FACULTY OF ENGINEERING AND ARCHITECTURE

Fungal susceptibility of bio-based building materials

Using X-ray micro CT as a tool for monitoring the moisture distribution and mass loss during lab-based fungal degradation testing

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PREAMBLE

Due to the outbreak of the corona epidemic in early March 2020, the Belgian government announced several recommendations to limit the further spread of the coronavirus COVID-19. For educational institutions such as UGent, activities with physical presence of students and lecturers were cancelled and a change to online learning was implemented. Since almost all students were not allowed to enter buildings of UGent, these measures had various consequences for master students whose research required lab work, surveys, interviews, etc.

In this thesis, three different experiments would have been executed. However, the experiment concerning the monitoring of the moisture distribution of several bio-based materials using MRI and CT scanning could not be executed because the weekly follow-up would require physical presence, which infringes the general measurements stated by the Belgian government. After consultation with my promotors and tutor, it was decided to replace the original experiment involving MRI and X-ray CT assessment on several bio-based materials with an analysis of the moisture distribution of four different wood species that are exposed to a basidiomycete fungus using X-ray CT. The analysis is performed on an existing data set, so no physical presence was required.

The control experiment regarding the monitoring of the moisture content of several engineered wood materials without exposure of fungi could be executed thanks to my tutor. She weighed the samples on a weekly basis, making it possible to analyse the evolution of the moisture content over time.

PREFACE

During my civil engineering studies, I often encountered courses dealing with the design of concrete and steel structures. Unfortunately, courses regarding the behaviour of wood-products and wood design were rather rare. I always liked to have a better understanding in the behaviour of wood-based products, so choosing the thesis topic 'Fungal susceptibility of bio-based building materials' was an ideal opportunity.

The elaboration of this master's dissertation was a challenge, which I could not have accomplished without the support of a few people. First of all, I would like to thank my tutor, ir. Liselotte De Ligne, for giving me the necessary instructions, for helping the realisation of multiple experiments and providing me with useful feedback on a regularly basis. I would also like to thank my promotors, prof. dr. ir.-arch. Nathan Van Den Bossche, prof. dr. ir.-ach. Marijke Steeman and prof. dr. ir. Jan Van den Bulcke, for giving me feedback, making it possible to realize this research and for defining the main topics of this thesis.

Furthermore, I want to thank my friends, with whom I have shared countless great moments that I will always remember. Their enthusiasm made the years spent in Ghent an unforgettable period.

Lastly, I want to express my gratitude to my parents for tolerating me in stressful times, but especially for supporting me during the long days and nights I spent working on this thesis and for their unconditional love. I am also aware that I have two wonderful siblings and a loving family, who are always by my side and made me the person I am today.

ABSTRACT

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Summary: Since climate change and resource depletion are two of the main issues in today's world, the focus of the building industry is changing towards sustainable designs. As a consequence, bio-based materials are gaining more and more importance. However, they are sometimes susceptible to wood-destroying fungi, which not only cause aesthetical damage, but also degrade the material, possibly compromising the structural integrity. In order to improve the moisture dynamics and biological resistance of bio-based materials, a proper understanding of the relation between fungus and material is required, in particular how the material's structure and wood anatomical features affect the degradation process. As the current standardized tests are not suitable to investigate how material's structure affects the degradation process and for the durability assessment of bio-based materials, whose natural durability is enhanced by new technologies, new test methods need to be developed.

In this thesis, we performed a preliminary experiment based on the mini-block test, in which the samples were brought to an initial moisture content ranging between 20-30% MC to increase the degradation potential. Based on the results, we can conclude that most bio-based materials are not durable when they are exposed to conditions favouring fungi (i.e. the samples are initially wet and remain wet during the degradation process and the hyphae can enter the samples from the sides. In case of thermally modified spruce, bringing the samples to 20-30% MC did not make them susceptible to decay.

In order to have a better insight in how the material's structure and moisture properties affect the degradation process, we performed a second experiment, in which the moisture distribution and mass loss of four solid woods were monitored on a weekly basis using the non-destructive technique X-ray CT. For most materials, a density increase of 10-25% was observed due to the moisture uptake from the malt agar medium. The samples exposed to *Coniophora puteana* showed an additional 10-50% density increase, due to the moisture production of the fungus. The material's structure of Scots pine seemed to have big impact on the degradation process. The samples with more latewood have smaller lumina and thicker cell walls, therefore obstructing the fungal colonization, and resulting in less degradation after ten weeks of exposure to *C. puteana*. Based on this result, there can be concluded that X-ray CT is a suitable technique to investigate the influence of material's structures on the degradation process.

Keywords: Bio-based materials, mini-block test, fungal degradation, X-ray CT, *Coniophora puteana*

EXTENDED ABSTRACT

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Jordy Caes

Supervisors: Ir. L. De Ligne, Prof. Dr. Ir.-ach. N. Van Den Bossche, Prof. Dr. Ir.-ach M. Steeman and Prof. Dr. Ir. J. Van den Bulcke

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Keywords*: bio-based materials, mini-block test, fungal degradation, X-ray CT, *Coniophora puteana

I. Introduction

In today's world, climate change and resource depletion are two main issues, so awareness of renewability, recyclability and a small carbon footprint is becoming increasingly important. The building industry has a considerable impact on the environment as it consumes a lot of energy, water and raw materials [1]. Since there is a trend towards sustainable building, bio-based materials are gaining more and more importance. Bio based materials are renewable, recyclable and consume significantly less energy during production compared to steel, aluminium and concrete [2]. However, when bio-based materials are not well applied, and therefore exposed to certain conditions that favour insects, fungi or bacteria, decay can occur when the material is not durable in itself or when the durability was not increased with protection products or enhancement methods [3]. Fungal degradation not only leads to aesthetical damage, but can also compromise the structural integrity of buildings. As a consequence, it is necessary to have a better understanding of the degradation process and its influencing factors.

The most widely used laboratory method for determining the natural durability of solid wood against wood-destroying fungi is the CEN TS 15083-1 test method [4]. This test method adequately assesses the natural durability of various wood species and the efficacy of preservatives. However, this method is not suitable for the durability assessment of bio-based materials, whose natural durability is enhanced by new technologies, such as glued laminated timber, thermally modified wood, chemically modified wood and wood treated with water repellents [5]. These (engineered) materials have the ability that the material resistance can be improved by changing the material's components during manufacture, for instance the glue type in plywood, or by modifying the wood structure, for instance by thermal modification, chemical modification, etc. As the moisture properties of these materials are enhanced, it might be possible that the strict interpretation of the standard test leads to invalidated results. For example, a thermal treatment leads to a reduced hygroscopicity, wettability and equilibrium moisture content [6]. As a consequence, the material may become insufficiently wet in the prescribed 16 weeks for fungal degradation. This does, however, not mean that the material can eventually not be degraded. For this reason, we need to find out if a modified

version of the standard test leads to more accurate results, for instance by prolonging the exposure time or increasing the initial moisture contents. We therefore perform a preliminary experiment, in which the samples are brought to an initial moisture content ranging between 20-30% MC (to increase the degradation potential) before exposure to basidiomycete fungi.

In addition, this standardized test does not allow to gain insight in what is going on at the inside of the material. A thorough knowledge about the relationship between fungus and material is still lacking, in particular how the material's structure and moisture properties affect the degradation process. In order to be able to design new materials and apply them optimally, it is necessary to understand how the material's structure affects the fungal degradation. X-ray CT seems a suitable technology to monitor the moisture distribution and mass loss, because it is a non-destructive technique that allows to assess the density of wood in three dimensions, thus enabling us to obtain localized information inside the wood during the degradation process [7]. We therefore perform a second experiment, in which the moisture distribution and mass loss of four solid woods are monitored on a weekly basis using X-ray CT.

II. Materials and Methods

A. *Adapted mini-block test for bio-based materials*

In this experiment several (engineered) wood products are exposed to two basidiomycete fungi, in order to investigate their resistance against fungal decay. Before exposure, the materials are brought to moisture contents ranging between 20-30% MC to increase the degradation potential. Two different types of fungi are used, the brown-rot fungus *Coniophora puteana* and the white-rot fungus *Trametes versicolor*. The main objective of this experiment is to assess if bio-based materials are degraded in a mini-block test, when the samples have a moisture content of 20-30% before fungal exposure.

1) *Materials*

In total, six different wood materials are tested, of which the components are shown in Table 1. For each material, 21 samples are prepared with a size of 3 x 1 x 0.5 cm³ and marked with a code and number for distinction.

Table 1: Abbreviation and components of tested materials

Material type and abbreviation	**Components**
Scots pine sapwood (*Pinus sylvestrus L.)* (SPS)	
Radiata pine plywood (RPP)	Radiata pine veneers, glue type: non-specified
Wood insulation (WI)	Norway spruce and Scots pine fibres, PUR resin, paraffin
Porous fibreboard (PF)	Norway spruce and Scots pine fibres, bitumen emulsion
Oriented strand board (OSB)	Scots pine fibres, PUR resin, formaldehyde-free glueing
Thermally modified spruce (TMS)	Process: 1) Hydrothermolysis up to 170°C 2) drying 3) heated again to up to 180ºC in dry conditions without oxygen

2) *Method*

C. puteana and *T. versicolor* are grown in Petri dishes (diameter 9 cm) filled with 20 ml of a growth medium (2% agar and 3% malt). All mini-blocks are oven dried (for 24 hours at 103°C), weighed and sterilized under steam at 121°C (Fedegari Autoclavi Spa). After sterilization, the mini-blocks are placed in a vacuum desiccator and ballasted with weights such that they do not float during the wetting procedure. This operation is done in the laminar flow, while ensuring that all elements of the experiment set-up are sterilized with ethanol.

A sterilized glass piece containing a valve is installed in the desiccator. After closing the outlet of the valve, which ensures the maintenance of sterility at the inside, the desiccator is taken out of the laminar flow and connected to a vacuum pump. After establishing a vacuum corresponding to a pressure of 0.8 bar for 30 minutes, the valve is closed and disconnected from the vacuum pump. The desiccator is then returned in the laminar flow and connected to a sterile water supply. Before opening the valve and filling the desiccator, the tube is made air-free. When the samples are completely submerged, the valve is closed and the desiccator is subjected to vacuum again for 30 minutes. After a day, the samples are taken out of the desiccator and placed on sterilized racks for drying in the laminar flow. The samples are weighed regularly until a moisture content of 20-30% is reached. Next, the samples are wrapped in aluminium foil, to prevent dehydration, and put in the fridge until the start of the experiment.

When the fungi have overgrown the Petri dishes, sterilized metal grids are placed on the fungi, this to avoid that the samples take up water directly from the malt agar medium. After checking the moisture contents one last time, three mini-blocks are placed in each Petri dish. The Petri dishes are closed with surgical tape, to prevent mites from contaminating the growth medium, and put in a climate chamber at 20°C and 70% RH. The tape also has the property to allow gas exchange between the interior environment of the Petri dish and the exterior environment, which is required for those fungi that need oxygen to survive.

After 8 weeks, the samples are taken out of the petri dishes, cleaned, weighed, oven dried and weighed again, in order to determine the moisture content at the end of the experiment and the mass loss due to fungal degradation.

B. *Using X-ray CT as a tool for monitoring the moisture distribution and mass loss of solid woods*

1) *Degradation test*

In this experiment, a durability test is performed according to the mini-block method of Bravery, because a smaller sample size and a shorter test period seem beneficial for experimenting with X-ray CT [7]. Twenty mini-blocks of beech (*Fagus sylvatica L.*), Scots pine (*Pinus sylvestris L.*), gaboon (*Aucoumea klaineana* Pierre) and Norway spruce (*Picea* abies *(L.)* Karst) with dimensions 30 x 10 x 5 mm³ are oven dried for 24 hours at 103°C and weighed. Next, they are sterilized using gamma irradiation and placed in Petri dishes (diameter 9 cm), filled with a malt agar growth medium (40% malt, 2% agar) and inoculated with the brown-rot fungus *Coniophora puteana* [7]. Fifteen samples are exposed to the fungus when the mycelial area has a radius of approximately 1.5 cm. The other five mini-blocks are not exposed to fungi

and serve as control samples to compare the moisture behaviour. The samples are placed on plastic grids to avoid direct contact with the growth medium. The choice for plastic grids can be explained by the fact that metal objects affect the density of adjacent tissues during CT scanning [8]. Furthermore, a reference material was placed on top of each sample. The reference material is required for calculating the density from an X-ray CT image. The Petri dishes are kept in a climate chamber (20°C and 75% RH) for 10 weeks and scanned on a weekly basis with X-ray CT. After 10 weeks of degradation, the samples are weighed immediately to check the final moisture content and oven dried to assess the mass loss. The blocks were scanned one last time after oven drying [7].

2) *X-ray CT set-up*

The Environmental Micro-CT (EMCT) system at the Centre for X-ray Tomography at Ghent University (UGCT, www.ugct.ugent.be) is used to obtain X-ray CT scans of the wood samples. The energy source emits X-rays, which partially penetrate through the stack of Petri dishes and hit the 2D pixelated detector. The detector detects how much of the incoming X-ray energy is able to pass through the stack, and therefore also how much energy has been absorbed by the wood, air and other elements in the Petri dish [7]. The denser the wood is, the more energy it will absorb. During the measurements, the stack of Petri dishes remains in place, while the table on which the source and detector are mounted rotates (0-360°). The scan settings used in this set-up allow for a resolution of 68 µm (Table 2).

Table 2: Scan settings EMCT [7]

Voltage	+80 kV	**Exposure time**	100 ms
Wattage	12 W	**Number of averages**	3
Filter	No filter	**Rotation**	360°
Resolution	68 μm	**Number of images**	2200

Since the detector has a limited field of view, the entire stack of Petri dishes cannot be scanned at once. Consequently, we make use of a motor allowing for vertical movements, such that the height of the stack of Petri dishes automatically changes after each scan cycle [7]. In order to avoid unnecessary X-ray radiation exposure of the fungal cultures that are not in the field of view, a PVC tube with lead cladding is positioned around the stack of Petri dishes. This ensures a blockage of X-rays, except at a central slit that allows X-ray passage only through one Petri dish [7].

3) *Image reconstruction*

The detector takes 2200 images during one rotation cycle of 360°. The images are then reconstructed with the Octopus reconstruction software package (licensed by TESCAN-XRE: www.xre.be) [9]. A software beam hardening correction was applied (BHC-values 0.12 0 0). We loaded the resulting greyscale volume for each Petri dish in Fiji and extracted the mini-blocks [10]. A greyscale profile was taken along the longitudinal direction of the mini-block, where the average grey value was calculated for each slice. Afterwards, this greyscale profile was converted to a density profile by rescaling with a reference material (which has a similar elemental composition to wood and a known density (1400 kg/m³)), and air (1.2 kg/m³) [7].

III. Results and Discussion

A. *Adapted mini-block test for bio-based materials*

A first observation that can be made is that both fungi are sufficiently virulent to degrade all bio-based materials, except for thermally modified spruce (Figure 1). Considering that all materials are based on softwoods, it is logical that the brown-rot fungus *C. puteana* causes larger mass losses than the white-rot fungus *T. versicolor* [11].

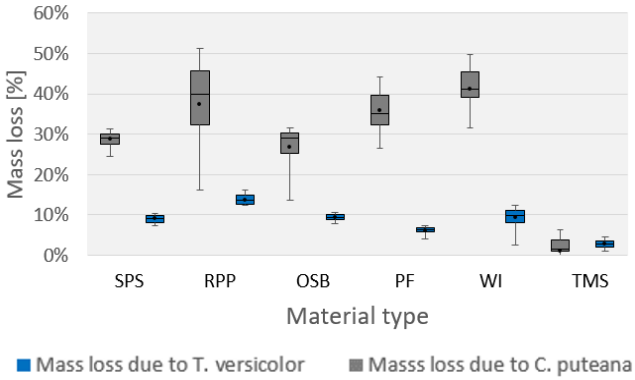


Figure 1: Mass losses due to *C. puteana* and *T. versicolor*

Since the mass loss of thermally modified spruce is limited, i.e. ranging between -1.16% and 4.49% ML, this could possibly indicate that the samples of thermally modified spruce are too dry or wet for degradation. The first explanation is rejected because all samples had an initial moisture content between 20-30%. Being too wet is most likely not the explanation either, as most samples of thermally modified spruce had a moisture content below 80% (Figure 2).

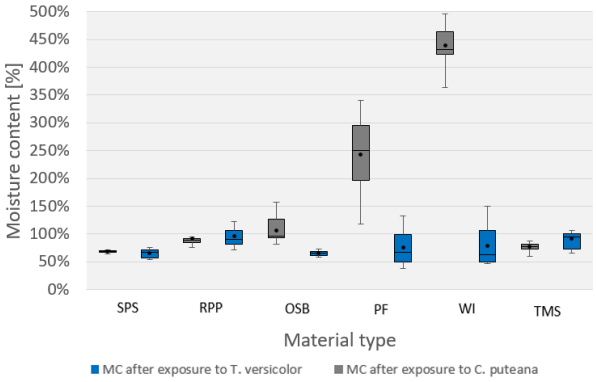


Figure 2: Moisture contents after fungal degradation

The reason for the limited degradation can be found in the thermal modification. Due to the thermal treatment at 200°C, a multitude of different reactions take place (e.g. hydrolytic splitting of polysaccharides, oxidation and radical reactions and several condensation reactions) [3]. As a consequence, the material's characteristics undergo several changes: a reduction of the equilibrium moisture content of the wood; a better biological durability and an improved dimensional stability [12]. In a survey of Weiland and Guyonnet, the chemical modification and fungal degradation of thermally modified wood was studied. They mentioned several factors that contributed to an increased fungal resistance [13]:
(1) The thermal treatment causes the creation of new free molecules in the wood, acting as fungicides.

(2) The formation of some molecules, e.g. furfural, may blend in the lignin network. This makes sure that the fungi cannot longer recognize the wood substrate and is thus incapable of degrading it.
(3) The thermal treatment eliminates the pentanes (hemicelluloses), the elementary nutritive substances of wood, hence inhibiting the initial fungal colonization.
In a study of De Ligne et al., the existence of fungicidal properties was tested for thermally modified spruce with the same properties as in this experiment. Based on their results, there can be concluded that there were no molecules acting as fungicides, so the first explanation of Weiland and Guyonnet can be rejected [5].

In order to determine whether a material is durable or not, the durability rating scale according to CEN/TS 15083-1 can be used. This rating scale assigns a durability class based on the highest median mass loss. An overview of the assigned durability classes can be found in Table 3. Note that the application of this durability rating is, strictly speaking, only valid when the dimensions of the samples are as prescribed (5 x 2.5 x 1.5 cm^3) and when the mass loss caused by *C. puteana* of the reference material is higher than 30% after an exposure time of 16 weeks [4].

Table 3: Assigned durability classes

Material type	**Highest median mass loss [%]**	**Durability class**
Scots pine sapwood	29.03	4
Radiata pine plywood	39.96	5
Oriented strand board	29.07	4
Porous fibreboard	35.08	5
Wood insulation	41.24	5
Thermally modified spruce	2.68	1

Wood insulation and porous fibreboard are both heavily deteriorated by *C. puteana*. This indicates that the non-wood components, bitumen emulsion for porous fibreboard and PUR resin and paraffin for wood insulation, barely affected the fungal degradation in this experiment set-up. The results for OSB correspond with findings of Amusant and Fojutowsko, who found mass losses ranging between 20-45% for different OSB panels in case of exposure to *C. puteana* [14], [15]. The pine plywood samples were also severely degraded by *C. puteana*. Note that this will most likely not occur in practice, where the edges are often shielded by other elements, thus preventing fungal penetration at the sides. This is confirmed by a study from Van den Bulcke et al., in which the sealing of plywood edges has shown to have an important impact on the fungal degradation. [16].

It is important to realize that the mini-block test is not adequate for assessing the actual durability of these bio-based materials. Because of the mini-blocks' dimensions, the non-wood components could not affect the degradation process. The mini-block test, however, does show that the bio-based materials are not durable when they are exposed to conditions favouring fungi (the samples are initially wet and remain wet during the degradation process, and the hyphae can enter the samples from the sides. In case of thermally modified spruce, bringing the samples to 20-30% MC did not make them susceptible to decay. Therefore, it would be interesting to find out if the fungus is capable of degrading thermally modified wood using capillary or loosely bound water and if a longer wetting duration will result in the wood reaching a minimum threshold of bound water in the cell wall which allows decay [5].

In this experiment, it is unknown how much of the material moisture content is due to the moisture absorption from the agar medium and how much is due to moisture production by the fungus. Therefore, a control test (with similar set-up) was performed in which the moisture content is monitored on a weekly basis without the presence of basidiomycete fungi. In this control experiment, contamination occurred, so only the results of the samples without contamination are included (Figure 3).

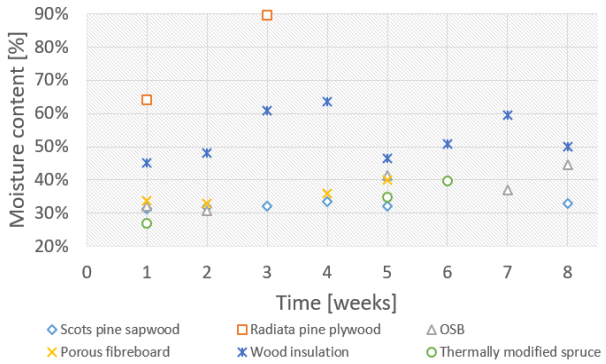


Figure 3: Variation of median moisture content for samples without fungus

By comparing Figure 2 and Figure 3, one can observe that the moisture contents of the samples exposed to *C. puteana* are higher than those without fungus. This is logical because sugars are metabolized into water and carbon dioxide during fungal deterioration [17]. Since more degradation occurred in case of *C. puteana*, it is logical that the moisture contents of the samples exposed to *T. versicolor* are lower than those exposed to *C. Puteana* and higher than those without fungus. For the insulation materials, an increase of at least 200% in MC was found in case of exposure to *C.* puteana, whereas the other materials showed moisture content increases of 20% to 40%. Hence, there can concluded that the presence of fungi has a significant impact on the material moisture content.

B. Using X-ray CT for the monitoring of the moisture distribution and mass loss of solid woods

In this data analysis, the main goal is to find out how the moisture distribution varies over time during fungal degradation and how the material's structure affect the degradation process. Before proceeding with the actual analysis, we compare the mass losses of the CT experiment to those of a parallel experiment. This parallel experiment was executed at the same time as the CT experiment, the only difference is that the samples were not exposed to X-ray radiation. The goal of this comparison is to find out whether X-ray radiation has an influence on the degradation process and if the amount of degradation suffices to further investigate the factors influencing the degradation process.

As it is known that water and carbon dioxide are produced during fungal deterioration, it would be interesting to find out how much of the material moisture content is due to the moisture production by the fungus and how much due to the moisture absorption from the agar medium. Therefore, the relative mean density variations of the samples exposed to *C. puteana* are compared to those of the control samples.

Lastly, the densities of the oven dry samples before degradation are compared to those after degradation. This because it would be interesting to find out which phenomena can be observed. Firstly, a density loss is expected due to the fungal degradation. However, it might be possible that there is densification because of the shrinkage as a result of the loss of wood structural elements. Since CT-images are available, both phenomena can be observed visually.

1) Influence of X-rays in CT on fungal degradation

Since high amounts of X-rays can harm living organisms, it is important to test whether X-ray radiation in CT has an influence on the behaviour of *C. puteana*. In a study of De Ligne et al., it was found that *C. puteana* showed a clear recovery potential after X-ray treatment, thus enabling the use of X-ray CT scanning to track fungal degradation [7]. The fungus *C. puteana* is weekly exposed to X-ray radiation for only 14 minutes, so we do not expect that the degradation is significantly inhibited. In order to find out if the X-rays have an influence on the magnitude of the mass losses, the results in the CT experiment are compared to those in the parallel experiment without CT (Figure 4). Note that both experiments were executed in such way that the same conditions were applicable as much as possible. For example, the Petri dishes were put in the same climate chamber, both experiments were executed at the same time and lasted 10 weeks, etc. This is important because that way, the X-ray radiation is the only influencing factor.

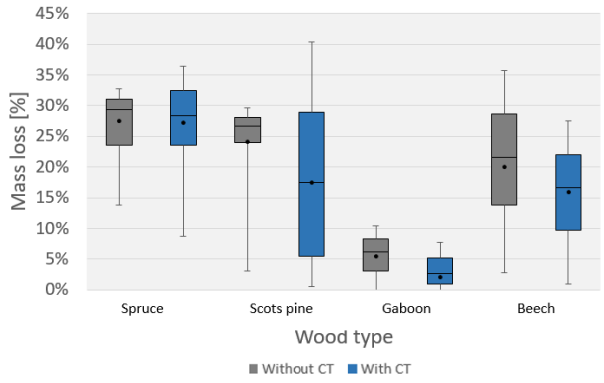


Figure 4: Comparison mass losses due to *C. puteana*

Based on the results for spruce, one could conclude that X-rays do not have an adverse effect on the fungal degradation and X-ray CT is a proper tool to assess the fungal degradation of bio-based materials. However, the mass losses of beech and gaboon are a bit smaller than those of the parallel experiment. Therefore, it could be possible that X-rays have an influence on the fungal degradation. In order to find this out, it is recommended to execute a new experiment. However, for the purpose of this experiment, the amount of degradation suffices to further investigate how the density varies over time and how the material's structure affects the degradation process.

2) Evolution of density over time

It could be interesting to find out how the density of the samples changes over time during exposure to *C. puteana* and how the material's structure influences the moisture distribution and fungal degradation. As the samples vary in density at the start of the experiment, it was decided to plot the relative mean density variation with respect to the oven dry density. The resulting graphs are not straightforward to interpret because there are several factors affecting the results. On the one hand, there is moisture uptake from the malt agar medium and moisture production by the fungus, resulting in an increase of the mean density profile. On the other hand, there is water evaporation and fungal degradation, resulting in a decrease of the mean density profile. In order to find out the influence of some of these factors, the relative density changes are also analysed for the mini-blocks subjected to the same set-up, but without fungus. The results of the analyses can be found in Figure 5 to Figure 9. Before analysing the results, some important comments are given regarding the indices and the colour codes in the graphs of the samples exposed to *C. puteana*. 'Oven dry' represents the results of the oven dry dataset before degradation, week 0 the results of the samples placed in Petri dishes for several minutes, and week 1 to 10 the results of the samples exposed to the fungus for 1 to 10 weeks. The colour code refers to which samples were placed together in a Petri dish, and therefore subjected to the same conditions.

In Figure 5 and Figure 6, the relative mean density variation is showed for spruce and Scots pine respectively. The density increases sharply until week 1-2, which indicates that the malt agar medium provides sufficient nutrients for the fungus. The peak can therefore be explained by a high fungal activity and its associated moisture production. After reaching the peak, the density decreases again (for spruce and pine) or stagnates (for pine). The most degraded samples usually have a lower density. This is logical because a higher mass loss indicates that the fungus degraded more of the wood substances.

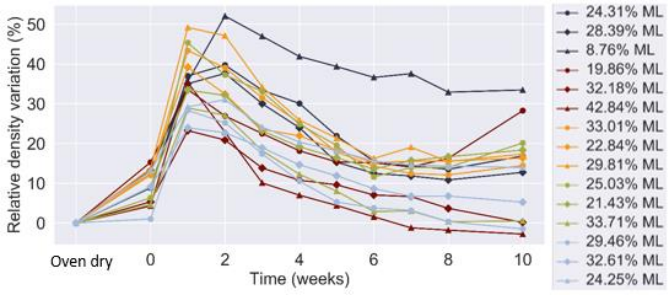


Figure 5: Relative density variation of spruce during exposure to *C. puteana*

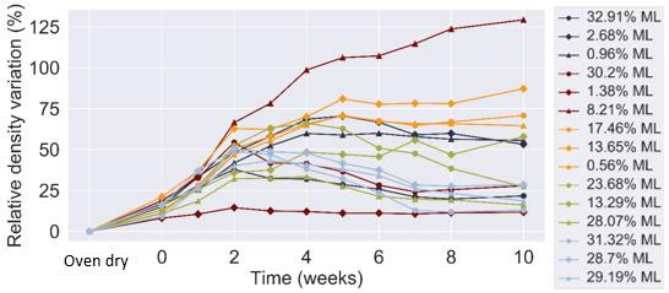
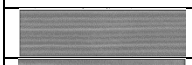
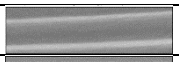
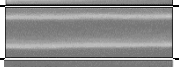
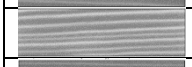
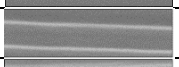
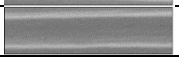


Figure 6: Relative density variation of Scots pine during exposure to *C. puteana*

After week 2, two different density patterns can be distinguished for Scots pine. This could indicate that the material's structure has an important influence on the fungal degradation. Typically, two zones of growth can be distinguished within a tree ring: earlywood and latewood. Earlywood cells have larger radial diameters and thinner cell walls, whereas latewood is denser, has thicker cell walls and smaller lumens [18]. Based on the mass losses of Scots pine mini-blocks, it seems that the amount of latewood regions has an influence on the fungal degradation. Mini-blocks with more latewood regions had a significantly smaller mass loss (Table 4). This implies that the samples with more earlywood, which have larger lumina, are more invasive. The samples with more latewood have smaller lumina and thicker cell walls, therefore obstructing the initial fungal colonization, and resulting in less degradation after ten weeks of exposure to *C. puteana*.

Table 4: Influence of latewood regions on fungal degradation for Scots pine

Mini-block	Mass loss [%]	Mini-block	Mass loss [%]
	2.68		32.91
	0.96		30.20
	1.38		31.32
	0.56		23.68

For beech and gaboon (Figure 7 and Figure 8), the density increases until week 1 or 2. After reaching the peak, the density decreases again when the samples are being moderately degraded, and stagnates when the samples are barely degraded. Since the median mass loss of gaboon is 2%, this indicates that the density increase is due to the moisture uptake from the malt agar medium and the moisture production of *C. puteana* when sugars are metabolized from the agar medium.

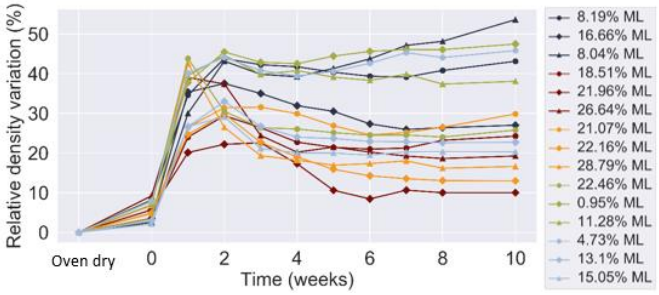


Figure 7: Relative density variation of beech during exposure to *C. puteana*

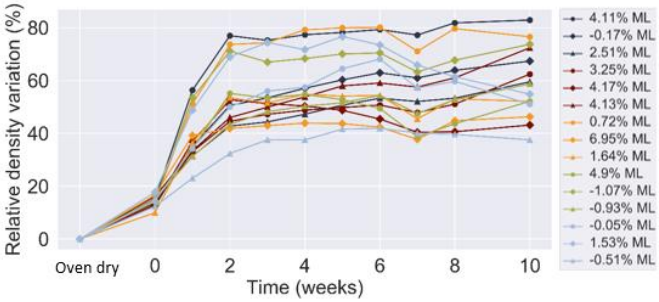


Figure 8: Relative density variation of gaboon during exposure to *C. puteana*

In Figure 9, the relative mean density variation is shown for the control samples. The density increases until week 1-2 and remains approximately constant for the other weeks. Since the density increase varies between 10-25%, there can be concluded that the moisture production of the fungus is responsible for an additional density increase of 10-50%.

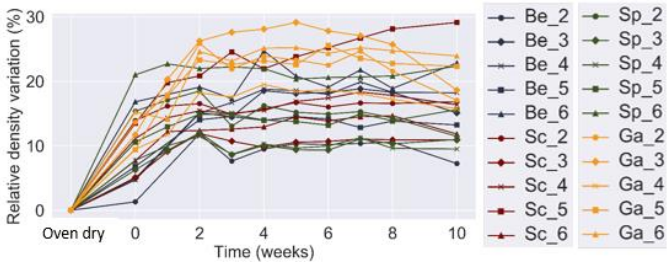


Figure 9: Relative density variation in case there is no exposure to *C. puteana*

3) Moisture infiltration during fungal degradation

In the graphs above, it is not possible to see how the moisture enters the mini-blocks. A possible way to visualize this is by taking snapshots of the degradation process over time (Figure 10). Darker pixels correspond to regions with low densities, such as air, whereas white pixels correspond to regions with high density.

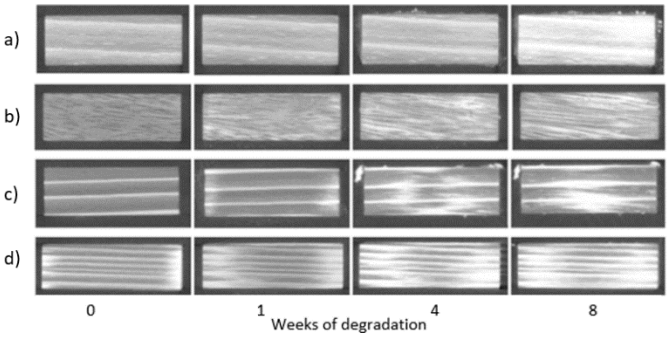


Figure 10: CT slices showing the fungal degradation of (a) beech; (b) gaboon; (c) Scots pine and (d) Norway spruce over time by *C. puteana* [21]

In this figure, the white stripes in beech, Scots pine and Norway spruce indicate the latewood zones of the growth rings, which are denser than earlywood [21]. As mentioned before, week 0 corresponds to the moment where the samples are placed in the Petri dishes for several minutes, so no degradation has occurred yet. As more weeks pass, some regions become more whitish, which indicates moisture production of the fungus. For Scots pine (c) and spruce (d), the fungal degradation starts at the sides and propagates towards the centre of the samples. This is logical because the mini-blocks were cut in such way that the transverse plane is on the sides, causing water and hyphae to enter the wood through the tracheid openings [21]. For beech (a) and gaboon (b), however, the density increases more uniformly in the mini-blocks.

This can be confirmed by plotting the density profile along the length of the sample. The density of Scots pine (Figure 11) is constant at the start of the experiment (week 0). As the weeks pass (week 1 - week 10), one can see that the density peak moves from the sides towards the centre of the mini-block. Furthermore, one can observe that the density decreases again when the peak has been reached. This indicates that the

fungus is degrading the wood components during its travel towards the centre. A different pattern can be observed for beech (Figure 12). The density is almost constant along the length, so it is more likely that the fungus attacks the sample uniformly at the bottom towards the top.

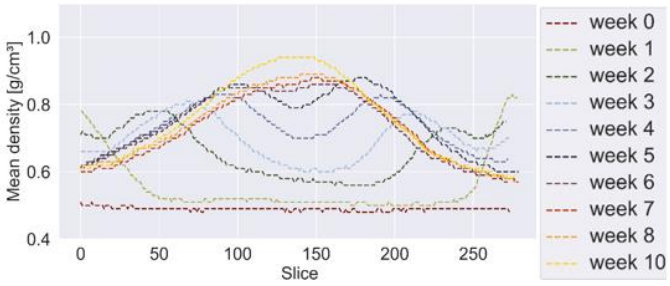


Figure 11: Density profile in longitudinal direction for a Scots pine mini-block with 17% ML

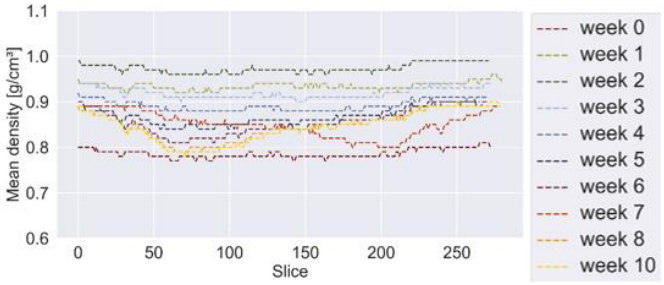


Figure 12: Density profile in longitudinal direction of a beech mini-block with 22% ML

4) Comparison oven dry densities before and after fungal degradation

During the degradation process, fungi break down structural elements of the wood. As a consequence, one would expect that the density decreases. However, it may also be possible that the wood densifies due to shrinkage as a result of the degradation. In order to find out which phenomenon occurs, or if there is a combination of both phenomena, the oven dry densities before and after degradation are compared (Figure 13).

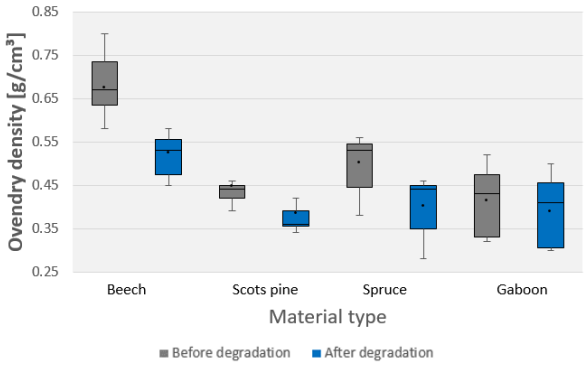
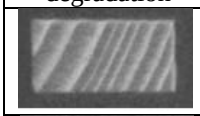
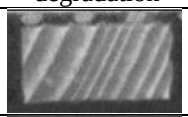
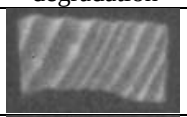
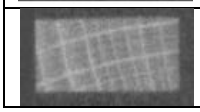
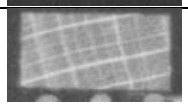
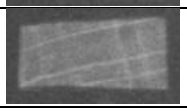


Figure 13: Comparison oven dry densities before and after degradation

For all wood species, one can see that the oven dry densities before degradation are higher than after degradation, which indicates that the density loss is due to the fungal degradation. In order to find out if there is densification, we take snapshots of the CT images of the mini-blocks after ten weeks of degradation and of the oven dry mini-blocks before and after degradation (Table 5). It can be observed that he shrinkage effect is more pronounced after oven drying the degraded samples. This is logical because the moisture, which is produced when the fungus metabolizes sugars or when the sample takes up moisture from the growth medium, evaporates. However, shrinkage was only noticeable for the most degraded mini-blocks. This can be explained by the fact that the more wood substances are degraded, the less remains of the wood structuring elements and the more the samples will shrink.

Table 5: Shape of mini-blocks before and after degradation: (a) spruce (33% ML); (b) beech (21% ML)

	Oven dry shape before degradation	Shape after 10 weeks of degradation	Oven dry shape after degradation
a)			
b)			

The degraded mini-blocks clearly shrunk, but since the graph in Figure 13 shows that there are only density losses, one can conclude that the densification is of minor importance to the overall density loss.

IV. Conclusion

Since the current standardized tests are not suitable to investigate how material's structure affects the degradation process and for the durability assessment of bio-based materials, whose natural durability is enhanced by new technologies, we performed two experiments. The first experiment is based on the mini-block test, and assessed the fungal susceptibility of several bio-based materials, of which the samples were brought to an initial moisture content ranging between 20-30% MC (to increase the degradation potential). Based on the results, we can conclude that most bio-based materials are not durable when they are exposed to conditions favouring fungi (i.e. the samples are initially wet and remain wet during the degradation process and the hyphae can enter the samples from the sides. In case of thermally modified spruce, bringing the samples to 20-30% MC did not make them susceptible to decay.

In a second experiment, the moisture distribution and mass loss of four solid woods were monitored on a weekly basis using X-ray CT. For most materials, a density increase of 10-25% was observed due to the moisture uptake from the malt agar medium. The samples exposed to *Coniophora puteana* showed an additional 10-50% density increase, due to the moisture production of the fungus. The material's structure of Scots pine seemed to have a big impact on the degradation process. The samples with more latewood have smaller lumina and thicker cell walls, therefore obstructing the fungal colonization, and resulting in less degradation after ten weeks of exposure to *C. puteana*. Based on this result, there can be concluded that X-ray CT is a suitable technique to investigate the influence of material's structures on the degradation process. Especially for bio-based materials, this technology looks very promising, as a better understanding of the influence of non-wood components (e.g. glue, bitumen emulsion, etc.) on the degradation process facilitates their design.

ACKNOWLEDGEMENTS

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1 INTRODUCTION

Nowadays, a wide range of building materials is available on the market. In order to choose which building materials to use in a specific construction project, a balance should be made between the benefits and limits of the available materials. These days, concrete, steel, masonry, glass and bio-based materials are the most commonly used building materials. However, the important issues regarding climate change and resource depletion may lead to a shift in the ranking of the building materials. Therefore, it is important to have a better understanding of the parameters that might affect the choice of building material. These parameters mainly consist of the carbon footprint and uptake, renewability, recyclability, energy consumption and need for maintenance. Table 1 gives an overview of the carbon footprint and uptake of several building materials. In this table, CO_{2e} is the sum of fossil based emissions calculated taking into account IPPC weighing factors (for 100 years). The components of the CO_{2e} are CO_2, CH_4 and N_2O [1].

Table 1: Overview of carbon footprint and uptake of several building materials [1]

Building material	Carbon footprint: CO_{2e} emission [g/kg]	Carbon uptake CO_2 uptake [g/kg]
OSB	208	1692
Plywood	229-718	1188-1731
Cross laminated timber (CLT)	362-408	1610-1611
Fresh timber	44-49	1182-1184
Wood fibre insulation	243	1240
Glass wool	3148	-
Stainless steel	3778	-
Float glass	1230	-
Ceramic tile	613	-
Aluminium sheet	2980	-
Reinforced aerated concrete block	511	-

Note that the carbon footprint of concrete in this table is relatively small compared to steel, glass and aluminium. The reason for this is that the cement production is not taken into account. According to a study of Shao et al., the cement and steel industry are responsible for the highest equivalent carbon emissions of all commonly used building materials [2]. In 2012, the production of cement was accountable for approximately 5% of the total carbon dioxide emission [3]. Figure 1 shows the evolution of the CO_2-emissions from cement production over time. In this figure, CCS stands for 'Carbon capture and storage', which is a technology that prevents carbon dioxide from entering the atmosphere. Based on the results of the mentioned studies and the trend of carbon dioxide emissions for cement, one can conclude that the production of concrete is not environmentally friendly.

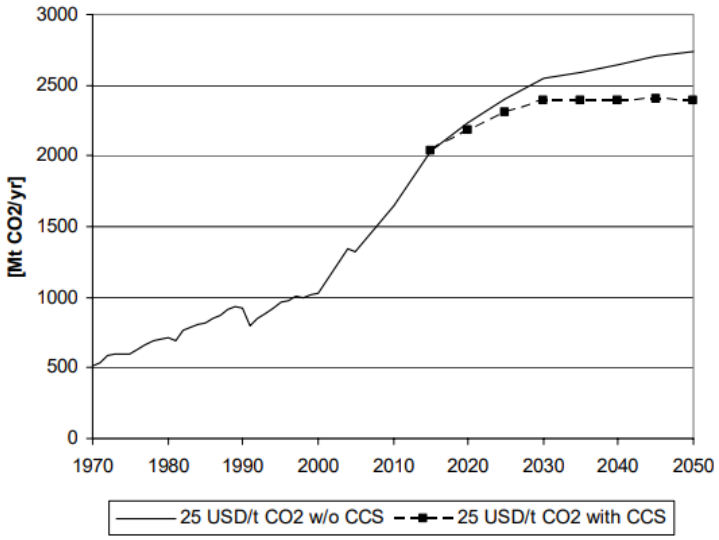

Figure 1: CO_2 emissions due to cement production [4]

The relative share of the energy consumption is visualized in Figure 2. In this figure, one can see that the steel and concrete industry are the most energy intense. This is also confirmed in a study of Schmitz et al., who found that the production of one ton cement requires 3-6 GJ and one ton of steel 20-30 GJ [5].

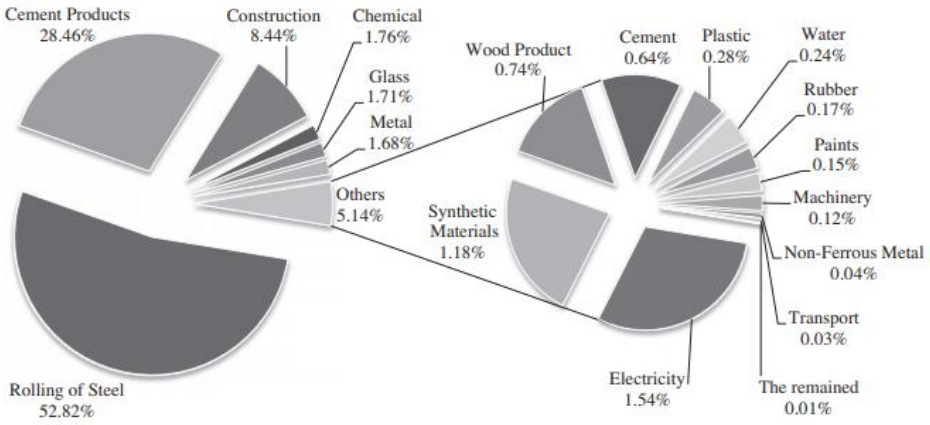

Figure 2: Energy consumption components in building industry [2]

Apart from the carbon footprint and energy consumption, there are a lot of other parameters that may affect the choice of building material. For the most commonly used building materials, an overview of the maintenance costs, recyclability and renewability is presented in Table 2.

Table 2: Overview of maintenance costs, recyclability and renewability for commonly used building materials

Material	Maintenance costs	% Recyclability	Renewable?
Concrete	negligible	30% [6]	No (aggregate depletion)
Steel	High (due to corrosion)	100% [6]	No (depletion of iron ore)
Glass	High (due to cleaning)	Theoretically 100% [6], in reality 10-40% due to waste and damage [7]	No (depletion of silica [8])
Wood	Depends*	100% [9]	Yes [9], [10]

* The maintenance costs depend on multiple variables: exterior or indoor application, allowance to weathering and fungal degradation, etc. Usually, wooden elements require attention every year when exposed to direct sunlight

A main challenge of the 21th century involves the reduction of the emission of greenhouse gases. In order to satisfy this challenge, the fabrication process of some materials has to improve and/or one could choose to make use of environmentally friendly building materials, for instance bio-based materials, such that the CO_2 emission and the energy consumption decrease.

The use of concrete or steel has become indispensable in almost all constructions, but at the same time these materials are also responsible for huge amounts of CO_2 emissions. In addition, one should be aware of the fact that many materials, including aggregates and iron ore, two important substances of respectively concrete and steel, are finite resources. The depletion of the latter often results in rising costs of raw materials, so using other materials such as bio-based products can be advantageous. However, a change in perspective is necessary in order to tackle the climate issues. One possibility is to reduce the use of concrete and steel and utilize bio-based materials instead when possible.

Bio-based materials will never fully replace concrete or steel, because extensive knowledge about their structural behaviour is already available and is still expanding. Furthermore, both the steel and concrete industry are focusing on recyclability and lowering their carbon footprint by innovative concepts. Architects and engineers should, however, try to implement bio-based materials more, such that a harmonious design between steel, concrete and wood is obtained and the overall carbon footprint is reduced. The technology already allows the use of hybrid structures, as can be seen in Figure 3.

Figure 3: Hybrid concrete-timber structure: Illwerke Zentrum Montafon, Austria [11]

From now on, the focus is put on bio-based materials, as many people are still unaware of their benefits and properties. Depending on several properties (thermal, structural, acoustic, etc.), a specific type of material could be interesting for a certain application. Therefore, the first chapter of the literature study deals with some general information regarding bio-based materials and its properties. Since all materials have their limits, it is important to understand to which threats bio-based materials can be exposed. After discussing the threats, good building practice and protection measures for bio-based materials are tackled. This because it is important to prevent fungal growth as much as possible, but also to have an idea of how the material's characteristics can be enhanced when moisture cannot be avoided, therefore exposing bio-based materials to conditions favouring fungi. The final chapter of literature study describes the procedure and problems of the most widely used laboratory method for determining the natural durability against wood-destroying fungi. In order to tackle these problems, we execute two experiments to obtain more insight in the degradation process.

As a preliminary experiment, the natural durability of several bio-based materials is tested. Since these materials consist of non-wood components, such as glues, coatings, resins, etc., that most likely affect the fungal susceptibility, a modified version of the standardized procedure is used to find out if the materials are sufficiently degraded after 8 weeks of exposure to *Coniophora puteana*. Additionally, an experiment set-up is proposed such that the effect of the material structure and wood anatomical features on the progress of decay can be investigated using both MRI and X-ray CT. Finally, an analysis is made of the results of a previously executed experiment, in which the moisture distribution of four solid woods is monitored using X-ray CT. In this analysis, we investigate whether the X-rays have an influence on the fungal degradation, how the density varies over time and how the material's structure affects the fungal susceptibility.

2 LITERATURE STUDY

2.1 Properties of bio-based materials

Throughout human history bio-based materials such as wood, straw, bamboo, reed, hemp, etc. always have had an important role. They have been and are still widely used as construction materials and have important advantages. Wood is, for instance, a very versatile material. After cutting the tree, wood can be adapted to the desired needs and use, as can be seen in Figure 4. The Mannheim Multihalle was initially built as a temporary structure for a horticultural exhibition in Mannheim. It has a roof area of almost 10 000 m² and is one of the largest self-supporting timber grid shell structure in the world [12].

Figure 4: Mannheim Multihalle, wooden roof structure (left); Metropol Parasol, Seville (right) [12], [13]

One of the main advantages of bio-based materials in today's world is the fact that they are sustainable and recyclable [9]. When bio-based materials are being used in construction applications, they offer some extra advantages [10]:

- Exceptionally low linear coefficient of thermal expansion
- Capture and storage of carbon extracted from atmospheric CO_2 through photosynthesis
- Buffering capacity of insulation materials
- Biodegradability at the end of the material's service life (production of an organic fertilizer and biomethane to supply energy)
- Excellent performance-to-weight ratios
- Lower embodied energy than most man-made materials
- Relatively high specific heat capacity
- Beneficial contributions to health and indoor air quality

Note that the way of producing bio-based materials is important for the ecological footprint. Using chemical fertilizers or pesticides has negative consequences for the quality of the environment. Cutting down trees should be done in sustainably managed forests in order to have a low ecological footprint [9].

Bio-based materials also have their limits. When not well applied and therefore exposed to certain conditions that favour insects, fungi or bacteria, decay can occur when the material is not durable in itself or when the durability is not increased with protection products or enhancement methods [14]. In structural applications, this can lead to loss in strength and ultimately compromise the structural stability of the building. Bio-based materials are composed of organic compounds, which is often regarded as at risk for fire. If fire safety is a concern due to the purpose of the building, bio-based materials are not

preferred, unless a fire protection system is implemented. Foreseeing such a protection is costly and the treatment usually has a negative impact on the environment. Abiotic factors such as the sun, water and wind can cause deterioration of bio-based materials as well [14]. Wood is a hygroscopic material, which means that it absorbs and desorbs moisture. This causes swelling and shrinkage, which can lead to damage of the structure when not taken into account.

The type of bio-based material used in a construction project mainly depends on its function in the built facility. An important distinction is made whether the material is used in the main load-bearing structure or not, because the required properties are very different.

2.1.1 Structural function

In case the bio-based material is used in the main load-bearing structure, the most important properties are the strength and stiffness that need to be sufficient to maintain structural integrity. Contrarily to steel and concrete, the variability in strength and stiffness is larger, hence several safety factors should be included in the design. Furthermore, it is important to take into account the fact that the strength of wood is not constant in time. The strength varies with the moisture content and the load duration. From literature, it is found that the strength-load duration relationship approximately follows a hyperbolic curve, which can be seen in Figure 5 [15].

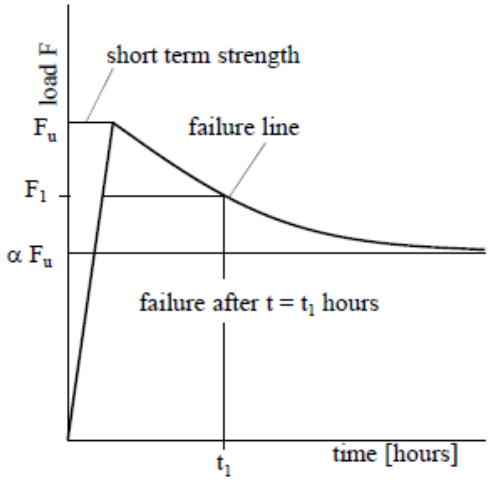

Figure 5: Hyperbolic relation between strength and load duration [16]

In wood design, it is impractical to implement this strength-load duration relationship exactly. It is, however, very important to take this influence into account. For that reason, Eurocode 5 defines five load-duration classes and three service classes, which determine the value of a safety factor taking into account the effect of the moisture content and load duration.

2.1.2 Non-structural function

In case the function of building element is non-structural, the strength and stiffness are of less importance. The application area of bio-based materials is still quite large: thermal insulation, furniture, doors, gates, ships, façade cladding, etc. These specific applications all require some properties that can affect the thermal or acoustic performance, the comfort, the aesthetical appearance, etc. An overview of the most important properties can be found in Table 3.

Table 3: Properties of bio-based materials

Property	Influence on
Moisture content	Shrinkage and swelling, durability, biological resistance, strength and stiffness, density, thermal performance
Conduction coefficient	Thermal performance
Heat capacity	Comfort
Electrical conductivity	Safety
Type of wood	Weathering resistance
Chemical resistance	Strength and stiffness, aesthetics
Durability	Maintenance costs
Biological resistance	Strength and stiffness, aesthetics, health

The moisture content is a very important property, as it influences a lot of other parameters (Table 3). Since bio-based materials are hygroscopic materials, the density depends on the weight of water in a given volume, the weight of wood substance in a given volume and the volume of the material at a specified moisture content. Consequently, the density of wood-based products will vary with moisture content θ.

$$\rho_\theta = \frac{m_\theta}{V_\theta} \tag{2-1}$$

The moisture content of the bio-based material has to be specified when reporting a density value. In engineering design, the density is mostly based on an oven dry weight and a volume at 12% moisture content [17].

The type of wood, coniferous or deciduous, can have an influence on the weathering resistance. Most coniferous trees are adjusted to the warm temperatures and humid environment. Consequently, bio-based materials originating from conifers are less sensitive to warm weather than deciduous trees [18].

2.2 Threats for bio-based materials

Although bio-based materials have many advantages in structural applications compared to other building materials, they can be exposed to different sources of threats during their service life. It is important to have sufficient knowledge about the different types of threats and how to avoid them, as they usually deteriorate the quality of the material. An overview of the most important biological organisms causing damage are listed in Table 4.

Table 4: Biological damage of wood by organisms [19]

Type of organism	Character of wood damage
Bacteria	(Less intense) decomposition of wood cells
Fungi	Rot of wood cells
Insects	Damage by feeding marks produced by larvae
Marine borers	Damage by boreholes and tunnels
Humans	Bad protection and maintenance, unsuitable processing of wood, etc.

By imprudent handling, humans can easily damage wood, but also indirect damage can arise when conditions that favour insects, fungi or bacteria are created. Besides the biological organisms, two other damage origins are of importance, namely fire and weathering.

2.2.1 Fire

When bio-based materials are part of the main load bearing structure, attention should be given to fire resistance, as fire adversely affects several wood properties. In case the wood is dry, the modulus of elasticity parallel to grain decreases linearly with increasing temperature to approximately 200°C. Above this temperature, which is usually the case for fires, a non-linear decrease is found [20]. Furthermore, the compressive and tensile strength also decrease with increasing temperature. Note that the moisture content plays an important role in the fire design, as an increasing moisture content results in sharper decreases of the strength [20]. When fire is considered in the design, it is important that all members still have sufficient (residual) strength to safely evacuate all inhabitants and to prevent partial or total collapse. In case the natural fire resistance is not sufficient, water or solvent based fire retardant coatings can be applied to protect the surface against the extensive heat from fires and slow down the process of strength loss [21].

2.2.2 Weathering

When wood products are used in outdoor applications, they are often susceptible to weathering, which includes solar radiation (UV-light), moisture (dew, rain, snow and humidity), oxygen and temperature [22]. Depending on the wood species, weathering can result in a loss of brightness and change of colour when unprotected wood is exposed outdoors [23]. In a study where several hardwoods were tested against weathering, structural changes in their cell walls were detected after being exposed to sunlight and UV light for respectively 30 days and 500 hours. Furthermore, deterioration and discoloration of the wood surfaces were observed. These phenomena were caused by a loss of lignin and generation of carbonyls, carboxylic acids and quinones [24]. In case appearance is importance, a suitable protective measure should be provided, for instance by applying a coating or by performing a surface treatment [22].

2.2.3 Marine borers

Marine borers, which attack both softwoods and hardwoods for shelter, can be found in salt water and are divided in two categories, namely the molluskan borers (shipworms and pholads) and crustacean borers (gribble). Wood is used in marine environment for multiple purposes such as groynes, piers, dolphins, but also plays an important role in the construction of ships [25]. Multiple reports exist of marine borer damage in wooden piling of dikes and ships, risking the stability of the construction [26]. Since this could eventually endanger the human safety, it is important to understand the conditions in which marine borers are active. Warm climates are favourable for their life process, whereas cold temperatures reduce their activity. Since a salt content between 0.9% and 3.5% is required to survive, one can state that most European coastal areas are an ideal breeding place [10]. In a study of Sivrikaya, they found that commonly used wood species such as Scots pine, beech, ash, etc., were severely attacked by marine borers, whereas tropical species showed a high resistance [25]. Based on these results, one can conclude that tropical species should be used for important coastal structures, or that the less resistant wood species should be enhanced. The presence of marine borers should, in any case, not be ignored, as they can cause severe damage (Figure 6).

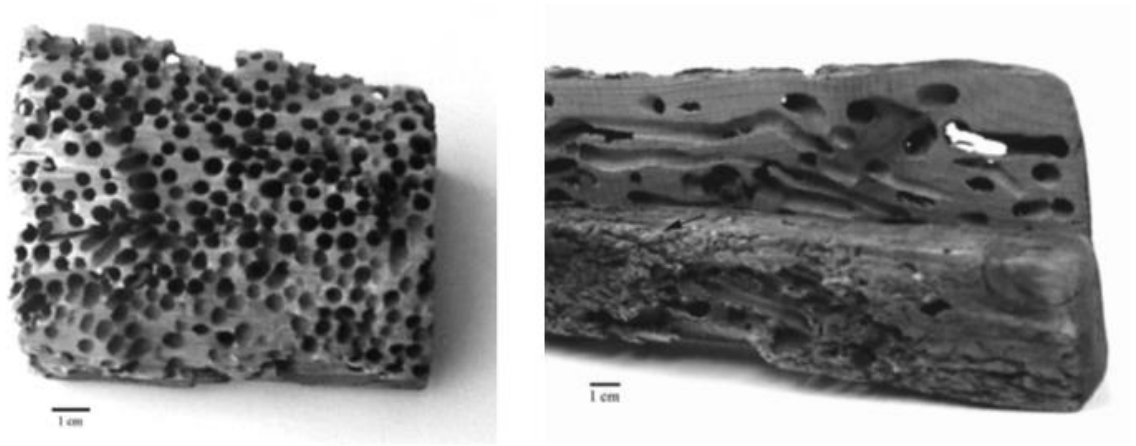

Figure 6: Pholad attack of wood (left) and wood damaged by shipworms and gribble (right)

2.2.4 Insects

There are several insects that use wood as food, shelter or breeding place. The most common insects that destroy lignocellulose are beetles and termites [10]. Mature beetles do not consume wood, but they lay eggs in the large pores of woods, and the larvae do eat wood. After pupation, the adult beetles bite their way out of the wood and continue their lives [26]. In tropical climates, termites are often a problem because they prefer temperatures between 26°C and 32°C [10]. They can be divided in three groups: dampwood termites, subterranean termites and drywood termites. The first group can be found in damp and decaying wood, but they cause little damage compared to other organisms [27]. Subterranean termites cause the most significant economic damage of all groups. They build nests in the earth up to 2.5 to 3 m below the ground level and use tunnels to buried or above-ground sources of wood [26]. Most termite attacks are detected too late, because they leave the outer surface of the wood intact while destroying the entire interior [10]. In Southern European countries, subterranean termites can be a significant problem, so a protection should be applied. Two main physical barriers can be used, either a barrier made of plastic or a steel net (Figure 7). Note that climate change can contribute to higher temperatures in Central and West Europe, possibly making termites a bigger threat to bio-based materials.

Figure 7: Steel net as barrier against termites [10]

2.2.5 Bacteria

Wooden pile foundations along coastal areas were often regarded as free from biological decay because of the low oxygen concentration. Low oxygen levels, indeed, prevent fungal activity, but there exist bacteria that cause significant degradation in low oxygen concentrations [28]. Most aerobic and anaerobic bacteria cause selective degradation of non-lignified cells and pit membranes. However, this type of degradation is not characterized by penetration into the cell walls, in which the cellulose and

hemicellulose are protected by the lignin-matrix [29]. Actual wood-decaying bacteria do penetrate the cell walls and attack the lignified fibres in both soft- and hardwoods. They can be categorized in two groups, namely the erosion and tunnelling bacteria. The latter bacteria are found in environments similar to soft-rot fungi, whereas erosion bacteria can continue to survive in environments characterized by low oxygen levels [30]. Chemical preserved wood is not always resistant to these bacteria, so bacterial decay can eventually cause significant strength loss. The rate of decay is very slow, which means that bacterial decay does not have a great economic impact [27]. One should, however, be critical and possibly consider to take action against bacterial decay as most structures have a service life of multiple decades and loss in strength can be detrimental for the global stability of structures.

2.2.6 Fungi

In temperate climates, including Belgium, fungi are the most important organisms that damage wood [27]. For this reason, it is important to have a better understanding of the different types of fungi and how they affect the quality of the wood product. Fungi form a separate group of organisms in the kingdom of fauna and flora and include microorganisms such as yeasts and moulds. They feed on organic material and grow by extension and branching of the hyphae [27]. Many different fungi with different characteristics exist and are therefore categorized in different groups, of which an overview can be found in Table 5.

Table 5: Categories of fungi [10]

	Wood-destroying fungi			**Staining fungi**		**Surface moulds**
Types of fungi	Brown-rot fungi	White-rot fungi	Soft-rot fungi	Blue-stain fungi	Other stain fungi	Ascomycetes, deuteromycetes
	Basidiomycetes	Basidiomycetes (ascomycetes)	Ascomycetes deuteromycetes	Ascomycetes, deuteromycetes	Ascomycetes, deuteromycetes	
Example	*Coniophora puteana*	*Trametes versicolor*	*Chaetomium globosum*	*Aureobasidium pullulans*	*Arthrographis cuboidea*	*Paecilomyces variotii*

In order for surface moulds and staining fungi to produce spores, a high relative humidity is required. They live on non-structural components of wood and can thus be found more frequently in sapwood. Moulds typically have a pale coloured or colourless mycelium. The spores of moulds can have different colours, depending on the type of mould. The main economic impact of moulds is the discoloration of the surface, but they also have the potential to cause allergic reactions for inhabitants, e.g. breathing difficulties [10].

In contrast to moulds, staining fungi can be recognized by a coloured mycelium, typically brown or black. Staining fungi can be divided in two groups, namely sap-stain fungi and blue-stain fungi (Figure 8). The first group is characterized by a rapid and deep penetration in the sapwood, whereas the latter category mainly grows near the surface of wood [27]. Furthermore, blue-stain fungi also have the ability to penetrate through paint films and hence increase the permeability of otherwise refractory lignocellulosic materials [10].

Figure 8: Blue staining fungi invading the sapwood and not the inner heartwood [31]

Soft-rot fungi, which are closely related to moulds and stain fungi, are capable of degrading the lignocellulose and hence causing significant strength loss. However, the wood has to be almost permanently moist in order for them to cause degradation. This means that soft-rot fungi are mainly present when wood-based materials are used in ground contact, cooling towers or other coastal applications. In untreated wood above the ground, soft-rot fungi are rarely present because they are subdued by wood-rotting basidiomycete fungi [27].

Basidiomycetes are the most damaging in constructions and consist of brown- and white-rots. White-rot fungi degrade hemicellulose, cellulose and lignin and typically leave a bleached a whitish colour after degradation of the lignocellulose (Figure 9) [10]. White-rots cause strength loss of the wood, but it occurs relatively slow.

Figure 9: Whitish colour after degradation of OSB due to white-rot fungus

Brown-rot fungi degrade hemicellulose and cellulose, but not lignin. After degradation, the wood is left with a brow colour because of the absent cellulose and the remaining brown lignin, as can be seen in Figure 10 [10].

Figure 10: Brown-black colour after degradation of pine sapwood due to brown-rot fungus

In the degradation process of brown-rot fungi, the cellulose is chopped by a non-enzymatic process, resulting in a rapid strength loss. Furthermore, this causes the wood to crack across the grain as it shrinks when the cellulose is degraded and removed. The cross-grain cracks combined with longitudinal cracks typically result in a cubical cracking pattern, which can be seen in Figure 11 [27].

Figure 11: Cubic fracture pattern after brown-rot decay [10]

Basidiomycete fungi have the same basic needs as many other organisms in order to decay wood: nutrients, oxygen, a suitable temperature and water. In case of wood-products, the elementary nutrients are usually present. Considering that a normal atmosphere contains approximately of 21% oxygen, wood-rotting fungi can still grow at oxygen concentrations of 1-2% and oxygen is ubiquitous, the presence of sufficient oxygen is usually no limiting factor [27]. Unfortunately, the comfortable temperatures for inhabitants correspond to the ideal temperatures for the growth of wood-decaying fungi, as can be seen in Figure 12. Since human comfort is essential, temperature regulation is not an option to avoid wood rot.

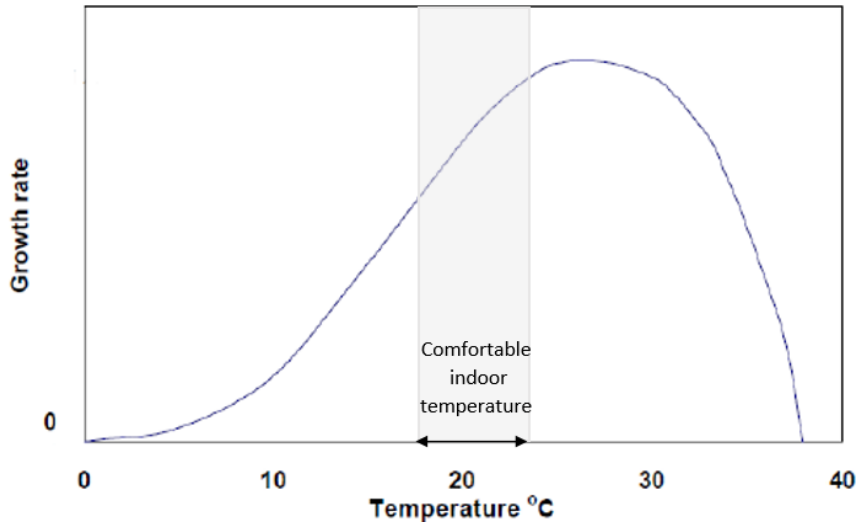

Figure 12: Temperature-dependent growth rate for wood-decaying fungi [27](

Water is the fourth requirement for fungal decay and is the key factor in the design of durable structures. The moisture content (MC) of wood is an important factor for controlling fungal decay and is defined as the ratio of the difference in weight of the wet and oven dried sample on the weight of the oven dry sample. In living trees, the moisture content varies from 150% to 200% MC in sapwood and 30% to 40% MC in heartwood. The great difference can be explained by the fact that the sap flow only occurs in sapwood, and not in heartwood [32].

Two different types of water can be distinguished in the inner structure of wood, namely free-flowing water in the pores and water in the cell walls. The critical moisture content, also called the fibre saturation point (FSP), is defined as the moisture content at which there is no free-flowing water in the pores. When wood dries below the FSP, the cell walls loose water, shrink and stresses arise, possibly resulting in cracks [27]. For moisture contents below the FSP, the mycelium experiences more difficulties to extract water from the cell walls because of the high capillary forces. The minimum

moisture content for fungi to colonize is higher than the one to decay. Morris found that the minimum moisture content for persistent fungal decay lies between 22% and 24% MC. However, 20% MC is often indicated as the safe maximum moisture content under which no fungal decay can occur [27].

2.3 Good building practice

Interrupting overlap of vapour barriers, air leakage, thermal bridges, etc should be avoided, yet occur. Hence, air and moisture can infiltrate towards the inner environment and damage could occur. Several types of damage phenomena can be distinguished, see Figure 13.

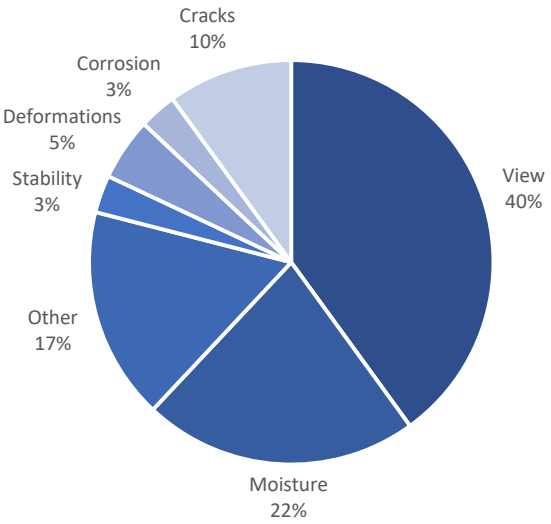

Figure 13: Causes of building damage [33]

It is important to realize that moisture is responsible for more than 50% of the total damage, either directly or indirectly. Moisture is a catalyst for different damage mechanisms and is only unacceptable when it adversely affects a part of the building [33]:

- Reduction of the durability of construction elements: frost damage, wood rot, corrosion, decrease of stiffness, etc.
- Changes in view: damp spots, fungi, algae, salt efflorescence, etc.
- Economic damage: hindering the building function, increase of energy losses, etc.

In practice, most problems can be avoided by implementing good building practice. Moisture protection is thus a key issue for many building materials, especially for wood-based products. Two principle rules should be used. First of all, liquid water should be avoided in the building, and when this is not possible, the water should be removed as fast and effective as possible [10]. One way to achieve these principle rules is by design, i.e. good building practice. Another way is to apply coatings and hydrophobing agents or to enhance the material's characteristics by modification. The latter method is often very efficient, but is also accompanied by an increase in costs. Hence, the focus in this section is put on good building practice. In case the wood-based elements are exposed to moist from the exterior, the design rules presented below should be applied.

1. **Avoid direct contact with wet walls**

In the left part of Figure 14, there is a physical separation between the end of the beam and the wet wall, thus allowing drying of the wood. In the right part, the end of the beam is in direct contact with the wet wall. Consequently, drying is inhibited and conditions favouring fungi are created.

Figure 14: Design rule 1: avoid direct contact with wet walls [10]

2. Provide a gutter to avoid moistening of an exterior wall due to splash

In the left part of Figure 15, moistening of the exterior wall can be observed due to the lacking of a gutter. This mainly results in aesthetical damage, but also wood rot can be encouraged. In the right part of Figure 15, cascaded eaves can be observed. That way, water accumulation and moistening of exterior walls is avoided.

Figure 15: Design rule 2: provide a gutter to avoid splash

3. Provide end grain protection

In the left part of Figure 16, one can see that the end of the beam is unprotected, with decay as a consequence. In the design at the right, a metal cover is provided at the end grain wood. In section 4.2.4, it will become clear that fungal degradation starts at the sides for some wood species. Due to the cutting operation, the transverse plane is on the sides, causing water and hyphae to enter the wood through the tracheid openings. In the left design, this is clearly the case. Consequently, there can be concluded that providing an end grain protection is required for a good design.

Figure 16: Design rule 3: provide an end grain protection

4. Provide covers at top of main beams

In the left part of Figure 17, it can be noticed that no physical protection is applied on top of the main beam, resulting in severe decay. The right part of Figure 17 shows a good design, where a proper coating is applied on the main beams as protection against weathering.

Figure 17: Design rule 4: provide cover at the top of main beams

5. Protection from water accumulation by providing a good separation between beams

In both left and right parts of Figure 18, the beams are installed with a good separation to avoid water accumulation. In addition, it can be seen that the beams in the right design are inclined to allow for water drainage.

Figure 18: Design rule 5: prevent water accumulation by providing good separation between beams and allowing for water drainage

6. Protection from ground contact

The wooden pillars are in direct contact with the ground in the left part of Figure 19, creating favourable conditions for fungi and other organisms, whereas the pillars are installed in a concrete foundation in the right design of Figure 19, hence avoiding moisture due to direct ground contact.

Figure 19: Design rule 6: avoid direct ground contact

The design rules mentioned above are mainly important for exterior elements. In the indoor environment, it is important to avoid moisture because surface condensation and surface fungi are common problems. Surface fungi grow on porous surfaces when the following three conditions are satisfied [33].

1. The surface contains nutrients for fungi
2. The surface temperature and humidity is sufficiently high
3. Conditions 1 and 2 are fulfilled for a sufficiently long period

Fungal growth at surfaces is highly depending on the surface temperature and relative humidity, as can be seen in Figure 20. In this figure, index 0 corresponds to no fungal growth; index 1 to growth initiation; index 2 to moderate growth, visible under the microscope; index 3 to visible growth, fungal spores; index 4 to fungal deterioration over 10% of the surface and 5 to fungal deterioration over 50% of the surface.

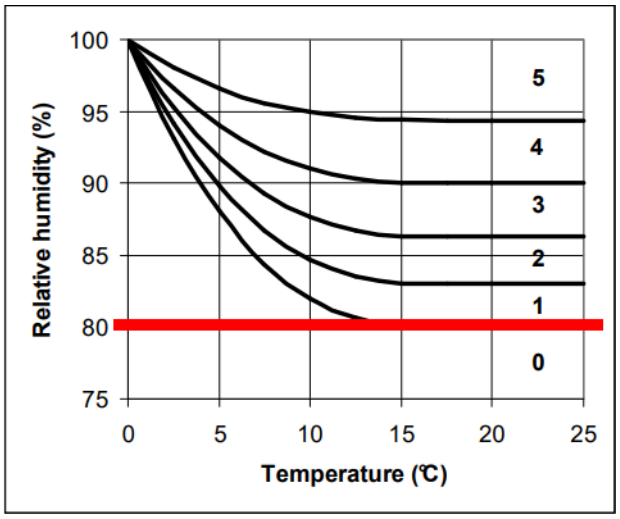

Figure 20: Isoline-diagram in function of fungal index [33]

In this figure, one can clearly see that there is no fungal growth on surfaces when the relative humidity is lower than 80%, independent of the surface temperature. Hence, the following requirement can be formulated.

$$\phi_{si} = \frac{p_i}{p_{sat}(\theta_{si})} < 0.8 \qquad (2\text{-}2)$$

Where ϕ_{si} is the relative humidity at the surface, p_i the vapour pressure of the indoor air and $p_{sat}(\theta_{si})$ the saturation vapour pressure at a temperature equal to the surface temperature. Hence, one can conclude that the probability of the fungal growth and surface condensation increases when:

- The room is insufficiently ventilated (the produced vapour cannot be drained)
- The room is insufficiently heated (low average indoor temperature)
- The walls are badly insulated (high U-value)
- The heat transfer by convection and radiation is small (at corners and behind furnishing)

The first and second parameter are mainly dictated by the behaviour of the inhabitants, but by installing regulated ventilation, the design engineer can have a small influence. The third parameter, for one, is the most important because the engineer can easily adapt the material's characteristics such that a low U-value is obtained.

In case of elements in the building envelope, drying is usually inhibited as they are positioned in between two other materials. Once again, moisture should be avoided in order to prevent fungal growth

and to make sure that the thermal performance is maintained. Moistened insulation typically has a higher heat conduction coefficient, hence an increased U-value and a reduced thermal performance. Typically, a building is characterized by a thermal and vapour pressure gradient over its envelope. When the temperature of a material is lower than the dew point of air, internal condensation occurs. In case one is interested where and how much internal condensation will occur and how the vapour pressure varies over the envelope, the method of Glaser can be used [33].
In order to prevent internal condensation, and thus fungal growth, one can apply one or more of the following measures.

- Limiting the moistening by applying a vapour barrier at the hot side of the insulation
- Facilitating drying by applying vapour-permeable materials at the cold side of the insulation
- Limiting the vapour flow by ventilation

Typically, a vapour barrier is chosen as a means to avoid internal condensation. It is, however, important to note that the execution should be done carefully, as staple holes and bad overlapping negate its intended effect. Figure 21 shows a bad execution of the vapour barrier.

Figure 21: Bad execution of the vapour barrier

In some cases, providing good building practice is not sufficient to prevent fungal growth, e.g. in buildings at locations with a high flooding probability, when there is a leak in the pipes, when there are clogged drains on flat roofs, etc. In these situations, wood-based materials have a high probability to remain wet for a sufficiently long period in order for fungal growth to occur. Consequently, other measures have to be taken to make sure that the quality of the wood product is maintained.

2.4 Protective measures for bio-based materials

When bio-based materials are used in construction projects, the design engineers have to determine the use class, as the damage sources depend on it. Subsequently, a suitable protection against weathering and/or wood-destroying organisms can be applied. Table 6 shows the use classes according to EN 335. Note that there exist extreme cases for the use of wood products, which can cause another use class than defined in this standard.

Table 6: Use classes according to EN 335 [34]

Use class	General service conditions	Occurring organisms				
		Wood-disfiguring fungi	Wood-destroying fungi	Beetles	Termites	Marine organisms
1	Interior, dry	-	-	U	L	-
2	Interior or under roof, not exposed to weather, possibility of condensation	U	U	U	L	-
3.1	Exterior, without soil contact, exposed to weather, limited moisture conditions	U	U	U	L	-
3.2	Exterior, without soil contact, exposed to weather, persistent moisture conditions	U	U	U	L	-
4	Exterior, in contact with soil or freshwater	U	U	U	L	-
5	Permanently or regularly immersed in salt water	U	U	U	L	U

U = spread all over Europe

L = occurs locally all over Europe

There are multiple approaches to provide a protective measure against decay and moisture. The easiest way to do this is by using a durable wood species. However, considering that most European wood species have an insufficient durability for outdoor applications according to EN 350-2 (Table 7) and transporting durable wood species increases the ecological footprint, other protective measures can be recommended.

Table 7: Durability classes according to EN 350-2 [34]

Durability class	Time [years] without degradation in ground contact	Examples of wood species (heartwood)
I	≥ 25	Azobé, piquia, cumaru, afzelia, bilinga, iroko, masseranduba, teak
II	≥ 15	European oak, basralocus, chestnut, magogany, merbau, robinia, wengé, western red cedar
III	≥ 10	Larch, douglas fir, American whitewood, meranti, pitch pine
IV	≥ 5	Pine, American oak, hemlock, spruce
V	< 5	Beech, poplar, birch, radiata pine

The protective measures can be divided into three main categories: chemical preservation, modification and the application of coatings. The procedure, advantages and disadvantages of these technologies are outlined in section 2.4.1 to 2.4.3.

2.4.1 Chemical preservation

Depending on the intended use and the duration of the activity, the preservative's characteristics should fulfil some requirements. Wood preservatives should be chemically stable, penetrate the wood easily and be resistant to leaching out, evaporation, and light. Furthermore, the environmental pollution coming from the use and processing of the preserved wood products should be minimal [35]. Other important considerations for choosing a preservative are its corrosion resistance and its compatibility with paints and coatings, adhesives and other building materials. However, no preservatives have yet been established that meet all of these criteria [35].
Wood preservatives are mostly used in forestry and sawmills, but also have some applications in structural engineering. In case the wood cannot be dried immediately after sawing, some wood species are susceptible to discolouring fungi. When stored or transported, repeated treatment can be required to protect the wood and maintain its quality [10]. In addition, preservatives are used to protect non-load bearing building components, such as windows and outer doors [35]. In structural engineering, flame retardants should be used because early collapse must always be prevented.
Chemical preservation is believed to remain the prevailing wood-protection process in Europe in the coming decades. Toxic active ingredients will be replaced with more environmentally friendly alternatives. Modified wood is believed to replace biocidal treated wood in less challenging applications when the price of the modified wood decreases and its effectiveness is proven [10].

2.4.2 Modification

A potential alternative to chemical preservation and the use of durable exotic wood species is modification of less-durable wood (e.g. beech, pine, spruce, etc.). The main goal of modifying the wood structure is to improve its properties, such as fungal susceptibility, resistance to fire and water, etc. That way, cheaper and local wood species can be used that have similar properties to the exotic species. However, modifying the species' structure is not always preferred because of the following reasons [36]:

- Complicated production processes
- High production costs
- Decrease in strength and increase in fragility of wood due to thermal and some chemical modifications
- Only well-impregnable wood species can be chemically modified

Various methods can be used to modify the wood structure and can be divided into four categories: mechanical, physical, chemical and biological modification. Table 8 shows the occurring changes for these modification techniques.

Table 8: Methods for modification of wood [36]

Modification method	Change in the wood structure		
	Geometric	Anatomical and morphological	Molecular
Mechanical	+	+	-
Plasma, laser	(+)*	+*	+*
Thermal	-	(+)	+
Chemical: filling lumens	-	+	-
Chemical: blocking –OH groups	-(+)	(+)	+
Biological	-(+)	-(+)	+

+ a significant change; (+) an insignificant change; - without an apparent change, * changes in surfaces

2.4.2.1 Mechanical modification

In mechanical modification, the wood is first plasticized, to reduce the glass transition temperature of lignin, and compressed in a metal mould afterwards. As a result, the compressed wood has a higher density, hardness and compression strength [36]. However, this type of modification does not increase the hygroscopicity and fungal resistance [36], [37]. Consequently, densified wood is typically used for interior application (because of its good fire resistance), products exposed to increased stresses or for musical instruments. Exposing densified wood to exterior environments is not recommended, as it should be protected against weathering and fungi.

2.4.2.2 Plasma and laser treatments

Since plasma and laser treatments are confined to the outermost surface layer and do not affect the bulk properties, they are used for modifying the surface characteristics of wood [38]. Typically, such treatments positively affect surface characteristics by increasing the polar component of the surface energy [38]. On the one hand, plasma treatments can be used to increase the hydrophilicity and wettability such that the adhesive strength of for example laminated wood is improved. On the other hand, plasma treatments can also be used to increase the hydrophobicity [39]. That way, the wettability decreases and the resistance against wood-decaying fungi increases. Furthermore, plasma and laser modification usually improves the weather resistance [36]. Laser and plasma modification also have their limits. Firstly, the technique is relatively expensive. In addition, surface damage can negate the positive effects of the treatment, as only the surface characteristics are modified.

2.4.2.3 Thermal modification

Of all different types of modification, thermal modification is the most advanced commercially. The first report about the effects of high-temperatures on the physical properties dated from 1915, so a lot of space was available for improvements [36]. During thermal modification, the temperature typically reaches values between 160°C and 240°C. Temperatures lower than 140°C only result in slight changes in material properties, whereas temperatures higher than 300°C cause severe deterioration [10], [40]. At these elevated temperatures, a multitude of different types of reactions take place, e.g. hydrolytic splitting of polysaccharides, oxidation and radical reactions and several condensation reactions [10]. These chemical reactions are responsible for altering many different properties, of which an overview is listed in Table 9. Note that the extent of change depends on many process variables, e.g. time and temperature of the treatment, wood species, sample dimensions, use of catalysts, closed versus open systems, etc. [40].

Table 9: Overview of advantages and disadvantages of thermal modification [40]

Advantages	Disadvantages
Improved dimensional stability	Decrease of Young's modulus
Reduced hygroscopicity: decrease in equilibrium moisture content and reduced wettability	Reduced impact toughness, modulus of rupture and work to fracture
Improved microbiological resistance	Tendency for cracks and splits to form
Darkening of the material	Darkening of the material

In Europe, different processes exist to thermally modify wood products: ThermoWood process, Plato process, OHT process and the rectification process [36]. In a preliminary experiment, which is described later in section 3.1, thermally modified spruce is tested to investigate its resistance against fungal decay. Since the thermal treatment is done according to the Plato process, this process is explained in more detail. The Plato process has four stages, which are implemented at atmospheric as well as increased pressure [36] :

1. Hydrothermolysis: fresh or air-dried wood is modified hydrothermally by using water vapour or hot air at a temperature of 150-190°C and an increased pressure of 0.6-1 MPa for a period of 4-5 hours.
2. Drying: the wood is dried in an oven until the humidity is decreased to approximately 8-10% (which usually lasts 3 to 5 days).
3. Curing: the wood is stabilized at a temperature of 150-190°C and an atmospheric pressure of 0.1 MPa for a period of 12-16 hours. In this stage, the access to oxygen is limited. The cured wood has a humidity of than less 1%.
4. Conditioning: the wood is humidified again in the oven chamber until a humidity of 4-6% is reached (which typically last 3 days).

Thermally modified wood can be used for indoor and outdoor applications, e.g. saunas and bathroom furniture, noise barriers, flooring, garden furniture, etc. [36]. As mentioned before in section 2.2.1, the strength properties decrease with increasing temperature, hence thermally modified wood is not recommended for structural applications, where strength and stiffness are the most important properties.

2.4.2.4 Chemical modification

Many of the available chemical preservatives are toxic or corrosive and are hence harmful to the environment. As an alternative, chemical modification can be used to change the material's structure, resulting in an improved dimensional stability, decay resistance, fire retardancy, resistance to UV radiation, etc. depending on the type of chemical modification [41]. When the wood is being chemically modified, the chemical substances can be located in different parts of the wood [36]: (1) lumina of the cells and (2) cell walls, with or without covalent bonds to the structural wood components.
In the first case, the large pores (lumina) are filled with various types of natural or synthetic substances, e.g. amino resins, epoxides, acrylates, styrene, etc. They affect the material's properties by creating a film which mechanically prevents the entry of microorganisms into the cell walls. Furthermore, they form a compact filling in the lumina that prevents the entry of microorganisms deeper in the wood [36]. In the second case, hydroxyl groups or other functional groups are bounded with the structural wood components, causing a decreased equilibrium moisture content of the wood and thus a better fungal resistance [36]. Furthermore, the changed molecular structure makes it more difficult for wood-decaying fungi to identify nutrition sources [42].

2.4.2.5 Biological modification

In the animal kingdom, fights for food and territory are one of the most natural things in the world. This creates antagonistic and synergic relationships between them. In particular, the principle of antagonism can be used deliberately in wood-based products. It is based on the ability of some organisms, e.g. moulds and bacteria, to produce biocidal substances that kill, suppress or stop the growth or enzymatic activity of organisms more dangerous to wood (e.g. wood-decaying fungi) [36]. During biological modification, the wood-based material is infected with a biological organism that produces fungicidal substances. An example of an organism that produces fungicidal substances is the fungus *Trichoderma viride*, which prevents the growth of wood-decaying fungi by producing viridin [43]. The main application field of biological modification can be found in agriculture, where biological control agents are used to prevent plant diseases and insect attacks [44].
Although biological modification can increase the resistance against wood-decaying organisms, the technology is not always compatible with the questions that arise about health and ecology [36]:

- What is the specificity of the infecting organism? (Does it repel all biological pests of wood or only a particular wood-decaying fungus?)
- What is the negative effect of the organism on the environment and human health?

Since the answers on these questions cannot be answered accurately, the method is quite expensive and other modification techniques have proven their effectiveness, biological modification is mostly not recommended.

2.4.3 Coatings

When bio-based materials are properly designed, there is no need to apply a surface coating to ensure that the material reaches its expected service life [10]. The choice for coatings is often due to the importance of the aesthetical appearance, but coatings can also serve as protection against abiotic factors such as liquids (for instance, in the case of floors), weathering, etc. Typically, two aspects have to be considered for coatings: (1) the coating has to produce a protective and/or decorative film and (2) the coating material has to be applied to a substrate before forming a film. [10]. This indicates that the coating material has to be in a liquid state before hardening. The transition from liquid to solid state is an important step, as it influences the manufacture, application and the coating's behaviour. The surface finishing can be classified based on the application process (spraying, roller coating, etc.), drying/curing process, its intended use, film thickness, etc. [10]. Adhesion and cohesion are two important aspects of a coating because failure might occur when coatings are exposed to environmental loads. In case the adhesive strength (strength of the interlayer between the material and the film) is insufficient, delamination of the film might occur. In case the cohesive strength of the coating is insufficient, cracks can arise in the film, thus exposing the wood to the environmental loads [45].

2.5 Testing methods for fungal susceptibility

In literature, detailed information can be found about the properties of solid wood and Eurocodes exist to perform standardized tests to assess the resistance against wood-destroying fungi. Multiple tests exist to determine the natural durability of timber, but since the most widely used laboratory method for determining the natural durability of solid wood against wood-destroying fungi is the CEN TS 15083-1 test method, this method is explained in more detail.

The laboratory method presented by CEN/TS 15083-1 provides one criterion by which the durability of timber can be assessed. It is, however, recommended to use other relevant tests, e.g. CEN/TS 15083-2, and above all practical experience [46]. In this procedure, test specimens prepared from the wood to be tested and the reference wood are exposed to attack of wood-destroying basidiomycete fungi. After a prescribed incubation time under defined conditions, the percentage of dry mass loss is used to estimate the fungal resistance on the basis of a durability rating, which is presented in Table 10.

Table 10: Durability rating scale [46]

Durability class	Description	Mass loss [%]
1	Very durable	≤ 5
2	Durable	≥ 5 to ≤ 10
3	Moderately durable	≥ 10 to ≤ 15
4	Slightly durable	≥ 15 to ≤ 30
5	Not durable	≥ 30

This standardized procedure adequately assesses the natural durability of various wood species and the efficacy of preservatives. However, this method is not suitable for the durability assessment of bio-based materials, whose natural durability is enhanced by new technologies, such as glued laminated timber, thermally modified wood, chemically modified wood and wood treated with water repellents [47].

In the standard laboratory tests, optimal conditions for fungi are used. The growth medium has a double function: (1) serving as nutrient for the fungus and (2) maintaining a sufficiently high moisture content for fungal activity [47]. In case of thermally or chemically modified wood, it might be possible that the wood does not reach the moisture content required for fungal degradation because the moisture dynamics are changed in such way that the equilibrium moisture content is decreased. As a consequence, the material may become insufficiently wet in the prescribed 16 weeks for fungal degradation, resulting in invalidated results according to the strict interpretation of the standard. This does, however, not mean that the material can eventually not be degraded.

The different enhancement technologies each affect the fungal susceptibility differently, making it difficult for the current standard of solid woods to be adequate for all bio-based materials. There does not yet exist standardized tests for these types of materials, although a standard exists for wood-based panels (CEN ENV 12038). These (engineered) materials have the advantage that the material resistance can be improved by modifying the characteristics of its components during manufacture, for instance the glue type in plywood, or modifying the wood structure according to one of the methods presented in section 2.4.2. In order to design these material to fit a specific purpose, it is necessary to have a standard test procedure of which the results correspond to reality. Since the current standardized test is not adequate, we need to find out if a modified version leads to more accurate results, for instance by prolonging the exposure time or increasing the initial moisture contents. We therefore perform a preliminary experiment, in which the samples are brought to an initial moisture content ranging between 20-30% MC before exposure to basidiomycete fungi.

In addition, this standardized test does not allow to gain insight in what is going on at the inside of the material. A thorough knowledge about the relationship between fungus and material is still lacking, in particular how the material's structure and moisture properties affect the degradation process. In order to be able to design new materials and apply them optimally, it is necessary to understand how the material's structure affects the fungal degradation. X-ray CT seems a suitable technology to monitor the moisture distribution and mass loss, because it is a non-destructive technique that allows to assess the density of wood in three dimensions, thus enabling us to obtain localized information inside the wood during the degradation process [48]. We therefore perform a second experiment, in which the moisture distribution and mass loss of four solid woods are monitored on a weekly basis using X-ray CT.

3 MATERIAL AND METHODS

3.1 Preliminary experiment: Adapted mini-block test for bio-based materials

In this experiment several (engineered) wood products are exposed to two basidiomycete fungi, in order to investigate their resistance against fungal decay. Before exposure, the materials are brought to moisture contents ranging between 20-30% MC to increase the degradation potential. Scots pine sapwood is included in the test to serve as a reference and to see whether the applied fungal strains are sufficiently virulent. Two different types of fungi are used, the brown-rot fungus *Coniophora puteana* and the white-rot fungus *Trametes versicolor*. The main objective of this experiment is to assess if bio-based materials are degraded in a mini-block test, when the samples have an initial moisture content of 20-30% before fungal exposure. In addition, a comparison is made between the fungal degradation due to *C. puteana* and *T. versicolor*.

3.1.1 Sample preparation

In total, six different wood materials are tested, of which the components and abbreviations are shown in Table 11. For each material, 21 samples are prepared with a size of 3 x 1 x 0.5 cm^3 and marked with a code and number.

Table 11: Components of the tested materials

Type of bio-material	Components
Scots pine sapwood (*Pinus sylvestris L.)*	
Radiata pine plywood	Radiata pine veneers, glue type: non-specified
Wood insulation	Norway spruce and Scots pine fibres, PUR resin, paraffin
Porous fibreboard	Norway spruce and Scots pine fibres, bitumen emulsion
Oriented strand board	Scots pine fibres, PUR resin, formaldehyde-free glueing
Thermally modified spruce	Process: 1) Hydrothermolysis up to 170°C 2) drying 3) heated again to up to 180ºC in dry conditions without oxygen

The durability of each bio-based material is assessed as the mass loss after eight weeks of degradation by *Trametes versicolor* and *Coniophora puteana*. Therefore, we have to weigh the samples before and after degradation. Since the weight varies with the moisture content, it is important to oven dry the samples at a temperature of 103°C for a period of 18 to 24 hours, before weighing. After oven drying, the samples are placed quickly into a desiccator containing silica drying crystals to ensure the samples to remain dry while cooling down, and weighed.

3.1.2 Optimal conditions for fungal growth

In order to create optimal growth conditions for fungi, three aspects are of importance: (1) the presence of easily available nutrients; (2) optimal moisture conditions and (3) optimal temperature conditions. It is important to have sterile conditions during operations involving the wood samples and growth medium to prevent contamination. For this reason, the agar medium, the wood samples, the metal grids and several bottles of demineralized water are autoclaved (Fedegari Autoclavi Spa) for steam sterilization at a temperature of 121 °C. After autoclavation, all operations involving the sterilized materials are done in the laminar flow to maintain the sterility.

3.1.2.1 Nutrients

In this experiment, nutrients are provided by making a fungal growth medium of demineralized water, malt and agar. A commonly used mixture exists of demineralized water, 3% weight percentage of malt and 2% weight percentage of agar. Considering that one Petri dish contains three mini-blocks and all Petri dishes need approximately 20 ml of the mixture, one can easily calculate the amount of water needed:

$$V_{water} = 7 \cdot 6 \cdot 20\ ml = 840\ ml$$

Taking in mind that pouring exactly 20 ml per Petri dish is difficult, it is recommended to make some extra. In this case, the mixture consists of 1 L of water, 30 grams of malt and 20 grams of agar.

3.1.2.2 Moisture conditions

Jones and Brischke found that the optimal growth conditions of most fungi are found between 25°C and 30°C at fibre saturation point, which corresponds to a wood moisture content around 21% [10]. The fibre saturation point actually varies for different wood species, but since 20% MC is a lower limit for fungal degradation, the goal is to bring the samples to an initial moisture content ranging between 20-30% MC.

A possible method to obtain these moisture conditions is by submerging the mini-blocks in sterile demineralized water such that all pores are filled, and drying them afterwards until the desired moisture content is reached. The applied wetting procedure can be explained as follows.
After sterilization, the mini-blocks are placed in a vacuum desiccator and ballasted with weights to prevent floating during the wetting procedure. This operation is done in the laminar flow, while ensuring that all elements of the experiment set-up are sterilized with ethanol. A sterilized glass piece containing a valve is installed in the desiccator. After closing the outlet valve, ensuring the inside of the desiccator to remain sterile, the desiccator is taken out of the laminar flow and connected to a vacuum pump (Figure 22). After establishing a vacuum corresponding to a pressure of 0.8 bar for 30 minutes to extract the air of all pores, the valve is closed and disconnected from the vacuum pump, and the desiccator is brought back in the laminar flow and connected to a sterile water supply.

Figure 22: vacuum pump connected to desiccator

Before opening the valve and filling the desiccator, the tube is made air-free. The filling has to be done continuously in order to avoid air infiltration. When the samples are completely submerged, the valve is closed and the desiccator is subjected to vacuum for another 30 minutes. Figure 23 shows the wetting procedure of porous fibreboard.

Figure 23: Wetting procedure of porous fibreboard

After a day, the samples are taken out of the desiccator and placed on sterilized racks for drying in the laminar flow. The samples are weighed regularly until a moisture content of 20-30% is reached. The moisture content can be determined according to equation 3-1, in which θ is the moisture content [%] and w_{dry} and w_{wet} respectively the oven dry and wet weight of the sample.

$$\theta = \frac{w_{wet} - w_{dry}}{w_{dry}} \cdot 100 \qquad (3\text{-}1)$$

The samples do not dry out at the same rate, so when the desired moisture content is reached, the samples are wrapped in aluminium foil, to prevent dehydration, and put in the fridge until the start of the experiment.

3.1.2.3 Temperature conditions

As mentioned before, the growth rate of wood-decaying fungi depends on the temperature. It is important that the fungi grow fast enough in order to be able to degrade a wood species after a prescribed period. For this reason, the Petri dishes are placed in a climate chamber at a temperature of 20°C and a relative humidity (RH) of 70% during the experiment, as prescribed by the standard natural durability test [46].

3.1.3 Order of operations

As mentioned before, the agar medium, the wood samples, the metal grids and several bottles of demineralized were autoclaved after preparing the samples and making the agar medium. After autoclavation, approximately 20 ml agar medium is poured in Petri dishes. After solidifying, the Petri dishes are inoculated with fungi *Trametes versicolor* and *Coniophora puteana*, closed off with surgical tape and placed in a climate chamber at a relative humidity of 70 % and a temperature of 20°C. The surgical tape is a precaution to prevent mites from entering the Petri dish and contaminate the malt agar medium. The tape also has the property to allow gas exchange between the interior environment of the Petri dish and the exterior environment, which is required for those fungi that need oxygen to survive.

A week after inoculation, the fungi have overgrown the Petri dishes and sterilized metal grids are placed onto the fungus. This ensures that direct contact between the wood samples and the agar is avoided, so the wood samples cannot directly take up moisture from the agar medium. After checking the moisture content of the mini-blocks one last time, three samples are placed in each Petri dish. Contact between the samples is not allowed as this could obstruct the fungal growth at some sides. After taping the Petri dishes, they are placed in a climate chamber for a period of eight weeks such that the fungus can grow and degrade the mini-blocks.

After eight weeks, the wood samples are removed from the Petri dishes and the adhering mycelium is scraped off to limit the share of the fungal biomass in the wet and oven dry weight. After weighing the wet weights, the samples are oven dried for 18 to 24 hours at a temperature of 103°C. The wet weights before (after) fungal decay are determined because a moisture content lower than 20% (higher than 80%) together with a mass loss under 3% could imply that the samples were too dry (wet) for degradation. Finally, applying the same weighing methodology as before, one can determine the mass loss of each sample according to equation 3-2, in which $w_{dry,0}$ and $w_{dry,1}$ are respectively the oven dry weight before and after fungal decay.

$$Mass\ loss = \frac{w_{dry,0} - w_{dry,1}}{w_{dry,0}} \tag{3-2}$$

3.1.4 Control test

Using the procedure described above, the moisture contents and mass losses of several bio-based materials are calculated after a period of eight weeks of exposure to *C. puteana*. In that experiment, however, it is unknown how much of the material moisture content is due to the moisture absorption from the growth medium and how much due to moisture production by the fungus. Therefore, a control test is performed in which the moisture content of samples without exposure to basidiomycete fungi is determined on a weekly basis. In order to be able to compare the final moisture contents in the control test to those of the samples exposed to *C. puteana*, the same bio-based materials are used.

The samples have the same dimensions as before, namely 3 x 1 x 0.5 cm³. The experiment has to last 8 weeks in order to compare the results to those of the preliminary experiment, so 24 samples are required for each material type. Considering that each Petri dish is filled with 20 ml of the growth medium (to have similar conditions), producing a mixture containing 1000 ml water, 20 g agar and 30 g malt is sufficient.

The samples are oven dried for 24 hours at a temperature of 103°C and the oven dry weights are determined. Next, the bottles of agar medium, metal grids and samples, which are packed in aluminium foil to prevent them from moistening, are autoclaved (Fedegari Autoclavi Spa) for steam sterilization at

a temperature of 121°C. The Petri dishes are then filled with the growth medium in the laminar flow. After solidifying, the metal grids are placed in the Petri dishes together with three samples of each material type. Finally, stacks of six Petri dishes are made, taped with surgical tape to prevent microorganism from entering, and put in the climate chamber at 20°C and 70% RH. On a weekly basis, a stack of Petri dishes is removed from the climate chamber, and the moisture contents are determined.

3.2 Experiment 2: Monitoring of the moisture distribution of bio-based materials using MRI and X-ray CT

In most experiments regarding fungal susceptibility, the moisture content and mass loss are determined at the end of the experiment or a weekly follow-up is applied. However, it is impossible to remove samples from the experiment and placing them back without influencing the fungal growth (the mycelium is often adhered to the surface of the samples). To gain insight into how different material's structures and wood anatomical features affect degradation, non-destructive methods are needed. There exist multiple techniques to visualize the internal structure of wood-based materials. We select two of them that are non-destructive and are interesting for the visualization of water, magnetic resonance imaging (MRI) and computed tomography (CT). Both methods are based on different principles and have several benefits and limits, which will be outlined in section 3.2.1 to 3.2.3.

3.2.1 Working principle of MRI

MRI is usually used to visualize the internal structure of an object. Since magnetic gradients have to be created to visualize the inner structure, the object to be scanned has to be placed in the inner hole of the MRI machine. Depending on the size of the object, different types of MRI machines can be used, as can be seen in Figure 24.

Figure 24: MRI machine for people (left) and for mice (right) [49],[50]

In order to understand how MRI works, an understanding of several physics principles is required. MRI is based on nuclear magnetic resonance (NMR), of which the basic principle deals with the interaction of certain atomic nuclei, radio frequency energy and a strong magnetic field [51]. Radio waves are characterized by frequencies as high as 300 GHz to as low as 3 kHz, corresponding to wavelengths from 1 mm to 100 km, which can be seen in Figure 25. Typically, radio waves used in MRI have wave lengths of approximately 1 m [52].

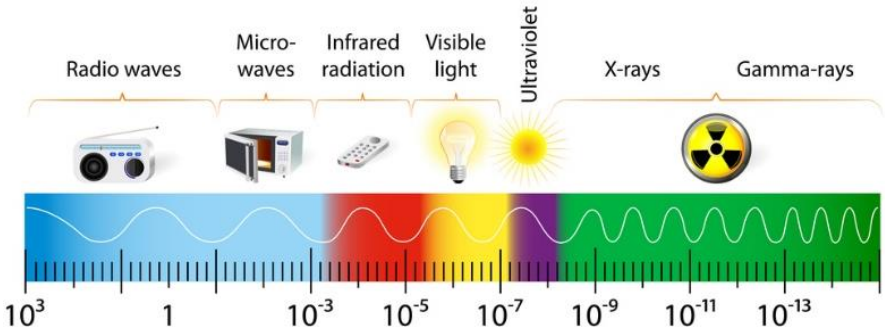

Figure 25: The electromagnetic spectrum [53]

Since the interaction of atomic nuclei is important in the understanding of MRI, a closer look is taken at the most simple element, namely hydrogen, which consist of one proton and one electron orbiting the proton. It is important to realize that the proton is not static in the centre of the atom, but actually rotates on its axis [51]. As a proton is positively charged, a small magnetic field arises in a specific direction than can be determined with the right hand rule. A lot of protons can be found in the human body, for example in water, fats and carbohydrates. In normal situations, the rotation of the protons is randomly oriented, so they cancel each other out and there is no net magnetic moment. However, when a strong magnetic field is present, which is the case in a MRI machine, the spins of the protons line up and two different situations emerge. Most of the protons line up with the main magnetic field, but a few line up directly opposite to the main magnetic field. The orientation of the magnetic field is determined by the amount of energy associated with each of the individual atoms or protons. The protons that line up opposite to the main magnetic field have a little extra energy, for example due to a local increase of heat, and are therefore considered to be in a high-energy state, whereas the ones that line up with the main magnetic field are in a low-energy state.

When a strong magnetic field is present, the protons do not simply point in the direction of the main magnetic field, but actually precess, just like a spinning top. The rate of precession can be determined by the Larmor frequency equation [54]:

$$f = \gamma \cdot B_0 \tag{3-3}$$

This equation states that the rate of rotation f is proportional to the strength of the local magnetic field B_0. At 1.0 T, the Larmor frequency of a hydrogen nucleus is 42.6 MHz, so the constant in the equation equals 42.6 MHz/T. This will be important when the imaging part is discussed.

As stated before, the protons line up with or opposite to the direction of the main magnetic field. Even though they all precess in space, when all opposing vectors are cancelled out, a net magnetisation along the main external magnetic field arises, which is called longitudinal magnetization. Since this net magnetisation is directed in the same direction as the main external magnetic field, it cannot be measured directly. For that reason, energy is put into the protons. When a radio frequency pulse of exactly 42.6 MHz is transmitted in the vicinity of the protons, they will absorb the energy, causing some of them to switch towards the high-energy state. When sufficient energy is transmitted to flip some of the protons from the low-energy state to the high-energy state such that both states are equally distributed, there is no net longitudinal magnetisation left.

In addition, the radio frequency pulse pushes the protons to synchronize and spin together, creating a net magnetic force perpendicular to the longitudinal magnetization which is called transverse magnetization [54]. This magnetization can be detected with an antenna or coil because a small current

is generated by the rotation of the transverse magnetization, which is caused by the precession of the protons.

After removal of the radiofrequency pulse, the protons will relax back to their original position. First, the protons repel each other and move apart, which causes the transverse magnetisation to disappear. This action is referred to as the T_2 or spin-spin relaxation [51]. Secondly, the protons in the high-energy state return to the low-energy state, creating once again a net longitudinal magnetization. This action is called the T_1 or spin-lattice relaxation, as energy is transferred from the spins to the surrounding tissue or lattice. Because protons in human bodies originate from different sources, such as free flowing water and fats and proteins that are fixed in position, their T_1 and T_2 relaxations differ. This will also be the case for different types of water in bio-based materials. In a study of Hernandez and Caceres, in which MRI is used to visualize the liquid water distribution of sugar maple, three different T_2 relaxations could be differentiated. A fast T_2 that represents all cell-wall water, a medium T_2 that represents water in earlywood lumina and a slow T_2 that corresponds to water in latewood lumina [55].

The T_1 and T_2 relaxations can be measured by changing how quickly the radiofrequency pulses are emitted, which is called the repetition time T_R, and how quickly is listened to the return signal emitted by the protons, which is called the echo time T_E [51]. The image contrast depends on three factors, namely the T_1 relaxation, the T_2 relaxation and the proton density in the sample, but it is possible to minimize the effects of two parameters to obtain a specific image contrast. By rapidly emitting radiofrequency pulses (a low T_R), which causes the spins to remain in the high-energy state, and having a low T_E, which mitigates the T_2 effects, the T_1 effects can be accentuated. These characteristics are typically applied in T_1 weighed images. T_2 weighed images, on the other hand, are obtained by a longer echo time to allow the protons to move away from each other and a long repetition time to mitigate the T_1 effects.

In order to create an NMR image, also called MRI, magnetic gradients are created. First, a gradient is created along the length of the body. Since the frequency is proportional to the strength of the magnetic field, the resonant frequency of the hydrogen nuclei indicates their location in the gradient [54]. At an interesting part of the object under study, a slice is made and emitted with a radiofrequency pulse that causes the protons to resonate. However, since the local magnetic gradient is homogeneous, typically with strengths of 1 T, 1.5 T and 3 T, the net magnetic moments in that slice are in phase spinning together in sync and cannot be distinguished from each other. To solve this problem, two other gradients can be applied to further localize the magnetic moments [51]. By briefly exposing the slice to a gradient along the direction of gravity, the magnetic moments at the bottom of the gradient slow down because of the lower magnetic field strength and a phase shift along this axis a created. The third gradient, with its direction perpendicular to the directions of the other gradients, is used to localize each of the signals. This frequency encoding gradient remains on while recording the signals. Each of the signals has a unique phase and frequency and can thus be localized in 3D space. By giving a greyscale value to the volume elements in a slice (voxels) corresponding to the strength of the local signal, the hydrogen concentration can be measured. Since a standard MRI is built up by a 256 x 256 or 512 x 512 matrix, a lot of details can be observed in the structure of an object. By applying this technique on samples of bio-based materials, the hydrogen distribution corresponding to H_2O molecules inside the sample can be visualized.

3.2.2 Working principle of X-ray CT

In contrast to the radio waves used in MRI, X-ray CT uses X-rays, which typically have wavelengths of 0.01 nm to 10 nm. In order to explain the main principles of CT, a simple example is elaborated. Assume that an object is composed of three materials with different densities and is put in the centre of the

gantry, the device that contains the X-ray source and sensors (Figure 26). The black represents a high density, the light grey a low density and the dark grey a medium density [56].

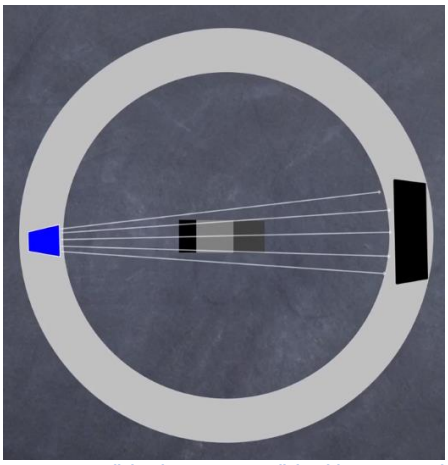

Figure 26: X-ray source (blue), sensors (black), X-rays (grey lines) [56]

In case the source emits X-rays at an angle of 0°, which is the instance in Figure 26, the high density material obstructs part of the X-rays. As the X-rays are only blocked by the densest material, the image can be presented as follows.

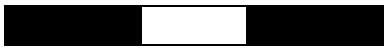

Figure 27: Intensity image at 0°

In case the X-rays are emitted at a 90° angle, the X-rays pass through all three materials, but since they have a different density, the share in obstructing will differ. An image of the intensities received by the sensor will thus be different, see Figure 28.

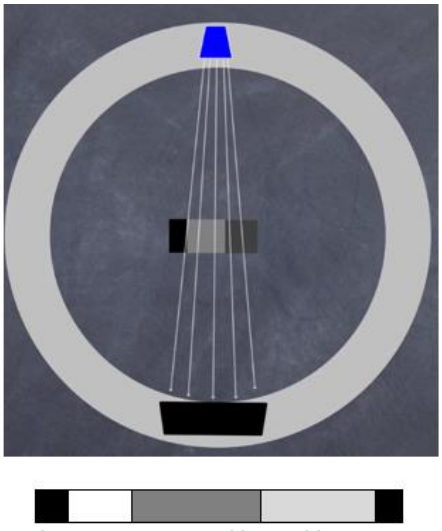

Figure 28: Image of intensities at 90°

The lighter the colour in this figure, the more the X-rays are obstructed. The colour code is thus logical, as a denser material can hinder the X-rays more easily. By comparing intensities at 0° and 90°, one can deduce that the object has a rectangular shape as the length of the intensity zones of both angles differ.

When the X-rays are emitted at an angle of 45°, the following occurs (Figure 29).

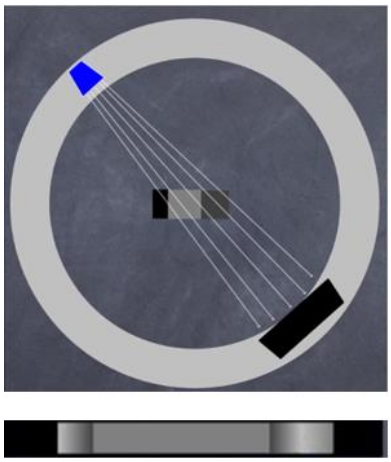

Figure 29: Image of intensities at 45°

A part of the X-rays only go through the middle material, which explains the dark grey at the middle. The light grey can be explained by X-rays passing only through the medium dense material. The darkening at the right of the light grey can be explained by the fact that the X-ray have to pass through less and less material, so less obstruction occurs. The same can be said at the colours at the left side. The X-rays that only pass through the densest material explain the white colour. By moving to the right, more X-rays pass through the less dense material. Hence, less X-rays are absorbed and more X-rays reach the sensors than when they only pass through the densest material, resulting in a darkening of the colour in the scan. Note that the colour code of Figure 29 is not 100% accurate, but it is the concept that is the most important.

The most important observation of this example is that there will be differences in intensities as the X-rays pass materials with different densities, and thus get absorbed at different amounts [56]. Typically, a more dense material will turn up more white in the scan, whereas less dense materials are characterized by a darker colour. By taking scans at many points around the gantry, and by using complex mathematics, which will not be discussed here, a computer will be able to reconstruct the object and its different substances in 3D.

3.2.3 Advantages and disadvantages of MRI and CT

Usually, MRI and CT scanning are used for the visualization of internal organs of humans and animals. Using these technologies for the visualization of the inner wood structure during fungal deterioration is quite innovative and consequently brings forth some challenges. The limits and benefits of both techniques for this specific application are listed in Table 12 and Table 13.

Table 12: Benefits and disadvantages of MRI [54]

Benefits	Disadvantages
- MRI can differentiate water and other soft tissue better than CT - Any plane can be imaged - No ionizing radiation - No contrast medium is required - Ability to provide tissue characterization beyond simple hydrogen distribution	- Petri dishes do not fit in the MRI machine → Other set-up has to be made, with maintenance of the sterile conditions - Only one tube can be scanned at the time → Time consuming procedure - The samples have to be centred manually - Complex and high costs - Noise during the scan - No metals are allowed in the proximity*

* Since the MRI machine has a strong magnet, metals could damage the machine.

Table 13: Benefits and disadvantages of CT [54]

Benefits	Disadvantages
- Petri dishes fit in the machine set-up - The procedure can be automated so that stacks of Petri dishes can be scanned without supervision	- Ionization radiation is used - Metals should not be near the samples * - Wood and water are difficult to differentiate based on X-ray CT images**

* Metals are not allowed because metal artefacts appear as a streaking effect on an image, with areas of increased and decreased density obscuring adjacent structures. Hence, the CT images can be degraded [57].

** The differentiation is difficult when the density of the wood is close to the density of water.

Dealing with the disadvantages of MRI and CT is important in the preparation phase of the experiment. The specific measures that were taken for the execution of the experiment are described in section 3.2.4.

3.2.4 Materials and methods

In this experiment, several bio-based materials are exposed to the brown-rot fungus *Coniophora puteana* and monitored on a weekly basis using MRI and X-ray CT. By using X-ray CT, the densities of the different substances can be determined based on the attenuation. However, the density of some components are close to the density of water, therefore making it harder to differentiate wood from water. This problem does not occur in MRI, where the free water is the only component that is visualized. By combining both X-ray CT and MRI scans and comparing the results, it will be easier to understand how the material's structures and wood anatomical features affect the degradation process.

The main objective of this experiment is to see how the moisture distribution and degradation evolve during the exposure to *Coniophora puteana*. Additionally, a comparison is made between the moisture distribution of the sealed samples and the samples of which the initial moisture content was brought in the range of 20-30%. Finally, the difference in moisture behaviour between samples exposed to *Coniophora puteana* and samples without exposure to fungi is examined.

3.2.4.1 Sample preparation

In this experiment, four commonly used bio-based materials (Norway spruce, gaboon, OSB and radiata pine plywood) are tested. Spruce is included to serve as a reference and to see whether the fungal strains are sufficiently virulent. In the preliminary experiment described in section 3.1, all samples underwent a wetting procedure. In order to find out whether this wetting procedure has an influence on the behaviour of the fungus and the moisture distribution, two types of samples are distinguished. A first type is characterized by an initial moisture content ranging between 20% and 30%, such that the degradation potential is increased. The second type is conditioned for two weeks in a climatic chamber at 20°C and 65% RH. In reality, the header and stretcher faces of plywood and OSB are often not exposed and therefore not vulnerable to attack. For this reason, the header and stretcher faces of part of the samples are sealed with a waterproof mixture such that the fungus can only penetrate through the bottom surface. An overview of the different types of samples and their specificities can be found in Table 14.

Table 14: Specificities of the tested samples

Type of material	Number of samples exposed to *C. puteana*	Number of samples without exposure to fungi	Sealed?
Spruce (*Picea abies (L.)H. Karst.)*	12	2	No
Gaboon (Aucoumea klaineana Pierre)	6	2	No
'Dry' OSB	4	2	Yes
'Wet' OSB	4	0	No
'Dry' radiata pine plywood	4	2	Yes
'Wet' radiatapine plywood	4	0	No

The samples of spruce and gaboon have dimensions 3 x 1 x 0.5 cm^3, whereas the samples of OSB and pine plywood have dimensions 3 x 2 x 0.75 cm^3. That way, the effect of the glue on the moisture distribution and fungal decay can become more clear. Note that these larger samples originate from a thicker plate of 1.5 cm. However, the experimental set-up does not allow for a sample height larger than 0.75 cm. Sawing the thick plate in two halves solves this problem, but this also means that either the top or bottom face of the samples is characterized by an irregular surface. To mitigate the possible influence of this effect during the degradation process, the irregular surface is marked and will be located at the top.

3.2.4.2 Sealing procedure

Due to the cutting operation, it might be possible that the transverse plane is on the sides, causing water and hyphae to enter the wood through the tracheid openings. By providing a sealing, one can make sure that the degradation process does not start at the sides. In this experiment, we choose to apply a sealing to part of the OSB and pine plywood samples because their sides are often shielded by other building elements in practice. Furthermore, we can compare the results and find out if the sealing has a significant impact on the degradation process. The sealing consists of two products that are mixed according to prescribed proportions. The first ingredient is a solvent-borne paint that contains a polyisocyanate curing agent and is based on aliphatic acrylic polyurethane [58]. The second product is a hardener that mainly consists of isocyanic acid, hexamethylene ester and polymers. In order for the sealing to be effective, the side faces are repainted three times, with a drying period of 24 hours. Figure 30 shows how he samples should be sealed.

Figure 30: Sealing application on OSB and pine plywood samples

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3.2.4.3 Wetting procedure

The wetting procedure of the samples is the same as for the preliminary experiment. In sterile conditions, the samples are first subjected to vacuum suction such that the air is extracted from all pores. After pouring water in the desiccator, they are subjected to vacuum once again. By letting the samples dry on racks in the laminar flow and regularly weighing them, an initial moisture content of 20-30% is reached. The samples dry out at a different rate, so the samples with a 20-30% MC are packed in a double layer of aluminium foil and put in the fridge until the start of the experiment.

3.2.4.4 Experiment set-up

As mentioned before, Petri dishes do not fit in the MRI machine [50]. Hence, another experiment set-up should be realized without compromising the sterility and the gas exchange with the outer environment. A possible solution for this problem is the use of tubes made of polypropylene (PP) and caps made of high-density polyethylene (HDPE). Since completely closing the cap prevents the gas transport between the inner and outer environment, a small hole is bored in the cap and covered with a double layer of surgical tape to prevent microorganisms from infiltrating and contaminating the growth medium (Figure 31). The tubes have an opening diameter of 3 cm, a conical bottom and are marked till a volume of 50 ml.

Figure 31: Tubes with hole in cap to allow gas exchange

By making a hole in the cap, the inside is no longer sterile. Hence, the tubes are autoclaved (Fedegari Autoclavi Spa) for steam sterilization at a temperature of 121 °C. Note that the caps are made of HDPE and are not allowed inside the autoclave. Therefore, the caps are sterilized by ethanol in the laminar flow.

3.2.4.5 Order of operations

Fungi need nutrients to grow, so a growth medium should be produced. As in the other experiments, a mixture is produced containing 2% agar and 3% malt per 100 ml demineralized water. In total, 31 tubes are required containing each 25 ml of the mixture, which means that combining 1 L water, 20 g agar and 30 g malt is sufficient. After autoclaving the tubes and bottles containing the malt agar medium, the tubes are filled in the laminar flow, the caps are screwed on the tubes in such way that the hole is located above the liquid, and the tubes are placed horizontally on racks until the growth medium is solidified.

After inoculating with *Coniophora puteana*, the tubes are put in the climate chamber at 20°C and 70% RH. After a few days, it became clear that some microorganisms were able to enter the tubes through the hole in the cap, preventing the further growth of *C. puteana*. The contamination could be observed in the tubes both with and without fungus, as can be seen in Figure 32.

Figure 32: Contamination in tube with inoculated fungus (left) and solidified malt agar medium (right)

In this figure, it can clearly be seen that the contamination starts at the cap of the tube. This means that the double layer of surgical tape over the hole does not maintain the sterility at the inside of the tube. Consequently, the experiment set-up should be adjusted.

In a modified experiment set-up, no holes are bored in the caps. This ensures that the sterility can be preserved at both the inside and outside of the tube. In order to keep the growing fungus alive, allowing gas transport is very important. This can for example be done by unscrewing the cap a little bit.

When all samples have the desired specificities (sealed, moistened or conditioned in the climate chamber for two weeks) and the *Coniophora puteana* has grown for at least a week, the samples are placed on plastic grids in the tubes. The choice for plastic grids can be explained by the fact that metals are not allowed in the vicinity of the MRI machine (due to the strong magnet) or in the vicinity of the test sample during CT scanning (due to the influence on the density of adjacent elements). The tubes are then put in the climate chamber at a constant temperature of 20°C and a relative humidity of 70%. Once again, the caps are opened a little bit to allow for gas transport.

As mentioned in the preamble, the corona epidemic prevented further execution of this experiment. This experiment will be executed in a future period, when the corona measurements are cancelled.

3.3 Data-analysis: Using X-ray CT as a tool for monitoring the moisture distribution and mass loss for solid woods

Bio-based based materials, including solid woods and wood-engineered products, are susceptible to fungal degradation. There exist standard tests to determine the natural durability based on the measured mass loss after sixteen weeks of exposure to basidiomycete fungi. However, a thorough knowledge about the relationship between fungus and material is still lacking, in particular how the material's structure and moisture properties affect the degradation process. The standard tests are adequately assess the natural durability of several wood species and the efficacy of preservatives. However, they do not allow to gain insight in what is going on insight the material. As a consequence, different testing methods have to be developed, such that a better understanding is obtained of the degradation process inside the material. X-ray CT seems a suitable technology to monitor the moisture distribution and mass loss, because it is a non-destructive technique that allows to assess the density of wood in three dimensions, thus enabling us to obtain localized information inside the wood during the degradation process [48].

3.3.1 Degradation test

In this experiment, a durability test is performed according to the mini-block method of Bravery, because a smaller sample size and a shorter test period seem beneficial for experimenting with X-ray CT [48]. Twenty mini-blocks of beech (*Fagus sylvatica* L.), Scots pine (*Pinus sylvestris L.*), gaboon (*Aucoumea klaineana* Pierre) and Norway spruce (*Picea* abies (L.) Karst) with dimensions 3 x 1 x 0.5 cm³ are oven dried for 24 hours at 103°C and weighed. Next, they are sterilized using gamma irradiation and placed in Petri dishes (diameter 9 cm), filled with a malt agar growth medium (40% malt, 2% agar) and inoculated with the brown-rot fungus *Coniophora puteana* [48]. Fifteen samples are exposed to the fungus when the mycelial area has a radius of approximately 1.5 cm. The other five mini-blocks are not exposed to fungi and serve as control samples to compare the moisture behaviour. The samples are placed on plastic grids to avoid direct contact with the growth medium. The choice for plastic grids can be explained by the fact that metal objects affect the density of adjacent tissues during CT scanning. Furthermore, a reference material with known density was placed on top of each sample (Figure 33). The reference material is required for calculating the density from an X-ray CT image. The Petri dishes are kept in a climate chamber (20°C and 75% RH) for 10 weeks and scanned on a weekly basis with X-ray CT. After 10 weeks of degradation, the samples are weighed immediately to check the final moisture content and oven dried to assess the mass loss. The mini-blocks were scanned one last time after oven drying [48].

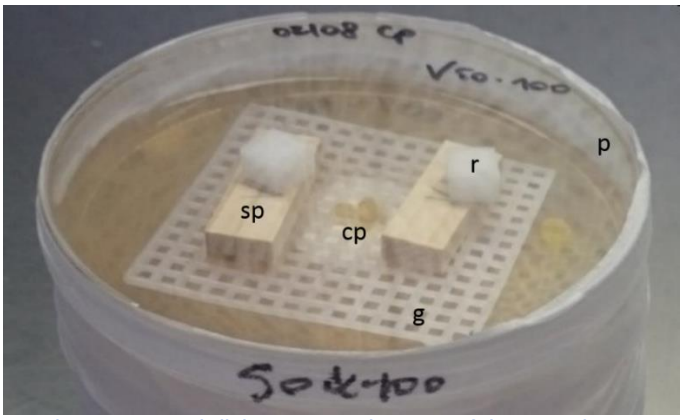


Figure 33: Petri dish set-up at the start of the experiment, cp = C. puteana; g = plastic grid; p = Petri dish; r = reference material and sp= Scots pine sapwood [48]

3.3.2 X-ray CT set-up

The Environmental Micro-CT (EMCT) system at the Centre for X-ray Tomography at Ghent University (UGCT, www.ugct.ugent.be) is used to obtain X-ray CT scans of the wood samples. The EMCT is a rather unique high-resolution setup developed for fast CT scanning and in-situ monitoring [48]. The energy source (Figure 34.1) emits X-rays, which partially penetrate through the stack of Petri dishes (Figure 34.3) and hit the 2D pixelated detector (Figure 34.2). The detector detects how much of incoming X-ray energy is able to pass through the stack, and therefore how much energy has been absorbed by the wood, air and other elements in the Petri dish [48]. The denser the wood is, the more energy it will absorb. During the measurements, the stack of Petri dishes remains in place, while the table on which the source and detector are mounted (Figure 34.5) rotates. While rotating, the Petri dish is thus scanned from every angle (0-360°). The scan settings used in this set-up allow for a resolution of 68 µm (Table 15).

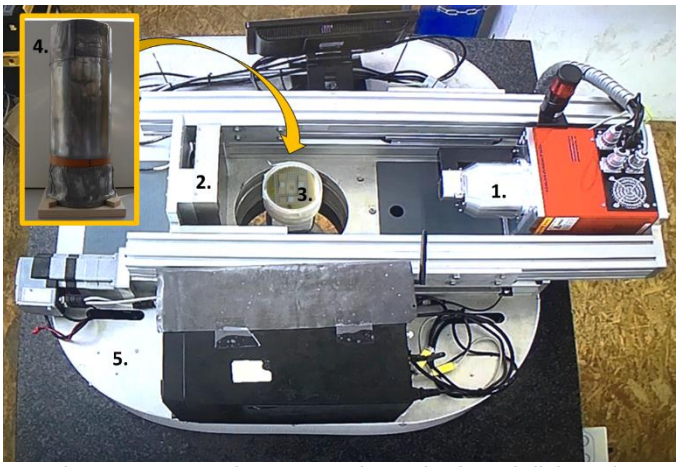


Figure 34: X-ray CT set-up, 1) X-ray source 2) Detector 3) Stack of Petri dishes 4) PVC tube with lead cladding 5) rotation table [48]

Since the detector has a limited field of view, the entire stack of Petri dishes cannot be scanned at once. Consequently, we make use of a motor allowing for vertical movements such that the height of the stack of Petri dishes automatically changes after each scan cycle [48]. In order to avoid unnecessary X-ray radiation exposure of the fungal cultures that are not in the field of view, a PVC tube with lead cladding is positioned around the stack of Petri dishes (Figure 34.4). This ensures a blockage of X-rays, except at a central slit that allows X-ray passage only through one Petri dish [48].

Table 15: Scan settings EMCT [48]

Voltage	+80 kV	**Exposure time**	100 ms
Wattage	12 W	**Number of averages**	3
Filter	No filter	**Rotation**	360°
Resolution	68 μm	**Number of images**	2200

3.3.3 Image reconstruction and analysis

The detector takes 2200 images during one rotation cycle of 360°. The images are then reconstructed with the Octopus reconstruction software package (licensed by TESCAN-XRE: www.xre.be) [59]. A software beam hardening correction was applied (BHC-values 0.12 0 0). We loaded the resulting greyscale volume for each Petri dish in Fiji and extracted the mini-blocks [60]. A greyscale profile was taken along the longitudinal direction of the mini-block, where the average grey value was calculated for each slice (Figure 35). Afterwards, this greyscale profile was converted to a density profile by rescaling with a reference material (which has a similar elemental composition to wood and a known density (1400 kg/m³)), and air (1.2 kg/m³) [48].

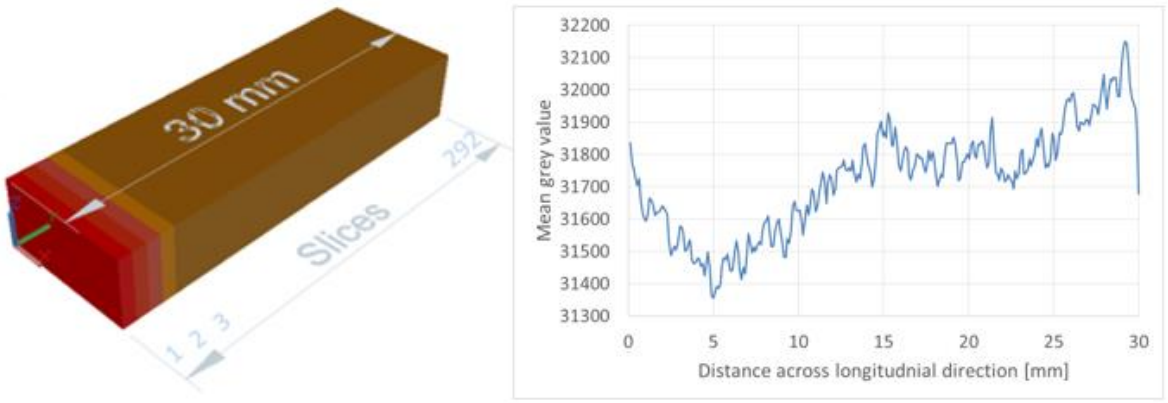


Figure 35: For 292 slices along the longitudinal direction of the mini-block (left); the average grey value is determined with Fiji, resulting in a grey scale profile of the mini-block (right)

4 RESULTS AND DISCUSSION

4.1 Preliminary experiment: Adapted mini-block test for bio-based materials

4.1.1 General results

The mass losses caused by the fungi *C. puteana* and *T. versicolor* are respectively shown in Figure 36 and Figure 37. A first observation that can be made is that both fungi are sufficiently virulent to deteriorate all bio-based materials, except for thermally modified spruce. This means that the applied procedure of this experiment, which is adapted from the standardized test intended for solid woods, generates useful results for the engineered wood products and insulation materials, except for thermally modified spruce.

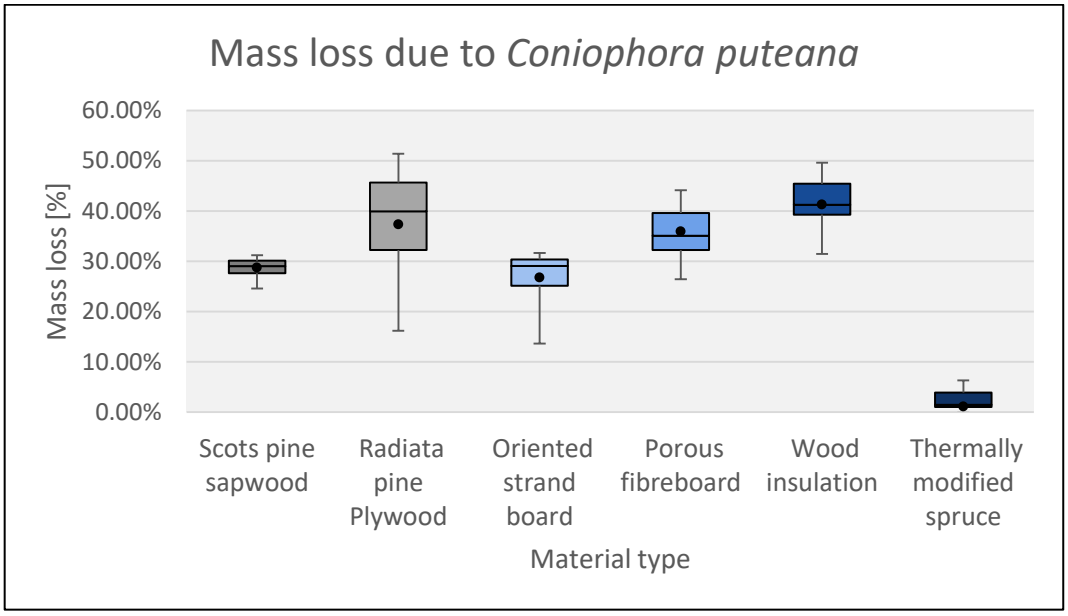


Figure 36: Mass losses due to Coniophora puteana

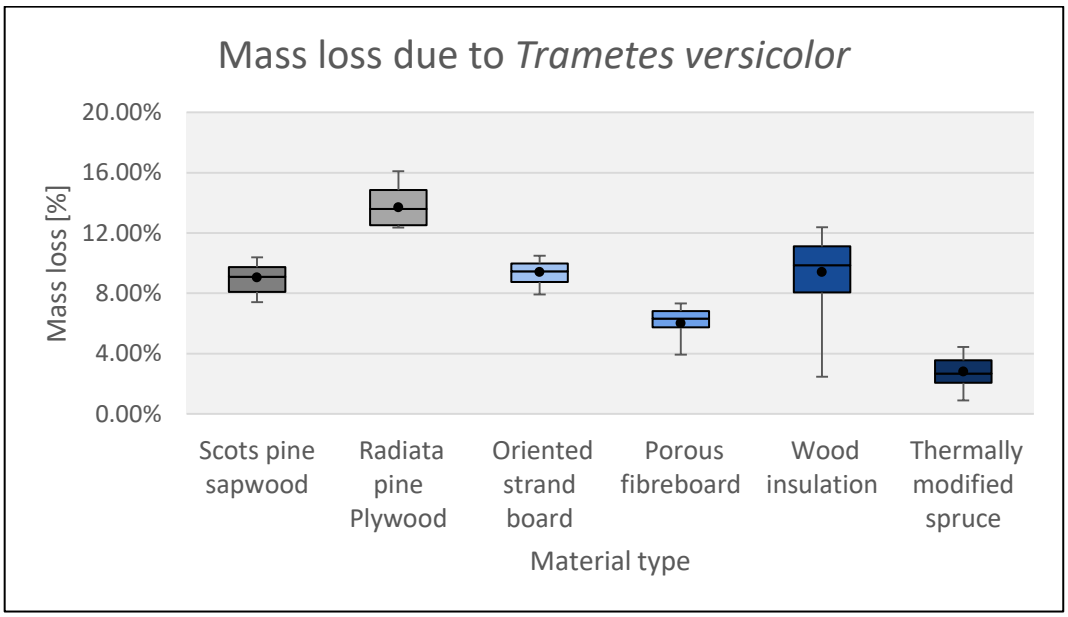


Figure 37: Mass losses due to Trametes versicolor

By comparing Figure 36 to Figure 37, one can observe that the brown-rot fungus *C. puteana* is, in general, responsible for greater mass losses than the white-rot-fungus *T. versicolor*. Considering that brown-rot fungi typically tend to attack softwoods and that all materials in this experiment are based on softwoods, this observation is logical. The mass loss for thermally modified spruce varies between -1.16% and 4.17% in case of *C. puteana* and 0.89% and 4.49% in case of *T. versicolor*. This could possibly indicate that the samples of thermally modified spruce are too dry or wet for degradation. Therefore, the moisture contents before and after fungal decay are calculated and displayed in boxplots (Figure 38 to Figure 40).

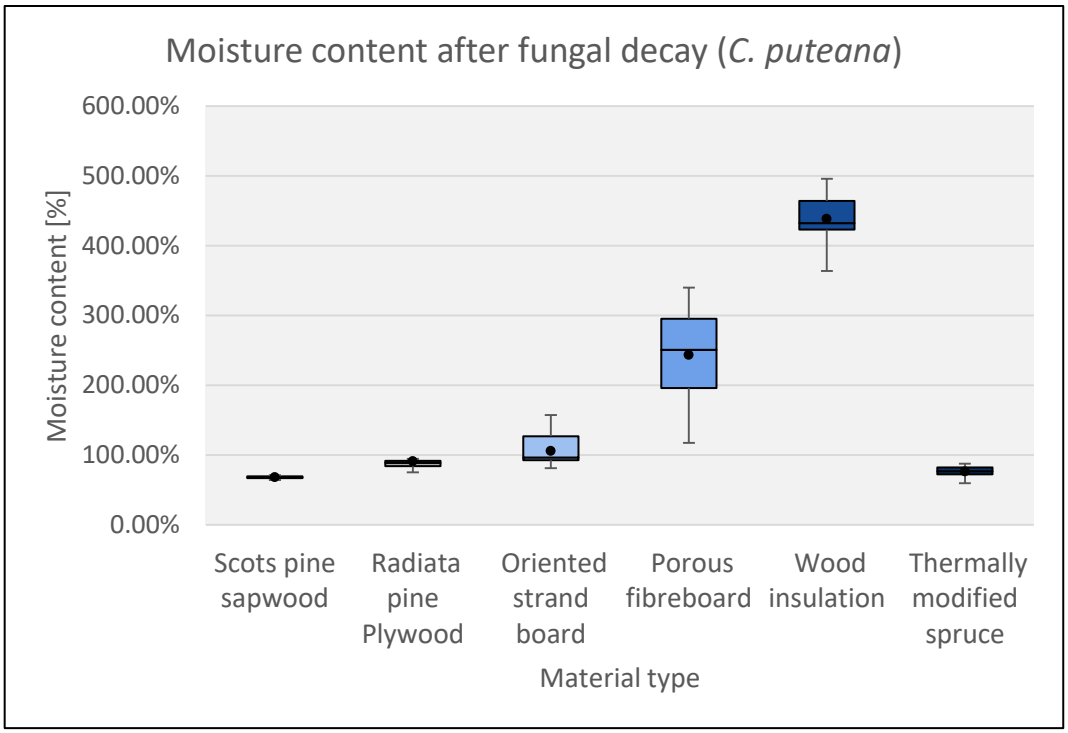


Figure 38: Moisture contents after fungal degradation (C. puteana)

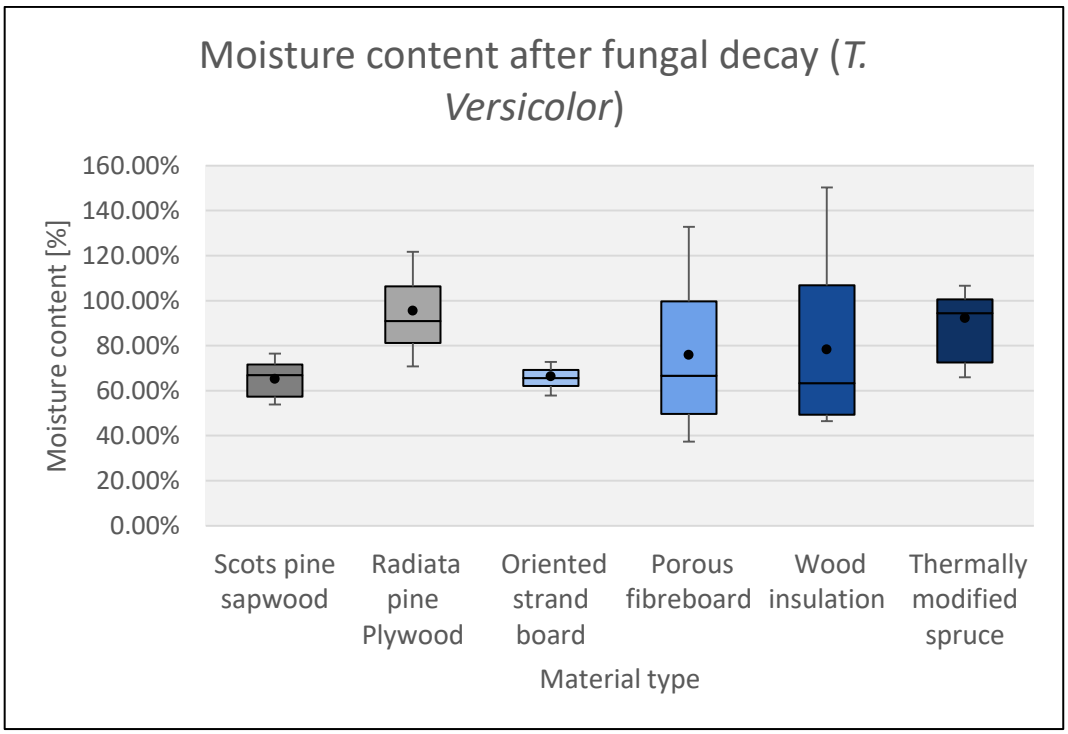


Figure 39: Moisture contents after fungal degradation (T. versicolor)

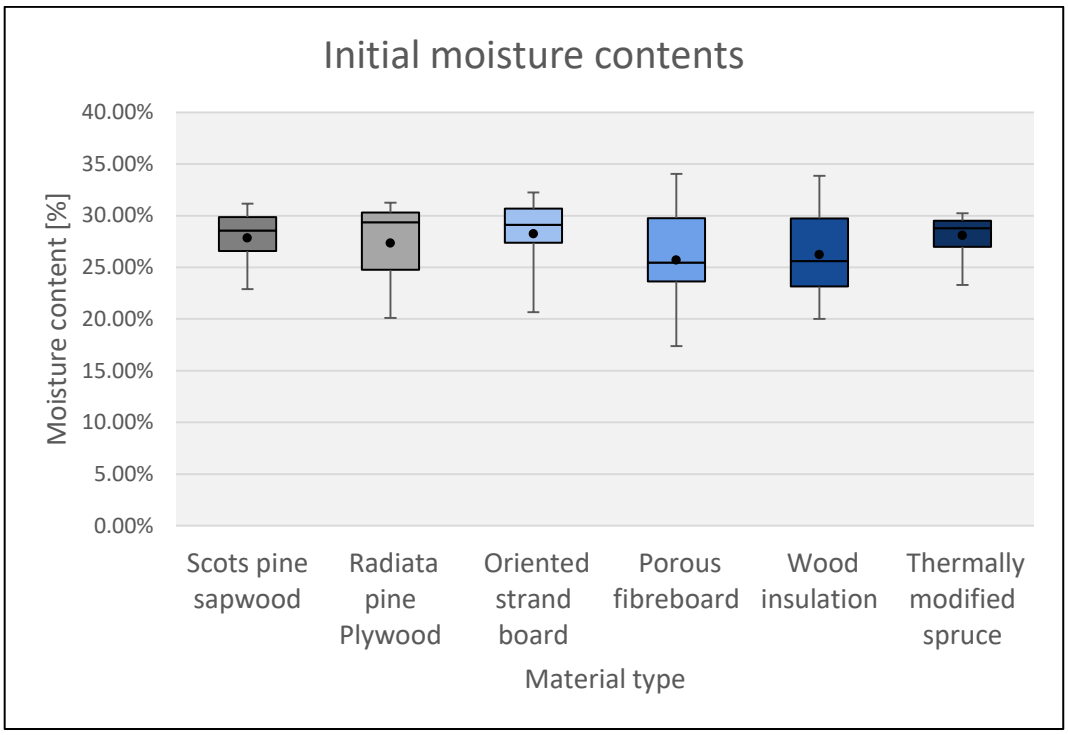


Figure 40: Initial moisture contents

One can observe that the initial moisture content of thermally modified spruce is higher than 20% and the moisture content after fungal decay varies between 59.70% and 131.88%. This indicates that the samples are certainly not too dry for deterioration. It could be possible that the samples are too wet for deterioration, but since there are multiple samples with a final moisture content below 80%, being too wet is most likely not the reason for little deterioration. Possible reasons for the limited mass loss are explained in section 4.1.2, where the results are compared to findings in literature. With regard to the other types of bio-based materials, the mini-blocks easily degrade in the set-up of this experiment regardless of the moisture content before and after fungal decay. Consequently, one can conclude that that the adapted mini-block test shows that the bio-based materials, except for thermally modified spruce, are not durable when they are exposed to conditions favouring fungi, i.e. the samples are initially wet and remain wet during the degradation process and the hyphae can enter the samples from the sides.

Based on the graphs of the moisture contents after degradation, it is not possible to determine how much of the moisture content is due to the water uptake from the malt agar medium and how much due to the water production of the fungus. For this reason, a control experiment was performed in a similar set-up, but without the presence of fungi. The results of this experiment can be seen in Figure 42, where the median moisture content is plotted over time. Unfortunately, there was some contamination, as can be seen in Figure 41. Since contamination can have a huge influence on the results, only the samples without contamination are included in the graph. The complete results, in which the contaminated samples are included as well, can be found in Appendix A.

Figure 41: Contamination of OSB (left) and pine plywood (right)

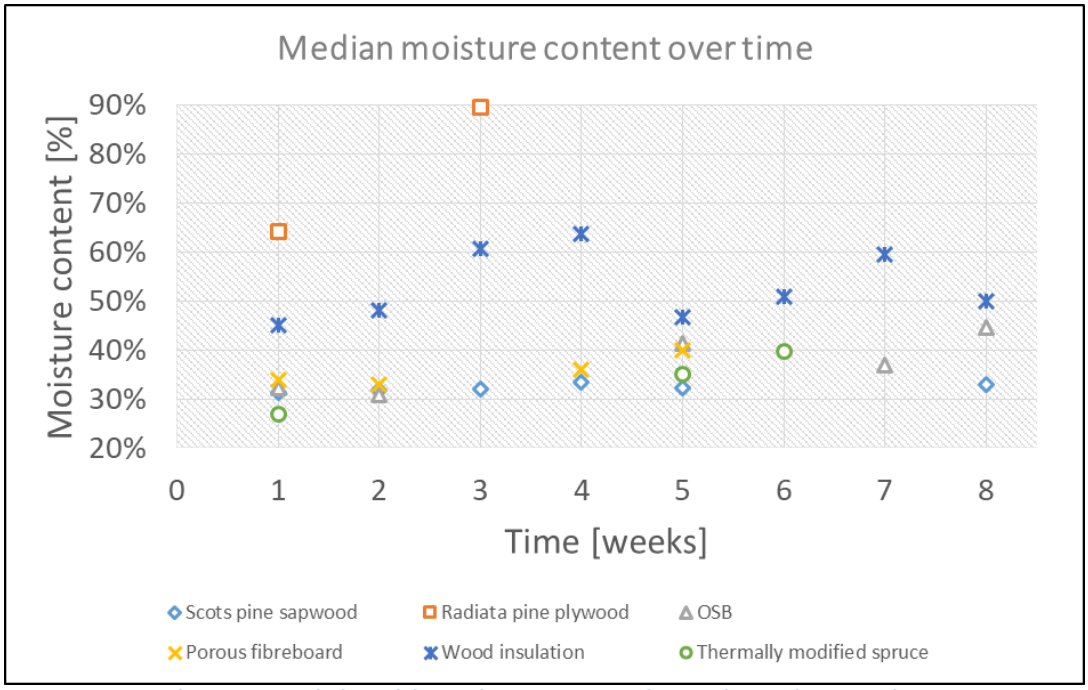


Figure 42: Variation of the moisture content of control samples over time

By comparing Figure 42 to Figure 38 and Figure 39, one can observe that the moisture contents of the samples exposed to the brown-rot fungus *C. puteana* are a lot higher than the moisture contents of the control samples. This is logical because during fungal degradation, sugars are metabolized in water and carbon dioxide [61]. This also explains why the moisture contents of the samples exposed to the white-rot fungus *T. versicolor* are lower than those exposed to *C. puteana* and higher than those of the control samples. Especially for the insulation materials, the moisture contents after exposure to *C. puteana* are extremely high compared to the control samples (a moisture content increase of approximately 370% for wood insulation and 210% for porous fibreboard). For the other materials, an increase of at least 30% MC is observed. Consequently, there can be concluded that the presence of a fungus has an important effect on the material moisture content.

4.1.2 Comparison with findings in literature

In order to have an idea whether a certain material is labelled as durable or not, the Eurocode, more specifically CEN/TS 15083-1, provides a durability rating scale based on the highest median mass loss determined for all specimens exposed to two fungi. An overview of the median mass losses and the corresponding durability classes can be seen in Table 16.

Table 16: Overview median mass loss and corresponding durability class

Material type	Median mass loss due to *C. puteana* [%]	Median mass loss due to *T. versicolor* [%]	Durability class
Scots pine sapwood	29.03	9.09	4
Radiata pine plywood	39.96	13.59	5
OSB	29.07	9.46	4
Porous fibreboard	35.08	6.32	5
Wood insulation	41.24	9.86	5
Thermally modified spruce	0.29	2.68	1

Note that applying the durability scaling is, strictly speaking, only valid when the dimensions of the samples are as prescribed (5 x 2.5 x 1.5 cm^3) and when the mass loss caused by *C. puteana* of the reference material is higher than 30% after an exposure time of 16 weeks [46]. Since the experiment does not fulfil these requirements, applying the durability rating scale is actually not valid. In a study of Deklerck et al., the durability assessment was done according to the same procedure. In order to find out if the obtained durability class corresponds to reality, they compared the results to findings in Houtvademecum and Tropix 7 technical sheets [62].

One should, however, be careful to draw conclusions about the fungal resistance of the materials. This experiment is based on the mini-block test using relatively small samples. The influence of the non-wood components, e.g. the glue layers in plywood, the bitumen emulsion in porous fibreboard, etc. may become more clear in case bigger samples are used. Additionally, the hyphae can sometimes not infiltrate the material from the sides, for instance in case of OSB and pine plywood, as they are often shielded by other building elements. Consequently, the assigned durability classes may not correspond with the actual durability.

4.1.2.1 Scots pine sapwood

The deterioration of Scots pine sapwood caused by *Coniophora puteana* (29% median ML) is a commonly found outcome in literature. In a study of the VTT Technical Research Centre of Finland, *Coniophora puteana* causes a median mass loss of 16.9% for an incubation time of 6 weeks and a median mass loss of 34.1% for an incubation time of 10 weeks [63]. Note that the specimens in this study are longer (10 cm) than the samples in the preliminary experiment (3cm), which could explain the difference. A study of Eslyn and Highley investigated the fungal susceptibility of different types of pine sapwood. They found an average mass loss ranging from 8.4% to 21.8% for white-rot fungi and 25.0% to 37.8% for brown-rot fungi [64]. The mass losses of Scots pine sapwood in the preliminary experiment lie in the ranges of this study, so one can conclude that the results are in accordance with findings in literature.

4.1.2.2 Radiata pine plywood

The plywood in the preliminary experiment consists of four layers of pine and a waterproof glue that accounts for the bonding. In Figure 43, the deterioration of the plywood is visualized.

Figure 43: Deterioration of radiata pine plywood samples

One can clearly see that cracks occur in an alternating pattern. During the manufacturing process of plywood, the composition of the layers is typically arranged such that the grain direction has an alternating pattern because this offers several benefits (improved stability, making the strength more consistent, etc.). A study of Kljak et al. showed that the grain direction has a great influence on bending properties of the sandwich panel, as well as on stress values in each layer [65]. The alternating crack pattern can thus be explained by the different orientation of the grains.

A study of Laks, Richter and Larkin shows that the mass loss of pine plywood samples caused by the brown-rot fungus *Gloeophyllum trabeum* is higher than the one of the white-rot fungus *T. versicolor* [66]. This corresponds with the findings that white-rot fungi typically decay softwoods at a lower rate than brown-rot fungi. Another interesting result of this study is that the decay resistance of plywood is higher than the one of oriented strand board [66], whereas the preliminary experiment shows opposite results. The difference in results can be due to several factors: difference in sample dimensions, different types of glue, different initial moisture conditions, etc.

Although the pine plywood samples are easily degraded in this specific set-up, this will probably not be the case in practice, where the edges from laminated floors are often shielded by other elements, thus preventing fungal penetration at the sides. This is confirmed by a study from Van den Bulcke et al., in which the sealing of plywood edges has shown to have an important impact on the fungal degradation. By sealing the edges, the glue layers were able to limit the fungal degradation compared to the degradation of non-sealed samples [67]. Additionally, a study from De Windt et al. showed that many plywood types have excellent moisture dynamics, therefore preventing fungal growth most of the time. Two mechanism were found to influence the durability, namely the adhesive that might act as fungicide and the adhesive that acts a physical barrier and therefore preventing water uptake [68].

4.1.2.3 Porous fibreboard

The porous fibreboard samples are heavily deteriorated by *C. puteana*. This indicates that the bitumen emulsion barely had an influence on the fungal degradation in this experiment set-up. In order to make the effect of the bitumen emulsion more pronounced, two adjustments can be made to the experiment set-up: (1) larger sample sizes and (2) not exposing the samples to vacuum.
Before the determination of the wet and oven dry weight after degradation, the adhering mycelium should be removed. As porous fibreboard is composed of wood fibres and bitumen emulsion, the removal of the adhering mycelium is a difficult task because it was affixed to the surface of the samples (Figure 44). This operation sometimes resulted in the occurrence of cracks and loosening of wood fibres at the surface. A possible explanation for these phenomena is that the effect of the bitumen emulsion, which acts as a glue between the wood fibres, is partly negated after the wetting procedure.

Figure 44: Adhered mycelium to porous fibreboard sample

The sample in this figure shows a mass gain of 3.7%, which can be due to two explanations: (1) the fungal biomass of *T. versicolor* is higher than the mass loss of the sample or (2) the mycelium was not removed properly. Considering that the sample in Figure 44 was the only sample with a mass gain, it was treated as an outlier in the analysis.

4.1.2.4 Oriented strand board

The deterioration caused by *C. puteana* is visualized in Figure 45. A clear phenomenon that can be perceived is the occurrence of multiple cracks along the division line of two layers. Possibly, this can be explained by the wetting procedure, where the samples were subjected to vacuum suction. This action could have weakened the bonding of the separate layers. Another possible explanation for the cracks is the growth and water production of the fungus in the mini-block itself.

Figure 45: Deterioration of OSB samples caused by C. puteana

The results for OSB correspond with findings of Amusant and Fojutowsko, who found mass losses ranging between 20-45% for different OSB panels [69],[70]. A study by Ayriilmis et al. found that the average mass loss caused by *T. versicolor* of the OSB samples with dimensions 20 by 20 by 10 mm was 16.23%, which indicates that the OSB was slightly durable. In case the OSB samples were chemically treated by spraying Disodium octaborate tetrahydrate (DOT), boric acid (BA), melamine phosphate (MP), and a BA/DOT mixture, both brown-rots and white-rots did not cause any significant weight loss after 12 weeks [71]. This indicates that the applied protection mechanism is an effective way to increase the fungal resistance. This study, however, also found that the chemical treatment had an adverse effect on other parameters, e.g. a lower resistance to termites and thickness swell and an increased water absorption [71].

4.1.2.5 Wood insulation

During the removal of the adhering mycelium, the same difficulties as with porous fibreboard were experienced, see Figure 46.

Figure 46: Adhering mycelium to wood insulation samples

As mentioned before, the final moisture contents of the insulation materials (wood insulation and porous fibreboard) are remarkably higher than these of the other materials when exposed to *Coniophora puteana*. This can possibly be explained by the low density and high porosity of the insulation materials. Since the samples in the experiment have the same volume, namely 1.5 cm^3, and the oven dry weights are known, the densities can be determined (Table 17).

Table 17: Mean densities of the bio-based materials

Material	Mean density [kg/m^3]
Scots pine sapwood	555
Radiata pine plywood	485
Oriented strand board	595
Porous fireboard	200
Wood insulation	140
Thermally modified spruce	440

One can see that the density of wood insulation and porous fibreboard is approximately two to four times smaller than the one of the other materials. This means that the inner structure has more pores, so when water is created as a by-product during fungal degradation, more specifically when sugars are metabolized in water and carbon dioxide, the insulating materials contain more free water, which explains the higher moisture contents.

4.1.2.6 Thermally modified spruce

Although the samples had initial moisture contents between 20-30% MC, the mass loss of thermally modified spruce was limited compared to the other materials. Due to the thermal treatment at 200°C, the wood structure underwent several changes: a reduction of the equilibrium moisture content of the wood; a better biological durability and an improvement of the dimensional stability [63].

In literature, several reasons exist for the increased biological durability of thermally modified wood. A first explanation is that the wood becomes more hydrophobic, limiting the water absorption and thus mitigating fungal growth [72]. The limited water absorption is confirmed in the wetting procedure of this experiment. After wetting the samples of each material type for a day, they all sunk to the bottom of the desiccator, except for thermally modified spruce (Figure 47). This can be explained by the fact that the microscopic structure is changed in such way that vacuum pumping does not result in the extraction of the air in all pores. This did not have an effect on acquiring the initial moisture range, since the samples are sufficiently water saturated, but it could have an effect on the type of pores in which the moisture is located. This in turn might affect fungal growth, since the affinity for water differs between different pore types.

Figure 47: Floating samples of thermally modified spruce after wetting them for a day

In a survey of Weiland and Guyonnet, the chemical modification and fungal degradation of thermally modified wood was studied. They mentioned several factors that contributed to an increased fungal resistance [73]:

- The thermal treatment causes the creation of new free molecules in the wood, acting as fungicides.
- The formation of some molecules, e.g. furfural, may blend in the lignin network. This makes sure that the fungus cannot longer recognize the wood substrate and is thus incapable of degrading it.
- The thermal treatment eliminates the pentanes (hemicelluloses), the elementary nutritive substances of wood, and hence the initial colonization of fungi is inhibited.

In a study of De Ligne et al., the existence of fungicidal properties was tested for thermally modified spruce with the same properties as in the preliminary experiment. Based on their results, it can be concluded that there were no molecules acting as fungicides [47]. Hence, the first explanation of the study of Weiland and Guyonnet for the increased fungal resistance can be rejected.

In this experiment, some samples showed a mass gain instead of loss. This is a phenomenon that sometimes can be observed with durable materials. In literature, there is little information about this phenomenon. During fungal colonisation, the hyphae form a 3D network by extension and branching. This mycelium secretes enzymes that convert polymers in the wood into breakdown products that mainly serve as nutrients. As a result, the organic material is degraded in time, while being replaced by the fungal biomass within the wood [74]. The fungal growth can be stopped by drying and/or heating the material. By drying, the fungus is brought in a ‘hibernated’ state, whereas heating will kill the fungus. By heating/drying the sample during fungal colonisation, a mycelium-based composite is created [74]. In this experiment, the fungus was able to degrade some wood substances in thermally modified spruce, but as a mass gain was observed, this would mean that the fungal biomass was higher than the resultant mass loss of the degraded wood substances. Note that the samples were only exposed to basidiomycete fungi for 8 weeks. In case the test period would be prolonged, the fungi would have more time to degrade the material.

4.1.3 Conclusion and recommendations

This experiment examined the fungal susceptibility of several bio-based wood materials in optimal conditions, more specifically when the materials are initially moistened and remain wet during the incubation period. Based on the results, we can conclude that most of the tested bio-based building materials are not durable in the absolute worst-case scenario. In practice, however, those worst-case conditions seldom occur. Additionally, some of these materials have excellent moisture dynamics, probably causing them to remain insufficiently wet for fungal deterioration [47], [68].

The median mass losses are found to be of the same order of magnitude as for pine sapwood, except for thermally modified spruce. For the latter material, there can be concluded that bringing the samples to a MC of 20-30% did not make the samples susceptible to decay by *C. puteana* and *T. versicolor*. Thermal modification leads to a decrease of the equilibrium moisture content by limiting the amount of water that can bind to the cell wall. Hence, it is expected that the water in the thermally modified spruce samples is mainly present as capillary or loosely bound water [47]. Consequently, it would be interesting to find out if the fungus is capable of degrading thermally modified wood using capillary or loosely bound water and if a longer wetting duration will result in the wood reaching a minimum threshold of bound water in the cell wall which allows decay [47].

Based on the results, most materials were labelled as not durable. One should, however, be careful to draw conclusions about the fungal resistance of some materials. Due to the smaller sample size, the glue layers and bitumen emulsion were not able to affect the degradation process. Additionally, the samples were not sealed at the sides, so the mycelium could infiltrate at all sides. This does not correspond to reality, where the sides of some materials are often shielded by other elements, thus preventing fungal attack from the sides. Consequently, there can be concluded that the preliminary experiment is not suitable to assess the durability adequately. Hence, another test set-up should be developed, in which the effect of glue layers, bitumen emulsion, etc. can become clear and in which the shielding aspect can be taken into account. Furthermore, it should be investigated whether the wetting procedure, during which the samples were exposed to vacuum suction, affects the material's structure (e.g. the effectiveness of the glue layers, bitumen emulsion, etc.).

4.2 Data analysis: Using X-ray CT for the monitoring of the moisture distribution and mass loss of solid woods

In this data analysis, the main goal is to find out how the moisture distribution varies over time during fungal degradation and how the material's structure affect the degradation process. As X-ray CT measures attenuation coefficients, converting them to densities is the best way to obtain meaningful (quantitative) information. Before proceeding with the actual analysis, we compare the mass losses of the CT experiment to those of a parallel experiment. This parallel experiment was executed at the same time as the CT experiment, the only difference is that the samples were not exposed to X-ray radiation. The goal of this comparison is to find out whether X-ray radiation affects the degradation process and if the amount of degradation suffices to further investigate the factors influencing the degradation process.

In order to make statements about the density variation, it is necessary to check whether the densities based on the attenuation coefficients correspond to the actual densities of the samples. A possible method to verify this is by doing a linear regression, resulting in the best possible relation between both densities, and subsequently doing a paired T-test, which investigates if the obtained relationship is valid. In the CT experiment, five control samples were included for each wood species in order to be

able to compare the moisture behaviour. As it is known that water and carbon dioxide are produced during fungal deterioration, it would be interesting to find out how much of the material moisture content is due to the moisture production by the fungus and how much due to the moisture absorption from the growth medium.

Lastly, the densities of the oven dry samples before degradation are compared to those after degradation. This because it would be interesting to find out which phenomena can be observed. On the one hand, a density loss is expected because of the fungal degradation. On the other hand, it might be possible that there is densification because of the shrinkage as a result of the loss of wood structural elements. Since CT-images are available, both phenomena can be observed visually.

4.2.1 Influence of X-ray radiation in CT on fungal degradation

As mentioned before in section 3.2.3, ionization radiation is used in X-ray CT. Since high amounts of X-rays can harm living organisms, it is important to test whether X-rays have an influence on the behaviour of *C. puteana*. In a study of De Ligne et al., it was found that *C. puteana* showed a clear recovery potential after X-ray treatment, enabling the use of X-ray CT scanning to track fungal degradation [48]. The fungus *C. puteana* is weekly exposed to X-ray radiation for only 14 minutes, so we do not expect that the degradation is significantly inhibited. In order to find out if the X-rays have an influence on the magnitude of the mass losses of the materials, the results of the CT experiment are compared to those of the parallel experiment without CT. Note that both experiments were executed in such way that the same conditions were applicable as much as possible. For example, the Petri dishes were put in the same climate chamber, both experiments were executed at the same time and lasted 10 weeks, etc. This is important because that way, the X-rays are the only influencing factor. The results of both experiments are shown in Figure 48.

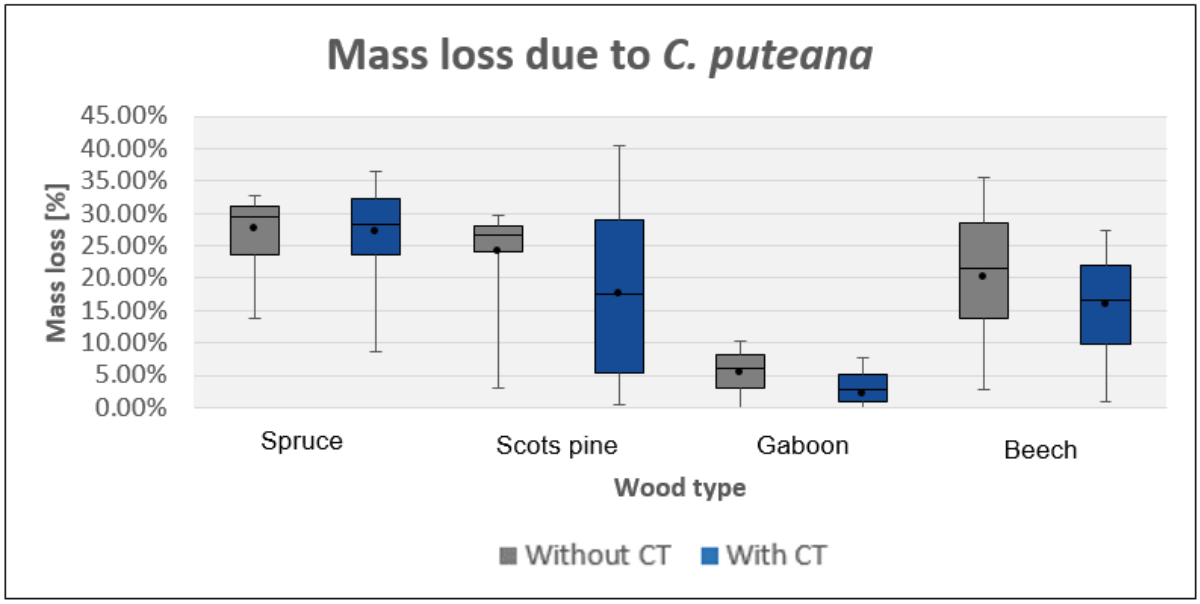


Figure 48: Comparison mass losses of samples in CT experiment to parallel experiment

In Figure 48, it can be seen that the mass loss for spruce is almost the same for both experiments. The results for spruce are in agreement with the results in a study of Metsä-Kortelainen et al., who found a median mass loss of 31.3% for spruce samples with dimensions 5x20x35 mm³ after 10 weeks [63]. The mass losses of beech and gaboon in the CT experiment are close to those of the parallel experiment, but a little smaller. Gaboon showed little degradation compared to the other materials. This is a commonly found result in literature. In a study of Reinprecht et al., a median mass loss of 6.48% was found for gaboon samples with dimensions 25x25x3 mm³ after 6 weeks [75]. The results of beech

correspond quite well to findings of Ayata et al., who found a median mass loss of 23.74% for beech samples with dimensions 20x20x10 mm³ after 12 weeks of exposure to *C. puteana* [76]. The results of Scots pine show high variability for both experiments and are not commonly found in literature. The median mass loss for Scots pine in the CT experiment correspond to findings in literature, but only when bigger samples are tested. For example, a study of Irbe et al. found a mean mass loss of 21% after two months of exposure to *C. puteana*, in case the sample size was 5 x 2.5 x 1.5 cm³ [77].

Based on the results of spruce, one could conclude that X-rays do not have an adverse effect on the fungal degradation and X-ray CT is a proper tool to assess the fungal degradation of bio-based materials. However, the mass losses of beech and gaboon are a bit smaller than those of the parallel experiment. Therefore, it could be possible that X-rays have an influence on the fungal degradation. In order to find this out, it is recommended to execute a new experiment. However, for the purpose of this experiment, the amount of degradation suffices to further investigate how the density varies over time and how the material's structure affects the degradation process.

4.2.2 Relation between actual density and density based on grey values

As mentioned before, X-ray CT measures the attenuation coefficients, and not the density. Therefore, one should verify if the derived density of the CT experiment corresponds to the actual density. The easiest way to do this is by comparing the oven dry densities. Since the oven dry samples are weighed before the first scan moment and the volumes of the mini-blocks are known (1.5 cm³), it is possible to compare the density to the results of the oven dry samples after taking CT scans. After CT scanning, the mean attenuation coefficients (grey values) can be calculated and transformed to densities according to equation 4-1, in which a reference material with known density and air are used as reference materials.

$$\frac{\rho_{sample} - \rho_{air}}{\rho_{ref} - \rho_{air}} = \frac{GV_{sample} - GV_{air}}{GV_{ref} - GV_{air}} \tag{4-1}$$

Since the density of the reference material is approximately 1.4 g/cm³ and the density of air 0.001 g/cm³ ($\approx 0\ g/cm^3$), equation 4-1 can be simplified.

$$\rho_{sample} = 1.4 \cdot \frac{GV_{sample} - GV_{air}}{GV_{ref} - GV_{air}} \tag{4-2}$$

In this equation ρ_{sample} is the mean density of the sample in g/cm³ and GV is the mean grey value. By plotting the oven dry densities of corresponding samples, performing a simple linear regression and calculating the square of the correlation coefficient, one can check if the densities are correlated. Figure 49 and Figure 50 show the linear regression of the wood species.

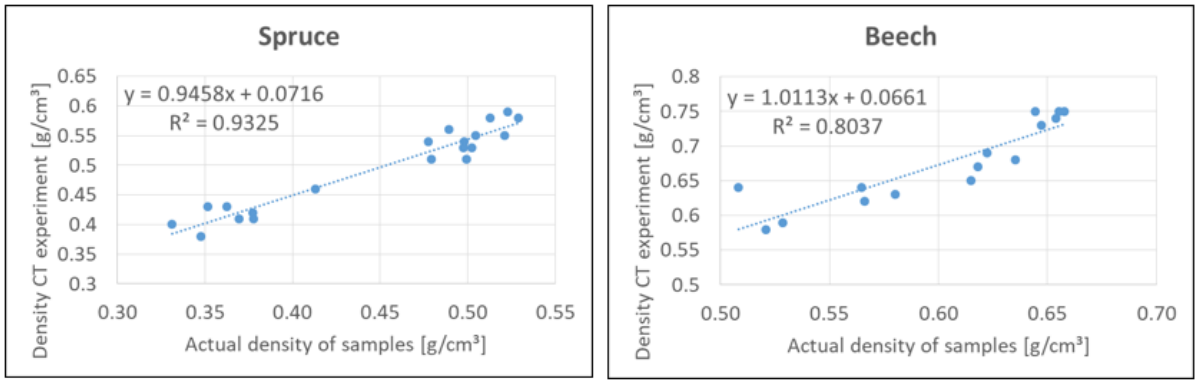


Figure 49: Linear regression of densities of spruce (left) and beech (right)

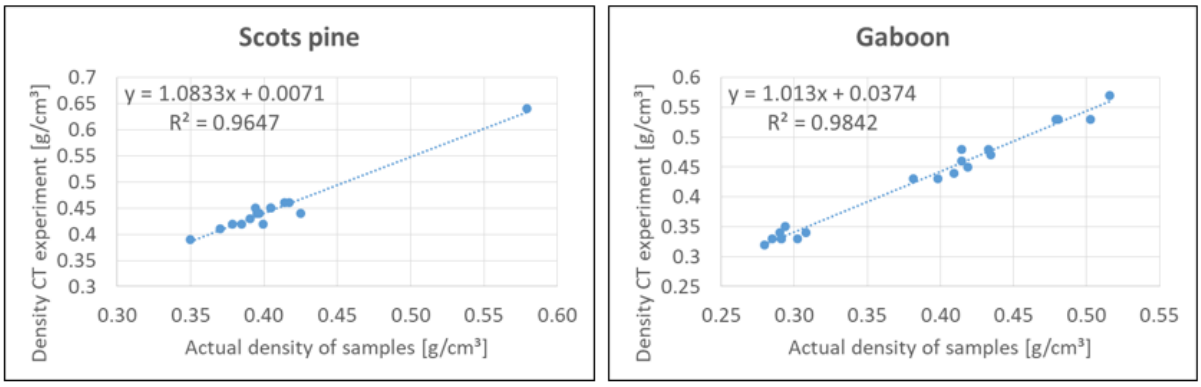


Figure 50: Linear regression of densities of Scots pine (left) and gaboon (right)

In these figures, one can observe that the slopes of the regression lines are close to one, which indicates that both densities are close to each other. Furthermore, one can see that the square of the correlation coefficient varies between 0.8037 and 0.9842, which indicates that there exist a strong relationship between the two parameters.

For beech, the following relation holds according to Figure 49:

$$\rho_{CT,beech} = 1.0113 \cdot \rho_{actual,beech} + 0.0661 \tag{4-3}$$

We can check if this equation can be used in the further analysis by executing a paired T-test. In order to execute a paired T-test, several assumptions must be verified:

- The dependent variable must be continuous: OK
- The observations are independent of one another: OK
- The dependant variable should be normally distributed.

This last condition can be verified using a Shapiro-Wilk test in SPPS, of which the results are shown in Figure 51. As the significance level is higher than 5%, one can accept the null hypothesis of the Shapiro-Wilk test and conclude that the variable follows a normal distribution.

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Density	,157	20	,200*	,916	20	,082

Figure 51: Normality test for dependent variable

In the paired T-test, the null hypothesis H_0 is: $\mu_{CT} = 1.0113 \cdot \mu_{actual} + 0.0661$ and the alternative hypothesis H_1 is $\mu_{CT} \neq 1.0113 \cdot \mu_{actual} + 0.0661$. The null hypothesis can be accepted when the following requirement is fulfilled.

$$-t_{n-1;1-\frac{\alpha}{2}} \cdot \frac{S_d}{\sqrt{n}} \leq \bar{D} \leq t_{n-1;1-\frac{\alpha}{2}} \cdot \frac{S_d}{\sqrt{n}} \tag{4-4}$$

In which $\bar{D}$ and S_d are respectively the mean value and standard deviation of the differences between the left hand side and ride hand side of equation 4-3. In total, there are 20 samples and a confidence level of 5% is assumed. Hence, equation 4-4 can be simplified to:

$$-0.0126 \leq \bar{D} \leq 0.0126 \quad (4\text{-}5)$$

Since $\bar{D}$ equals 0.002, one can accept the null hypothesis. Consequently, analyses of the actual densities can be done by using the following relationship:

$$\rho_{actual,beech} = 0.9888 \cdot \rho_{CT,beech} - 0.0654 \quad (4\text{-}6)$$

Similar calculations can be done for the other wood species. Since the null hypothesis can be accepted each time, the relationships in Figure 49 and Figure 50 can be used to transform the density obtained in the CT experiment to the actual density. Consequently, interpretations regarding density changes based on the CT analysis are also valid for the actual density changes.

4.2.3 Impact of moisture on density

Since both the wet and oven dry density are known at the end of the experiment, it is possible to determine the relative share of moisture using equation 4-7. The results can be found in Figure 52.

$$\text{Relative share of moisture [\%]} = \frac{\rho_{wet} - \rho_{ovendry}}{\rho_{ovendry}} \cdot 100 \quad (4\text{-}7)$$

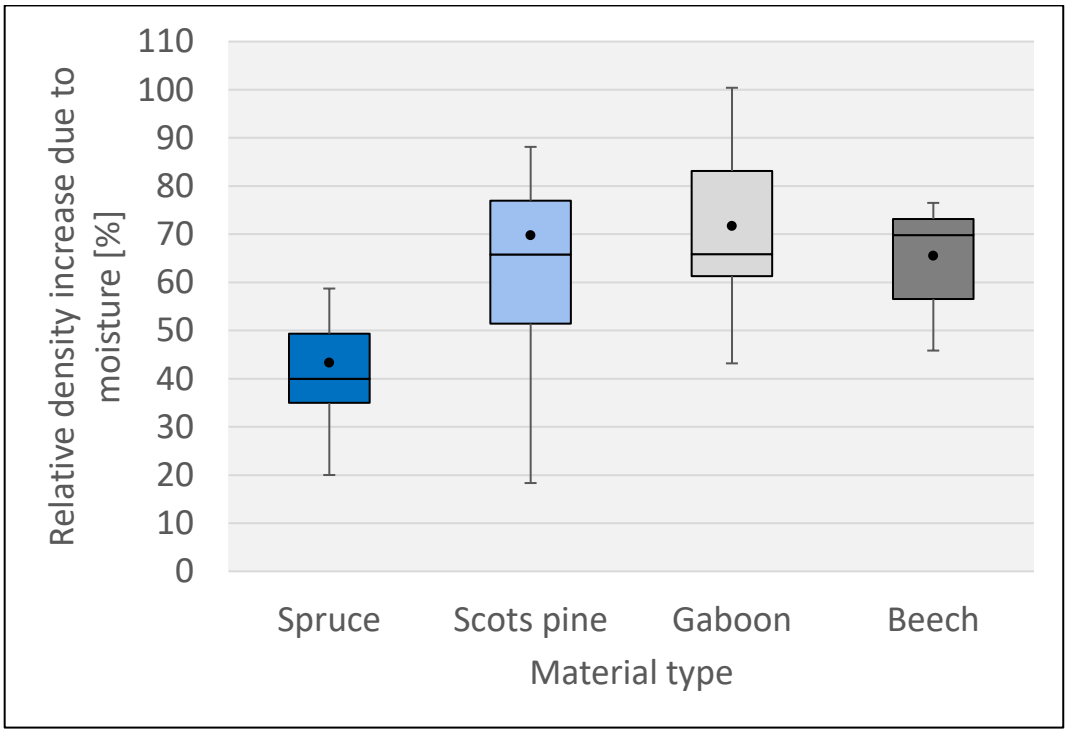


Figure 52: Density increase due to moisture in the mini-blocks

It can be observed that the relative share of water is the highest for gaboon. Considering that the fungus barely degraded the samples, and a large proportion of the wet density is due to moisture, this indicates that the fungus mainly gets its nutrients from the malt agar medium and that the available nutrients in the growth medium are most likely not depleted after ten weeks.

In addition, the moisture contents were determined after ten weeks of degradation (Figure 53). Since the moisture content of most gaboon samples are lower than 80%, it can be concluded that the samples were not too wet for degradation.

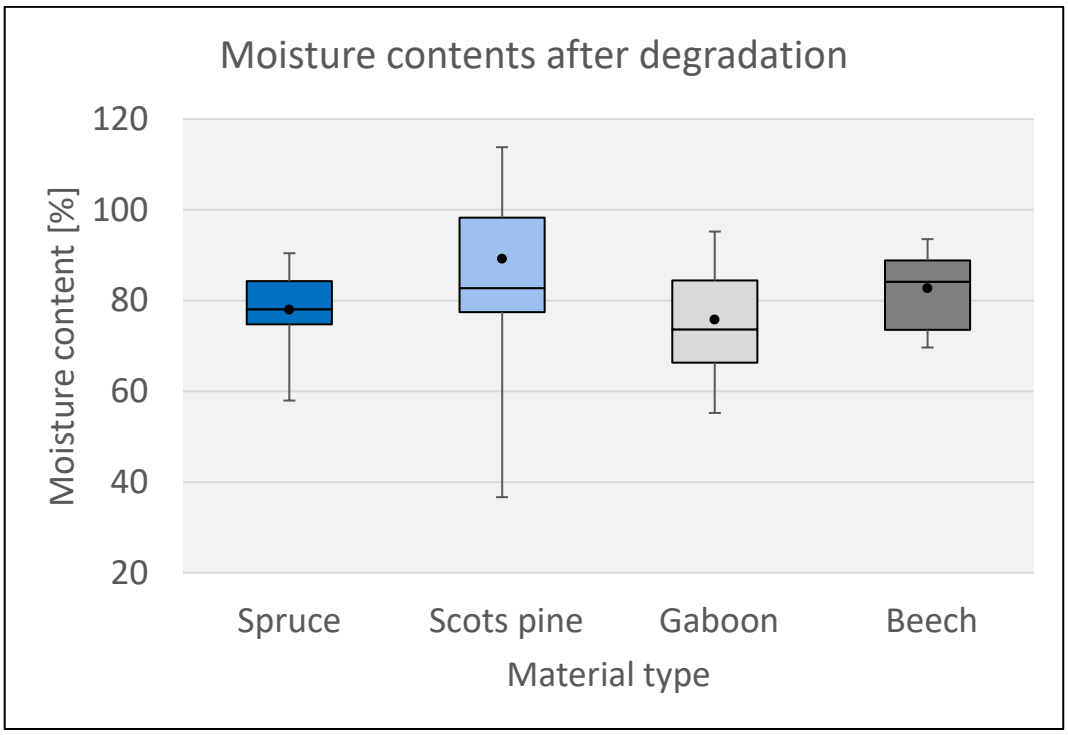


Figure 53: Moisture contents after fungal degradation

4.2.4 Evolution of density over time

As most wood species have different inner structures, it would be interesting to see how the density of the samples changes over time during exposure to the basidiomycete fungus *C. puteana* and how the material's structure influences the moisture distribution and degradation process. Since the mini-blocks vary in density at the start of the experiment, it was decided to plot the relative mean density variation, which is calculated according to equation 4-8, instead of the mean density.

$$\text{Relative density change [\%]} = \frac{\rho_{sample} - \rho_{ovendry}}{\rho_{ovendry}} \cdot 100 \qquad (4\text{-}8)$$

The resulting graphs are not straightforward to interpret because there are several factors that affect the results. On the one hand, there is moisture uptake from the agar medium and moisture production by the fungus, which metabolize sugars into water and carbon dioxide, resulting in an increase of the mean density profile. On the other hand, there is water evaporation and fungal degradation, resulting in a decrease of the mean density profile. In order to find out the influence of some of these factors, the relative density changes are also analysed for the mini-blocks subjected to the same set-up, but without fungus. The results of both analyses can be found from section 4.2.4.1 to 4.2.4.4.
Before analysing the results, some important comments are given regarding the indices and the colour codes in the graphs.

- 'Oven dry' represents the results of the oven dry dataset before degradation.
- Week 0 represents the results of the samples placed in the Petri dish for several minutes.
- Week 1 to 10 represent the results of the samples exposed to *C. puteana* for 1 to 10 weeks.
- The colour code in the graphs refers to which samples were placed together in a Petri dish and therefore subjected to the same conditions.

Note that no data points are shown in week 9. The reason for this is that the settings of the CT machine were not the same as for the other weeks, causing blurred images.

4.2.4.1 Norway spruce

In Figure 54, a global trend can be observed. The density increases sharply until week 1 and then decreases again until week 10. During week 0-2, the malt agar medium provides sufficient nutrients for the fungus. Consequently, the peak of increasing density could be explained by a high fungal activity and its associated moisture production. After reaching the peak, the density decreases again, possibly because the fungus reaches its active phase and starts assimilating nutrients from the wood. In week 10, one can see that the samples with large mass losses usually have a lower density. Note that the most severely degraded samples even have a final density lower than the oven dry density. It seems logical that the most degraded samples have lower densities because a higher mass loss indicates that the fungus degraded more of the wood substances. Most samples that are heavily degraded (32-42% ML) have a moisture content ranging between 70-79% MC. However, the less degraded samples (9-25% ML) have similar moisture contents that vary between 68-89%, so it seems that the fungus is still degrading wood components in most samples, regardless of the wood degradation that has already occurred.

Furthermore, it can be seen that the mass losses of the samples of the same Petri dish differ much, although the samples were exposed to the same conditions. In Figure 55, the density variation is shown for samples that are not exposed to fungi. The samples take up moisture from the agar relatively quickly after being placed in the Petri dishes (week 0). In the following weeks, the density first increases a little bit and then stays approximately constant. By comparing Figure 54 and Figure 55, one can conclude that the fungus is the cause of density increases higher than 20%.

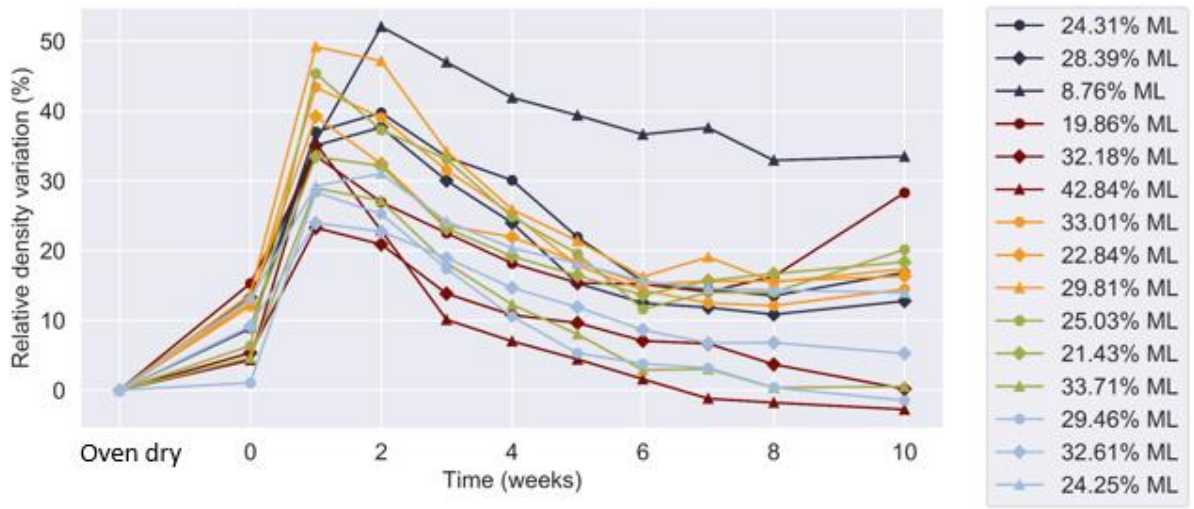


Figure 54: Relative mean density variation of spruce during exposure to C. puteana

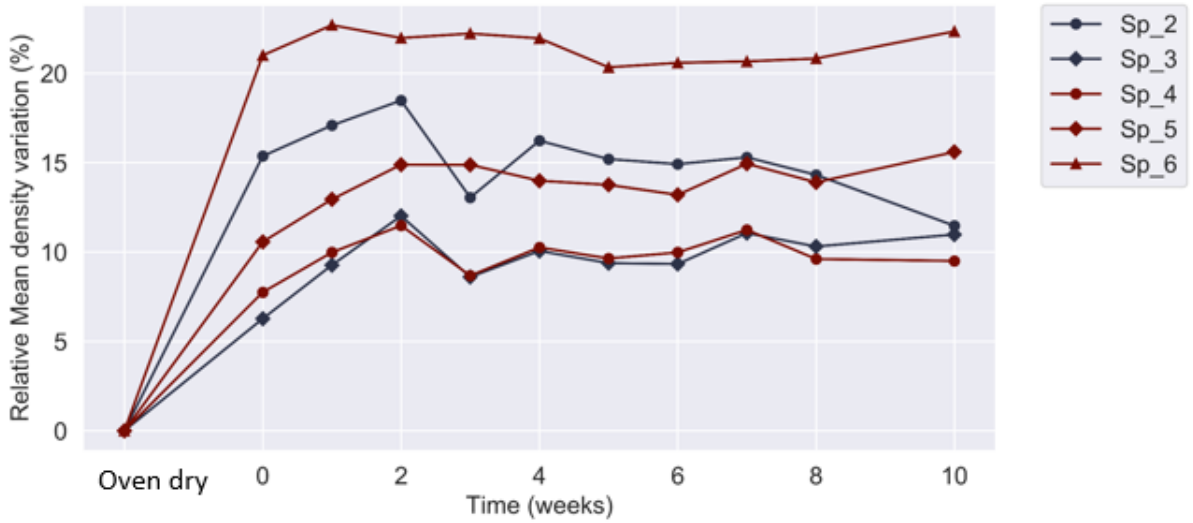


Figure 55: Relative mean density variation of spruce (control samples)

4.2.4.2 Scots pine

The evolution of the relative mean density of Scots pine (Figure 56) is clearly different than for spruce. For all samples, the density increases in the first two weeks. Afterwards, two major phenomena can be observed, namely a density decrease and stagnation. In case the density decreases, severe degradation ($\geq 20\%\ ML$) is observed, whereas little to moderate degradation occurs for samples with a density stagnation (or small increase). Furthermore, it can be observed that, in contrast to spruce, the severely degraded samples do not decrease below the oven dry density. This can possibly explained by the fact that these samples have a higher moisture content (78-98% MC) and therefore making the samples too wet for degradation.

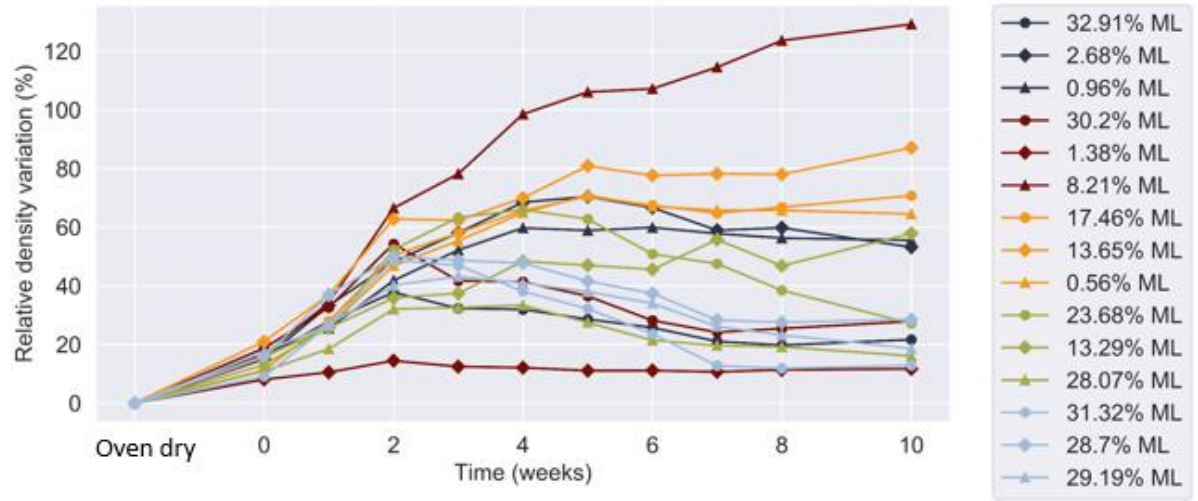


Figure 56: Relative mean density variation of Scots pine during exposure to C. puteana

The same comment as for spruce can be made about the Scots pine samples in the same Petri dishes. The graph patterns and final mass loss differ a lot, although the samples are exposed to the same conditions. This could possibly mean that the inner structure has an important influence on the fungal degradation. Typically, two zones of growth can be distinguished within a tree ring: earlywood and latewood. Earlywood is formed in the spring, whereas latewood is formed when the growth rate of the tree slows down and stops in autumn, resulting in different types of cells [78]. Earlywood cells have larger radial diameters and thinner cell walls, whereas latewood is denser, has thicker cell walls and smaller lumens [78]. Based on the mass losses of Scots pine mini-blocks, it seems that the amount of latewood regions of the growth rings had an influence on the fungal degradation. In case the mini-block

had more latewood regions, and thus growth rings, a lower mass loss was observed (Table 18). In several studies, it was observed that most brow-rot fungi colonize the earlywood first, while the thicker cell walls and narrower lumina hamper the colonization in latewood [79], [80]. This implies that the samples with more earlywood, which have larger diameters, are more invasive, whereas the initial fungal colonization is obstructed when the wood has higher amounts of latewood, resulting in less degradation after ten weeks of exposure to *C. puteana*.

Table 18: Influence of the amount of latewood regions on fungal decay for Scots pine

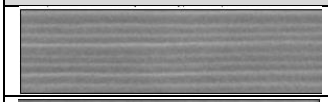
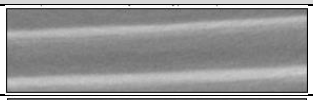
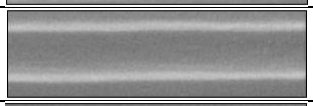
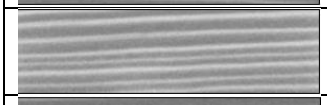
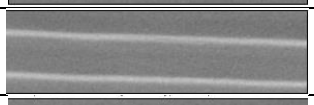
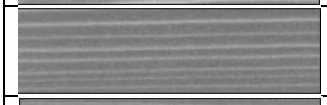
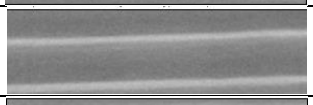
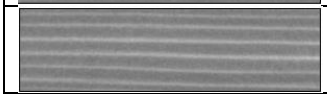
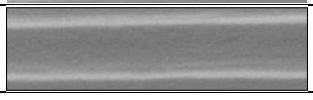
Mini-block	Mass loss [%]	Mini-block	Mass loss [%]
	2.68		32.91
	0.96		30.20
	1.38		31.32
	8.21		28.70
	0.56		23.68

Figure 57 shows the density variation for the control samples. The same patterns can be observed as for spruce. First, there is density increase of 10-20% until week 2, and a stagnation afterwards. The moisture production of the fungus is thus responsible for density increases above 20%.

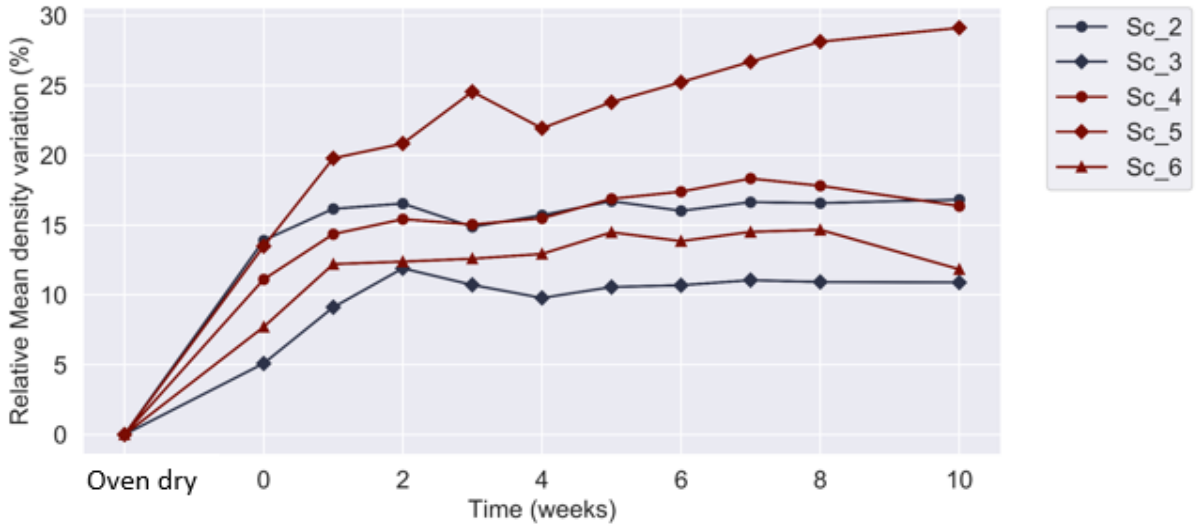


Figure 57: Relative mean density variation of Scots pine (control samples)

4.2.4.3 Beech

For beech, the density increases sharply and reaches a peak in week 2 (Figure 58). Then, two phenomena can be distinguished, namely a stagnation or a decrease. The stagnation mainly corresponds to samples that show a relatively small mass loss (≤ 11% ML), whereas the decrease corresponds to samples with larger mass losses (20-30% ML). The density increase of the samples without fungus (Figure 59) lies approximately between 10-20%, which indicates that the moisture production of *C. puteana* causes an additional 30-40% density increase.

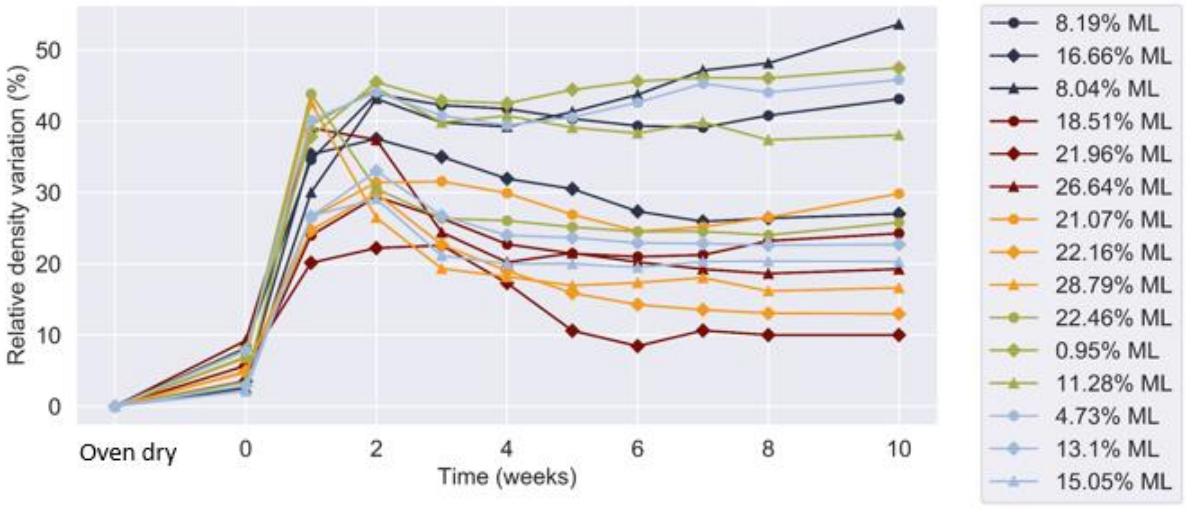


Figure 58: Relative mean density variation of beech during exposure to C. puteana

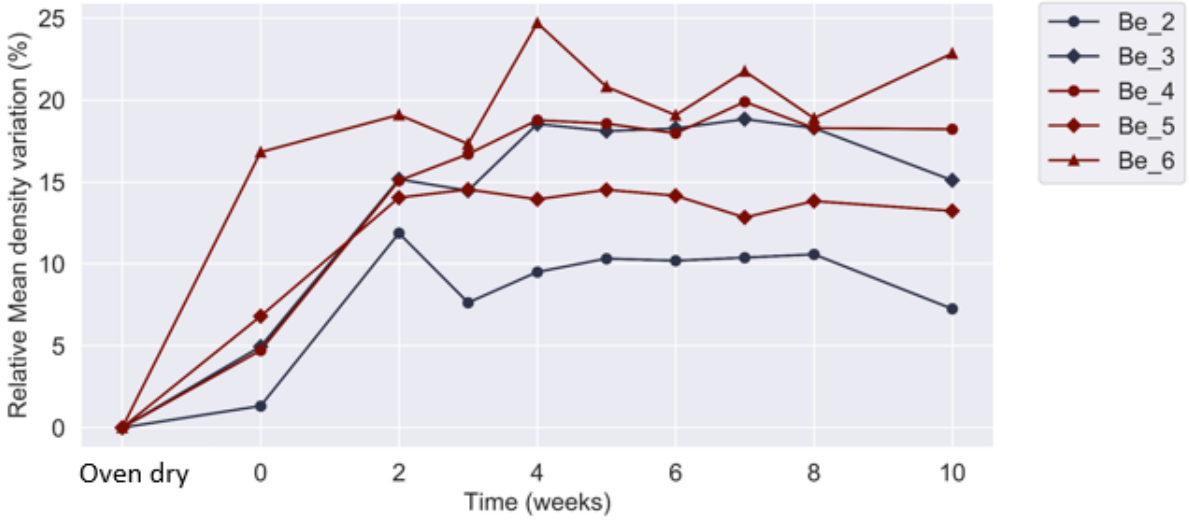


Figure 59: Relative mean density variation of beech (control samples)

4.2.4.4 Gaboon

For gaboon, one can see that the densities increase until week 3-4 and then stagnates (Figure 60). For the samples without fungus, the maximum density change is approximately 30% (Figure 62). This means that the moisture production of the fungus is responsible for another 15-50% density increase. However, the increased densities do not imply that the fungus is degrading wood components (the median mass loss is equal to 2%). An increased density indicates that moisture is absorbed from the malt agar medium and/or moisture is produced when *C. puteana* metabolizes sugars from the growth medium

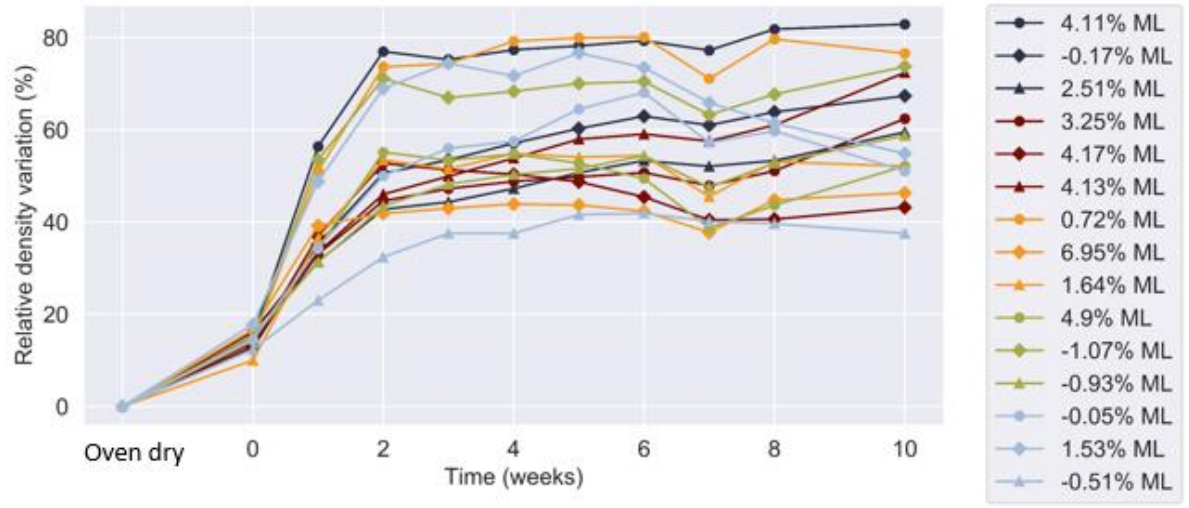


Figure 60: Relative mean density variation of gaboon during exposure to C. puteana

One can see that the orange and green lines show a dip in week 7. This can be explained by the fact that the mean grey value of the reference material, used for gaboon samples, did not correspond to the 'usual' values, as can be seen in Figure 61. This outlier is also used for the yellow and light green curves of week 7 for Scots pine (Figure 56), but this effect is not as noticeable as for gaboon.

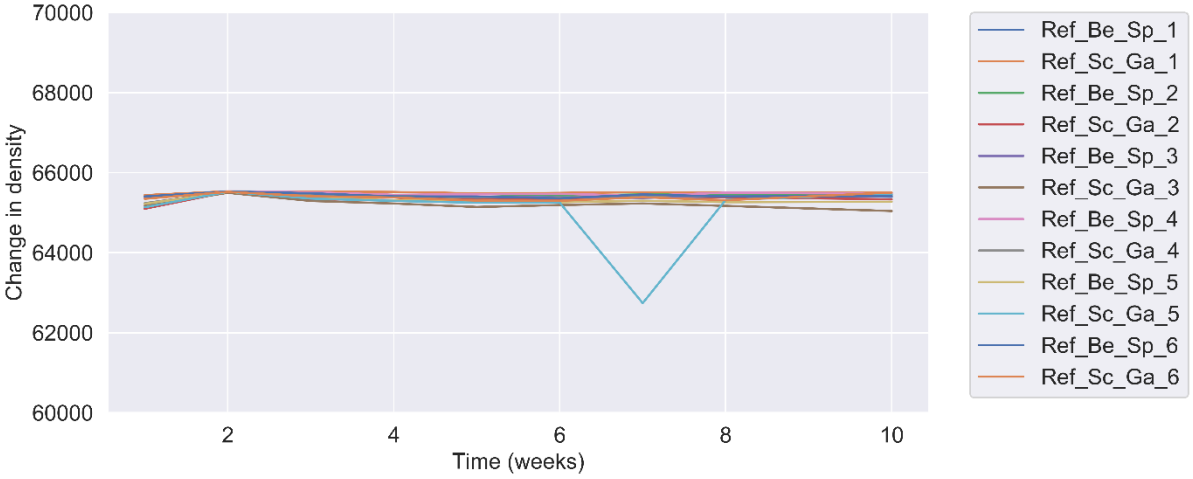


Figure 61: Evolution of the grey values of the reference material over time

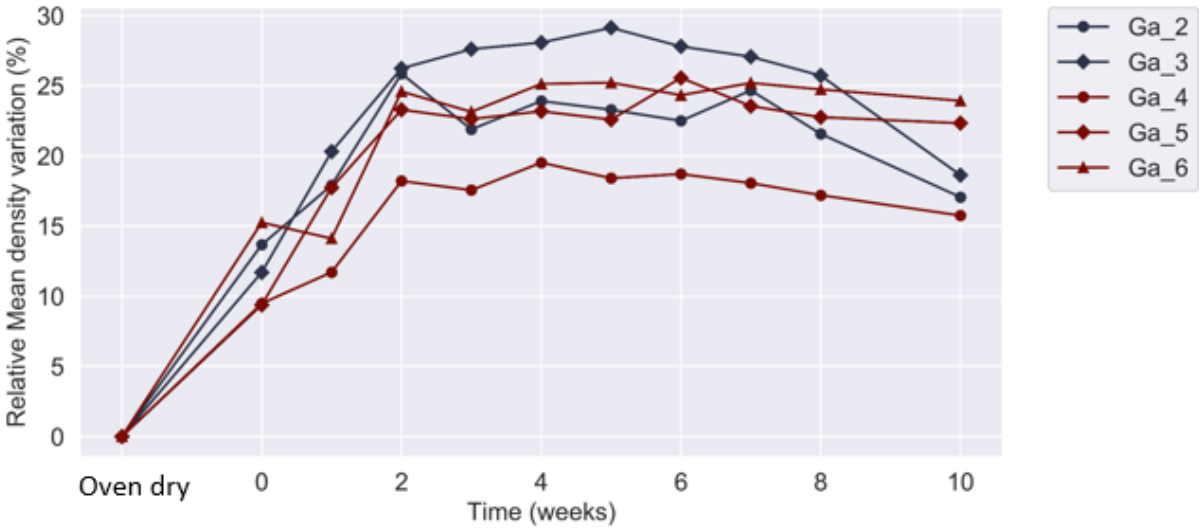


Figure 62: Relative mean density variation of gaboon (control samples)

4.2.5 Moisture infiltration during fungal degradation

In the graphs above, it is not possible to see how the moisture enters the mini-blocks. A possible way to visualize how the moisture infiltrates the sample, is by taking snapshots of the degradation process over time (Figure 63). Darker pixels correspond to regions with low densities, such as air, whereas white pixels correspond to regions with high density.

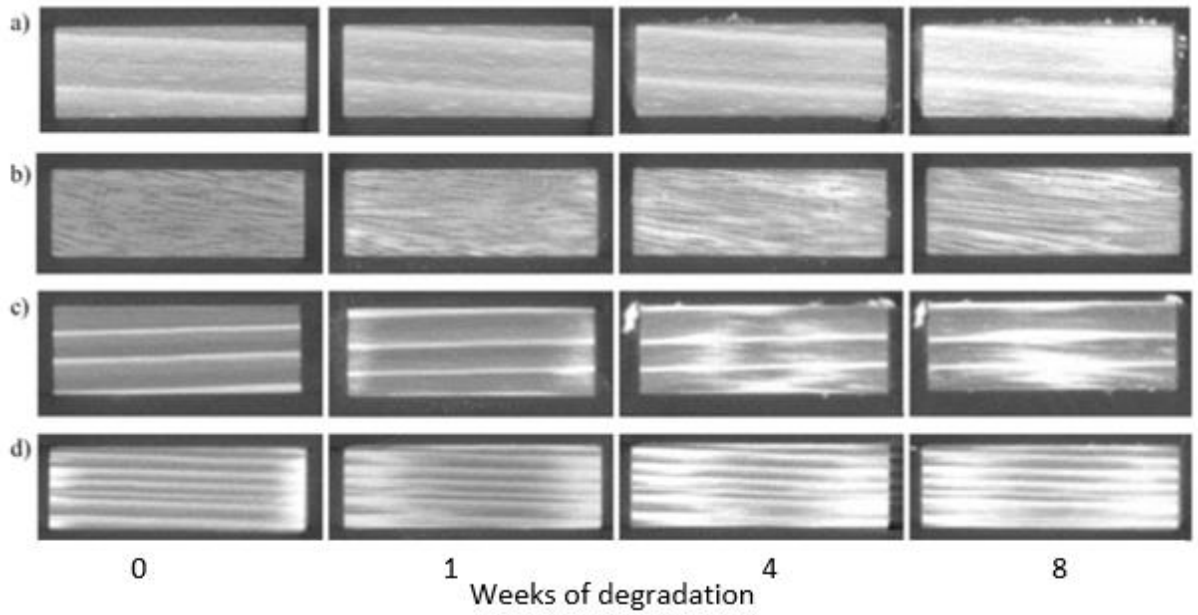


Figure 63: CT slices showing the fungal degradation of (a) beech; (b) gaboon; (c) Scots pine and (d) Norway spruce over time by C. puteana [81]

In this figure, the white stripes in beech, Scots pine and Norway spruce indicate the latewood zones of the growth rings, which are denser than earlywood [81]. As mentioned before, week 0 corresponds to the moment where the samples are placed in the Petri dishes for several minutes, so no degradation has occurred yet. As more weeks pass, some regions become more whitish, which indicates moisture production of the fungus. For some wood species, a different pattern can be distinguished. For Scots pine (c) and spruce (d), the fungal degradation starts at the sides and propagates towards the centre of the samples. This is logical because the mini-blocks were cut in such way that the transverse plane is on the sides, causing water and hyphae to enter the wood through the tracheid openings [81]. For beech (a) and gaboon (b), however, the density increases more uniformly in the mini-blocks.

This can be confirmed by plotting the density profile along the length of the sample. Figure 64 shows the density variation of a Scots pine mini-block with 17% ML. The density is constant at the start of the experiment (week 0). As the weeks pass (week 1 - week 10), one can see that the density peak moves from the sides towards the centre of the mini-block. Furthermore, one can observe that the density decreases again when the peak has been reached. This indicates that the fungus is degrading the wood components during its travel towards the centre. A different pattern can be observed for beech (Figure 65). The density is almost constant along the length, so it is more likely that the fungus attacks the sample uniformly at the bottom towards the top.

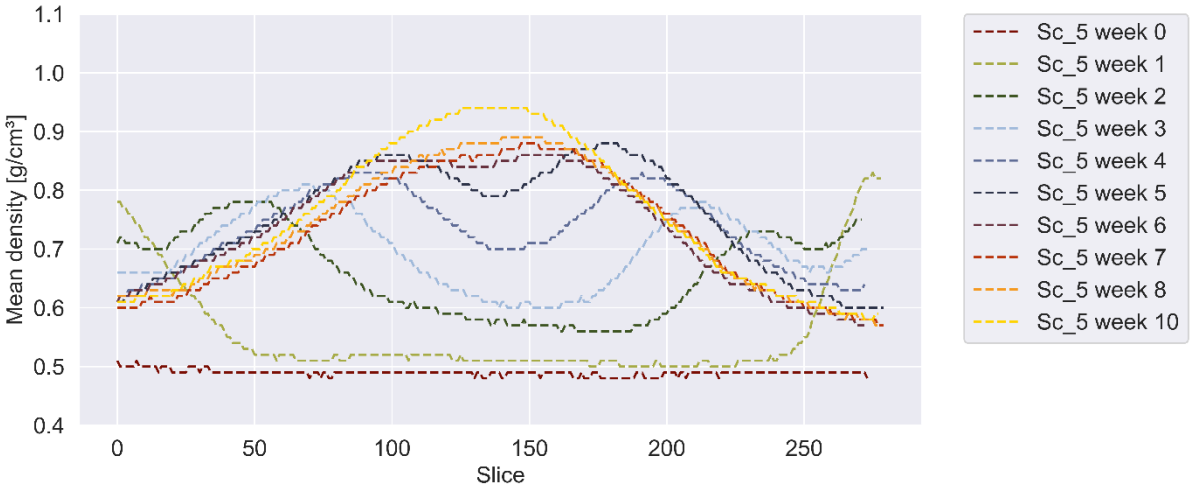


Figure 64: Longitudinal density profile a Scots pine mini-block with 17% ML

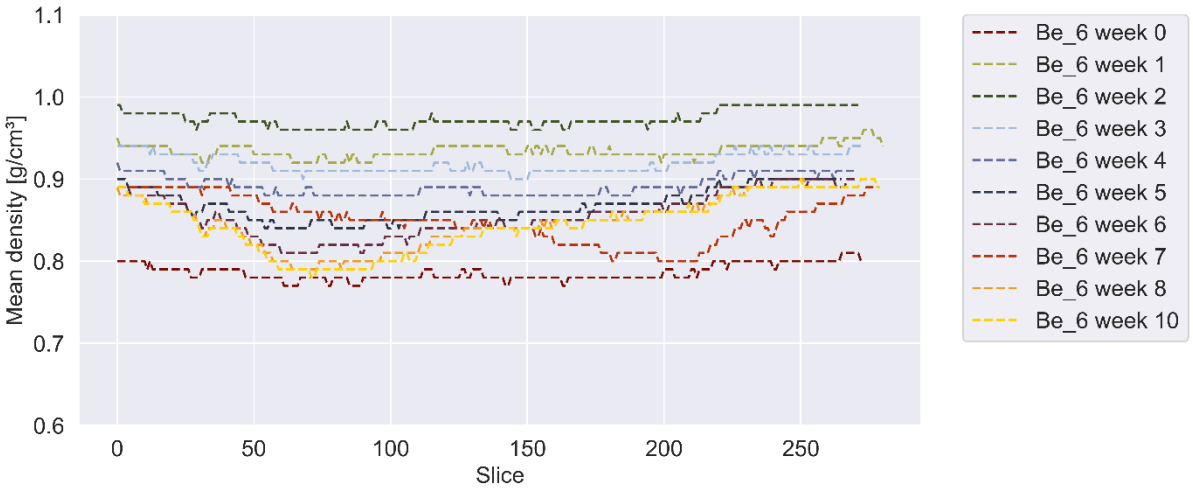


Figure 65: Longitudinal density profile of a beech mini-block with 22% ML

The density profile of Scots pine and Norway spruce are different when the samples are not exposed to *C. puteana*. Figure 66 shows the density variation of a Scots pine mini-block. The density profile is quasi uniform along the longitudinal direction. In this specific experiment, the mini-blocks were cut in such way that the transverse plane is on the sides. Since the tracheid openings are the largest at those sides, this indicates that the fungus *C. puteana* is attacking the mini-block at the 'weakest' parts of the mini-blocks.

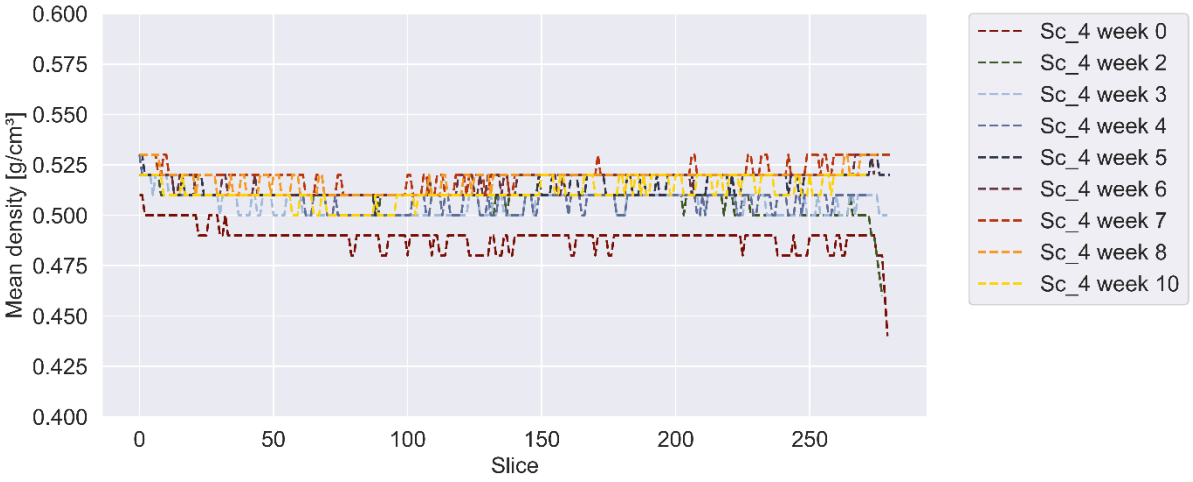


Figure 66: Longitudinal density profile of a control sample of Scots pine

4.2.6 Comparison of oven dry densities before and after degradation

During the degradation process, fungi break down structural elements of the wood. As a consequence, one would expect that the density decreases. However, it may also be possible that the wood densifies due to shrinkage as a result of the degradation. In order to find out which phenomenon occurs, or if there is a combination of both phenomena, the oven dry densities and volumes are compared. In Figure 67 , the oven dry densities before and after degradation are visualized.

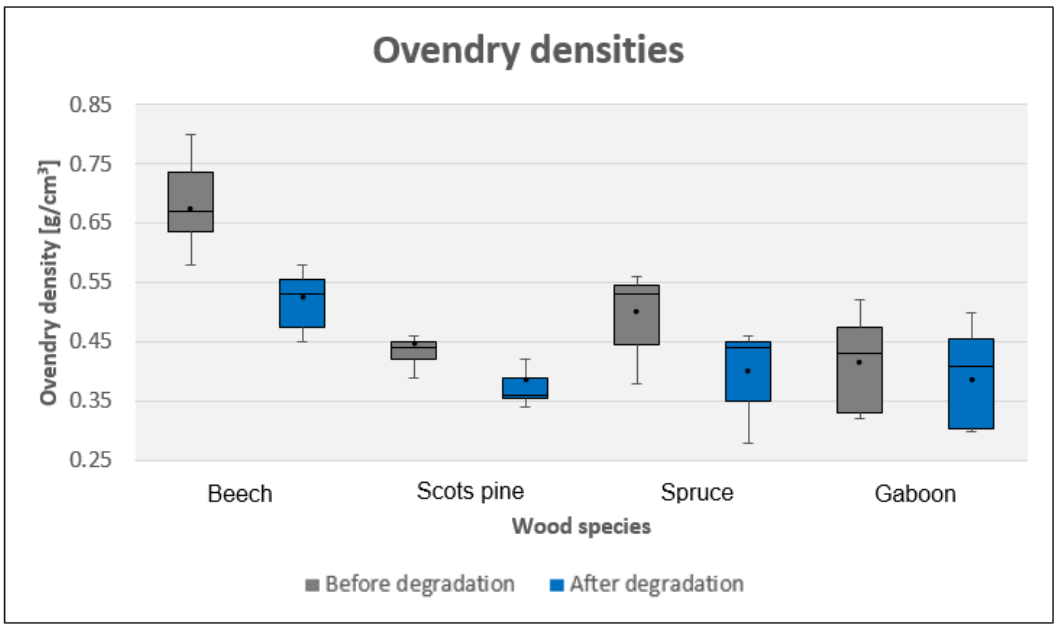


Figure 67: Comparison of oven dry densities before and after degradation

For all wood species, one can see that the oven dry densities before degradation are larger than after degradation, which indicates that the density loss is due to the fungal degradation. The magnitude of the density loss is mostly in line with the mass loss. The samples with a large (small) mass loss also have a large (small) loss in density, as can be seen in Figure 68

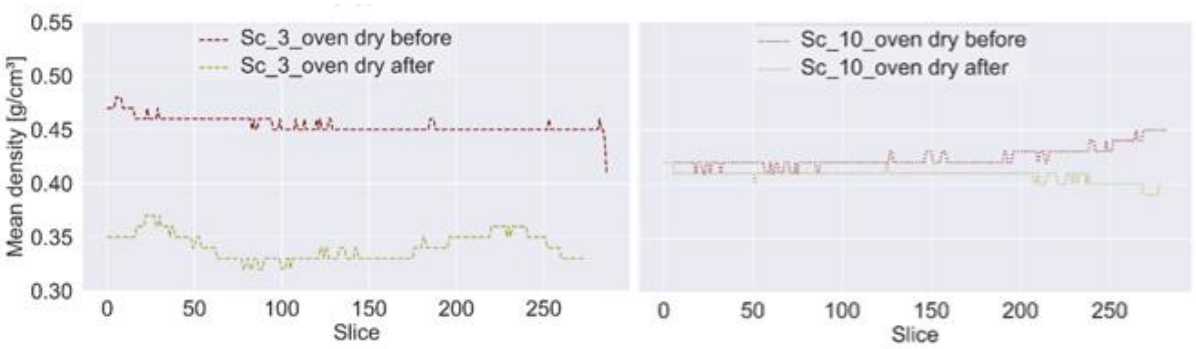
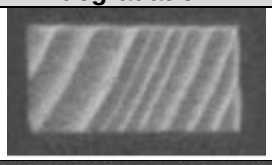
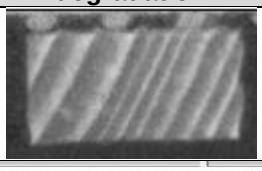
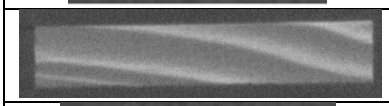
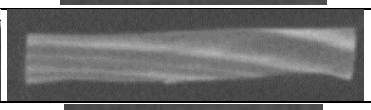
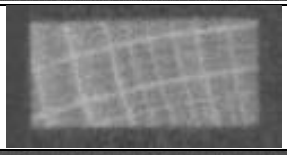
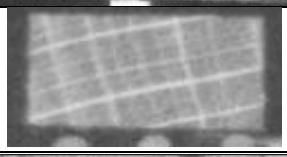
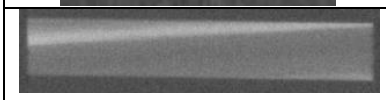
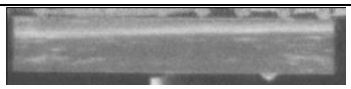


Figure 68: Mean density profile in longitudinal direction: sample with 30% ML (left), sample with 1% ML (right)

Based on the results presented in Figure 67, it does not seem that there occurs any densification due to shrinkage as a result of fungal degradation. Therefore, we take snapshots of the CT images of the mini-blocks after ten weeks of degradation and of the oven dry mini-blocks before and after degradation (Table 19). It can be observed that the edges of some mini-blocks are degraded a little bit. This indicates that shrinkage occurs due to fungal degradation, and results in (a small) densification. The shrinkage effect is more pronounced after oven drying the degraded samples. This is logical because the moisture, which is produced when the fungus metabolizes sugars or when the sample takes up moisture from the growth medium, evaporates. However, shrinkage was only noticeable for the most degraded mini-blocks. This can be explained by the fact that the more wood substances are degraded, the less remains of the wood structuring elements and the more the samples will shrink.

Table 19: Shape of mini-blocks before and after degradation: (a) spruce (33% ML); (b) beech (21% ML); (c) Scots pine (33% ML)

	Oven dry shape before degradation	Shape after 10 weeks of degradation	Oven dry shape after degradation
a)			
a)			
b)			
c)			

The degraded mini-blocks clearly shrunk, but since the graph in Figure 65 shows that there are only density losses, one can conclude that the densification is of minor importance to the overall density loss.

4.2.7 Conclusion and recommendations

In this data-analysis, several interesting topics were investigated. By comparing the mass losses of samples exposed to X-ray radiation to those in a parallel experiment, we found that the mass losses of spruce were almost the same for both experiments. Furthermore, the mass losses of beech and gaboon were slightly smaller in case of the CT experiment, which indicates that the X-ray radiation could have an influence on the fungal degradation. Based on the obtained results, it is not possible to make a clear statement about this matter. Therefore, it is recommended to execute new experiments. However, for the purpose of this experiment, sufficient mass loss was reached in the CT experiment to further investigate how the density varies over time and how the material's structure affects the degradation process.

Next, it was examined if the densities based on X-ray attenuation coefficients correspond to the actual densities of the samples. Therefore, a linear regression was done, resulting in the best possible linear relationship. Since the slopes were close to one and the determination coefficient was each time higher than 80%, there could be concluded that the 'CT densities' correspond quite well to the actual densities of the samples. By additionally doing a paired T-test, which gave positive results, there could be concluded that it is allowed to convert the 'CT density' to the actual density using the relationships obtained from the linear regression.

After evaluating the density variation profiles, it became clear that the presence of *C. puteana* had a big impact on the density, as it caused a density increase of approximately 20-45%, in addition to the 10-20% increase due to moisture absorption from the growth medium. For the four wood species, two different fungal behaviour patterns could be distinguished. For Norway spruce and Scots pine, the fungal degradation starts at the sides and moves towards the centre of the mini-blocks as the weeks pass. For beech and gaboon, in contrast, the degradation is quasi uniform along the longitudinal direction. In case of Scots pine, the material structure seemed to have an impact on the degradation. When the sample had many latewood regions, the resultant mass loss was remarkably smaller. This could be explained by the fact that latewood is denser, has thicker cell walls and smaller lumina compared to earlywood. The samples with more latewood are thus less invasive, resulting in less degradation after ten weeks of exposure to *C. puteana* compared to samples with mainly earlywood. This indicates that X-ray CT is a suitable technique to investigate how the material's structure affects the degradation. Especially for bio based materials, X-ray CT looks very promising, since thorough knowledge about the influence of non-wood components such as glue layers in plywood, bitumen emulsion in porous fibreboard, etc. on the degradation process is still lacking.

Finally, by comparing the oven dry densities before and after fungal decay, all materials showed a loss in density due to wood degradation. Based on the CT images, it could be observed that the most degraded samples shrunk, as a result of fungal degradation, resulting in densification. However, the densification due to shrinkage is of minor importance compared to the overall density loss due to degradation.

In this data-analysis, only X-ray CT was used to monitor the mass loss and moisture distribution. In the CT images, however, it is not easy to differentiate free water from other components. This problem can be solved by additionally using MRI. This technique makes it possible to visualize the free water, so additional insights can be obtained in how the moisture is actually distributed in the mini-blocks and where the fungal activity is the highest.

5 CONCLUSION

The current standardized laboratory tests are excellent to assess the natural durability of various wood species against wood-destroying fungi and the efficacy of preservatives. However, these tests are not suitable for the durability assessment of bio-based materials, whose natural durability is enhanced by new technologies, such as glued laminated timber, thermally modified wood, chemically modified wood and wood treated with water repellents. The moisture dynamics of these materials are changed in such way that the equilibrium moisture content is decreased. As a consequence, the material may become insufficiently wet for fungal degradation when tested according to the standardized procedure. This does, however, not mean that the material can eventually not be degraded.

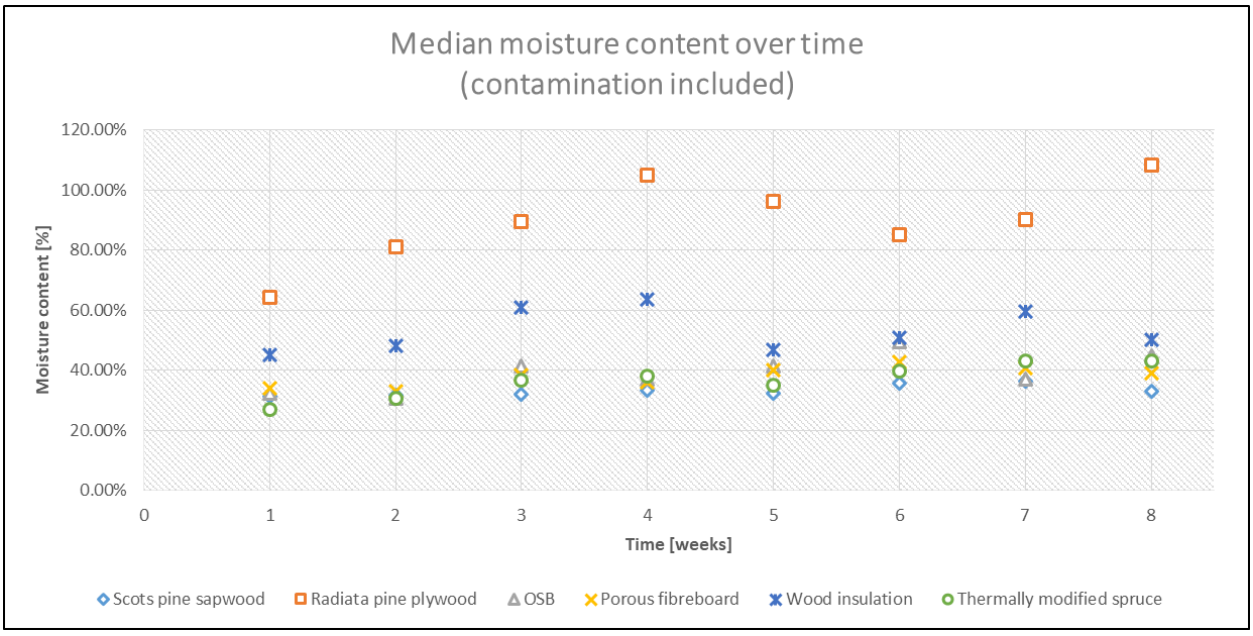
In addition, these standardized tests do not allow us to gain insight in what is going on at the inside of the material. A thorough knowledge about the relationship between fungus and material is still lacking, in particular about how the material's structure and moisture properties affect the degradation process. In order to be able to design new materials and apply these bio-based materials in an optimal way, it is necessary to have a better understanding in how the material's structure affects the fungal degradation. To overcome these problems of the standardized test, new test methods need to be developed.

In this thesis, we therefore performed a preliminary experiment based on the mini-block test, in which the samples (3 x 1 x 0.5 cm^3) were brought to an initial moisture content ranging between 20-30% MC before exposure to basidiomycete fungi. Based on the results, there can be concluded that most bio-based materials are not durable when they are exposed to conditions favouring fungi (the samples are initially wet and remain wet during the degradation process and the hyphae can enter the samples from the sides. In case of thermally modified spruce, bringing the samples to 20-30% MC did not make them susceptible to decay. This adapted mini-block test is, however, not useful to determine the natural durability of the bio-based materials. Due to the smaller sample size, the glue layers in pine plywood and the bitumen emulsion in porous fibreboard were not able to have a significant impact on the degradation process. Furthermore, the samples were not sealed at the sides, so the mycelium could infiltrate at all sides. This does not correspond to reality, where the sides of some materials are often shielded by other elements, thus preventing fungal attack from the sides. To further enhance the performance of bio-based building materials in terms of moisture and biological durability, we need to understand how the different material characteristics affect these factors. The preliminary experiment is not suitable to assess the durability adequately, so another test set-up should be developed, in which the effect of glue layers, bitumen emulsion, etc. can become clear and in which the shielding aspect can be taken into account.

In order to obtain a better understanding in how the material's structure affects the fungal degradation, we performed a second experiment, in which the moisture distribution and mass loss of four solid woods were monitored on a weekly basis using the non-destructive technique X-ray CT. The resulting data show the relative density variation over time for beech, Norway spruce, Scots pine and gaboon. The density variation is affected by several factors: a mean density increase is expected due to the moisture production of the fungus and moisture absorption from the growth medium, whereas a mean density decrease is expected due to fungal degradation and water evaporation. For most materials, a density increase of 10-25% was observed due to the moisture uptake from the malt agar medium. The samples exposed to *Coniophora puteana* showed an additional 10-50% density increase, due to the moisture production of the fungus. The material's structure of Scots pine seemed to have a big impact on the degradation process. The samples with more earlywood have larger lumina, and therefore more invasive, whereas the samples with more latewood have narrower lumina and thicker cell walls, therefore obstructing the initial fungal colonization, and resulting in less degradation after ten weeks of exposure to *C. puteana*. Based on this result, there can be concluded that X-ray CT is a suitable

technique to investigate the influence of material's structures on the degradation process. Especially for bio-based materials, this technology looks very promising, as a better understanding of the influence of non-wood components such as glue layers in plywood, bitumen emulsion in porous fibreboard, etc. on the degradation process facilitates their design.

APPENDIX A: COMPLETE RESULTS OF CONTROL TEST



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