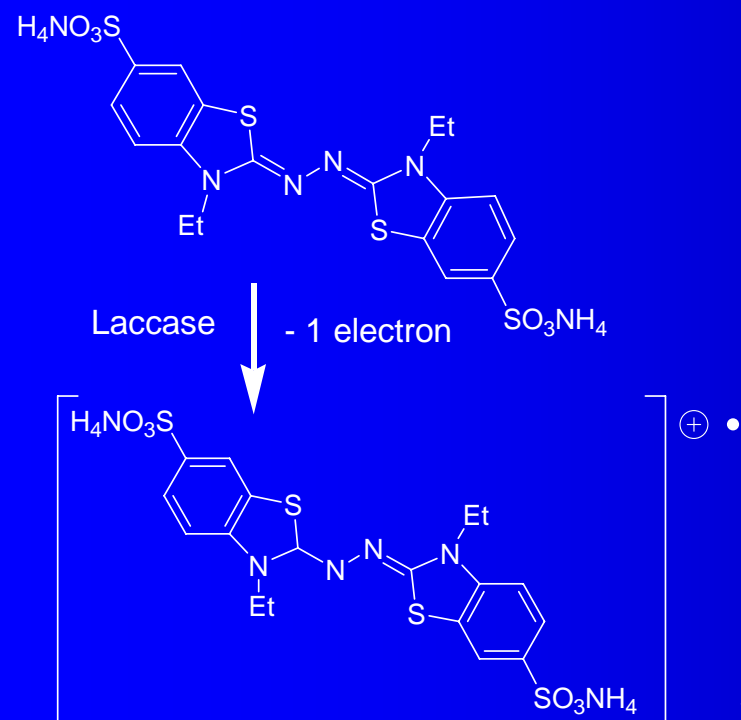


- Controls are deeply colonized and degraded
- Decaying process proceeds in two stages
- Heat treated blocks are weakly colonized and degraded

Mass losses of blocks exposed to *T. versicolor*

Oxidizing activities

Oxidation of ABTS followed by measurement of absorbance at 420 nm

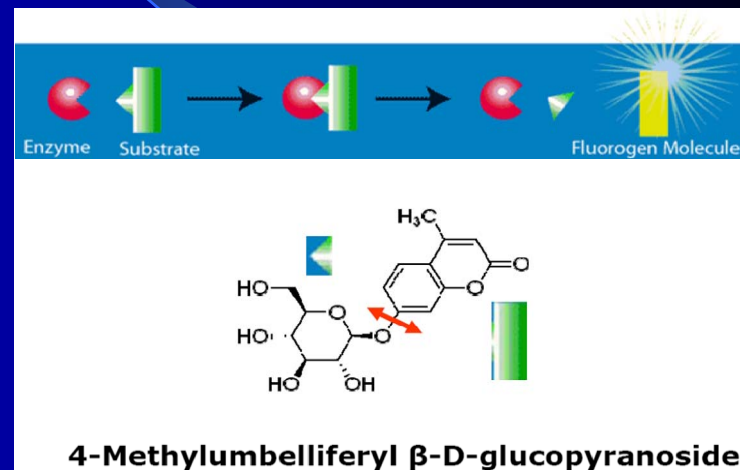


- Important activities during the first incubation days in both cases
- Lower activities in the case of heat treated samples during the the second stage of the experiment

Hydrolytic activities

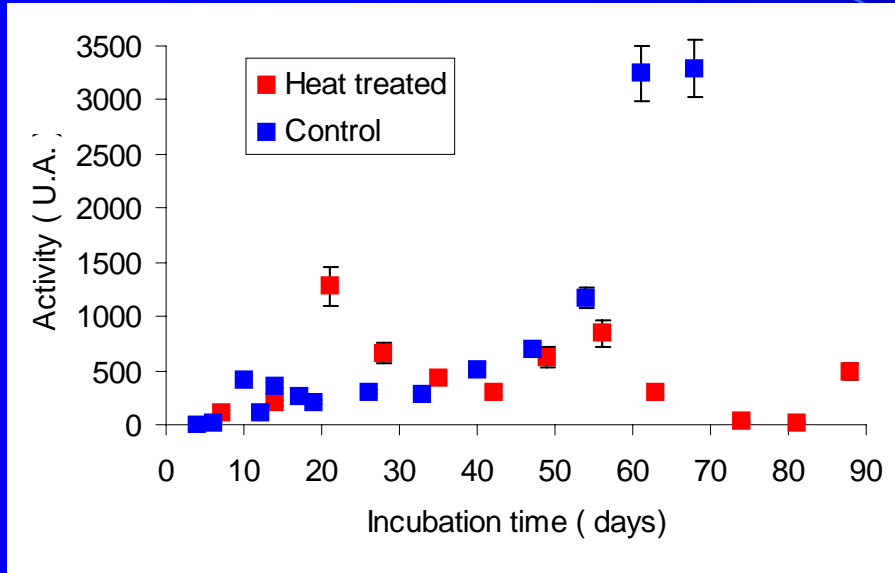
Glucosidase, cellobiohydrolase and chitinase activities were investigated using fluorogenic assays based on marked substrates

- MU- β -D-glucopyranoside for β -glucosidase
- MU- β -D-cellobioside for cellobiohydrolase
- MU-N-acetyl- β -D-glucosaminide for chitinase

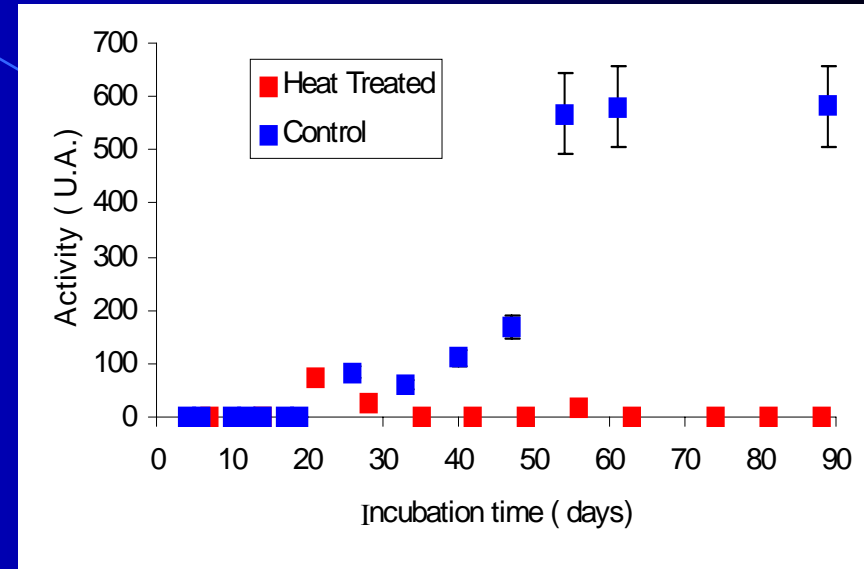


Fluorescence emission is recorded on Fluorescence Spectrophotometer with an excitation wavelength of 360 nm and an emission wavelength of 450 nm

Hydrolytic activities



Glucosidase activity

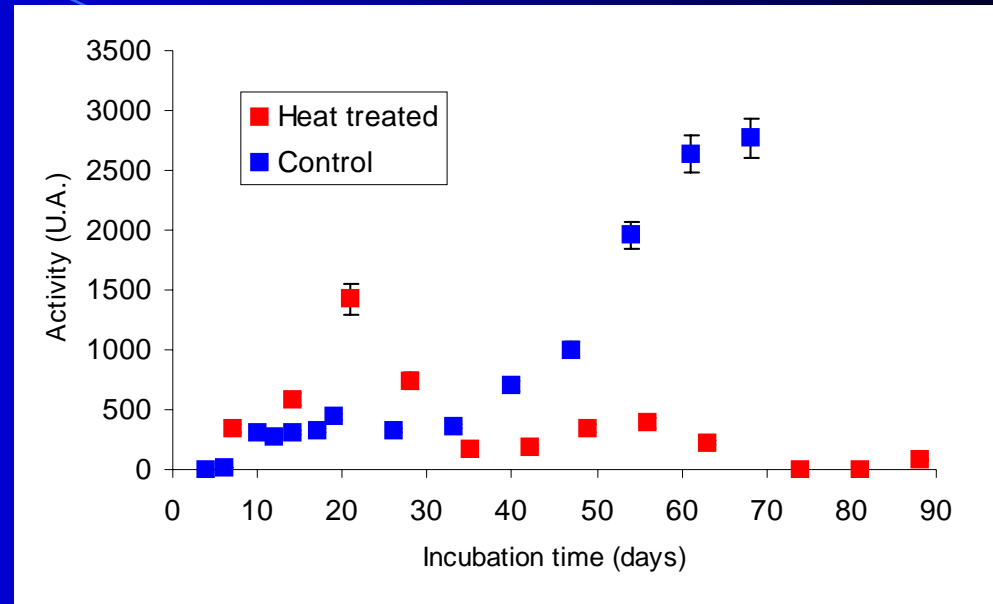


Cellobiohydrolase activity

- Weak activities for both enzymatic activities tested during the first weeks of incubation : previous lignin removal or depletion of easily assimilable malt-agar nutrients
- Increase of activity after approximately 40 days of incubation in the case of controls, while no or weak activities were detected in the case of heat treated samples

Hydrolytic activities

Chitinase activity



- Different behaviour between control and heat treated blocks
- Chitinase activities are more important during the first weeks of incubation and decrease in a second time in the case of heat treated blocks
- Chitinase activities increase slowly in a first time and more rapidly in a second time in the case of controls
- Increase of activity is concomitant with that of glucosidase and cellobiohydrolase activities

Enzymatic activities - Partial conclusions

- Enzymatic activities are strongly correlated with the nature of wood used for the test (control or heat treated)
- Laccase activity is similar in a first time for both types of samples, but decrease strongly in the second time for heat treated samples
- Glucosidase and cellobiohydrolase activities are weak or not detected in the case of heat treated blocks
- Chitinase activities are also strongly modified according to the nature of the sample

Reasons of lower susceptibility to fungal enzymes?

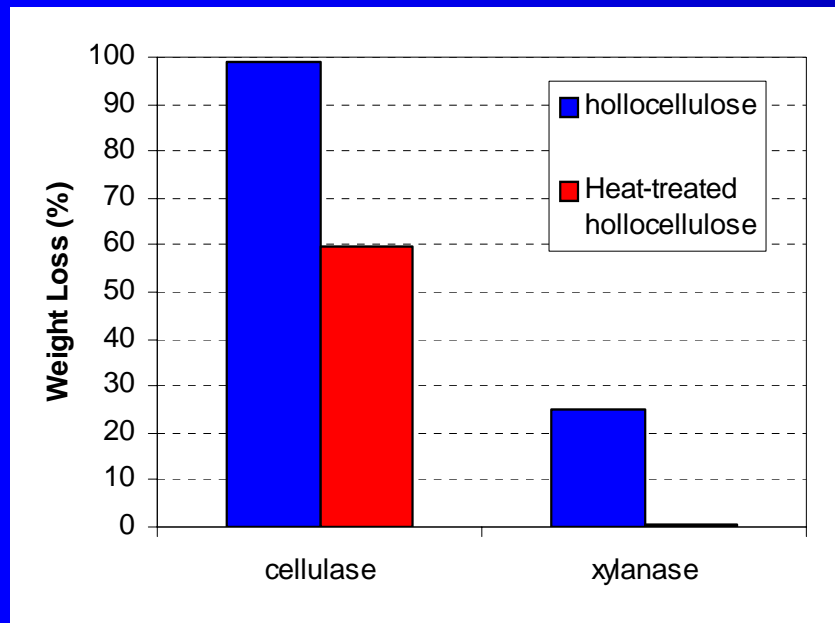
- Non recognition of wood polymers by enzymes due to chemical modification resulting from heat treatment
- No production of enzymatic activities due to the death of the fungus resulting from the production of biocidal compounds during treatment

In vitro degradation of holocellulose by different commercially available enzymes

Cellulase from *Trichoderma reesei* (ATCC 26921)

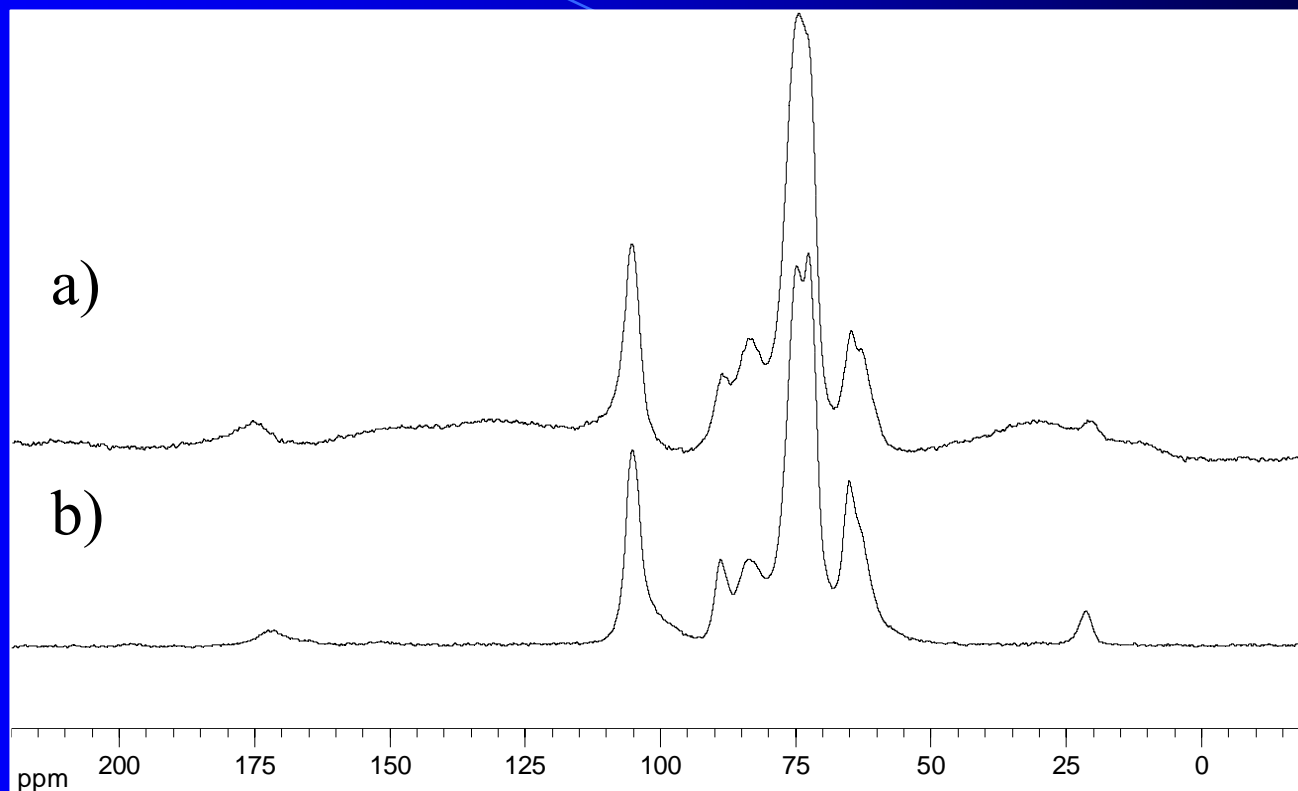
Xylanase from *Thermomyces lanuginosus* (EC 253-439-7)

Delignification of holocellulose using sodium chlorite method followed by heat treatment of the resulting material under nitrogen at 240°C



- Holocellulose from untreated wood is strongly degraded
- Holocellulose from heat treated wood is less susceptible to degradation
- Chemical modifications of wood components seems responsible of the lower susceptibility of wood to enzymatic degradation

Characterization of chemical modifications resulting from heat treatment



CP/MAS ^{13}C NMR spectra of holocellulose

a) unmodified holocellulose b) heat treated at 240°C during 21h, WL = 41%

Apparition of new aliphatic carbons between 10-50 ppm and aromatic or alkenic carbons between 110-160 ppm ascribable to dehydration products

Conclusions

- Different methods have been developed to characterize enzymatic activities during degradation of wood by fungi
- Wood degradation processes occur in two stages under experimental conditions used
 - a first stage characterized by strong oxidizing activities
 - a second stage characterized by a strong increase of hydrolytic activities
- Enzymatic activities recorded in the case of heat treated blocks are significantly weaker than those determined on control samples
- Tests realized with commercial enzymes corroborate the fact that chemical modifications are responsible of improved decay resistance of heat treated wood
- The exact effect of chemical modification is still not completely elucidate
 - non recognition of modified wood polymers by enzymes?
 - reduction in cell wall permeability limiting access of enzymes?

Acknowledgement
to all the co-authors of this work
and especially to Serge Lekounougou, who is now looking
for a post doctoral position



lekous@hotmail.com

Thank you for your attention