Strategies for Cellulose Fiber Modification

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The Royal Institute of Technology (Kungliga Tekniska Högskolan), KTH, was founded in 1827 and is the largest of Sweden’s universities of technology. The university has over 11,000 undergraduate students, 1,500 active postgraduate students and a staff of 3,100 people.

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ABSTRACT

This thesis describes strategies for and examples of cellulose fiber modification. The ability of an engineered biocatalyst, a cellulose-binding module fused to the Candida antarctica lipase B, to catalyze ring-opening polymerization of ε-caprolactone in close proximity to cellulose fiber surfaces was explored. The water content in the system was found to regulate the polymer molecular weight, whereas the temperature primarily influenced the reaction rate. The hydrophobicity of the cellulose sample increased as a result of the presence of surface-deposited polyester.

A two-step enzymatic method was also investigated. Here, Candida antarctica lipase B catalyzed the acylation of xyloglucan oligosaccharides. The modified carbohydrates were then incorporated into longer xyloglucan molecules through the action of a xyloglucan endotransglycosylase. The modified xyloglucan chains were finally deposited on a cellulose substrate.

The action of Candida antarctica lipase B was further investigated in the copolymerization of ε-caprolactone and D,L-lactide. Copolymerizations with different ε-caprolactone-to-D,L-lactide ratios were carried out. Initially, the polymerization was slowed by the presence of D,L-lactide. During this stage, D,L-lactide was consumed more rapidly than ε-caprolactone and the incorporation occurred dimer-wise with regard to the lactic acid units.

Morphological studies on wood fibers were conducted using a sol-gel mineralization method. The replicas produced were studied, without additional sample preparation, by electron microscopy and nitrogen adsorption. Information concerning the structure and accessibility of the porous fiber wall was obtained. Studies of never-dried kraft pulp casts revealed micro-cavities and cellulose fibrils with mean widths of 4.7 (±2) and 3.6 (±1) nm, respectively.

Finally, cationic catalysis by simple carboxylic acids was studied. L-Lactic acid was shown to catalyze the ring-opening polymerization of ε-caprolactone in bulk at 120 °C. The reaction was initiated with methyl β-D-glucopyranoside, sucrose or raffinose, which resulted in carbohydrate-functionalized polyesters. The regioselectivity of the acylation was well in agreement with the corresponding lipase-catalyzed reaction. The polymerization was also initiated with a hexahydroxy-functional compound, which resulted in a dendrimer-like star polymer. The L-lactic acid was readily recycled, which made consecutive reactions using the same catalyst possible.

Keywords: Candida antarctica lipase B, cationic catalysis, cellulose-binding module, dendrimer, enzymatic polymerization, fiber modification, silica-cast replica, sol-gel mineralization, organocatalysis, xyloglucan endotransglycosylase
LIST OF PUBLICATIONS

This thesis is based on the following publications, which in the text are referred to by their Roman numerals:

I polyester coating of cellulose fiber surfaces catalyzed by a cellulose-binding module – Candida antarctica lipase B fusion protein
M.T. Gustavsson, P.V. Persson, T. Iversen, K. Hult, M. Martinelle
Biomacromolecules 2004, 5, 106-112

II A two-step strategy for the enzymatic modification of cellulose fiber surfaces using Candida antarctica lipase B and the Populus tremula \times tremuloides xyloglucan endotransglycosylase
M.T. Gustavsson, P.V. Persson, T. Iversen, M. Martinelle, K. Hult, T.T. Teeri, H. Brumer
Manuscript

III Structural characterization of a lipase-catalyzed copolymerization of \( \varepsilon \)-caprolactone and D,L-lactide
J. Wahlberg, P.V. Persson, T. Olsson, E. Hedenström, T. Iversen
Biomacromolecules 2003, 4, 1068-1071

IV Silica-cast replicas for morphology studies on spruce and birch xylem
P.V. Persson, A. Fogden, J. Hafrán, G. Daniel, T. Iversen
IAWA Journal 2004, 25, in press (issue 3)

V Silica nanocasts of wood fibers: a study of cell wall accessibility and structure
P.V. Persson, J. Hafrán, A. Fogden, G. Daniel, T. Iversen
Biomacromolecules 2004, 5, in press (issue 3)

VI Selective organocatalytic ring-opening polymerization: a versatile route to carbohydrate-functionalized poly(\( \varepsilon \)-caprolactones)
P.V. Persson, J. Schröder, K. Wickholm, E. Hedenström, T. Iversen
Manuscript

VII Direct organocatalytic synthesis of dendrimer-like star polymers
P.V. Persson, T. Iversen, A. Córdova
Manuscript
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1 INTRODUCTION

Composites of natural fibers and man-made polymers are desirable from an environmental point of view. Aliphatic polyesters are often biodegradable and biocompatible, and have good mechanical properties, and they are therefore particularly interesting. Polylactide (PLA) and poly(\(\varepsilon\)-caprolactone) (PCL) are two examples of such aliphatic polyesters. PLA is produced from lactic acid or lactide, and has mechanical properties that make the polymer promising as a matrix component in natural fiber composites. Some applications, such as automotive interior components, are already commercially viable.\(^1\) PCL, made from petroleum-derived \(\varepsilon\)-caprolactone (\(\varepsilon\)-CL), is a hydrophobic polyester with a low glass transition temperature.\(^2\) It is also notable that this cyclic monomer is liquid at room temperature and a good solvent for many compounds.

Ring-opening polymerization (ROP) of cyclic esters is carried out mainly in bulk or in solution, and is normally catalyzed/initiated by an organometallic compound.\(^3\) However, ROP performed in the presence of cellulose fibers is somewhat delicate. The fibers are moist and carry numerous reactive hydroxyl groups. Tin (II) 2-ethylhexanoate, which is otherwise a common and efficient initiator/catalyst for ring-opening polymerization, is hygroscopic, hydrolyzes easily to stannous hydroxide, and is susceptible to undesired reactions with hydroxyl groups.\(^4\) A more suitable choice is therefore a biocatalyst. Lipases have been shown to efficiently catalyze transesterification reactions.\(^5,6\) The conditions need not be extremely dry; in fact, small amounts of water can be favorable for the polymerization.

In Chapter 2 (papers \(\text{I–III}\)), lipase catalysis in fiber-modifying processes is summarized. Paper \(\text{I}\) describes the use of an engineered biocatalyst – the \textit{Candida antarctica} lipase B equipped with a cellulose-binding module (CBM-CALB)\(^7,8\) – in the polymerization of \(\varepsilon\)-CL in close proximity to cellulose fibers. Paper \(\text{II}\) elaborates on this with a different approach; here xyloglucan oligosaccharides are acylated in a lipase-mediated step using a vinyl ester or a thiolactone, and are then incorporated into longer xyloglucan chains through the action of a xyloglucan endotransglycosylase. In the final step, the modified xyloglucan is deposited on a cellulose substrate. In paper \(\text{III}\), the CALB-catalyzed copolymerization of \(\varepsilon\)-CL and D,L-lactide is examined with respect to mechanism and product pattern. The study also provides a hint that CBM-CALB would also be capable of catalyzing the ROP of D,L-lactide.
The wood ultrastructure, including the pore system, is altered during the pulping process. It is important to take these changes into account, especially those affecting the accessibility to reactants and catalysts, when designing systems for fiber modification. In Chapter 3 (IV, V), a casting method based on silica sol-gel is described. This technique allows the nanostructure of wet fibers to be transferred to a hard silica cast, which can be studied under the electron microscope or by adsorption measurements. Paper IV is primarily a reference study to demonstrate the usefulness and versatility of the casting method. Paper V deals mainly with delignified fibers, and provides a rationale for the results of the biocatalytic modifications (I, II). One important insight is that polymerization inside the fiber cell wall is unlikely to be accomplished using enzymes such as lipases due to the restricted pore size. A natural step forward is to search for a catalyst by which this could be achieved.

There are several demands that have to be met before choosing a catalyst for modification inside the fiber wall. In addition to being sufficiently small for cell-wall penetration, the catalyst should also be non-toxic, easily handled, and not detrimental to the wood polysaccharides. These requirements are theoretically fulfilled by some low-molecular weight organic carboxylic acids. Among the acids that were screened, L-lactic acid was found to be the most promising candidate. In Chapter 4, the catalytic action of this naturally occurring organic acid is closely examined, and is found to provide the basis for a novel organocatalytic route to end-functionalized polyesters. In paper VI, the synthesis of mono-, di-, and trisaccharide-functionalized PCL is described. This can be regarded as a model system for polysaccharide modification. In paper VII, a hexahydroxy initiator is employed in the synthesis of a dendrimer-like star polymer. The initiator is itself a triester and susceptible to acid-catalyzed transesterification reactions, and this makes it possible to assess the selectivity of the catalyst.
2 ENZYMATIC STRATEGIES FOR FIBER MODIFICATION

2.1 Lipase catalysis

Lipases (acylglycerol acylhydrolases, EC 3.1.1.3) are enzymes whose native function is to catalyze the hydrolysis of fatty acid esters in aqueous biological systems. In the early 1980s, Žaks and Klibanov studied the activity of lipases in organic solvents. They showed that the enzymes remained active in organic media and that transesterification reactions were efficiently catalyzed. In addition, the biocatalyst exhibited good thermostability and selectivity.

If a cyclic ester is employed in such a reaction system, subsequent transesterifications lead to the formation of a polymer. A number of lipases from different sources have been shown to catalyze ring-opening reactions in organic solvents or in bulk. An advantage over condensation polymerization (for example the polymerization of hydroxy acids) is that no leaving group such as water is generated that would restrict monomer conversion or the molecular weight of the polymer product.

The lipase B from the yeast Candida antarctica (CALB), marketed as Novozym 435 by Novo Nordisk A/S immobilized on a polyacrylate resin, has frequently been utilized as catalyst in ROPs. CALB, a well-characterized serine hydrolase, catalyzes hydrolysis and esterification according to a bi-bi ping-pong reaction mechanism. In Figure 2.1, the transesterification of a cyclic ester, the seven-membered ε-caprolactone (ε-CL), is shown. The first step is an acylation reaction between the serine 105 residue and the ester. In the subsequent stages, the alcohol R-OH attacks the acyl enzyme, the product is released in a deacylation step, and the enzyme returns to its ground state.
2.2 Xyloglucan endotransglycosylase catalysis

Xyloglucan endotransglycosylases (XETs) are enzymes that mediate the cleavage and re-ligation of xyloglucan molecules during plant cell wall growth. In plants, coordination of xyloglucan with cellulose is thought to give rise to greater strength through an extensive hydrogen bond network. Xyloglucan consists of a $\beta(1\rightarrow4)$-linked glucan backbone with branches of xylose and galactose. The seed of the tropical tamarind tree is a rich source of xyloglucan and has been used on a large scale since World War II as a sizing additive in the cotton textile industry.

The repeating units of xyloglucan, referred to as xyloglucan oligosaccharides (XGOs), are readily prepared by a digestive endoglucanase treatment. The XGOs formed in the degradation of tamarind xyloglucan are shown in Figure 2.2. The reaction mechanism of XET is shown in Figure 2.3. In contrast to xyloglucan chains and XGOs, water is excluded from the active site of the enzyme, and this prevents hydrolysis of the biopolymers. XET degrades xyloglucan by cleaving the carbohydrate backbone in an endo-manner and linking the new chain to the 4-$O$ position at the non-reducing end of another xyloglucan chain or XGO.
Figure 2.2 The four repeating units of tamarind seed xyloglucan. The number of primary hydroxyls (indicated by asterisks) is dependent on the degree of galactosyl substitution.

Figure 2.3 The reaction mechanism of transglycosylation catalyzed by XET (R ≠ H).

2.3 Polyester modification of cellulose fiber surfaces

In paper I, an enzymatic approach to introduce polymers to the surface of a cellulosic material is described. Lipase-catalyzed ROPs were carried out in close proximity to cellulose fiber surfaces (filter paper) utilizing CBM-CALB. The CBM efficiently bound the fusion protein to the cellulose surface. The ring-opening polymerization was performed in a closed reaction chamber with different water activities (\(a_w = 0.11\) or 0.84). The monomer \(\varepsilon\)-CL was added and the chamber was incubated at 20 or 60 °C. The addition of monomer from the gaseous phase was also studied.

The monomer conversion, monitored by gel permeation chromatography (GPC), is shown in Figure 2.4. The reaction temperature had a stronger impact than water activity on the monomer consumption rate. After 8 h at 60 °C, all the \(\varepsilon\)-CL had been converted, whereas the conversion in this time at 20 °C was less than 50%.
The molecular weight of the polymer produced was highly dependent on the water activity. With a low water activity, high-molecular weight polymers were formed, whereas mainly oligomers or polymers of low molecular weight were detected at the higher water activity level (Figure 2.5). In agreement with previous studies, it was found that the temperature primarily influenced the polymerization rate, whereas the water activity mainly regulated the polymer molecular weight.

Figure 2.4 ε-CL consumption at $\omega_w=0.11$ (■, ●) and $\omega_w = 0.84$ (□, ○) monitored by GPC.

Figure 2.5 Molecular weight distribution (GPC) of extracted polyester after 48 h reaction time at 60 °C. Mainly oligomers and polymers of low molecular weight were produced at high water activity, whereas the lower water activity afforded polymers of $M_w = 41,000$ ($M_w/M_n = 3.1$).
Infrared spectroscopy on the modified paper samples before and after extraction with organic solvent revealed no covalent attachment of polyester to the cellulose surface. The appearance of the polymer-coated paper is shown in Figure 2.6.

A water drop test was carried out to demonstrate the increase in hydrophobicity as a result of the polymerization on the paper surface. The best result was observed with samples prepared at a high temperature combined with low water activity (Figure 2.7).

![Unmodified paper vs Polymer-coated paper](image)

**Figure 2.6** Micrographs (100×) of cellulose fibers, untreated and after polyester deposition.

![Water drop test](image)

**Figure 2.7** Water drop test on polymer-coated filter paper after 48 h reaction time at different water activities and different temperatures.
The polymerization was also carried out by the gas-phase addition of monomer at 80 °C. This kind of addition of monomers to immobilized enzymes has been reported for several enzymes including lipases. Again it was found that more and larger polymers were produced at the lower water activity level.

2.4 Fiber modification using modified xyloglucan oligosaccharides

In publication II, another enzymatic procedure for the modification of cellulose fiber surfaces is explored. The strategy utilizes the inherent ability of the water-soluble hemicellulose xyloglucan to adsorb onto cellulose surfaces. XGOs, prepared from high-molecular weight tamarind xyloglucan, were first acylated in a CALB-mediated reaction. The modified XGOs were then transferred to longer xyloglucan chains in a reaction catalyzed by PttXET16A, a XET from the hybrid aspen *Populus tremula x tremuloides*. Finally, the modified xyloglucan was adsorbed onto a cellulose substrate. The different stages in the process are shown in Figure 2.8.

In the first stage, catalyzed by CALB, stearoyl or mercaptobutanoyl XGOs were synthesized using vinyl stearate or γ-thiobutyrolactone as acyl donor. The reaction was performed in a mixture of dimethyl sulfoxide and tert-amyl alcohol according to the method developed by Ferrer et al. The choice of solvent system is a compromise and it is by no means perfect; high lipase activity and reasonable carbohydrate solubility are difficult to achieve simultaneously in non-aqueous media.
XGO monoesters were formed regardless of galactosyl substitution, but di- and triesters were obtained only for the XGO variants with respectively two/three and three primary hydroxyls. This selectivity of lipases towards the primary hydroxyl groups of carbohydrates has been reported previously.36-42

The second stage is a XET-catalyzed transfer of the modified XGOs to longer xyloglucan chains. A lower reactivity than that of unmodified XGOs was observed, possibly as a result of increased steric hindrance. Figure 2.9 shows a MALDI-TOF mass spectrum of the products obtained from PttXET16A-mediated transglycosylation with monostearoyl XGOs as glycosyl acceptors.

Finally, xyloglucan, modified with either long alkyl chains or thiol groups, was deposited onto a cellulose substrate. The presence of modified xyloglucan on the cellulose surface was confirmed by Fourier transform infrared spectroscopy for the detection of fatty acid ester or by staining with the thiol-specific reagent sulforhodamine methanethiosulfonate.

![MALDI-TOF mass spectrum of the stearoyl-modified XGOs incorporated into larger xyloglucan chains. The number of glucose residues is indicated. The splitting of signals into clusters is a result of different degrees of galactosylation.](image)

**Figure 2.9** MALDI-TOF mass spectrum of the stearoyl-modified XGOs incorporated into larger xyloglucan chains. The number of glucose residues is indicated. The splitting of signals into clusters is a result of different degrees of galactosylation.
2.5 Copolymerization of ε-caprolactone and D,L-lactide

In paper III, the lipase-catalyzed copolymerization of ε-CL and D,L-lactide is reported. It has been demonstrated that CALB is efficient in catalyzing the polymerization of ε-CL, in organic solvent or in bulk.\textsuperscript{40,41,43-52} On the other hand, it has been suggested that D,L-lactide acts as a reversible inhibitor of the lipase,\textsuperscript{53} without being polymerized.\textsuperscript{54,55} It was, indeed, found that D,L-lactide is incorporated into the resulting polymer. Furthermore, the ability to slow the copolymerization made it possible to study the structure of the polymer and how it changed during the course of the reaction. The lipase-catalyzed copolymerization is shown in Figure 2.10. The reaction between one molecule each of ε-CL and D,L-lactide thus yields one caprolactone unit connected to two lactic acid units.

The ability of D,L-lactide to slow the polymerization is evident in Figure 2.11. After one week, the conversion of ε-CL in the reference reaction was complete but only 42% in one of the D,L-lactide-containing reactions (ε-CL to D,L-lactide molar ratio 10:1). During the first hours, a rapid conversion of D,L-lactide was detected (paper III: Figure 5). After this initial phase, both monomers were converted at approximately the same rate. The presence of D,L-lactide thus influenced the reactivity of ε-CL negatively, which was also reflected in a lower molecular weight of the polymer. It is also worth noting that macrocycles of pure PCL and copolymers with up to two lactic acid units in the ring were initially formed. At longer reaction times, these cycles were transformed into a linear product.

The change in polymer structure during the course of reaction was studied in experiments with a high D,L-lactide content. MALDI-TOF mass spectra (oligomeric region) after one, three and eight weeks are shown in Figure 2.12. During the first week, the D,L-lactide was preferentially incorporated as the lactic acid dimer (see signals inside frames). This non-random product pattern as a result of the initial dimer-type incorporation of lactide was gradually destroyed by intermolecular transesterification reactions as the reaction proceeded.

![Figure 2.10](image)

**Figure 2.10** Lipase-catalyzed copolymerization of ε-CL and D,L-lactide. The ring opening of one lactide molecule leads to the integration of two lactic acid units into the polymer chain.
Figure 2.11 Consumption of ε-CL at 60 °C in (○) neat conditions, ε-CL to D, L-lactide molar ratio 20:1 (■) and 10:1 (●).

Figure 2.12 MALDI-TOF MS of the copolymerization after one, three and eight weeks (I, II and III, respectively) in a reaction with a high D,L-lactide content (ε-CL to D,L-lactide molar ratio 2:1, 70 °C). The marked signals show oligomers containing 0, 1, 2, 3 and 4 caprolactone units (a-e, respectively). The relatively more frequent occurrence of oligomers with an even number of lactic acid units is indicated (frames).
3 SILICA MINERALIZATION OF WOOD

3.1 Chemistry and structure of wood

In order to accurately describe and predict the properties of a wood fiber, chemistry and structure are interconnected and equally important. Chemically, a wood fiber is composed mainly of cellulose, hemicellulose and lignin (for Norway spruce 42, 25 and 27%, respectively).\(^5^6\) Cellulose, the major biopolymer on Earth, is a linear homopolymer of high molecular weight, composed of β-D-glucopyranose units linked in a (1→4)-glycosidic fashion. The hemicelluloses are branched heteroglycans containing different types of carbohydrate units. The dominating hemicellulose species in softwoods (e.g. spruce and pine) is O-acetyl-galactoglucomannan, whereas hardwoods such as birch and aspen contain predominantly O-acetyl-4-O-methylglucuronoxylan. Lignin, the third major component of wood, is an aromatic three-dimensional macromolecule built up of phenylpropane units which functions as a glue that fixes the wood structure. Softwood lignin is built up mainly of coniferyl alcohol, whereas hardwood lignin is formed from a mix of coniferyl and sinapyl alcohol. The chemical structures of cellulose, one of the hemicelluloses, and lignin are shown in Figure 3.1.

Cellulose, hemicellulose and lignin are distributed in the cell wall in a way that makes the material both strong and flexible. Several proposals regarding the softwood cell wall architecture have been put forward over the years, and researchers agree that the wood fiber wall consists of a lignin-rich middle lamella, a primary wall, a secondary wall, and, sometimes, a warty layer. One of the most frequently cited models, proposed in 1984 by Fengel and Wegener,\(^5^7\) is shown in Figure 3.2.
Figure 3.1 Schematic structures of cellulose, hemicellulose (here represented by \(O\)-acetyl-galactoglucomannan which is abundant in softwoods) and lignin.

Figure 3.2 Schematic illustration of a typical softwood fiber (tracheid).\textsuperscript{57}  
S = secondary layer, P = primary layer, W = warty layer and ML = middle lamella.
The S2 layer forms the main fraction of the cell wall and hence contributes to a large extent to the properties of the fiber. The cellulose fibril and fibril aggregates of the S2 layer, the major load-bearing parts of the fiber wall, are aligned almost parallel to the fiber axis. These structures have a core of highly ordered cellulose surrounded by less ordered domains,\textsuperscript{58,59} bordering on a matrix of hemicellulose and lignin. A model of this assembly is shown in Figure 3.3.

![Diagram of fiber structure](image)

**Figure 3.3** Hierarchical representation of the secondary wall. The illustration is a combination of models proposed by Kerr and Goring\textsuperscript{60} and Marchessault and Sundararajan.\textsuperscript{61}

Electron microscopy is frequently used in the study of fiber structure. Electrons, generated from an emission source, interact with the sample. Transmitted, back-scattered or secondary electrons are detected, and an image is created. High vacuum combined with a dried and fairly conductive sample is generally needed in order to obtain high resolution. Native wood is moist and non-conductive and it is therefore usually dried and metal-coated prior to electron microscopy. Such measures are likely to cause some degree of distortion of the native structure. It is hence desirable to find methods where drying and coating steps can be avoided. One such approach, a casting technique based on silica sol-gel, has been presented in papers IV and V. The technique is summarized in the next section.
3.2 Silica sol-gel

Silica sol-gels can be produced by the concurrent hydrolysis and condensation of silicon alkoxides. Studies in the field began as early as the mid-1800s, and have, for example, led to the commercial development of colloidal silica powders of uniform particle sizes. In 1992, it was demonstrated that silica with uniform pore channels varying from 1.5 nm to greater than 10 nm could be synthesized in a controlled manner in a liquid-crystal templated process using a surfactant. The merging of this knowledge with the well-established sol-gel technology has initiated a wave of research activities in the field of biomimetic materials chemistry. Hierarchically structured inorganics templated by living cells, bacteria, virus particles, cuttlefish bone, sea urchins, diatoms, and hairs from dogs or humans are some examples. Cellulose tissue and fibers have also been investigated in the making of such materials, sometimes called biomorphous ceramics.

The in situ silica mineralization procedure developed by Shin et al. has been employed to study the structure and the pore system of the wood fiber cell wall. The sol-gel is formed from tetraethyl orthosilicate (TEOS) in an ethanolic solution at an acidic pH. Under these conditions, in the presence of a cationic surfactant, hexadecyltrimethylammonium chloride (CTAC), rapid hydrolysis and condensation occur, and this results in low-molecular weight polysilicic acid. A micellar phase is created, which prevents the silica particles from growing and aggregating into a gel. The chemistry of the sol-gel formation is outlined in Figure 3.4.

![Figure 3.4](image)

**Figure 3.4** Nanometer-sized silica particles are formed during the surfactant-templated hydrolysis and condensation of TEOS.
3.3 Mineralization of wood fibers

Wood samples were immersed in the sol-gel and mineralized at 60 °C. The efficiency of the mineralization can be discussed in terms of fiber cell wall accessibility. Mineralized wood and pulp samples were compared using energy-dispersive X-ray analysis (EDXA). The result is shown in Figure 3.5. The different silicon signal intensities, for wood and pulp, expressed as the ratios $A_w/B_w$ and $A_p/B_p$ (0.2 and 0.4 respectively), indicate that considerably more silica has entered the cell wall of the pulp sample.

Figure 3.5 Field-emission environmental scanning electron microscopy (FE-ESEM) images of cross sections of wood and kraft pulp (kappa 18) impregnated with silica sol-gel. The spectra show the relative intensities of carbon and silicon measured along the dashed lines using X-ray detection (EDXA). Scale bars 5 µm.
3.4 Silica casts studied by electron microscopy

The silica-mineralized wood and pulp samples were calcined at 575 °C in air to remove the organic material and leave a cast of pure silica. The casts were studied with environmental scanning electron microscopy (ESEM) without further sample preparation. Figure 3.6 shows casts of spruce wood and kraft pulp. Features such as tracheids, ray cells and bordered pits were well reproduced with respect to both structure and dimensions in the wood and pulp samples. An obvious difference is the loss of pit membranes and sometimes pit chambers in the kraft pulp fibers as a result of the quite harsh delignification process.

![Figure 3.6 ESEM of silica casts](image)

The wood and pulp casts were also studied by transmission electron microscopy (TEM). The removal of lignin and hemicellulose from the fiber wall during the kraft pulping process leads to an increase in total pore volume. Ultrastructural changes are also induced, some of which are visible in Figure 3.7. In contrast to the dense and rather irregular structure of the lignified cell wall, the kraft pulp cast exhibits a regular, highly oriented structure. The dark regions are the original fiber cell-wall pore structure that has been filled with silica. The light (unfilled) structures, oriented and uniform in size, probably originate from the cellulose fibrils. The silica thus seems to be distributed on the surface of (and not inside) the compact solid cellulose fibrils, which creates pores of the corresponding size during calcination. The image of the pulp cast reveals that the porous structure of the pulp fiber cell wall at the nanometer level can be described as an anisotropic ordered structure oriented in the
direction of the cellulose fibrils. These pores seem to be rather long compared to their width; unbroken structures of up to a micrometer in length were observed, which is in accordance with previously reported results from NMR relaxation measurements.\textsuperscript{81,82}

\textbf{Figure 3.7} TEM images of silica casts from spruce wood and kraft pulp. Scale bars 200 nm. Inset is a magnification of the pulp cast, scale bar 15 nm. The parallel white lines define the locations where the widths of the cellulose structures and pores was measured.
3.5 Image analysis

The kraft pulp cast was measured manually to quantify the widths of both the light and dark structures (Figure 3.7, parallel lines). The result is shown in Figure 3.8. The mean value of the width of the light-shaded structures assigned to cellulose fibrils was found to be 3.6 (±1) nm. Similar results have been reported using rapid-freeze deep-etched softwood and pulp samples, embedded and stained samples, and from NMR measurements. The mean width of the dark elongated structures, i.e. the pores of the original pulp, was determined to be 4.7 (±2) nm. Results in this size range have been reported using solute exclusion, NMR relaxation, and inverse size exclusion chromatography.

![Figure 3.8](image)

Figure 3.8 Analysis of the pulp cast structure measured along the white lines of Figure 3.7.
3.6 Nitrogen adsorption

The pore distribution of the silica casts was also measured using the nitrogen adsorption technique. The results for the calcined wood, pulp and reference silica samples are shown in Figure 3.9. Even though the total surface area of the pulp cast was lower than that of the reference (873 and 1224 m² g⁻¹, respectively), the contribution from the region where the cellulose fibril and fibril aggregate surfaces are expected to lie (d > 3.5 nm)⁸⁴,⁹¹ is much higher (128 and 24 m² g⁻¹, respectively). This difference is visible in the figure, with a distinct peak in the distribution at a mean diameter of approx. 4 nm. This agrees with the diameter of the fibril structures observed in the TEM image of the pulp cast (cf. Figure 3.7).

![Figure 3.9](image_url)  
*Figure 3.9* The pore diameter distribution calculated from nitrogen adsorption measurements on silica casts. ● Reference; ♦ Wood; ■ Kraft pulp.
3.7 Image gallery

In order to demonstrate the versatility of the sol-gel casting method, some assorted electron micrographs of different wood species and tissue types are shown in figures 3.10–3.13. All the images with the exception of that of poplar show silica casts after calcination.

**Figure 3.10** Top: Image of a cross section of mineralized poplar tension wood before calcination. The so-called gelatinous layer (G-layer) consists of un lignified, almost pure cellulose. The absence of lignin in the G-layer simplifies silica penetration, and gives rise to a higher silicon signal than in the secondary wall. FE-ESEM/EDXA. Bottom: Spruce pulp, kappa number 18. The structural integrity of the cell walls after calcination indicates successful mineralization. ESEM.
Figure 3.11 Spruce compression wood tracheids. The twisted appearance resembles the characteristic helical orientation of the cellulose fibrils from the secondary cell wall of compression wood.\textsuperscript{93} FE-ESEM (top) and ESEM.
Figure 3.12 Birch (ESEM). Top: Fibers, ray cells and the remnant of a vessel (the large cavity to the left). Bottom: Released cell wall-material, probably from a vessel.
Figure 3.13 Birch (ESEM). Top: Fibers and ray cells with numerous apparently slightly distorted half-bordered pits. Bottom: Expansion of frame.
4 ORGANOCATALYTIC POLYMERIZATION

The CBM-CALB-catalyzed modification (paper I) resulted in a polyester coating of the fiber surface. Given the reported dimensions of CALB, $3 \times 4 \times 5$ nm,\(^9\) and the shape and pore size distribution of the fiber wall, according to paper \(V\) and previous studies,\(^8\, 88-90\) this outcome was rather expected. In addition, the high affinity of the CBM towards cellulose surfaces probably makes it even more difficult for the enzyme to reach interior domains of the cell wall. A smaller catalyst is therefore needed to achieve cell wall penetration and subsequent polymerization.

4.1 Organocatalysis

There is currently a renewed interest in using rather simple organic molecules as catalysts in traditional organic-chemical transformations.\(^95-105\) Related to this thesis are the reaction systems of Hedrick and coworkers, where heterocyclic carbenes or phosphines are employed as nucleophilic catalysts in transesterification reactions, including the ring-opening polymerization of cyclic esters.\(^106-111\)

Inspired by the progress in this field, some low-molecular carboxylic acids were screened in order to find a suitable catalyst for the ROP of \(\varepsilon\)-CL. Propanoic, hexanoic, 6-hydroxyhexanoic and L-lactic acid were tested, and the latter was shown to be the most promising. In this chapter, general aspects of L-lactic acid-catalyzed ROP are described. The versatility of the reaction system has been explored, demonstrated by the successful synthesis of end-functionalized PCL. This was accomplished by performing the polymerization in the presence of carbohydrates (paper \(VI\)), or a multifunctional initiator (paper \(VII\)).

4.2 Ring-opening polymerization catalyzed by L-lactic acid

The bulk polymerization of \(\varepsilon\)-CL at 120 °C was carried out with different concentrations of L-lactic acid present. The monomer consumption is shown vs. time for reactions with different concentrations of L-lactic acid in Figure 4.1. It can be seen that a higher L-lactic acid concentration increases the polymerization rate. In the absence of L-lactic acid, no consumption of \(\varepsilon\)-CL was detected (not shown).
The concentration of L-lactic acid also influenced the polymer molecular weight and the polydispersity. A high L-lactic acid concentration resulted in a relatively low molecular weight and a broad polymer distribution, as shown in Figure 4.2. This indicates that the number of nucleation sites increases with increasing L-lactic acid concentration.
In the ring-opening polymerization of ε-CL, a nucleophilic initiator must be present to open the first lactone of each polymer chain. Normally, in enzyme catalysis, water or some added nucleophile, e.g. an alcohol, executes this step. The polymerization described here, however, was performed under fairly dry conditions (120 °C) and without addition of initiator. Control reactions with propanoic or hexanoic acid instead of l-lactic acid gave no polymer product. This strongly indicates that the secondary hydroxyl group of L-lactic acid, in the absence of competition from more reactive species, also adopts the role of initiator for the polymerization. The MALDI-TOF mass spectra in Figure 4.3 reveal that this is indeed the case. After complete consumption of monomer, termination reactions involving the carboxylic acid function of l-lactic acid were observed. The l-lactic acid should nevertheless be regarded foremost as a catalyst, since no insertion of additional l-lactic acid units seems to occur as long as unreacted ε-CL remains in the system.

![MALDI-TOF mass spectra](image)

**Figure 4.3** MALDI-TOF mass spectra of the polymerization of ε-CL catalyzed by l-lactic acid (120 °C, ε-CL to l-lactic acid molar ratio 10:2) taken after 1.5 h and 5 h. The polyester was initiated with l-lactic acid (a and b; different adducts) or water (c). After complete consumption of ε-CL, termination reactions were observed, involving the carboxylic acid group of l-lactic acid (d).

### 4.3 Selective acylation of carbohydrates

The lipase-catalyzed acylation of carbohydrates,36-39,112 and reactions aiming at carbohydrate-functionalized PCL,40-42 all face the challenge of simultaneously achieving a decent sugar solubility and high enzyme activity. Since the l-lactic acid-catalyzed reactions yielded end-functionalized polyester at a relatively high temperature, a natural extension was to study the behavior of various low-molecular carbohydrates in the reaction system in comparison with the lipase-mediated systems. Methyl β-D-glucopyranoside and the non-reducing sugars sucrose and raffinose were chosen. All the reactions were performed
at 120 °C with a monomer to L-lactic acid to carbohydrate molar ratio of 10:1:1. The chemical structures of the sugars and their initiating reaction with ε-CL are shown in Figure 4.4.

The monomer consumption of the carbohydrate-containing polymerizations is shown in Figure 4.5. All reactions with carbohydrates present were faster than the corresponding control reaction. The unexpectedly rapid conversion of the raffinose-containing reaction can probably be explained by the relatively high amount of crystal water, which was not completely removed during drying. These water molecules gave rise to a water-initiated product, which was confirmed by MALDI-TOF MS. The molecular weight and polydispersity according to GPC are shown in Figure 4.6. The results are similar to those reported for the lipase-catalyzed reactions.

Figure 4.4 End-group functionalization using carbohydrates. Asterisks indicate the primary hydroxyls, the most reactive nucleophilic sites of the sugars.
Figure 4.5 Conversion according to GPC of ε-CL catalyzed by L-lactic acid in the presence of methyl β-D-glucopyranoside (▲), sucrose (●) or raffinose (♦) compared with the control reaction (■, only ε-CL and L-lactic acid; same as in Figure 4.1).

Figure 4.6 Molecular weight and polydispersity of carbohydrate-functionalized PCL according to GPC. ▲ Methyl β-D-glucopyranoside; ● sucrose; ♦ raffinose.
MALDI-TOF spectra of the carbohydrate-functionalized PCLs are shown in Figure 4.7. In the raffinose-initiated sample small amounts of a disaccharide-initiated product were detected. This was probably a result of either degrading acidolysis or the presence of disaccharide impurities in the starting material.

The acylation position was determined by NMR and methylation analysis. The main products were acylated on the primary hydroxyl groups of the carbohydrate end-groups, for the methyl β-D-glucopyranoside-initiated product with a regioselectivity well in agreement with corresponding lipase-catalyzed acylations of mono- and disaccharides.36-42,112

![Figure 4.7 MALDI-TOF MS of purified fractions of PCL functionalized with a) methyl β-D-glucopyranoside, b) sucrose and c) raffinose.](image-url)
4.4 Synthesis of dendrimer-like poly(ε-caprolactone)

Dendrimers (from the Greek dendros, tree) are polymers characterized by a highly branched, well-defined structure built around a central core. One example is the synthesis of dendritic aliphatic polyesters based on a hexahydroxy-functional initiator. The schematic appearance of a dendrimer and the chemical structure of the initiator are shown in Figure 4.8.

The first organocatalytic synthesis of dendrimer-like PCL is described in paper VII. The reaction is outlined in Figure 4.9. After 1 h, complete conversion of monomer catalyzed by L-lactic acid had occurred according to GPC. NMR revealed that all hydroxyl groups of the initiator had been acylated. The product was purified by precipitation and isolated in high yield (90%) with an average molecular weight (M_w) of 12 400 Da (M_w/M_n = 1.4). Furthermore, the L-lactic acid was readily recovered and consecutive reactions were performed with no apparent decrease in catalytic activity or molecular weight of the polymer product. These star-shaped polymers are compact, well-defined, and could perhaps be used for fiber modification analogous with the impregnation using polysilicic acid particles described in papers IV and V.

Figure 4.8 Left: typical dendrimer structure. Right: hexahydroxy-functional initiator based on 1,1,1-tris(hydroxyphenyl)ethane coupled to three 2,2-bis(hydroxymethyl)propanoic acid residues.
Figure 4.9 Synthesis of dendrimer-like PCL catalyzed by L-lactic acid at 120 °C.
5 CONCLUDING REMARKS

In this thesis, some aspects of cellulose fiber modification have been presented.

The CBM-CALB methodology for surface modification of cellulose surfaces has a great potential, and I believe that high-performance applications such as biosensors or targeted filter systems are within reach.

The XET approach, developed and patented by Teeri, Brumer et al., is an elegant route to functionalized oligo- and polysaccharides with a high affinity to cellulose fibers. This technique should serve as an inspiration for the development of cost-efficient processes to obtain modified short-chain polysaccharides for use in fiber surface modifications. Such modified carbohydrates could perhaps be prepared from cellulose, hemicellulose or cellulose derivatives.

The use of carboxylic acid catalysts, such as L-lactic acid, makes it possible to reach interior parts of the fiber wall that are inaccessible to lipases. In future work, the concept of organocatalysis in ring-opening polymerization ought to be studied in actual fiber modification experiments.
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>$a_w$</td>
<td>water activity</td>
</tr>
<tr>
<td>CALB</td>
<td><em>Candida antarctica</em> lipase B</td>
</tr>
<tr>
<td>CBM</td>
<td>carbohydrate-binding module</td>
</tr>
<tr>
<td>CBM-CALB</td>
<td>cellulose-binding module fused to <em>Candida antarctica</em> lipase B</td>
</tr>
<tr>
<td>$\varepsilon$-CL</td>
<td>$\varepsilon$-caprolactone</td>
</tr>
<tr>
<td>CTAC</td>
<td>hexadecyltrimethylammonium chloride</td>
</tr>
<tr>
<td>EDXA</td>
<td>energy-dispersive X-ray analysis</td>
</tr>
<tr>
<td>ESEM</td>
<td>environmental scanning electron microscopy</td>
</tr>
<tr>
<td>GPC</td>
<td>gel permeation chromatography</td>
</tr>
<tr>
<td>MALDI-TOF MS</td>
<td>matrix-assisted laser desorption ionization time-of-flight mass spectrometry</td>
</tr>
<tr>
<td>PCL</td>
<td>poly($\varepsilon$-caprolactone)</td>
</tr>
<tr>
<td>PLA</td>
<td>polylactide</td>
</tr>
<tr>
<td>PttXET16A</td>
<td><em>Populus tremula x tremuloides</em> xyloglucan endotransglycosylase 16A</td>
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<tr>
<td>ROP</td>
<td>ring-opening polymerization</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>TEOS</td>
<td>tetraethyl orthosilicate</td>
</tr>
<tr>
<td>XET</td>
<td>xyloglucan endotransglycosylase</td>
</tr>
<tr>
<td>XGO</td>
<td>xyloglucan oligosaccharide</td>
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REFERENCES


