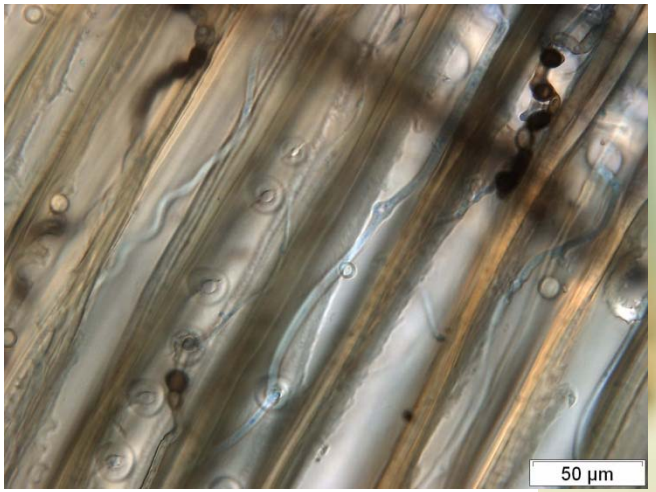
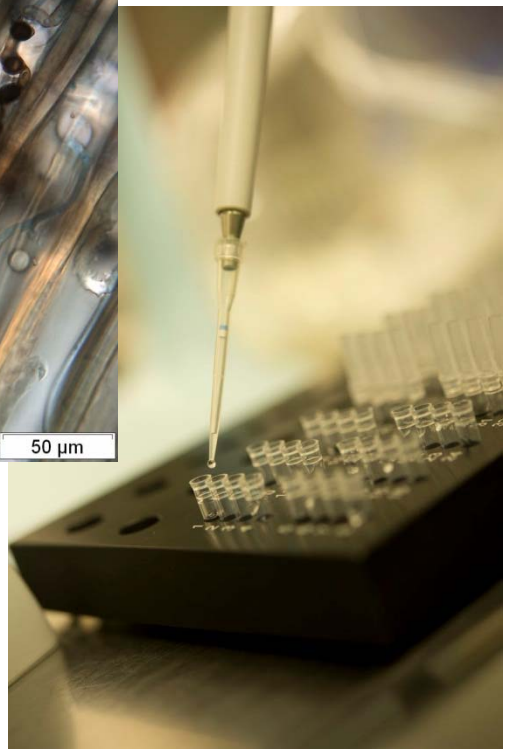
Annica Pilgård

Understanding fungal decay processes – How and why





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UNDERSTANDING FUNGAL DECAY PROCESSES – Why?

Fundamental research on fungal degradation

Understand **where and how** fungi attack modified wood

Why is the degradation delayed?

Hypothesis for the mode of action of modified wood

- Enzyme non-recognition
- Micropore blocking
- Moisture exclusion due to blocking of OH-groups
- Moisture exclusion due to decrease in void volume

…But no one really knows how it works…

UNDERSTANDING FUNGAL DECAY PROCESSES – Why?

Fundamental research on fungal degradation

Understand **where and how** fungi attack modified wood

If we understand the protection mechanisms of modified wood, we can improve them more efficiently

Hy

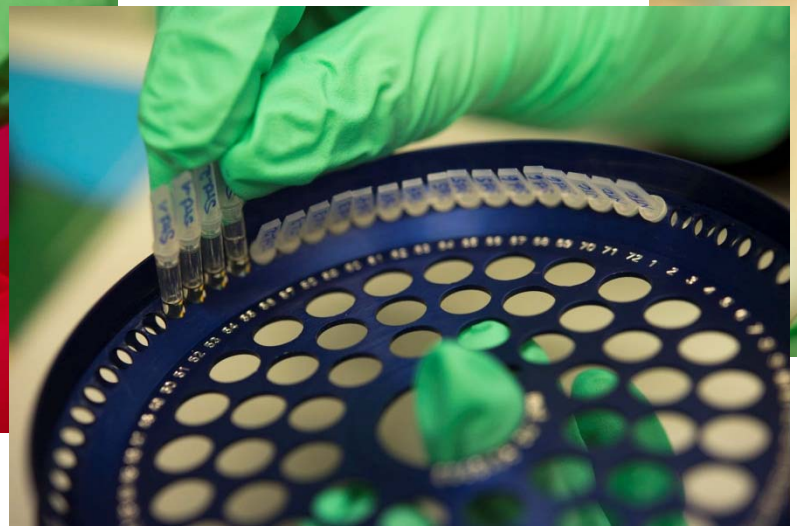
- Micropore blocking
- Moisture exclusion due to blocking of OH-groups
- Moisture exclusion due to decrease in void volume

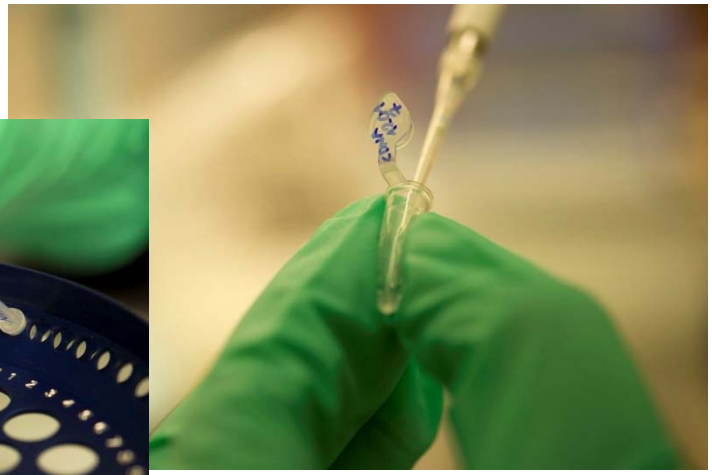
…But no one really knows how it works…

UNDERSTANDING FUNGAL DECAY PROCESSES

How?

- Ongoing research





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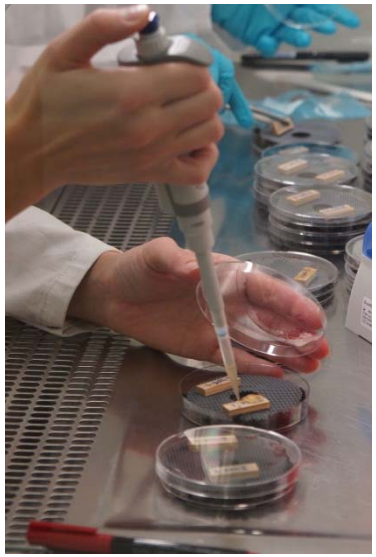
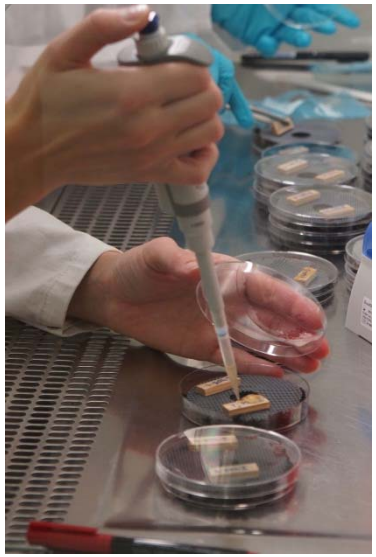
RESEARCH GOAL

Understand the mechanisms of decay resistance in modified wood



OBJECTIVE 1

How do fungi colonize modified wood?



OBJECTIVE 1 – How do fungi colonize modified wood?

COLONISATION PATTERNS

Lab tests and field tests

Are fungi able to colonize the wood?

How do they colonize the wood?

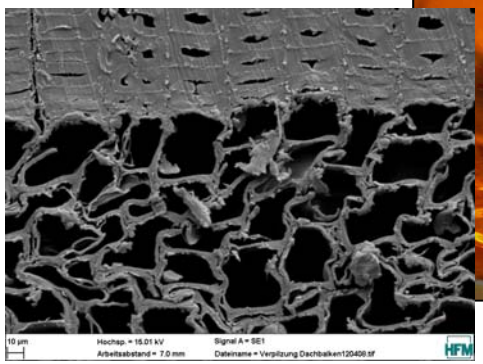
How much fungi are in the wood sample at a specific time point?

qPCR - Species specific or groups of species

Chemical analysis (ergosterol/chitin assay)

Microscopy

- Light microscopy
- Electron microscopy



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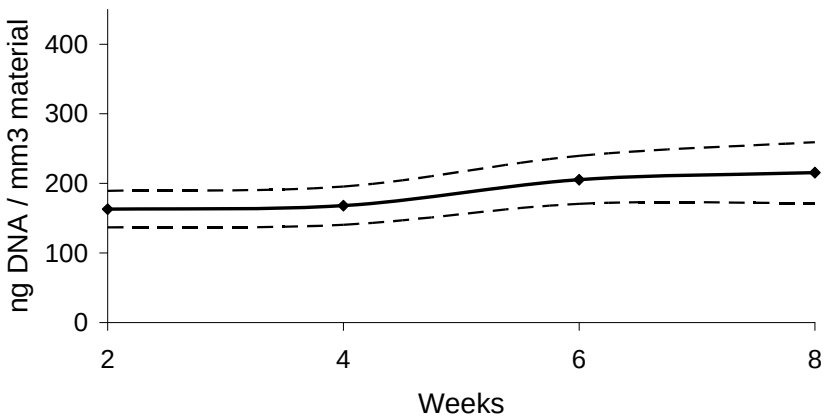
OBJECTIVE 1 – How do fungi colonize modified wood?

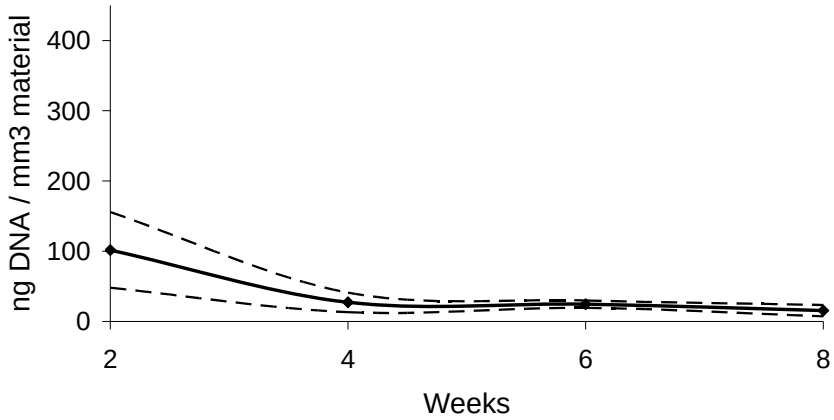
COLONISATION PATTERNS (LAB TEST)

Amount of *Trametes versicolor* (white rot) over eight weeks

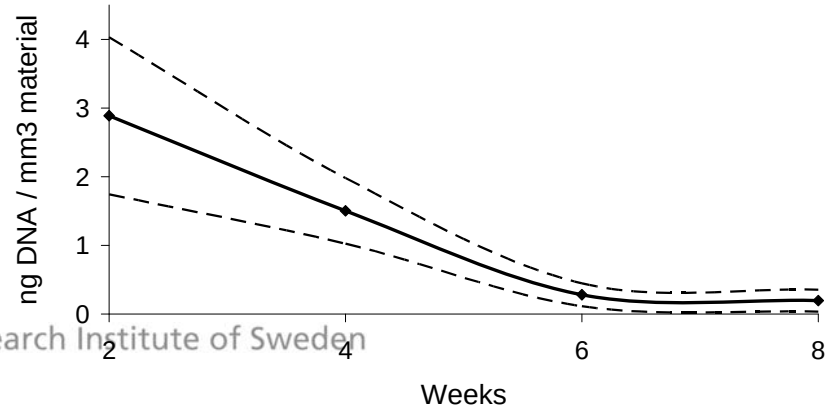
Control *P. sylvestris*



Furfurylated *P. sylvestris*

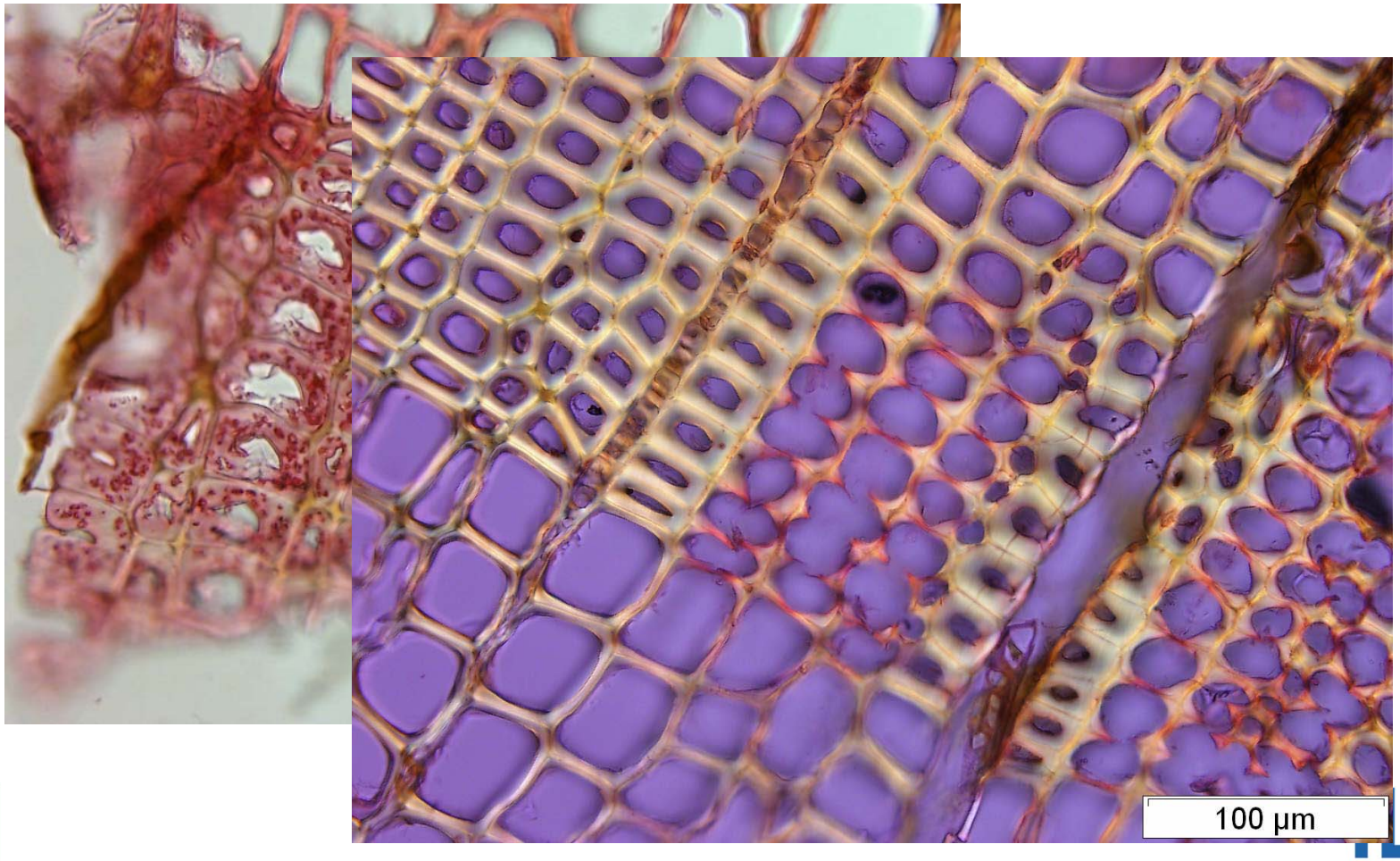


Cu-HDO impregnated *P. sylvestris*



OBJECTIVE 1 – How do fungi colonize modified wood?

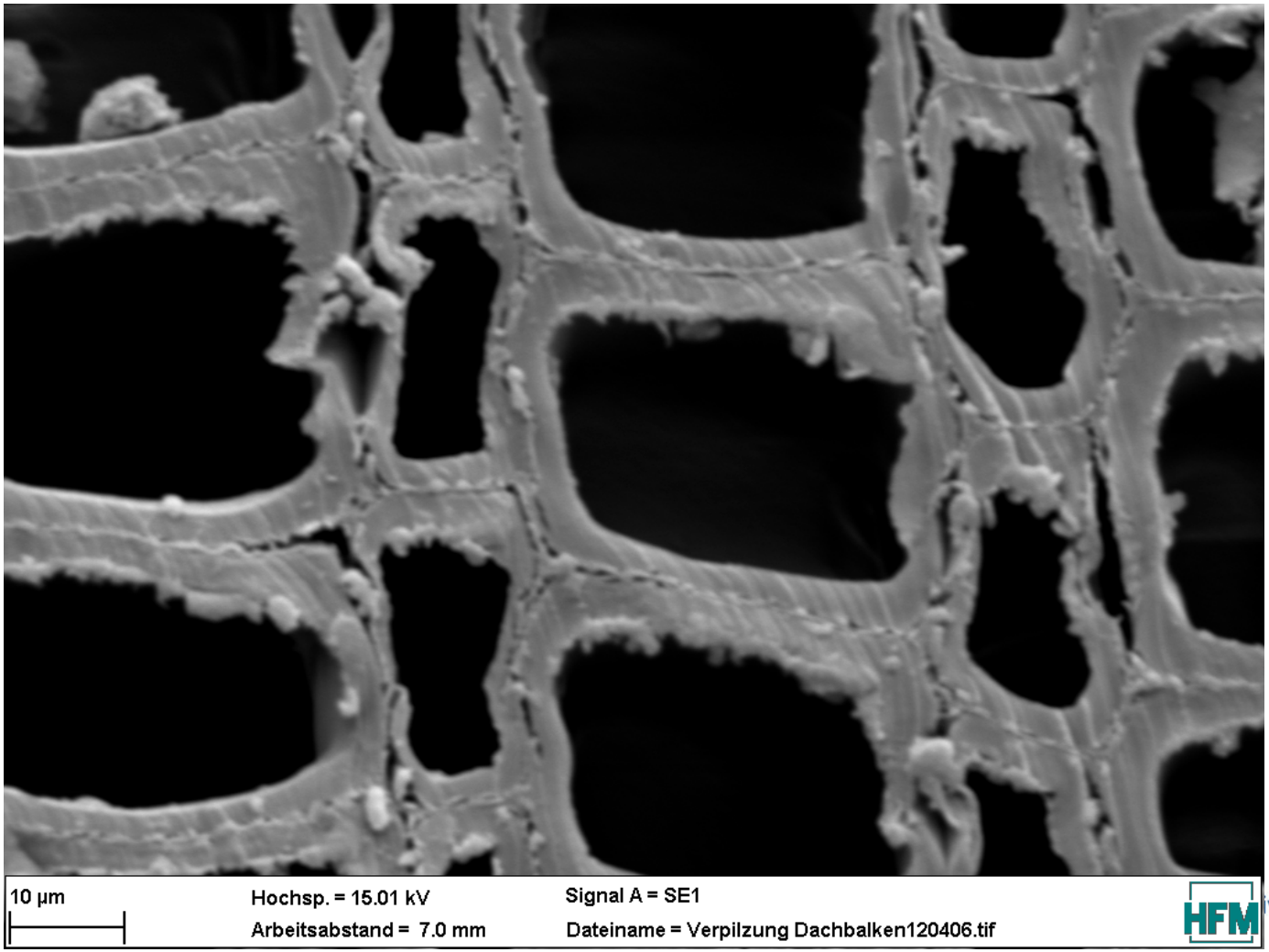
COLONISATION PATTERNS (FIELD TEST)



OBJECTIVE 1 – How do fungi colonize modified wood?

COLONISATION PATTERNS

SIGNS OF EARLY DEGRADATION



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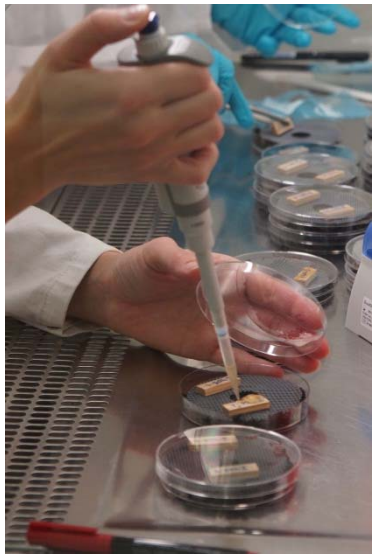
OBJECTIVE 2

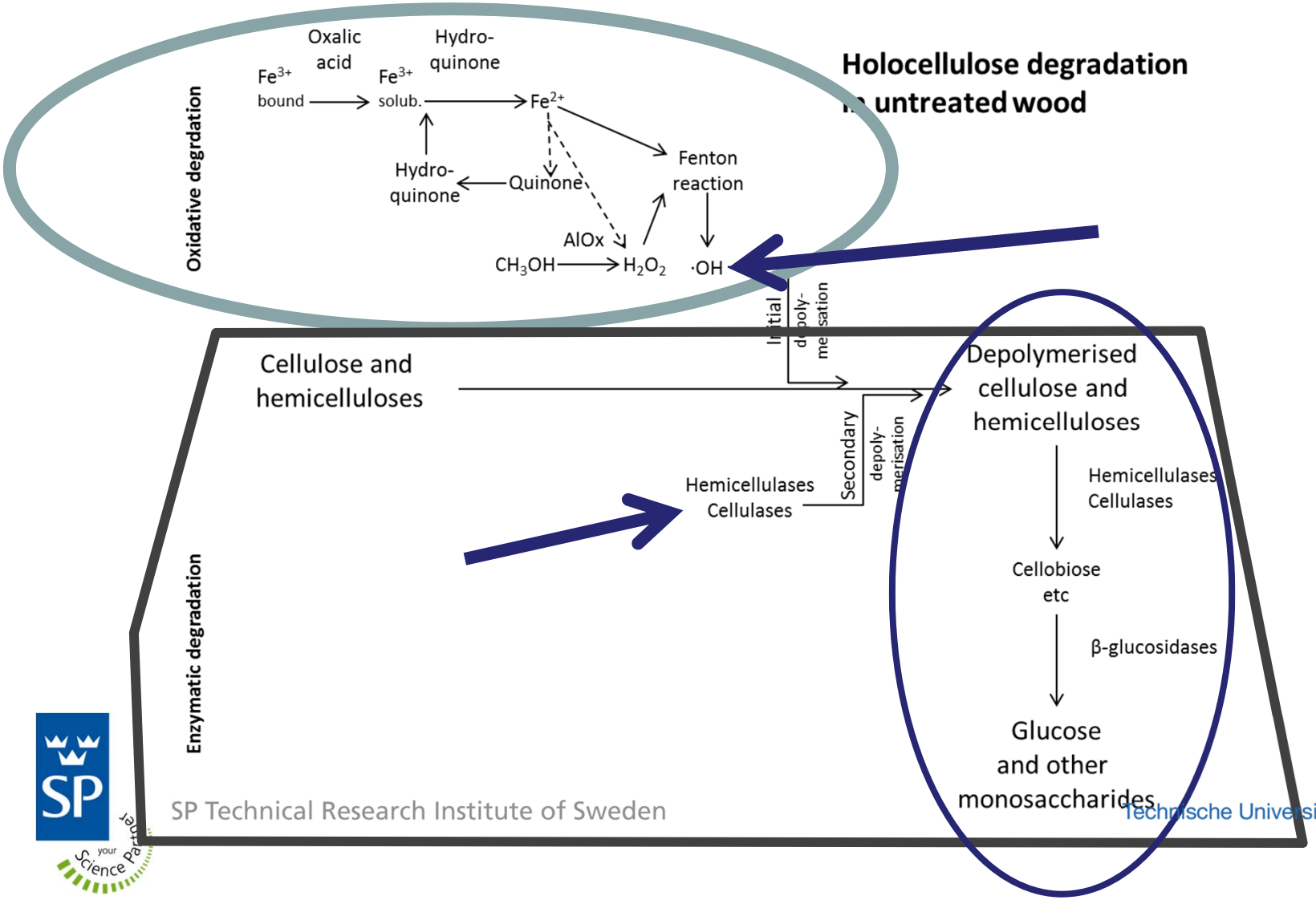
What is the reaction of the fungus to modified wood?

A) At the RNA level



Objective 2 - What is the reaction of the fungus to modified wood?
A) RNA

BROWN ROT DEGRADATION

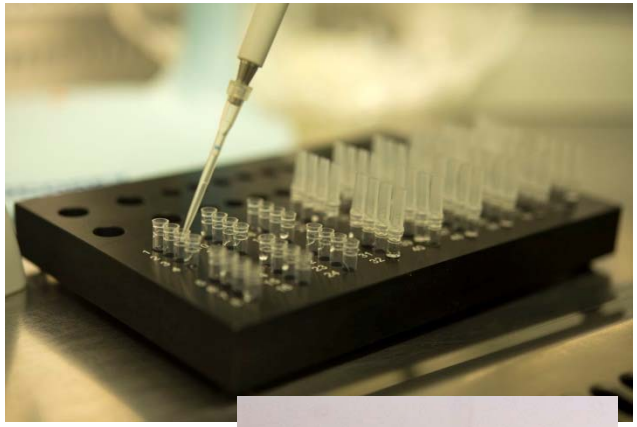


Objective 2 - What is the reaction of the fungus to modified wood?
A) RNA

GENE EXPRESSION

Depicts intention of the fungus

Gene → **RNA** → protein → action

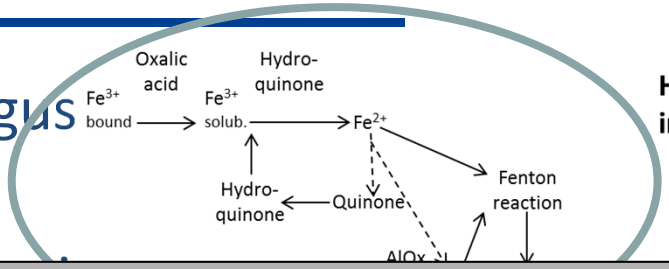


What action is the fungus attempting to take?
How does the gene expression vary over time?
Does the fungus behave different depending on wood material?

Objective 2 - What is the reaction of the fungus to modified wood?
A) RNA

GENE EXPRESSION

Depicts intention of the fungus



Holocellulose degradation in untreated wood

Ge

Seems like the fungi attempts to bombard the material with radicals – wood modification protects against oxidative degradation

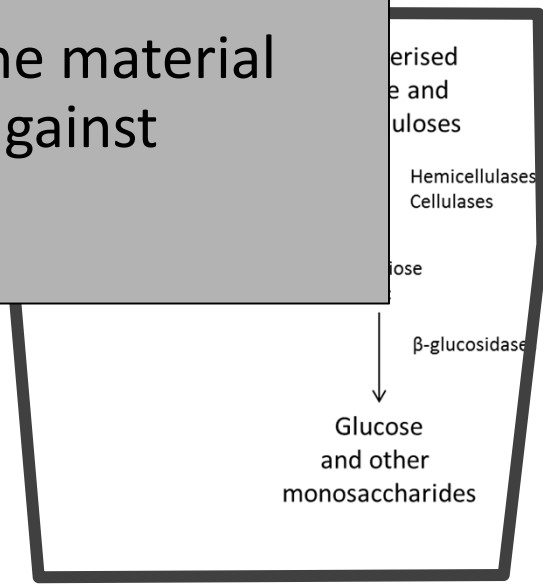
In

Genes involved in oxidative degradation

Up-regulated in modified wood materials

Genes involved in enzymatic degradation

Uninfluenced in modified wood

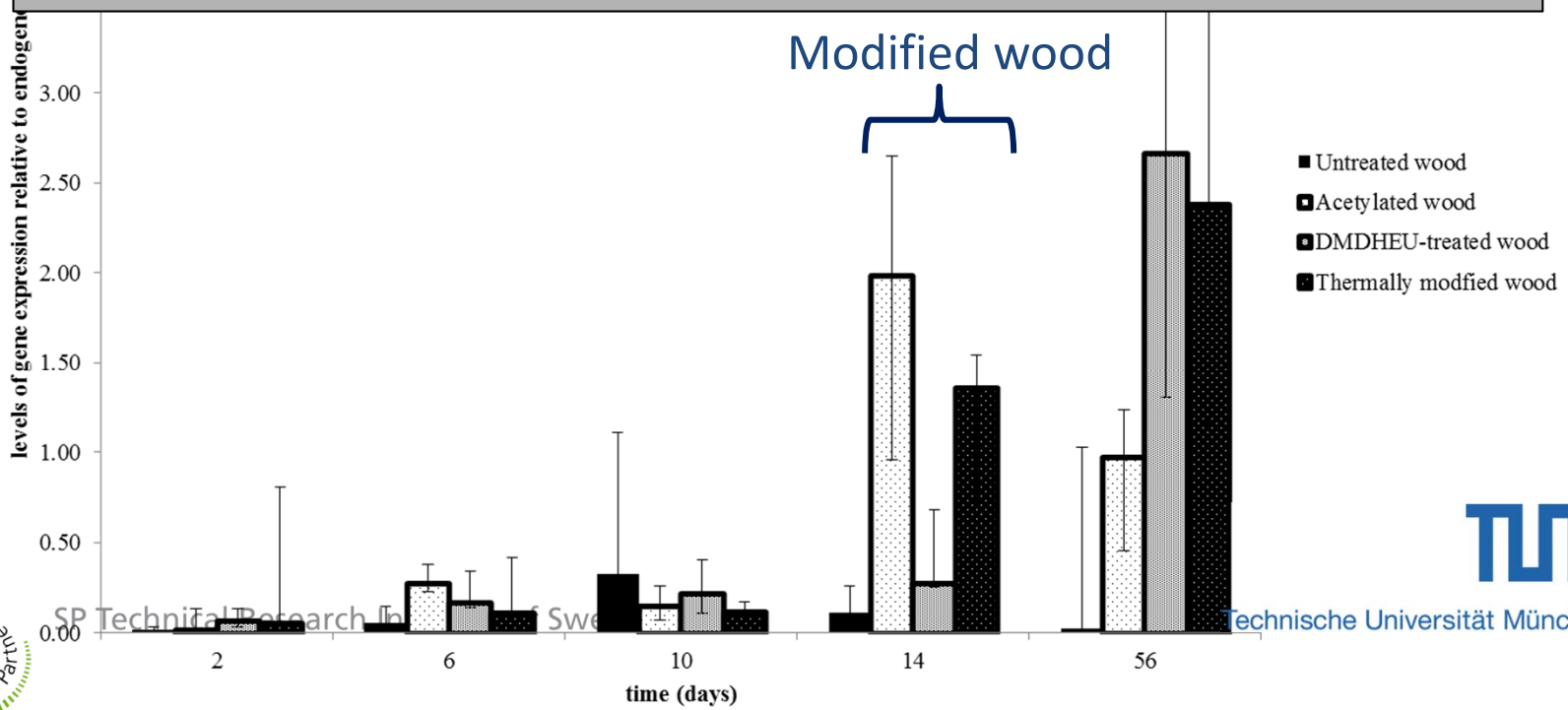


Objective 2 - What is the reaction of the fungus to modified wood?
A) RNA

GENE EXPRESSION

Ex[illegible]n)
in [illegible]s
[illegible]wood

Seems like the fungi attempts to bombard the material with radicals – wood modification protects against oxidative degradation



Objective 2 - What is the reaction of the fungus to modified wood?
A) RNA

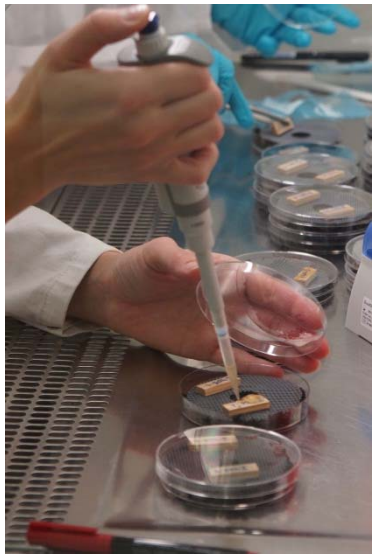
SEQUENCING

Sequencing the *Postia placenta* transcriptome during different degradation stages of acetylated wood

Are there other important genes – other intentions?

OBJECTIVE 2

What is the reaction of the fungus to modified wood?
B) At the protein level

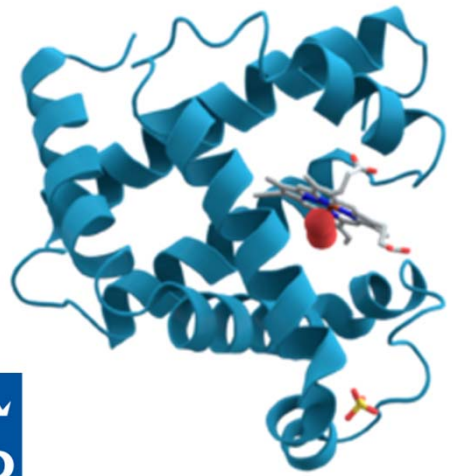


Objective 2 - What is the reaction of the fungus to modified wood?
B) Protein

PROTEOMICS

Gene → RNA → **protein** → **action**

Can we find the actual proteins/enzymes?
Are they really produced or did the fungi only intend to produce them?
Consistent with the sequencing results?



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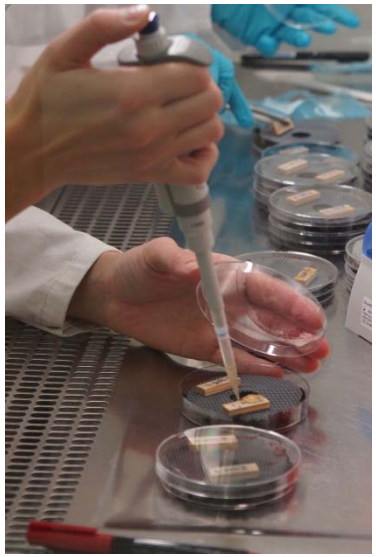
OBJECTIVE 2

What is the reaction of the fungus to modified wood?
C) At an extracellular level



Objective 2 - What is the reaction of the fungus to modified wood?
C) At an extracellular level

OXIDATIVE DEGRADATION

Seems like the fungi attempts to bombard the material with radicals – wood modification protects against oxidative degradation

But are radicals really formed?

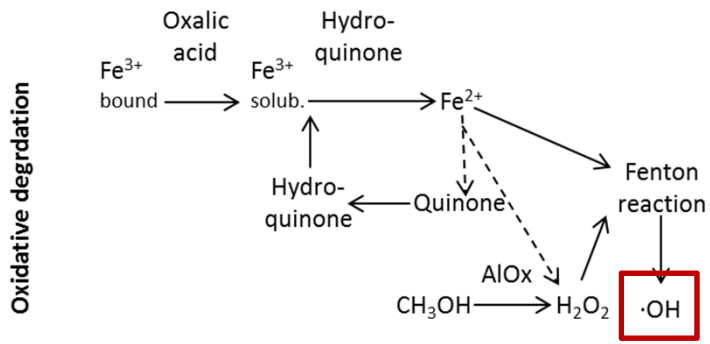
Objective 2 - What is the reaction of the fungus to modified wood?
C) At an extracellular level

OXIDATIVE DEGRADATION

Seems like the fungi attempts to bombard the material with radicals – wood modification protects against oxidative degradation

But are radicals really formed?

Are hydroxyl radicals formed inside the wood cell wall?



To what extent are they able to degrade modified wood?

Hydroxyl radicals can cleave modified wood polysaccharides in DMDHEU treated wood and acetylated wood

Objective 2 - What is the reaction of the fungus to modified wood?
C) At an extracellular level

OXIDATIVE DEGRADATION

Seems like the fungi attempts to bombard the material with radicals – wood modification protects against oxidative degradation

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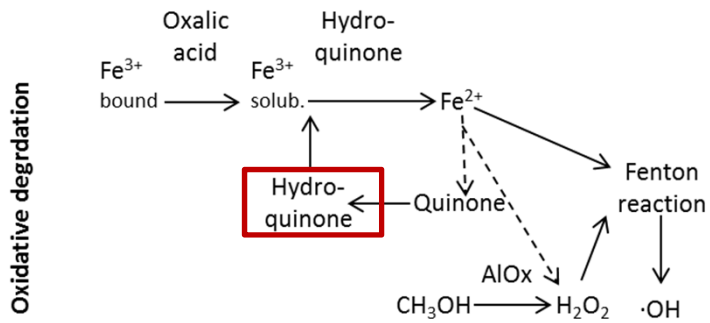
Localization of H_2O_2



To what extent are they able to degrade modified wood?

Can the fungal ion-reductants diffuse into the wood cell wall?

Objective 2 - What is the reaction of the fungus to modified wood?
C) At an extracellular level

OXIDATIVE DEGRADATION

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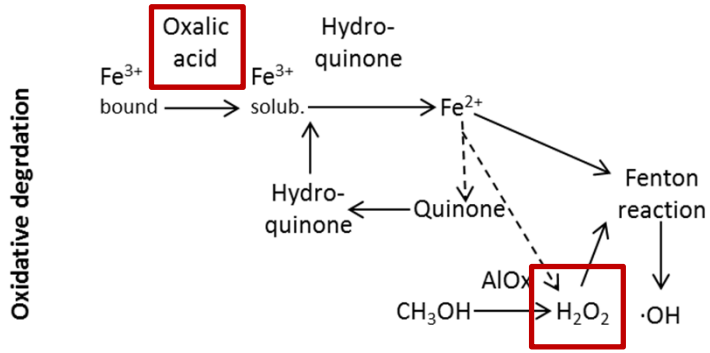


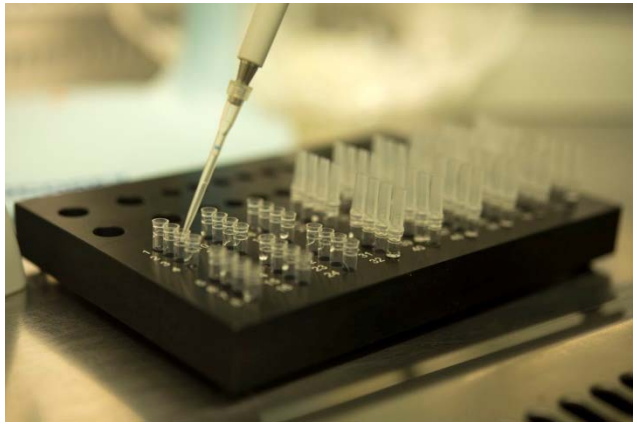
To what extent are they able to degrade modified wood?

Can the fungal reductants diffuse into the wood cell wall?

Is oxalic acid and hydrogen peroxide formed and secreted?

Thank you!

CONCLUSIONS



We know:

Fungi can colonize modified wood

Fungi recognize modified wood as food

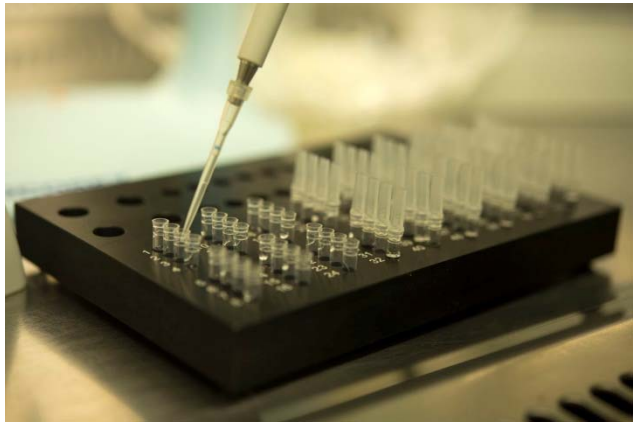
Some genes involved in oxidative degradation are induced in modified wood

Fungal cellulases are secreted in modified wood

Fungal cellulases can degrade modified wood polysaccharides

Fungi should be able to absorb and metabolise glucose from modified wood

CONCLUSIONS

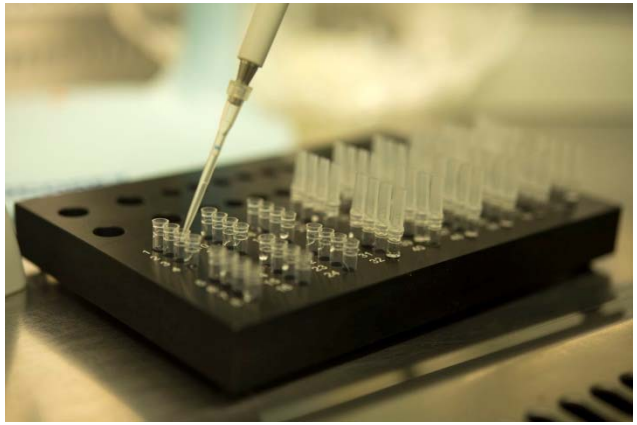


We kind of know (but want to make sure):

Hydroxyl radicals can cleave modified wood polysaccharides (in DMDHEU and acetylated wood)

Fungal cellulases can degrade modified wood polysaccharides (in DMDHEU and acetylated wood)

CONCLUSIONS



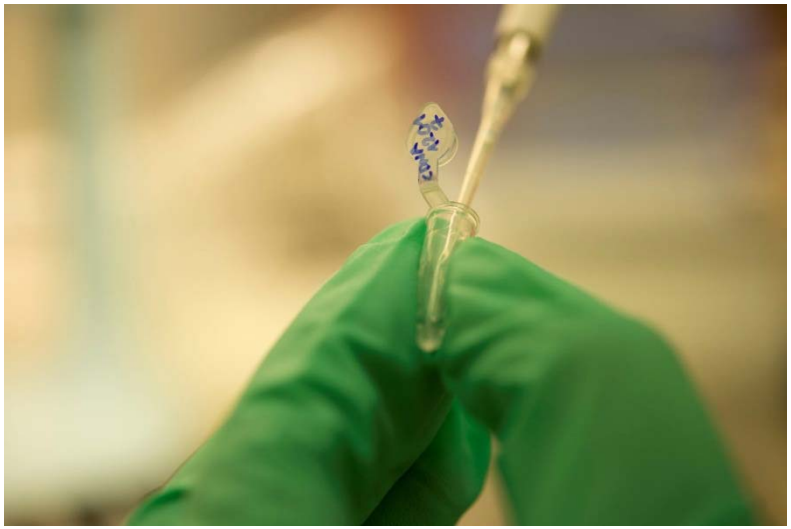
We don't know (but plan to find out):

If all genes involved in oxidative degradation are induced in modified wood

If enzymes and molecules needed for degradation (other than cellulases) are translated into proteins and secreted

If molecules needed for degradation are able to diffuse into the wood cell wall

If the Fenton reaction works in modified wood



Objective 2 - What is the reaction of the fungus to modified wood?
C) At an extracellular level

ENZYMATIC DEGRADATION

Can fungal hemicellulases and cellulases depolymerize modified wood?

Fungal cellulases can degrade modified wood polysaccharides in DMDHEU, acetylated wood, thermally modified wood and furfurylated wood