Biochemical modification of wood components

PETER JOSEFSSON

Licentiate Thesis in Fibre and Polymer Technology
Stockholm, Sweden 2006
Biochemical modification of wood components

Peter Josefsson

Licentiate Thesis

KTH, the Royal Institute of Technology
Department of Fibre and Polymer Technology

Stockholm 2006
Akademisk avhandling som framlägges till offentlig granskning för avläggande av teknologie licentiatexamen fredagen den 24 november 2006 klockan 10.00 i STFI-salen, Drottning Kristinas väg 61, Stockholm
Abstract

The degradation of cellulose found in wood is one of the most important degradation processes for the carbon flux on earth. The degradation is performed by microorganisms that typically use enzymes. Since the cellulose in wood is crystalline and embedded in other polymers, making it inaccessible and durable, the enzymatic methods of cellulose degradation is also complex.

In this thesis, the action of some of these enzymes, called cellulases, have been studied both fundamentally and for industrial purposes. By using model cellulose films and a quartz crystal microbalance it was found that endoglucanases not only depolymerize but also swell model cellulose films. Most probably, this contributes to the synergy seen between endoglucanases and exoglucanases.

When an pulp fibers were pre-treated with endoglucanases and beaten subsequently, the fibers became more swollen than reference fibers. The effects of beating enzyme pre-treated fibers were investigated, indicating that endoglucanases improves the fiber/fiber interaction but also alters the behavior of the fibers in the beating process to become more susceptible to the beating.

The second part of the thesis has been focused on the use of an albino fungi in order to decrease the amount of wood extractives in wood chips prior to thermo mechanical pulp production. The fungus decreased the most troublesome component, the triglycerides, by more than 90 percent in two weeks without any detrimental effects on pulp properties. On the contrary, pulp strength and optical properties were improved.
List of Publications

This thesis represents a summary of the papers below, which are found appended at the end of the thesis.

Paper I

The Action of Fungal Cellulases studied using Model Cellulose Films and a Quartz Crystal Microbalance
Peter Josefsson, Gunnar Henriksson and Lars Wågberg
Manuscript

Paper II

Endoglucanase Treatment of Cellulose Fibers Improves the Fiber/Fiber Interaction, but Weakens the Fiber Strength
Peter Josefsson, Katarina Karlström, Gunnar Henriksson and Lars Wågberg
Manuscript

Paper III

Controlled Seasoning of Scots Pine Chips Using an Albino strain of Ophiostoma
Peter Josefsson, Fredrik Nilsson, Lars Sundström, Christin Norberg, Ewa Lie, Marianne Björklund Jansson and Gunnar Henriksson
Industrial and Engineering Chemistry Research 2006 45(7) 2374–2380
3 The experiments

3.1 The action of fungal cellulases (Paper I) ........................................... 27
  3.1.1 The model cellulose films ..................................................... 27
  3.1.2 Quartz crystal microbalance with dissipation ............................ 28
  3.1.3 Experimental procedure ....................................................... 29

3.2 Endoglucanase beating of kraft pulp (Paper II) ................................. 30
  3.2.1 Pulp .................................................................................. 30
  3.2.2 Cellulase ........................................................................... 30
  3.2.3 Treatment procedure ............................................................ 30
  3.2.4 Pulp properties ................................................................. 30

3.3 Fungal wood chip seasoning (Paper III) .............................................. 31
  3.3.1 Seasoning and pre-treatment .................................................. 31
  3.3.2 Analysis of wood extractives ................................................ 31
  3.3.3 Analysis of pulp properties ................................................... 31

4 Results and discussion

4.1 The action of cellulases (Paper I) ...................................................... 33
  4.1.1 Results ............................................................................... 33
    4.1.1.1 The endoglucanases ....................................................... 33
    4.1.1.2 The exoglucanases and synthetic mixture .......................... 35
  4.1.2 Discussion .......................................................................... 35
    4.1.2.1 The adsorption phase ..................................................... 36
    4.1.2.2 The action of the cellulases ............................................ 37

4.2 Endoglucanase beating of kraft pulps (Paper II) ................................. 39

4.3 Seasoning of wood chips (Paper III) ................................................ 43
  4.3.1 Results ............................................................................... 43
  4.3.2 Discussion .......................................................................... 43

5 Conclusions

5.1 Future work .............................................................................. 47

6 Acknowledgements
CONTENTS

Bibliography
# Glossary

<table>
<thead>
<tr>
<th><strong>Word</strong></th>
<th><strong>Description</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>ascomycota</td>
<td>Fungi that produce spores called an ascus (Greek for bladder or wineskin)</td>
</tr>
<tr>
<td>basidiomycota</td>
<td>Fungi that produce spores with a club-shaped structure called a basidium</td>
</tr>
<tr>
<td>cellulase</td>
<td>An enzyme that hydrolyze 1,4-β-glucosidic linkages found in cellulose</td>
</tr>
<tr>
<td>cellubiohydrolase</td>
<td>See exoglucanase</td>
</tr>
<tr>
<td>cellulolytic</td>
<td>Causing hydrolysis of cellulose</td>
</tr>
<tr>
<td>CBM</td>
<td>“A carbohydrate-binding module (CBM) is defined as a contiguous amino acid sequence within a carbohydrate-active enzyme with a discreet fold having carbohydrate-binding activity” [1]</td>
</tr>
<tr>
<td>Carbohydrate binding module</td>
<td>See CBM</td>
</tr>
<tr>
<td>dissipation</td>
<td>The loss of energy of a system in oscillation due to friction of turbulence</td>
</tr>
<tr>
<td>endoglucanase</td>
<td>A cellulase causing hydrolysis within the cellulose chain</td>
</tr>
<tr>
<td>exoglucanase</td>
<td>A cellulase causing hydrolysis at the end of the cellulose chain</td>
</tr>
<tr>
<td>QCM</td>
<td>The Quartz Crystal Microbalance, QCM is an ultra-sensitive mass sensor utilizing the piezoelectric properties of quartz crystals.</td>
</tr>
<tr>
<td>Quartz crystal microbalance</td>
<td>See QCM</td>
</tr>
<tr>
<td>wood resin</td>
<td>Chemical compounds that are extractable from wood using a solvent of low polarity, for example hexane or diethylether</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction and objective

1.1 Introduction

A freshly cut branch is flexible and strong. After a few months under humid conditions at room temperature, the branch has lost most of its strength properties and easily breaks when force is applied.

This loss of strength is caused by microorganisms that degrade the wood polymers, using them in their metabolism. These microorganisms have developed efficient, typically enzymatic, methods for degrading wood polymers. Since wood is a complex structure comprising crystalline cellulose embedded in hemicellulose and lignin, which are amorphous, and with a high degree of polymerization, its degradation is also complex. For example, the degradation of cellulose does not utilize only one kind of enzyme but several that work together synergistically.

Another component of wood is wood extractives, which among other compounds contain triglycerides and sterol esters. Also these components can be used in the metabolism, why enzymes capable of degrading these are present in many species, from microorganisms to humans.

Cellulases and lipases are enzymes that hydrolyze bonds in cellulose and in triglycerides respectively. These two groups of enzymes are commonly used in our every day life, as an example when when added to laundry detergent, the lipases enhance the removal of triglycerides by hydrolyzing them, while cellulases are used for cutting of fibrils on the surface of clothes in order to eliminate the “worn” look after washing. Cellulases and lipases have also been implemented in the pulp and paper industry, lipases to reduce problems with wood resin and cellulases as deinking- and dewatering-enhancing agents.
1.2 Objective

The objective of this thesis has been to study biochemical methods for modification of wood components. In this scope, two main paths of investigation have been followed. The first part focuses on determining the mode of action of different types of cellulases using thin model cellulose films and then trying to relate the molecular action to macroscopic properties of fibers, in order to determine how to apply cellulases industrially.

The second part focuses on how to reduce problems associated to wood resin from wood chips, using an albino fungus.
Chapter 2

Background

Since the enzymes used in this investigation originate from fungi and bacteria, a short background describing them are given followed by a specific description of the enzymes that hydrolyze cellulose and the interaction of enzymes with surfaces.

2.1 Wood degrading microorganisms

Wood has since long been an abundant biological material. Because of this, wood has been an important source of energy for microorganisms, why several species have developed the ability to efficiently acquire energy by degrading some or all wood components. Below is a brief introduction to some of these microorganisms given, for a more complete description, see [2, 3].

2.1.1 Brown rot fungi

Brown rot fungi belong to the phylum basidiomycota and typically color the wood brown during the decay. Brown rot fungi are capable of degrading hemicellulose and cellulose but leave the lignin only slightly modified [2]. Hydroxyl radical producing enzymes are important in the degradation among brown rot fungi, although some brown rot fungi produce hemicellulases and cellulases [4].

2.1.2 White rot fungi

White rot fungi also belong to the phylum basidiomycota. They are generally able to degrade wood completely, as they are efficient in degrading cellulose, hemicellulose and also in mineralizing lignin. They have therefore been intensively studied for example for biopulping of wood chips [5, 2].
2.1.3 Sapstain fungi

Sapstain fungi are either belong to Ascomycota or Deuteromycota [2]. When growing on wood, these fungi use wood extractives and simple sugars are their major source of nutrition [5]. Sap stain fungi are capable of penetrating wood fiber walls. They degrade pit membranes, resulting in an increased ability to absorb water [2]. These fungi, however, are incapable of degrading lignified wood cells or cellulose [5]. The staining produced by the fungus range from light brown to blue and nearly black [2]. *Ophiostoma piliferum* is an example of a sapstainer [2], which is more thoroughly described below.

2.1.3.1 *Ophiostoma piliferum*

*Ophiostoma piliferum* is an ascomycete and is considered a saprophyte [6]. The fungus is an aggressive pioneer species that rapidly colonize sapwood penetrating deeply into sapwood, by colonizing ray parenchyma cells [7] and growing prolifically in transversal and longitudinal resin canals [6, 8].

The fungus forms hyphae that can grow from one cell to another via pit apertures disrupting the thin walled parenchyma cells [6]. Although the hyphae can bore through cell walls, they do not degrade tracheid cell walls, and thus no loss of wood strength is evident [7, 3].

During the colonization, simple carbohydrates, fatty acids, triglycerides and other components in parenchyma cells and in resin canals are used as carbon sources [6]. When *Ophiostoma piliferum* colonizes wood, it normally causes a blue to grey–black discoloration in and on the wood. This staining is primarily caused by melanin associated with carbohydrates and proteinaceous material [9].

In the beginning of the 1990s, Farrel et al developed an albino *Ophiostoma piliferum* using traditional crossings of *Ophiostoma piliferum* [10, 6, 11, 12]. This fungus was then used as an aid in seasoning of wood chips, to avoiding staining and yield losses because of other wood degrading microorganisms.

2.1.4 Moulds

Moulds belong to the ascomycetes or the deuteromycetes [2], and use wood extractives and simple sugars as their source of nutrition from wood. They generally do not penetrate deeply into wood but they are capable of penetrating the fiber cell walls and some are cellulolytic [2].
2.2. CELLULASES

2.1.5 Bacteria

Bacteria are capable of rapidly invading wood, but cannot compete with the more rapid degradation caused by fungi. In degrading wood components, bacteria occurring in the rumen of cewing animals, employ cellulosomes for degradation of wood components [13, 2], described in section 2.2.2.

2.2 Cellulases

To use the energy stored in cellulose, microorganisms must hydrolyze the cellulose molecule to glucose. Fungi and bacteria use enzymes for this, and the kind of enzymes that are capable of hydrolysing 1,4-\(\beta\)-glucosidic linkages found in cellulose are generally called cellulases. Most of these are glycoside hydrolases. The fact that there exist enzymes capable of degrading or modifying cellulose has been known since the beginning of the twentieth century [14] and has attracted a vast research attention over the decades, due to the industrial potential of using enzymatic means to modify cellulose.

The general mechanism of cellulases is the hydrolysis of the \(\beta\)-1,4-glucosidic bond between glycosyl moieties by an acid catalysis as shown in figure 2.1. However, the degradation of cellulose in wood is more complex than the process shown in figure 2.1 because cellulose in wood is embedded in other wood carbohydrates and lignin. Also, the cellulose in wood is of rather high degree of polymerization, at least 15000 [15], and occurs in various crystalline structures.

Because of the complex substrate presented by wood, different strategies have evolved for degrading the cellulose in it. Among anaerobic bacteria occurring in the rumen of cewing animals, complexes of hydrolytic enzymes, called cellulosomes, are common while aerobic bacteria usually secrete individual enzymes extracellularly. A third group of cellulases that produce radicals in order to achieve depolymerization is also common, more described in section 2.2.3.

2.2.1 Cellulases from aerobic microorganisms

The cellulases used among most aerobic microorganisms are secreted outside the cell wall in order to produce monomers or oligomers that can be transported into the cell and then used as nutrition within the cell.

These cellulases are typically modular, often comprising not only a catalytic core module but also a linker with an attached carbohydrate binding module attached to, as shown in figure 2.2.
Figure 2.1: A scheme of the hydrolysis of $\beta$-1,4-glucosidic bond in cellulose [16] by a cellulase.

Figure 2.2: Cellulases are typically modular, often consisting of a catalytic core module and a carbohydrate binding module, CBM, connected to each other via a linker [16].
2.2. CELLULASES

2.2.1.1 Endoglucanases and exoglucanases

In principle, cellulases from aerobic fungi can be divided into four functional groups, as shown in table 2.1 [17]. The table shows that there are two functionally different catalytic units, called endoglucanases and exoglucanases respectively, see also figure 2.3, which either have or lack a module that facilitates the binding to cellulose.

Figure 2.3: A schematic description of the main types of cellulases; endoglucanases and exoglucanases [16].

Generally, the endoglucanases have a cleft-shaped active site enabling hydrolysis within the cellulose molecule whereas the exoglucanases have a more tunnel-like shaped active site [18]. This tunnel shape restricts the exoglucanases to act at the ends on the cellulose molecule, either from the non-reducing or from the reducing end depending on the structure of the tunnel. However, it should be noted that table 2.1 presents a simplified classification and that for example exoglucanases can display some activity corresponding to that of endoglucanases [19]. For an update classification see [20] and for a more complete description see [21, 22, 19].

2.2.1.2 The carbohydrate binding module (CBM)

The module often connected to the catalytic core module of cellulases is called the carbohydrate-binding module (CBM). In the case of cellulases from aerobic fungi, most of the CBMs belong to the type 1 CBMs. For an update classification, see [20].

The CBM is believed to increase the hydrolytic activity of the enzyme by bringing the catalytic core module into close contact with the substrate, thus increasing the substrate concentration to the enzyme. It has been shown that cellulases lacking a CBM show less catalytic activity than do cellulases possessing one [23]. CBMs have also been suggested to display a “sloughing off” effect on cellulose surfaces [24, 1]. A similar non-hydrolytic effect has also been found in chitinases [25].
Table 2.1: A simplified classification of cellulases based on their mode of action from [17].

<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>Mode of action for degradation of cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Exoglucanase</td>
<td>Progressively from reducing end of cellulose</td>
</tr>
<tr>
<td>B</td>
<td>Exoglucanase</td>
<td>Progressively from non-reducing end of cellulose</td>
</tr>
<tr>
<td>C</td>
<td>Endoglucanase with CBM</td>
<td>Attack less ordered cellulose, bind to and possibly also nick crystalline cellulose</td>
</tr>
<tr>
<td>D</td>
<td>Endoglucanase without CBM</td>
<td>Negligible binding to and negligible activity towards crystalline cellulose, but activity on less ordered cellulose</td>
</tr>
</tbody>
</table>

CBMs are typically between 4 to 20 kDa in size and bind to the cellulose using three aromatic and often polar residues [26, 1]. The aromatic groups are often tryptophans or tyrosine, and are arranged to correspond to the length of the unit cell in cellulose, 10.4 Å. The polar groups may further facilitate binding by forming hydrogen bonds with the substrate [27].

The binding of the CBM to cellulose has been shown to be slightly exothermic but mainly entropy driven [28]. This binding has been suggested to be both reversible and irreversible [29], but it has also been argued that it cannot be irreversible since this would be fatal to the enzyme activity [30]. However, CBMs have been shown to be mobile on cellulose surfaces [31].

2.2.1.3 Synergy

“Synergy or synergism (from the Greek synergos meaning working together) refers to the phenomenon in which two or more discrete influences or agents acting together create an effect greater than that predicted by knowing only the separate effects of the individual agents’ [32].

Among cellulases, synergy is common, resulting in a more rapid degradation when different types of cellulases are allowed to act together. Four different types of synergies are believed to exist, namely, endo–exo synergism, exo–exo synergism, endo–endo synergism and endoglucanase–β-glucosidase synergism.

**Endo–exo synergism**

Endo–exo synergism is the phenomenon occurring when endoglucanases cleaves the cellulose molecule in the middle, thus increasing the number of available end groups on which the exoglucanases can act on [33].

**Exo–exo synergism**
2.2. CELLULASES

Native cellulose $\xrightarrow{C_1}$ Shorter, linear cellulose chains $\xrightarrow{C_x}$ Soluble products

Figure 2.4: An interpretation of the model of Reese et al [36] in which the degradation was split into a swelling phase ($C_1$) and a hydrolytic phase ($C_x$) in which the enzymes were active.

The two types of exoglucanases, one acting from the non-reducing end and the other from the reducing end, show synergy [34, 35]. The synergy has been explained as the process by which exoglucanases acting on the reducing ends open up the structure beneath the chain it is hydrolyzing. The chain beneath may consist of a non-reducing end, and if so, the action of the exoglucanase acting on reducing ends will have created an end-group for the exoglucanase acting from the non-reducing end of cellulose.

**Endo–endo synergism**

There exist small endoglucanases, such as EG28, which lack CBM. These display high activity on amorphous, or at least less ordered, cellulose but low activity on crystalline cellulose. It has therefore been suggested that these cellulases diffuse into pores and nick the less ordered cellulose material, resulting in a separation of the fibrils. Thus, the larger endoglucanases will gain access to the surface and be assisted synergistically by the small endoglucanases [17].

**Exoglucanase–$\beta$-glucosidase synergism**

The product of exoglucanase hydrolysis of cellulose is almost exclusively cellobiose, which acts as an inhibitor of exoglucanase activity. Since $\beta$-glucosidase cleaves cellobiose to glucose, it promotes the action of exoglucanases.

2.2.1.4 Mode of action of cellulases

In 1950, Reese et al presented the first model of cellulose degradation [36]. This model, presented in figure 2.4, divided cellulose degradation into two steps. The first step was believed to be a non-hydrolytic step, called $C_1$, in which the cellulose became swollen which was followed by an enzymatic hydrolysis step, called $C_x$. The suggestion of swelling as a part of the degradation has during the years been discussed and discarded, but considering the results of this thesis, this hypothesis might be brought back from the cold.

Since the model of Reese et al., several other models on the topic of cellulose hydrolysis have been presented [37, 38, 39, 40, 41]. In the model suggested by Henriksson et al [17], presented in figure 2.5, the degradation is initiated by small endoglucanases lacking CBMs.
that attack the amorphous regions of the cellulose, resulting in separation of the fibrils. Endoglucanases with CBMs then attach, nick and degrade parts of the chains increasing the number of available end-groups on which the exoglucanases can act processively. This model was suggested using results from experiments in which purified mono-components of cellulases were mixed in different ratios and the actions of the cellulases were measured as the production of reducing sugars.

Figure 2.5: A model of cellulose degradation by cellulases from white rot fungi [17]. (1) The microfibrils are kept together by the surrounding matrix of a less crystalline material. (2) Small endoglucanases lacking CBMs (striped circles) separate the fibrils by nicking and hydrolyzing the amorphous material. (3) Endoglucanases with CBMs (filled circles) create nicks and degrade parts of the chains on the microfibrils after which (4) exoglucanases (patterned and open boxes) acting from the non-reducing and reducing chain ends respectively, attach and act processively on the available chains.
2.2.2 Cellulosomes

Cellulases occurring among anaerobic microorganisms, in the rumen of chewing animals, are designed somewhat differently from cellulases from aerobic fungi. These cellulases are not released from the cell but are anchored to the exterior of the cell wall and are typically ordered in complexes of enzymes with different catalytic activity [42, 43].

2.2.3 Radical-producing cellulase

A less specific method for degrading cellulose is through the action of radicals. This method is common especially among brown and white rot fungi, which produce enzymes capable of generating radicals that can depolymerize the cellulose.

Cellulobiosehydrogenase is an example of a radical producing enzyme. Briefly stated, the enzyme generates hydroxyl radicals by reducing $\mathrm{O}_2$ to $\mathrm{H}_2\mathrm{O}_2$ and $\mathrm{Fe}^{3+}$ to $\mathrm{Fe}^{2+}$, chelated by an acid. When $\mathrm{Fe}^{2+}$ and $\mathrm{H}_2\mathrm{O}_2$ diffuse into wood pores and meet, they form hydroxyl radicals in a Fenton-type reaction. These radicals are then believed to cause depolymerization of lignin and cellulose [16].

In some fungi, the binding of cellulobiosehydrogenase to cellulose is facilitated by a CBM, while in other cases a surface of the enzyme, separated from the active site, binds to the cellulose [16].

2.3 Lipases

Lipases, also called glycerol ester hydrolases, are enzymes that catalyze the hydrolysis of ester bonds in glycerides and steryl esters [16]. The typical action of lipases is shown in figure 2.6. Lipases have been intensively studied for use in the pulp and paper industry for diminishing problems related to triglycerides [44, 45, 46].

2.4 Enzymes-substrate interactions

Polymer adsorption occurs ubiquitously, and proteins are the most surface active among the bio-polymers. In many situations, protein adsorption is advantageous, for example, in protein purification, biosensor applications and intravascular thrombosis. In other situations, protein adsorption is undesired, for example, on contact lenses, on food processing equipment and on tubings during blood dialysis.

Proteins are large polymers of amino acids linked together by peptide bonds. Proteins occur in a wide range of sizes, with molecular masses ranging from a few kDa to thousands
When the lipase is converted to an oil drop the lid is opened, and expose an hydrophobic active site. The "inside" of the open lid make the surface of the lipase more hydrophobic.

Figure 10-16: The substrate specificity of lipases. The specificity of different lipases varies; some lipases hydrolyse triglycerides and diglycerides (reactions a and b), but cannot hydrolyse the central ester bond (reaction c), whereas other lipases also can hydrolyse the ester bond in steryl esters (reaction d), in addition to triglycerides.

Figure 10-17: Structural conformation change in lipases during catalysis.

A wide range of organisms, from plants and animals, to filamentous fungi, yeasts and bacteria, produce lipases. The lipases of wood degrading fungi have not been intensively investigated, but there is no reason to believe that they differ drastically from other lipases. The technically most used lipases are produced by yeasts as Candida antarctica and eubacteria as Pseudomonas. Lipases have a number of properties that are unusual among enzymes; they are often unusually stability in organic solvents that denaturates and inactivate most other enzymes, and have often a tolerance for extreme pH and high temperatures.

Proteins are normally folded in a way that the inside of the protein consists of hydrophobic amino acids and the surface of more hydrophilic amino acids that can interact with water. If the water is replaced with a non-

Figure 2.6: A schematic description of the substrate and products of Lipases hydrolysis [16].
of kDa. Water is the natural solvent for proteins except for example for those that are membrane bound.

2.4.1 Surfaces

The structural composition of the interface often differs from that of the bulk phase. This difference originates from the minimization of the free energy at the surface; to create a surface in vacuum a certain amount of energy is required—the surface energy or surface tension, $\gamma$ (J/m$^2$). In a liquid, this energy is modified by the interaction with the liquid or by the adsorption of for example surfactants or proteins.

2.4.2 Protein-surface interactions

No adsorption will occur unless the adsorbed state is energetically favorable, so that the free energy decreases due to adsorption:

$$\Delta G_{\text{Adsorption}} = \Delta H_{\text{Adsorption}} - T \cdot \Delta S_{\text{Adsorption}}$$

(2.1)

Typically, the enthalpy change upon protein adsorption is relatively small, and hence the entropy contribution dominates the adsorption process. Generally, the adsorption is mainly affected by four driving forces, as follows:

1. Ionic interactions between surface and protein [47, 48] Charges on the protein and on the adsorption surface affects the adsorption similarly to the adsorption of polyelectrolytes, where in the simpler cases opposites charges attract and equal charges repel. The adsorption maxima of proteins are pH-dependent. The maximum is often said to be found close to the isoelectric point of the protein, though perhaps it should be at a pH at which the surface charge + the protein charge (per unit area of adsorption) is minimized. However, it has been demonstrated using a positively and negatively charged protein and two negative surfaces, that the negatively charged protein can adsorb more than the positively charged protein can [48]. Thus, factors other than ionic can dominate the adsorption process.

As an example, the binding of a cellobio dehydrogenase and a cellobiohydrolase 1 has been shown to be unaffected by the salt concentration, suggesting that the ionic interaction is of lesser importance among cellulases [49].

2. Non–ionic interactions between surface and protein [47, 48] Also contributing to the adsorption are non–ionic interactions, often characterized by $\chi_s$. To exemplify non–ionic interactions, the case of the carbohydrate binding module is chosen. As
previously mentioned, the binding site of the module consists of aromatic groups. These are rather hydrophobic and have a high polarizability leading to the formation of significant van der Waals interactions. For these groups, the cellulose surface is a better ‘solvent’ and it will thus be energetically more favorable for the carbohydrate binding module to be adsorbed than it would be in solution.

3. The interaction of the enzyme with the solvent The solubility of a protein in the solvent can be of importance for adsorption. The less soluble the protein, the more favorable it is adsorption. The solubility of a species in a solvent is often described by the $\chi$-parameter, where $\chi < 0.5$ indicates a good solvent and $\chi > 0.5$ indicates a poor solvent. This indicates whether it is favorable to interact with the solvent or with another protein molecule or a surface [50].

4. Conformational changes of the protein structure upon adsorption Upon adsorption, the interaction of the protein with the surface might induce structural changes in the protein. The stability of proteins in water is governed by hydrophobic interactions between amino acid residues. Apolar residues that have been hidden from the water can interact with a surface, inducing a three-dimensional change in the protein structure in the adsorbed state [48].

2.5 Technical applications

2.5.1 Endoglucanase beating of chemical pulps

Chemical pulps are not suitable for papermaking immediately after pulping, since the fibers are stiff and have poor joint forming abilities. Therefore, in order to produce paper with acceptable strength properties the pulp is beaten. The beating process consumes appreciable amounts of energy and also leads to production of fines, free fine material, which significantly increases the dewatering resistance and also consumes paper chemicals. A low dewatering resistance is crucial for an efficient papermaking process, since high machine speed is necessary to maintain profitability, and the drying and pressing sections often present production bottlenecks. Furthermore, beating decreases the bulk of the paper, which is seldom desirable.

With this as a background, along with the fact that energy prices have recently increased dramatically, there is much interest in finding alternatives to traditional beating. In this context, enzymatic treatments can be of great interest since they offer specific interactions with the components of the fiber wall without a large number of negative side effects.
2.5.2 Fungal degradation of wood resin

Trees consist not only of polymers but also of 1–5 percent of the dry weight of wood resins. The wood resins function as energy storage and/or protection of the tree. When paper is to be produced from mechanical pulp wood resins can cause great problems. These problems may appear as dark spots or holes in the paper and in worst case–web breaks on the paper machine that decrease the paper machine availability. Thus, wood resins are unwanted in mechanical pulping [51]. Among the components of wood resin, triglycerides and sterol esters are believed to be the most troublesome.

Several methods can be used to reduce wood resin–related problems. For example, lipases that hydrolyze triglycerides or talc, alum or cationic polymers can be added to stabilize the pitch. Also, the pulp can be washed to remove wood resins. Another method for diminishing pitch problems is to season the wood by storing it and waiting for microorganisms and oxidative processes to occur.

Another approach to seasoning was introduced by Roberta Farrell [12], who bred an albino *Ophiostoma piliferum* using traditional crossings. Since sap stain fungi lack the ability to degrade wood polymers, yet can use the wood resins as a source of energy, by applying this fungus to wood chips followed by seasoning this should decrease the overall amount of wood resins.

The degradation of wood resins by the fungus has been shown in several experiments [10, 52, 53] without having any deleterious effects on the wood properties [6, 54]. It has furthermore been shown that the fungus can out compete other wood degrading fungi, thus increasing the yield and brightness of pulp made from fungus-seasoned chips [55]. It has also been suggested that pores in the wood are opened by the fungus, leading to enhanced steam impregnation [52].
Chapter 3

The experiments

This chapter provides an overview description of the experiments conducted in this research while more detailed information concerning them can be found in the related papers.

3.1 The action of fungal cellulases (Paper I)

This section describes the methods and experimental setup for the studies on the cellulase-cellulose interaction.

3.1.1 The model cellulose films

Model cellulose films have previously been developed to enable fundamental studies of cellulose swelling and of the interaction between cellulose and other polymers. These films are appealing to use because the fibre surface is difficult to study fundamentally due to the great variability of the fibers themselves and the small size, porosity and roughness of the fiber surface. Also, the influence of other wood polymers and of lignin can be difficult to estimate and to take account of when studying the fibers.

The model cellulose films are smooth, non-porous and can be reproduced, see figure 3.1. They are also similar to fibers in known properties of fibers such as swelling and the fibrillar structure. The crystallinity of the films has been difficult to determine, however, fibers spun from the same dissolution, as the films are made from, have been shown to be partly crystalline of cellulose II. [56, 57, 58].

The films are obtained by anchoring a cationic polymer on a silicon oxide surface, then cellulose, dissolved in N-methylmorpholine oxide and diluted by dimethylsulfoxide, is spin coated on top of this layer. The temperature and concentration of the solution of the dissolved cellulose can be used to affect the viscosity of the solution which is what determines
the film thickness and surface roughness upon spin coating [57].

### 3.1.2 Quartz crystal microbalance with dissipation

The quartz crystal microbalance (QCM) technique is based on measuring the frequency changes of a vibrating piezoelectric quartz crystal. In the case of a film that is flat, uniform, firmly attached to the crystal and fairly rigid, the change in mass is directly proportional to the change in frequency, as stated by the Sauerbrey equation as follows: [59]:

\[
\Delta \text{mass} = C_{\text{crystal}} \cdot \frac{\Delta \text{frequency}}{n}
\]  

(3.1)

In this equation, \( C_{\text{crystal}} = 0.177 \) represents the sensitivity constant of the QCM crystal, \( n \) is the overtone number and \( \Delta \text{frequency} \) is the resonance frequency change of the crystal. Using equation 3.1, the mass can be determined for a given adsorbed layer. However, when the film is not rigid, but somewhat viscous, equation 3.1 underestimates the film mass. In such cases, by measuring the energy dissipated, lost, in the system when no voltage is applied, see equation 3.2, an estimate of the viscoelastic properties can be found.

\[
D = \frac{E_{\text{dissipated}}}{2\pi \cdot E_{\text{stored}}}
\]  

(3.2)

Using the dissipation, the mass of the adsorbed layer, even when viscous, can be calculated using a model derived by Vionova et al [60]. Experimentally, the dissipation is determined by measuring the time constant, \( \tau \), for the exponential decay of the amplitude of the frequency, \( f \), when the driving voltage is turned off, see equation 3.3.

\[
D = \frac{1}{\pi \cdot f \cdot \tau}
\]  

(3.3)

This technique, called quartz crystal microbalance with dissipation (QCM–D) allows the measurement of polymer adsorption on different substrates on the silica crystal [57, 58].
3.1. **THE ACTION OF FUNGAL CELLULASES (PAPER I)**

3.1.3 **Experimental procedure**

Table 3.1: The cellulases used in the current investigation.

<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>Mode of action</th>
<th>In this study</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Exoglucanase</td>
<td>Progressively from reducing end</td>
<td>CBH 58</td>
<td><em>Phanerochaete cryosporium</em></td>
</tr>
<tr>
<td>B</td>
<td>Exoglucanase</td>
<td>Progressively from non-reducing end</td>
<td>CBH 50</td>
<td><em>Phanerochaete cryosporium</em></td>
</tr>
<tr>
<td>C</td>
<td>Endoglucanase with CBM</td>
<td>Attack amorphous, less ordered, cellulose and bind to and possibly nick crystalline cellulose</td>
<td>EG I</td>
<td><em>Trichoderma reesei</em></td>
</tr>
<tr>
<td>C</td>
<td>Endoglucanase with CBM</td>
<td>Attack amorphous, less ordered, cellulose and bind to and possibly nick crystalline cellulose</td>
<td>EG V</td>
<td><em>Humicola insolens</em></td>
</tr>
<tr>
<td>D</td>
<td>Endoglucanase without CBM</td>
<td>Negligible binding to and negligible activity towards crystalline cellulose</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The cellulases presented in table 3.1 were used in this investigation. The cellulases were desalted to the used buffer and the light absorbances at 280 nm together with extinction coefficients of the cellulases were used to determine the cellulase concentrations. A synthetic mixture, consisting of 50 percent CBH 50, 25 percent CBH 58 and 25 percent EG I, was prepared using the determined concentrations.

For every cellulase, the following procedure was repeated. A dry model cellulose film was inserted into a QCM D300 (Q-Sense, Västra Frölunda, Sweden). Buffer, 50 mM NaAc, pH 4.5, was injected into the film and the swelling of the film was monitored. After approximately four hours, when the swelling had reached a plateau level, the cellulase was injected and allowed to adsorb for five minutes, which was followed by rigorous rinsing with buffer. Subsequently to rinsing, the action of the cellulase was monitored.

To simplify comparison, the respective levels in frequency and dissipation of every film just before the injection of the cellulase were set as zero levels. The experiments were conducted at room temperature.
3.2 Endoglucanase beating of kraft pulp (Paper II)

3.2.1 Pulp

Totally chlorine-free bleached softwood kraft pulp, in dry lap form, was obtained from SCA Forest Products, Östrand Pulp Mill, Sundsvall, Sweden.

3.2.2 Cellulase

The endoglucanase, EG V (also used above) from *Humicola insolens* was obtained from Novozymes A/S, Denmark, in a liquid preparation called Novozym 467.

3.2.3 Treatment procedure

The cellulase was diluted to the chosen concentrations in a phosphate buffer, 11 mM KH$_2$PO$_4$, and 9 mM Na$_2$HPO$_4$ prepared in deionized water and adjusted to pH 7.0 with 1 M NaOH. Pulp, 30 g, was disintegrated according to ISO 5263-1:2004, washed and diluted in pre-warmed phosphate buffer to 5 percent consistency. The appropriate amount of cellulase was then added and the pulp suspension was incubated in polyethylene bags at 53 ± 2 °C for 2 hours. The incubation was followed by heat deactivation by adding boiling deionized water to 2.5 percent consistency and further incubation of the pulp at 90 ± 2 °C for 35 minutes.

3.2.4 Pulp properties

After treatment and cooling, the pulp was characterized in terms of Schopper–Riegler number, Water Retention Value and viscosity using the ISO 5267-1:1999, SCAN–C 62:00 and SCAN–CM 15:99 standard methods, respectively. PFI-beating was performed according to ISO 5264–2:2002 at 10 percent consistency in deionized water. Laboratory sheets were prepared according to ISO 5269–2:2004 and conditioned according to ISO 187. The sheets were then analyzed for density (ISO 5270), tensile index (SCAN–P 67), tear index (ISO 1974), formation number (Formation evaluation with radiographs BA001 I–E) and air permeability (ISO 5636–5:1986).
3.3 Fungal wood chip seasoning (Paper III)

3.3.1 Seasoning and pre–treatment

Cartapip, a commercial albino strain of *Ophiostoma piliferum*, was supplied by Agrasol Inc. (Raleigh, USA). Fresh Pine and Spruce chips were sprayed with a suspension of fungal mycelium and then stored under controlled temperature and humidity conditions. Samples were taken out after 0, 5, 8, 11 and 14 days and dried on aluminium foil for three days.

3.3.2 Analysis of wood extractives

The dried chips were ground and extracted in an acetone–cyclohexane (9:1) mixture, and then dried in order to determine the total amount. The samples were then derivatized and quantified using a gas chromatograph according to the method described by Örsä and Holmbom [61].

3.3.3 Analysis of pulp properties

Laboratory sheets were produced, according to ISO 536:1995, and analyzed for brightness (Scan–P 3:93), tear strength (EN 21974:1995) and tensile strengths (Scan–P 67:93). Bulk (EN 20534:1994) and freeness (Scan–C 24:65) were also measured.
Chapter 4

Results and discussion

4.1 The action of cellulases (Paper I)

4.1.1 Results

4.1.1.1 The endoglucanases

The endoglucanases investigated in this study were EG V (both with active and inactivated catalytic module) and EG I. The initial phase of the experiments detected as a decrease in frequency and increase in dissipation was interpreted as an adsorption of the enzyme to the cellulose surface. The adsorptions of the endoglucanases, presented in figure 4.1, showed rather similar trends in terms of frequency and dissipation change.

The actions of the endoglucanases after rinsing are presented in figure 4.2. The active endoglucanases, EG I and EG V, caused a continuous increase in dissipation by 7 and 12 units respectively over ten hours. As well over this time, the frequency decreased by 100 and 150 Hz respectively. The frequency of the inactive EG V was increased by 20 Hz over ten hours, accompanied by a 1-unit decrease in dissipation.
CHAPTER 4. RESULTS AND DISCUSSION

Figure 4.1: The frequency shift and the dissipation during adsorption of the endoglucanases.

Figure 4.2: The frequency shift and the change in dissipation due to the action of the adsorbed endoglucanases.
4.1.1.2 The exoglucanases and synthetic mixture

The trends of the adsorption of the exoglucanases were similar although the levels differed slightly. The frequency change was $-70$ Hz for CBH 50 and $-140$ Hz for CBH 58, as presented in figure 4.3. In terms of the dissipation signal, CBH 50 and CBH 58 were similar, with increases of 1.6 and 1.2 units respectively. The synthetic mixture caused a frequency change of $-160$ Hz, while the dissipation increased by 9.3 units during the adsorption.

![Graph 1](attachment:image1.png)  
(a) The frequency shift and the dissipation change during adsorption of two exoglucanases and a synthetic mixture of cellulases.

![Graph 2](attachment:image2.png)  
(b) The dissipation

Between 5 and 15 minutes after adsorption, it can be seen that the dissipation and frequency signals of the exoglucanases were linear, while the signals of the synthetic mixture had a more exponential appearance.

The action of the exoglucanases and the synthetic mixture can be seen in figure 4.4. The frequency signal increased to reach a plateau level while the dissipation initially increased to a maximum after which it decreased back to the starting level.

4.1.2 Discussion

To study the action of cellulases, model cellulose films were chosen as the model system. Any model system chosen will have its advantages and disadvantages. The greatest disadvantage of the model films chosen was likely the fact that the degree of polymerization of the cellulose in the films was around 470 while wood consist of cellulose with a degree...
of polymerization of at least 15000 [15] and pulp around 1200 [58]. The degree of polymerization in the films would thereby correspond to 3 percent and 40 percent as compared with cellulose in wood fibers and in pulp fibers respectively. Because of this, the synergy between endo- and exoglucanases will most probably have been shifted, reducing the importance of the effects of the endoglucanases. Therefore, transfer of knowledge from model experiments to wood and pulp fibers has to be done cautiously. However, the advantages, mentioned in section 3.1.1 were considered to outweigh the disadvantages.

The setup of the experiments also relied on another important feature, i.e., the rinsing of the flow cell. Experiments run without rinsing gave results that were hard to interpret. Rinsing removed the enzymes in the bulk phase and since cellulases with CBM bind strongly to the cellulose, bulk dilution was of less importance. This was also detected in the case of the inactive endoglucanase, where the adsorption change during ten hours was limited.

### 4.1.2.1 The adsorption phase

Using the Sauerbrey relation (equation 3.1), the adsorption of the endoglucanases was found to be 3.7 ±0.3 mg/m², which can be regarded as a rather high adsorption [48]. The adsorption of the exoglucanases were calculated to be 4.1, 8.3, and 9.4 mg/m² for CBH 50, CBH 58 and the synthetic mixture respectively. However, the kinetics for the enzymatic action of the exoglucanases and especially of the mixture most likely affected the signal during the adsorption phase, hence the magnitude of the adsorption should not be directly compared to the magnitude of the adsorption of other proteins.
4.1.2.2 The action of the cellulases

The dissipation of the inactive endoglucanase decreased by one unit while the frequency increased 20 Hz over ten hours. According to the Sauerbrey relation (equation 3.1), this would correspond to a decrease in film mass of 1.2 mg/m², which is approximately 25 percent of the calculated adsorbed protein mass. This mass decrease could have been caused by either desorption due to bulk dilution or by deswelling of the film. The most probable explanation is desorption, indicating that the cellulases are not totally irreversibly bound to the cellulose but governed by an equilibrium process, which however, is strongly driven to the adsorbed state.

Increasing dissipation and decreasing frequency, as seen for the active endoglucanases, can be interpreted as a mass increase. Since only buffer was present in the flow cell, the mass increase must have been caused by water uptake of the cellulose. Thus, the adsorbed endoglucanases caused a swelling of the cellulose film. Using Voinova modeling [60], the decrease in frequency and increase in dissipation were found to be equivalent to a mass increase of 5–10 mg/m², corresponding to approximately 20–50 percent increase in the total film mass.

The most probable reason for this swelling is a change in the balance between swelling and restraining forces in the thin film. The swelling forces can be traced back both to charges and to hydrophilic hydroxyl groups in the film. The charges, likely carboxyl groups [56], probably originating from small remaining fractions of hemicellulose and possibly also oxidized cellulose end-groups create a swelling pressure, which is counteracted by the interaction between the cellulose molecules in the film. When the film becomes depolymerized by the endoglucanase, these counteracting, restraining forces decreases resulting in an increased film swelling.

The increase in frequency, as seen for the exoglucanases, can be interpreted as mass loss caused by cellulose hydrolysis, resulting in water soluble oligomers leaving the film. The initial increase in dissipation followed by a decrease back to the starting level can be explained as follows. First, it is suggested that the exoglucanases form small cavities in the film, resulting in a more loosely assembled film. Then, at a breaking point, the exoglucanase continues to degrade the film surrounding the cavities, resulting in a flatter film, of less dissipation.

The action of the synthetic mixture of cellulases was rather similar to that of the exoglucanases, however, the dissipation maximum was reached at one third of the time it took for the exoglucanases to reach the maximum.

From these results, a model of the action of cellulases is presented in figure 4.5. The endoglucanases first make the cellulose accessible to the exoglucanases by producing new end-groups and by swelling the film. The exoglucanases then efficiently degrade the avail-
The cellulose contain both crystalline fibrils ( ), and less ordered chains ( ), where the latter in part mediate the interaction between the crystalline fibrils. Some fibrils are extended, as "hairs", from the fiber surface. The hydrophilic surface binds water ( ).

Endoglucanases attack specifically the less ordered cellulose ( ), which leads to that some of the crystalline fibrils ( ) are more exposed to the surrounding solution, i.e., the surface become more "hairy", and thus bind more water ( ). The weaker interaction between crystalline fibrils also lead to an increased energy dissipation of the film.

Exoglucanases can degrade crystalline fibrils ( ), but are dependent of free available chain ends. The "hairs" act as good substrate and will therefore be quickly degraded. Thereafter the activity declines due to lack of possible attack sites. The loss of "hairs" leads to less water ( ) binding and a decrease in the energy dissipation of the film.

Figure 4.5: A suggested model of cellulose degradation by cellulases from aerobic fungi.

able cellulose chains.
4.2 Endoglucanase beating of kraft pulps (Paper II)

Softwood kraft pulps treated with endoglucanases prior to beating displayed enhanced tensile strength properties at low beating levels. On the other hand, at higher beating levels the endoglucanase pre-treatment seemed to reduce the tensile strength, as compared with the reference, as shown in figure 4.6.

![Figure 4.6: Tensile index (A) and tear index (B) of endoglucanase treated TCF-bleached kraft pulp, 0.8 ECU/g, and untreated pulps beaten for various numbers of revolutions.](image)

The tensile strength of paper depends on a number of parameters, among which fiber strength, fiber-fiber joint strength and the formation of the fibers in the sheet are important. As shown in figure 4.7, the formation number decreased (i.e. improved) for endoglucanase pre-treated pulp. Therefore, the formation was not the cause for the observed strength loss.

The capacity of fibers to retain water is presented in figure 4.8. These parameters typically increase with increasing fibrillation of fibers, resulting in increasing joint strength. As seen in figure 4.8, these parameters do increase more for endoglucanase treated pulp than for untreated pulp. This in turn suggests that the ability if the fibers to form strong fiber-fiber joints is improved because of the endoglucanase treatment.

Thus, since the formation was improved and the fibrillation seemed to increase, the only left is the individual strength of the fibers. As indicated in figure 4.7, the viscosity of the endoglucanase pre-treated fibers was initially lower than that of untreated fibers, which is not surprising since endoglucanases can efficiently reduce the degree of polymerization. However, the continued decrease in viscosity as the degree of beating increases is interesting. It has to be pointed out that this is the average viscosity and it is likely that the
Figure 4.7: The formation numbers decreased slightly indicating a marginal improvement in formation for endoglucanase treated pulp. The degree of polymerization expressed as viscosity, decreased upon beating, of endoglucanase pre-treated and untreated pulps during beating.

endoglucanases might attack weak zones of the fibers, without causing a significant decrease in the average viscosity. Thus, the fiber surface was probably more depolymerized than cellulose within the fiber wall. When beating is applied, the fibers are more susceptible because of the lower degree of polymerization resulting in a shifted beating process with fiber cutting as one example.

Therefore, from the results presented above, it seems as endoglucanase treatment prior to beating has two effects, on one hand the pre-treatment causes external fibrillation of the fiber, which enhances strength properties, on the other hand, the treatment makes the fibers more susceptible to beating, which weakens the individual fiber strength, when heavier beating is applied.

A model of the effects of endoglucanase pre-treatment is given in figure 4.9.
4.2. ENDOGLUCANASE BEATING OF KRAFT PULPS (PAPER II)

Figure 4.8: Schopper–Riegler numbers (A) and water retention values (B) of endoglucanase pre-treated and untreated pulps with different amounts of beating.

Figure 4.9: Suggested model of the effects of endoglucanase pre-treatment in combination with beating.
4.3 Seasoning of wood chips (Paper III)

4.3.1 Results

During two weeks of controlled seasoning with the addition of fungi, the content of wood extractives decreased by approximately 40 percent for Scots pine and 25 percent for Norway spruce, while the triglyceride contents were degraded to an even greater degree, as shown in figure 4.10.

(a) The total amount of wood extractives during three weeks of seasoning.

(b) The amount of the main components of wood resin during two weeks of seasoning.

Figure 4.10: The effect of seasoning on the wood resin amounts and composition.

Both fresh and seasoned chips were refined and the properties of the pulp were investigated. As shown in figure 4.11 and 4.12, the pulp produced from seasoned pine chips possessed fibers that were less shortened fibers and that displayed less light absorbance and improved strength properties at similar energy input.

4.3.2 Discussion

The reduction in the amount of wood resins during seasoning, presented in figure 4.10, behaved as expected. Similar reductions would most likely have been found even had the chips not been sprayed with fungi. However, the pulp would also likely have lost strength and brightness and the yield would have declined due to the uncontrolled action of cellulytic microorganisms. The losses in yield, brightness and strength properties and the cost for storage are some of the reasons why pulp mills today avoid seasoning.
CHAPTER 4. RESULTS AND DISCUSSION

Figure 4.11: The average fiber length of seasoned pine wood fibers was approximately 0.15 mm less shortened than that of fresh pine, and the tear–tensile relationship was positively affected.

However, as seen in figure 4.11 and 4.12, the seasoning with this albino fungi did not deteriorate the strength properties, neither were the optical properties of the pulp deteriorated. On the contrary, the strength– and the optical properties were improved.

The improved strength properties can be related to the fact that the average length of the fibers from seasoned wood chips was less shortened than that of fibers from untreated wood chips. The reason why the average fiber length was longer than the not seasoned may be explained by the decrease in wood resin amount. The wood resins might have affected the surface properties of the fibers in the refiner, acting as a lubricant. When the lubricants are absent or at least reduced in amount, the refiner blades will separate the fibers more efficiently. Another explanation, that does not contradict the previous one, would be that the fungal growth produces pores in the chips, which could result in improved steam impregnation and thus improved refining.

The improvement in the optical properties was rather small, yet significant. The lower light absorbance can perhaps be explained by the fact that some of the wood resin components were yellow and that removal of these should result in less light absorbance.
Figure 4.12: The effect of wood chip seasoning on the optical properties of the paper produced, displaying less light absorbance but no change in light scattering.
Chapter 5

Conclusions

An inactivated and two active endoglucanases adsorbed to model cellulose films in similar amounts. The active endoglucanase caused film swelling, while the inactive endoglucanase partly desorbed, displaying no tendency to swell or degrade the film. The CBM of EG V was therefore suggested to be incapable of separating fibrils or to bind irreversibly to cellulose. The investigated also showed that exoglucanases degraded the cellulose films, and that the rate of degradation was significantly higher when exoglucanases were used in combination with endoglucanases.

When pulp fibers were pre-treated with endoglucanases and beaten subsequently, the tear index decreased significantly. The tensile index increased at low beating levels but decreased at higher beating levels, as compared with an untreated reference. This was explained by the action of the endoglucanases, which swelled the fibers, thus altering the beating properties of the fibers.

The spraying of pine chips with an albino sap stain fungus followed by seasoning resulted in a significant reduction in the amount triglycerides. Pulp produced from seasoned pine chips displayed improved strength- and optical properties.

5.1 Future work

It was suggested that the cause of the swelling of the model cellulose films, caused by the action of endoglucanases, was due to the depolymerization of cellulose chains, resulting in a decrease in the restaining forces of the film, shifting the balance between the restraining and swelling forces to an extended swelling. Future research should investigate the importance of the swelling pressure of the film on the degree of swelling. Also, this should be related to applications where fibers are heavily beaten, for example in greas proof paper production or in industrial separation of microfibrils.
Chapter 6

Acknowledgements

Lars and Gunnar, thank you for creating a creative and inspiring environment in which it is very exciting and enjoyable to work.
Anna, I would like to thank you for your support in the slow periods and for critical thinking in the fast periods.
Finally, I would like to thank Bo Rydins stiftelse for financial support.
Bibliography


