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Risk for mould growth in insulation materials

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Preface

This project has been carried out in collaboration with the Paper and Fiber Research Institute (PFI), as part of their iWood project. The preparation of the material used in this project and some of the testing has been done in their laboratory at Gløshaugen. Other parts of the testing was done in collaboration with Sintef Byggfork in their laboratory at Gløshaugen.

I would like to thank Engineer Bente Wallervand Ofte who has been most helpful during the testing done at Sintef Byggforsk.

At PFI I would like to thank Research sceientist Malin Brodin, Senior Research scientist Kai Toven and Senior Research scientist Kristin Syverud for appreciated academic guidance.

Declaration of compliance

I declare that this is an independent work according to the exam regulations of the Norwegian University of Science and Technology (NTNU).

Place and date: Trondheim, 16.06.2014

Signature: 

Abstract

The aim of this project has been to evaluate what effect different types of fire retardant additives have on mold growth in cellulose insulations. The cellulose insulation materials used have been prepared from two different virgin fiber thermo mechanical pulps and recovered newspaper. Fire retardants additives used has been boric acid, borax pentahydrate, ammonium polyphosphate, magnesium hydroxide and aluminum hydroxide. This have been added to the different cellulose pulps, alone and in combination, to evaluate whether they prevent or provide better conditions for mold growth. A Glava glass wool was also tested to compare the cellulose materials with a commercial product. To insure the results from the Glava testing, untreated glass wool was also tested.

Two different test methods, referred to as Method 1 and Method 2, have been used to determine the extent of mold growth in the prepared samples. Method 1 was developed as a simple pretest to determine what samples was to be tested further in Method 2. Method 2 was based on a previous master thesis with a similar topic to the one in this project, and included incubating the samples with a spore suspension.

As it turns out, Method 1 gave rapid results that were easy to interpret, and provided most of the results used in the conclusion of this report, while Method 1 had a slower development in the mold growth, and further study has been recommended.

From the results, it was found that ammonium polyphosphate provides good conditions for mold growth in cellulose fiber, but that this can be prevented by addition of boric acid, borax pentahydrate, or also magnesium hydroxide. Aluminum hydroxide used alone do not lead to mold growth, but when used in combination with ammonium polyphosphate it increases the growth.

Some mold growth was found on the surface of the tested glass wools, but it was assumed that this was due to moist debris on the surface and that the glass wool only acted as a support matrix.

Sammendrag

Målet med dette prosjektet har vært å evaluere hvilken effekt ulike typer brannhemmende tilsetningsstoffer har på muggvekst i celluloseisolasjon. Celluloseisolasjonsmaterialer som ble brukt er laget av to forskjellige nyfiber, termomekaniske masser og gjenvunnet avis. Brannhemmende tilsetningsstoffer som ble benytt var borsyre, borax-pentahydrat, ammoniumpolyfosfat, magnesiumhydroksid og aluminiumhydroksid. Disse har blitt tilsatt de ulike cellulosemassene, alene og i kombinasjon, for å vurdere om de forebygger eller gir bedre vilkår for muggvekst. En Glava glassull ble også testet for å sammenligne cellulosematerialene med et kommersielt produkt. For å sikre resultatene fra Glava testing, ble ubehandlet glassull også testet.

To forskjellige testmetoder, kalt Metode 1 Metode 2, ble benyttet for å bestemme omfanget av muggvekst på de preparerte prøvene. Metode 1 ble utviklet som en enkel forundersøkelse for å finne ut hvilke prøver som skulle testes ytterligere i Metode 2. Metode 2 ble basert på en tidligere masteroppgave med et lignende tema som dette prosjektet, og inkluderte å inkubere prøvene med en sporesuspensjon.

Det viste seg at Metode 1 ga raske resultater som var lette å tolke og ga det meste av resultatene som ble brukt i konklusjonen i denne rapporten, mens Metode 1 hadde en tregere utvikling i muggveksten, og videre studier har blitt anbefalt.

Fra resultatene ble det funnet at ammoniumpolyfosfat gir gode betingelser for muggvekst i cellulosefibre, men at dette kan forhindres ved tilsetning av borsyre, borax-pentahydrat eller magnesiumhydroksid. Aluminiumhydroksid anvendt alene fører ikke til muggvekst, men når det brukes i kombinasjon med ammoniumpolyfosfat det øker veksten.

Noe muggvekst ble funnet på overflaten av glassullen som ble testet, men det ble antatt å være på grunn av fuktig smuss på overflaten og at glassull kun fungert som en støttematrise.

Table of Content

Innhold

1 Introduction

This project has been part of the iWood project at PFI, where the final goal is to make a cellulose insulation based on loose fibers that do not contain harmful additives. Challenges related to cellulose insulations' resistance to fire and biological degradation have to be met to reach this goal.

Cellulose insulation materials are added different components to become more resistant to fire. Boric acid and Borax are widely used, as these also prevent biological degradation and mold growth. The problem with these additives is that they are classified as toxic, and thus, the iWood project desires to develop a cellulose insulation material using other additives to prevent fire.

Alternatives to the toxic borates are ammonium polyphosphate and inorganic hydroxides. Previous it has been shown that these use different mechanisms to prevent fire, and it was recommended to add both in a cellulose insulation material to ensure a better resistance to fire (Heggebø, 2013).

The purpose of this project was to explore the risk for microbiological growth in virgin fiber based and recycled fiber based cellulose insulation materials with and without fire retardant additives. If some of the alternative fire retardants shows the same preventing effect on mold growth as the borates, the iWood project is on step closer to producing a non-toxic cellulose insulation

The amounts of fire retardants added is based on the previous study on fire retardancy mentioned above. The methods used to determine mold growth in the cellulose insulation materials prepared was based on a previous master thesis with a similar topic and a standard test method issued under the fixed number C1338 (ASTM Int'l, 2008).

2 Mold Growth in Insulation Materials

Microorganisms can be divided in to several major groups, such as bacteria, viruses, fungi and algae (Talaro, 2009a) .Some of these microorganisms, including fungi, produces the enzyme cellulase, which makes them capable of digesting cellulose. In nature, these microbes work as decomposers and play an important role in breaking down and recycling plant materials (Madigan, et al., 2012). This means that cellulose insulation is exposed to a degradation by microorganisms if the right growth conditions are established. The scope of this project was to explore the risk for microbiological growth in virgin fiber based and recycled fiber based cellulose insulation materials with and without fire retardant additives, and knowledge of the lifecycle of fungi (mold) can help prevent degradation of the cellulose insulation. Some fungi can also cause allergies and other medical conditions in humans, another reason why fungal growth is not wanted in a house's insulation (Talaro, 2009b).

2.1 The Lifecycle of Fungi

The Kingdom of fungi is vast and stocked with a great variety of complex organisms. It is divided into two groups: the macroscopic fungi, which includes mushrooms, puffballs and gill fungi, and the microscopic fungi, which includes molds and yeasts. The bodies of mold is made up of long, threadlike cells called hyphae. Normally one thinks of mold as cotton like or hairy textures growing on food that has been left in the fridge for too long. This hairy texture occurs when hyphae grow together across a surface and woven together forms a colony of mold, or a *mycelium* (Talaro, 2009b). Figure 2.1 shows the development of a fungal colony.

There are several reproducing strategies for fungi. One of them is simply outward growth of existing hyphae, and another is fragmentation, where a piece of mycelium separates and generate a new colony. However, the main approach to reproduction is done by the production of spores. Spores can, for simplicity, been thought of as the seed of the fungi, and the formation can be both asexual and sexual. The spores as compacted and light weighted, which makes them easily dispersed through the environment by water, air, and living things. If the spore lands on a suitable substrate, it will germinate and produce a new fungus colony. Figure 2.2 describes the lifecycle of mold (Talaro, 2009b); (Madigan, et al., 2012).

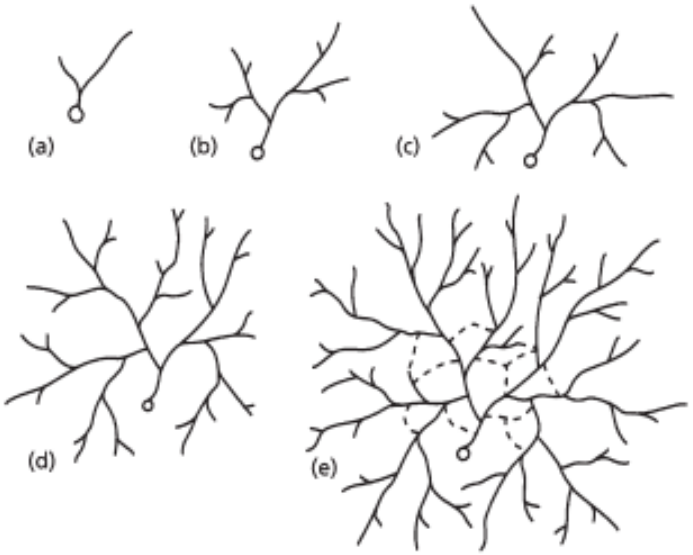

Figure 2.1(a-e): A schematic overview of the stages in the development of a mycelium, from germination to spore (Deacon, 2006b).

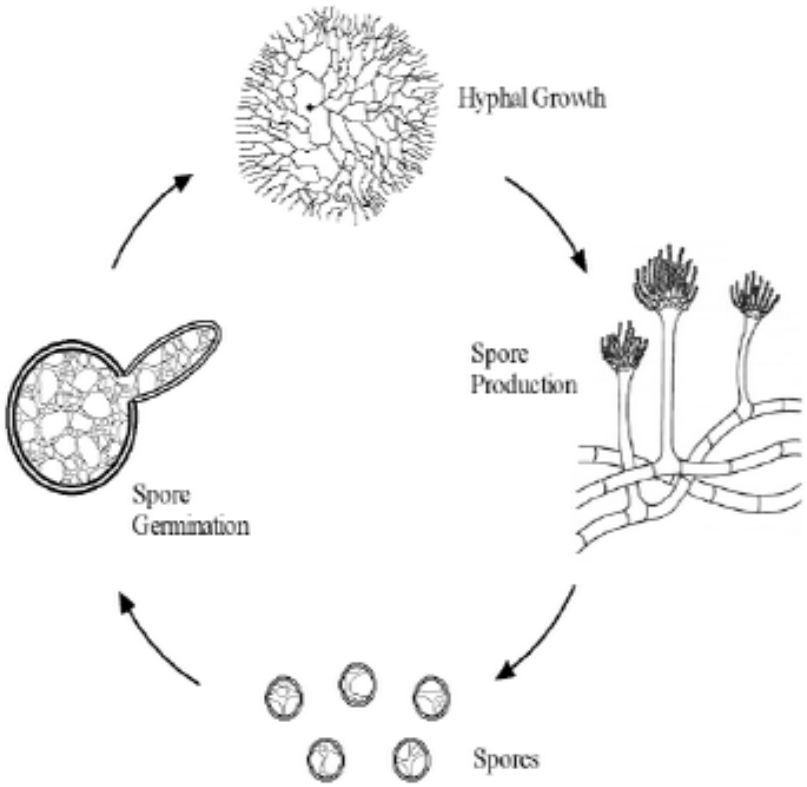

Figure 2.2: Simplified lifecycle of mold. The figure shows how spores are produced in the mycelium and then dispersed to germinate in new environment and start a new colony (ToxicBlackMold, n.d.).

2.2 Nutrients for Microbiological Growth

Fungi can acquire nutrients from a broad variety of organic materials, or *substrates*. These are needed for cellular activities, like metabolism and growth (Talaro, 2009c). To access the nutrients, the fungus penetrates the substrate and secretes enzymes that reduces the nutrients to small molecules. With this "technique", fungi can utilize nutrients from several substrates, including feathers, petroleum products, wood and cellulose (Talaro, 2009b). Figure 2.3 illustrates the wide range of organic compounds that can be utilized by fungi, from the simple organic compound methane, to the more complex compound lignin.

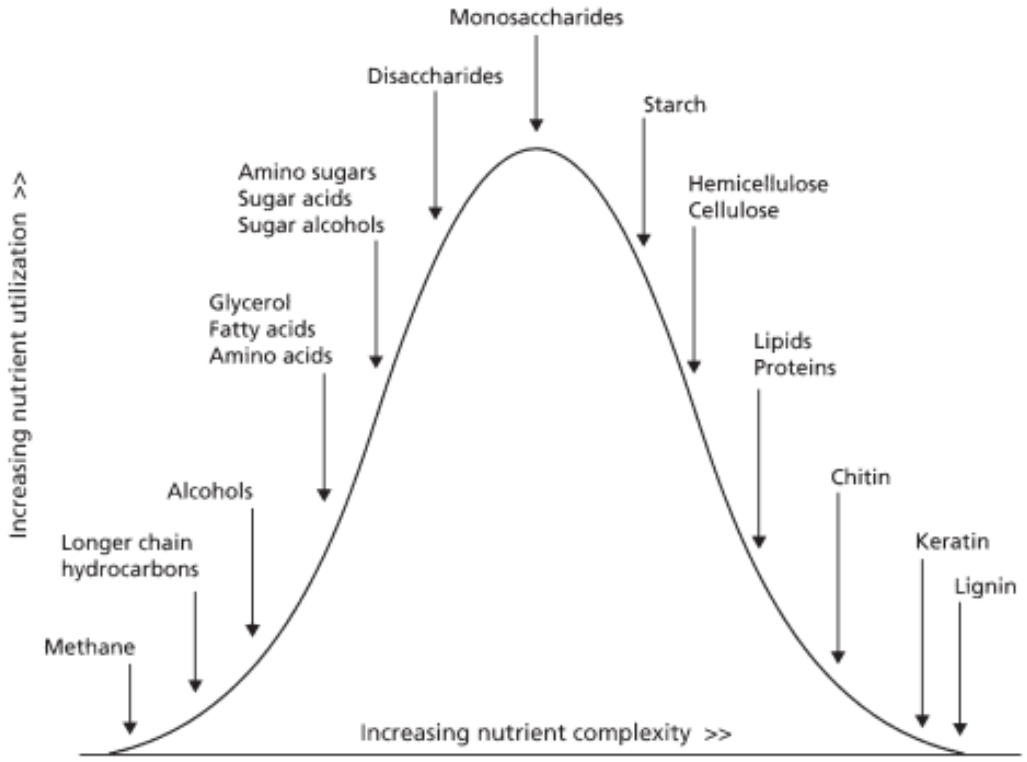

Figure 2.3: Some representative carbon substrate of fungi. The complexity of the nutrients are increasing from left to right, and the vertical arrangement gives the share of fungi that can utilize the nutrients (Deacon, 2006a).

The essential nutrients for microbiological growth can be divided into macronutrients and micronutrients. Macronutrients include compounds containing carbon, hydrogen and oxygen. These components are used in key roles in the cell structure and metabolism, and are thus needed in relatively large amounts. Manganese, zinc, and nickel are examples of micronutrients. These are trace elements needed in a much smaller amount, but are still important, as they are involved in enzyme functions and maintenance of protein structure. What constitutes as a micronutrient varies between microorganisms (Talaro, 2009c).

Many mineral nutrients are also important for the fungal activities, especially nitrogen, phosphorus, and iron. Nitrogen can often be the limiting factor in fungal growth. Even if the atmosphere consist of 79% nitrogen (Talaro, 2009c), fungi cannot fix this nitrogen and have to find other sources. Amino acids is an alternative source of this nutrient, and many fungi can use ammonia as a source. Phosphorus is needed of all organisms, in the form of phosphates, as it is used in several activities e.g. energy production. Soil can be a source for phosphorus, but fungi can also use enzymes to cleave phosphorus from organic sources, or even release organic acid to solubilize inorganic phosphates. Fungi can in addition accumulate and store phosphates for later requirements. Iron can be found from ferric oxides or hydroxides, and has a key part as a donor and acceptor of electrons in cellular processes (Deacon, 2006a).

2.3 Environmental Factors

Apart from nutrients, several environmental factor has to be in place for fungal growth to occur. Presence of oxygen is important, as most fungi are strictly aerobe and dependent on oxygen at least in some stages of their lifecycle. Carbon dioxide is also needed in a small amount to generate different proteins and lipids. Fungi has a relatively high tolerance for growth in acidic environments, some can even grow at pH 2.0. Nonetheless, the optima for growth is pH 5.0-7.0, but many fungi will grow in the range pH 4.0-8.5 (Deacon, 2006c).

Most fungi are mesophilic, meaning they grow at moderate temperatures. These types of fungi grow in the temperature range from 10-40°C, but has different tolerances within these temperatures and are usually grown at room temperature. Some fungi, so called psychrophiles, can grow at lower temperatures, and thermophiles and hyperthermophiles are fungi that can grow at higher temperatures. Combined, fungi has a growth range in terms of temperature from about -5°C and up to 62°C (Deacon, 2006c).

Water is important for all fungi as it helps to release extracellular enzymes that degrades the nutrients needed for fungal growth. The presence of water is also required in the uptake of nutrients through the cell wall of the fungi (Deacon, 2006c). The availability of water is given by the water activity (Aw), which indicates in what degree the water is bound by external forces. The water activity is given by a decimal number between 0 and 1, where 1 implies no external forces. It is equivalent to relative humidity (%RH) by Equation 2.1:

$$\%RH = 100 \times A_w \tag{2.1}$$

The water activity needed for fungal growth vary between different species. For mold found in building materials the minimum water activity has to be in the interval 0.76-0.96 for growth to occur (Grant, et al., 1989).

Not all these environmental factors needs to be present in their optimal conditions to give fungal growth. Usually fungi can tolerate one suboptimal factor as long as the others are near their optimal range. On the other hand, a combination of suboptimal conditions will lower the chances of growth and even prevent it. Competition between different species of fungi growing on one material can also restrict growth (Deacon, 2006c).

3 Insulation Materials

There are many commercial insulation materials on the marked today. The most common and well known insulation materials are mineral wools, like rock wool and glass wool (Glava), but there are also commercial cellulose insulations. Wanted properties from an insulation material depends on where it is going to be applied, where in a building and how the climate is in the surrounding area. Still the most important property of an insulation material is the thermal conductivity. This is also called the lambda (λ) value of the material, and is a measure of how efficient the heat transport is limited, given in W/m·K. A lower lambda value gives less heat loss, and thus a better insulation material. Typical lambda values for mineral wools ranges from 0,034 W/m·K to 0,040 W/m·K, while for cellulose insulation the values lie between 0,038 W/m·K and 0,043 W/m·K (Bakalders, 2011). In the terms of heat conductivity, cellulose insulation seems like a worthy opponent to mineral wool insulations.

Other aspects that are important for insulation materials are their fire retardancy. According to § 7.24 in "Ren veiledning til teknisk forskrift til plan- og bygningsloven 1997, utgave 2" (Statens bygningstekniske etat, 1999): "*Insulation in constructions must not contribute to increased risk of spread of fire in a building. Insulation must initially be incombustible*". An insulation material's reaction to fire are classified in Euro Classes accordance with EN 13501-1. Mineral wools are mostly classified as A1 or A2, as they have no contribution by fire, whereas cellulose insulations have the lowest classification and their contributions by fire are not specified (Byggforsk, 2004). Paragraph 7.24 also states that cellulose insulation can be used as long as it do not contribute to fire spreading. This can be done by cementing or casting it in place (Statens bygningstekniske etat, 1999). Addition of fire retardants may also give cellulose insulation a higher classification in the Euro Classes (Byggforsk, 2004). Further, the paragraph gives specifics on what type of building and which risk classes the cellulose insulation has to be approved for that opens for a wider use, even with low reaction to fire classification (Statens bygningstekniske etat, 1999).

Being an organic material, cellulose is naturally exposed to degradation by microorganisms, like bacteria and fungi (Madigan, et al., 2012), and a degradation is likely to affect the insulation properties of the material. In addition, microbial growth (mold) is typically not wanted in buildings and houses as it can produce allergens and lead to allergies and other medical conditions (Talaro, 2009b). A common perception seems to be that mineral wool is less prone to microbial attack. The scope of this project was to explore the risk for microbiological growth in cellulose insulation materials. In addition, the risk for microbiological growth in glass wool has been studied to compare the prepared cellulose insulation with an existing, commercial insulation material, and to test the perception on microbiological growth in these types of materials. In the following sections a more thorough description of glass wool, cellulose insulation and fire retardant additives is given.

3.1 Glass Wool

Glass wool is a material based on a mix of quartz sand, feldspar and/or dolomite and recycled glass that has been melted together at 1400 °C. The molten mass is blown through a rotating nozzle, resulting in thin glass fibers (Bakalders, 2011).

Two types of glass wool was used in this project, Glava Extrem 33 and an untreated glass wool from Antech. Glava A/S produce a range of glass wool for different use. In general, Glava glass wool is based on silicat glass containing vitreous (silicate) fiber with an alkaline oxide and alkali earth oxide content greater than 18%. These oxides include Na2O, K2O, CaO, MgO and BaO. The silicate glass constitutes 88-97% of the weight of Glava glass wool. Cured urea-modified Phenol/formaldehyde resin are added in amount from 2-11% to bind the fibers together and emulsified oil is added in 0-1% for dust preventions. The untreated glass wool was included in the testing to see if it is the additives in Glava Extrem 33 that prevents potential mold growth or if the glass wool itself is an unfavorable material. The exact amounts of the different components used in Glava Extrem 33 is not given. According to the Classification, Labeling and Packaging of substances and mixtures (CLP) regulations Glava glass wool is not classified hazardous, but coarse fibers may cause mechanical irritation on the skin (GLAVA A/S, 2013).

3.2 Cellulose Insulation Materials

The term cellulose insulation include insulation made out of wood fibers or recovered newspaper. The raw material for both is debarked stemwood that has gone through a pulping process. The cellulose materials are grounded to a wool-like consistence before they are used as insulation (Byggforsk, 2004). It is produced from environmentally friendly materials and relatively easy to install (Shen K., et al., 2010). The installation is done by using compressed air to blow the loose fill material in place. An alternative is making insulation panels or mats of the cellulose material (Byggforsk, 2004).

3.2.1 Components in Cellulose Insulation Materials

Wood is a nonuniform material, made from various types of cells, giving trees their physical, anatomical and chemical properties. Since wood is a biodegradable material, and to better understand how microorganisms can utilize the different components in wood, a closer description of the components follows.

The cells in the stemwood is built up of a polymeric matrix of carbohydrates (cellulose and hemicellulose) and lignin, which are the "structural components" of the cells. There are also a minor part of "nonstructural components" such as extractives, inorganics and proteins present in the wood cell wall. The percentage content of the different components varies between different types of wood. Table 3.1 shows how the components usually vary in a woody feedstock (Alén, 2000).

Table 3.1: Typical chemical composition of woody feedstock used for pulping (% of the feedstock dry solids) (Alén, 2000).

Component	**Woody feedstock** (% of the feedstock dry solids)
Carbohydrates in total	65 – 80
- Cellulose	40 – 45
- Hemicellulose	25 – 35
Lignin	20 – 30
Extractives	2 – 5
Inorganics	0.1 – 1

According to (Alén, 2000), cellulose is the world's most abundant and important biopolymer, which is easy to imagine, as it is the main component in wood. From Table 3.1 it can be seen that cellulose is the main component of wood, as up to 45% of the wood can be cellulose. To utilize cellulose from wood, the stemwood can be debarked and used for pulping. The resulting cellulose pulp can be used in a wide range of products, such as cellulose insulation and newspapers. This polysaccharide consists of β-1,4-D-glucopyranose units. These units are linked together by 1,4-glucosidic bonds, giving long, linear chains (Price & Horrocks, 2010). At the C_1-end of the cellulose chain there is a hemiacetal structure that has reducing properties, while at the C_4-end the C_4-OH is a nonreducing alcoholic hydroxyl group. This gives the cellulose chain both a reducing and a nonreducing end (Alén, 2000). Figure 3.1 shows the stereochemical structure of cellulose where the C_1 and C_4 are marked.

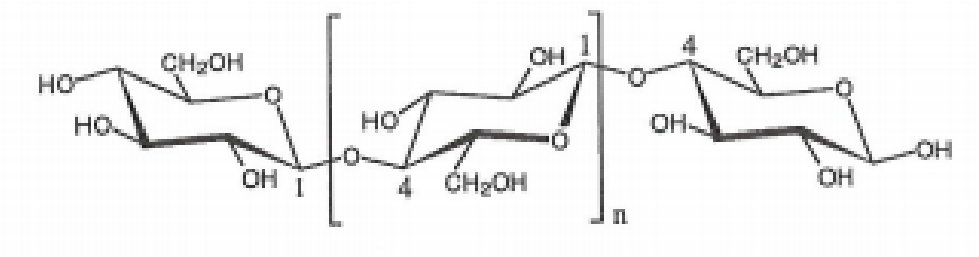

Figure 3.1: Stereochemical structure of cellulose (Alén, 2000).

Native wood cellulose has a degree of polymerization in the order of 10 000. This decreases somewhat during technical processes and can become as low as 500-2000. There is a strong tendency of intra- and intermolecular hydrogen bonding between the cellulose chains, which results in an aggregation into microfibrils. These bundles of microfibrils can form either crystalline or amorphous regions. Further aggregation of microfibrils results in "fiber wall cellulose", with a degree of 60 – 75% crystallinity. The high degree of crystallinity gives the wood its mechanical strength. It also means that during chemical treatments, cellulose is relatively inert, only soluble in a few solvents (Alén, 2000). The crystallinity of cellulose also makes it harder for enzymes of fungi (and other microorganisms) to access the glucose units, but some fungi have developed a system using several enzymes in synergy to degrade the cellulose molecule and utilize the sugars (Deacon, 2006a).

Hemicellulose is the second largest component of wood, with up to 35% of the wood consisting of hemicellulose, as seen in Table 3.1. The building blocks of these polysaccharides are hexoses, pentoses, or deoxyhexoses, with the presence of a small amount of certain uronic acids, see Table 3.2. Usually these units exist as six-membered (pyranose) structures.

Table 3.2: Building blocks in hemicellulose, (Alén, 2000).

Hexoses	Pentoses	Deoxyhexoses	Uronic acids
D-glucose	D-xylose	L-rhamnose	4-O-methyl-D-glucoronic acid
D-mannose	L-arabinose	L-fucose (rare)	D-galacturonic acid
D-galactose	D-arabinose		D-glucoronic acid

Hemicellulose is not crystalline and has a much lower degree of polymerization (100 – 200) than cellulose, which leads to a generally lower chemical and thermal stability. The fact that hemicellulose is soluble in alkali, as opposite to cellulose, can be exploited to fractionate different polysaccharides in lignin-free samples (Alén, 2000). Like for cellulose, some fungi have developed methods using enzymes to utilize the carbohydrates in hemicellulose, making it prone to biological degradation in the same manner as cellulose (Deacon, 2006a).

Lignin differs from cellulose and hemicellulose by being an amorphous polymer consisting of phenylpropane units that are not linked together in any systematical order. This gives lignin a more flexible structure and makes it work as a binder in wood (Alén, 2000). The complex structure of lignin makes it hard to degrade biologically (Deacon, 2006a).

3.2.2 Production of Cellulose Pulps

In this project two cellulose insulation materials based on wood fiber are studied. They are produced by thermo mechanical pulping (TMP) of wood, and are from now on referred to as TMP1 and TMP2. These cellulose materials are developed especially for the iWood project, and therefore details of the production will not be given here. In general the production of thermo mechanical pulps involves steaming of the raw material under pressure before and during mechanical fibrillation in a refiner. With this technique the fibers keep all the wood components (Smook, 2002). The TMP1 and TMP2 are processed differently after fibrillation in a way that affects the content of cellulose, hemicellulos and lignin in the two pulps. This is thought to have a possible effect on the foundation of microbiological growth.

A cellulose insulation based on recovered newspaper has also been studied in the project. Newspapers are commonly made from thermo mechanical pulps, but also from recovered newspaper that has been cleaned and re-pulped (Sixta, 2006). The recovered newspaper used here will most likely contain more oils and biological spore than the unused thermo mechanical pulps, and contain ink and fillers. This might make it more subjected to biological growth.

3.3 Fire Retardants

A downside of cellulose insulation materials is that they are flammable and biologically degradable (Hansen & Eriksen, 2000). Fire retardants are thus added to slow down the degradation process or delay the ignition of the material. The types of fire retardants added to the cellulose insulation materials prepared in this project are based on the results from the reaction to fire tests done in in conjunction with the project "Alternative fire retardants in wood fiber insulation – Effects on ignitability" (Heggebø, 2013). A recommendation from that project was to mix different fire retardants to get an increased combined effect. Several combinations were tried in this project, but the combined effect was evaluated in terms of fungal growth, and not in terms of fire retardancy. A description of the applied fire retardants follows.

3.3.1 Ammonium polyphosphate

Ammonium polyphosphate (APP) is a stable, non-volatile, inorganic salt that is relatively insoluble in water (SpecialChem, 2014a). It is commonly used as fire retardants in polymer materials, also cellulose insulation. APP exists in different crystalline forms and the commercial APP products varies in molecular weight, particle size, solubility, etc. The phosphorus content also vary between the products, and can be as high as 30% (Joseph & Ebdon, 2010). In this project Exolite AP422 (Clariant) is the applied form of APP. Figure 3.2 shows the chemical structure of APP.

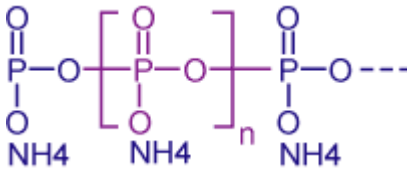

Figure 3.2: The chemical structure of ammonium polyphosphate (SpecialChem, 2014a)

When applied as a fire retardant, APP will decompose to ammonium and phosphoric acid when exposed to heat. When used in cellulose material, the phosphoric acid will react with the hydroxyl groups of the cellulose, and form a non-stable phosphate ester. This leads to a dehydration of the cellulose substrate and an enhanced char formation. The char builds up on the surface of the material and act as a barrier from both heat and oxygen, and thus prevents further decomposition of the material. In addition, the char layer reduces smoke emission (Joseph & Ebdon, 2010). Figure 3.3 illustrates this mechanism.

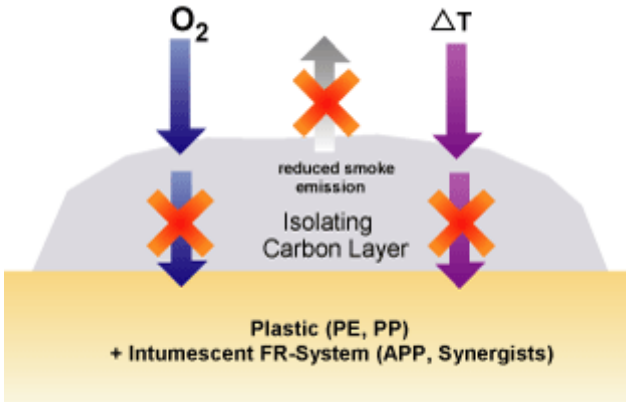

Figure 3.3: The mechanism of ammonium polyphosphate (APP) as a fire retardant. The carbon layer is formed by the decomposed APP in reaction with hydroxyl groups in the material, and functions as a barrier from both heat and oxygen, prevention further decomposition (SpecialChem, 2014b).

Ammonium polyphosphate is also used to produce complete liquid fertilizers (Krishnamurthy, et al., 2009), which has led to the concern that it will work as a nutrient to fungal growth when applied in cellulose insulation materials.

3.3.2 Boric acid based fire retardants

In commercial loose fill cellulosic insulation, APP is often used in combination with boric acid based fire retardants to improve the fire retardancy. Boric acid has properties that limit biodegradation (Hansen & Eriksen, 2000).

The relatively weak boric acid ($B_2O_3 \cdot 3H_2O$) is a triclinic crystal, soluble in water, alcohols and glycerin. The boric acid used in this project is Optibor (20 Mule Team Borax).When heated, boric acid undergoes an endothermic reaction, thus absorbing heat and slowing down ignition if it is used as a fire retardant. If used in cellulose insulation materials the water released in the endothermic reaction work as a diluent in the gas phase of the heated cellulose material. Boric acid is an effective smoldering inhibitor (Shen K., et al., 2010).

Often the crystalline borax pentahydrate ($Na_2O \cdot 2B_2O_3 \cdot 5H_2O$) is used in addition to boric acid as a fire retardant. The compound used in this project is the Neobor (20 Mule Team Borax). Borax pentahydrate also dehydrates during heating, giving off water and slowing down ignition. Borax pentahydrate is an efficient fire retardant in cellulose materials in terms of surface flammability, but due to the Na_2O content it can promote smoldering combustion (Shen K., et al., 2010).

According to the Classification, Labeling and Packaging of substances and mixtures (CLP) regulations boric acid is classified as toxic for reproduction, with a specific concentration limit at ≥ 5.5% (European Chemicals Agency, 2014a). Borax pentahydrate has the same classification, with a specific limit concentration of ≥ 6.5%. The same regulation states that it is eye irritating at a specific limit of ≥ 6.5% (European Chemicals Agency, 2014b). For these reasons, this project aims to find another compound that can act as a fire retardant in symbioses with APP. In the project "Alternative fire retardants in wood fiber insulation – Effects on ignitability" (Heggebø, 2013) it was found that samples with APP had little to no smoldering during the test, but obvious surface flammability, while the opposite was true for samples containing boric acid and borax. Thus, to replace these toxic boric acid based fire retardants, a compound that is able to inhibit flammability and limit biological degradation has to be found.

3.3.3 Inorganic hydroxides

More than half of the fire retardants used globally are inorganic hydroxides. Compared to phosphorus containing fire retardants they are low in cost, and they are also low in toxicity. As fire retardant, aluminum hydroxide is the largest selling inorganic hydroxide, closely followed by magnesium hydroxide. The use of these types of fire retardants is increasing, as their impact on the environment is thought to be less harmful compared to halogen and phosphorus containing fire retardants. Like the boric acid based fire retardants, the fire retardancy effect originates from the endothermic decomposition of the compound when exposed to heat (Hornsby, 2010).

A drawback is that as much as 40-60% of the material has to be inorganic hydroxide for it to be an effective fire retardant, and will most likely affect the material it is used in. Halving the fiber content of cellulose insulation would have a definite negative effect on the insulation properties (Hornsby, 2010). In this project aluminum hydroxide and magnesium hydroxide have been added to cellulose insulation materials, also in combination with APP, to see what effect they have on microbial growth. The amounts added have not been as high as recommended, as they are thought to be used in combination with APP in further applications. Their joint effect might lower the need for such large amounts of inorganic hydroxides.

4 Methods

In this project, two different methods were used to find what effects the different fire retardants had on the insulation materials in terms of mold growth. The first method described under, Method 1, was a basic test to give a quick indication on how the different fire retardants affected the pulps and in what degree they prevented or enhanced mold growth. The amounts used here were based on the result from the fire testing done in ““Alternative fire retardants in wood fiber insulation – Effects on ignitability” (Heggebø, 2013). Results from the first 3 weeks of Method 1 were used to decide what amounts of fire retardants should be used in the second method, Method 2. The insulation materials used in the two different methods were prepared as described below.

4.1 Preparation of the Tested Insulation Materials

The two pulps that were used in this project were stored frozen. They were dried in a heating cabinet, holding 50˚C, overnight. This was to decrease the drying time and thereby minimizing contamination of microorganisms from air, compared to if the pulps had been air-dried over a longer period. To give the pulps a similar texture and basis for comparison they were run through a Hammer mill, resulting in a wool-like and fluffy consistency of the pulps. This was also done to increase the surface area of the fibers and give the mold a bigger area to grow on. Fire retardants were blended in with the pulps in a plastic bag. The dry content of the cellulose materials were taken into account to get the correct mixing ratios, see Appendix A.1.

The recovered newspaper was also run through the Hammer mill to give it a wool-like consistency, as described in section 3.2 Cellulose insulation Materials, so that it could be compared to the commercial cellulose insulation made from recovered newspaper. No fire retardants were added.

The mineral wools, both the Glava Extrem 33 and the untreated glass wool, were cut into suitable pieces but other than that remained untreated.

Nitrile gloves and facemask were used in the preparation of all the samples.

4.2 Method 1

This method was based on the conditions given in “Standard Test Method for Determining Fungi Resistance of Insulation Materials and Facings”, a standard issued under the fixed number C1338 (ASTM Int'l, 2008). The standard gives that equipment used during the test period should maintain a temperature of 28-30°C and a relative humidity of 95% (± 4%), normally achieved by using an environmental chamber or cabinet. Such equipment was not available for this method, so instead the prepared samples were added water to a dry content of 20%, sealed in a plastic bag, and placed in a water bath keeping 30 ◦C. The low percentage of dry content may seem severe, but this was to ensure mold growth in a short amount of time so the results could be applied in Method 2. The standard method also involves incubating the test material with a specified spore suspension, but this step was skipped to keep the test simple. Since spores from mold are dispersed in nature in huge numbers (Rylander, 2008), some types of spores will most likely have infected the test materials, as it was not kept in sterile surroundings. From this, it can be assumed that there has been a “natural” incubation of the samples. A test period of minimum 28 days ± 8 hours is recommended in the standard (ASTM Int'l, 2008).

As mentioned, this method was used as a pretest to decide which amounts of fire retardants was going to be added to the different pulps in a more thorough method, see section 4.3 Methode 2. This part of Method 1 is from now referred to as Part 1. Part 1 was continued after Method 2 was started, and had a total test period of 17 weeks.

TMP1 was used as the main test material in Part 1 and was mixed with different amounts of APP, magnesium hydroxide, boric acid and borax. The TMP2 was mixed with APP and magnesium hydroxide (Mg(OH)2). Two samples with clean TMP1 and TMP2 were also tested, as well as recovered newspaper and Glava Extreme 33. Table 4.1 shows the prepared samples and the different amounts of pulp, fire retardant(s) and water in each of them. Two parallels were made for each sample and each sample weighed 15 grams. To add the right amount of water and fire retardants, the dry content of the pulps were taken into account, see Appendix A.2 for calculations.

Table 4.1: Prepared samples for Method 1 – Part 1. Percentage amount of added fire retardant(s) is based on the weight of the whole sample, 15 grams.

Insulation Material	APP (%)	Borax (%)	Boric acid (%)	$Mg(OH)_2$ (%)
TMP1	7.5			
TMP1	15			
TMP1	7.5	0.5		
TMP1	7.5	2		
TMP1	7.5			5
TMP1	7.5			20
TMP1	7.5	2	3	
TMP1	7.5			
TMP2				
TMP2	7.5	2		
Glava Extrem 33				
Recovered newspaper				

Method 1 was redone with some of the same samples used in Method 2, from now on referred to as Part 2. This was to easier compare the results from Method 1 and Method 2, and see if this simple method was just as applicable to evaluate mold growth on cellulose insulation materials. Table 4.2 shows which samples was used in Part 2. The test period of Part 2 was 7 weeks in total.

In Method 1, the samples were monitored once a day the first week to closer follow the development of growth/degradation. Notations on the development for each sample were taken once a week and supplemented with pictures of the samples documenting the surface coverage of mold growth.

Table 4.2: Prepared samples for Method 1 – Part 2. Percentage amount of added fire retardant(s) is based on the weight of the whole sample, 15 grams.

Insulation Material	**APP (%)**	**Borax (%)**	**Boric acid (%)**	**$Mg(OH)_2$ (%)**	**$Al(OH)_3$ (%)**
TMP1					5
TMP1					10
TMP1	7.5				1
TMP1	7.5				5
TMP1	7.5				10
TMP1	7.5		1		
TMP1	7.5		2		
TMP1	7.5			1	
TMP1	7.5			5	
TMP1	7.5	2		10	
TMP1					5
TMP1					10
TMP2					5

4.3 Method 2

This method is based on a previous master thesis «Studie av mugsopp på bygningsmaterialer – risiko for vekst av muggsopp på vindsperre» (Myklebost & Jansen, 2007), done in collaboration with Sintef Byggforsk. The temperature and relative humidity conditions are based on the "Standard Test Method for Determining Fungi Resistance of Insulation Materials and Facings", similar to those in Method 1.

Table 4.3 shows which samples were prepared for this method based on the result from part 1 in Method 1. In addition to magnesium hydroxide, aluminum hydroxide (Al(OH)3) was also tested in this method. Three parallels were made for each sample. A forth parallel was made for all the 5 different insulation materials as a reference. The reference parallels were not incubated with the spore suspension.

About 5 grams of the prepared insulation samples were placed in perforated (7.5 cm × 7.5 cm) aluminum form. The samples were conditioned for a week in a climate room keeping 50% relative humidity and 25˚C, before they were incubated with a suspension of mold spores.

Table 4.3: Prepared samples for Method 2.. Percentage amount of added fire retardant(s) is based on the weight of the whole sample, 5 grams.

Insulation Material	**APP (%)**	**Borax (%)**	**Boric acid (%)**	**$Mg(OH)_2$ (%)**	**$Al(OH)_3$ (%)**
TMP1					
TMP2					
Recovered newspaper					
Glava Extrem 33					
Untreated glass wool					
TMP1	7.5				
TMP1	15				
TMP1	7.5				1
TMP1	7.5				5
TMP1	7.5				10
TMP1	7.5			1	
TMP1	7.5			5	
TMP1	7.5			10	
TMP1	7.5		1		
TMP1	7.5		2		
TMP1	7.5	2			
TMP1	7.5	2	3		
TMP1					5
TMP1					10
TMP1				5	
TMP1				10	
TMP2	7.5			5	

The samples was incubated with a spore suspension containing spores from mold types listed in Table 4.4, and are all typically found in building materials (Mattsson, 2004). The spores have been collected from VTT Technical Research Center of Finland, and the VTT numbers of the molds are given in Table 4.4.

Table 4.4: Species of mold used in this study, and their corresponding VTT number.

Species	**VTT number**
Aspergillus versicolor	D-96660
Cladosporium cladosporioides	D-96646
Penicillium chrysogenum	D-96661

The spore suspension was produces by adding 20 mL sterile water with a content of 0.02% Tween 80 to an agar plate containing a week old mold growth. The liquid was carefully mixed with the mold spores and the suspension was filtered through a cotton ball into an Erlenmeyer flask to remove large particles. The resulting suspension was stirred for 30-60 seconds to avoid formation of lumps. A drop of the suspension was transferred to a counting chamber, coated with a coverslip and a microscope was used to controlling spore concentration. From the observed concentration, a conservative dilution factor was calculated, and the suspension was diluted until wanted concentration of 30 spores per square in the counting chamber was observed. The procedure was done for all the three mold species, and 50 mL of each suspension was mix together to the final spore suspension. The spore suspension was then applied to the samples using 10 sprays from a spray flask, see Figur 4.1.

A control of the viability of the spores in the four suspension was done by growing each suspension on an agar plate that was kept in a climate chamber holding room temperature. All the grown suspension showed viable spores after 1 week.

Figure 4.1: Application of the spore suspension using a spray flask.

To achieve the wanted conditions the samples were placed in static climate chambers. These chambers were made using plastic containers (600 mm × 400 mm × 120 mm) placed in a heating cabinet holding 30˚C. To ensure an airtight environment the containers were closed off using rubber strips and a glass plate (4 mm thick), see Figure 4.2. To get a relative humidity (%RH) of 93% the base of the chambers were filled with a saturated salt solution. According to Quantifoil Instruments a saturated solution of potassium nitrate (KNO_3) in distilled water gives a %RH of 92.31±0.60 at 30˚C in an enclosed chamber. To measure the relative humidity the plastic containers were provided with a small hole at the side to enable the measuring apparatus to be inserted. The hole was closed with clay. A visual control of the saturated solution was done weekly and the relative humidity was measured regularly along with the temperature. An aluminum grate was mounted 50 mm from the base of the containers for the samples to be placed on and thus avoid contact with the salt solution. A total of 18 samples were placed in each container, see Figure 4.2.

Figure 4.2: Setup of climate chambers.

The test period lasted for 13 weeks and each week the samples were observed using a stereomicroscope (Olympus SZX12). Observations were noted and a picture was taken (Olympus DP71 attached to the Olympus SZX12) if growth was spotted. In addition the samples was observed without using a stereomicroscope, and pictures of the whole sample was taken using DigiEye photo chamber to ensure comparable light conditions each time a photo was taken.

5 Results and Discussion

5.1 Results and Discussion – Method 1

The results from Method 1 are presented as percentage mold growth coverage on the surface of the samples, determined from the photos takes of the samples each week. There were two parallels of each set of samples, and the results are presented as an average of the two. Since this method had a "natural" incubation of spores, a difference in mold growth between the parallels was expected, but most of the parallels show a similar trend. For a more detailed description of the observation made during the test period, see Appendices B.1 and B2.

5.1.1 Part 1

Figure 5.1 shows the results from Part 1 for the samples where growth was observed. The samples where no growth was observed are listed in Table 5.1.

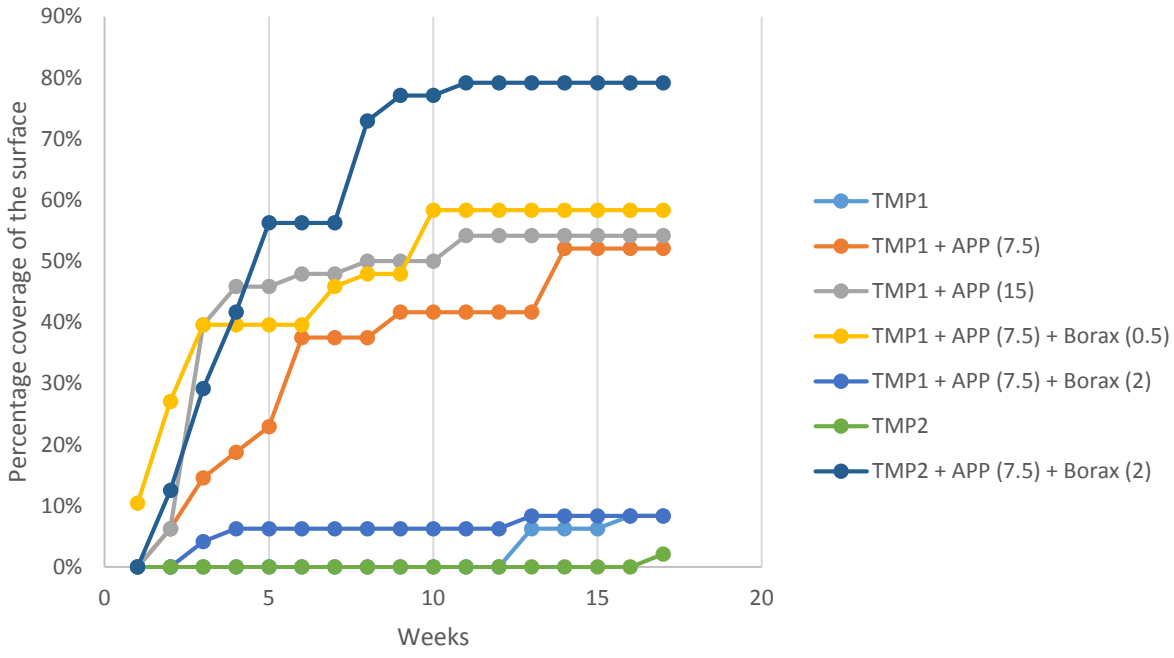

Figure 5.1: Samples in Method 1 – Part 1 where mold growth was observed, given as percentage mold growth coverage on the surface during the-17 weeks test period. The numbers in parentheses in the sample names are percentage amounts of fire retardants added, based on weight.

Table 5.1: Samples in Method 1 – Part 1 where mold growth was not observed during the 17-week test period. The numbers in in brackets in the sample names are percentage amounts of fire retardants added, based on weight.

Samples where no growth was observed
TMP1+APP+$Mg(OH)_2$ (5)
TMP 1+APP+$Mg(OH)_2$ (20)
TMP 1+Boric acid (3) + Borax (2)
Glava Extrem 33
Recovered newspaper

5.1.1.1 Results after 3 Weeks – Deciding Samples for Method 2

The development of mold growth in the samples of Part 1 had been observed for 3 weeks when the results were used to determine what samples should be prepared for Method 2. From Figure 5.1 it is clear that already after these 3 weeks the samples with APP are exposed to mold growth. The samples of pure TMP1 and TMP2 show no sign of mold growth at this time, but the surface of the sample with 15% APP added to TMP1 and the sample with 7.5% APP and 0.5% Borax added to TMP1 have a coverage of about 40% mold. The sample added 7.5% APP to TMP1 has a lower mold coverage of 15%. From this, a higher addition of APP seems to give a higher risk of mold growth, and borax in the amount of 0.5% does not seem to have a preventing effect on mold growth. A higher addition of Borax (2%), combined with 7.5% APP, seems more effective in the TMP1 sample, with only 4% mold coverage after 3 weeks. On the other hand, the TMP2 sample with the sample amount of Borax and APP have a coverage of 29% at that time.

The TMP1 samples added APP and magnesium hydroxide do not show any sign of growth after 3 weeks, neither does the TMP1 sample added boric acid and Borax. The Glava Extrem 33 and the recovered newspaper are also free of mold growth after this period.

From these results after 3 week of observations, it was decided that all samples without additives should be tested in Method 2. Samples with different amount of APP (7.5% and 15%) were to be tested to confirm the effect APP had on mold growth. Since the magnesium hydroxide seemed to have a preventing effect on mold growth also in smaller amounts (5%),

different amounts of this additive were to be tested in Method 2. Because of the positive effect of magnesium hydroxide, it was decided that aluminum hydroxide should be tested as well. Borax in 0.5% addition did not seem to have a preventing effect, so only an additive of 2% was tested further. The combination of boric acid (3%) and Borax (2%) was tested further. In addition, different amounts of boric acid in combination with APP were tested, to see if the acid had a better preventing effect that Borax. It was also decided to continue these samples in Method 1 to see if mold growth would evolve in the samples that had not shown signs of growth yet and to see if the results from this method could compare with the results from Method 1.

5.1.1.2 Results after 17 Weeks

At the end of the test period of 17 weeks, it is clear from Figure 5.1 and Table 5.1 that the trends of mold growth are similar to those after 3 weeks. Most coverage of mold is seen in the TMP2 sample added APP (7.5%) and Borax (2%), with as much as 79% coverage. TMP2 without any additives shows the first sign of growth in the last week of the test period, with only 2% coverage. TMP1 without any additives shows sign of growth in week 13, but has a coverage of only 8% at the end of the test period. With additives, the TMP1 seems less affected than the TMP2, with most coverage on the sample added 0.5% Borax and 7.5% APP, where the coverage is 58%. TMP1 with 15% and 7.5% addition of APP lies in the same range, with 54% and 52% coverage respectively. With addition of 2 % Borax and APP (7.5%), the TMP1 has a coverage of 8% after 17 weeks, the same as pure TMP1.

The samples where no mold growth was observed after 3 weeks still have no signs of growth after 17 weeks. When that is said, there was observed a slight change of color in the samples added magnesium hydroxide, as these got more of an orange color already after 1 week, see Figure 5.2.

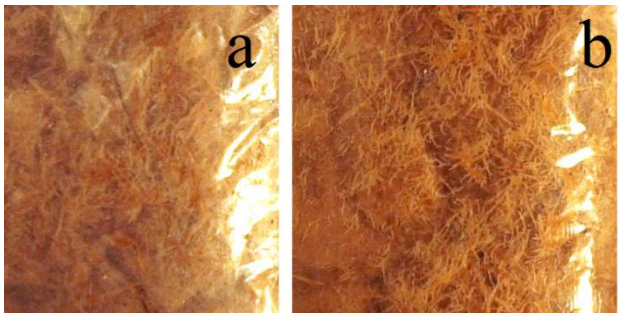

Figure 5.2(a-b): a) A sample of TMP1 without any additives after 1 week of testing. b) A sample of TMP1 added 5% magnesium hydroxide after1 week of testing. Notice the slightly more orange color compared to the sample in a).

5.1.2 Part 2

Some of the samples prepared for Method 2 were also used in this part of Method 1 to get a better basis for comparison of the two methods. The results from Part 2 for the samples where growth was observed are shown in Figure 5.3. The samples where no growth was observed are listed in Table 5.2.

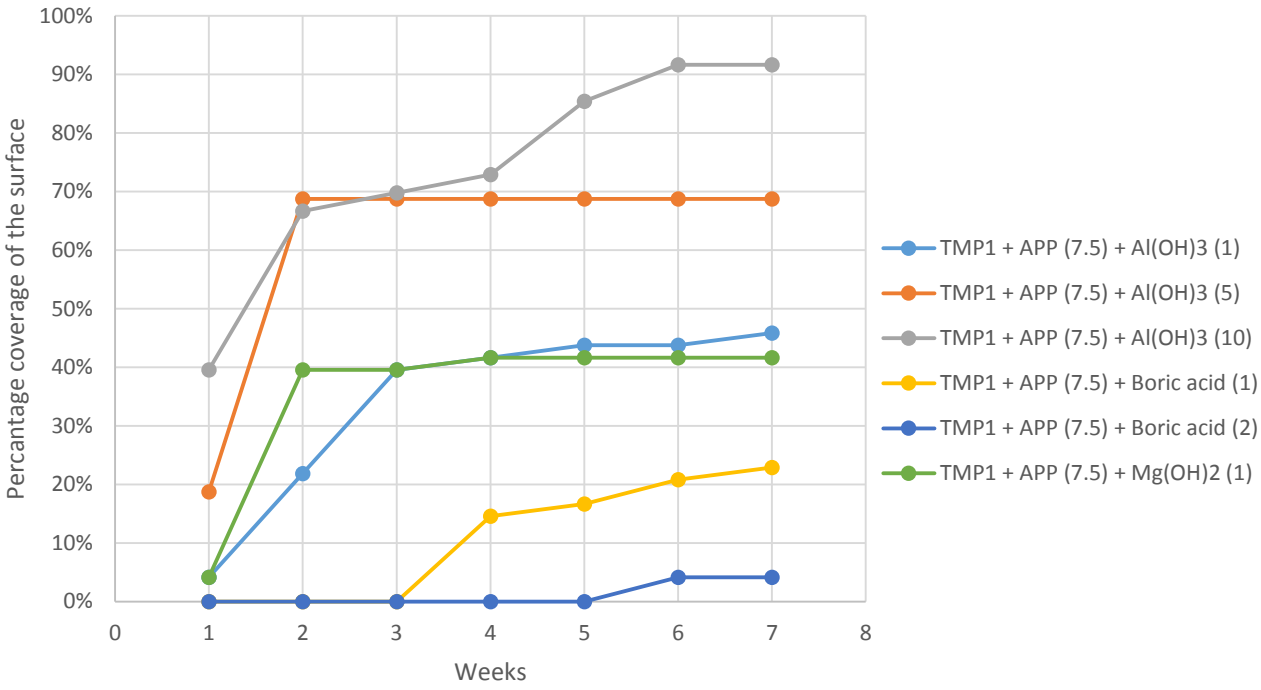

Figure 5.3 Samples in Method 1 – Part 2 where mold growth was observed, given as percentage mold growth coverage on the surface during the 7-week test period. The numbers in in parentheses in the sample names are percentage amounts of fire retardants added, based on weight.

Table 5.2: Samples in Method 1 – Part 2 where mold growth was not observed during the 7-week test period. The numbers in in brackets in the sample names are percentage amounts of fire retardants added, based on weight.

Samples where no growth was observed
TMP1 + Al(OH)3 (5)
TMP1 + Al(OH)3 (10)
TMP1 + APP (7.5) + Mg(OH)2 (5)
TMP1 + APP (7.5) + Mg(OH)2 (10)
TMP1 + Mg(OH)2 (5)
TMP1 + Mg(OH)2 (10)
TMP2 + Mg(OH)2 (5)

From Figure 5.3 the results show that addition of 7.5% APP in combination with aluminum hydroxide does not have a preventive effect on mold growth, the results can even point to that a larger addition of aluminum hydroxide leads to an increase in growth. These samples have severe coverage of growth after only 1-2 weeks. Addition of 10% aluminum hydroxide in combination with APP gives a coverage of mold growth at 92% after 7 weeks on the TMP1. TMP1 added 5% aluminum hydroxide and APP gives 69% coverage, and 1% aluminum hydroxide in combination with APP has a coverage of 46% on TMP1 at the end of the test period.

The samples of TMP1 added 7.5% APP in addition to 1% aluminum hydroxide or 1% magnesium hydroxide have about the same coverage after 7 weeks, 46% and 42% respectively. This imply that addition of hydroxide in such small amount does not have a significant effect on the mold growth, neither positive nor negative.

The samples added 7.5% APP and boric acid show a lower degree of mold growth. The sample added 1% boric acid has a coverage of 23% after 7 weeks, and the sample added 2% has a coverage of only 4% at the end of the test period. These samples also show sign of growth later than the other samples with growth.

When aluminum hydroxide is added to TMP1 without APP, there are no signs of mold growth during the 7-week test period. This is also true for all the TMP1 and TMP2 samples with magnesium hydroxide, and in these there are no growth even in the samples added APP. As in Part 1 there are some color changes in the samples with magnesium hydroxide.

5.1.3 Results from Method 1 as a Whole

The results from Method 1 imply that ammonium polyphosphate does in fact have an increasing effect on mold growth in cellulose materials. When TMP1 was added 15% of APP it had a coverage of mold of 40% after only 3 weeks, and after 10% it reached its maximum coverage of 58%. The TMP1 sample with 7.5% APP had a slower development in the mold growth: it reached 40% coverage after 6 weeks, but after 14 weeks it had 54% coverage, which is comparable with the sample added 15% APP. Since both nitrogen and phosphates are present in APP, and are nutrients to fungi (Deacon, 2006a), it seems likely that the mold utilizes the APP as nutrition, giving better conditions for the mold and increased growth.

Borax seems to prevent some of the growth when added in addition to APP, but needs to make up at least 2% of the material based on weight to have an effect. Boric acid seems to have the same preventing properties as Borax. As boric acid is often added to cellulose insulation to prevent microbial growth (Hansen & Eriksen, 2000), these results seem accurate. The preventing effect borates have on mold growth has been verified in previous reports, and it has been found that the presence of borates significantly decrease the amount of mold growth (Fogel & Lloyd, 2002).

The inorganic hydroxides added do not seem to affect the TMP1 in terms of mold growth when added alone, as the development, or absence, of mold growth are comparable to the results for pure TMP1. Magnesium hydroxide also seems to prevent mold growth when added in addition to APP, as these samples do not show signs of mold growth, neither in Part 1 nor in Part 2. Even so, there are color changes in these samples, which can be caused by bacteria, or other microbial growth, (Talaro, 2009c). Another possibility is that the magnesium hydroxide reacts with the cellulose material, causing a discoloring.

Aluminum hydroxide seems to have an increasing effect on mold growth when combined with APP, as increasing the amount of aluminum hydroxide gives a higher coverage of mold growth. Aluminum hydroxide is commonly used in wastewater treatment to remove phosphorus. It has been shown that it has a significant sorptive capacity for phosphates (Galarneau & Gehr, 1997).

Considering this, it is likely that the aluminum hydroxide absorbs the phosphate from the APP, making either that or the nitrogen more accessible for the fungi to utilize.

When comparing the two pulps used as base for the insulation material, it seems that without additives, they are both quite resistant to mold growth. TMP2 shows a higher percent of mold growth covering than TMP1 when added 7.5% APP and 0.5% Borax, but when added magnesium hydroxide there are no signs of growth. The TMP2 is darker in color than TMP1 and the results might have been affected by the fact that the mold growth was harder to spot in the darker material.

From the results in this test, the Glava Extrem 33 does not show any sign of mold growth. The recovered newspaper did not show signs of mold growth, but as the material was rather dark and colorful due to the ink prints on the paper, possible growth might have been overlooked. Method 1 may not have been the best way of testing these two materials in terms of visual mold growth.

5.2 Results and Discussion – Method 2

The results from Method 2 are not presented as suggested in «Studie av mugsopp på bygningsmaterialer – risiko for vekst av muggsopp på vindsperre». The reason is that there are prominent mold growth only in a few samples, and a description on observation made in the samples during the 13-week observation period seems like a better way to present the results. In general, all three parallels of each set of sample showed comparable results and will be considered as a whole in the following sections. Only samples containing recovered newspaper showed visible growth without using a stereomicroscope.

5.2.1 Results from Samples Containing Pure Insulation Materials

5.2.1.1 TMP1

The incubated sample containing TMP1 without any additives showed signs of growth after 1 week. Figure 5.4a) shows example of hyphae present at that time. Growth was only spotted in some parts of the sample. After 3 week the growth had spread in some extent, but during the remaining weeks of the test period, the growth spread in minor amounts.

The reference sample of TMP1 that had not been incubated also showed growth of hyphae after only 1 week, see Figure 5.4b), but the spreading was small also in this sample.

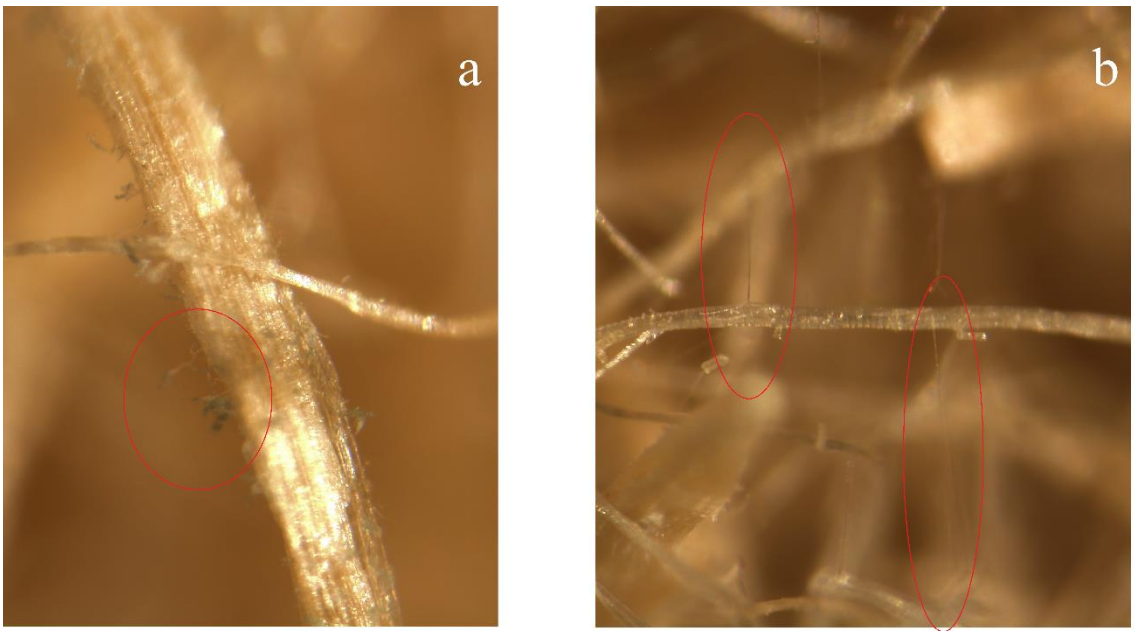

Figure 5.4 (a-b): Example on mold growth in TMP1 samples 1 week into the test, 90 times enlargend. a) Red markings points out signs of mold growth on an incubated TMP1 sample. b) Red marking shows hyphae treads on the reference sample.

5.2.1.2 TMP2

The incubated samples containing TMP2 without any additives showed sign of growth after 1 week, see Figure 5.5a). Similar to TMP1 without additives, growth only spread in minor extent during the test period.

The reference sample of TMP2 that had not been incubated showed growth after 1 week, and during the test period, it spread in larger extent and could be spotted through the whole sample using a stereomicroscope, see Figure 5.5b-c). Even so, no growth became visible on the surface without using a stereomicroscope.

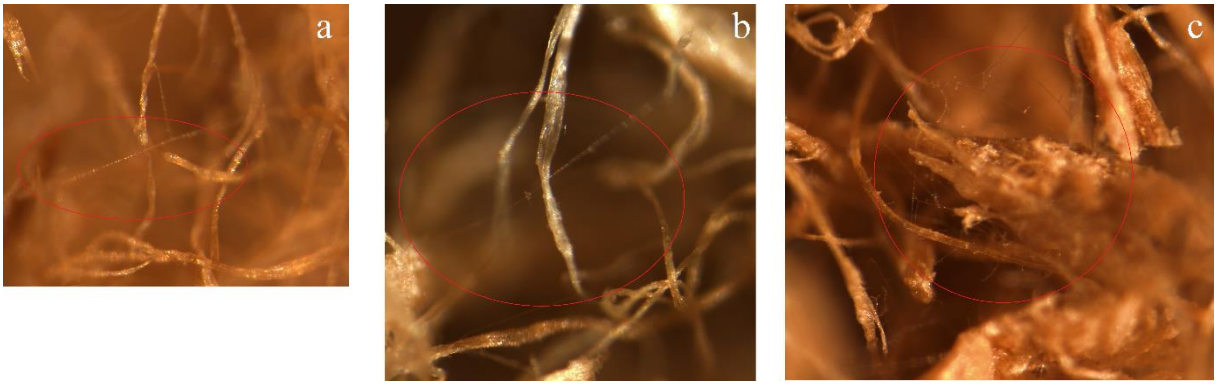

Figure 5.5 (a-c): a) Red markings points out hyphae treads on an incubated TMP1 sample, 90 times enlarged. b) Red marking shows hyphae treads on the reference sample, 90 times enlarged. c) Red marking shows a denser occurrence of hyphae in the reference sample after 11 weeks, 90 times enlarged.

5.2.1.3 Recovered Newspaper

The incubated samples containing recovered newspaper showed signs of growth after 3 weeks, and mycelium was observed in week 8, see Figure 5.6a-b). At this time, the growth was also starting to show without using a stereomicroscope, as the sample looked more "hairy" than at the beginning of the test. The growth spread through most of the sample during the test period.

The reference sample that had not been incubated showed growth signs after 4 weeks, and mycelium after 5 weeks, comparable to that observed in the incubated sample, see Figure 5.7a-b). The growth was at this time visible without using a stereomicroscope, and had the same "hairy" texture as the incubated sample.

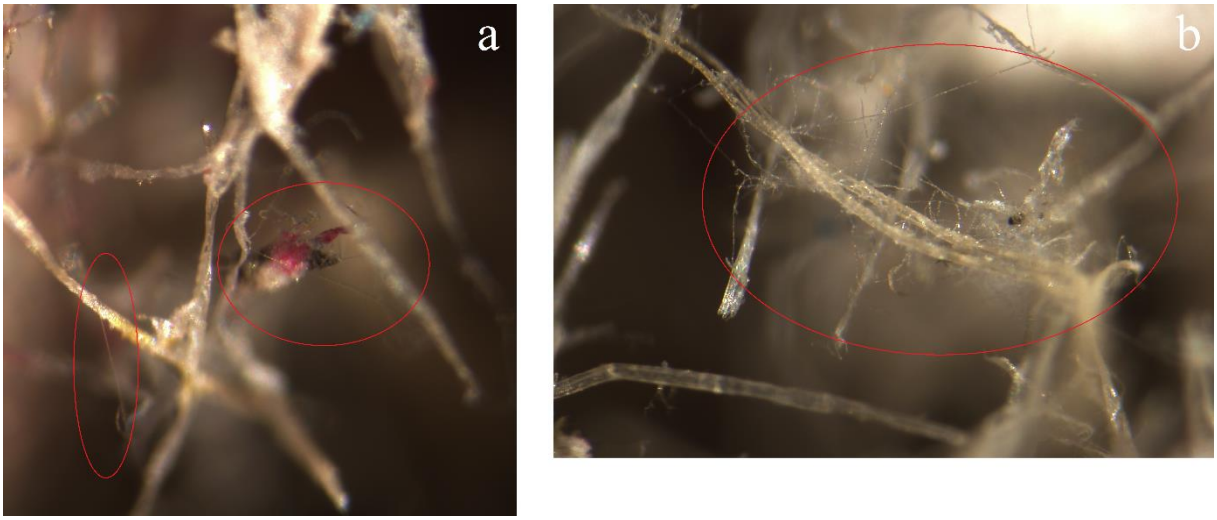

Figure 5.6 (a-b): a) Red markings shows presence of hyphae after 3 weeks on an incubated sample containing recovered newspaper. b) Red markings shows presence of mycelium after 3 weeks on an incubated sample containing recovered newspaper.

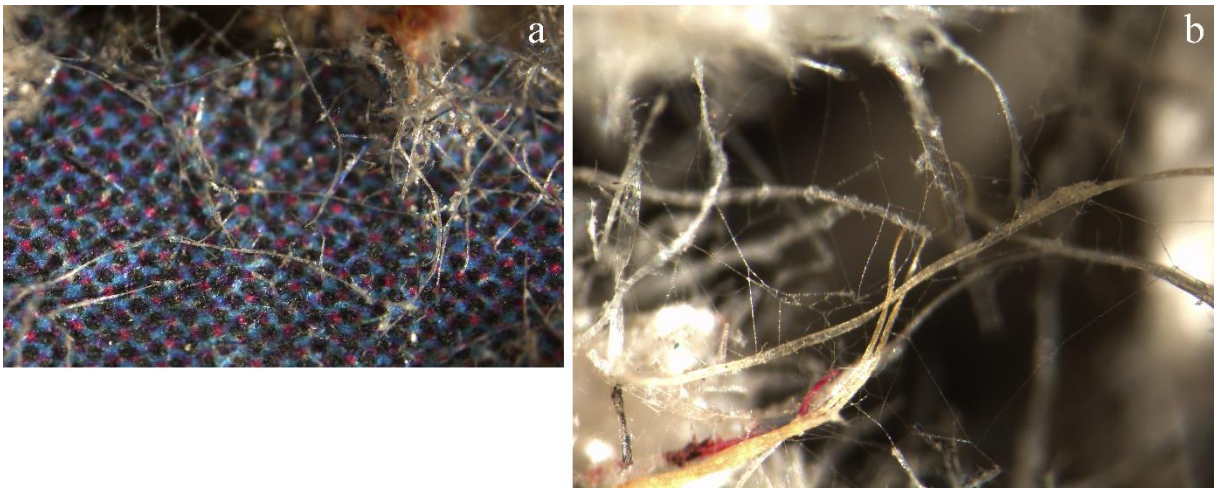

Figure 5.7 (a-b): a) Presence of hyphae after 4 weeks on the reference sample of the recovered newspaper, 25 times enlarged. b) Presence of mycelium on the same sample after 5 weeks, 90 times enlarged.

The fact that mold growth is present in such extent in the recovered newspaper samples might be due their higher content of oil and biological spores, as described in section 3.2.2 Production of Cellulose Pulps.

5.2.1.4 Glava Extrem 33 and Untreated Glass Wool

The incubated samples containing Glava Extrem 33 showed signs of growth after 2 weeks, see Figure 5.8a) and 5.9a). At first, this was believed to be dust or other impurities, but the development in the sample the following weeks made it evident that this was some sort of mold growth. The same type of growth was observed in the incubated sample containing untreated glass wool, see Figure 5.9b). The reference samples of the glass wool types showed no signs of growth during the test period, see Figure 5.8b).

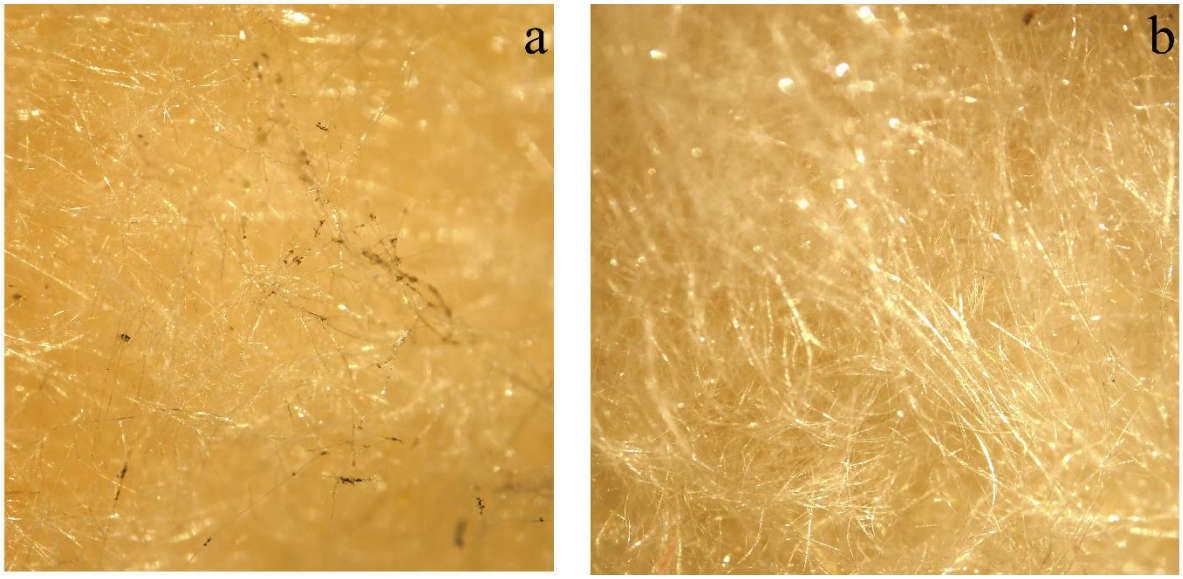

Figure 5.8 (a-b): a) Black spots on an incubated sample Glava Extrem 33 indicates mold growth after 2 weeks,, 25 times enlarged. b) Reference sample of Glava Extrem 33 after 13 weeks shows no indication of mold growth, 25 times enlarged.

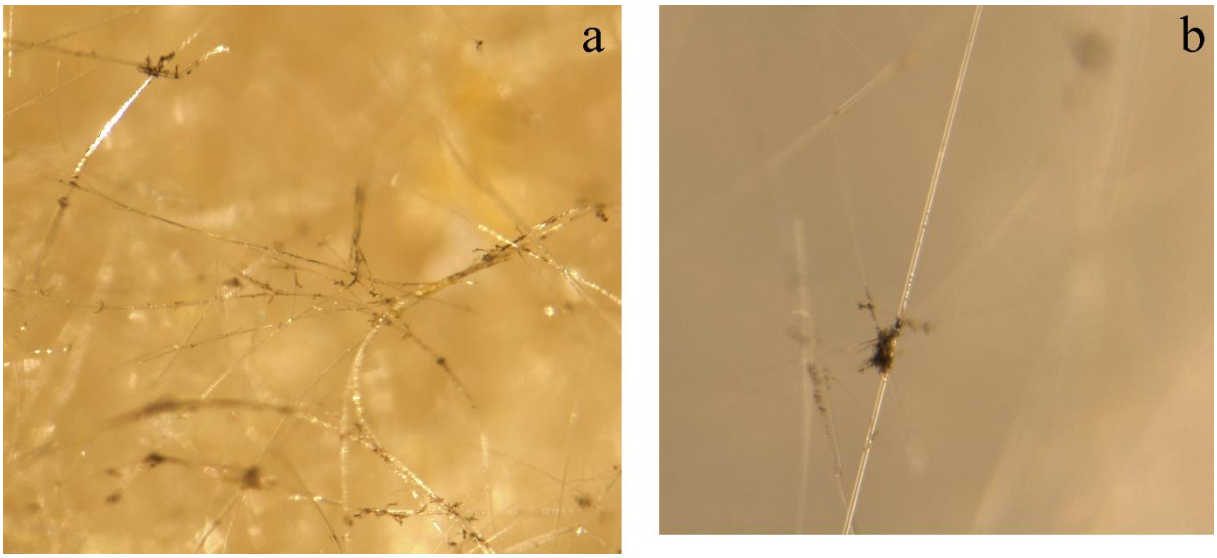

Figure 5.9 (a-b): a) Black growth on an incubated Glava Exteme 33 sample 90 times enlarged. b) Black growth on an incubated untreated glass wool sample 90 times enlarged.

The explanation for the growth in the incubated samples can be that glass wool can serve as a support matrix for collection of debris, which have the capability of supporting mold growth when moist (Van Loo, et al., 2004). As the spores are applied in a suspension of distilled water and Tween 80, this might give enough moist for the glass wool to be a support material for growth. When compared to the absence of growth in the samples that were not incubated and supplied moist, this seems like a plausible explanation.

5.2.2 Results from Samples Containing TMP1 and APP

The amount of growth in the samples containing TMP1 and APP were surprisingly low compared to the results from Method 1. Growth was observed after 1 week, but only in some parts of the samples and was hard to detect. There were little development in the mold growth during the test period and no clear distinction between the sample containing 7.5% APP and the sample containing 15% APP. Figure 5.10a-b) shows growth found in these samples after 11 weeks and 13 weeks.

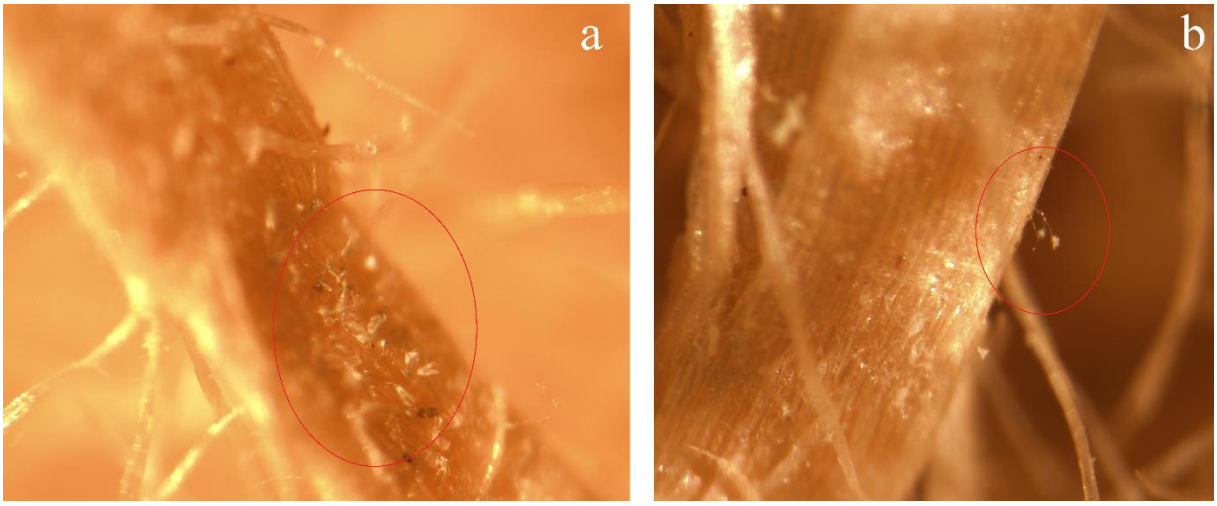

Figure 5.10 (a-b): a) Example on growth found after 13 weeks in a sample containing TMP1 and 15% APP, 90 times enlarged. b) Example on growth found after 13 weeks in a sample containing TMP1 and 7.5% APP, 90 times enlarged.

5.2.3 Results from Samples Containing TMP1, APP (7.5%) and Boric Acid Based Fire Retardants

Some minor growth was present in the samples containing TMP1, 7.5% APP and 1 % boric acid after 1 week, but the growth did not spread in much extent during the test period. The same is true for the sample containing TMP1, 7.5% APP and 2% Borax. The sample containing TMP1, 7.5% APP and 2% boric acid did not show signs of growth until week 8, see Figure 5.11, and after this the spreading was minor.

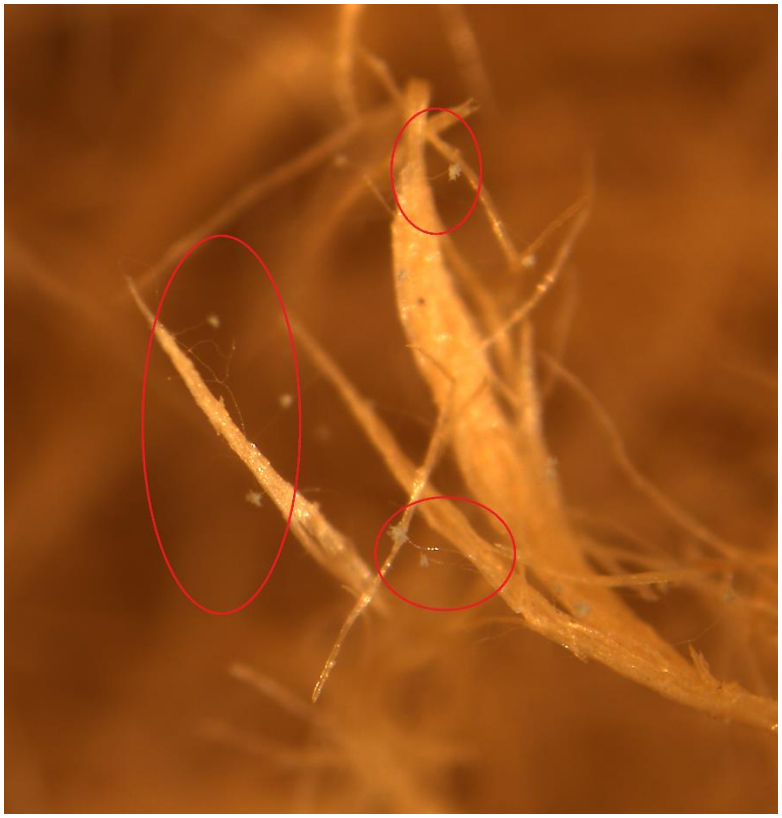

Figure 5.11: Red markings show examples on mold growth observed after 8 weeks in a sample containing TMP1, 3% boric acid and 2% Borax, enlarged 90 times.

The sample containing TMP1, 7.5% APP, 3% boric acid, and 2% Borax did not show any signs of growth during the test period. These results supports the previously reported preventing effects boric acid based fire retardants have on mold growth (Hansen & Eriksen, 2000).

5.2.4 Results from Samples Containing TMP1, APP, and Inorganic Hydroxides

Like the samples containing TMP1 and APP, the samples also including inorganic hydroxides shows growth of hyphae after 1 week, and have a low growth development during the test period. There are not obvious differences depending on amount of inorganic hydroxide added. Obvious differences between the samples containing magnesium hydroxide and the sample containing aluminum hydroxide are not present in terms of growth. In the samples with magnesium hydroxide white spheres was formed on the surface of the fibers, see Figure 5.12. These spheres are assumed to be magnesium hydroxide grains and not mold growth.

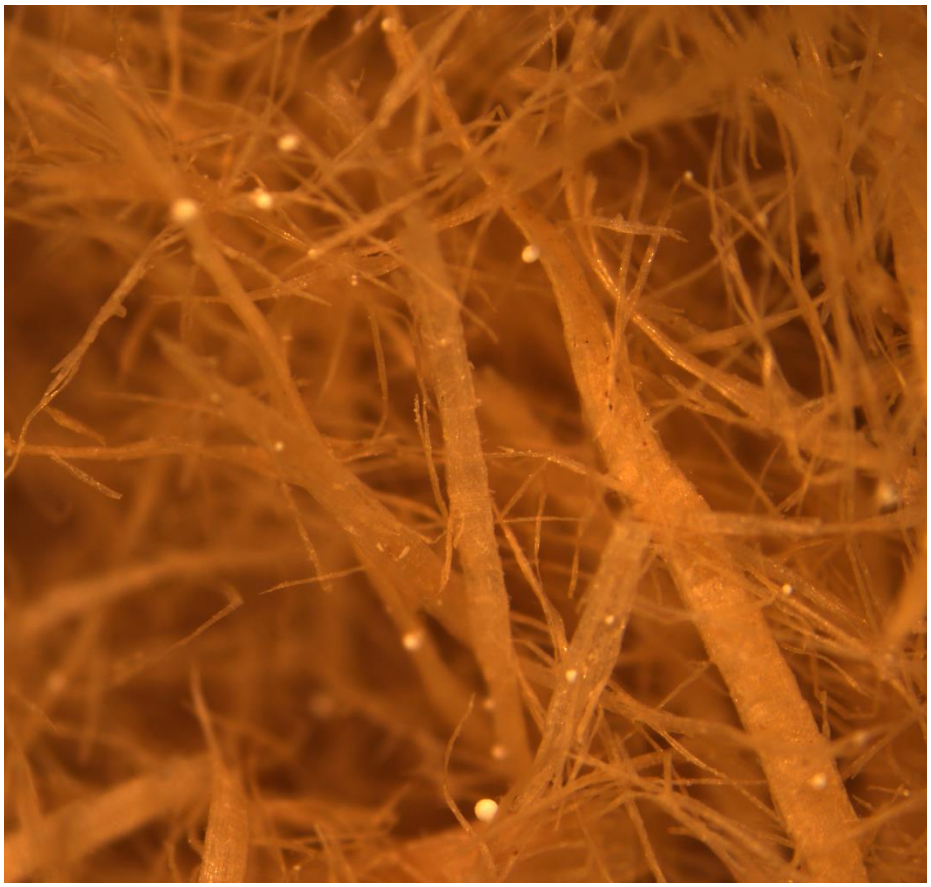

Figure 5.12: A sample containing TMP1, 7.5% APP and 5% magnesium hydroxide after 13 weeks. The white spheres are assumed to be magnesium hydroxide.

5.2.5 Results from Samples Containing TMP1 and Inorganic Hydroxide

The recurrent trend of some hyphae growth after 1 week and no particular development of the growth during the test period is viable also for the samples containing TMP1 and inorganic hydroxide. In terms of growth, there is little difference between the samples containing magnesium hydroxide and the samples containing aluminum hydroxide. The effect of the amounts of inorganic hydroxide it also hard to differentiate.

In the samples with magnesium hydroxide, the same white spheres were formed on the surface of the fibers, as for the samples containing 7.5% APP in addition to the TMP1 and magnesium hydroxide. This support the assumption that these spheres are magnesium hydroxide grains, as they are present only in the samples containing magnesium hydroxide.

Like the samples containing magnesium hydroxide in Method 1, it was detected a color change in the samples containing TMP1 and magnesium hydroxide, see Figure 5.13a-b). This color change occured after 4 weeks. From the figure, the color change is not obvious, it was clearer when directly observed.

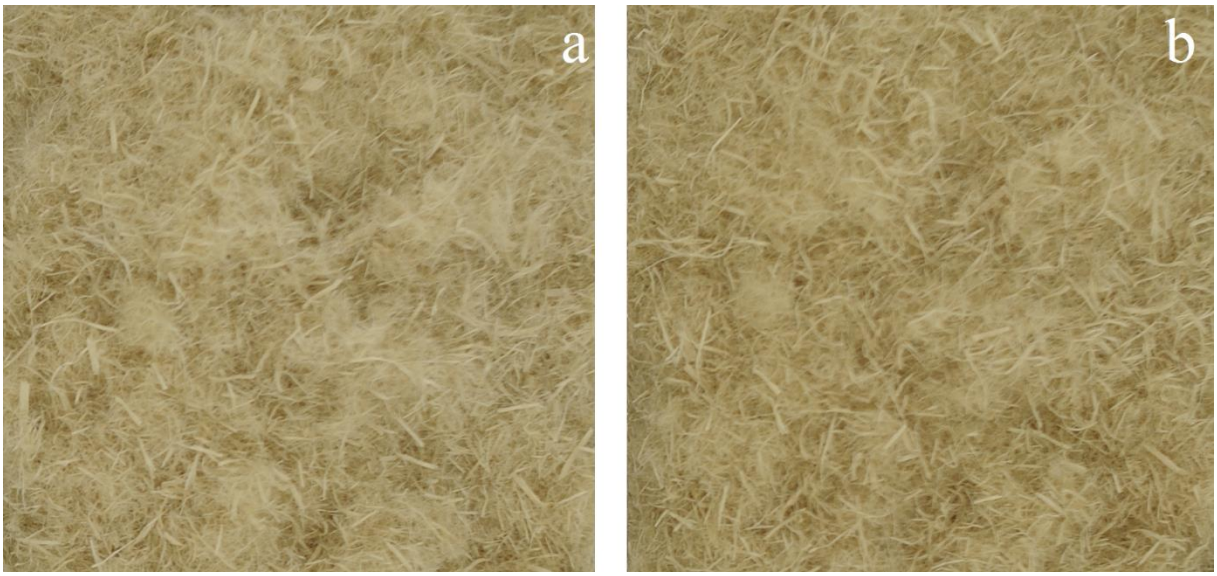

Figure 5.13 (a-b): a) A sample containing TMP1 and 5% magnesium hydroxide at the beginning of the test period. b) The same sample after 13 weeks, now with a slightly darker color.

5.2.6 Results from Samples Containing TMP2 and Magnesium Hydroxide

The sample containing TMP2 and 5% magnesium hydroxide shows prominent growth of hyphae after 1 week, and is one of the few samples where there is an obvious increasing development of mold growth. Figure 5.14a) shows growth found after 1 week, and Figure 5.14b) shows growth found after 13 weeks.

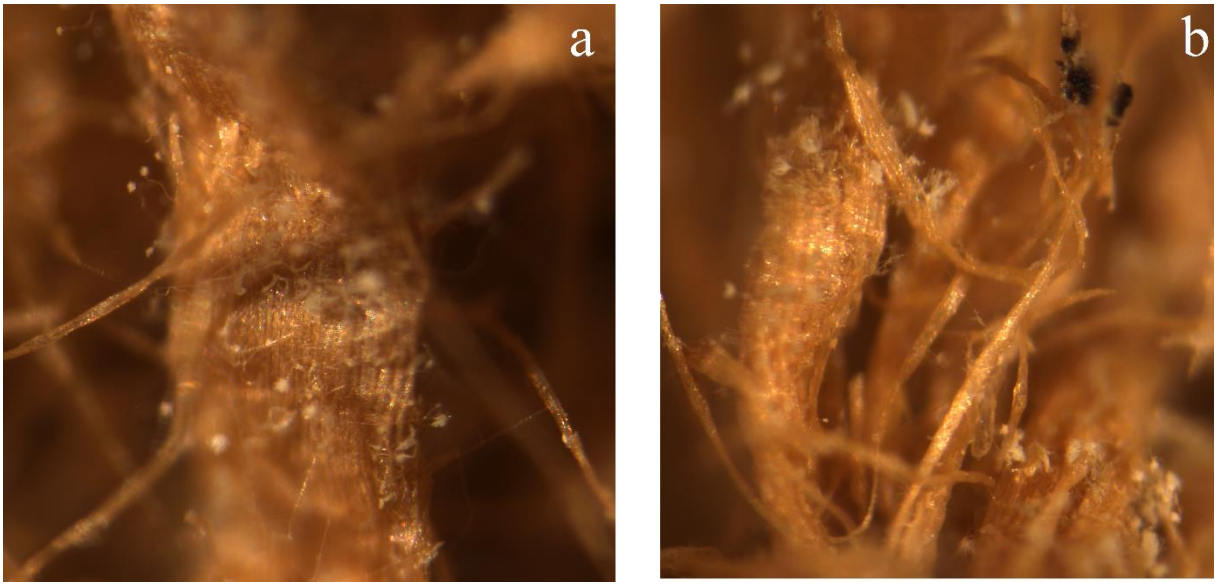

Figure 5.14 (a-b): a) Example of observed mold growth after 1 week in a sample containing TMP2 and 5% magnesium hydroxide, enlarged 90 times. The cellulose fibers are cover in white mold growth. b) Example of observed white mold growth after 13 week in a sample containing TMP2 and 5% magnesium hydroxide, enlarged 90 times. The cellulose fibers are cover in white mold growth.

It was observed, using the stereomicroscope, that the surface of the TMP2 seems rougher than the surface of TMP1. This might give a larger surface area of the TMP2, thus a larger area for the mold to grow on, which might explain the more prominent growth on TMP2. As stated in section 3.2.2 Production of Cellulose Pulps, the different treatment TMP1 and TMP2 had after fibrillation, altered the content of cellulose, hemicellulos and lignin in the two pulps. This alteration might have removed some of the content that is harder to utilize for fungi from TMP2, and made the more easily degradable content more available. This could also be an explanation for the more prominent growth in the TMP2 sample compared to the samples containing TMP1.

5.3 Comparison and Evaluation of the two Methods

In terms of mold growth, it is difficult to compare the two methods conducted in this project. In Method 1 the mold growth is severe in most of the samples where growth occurs, and conclusions on what effects the different materials and additives have on the mold can easily be drawn. As there are little results to assess from Method 2, conclusion drawn in this project will mostly be based on results from Method 1.

Still Method 2 gives some understanding of mold growth in some of the materials. As mentioned in section 5.1 Results and Discussion from Method 1, it was harder to spot growth in the darker TMP2 material, and the ink print on the recovered newspaper made it hard to distinguish mold growth from ink. When samples of these materials was observed in the stereomicroscope in Method 2, it was clear that growth was present in bigger amount than in the TMP1 samples. This supports the assumption that Method 1 is not as suitable for darker materials.

The slow mold growth in Method 2, compared to the rapid and prominent growth in Method 1, might be because of the lower availability of water. A relative humidity of about 90% corresponds to a moist content of 0.12 kg water per kg dry cellulose (Earle, 1983). Compared to 4 kg water per kg dry cellulose (60 gram water per 15 gram dry cellulose), used in method gives a striking difference in water availability between the two methods. In further testing, it could be wise to increase the dry content of the materials tested in Method 1 if a slower development in mold growth is wanted. This can be done by simply adding less water to the samples. A slower development might make it easier to distinguish the effects additives have on the mold growth and make it more comparable with Method 2, at least based on the results from this project. The dry content should thus be assessed depending on the objective of the testing.

As the sample in Method 2 are incubated with a specified spore suspension, it was expected that the mold growth in these sample would be at least as prominent as in Method 1. The slower mold growth in Method 2 could be because the conditions were not optimal, as the mentioned lower activity of water or because of competition between the different species of fungi added (Deacon, 2006c). According to "Standard Test Method for Determining Fungi Resistance of Insulation Materials and Facings", which has many similarities to Method 2, the test should be repeated if the control specimens do not show an abundance of growth after 3-7 days (ASTM Int'l, 2008). From growth test of the spore suspensions on agar plates it is clear that this requirement is met, and there is no reason to believe that the mold should not be able to grow under the given conditions. In the same standard method, it is also stated that some materials may need a longer incubation period that 28 days, and the test might be extended.

6 Conclusion and Recommendations

The scope of this project was to explore the risk for microbiological growth in virgin fiber based and recycled fiber based cellulose insulation materials with and without fire retardant additives. To determine risk of mold growth in the cellulose insulation materials prepared, two methods were used and the results compared.

Method 1 was conducted under conditions giving a temperature of 30 ˚C and a dry content of 20% in the tested insulation materials. This gave a rapid growth of mold and apparent results after only a few days. Thus this method was used to determine what samples was prepared for the more thorough Method 2. In further work the dry content of the samples can be considered altered, depending on what types of results is wanted.

In Method to the temperature was also 30 ˚C, and the relative humidity was about 92%. This method also included incubating the samples with a spore suspension, and thus, the result was expected to be a relatively high coverage of mold growth, at least in the samples where this had been the case in Method 1. After a 13-week test period, the results from this test were hard to determine, as there were little difference between the tested samples. Still, growth has been detected in almost all the samples, and it is recommended that the test period is elongated and that observation of these samples is continued for some time after this project has been concluded.

From the results from the two methods used in this project it can be concluded that ammonium polyphosphate have properties that makes it easier for mold to grow on cellulose insulation materials. As expected the boric acid based fire retardants prevented mold growth. Even when added in only 2%, in addition to 7.5% APP, boric acid delayed the first signs of mold growth with 4 weeks, compared to samples containing only 7.5% APP. It also reduced the extent of the mold growth from 54% coverage to 4% coverage.

To replace boric acid based fire retardant it is clear that additives have to delay and reduce the mold growth caused by APP quite effectively. From the results it seems that magnesium hydroxide can do this even better than boric acid, as no growth were observed in the samples added APP in addition to magnesium hydroxide. There is on the other hands a slight color change in the samples added magnesium hydroxide, so in further work it will be good to rule out that this is caused by bacteria or other microbial growth.

When used alone aluminum hydroxide do not seem to have any effect, neither positive nor negative on the extent of mold growth, but in combination with APP it is plausible that it makes it easier for the fungi to utilize the nutrition found in APP. What causes this would be interesting to do in further work.

In a recent issue of "Teknisk Ukeblad" there was an article about the American company Ecovative. They have developed a house where the insulation is based on mycelium. Accordingly this gives good insulation properties, is a sustainable method to build houses and good for the environment (Garathun, 2014). With this in mind, the aspect of this project can have a change of perspective in the future and evaluate if some kind of mold growth actually can work in synergy with the cellulose insulation.

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Appendix

A Calculations

A.1 Calculations of the Amount of Pulp and Fire retardant(s) in the Prepared Samples

The amount of the test samples before adding water was to be 15 grams. The dry matter content, DM, of the pulps needs to be taken into account when the amount of pulp in the sample is calculated, as seen from Equation A1. An example calculation for the sample with 85 % TMP1 and 15 % APP is shown. Table A.1 shows the dry matter content of the three pulps.

$$\text{Amount}_{\text{pulp}} = (\text{Amount}_{\text{dry matter}} \cdot (2\text{-DM\%})) \cdot \text{Amount}_{\text{pulp, percent}} \quad \text{(A1)}$$

$$\text{Amount}_{\text{TMP1}} = (15g \cdot (2 - 88,28\%)) \cdot 85\% = 14,24g$$

Table A.1: Dry matter content of recovered newspaper, TMP1, and TMP2.

Pulp	**Dry matter content, DM% [%)**
Recovered newspaper	92,0
TMP1	88,28
TMP2	94,18

The amount of fire retardant(s) are based on the dry matter mass of 15 grams, and given by Equation A2. An example calculation for the amounts of APP used in the samples is shown.

$$\text{Amount}_{\text{Fire retardant}} = 15g \cdot \text{Amount}_{\text{Fire retardant, percent}} \quad \text{(A2)}$$

$$\text{Amount}_{\text{APP}} = 15g \cdot 15\% = 2,25g$$

B.1 Reporting From Method 1 – Part 1

Observations made during the test period of Method 1, Part 1.

	start	1 week	2 weeks	3 weeks	4 weeks	5 weeks
TMP1						
TMP1+APP (7,5)			black and green growth	some spreading	spreading, mostly green growth	spreading, mostly green growth
TMP1+APP (15)			black growth in one parallel, green growth in the other	some spreading	some spreading	some spreading, same black/green difference
TMP1+APP+Borax(0,5)		Black spots -> microbiological growth	still growth -> spreading out in the sample	some spreading	some spreading, mostly black growth	some spreading, mostly black growth
TMP1+APP+Borax(2)			some green growth in one of the parallel	some spreading	some spreading, mostly green growth	some spreading, mostly green growth
TMP1+APP+Mg(OH)2(5)		The salt has gatherd in some extent at the bottom of the sample				
TMP1+APP+Mg(OH)2(20)		The salt has gatherd in some extent at the bottom of the sample				
TMP1+Boric acid+Borax						
TMP2						
TMP2+APP+Borax			Black growth	Black and green growth	spreading	spreading
Glava		Water unevenly distributed				
Recovered newspaper		Hard to tell because of the look of the paper	possible some black growth	assumed growth has not spread		

	6 weeks	7 weeks	8 weeks	9 weeks	10 weeks	11 weeks
	no significant differeence from last week			36,5C	38C	38C, nothing new
TMP1		one parallel is darker in one corner	no significant spreading from the dark corner	the parallels are darker		
TMP1+APP (7,5)		additional white growth in one parallel	more of the white growth			
TMP1+APP (15)		additional white growth	more of the white growth			
TMP1+APP+Borax(0,5)		more orange in the color				
TMP1+APP+Borax(2)		more orange in the color				
TMP1+APP+Mg(OH)2(5)						
TMP1+APP+Mg(OH)2(20)						
TMP1+Boric acid+Borax						
TMP2						
TMP2+APP+Borax					Some green growth too	
Glava			darker yellow color - most likely because the water is spread evenly now			
Recovered newspaper		A crispier texture, "vakuum packed"				

	12 weeks	13 weeks	14 weeks	15 weeks	16 weeks	17 weeks
	easter	39,4C	39,1C	39,1C, lights has changed	38,5C	39,6C
TMP1		darker/more grey in color, some black growth			some green growth	Darker in color and some green growth
TMP1+APP (7,5)		one black and one green parallell, white lumps				Green, some white and black growth, where one parallel is more black and "vakuum packed"
TMP1+APP (15)		one black and one green parallell, white lumps. The black parallell is "vakuum packed"				Green, some white and black growth, "vakuum packed" tendencies
TMP1+APP+Borax(0,5)		black growth				Black spots
TMP1+APP+Borax(2)		some green growth		indications on green and black growth	darker in color	Some green and black growth, darker in color
TMP1+APP+Mg(OH)2(5)						More orange in color and some salt has gatherd at the bottom
TMP1+APP+Mg(OH)2(20)						More orange in color and some salt has gatherd at the bottom
TMP1+Boric acid+Borax			more orange in color?		some darker areas	No apparent growth
TMP2						some green growth in one parallel
TMP2+APP+Borax		black and green growth				Black growth
Glava						No apparent growth
Recovered newspaper				Loose fibers or growth?		Loose fibers or growth?

B.2 Reporting From Method 1 – Part 2

Observations made during the test period of Method 1, Part 1.

	start	1 week	2 weeks	3 weeks	4 weeks	5 weeks
TMP1+Al(OH)3(5)						
TMP 1+Al(OH)3(10)						
TMP 1+APP+Al(OH)3(1)		some black growth		black growth, one parallel also has green/white growth	green, black and white growth, more in one parallel	more of the white growth
TMP 1+APP+Al(OH)3(5)		black spots and som light yellow growth	spreading		black and green/white growth	black and some white
TMP 1+APP+Al(OH)3(10)		black spots and som light yellow growth	spreading		almost all black	black and some white
TMP 1+APP+Boric acid(1)				black growth on the top of the samples	black areas, some yellow spots	some spreading of the black, but still only at the top
TMP 1+APP+Boric acid(2)						a black spot or two
TMP 1+APP+Mg(OH)2(1)		some green growth		some black growth in one parallel, the other is all green and smells	One parallel is almost fine, the other is all covered in green and white growth	one parallel is a little black one the backside, the other is worse than week 4
TMP 1+APP+Mg(OH)2(5)					more orange in color	
TMP 1+APP+Mg(OH)2(10)					more orange in color	
TMP 1+Mg(OH)2(5)				more orange in color		
TMP 1+Mg(OH)2(10)				more orange in color		
TMP 2+Mg(OH)2(5)						

	6 weeks	7 weeks	8 weeks
TMP1+Al(OH)3(5)		No apparent growth	
TMP 1+Al(OH)3(10)		No apparent growth	
TMP 1+APP+Al(OH)3(1)	ugh	Apparent growth in both parallels: blavk, green and some white	
TMP 1+APP+Al(OH)3(5)		some green growth too	Mostly black growth, some green and white
TMP 1+APP+Al(OH)3(10)		some green growth too	Mostly black growth, some green and white (more in one)
TMP 1+APP+Boric acid(1)	some green growth too	black on top, som green	
TMP 1+APP+Boric acid(2)			some black growth
TMP 1+APP+Mg(OH)2(1)	ugh	Mostly green growth, but one parallel is also black, white growth in both	
TMP 1+APP+Mg(OH)2(5)			more orange in color, but no apparent growth
TMP 1+APP+Mg(OH)2(10)			more orange in color, but no apparent growth
TMP 1+Mg(OH)2(5)		more orange in color, but no apparent growth	
TMP 1+Mg(OH)2(10)		more orange in color, but no apparent growth	
TMP 2+Mg(OH)2(5)			more orange in color, but no apparent growth