Doctoral Thesis

Durability and physical properties of thermo-hygro-mechanically (THM)-densified wood

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Durability and Physical Properties of Thermo-Hygro-Mechanically (THM)-densified Wood

A dissertation submitted to

ETH ZURICH

for the degree of

Doctor of Sciences

presented by

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2008
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SUMMARY

Due to its polymeric nature, wood is susceptible both to fluctuations in humidity and to biodeterioration by micro-organisms and insects. Traditionally, wood durability has been improved by using preservatives containing toxic chemicals, such as arsenic, zinc, copper, chromium or creosote oils etc. In Europe there is increasing environmental, legislative and consumer pressure on the use of traditional wood preservatives and also tropical timber species. Apart from the risks involved in using such substances for treatments, there is increasing concern over the problems arising in the disposal of the timbers at the end of their commercial lifetime. In some European countries there are even legal bans on the use of selected chemicals. Consequently, there is a need to develop alternative, more environmentally-benign wood preservation methods that adequately protect wood at a reasonable price. It is envisaged that new approaches based on wood modification with heat or environmental friendly chemicals will replace the use of traditional preservatives.

Three methods of wood modification have been investigated in recent years: chemical, enzymatic and thermal modification. All processes lead to alterations in the wood structure and their properties. For several decades, worldwide, research has been carried out on methods of mechanically and thermally treated wood. Considerable effort has been undertaken in a number of countries e.g. USA, Japan, France, Denmark, Germany, Sweden, Netherlands, Finland etc., and to some extent in Switzerland, to investigate and improve wood modification processes.

A process which combines wood densification and post-treatment with heat and steam, termed thermo-hygro-mechanical (THM) densification has been developed in recent years at EPFL, Lausanne. Preliminary studies indicate that THM-densified wood possesses reduced hygroscopicity, improved mechanical performance and little set-recovery. The use of THM-densified wood of European trees with enhanced durability and qualities, without the need for biocides, would be an ideal solution to the environmental problem.

The emphasis of this thesis has concentrated on establishing the fundamental understanding of the principles and mechanisms which determine the durability of THM-densified against fungal decomposition. Particular attention is paid to the interactions that exist between the altera-
tions of the wood constituents, its physical properties, the micro-morphology of the wood structure during the processing of THM-densified wood and the colonisation and degradation by wood decay fungi.

To assess the durability of THM-densified wood, differently treated Norway spruce (*Picea abies* L.) and beech (*Fagus sylvatica* L.) specimens were incubated with various wood decay fungi causing brown-, white- or soft rot degradation. Alterations in the chemical constituents arising from the treatments and other selected physical properties of the specimens were analysed.

In conclusion, it can be stated:

- **THM-densification of wood at relatively high temperatures not only improves the dimensional stability, compared to densified and non post-treated specimens, but also renders conditions less conducive to hyphal growth within the secondary walls of tracheids and xylem ray parenchyma.**

- The process of THM-treatment enhances the durability of Norway spruce wood against colonization and degradation by brown rot fungi.

- THM-treatment did not enhance the durability of Norway spruce and beech wood against white rot and soft rot fungi. Hyphae of both types of decay managed to circumvent conditions restricting hyphal growth by hyphal tunnelling in secondary walls of fibre tracheids of beech or by forming a bore holes that transversally penetrate cell walls of early wood tracheids.

- TH-treated Norway spruce and THM-treated beech wood are highly susceptible to colonization and degradation by soft rot fungi.

- **THM-densified wood may have some potential for utility class 3 but on the basis of the current findings it is inappropriate for application in utility class 4 (e.g. railway sleepers).**

It is envisaged that the new knowledge acquired within the PhD-thesis will provide a sound basis for further investigations in the area of wood modification and preservation. Such work should focus on specific end-uses of the product and include considerations of environmental and economical aspects as well as sustainability issues.
ZUSammenfassung


Der Forschungsfokus im Bereich präventiver Holzbearbeitung lag in den vergangenen Jahren vor allem auf chemischer, enzymatischer und thermaler Modifikation. Durch diese Prozesse gelangen Veränderungen in Struktur und Eigenschaft des Holzes. Achtbare Erfolge wurden weltweit im Bereich der mechanischen und thermischen Modifikation erzielt. Über Jahrzehnte taten sich in diesem Bereich vor allem Länder wie die USA, Japan, Dänemark, Deutschland, Schweden, die Niederlande, Finnland etc. und zu einem gewissen Anteil auch die Schweiz hervor.


Die wichtigsten Schlussfolgerungen sind:

- Hygrothermische Behandlung von Holz, das bei relativ hohen Temperaturen verdichtet wurde, verbessert, im Vergleich zu verdichtetem aber nicht nachbehandeltem Holz, nicht nur die Dimensionsstabilität sondern wirkt auch einer Besiedlung der Holzzellen durch Pilzhyphen entgegen.
- Die THM-Verdichtung erhöht die Resistenz von Fichtenholz gegenüber Braunfäuleerreger.
- TH-behandeltes Fichten- und THM-behandeltes Buchenholz weisen eine hohe Anfälligkeit gegenüber Moderfäuleerreger auf.
ACKNOWLEDGEMENTS

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Oleksandr Skyba

St.Gallen, 02.03.2008
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>One-way analysis of variance</td>
</tr>
<tr>
<td>ASE</td>
<td>Accelerated solvent extraction</td>
</tr>
<tr>
<td>BSTFA</td>
<td>N,O-Bis (Trimethylsilyl) trifluoroacetamide (derivatization reagent)</td>
</tr>
<tr>
<td>COST</td>
<td>European Cooperation in the Field of Scientific and Technical Research</td>
</tr>
<tr>
<td>CC</td>
<td>Chromium copper salt</td>
</tr>
<tr>
<td>DIN</td>
<td>Deutsches Institut für Normung</td>
</tr>
<tr>
<td>DP</td>
<td>Degree of polymerisation</td>
</tr>
<tr>
<td>DS</td>
<td>Decay susceptibility</td>
</tr>
<tr>
<td>EMC</td>
<td>Equilibrium moisture content</td>
</tr>
<tr>
<td>EMPA ***</td>
<td>strain number, followed by number</td>
</tr>
<tr>
<td>EN ***</td>
<td>European standard, followed by number</td>
</tr>
<tr>
<td>FBE</td>
<td>Fluidised bed extraction</td>
</tr>
<tr>
<td>FID</td>
<td>Flame ionization detector</td>
</tr>
<tr>
<td>GS-FID</td>
<td>Gas-supported flame ionization detector</td>
</tr>
<tr>
<td>HB</td>
<td>Brinell hardness</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HSD</td>
<td>Tukey's Honestly Significant Difference (statistics; analysis)</td>
</tr>
<tr>
<td>IS</td>
<td>Internal standard</td>
</tr>
<tr>
<td>L</td>
<td>Longitudinal direction</td>
</tr>
<tr>
<td>LVL</td>
<td>Laminated veneer lumber</td>
</tr>
<tr>
<td>MC</td>
<td>Moisture content</td>
</tr>
<tr>
<td>MDF</td>
<td>Medium density fibreboard</td>
</tr>
<tr>
<td>MEA</td>
<td>Malt-extract agar</td>
</tr>
<tr>
<td>MOE</td>
<td>Modulus of elasticity</td>
</tr>
<tr>
<td>OPA</td>
<td>o-phthalaldehyde</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>PC</td>
<td>Personal computer</td>
</tr>
<tr>
<td>R</td>
<td>Radial direction</td>
</tr>
<tr>
<td>RH</td>
<td>Relative humidity</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical package for the social sciences</td>
</tr>
<tr>
<td>STSM</td>
<td>Short term scientific mission</td>
</tr>
<tr>
<td>T</td>
<td>Tangential section</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermogravimetric analysis</td>
</tr>
<tr>
<td>TH</td>
<td>Thermo-Hygro treatment</td>
</tr>
<tr>
<td>THM</td>
<td>Thermo-Hygro-Mechanical treatment</td>
</tr>
<tr>
<td>TLS</td>
<td>Tangential longitudinal section</td>
</tr>
<tr>
<td>TS</td>
<td>Transverse section</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>WHS</td>
<td>Water-holding capacity</td>
</tr>
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</table>
CHAPTER 1
INTRODUCTION

1.1 Background

Wood products are used extensively in residential construction and other outdoor applications where the wood is exposed to the environment and can be colonized and degraded by a range of microorganisms. To prevent degradation, non-durable wood products used in applications where they are susceptible to biodeterioration are treated with biocides. Treated wood is a sustainable, inexpensive and an effective building material that requires relatively little energy to manufacture. In addition trees sequester carbon dioxide from the atmosphere as they grow. Therefore, treated wood is an economical construction material for homeowners and provides ecological benefits to society. However, wood preservation has recently undergone dramatic changes worldwide. These changes have been driven by both real and perceived environmental concerns and governmental regulations (Preston, 2000; Archer and Lebow, 2006).

Prevention of wood decay has long been a concern for the wood and forestry industry, since Hartig (1878) showed that fungi are the casual agents of wood decay. The preservatives employed for wood preservation must be effective against a wide variety of organisms and must last for many years expected from treated wood. The worldwide market for wood-preserving biocides at the active supplier level is relatively small, about $US 0.6 billion compared with the $US 36 billion market for agrochemicals (Schultz et al., 2007).

In addition to biodegradation, another challenge with wood in outdoor exposure is that wood is a hygroscopic material that swells when wetted and shrinks as it dries, although such effects are species dependent. This can lead to undesired dimensional changes that result in lumber splitting or warping over time (Evans, 2003). Furthermore, the surface of wood exposed to sunlight can suffer photo degradation, and exterior lumber can be colonized by moulds or blue stain fungi if not treated with primers. Therefore, certain species of wood decking gradually lose their initial attractive appearance and require maintenance over time.

The wood protection industry has traditionally relied on only a few first-generation preservatives that have a broad range of activity against many wood colonizing and degrading organ-
isms, which are inexpensive and remain effective for many years. These first-generation preservatives are creosote, oil-borne pentachlorophenol and the water-borne arsenicals, principally chromated copper arsenate (CCA). Prior to the introduction of creosote, the first industrial preservative which was developed some 170 years ago, the choice was to use the naturally durable heartwood from certain tree species or to accept rapid replacement cycles of non-durable wood materials. A small but lively market still exists for naturally durable lumber, but harvesting restrictions of durable timber led to the development of manufactured preservatives for non-durable, treatable wood species.

The major wood preservatives during the last half of the twentieth century were the water-borne metallic arsenicals, principally CCA (Preston, 2000; Freeman et al., 2003). Being water borne, treatment with CCA treated wood did not have an oily odour or surface residues, and proved to be a long-term dependable product even in the most severe hazard environments and applications. The relatively low chemical costs of CCA made it an excellent preservative for residential applications such as decking. Owing to many benefits of CCA and the increased demand in residential applications, up to the end of the 20th century CCA-treated wood accounted for about 80% of the total volume of treated wood (McDonald et al., 2004). The consumption of preservative treated wood in Western Europe is estimated at 6 million m$^3$ per year with about 30000 tonnes of toxic preservatives used (McDonald et al., 2004).

During the 1990s, concerns increased over arsenic and chromium exposure and disposal issues of CCA-treated wood. The use of traditional wood preservatives is being subjected to increasing environmental, legislative and consumer pressure. Apart from the risks involved in using such substances for treatments, there is increasing concern over the problems arising in the disposal of the timbers at the end of their commercial lifetime. Biocides that replaced CCA for residential applications in Europe, parts of Asia and North America are the second-generation water-borne copper-rich systems that contain complexed copper (II) and an organic co-biocide e.g. boron to control copper-tolerant fungi (Preston, 2000; Evans, 2003). These preservatives have a similar efficiency as CCA for the intended applications and, since they are water borne, are suited for residential applications. However, concerns exist with issues such as potential for copper impacts on aquatic systems, potential for metal fastener corrosion and different patterns of mould growth from that seen on CCA-treated lumber, as well as general concerns over the ultimate disposal of wood treated with inorganic preservatives. The environmental concerns with any metals have led some European countries to move towards non-metallic systems for residential applications, and this trend can be expected to in-
crease. This has also been driven by European initiatives funding research into non-biocidal protection systems for such applications, regardless of cost or overall environmental benefit.

Another non-metallic water-borne preservative type currently available for residential applications is borate formulations such as disodium octaborate (Micales-Glaeser et al., 2004). Borates are inexpensive, have an extremely low mammalian toxicity and a broad range of activity against decay fungi and insects. They are not corrosive to metal fasteners, are colourless and odourless and have a long history as wood preservatives.

Organic biocides/agrochemicals employed or being considered for wood preservation for residential exterior applications include the family of biocides known as triazoles such as tebuconazole or propiconazole which are highly effective against a broad range of Basidiomycetes and exhibit good stability and leach resistance in wood (Diouf et al., 2002; Schultz et al., 2002), and the synthetic pyrethroids such as permethrin or the highly active neonicotinoids like imidacloprid or thiamethoxam to prevent termite/insect damage (Lekounougou et al., 2008).

Economical and effective water-borne organic systems is difficult to develop for some applications such as ground-contact uses in areas that have high deterioration hazards. This is because organics are relatively expensive compared to metallic biocides and undergo various biotic and abiotic degradation reactions that deplete an organic biocide over the long service life expected from treated wood.

Other non-biocidal additives that have been extensively studied are water repellents for above-ground applications (Williams and Feist, 1999). Water repellents provide multiple advantages to wood products, including reducing the decay potential and leaching of biocides from treated wood and improve dimensional stability in exterior situations. Most current water repellents are based on hydrocarbon waxes and/or thermoplastics, with the wood product treated using a water-based emulsion formulation (Englund et al., 2004).

It is likely that some future generation of wood protection products will be based on non-biocidal methods to prevent biodeterioration. Indeed, some non-biocidal preserved wood are already available, primarily in Europe, and this is mostly chemically modified acetylated wood and heat-treated wood (Evans, 2003; Rowell, 2006). Chemical modification has been studied for over 50 years by various researchers who have found that, if the wood is properly treated to a certain weight gain, it has greatly increased decay and insect resistance and en-
hanced weathering properties. Due to the recent interest in biocide-free wood, chemical modification has seen renewed interest, with most recent impetus driven by European research.

Other non-biocidal methods include treating wood with various polymers or monomers that polymerize in situ, either bonding alone and/or with the wood structural components (Rowell, 2006). This approach also enhances the weathering properties of wood. The cost effectiveness of such methods is of a higher order than that of any of the biocide approaches outlined above, and the treatment process requires more attention.

It is possible to impart a degree of durability of wood without use of toxic components. Such treatments employ strategies that limit water movement into the wood and/or render the wood structure unsuitable for microorganisms. The interest is largely focused on two areas: wood modification and heat treatments. Both approaches are more expensive than conventional wood preservation, however increasing environmental concern together with legislative and consumer pressure have made these alternative approaches more attractive.

The goal of heat treatment is both to volatilise wood components that are used as source of nutrients for decay fungi and to alter the wood structure (Rapp, 2001). For this purpose, wood is heated in a non-oxidative atmosphere, with the resulting product claimed to have increased decay resistance and dimensional stability. However, termite susceptibility remains a major problem, so heat-treated lumber is not suitable for regions where termites are present, or for exposed or ground-contact applications. Furthermore, depending on the process used, wood suffers some loss in mechanical properties and is hence not appropriate for critical structural applications (Rusche, 1973; Militz, 2002).

In the last few years the impact of Thermo-Hygro-Mechanical (THM) treatment of wood on its mechanical and physical properties has been investigated. The first thermo-hygro reactor that was developed at the laboratory of the EPFL in 1995 was a closed system with a single chamber in which small wood elements were compressed (densified) under saturated vapor at a temperature of approx. 150°C. A piston was used to load the wood specimen transversally under a controlled displacement mode (Figs. 2.1 - 2.3). Small specimens of beech, spruce and maritime pine, were compressed in the transverse direction. The resulting product is known as THM-densified wood to distinguish it from densified wood fabricated in an open system also known as “TM” densified wood. Preliminary studies indicate that THM densified wood is less hygroscopic and has superior mechanical and physical properties when compared to untreated wood and industrially densified TM wood (Navi and Girardet, 2000). Microscopic ob-
servations of wood before and after densification revealed clear differences at the cell level in wood densified after TM and THM processing (Navi and Girardet, 2000; Heger et al., 2003; Navi and Heger, 2005). However, the resistance of THM-densified wood to degradation by individual groups or a combination of microorganisms has so far not been assessed.

1.2 CONSTRUCTION OF THE THESIS

The thesis is arranged in seven chapters (Fig. 1.1). The first part of Chapter 1 gives an introduction to the wood substrate outlining the structure, chemistry and physical properties investigated in the study. It also contains general information on wood decay fungi and their modes of degradation. The second part of Chapter 1 covers aspects of wood modification summarising major research work in the field of thermal treatments and wood densification. The second chapter contains a description of detailed methods and materials used in the study. Chapter 3 presents the results of the investigations on the resistance of THM-densified wood to degradation by brown-, soft-, white-rot and pyrophillic fungi. Mechanical properties and water-related behaviour of THM-densified wood is described in Chapter 4 and a detailed account on the assessment of the chemical alterations of the cell wall constituents provided in Chapter 5. Chapter 6 provides a synthesis of the main results in relation to the work hypotheses presented in this section. Finally, the conclusions are presented in Chapter 7 together with future objectives and suggestions for further investigations.
Figure 1.1 Schematic structure of the project.
1.3 Anatomy, Chemical Composition and Physical Properties of Wood

Although wood continues to be used for many applications because of its numerous excellent material properties, it also suffers from a number of disadvantages. Dimensional changes in response to altering atmospheric conditions, susceptibility to biological attack and changes in appearance when exposed to weathering restrict the potential use of wood. The properties of wood reflect are governed by its structure at the macroscopic, microscopic and molecular levels. This chapter gives a brief overview of the structure and chemical composition of wood.

1.3.1 Wood Structure

The wood structure of gymnosperms is relatively homogenous. The xylem consists mainly of tracheids and, to a small extend, of parenchyma cells. The latter are generally arranged as uniseriate xylem rays aligned radially in the wood. In addition to ray parenchyma, axially aligned parenchyma cells and resin canals surrounded by epithelial cells also occur in the wood of various genera (Fig. 1.2). Tracheids are elongated dead cells, which provide water conduction and strength and support i.e. have a dual function in the wood of conifers.

Figure 1.2 Types of cells present in hardwoods and softwoods (Encyclopaedia Britannica, 2007).
The wood of more evolved angiosperms has a more heterogeneous structure and cells show a functional differentiation. Vessels provide water conduction and fibres strength and support, whereas parenchyma cells store, convert and transport nutrients. The different cell types are often distinguished by macroscopic features (Fig. 1.2). This applied, in particular to angiospermous wood which may possess broad multiseriate xylem rays and/or a characteristic arrangement and concentration of vessels or axial parenchyma (Schwarze et al., 2000).

Kerr and Bailey (1934) proposed a cell wall model distinguishing between five different cell-wall layers. These five layers are the middle lamella, the primary wall and a three-layered secondary wall (Fig. 1.3).

1.3.1.1 Middle lamella

In all plant tissues, neighbouring cell elements are attached by a middle lamella to another. The middle lamella is largely isotropic and appears homogenous under the scanning electron microscope, as it consists largely of amorphous, i.e. shapeless substances like pectin and lignin (matrix) (Fig. 1.3). Because of its amorphous structure and the lack of cellulose, it does not exhibit birefringence under polarized light, and appears dark. Pectin is a constituent of the middle lamella and as a cementing substance (ultra adhesive) it has an important task of connecting neighbouring cells to one another. Pectin is a high polymer substance which contains building blocks of galacturonic acid molecules with carboxyl groups partially esterified by methanol. The thickness of the middle lamella ranges from a few tenths of a micrometer up to 5 µm in the cell-wall corners. For various reasons the middle lamella plus the primary wall is also called the compound middle lamella. The mechanical properties of the compound middle lamella provide the compression strength and stiffness of the cell wall. The higher compression strength derives from smaller proportion of microfibrils laid down in the compound middle lamella and with the associated higher lignin concentrations. During maceration, i.e. the chemical dissolution of the middle lamella, individual cells in the wood tissue are separated from each other. Thus the mechanical properties of the wood are altered. An identical effect can also be induced by the enzymatic action of fungal hyphae. Selective delignification by particular fungal species is repeatedly observed in nature. In contrast, other fungi either do not break lignin down, or do so only together with hemicelluloses and cellulose, depending on different enzyme systems, and the conditions in a particular host.
1.3.1.2 PRIMARY WALL

As already described, the primary wall is difficult to distinguish from the adjacent middle lamella under a light microscope or an electron microscope, and therefore it is evaluated jointly with it from a biomechanical standpoint. Like the secondary wall layers, the primary wall exhibits a framework substance of cellulose fibrils besides the matrix. This cell wall layer is characterized by its cellulose forming only ca 2.5 % of the total, and the fibrils run scattered, mainly transversely to the cell axis.

1.3.1.3 SECONDARY WALL

The secondary wall forms the largest layer of the cell wall. It is immediately attached to the compound middle lamella and it demarcates cell wall from the cell lumen. In contrast to the compound middle lamella, cellulose makes up 94% (Fig. 1.3) of the defining chemical substances (Fengel and Wegener, 1984). Its biomechanical function is primarily to provide high tensile strength to the cell wall. The secondary wall has prominent layers, usually in the form of an outer (S₁), middle (S₂), and an inner (S₃) secondary wall (Fig.1.3). Both the thickness of each layer and the arrangement of its cellulose fibrils differ as follows.
The outer secondary wall (S₁ layer) lies next to the primary wall. Its cellulose fibrils exhibit a weak parallel arrangement, being oriented approximately transversely to the longitudinal axis of the cell.

The central secondary wall (S₂ layer) is several micrometers thick, is the thickest wall layer and it forms the bulk of the cell wall. In spruce tracheids it is between 1 and 5 μm thick and thus makes up 74-84% of the whole cell wall. The fibrils run parallel to another at a low angle (parallel helical arrangement) to the direction of the cell wall axis. Thus, individual lamella composed of cellulose; lignin and hemicelluloses follow one another, together forming the S₂ layer. The cell wall, thus consist of concentrically arranged lamella. From the high content of cellulose of the S₂ layer of the cell wall, it is clear that it provides tensile strength to the wood (Fig. 1.4). Also, the S₂ layer, being very rich in carbohydrates (cellulose), is preferentially broken down by brown-rot and soft-rot fungi. It is brown-rot fungi especially that destroy the cellulose during initial stages of degradation. This has the consequence that even during incipient stages of decay a drastic reduction in wood strength can be measured.

The inner secondary wall (S₃ layer) separates the cell wall from the lumen (Fig. 1.3). It is relatively thin, and most cell-wall researchers believe it consists only of a single lamella (Jayme and Fengel, 1961). The microfibrils are arranged either parallel or slightly scattered, the texture resembling that of the primary wall. On the basis of its chemical composition, the S₃ layer of tracheids certainly occupies a special position within a secondary wall. It has a lower concentration of cellulose than the S₂ layer and in gymnospermous wood it exhibits a higher degree of lignification. Moreover, especially in the tracheids of conifers, it shows a high re-

![Diagram of cell wall layers](image-url)
sistance against to certain decay types e.g. simultaneous rot. This becomes particularly apparent for wood decay fungi that cause a simultaneous rot and thus degrade the cell wall in the immediate vicinity of their hyphae. If such hyphae grow in the cell lumen of tracheids then they will automatically come into contact with the resilient S₃ layer, which hampers degradation. The selective pressure that is exerted is so strong that fungal species that cause a simultaneous rot are very rarely recorded in wood of conifers. In contrast brown-rot fungi have adapted better to the conifer wood substrate. Admittedly the hyphae of the brown rot fungi are also incapable of degrading the resilient S₃ layer directly, but it is penetrated by low molecular weight substances so that the regions of the secondary wall lying beyond it (S₂) are preferentially degraded. Only in some exceptional cases a S₃ layer is not formed, for example in certain types of tension wood fibres in angiospermous wood and in compression wood of conifers.

1.3.2 WOOD CHEMISTRY

For the interpretation of alterations in the physical and chemical structure of wood during the process of THM-densification a good basic knowledge of its chemical composition, structure and physical properties are required. The chemical composition of the cell wall plays a key role in the thermally activated chemical processes softening and degradation of cell wall constituents have an important influence on the compression of solid wood.

There are two major chemical components in wood: lignin (18 - 35 %) and carbohydrates (65 - 76 %). Both are complex polymeric materials. Minor amounts of extraneous materials, mostly in the form of organic extractives and inorganic minerals (ash) also consist in wood (usually 4 - 10%). Overall, wood has an elemental composition of approx. 50% carbon, 6% hydrogen, 44% oxygen and trace amounts of several metal ions (Pettersen, 1984).

1.3.2.1 CELLULOSE

Cellulose (C₆H₁₀O₅)ₙ is a high molecular and well defined polymer composed purely of anhydroglucopyranose linked to each other by β-1,4-glycosidic bonds. Therefore cellulose can be described as a linear polymer glucan with a uniform chain structure. Each molecule contains from 800 to 1500 glucose monomers depending on their origin. The cellulose chains are 0.25 to 5 µm long. Accordingly, the molecular weight varies widely (5 x 10⁴ – 2 x 10⁶ g/mole). The type of bonding causes the molecules to be flat and ribbon-like and it allows the formation of internal hydrogen bonds which stabilise the molecule. The molecules are situated par-
allel to each other and linked with hydrogen bonds laterally. They can crystallise and produce aggregates called microfibrils. According to the rosette theory (Giddings et al., 1980) each microfibril contains 36 chains, lying side by side. 40-50% of the mass of wood consists of cellulose.

Cellulose microfibrils are partly crystalline and partly amorphous with a degree of approx. 60%. The exact nature of the arrangement of glucan chains within the microfibrils is still a point of controversy and several models have been suggested. The models differ mainly in the presentation of the less ordered amorphous cell wall regions (Fengel and Wegener, 1984). The mode of synthesis is though to be the basis of the crystallisation: complexes of cellulose-synthesizing enzymes are embedded in the plasmalemma in the form of rosettes (Giddings et al., 1980).

The bonding within the cellulose and in the microfibril is of relevance for many of the properties of wood. Covalent bonding both within the glucose rings and between the rings to form the chains contribute to the high axial tensile strength of wood. Laterally within a microfibril the cellulose chains are bonded by hydrogen bonds and van der Waals forces.

Due to the non-branched nature and high degree of crystallinity, cellulose is insoluble in most solvents. Swelling of cellulose may however occur, which in the case of most popular solvents results in hydrogen bonding of solvent molecules between the cellulose chains. The swelling is mostly highly dependant on temperature (Niemz, 1993; Navi and Heger, 2005). Water can be bound by hydrogen bonds to the hydroxyl groups on the surfaces of microfibrils but it cannot penetrate the crystalline cellulose, since the hygroscopic hydroxyl groups are mutually stabilized by hydrogen bonds within this region (Zimmermann et al., 2004).

1.3.2.2 Hemicelluloses

Hemicelluloses are non-cellulotic polysaccharides with a low degree of crystallization. Contrary to cellulose, hemicelluloses are often branched polysaccharides. In addition they usually have much smaller molecular chains attaining a degree of polymerisation values in the range of only 200. Large deviations between hemicelluloses in softwood and hardwood are recognized. However, in both softwood and hardwood the hemicelluloses amount to approximately 20-30% of the dry wood weight.

Hemicelluloses are composed of various sugar units. These sugar units can be subdivided into groups such as pentoses, hexoses, hexuronic acids and deoxy-hexoses. The main proportion
of hemicellulose in angiospermous wood is a xylan of the type 0-acetyl-4-0-methylglucorono-β- xylan. The sugar units often consist of side groups of this main chain (Dinwoodie, 2000). Branched polymers contain neutral and/or acidic acid groups. These groups render hemicelluloses noncrystalline or poorly crystalline, so that they exist more like a gel than as oriented fibres (Watanabe et al., 1989). The OH-groups of the xylan main chain are partially acetylated. The 4-O-methylglucuronic acid residues are linked to the main chain by α-1,2-linkages. On average the hardwood xylans contain one uronic for each of the xylose residues, however, the distribution of acidic residues is found to be highly uneven (Fengel and Wegener, 1984).

Due to the heterogeneous and branched nature with many side groups, hemicelluloses form a predominantly amorphous substance with easy access to OH-groups and other reactive groups. Hemicelluloses are therefore relatively hydrophilic and sensitive to thermal degradation (Shiraishi and Hon, 2001).

### 1.3.2.3 Lignin

Lignin is a three dimensional molecular polymer mainly consisting of phenylpropane units which dictate a completely amorphous and highly cross-linked network. Due to its cross-linked nature lignin is quite stable and relatively hydrophobic compared to cellulose and hemicelluloses. Taking the cell wall as a whole, the typical composition is approx. 50% cellulose (of the dry weight of wood), 25 % lignin, 20-25 % hemicelluloses and 1-4 % pectin (Donaldson, 2001).

The lignin structure cannot be described by a simple combination of one or few monomeric units or by one or few types of linkages. Therefore the lignin structure is still a matter of controversy (Adler, 1977), several models have been suggested. The structural concept of softwood lignin is illustrated in Figure 1.5 (Brunow, 1998).
The nature of the lignin itself also has a great influence on wood decomposition of fungi, as lignin is not a well defined chemical substance but rather a heterogeneous class of compounds. Lignins are polymers of phenylpropene units: guaiacyl (G) units from the precursor *trans*-coniferyl-alcohol, syringyl (S) units from *trans*-sinapyl-alcohol, and p-hydroxyphenyl (H) units from the precursor *trans*-p- coumaryl alcohol. The exact composition of lignin varies greatly within species. Gymnospermous lignin consists almost exclusively of guaiacyl monomers, whereas angiospermous lignin consists of approximately equal ratios of guaiacyl and syringyl (Whetten and Seredoff, 1995). For Norway spruce (*Picea abies*) a ratio G:S:H = 94:1:5 has been reported and for pine (*Pinus taeda*) G:S:H = 86:2:13 (Erickson et al., 1973). Knowledge of the lignin composition of different cell types of wood allows interpretation of degradation modes and facilitates the prediction whether the xylem of a specific host is resistant or susceptible to a certain decay type (Obst et al., 1994; Schwarze, 2007).

**1.3.2.4 LIGNIN-POLYSACCHARIDE LINKAGES**

An element contributing to the complex structure of lignin is the existence of lignin-polysaccharide linkages. True covalent bonds between lignin and the polysaccharides have been detected in many wood species. In addition multiple secondary interactions (hydrogen bonding, van der Waals forces etc.) exist. The compounds formed by the covalent linkages are referred to as LCC’s (Lignin-carbohydrate complex) or LPC’s (Lignin-polysaccharide complex). The polysaccharides involved may be both hemicelluloses as well as cellulose, yet the former seems to be by far more abundant (Fengel and Wegener, 1984).
The results obtained by Eriksson (Eriksson and Goring, 1980) indicate that lignin is bound to all kinds of sugar units in the hemicelluloses. Linkages to the side chain units seem to be the rule. The most dominant types of LPC’s are believed to be ether linkages, ester linkages and glycosidic-like linkages. Direct evidence of the latter has not yet been proven. The linkages between lignin and polysaccharides have presumably a high impact on the compressive behaviour particularly on the processes of softening and degradation.

**1.3.2.5 Extractives**

The extraneous components of wood (extractives and ash) are the substances other than cellulose, hemicelluloses and lignin. They do not contribute to the cell wall structure and are mostly soluble in neutral solvents (Pettersen, 1984). Extractives are the varieties of organic compounds including lipids, waxes, alkaloids, proteins, simple and complex phenolics, simple sugars, pectin, mucilage, gums, resins, terpenes, starches, glycosides, saponins and essential oils (Shiraishi and Hon, 2001). Many of these function as intermediates in tree metabolism, as energy reserves or as part of the tree’s defence mechanism against microbial attack. They contribute to wood properties such as colour, odour and decay resistance.

Pectin is a common name for a group of amorphous polymers that exist in plant cell walls, especially in the middle lamella between xylem cells. Pectic polymers rich in galacturonic acid are structurally important especially during cell wall formation. They exist in nature both in a methylesterified and in a free acidic form. Polymers also contains neutral sugars, notably D-galactose, L-arabinose, L-rhamnose, D-xylose, L-fucose and D-apiose (Aspinall, 1981). Pectin in form of a calcium salt forms crosslinks that affect the structural properties of the amorphous matrix. The content of galacturonans is less then 1% in lignified soft- and hardwoods (Fengel and Wegener, 1984) Wood decay fungi can utilize pectin as a carbon source by producing pectinolytic enzymes.

Pectin is a good chelator for Ca$^{2+}$ and acts as a selective binder for Ca$^{2+}$ ions in non-lignified tissue. One key to pectin hydrolysis by plant pathogens has been shown to be fungal production of oxalic acid, which lowers the pH of substrate and chelates calcium ions. Production of oxalic acid may serve a similar role during incipient wood decay as calcium oxalate has been found by light- and scanning electron microscopy, during both brown- and white rot decay (Green III et al., 1996; Green III and Clausen, 1999; Schwarze et al., 2006)
1.3.3 Wood Physical Characteristics

1.3.3.1 Orthotropic Nature of Wood Properties

The cellular structure of wood and the physical organization of the cellulose chain within the cell wall affect the physical and mechanical properties of wood depending on the direction of loading. Wood may be described as an orthotropic material; i.e. it has unique and independent mechanical properties in the directions of three mutually perpendicular axes (longitudinal, radial and tangential) (Niemz, 1993). The properties of wood parallel to the grain are higher than perpendicular to the grain, since the grain direction is also the direction of the primary bonds of major chemical constituents of the cell wall.

1.3.3.2 Hygroscopicity

Wood can absorb water as a liquid, if in contact with it, or as vapour from the surrounding atmosphere. The chemical nature of the wood substance particularly that of polysaccharides, renders wood cell walls hygroscopic (or hydrophylllic) (Siau, 1984). The hydroxyl groups of cellulose and hemicellulose molecules are responsible for its great affinity to water and have a very strong tendency to form hydrogen bonds. Lignin, on the other hand, possesses comparatively few free hydroxyls and as a result is much less hygroscopic. In an atmospheric environment containing moisture, dry wood will absorb moisture until equilibrium with the surrounding atmosphere exists. Similarly, saturated wood, when placed in an atmosphere of lower relative humidity (RH), will lose moisture until equilibrium is attained (Wolcott and Shutler, 2003).

Hygroscopicity is of primary importance because moisture in wood affects all wood properties. For example, moisture content can increase by 100% or more of the dry weight affecting eg. transport costs of timber. Variation in moisture content causes wood to shrink or swell, altering its dimensions. Resistance to decay and insects is also greatly affected. The working, gluing, and finishing of wood and its mechanical, thermal, and acoustic properties are all influenced by the moisture content. Processing operations, such as drying, preservative treatment, and pulping are also affected (Muszynski et al., 2005).

The moisture properties of wood result from both its chemical constituents and the capillary nature of the amorphous zones of the microfibrils that make up the cell wall. Water may occur in wood in liquid or vapour phases in the lumina of the cells and pit cavities. Water may also occur as bound (hydrogen bonding) water on the surfaces, within or between the microfibrils.
in the amorphous zones. The zone between microfibrils is termed *transient capillary system* since it contracts when wood is dried and expands when wood is moistened. Changing moisture levels in this zone affect many wood properties such as strength, swelling and shrinking, and electrical conductivity. This zone is also the point where fungal enzymes access inside the cell wall and initiate decay. The water availability is influencing the degradation processes to a large extend and is therefore the most important factor in wood protection. The fungi need water in order to take up the nutrients and for transport processes within mycelium. To grow effectively in wood, fungi need some free water present in the cell lumen. This means that fungi are unable to grow effectively in wood below the fibre-saturation point, which varies within different wood species but an average value of 28 to 30% is generally assumed (Niemz, 1993; Schmidt, 2006).

**1.3.3.3 Dimensional Instability of Wood**

Wood undergoes dimensional changes when its moisture sinks below the fibre saturation point. Loss of moisture results in shrinkage, and gain in swelling. It is characteristic that these dimensional changes are anisotropic i.e. different in axial, radial, and tangential directions. Average values for shrinkage vary strongly for different wood species but are assumed to be approx. 0.4 %, 4 % and 8 %, respectively (Kollmann and Côté, 1995). Shrinkage in volume is approx. 12 %, but large variations are exhibited among wood species. These values refer to changes from the green to oven-dry condition and are expressed in percentage of green wood.

![Figure 1.6 Distortions of the sawn wood due to shrinkage and swelling (Encyclopaedia Britannica, 2007).](image-url)
dimensions. The differential shrinkage and swelling in different growth directions are attributed mainly to the cell wall structure. The difference between axial and the two lateral (radial and tangential) directions can be explained on the basis of respective orientation of microfibrils in the layers of the secondary cell wall, but the reasons for the differences between radial and tangential directions are not well understood.

In general, the factors that affect shrinkage and swelling are moisture content, density, content of extractives and abnormalities in wood structure (Mantanis et al., 1994). The amount of shrinkage or swelling that occurs is approximately proportional to the alterations in moisture content. The higher the wood density, the greater is its shrinkage and swelling, because denser (heavier) woods contain more hemicelluloses (substance which is mainly responsible for shrinkage/swelling). Extractives reduce EMC as well as shrinkage and swelling because they occupy spaces within cell walls that otherwise could filled with water (Popper et al. 2006).

Mechanical stresses (compression or tension) may cause permanent deformation of wood cells, which in turn affects shrinkage and swelling (Skaar, 1972).

Dimensional changes in wood caused by shrinkage and swelling can result in opening or sealing of joints, changes in cross-sectional shape, warping, checking (formation of cracks), case-hardening (release of stresses in resawing or during other processing with consequent warping) (Fig. 1.6). Thus, the fact that wood shrinks and swells causes a great problem for the utilization of wood and many attempts have been made over the years to find solutions for this problem. Methods described in the literature can be classified in one of the following five groups:

1. Laminating of anisotropic sheets (reconstruction into such products as plywood, particleboard, and fibreboard) (Kollmann and Côté, 1995).
2. Applying water repellent surface and internal coatings to retard moisture adsorption or moisture loss (Williams and Feist, 1999).
4. Bulking the fibres with chemicals (salts, sugars, polyethylene glycol, synthetic resins, or other substances), thus reducing the moisture uptake capacity of wood (Militz et al., 1997).
5. Cross-linking the cellulose chains of the fibres so that their separation by water is minimized (Dwianto et al., 1996).
1.4 Types of Wood Decay

Wood decay fungi are conventionally classified into three main groups i.e. brown-, white- and soft rot fungi each possessing its own degradation pattern (Schwarze et al., 2000).

1.4.1 Brown Rot

Brown rot is a type of wood decay caused exclusively by fungi of the Basidiomycetes. This class contains many families, though the overwhelming majority of the brown-rot fungi belong to the family Polyporaceae. Interestingly, only 6% of all the known wood decay fungi are now known to cause a brown rot. Moreover they are mainly associated with gymnosperms, whereas white rot fungi are associated with angiosperms (Gilbertson, 1980).

Brown rot fungi are characterized by their extensive and rapid depolymerisation of cellulose leading to a rapid loss in wood strength at early stages of decay. Of all wood decay types brown rot fungi are generally considered to be the most important decay type for wood in-service. Brown rot is normally characterized by an excessive removal of cellulose and hemicelluloses and although demethylation of lignin occurs (degree dependant on fungal species) lignin normally persists as a weak amorphous residual skeleton which easily fractures cubically and crumbles into powder when dry. The modified lignin remaining gives the decayed wood its characteristic colour and consistency. The degradation of cellulose and hemicelluloses takes place at different stages (Fig. 1.7). It is assumed that hydrogen peroxide is probably formed in a pre-cellulototic phase, and easily penetrates the cell wall and together with iron ions, overcomes the lignocellulose matrix by oxidative depolymerisation. This assumption seems necessary, as cellulose-decomposing enzymes are relatively large and much smaller cell-wall capillaries cannot be simply penetrated without further ado. Clearly, the hemicelluloses surrounding the cellulose are also affected, so that the cellulose then becomes accessible for cellulases. This is followed by indiscriminate cleaving of the

Figure 1.7 Development stages of brown rot (Schwarze et al., 2000).
cellulose chain molecules (starting at many places), quickly forming many individual cellulose chain fragments. The combination of the preferential and indiscriminate degradation of cellulose is closely associated with a drastic loss of bending strength after only a very short period of degradation. According to latest studies by (Green III et al., 1992) this strength loss is mainly attributed to degradation of hemicellulose, because far-reaching cellulose degradation could not be determined at the very early stages of wood decay.

1.4.2 Soft rot

The term “soft-rot” is used to describe a very specific type of decay caused by a large number of Ascomycetes and Deuteromycetes which typically produce chains of cavities with conical ends within the S2 cell wall layers of soft- and hardwoods exposed to terrestrial and aquatic environments. The term was originally used by Savory to describe the characteristic softening of wood by cellulose-destroying microfungi and to distinguish it from decay caused by white- and brown rot fungi (Savory, 1954). Although softening of wet wood is typical, studies on the soft-rot attack of CCA (copper-chromium-arsenic) treated timber has shown degraded wood to be hard, nevertheless the term is widely accepted (Courtois, 1963; Liese, 1964; Rayner and Boddy, 1988). Economically soft rot attack is of considerable importance since it causes great damage to wooden constructions and preservative treated wood, especially in ground contact. Although cavity formation remains the most characteristic feature for the microscopic detection of soft rot, many of the fungi involved also produce cell wall erosion similar to simultaneous white rot particularly in hardwoods. Cavity formation and cell wall erosion are normally referred as Types 1 and 2 respectively (Schwarze et al., 2000).
Considerable research has been directed towards understanding the manner of cavity formation within the S<sub>2</sub> cell walls of fibres (Fig. 1.8). Studies on the mechanism of soft rot attack have shown the involvement of specialized microhyphae (0.3 - 0.4 µm thick) which traverse the S<sub>2</sub> layer from the wood cell lumen and then reorientate along the cellulose microfibrils by characteristic T-branching (growth in two directions) or L-bending (growth in one direction). In fact the characteristic alignment of soft rot cavities parallel to the axis of the cellulose microfibrils is well-defined and can be used as a precise means for determining the microfibril angle of individual cell wall layers in wood fibres cite authors. Direct penetration of cell walls as described for blue stain fungi may also occur. Following growth of the microhyphae, T-branching or L-bending occurs over a finite distance, and cavities then develop around the hyphae by the release of enzymes (putatively endocellulase) along what is then described as a proboscis hyphae. After cavity formation - size, shape and form dependant on wood species in fungal enzymes – the hyphae produce further microhyphae and the sequence is repeated citation. Particularly, the mechanism of cavity formation in relation to the cellulose structure of the cell wall and T-branching/L-bending has fascinated scientists for years although the process remains poorly understood. An understanding of the mechanism of T-branching and L-bending could lead to methods to inhibit this process, preventing cavity formation and resulting in simple cell penetration similar to that of blue stain fungi with less economic consequence. Continuous enlargements of existing cavities and the formation of new cavities normally leads to the total destruction of the S<sub>2</sub> layer.

While the middle lamella and S<sub>3</sub> layers appear easily penetrated by microhyphae, the former is not degraded by soft rot fungi and the S<sub>3</sub> is only poorly attacked in softwoods. Both experience this limited attack because of the thickness of the layer and lignin content. SEM and TEM observations on cavity hyphae show the hyphae normally associated with a variety of granular and fibrillar materials including extracellular slime, melanin and lignin break down products. These residual materials remain even after death and lysis of cavity hyphae forming a skeleton in a highly degraded S<sub>2</sub> cell wall matrix. Soft rot erosion (Type 2) causes a characteristic thinning of wood fibre walls from the cell lumen similar to that caused by white rot decay and higher ascomycete fungi like *Hypoxylon, Xylaria, Daldinia* (Rayner and Boddy, 1988; Zabel and Morrell, 1992).

Possibly the most important factor affecting the micromorphology of soft-rot attack is the type and concentration of lignin. Soft rot fungi generally produce greater decay in hard – than in softwoods which is thought to be related to the higher lignin content and guaiacyl type of
lignin in softwoods compared to the lower and syringyl-guaiacyl nature of hardwood lignin (Liese, 1961; Nilsson et al., 1989; Schwarze et al., 1995; Schwarze et al., 2004). Soft rot susceptibility appears inversely correlated with lignin content. As described above, the structure of wood cell walls and particularly the molecular arrangement of the cellulose and hemicelluloses and its protection by ligning probably “regulates” the decay process. Just as the high lignin containing middle lamella layers are not degraded by soft rot fungi, high lignin containing thin concentric layers within the $S_2$ layers of polylaminate cell walls are initially also poorly attacked.

1.4.3 White rot

White rots are caused by basidiomycetes and by certain ascomycetes. The common feature of all these fungi is that they can degrade lignin as well as cellulose and hemicelluloses. However, the relative rates of degradation of lignin and cellulose vary greatly according to the species of fungi and the conditions within the wood. As with brown rots, there is additional variation related to the preferential decay of different zones within the annual ring. The adaptation of white rot fungi to the much more heterogeneous structured wood of angiosperms, plus their ability to degrade all the cell wall constituents extensively, leads to a multiple patterns of wood decay. Within this range of variation, two broad divisions are widely accepted: selective delignification and simultaneous rot (Rayner and Boddy, 1988; Zabel and Morrell, 1992). White rot fungi degrade lignin by oxidative processes, which involve phenoloxidases such as laccase, tyrosinase and peroxidase. They degrade cellulose in a less drastic way than brown rot fungi, since their cellulolytic enzymes attack the molecules only from the ends, splitting off glucose or cellobiose units.

1.4.3.1 Selective delignification

In selective delignification, at the early stage of decay, lignin is broken down more than hemicelluloses or cellulose. The hyphae grow in the cell lumina in some cases, so that the lignin is dissolved out of the adjacent cell wall (Fig. 1.9). In other cases, hyphae penetrate the cells and initially delignify the middle lamella so that the cells tend to separate. As indicated above, cellulose is left relatively unaltered during selective delignification, at least during early stages of decay. The resulting residual material is stringy in texture, having lost much of its stiffness and hardness while retaining considerable tensile strength (Schwarze et al., 1995). This contrasts very much with brown rots in which cellulose is removed while lignin remains, leaving a very brittle residue. White rot fungi degrade lignin by oxidative processes by means
of phenoloxidases formed and released by the hyphae, such as laccase, tyrosinase and peroxidase. Cellulose is broken down more slowly than in brown rot, so that reduction in wood strength properties is less drastic. One reason for this could be the fact that glucose or cellobiose is split off only from the ends of the cellulose chain molecules and thus large coherent cellulose fibrils are preserved longer. Another reason could be longer persistence of radial structures in the S₂ layer which contributes to wood strength properties (Schwarze and Engels, 1998). As mentioned, the cellulose remains relatively unaltered during selective delignification, at least during early stages. Due to dissolution of lignin-rich middle lamella and the separation of individual cell elements from their matrix, the consistency of the decayed wood becomes increasingly fibrous or stringy and it loses stiffness and compression strength, although its toughness persists at first.

1.4.3.2 SIMULTANEOUS ROT

Fungi causing a simultaneous rot comprise a large group of species which occur commonly in angiosperm, but only rarely in gymnospermous wood. One reason for this selection process may be related to the extremely resilient S₃ layer of tracheids that hampers degradation by hyphae from within the cell lumen outwards. By contrast, enzymes in brown rot and selective delignification, low molecular weight substances, simply diffuse through the S₃-layer into the secondary wall (Figure 1.10). The S₃ layer of conifer tracheids is more resistant to degradation than that of the wood fibres in angiosperms. This does not deter brown rot fungi, which are able to degrade the cell wall by means of diffusible secretions from hyphae within the tracheid lumen, but a resistant S₃ layer is a considerable barrier to fungi that cause a simultaneous rot. This may account for the fact that this type of white rot fungi occurs mainly in angiospermous wood, while brown rot fungi predominate in gymnospermous wood.
In simultaneous rot, lignin and structural polysaccharides, including cellulose, are degraded at similar rates by enzymes secreted by hyphae growing on the $S_3$ layer in the cell lumen. This form of degradation takes place close to the hyphae involved, and results in the formation of erosion troughs (Fig. 1.10). The enzymes that they secrete decompose all cell wall constituents of the lignified cell wall (Eriksson et al., 1990). As the degradation of cellulose, hemicellulose, and lignin occurs at nearly the same rate, the term is appropriate, although the general term white rot is often applied. As mentioned above, simultaneous rot occurs by the erosion of troughs where hyphae grow on the cell wall, in contrast to the general dissolution of cellulose within the cell wall that occurs in brown rots. The coalescence of the erosion troughs induced by numerous hyphae results in a general cell wall thinning from the lumen outwards (Liese, 1970; Schwarze et al., 1995).

### 1.4.4 Pyrophillic fungi

Many boreal organisms are associated with fire including special species of fungi (Esseen et al., 1992). The specific set of soil dwelling discomysetes that characterise fungal community after fire is well presented in the literature, whereas only few studies have been devoted to the effect of forest fires on wood inhabiting fungi (Penttilä and Kotiranta, 1996). The post-fire fungus *Daldinia loculata* (Lév.), a member of the xerophytic genus *Daldinia* is a central species utilising post fire habitats since it is associated with the fauna of rare pyrophilous insect species (Dahlberg, 2002). As mentioned by (Johannesson, 2000), several *Daldinia* species are documented to exhibit a preference for burned woody substrate (Ju et al., 1997) and therefore may be termed as anthracophilous (literally, “coal-loving”), pyrophilous (“fire-loving”), py-
rophillic, carbonicolus ("coal-inhabiting"), phoenicoid or pyroxyphilous fungi (Johannesson, 2001; Dahlberg, 2002; Mueller et al., 2004; Maheshwari, 2005).

According to Rogers (1979), *Daldinia* sp. cause a white rot decay employing enzymatic systems that degrade cellulose and lignin. *Daldinia* is reported to be a rapid wood decomposer i.e. after eight weeks incubation it has been shown to induce weight losses of 62.9% in sapwood blocks of birch (Nilsson et al., 1989). Wood decayed by *Daldinia loculata* characteristically has a gross appearance showing concentric dark speckled wood zones, resulting from the dark coloured mycelium growing in the early wood vessels, a phenomenon also known as Calico wood (Panisset, 1929). At advanced stages of decomposition, the wood exhibits a patchy appearance as some localised regions are stronger decayed than others (Boddy et al., 1985).

### 1.4.5 Decay Susceptibility

Studies on wood preservation and wood decay often involve laboratory decay experiments. The results are almost always expressed in percent dry weight loss. As suggested by Nilsson and Daniel (1992), the percent weight loss provides a useful measure of the amount of the decay put problems may arise when comparisons are being made between timbers of varying density. Wood is structurally a very variable tissue and structural variations are reflected in variations in density. Extractives may also contribute to density. Since most laboratory experiments are conducted with wood blocks of a certain size, and in most cases a uniform size, density of a particular specimen will determine the actual weight of the wood blocks. Percent weight loss is calculated from the simple formula (Eq. 2.1). It is obvious that \( W_0 \) will be determined by the size and the density of the wood specimens used in experiments. \((W_0-W_1)\) represents the weight of the wood substance that has been degraded by wood decay organism. By using the wood blocks of a certain size, decay activity will be limited to a predetermined volume of wood. This may be regarded as a special case of microbial growth in a closed environment. All the main nutrients are present in the form of water-insoluble structural wood components, i.e. cellulose, hemicelluloses embedded in lignin.

If we take a wood specimen, the amount wood substance consumed by the fungus \((W_0-W_1)\), will be determined by 1) volume of the wood block and 2) the decay rate. If a uniform block size is used, \((W_0-W_1)\) will be determined solely by the decay rate. Since \((W_0-W_1)\) represents
actual absolute weight loss for a given volume (g/cm$^3$), this appears to provide a good measure of decay rate during the time of exposure. There are no problems converting weight loss/volume figures to percent weight loss (WL%) if the wood blocks are of uniform size and density. The problems arise when comparisons are required for wood blocks of the same size but varying density. Here it is obvious that $W_0$ is determined by the density of specimen, i.e. high density specimens weigh more than low density ones. It is obvious that if $W_0$ is high and $(W_0-W_1)$ is constant, that high density specimens are more durable than low density ones simply because more wood substrate is present at the beginning of the decay process.

Assessment of decay rates of various timbers provide information on decay susceptibility or as proposed earlier by Butcher and Nilsson (1982) decay potential. It is calculated using Eq. 2.2. The results are expressed in g/cm$^3$ and support the point of view that the amount of wood consumed per unit volume provides a better assessment of decay organism degrading activity than percent weight loss when comparisons are required for wood blocks of varying density.

1.5 Wood modification

Due to environmental concerns regarding the use of certain types of preservatives, there has recently been a renewed interest in wood modification. Wood modification represents a process that is used to improve the material properties of wood, but produces a material that can be disposed of at the end of a product life cycle without presenting an environmental hazard. Modification of wood can involve active modifications, which result in a change of the chemical nature of the wood substrate or passive modifications (Hill, 2006), where a change in properties is affected but without an alteration in the chemistry of the material.

The concept of chemical modification of wood was developed primarily to improve its dimensional stability. Improvements in physical and mechanical properties, enhancement of durability against physical, chemical and biological degradation are a further advantage of such treatments. These treatments range from simple application of heat after impregnation with monomers for in situ polymerization or alteration of chemical composition of wood by chemical reactions (Fig. 1.11). Wood treated with conventional wood preservatives like creosote, pentachlorphenol, copper-chrome-arsenic (CCA) and fire retardants does not fall under the strict definition of chemically modified wood (Kumar, 1994). The chemical modification treatments can be classified broadly into two different types:
1. Chemical modification of the cell wall.

2. Densification and/or resin impregnation.

1.5.1 Chemical modification of the cell wall

Dimensional movement as well as biological degradation of wood has been attributed to the presence of numerous hydroxyl groups in various wood components. Blocking these sites by chemical substances causes swelling of the wood and does not only eliminate the moisture adsorption sites but also prevents the highly specific enzymatic reactions. A number of reagents capable of forming stable covalent bonds have been studied to substitute the reactive hydroxyl groups. The major types of linkages formed by reaction with wood are ether, acetyl, ester, etc. Chemical investigations on stable chemical bonds have involved alkyl or acid chlorides, anhydrides, carboxylic acids, epoxides, isocyanates, lactones, nitriles, etc. Since the protection induced is related to alterations in the chemical nature of wood, neither the reacting chemicals nor the wood should be toxic. Nevertheless, chemicals that react readily with wood hydroxyls can easily react with wood and tissue hydroxyls and thus can be hazardous (Rowell, 2006).

Figure 1.11 Modification of cell wall components (adapted from Rapp et al., 2000).
1.5.2 WOOD DENSIFICATION

Compressed wood, Impreg (resin-impregnated wood) and Compreg (resin-impregnated compressed wood) were developed during the first half of the 20th century (Stamm et al., 1946; Stamm and Baechler, 1960). Thereafter, German researchers successfully modified the distinctive feature of wood by compressing it in a hot-press under heat treatment. A new product was manufactured which was given the trade name “Lignostone” (Kollmann et al., 1968). During world war II a similar product was produced in US under the trade name “Staypak” (Seborg et al., 1945). This type of technology still exists in Switzerland, where until recently the company Stahel + König AG manufactured densified wood for textile shutters. The process is simple: wet wood samples with a moisture content not exceeding 15% are placed between heated plates of a hydraulic jack and compressed with a force of up to 245.2 bar in the radial direction. The temperature increases to approximately 140°C during a treatment period of two hours. This process is an open system and no control of the wood moisture content is possible during processing. This treatment method undoubtedly improves certain mechanical and physical properties of wood but the transformed shape (compression deformation) produced during densification is unstable and it recovers totally or partially after re-moistening and heating. The tendency to recover the original shape has limited the application of compressed products. To maintain permanent fixation, compressed wood must be modified by chemical substances such as maleic acid-glycerol (chemical modification) or impregnated with thermosetting resins as polymer binding agents, or by heating for a long time under high temperature. Since wood is a natural material, thermal degradation occurs when it’s overexposed to thermal treatment. It is also well documented that application of heat during a long period damages the polymeric constituents of wood; hemicelluloses degrade most rapidly, followed by lignin and then cellulose. A review on the thermal degradation of cell wall constituents has been provided by Fengel and Wegener (1984) and Levan and Windandy (1990).

During production of particle boards and MDF a strong densification takes place. The density of native spruce wood is approx. 450 kg/m$^3$ and the average density of particle boards made of spruce is approx. 700 kg/m$^3$. 
1.5.2.1 Wood Stabilization

One of the main problems associated with most types of densified wood (except those with high resin content) is the lack of dimensional stability. When soaked in water or exposed to high relative humidity, compressed products tend to exhibit irreversible swelling or spring back. This can be a serious problem when densified wood is used in high humidity environments. Thus, it is important to determine pressing conditions under which the recovery from compression for untreated compressed wood is minimized. There have been many studies related to wood stabilization by various treatments. Hillis (1984) reviewed the literature on stabilization of wood by heating process. The effect of steam pre-treatment was investigated by Hsu et al. (1988); Inoue et al. (1993); Inoue et al. (1998). More recently the effect of heat on the dimensional stability of compressed wood was evaluated by Norimoto et al. (1993).

Dwianto et al. (1996) found that preheating had a great influence on the permanent fixation. According to their results, the permanent fixation of compressive deformation in wood resulted from the release of stress stored in microfibrils and the matrix substance of the cell wall due to their degradation.

Hsu et al. (1988) developed a steam pre-treatment process to produce highly dimensionally stable wood based composites. They found that steam pre-treatment caused partial hydrolysis of hemicelluloses for both hard- and softwoods, which greatly increases compression of wood (i.e. reduces the tendency of internal stresses to build up in composites during hot pressing).

Inoue et al. (2000) found that almost complete fixation can be achieved by post-steaming compressed wood for 1min at 200°C or 8min at 180°C. There was a large increase in hardness and only a slight decrease in modulus of elasticity (MOE) and modulus of rupture (MOR). Inoue et al. (1996) also investigated the effect of pre-steaming. They found that the degree of recovery decreases if the compression time and temperature increase. Pre-steaming increases the compressibility of wood and reduces the amount of stored stress due to the viscous flow of wood substances.

Nakata et al. (1992) produced laminated veneer lumber (LVL) by steam injection pressing. They found that MOR and MOE of compressed LVL increased with increasing density. The dimensional stability of LVL was improved considerably. They also proposed a mechanism for fixation of compressive set by steam treatment. They hypothesize that relaxation of the stresses stored in the microfibrils and fixation of the compressive set is due to:

1) rapid hydrolysis of hemicelluloses and partial modification of lignin;
2) partial hydrolysis in cellulose of amorphous and paracrystalline region and reorientation in the crystalline region by steam treatment.

The stabilization of compressive deformation might be a problem in the application of densified wood; however, this problem can be solved by various treatments of wood.

1.5.2.2 Influence of High Temperature and Steam Treatment on Wood

Wood can be characterized as a polymeric cellular material (Wolcott et al., 1994). The properties of cellular materials are influenced by properties of the cell wall and the cellular geometry. The effect of moisture and temperature on the cellular structure is not very significant, whereas properties of the cell wall components are greatly affected by these factors. The dependence of the properties of the cell wall material on the moisture and temperature can be characterized by the viscoelastic behaviour of wood.

Viscoelastic behaviour of wood has been characterized by many studies (Panshin and Zeeuw, 1970; Niemz, 1993). Amorphous polymers of wood (lignin and hemicelluloses) as viscoelastic materials, can behave as viscous fluids and as linear elastic solids, depending on the time, temperature and diluents concentration. For isolated amorphous polymers the transition between the glassy and the rubbery state is defined as a glass transition temperature \( T_g \). Many properties of these polymers, such as the modulus of elasticity, changes dramatically when the material passes this softening point.

The effect of moisture and temperature on individual wood polymers has been investigated in many studies. Moisture has been found to change the glass transition temperature of amorphous polymers. Plasticization of cell wall compounds by water causes a reduction of the energy required to initiate chain mobility (Kelley et al., 1987). Temperature does not have a great effect on mechanical properties when the polymer is below \( T_g \), because molecular motion is limited. However, when the temperature of the polymer reaches \( T_g \), stiffness of the material decreases rapidly. This is attributed to the increase in thermal energy available for molecular motion (Cowie, 2007).

The behaviour of whole wood, however under various conditions can not be explained wholly by the glass transition points of isolated wood polymers, because the chemical and physical nature of isolated components may differ from their original form. Moreover, the combinations between polysaccharides and lignin may influence the behaviour.
Gardner et al. (1993) investigated the changes in polymer structure of wood flakes under hot pressing conditions. The data obtained indicates that an increase in cellulose crystallinity with heat and steam treatment results in an increasing the modulus of elasticity. Increase in crystallinity is attributed to the transition of amorphous polymers from the glassy to the rubbery state, when the increased mobility of lignin and hemicelluloses permits reorientation and crystallization of the cellulose microfibrils. It is also established that low pressing temperatures result in flake strength and stiffness, whereas, high pressing temperatures result in an increase in these properties.

Strength properties are proportional to wood density thus wood with high density is particularly used in circumstances where wear resistance is important e.g. for joineries and wooden floors. The price of such wood is often high and availability is limited. By densifying wood by compression or resin impregnation it is possible to increase strength and wear resistance. After densification, low density woods can be used to substitute harder wood species. Also woods with high density could be improved further in strength and hardness through densification (Blomberg et al., 2005). Thereby they can be competitive for purposes where wood is considered to be too soft e.g. for wood floors in public environments.

Nowadays an extensive work is carried out at the TU Dresden by Haller aimed at shaping of wooden parts by compression (Haller and Wehsener, 2001; Haller, 2004) and using the set recovery effect of compressed wood for obtaining desired shapes (Ziegler and Haller, 2006).

**Panzerholz** (DELIGNIT®) is a commercially available product. Multilaminated material (density 1.35-1.4 g/m³) and is manufactured from beech veneers and synthetic resins under high pressure and heat. It combines metal-like hardness and a high mechanical strength with the advantages of easy workability, low weight and low cost. The main characteristic properties of the material are its high mechanical strength, hardness and wear resistance, relatively low thermal conductivity compared to metals, favourable sliding characteristics, and corrosion proof and resistance to many chemicals. It’s applied as an alternative to metals particularly for tooling, jigs, and moulds; for transformer parts (if low electrical conductivity is required); for liquefied gas tank supports (if low thermal conductivity and high mechanical strength are required); for antimagnetic parts; for sliding surfaces not requiring lubrication as well as for looms in textile industry (if a particular MOE as well as cushioning effect are required).
Calignum is a novel and patented process for wood densification through semi-isostatic compression in a Quintus press (Flow Pressure System, Sweden). Calignum is also the name of the product. In the Quintus press wood is placed on a rigid table and compressed under a flexible oil-filled rubber diaphragm. The process is rapid. Maximum pressure of 140 MPa is reached after approximately two minutes and is then immediately lowered to atmospheric pressure. The wood is compressed at 20°C and preferable moisture content is between 5 and 15%. In the process, the rubber diaphragm surrounds the wood pieces tightly except against the steel table. As pressure increases, the weakest structures collapse in their weakest direction. Harder structures are not crushed or dislocated and can protrude. Consequently the densified wood may have an uneven shape. Once the weak parts of the wood structure have collapsed, further stress causes minor, predominantly elastic strain that will spring back immediately. Because wood is a viscoelastic material, the degree of deformation depends on the duration of load and part of the spring back is delayed (Blomberg and Persson, 2004). Crushing and checking can then be lowered and the structure can be less disturbed than when wood is densified between steel plates. The advantage of the process is that it is rapid; maximum pressure is obtained after approximately 2 minutes and is then immediately lowered to atmospheric pressure. Compression is conducted at 20°C and wood moisture contents range between 5% and 15% (Blomberg et al., 2005).

Wood compression techniques have been also applied by Adachi et al. (2004) to improve the drying behaviour of wood. Transversal compression of wood results in a very rapid removal of water from the compressed lumina and large amounts of water can be removed very quickly. High moisture content flat sawn Japanese cedar (Cryptomeria japonica D. Don) veneer was compressed using a roller press to mechanically remove water. The amount of the water removed depended on the amount of compression applied. At 60% compression, 400 kg/m$^3$ of water was removed. The process was not dependant on the size of the wood, the degree of compression or the feed speed of the specimen. After compression the remaining water contents were evenly distributed throughout the veneer regardless of the length of the specimen. The specimens did not completely recover to original thickness. High compression ratio and low temperature intensified the reduction of thickness. The bending strength after compression decreased in an intensively proportional manner with the thickness of the specimen and the compression degree.
1.5.2.3 **Plasticization**

Favot (1986) invented a method of densifying low quality, low density woods to produce wood products with features of high quality natural hardwoods. A green solid wood specimen with high moisture content is impregnated with anhydrous ammonia to plasticize the wood into a sponge-like form saturated with water and ammonia. This plasticized wood is placed between press plates of a cyclic press and, while maintaining the temperature of the wood below 100°C, it is subjected to a plurality of low pressure compression cycles each of approx. 30 seconds to 1 minute duration in which the wood is compressed to a predetermined thickness of up to 50% less than its original thickness. It is held at the reduced thickness for a short time and released during each cycle, whereby water and ammonia are squeezed out of the wood down to a moisture content of less than 30%. The moist wood specimen of reduced thickness obtained is then a kiln dried, densified solid wood product. This method may also be used to densify wood planks, pre-glued laminates and thin veneers. For veneers, a simpler pressing system and higher temperatures may be used. The wood used in invention has a low quality i.e. low density wood preferably of the hardwood type, typically having a density of about 300-400 kg/m³. Coniferous woods with high resin content are generally not suitable. Particularly useful woods for the process of this invention, poplar, alder, cottonwood, rubber
tree and soft maple can be classified. With some woods, such as poplar, it is preferable to use sapwood only and to neglect heartwood (Favot, 1986).

Bending wood by employing the ammonia plasticization technique in manufacturing bent wood articles like walking sticks, stairs, trays, peg tables etc is a very promising area for the better utilization of rubber wood. Rubber wood can also be used for producing various suitable curved components for the manufacturing of furniture, doors and other fancy, ornamental and utility items. Anhydrous ammonia is a strong, hydrogen bonding, low molecular weight solvent which penetrates not only into amorphous areas of the fibre cell wall but also into the lignin binding material of a wood member (Rosca et al., 2003). Some of the hydrogen bonds responsible for the rigidity of wood are broken by the ammonia. This results in a softening or plasticizing of the fibre structure so that the wood has sponge-like properties and can be compressed at moderate pressure. As the ammonia is removed from the wood, hydrogen bonds are again formed between the polymer chains, although not necessarily at the same locations in the polymer chains or between the same microfibrils, resulting once again in a rigid wood structure. In order to achieve a close contact between the ammonia and the fibre structure, wood member impregnated with ammonia generally requires moisture content of 20-30%. However, if the moisture content is too low, a proper plasticization will not be achieved (Berzinš et al., 1970).

In recent years a great effort has been made to utilize low-grade woods as a new source of raw materials for preparing resins and a number of new technologies have been developed for the liquefaction of wood. Many studies have pursued the application of liquefied wood to make wood adhesives and some moulding materials. Kobayashi et al. (2005) prepared liquefied wood from Japanese cedar and investigated the preparation of liquefied wood/epoxy resin and its application to wood adhesives. One of the most important ways of the application of the liquefied wood is increasing the wood contents within final products. It was found that wood treated with ozone in gas and liquid phases could be liquefied easier than untreated wood. It was suggested that one of the main effects of ozone treatment of wood was the decomposition of amorphous cellulose. On the other hand the lignin condensed together during liquefaction when it was ozonized in the liquid phase. This indicated that ozone treatment gave lignin reactive functional groups, followed by the condensation reaction. Although lignin was converted to a more condensable structure via ozone treatment, the condensation reaction was suppressed during liquefaction (Kobayashi et al., 2005).
1.5.3 Heat Treatment

The application of heat to wood results in modifications associated with chemical alterations in the material (Fig. 1.11). If carefully controlled, the property changes that are obtained due to thermal modification can be used for certain applications. There has been a great deal of commercial activity in this sector. Thermal modification of wood has long been recognized as a potentially useful method to improve dimensional stability of wood and increase its decay resistance (Militz, 2002). In recent studies Pfem (2006) investigated the possibility of using thermally treated wood for the production of music instruments.

As a result of thermally induced chemical changes to the macromolecular constituents, the physical and biological properties of the wood are altered. These changes include the following:

- improvements of dimensional stability, depending on the treatment conditions (Hsu et al., 1988; Popper et al., 2005);
- reduced hygroscopicity (a decrease in EMC at a given RH and reduced hygroscopicity) (Alén et al., 2002);
- improved resistance to microbial attack (Welzbacher et al., 2004);
- often an increase in modulus of elasticity during the initial stages of heating with a reduction thereafter (Hill, 2006);
- a reduction in impact toughness, modulus of rupture and work to fracture (Kubojima et al., 2000);
- reduced abrasion resistance (Ohtani et al., 2002);
- reduced hardness;
- a tendency for cracks and splits to form, knots to come loose;
- a darkening of the material (Sundqvist and Morén, 2002).

The properties of thermally treated wood highly depend upon the thermal treatment method applied, and it is very important to take them into account when comparing the various treatment methods.

The presence of water or water vapour affects the chemistry of thermal modification and heat transfer within the wood. Under dry treatment conditions, the wood is dried prior to thermal modification, or water is removed by the use of an open system, or a recirculating system equipped with a condenser. In closed systems, water evaporated from the wood remains as a
high pressure steam during the process. Steam can also be injected into the reactor to act as a heat-transfer medium and can additionally act as an inert blanket to limit oxidative processes. Such steam treatments processes are referred to as hygro-thermal treatments. A process where wood is heated in water, this is termed a hydro-thermal process. High temperature steam treatment of wood results in several changes in its chemistry (Rowell et al., 2002). These include:

- degradation of hemicelluloses to produce simple sugars which may undergo reversion reactions to form highly branched polysaccharides;
- degradation of both hemicelluloses and part of the cellulose to form furan type compounds;
- thermal softening of the cell wall matrix, mainly lignin;
- degradation of hemicelluloses to form volatile breakdown products;
- crosslinking between carbohydrate polymers and/or between lignin and carbohydrate polymers;
- increase in cellulose crystallinity.

1.5.4 Thermo-Hygro-Mechanical (THM) Treatment

To avoid wood deterioration due to heating, researchers have considered both moisture and heat treatment for improving wood dimensional stability. In Japan, in particular, different researchers e.g. Tanahashi (1990), Inoue et al. (1993), Norimoto et al. (1993) have made considerable progress towards the permanent fixation of compression set by using high temperature steam. Ito et al. (1998) have successfully developed an innovative process with high temperature steam, for the permanent fixation of transformed and compressed logs of sugi into square samples. Their method consists of several steps of steaming. In this process the log was profiled under saturated vapour at 150°C and then the product was steamed at 200°C for two minutes. Morsing (1998) demonstrated that permanent fixation of compression deformation could be achieved at high temperature e.g. 190°C for 20 hours or 5 hours at 200°C. He also compared the total fixation time of set-recovery using heat and steam treatments with beech and demonstrated that steam treatment could improve the hardness and dimensional stability of wood much faster than heat treatment alone.
THM-densified wood is a unique material in the field of engineered wood products. High temperature, moisture and compression are used to make a wood product with higher strength properties than those of natural wood. Compressed densified wood has not become a common wood product due to its instability in the presence of moisture. An improved densification method was presented in 1998 that included steaming to give shape fixation (Ito et al., 1998). Further improvements to densification were published by Navi and Girardet (2000) that led to production of a stable compressed wood with improved set-recovery. Small cylindrical specimens of spruce and maritime pine were densified in the radial direction under saturated steam in a multiparameter reactor. The densification consisted of two steps: at first the samples were plasticized in saturated steam at 140°C and then densified under controlled displacement mode. After densification, the samples were post-treated with steam under different conditions (Heger et al., 2003). Navi and Heger (2004) found that wood compressed by THM-densification showed increased shear and strength parallel to the grain as well as increased surface hardness. Increase in tensile strength after densification has also been reported by Navi and Girardet (2000). However, one study was found to contradict the previous research (Perkitny and Jablonski, 1984). It reported densified wood to be weaker than natural wood when assessed in bending and compression tests.

As an alternative to a hygro-post treatment, Norway spruce wood was densified in a common industrial scale process and afterwards thermally modified with an oil-heat treatment process (Welzbacher et al., 2005). According to the classification of natural durability (EN350-1, 1994) densified and oil-teat treated material was classified as very durable (durability class 1). The bending strength of densified and oil-heat treated material was only slightly reduced compared to untreated Norway spruce wood. Densified and thermally modified samples showed improved dimensional stability compared to untreated densified material. In contrast to steam post-treatment, significantly increased durability was a result of an oil-heat treatment. As a drawback, the dynamic mechanical properties of densified and oil-heat treated spruce were reduced by 40% compared to controls, though static bending strength was equal to untreated spruce wood.

Inoue et al. (2000) conducted tests to stabilize the compressive deformation of wood by wet heating under atmospheric pressure. By impregnating wood with organic liquids such as ethylene glycol and glycerine and heating at about 80°C, high swelling was obtained in a short period. The wood was then compressed in the direction perpendicular to the grain and heated at 180°C for more than 60 min to produce compressed wood with a high dimensional stabil-
It was confirmed that liquid remained in the wood cell wall after heating at high temperature, indicating that deformation fixation occurred because wood constituents were heated under swelling conditions at high temperature. Treatment with aqueous solutions was advantageous with regard to efficiency of impregnation, rinsing after processing, and reduction of costs. Also high dimensional stability was obtained by treatment with aqueous solutions of 40% or more glycerine as a swelling agent. Using an aqueous solution of 60% glycerine supplemented with 0.2% sulphuric acid, complete fixation of deformation was obtained by heating at 180°C and compression for shorter than 10 min.

The principle of densification and TH-post-treatment is also used in the manufacturing of wood based panels (particleboards and fibreboards), this results in the lower EMC of particleboards compared to solid wood. In case of fibreboards the hydro-thermal modification takes place during defibration (Walker, 2006).

As one potential field of application, bond performance of THM-densified yellow-poplar wood (*Liriodendron tulipifera* L.) was studied by Jennings *et al.* (2005). Phenol-formaldehyde film (PF) and polymeric diphenylmethane diisocyanate (pMDI) adhesives were used to bond specimens. THM samples bonded with PF film showed significantly higher fracture toughness than controls while no difference was found in controls. Fracture toughness of densified samples bonded with pMDI was higher compared to controls; however no change was noted for THM-densified wood samples. Results obtained by Jennings *et al.* (2005) showed that a densified yellow-poplar wood could be a suitable raw material for producing composite wood products and depending on the adhesive type may be considered a superior material in some cases.

### 1.6 Objectives and Hypotheses

**Principle objective**

The principal objective of the research project is to establish a fundamental understanding of the principles and mechanisms, which determine the durability of THM densified wood against fungal decomposition. Particular attention is paid to the interactions that exist between the alterations of the wood constituents, the micro-morphology of the wood structure during processing of THM densified wood and the colonization and degradation by wood decay fungi. The dynamic structure of the project and the synthesis of the proposed studies provide a
sound basis for the development of an environmentally friendly product that may have the potential to substitute the utilization of traditional wood preservatives.

Within the scope of the multidisciplinary studies the secondary objectives can be defined as follows.

**Secondary objectives**

1. **Investigation of interactions between wood decay fungi and the wood substrate modified after THM-treatment.** The use of THM-densified wood is only practical if its durability is comparable to that of wood treated with conventional wood preservatives and if it can be used in utility class 3 (above ground, not covered, exposed to frequent wetting) and 4 (in contact with ground or water, permanently exposed to wetting). For this purpose the performance of THM-densified wood against a broad range of micro-organisms was evaluated as wood used in different utility classes is susceptible to different types of wood decay. As wood used in utility class 4 is susceptible to attack by individual groups or a combination of different micro-organisms, studies of THM-densified wood in ground contact are required. In this context soft-rots are particularly prevalent during early stages of decay and under conditions of high moisture content of wood in contact with the soil. In this context, numerous studies show that white-rot fungi can possess dual modes of degradation and often switch from white- to a soft-rot mode of attack. The resistance of THM-densified should be also assessed against brown-rot as the decays type associated with wood in service. Fungi that have adapted to pyrolyzed wood substrate (pyrophillic fungi) will be included in the study.

2. **Characterization of the mechanical properties of THM-densified wood.** During the densification process compression forces induce major alterations in the wood microstructure. Increase in density of the wood specimens induces changes in the mechanical and wood strength properties. Moreover densification and subsequent TH post-treatment alter wood chemistry and individual wood constituents resulting in the elimination of shape memory which impedes fungal colonization.

3. **Assessment of chemical alterations induced by THM-densification.**
Hypotheses

Within the scope of the objectives defined above, the following hypotheses are proposed.

1. THM densification reduces the susceptibility of wood against wood decay fungi.

2. The alterations in decay resistance are related to the modification of the chemical composition of wood after TH-treatment and the changes of the wood structure induced by densification. Restriction of fungal growth by the occlusion of the cell lumina and the chemical modification of the wood substrate is associated with the depolymerisation of polysaccharides and modification of lignin.

3. TH-treated wood is more susceptible to pyrophillic fungi.

4. Wood decay fungi may switch their mode of action to circumvent unfavourable conditions induced by THM-densification and grow within cell walls.

5. TH-treatment substantially affects wood hygroscopicity, resulting in the elimination of spring-back.

6. THM-densification improves mechanical properties as well as hygroscopicity (water related behaviour).
CHAPTER 2
MATERIALS AND METHODS

2.1 PREPARATION OF THE WOOD SPECIMENS

Wood specimens were cut from the heartwood of living 40-50 year old trees of Norway spruce (*Picea abies* Karst.) and beech (*Fagus sylvatica* L.) originated from central Switzerland. Before treatment all wood samples were conditioned at 20°C and 65% relative humidity. The specimens were cut from the same stem and were free of knots and visible concentrations of resin and showed no visible signs of microbial infection.

2.2 PRODUCTION OF THM-DENSIFIED WOOD SPECIMENS

Seven different optimised treatments on the basis of the THM-process were selected for further investigations (Table 2.1). Non-treated control specimens were included for all test series.

Table 2.1 Types of treatments applied for wood modification.

<table>
<thead>
<tr>
<th>Treatment type</th>
<th>Treatment conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Control</td>
<td>Untreated reference specimens</td>
</tr>
<tr>
<td>2 TH 160°C</td>
<td>Not densified, heat treated at 160°C under saturated steam</td>
</tr>
<tr>
<td>3 TH 180°C</td>
<td>Not densified, heat treated at 180°C under saturated steam</td>
</tr>
<tr>
<td>4 Densified</td>
<td>Mechanically densified under saturated steam, without post-treatment</td>
</tr>
<tr>
<td>5 THM 140°C</td>
<td>Densified and post-treated at 140°C under saturated steam</td>
</tr>
<tr>
<td>6 THM 160°C</td>
<td>Densified and post-treated at 160°C under saturated steam</td>
</tr>
<tr>
<td>7 THM 180°C/80% RH</td>
<td>Densified under saturated steam and post-treated at 180°C under 80% relative humidity</td>
</tr>
<tr>
<td>8 THM 180°C</td>
<td>Densified and post-treated at 180°C under saturated steam</td>
</tr>
</tbody>
</table>
2.2.1 THM TREATMENT EQUIPMENT

The THM-reactor together with the heating circuits are all made of stainless steel and weigh altogether approx. 100 kg (Heger, 2004). The main parts of the reactor are presented on Figures 2.1 and 2.2. The treatment chamber is the most sophisticated part of the THM equipment. It consists of the removable cover, piston, isothermal mantle, isothermal cylinder and mould.

Figure 2.1 a) THM-reactor closed by the compression piston. b) Lid and the piston of the THM-reactor. c) Open THM-reactor. d) Isothermal cylinder. e) Mould-cylinders distributing the steam on the wood specimen (round and square).
Figure 2.2 Schematic drawing of the THM treatment chamber (Heger, 2004).
2.2.2 DENSIFICATION

During the first stage of the THM procedure the specimens were mechanically densified in the multiparameter reactor under saturated steam and controlled displacement mode in the radial direction (Fig. 2.1a, c; 2.2; 2.3). A diagram representing the THM-densification procedure is provided in Fig. 2.4. The process consists of 2 steps: at first, the sample is steam heated for 10 min. at 140°C and 3.61 bar steam pressure (plasticisation) and then densified under controlled displacement mode at 5 mm/min until 5kN, 2.5 mm/min until 10 kN, 1.25 mm/min until 20 kN, 0.5 mm/min until 22 kN. When 22 kN is reached for the second time, the piston (Fig. 2.1b) is stopped and the sample cooled down to 60°C and removed from the reactor if not subjected to further post treatment.

The value of the compression set (C) varied between approx. 70% for spruce and 45% for beech wood specimens (Table 2.2).
2.2.3 **Post-treatment**

After densification, the samples were post-treated under different conditions (Table 2.2). The post-treatments were carried out either under saturated steam (6.18 bar) or 80% relative humidity, which made it easier to control the temperature by varying the pressure. After densification the piston was stopped and maintained in the same position throughout the post-treatment stage. Afterwards the reactor was cleaned, the samples immediately cooled to 60°C and removed from the reactor.
Table 2.2 Condition and mean density of beech and Norway spruce wood samples before incubation (n=6).

<table>
<thead>
<tr>
<th>Condition of wood</th>
<th>Temperature (°C)</th>
<th>Duration (min)</th>
<th>Compression set (%)</th>
<th>Density (g cm⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Densification</td>
<td>Post-treatment</td>
<td>Densification</td>
<td>Post-treatment</td>
</tr>
<tr>
<td>Control</td>
<td>Untreated</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TH 160°C</td>
<td>Heat treatment</td>
<td>160</td>
<td>75</td>
<td>-</td>
</tr>
<tr>
<td>TH 180°C</td>
<td>Heat treatment</td>
<td>180</td>
<td>35</td>
<td>-</td>
</tr>
<tr>
<td>Densified</td>
<td>Mechanical</td>
<td>140</td>
<td>20</td>
<td>71.85 45.29</td>
</tr>
<tr>
<td>THM 140°C</td>
<td>Densification</td>
<td>140</td>
<td>20</td>
<td>150 72.85 46.21</td>
</tr>
<tr>
<td>THM 160°C</td>
<td>Densification</td>
<td>140</td>
<td>20</td>
<td>75 73.18 42.83</td>
</tr>
<tr>
<td>THM 180°C/80%RH</td>
<td>Densification</td>
<td>140</td>
<td>20</td>
<td>65 73.18 45.84</td>
</tr>
<tr>
<td>THM 180°C</td>
<td>Densification</td>
<td>140</td>
<td>20</td>
<td>35 73.33 44.74</td>
</tr>
</tbody>
</table>

SSC, saturated steam conditions. RH, relative humidity.
CHAPTER 2
MATERIALS AND METHODS

2.3 Resistance to Wood Decay Fungi

The resistance of THM-densified wood to fungal decay was assessed according to the procedures stated in the European Standards EN 113 and EN 807. These tests use the mass loss of solid wood blocks as the main criteria for decay and can be regarded as basis for evaluation of durability against three major decay types.

2.3.1 Durability of THM Treated Wood against Brown Rot Fungi

The fungi used were as follows:

- *Coniophora puteana* (Schum.: Fr.) Karst (isolate EMPA 62)
- *Gloeophyllum trabeum* (Pers.: Fr.) Murrill (isolate EMPA 100)
- *Poria placenta* (Fr.) Cooke (isolate EMPA 229)

Identification of pure cultures was confirmed using mycelial characters as observed on plates of malt extract agar (MEA). Test wood blocks, 50 x 25 x 15 mm³, were obtained from THM-treated specimens of Norway spruce wood and prepared according to EN 113 (1996). The blocks were dried at 100°C for 24 H, cooled in a desiccator and weighed, then sterilized with ethylene oxide for approximately 5 hours, dried at 65 °C for 48 hours and cooled down. They were then inoculated and incubated in Kolle flasks in units with fungi according to EN 113 (1996).

For each treatment/fungus combination and incubation period, 6 wood blocks were set up, together with 6 controls. The units were incubated in the moisture content between 6% and 10% in a random array at 22 ± 1 °C and 70 ± 5 % relative humidity for 16 weeks. After incubation, wood blocks were removed from Kolle flasks, cleaned from the adjusting mycelium and weighed. Before the incubated wood blocks were dried for measurement of weight loss, they were sampled at random points by removing chips of negligible weight. These were placed onto MEA to check whether the decay fungi were only micro-organisms present and this was confirmed in all cases. The weight loss was calculated as follows:

\[
WL_\% = \frac{W_0 - W_1}{W_0} \times 100\% \quad \text{(Eq. 2.1)}
\]

where:

- WL_\% weight loss
- W_0 original dry weight of the wood specimen before incubation
2.3.2 Durability of THM Treated Wood Against White Rot Fungi

The fungal cultures used for the determination of white rot resistance were as follows:

- *Trametes versicolor* (L.:Fr.) Quél (isolate EMPA 159*)
- *Trametes pubescens* (Schumach.) Pilát (isolate EMPA 229)

The white rot test was performed on THM-treated beech and spruce wood blocks according to EN 113 similar to brown rot test described in 2.3.1. Dry weight losses (Eq. 2.1) and decay susceptibility (Eq. 2.2) were assessed after the test.

2.3.3 Durability of THM Treated Wood Against Soft Rot Fungi

The efficiency of THM-treatment against soft rot and other soil inhabiting micro-organisms was evaluated according to European Standard EN 807 (2001). This method defines the requirements of the biological use class 4 (wood in ground or fresh water contact (EN 559-1, 1996). Two modifications of the experimental set allow a comprehensive assessment of soft rot degradation. The soil-bed test reveals resistance against soft rot fungi and spoil-inhabiting microorganisms. The optional screening test, however, is performed in vermiculite using a spore suspension of pure cultures of soft rot fungi.

2.3.3.1 Soil-bed Test

Six wood specimens for each treatment method were placed in a soil substrate. Before the test, moisture content (MC) and water holding capacity (WHC) of the substrate were determined according to EN 807. Soil substrate used in the study was a natural top soil from a test field in Thurgau, Switzerland. The amount of water required to bring the substrate to 95% of its WHC was calculated and added to the soil. Twelve containers (35 x 20 x 25 cm³) were filled with a total of 13.5 kg of soil and sealed with lids. In order to maintain enough moisture in the soil, the containers were monitored weekly and MC was adjusted by adding water or removing lids of the containers. The moisture content of wood was monitored on wood specimens prepared from Norway spruce sapwood. For the containers incubated with beech specimens – moisture controls prepared from beech were used.
Wood specimens of three dimensions were investigated: (1) beech 5 x 50 x 10 mm³ (L x R x T), (2) spruce 5 x 40 x 10 mm³ (L x R x T), (3) beech and spruce 30 x 10 x 5 mm³ (L x R x T). Six virulence control wood specimens, also prepared from spruce, were positioned in each container. The wood specimens were leached according to EN 84 (1996) before determining their initial dry mass to the nearest 0.001 g and incubation in the containers. All wood test specimens and virulence specimens were placed vertically into the soil with 20 mm of their length above the surface of the substrate and with a minimum of 20 mm apart and from the sides of the container (Fig. 2.6). Test wood specimens, virulence control wood specimens and moisture monitoring wood specimens were randomly distributed.

For comparison a chromium/copper (CC) salt solution was used as a reference wood preservative (CuSO₄ x 5H₂O – 50%, K₂Cr₂O₇ – 48%, CrO₃ – 2%). Concentrations of CC solution 0.4% and 1.6% were selected for beech wood specimens, 0.16% and 0.4% for spruce wood specimens respectively.

All test, reference and virulence control wood specimens were incubated for 8, 16, 24 and 32 weeks in a conditioning room at 28±1°C and 95-100% RH (Fig. 2.7). After
incubation the specimens were removed from the soil substrate, cleaned of adhering soil particles and weighed. Determination of the initial dry mass and the final dry mass after soil exposure was carried out by oven drying the wood specimens at 103°C and weighing to the nearest 0.001 g. The weight loss of each specimen was calculated as a percentage of the initial dry mass (Eq. 2.1). Decay susceptibility values were assessed taking into account the density of specimens (Eq. 2.2).

### 2.3.3.2 Optional screening test

The effectiveness of the wood modification treatment to prevent decay of the soft rot type was also assessed using the screening test in vermiculite (EN 807, 2001). This method uses a mixture of five fungi, which are all capable of causing soft rot. Test specimens are therefore not exposed to the wide range of organisms in the soil used in soil-bed test and which are known to influence the overall performance of a wood preservative in the ground contact service situation.

Six specimens of each treatment method (TH 160, TH 180, densified, THM 140, THM 160, THM 180, THM 180/80 and reference controls) were included in the test. Tested species were Norway spruce and beech. All specimens were sterilized with ethylene oxide for approx. 5 hours, dried at 65°C for 48 hours and cooled. They were then incubated in containers (10 x 10 x 6 cm³) with vermiculite (Fig 2.8) and inoculated with a mixed spore suspension of five fungi capable of causing soft rot. The following fungi were included in the test:

- *Chaetomium globosum* Kunze (isolate EMPA 569)
- *Humicola grisea* Traaen (isolate EMPA 571)
- *Petriella setifera* (Alf.Schmidt) Curzi (isolate EMPA 572)
- *Phialophora mutabilis* (van Beyma) W.Gams and Mc Ginnis (isolate EMPA 573)
- *Trichurus spiralis* Hasselbr (isolate EMPA 574)

Before the test MC and WHC of vermiculite were determined (EN 807, 2001). The amount of water needed to bring the substrate to 95% of its WHC was calculated and added to the ver-
miculite. All the containers were monitored weekly and MC was adjusted by adding sterile water. Only specimens with dimensions of $30 \times 10 \times 5 \text{ mm}^3$ ($L \times R \times T$) were included in this test. All specimens in containers were incubated for 16 weeks in a climate chamber with $29 \pm 1^\circ\text{C}$ and 65% RH. After incubation, the specimens were removed from the containers, cleaned and dried for the determination of the dry weight losses (Eq. 2.1).

**2.3.4 Durability of thermally treated wood against pyrophillic fungi**

The isolate used for the assessment of resistance of thermally treated wood specimens against pyrophillic fungi was *Daldinia loculata* (Lév.) (isolate EMPA 663).

Specimens of thermally treated wood were provided by Balz Holz AG (Switzerland) (types: II, III) and Mitteramskogler GmbH (Austria) (types: mezzo, forte, forte-exterior). The detailed account of treatment conditions is omitted due to commercial reasons. The only available information provided by the manufacturers was the gradient of temperature increase, mentioned as “intensity” in Table 2.3 and the treatment atmosphere. The wood from Balz Holz AG was treated in the autoclave at temperatures not exceeding 170 °C in the atmosphere of nitrogen (Bächle and Niemz, 2007). The samples of Mitteramskogler were treated in the dry chamber at temperatures up to 220°C. Selected physical properties of all products can be found in Bächle and Niemz (2007) and in Junghans and Niemz (2006). The fungal test was performed on thermally treated wood specimens according to EN 113 (1996) as described in 2.3.1. Six specimens were used for every treatment/fungus combination. The incubation period was 8 weeks.

**Table 2.3 Wood species and treatment types for assessing the durability of thermally treated wood against *Daldinia loculata***

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wood species</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beech II</td>
<td>Beech</td>
<td>Spruce II</td>
</tr>
<tr>
<td>Ash II</td>
<td>Maple</td>
<td></td>
</tr>
<tr>
<td>Maple forte</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spruce II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beech III</td>
<td>Beech</td>
<td>Spruce II</td>
</tr>
<tr>
<td>Ash mezzo</td>
<td>Maple</td>
<td></td>
</tr>
<tr>
<td>Mezzo</td>
<td>F-exterior</td>
<td></td>
</tr>
<tr>
<td>Ash forte</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maple F-exterior</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ash III</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Table 2.3 Wood species and treatment types for assessing the durability of thermally treated wood against *Daldinia loculata*.*
2.3.5 Decay Susceptibility

Decay susceptibility which is used for assessment of decay rate in wood of different densities can be calculated as follows:

\[
DS = \frac{W_0 - W_1}{V}
\]  
(Eq. 2.2)

where:

DS decay susceptibility

\(W_0\) original dry weight of the wood specimen

\(W_1\) dry weight after decay

\(V\) volume of the specimen

The results represent the amount of wood consumed per volume and are expressed in g/cm\(^3\).

2.4 Light Microscopy and SEM

The incubated test blocks were cut into sub-samples of approximately 20 x 5 x 5 mm\(^3\), with transverse, radial and tangential surfaces exposed to examination. These were fixed in a 2% glutaraldehyde buffered at pH 7.2 -7.4, dehydrated with acetone and embedded in a methacrylate medium (Schwarze and Fink, 1998). They were then sectioned at approximately 2 and 4 \(\mu\)m using a rotary microtome (Leica\® 2040 Supercut) fitted with a diamond knife. For general observations of cell wall degradation and hyphal growth, sections were stained for 12 h in safranine and then counter-stained for 3 min in methylene blue and in 30 min in auramin. Micrographs were taken with a microscope fitted with camera system (Leica\® DC 50, TWAIN). Longitudinal sections were cut in depth of samples (10 mm) and at the surface.

Radial longitudinal specimens from wood blocks were extracted from THM-densified samples. These were then prepared for scanning electron microscopy (SEM), dried in a vacuum oven at 40°C and 10 mbar for 12 hours, glued on a specimen holder using carbon adhesive and sputtered with a platinum layer of approx. 10 nm. The specimens were investigated in a Field Emission SEM (Jeol 6200F) at an acceleration voltage of 5 kV and working distance of 24 mm.
2.5 **TOTAL PROTEIN CONTENT**

Various indirect methods are applied for measuring fungal bioactivity in wood, such as dry weight loss, increased mass of fungus, linear hyphal growth, CO$_2$-production, heat production, O$_2$-consumption. Direct methods are based on the measurement of various specific fungal biomolecules such as ergosterol, phenol oxidase activity, enzyme-linked immunosorbent assay (ELISA), NMR- measurements, immunological methods, or the determination of chitin (Mohebby *et al.*, 2003).

The estimation of fungal biomass in THM-densified wood was performed in the department of wood technology of the Skogforsk (Norwegian Forest Research Institute) during a short-term scientific mission (STSM) within a framework of the COST E37. TH- and THM-treated Norway spruce specimens after brown rot test (see 1.5.1) were subjected to analysis of chitin, ergosterol contents and amount of cellulose remained after brown rot degradation.

**2.5.1 DETERMINATION OF CHITIN**

Chitin is found in the cell walls of all higher fungi and is used to estimate total fungal colonisation in wood substrates. Although chitin content has been reported to vary in relation to different growth conditions, culture media, incubation times, fungal strains and analytical methods, chitin is considered to be more refractory thus persisting after death and is regarded as a reliable parameter of both living and dead hyphae in wood (Eikenes, 2005).

A sensitive method for the determination of chitosan in colonised wood specimens was assessed using acidic hydrolysis and online derivatization with o-phthalaldehyde in a liquid chromatographic system with fluorescent detection.

*Hydrolysis of chitosan in wood samples*

The ground wood samples were hydrolyzed by 1.5 ml of 6M hydrochloric acid in a 2ml centrifuge tube for 64-70 hours and stored at 4°C until further processing. The hydrolyzed samples containing glucosamine were diluted 1:5000 (v/v) by deionized water and analyzed for the content of glucosamine by high performance liquid chromatography (HPLC) with on-line fluorimetric derivatization by o-phtalaldehyde. Homocysteic acid was used as internal standard (IS).

*Preparation of o-phthalaldehyde (OPA) solution*
270 mg o-phthalaldehyde (OPA) was added to a 50 ml volumetric flask and dissolved in 5 mL methanol. Then 200 µl 2-mercaptoethanol was added and diluted to the final volume with 0.4 M borate buffer at pH 9.5. The solution was then filtered (0.45 µm, Non-Sterile Acrodisc, Gelman Sciences), transferred to 2 ml dark coloured HPLC vials. The vials were then immediately purged with argon and sealed with snap caps with rubber septa. The solutions were stored in the dark at 4°C.

**Preparation of eluent for HPLC**

To 1 L volumetric flask 2.05 g sodium acetate (0.025 mol) and 5.25 g citric acid monohydrate (0.025 mol) were added and dissolved in 900 ml deionised water. The pH was adjusted to 5.3 with 50% (w/v) sodium hydroxide before the solution was diluted to the final volume and filtered (0.45 µm).

The eluent for the HPLC was prepared by mixing seven volumetric units of the buffer solution with three volumetric units of methanol (HPLC quality). The eluent was degassed by purging with helium (> 99.996%) for 5 min.

**HPLC method**

An Agilent 1100 series liquid chromatograph (Agilent technologies, Palo Alto, CA, USA) equipped with a Shimadzu RF-551 fluorescence detector (Shimadzu, Duisburg, Germany) was used for separation at 50 °C on a Zorbax Eclipse XDB-C8 (4.6 × 75 mm, 3.5 µm particle size, Agilent technologies, USA) with an analytical guard column Eclipse XDB-C8 (4.6 × 12.5 mm, Agilent technologies). The eluent (see above) was continuously purged with helium, and the flow was set to 2.0 ml/min.

Prior to injection the compounds were derivatized by the Agilent 1100 series auto injector/sampler (Agilent technologies) by mixing 5 µl of sample with 10 µl of OPA derivatization agent prior to injection, and the product was detected after chromatographic separation using λ_{\text{excitation}} = 340 nm and λ_{\text{emission}} = 445 nm. Peak height was used for quantification by means of an internal standard (4 µmol/l homocysteic acid) and a seven point calibration with glucosamine ranging from 0 to 4 µmol/l. The standard equation was obtained by a linear regression of the ratio of the height of the internal standard and the glucosamine peaks versus the ratio of the amount of internal standard and glucosamine in the standard samples. The regression coefficient was typically better than \( r = 0.999 \).
**Stability of OPA during repeated injections of a control sample**

The chromatographic system was set up to inject a control sample containing 4 mmol/l internal standard (homocysteic acid) and 4 mmol/l glucosamine every 4 min for 10 h (150 injections). Every ten injections were made from each of 15 separate vials containing uncontaminated control solution (as above).

**2.5.2 Ergosterol Assay**

Ergosterol is the most prominent sterol in most fungi and is found mainly in cell membranes. The chromatographic system used for the ergosterol assay fully separated both ergosterol and the internal standard (cholesterol) from other compounds in the matrix: these were detected 16.9 and 18.0 minutes after injection respectively. Cholesterol was used as an internal standard due to its similarity to ergosterol.

Grinded wood samples (400 mg) were placed in an extraction column and sealed. Methanol (25 ml) and stock solution of internal standard (10 µl of a solution of 0.012 g cholesterol dissolved in 100 ml methanol) were added to the extraction flask. The column and the extraction flask were placed in the fluidized bed extraction (FBE) unit (FexIKA 50, IKA Werke GmbH&Co, Sauafen, Germany). The extraction unit (Fig. 2.9a) was heated to 92°C followed by an isocratic period of 7 minutes to allow samples to be fully covered by methanol. The solvent was then returned to the evaporation flasks by cooling step at 60°C. The heating and cooling cycles were repeated 12 times. The solvent was removed by vacuum evaporation in a rotary evaporator at 70°C and 250 mbar. The extractives were re-dissolved in 1.0 ml dry pyridine (water content below 0.05%) and derivatized with 500 µl BSTFA at ambient temperature for a minimum of 4 hours before being analyzed by GC-FID.

![Figure 2.9 a) Automated fluidized bed extraction unit. b) Agilent 1100 series liquid chromatograph. c) Samples before derivatisation in Agilent 1100 injector.](image)

The separation and quantification of the compounds were achieved using a gas chromatograph (Agilent 6890 Series, Agilent Technology) with a FID detector and an autosampler.
(Agiler 7683 Series autosampler and Agilent 7683 Series injector). The gas chromatograph (Figs. 2.9 b-c) was equipped with CP-SIL low bleed MS (5% phenyl: 95% demethyl polysiloxilan) capillary column of 20 m x 0.25 mm i.d. (0.25 μm film thickness). Helium carrier gas was maintained at a constant flow rate of 1.0 ml min⁻¹. Following the pulsed split less injection (90 psi for 1 minute) of 5 µl sample, the GC column temperature was programmed as follows: isothermal at 200°C for 2 minutes, 10°Cmin⁻¹ from 200°C to 300°C, followed by an isothermal period at 300°C for 16 minutes. The FID was operated at 300°C with a gas flow of hydrogen at 40ml min⁻¹ and of artificial air at 450 ml min⁻¹. Peak area was used for the quantification of ergosterol with a four level calibration (cholesterol as an internal standard).

2.5.3 THERMOGRAVIMETRIC ANALYSIS
Thermogravimetric analysis (TGA) was carried out using a Thermogravimetric Analyzer PYRIS 1 TGA from Perkin Elmer module supported with a PC and software for control and data processing. Approximately 6 mg of the samples powder has been introduced into an open sample pan (Fig. 2.10) and heated according to the following program:

1. Hold for 1min at 35.00°C
2. Heat from 35.00°C to 230.00°C at 30°C/min
3. Hold for 40min at 230°C
4. Heat from 230°C to 300°C at 20°C/min
5. Hold for 45min at 300°C
6. Heat from 300°C to 600°C at 40°C/min
7. Hold for 10min at 600°C

Figure 2.10 Plate of TGA analyzer with sample pans.

2.6 PHYSICO-MECHANICAL PROPERTIES
In most cases wood modification results in changes of its mechanical and physical properties. Alterations in wood density, Brinell hardness, compression strength and MOE as well as moisture-related properties were investigated on THM-densified beech and Norway spruce wood specimens. Due to the restrictions of the specimens’ sizes, it was not possible to assess additional parameters.
2.6.1 DENSITY

Density, being one of the most important properties of wood, increases under compression proportionally to the compression strength. The density of the specimens was assessed according to DIN 52-182 (1976) after conditioning in the normal climate (20°C and 65 % relative humidity) until the constant mass was reached.

2.6.2 COMPRESSION SET

The value of compression set ($C$) was defined as follows:

$$C = \frac{R_o - R_c}{R_o} \times 100 \% \quad \text{(Eq. 2.3)}$$

where:

- $R_o$ and $R_c$ dimensions of the samples in the direction of compression before and after densification respectively.

2.6.3 COMPRESSION SET RECOVERY

Compression set recovery ($C_{rec}$) (synonyms: spring-back, set-memory, set-recovery, shape-recovery) determines the irreversibly recovered thickness in the direction of compression due to shape-memory effect. It is calculated as follows:

$$C_{rec} = \frac{R_{c_{wet}} - R_c}{R_o - R_c} \times 100\% \quad \text{(Eq. 2.4)}$$

where:

- $R_o$ size of specimen before densification
- $R_c$ size of specimen after densification
- $R_{c_{wet}}$ size of specimen in the direction of compression after soaking in water for 3 days at 20°C, followed by oven-drying.
2.6.4 MOISTURE CONTENT

The percentage of moisture content was determined according to DIN 52-183 (1977).

2.6.5 LINEAR SWELLING

All specimens (dimensions approx. 10 mm x 25 mm x 30 mm, R x T x L) were placed into the climate chamber with a constant temperature of 20°C and a relative humidity of 35%. Each specimen was weighed at regular intervals until the alterations in weight over a 2 day period were less then 0.1%. After such a defined equilibrium was reached the dry weights as well as the dimensions were recorded. The relative humidity was subsequently increased according to the scheme: 35% → 50% → 65% → 80% → 93% (DIN 52-184, 1979). Finally the specimens were dried in the oven at 103 ± 2°C for 2 days and both the oven dry weight and the dimensions were measured. The linear swelling was calculated as follows:

\[ \alpha_u = \frac{a_u - a_o}{a_o} \times 100\% \]  \hspace{1cm} (Eq. 2.5)

where:

- \( a_u \) size of the specimen in the particular anatomic direction at the moisture content \( u \)
- \( a_o \) size of the oven dry specimen.

2.6.6 BRINELL HARDNESS

In order to estimate Brinell hardness (HB) a steel sphere with a diameter of 10mm was pressed against the surface of the wood specimen for 10 seconds with a defined force (500 N). The surface of indentation was measured, and the load divided by the area of the impression surface upon the material. Since different spheres produce different impressions, to maintain the same HB the ratio of the load to the square of sphere diameter needs to be constant. The test was conducted on all specimens in radial and longitudinal direction according to DIN 1534 (2000). The Brinell hardness (HB) is calculated as follows:

\[ HB = \frac{2 \times F}{\pi \times D \times (D - \sqrt{D^2 - d^2})} \]  \hspace{1cm} (Eq. 2.6)
where:

\[
\begin{align*}
F & \quad \text{applied force (N)} \\
D & \quad \text{diameter of indenter (10 mm)} \\
d & \quad \text{diameter of indentation (mm)}
\end{align*}
\]

The measurements were conducted with the Universal Testing Machine “Zwick” equipped with “Universalhärtemesskopf”. This device gives an opportunity to measure the depth of indentation and calculate the diameter of indentation out of it. This simplifies the procedure considerably and averts time consuming and inaccurate indentation diameter measurements. The HB was calculated as follows:

\[
HB = \frac{F}{\pi \times D \times h}
\]

where:

\[
\begin{align*}
F & \quad \text{applied force (N)} \\
D & \quad \text{diameter of indenter (10 mm)} \\
h & \quad \text{depth of indentation (mm)}
\end{align*}
\]

**2.6.7 COMPRESSION STRENGTH PERPENDICULAR TO THE GRAIN**

The compressive strength of a material is that value of uniaxial compressive stress reached when the wood fails completely. The compression strength value was obtained experimentally in a compressive test according to DIN 52-192 (1979). Wood specimens (dimensions 50 x 25 x 15 mm³) were loaded in the Zwick (100 kN) Universal Testing Machine longitudinally or radially to the grain, applying the load at a displacement rate of 0.5 mm/min. The radial loading referred to loading applied in the radial direction (loading applied to the tangential surface). The compression strength (\(\sigma_{\text{max}}, \text{MPa}\)), Young’s modulus in compression (MOE) were both determined from the recorded strain – stress curves using the pre-calibrated Zwick software. The maximum compression of 2% was defined for all wood specimens. The Young’s modulus was calculated between 10 and 30% of the maximum load of each specimen.
2.6.8 Porosity

Mercury intrusion porosimetry was used for assessment of the porosity of the specimens (Junghans et al., 2005; Moura et al., 2005). Specimens of untreated, TH 180 and THM180 Norway spruce wood were cut into sub-samples of about 10 mm x 7 mm x 5 mm (R x T x L). The specimens were fixed in dilatometer which was later filled with 450 ml of mercury. During the measurement process the pressure on mercury was constantly raised. The alterations in the mercury level in the column were registered via the change of electrostatic capacity. The measurements were conducted on the PASCAL 140/440 (ThermoFinnigan, UK).

2.7 Chemical composition

Alterations in the wood chemistry caused by the THM-treatments were assessed during an STSM at the Institute of Wood Chemistry and Technology (Hamburg University). Carbohydrates composition and content were analysed chromatographically as described below, while the extractives were analysed only gravimetrically due to restrictions of equipment.

2.7.1 Carbohydrate analysis

The carbohydrate content of wood comprises cellulose and hemicelluloses. Hemicelluloses consist of a mixture of polysaccharides synthesized in wood almost entirely from glucose, mannose, galactose, xylose, arabinose, 4-O-methylglucuronic acid and galacturonic acid residues. Generally, hemicelluloses are of much lower molecular weight than cellulose and some are branched. They are closely associated with cellulose and appear to contribute as a structural component to the plant. Hemicelluloses are soluble in alkali and easily hydrolyzed by acids. This property is used for their chromatographic analyses.

Two-step hydrolysis consisting of pre- and post-hydrolysis

Prior to hydrolysis all wood samples were grinded in a vibration mill (Herzog, Germany), cellulose samples were conditioned and fluffed (Ika Labormühle, Germany). All samples were conditioned at 20°C and 65% rH. The moisture content was measured and the results recorded.

Pre-hydrolysis (depolymerisation of carbohydrates)

2 ml 72% H₂SO₄ is added to 200 µg (± 10 µg) of the wood sample. The sample was hydrolysed for 1 hour at 30°C (incubated in a water bath). The reaction vessel is a short test tube. The sample has to be stirred during the reaction.
During hydrolysis the sample was pressed against the wall of the test tube with a glass stick. This reduces the particle size and makes the sample more accessible to the acid.

After 1 hour the reaction was terminated by addition of 6 ml distilled H$_2$O. Each sample is transferred into a 100 ml volumetric flask using 50 ml of distilled water. The vessel was closed with a small condenser (a glass ball).

**Post-hydrolysis** (further hydrolysis of reversion products)

Samples were hydrolysed under pressure (0.12 MPa/1.2 bar) in an autoclave for 40 min at 120°C. After cooling down to room temperature, the flask was filled up to a volume of 100 ml. The flask was shaken and the condensed lignin residue removed by filtration through a G4 sinter glass crucible.

1 ml of the solution was transferred to a sample vial for the analysis on the Borate system. 1.5 ml has to be saved in a plastic tube for a second analysis. Thereafter the sample was frozen.

The solid residue was washed intensively with distilled water and then the crucible was dried at 105°C and the lignin amount determined gravimetrically (Klason lignin).

**Borate anion exchange chromatography**

*Stationary phase:* anion exchange MCI Gel CA08F (Mitsubishi) packed in Omnifit empty columns 7 x 11.5 mm (60°C).

*Mobile phase:* 07. ml/min gradient elution with A: 0.3 M potassium tetraborate B: 0.9 M potassium tetraborate

Programme: 0 min : 90% A, 10% B

35 min : 10% A, 90% B

47 min : end

Post-column derivatization was obtained by adding cubicinchoniate (0.35 ml/min) and subsequent heating to 105°C in 0.3 mm, 30 m Teflon coil. Detection was conducted at 560 nm with an UV-detector.

2.7.2 KLASON LIGNIN

Klason lignin was determined gravimetrically as the residue after hydrolysis of hemicelluloses.
2.7.3 Determination of Extraneous Compounds

The solubility of wood in various solvents is a measure of the extraneous components content. No single solvent is able to remove all of the extraneous materials. Ether is relatively nonpolar and extracts fat, resins, oils sterols and terpenes. Ethanol/benzene is more polar and extracts most of the ether-solubles plus most of the organic material insoluble in water. Hot water extracts some inorganic salts and low molecular weight polysaccharides including gums and starches. Water also removes certain hemicelluloses such as the arabinogalactan and gum. In this study the acetone/water (9:1) solvent has been used.

Extraction of extraneous compounds

For extraction, shavings of Norway spruce and beech wood were prepared from TH-treated and THM-densified wood samples. The samples were immediately freeze-dried and ground in a mill with a rotating knife (Retsch) using a 3-mm screen.

Extraction was performed using an accelerated solvent extraction (ASE 200, Dionex). Extractions with acetone/water (9:1) at 70°C at a constant pressure of 10 atm and a static equilibration treatment of 7 min is the most suitable method for extraction of phenolic compounds from beech and spruce wood. For each treatment, 2 g of freeze-dried wood powder was extracted once and the extract was made up to 25 ml by addition of acetone/water (9:1) solution.

Determination of extractive content

The extractive content was determined gravimetrically after the solvent was removed using a rotary evaporator under vacuum.

2.8 Statistical Analysis

One-way analysis of variance (ANOVA) of the recorded valued was performed for all wood samples in Excel with the significance level set at p<0.05. A Tukey HSD post hoc test was performed in SPSS to demonstrate differences in mean values.
CHAPTER 3

RESISTANCE TO WOOD DECAY FUNGI

3.1 Resistance to Brown Rot

3.1.1 Results

Fungal colonization and wood degradation

Most test blocks in all the wood treatments were completely colonized by external mycelia after the 16-week incubation period, as assessed by EN 113 criteria. (Fig. 3.1)

For each fungus, the weight losses were significantly different (p<0.05) amongst the different wood treatments (Fig. 3.2). For all the fungi, the highest weight losses were observed in untreated and TH-treated (160°C and 180°C) wood. The lowest weight losses occurred in THM-densified wood post-treated at 180°C. Also, the fungi differed in the amount of weight loss that they induced.

Densification resulting from wood treatment

THM treatments resulted in increases of density ranging from 3- to 4-fold (Table 2.2). The density of wood blocks exposed to TH-treatment was similar to that of the untreated controls.
THM-treatment, with post-treatment at 140°C, compressed the tracheid lumina to a radial width ranging from 1 to 5 µm in the late-wood and from 1 to 10 µm in the early-wood (Fig. 3.4).

Figure 3.2 Dry weight losses of wood blocks in untreated controls, TH treated wood (160°C and 180°C), densified wood without thermal treatment and THM densified wood (140°C, 160°C and 180°C) incubated with Coniophora puteana, Gloeophyllum trabeum and Poria placenta. Bars, standard deviations. All columns with identical letters show no significant differences (p>0.05), those with different letters show significant differences (p<0.05).
Figure 3.3 Decay susceptibility of TH- and THM-treated Norway spruce wood specimens incubated with three brown rot fungi. Bars, standard deviations. Columns marked with asterisk denote significant difference in comparison to untreated controls (p<0.01).
With post-treatment at 160°C, the corresponding values were 0.5 to 2 µm in the late-wood and 1 to 5 µm in the early-wood (Fig. 3.4). With post-treatment at 180°C, most of the late-wood tracheid lumina were completely occluded, whereas those of early-wood tracheids were reduced to a width of 0.5 to 3 µm (Fig. 3.4). Such differences were noted by (Navi and Girardet, 2000), who also showed that ‘set-recovery’ (the tendency for cells partially to re-expand after densification) was reduced by raising the temperature of post-treatment).

Decay susceptibility, calculated according to Eq. 2.3, was not altered significantly for both TH-treatments in case of all three brown rot fungi (Fig. 3.3). Densified and not post treated Norway spruce specimens showed a two-fold significant increase in decay susceptibility against brown rot decay. THM-densification, however, did not result in improvements in decay susceptibility, on the contrary, most of the registered values for THM-densified specimens were significantly higher in comparison to untreated controls (Fig. 3.3).

3.1.1 DISCUSSION

Microscopical examination of the wood blocks showed that the differences between treatments regarding weight loss could be partly attributed to the restriction of fungal growth by the occlusion of tracheid lumina. In wood subjected to THM-treatment and post-treated at 180°, fungal hyphae failed to grow in cell types with completely occluded lumina and were confined to the partially occluded lumina of the early-wood tracheids. Comparisons of unoccluded and partially or fully occluded lumina and of the fungal growth within them are illustrated in Figures 3.4a and 3.6b for P. placenta and Figures 3.4b-c for G. trabeum. Complete occlusion of all cell lumina would be needed to prevent brown-rot activity, since degradative substances can diffuse from a single hypha in a cell lumen. However, if a high proportion of lumina are completely occluded, this will clearly restrict fungal ingress overall, sufficiently to explain why the rate of decay was reduced.

Although fungal hyphae were unable to enter fully occluded lumina and showed restricted development when occlusion was almost complete, the species tested in this study were able to produce narrow hyphae, which may enable them to enter cells with partially occluded lumina. Coniophora puteana produced much narrower hyphae in wood than in agar media and all three fungi showed some effect of substrate (agar versus wood) on their hyphal widths and morphology (Table 3.1). Poria placenta produced wider hyphae than C. puteana, even in wood, and may therefore be less able to enter partially occluded cell lumina. In wood, the hyphae of C. puteana all bore simple clamp connections, whereas those of P. placenta were
either very fine (1 - 2 µm), bearing simple clamps or somewhat wider (3 - 9 µm), with either medallion clamps or simple clamps. The hyphae of *G. trabeum*, which were abundant within the lumina of tracheids and xylem ray parenchyma in undensified wood, possessed numerous clamp connections, sometimes in the form of medallions (Fig. 3.5).

Figure 3.4 Transverse sections (TS) of densified Norway spruce wood; Bar = 10µm. a) Post treated at 140°C, and incubated with *Poria placenta*. Note: Cell lumina of all tracheids are only partially closed. b) Post treated at 160°C and incubated with *Gloeophyllum trabeum*. Note: Both in a) and b) fine hyphae (arrows) are visible within the lumina of earlywood growing on the S₃. Note for b): cell lumina of some tracheids are completely sealed. c) Post treated at 180°C and incubated with *Gloeophyllum trabeum*. Note: Hyphal growth (arrow) is very sparse and restricted to the early wood tracheids. Cell lumina of most tracheids are completely sealed.

Hyphal tunneling through cell walls is another means by which certain fungi may be able to grow through wood in which the lumina are inaccessible (Daniel *et al.*, 1992; Schwarze *et al.*, 1995; Schwarze and Fink, 1997; Worrall *et al.*, 1997; Schwarze and Fink, 1998; Schwarze, 2004). Only a few brown-rot fungi have been observed to grow in this way (Duncan, 1960; Schwarze *et al.*, 2000; Kleist and Schmitt, 2001; Kleist *et al.*, 2002) but *G. trabeum* was found to do so in the present study (Fig. 3.5).
Figure 3.5 TH treated (160°C) Norway spruce wood incubated with *Gloeophyllum trabeum*; Bar = 10 µm. a) Radial longitudinal section showing boreholes, multiple hyphal branching (arrows) and cavity formation within the secondary wall of tracheids. Mc = medallion clamp. b) Transverse section showing hyphal growth (arrows) along the spiral alignment of the cellulose microfibrils. Cavity formation is apparent around the hyphae. c-d) Radial longitudinal sections showing hyphal growth (arrows) within the secondary walls of xylem ray parenchyma. In regions of the secondary wall where hyphal tunnelling (arrows) has formerly occurred, secondary walls are completely degraded (asterix).

It penetrated the secondary walls transversely with very fine hyphae, < 0.5 µm in diameter. Tunnelling within the secondary walls began when these hyphae reached the compound middle lamella and changed direction without penetrating it (Fig. 3.5). Enzymatic activity along the length of the tunnelling hyphae produced individual cavities 0.5 - 2 µm in diameter within the S2-layer, following the alignment of the microfibrils (Fig. 3.5). Longitudinal sections revealed the early stages of cavity-formation, which began within the chambers of simple pits, together with localized multiple branching of the hyphae (Fig. 3.5). Subsequently, individual cavities coalesced to form larger cavities (Fig. 3.5).

Despite the hyphal tunnelling activity of *G. trabeum*, the weight loss caused by this fungus was reduced significantly by THM densification, as in the case of the other two fungi (Fig. 3.2). However, the tunnelling activity was predominantly observed in thermally treated wood, rarely in untreated and densified wood and in THM-densified wood post-treated at 140°C and 160°C. It was never observed in THM densified wood post-treated at 180°C. The effect of heat treatment is discussed below in relation to the availability of low-molecular weight carbohydrates and of moisture.
When cell lumen occlusion was absent (i.e. in untreated wood and in TH-treated non-densified wood), *C. puteana* and *P. placenta* preferentially degraded hemicelluloses and cellulose in the tracheid walls, as demonstrated by histochemical staining (red instead of blue; Fig. 3.6). A partial loss of birefringence under polarized light revealed cellulolysis in the early-wood tracheids, but not to any significant extent at this stage in the epithelial cells of the resin canals and in the late-wood tracheids. Hyphae were visible within the cell lumina (Fig. 3.6) and there was typical cracking of the cell walls extending from the S$_3$ layer into the S$_2$ and S$_1$ layers.

It seems likely that, in addition to causing occlusion of cell lumina, densification reduces the size of voids within cell walls. If so, the diffusion of fungal enzymes through the walls of densified wood may be retarded. A slow rate of diffusion may explain why tree species with naturally dense and highly lignified wood are relatively resistant to decay (Rayner and Boddy, 1988; Schwarze, 2004). This may be particularly important in resistance to brown-rot and selective delignification, which mainly involve the diffusion of degradative substances, rather than the direct erosion of cell walls by fungal hyphae. Even without densification, pores within undecayed woody cell walls are no more than 2 nm in diameter (Hill and Popadopoulos, 2001) and are therefore too small to allow the diffusion of cellulolytic enzymes (Cowling and Kirk, 1976). For this reason, non-enzymatic systems involving substances of low molecular weight have been implicated in the early stages of brown-rot, prior to pathways being opened up for the diffusion of enzymes (Koenigs, 1974; Murmanis and Highley, 1987). Evidence for the operation of such systems has, however, been challenged (Kerem *et al.*, 1999).

Apart from the effects of densification on the size of cell lumina and voids within cell wall, TH treatment may affect the suitability of wood as a substrate for fungal growth. In the present study, the moisture content of TH-treated wood was found to be far below the fibre saturation point by the end of the 16-week incubation period. Thus, no free water was available within the cell lumina, so that conditions for hyphal growth and wood degradation were unfavourable. These conditions were, however, circumvented by *G. trabeum*, which switched its mode of

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**Table 3.1 Hyphal width measured in agar media culture and in wood.**

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Hyphal width (µm)</th>
<th>Medallion clamps</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In agar</td>
<td>In wood</td>
</tr>
<tr>
<td><em>Coniophora puteana</em></td>
<td>2.5-8</td>
<td>0.5-2</td>
</tr>
<tr>
<td><em>Gloeophyllum trabeum</em></td>
<td>1-4.5</td>
<td>1-5</td>
</tr>
<tr>
<td><em>Poria placenta</em></td>
<td>2-8</td>
<td>1-9</td>
</tr>
</tbody>
</table>

---

69
action to hyphal tunnelling within the secondary wall so as to utilize the water that was bound there. The relatively rapid degradation of such wood may be explained also because its density is reduced due to the depolymerization of polysaccharides; this is estimated to amount to approximately 5 - 25% in coniferous wood.

Figure 3.6 Transverse sections of Norway spruce wood a) Untreated wood incubated with *Coniophora puteana*. Note: fine hyphae (arrows) within the cell lumina of tracheids; Bar = 50 µm. b) Untreated wood spruce incubated with *Poria placenta*. Note: abundant, fine - large hyphae (arrows); Bar = 50 µm. c) Densified wood incubated with *Poria placenta*. Note: Early wood tracheids are only slightly compressed. Hyphae (arrows) growing within the cell lumen of xylem ray parenchyma; Bar = 10 µm. d) Untreated wood incubated with *Gloeophyllum trabeum*. Note: Formation of bore holes and cavities (arrows) within the secondary walls of tracheids; Bar = 10 µm.

Despite of inducing significantly lower weight losses, THM-densification did not show improvements in durability when compared to untreated controls taking into account the density of the specimens.
3.2 Total protein content

3.2.1 Results

3.2.1.1 Chitin

The chitin content in decayed wood is usually expressed in relation to the dry mass of wood. The chitin assay had an average background level of 0.28 mg/g, which was subtracted from all values in the analysis. These corrected values increased with increasing weight loss of the wood samples after 16 weeks incubation with brown rot fungi.

For all wood decay fungi the highest chitin content was determined in TH-treated (160 and 180°C) wood. The lowest amount of chitin occurred in THM-densified wood post-treated at 180°C. All wood decay fungi also showed differences in the amount of detected chitin (Fig. 3.7). Wood specimens incubated with *P. placenta* had the lowest chitin content in the controls and in TH- and THM-treated wood specimens. The highest chitin content was recorded in specimens incubated with *G. trabeum*.

Figure 3.7 Chitin content in TH- and THM-treated Norway spruce wood specimens after 16 weeks incubation with three brown rot fungi. (n=5) Bars, standard deviation. Columns marked with an asterisk denote significant difference in comparison to untreated control (p< 0.01).
The major dependency between the chitin content and the weight loss induced by the fungus is apparent in Figure 3.8. The weight losses in wood specimens are proportional to the biomass of the fungal species. For all test organisms the amount of fungal biomass increases with the increasing weight losses.

### 3.2.1.2 Ergosterol

For untreated controls, only specimens incubated with *P. placenta* showed a low amount of ergosterol. Ergosterol of *G. trabeum* was recorded in TH160, THM 140 and THM 160 treated wood. Ergosterol of *Coniophora puteana* was determined in THM 140 and THM 180 treated wood and for *P. placenta* in TH 180 and THM 140 Norway spruce specimens respectively.

#### Table 3.2 Ergosterol content (µg/g) in untreated controls, TH- and THM-treated spruce wood after 16 weeks incubation with brown-rot fungi (n=1).

<table>
<thead>
<tr>
<th></th>
<th><em>G. trabeum</em></th>
<th><em>C. puteana</em></th>
<th><em>P. placenta</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>20.4</td>
</tr>
<tr>
<td>TH 160°C</td>
<td>76.55</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TH 180°C</td>
<td>0</td>
<td>0</td>
<td>46.00</td>
</tr>
<tr>
<td>THM 140°C</td>
<td>94.20</td>
<td>38.30</td>
<td>60</td>
</tr>
<tr>
<td>THM 160°C</td>
<td>59.43</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>THM 180°C</td>
<td>0</td>
<td>82.55</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 3.2 Positive (+) and negative (-) isolations of casual decay fungus from TH- and THM-treated Norway spruce wood specimens used for ergosterol determination.

<table>
<thead>
<tr>
<th></th>
<th>Gloeophyllum trabeum</th>
<th>Coniophora puteana</th>
<th>Poria placenta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>TH 160°C</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TH 180°C</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>THM 140°C</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>THM 160°C</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>THM 180°C</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Additional experiments were conducted in order to confirm the presence of fungal hyphae in wood specimens. The isolates were excised to determine the activity of the fungi. Shavings of specimens were placed onto Petri dishes with MEA and the growth activity of the causal decay fungi were monitored over a period of 3 weeks. Identification of cultures was confirmed using mycelial character as observed on plates of MEA. *Gloeophyllum trabeum* was present in all THM-treated (140, 160, 180°C) wood specimens, *C. puteana* exclusively occurred in THM 160 and THM 180, and *P. placenta* was the only species that could be isolated from untreated controls and thermally-treated (TH 180) Norway spruce wood (Table 3.3).

### 3.2.1.3 Cellulose after brown rot degradation

Cellulose content (analysed with TGA) was assessed on TH and THM-treated wood specimens after 16 weeks incubation with brown rot fungi. All samples revealed a certain degree of cellulose degradation by the enzymatic system of the brown rot fungi (Fig. 3.9). The lowest cellulose content was recorded for untreated controls incubated with *C. puteana*. THM-treated specimens incubated with *P. placenta* showed a slightly higher percentage of cellulose, whereas TH-treated specimens had the highest cellulose content comparable to that of untreated specimens. No remarkable difference in cellulose content has been recorded within THM-treated specimens (THM 140, THM 160 and THM 180).
3.2.2 DISCUSSION

A comparison of the recorded weight losses after 16 weeks incubation with brown rot fungi (Fig. 3.2) and the total chitin content showed that *G. trabeum* produced the highest biomass in all TH- and THM-treated specimens (Fig. 3.7). *Coniophora puteana* caused the highest weight losses in Norway spruce; however it did not show the highest amount of biomass in tested specimens. This may be explained by the morphological differences between the three fungal species used for the incubation of wood blocks according to EN 113 (EN113, 1996). One of the factors which presumably affects the chitin assay results is the hyphal diameter of the fungal species. Due to occlusion of the cell lumina after densification, fungal growth and colonization of wood was suppressed.

Our previous studies show that some of the tested fungal species produce small diameter hyphae that enabled them to enter partially occluded cells (Schwarze and Spycher, 2005). The lowest chitin content and weight losses did not exceeding 25 % in untreated controls were recorded for *P. placenta* which developed the largest diameter hyphae in wood (1-9 µm) (Fig. 3.2). *Gloeophyllum trabeum*, which produced larger diameter hyphae than *C. puteana* (1-5
µm vs. 0.5-2 µm, Table 3.1) could also adapt to adverse conditions by switching its degradation mode to hyphal tunnelling. This strategy allowed it to colonise larger areas within the wood specimens, which was indicated by increased chitin content compared to *C. puteana*.

Another explanation for lower biomass recorded for *C. puteana* is that mycelia formed at later stages of degradation contained minor amounts of chitin. This is also supported by the observation that brown and white rot fungi also colonize the wood with wall-less hyphae (Palmer *et al.*, 1984). Thus as a result of the depletion of available nitrogen sources at the later stages of degradation, autolysis of the older parts of the hyphae may occur (Levi and Cowling, 1969). Furthermore, self-parasitism as described by Rayner and Boddy (1988) may possibly result in a reduction of chitin content during more advanced stages of decomposition, allowing reallocation of resources between different development phases. The phenomena of self-parasitism has been mentioned in the literature for different Trichoderma spp. and for Stereum hirnsum (Rayner and Boddy, 1988), although it was never observed for any of the species included in this test. In this regard, further studies are necessary in order to gain more information on the brown rot decay fungi and variability in their development patterns.

TGA analysis of cellulose demonstrated that the induced substrate modification is partially attributed to the effects of thermal treatment during THM-modification of wood specimens (see 1.6.2, Fig. 1.11) and, to a less extend, to decomposition by decay fungi. The higher cellulose content of THM-densified wood incubated with *C. puteana* may be explained by the exceptional substrate specificity of the enzymatic systems of the fungus hence the inability to degrade cellulose modified by thermal treatments. This assumption appears to be supported by the fact that in untreated controls *C. puteana* induced the highest weight losses and showed the lowest cellulose content although no alterations in composition of chemical constituents were recorded.

Ergosterol determination, which is used for quantification of biomass in living hyphae, was performed on the specimens after they have been oven dried for the estimation of WL. This methodological inaccuracy might be the reason for inconsistent obtained values presented in Table 3.2. Despite the fact, that the presence of fungal mycelia was confirmed and assessed in all specimens by the chitin assay, ergosterol was not detected in all tested samples (Table 3.2). Positive results of isolations together with high amounts of detected ergosterol might lead to assumption that fungi may circumvent adverse conditions in the deeper parts of THM-densified wood that has been also reported in previous studies of Xie *et al.* (1997). Gloeophyl-
lum trabeum has been reported to show resistance against prolonged desiccation in low moisture environments (10 years in air dry wood at 12% moisture content) (Cartwright and Findlay, 1958). Coniophora puteana, however, was not viable after wood blocks were incubated for 81 days at 21.5% relative humidity (Rayner and Boddy, 1988). Production of chlamydospores by G. trabeum and P. placenta (Stalpers, 1978) may explain positive isolations after 12 months after incubation. Alternative explanations could be related to the fact that the amount of ergosterol of the remaining hyphae was below the detection limit (Eikenes, 2005) or that the hyphae did not contain ergosterol due to metabolic degradation, autolysis and oxidation after cell death (Bjurman and Viitanen, 1996). Additional experiments have to be conducted in the field of biomass quantification; particular attention should be paid to implementation of existing techniques to a modified substrate of THM-densified wood.
3.3 Resistance to White Rot

3.3.1 Results

After 16 week incubation most wood blocks were completely colonised by surface mycelium as assessed by EN 113 (1996). Beech wood showed lower resistance to white rot decay than wood of Norway spruce (Figs. 3.10 and 3.11). The dry weight losses were significantly higher in the case of beech wood (Fig. 3.10). The highest weight losses for beech were recorded in untreated controls, TH-treated (160 and 180°C) and densified specimens without post treatment incubated with *Trametes versicolor* (Fig. 3.10). All THM-treatments of beech (140, 160, 180/80, 180°C) induced significantly lower dry weight losses compared to untreated controls for both test fungi.

![Figure 3.10](image_url)

*Figure 3.10 Dry weight losses of beech and Norway spruce wood specimens in untreated, TH-treated wood (160 and 180°C), densified wood without thermal post-treatment, THM-densified wood (140, 160, 180°C and 180°C/80% RH) after 16 weeks incubation with *Trametes versicolor*. (n=6) Bars, standard deviations. Columns marked with an asterisk denote significant differences in comparison to untreated controls (p< 0.01).*

The lowest weight losses in Norway spruce wood occurred in all variations of THM-densification treatments (Figs. 3.10 and 3.11). The moisture content of beech specimens after
16 weeks incubation with white rot fungi was in the range of \(70 \pm 10\%\) for TH-treated and \(35 \pm 10\%\) for THM-treated specimens of beech, whereas that of TH- and THM-treated specimens of Norway spruce was \(58 \pm 8\%\) and \(30 \pm 5\%\) respectively.

Decay susceptibility, which is considered to be a more reliable value when comparing specimens of different density (Nilsson and Daniel, 1992), was calculated according to Eq. 2.3. All treatments of Norway spruce wood included in the study showed a higher decay susceptibility against both \(T. versicolor\) and \(T. pubescens\) compared to untreated controls. However, for beech wood a significant improvement of decay susceptibility was recorded for THM-densified (180 and 180/80) beech wood specimens incubated with \(T. pubescens\) (Fig. 3.12). All the remaining treatment/fungus combinations did not lead to significant changes in susceptibility, even more, densified and non-post treated beech incubated with \(T. pubescens\) showed a two-fold increase in decay susceptibility when compared to controls (Fig. 3.12).

![Figure 3.11](image_url)

**Figure 3.11** Dry weight losses of beech and Norway spruce wood specimens in untreated controls, TH-treated wood (160 and 180°C), densified wood without thermal post-treatment, THM-densified wood (140, 160, 180°C and 180°C/80% RH) after 16 weeks incubation with \(T. pubescens\). (n=6) Bars, standard deviations. Columns marked with an asterisk denote significant differences in comparison to untreated control (p<0.01).
Figure 3.12 Decay susceptibility of beech and Norway spruce wood specimens in untreated controls, TH-treated wood (160 and 180°C), densified wood without thermal post-treatment, THM-densified wood (140, 160, 180°C and 180°C/80%RH) after 16 weeks incubation with *Trametes versicolor* and *Trametes pubescens*. (n=6). Bars, standard deviations. Columns marked with an asterisk denote significant difference in comparison to untreated control (p< 0.01).
3.3.2 DISCUSSION

Control specimens of beech and Norway spruce wood, were completely colonized by surface mycelium after 16 weeks incubation. Microscopical studies revealed the presence of both types of white rot. Selective delignification was mostly associated with *T. pubescens* (Figs. 3.13 b, d), whereas *T. versicolor* induced a simultaneous rot, characterized by cell wall erosion and formation of bore holes in the secondary wall of tracheids (Figs. 3.13 a, c). This observation is in good agreement with previous studies of Schwarze and Baum (2000) and Schwarze (2007). In selective delignification, lignin and hemicelluloses are degraded earlier in the decay process than cellulose. Delignification of the secondary walls commenced from within the lumen towards the middle lamella and occurred in the immediate vicinity to hyphae growing on the surface of the cell lumen (Fig. 3.13 a) Cell wall delignification resulted in a distinct colour change of inner secondary wall from light blue to dark blue (Figs. 3.13 a, d) At a more advanced stage of degradation, selective delignification resulted in the separation of the outer secondary wall from the compound middle lamella, so that the cells tended to separate from one another (Fig. 3.13 b). The first effects on outer secondary wall were located in the area between cells, whereas cell wall corners resisted degradation the longest (Fig. 3.13 b). At the advanced stage of decay, localised degradation of lignin, hemicellulose and cellulose resulted in the formation of small cavities within secondary wall (S2) of fibre tracheids in beech (Fig. 3.13 c).

Formation of cavities in the cell walls of wood artificially infected with selected white rot basidiomycetes which was previously reported by Schwarze and Engels (1998) was reminiscent of a soft rot attack. In the past, cavity formation in the secondary wall of lignified cell walls was regarded as a distinct, relatively reliable character of a soft rot, which can be used to readily differentiate it from other modes of degradation (Hale and Eaton, 1985; Hale and Eaton, 1985; Eriksson *et al.*, 1990; Zabel and Morrell, 1992). The distinguishing feature of soft rot is its pattern of development, involving T-branching and L-bending (Nilsson *et al.*, 1989). The observed phenomena, however, differed from a soft rot mode of attack as T-branching, L-bending and hyphal growth was not ascertained within the cell wall. Atypical for a soft rot, or any other commonly described degradation pattern, substantial selective delignification of the cell wall always proceeded cavity formation.

The factors that appear to be most decisive in governing the delignification of THM-densified wood by white rot fungi are the quantity and type of lignin in tree species (Blanchette, 1991;
Schwarze et al., 2004). Conifer wood has a high concentration of guaiacyl lignin and the S₃ layer of tracheids and is very resistant to white rot fungi that cause a simultaneous rot (Liese, 1961; Blanchette et al., 1988; Nilsson et al., 1989; Obst et al., 1994), whereas beech wood that consists predominantly of syringyl-guaiacyl rich fibre tracheids is more susceptible to the latter type of decay (Baum et al., 2000; Schwarze et al., 2000).

![Figure 3.13](image_url)

**Figure 3.13** a) TS of Norway spruce wood incubated with *Trametes pubescens*. Note early stages of selective delignification in secondary walls by hyphae (arrows) growing in the cell lumina of tracheids results in a distinctive colour change. b) Transverse section (TS) of untreated beech wood incubated with *Trametes versicolor* showing selective delignification by hyphae (arrows) growing within the cell lumen. Note separation (*) of fibre tracheids from one another at advanced stages of degradation. c) TS of beech wood incubated with *Trametes versicolor* showing selective delignification in secondary walls of fibre tracheids caused by hyphae (arrows) growing in the cell lumen. Note small cavities in the secondary walls, that are separated from one another by radial structures (pointers). d) TS of Norway spruce wood after TH treatment at 180°C and incubation with *Trametes pubescens*. Note selective delignification commenced by hyphae growing on the S3-layer within the cell lumen (arrows).
Difference in the lignin composition may partly explain higher weight losses recorded in beech wood specimens incubated with *T. versicolor* and *T. pubescens*.

Direct correlation of the decay behaviour of various woods and syringyl content of in the lignin polymer is complex (Obst *et al.*, 1994) because many significant differences exists in amount of extractives, the composition and distribution of polysaccharides, the amount of lignin, the distribution of lignin, the distribution of S and G units in the lignin, the relative proportion of fibres, vessels and parenchyma cells, cell wall thickness, vessel element size, the presence of tyloses and the ratio of earlywood to latewood.

Microscopical examination of THM treated wood showed that differences between treatments regarding weight loss could be partly attributed to the restriction of fungal growth by the occlusion of the tracheids lumina. In wood subjected to THM 180 treatment, fungal hyphae were absent from latewood tracheids with completely occluded lumina of and only occurred in the partially occluded lumina of earlywood tracheids.

Bore holes formation within tracheids is another means by which certain wood decay fungi may be able to grow through wood where the lumina is inaccessible (Rayner and Boddy, 1988; Schwarze *et al.*, 2004). *Trametes pubescens* could circumvent conditions restricting fungal growth by producing bore holes in fibre tracheids of beech wood (Fig. 3.14 c) and *T. versicolor* was recorded to form bore hyphae that transversely penetrated cell walls of early wood tracheids in Norway spruce wood. Such adapting behaviour of decay fungi in adverse conditions is known under the term *plasticity of metabolism* and has been mentioned before by (Schwarze *et al.*, 1995; Schwarze *et al.*, 2000; Schwarze and Spycher, 2005).

Previous study (Skyba *et al.*, 2008) on soft-rot resistance of THM-densified wood indicated that a pronounced effect of THM treatment was only recorded in the superficial areas of the wood specimens. The same phenomenon was observed in this study where hyphae were not detected in deeper regions of the wood sections and cell wall degradation was restricted to outer surfaces of THM-densified wood.

Many fungal species cause simultaneous rot in hardwoods but only rarely in conifers (Schwarze, 2004). This preferential selection can be related to the extremely resilient S₃ layer of tracheids that hampers degradation by hyphae from within the cell lumen outwards. By contrast, low molecular weight substances causing brown rot and selective delignification, simply diffuse through the S₃ layer of the secondary wall (Koenigs, 1974; Murmanis and
Highley, 1987; Kerem et al., 1999; Schwarze and Spycher, 2005). THM treatment may modify the chemical composition of S3 layer and render it less resilient to penetration of fungal enzymes and hence increase the susceptibility of softwood to white rot degradation.

Figure 3.14 a) TS of THM densified Norway spruce post treated at 180°C incubated with *Trametes versicolor*. Note bore hyphae (arrow) growing transversely through the cell wall of earlywood tracheids. b) TS of THM densified Norway spruce post treated at 160°C incubated with *Trametes versicolor*. Note bore hyphae (arrow) branching within the secondary wall. c) TS of THM densified beech wood post treated at 160°C incubated with *Trametes pubescens*. Note cavities (arrows) within the secondary walls of fibre tracheids.

Decay susceptibility calculated taking into account dry weight losses and density of tested specimens did not reveal any improvement of the decay resistance in Norway spruce and only a slight reduction of susceptibility of beech wood against *T. pubescens* (Fig. 3.12). This fact questions the whole idea of wood modification by compression in general and THM-densification in particular. This issue will be discussed further in Chapter 6.
3.4 Resistance to Soft Rot

3.4.1 Results

3.4.1.1 Soil-Bed Test

THM wood post-treated at 140°C compressed the vessels of beech wood to a radial width of 20 - 24 µm to 2 - 7 µm in the late- and to 5 - 10 µm in the early wood. After post treatment at 180°C the corresponding values were 0.5 - 5 µm in the latewood and 4 - 8 µm in the early wood. Occlusion of the cell lumina of tracheids in spruce wood was within the range previously described in 3.1.1.

After 32 weeks the lowest dry weight losses were recorded in wood treated with CC (Figs. 3.15 and 3.16). All specimens modified by THM-treatment showed an inverse relationship between susceptibility to fungal degradation and increase in density (Figs. 3.15 and 3.16). In beech wood most THM-treatments resulted in a significant reduction in weight losses (p<0.05) by soft rot during initial stages of soil exposure but after 32 weeks failed to inhibit degradation (Figs. 3.15 and 3.16). In spruce wood all THM treatments resulted in a significant reduction (p<0.05) in weight losses regardless of the incubation period.

The density of wood specimens exposed to TH-treatment was slightly reduced in comparison to untreated controls (Table 2.2) and resulted in higher weight losses by soft-rot fungi than in the untreated controls in both beech and spruce wood specimens after 32 weeks incubation.

Fungal colonisation and degradation

In THM and TH-treated wood of spruce and beech the impact of thermal treatment resulted in distinct alterations in the micro-morphology of cell walls in close proximity to the specimen surface. The combustion of cellulose and hemicelluloses resulted in a conspicuous reddish appearance of the outer cell rows due to staining of lignin with safranine (Fig. 3.19). Moreover, loss of birefringence was apparent from the cell walls in this region which appeared dark when viewed between crossed Nicol prisms. In THM-treated wood of spruce modified regions were superficial and were merely recorded in a depth of 40 - 45 µm, whereas in beech modifications by thermal treatment were more extensive and apparent in a depth of 170 - 220 µm (Fig. 3.19). Even in cell regions close to the surface, parenchyma cells of multiseriate xylem rays in beech wood did not appear to be affected by heat treatment and cellulose maintained a distinct birefringence.
Results of decay susceptibility for beech and Norway spruce wood after 32 weeks incubation in soil are presented on Figures 3.17 and 3.18. For Norway spruce wood the lowest values occurred for CC –impregnated specimens. TH-treated specimens did not show any significant difference to controls. The highest decay susceptibility, however, was recorded for densified and THM-densified Norway spruce specimens.

Beech wood specimens of both dimensions included in the test showed to be susceptible to soft rot attack. All “bigger” TH and THM-densified specimens (75 x 10 x 5 mm$^3$) did not reveal any significant difference from untreated controls; even preservative treated test blocks (CC 0.4) did not differ from reference controls. Only in case of higher concentration of preservative (CC 1.6) susceptibility to soft rot was decreased (Fig. 3.18). The “smaller” specimens were all susceptible to soft rot degradation, whereas THM-densified specimens occurred...
to be highly susceptible showing significantly higher values compared to untreated controls (Fig. 3.18).

Figure 3.16 Dry weight losses of Norway spruce wood specimens in untreated controls, TH-treated wood (160 and 180°C), densified wood without thermal treatment, THM-densified wood (140, 160, 180°C and 180°C/80% RH) and CC (0.4 and 0.16 w/w %) impregnated wood incubated for 8, 16, 24 and 32 weeks. (n=6) Bars show standard deviations. Columns marked with asterisk show significant difference in comparison to untreated control (p< 0.01).
Figure 3.17 Decay susceptibility of Norway spruce wood specimens (45 x 10 x 5 mm³ and 30 x 10 x 5 mm³) in TH-, THM-treated and CC (0.4 and 0.16 w/w %) impregnated wood incubated with soil according to EN 807 for 32 weeks. (n=6) Bars, standard deviations. Columns marked with asterisk show significant difference in comparison to untreated reference (p<0.01)
Figure 3.18 Decay susceptibility of beech wood specimens (75 x 10 x 5 mm³ and 30 x 10 x 5 mm³) in TH-, THM-treated and CC (1.6 and 0.4 w/w %) impregnated wood incubated with soil according to EN 807 for 32 weeks. (n=6) Bars, standard deviations. Columns marked with asterisk show significant difference in comparison to untreated reference (p<0.01)
3.4.1.2 Optional Soft Rot Screening Test

Values recorded for Norway spruce wood specimens in optional screening test revealed after 16 weeks of incubation slightly higher weight losses in untreated controls compared to specimens incubated in soil (Figs. 3.16 and 3.19). TH 160 together with all THM-treated Norway spruce specimens showed a higher resistance; however, no significant improvement of soft rot resistance was recorded for TH180 treated specimens.

![Dry weight losses of spruce wood specimens in TH- and THM-treated wood incubated with soft rot fungi in vermiculite for 16 weeks (n=6) Bars, standard deviations. Columns marked with asterisks show significant difference in comparison to untreated reference (p<0.01).](image)

**Figure 3.19** Dry weight losses of spruce wood specimens (30 x 10 x 5 mm³) in TH- and THM-treated wood incubated with soft rot fungi in vermiculite for 16 weeks (n=6) Bars, standard deviations. Columns marked with asterisks show significant difference in comparison to untreated reference (p<0.01).

Soft rot fungi in the optional screening test induced more pronounced effect on beech specimens, compared to those embedded in soil (Figs. 3.15 and 3.20). Weight losses recorded for untreated controls were approx. 2-fold higher (35 % in screening test vs. 16% in soil-bed test) when inoculated with pure cultures. Both TH 160 and TH 180 treatments induced the highest weight losses in beech wood (Fig. 3.19). At the same time, THM-densification rendered beech wood more resistant compared to controls.
Figure 3.20 Dry weight losses of beech wood specimens (30 x 10 x 5 mm³) in TH- and THM-treated wood incubated with soft rot fungi in vermiculite for 16 weeks. (n=6) Bars, standard deviations. Columns marked with asterisks show significant differences in comparisons to untreated reference (p<0.01).

Figure 3.21 Moisture content in TH- and THM-treated spruce and beech wood specimens (30 x 10 x 5 mm³) in wood incubated with soft rot fungi in vermiculite for 16 weeks. (n=6) Bars, standard deviations.
Moisture content of wood specimens, which is one of the major factors influencing decay activity, was markedly higher for untreated and TH-treated spruce wood compared to the same treatments of beech. Reverse effect occurred in THM-densified wood where beech specimens had a relatively higher MC. Sterile controls (untreated specimens that were buried in vermiculite but not inoculated with spore suspension) had much lower MC, that could be compared to that of THM treated specimens (Fig. 3.21).

### 3.4.2 Discussion

In control specimens of beech and spruce wood, hyphae had completely colonized the wood specimens. Cell wall degradation by soft-rot fungi was induced either by formation of cavities by hyphae in the S₂ layer (type 1) or erosion troughs (type 2). Soft rot type 1 was characterized as a series of successive cavities with conical pointed ends which followed the direction of microfibrils within S₂ layer (Fig. 3.22). Soft rot type 1 was most prevalent in control and treated wood specimens of spruce. The latter observation is in good agreement with former studies showing that soft rot type 1 occurs in secondary walls with high concentration of guaiacyl, whereas soft rot type 2 is often associated with syringyl rich cell walls (Liese, 1961; Nilsson et al., 1989; Anagnost, 1998; Schwarze, 2004). Norway spruce wood has a high concentration of guaiacyl, and thus is moderately resistant to soft-rot fungi (Liese, 1970; Blanchette et al., 1988; Nilsson and Daniel, 1990; Baum et al., 2000), whereas wood of beech that consists predominantly of syringyl-rich fibre tracheids is which is more susceptible to soft-rot fungi (Baum et al., 2000; Schwarze and Baum, 2000; Schwarze, 2004). The latter differences in the lignin composition partly explains the higher weight losses recorded by soft-rot fungi in beech wood.

Interestingly, soft rot type 2 was exclusively observed in TH-treated wood of spruce (Fig. 3.23). Former studies show that many species that only cause soft rot type 2 in hardwoods, failed to exhibit any decay features or weight losses in softwoods (Anagnost, 1998). One reason for this selection process appears be related to the extremely resilient S₃ layer of tracheids that hampers degradation by hyphae from within the cell lumen outwards (Liese, 1970; Schwarze, 2004). This does not deter brown rot fungi, which are able to degrade the cell wall by means of diffusible secretions from hyphae within the tracheid lumen, but a resistant S₃ layer is a considerable barrier to fungi that cause soft rot type 2 or a simultaneous rot (Schwarze, 2004). In TH-treated wood it seems that thermal treatment induces chemical al-
interations in the S₃ layer which strongly reduces its resistance to soft-rot type 2 attack i.e. formation of erosion troughs by hyphae growing within the lumen (Fig. 3.23).

The optional soft rot test is conducted in order to screen the effect of pure soft rot cultures on the wood specimens and to relate it to the test unsterile soil. Higher weight losses induced by soft rot in all beech and Norway spruce specimens in screening test after 16 weeks, proved that most of the degradation in soil substrate is attributed to soft rot.

Figure 3.22 a) Tangential longitudinal section (T.L.S.) of TH-treated beech wood at 160°C. Note reddish staining of fibre-tracheids and multiseriate xylem ray parenchyma (arrows) with safranin astra-blue due to combustion of polysaccharides. b) Transverse section (T.S.) of TH-treated beech wood showing cavities (arrows) within secondary walls of fiber-tracheids. c) T.S. of untreated Norway spruce wood showing cavities (arrows) within the secondary walls of tracheids. d) T.L.S. of untreated Norway spruce showing bore holes (pointers) and lenticular cavities (arrows) with conical ends that follow the orientation of microfibrils in secondary walls of tracheids.
In THM-treated spruce wood colonisation of tracheids by soft-rot fungi was evidently hampered by densification. Hyphae were not detected in deeper parts of the wood specimens and cell wall degradation was restricted to the outer surface of the wood specimens (Fig. 3.23). Interestingly, THM–treated beech wood post treated at 180°C showed similar weight losses as controls. Thus, even a complete occlusion of cell lumina could not inhibit decay by soft-rot fungi as the occlusion was simply counteracted by directional growth within the cell wall (Fig. 3.23).

Figure 3.23 a) T.S. of TH-treated Norway spruce wood showing soft rot type 2 attack in secondary walls of tracheids. Note formation of hyphal sheaths and cell wall erosion troughs (arrows). b) T.S. of THM-treated beech wood post-treated at 160°C showing soft rot attack within a multiseriate xylem ray. Note complete degradation of the secondary walls (arrows) in xylem ray parenchyma. c) T.L.S. showing THM-treated spruce wood post-treated at 180°C showing non-occluded xylem ray parenchyma cells. Note: cavity formation (arrows) in the secondary walls of xylem ray parenchyma. d) T.S. of THM-treated spruce wood post treated at 180°C showing cavities within the secondary walls of tracheids (arrows).
Another important factor affecting resistance of THM-treated wood is the impact of the wood structure on compression and vice versa on hyphal colonisation and degradation. Norway spruce wood has a very homogenous structure and consists predominantly of axially aligned tracheids (90 – 95 %) and a low proportion of radially aligned uniseriate xylem rays (Panshin and Zeeuw, 1970). Thus, in THM-treated wood of spruce, post treated at 180°C most cells were almost completely occluded, regardless whether wood specimens were compressed in the radial or tangential direction. In contrast the structure of beech wood is more heterogeneous as it consists of vessels, fibre-tracheids, axial parenchyma and prominent multiseriate xylem rays. In THM-treated wood of beech, post treated at 180°C the multiseriate xylem rays counteracted compression in the radial direction and cell lumina of xylem ray parenchyma were only slightly occluded, facilitating hyphal colonisation and access from the surface of the deeper wood regions. Thus, the strongest soft rot attack in beech was observed in the secondary walls of xylem ray parenchyma and in fibre tracheids in close proximity to multiseriate xylem rays (Fig. 3.23).

The adverse affect of TH-treatment on wood resistance was previously also recorded for brown rot fungi (Schwarze and Spycher, 2005). It is well established that thermal treatment decreases the amount of accessible hydroxyl groups and results in a reduction in the hygroscopicity of treated wood (Tjeerdsma et al., 2000). TH treatment contributes to depolymerisation of polysaccharides and shifts the $T_g$ of lignin (Heger et al., 2003). However, in the present study, the moisture content of incubated TH-treated wood specimens was found to be well above the fibre saturation point. During incubation higher moisture content developed in spruce than in beech wood specimens. After eight weeks, the moisture content of the modified wood specimens was in the range of 130 ± 10% for TH-treated and 48 ± 10% for THM-treated beech specimens, whereas TH- and THM-treated spruce wood specimens had a moisture content of 225 ± 40% and 51 ± 15% respectively.

The results show that before selecting potential wood species for THM-treatment for utility class 4 it is essential to consider whether the lignin composition and anatomical features such as multiseriate xylem rays may promote wood colonisation and degradation by soft-rot fungi despite thermal treatment and/or considerable increases in density.
3.5 Resistance to Pyrophillic Fungi

3.5.1 Results

According to the EN 113 criteria (EN113, 1996) the majority of wood blocks were completely colonized by surface mycelium of *D. loculata* after 8 weeks incubation regardless of the treatment method applied (Fig. 3.24). For each wood species, the weight losses were significantly different (p<0.05) amongst various treatments (Fig. 3.25). For all hardwood species, the highest weight losses were recorded from untreated controls. The lowest weight losses were recorded from wood thermally treated at higher temperatures. For thermally treated Norway spruce wood significantly higher weight losses were recorded than from controls (Fig 3.25).

![Figure 3.24 Untreated controls and thermally treated maple wood specimens (Forte and F-exterior) after 8 weeks incubation with Daldinia loculata. Note that all specimens were colonised by surface mycelium whereas the degree of colonisation was higher for the untreated controls.](image)

Control specimens of beech, ash and maple showed comparable weight losses after 8 weeks, whereas controls of Norway spruce wood had 9-fold lower mass losses. Among all treatments the most effective was “forte-exterior” treatment for beech, ash and maple, which was also the treatment conducted at the highest temperature (Fig. 3.25). Thermally treated spruce, on the contrary, appeared to be more susceptible to colonization by *D. loculata*. That can be confirmed by visual observation of Fig. 3.26 showing sparse growth of surface mycelium on control specimens and strong colonisation of thermally treated ones. Recorded dry weight losses confirmed this fact quantitatively occurring to be substantially higher in thermally treated wood.
Figure 3.25 Dry weight losses of thermally treated wood specimens of beech, ash, maple and spruce after 8 weeks incubated with *Daldinia loculata*. (n=6) Bars show standard deviation. Columns marked with asterisk indicate significant difference in weight losses compared to untreated control within the group of the same wood species (p < 0.05).

Figure 3.26 Untreated controls and thermally treated spruce wood specimens after 8 weeks incubation with *Daldinia loculata*. Note sparse growth of surface mycelium on control specimens, whereas treated specimens (Spruce II) are strongly colonized.
3.5.2 DISCUSSION

Differences in weight losses induced by *D. loculata* attributed mostly to treatment temperature and conditions. No difference in decay resistance has been recorded between beech, ash, maple after “forte-exterior” treatment. In Norway spruce *D. loculata* caused a soft rot type 2 wood producing erosion troughs in the secondary walls of tracheids (Fig. 3.27). The same has been observed by (Schwarze, 2007) for other soft rot causing species. Colonization and degradation of the *S*₃ layer appeared to be facilitated by thermal treatment inducing higher weight losses in comparison to untreated controls. Improved resistance of hardwood species against *D. loculata* could be partially attributed to chemical alterations of wood constituents caused by thermal treatments.

![Figure 3.27](image)

Figure 3.27  a) Transverse section (T.S.) of thermally treated spruce wood “Spruce II” after 8 weeks incubation with *Daldinia loculata* showing type 2 soft rot erosion troughs (arrows) in the secondary walls of tracheids. b) T.S. of sound maple wood incubated after 8 weeks with *Daldinia loculata* showing hyphae (arrows) on the *S*₃ layer of the secondary wall of libriform wood fibres.

Existing European and American test methods to assess the efficiency of traditional wood preservatives were developed primarily for solid wood. A standard method of testing wood preservatives is performed according to EN 113 (1996) and is based on weight losses induced after 16 weeks by the brown rot fungi *C. puteana, P. placenta* and *G. trabeum* and the white rot fungus *T. versicolor*. Development of useful and reproducible test methods to assess the wood durability of modified wood, such as thermally treated wood, may require the use of wood decay fungi that have adapted to heat-treated or pyrolysed wood. Despite our assumption about the substrate preference of *D. loculata*, the fungus did not reveal any pyrophillic
behaviour on thermally treated hardwood species (beech, ash, maple) presumably due to low temperatures of treatments used, compared to temperatures during fire (400-500°C). However, pyrophillic nature of *D. loculata* was obvious in thermally treated Norway spruce, where it induced significantly higher weight losses and caused a soft rot type 2. The unique properties of thermally treated wood and new endure requirements suggest that *Dalldinia* sp. have high potential as the new test organism, especially for softwoods, however more strains should be evaluated and a new methodology should be established.
CHAPTER 4

PHYSICAL AND MECHANICAL PROPERTIES

4.1 RESULTS

4.1.1 COMPRESSION-SET, DENSITY AND SPRING-BACK

THM-densification induced a high degree of densification in treated wood specimens. Compression sets of approx. 73% and 45% for Norway spruce and beech wood, respectively, were obtained with the THM-densification process (Table 4.1). The values differed within treatments depending on the morphology of each treated sample. Densification and post-treatment of Norway spruce resulted in almost complete occlusion of the cell lumina in both early- and latewood tracheids (Fig. 4.8).

The density of the specimens was significantly increased by THM-densification and slightly reduced by the TH-treatments. The maximum density of 1.32 g/cm³ for spruce wood and 1.24 g/cm³ for beech wood was achieved by THM 140 and THM 160 treatments respectively (Table 4.1).

Table 4.1 Compression set and density of THM-densified beech and spruce wood specimens (mean values, n=12).

<table>
<thead>
<tr>
<th>Treatment of wood</th>
<th>Compression set (%)</th>
<th>Density (g/cm³)</th>
<th>Compression-set recovery (%)</th>
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</table>

THM-densification followed by post-treatment induced the elimination of compression-set recovery. THM 180 treated specimens showed the lowest compression set recovery values.
(Table 4.1). Densified and not post-treated specimens had a strong tendency of spring-back, although they did not fully recover to their initial shape. Thus, Norway spruce samples only recovered to approx. 45%, whereas beech wood showed higher shape memory effect (approx. 62%).

### 4.1.2 Water Sorption

Table 4.2 EMC in THM-densified beech wood (*Fagus sylvatica*) as a function of relative air humidity at 20°C (mean values, n = 6).

<table>
<thead>
<tr>
<th>Relative air humidity (%)</th>
<th>Treatment/Density (g/cm³)</th>
<th>TH 160</th>
<th>TH 180</th>
<th>Densified</th>
<th>THM 140</th>
<th>THM 160</th>
<th>THM 180</th>
<th>THM 180/80</th>
<th>Control</th>
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<td>18.3</td>
<td>18.2</td>
<td>16.9</td>
<td>26.4</td>
</tr>
</tbody>
</table>

**Figure 4.1** Relationship between EMC, relative air humidity and treatment of THM-densified beech wood specimens at 20°C.

All TH- and THM-treatments altered sorption behaviour of beech wood, resulting in reduction of wood moisture content in humid environment. This effect was least pronounced in densified samples that were not post-treated (Fig. 4.1). However, the THM 180/80 treatment markedly reduced the hygroscopicity of beech specimens.
Table 4.3 EMC in THM-densified Norway spruce wood (*Picea abies*) as a function of relative air humidity at 20°C (mean values, n = 6).

<table>
<thead>
<tr>
<th>Relative air humidity (%)</th>
<th>Treatment / Density (g/cm³)</th>
<th>Equilibrium moisture content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TH 160</td>
<td>TH 180</td>
</tr>
<tr>
<td>35</td>
<td>5.9</td>
<td>5.5</td>
</tr>
<tr>
<td>50</td>
<td>7.8</td>
<td>7.2</td>
</tr>
<tr>
<td>65</td>
<td>10.9</td>
<td>10.1</td>
</tr>
<tr>
<td>80</td>
<td>16.7</td>
<td>15.1</td>
</tr>
<tr>
<td>93</td>
<td>18.7</td>
<td>18.8</td>
</tr>
</tbody>
</table>

Figure 4.2 Relationship between EMC, relative air humidity and treatment of THM-densified Norway spruce wood specimens at 20°C.

THM180/80 treatment of Norway spruce wood rendered it less sensitive to fluctuations in the relative humidity. Increasing temperatures applied during the post-treatment phase led to reduction of EMC and decreased the rate of moisture-uptake compared to untreated control samples (Table 4.3, Fig. 4.2).

4.1.3 Swelling

Maximum swelling of the densified wood specimens was affected by TH-post-treatment, which markedly decreased it. However, the lowest swelling was recorded for TH160 and TH 180 -treated beech (Tables 4.4 and 4.5) and Norway spruce specimens (Tables 4.6 and 4.7).
Table 4.4 Tangential swelling of THM-densified beech wood (*Fagus sylvatica*) as a function of relative air humidity at 20°C (mean values, n = 6).

<table>
<thead>
<tr>
<th>Relative air humidity (%)</th>
<th>Treatment</th>
<th>Tangential swelling (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TH 160</td>
<td>TH 180</td>
</tr>
<tr>
<td>35</td>
<td>1.25</td>
<td>0.85</td>
</tr>
<tr>
<td>50</td>
<td>1.68</td>
<td>1.44</td>
</tr>
<tr>
<td>65</td>
<td>2.58</td>
<td>2.37</td>
</tr>
<tr>
<td>80</td>
<td>6.29</td>
<td>6.02</td>
</tr>
<tr>
<td>93</td>
<td>8.69</td>
<td>8.59</td>
</tr>
</tbody>
</table>

Table 4.5 Radial swelling of THM-densified beech wood (*Fagus sylvatica*) as a function of relative air humidity at 20°C (mean values, n = 6).

<table>
<thead>
<tr>
<th>Relative air humidity (%)</th>
<th>Treatment</th>
<th>Radial swelling (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TH 160</td>
<td>TH 180</td>
</tr>
<tr>
<td>35</td>
<td>0.82</td>
<td>0.86</td>
</tr>
<tr>
<td>50</td>
<td>1.09</td>
<td>1.06</td>
</tr>
<tr>
<td>65</td>
<td>1.41</td>
<td>1.38</td>
</tr>
<tr>
<td>80</td>
<td>2.67</td>
<td>2.66</td>
</tr>
<tr>
<td>93</td>
<td>3.55</td>
<td>3.57</td>
</tr>
</tbody>
</table>

Table 4.6 Tangential swelling of THM-densified spruce wood (*Picea abies*) as a function of relative air humidity at 20°C (mean values, n = 6).

<table>
<thead>
<tr>
<th>Relative air humidity (%)</th>
<th>Treatment</th>
<th>Tangential swelling (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TH 160</td>
<td>TH 180</td>
</tr>
<tr>
<td>35</td>
<td>1.80</td>
<td>0.91</td>
</tr>
<tr>
<td>50</td>
<td>2.06</td>
<td>1.41</td>
</tr>
<tr>
<td>65</td>
<td>3.56</td>
<td>2.84</td>
</tr>
<tr>
<td>80</td>
<td>4.95</td>
<td>4.63</td>
</tr>
<tr>
<td>93</td>
<td>6.61</td>
<td>6.12</td>
</tr>
</tbody>
</table>

Table 4.7 Radial swelling of THM-densified spruce wood (*Picea abies*) as a function of relative air humidity at 20°C (mean values, n = 6).

<table>
<thead>
<tr>
<th>Relative air humidity (%)</th>
<th>Treatment</th>
<th>Radial swelling (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TH 160</td>
<td>TH 180</td>
</tr>
<tr>
<td>35</td>
<td>0.99</td>
<td>0.40</td>
</tr>
<tr>
<td>50</td>
<td>1.41</td>
<td>0.74</td>
</tr>
<tr>
<td>65</td>
<td>1.90</td>
<td>1.70</td>
</tr>
<tr>
<td>80</td>
<td>2.64</td>
<td>2.80</td>
</tr>
<tr>
<td>93</td>
<td>4.58</td>
<td>4.46</td>
</tr>
</tbody>
</table>
Figure 4.3 Radial and tangential swelling of untreated controls, TH- and THM-treated beech wood specimens at 20°C (mean values).
Figure 4.4 Radial and tangential swelling of untreated controls, TH- and THM-treated Norway spruce wood specimens at 20°C (mean values).
Densification without TH-post treatment did not markedly improve the swelling behaviour of both species; furthermore, the values recorded for the radial swelling are approx. 4 times higher than those recorded for controls.

### 4.1.4 Brinell hardness

Table 4.8 Density, Brinell hardness parallel and perpendicular to the grain (n=20) in untreated controls, TH- and THM-treated beech and spruce wood specimens (wood moisture content 13%).

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Density (g/cm³)</th>
<th>( \text{HB}_\perp \text{ radial} ) mean value (N/mm²)</th>
<th>( \text{HB}_\parallel \text{ longitudinal} ) mean value (N/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>0.65</td>
<td>33.5</td>
<td>79.3</td>
</tr>
<tr>
<td></td>
<td>TH 160</td>
<td>0.65</td>
<td>40.4</td>
<td>109.9</td>
</tr>
<tr>
<td></td>
<td>TH 180</td>
<td>0.67</td>
<td>40.2</td>
<td>100.7</td>
</tr>
<tr>
<td></td>
<td>Densified</td>
<td>1.14</td>
<td>137.8</td>
<td>180.8</td>
</tr>
<tr>
<td></td>
<td>THM 140</td>
<td>1.22</td>
<td>181.8</td>
<td>192.2</td>
</tr>
<tr>
<td></td>
<td>THM 160</td>
<td>1.24</td>
<td>183.8</td>
<td>188.5</td>
</tr>
<tr>
<td></td>
<td>THM 180</td>
<td>1.19</td>
<td>179.8</td>
<td>191.6</td>
</tr>
<tr>
<td></td>
<td>THM180/80</td>
<td>1.18</td>
<td>175.3</td>
<td>178.0</td>
</tr>
<tr>
<td>Beech</td>
<td>Control</td>
<td>0.36</td>
<td>16.2</td>
<td>47.4</td>
</tr>
<tr>
<td></td>
<td>TH 160</td>
<td>0.36</td>
<td>15.9</td>
<td>49.1</td>
</tr>
<tr>
<td></td>
<td>TH 180</td>
<td>0.35</td>
<td>9.4</td>
<td>32.6</td>
</tr>
<tr>
<td></td>
<td>Densified</td>
<td>1.27</td>
<td>127.8</td>
<td>159.3</td>
</tr>
<tr>
<td></td>
<td>THM 140</td>
<td>1.32</td>
<td>166.2</td>
<td>159.1</td>
</tr>
<tr>
<td></td>
<td>THM 160</td>
<td>1.3</td>
<td>151.2</td>
<td>159.6</td>
</tr>
<tr>
<td></td>
<td>THM 180</td>
<td>1.28</td>
<td>158.1</td>
<td>161.1</td>
</tr>
<tr>
<td></td>
<td>THM 180/80</td>
<td>1.26</td>
<td>157.5</td>
<td>169.9</td>
</tr>
<tr>
<td>Spruce</td>
<td>Control</td>
<td>0.36</td>
<td>16.2</td>
<td>47.4</td>
</tr>
<tr>
<td></td>
<td>TH 160</td>
<td>0.36</td>
<td>15.9</td>
<td>49.1</td>
</tr>
<tr>
<td></td>
<td>TH 180</td>
<td>0.35</td>
<td>9.4</td>
<td>32.6</td>
</tr>
<tr>
<td></td>
<td>Densified</td>
<td>1.27</td>
<td>127.8</td>
<td>159.3</td>
</tr>
<tr>
<td></td>
<td>THM 140</td>
<td>1.32</td>
<td>166.2</td>
<td>159.1</td>
</tr>
<tr>
<td></td>
<td>THM 160</td>
<td>1.3</td>
<td>151.2</td>
<td>159.6</td>
</tr>
<tr>
<td></td>
<td>THM 180</td>
<td>1.28</td>
<td>158.1</td>
<td>161.1</td>
</tr>
<tr>
<td></td>
<td>THM 180/80</td>
<td>1.26</td>
<td>157.5</td>
<td>169.9</td>
</tr>
</tbody>
</table>
Figure 4.5 Brinell hardness of untreated controls, TH- and THM-treated beech wood specimens. Columns marked with asterisk (*) denote significant differences in comparison to untreated controls (p<0.01).

Figure 4.6 Brinell hardness of untreated controls, TH- and THM-treated Norway spruce wood specimens. Columns marked with asterisk (*) denote significant differences in comparison to untreated controls (p<0.01).
In comparison to the radial direction, control specimens of beech and Norway spruce wood showed a hardness approx. 3-fold higher in the tangential direction. The highest HB values were recorded for all densified samples i.e. for post-treated and non post treated wood specimens. TH-treatments, on the contrary, did not noticeably affect Brinell hardness; hence both species did not show significant difference from controls. THM-densification in radial direction resulted in substantial improvement of HB and rendered spruce wood samples equally hard in both tangential and radial directions (Figure 4.6). Similar tendency was observed for beech wood specimens (Figure 4.5).

### 4.1.5 Compressive strength perpendicular to the grain

TH-treatments decreased both the compressive strength and MOE in compression of beech and Norway spruce specimens. THM-densification, on the other hand, substantially augmented compression strength by approx. 20-fold in spruce and approx. 3-fold in beech (Table 4.9). Significant improvement of compressive strength induced by THM-densification of Norway spruce is demonstrated in Figure 4.7. Two strain-stress curves on the figure represent behaviour of TH- and THM-treated specimens in the compression test.

Observations of the specimens behaviour under compression showed that till the very moment of fracture of the THM-densified specimen no evidence of any deformation are visible to unaided eye. TH-treated wood blocks, however, behave as control specimens developing cracks and deformations at the early stages of compression.
Figure 4.7 Strain-stress diagram of THM 180 and TH 180 Norway spruce wood specimens in compression test.
Table 4.9 Compressive strength perpendicular to the grain (σ_{max}, 2% max. compression) and the modulus of elasticity in compression (MOE) of THM-densified beech and Norway spruce wood (n=3, wood humidity 13%).

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>σ_{max} (N/mm²)</th>
<th>st. dev</th>
<th>Change (%)</th>
<th>MOE (N/mm²)</th>
<th>st. dev</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beech</td>
<td>Control</td>
<td>12.96</td>
<td>2.45</td>
<td>0</td>
<td>1388</td>
<td>86</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>TH 160</td>
<td>10.87</td>
<td>3.74</td>
<td>-16.1</td>
<td>1251</td>
<td>124</td>
<td>-9.9</td>
</tr>
<tr>
<td></td>
<td>TH 180</td>
<td>8.42</td>
<td>1.52</td>
<td>-35.0</td>
<td>986</td>
<td>120</td>
<td>-29.0</td>
</tr>
<tr>
<td></td>
<td>Densified</td>
<td>50.16</td>
<td>2.18</td>
<td></td>
<td>1658</td>
<td>127</td>
<td>19.5</td>
</tr>
<tr>
<td></td>
<td>THM 140</td>
<td>56.73</td>
<td>2.12</td>
<td>337.7</td>
<td>1763</td>
<td>102</td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td>THM 160</td>
<td>51.31</td>
<td>3.76</td>
<td>295.9</td>
<td>1742</td>
<td>147</td>
<td>25.5</td>
</tr>
<tr>
<td></td>
<td>THM 180</td>
<td>53.67</td>
<td>3.03</td>
<td>314.1</td>
<td>1884</td>
<td>115</td>
<td>35.7</td>
</tr>
<tr>
<td></td>
<td>THM 180/80</td>
<td>49.48</td>
<td>3.39</td>
<td></td>
<td>1818</td>
<td>152</td>
<td>31.0</td>
</tr>
<tr>
<td>Spruce</td>
<td>Control</td>
<td>3.45</td>
<td>1.22</td>
<td>0</td>
<td>1123</td>
<td>131</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>TH 160</td>
<td>3.25</td>
<td>1.43</td>
<td>-5.8</td>
<td>951</td>
<td>154</td>
<td>-15.3</td>
</tr>
<tr>
<td></td>
<td>TH 180</td>
<td>2.62</td>
<td>2.09</td>
<td>-24.1</td>
<td>973</td>
<td>203</td>
<td>-13.4</td>
</tr>
<tr>
<td></td>
<td>Densified</td>
<td>68.71</td>
<td>12.28</td>
<td>1891.6</td>
<td>2567</td>
<td>112</td>
<td>128.6</td>
</tr>
<tr>
<td></td>
<td>THM 140</td>
<td>83.3</td>
<td>7.64</td>
<td>2314.5</td>
<td>3470</td>
<td>165</td>
<td>209.0</td>
</tr>
<tr>
<td></td>
<td>THM 160</td>
<td>72.42</td>
<td>11.46</td>
<td>1999.1</td>
<td>3774</td>
<td>258</td>
<td>236.1</td>
</tr>
<tr>
<td></td>
<td>THM 180</td>
<td>85.39</td>
<td>9.17</td>
<td>2375.1</td>
<td>3078</td>
<td>123</td>
<td>174.1</td>
</tr>
<tr>
<td></td>
<td>THM 180/80</td>
<td>81.05</td>
<td>10.96</td>
<td>2249.3</td>
<td>3719</td>
<td>235</td>
<td>231.2</td>
</tr>
</tbody>
</table>

The elastic behaviour is more pronounced in spruce wood than in beech. It is advisable to further investigate elastic properties for the potential application of THM–densified wood.

4.1.6 Porosity

Table 4.10 Porosity of Norway spruce wood specimens.

<table>
<thead>
<tr>
<th></th>
<th>untreated</th>
<th>TH 180</th>
<th>THM 180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pore diameter (µm)</td>
<td>1.69</td>
<td>5.16</td>
<td>0.26</td>
</tr>
<tr>
<td>Total porosity (%)</td>
<td>77.02</td>
<td>86.01</td>
<td>9.98</td>
</tr>
<tr>
<td>Total pore volume (mm³/g)</td>
<td>583.3</td>
<td>125.1</td>
<td>49.5</td>
</tr>
<tr>
<td>Density (g/cm³)</td>
<td>0.42</td>
<td>0.34</td>
<td>1.36</td>
</tr>
<tr>
<td>Relative pore volume (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class 1 (1000-1 µm)</td>
<td>54</td>
<td>62</td>
<td>39</td>
</tr>
<tr>
<td>Class 2 (1-0.001 µm)</td>
<td>46</td>
<td>38</td>
<td>61</td>
</tr>
</tbody>
</table>
THM-densification induced major alterations in the wood porosity markedly reducing the mean pore diameter and total porosity values. However, TH180 treatment without densification resulted in a slight increase of the porosity (Table 4.10). The pore size distribution indicates the impact of the treatment. The trend could be observed by dividing the pore’s radius into two classes (class 1: \( r > 1 \mu m \) and class 2: \( r < 1 \mu m \)). The amount of class 1 pores increased after TH treatment and decreased after THM-densification; on the contrary, the relative volume of smaller (class 2) pores decreased after TH process. However, the THM-densification induced the increase of relative amount of class 2 pores (Table 4.10).

![Figure 4.8](image)

Figure 4.8 Transverse section of Norway spruce densified and post-treated at 180°C. a) Latewood tracheids. Note a complete occlusion of the cell lumina. b) Earlywood tracheids. Note that the cell lumina of most tracheids is completely occluded.

4.2 DISCUSSION

THM-treatment of Norway spruce and beech rendered both species much denser than most of the central European species. The differences in values for compression set and density between two species can be explained by structural dissimilarities of two selected species (higher resilience of the hardwood) and a unique nature of every single test specimen. Compared to the strong impact of the temperature during plasticization and compressive force during densification phase on the density of the final product, the influence of post-treatment conditions on density was not significant.

The impact of temperature, duration and degree of steam saturation during post-treatment phase was more important for the elimination of shape memory than any other step of the THM procedure. Post-treatment under 180°C appears to be the most conducive for the elimi-
nation of the spring-back effect for both species with respect to mechanical requirements and constant strength properties of the densified product.

The spring-back effect of THM-densified wood decreased significantly with elevated post-treatments temperatures and shorter treatments durations, which is in agreement with results of Tabarsa and Chui (2000), Heger et al. (2003), Welzbacher et al. (2007). A comparable elimination of the shape memory and hence improved dimensional stability by steam post-treatment at the temperatures above 180°C has been mentioned by several authors before (Inoue et al., 1993; Dwianto et al., 1996; Ito et al., 1998). The mechanism by which steam heat treatment fixes the compressive set is thought to be due to softening of lignin combined with degradation of amorphous polysaccharide content (Morsing, 1998). Dimensional stabilisation is most likely caused by increasing cross linkage and relaxation of stored stresses by partial hydrolysis of hemicelluloses and degradation of lignin at elevated temperatures (Nornimo et al., 1993; Navi and Heger, 2005). However, macrobuckling and damage of the wooden structure in densified and not post-treated specimens imparted wood cells being unable to recover completely to the initial dimensions (44% and 61% compression set recovery for beech and spruce wood, Table 4.1).

Our study has shown that hygroscopicity of THM-densified specimens is markedly reduced as a result of modification by steam post treatment. This reduction is related to the temperature of the process. Other studies have indicated that the time and treatment atmosphere during the process also affect the sorption behaviour (Alexiou et al., 1990; Poblete et al., 2005; Popper et al., 2005; Welzbacher et al., 2008).

In steam post-treatments the inserted moisture contributes to the hydrolysis of the paracrystalline regions of celluloses (Ito et al., 1998) and furthermore causes inner stress relaxation by hydrolysis of hemicelluloses that results in fixation of compression set (Heger et al., 2003). Navi and Heger (2005) have calculated the amount of –OH groups using the Dent model and showed that half of the –OH groups were suppressed after TH-post-treatment at 180°C during 16 min making the wood less hydrophilic.

Swelling of THM-densified beech and Norway spruce was significantly reduced by implementation of steam post-treatment. However, the lowest swelling was recorded for TH-treated beech and spruce specimens (TH 160 and TH 180), which is related to the fact that these specimens were not densified and hence did not exhibit any set recovery, that contributed to the swelling behaviour especially in the radial direction (the direction of compression). Steam
post-treatment, in contrast to a dry post-treatment (in the environment excluding any contact with moisture) or oil-heat treatment (Treu et al., 2003), contribute to the complete fixation of compression set at lower temperatures and shorter durations, which is reflected in the energy demands for the production and consequently on the cost of the final product.

Reduction of MOE values in TH treated specimens are in good agreement with the work of Yilgor et al. (2001) where compression strength decreased by 13.3 % and MOE by 16.5%. However, many studies have shown that there is a slight increase of MOE when wood is thermally treated for short periods. In a study of the thermal degradation of beech and pine at various temperatures and time periods, it was found that although the decrease of strength and work to maximum load was proportionate to mass loss, irrespective of treatments and species the decrease in MOE only became significant when the mass losses exceeded 8% (Rusche, 1973). In the work of Kubojima et al. (2000) where wood samples were heated in a sealed reactor at 160°C under nitrogen or air for various time periods, the MOE increased over short treatment periods and then remained relatively constant. Also Bekhta and Niemz (2003) found little change in MOE for spruce wood heated at 200°C. Chang and Keith (1978) reported on the increase of MOE for wood samples (elm, beech, aspen and maple) after thermal treatment, but also noted that more severe treatments resulted in reduction of MOE. Radial compression of Norway spruce and beech, on the other hand, increased the density and led to the dramatic increase of the MOE. This correlates with the trends reported by Beaud et al. (2008) though with somewhat larger specimens. The same trend has been reported by Kubojima et al. (2004) who assessed the MOE and shear modulus of densified Japanese cedar (Cryptomeria japonica D. Don) in the free-free flexural vibration test. Moreover, due to the anisotropic character of crystalline cellulose, the compressive strength is limited in the radial and tangential direction. The slight decrease of the radial compression strength after TH-treatment may be caused by small radial fissures. Another reason might be the damage of parenchyma cells in the rays and epithelia cells around resin canals during heat treatment. Rays contribute to the compressive strength perpendicular to the grain and their damage might decrease the compressive strength.

However, an approx. 25-fold dramatic increase in compressive strength perpendicular to the grain (direction of densification) for Norway spruce might be additionally explained by the fact that all the specimens were carefully selected prior to treatments in order to achieve an exceptional efficiency of densification. In the study of Beaud et al. (2008a, b, c) with the industrial scale densification of Norway spruce wood panels (dimensions, 1 m x 3 m x 10 cm),
test specimens showed a 5-fold increase of compressive strength in the radial direction having a compression set of approx. 50% and density between 0.88 and 0.92 g/cm$^3$. 
CHAPTER 5
CHEMICAL ALTERATIONS

5.1 RESULTS

5.1.1 POLYSACCHARIDES

Hydrolysis of beech and Norway spruce revealed the presence of glucose, xylose, mannose, galactose, arabinose, ramnose and 4-O-methylglucuronic acid (4-O-Me) in both investigated wood species. In controls of spruce samples, glucose, mannose and xylose dominated with approx. 70%, 18% and 7% of all sugars respectively, whilst in beech wood samples the content of glucose, xylose, mannose was approx. 65%, 28% and 3% respectively (Tables 5.1 and 5.2).

![Figure 5.1 Absolute content of sugars in TH- and THM-treated Norway spruce wood.](image)

All TH and THM treatments reduced the amount of carbohydrates in spruce wood with an exception of the absolute amount of sugars in densified and non post-treated spruce wood samples, where the values did not alter significantly in comparison to controls. TH180 treatment resulted in an increase of glucose from 70% to 81% and decomposition of both mannose (from 18% to 13%) and xylose (from 7% to 5%). THM-densified wood post treated at 180°C
showed increased glucose content (77%) and a slightly altered mannose (16%) and xylose (5.7%) content.

Figure 5.2 Relative content of sugars in TH- and THM-treated Norway spruce wood.

Figure 5.3 Absolute content of sugars in TH- and THM-treated beech wood.
Table 5.1 Results of HPLC carbohydrate analysis of TH- and THM-treated Norway spruce wood specimens.

<table>
<thead>
<tr>
<th>Probe</th>
<th>Hydrol.-method</th>
<th>Hydrolysis-residue</th>
<th>∑ Sugar</th>
<th>Glucose</th>
<th>Xylose</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Arabinose</th>
<th>Ramnose</th>
<th>4-O-Me</th>
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<td>29.0</td>
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<td>46.6</td>
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<td>0.9</td>
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<td>46.7</td>
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<td>0.3</td>
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<td>-</td>
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<table>
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<th>Mannose</th>
<th>Galactose</th>
<th>Arabinose</th>
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<td>-</td>
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<td>-</td>
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Table 5.2 Results of HPLC carbohydrate analysis of TH- and THM-treated beech wood specimens.

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<tr>
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<th>Hydrolysis-residue</th>
<th>Σ Sugar</th>
<th>Glucose</th>
<th>Xylose</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Arabinose</th>
<th>Ramnose</th>
<th>4-O-Me</th>
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</thead>
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<td>0.2</td>
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<table>
<thead>
<tr>
<th>Probe</th>
<th>Hydrol.-method</th>
<th>Glucose</th>
<th>Xylose</th>
<th>Mannose</th>
<th>Galactose</th>
<th>Arabinose</th>
<th>Ramnose</th>
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<td>27.9</td>
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<td>0.5</td>
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<td>27.1</td>
<td>2.9</td>
<td>0.7</td>
<td>0.3</td>
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<tr>
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<td>27.3</td>
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<td>27.5</td>
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<td>THM 180</td>
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<td>18.2</td>
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<td>2.4</td>
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<td>0.6</td>
<td>0.8</td>
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<td>THM 180/80</td>
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<td>27.7</td>
<td>1.9</td>
<td>0.7</td>
<td>-</td>
<td>0.4</td>
<td>1.4</td>
</tr>
</tbody>
</table>
TH treatments did not significantly alter the chemical composition of sugars in beech wood samples (glucose 67%, xylose 27%, mannose 3%) whilst THM 180 resulted in an increase in glucose content of 77% and a reduction in xylose (17%) and mannose (0.5%) content.

5.1.2 KLASON LIGNIN

Klason lignin which is determined as the residue after hydrolysis of hemicelluloses did not show significant differences after TH and THM treatment of beech wood i.e. guaicyl-syringyl units. The effect of treatments was particularly pronounced in spruce wood specimens especially the ones post-treated at elevated temperatures (Fig. 5.5). The values for the untreated control specimens are in good agreement with reference values found in the literature (Fengel and Wegener, 1984; Kamdem et al., 2002). TH 180 increased the Klason lignin content in Norway spruce from 27% to 32%, the same was observed for THM 180 (32% of Klason lignin).
5.1.3 Extractives

In this study the acetone/water (9:1) solvent has been used for extraction of extraneous compounds. Catechin, taxifolin, aromadendrin, eriodictyol, naringenin and prunin in acetone/water extracts were first separated by reversed-phase HPLC and later detected with a photo-diode array detector as described by Mayer et al. (2006).

Generally, softwood has a higher extractive content than hardwood (Pettersen, 1984), the same was also demonstrated in this study. For both species the acetone/water (9:1) extract from the TH- and THM treated samples appeared considerably darker to the unaided eye compared to the extract of untreated controls and densified wood samples (Fig. 5.6). The data presented in Table 5.3 together with the visual observations of dark extractive residue obtained from each specimen, indicate that amount and composition of extractives are significantly influenced by conditions (temperature and the degree of steam saturation) of the wood modification process and that this influence is species-dependant.
Table 5.3 Results of the extraneous compounds analysis.

<table>
<thead>
<tr>
<th>Treatment</th>
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<th>Norway spruce</th>
</tr>
</thead>
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<tr>
<td></td>
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<td>Extract content (%)</td>
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<td>Control 2</td>
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<td>THM 180/80</td>
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</table>

Figure 5.6 Acetone/water (9:1) extracts in untreated controls and in TH- and THM-treated specimens of Norway spruce and beech wood.
Figure 5.7 Content of extraneous compounds in TH- and THM-treated Norway spruce and beech wood samples.

In spruce wood specimens the total amount of extractives increased for all treatments included in the study. The highest extractive content was recorded for TH 180 - treated Norway spruce wood at 180°C that was not densified. Densified spruce post-treated at 180°C under saturated steam conditions showed a significant increase in extractive content.

All TH- and THM-treated beech wood samples showed increased extractives content, no significant changes were recorded for densified and non post-treated specimens. It is of particular interest that reference controls showed a higher extractive content in spruce when compared to beech wood specimens. However, the TH treatments under saturated steam conditions induced the modification of phenolic (polyphenolic) compounds to a greater extent in beech wood.

5.2 DISCUSSION

It is well established that celluloses are degraded at higher temperatures than hemicelluloses and that the rate of cellulose degradation is reduced if the water (saturated steam in case of THM-treatment) is present, which is assumed to be due to the enhanced ability of the amorphous regions to change its structure to produce more thermally stable crystalline regions.
Steam accelerates the formation of organic acids (acetic acid) that catalyses the hydrolysis of hemicelluloses and to a lesser extend of the amorphous cellulose (Mitchell, 1988). Hydrothermal processing results in the hydrolysis of polysaccharides due to the action of hydronium ions generated by autoionisation of the water, although the formation of hydronium ions from acetic acid is more important (Garrote et al., 1999). Temperature ranges between 150°C and 230 °C are generally used, because hydrolysis is very slow at lower temperatures, whereas cellulose degradation begins to occur in the region 210-220 °C and becomes predominant at 270 °C (Hillis, 1984). Hardwoods are less thermally stable than softwoods and this is attributable to the hemicellulosic content and composition. Pentosans (that are found in a greater proportion in hardwood hemicelluloses) are more susceptible to thermal degradation than hexans (Fengel and Wegener, 1984). Additionally, hardwoods, in general, have a higher proportion of hemicelluloses and the hemicelluloses of hardwoods have a higher acetyl content compared to softwoods.

Cellulose degradation occurs at higher temperatures than that of hemicellulose. It is likely that minor thermal degradation does take place at relatively low temperatures but at a much slower rate than with the hemicelluloses. The amorphous regions of cellulose are more susceptible to thermal degradation and these regions probably exhibit similar thermal properties to the hexose properties of hemicelluloses (Kim et al., 2001). Presence of water reduced the rate of cellulose degradation, which is assumed to be due to the enhanced ability of the amorphous regions to change structure to produce more thermally stable crystalline regions (Fengel and Wegener, 1984; Dwianto et al., 1996). With extended heating, chain scission of the cellulose occurs, producing alkaline-soluble oligosaccharides, with a concomitant decrease in the cellulose degree of polymerisation and degree of crystallinity. It should be noted that this production of alkaline-soluble cellulose breakdown components can lead to errors in estimating the hemicellulose content (Shafizadeh, 1984).

It is generally accepted that lignin is the most stable component of the woody cell wall but some thermal degradation of lignin may occur at relatively low temperatures with the production of various phenolic breakdown products (Sandermann and Augustin, 1964). An increase in lignin content does not necessarily imply the formation of lignin during the process but decomposition of other wood components and their transformation into a lignin-like body (Campbell and Taylor, 1932). Hardwood lignin is more easily softened than softwood lignin due to the easy breakdown of cross-linkages in softwood lignin, or the lower lignin content in hardwoods or due to microstructural differences. Another reason for this could be the fact that
the pentoses of hardwoods are more unstable than the hexoses of softwoods (Sandermann and Augustin, 1964). It is reasonable to assume that an increase in the lignin content can only be obtained when the pentosan units condense to a product which is insoluble in concentrated $\text{H}_2\text{SO}_4$. If, however, pentosan units undergo hydrolysis there is no increase in the apparent Klason lignin content.

Furthermore, beech has a large amount of xylem ray cells in the radial direction and a more heterogeneous structure which restricts a uniform penetration of saturated steam during post-treatment and results in heterogeneous alterations in chemistry at surface areas of the specimens compared to deeper regions.

Sudo et al. (1985) demonstrated that lignin from beech wood steamed at temperatures below 200°C was richer in syringyl units and only slightly modified compared to treatments at higher temperatures where significant losses in metoxyl groups occurred. TH-treatment is shown to result in the production of soluble lignin fragments initially, but re-polymerisation was more pronounced as the reaction time progressed, possibly involving furfural and other polysaccharide degradation products. Garrote et al. (1999) also reported that extended heating with steam resulted in cross-linkage formation within lignin.

Tjeerdsma et al. (1998) studied the molecular changes occurring in wood (when treated according to the two-step Plato process) using CP-MAS NMR. Evidence was presented showing that wood modification was associated with the lignin degradation and production furfural. Although it seems likely that there are new cross-links formed in the lignin network structure as result of thermal treatment of wood, as noted previously by Seborg et al. (1953).

One more explanation of lower lignin values for spruce wood could be that under hot and slightly acidic conditions (pH of water at 180°C is 6), low molecular-weight phenolic compounds can be converted into highly condensed insoluble compounds with reduced solubility in acetone/water.

Several researchers have shown that the yield of free sugars formed from hemicelluloses during steaming of lignocellulosics undergoes a maximum in concentration and then decrease with increasing time of steaming (Lawther et al., 1996; Rowell et al., 2002). This would indicate that free sugars released from the degradation of hemicelluloses then undergo further reactions to other products. Pectic polysaccharides have also been shown to influence the production of free sugars upon heating (Overend and Chornet, 1987).
TH-treatment under saturated steam conditions, on one hand, intensifies the brown colour presumably due to polymerisation of catechin and other flavonoid monomers. On the other hand, TH-treatment presumably dissolves some extractives and degrades certain easily hydrolysable components of wood. Nuopponen et al. (2004) observed the migration of fats and waxes along axial parenchyma cells to the surface of steam heat treated Scots pine wood at temperatures between 100-160°C whereas at temperatures above 180°C fats and waxes disappeared from the wood surface.
CHAPTER 6
DISCUSSION

The main objective of this study was to establish a fundamental understanding of the principles and mechanisms, which determine the durability of THM densified wood against fungal decomposition. Particular attention was paid to the interactions that exist between the alterations of the wood constituents, its physical properties, the micro-morphology of the wood structure during processing of THM densified wood and the colonization and degradation by wood decay fungi.

6.1 DISCUSSION OF HYPOTHESES

6.1.1 HYPOTHESIS 1

THM-densification reduces the susceptibility of wood against wood decay fungi.

Subject to suitable conditions, wood is susceptible to degradation by microorganisms (Eriksson et al., 1990; Zabel and Morrell, 1992; Schwarze et al., 2004). In order for fungal attack to occur, three components are required: water, oxygen, temperature and source of nutrients. Altering one or a combination of these factors impedes wood degradation by wood decay fungi.

One of the most efficient ways of preventing fungal attack is to prevent the wood from reaching sufficiently high moisture content. This is easily achievable when wood is used in interior applications, but seems to be a major problem in case of the outside exposure. It is well established that wood will not decay if it is maintained in the environment where the moisture content is below a threshold value of around 20-25% of the dry wood weight, depending on the species (Cartwright and Findlay, 1958; Zabel and Morrell, 1992). As demonstrated in this study, thermo-hygro-treatment reduced the hygroscopicity of TH- and THM-densified beech and Norway spruce wood (Chapter 4), as a result the moisture content of the THM-densified wood specimens after 16 weeks incubation with white rot fungi was significantly lower than that of untreated controls (Fig. 3.21). Nevertheless, the values still exceeded the fibre saturation point, hence being not sufficiently low to decrease the susceptibility of THM-densified wood against decay fungi.
Temperature and oxygen availability are another two factors that affect fungal growth. Although the reduction of oxygen offers a certain protection potential, the method involves in practice creating very high water content in wood and its surroundings in order to replace the oxygen in the timber. This phenomenon mainly occurs in marine or aquatic environments and also in artificially created anaerobic conditions. Therefore, the exclusion of oxygen together with temperature regulation is primarily dependant on the surrounding but not on the substrate and hence was not affected by THM-densification.

Another possibility to increase the decay resistance of wood is to inhibit the access of decay fungi to the cell wall nutrients. This can be achieved by two different methods: mechanical restrictions of hyphal growth within wooden substrate and modification of the woody substrate leading to inability of fungal enzymatic systems to recognise cell wall constituents (see Hypothesis 2).

The process of THM treatment in the present execution with temperatures not exceeding 180°C and high degree of steam saturation (80% or 100%) did not improve decay susceptibility of neither Norway spruce nor beech against brown-, white- and soft rot decay. Considering the dry weight losses (WL%), though, as the assessment factor of decay resistance, we can assume the improvement of the resistance of Norway spruce and beech against brown- and white rot fungi (Chapter 3).

There appear to be different reasons for enhanced decay resistance of THM-densified Norway spruce wood to brown rot fungi. One simple explanation may be that during the process of densification the exerted compression force results in a collapse of the cell lumina, restricting the pathway of least resistance for hyphal growth. Another explanation may be associated with the chemical and physical blockage of cell wall micropores for the diffusion of low molecular weight enzymes. In brown rot the degradation of cellulose and hemicellulose takes place in different stages. It is assumed that hydrogen peroxide is probably formed in a pre-cellulolytic phase, and easily penetrates into the cell wall and, together with iron ions, overcomes the lignocellulose matrix by oxidative depolymerisation (Schwarze et al., 2003; Schwarze et al., 2004). This assumption seems valid, as cellulose-degrading enzymes are relatively large and the much smaller cell-wall capillaries cannot be simply penetrated without loosening of the cell wall matrix. For this reason, cell-wall degradation occurs not in the immediate vicinity of the hyphae or out from the lumen, but the ectoenzymes of the brown rot fungi must first diffuse very deeply into the cell wall through the S₃ layer in order to degrade
the cellulose-rich S_2 layer. Thus, high lignin content i.e. high density tends to delay the diffusion of the large molecules of the cellulose-degrading enzymes into the cell wall (Schwarze and Baum, 2000; Schwarze et al., 2000). It appears possible that after wood densification and heat treatment cell wall accessibility to low molecular weight enzymes is hampered. This possibly limits the ability of cellulolytic enzymes to diffuse quickly and extensively into the cell wall, resulting in delayed degradation of THM-densified Norway spruce wood by brown rot fungi.

In the case of degradation by soft rot fungi, Norway spruce and beech specimens showed enhanced resistance at the initial stages of decay; however, THM-treated beech wood specimens succumbed to the degradation after 32 weeks. Microscopical examinations showed that in THM-treated wood of Norway spruce soft rot commenced from the outer wood surfaces and cavity formation was not found in deeper regions of the wood specimens. In beech wood, hyphal colonisation and degradation was facilitated by the non-occluded lumina of parenchyma cells in muliseriate xylem rays. Moreover, the higher syringyl lignin content of beech wood rendered it more susceptible to soft rot attack than spruce wood. TH-treated wood of beech and spruce was highly susceptible to soft rot type 2 attack in the latter host.

### 6.1.1 Hypothesis 2

The alterations in decay resistance are related to the modification of the chemical composition of wood after TH-treatment and the changes of the wood structure induced by densification. Restriction of fungal growth by the occlusion of the cell lumina and the chemical modification of the wood substrate are associated with the depolymerisation of polysaccharides and modification of lignin.

Since the TH-modification of the wood substance reduces the moisture content in the wooden cell wall, it is not clear whether decay resistance is due to a hindering of enzyme action or disruption of necessary water relations. Sailer and van Etten (2007) suggest that the blocking of free space in the cell wall (which in this study was achieved by mechanical densification) might be one of the effective methods of hampering fungal access to cell wall nutrients. This, however, implies that the efficiency of wood modification is not necessarily based on chemical changes of the wood substrate but also on limitation of accessibility of the wooden cell wall. Mechanical densification of wood specimens reduces the size of voids and cell wall mi-
cropores thereby preventing the ingress of degradative agents. The decay of wood by microorganisms requires the access of degradative agents to the interior of the cell wall. In brown rot decay, the polysaccharide component of the cell polymers is extensively depolymerised before much weight loss occurs (Kirk and Highley, 1973). With white rot selective delignification, no extensive cellulose degradation occurs at the initial stages of decay, with the lignin being preferentially decomposed and removed. Various enzymes are considered to be responsible for the decomposition of the cell wall polymers, but before they become involved in the decay process they should gain access to the cell wall. Cellulases isolated from different decay fungi vary in molecular weight but typical dimensions, based on these molecular weights, are approx. 5.0 nm if spherical and 3.3 x 20 nm if ellipsoidal (Cowling and Kirk, 1976). Xylanase has been shown to have an approx. diameter of 7 nm (Green III et al., 1992). Lignin peroxidases have dimensions of 4.7 nm (spherical) or 4.3 x 6.0 nm (ellipsoidal) (Fluornoy et al., 1991). However, it has been established in numerous studies that the cell wall micropores of sound wood have diameters no greater than 2 nm (Hill and Popadopoulos, 2001). It is therefore apparent that during the initial stages of decay these enzymes are not capable of penetrating the cell wall. This has been verified by various studies of the distribution of enzymes using electron microscopy combined with labelling methods. By using immuno-gold labelling, it has been shown that lignin peroxidase does not penetrate the cell wall of non-degraded wood (Daniel et al., 1989; Srebotnik and Messner, 1991). For this reason low molecular weight diffusible agents (LMWDA) have been suggested as being responsible for initiating decay (Murmanis and Highley, 1987; Srebotnik and Messner, 1991). It seems likely that in addition to causing occlusion of the cell lumina, THM-densification also reduces the size of voids within cell walls inducing retarded diffusion of fungal LMWDA through the cell walls. This may be particularly important in resistance to brown rot which mainly involves diffusion of degradative substances (LWMDA) rather than the direct erosion of cell wall by fungal hyphae (Kerem et al., 1999).

Chemical analysis revealed that when wood is treated in a THM reactor, it appears to undergo slight alterations in the composition of its chemical constituents. Degradation of hemicelluloses results in the production of simple sugars, which might undergo further dehydration reactions to form highly reactive furfural, hydroxymethylfurfural and other substances. These substances rapidly develop linkages with lignin, thus apparently increasing the lignin content and altering its structure and chemical composition. The syringyl-guaiacyl unit ratio is also presumably affected by the THM-treatment, which induces alterations in the resistance
against white- and soft-rot decay fungi. Mobilisation of extractives during THM-densification is responsible for darkening of the wood substrate, however partial removal of extractives allow greater access of water molecules to the cell wall and renders wood susceptibility.

The dimensional stability of THM-treated wood is improved compared with normal kiln dried spruce and beech wood as a result of reduction of concentration of water absorbing hydroxyl groups after the degradation of hemicelluloses.

6.1.1 Hypothesis 3

TH-treated wood is more susceptible to pyrophillic fungi.

Despite our assumption about the substrate preference of pyrophillic fungi, the investigations with *Daldinia loculata* did not reveal any alterations in behaviour on thermally treated wood of beech, ash and maple. Even after a shortened incubation period of 8 weeks, all the hardwood control species showed much higher weight losses than treated specimens. Significantly higher weight losses and hence higher susceptibility were recorded for thermally treated Norway spruce. This fact on one hand indicates the improved resistance of thermally treated wood to *D. loculata*, but on the other hand shows that the conditions of the treatment are not strong enough for the pyrophillic nature to become apparent.

In order to develop new and useful test methods to assess the durability of thermally modified wood, more strains of pyrophillic fungi have to be tested.

6.1.1 Hypothesis 4

Wood decay fungi may switch their mode of action to circumvent unfavourable conditions induced by THM-densification and grow within cell walls.

Adaptability to changing environment is a feature that enables living organisms to cope with environmental stresses and pressures. Adaptations can be structural, behavioral or physiological. Structural adaptations are special body parts of an organism that help it to survive in its natural habitat (e.g., skin colour, shape, body covering). Behavioral adaptations are special ways a particular organism behaves to survive in its natural habitat. Physiological adaptations
are systems present in an organism that allow it to perform certain biochemical reactions e.g. producing venom, secreting slime, being able to keep a constant body temperature (Deacon, 2006).

In the present study the organisms that were included in the tests showed clear evidence of adaptability to unfavourable growth conditions. In the brown rot resistance test, the fungal species produced narrow hyphae, which enabled them to enter cells of THM-densified wood with partially occluded lumina. In wood, the hyphae of *C. puteana* all bore simple clamp connections, whereas those of *P. placenta* were very fine (1-2 µm) bearing simple clamps or somewhat wider (3-9 µm) with either medallion clamps or simple clamps.

Hyphal tunnelling through cell walls is another means by which certain fungi may be able to grow through wood in which the lumen is inaccessible (Daniel *et al.*, 1992; Schwarze *et al.*, 1995; Worrall *et al.*, 1997; Schwarze *et al.*, 2004). Only few brown rot fungi have been reported to grow in this way (Schwarze *et al.*, 2000; Kleist and Schmitt, 2001; Kleist *et al.*, 2002) and *Gloeophyllum trabeum* which switched its mode of action exhibited such behaviour in the present study. It penetrated the secondary wall transversely; tunnelling began when these hyphae reached the compound middle lamella and changed direction without penetrating it.

In the study on white rot resistance, *T. pubescens* could circumvent conditions restricting fungal growth by producing bore holes in the fibre tracheids of beech wood and *T. versicolor* was recorded to form bore hyphae that transversely penetrated cell walls of early wood tracheids in THM-densified Norway spruce wood. Thus, even a complete occlusion of cell lumina could not inhibit decay by soft rot fungi as the occlusion was simply counteracted by directional growth within the cell wall.
6.1.1 **Hypothesis 5**

**TH-treatment substantially affects wood hygroscopicity, resulting in the elimination of spring back.**

The biggest problem associated with most of wood modification methods that include densification/compression is the lack of dimensional stability. When soaked in water or exposed to high relative humidity, compressed products tend to exhibit irreversible swelling or spring back (Hillis, 1984; Inoue et al., 1998; Schrepfer and Schweingruber, 1998). In this study thermo-hygro post-treatment was used as a method of dimensional stabilization of densified wood. An almost irreversible compression of sugi (Cryptomeria japonica D. Don) wood was reported by Inoue et al. (1993). The authors subjected small sugi specimens (20 x 20 x 30 mm) to steaming for 1 min at 200°C or for 8 min at 180°C. In this study somewhat bigger beech and Norway spruce specimens were subjected to TH-post-treatment at the highest 180°C for 35 min (Table 2.2). Results presented in Chapter 4 indicate the importance of TH-post-treatment phase, although the set-recovery was significantly reduced, it was not completely eliminated, causing re-expanding of cell lumina as the humidity in the environment increased. However, the spring-back effect of THM-densified samples decreased strongly with rising temperatures during post-treatment phase. The fixation of the compressed wood structure and hence, the elimination of spring back is presumably a result of thermal modification of the hygroscopic components in the cell wall (hemicelluloses and lignin-hemicelluloses matrix, in which the crystalline cellulose microfibrils are embedded). Moreover, another aspect contributing to the elimination of spring back is the alterations in the wood macromorphology. Macro buckling and mechanical damage of the wooden structure by densification imparts wood cells being unable to recover completely to the initial shape.

It is anticipated that in order to accomplish complete elimination of the spring back effect post-treatment at higher temperatures and addition of supplementary substances (oils, resins or gases) should be considered.
6.1.1 Hypothesis 6

THM-densification improves mechanical properties as well as hygroscopicity of Norway spruce and beech.

THM-densification procedure allows compressing of wood specimens in the radial direction to approx. 70% (Norway spruce) and 45% (beech) of their initial size inducing the consequent increase of density to approx. 1.3 g/cm$^3$ and 1.25 g/cm$^3$ for spruce and beech respectively. Increase in density of the wood specimens induces changes in the mechanical and wood strength properties. Moreover, TH-post-treatment slightly alters wood chemistry and individual wood constituents (Chapter 5) contributing to changes in the mechanical properties (decrease of HB, MOE and compressive strength) and improvement of dimensional stability.

During the densification process compression forces induce major alterations in the wood microstructure.

The compression-set recovery of THM-densified specimens was not completely eliminated by the TH-post-treatment for 35 min at the temperatures not exceeding 180°C. TH- and THM-treatments reduced hygroscopicity of beech and spruce specimens. Increasing temperatures applied during the post-treatment phase led to reduction of EMC and decreased the rate of moisture-uptake.

Increased radial swelling values in densified and THM-densified wood could be explained by the incompletely eliminated shape-memory effect. TH-treated specimens, however, possessed the strongest dimensional stability compared to controls and THM-densified specimens. Elevating the temperatures applied during steam post-treatment leading to the increase of the cellulose crystallinity and results in decreased swelling in tangential and radial directions.

It is apparent that TH-treatment has an adverse effect on strength properties wood through its effect on mechanical properties. However, densification in combination with TH-post-treatment improves mechanical performance of both tested species, rendering wood more rigid but less elastic.
6.2 Remarks on Wood Modification

Currently the production of modified wood is very small compared to the consumption of timber in Europe. However, it is anticipated to increase exponentially in the coming years. The preservatives commonly used today can achieve a 50-year service life. However, the importance of a long service life should be questioned. Does the life expectancy of garden furniture have to be so long, for instance? Could a 10-year service life be long enough? Even if life expectancy of the railway sleepers is 50 years, mechanical wear breaks them down much earlier than this. Telegraph poles become hollow during ageing, since the biocide impregnated sapwood is undamaged, but the naturally durable, hard to impregnate hardwood is totally destroyed. An additional consideration is that wood treated with conventional preservatives is classified as hazardous waste. Timber could therefore be protected in environmentally benign way, in order to meet the requirements of various end use situations, so that the treated/modified wood is “fit for the end-use” and to ensure that it has a beneficial and safe life cycle, including production, use and eventual disposal.

The standards that have been developed to date are concerned with determining the properties of conventional wood products. At present, claims made with respect to modified wood are based upon the current set of standards which may not always be appropriate to the performance of these new materials in real-life situations. This is an area that has to be addressed in the near future. There is a need to develop appropriate standards and agree them on international level. In this study the attempt has been made to investigate the susceptibility of thermally treated wood against a pyrophillic fungus *D. loculata*. Adding pyrophillic fungi to the obligatory test organisms for the assessment of biological resistance of thermo wood would provide more realistic basis for the product’s biological performance evaluation.

Economic and sustainability aspects should not be neglected at the early stages of the development of new wood modification methods. There are inevitably going to be additional costs associated with mood modification, compared to conventional preservative –treated wood. It is essential that these costs are not so high that the product is no longer competitive on the market. However, provided that the benefits associated with using of modified wood are apparent, it could be possible to sell products at the premium rate.
CHAPTER 7
CONCLUSIONS

7.1 CONCLUSIONS

Hygro-thermal treatment of wood densified at relatively high temperatures not only increases dimensional stability but also seems to make conditions less conducive to hyphal growth within the secondary walls of tracheids and xylem ray parenchyma. The extent to which fungal decay is retarded in this way may be influenced by the hyphal width and colonisation strategies of the fungi concerned.

The process of THM-treatment increased the resistance of spruce but not of beech wood to degradation by soft-rot fungi. Microscopical examination showed that in THM-treated wood of Norway spruce soft rot commenced from the outer wood surfaces and cavity formation was not found in deeper regions of the wood specimens. In beech wood hyphal colonisation and degradation was facilitated by the non-occluded lumina of parenchyma cells in multiseriate xylem rays. Moreover, the higher syringyl lignin content of beech wood renders it more susceptible to soft-rot attack than spruce wood. TH-treated wood of beech and spruce was highly susceptible to soft-rot fungi and cell wall modification induced soft rot type 2 attack in the latter host. The conclusion is that TH-treated Norway spruce wood and THM-treated beech woods are highly susceptible to soft-rot attack and therefore inappropriate for application in utility class 4.

The THM-treatment did not improve the resistance of Norway spruce and beech specimens against white rot degradation either. White rot fungi circumvented conditions restricting hyphal growth by producing hyphal tunnelling in secondary walls of fibre tracheids of beech or by forming a bore holes that transversally penetrated cell walls of early wood tracheids in THM-densified Norway spruce.

The THM-treatment in saturated steam at temperatures not exceeding 180°C does not completely eliminate set recovery of Norway spruce and beech, however it improves the dimensional stability and mechanical performance due to slight alterations in the chemical composition and changes in the micro-morphology of the wood structure.
It is therefore concluded that THM-densification in the present execution is not an appropriate method of wood modification considering the improvements of biological resistance, enhancements of physical performance and the economical aspect of production.

7.2 **FUTURE PERSPECTIVES**

Even though the THM-densification did not affect durability of Norway spruce and beech significantly enough to consider further up-scaling and implementation of the process on the industrial level, it provided a sound basis for future investigations in the area of wood modification and preservation.

It is anticipated that substantial improvements to the THM-treatment procedure, followed by the detailed assessment of its various characteristics could contribute to developing of new products. In this light, following aspects should be taken into consideration for further investigations:

- increasing the range of temperatures applied during post-treatment phase in order to reach a complete elimination of set recovery and dimensional stability;
- utilization of additional substances during post-treatment phase to improve decay resistance and selected mechanical properties;
- due to high energy costs of THM-treatment, it is reasonable to consider surface densification of the material instead of densification of the entire wood block;
- careful selection of assessment criteria and test organisms during biological resistance evaluation is essential for the objective determination of the product’s performance.
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REFERENCES


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### EDUCATION

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<td>03. 2005 - 10. 2008</td>
<td>PhD</td>
<td>Sustainability through new technologies for enhanced wood durability, Department of Civil Engineering (ETH), Wood Protection Group (EMPA)</td>
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<tr>
<td>09. 2002 - 02. 2004</td>
<td>MSC in Industrial Biotechnology</td>
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<tr>
<td>09. 1998 - 07. 2002</td>
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<td>National Technical University of Ukraine “Kiev Polytechnic Institute”</td>
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<td>09. 1988 - 06. 1998</td>
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### PROFESSIONAL EXPERIENCE

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### RELEVANT SCIENTIFIC (RESEARCH) TECHNIQUES

**Analytical techniques**
- Spectrophotometry, polarimetry, fluorimetry, TGA Chromatography: ion exchange, HPTLC, CPG Microscopy: SEM, ESEM, FESEM, EDX, REM image analysis

**Biochemistry**
- Enzymes purification, immobilization, enzymatic activity assays

**Microbiology, Mycology, Wood Science**
- Bacteria and fungi cultivation and identification, Fungal modification of wood, Wood durability and hazard class assessment, Testing according to European Norms EN152, EN113, EN807

**Project Management**
- Materials, time, people management, motivation & communication, reporting

**PC MS office, SPSS, EndNote, Photoshop, Illustrator, Origin, Image Access**
APPENDIX

I. COMPOSITION OF NUTRIENT MEDIUMS

*Malt extract agar (MEA)*

2,5% Agar N3 (Oxoid)
4% Malt (Oxoid)

*Nutrient solution for the soft rot screening test*

\[
\begin{align*}
\text{NH}_4\text{NO}_3 & \quad - 6 \text{ g} \\
\text{K}_2\text{HPO}_4 & \quad - 5.12 \text{ g} \\
\text{MgSO}_4 \times 7\text{H}_2\text{O} & \quad - 2.04 \text{ g} \\
\text{KCl} & \quad - 0.5 \text{ g} \\
\text{NaCl} & \quad - 0.01 \text{ g} \\
\text{FeSO}_4 & \quad - 0.002 \text{ g} \\
\text{MnSO}_4 & \quad - 0.001 \text{ g} \\
\text{ZnSO}_4 & \quad - 0.001 \text{ g} \\
\text{H}_2\text{O} & \quad - 2 \text{ L} \\
\end{align*}
\]

*ANTAROX – 1 drop per 1 L

*Medium I for the soft rot screening test*

1.5% Agar N1 (Oxoid)
1% Malt (Oxoid)
0.5% oat flower (Coop)

*Medium II for the soft rot screening test*

0.1% Malt (Oxoid)
II. Determination of Chitin

**Chemicals**
- 6.0 mol/l hydrochloric acid
- 2 mmol/l homocysteic acid
- o-phtalaldehyde (OPA)
- methanol
- 2-mercaptoethanol
- 0.4 mol/l borate buffer
- argon gas
- sodium acetate
- citric acid monohydrate
- 50% (w/v) sodium hydroxide
- helium gas

**Reagents**

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<tr>
<td>6 M HCl (IS)</td>
<td>0.071 g of DL-homocysteic acid in 200 ml volumetric flask, 90 ml deionised water, 98 ml concentrated HCl. Final DL-homocysteic acid concentration: 2000 μM</td>
</tr>
<tr>
<td>9 M HCl (IS) for dilution 1:150</td>
<td>0.033g of DL-homocysteic acid in 200ml volumetric flask, 40 ml deionised water, 147 ml of concentrated HCl. Final DL-homocysteic acid concentration: 900 μM</td>
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<tr>
<td>9 M HCl (IS) for dilution 1:20</td>
<td>0.022 g DL-homocysteic acid diluted with deionised water in 50 ml volumetric flask. Final DL-homocysteic acid concentration: 120 μM</td>
</tr>
<tr>
<td>Borate buffer 0.4 M</td>
<td>6,183 g of boric acid in 250 ml volumetric flask. pH adjusted to 9.5 with approximately 36 ml 0.5 M NaOH. Solution diluted with deionised water</td>
</tr>
<tr>
<td>0.5 M NaOH</td>
<td>4 ml of concentrated 6 M NaOH diluted with deionised water in 50 ml volumetric flask (concentration of NaOH is 0.48 M)</td>
</tr>
<tr>
<td>0.25 M NaOH</td>
<td>2 ml of 50% NaOH solution diluted with deionised water in 100 ml volumetric flask</td>
</tr>
<tr>
<td>4μM DL-homocysteic acid</td>
<td>0.0733 g DL-homocysteic acid diluted with deionised water in 100ml volumetric flask. Final concentration 4002 μM</td>
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**Standard solutions**

Calibration curve: calibration solutions (0, 0.1, 0.25, 0.5, 1, 2, 4, 6, 8, 10 μM of glucosamine with 4μM of IS).

Stock solution of glucosamin [A]: 0.2156 g of glucosamine in 100 ml of water. Concentration: 10000μM.

Stock solution of internal standard (IS) [B]: 0.1832 g DL-homocysteine acid in 100 ml of water. Concentration: 10000 μM.

Standard solution of glucosamine [C]: 1 ml of solution [A] in 100 ml water. Concentration: 100 μM.

Standard solution of IS [D]: 2 ml of of solution [B] in 100 ml water. Concentration: 200 μM.

MW glucosamine (g/mol) 215.64

MW DL-homocysteine acid (g/mol) 183.18

| Table I Standard solutions for the determination of chitin. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Standard Nr.    | Solution [C], ml in 100ml volumetric flask | Solution [D], ml in 100ml volumetric flask | Glucosamine (μM) | IS(μM)          |
| 1               | 0               | 2               | 0               | 4               |
| 2               | 0.1             | 2               | 0.1             | 4               |
| 3               | 0.25            | 2               | 0.25            | 4               |
| 4               | 0.5             | 2               | 0.5             | 4               |
| 5               | 1               | 2               | 1               | 4               |
| 6               | 2               | 2               | 2               | 4               |
| 7               | 4               | 2               | 4               | 4               |
| 8               | 6               | 2               | 6               | 4               |
| 9               | 8               | 2               | 8               | 4               |
| 10              | 10              | 2               | 10              | 4               |

**III. ERGOSTEROL ASSAY**

**Chemicals**

- cholesterol solution in methanol (0.012 g cholesterol in 100 ml of methanol)
- ergosterol
- dry pyridine (water content less than 0.05%)
- bis-(trimethylsilyl)-trifluoracetamide (BSTFA)
- argon gas
**Standard solutions**

Solution A [internal standard for cholesterol]: 0.012 g Cholesterol in 50 ml dry pyridine  
0.24 mg/ml → 240 µg/ml → 160 µg/ml  

Solution B [pyridine with IS]: 5 ml of solution A is diluted with pyridine in 100 ml volumetric flask  
12.00 µg/ml → 8 µg/ml  

Solution C [ergosterol solution]: 0.01 g ergosterol in 100 ml dry pyridine 0.1 mg/ml → 100 µg/ml  

Cholesterol solution: 0.012 g cholesterol in 50 ml dry pyridine 0.24 mg/ml → 240 µg/ml

### Table II Standard solutions for the determination of ergosterol.

<table>
<thead>
<tr>
<th>Solution</th>
<th>C (µl)</th>
<th>A (µl)</th>
<th>Dry pyridine (µl)</th>
<th>BSTFA (µl)</th>
<th>Cholesterol (µl/l)</th>
<th>Ergosterol (µl/l)</th>
<th>Code</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>100</td>
<td>850</td>
<td>500</td>
<td>16</td>
<td>3,333</td>
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<td>800</td>
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