

**Elucidating the pulp properties that influence the ability of enzymes
to facilitate the conversion of hardwood Kraft pulp to dissolving-
grade pulps**

by

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Abstract

Dissolving pulp is characterized by its high cellulose/low hemicellulose content, minor amounts of residual lignin/extractives, high brightness and a uniform molecular weight distribution. Dissolving pulp can be produced through acid sulfite cooking or alkaline Kraft cooking. However, due to issues with chemical recovery and pollution, the predominant pulping process has globally shifted to the Kraft process. Kraft pulps retain hemicellulose and high molecular weight cellulose, which are undesirable for dissolving pulps. Therefore, steps such as prehydrolysis (PHK) and cold caustic extraction (CCE) aimed at removing hemicellulose and decreasing cellulose molecular weight are typically employed. However, these processes are chemically intensive, non-specific and pose operational challenges for mills.

The use of enzymes (hemicellulases and cellulases) is one potential alternative to chemical methods of facilitating mill conversion due to the high specificity of enzymes and their ability to function under more benign conditions. Initially, xylanase and oxalic acid treatments were assessed for their potential to convert Kraft-to-dissolving pulp. It was apparent that the accessibility of hemicellulose and cellulose to chemical or enzymatic reagents was critical. Compared to oxalic acid, enzymes were more specific in removing hemicellulose while boosting cellulose reactivity. Model substrates, varying in their hemicellulose accessibility and cellulose properties, were used to assess the influence of various pulp characteristics on enzymatic pulp modification. The influence of pulp characteristics imparted by PHK and CCE on the ease of enzymatic modification was also assessed. It appeared that CCE negatively impacted the accessibility of hemicellulose due to the solubilisation of low molecular weight carbohydrates fragments which acted as “spacers” between cellulose microfibrils, preventing fibril aggregation.

Lowering the acidity of the prehydrolysis or the alkalinity of Kraft pulping conditions increased the ease of enzymatic removal of the hemicellulose, presumably by increasing hemicellulose accessibility. Separating the fibres into various size fractions indicated that the shorter fibres within the Kraft pulp were more susceptible to enzymatic modification, likely due to their increased porosity. It was apparent that Kraft pulping conditions played a significant role in governing enzyme accessibility to the various pulp carbohydrates and thus the potential of using enzymes to enhance dissolving pulp production and properties.

Preface

List of publications

- Dou X, Chandra R, Saddler JN (2016). The influence of hemicellulose accessibility when converting kraft to dissolving grade pulp using enzymes. *In preparation*.
- Dou X, Chandra R, Saddler JN (2016). The influence of prehydrolysis and cold caustic extraction on the purity, accessibility and reactivity of dissolving-grade pulp. *In preparation*.
- Dou X, Chandra R, Choi L, Saddler JN (2016). Upgrading hardwood Kraft pulps to dissolving-grade pulps: Importance of fibre size for pulp purity, accessibility and reactivity. *In preparation*.
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For all the publications, Xiaoli Dou, Jack Saddler and Richard Chandra contributed to the planning of the experimental work, interpretation of the results and drafting of the manuscripts. Xiaoli Dou carried out most of the laboratory work.

Table of contents

Abstract.....	ii
Preface.....	iv
Table of contents.....	v
List of tables.....	ix
List of figures.....	xii
List of abbreviations.....	xviii
Acknowledgements.....	xxi
Dedication.....	xxii
1. Introduction.....	1
1.1 Background about dissolving pulp.....	1
1.2 Approaches to converting Kraft pulps to dissolving grade pulps.....	6
1.2.1 Traditional routes to producing dissolving pulp via the acid sulfite cooking process... 6	6
1.2.2 Kraft cooking process.....	8
1.2.3 Other potential prehydrolysis/pulping methods.....	16
1.3 Cellulose and cellulose derivatives.....	20
1.3.1 A brief introduction on the structure of cellulose.....	20
1.3.2 Pore structure and surface morphology of cellulose.....	23
1.3.3 Reactivity and accessibility of cellulose.....	28
1.4 Cellulose derivatives.....	33
1.4.1 Cellulose xanthate.....	33
1.4.2 Cellulose nitrate.....	35
1.4.3 Cellulose acetate.....	36
1.5 Dissolving pulp properties.....	37
1.5.1 The hemicellulose content.....	39

1.5.2 Alkaline solubility (S10/S18)	40
1.5.3 Intrinsic viscosity (DP)	41
1.6 Enzymes that might facilitate the conversion of Kraft to dissolving pulps	43
1.6.1 Introduction to xylanases	43
1.6.2 Enzymes applications in traditional pulp and paper industry	45
1.6.3 Enzymes applications in dissolving-grade pulp.....	47
1.7 Research approach	50
2. Materials and methods	56
2.1 Cellulosic pulps.....	56
2.2 Enzymes.....	56
2.2.1 Commercial enzyme preparations.....	56
2.2.2 Commercial enzyme activity measurement	56
2.2.3 Protein content quantified by modified ninhydrin assay	58
2.3 Model substrates	59
2.4 Prehydrolysis and Kraft cooking	60
2.4.1 Prehydrolysis.....	60
2.4.2 Kraft cooking	60
2.5 Chemical composition analysis.....	61
2.5.1 Chemical compositional analysis on pulps	61
2.5.2 Chemical composition analysis prehydrolysis liquor/enzymatic hydrolysate	61
2.6 Cold caustic extraction (CCE)	62
2.7 Chlorite delignification	62
2.8 Enzymatic/chemical (acid) hydrolysis	62
2.9 Pulp properties analysis	63
2.9.1 Intrinsic viscosity	63

2.9.2 Alkaline solubility S10/S18	64
2.9.3 Water retention value	65
2.9.4 Simons' staining	66
2.9.5 Crystallinity index.....	66
2.9.6 Fock's reactivity measurement	67
2.10 Fibre quality analyzer	68
2.11 Carbohydrate Binding Module (CBM) adsorption assay	69
2.12 Nitrogen adsorption	69
2.13 Solute exclusion technique	69
2.14 Solid state CP/MAS ¹³ C-NMR measurements	71
2.15 Pulp fractionation.....	71
2.16 Paper strength test.....	72
3. Results and discussion	73
3.1 Influence of chemical and enzymatic modification on the conversion of traditional Kraft pulp to dissolving pulp.....	73
3.1.1 Background.....	73
3.1.2 Results and discussion	76
3.1.3 Conclusions.....	91
3.2 Exploring the role of hemicellulolytic and cellulose modifying enzymes on model substrates.....	92
3.2.1 Background.....	92
3.2.2 Results and discussion	96
3.2.3 Conclusions.....	112
3.3 The influence of prehydrolysis and cold caustic extraction on the purity, accessibility and reactivity of dissolving-grade pulp	113
3.3.1 Background.....	113

3.3.2 Results and discussion	117
3.3.3 Conclusion:	135
3.4 Assessing the influence of varying prehydrolysis and pulping conditions on the action of enzymatic hydrolysis and the removal of hemicellulose on increasing cellulose reactivity ..	136
3.4.1 Background	136
3.4.2 Results and discussion	140
3.4.3 Conclusions.....	159
3.5 Upgrading hardwood Kraft pulps to dissolving-grade pulps: The importance of fibre size for pulp purity, accessibility and reactivity.....	160
3.5.1 Background.....	160
3.5.2 Results and discussion	163
3.5.3 Conclusions.....	182
4. Conclusions and future work	183
4.1 Conclusions.....	183
4.2 Future work.....	187
4.2.1 Improvements and proper quantification of the reactivity of cellulose hydroxyl groups in dissolving pulps	187
4.2.2 Employing steam explosion with acid catalyst to replace prehydrolysis step to produce dissolving pulp	188
4.2.3 The influence of mechanical refining on xylan removal by xylanase and cellulose reactivity improvement.....	189
References.....	190

List of tables

Table 1: Composition and characteristics of paper, viscose and cellulose acetate type pulps (Sjöström, 1981; Engström et al., 2006; Köpcke et al., 2010).....	2
Table 2: Derivatives and end-use products from dissolving-grade pulps (Hielt, 1985; Hinck et al. 1985; Floe, 2011).....	3
Table 3: Intra- and intermolecular hydrogen bonds of Cellulose I and Cellulose II (Sjöström, 1981; Klemm et al., 2004)	22
Table 4: Commercial grade of cellulose acetate (Ranby and Rydholm, 1956)	37
Table 5: Typical specifications for dissolving-grade pulp (Kaur et al., 2016)	38
Table 6: Characteristics of the different treated pulps. KP: Kraft pulp. DP: Dissolving pulp. O: Oxalic acid (0.057g/g) treated Kraft pulp. O-CCE: 7% sodium hydroxide extracted O. X: Xylanase (500BXU/g) treated Kraft pulp. X-CCE: 7% sodium hydroxide extracted X. X-CCE-EG: EG (45ECU/g) treated X-CCE. CCE: 7% sodium hydroxide extracted Kraft pulp	83
Table 7: Chemical composition (%) of various “model” xylan substrates.....	98
Table 8: Protein content (mg/ml), specific activities (U/ml), and xylanase activity on birch wood and oat spelt xylans (U/mg) results of Multifect and HTec xylanases. Protein content and specific activities were applied on commercial xylanases preparations. Activity on birch wood and oat spelt xylans were by purified xylanase.....	101
Table 9: Influence of pulp morphologies on cellulose reactivity after mechanical refining. Refining was conducted on CCE treated Kraft pulp. Fibril/fibre swelling and ASA were obtained by solute exclusion technique on wet samples, and surface area was based on BET analysis of nitrogen absorption isotherm. ASA: accessible surface area; DsP: dissolving-grade pulp.....	111
Table 10: Macroscopic properties of differently treated pulps. KP: Kraft pulp. DsP: Dissolving pulp made from PHK process. CCE KP: 9 % sodium hydroxide extracted Kraft pulp. ASA: Accessible surface area	120
Table 11: Fibril size and fibril aggregate size of different treated pulps. Quantifications were made by spectral fitting of the cellulose C ₄ region of the CP/MAS ¹³ C-NMR spectra. KP:	

Kraft pulp. DsP: Dissolving pulp. 4.5/6.5/9% CCE KP: 4.5/6.5/9% sodium hydroxide extracted Kraft pulp	124
Table 12: Curls and kinks results on different treated pulps	128
Table 13: Various post-treatments on KP obtained from CCE process to enhance end product properties. Steam explosion: 170°C, 5 min; Acid: 0.4% sulfuric acid hydrolysis, 5% pulp consistency, 121°C, 1 hour; PFI: 20K revolutions PFI refining; EG: 450 ECU/g Fibrecare R hydrolysis, 5% pulp consistency, 50°C, 2 hours; Curl: Kitchenaid stand mixer, 80% pulp consistency, 30 minutes. ASA: Accessible surface area.....	131
Table 14: Sugar analysis of prehydrolysis liquors from different conditions (based on wood, %). Water PHK was the autocatalyzed prehydrolysis. Acid PHK was the 0.4% sulfuric catalyzed prehydrolysis.....	143
Table 15: Chemical composition and yield analysis of pulps made from different prehydrolysis process after going through different chemical/enzymatic treatments. Water PHK was the autocatalyzed prehydrolysis. Acid PHK was the 0.4% sulfuric acid catalyzed prehydrolysis. Multifect/HTec xylanases used in this work were the commercial xylanase preparations.	145
Table 16: Chemical composition and yield analysis on pulps after going through different chemical/enzymatic treatments. Low/Med/High Alk, Kraft cooking process with low (16% NaOH), medium (20% NaOH) and high (24% NaOH) alkalinity. Multifect/HTec xylanases used in this work were the commercial xylanase preparations. Delignification was a 3-step chlorite delignification to remove most of the lignin from the pulps.	148
Table 17: Chemical composition of isolated fibre fractions from hardwood Kraft pulp. KP: Kraft pulp. Commercial DsP: Commercial dissolving pulp. Unbleached R48/100/200: Unbleached fibre fractions retained on 48/100/200 meshes of Bauer-Mcnett fibre classifier. Upgraded R48/100/200: Unbleached fibre fractions went through all of the treatments (bleaching, xylanase, CCE, and endoglucanase treatment) resulting in dissolving-grade pulp fractions. Mass ratio was the quantity of different unbleached fractions after fractionation. There was about 7.2% total fines loss during the fractionation process.....	165
Table 18: Fibre dimensions, fines content, coarseness and vessel results measured by FQA. KP: Kraft pulp. Commercial DsP: Commercial dissolving pulp. Upgraded R48/100/200: Fibres retained on 48/100/200 meshes of Bauer-Mcnett fibre classifier, they were all dissolving-grade pulps upgraded from the different unbleached Kraft pulp fractions. Fines were defied	

as fibre length in between of 0.07mm to 0.20mm. Fibre length was the length weighted average fibre length. Vessel: The ranges for of fibre length and fibre width of vessel measured in this study were 0.1-1.5mm and 80-400 μm 168

Table 19: Impact of short fibre fraction on the mechanical strength (tensile and tear index) of paper. There was an around 10% decrease in tensile index due to the removal of short fibre fraction. 181

List of figures

Figure 1: Sulfonation of lignin and substituted structures containing alpha-carbonyl groups (Sjöström, 1981).	7
Figure 2: Cleavage of the phenolic b-O-aryl-ether bond during Kraft pulping (Gierer, 1970).....	9
Figure 3: A) Primary condensation; B) Secondary condensation; C) Coupling of phenolate to formaldehyde during Kraft pulping (Gierer, 1970; 1980)	10
Figure 4: Peeling mechanism during the Kraft cooking process. The peeling reaction basically unzips the carbohydrates by removing terminal sugars one at a time. The reaction takes place from the reducing end of the glucose molecules (Fengel and Wegener, 1983)	12
Figure 5: The “stopping mechanism” during Kraft cooking process (Fengel and Wegener, 1983)	13
Figure 6: The main reactions involved in the viscose process. The first step, mercerization, is the reaction of the cellulose with sodium hydroxide, forming alkali cellulose. Step Two is pre-ripening, during which the oxygen from the air participated in the oxidative hydrolysis to reduce the intrinsic viscosity of cellulose. The xanthation step generates cellulose xanthate from alkali cellulose, which dissolves the cellulose pulp in alkali. This is followed by a ripening step during which the xanthation groups redistribute to form a more thermodynamically stable structure. The last regeneration step happed within an acid bath and the xanthation groups are removed (Schlotter, 1988).	34
Figure 7: Molecular weight distribution curve of one dissolving pulp sample and the theoretical fraction of alkaline solubility (Strunk, 2012).....	42
Figure 8: SDS-PAGE of commercial enzymes done by one of my colleagues Jinguang Hu: Fibrecare R: Fungal GH10 endo-glucanase (lane 1); 2: Multifect: Fungal GH11 endo-xylanase (lane 2); 3: Htec: Fungal GH10 endo-xylanase (lane 3); 4: Pulpzyme HC 2500: bacterial GH11 endo-xylanase (lane 4) (Hu, 2014)	58
Figure 9: Retaining mechanism for the cooperative action of the two carboxylic acid groups in the active site of cellulases. Oxalic acid has the same two carboxylic acid groups and could mimic the enzymes. One carboxyl group acts as a proton donor and the other carboxyl group could function as a nucleophile to mimic the retaining hydrolysis mechanism of cellulase (McCarte et al., 1994; Kayser et al., 2013).....	75

Figure 10: Impact of oxalic acid or xylanase treatment on the hemicellulose content of commercial Kraft pulp. Oxalic acid treatment was performed at a pulp solids loading of 5% and a temperature of 121°C in an autoclave for 1 hour using an oxalic acid loading of 0.054g/g. Xylanase treatment was performed at pulp consistency 5% at a temperature 60°C, 2 hours with a xylanase loading of 500 BXU. KP: Kraft pulp. DsP: Commercial dissolving-grade pulp; O-KP: oxalic acid treated Kraft pulp; X-KP: xylanase treated Kraft pulp. 78

Figure 11: The impact of oxalic acid loading during autoclave treatment on the residual hemicellulose content of hardwood Kraft pulp. Oxalic acid treatment was performed at a pulp consistency of 5%, a temperature of 121°C in an autoclave for 1 hour. As specified, some pulps were subsequently subjected to an alkaline extraction at a pulp consistency of 7% using a 7% NaOH charge at room temperature for 30 min in an orbital shaker rotating at 150rpm. 79

Figure 12: Impact of xylanase loading on xylose release from Kraft pulp and dissolving pulp measured using the dinitrosalicylic acid method. Xylanase hydrolysis was conducted at 60°C, 5% pulp solid loading for 2 hours at a xylanase loading of 500 BXU/g pulp. 79

Figure 13: Impact of sodium hydroxide concentration on the residual hemicellulose content of kraft pulp. The alkaline extraction was conducted using a pulp consistency of 7% at room temperature, for 30 minutes. 81

Figure 14: Relationship between cellulose reactivity, intrinsic viscosity and accessibility (WRV) of treated pulps. Oxalic acid treatment was performed at a chemical loading of 0.057 g/g for 1 hour at 121°C using a 5% pulp consistency. Xylanase treatment was performed at an enzyme loading of 500 BXU/g for 2 hours at 60°C using a 5% pulp consistency. The alkaline extraction as performed at a NaOH concentration of 7% for 30min at room temperature using a 7% pulp consistency. The endoglucanase treatment (EG) was performed at an enzyme loading of 45 ECU/g for 2 hours at 50°C using a 5% pulp consistency. KP: Kraft pulp. DsP: Dissolving pulp. O: Oxalic acid treated Kraft pulp. O-CCE: 7% sodium hydroxide extracted O. X: Xylanase treated Kraft pulp. X-CCE: 7% sodium hydroxide extracted X. X-CCE-EG: EG treated X-CCE. CCE: 7% sodium hydroxide extracted Kraft pulp. 87

Figure 15: FE-SEM pictures of the different treated pulps (a) Kraft pulp (b) CCE: 7% NaOH extracted Kraft pulp (c) O-CCE: Oxalic acid (0.057g/g) treated Kraft pulp, followed by 7%

NaOH extraction (d) X-CCE-EG: 7% NaOH extracted xylanase (500 BXU/g) treated Kraft pulp, followed by EG (45ECU/g) treatment.....	91
Figure 16: SDS-PAGE of purified enzymes: GH11 endo-xylanase (lane 1), GH10 endo-xylanase (lane 2) and marker (lane M). Proteins were identified by LC-MS/MS. Proteins are named according to their glycoside hydrolase family (Hu, 2014)	100
Figure 17: Impact of pH/temperature to the activity of commercial xylanases (a) Multifect xylanase (b) HTec xylanase. Xylanase hydrolysis was applied on birch wood xylan (Lingfeng Long, 2016).....	101
Figure 18: Solubilization of birch/oat spelt xylans with Multifect and HTec commercial xylanase preparations. Xylanase hydrolysis was applied on ground birch wood, isolated birch wood xylan, Kraft pulp, isolated xylan from Kraft pulp and isolated oat spelt xylan. Xylanase hydrolysis was conducted under 50°C, 150rpm shaking, and 5% substrate consistency for 2 hours. 10 mg/g protein loading was used in this work.	103
Figure 19: Results of the spectral of the cellulose C ₄ -region recorded by solid state CP/MAS ¹³ C NMR on Avicel microcrystalline cellulose and PASC (78% phosphoric acid swollen cellulose). Blue line was on Avicel, and red line was on the PASC. Phosphoric acid treatment was conducted under very low temperature (~0°C) according to Mélanie Hall and Andreas S. Bommarius (2010).....	106
Figure 20: Effect of phosphoric acid concentration on the crystallinity index of Avicel microcrystalline cellulose. The crystallinity index was determined by solid state CP/MAS ¹³ C NMR.	107
Figure 21: Influence of crystallinity index on Fock's reactivity of cellulose. The crystallinity index was determined by solid state CP/MAS ¹³ C NMR.	109
Figure 22: Schemes about cellulose microfibril aggregation after alkaline extraction	115
Figure 23: Residual hemicellulose content and Fock's reactivity of different alkaline extracted pulps. KP: Kraft pulp (Dried). 5/7/8/9% CCE: 5%/7%/8%/9% NaOH extracted Kraft pulp. DsP: Commercial dissolving pulp obtained from prehydrolysis process (undried).	118
Figure 24: CP/MAS ¹³ C NMR spectra of different treated pulps. (A) Undried dissolving pulp obtained by prehydrolysis process; (B) Kraft pulp; (C) 5% NaOH extracted KP; (D) 7% NaOH extracted KP; (E) 9% NaOH extracted KP.....	121

Figure 25: Results of the spectral fitting of the cellulose C₄-region recorded on dissolving pulp made using the prehydrolysis Kraft process. The black lines represent the experimental spectra. The fitted lines are shown in blue color. 124

Figure 26: Fock's reactivity of different post-treated dissolving pulps..... 129

Figure 27: BJH cumulative pore volume/adsorption surface area of Kraft pulp (KP), 7% NaOH extracted Kraft pulp (CCE KP) and PFI mechanical refined CCE KP (PFI CCE KP). The BET results showed the total pore volume and accessible surface area remained similar after alkaline extraction on Kraft pulp, but after PFI mechanical refining, there was a more than 50% and 60% improvement in total volume and accessible surface area respectively. 132

Figure 28: FE-SEM micrographs showing the microscopic structure of KP (Kraft pulp), CCE KP (9% NaOH extracted Kraft pulp), PFI (mechanical refined CCE KP) and DsP (Dissolving pulp obtained from prehydrolysis process)..... 134

Figure 29: Flow chart of the impact of varying prehydrolysis on the characteristics of substrates such as the accessibility of hemicellulose to enzymes or the reactivity of cellulose to derivatization reagents. Sulfuric acid, autocatalysis and without prehydrolysis were conducted to generate pulps varying in their different characteristics such as the accessibility of hemicellulose and cellulose molecular weight with subsequent Kraft pulping. Xylanase hydrolysis (Multifect and HTec commercial xylanase preparations) was employed before and after delignification steps. 138

Figure 30: Flow chart of the impact of varying Kraft cooking conditions on the characteristics of substrates such as the accessibility of hemicellulose to enzymes or the reactivity of cellulose to derivatization reagents. 24%, 20% and 16% of NaOH were employed to generate pulps varying in their different characteristics such as the accessibility of hemicellulose and cellulose molecular weight. Multifect commercial xylanase preparation of which the primary xylanase was from GH10, and HTec commercial xylanase preparation of which the primary xylanase was from GH11 were employed before/after the delignification steps, and after CCE treatment. 140

Figure 31: Fibre length, width and fines analysis by Fibre Quality Analyzer (FQA) on different pulps. Water PHK was the autocatalyzed prehydrolysis. Acid PHK was the 0.4% sulfuric acid catalyzed prehydrolysis. Low/Med/High Alk: Pulps obtained from Kraft cooking process with low (16% NaOH), medium (20% NaOH) and high (24% NaOH) alkalinity. All

the measurements were employed on the pulps that had been gone through all the conversion processes and became dissolving-grade pulps. Fines are defined as fibre length in between of 0.07mm to 0.20mm. L_w : weight-weighted average fibre length. L_n : length-weighted average fibre length..... 151

Figure 32: Accessibility of pulps varying in their prehydrolysis and pulping conditions to water (a) and direct blue dye (b). Water PHK was the autocatalyzed prehydrolysis. Acid PHK was the 0.4% sulfuric acid catalyzed prehydrolysis. Low/Med/High Alk: Pulps obtained from Kraft cooking process with low (16% NaOH), medium (20% NaOH) and high (24% NaOH) alkalinity. All the measurements were employed on the pulps that had been gone through all the conversion processes and became dissolving-grade pulps. 153

Figure 33: Xylan removal from different substrates by Multifect and HTec xylanases. Xylanase hydrolysis was conducted according to the different additional points shown in Figure 29 and Figure 30. Water PHK was the autocatalyzed prehydrolysis. Acid PHK was the 0.4% sulfuric acid catalyzed prehydrolysis. Low/Med/High Alk: Pulps obtained from Kraft cooking process with low (16% NaOH), medium (20% NaOH) and high (24% NaOH) alkalinity. Multifect/HTec xylanases used in this work were the commercial xylanase preparations..... 155

Figure 34: Intrinsic viscosity (a) and Fock's reactivity (b) of different pulps. Water PHK was the autocatalyzed prehydrolysis. Acid PHK was the 0.4% sulfuric acid catalyzed prehydrolysis. Low/Med/High Alk: Pulps obtained from Kraft cooking process with low (16% NaOH), medium (20% NaOH) and high (24% NaOH) alkalinity. All the measurements were employed on the pulps that had undergone through all the conversion process and became dissolving-grade pulps. 157

Figure 35: Relationship between cellulose reactivity and intrinsic viscosity. Pulp reactivity is inversely proportional to intrinsic viscosity, or the average cellulose molecular weight. .. 159

Figure 36: Schematic processing of various applications of different fractions from unbleached hardwood Kraft pulps 164

Figure 37: Porosity (a) and accessible surface area (b) of fibre fractions measured by solute exclusion technique. KP: Kraft pulp. DsP: Commercial dissolving pulp obtained from PHK process. R48/100/200: Fibres retained on 48/100/200-mesh screens of Bauer-Mcnett fibre classifier. All the measurements were on the pulp fractions that had gone through all the

conversion process to dissolving-grade pulps. Solute exclusion was used to investigated wet pulps asit is an accurate method to analyze the pore structure of pulps.	170
Figure 38: Accessibility of pulp fractions to water (a) and direct blue dye (b). KP: Kraft pulp. DsP: Commercial dissolving pulp made from PHK process. R48/100/200: Fibres retained on 48/100/200-mesh screens of Bauer-Mcnett fibre classifier. WRV: Water retention value. All the measurements were on the pulp fractions that had been gone through all the conversion process to dissolving-grade pulps.....	171
Figure 39: Xylanase hydrolysis on unbleached short fraction pulp; (A) before bleaching (B) after bleaching.....	173
Figure 40: Xylan hydrolysis by xylanases from different fibre fractions. KP: Kraft pulp. R48/100/200: Fibres retained on 48/100/200-mesh screens of Bauer-Mcnett fibre classifier. Multifect and HTec xylanases used in this work were the commercial xylanase preparations. Xylanase hydrolysis was conducted under 50°C, 5% solid loading in sodium acetate buffer (50mM, pH 4.8), and the protein loading was 10mg/g.	174
Figure 41: Degree of hemicellulose removal during CCE. CCE was conducted at 7% pulp consistency, 7% NaOH (w/v), room temperature for 30 minutes. CCE: Cold Caustic Extraction. R48/100/200: Fibres retained on 48/100/200-mesh screens of Bauer-Mcnett fibre classifier. CCE was conducted on the pulps that went through the delignification and xylanase hydrolysis steps.....	176
Figure 42: Fock’s reactivity of different fibre fractions. KP: Kraft pulp. DsP: Commercial dissolving pulp. R48/100/200: Fibres retained on 48/100/200-mesh screens of Bauer-Mcnett fibre classifier. Endoglucanase was applied after CCE treatment.	178
Figure 43: Relationship between cellulose intrinsic viscosity and reactivity (Fock’s method). KP: Kraft pulp. DsP: Commercial dissolving pulp. R48/100/200: Fibres retained on 48/100/200-mesh screens of Bauer-Mcnett fibre classifier. Endoglucanase was applied after CCE treatment.	179
Figure 44: Microscopy of different pulp fractions. KP: Kraft pulp. DsP: Commercial dissolving pulp. R48/100/200: Fibres retained on 48/100/200-mesh screens of Bauer-Mcnett fibre classifier.	180
Figure 45: Mechanical refining to enhance xylan removal by xylanase	189

List of abbreviations

ASA	accessible surface area
BET	Brunauer–Emmett–Teller
BXU	birch xylan unit
CBM	carbohydrate-binding module
CCE	cold caustic extraction
CED	cupriethylenediamine
DNS	3,5-dinitrosalicylic acid
DP	degree of polymerization
DS	degree of substitution
DsP	dissolving-grade pulp
DW	dry weight
ECU	endo-1, 4- β -glucanase unit
EG	endoglucanase
FQA	fibre quality analyzer
FSP	fibre saturation point
GH	glycoside hydrolase
GPC	gel permeation chromatography
HexA	hexenuronic acid

HPLC	high performance liquid chromatography
IU	international unit
kDa	kilodalton
KP	Kraft pulp
LCC	lignin-carbohydrate complex
L_n	number averaged fibre length
L_w	weight averaged fibre length
Me-GlucA	4-O-Methyl-D-glucuronic acid
Mn	number average molecular weight
Mw	weight average molecular weight
NBSK	northern bleached softwood kraft
NMMO	N-Methylmorpholine N-oxide
NMR	nuclear magnetic resonance
PASC	phosphoric acid swollen cellulose
PDI	polydispersity
PHK	prehydrolysis
<i>PI</i>	isoelectric point
PFI	laboratory Pulp Refiner
R48	fibres retained on the 48-mesh screen

S10/S18	alkaline solubility in 10/18% NaOH
SDS	sodium dodecyl sulfate
SE	steam explosion
PAGE	polyacrylamide gel electrophoresis
WRV	water retention value
XRD	X-ray diffraction

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Dedication

This thesis is dedicated to my beloved husband Yong Tang, who always unconditionally loves me and whose good examples have taught me to work hard for what I aspire to achieve and be a person of righteousness. Thanks for all of his constant encouragement and support during the challenges of my four-year PhD study. I also want to dedicate this thesis to our coming baby Tyson Tang. Carrying such an amazing baby in my belly during the past seven months when I was drafting the thesis is one of the happiest things I have ever experienced in my life.

1. Introduction

1.1 Background about dissolving pulp

Dissolving pulp is generally characterized by its high cellulose/low hemicellulose content, <1% residual lignin, extractives and minerals, high brightness and a uniform molecular weight distribution (Elg Christoffersson, 2004; Sixta, 2006) (Table 1). Dissolving pulp and cotton linters are viewed as the most important raw materials for the manufacture of viscose rayon and cellulose derivatives, such as cellulose esters and cellulose ethers (Strunk, 2012; Engström, et al., 2006). Unlike mechanical pulp which has become a depressed sector in the pulp and paper industry, resulting in some mills shutting down, dissolving-grade pulp production is experiencing an expanded market (Paper 360°, 2011). One of the reasons is due to the increased demand by nations experiencing increased prosperity such as China and India and a continued shortfall in cotton supply to the textile industry. This increased demand has been the main impetus for the recent surge in the worldwide production of dissolving pulp. Over the past three years, worldwide cotton production has decreased by 7% while over the next five years' global cotton consumption is projected to increase by 4% (Paper 360°, 2011). Due to these factors, the consumption of dissolving pulp has risen, approaching 6 million tonnes per year. Approximately 70% of this dissolving pulp has been used to produce viscose, a precursor for textile products such as rayon that can substitute for cotton (Table 2). In Canada alone, compared to the same period in 2011, it was reported that the volume of dissolving pulp exports rose 43% in the first 10 months of 2011 (<https://cfs.nrcan.gc.ca/selective-cuttings/4>). China was the major recipient of increased Canadian production, accounting for 49% of total dissolving pulp exports, followed by India (Paper 360°, 2011). Over this period, Canada's export value of dissolving pulp totaled

\$645 million, equivalent to 21% of total Northern Bleached Softwood Kraft (NBSK) exports. Over the long term, a conservative estimate suggests that the global demand for dissolving pulp could grow at around 6-7% per year, to feed the rising global textile demand. Therefore, an increasing number of pulp mills that used to make Kraft pulp have modified their traditional methods of making paper-grade pulp to now produce dissolving pulp (Paper 360°, 2011).

Table 1: Composition and characteristics of paper, viscose and cellulose acetate type pulps (Sjöström, 1981; Engström et al., 2006; Köpcke et al., 2010)

Parameters	Paper product	Viscose product	Cellulose acetate product
Hemicellulose %	11.1	3.0	2.5
Alkaline solubility R10 %	87.1	89.0	93.5
Viscosity ml/g	1000	500	820
Polydispersity Mw/Mn	29.7	4.5	4.0
Ash %	-	0.1	0.1
Acetone extractive %	0.3	0.2	0.05
Brightness	90	93	94

Table 2: Derivatives and end-use products from dissolving-grade pulps (Hiett, 1985; Hinck et al. 1985; Floe, 2011)

Derivatives	Applications	End product %
<i>Cellulosic fibres viscose rayon staple</i>		
Regular	Apparel fabric	42
high-wet modulus	Special fabric for apparel, furnishings	3
<i>Viscose rayon filament yarn</i>		
Regular tenacity	Apparel	10
High tenacity	Tire cord and belting, industrial uses	7
Acetate staple and tow	cigarette filters	8
Acetate filament yarn	Apparel, furnishing	7
<i>Others from viscose rayon</i>		
Cellophane	Packaging	7
Sponges, sausage casings	Packaging	1
Acetate plastics	Photographic films, sheets, moldings	1
Cellulose nitrates	Lacquers, film, explosives	8
Other cellulosic organic compounds	Additives in food, cosmetics	8
Special paper pulps	Filter, photographic papers	2

The traditional way to produce dissolving pulp is from upgrading sulfite cooking under acid conditions which remove hemicellulose and lignin while decreasing the molecular weight of cellulose (Sixta, 2006). However, due to issues with chemical recovery, environmental pollution and the inability to effectively cook some wood species during sulfite cooking, the predominant pulping process has globally shifted to the Kraft process. Several companies are currently exploring ways to convert their Kraft mills to produce dissolving pulp (Sixta et al., 2004; Elg Christoffersson, 2004). However, Kraft pulps retain a high residual hemicellulose content and high molecular weight cellulose, which are detrimental attributes for dissolving pulps. Therefore, aggressive chemical steps such as prehydrolysis prior to Kraft cooking (PHK) or cold caustic extraction (CCE) after pulping that aim to remove hemicellulose and tune cellulose molecular weight to promote pulp reactivity are often implemented (Wollboldt et al., 2010; Gehmayr and Sixta, 2011; 2012; Wan et al., 2010; Engström, et al., 2006; Köpcke et al., 2010). However, the process of converting Kraft pulps to dissolving pulps still experiences obstacles such as the inability of chemical reagents to specifically remove hemicellulose and the pulps poor reactivity with derivatization reagents due to the recalcitrant hydrogen bonds within the cellulose.

It has previously been suggested that the use of enzymes such as hemicellulases and cellulases is one attractive option to facilitate the conversion of Kraft pulps to dissolving pulps due to their specificity and ability to function at benign conditions compared to chemical processes (Engström et al., 2006; Köpcke et al., 2010; Gehmayr and Sixta, 2011; 2012; Östberg and Germgård, 2013; Östberg et al., 2012). Hemicellulases, such as xylanases, and cellulase monocomponents, such as endoglucanases (EG), have been successfully used to remove the residual hemicellulose and decrease dissolving pulp viscosity (Engström et al., 2006; Köpcke et al., 2010; Gehmayr and Sixta, 2011; 2012; Östberg et al., 2012). Xylanases have been added to

reduce hemicellulose content while endoglucanases have been added to provide a controlled decrease in cellulose molecular weight, thus increasing pulp reactivity during subsequent derivatization without increasing polydispersity (Östberg and Germgård, 2013). It has been shown that xylanase pretreatment of an unbleached oxygen delignified eucalyptus Kraft pulp combined with a 7% CCE could reach the similar level of a xylan removal as a CCE step employing 10% NaOH while also providing 10% higher pulp yield (Gehmayr et al., 2011). It has also been shown that xylanases combined with alkaline extraction can hydrolyze more xylan from birch wood derived commercial Kraft pulp than two stages of alkaline extraction. This implies that xylanases can cleave the hemicellulose and then enhance its subsequent alkaline extraction (Köpcke, 2010). However, hemicellulases added after the bleaching processes have shown limited success while the application of xylanases to reduce the chemical loading necessary to remove hemicellulose during a CCE treatment, has also been shown to compromise downstream reactivity of dissolving pulps. In several cases hydrolytic enzymes have been added to remove hemicellulose after an oxygen delignification step was applied to the Kraft pulp brown stock. This enzyme treatment has been shown to decrease the efficacy of hemicellulose removal since the “easily accessible” hemicellulose is removed or relocated (Kim and Paik, 2000). Overall it is apparent that the use of enzymes to modify dissolving pulps and Kraft pulps has shown mixed results (Rahkamo et al., 1998; Hakala et al., 2013; Schild and Sixta, 2011) and requires further investigation. This is a major focus of this thesis.

As described earlier, various chemo-enzymatic approaches can be employed to convert Kraft pulps to dissolving-grade pulps. It is evident that the application of different approaches results in pulps that vary in properties such as pore size, accessible surface area and microfibril aggregation, profoundly impacting cellulose accessibility and reactivity (Gehmayr and Sixta,

2011; 2012). In the following sections, more details about the chemical processes for converting Kraft pulps to dissolving pulps are discussed, with a focus on the impact of different treatments on the characteristics of the substrates such as chemical composition, cellulose accessibility and reactivity.

1.2 Approaches to converting Kraft pulps to dissolving grade pulps

1.2.1 Traditional routes to producing dissolving pulp via the acid sulfite cooking process

Sulfite pulping under acidic conditions is the traditional method used to produce dissolving pulp. The acid sulfite process simultaneously removes both lignin and hemicellulose from the cellulose and reduces the molecular weight of the cellulose (Engström et al., 2006). During acid sulfite pulping, the α -hydroxyl/ether groups are cleaved by the simultaneous formation of benzylium-ions (Sjöström, 1981). This reaction can take place regardless of whether the phenolic hydroxyls from the phenyl-propane units have been etherified or not. The cleavage of the α -aryl ether bonds results in the fragmentation of lignin during the acid sulfite pulping process (Figure 1) (Sjöström, 1981). The benzylium-ions are then sulfonated by reacting with the hydrated sulfur dioxide/bisulfite from the cooking liquor. The benzylium-ions formed from the 1, 2-diarylpropane structures can be easily converted to stilbenes by the elimination of hydrogen-ions at β -position. As a result, the γ -carbon atoms with high electrophilicity are sulfonated (Sjöström, 1981).

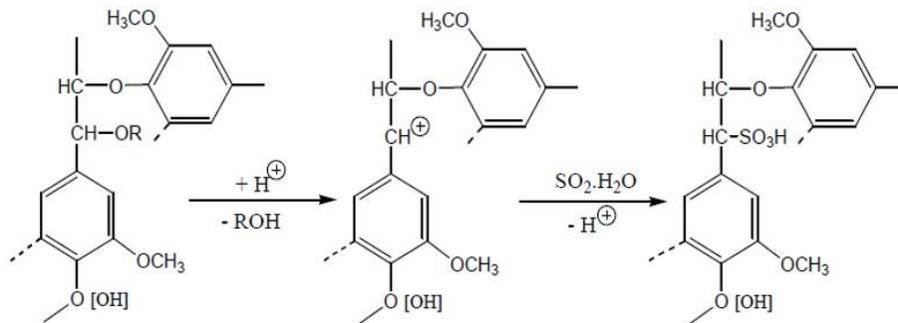


Figure 1: Sulfonation of lignin and substituted structures containing alpha-carbonyl groups (Sjöström, 1981).

With the removal of lignin during the acid sulfite cooking process, the concurrent depolymerization of cellulose and hemicellulose cannot be avoided due to the high susceptibility of the glycosidic bonds to acid attack. Since hemicellulose has an amorphous structure and a low DP, it can be hydrolyzed more easily than the complex and crystal cellulose during acid cooking (Sjöström, 1981). Typically, when hydrolysis has progressed, a high portion of hemicellulose will be solubilized in the cooking liquor (Sjöström, 1981). Although cellulose can also be cleaved during acid pulping, it has been reported that the losses in cellulose are not significant (Sjöström, 1981). To achieve a high cellulose content, dissolving pulps will have ideally been produced using an acid sulfite process that simultaneously removes both lignin and hemicellulose from the cellulose component (Engström et al., 2006). However, due to issues with pollution and chemical recovery, long cooking times, weaker fibres and the inability to effectively pulp some softwood species, the predominant pulping process has globally shifted towards the modified Kraft process. As mentioned earlier, the increasing demand for dissolving pulp has increased the number of pulp mills using the alkaline Kraft process (~80%), to convert from producing paper-grade Kraft pulp to dissolving pulp (Paper 360 by Tappi).

1.2.2 Kraft cooking process

Kraft pulping is generally performed in a digester with an alkaline solution composed of sodium hydroxide (NaOH) and sodium sulfide (Na₂S) at a pH in the range of 13-14, and this solution is termed the so-called “white liquor”. The digester with loaded wood chips and cooking chemicals is then heated to 160-170°C with the cooking generally lasting for approximately 2 hours. During the cooking process, the presence of hydrosulfide ions and hydroxyl ions can greatly facilitate delignification because of their strong nucleophilicity, resulting in the reaction with the α -carbon and β -carbon groups on the side chains of lignin, facilitating the final cleavage of β -O-4 linkages (Figure 2) (Gierer, 1970). Lignin condensations also occurs during the Kraft pulping process (Gierer, 1970; 1986; Sjöström, 1981). The primary condensation reactions occur when the quinone methides are formed by the elimination of the α -substituent. Secondary condensation occurs when quinone methides are formed after initial ether cleavage (Figure 3) (Gierer, 1970; 1986). These condensations result in the formation of C-C bonds (Figure 3), which are highly resistant structures to the subsequent pulping process, further decreasing the final delignification efficiency (Gierer, 1970; 1986).

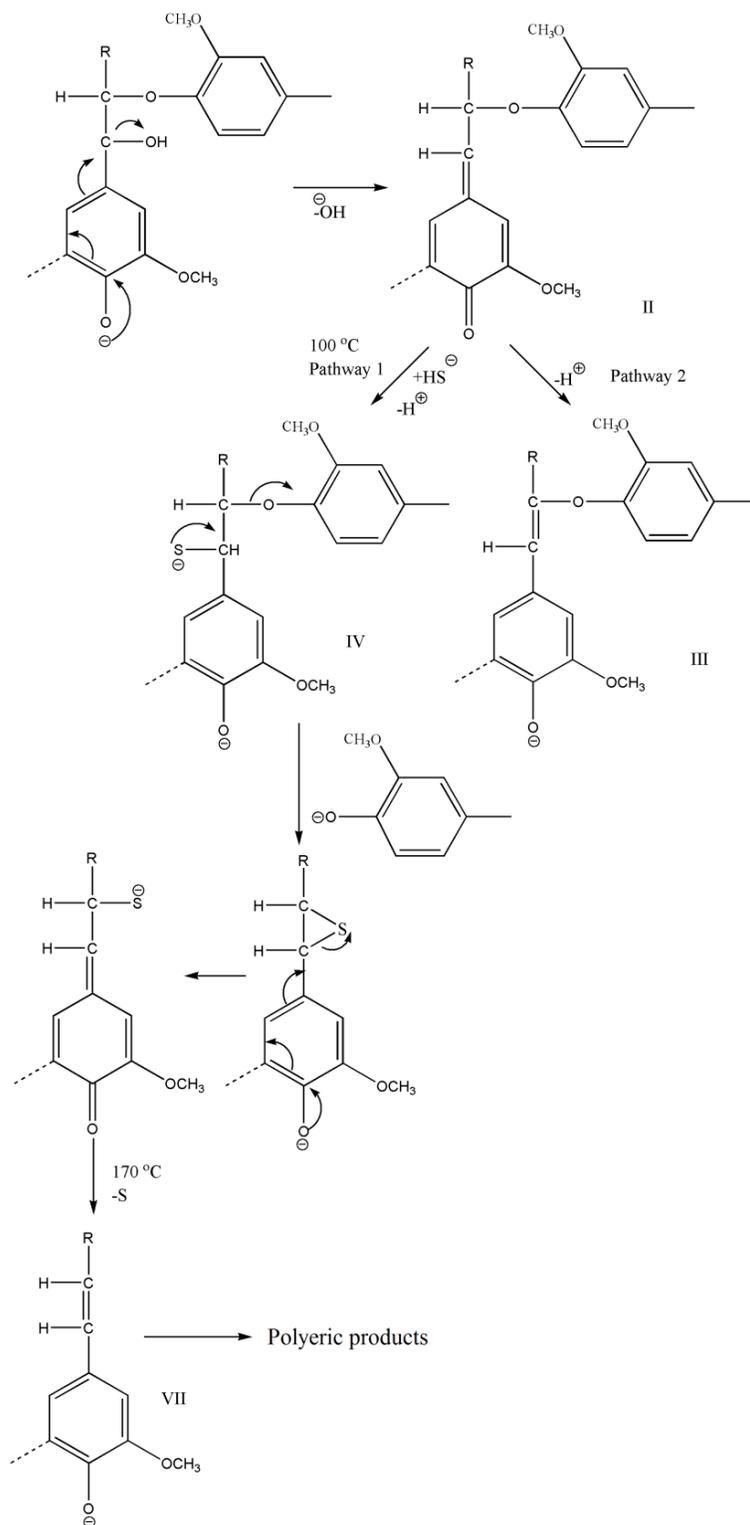


Figure 2: Cleavage of the phenolic b-O-aryl-ether bond during Kraft pulping (Gierer, 1970)

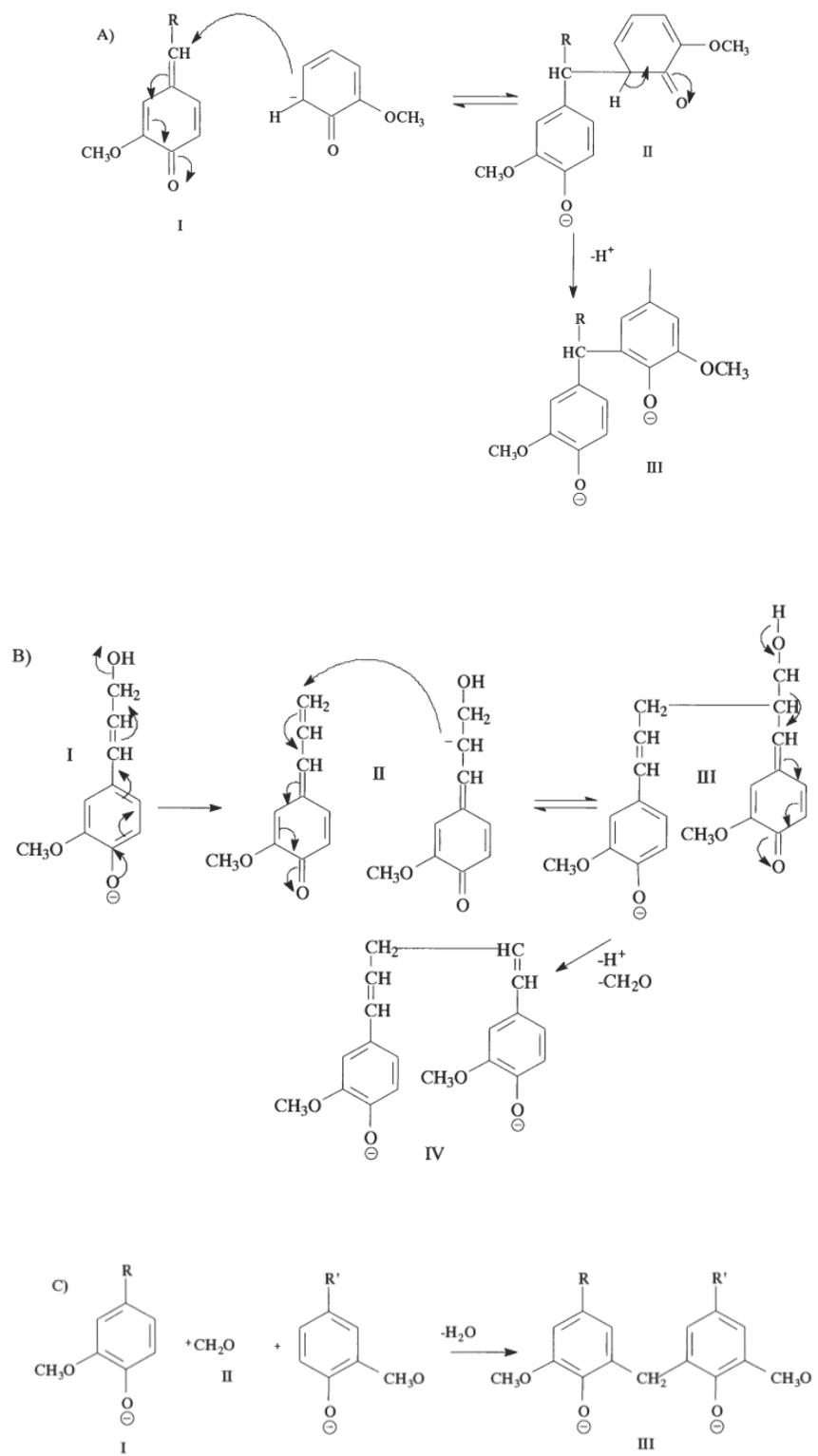


Figure 3: A) Primary condensation; B) Secondary condensation; C) Coupling of phenolate to formaldehyde during Kraft pulping (Gierer, 1970; 1980)

During delignification, alkaline degradation of the carbohydrates can also occur. These are referred to as “peeling” reactions, leading to a considerable loss in the final pulp yield. The peeling reactions start from the reducing ends of the polysaccharide chains (Figure 4) (Fengel and Wegener, 1983; Sjöström, 1981). As a result of alkaline hydrolysis occurring at high temperatures, new end groups are also formed, giving rise to additional carbohydrate degradation (secondary peeling) (Fengel and Wegener, 1983). This results in a loss in yield of cellulose and hemicellulose after the pulping process. Since hemicellulose is amorphous and have a lower molecular weight than cellulose, they undergo more extensive degradation during the Kraft pulping process (Sjöström, 1981). It has been reported that greater than 30% of the wood carbohydrates are lost during Kraft pulping (Sjöström, 1981). However, the peeling reactions are eventually interrupted, due to the end group stabilization (also called “stopping reactions”), during which the reducing end groups are converted to more stable carboxylic acid groups. The peeling reaction can then be stopped (Fengel and Wegener, 1983) (Figure 5).

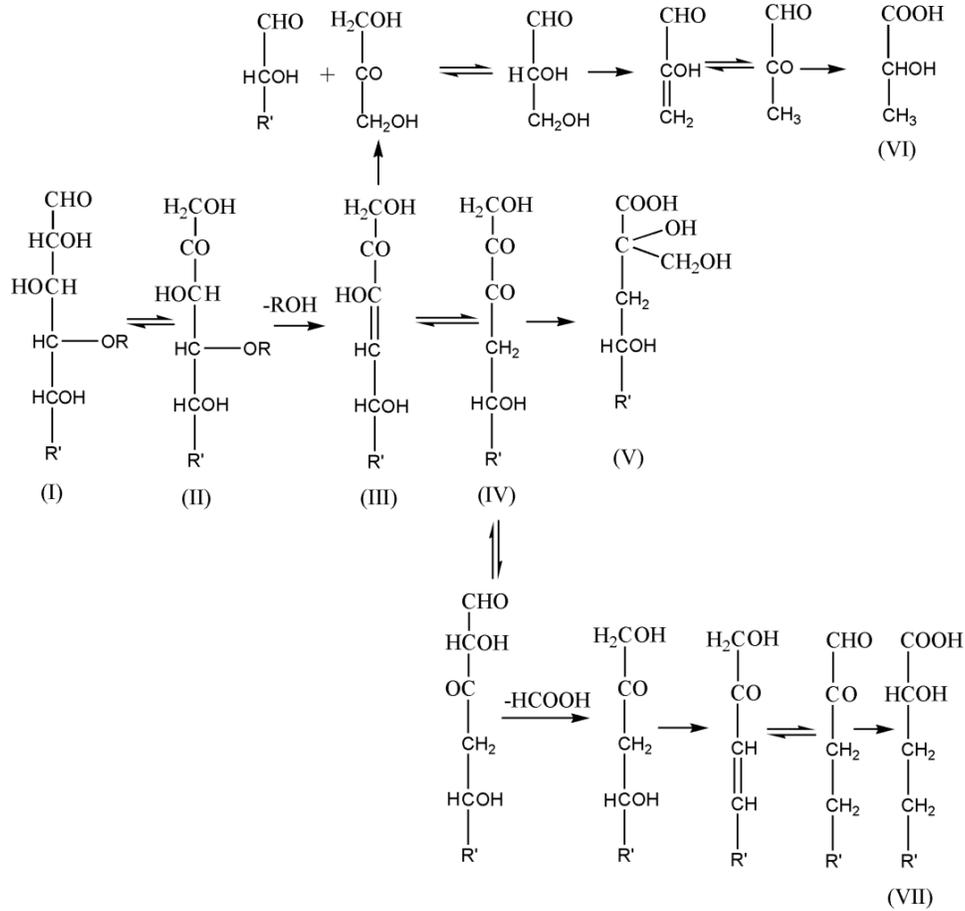


Figure 4: Peeling mechanism during the Kraft cooking process. The peeling reaction basically unzips the carbohydrates by removing terminal sugars one at a time. The reaction takes place from the reducing end of the glucose molecules (Fengel and Wegener, 1983)

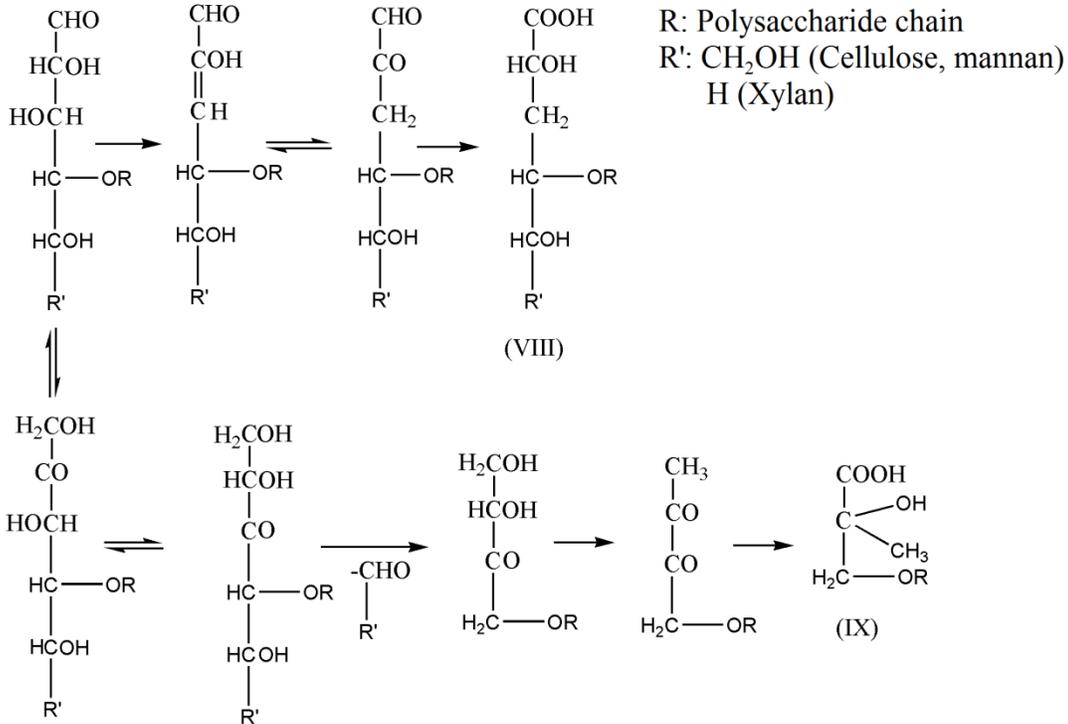


Figure 5: The “stopping mechanism” during Kraft cooking process (Fengel and Wegener, 1983)

Although a portion of the hemicellulose is degraded, the overall good retention of residual hemicellulose and the high molecular weight of the cellulose in the pulp have made Kraft pulping the method of choice for traditional pulp products. However, as mentioned earlier, some of the appealing attributes of Kraft pulp are problematic for dissolving pulp. This has resulted in significant challenges to the production of dissolving pulp using the Kraft pulping process. One of the main challenges is that the high hemicellulose content of Kraft pulps and the derivatization chemicals used for cellulose esterification and etherification result in inconsistencies in the final derivatized products (Strunk, 2012; Elg Christoffersson, 2004). The hemicellulose remaining after the pulping and bleaching process also has a high potential to form bonds and agglomerates between cellulose. This results in a decrease in the ease of filtering during the production of viscose and an increased in the particle content. These issues compromise end product qualities, such as fibre mechanical strength (Strunk, 2012). Another challenge is the poor cellulose

reactivity to derivatization chemicals of Kraft pulps (Strunk, 2012; Elg Christoffersson, 2004). In traditional Kraft pulping, in order to improve the mechanical strength of paper, the cellulose DP should be preserved. However, high cellulose DP in a dissolving pulp means it will be challenging to dissolve and show decreased reactivity during any subsequent derivatization reactions (Engström, et al., 2006; Köpcke et al., 2010; Granström, 2009). For the viscose process in particular, the unreacted fibres pose significant challenges regarding filterability after xanthation (Strunk, 2012). As described later in the thesis, these challenges were a major focus of the project. Possible solutions included reducing the high hemicellulose content of Kraft pulp, maximizing the reactivity of cellulose hydroxyl groups within the dissolving pulp and controlling the reduction in the degree of polymerization of the cellulose while limiting polydispersity.

In order to convert a conventional Kraft pulping process to produce dissolving grade pulp, there are many steps that must be implemented including, prehydrolysis Kraft pulping (PHK), cold caustic extraction (CCE) to reduce the hemicellulose content, acid washing to decrease the hexenuronic acid content and bleaching using a hypochlorite stage to reduce the cellulose's degree of polymerization (Köpcke et al., 2010; Wollboldt et al., 2010; Wan et al., 2010; Muhammad, 2012). Prehydrolysis involves cooking wood chips in water or dilute acid. During prehydrolysis, the high temperature employed (typically 160-180°C) liberates the acetyl groups that are attached to the hemicellulose backbone (Testova et al., 2014; Borrega et al., 2013; Borrega and Sixta, 2013). This lowers the pH to 2-3.5, promoting hemicellulose hydrolysis. It has been shown that, during the pre-hydrolysis step, more than 60% of the original hemicellulose could be solubilized from the starting chips (Testova et al., 2014; Borrega et al., 2013; Borrega and Sixta, 2013). In addition, the prehydrolysis step imparts increased permeability to the wood chips, facilitating the penetration of the alkaline pulping chemicals into the wood chips, thereby

accelerating delignification during subsequent Kraft pulping (Schild et al., 2010). The addition of a CCE step to the process further purifies cellulose by extracting the low molecular weight hemicellulose precipitated on the surface of fibres after the Kraft cooking (Gehmayr et al., 2011; 2012). However, the use of these chemical steps increases the cost of production and adds increased input to the Kraft recovery boiler. This in turn slows down the overall pulp production rates. For example, although the use of a prehydrolysis step can remove hemicellulose, it results in challenges for the pulping stage with regard to extractives accumulation and pH swings from the acidic pH utilized in prehydrolysis to the alkaline pH in Kraft pulping (Liu et al., 2011). Although the use of a CCE step also removes hemicellulose, the highly alkaline conditions employed in a CCE step can result in the formation of cellulose II. This can favor the realignment of cellulose to increase hydrogen bonding, resulting in the possible formation of cellulose fibril aggregates that exhibit lower reactivity (Gehmayr and Sixta, 2012; Köpcke et al., 2010; Schild and Sixta, 2011).

As mentioned earlier, another chemical step which can be utilized to convert Kraft to dissolving pulp is to employ a hypochlorite step to decrease the DP of the cellulose and improve cellulose reactivity (Köpcke et al., 2010; Wollboldt et al., 2010; Wan et al., 2010). However, as hypochlorite is a non-specific oxidizing agent, the decrease in cellulose DP can be accompanied by undesirable increases in cellulose polydispersity. As will be described in more details later, there have been several investigations which have assessed the ability of enzymes to modify pulp fibres with a goal of improving their potential for dissolving pulp applications.

1.2.3 Other potential prehydrolysis/pulping methods

1.2.3.1 Steam explosion

Unlike prehydrolysis, which requires high temperature, pressure and long cooking time, steam explosion is a relatively simple process that has been used to remove hemicellulose from wood chips, requiring relatively lower chemical and energy inputs (Taherzadeh and Karimi, 2008; Weil et al, 1998). Over the past few decades, steam pretreatment has received significant attention and it is the predominant pretreatment used at a commercial level (DuPont, DSM-Poet, BetaRenewables, etc.) to facilitate the enzymatic hydrolysis of lignocellulosic biomass to fermentable sugars (Taherzadeh and Karimi, 2008; Kumar et al., 2011; Chandra et al., 2016). During pretreatment, biomass is exposed to steam at high temperatures (170-210°C) and pressures (Weil et al, 1998). The high pressure is then released, during which the mechanical shearing force promotes the separation of fibres and increases the surface area of the biomass (Boehm, 1930). During the un-catalyzed steam pretreatment of hardwoods or agricultural residues, so called “autohydrolysis”, the inherent acetyl groups that decorate the backbones of the hemicellulose macromolecule provide acetic acid, thus “auto-catalyzing” the removal of hemicellulose (Chandra et al, 2007; Yang and Wyman, 2006; Kumar et al, 2009). In addition, under the high temperature and pressure, lignin can readily reach its glass transition temperature, migrate and then re-precipitate in the form of lignin droplets onto the surface of fibres during the final cooling stage (Shevchenki et al, 2001). Overall, the solubilisation of large amounts of hemicellulose and the formation of lignin droplets have been shown to aid in the exposure of cellulose, further enhancing the accessibility of cellulose to hydrolytic enzymes or other chemicals during the bioconversion or derivatization process (Shevchenki et al, 2001; Jeoh et al, 2007; Kabel et al, 2007). It has also been previously shown that, after a steam explosion, 90% of

the cellulose present in poplar chips could be hydrolyzed within 24 h (Grous et al, 1986). Compared with untreated poplar chips the glucan conversion was only 15% (Grous et al, 1986). It has also been reported that the lignocellulosic substrates become more porous after the removal of hemicellulose and lignin droplet formation, further promoting the enzymatic hydrolysis of cellulose (Shevchenki et al, 2001).

One of the attractive aspects of using the steam pretreatment process to potentially produce dissolving-grade pulps is that it allows the selective solubilisation of the hemicellulose component, especially when it is combined with an acidic catalyst such as sulfur dioxide or sulfuric acid. Steam pretreatment has also been shown to impart changes to the cellulose structure including a reduction in the molecular weight of the cellulose and a general increase in the accessibility of cellulose to downstream derivatization reagents (Chandra et al, 2007; Arantes and Saddler, 2011). It has also been reported that steam pretreatment can improve the accessibility of fully bleached softwood and hardwood paper pulps, as well as increasing the solubility of cellulose present in dissolving pulps treated in NaOH/urea/thiourea (Kihlman et al., 2012). Other work has also shown that, steam explosion at low temperature, improved the Fock's reactivity of an unbleached Kraft pulp but reduced its reactivity at high temperatures (Tikkanen, 2014). An increase in reactivity was noticed when adding an acid catalyst after the steam explosion of unbleached pulps. This work also showed that steam explosion of dissolving-grade pulps resulted in an overall increase in Fock's reactivity (Tikkanen, 2014). However, it has also been reported that the reduction in the degree of polymerization of cellulose that results from steam pretreatment occurs in an uncontrollable manner (Kihlman et al., 2012). Therefore, the potential improvements in reactivity and hemicellulose removal will likely come at the expense of increasing the polydispersity index (PDI) of cellulose (Kihlman et al, 2012).

1.2.3.2 Organosolv pulping

A wide range of organic solvents, such as methanol, ethanol or acetone, have been used to delignify lignocellulosic substrates. Organic chemicals were originally applied to try to separate wood into different components such as lignin and carbohydrates (Muurinen, 2000). Organosolv pulping processes involve treating wood chips with a mixture of water and organic solvents, sometimes with the addition of acid/base catalyst, at high temperatures and pressures, to remove the lignin/hemicellulose and fractionate the biomass (Kleinert and Tayenthal, 1931). In recent years it was found the properties the pulps obtained from organosolv pulping process could fulfill most of the requirements of Kraft pulps (Dahlmann and Schroeter, 1990), leading to a rapid development of various organosolv pulping processes.

During the organosolv pulping process, amounts of glycoside linkages, LCC bonds, and linkages in between of lignin units such as alpha-ether linkages and beta-ether linkages are cleaved, resulting in the removal of the majority of hemicellulose and lignin components (Muurinen, 2000). Therefore, a final cellulose-rich pulp feedstock can thus be obtained (McDonough, 1992; Sarkanen, 1990). Generally organosolv pulping can be carried out under different acidic or alkaline catalysts, and acid-catalyzed organosolv processes are more commonly employed especially in bioconversion area (Del Rio, 2012; Del Rio et al., 2010; 2011; Pan et al., 2005; 2006). Similar to steam explosion process, hardwoods or agricultural residues can be subjected to organosolv pulping process with or without adding catalysts due to the high content of acetyl groups. During the autocatalyzed organosolv pulping process, delignification is promoted by acetic acid formed by the release of acetyl groups that decorate the backbone of the hemicellulose macromolecule to provide acid thus “auto-catalyzing” the removal of lignin and hemicellulose (Sarkanen, 1990). However, without the addition of acid catalyst, higher

temperature ($>190^{\circ}\text{C}$) are generally required for the effective delignification to occur (Pye and Lora, 1991). With the input of acid during organosolv pulping process, it has been found that high acid concentration could result in a high removal of lignin and results in a low kappa number on the pulp but at the expense of consuming overall pulp yield. However, less lignin-content organosolv pulps may have a high potential for the production of dissolving pulps especially most of the extractives have been removed during this process (Johansson and Ylinen, 1987).

In the case of producing dissolving pulps by using organosolv process, various methods such as Acetosolv, Formacell and Milox have been previously suggested (Nimz et al. 1986; Nimz and Schone 1992; Parajo et al. 1995). Compared to the traditional acid sulfite cooking process to produce dissolving pulp, organosolv process has been proposed to possess some advantages regarding to the pulp purity and selectivity in delignification (Puls et al., 1999). It has also been found that pulp obtained from organosolv process possessed highly accessible cellulose feedstock which was an important attribute for dissolving-grade pulps (Puls et al., 1999). However, the high cost input and total energy consumption, and difficulty in recovering the solvents of organosolv pulping process decrease its overall commercialization (Zhao et al, 2009).

After discussing the traditional chemical processes that can be used or potentially used to produce dissolving-grade pulp, the next section will focus on the introduction of cellulose ultrastructure, its accessibility/accessibility and different derivatization products made from dissolving-grade pulps.

1.3 Cellulose and cellulose derivatives

1.3.1 A brief introduction on the structure of cellulose

Cellulose is a homogeneous polysaccharide composed of β -D-glucopyranose units that are connected by β -(1, 4)-glycosidic bonds. It is the main chemical component in the wood (45-50%) (Sjöström, 1981). Cellulose molecular chains have a linear structure and thus a strong tendency to form the intra- and intermolecular hydrogen bonds. Bundles of cellulose chains are aggregated with each other in the form of elementary fibrils, which are composed of 30-36 cellulose chains with a size of 3-5nm and are regarded as the smallest cellulose fibril units (Himmel et al., 2007; Muhlethaler, 1965; Fengel and Wegener, 1989). Bundles of elementary fibrils are associated together with hemicellulose and pectin to form the structure referred to as microfibrils, in which the highly ordered crystalline regions alternate with less ordered amorphous regions. Microfibrils then form the macrofibrils and finally the whole cellulose fibres (Himmel et al., 2007).

The details of cellulose's crystalline structure have mainly been determined by electron microscopic techniques, X-ray diffraction and neutron diffraction experiments (Chanzy et al., 1986; Nishiyama et al., 2002). Several different crystalline structures of cellulose have been suggested based on these techniques with different types of structures corresponding to the conformation and location of hydrogen bonds. Natural cellulose is generally composed of Cellulose I and includes both Cellulose I α and Cellulose I β . Cellulose I α has a triclinic unit cell containing one chain and Cellulose I β is comprised of a monoclinic unit cell consisting of two parallel chains (Nishiyama et al. 2002). Cellulose II is also called regenerated cellulose which can be obtained by treating native cellulose with a strong base. Cellulose II contains anti-parallel chains and has a lower free energy compared with Cellulose I. The change from Cellulose I to

Cellulose II is generally irreversible. Cellulose III, IV and X can be produced when Cellulose I and II are subjected to certain chemical treatments such as ammonia or concentrated hydrochloric acid (Ishikawa et al., 1997).

To constitute the unit cell of native cellulose (Cellulose I), a few individual cellulose chains are organized into the planar sheets by intra- and intermolecular hydrogen bonds. These planar sheets are associated with each other via Van der Waals forces (Sjöström, 1981). There are no hydrogen bonds in between the different sheets of parallel cellulose chains. Hydrogen bond networks only exist between the cellulose chains on the same planar sheet (Nishiyama et al., 2002). There are two types of intramolecular hydrogen bonds within each cellulose chain, one is in between of O (6) of one glucose residue and the H...O (2) of the adjacent glucose. The other intramolecular hydrogen bond is formed in between the H...O (3) and the O (5) (Table 3). Intermolecular hydrogen bonds exist between the O (3) and H...O (6). Even though there are no hydrogen bonds in cellulose I between the different layers while the individual Van der Waals force is weak, the large number of interactions between the adjacent planar sheets results in a strong cumulative force (Pizzi and Eaton, 1985). Finally, these sheets of parallel cellulose chains (around 36 cellulose chains) are stacked on top of each other via Van der Waals forces to form the structures called the elementary fibrils (Himmel et al., 2007).

Cellulose II has anti-parallel chains. The hydrogen bonds not only exist within the same layer but also between the different sheets of cellulose chains (Sjöström, 1981). Cellulose II can be obtained by treating native cellulose with strong alkali to swell the cellulose chains which destroys the previous lattices of Cellulose I (Ishikawa, 1997). The change in the lattice is permanent. The hydrogen bond network in Cellulose II is mainly formed by attaching the O (3) of one glucose residual with the H...O (5) in the adjacent glucose unit, and the O (6) with H...O

(2). Intermolecular hydrogen bonds are formed in between the O (3) and the H...O (6) (Table 3). All the hydrogen bond distances within Cellulose II are shorter than Cellulose I, indicating a more compact cellulose microfibril structure.

Table 3: Intra- and intermolecular hydrogen bonds of Cellulose I and Cellulose II (Sjöström, 1981; Klemm et al., 2004)

Cellulose	Conformation	Intramolecular hydrogen bonds		Intermolecular hydrogen bonds	
		Bonds	Bond distance /nm	Bonds	Bond distance /nm
Cellulose I	gt	O ₍₃₎ —H.....O _(5')	0.275	O ₍₆₎ —H.....O _(3')	0.279
		O _(2') —H.....O ₍₆₎	0.287		
Cellulose II	tg	O ₍₃₎ —H.....O _(5')	0.269	O ₍₆₎ —H.....O ₍₃₎	0.267
		O _(2') —H.....O ₍₆₎	0.273		

It has been previously reported that when the concentration of the NaOH solution during CCE treatment exceeded 8%, the initial cellulose I on Kraft pulps was gradually modified to cellulose II (Gehmayr and Sixta, 2012). As reported later in the thesis, in the case of a Kraft pulp treated with 9% sodium hydroxide during the production of dissolving pulp, the solid CP/MAS ¹³C NMR spectrum showed a distinct signal at 107ppm, originating from cellulose II (Larsson et al., 1999). The same peak was discernible on 7% CCE treated pulp, which indicates a portion of the cellulose underwent mercerization by the alkaline treatment during CCE, especially when the NaOH concentration exceeded 7%. It has also been shown that performing an alkaline extraction using an alkaline concentration of 9% could convert 13% of the cellulose in Kraft pulp to

cellulose II (Gehmayr and Sixta, 2012). Due to the configurational change of O₆ on Cellulose II, which was an irreversible conversion from trans-gauche structure on cellulose I to gauche-trans orientation, this resulted in an increase in the number of inter- and intra-planar hydrogen bonds. As a result, cellulose II is more densely packed after drying, resulting in a decreased surface area and a lower accessibility of the hydroxyl groups within the cellulose to reactants during downstream derivatization (Gehmayr and Sixta, 2012). The formation of cellulose II also increases the occurrence of “fibril aggregation”, sometimes resulting in the collapse of the cellulose structure upon removal of the hemicellulose and low molecular weight cellulose that act as “spacers” in between the cellulose microfibrils (Gehmayr et al., 2012). Since there is an increased possibility for cellulose II to aggregate during the drying process, due to the existence of hydrogen bonds in between planar sheets (Sjöström, 1981), the compact cellulose structure limits its accessibility towards downstream derivatization reagents. As a result, cellulose II is an undesired component for dissolving pulp. Although the amount of alkali used for CCE can be decreased in order to minimize the conversion of cellulose I to cellulose II, a compromise must be found to provide sufficient alkali to reduce the hemicellulose content to dissolving grade levels (~5%) without losing cellulose reactivity.

1.3.2 Pore structure and surface morphology of cellulose

The properties of cellulose fibres such as pore size/frequency/distribution and external surface area directly influence their ability to be accessed by enzymes and/or derivatization reagents during the downstream conversion process. Therefore, the attributes related to accessibility are regarded as crucial parameters for cellulose fibres (Strunk et al., 2011; 2012). Since cellulosic biomass is a heterogeneous material with many pores, its ability to be accessed and reacted via downstream derivatization reagents or enzymes is governed by the availability/accessibility of its

reactive sites such as hydroxyl groups or glucosidic bonds (Sjöström, 1981; Chandra et al., 2008a; Arantes and Saddler, 2010, 2011; Esteghlalian et al., 2001a). Generally, the higher the pore volume, porosity and surface area of a substrate the more this should increase the accessibility of the reactive sites on the cellulose to reagents (Strunk et al., 2011; 2012). Different pretreatments such as steam explosion, hot water extraction or alkaline swelling are able to aid in expanding capillaries and enlarging the pore volume of substrates. Mechanical refining is also an effective treatment to expand capillaries or pores, increase fibril aggregation, increase the accessible surface of fibril aggregates and disrupt the compact cellulose matrix (Tian et al., 2014; Wu et al., 2014). Other work also showed there was more than a 50% to 60% improvement in accessible surface area when an alkaline extracted Kraft pulp obtained from a hardwood after was subjected to mechanical refining in a PFI mill.

The crucial role that pore structure and surface area play during the derivatization or bioconversion process has been reported extensively (Chandra et al., 2008a; Arantes and Saddler, 2010, 2011; Esteghlalian et al; Wiman et al., 2012; Wang et al., 2012). It was first shown by Stone and Scallan (Stone and Scallan, 1967) that the initial hydrolysis rate by enzymes on phosphoric acid swollen cellulose (PASC) was related to the pore volume of the swollen fibres. It has also been reported that after a mild acid hydrolysis, regardless of the substrate used, the initial cellulase hydrolysis rate was linearly correlated with the pore volume of the substrate that was accessible to a solute with a diameter of 5nm. This is a similar sphere size as cellulase enzymes (Grethlein, 1985). The paper also indicated that, in addition to the pore volume and pore size distribution, the initial enzymatic hydrolysis rate strongly correlated with the accessible surface area that was available to the enzymes (Grethlein, 1985). The importance of the reactive surface area to enzymes during the bioconversion process has also been confirmed by using

chemical staining approach, protein adsorption technique and NMR techniques (Chandra et al., 2008; Arantes and Saddler, 2010; Foston and Ragauskas, 2012). This work showed that the accessible surface area was a key factor in controlling enzymatic hydrolysis. As reported in the results section of this thesis, after the pore size and surface area were enhanced by mechanical refining, the reactivity of a dissolving-grade pulp to NaOH and CS₂ during the xanthation derivatization process to make viscose fibres was improved from 31.6% to 54.5%.

Although the measurements of the pore structure and the surface area reflect enzymatic and chemical accessibility, accurate measurement of cellulose accessibility and reactivity is still an area full of challenges despite decades of research. To date, much of the pore structure and surface morphological analysis performed on the cellulosic substrates have employed mercury intrusion porosimetry, nitrogen/carbon dioxide adsorption, Simons' stain technique, protein adsorption such as cellulose binding module (CBM) adsorption and the solute exclusion method (Stone and Scallan, 1967; Chandra et al., 2008a; Gourlay et al., 2012; Wang et al., 2012; Roselli and Sixta, 2014). Mercury intrusion porosimetry and nitrogen/carbon dioxide adsorption are two similar techniques that are applied on the dried cellulosic samples to measure the pore size and available surface area (Moura et al., 2005). Basically both the techniques involve the quantification of the amount of mercury or nitrogen/carbon dioxide molecules that interacts with the test samples over a range of varying pressures. For mercury intrusion porosimetry, since mercury does not penetrate the pores of most substrates spontaneously by the capillary action, it is forced to enter into the pores by applying external pressure. Generally, the external pressure that is employed to equilibrate the capillary force has an inverse correlation with the pore size of the test samples. For the large macro-pores, only low pressure is sufficient to force the mercury into them, whereas high pressures are required to intrude the mercury into small pores. As for the

small pores, higher pressure is required to push the mercury to enter the pores. Measuring the amount of mercury that is adsorbed onto the cellulosic sample over a range of varying pressures can give the pore volume and pore size distribution (Dang-Vu and Hupka, 2005; Westermarck, 2000). In the case of N₂/CO₂ adsorption, the amount of gas that is adsorbed onto the dried cellulosic materials gives an indication of the pore size of the substrates. The surface area is obtained from adsorption sites in the cellulosic materials that are exposed to the varying gas pressure at a certain temperature. The pore structure analysis accounts for both the internal pores and outer surface area as well (Fan et al., 1980; Gharpuray et al. 1983; Chandra et al., 2008a). The interpretation of the adsorption isotherm for N₂/CO₂ adsorption requires a model. The Brunauer-Emmett-Teller (BET) model is often chosen for cellulosic samples. However, one of the main limitations in performing either of these measurements is that the test samples are first dried, during which the cellulosic samples might undergo hornification (Jayme, 1944; Luo et al., 2011). Hornification is generally irreversible and generally involves the collapse of pores within the substrate (Diniz et al., 2004). Thus, neither of these techniques might accurately quantify the pore structure and surface morphology of a cellulosic material (Chandra et al., 2008a).

Simons' stain is another approach used to quantify overall surface morphological properties such as the pore structure or accessible surface area of cellulosic pulps (Yu and Atalla, 1998; Chandra et al., 2008a). This method was originally developed as a staining method for light microscopy to evaluate the extent of mechanical damage undergone by lignocellulosic fibres during beating and refining (Joutsimo et al., 2005; Simons, 1950; Loeb et al., 1964; Moore, 1953). It also has been utilized to analyze the structure of wood fibres (Yu and Atalla, 1998; Chandra et al., 2008a). This method uses high molecular weight direct orange and low molecular direct blue dyes that are incubated with biomass substrates with subsequent evaluation of the adsorption of the dyes onto

the cellulosic fibres (Yu and Atalla, 1998; Chandra et al., 2008a). Since the direct orange has a strong affinity for cellulose and the direct blue dye a relatively weak affinity, the smaller molecular direct blue dye penetrates into both the big pores and the small pores of cellulosic fibres and binds weakly with cellulose. However, since the direct orange dye has a higher binding affinity to the cellulose than the direct blue dye, it can displace the blue dye, adsorbing onto the surface of the cellulosic substrates. This leaves the small direct blue dye within the small pores of the pulps, which are inaccessible to the larger orange dye (Yu and Atalla, 1998; Chandra et al., 2008a). The porosity of the substrate is indicated by the ratio of the bound orange dye to the blue dye. The total orange dye adsorbing on the sample predicts the accessible surface area to enzymes during the downstream bioconversion process (Chandra and Saddler, 2012). This modified technique has also been employed to accurately predict the enzymatic digestibility to cellulosic biomass (Chandra et al., 2008a). This technique also has some drawbacks, as the two sizes of dyes cannot accurately represent the overall pore size distribution of the substrates, but instead categorize the pores of the substrates into two groups. Recently it has been reported that the adsorption of orange dye alone is a capable indicator for cellulose that is accessible to cellulases since the orange dye has a similar size to the predominant cellulase enzymes (Chandra and Saddler, 2012). Since the derivatization chemicals of cellulose are much smaller than the dyes, even though Simons' stain is a useful technique to predict enzymatic hydrolysis, it might not be an ideal technique to evaluate the accessibility of cellulose during the derivatization process of cellulose (Gourlay, 2014).

The solute exclusion method was developed for quantifying the pore size, pore size distribution, porosity and accessible surface area on test samples without the need for drying (Stone and Scallan, 1968; Grethlein, 1985; Roselli et al., 2014). This technique involves soaking the samples

in a series of solutions containing a known concentration of a solute such as dextran or polyethylene which is required to have a linear structure and narrow molecular weight distribution (Aggebrandt and Samuelson, 1964). Basically, this method works by incubating the test sample with a dextran/polyethylene solution and then measuring the changing in the concentration of the solution which might occur after the solute or water molecules go into the pores of the samples (Stone and Scallan, 1968). Bigger solutes such as dextran with a size of 54nm, which is almost bigger than all of the pores in a cellulosic sample, are used to determine the total porosity of the sample (Mansfield et al., 1997; Grönqvist et al., 2014). During this measurement, since no drying is required, it is more accurate and a widely used method to analyze the pore structure and surface area of a cellulosic substrate. Some of the drawbacks of this technique are that it consumes significant time and effort to achieve repeatable results as well as an inability to accurately measure pores with irregular shapes (Converse, 1993).

1.3.3 Reactivity and accessibility of cellulose

Accessibility and reactivity are important parameters during the derivatization process of cellulose. In each anhydroglucose unit within the cellulose, there are three hydroxyl groups that can react with various chemicals to achieve etherification or esterification (Sjöström, 1981). The hydroxyl groups of cellulose can be partially or fully reacted with those chemicals to provide derivatives with useful properties (Sjöström, 1981). Most of the cellulose derivatives have the general formula Cellulose-O-R, where the oxygen can be any of the cellulose hydroxyls. Cellulose esters and cellulose ethers are the most important commercial materials. Of the different reactive hydroxyl groups, the one at the 6th carbon position acts as the primary alcohol (HO-6), whereas the hydroxyl groups at the 2nd and 3rd positions behave as secondary alcohols (HO-2 and HO-3) (Sjöström, 1981). Although various factors such as steric hindrance and

thermodynamic stability are involved during the derivatization procedure of cellulose, one prerequisite for etherification of cellulose is the pre-ionization of the hydroxyl groups (Strunk, 2012). Due to the inductive effects of neighboring substituents, HO-2 has the highest acidity and tendency to dissociate during the derivatization, followed by HO-3 and then HO-6. As a result, HO-2 is the most readily etherified hydroxyl group in comparison to the others (Sjöström, 1981). After the HO-2 has been substituted, the tendency for the dissociation of HO-3 is increased, as well as its reactivity. In the case of esterification, HO-6 possesses the highest reactivity due to the primary hydroxyl group. It has been reported that during the esterification process, the reactivity of HO-6 is ten times more than the other hydroxyl groups (Guthrie and Hebeish, 1981).

An important factor to be considered in the reactions of cellulose concerns the accessibility, meaning the relative ease by which the hydroxyl groups can be reached by the reactants. For instance, the HO-6 group shows a higher reactivity towards bulky substituents than do the other hydroxyl groups since it is the least sterically hindered (Sjöström, 1981). The reactivity of dissolving pulp is another important quality parameter since it signifies the processability or suitability of dissolving pulp to be used as raw material for the viscose and other derivatization processes (Strunk, 2012; Östberg and Germgård, 2013). The reactivity of a pulp directly affects the subsequent derivatizations such as acetylation, xanthation or nitration. Reactivity is mainly measured by the availability of the HO-2, HO-3 and HO-6 hydroxyl groups of the monomeric units of cellulose to the reactants (Sixta, 2006). Although the amorphous cellulose in a pulp may exhibit higher reactivity, if companies want to make a quality product, homogenous substitution along the cellulose molecule is desired (Strunk, 2012). A key issue with the study of dissolving pulps is the development of effective tests for the quantification of cellulose reactivity. This can

be regarded as one of the most critical parameters affecting the subsequent utility of a given pulp sample (Strunk, 2012). It has been reported that the reactivity of cellulose during derivatization is determined by the structure and morphology of the cellulose (Strunk et al., 2011; 2012). However, there have been few, if any, studies that have compared the various derivatization techniques for their ability to estimate the “reactivity” of cellulose hydroxyl groups. It is also unclear what role that cellulose accessibility and porosity might play in the ease of derivatization. Those studies that have looked at cellulose accessibility have focused on assessing cellulose accessibility as it relates to cellulose hydrolysis (Chandra et al., 2008a). For example, if the derivatization of cellulose is carried out under acidic conditions, such as during nitration and acetylation (Barkalow et al., 1989), the cellulose may be less swollen and accessible than reactions such as xanthation performed under alkaline conditions (Fock, 1959; Östberg, 2012; Östberg and Germgård, 2013). In addition, since viscose is the main product currently being made using dissolving pulp, most studies aiming to quantify cellulose reactivity have employed the Fock’s test (Fock, 1959). This technique is tedious and challenging and employs toxic chemicals such as carbon disulfide (Östberg, 2012).

The most widely used methods for quantifying reactivity include the Fock’s test, gamma number and filterability (filter clogging value, Kw). These assays primarily assess the “processability” of a given dissolving pulp (Fock, 1959; Strunk, 2012; Östberg and Germgård, 2013). The Fock’s test is a wet chemistry method that employs potassium dichromate that reacts with the cellulose to determine the amount of cellulose that has been reacted and dissolved during the xanthation reaction (Östberg, 2012). It measures the yield of regenerated cellulose after xanthation. However, it has been shown that there are some limitations with this method. For example, although the main oxidizing agent, potassium dichromate, is a strong oxidizing reagent, it may

not be able to oxidize all of the carbohydrate components, resulting in an underestimation of the unreacted cellulose (Östberg, 2012). During the xanthation step, there may also be parts of the pulp that do not react with carbon disulfide and are instead oxidized by potassium dichromate. In summary, this method is not accurate and also not friendly to the environment (Östberg and Germgård, 2013). Alternatively, the gamma number is determined by making spectrophotometric measurements of viscose dissolved in sodium hydroxide. It is defined as the number of substituted xanthation groups per 100 anhydrous units (Östberg, 2012; Östberg and Germgård, 2013). The theoretical maximum gamma number is 3, which means all the three hydroxyl groups are substituted by xanthate groups (Östberg, 2012; Östberg and Germgård, 2013). However, during the ripening process to make dissolving pulp, the xanthate groups move from the kinetically favored C₂ and C₃ positions to the more thermodynamically stable C₆ position. During this redistribution process some xanthate groups are released and form by-products (Strunk, 2012). As a result, the gamma number is usually less than 3 (Östberg, 2012; Östberg and Germgård, 2013). The gamma assay is also problematic as, in addition to the cellulose being substituted, the residual hemicellulose can also react, resulting in a high and inaccurate gamma number (Östberg, 2012; Östberg and Germgård, 2013). The storage time will also impact results since the solution in this test is erratic based on previous research that showed the gamma number will reduce with increasing storage time (Östberg, 2012; Östberg and Germgård, 2013). The filterability is another test performed by timing the flow of the xanthation solution out of a 150 mesh. This method is primarily used to determine the processability of viscose dope prior to spinning of the filament. Filterability is impacted by pore area, permeability and pore diameter (Strunk, 2012). Of the three methods described above, it has been reported that the Fock's test and the filter clogging value determine cellulose reactivity at the fibre level

while the gamma number analysis mainly measures the reactivity at a molecular level (Östberg, 2012; Östberg and Germgård, 2013). However, the most common tests used to assess pulp reactivity are the Fock's test, gamma number and filterability which are based on alkaline xanthation for viscose production. These methods may not be applicable to the production of other cellulose derivatives, such as cellulose acetate, that are produced in an acidic environment where the cellulose may vary in its accessibility. Therefore, more methods are needed if we are to quantify the reactivity of cellulose. The types of methods that could be used include, CP/MAS NMR (Christoffersson et al., 2002; Filpponen and Argyropoulos, 2008), cupriethylene diamine (CED) (Arnoul-Jarriault et al., 2015) or NaOH/urea dissolution (Cai et al., 2006; 2007; 2008; Chen et al., 2007; Wang, 2008; Kihlman et al., 2011; 2012; 2013), or water retention values.

Due to the challenging issues discussed above, new methods involving novel solvents have emerged that hope to measure reactivity by dissolving the cellulose. These ionic liquids are supposedly environmentally friendly, safe and able to accurately assess pulp reactivity. Examples include N-methylmorpholine-N-oxide (NMMO) (Bang et al., 1999), sodium hydroxide/urea/thiourea system and sodium hydroxide/zinc oxide system (Kihlman, et al., 2011; 2012; 2013). In the sodium hydroxide/urea system the cellulose has been shown to rapidly and completely dissolve. It was shown that optical micrographs could be used to quantify the amount of dissolved cellulose in the sodium hydroxide/urea/thiourea system under low temperature. Cellulose solubility was characterized by designating the dissolution from “very poor to very good” levels (Kihlman, et al., 2012). The structure and properties of cellulose after being dissolved into sodium hydroxide/urea were characterized by CP/MAS ¹³C NMR, wide/small-angle X-Ray diffraction and small-angle neutron scattering. This showed that the urea hydrates could be self-assembled at the surface of the sodium hydroxide hydrogen-bonded cellulose to

form an inclusion complex. This inclusion complex lead to the dissolution of cellulose into the sodium hydroxide and urea system without changing structure (Cai et al., 2006; 2007; 2008; Chen et al., 2007; Wang, 2008; Kihlman et al., 2011; 2012; 2013). However, this method is quite subjective, designating the dissolution levels among a relatively few samples. It has also been reported the dissolution is heavily influenced by the molecular weight of pulps (Kihlman et al., 2012). As a result, those pulp samples with a higher molecular weight were hard to completely dissolve.

1.4 Cellulose derivatives

1.4.1 Cellulose xanthate

Cellulose xanthate is mainly produced by treating the dissolving-grade pulps with aqueous sodium hydroxide and carbon disulfide. The resulting solution is then used to spin into the viscose rayon fibres (Sjöström, 1981). First the cellulose pulps are supplied in bales and then added to steeping tanks where sodium hydroxide lye initially reacts with cellulose to form the alkali cellulose (Figure 6). Most of the hemicellulose and low molecular weight cellulose components are thus solubilized by the caustic lye (Strunk, 2012). Meanwhile, cellulose is “activated” by caustic lye through the swelling of the fibres and the breaking of those intra-/intermolecular hydrogen bonds in between the cellulose (Sjöström, 1981). As a result, the alkali cellulose is more accessible than the native cellulose in being able to react with the derivatization reagents. The excess of sodium hydroxide lye and dissolved low molecular weight carbohydrates are removed after going through a subsequent pressing stage. After a shredding step, the pressing and activation of the cellulose occurs. Shredding can increase the surface area of alkali cellulose and thereby increase the accessibility of cellulose to derivatization chemicals (Strunk, 2012). The

next step is called “aging” during which the intrinsic viscosity of the alkali cellulose is adjusted after undergoing the oxidative hydrolysis reaction (Strunk, 2012). This reduction in cellulose DP is a vital step to allow for the better processability and reactivity of viscose dopes in the later derivatization process (Schlotter, 1988; Strunk, 2012). In the subsequent xanthation step, carbon disulfide (CS_2) is added to react with alkali cellulose to form cellulose xanthate solution which is generally orange due to the side reactions that happened during the redistribution of the xanthation groups (Strunk, 2012).

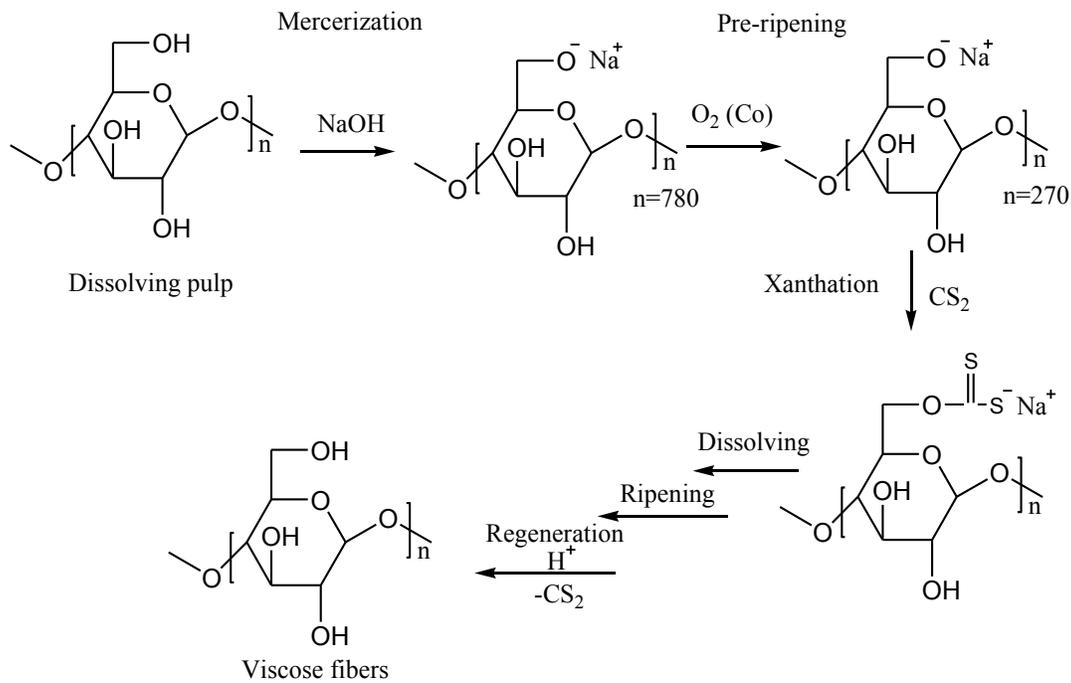


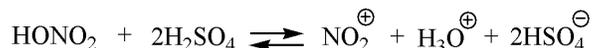
Figure 6: The main reactions involved in the viscose process. The first step, mercerization, is the reaction of the cellulose with sodium hydroxide, forming alkali cellulose. Step Two is pre-ripening, during which the oxygen from the air participated in the oxidative hydrolysis to reduce the intrinsic viscosity of cellulose. The xanthation step generates cellulose xanthate from alkali cellulose, which dissolves the cellulose pulp in alkali. This is followed by a ripening step during which the xanthation groups redistribute to form a more thermodynamically stable structure. The last regeneration step happened within an acid bath and the xanthation groups are removed (Schlotter, 1988).

After forming cellulose xanthate, caustic lye and water are then added under vigorous agitation to dissolve the cellulose and form the viscose dope. For viscose rayon fibres, the composition of

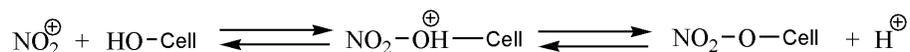
the dope is about 10wt% cellulose and 5wt% caustic lye (Strunk, 2012). The following ripening step aids in removing the entrapped air bubbles in the viscose dope, which could harm the spinning process and the quality of the end product. At the same time, ripening facilitates the redistribution of the xanthate groups from the kinetically favored C₂ and C₃ positions to the more thermodynamically stable C₆ position (Strunk, 2012). Even though the degree of substitution is reduced during the redistribution process, by a release of some xanthation groups to form by-products which make the whole solution orange, the re-arrangement of those xanthate groups forms a more homogeneous derivatization of the cellulose (Strunk, 2012). After filtration, the viscose dope is transported to the spinning step where the viscose dope is pressed through spinnerets into an acid spin, during which both carbon disulfide and hydrogen sulfide are released (Strunk, 2012).

1.4.2 Cellulose nitrate

Cellulose nitrate is generally prepared in an acid mixture which contains nitric acid and sulfuric acid (Sjöström, 1981). The first reaction involves the generation of the nitronium ion (NO₂⁺):



This reaction is an acid-base equilibrium in which the weak nitric acid acts as the base and sulfuric acid is the acid. In the next step, the electrophilic nitronium ion reacts with the hydroxyl groups of cellulose:



The concentration of nitric acid in the acidic nitration mixture is approximately 20-25%. The degree of nitration can be adjusted by changing the water content. However, cellulose sulfate might form during this nitration process as a by-product. As the formation of cellulose sulfate results in cellulose nitrate instability, the sulfate groups must be further removed (Sjöström, 1981).

1.4.3 Cellulose acetate

Cellulose acetate is the acetate ester of cellulose and it was first prepared in 1865. Cellulose acetate can be used as a film base in photography, as a component in coating and as a thermoplastic used to produce eyeglass frames and other products (Sjöström, 1981). It has also been used as a synthetic fibre in the manufacture of cigarette filters. Cellulose acetate successfully replaced cellulose nitrate for the production of photographic films since the 1950s. Its advantages include being less flammable and lower in cost to produce. The various products that are made from cellulose acetate have different requirements especially regarding its degree of substitution (DS) which can range from 1.5 to 3. Solvent systems are also important in determining the different final products obtained after the derivatization process (Table 4).

Table 4: Commercial grade of cellulose acetate (Ranby and Rydholm, 1956)

Degree of Substitution	Solvents	Applications
1.8-1.9	Water-propanol-chloroform	Composite fabrics
2.2-2.3	Acetone	Lacquer, plastics
2.3-2.4	Acetone	Acetone rayon
2.5-2.6	Acetone	X-ray and safety films
2.8-2.9	Methylene chloride-ethanol	Insulating foils
2.9-3.0	Methylene chloride	Fabrics

1.5 Dissolving pulp properties

Dissolving pulp is a high-quality pulp employed for the manufacture of various industrial products such as viscose fibres, cellulose nitrate and cellulose acetate. Although the end-use products define the chemical composition and the cellulose purity/accessibility/reactivity required, nevertheless there are certain fundamental requirements that must be fulfilled prior to use of dissolving pulp as a raw material to produce those derivatives (Table 5).

Table 5: Typical specifications for dissolving-grade pulp (Kaur et al., 2016)

Chemical properties	Description
α -cellulose	Represents undamaged long-chain cellulose content; should be >90%
Alkaline solubility	Measures solubility of pulp (S10, S18) in different concentrations of NaOH, represents the amount of hemicellulose and degraded cellulose present in the pulp
S10 %	Optimum swelling of pulp; both hemicellulose and degraded cellulose (up to <150 DP) are dissolved in 10% NaOH solution
S18 %	Hemiciellulose (<50 DP) dissolved in 18% NaOH solution. Sometimes degraded cellulose of very low DP (usually after cellulose treatment) also dissolves. Viscose process yield=0.88(100-S18) (Hinck et al. 1985)
Reactivity %	Most critical parameter; methods include viscose filter value (Treiber 1987) and more common Fock's method (Fock, 1959); Fock reactivity should be > 65%
Pentosan %	Represents major hemicellulose fraction of hardwood; should be 0.5-10%
Intrinsic viscosity ml/g	Corresponds to the length of cellulose chain; give a relative indication of the degradation of cellulose chains (decrease in cellulose molecular weight)
Molecular weight distribution	Determined by gel permeation chromatography (GPC); should be uniform
Degree of polymerization	Related to the molecular weight by the formula $DP=M/162$, where 162 is the molecular weight of the anhydroglucose unit (Elg Christoffersson, 2004) and to intrinsic viscosity for cellulose dissolved in cupriethylenediamine
Ash %	Should be <0.06%
Extractives %	Should be <0.5%
Residual lignin %	Should be <0.2%
Brightness % ISO	Should be >90%
Yield %	30-35%

1.5.1 The hemicellulose content

Generally the hemicellulose content indicates the amount of low molecular weight hemicellulose remaining in the pulp after various cooking and bleaching processes. The high retention of hemicellulose in a Kraft pulp is an attribute that has been shown to be beneficial for papermaking. However, as mentioned earlier, it is detrimental for dissolving pulp, especially for making cellulose acetate which has a higher requirement for mechanical strength. Thus, the hemicellulose content should be kept as low as possible (<2%). The main reasons why hemicellulose is detrimental to dissolving-grade pulps are because the hemicellulose negatively influences the impregnation of the alkaline solution into fibres to generate the homogeneous alkaline cellulose during xanthation. Compared to cellulose, hemicellulose is amorphous and have a greater susceptibility to react with the caustic lye and then dissolve in the alkaline solution. As a consequence of hemicellulose consuming the alkali, the alkali molecules become viscous and difficult to diffuse into cellulose microfibrils to react with the cellulose, leading to less homogeneous alkali celluloses being formed. As discussed earlier, one of the most crucial prerequisites for derivatization of cellulose is the pre-ionization (mercerization during the viscose process) of the hydroxyl groups on the cellulose. If the mercerization does not occur on the cellulose, the subsequent xanthation reactions are difficult, resulting in the poor dissolution of the cellulose pulps during the xanthation process (Strunk, 2012). The presence of hemicellulose also prolongs the pre-aging timing required during the viscose process. Generally, hemicellulose has a very low DP and plenty of short chains, resulting in a large number of reducing ends. During the pre-aging step, part of the oxygen is consumed by the large amount of reducing ends of the hemicellulose, leaving less oxygen to react with the cellulose, decreasing its intrinsic viscosity. As a result, longer pre-aging times are acquired to let the oxygen react with

the cellulose to make the final pulp reactive. In addition, the hemicellulose influences the xanthation process. The amorphous hemicellulose can react with carbon disulfide more quickly compared to the cellulose component. After the consumption of the carbon disulfide, there are limited chemicals available for cellulose during the esterification reaction, and the low degree of esterification leads to the poor final dissolution of the cellulose xanthate (Strunk, 2012). Hemicellulose also impacts the mechanical strength of final products. During the Kraft cooking process, most of the hemicellulose is de-branched, resulting in the remaining hemicellulose having a very linear structure. This increases its tendency for potential co-crystallization and association with cellulose which in turn can increase the resistance of the cellulose during the viscose process. Once the low molecular weight hemicellulose enters the final product such as viscose fibres, the mechanical strength of the final product, such as its tenacity, will be weakened (Strunk, 2012).

1.5.2 Alkaline solubility (S10/S18)

The alkaline solubility measures the solubility of the low molecular weight components of the pulps in solutions of 18 and 10% NaOH (Hinck et al., 1985). Generally these measurements represent the amount of hemicellulose and degraded low molecular weight cellulose present in the pulps after various pulping and bleaching processes (Strunk, 2012). Non-linear, low molecular weight impurities such as low molecular weight cellulose and hemicellulose have a high susceptibility to react with alkali, consequently deteriorating the uniformity of the alkaline cellulose (Strunk, 2012). As a result, the presence of these “impurities” compromises the strength of the polymer mixture, especially when the resulting dissolving pulp is spun into fibres for textile applications (Gehmayr and Sixta, 2012). Pulp “purity” is related to the molecular weight of the components in pulps and it can be characterized by the alkaline solubility in 10% and 18%

sodium hydroxide providing so-called S10 and S18 values (Elg Christoffersson, 2004). Typically, the 10% NaOH solubilizes both the hemicellulose and low molecular cellulose (S10) while the 18% NaOH primarily solubilizes the hemicellulose impurities (S18) in the pulp. It has been reported that sodium hydroxide at a concentration of 10% is capable of dissolving hemicellulose and cellulose with molecular weights less than 25000 (Strunk, 2012). Therefore, higher S10 and S18 values indicate a higher amount of low molecular fragments that are soluble in sodium hydroxide and thus lower pulp purity. As mentioned in section 1.4.1, the alkaline solubility of dissolving grade pulps is crucial to both the processability and the mechanical strength of cellulose derivatives such as viscose fibres (Strunk, 2012).

1.5.3 Intrinsic viscosity (DP)

The intrinsic viscosity is an important parameter for dissolving-grade pulps (Engström, et al., 2006; Köpcke et al., 2010; Strunk, 2012). Generally it correlates with the average length/degree of polymerization of cellulose. It can be measured using different solvents but cupriethylenediamine is the most widely used solvent (Immergut et al., 1953; Evans and Wallis, 1989; Kasai, 2002). A range of weights of the purified cellulosic pulp sample is dissolved in cupriethylenediamine solution and the viscosity of the solution is measured in a capillary viscometer. The intrinsic viscosity gives a measurement of the degree of polymerization of cellulose according to the formula:

$$[\eta] = 1.33 \times DP^{0.905}$$

The molecular weight of cellulose can then be calculated by the formula $DP = M/162$, where 162 is the molecular weight of the individual anhydroglucose unit (Elg Christoffersson, 2004). Generally the molecular weight of cellulose can be expressed in various ways. The number

average molecular weight (M_n) can be measured by determining the total amounts of the reducing ends or using an osmometry method. The weight average molecular weight (M_w) can be deduced from light scattering experiment (Sjöström, 1981). The ratio of M_w/M_n is the polydispersity index which is corresponding to the broadness of the molecular weight distribution of the cellulose. All of those parameters can be obtained from the gel permeation chromatography (GPC) measurement (Hill et al., 1995; Rousselle, 2002). Dissolving-grade pulp applications require a very narrow molecular weight distribution especially for high grade dissolving pulps such as for cellulose acetate production (Figure 7). As a result, to achieve a viscose fibre with the highest strength, the viscose producer should utilize a pulp with high R10, low S10-S18 and low S18 (Strunk, 2012).

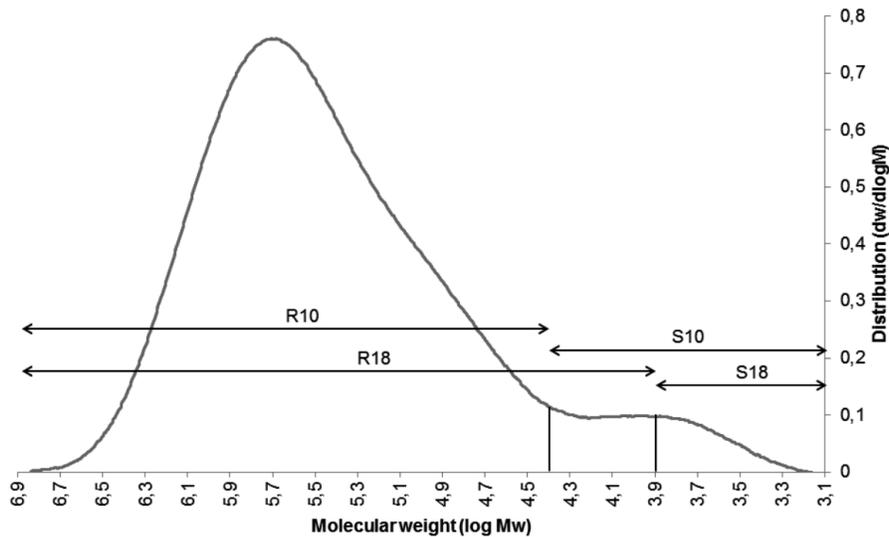


Figure 7: Molecular weight distribution curve of one dissolving pulp sample and the theoretical fraction of alkaline solubility (Strunk, 2012).

It has been shown that the reactivity of dissolving pulp is inversely proportional to the intrinsic viscosity, or the average cellulose molecular weight, in a given pulp sample (Engström et al., 2006). Decreasing the molecular weight of cellulose is also a key processing step in the

production of dissolving pulp. This decrease in molecular weight is achieved through steps such as adding a hypochlorite stage during the bleaching process which acts to both remove lignin and impart a slight decrease in the molecular weight of cellulose to improve the reactivity of cellulose.

1.6 Enzymes that might facilitate the conversion of Kraft to dissolving pulps

1.6.1 Introduction to xylanases

Enzymes are biological proteins produced by living organisms. They are generally known as “biocatalysts” that are used to specifically accelerate biochemical reactions (Gurung et al., 2013). Enzymes are highly substrate specific. It has been suggested that the main reason for this specificity is because both the enzymes and substrates have complementary geometric shapes which can fit exactly into each other. This is referred to as “the lock and key” model (Fischer, 1894). Due to their high specificity, enzymes have been exploited in various industries such as brewing, dairy, detergent, textile and bakery (Kaur et al., 2016). The growth in industrial enzymes has been triggered by various factors such as the rapid development in the technology to produce enzymes, the better understanding of the interaction of different substrates with enzymes and the increased requirement of the industry to apply more eco-friendly approaches (Kaur et al., 2016).

Since xylan is the most abundant hemicellulose especially in hardwood plant cell walls and pulps, a considerable part of the work published so far has been focused on the applications of xylanases (Suurnäkki et al., 1997; Collins et al., 2005; Kaur et al., 2016). Xylanases are glycosidases which catalyze the endo-hydrolysis of 1, 4- β -D-xylosidic linkages in xylan (Collins et al., 2005). The heterogeneity and complexity of xylan has resulted in an abundance of diverse

xylanases with varying specificities, primary sequences and folding. Thus, this has led to some limitations with the classification of these enzymes by substrate specificity alone. It was reported that xylanase sequences classified in families 5, 7, 8, 10, 11 and 43 contain truly distinct catalytic domains with a demonstrated endo-1, 4- β -xylanase activity (Collins et al., 2005). Glycoside hydrolase family 10 and 11 are the two xylanase families I that are discussed in detail here since they are widely used and have high activity on xylan.

Glycoside hydrolase (GH) family 10 consists of endo-1, 4- β -xylanases, endo-1, 3-xylanases and cellobiohydrolases (Coutinho and Henrissat, 1999). The major enzymes of this family are endo-1, 4- β -xylanases. However, substrate specificity studies have revealed that these may not be entirely specific for xylan and they may also be active on low molecular mass cellulosic substrate as well (Mansfield and Saddler, 2003; Gilkes et al., 1991). Furthermore, these enzymes are highly active on short xylo-oligosaccharides, indicating small substrate binding sites (Biely et al., 1997). Hydrolysis studies have shown that most family 10 xylanases can attack the xylosidic linkage on the non-reducing end of a substituted residue, but can only cleave at the third xylosidic linkage after a substituted residue. Members of this family typically have a high molecular weight and a low *PI* (Collins et al., 2005).

In contrast to family GH10 xylanase, GH11 xylanases are generally monospecific. These xylanases are “true xylanases” as they are exclusively active on D-xylose containing substrates. They have a lower catalytic versatility than family 10 xylanases and the products of their action can be further hydrolyzed by the family 10 enzymes (Biely et al., 1993; 1997). Furthermore, substituents or β -1, 3 linkages represent a more serious hindrance to their activity, resulting in the production of larger products than GH10 xylanases. Further differences between family 10 and 11 xylanases include Family 11 enzymes being generally characterized by a high *pI*, a lower

molecular weight. Family 11 also works more effectively on linear xylan due to the steric hindrance presented by hemicellulosic sugar substituents (Biely et al., 1997).

Earlier work has shown the existence of true “xylanases” that react with xylan with high specificity (Wong and Saddler, 1992). However, we anticipate that the ability of either GH10 or 11 xylanases to react with a given xylan sample will be dependent on a substrate's structural characteristics including its molecular weight and branching. Therefore, in the work reported here we assessed both GH10 and 11 endo-xylanases with various commercially available and in-house xylan preparations, including linear birch xylan, substituted oat spelt xylan, and xylan rich hemicellulose isolated from hardwood Kraft pulp using alkaline extraction, to determine their activity. It was hypothesized that the GH11 xylanase will be more effective on the less branched xylan from birch wood xylan while GH10 xylanases will be more active upon the more branched xylan from Oat spelts. This work on model substrates should provide some insights on how these xylanases will act on real pulps that have been treated by the different cooking or bleaching steps that have altered the structure of xylan.

1.6.2 Enzymes applications in traditional pulp and paper industry

The potential use of enzymes in the pulp and paper industry was realized in the late 1980s, shortly after the discovery of the xylanase-aided pre-bleaching concept (Viikari et al., 1994; Paice et al., 1992; Wong et al., 1997; Beg et al., 2001). In addition, novel enzymatic and microbial applications have been investigated for improving the processing of wood fibres as well as for improving the sustainability of the pulping, bleaching and papermaking process (Viikari et al., 1994; Wong et al., 1997; Beg et al., 2001).

One of the wider applications of xylanases is in the pre-bleaching of Kraft pulp to reduce the use of bleaching chemicals and the associated environmental problems along with an improved quality of product (Senior et al., 1991; Allison et al. 1993; Bajpai et al. 1994). Xylanases are used in the pulp and paper industry as a pre-bleaching reagent for unbleached hardwood Kraft pulp which contains up to 20% xylan resulting in 15% savings in bleaching chemicals and a 2% ISO gain in pulp brightness (Thakur et al., 2012). It has been hypothesized that the underlying mechanism behind xylanases ability to improve pulp bleach-ability is the removal of xylan-lignin complexes that become re-precipitated on the surface of fibres at the end of the Kraft cooking process as the cooking liquor becomes saturated. Thus the xylanase treatment removes a portion of the xylan and lignin concurrently. Xylanases can also aid in the removal of the chromophoric components such as hexeneuronic acids to save bleaching chemicals (Viikari et al, 1994; Wong and Saddler, 1992). Studies have also shown the applicability of xylanases in de-sizing of fabric (Csiszar et al., 2001), increasing water retention, reducing beating times in virgin pulps, restoring bonding and increasing freeness in recycled fibres (Dhiman et al., 2008b). Xylan-degrading enzyme systems also have considerable potential in other biotechnological applications including the bioconversion of lignocelluloses and agricultural wastes into fermentative products (Hu et al., 2011; 2013; 2014).

Other enzymes are also known to modify a number of pulp and paper properties. For example, lipases have been used for pitch control (Fischer et al., 1993) and laccases have been used in the bleaching process (Ibarra et al. 2006). Cellulases have been used for deinking (Heitmann et al., 1992; Prasad et al., 1993) and dewatering of recycled fibres (Verma et al, 2013). Endoglucanases have been applied for surface treatments in the textile and laundry industries (Miettinen-Oinonen et al., 2004).

Various enzymes have been assessed for aiding in dissolving pulps production. Xylanases have been assessed alone or in combination with alkaline extraction in dissolving-grade pulp production (Bajpai and Bajpai 2001; Gehmayr et al., 2011; Köpcke et al., 2010). Micro-organisms have also been investigated for bio-pulping and bio-bleaching to produce dissolving pulp (Christov et al., 1998; Ferraz et al 1998). In addition, one of the enzyme applications includes the reactivity improvement of dissolving pulp by cellulases (Cao and Tan 2002; Engström et al., 2006; Rahkamo et al., 1996; 1998).

1.6.3 Enzymes applications in dissolving-grade pulp

Due to their specificity and ability to function at benign reaction conditions compared to the processes mentioned above, the use of enzymes is an attractive option to facilitate the specific removal of the hemicellulose component, narrow the cellulose molecular weight and increase cellulose reactivity (Gehmayr and Sixta, 2012). In contrast to the application of xylanases as a pulp bleaching aid, where we would like to retain as much of the hemicellulose in the pulp to maximize yield and mechanical strength, dissolving pulp requires purified cellulose with a minimal amount of hemicellulose. Therefore, for dissolving pulp applications, xylanases are employed to remove xylan by cleaving xylan molecules to increase their solubility during subsequent alkaline extraction (Köpcke et al., 2010).

Hemicellulases such as xylanases and cellulase monocomponents such as endoglucanases have been applied to improve the conversion of Kraft to dissolving grade pulps (Engström et al., 2006; Köpcke et al., 2010; Gehmayr and Sixta, 2011; 2012; Östberg and Germgård, 2012; 2013). Xylanases have been added to reduce hemicellulose content while endoglucanases have been added to provide a controlled decrease in cellulose molecular weight without increasing

polydispersity and to increase pulp reactivity during subsequent derivatization (Engström et al., 2006; Köpcke et al., 2010; Gehmayr and Sixta, 2011; 2012; Östberg and Germgård, 2012; 2013). For example, it has been shown that xylanase pretreatment of an unbleached oxygen delignified Eucalyptus Kraft pulp combined with a 7% NaOH CCE could reach the similar levels of xylan removal as a CCE step employing 10% NaOH while also providing 10% higher pulp yield (Gehmayr et al., 2011). It has also been shown that xylanases combined with alkaline extraction can hydrolyze more xylan from commercial Kraft pulp from birch than two stages of alkaline extraction which means that xylanases can cleave the hemicellulose and then enhance the alkaline extraction (Köpcke et al., 2010). However, hemicellulases added after the bleaching processes have had limited success while the application of xylanases to reduce the chemical loading necessary to remove hemicellulose during a CCE treatment as mentioned above, has also been shown to compromise the downstream reactivity of dissolving pulp (Gehmayr et al., 2011; 2012). In addition, in several cases, the hydrolytic enzymes that were added to remove the hemicellulose after an oxygen delignification step (on a Kraft pulp brown stock) were shown to decrease the efficacy of hemicellulose removal since the “easily accessible” hemicellulose was already removed or relocated (Christov and Prior, 1993; Kim et al., 2012).

It is evident that the benefits of adding hemicellulases during the conversion of Kraft pulp to dissolving pulp remains unclear. On some occasions they have been shown to compromise the downstream reactivity of the pulp, even after the addition of endoglucanases which have been shown to improve pulp reactivity (Östberg et al., 2013; Östberg and Germgård, 2013). Overall the benefits of using enzymes to modify dissolving pulps and Kraft pulps have been mixed. These inconsistencies in enzyme efficiency are likely due to varying approaches and addition points within the pulping/bleaching processes and the use of different biomass substrates with

varying levels of cellulose/hemicellulose accessibility (Christov and Prior, 1993). It is evident that these previous studies have not firmly established the beneficial effects of the addition of enzymes for converting Kraft to dissolving pulp. It can be surmised that much of the inconsistency has resulted from a lack of consideration of the characteristics of the substrate to which the enzymes are being applied. For example, previous studies have not shown the effects of the location and type of hemicellulose and the condition of cellulose as determined by upstream pulping/bleaching conditions on the subsequent ease of removal/hydrolysis of hemicellulose by hemicellulases. The ability to enhance cellulose reactivity through endoglucanase addition has also not been assessed in detail (Kim et al., 2012; Östberg et al., 2013; Östberg and Germgård, 2013; Miao et al., 2014). As well as considering the influence of enzymes on process parameters, these past studies have mostly been limited to the addition of hemicellulases and endoglucanases (Gehmayr et al., 2011; 2012; Köpcke et al., 2010). However, as discussed below, there are several accessory proteins that can work synergistically to improve the accessibility of cellulose and hemicellulose to hydrolytic enzymes such as cellulases and hemicellulases (Hu et al., 2013; 2014; Berlin et al., 2007; Bura et al., 2003; Kumar and Wyman, 2009a; Harris et al., 2010; Quiroz-Castañeda et al., 2011; Gourlay et al., 2012). These, so called accessory enzymes, can potentially aid in improving the effects of these enzymes on facilitating the production of dissolving pulp.

Earlier work has shown that the use of high loadings of hemicellulases results in the removal of only 60% of the total hemicellulose from dissolving pulp, likely due to the limitations of accessing some of the residual hemicellulose after the many chemical treatments (Gübitz et al., 1997; 1998). Therefore, the work within this thesis first evaluated and compared the impact of the accessibility of the residual 20% hemicellulose in Kraft pulp to hemicellulases. These

enzymes were compared with a much smaller dicarboxylic acid (oxalic acid) which has been reported to biomimetically remove hemicellulose under mild conditions. Subsequent work then assessed the influence of various substrate characteristics on the action of xylanases and cellulases by using model substrates. It was hypothesized that in the process to produce Kraft pulp, the hemicellulose becomes less accessible as it is protected by the complicated cellulose matrix and residual lignin, thereby compromising its removal using enzymes. The model substrates with the xylan adsorbed to the surface were prepared and compared to the “real” substrates that are encountered at various points in the process toward the conversion of Kraft to dissolving pulp. Determining the substrate characteristics that had the greatest influence on the ease of enzymatic hemicellulose removal allowed us to determine the point in the process where enzymes could provide the greatest benefit. The pulps from various points in the process will also be studied to determine the substrate characteristics that have the greatest influence on the action of the enzymes in order to gain fundamental knowledge on the substrate-enzymes interactions. Subsequent work investigated the conditions that amplified a given substrate characteristic shown to influence enzyme activity, such as the enrichment of surface xylan generated by low alkaline cooking followed by the application of enzymes that could potentially improve the action of hemicellulases and cellulases.

1.7 Research approach

Some of the past work that applied enzymes to modify dissolving pulps involved assessing the ability of hemicellulases to remove the residual hemicellulose from dissolving grade pulps (Gübitz et al., 1997). It was shown that, when xylanases and mannanases were applied to the pulp, only 60% of hemicellulose could be removed using a high loading of hemicellulases. It was

hypothesized that, based on a complete enzymatic hydrolysis of the carbohydrate components, the remaining hemicellulose were linked with lignin as part of a lignin carbohydrate complex (LCC) that could not be accessed by the enzymes (Gübitz et al., 1997). This previous work demonstrated the possibility of LCC's playing a role in influencing the ability of hemicellulases to remove the hemicellulose from dissolving pulp. However, the challenge of removing the last 4% of the hemicellulose in the presence of >95% cellulose suggests that the lack of accessibility of the hemicellulose to hemicellulases plays a significant role. Therefore, the initial work described in Section 3.1 evaluated and compared the impact of the accessibility of the residual 20% hemicellulose in Kraft pulp to hemicellulases versus the much smaller oxalic acid molecule which has been reported to biomimetically remove hemicellulose under mild conditions (Lu and Mosier, 2007a; Lee et al., 2013; vom Stein et al., 2011). It was hypothesized that the oxalic acid would have a greater ability to access the hemicellulose in the pulp compared to the larger xylanase enzymes.

As well as investigating the effects of oxalic acid and xylanase, the effects of altering the alkaline extraction conditions employed after the enzyme treatment were also assessed. It has been demonstrated that, during the upgrading process of Kraft pulp to dissolving pulp, the combination of xylanase treatment, followed by the alkaline extraction can effectively remove the residual hemicellulose and decrease pulp viscosity (Hakala et al., 2013). Therefore, in the work reported in Section 3.1, oxalic acid and xylanase treatments were followed by alkaline extraction to assess the ability to upgrade a traditional Kraft pulp to a dissolving pulp. It was found that the combination of either oxalic acid or xylanase treatment with alkaline extraction could reduce the residual hemicellulose content in the Kraft pulp (20%) to the level of a commercial dissolving grade pulp (5-6%).

Although the initial hypothesis was that the dicarboxylic acid would have a greater ability to access the hemicellulose in hemicellulose-rich Kraft pulp compared to the larger xylanase enzymes, the results showed that the oxalic acid solubilized as similar amount of hemicellulose from the Kraft pulp as did the xylanase. The low hemicellulose solubilization results by oxalic acid and xylanase indicated there was still a limitation of the accessibility of hemicellulose on Kraft pulps which needed to be further studied and improved by modifying the upstream processes, to enhance the accessibility of hemicellulose and cellulose to chemical/enzymatic reagents. The influence of pulp characteristics such as the accessibility of the hemicellulose and cellulose on the ease of enzymatic action was further assessed using model substrates, as described in the second chapter (Section 3.2). We added xylanases to isolated xylans from different sources such as ground wood chips and Kraft pulp to compare xylan hydrolysis yields. It was anticipated that, after the isolation process, the accessibility of xylan should be significantly increased, resulting in a greater xylose release by xylanases. In addition, model cellulose substrates with varying crystallinities, molecular weights, surface area were also studied to assess the influence of these characteristics on the final cellulose reactivity.

The results on model substrates showed that the isolated xylans had a much higher xylose release by xylanases compared to when they were with the initial wood chips and pulps. The higher xylose release results on isolated xylans further indicated the accessibility of xylans was crucial for enzyme action. It was apparent that substrate characteristics such as the accessibility of hemicellulose, the crystallinity/DP/accessible surface area impacted the reactivity of enzymes and derivatization reagents with the fibres. Therefore, it was anticipated that in the conversion process from Kraft pulp to dissolving-grade pulp, the application of either a PHK or CCE treatment to remove hemicellulose should result in pulps that vary in properties such as pore size,

accessible surface area and microfibril aggregation that should impact cellulose accessibility and reactivity. As a result, the subsequent work (Section 3.3) investigated the influence of a PHK and CCE treatment on pulp characteristics and the ease of enzymatic hydrolysis. The work in Section 3.3 showed that CCE treatments compromised hemicellulose/cellulose accessibility and enzymatic digestibility due to the solubilisation of the highly accessible part of hemicellulose and low molecular weight cellulose. It was likely that these components acted as “spacers” between the cellulose microfibrils to prevent fibril aggregation. However, CCE is still regarded as an attractive treatment for the production of dissolving-grade pulps due to the mild reaction reactions and being able to recycle the chemicals, although it does lower cellulose reactivity, as described earlier. As a result, post-treatments were required to enhance the accessibility and reactivity of dissolving pulp treated with a CCE step prior to the addition of downstream derivatizing chemicals. The goal of activation is to open up the capillaries and pores, disrupt the complex crystalline cellulose matrix, shorten cellulose DP and break down the existing inner- or intramolecular hydrogen bonding. As a result, more hydroxyl groups are available and accessible for derivatization. Widely used post-treatment methods include degradative treatments such as chemical or enzymatic hydrolysis, mechanical treatments such as PFI refining or steam explosion, or combination thereof.

From this work it was apparent that the characteristics of the substrates at the various points in the process in the conversion of a Kraft to dissolving grade pulp (PHK vs. CCE) would influence the substrate factors that facilitate the ease of enzymatic hemicellulose removal. Key steps such as PHK and CCE likely resulted in substrate changes in both the accessibility of hemicellulose and cellulose contained in the pulp and the overall substrate accessibility to enzymes. The influence of the PHK conditions on the distribution/amount of hemicellulose, the degree of

polymerization and accessibility of cellulose and the ease of removal of the lignin component were assessed. Both the prehydrolysis and the pulping conditions were then altered (Section 3.4) to create substrates that were used to gain further insight into the characteristics that improve their susceptibility to enzymatic hemicellulose hydrolysis and cellulose modification by endoglucanases. These studies also determined if specific prehydrolysis/pulping conditions could be altered to increase hemicellulose and cellulose reactivity, using either enzymes and chemical reagents, during the subsequent cellulose derivatization process.

It has been previously reported that the different fibre fractions from hardwood Kraft pulp had distinct susceptibility towards enzymes and derivatization reagents in the bioconversion or pulp and paper making area (Jackson et al., 1993; Mansfield et al., 1996; Mooney et al., 1999). Most of the previous work indicated that short fibre fractions which mainly contained vessels and fines were the main target for enzymatic or chemical hydrolysis due to their high porosity and surface area. However, in the pulp and paper making industry, fines drastically decrease pulp freeness, result in poor dewatering of the pulp, and slow down the drying rate for paper (Seth, 2003). As a result, the short fibre fraction could be a potential pulp furnish to make dissolving-grade pulp due to its high accessibility. As the previous work had indicated that the short fibre fraction was more preferentially attacked by enzymes, we thought it would be worthwhile to investigate whether the action of xylanases could reach a higher xylan removal from the short fibre fractions, and endoglucanase could access the short cellulose fibres better to enhance the pulp reactivity. The application of fractionation concept for dissolving pulps, which aims to enhance the purity and reactivity of dissolving pulps, has not been widely reported and was studied in the last chapter of the thesis (Section 3.5). The objective of this part of the thesis was to investigate the influence of fibre size on the enzymes/chemicals ability to upgrade hardwood Kraft to dissolving pulp grade.

This work showed that the short fibre fraction had a higher hemicellulose content, higher specific surface area, larger pore diameter and lower coarseness due to the high content of vessels and fines than the long fibre fraction. These properties resulted in a higher hemicellulose removal by hemicellulases and enhanced cellulose reactivity to endoglucanase and derivatization reagents.

In summary, the potential conversion of Kraft to dissolving pulp can be facilitated through increasing the accessibility of hemicellulose and cellulose by enhancing the upstream pulping/PHK/CCE conditions. Enzymes can be successfully used to reduce the chemical intensive and non-specific nature of the current processes used to produce dissolving pulps from hardwoods.

2. Materials and methods

2.1 Cellulosic pulps

Commercially-dried, ECF-bleached hardwood Kraft pulp was generously provided by Alberta-Pacific Forest and commercially-dried, TCF-bleached hardwood dissolving pulp was generously supplied by Fortress Paper, Canada. Maple, aspen and birch chips (Section 3.4) were provided by Fortress Paper, Canada.

2.2 Enzymes

2.2.1 Commercial enzyme preparations

Multifect xylanase (Genencor UB Inc., Palo Alto, CA) and HTec/Pulpzyme HC 2500/ Fibrecare R (Novozymes, Franklinton, NC) were commercial xylanase and endoglucanase preparations.

2.2.2 Commercial enzyme activity measurement

In the Section 3.1, the activity of xylanase was measured by Birchwood Xylan Unit (BXU) development by Enzyme Development Corporation. Briefly, the xylanases hydrolyzed substrates such as dissolved birch wood xylan and the amount of released reducing carbohydrate was determined spectrophotometrically using dinitrosalicylic acid. One BXU is defined as the amount of enzyme that resulted in reducing carbohydrates having a reducing power corresponding to 1nmol xylose from birch wood xylan in one second under assay conditions (1BXU=1kcat). The xylanase activity (BXU/g) was obtained by multiplying the xylose concentration (determined by the standard curve in $\mu\text{mol/ml}$) by 1000, dividing by the reaction

time (300s) and the enzyme dilution concentration in g/ml. The BXU was calculated according to the following equation:

$$\text{BXU} = \frac{\text{Xylose Conc. (from curve)} \times 1000}{\text{enzyme conc. (g/ml)} \times 300 \text{ s.}}$$

A monocomponent endoglucanases (Fibrecare R) were provided by Novozymes, Franklinton, NC. The cellulolytic activity of the endoglucanase was expressed in Endo Cellulase Units (ECU). The endoglucanase hydrolyzes hydroxyethylcellulose, and the reducing sugars produced are assayed spectrophotometrically using dinitrosalicylic acid. The activity of Fibrecare R used in this work was 4500 ECU/g.

In Section 3.2, xylanase activity was determined by a method modified from Lin and Thomson (1991) using birch wood and oat spelt xylans. Briefly, birch wood and oat spelt xylans were dissolved in 50 mM sodium acetate buffer (pH 5.0) by stirring overnight at room temperature. To measure the activity, 70 μl substrates were added in microplates with 30 μl of the appropriately diluted enzyme samples and mixed in an incubator at 400 rpm for various incubation times at 50°C. The enzymatic reaction was stopped by adding 200 μl of 3, 5-dinitrosalicylic acid (DNS) reagent after exactly 15 min incubation. After that, the microplates were placed in an oven at 105 °C and boiled for 40 min. The reducing sugar content of the samples was analyzed by measuring the absorbency at 540 nm. Xylose and glucose standards were used for calibration. The reducing sugar released (μmol) at different hydrolysis times was plotted and the enzyme activities ($\mu\text{mol}/\text{min}$) were determined by the slope of the linear phase of the hyperbolic curve.

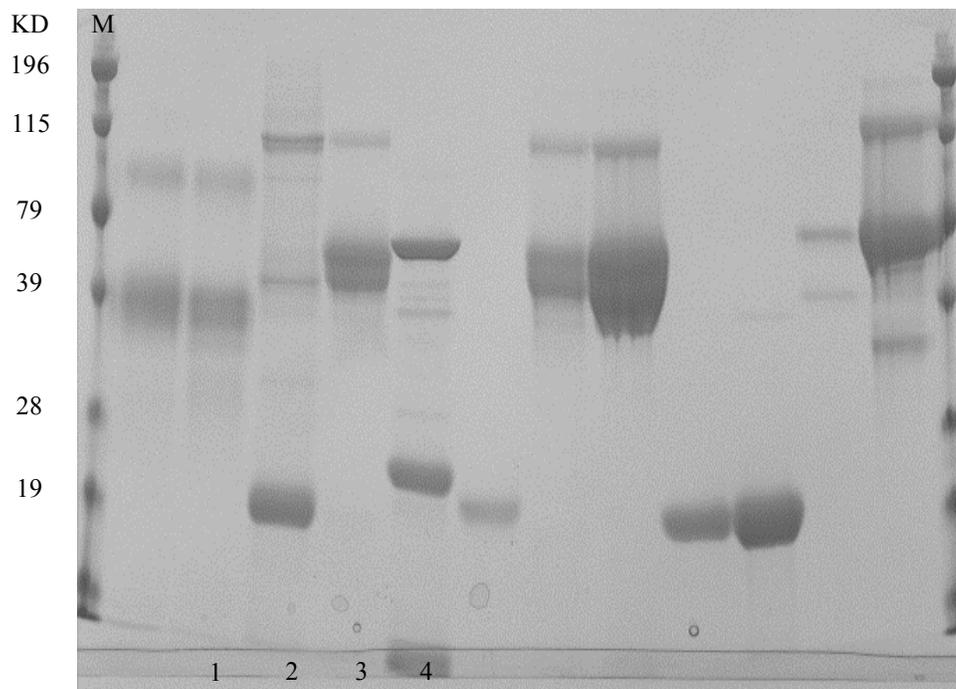


Figure 8: SDS-PAGE of commercial enzymes done by one of my colleagues Jinguang Hu: Fibrecare R: Fungal GH10 endo-glucanase (lane 1); 2: Multifect: Fungal GH11 endo-xylanase (lane 2); 3: Htec: Fungal GH10 endo-xylanase (lane 3); 4: Pulpzyme HC 2500: bacterial GH11 endo-xylanase (lane 4) (Hu, 2014)

2.2.3 Protein content quantified by modified ninhydrin assay

Protein content was quantified by the modified ninhydrin assay, which was developed to improve its overall accuracy and speed (Mok et al., 2015). Basically 100 μ l of protein containing samples was first incubated with 50 μ l of NaBH₄ for 60 min at a ratio of 1:3 NaBH₄/total sugar (w/w) in a screw cap micro-centrifuge tube (0.5ml) with BSA as a protein standard. This was followed by an addition of 300 μ l of 9M HCl and subsequent heating in a dry heating bath at 130 $^{\circ}$ C for 2 hours. After cooling down to the room temperature, 100 μ l of the hydrolysate was transferred to a 1.5ml micro-centrifuge tube and then neutralized with 100 μ l of 5M NaOH solution. Upon the neutralization, 200 μ l of 2% ninhydrin reagent was added and then heated at 100 $^{\circ}$ C for 10 min. Samples were then cooled to room temperature prior to the addition of 500 μ l

of 50% (v/v) ethanol. In the end, 200 μ l of the solution was transferred to the microplate and the samples were read at 560nm absorbance.

2.3 Model substrates

Phosphoric acid treatments were used to disrupt Avicel PH-101 (Sigma-Aldrich) to make PASC. Briefly, ice-cold concentrated phosphoric acid solutions were produced at various concentrations and 14.5ml was added to 50ml centrifuge tubes containing 0.2g Avicel pre-wetted with 0.5ml nanopure water to give final phosphoric acid concentrations of 0-78% w/w. Samples were incubated for one hour in the reactor under very low temperature with occasional mixing. Ice-cold nanopure water (35ml) was slowly added to the sample, followed by the centrifugation at 10000g for 15 minutes. The fibres were then re-suspended in 50ml nanopure water and washed for four times with 50ml nanopure water, followed by one wash with 50ml 20mM Na₂CO₃ and two subsequent washes in 50ml nanopure water.

The xylan was isolated from Kraft pulp using 9% NaOH extraction under room temperature for 30min with a 7% pulp consistency and 150 rpm shaking. The filtrate was then neutralized with 6M HCl to adjust the final pH to around 5.5, then centrifuge and wash the precipitation few times until the pH of washed water closed to 7.

Both birchwood xylan and Oat spelt xylan used in Chapter II were purchased from Sigma-Aldrich. The batch number of birchwood xylan was 101M0169.

2.4 Prehydrolysis and Kraft cooking

2.4.1 Prehydrolysis

The laboratory scale prehydrolysis was performed on a custom-built four vessels (2L each) rotating digester (Aurora products Ltd. Savona, BC, Canada) as described by Pan et al, (2005). Wood chips were pretreated in water under vacuum overnight to remove the air before conducting the prehydrolysis experiments. Two hundred grams of wood chips (maple: aspen: birch mass ratio is 7:2:1) and the required amount of water were added into the cooking vessels. The wood-to-liquid ratio was kept at 1:4. The system was pre-warmed to 80°C within 27 minutes, and then heated to the maximum temperature of 170°C within one hour. It was then, kept at this maximum temperature for another one and a half hours. After that, the vessels were cooled down in cold water until the pressure reached zero. The vessel was then opened up and the prehydrolysis liquor was taken out, filtered using filter paper, and then kept under 4°C for chemical composition analysis. For acid prehydrolysis, 0.4% sulfuric acid was loaded during the cooking process.

2.4.2 Kraft cooking

Kraft cooking was carried out within the same vessel as described in 2.4.1. Wood chips were pretreated in water under vacuum overnight to remove the air before the Kraft cooking process. Equivalent to 200g prehydrolyzed wood chips and the required amount of water were added into the vessel. The wood-to-liquid ratio was kept at 1:4. NaOH loading was 16%, 20% and 24% on chips. Sulfidity was 25%. The system was pre-warmed to 110°C within 37 minutes, then heated to a maximum temperature 170°C in 25 minutes. It was then maintained at the maximum

temperature until the final H factor reached 1000. After cooking, the unbleached brown stocks were washed, and then collected at 4°C for the next screening process.

2.5 Chemical composition analysis

2.5.1 Chemical compositional analysis on pulps

Oven-dried weights were determined by drying to constant weight at 105°C in a convection oven. The Klason lignin content of the all of the substrates was determined according to the TAPPI standard method T-222 om-98. The hydrolysate was retained for determination of monosaccharide composition and acid soluble lignin. Acid soluble lignin was determined on a Cary 50 UV-Vis spectrometer at 205nm as described by Dence (1992). The monosaccharides in the filtrate were determined with a DX-3000 HPLC system (Dionex, Sunnyvale, CA), equipped with anion exchange column (Dionex CarboPac PA1) and ED40 electrochemical detector, with the fucose as the internal standard. The column was reconditioned using 1M NaOH after each analysis. Monosaccharides including arabinose, galactose, glucose, xylose and mannose in the pulps were quantified by reference to standard sugars.

2.5.2 Chemical composition analysis prehydrolysis liquor/enzymatic hydrolysate

The monosaccharides in the liquid samples were determined by dilution of the liquor in deionized water followed by HPLC analysis as described in section 2.5.1. To hydrolyze the oligomers in the prehydrolysis liquor or the hydrolysate after enzyme hydrolysis into monomeric sugars, a septa bottle containing 5-15ml of the hydrolysate (depend on the concentration of sugars in samples) and calculated amount of 4% sulfuric acid (to fill up the final volume into

20ml) were sealed and put into an autoclave machine under 121°C for 1hour. After the bottles cooking down, the liquor was then taken out and centrifuge for chemical composition analysis.

2.6 Cold caustic extraction (CCE)

Cold caustic extraction (CCE) was carried out at 7% pulp consistency (the weight ratio of the amount of pulps to the pulp suspension), room temperature, 150rpm in an incubated benchtop shaker for 30 minutes. Different sodium hydroxide concentrations were used. After the extraction process the pulp was then taken out, filtered and washed several times until the final pH of filtrate was closed to 7.

2.7 Chlorite delignification

The delignification of cooked pulp brown stocks was achieved using sodium chlorite according to the procedure in the pulp and Paper Technical Association of Canada's (PAPTAC) useful methods G10. U. Approximately 30 g (DW) of never-dried pulps were suspended in a 300 ml solution composed of 5% (w/v) NaClO₂ dissolved in 1% (v/v) acetic acid and incubated overnight in the dark at room temperature. The delignified pulps were then filtered using a funnel and washed thoroughly with water. The resulting pulps with no detectable lignin as characterized by the Klason method were collected and kept at 4°C.

2.8 Enzymatic/chemical (acid) hydrolysis

The xylanase hydrolysis was performed in a flask in IST-4075 incubated benchtop shaker with a speed of 150 rpm at 60°C for 2h. The calculated amount of xylanase (500BXU/g) was added to deionized water (for Pulpzyme HC 2500) or sodium acetate buffer (50 mM, pH 4.8, for Multifect

and HTec), and then added to pulp to get a final 5% pulp consistency. After the xylanase treatment the reaction mixtures were heated at 100°C for 20 minutes to inactivate the enzymes. Supernatants were collected after centrifugation at 13000 rpm for 10 minutes and stored at -20°C for the sugar analysis.

The endoglucanase (Fibrecare R) hydrolysis was performed in a plastic bag. The calculated amount of enzyme (45ECU/g) was added to deionized water and then added to the pulps in the bag to get a final 5% pulp consistency with a pH of 7. The bag was then placed into a water bath at a temperature of 50°C for 2 h. After endoglucanase hydrolysis, the reaction mixtures were heated at 100°C for 20 minutes to inactivate the enzymes. Pulps were then washed and stored at 4°C for subsequent analysis.

Oxalic acid/sulfuric acid hydrolysis was carried out at a 5% pulp consistency in deionized water. A required amount of oxalic acid was added to the pulp. The pulp was allowed to react in an autoclave at 121°C for 1 hour. After treatment the pulps were washed three times with deionized water and stored at 4°C prior to testing.

2.9 Pulp properties analysis

2.9.1 Intrinsic viscosity

The viscosity of substrate solutions containing 0.06%, 0.1%, 0.125%, and 0.5% (w/v) pulps in 0.5 M cupriethylenediamine (CED) was measured on a capillary viscometer (Cannon Ubbelohde Viscometer, Cannon Instrument Co., State College, PA) according TAPPI standard method T230 om-99. The specific viscosity of the substrate solutions was determined according to the following equation:

$$\eta_{sp} = \frac{(\eta_c - \eta_o)}{\eta_o}$$

Where η_c is the viscosity of the sample at concentration c and η_o is the viscosity of the solvent. The intrinsic viscosity of each substrate was calculated by extrapolating a plot of η_{sp}/c as a function of c to $c = 0$ as described by Lapierre et al, (2009). The viscosity average degree of polymerization (DP_v) of cellulose was calculated from the intrinsic viscosity by the following equation:

$$DP_v = (1.65\eta_{int})^{1.11}$$

2.9.2 Alkaline solubility S10/S18

The alkaline solubility S10 and S18 were tested according to TAPPI 235. A test specimen was placed in a 300 ml tall form beaker and 75ml of the desired NaOH reagent (10% NaOH for S10 measurement, and 18% NaOH for S18 measurement) was added after adjusting previously to 25°C. The pulp was stirred with the dispersion apparatus until it was completely separated. When the pulp was completely dispersed, the adhered pulp fibres were then removed with an extra glass rod. The next 25ml NaOH was added to raise the agitator and shell. As a result, 100 ml of NaOH reagent was finally added into the beaker. The pulp suspension was then stirred for 10 seconds, and the beaker was kept in the water bath 25°C for 60 minutes. At the end of the reaction time, the pulp suspension was again stirred and then transferred to a filtering funnel. The first 10 to 20ml of the filtrate was discarded, and 50ml of the filtrate was collected in a clean and dry filtration flask. In the following step, 10ml of the filtrate and 10ml of 0.5N potassium dichromate solution were added into a 250ml flask. And 30ml of concentrated sulfuric acid was cautiously added into the flask. In the following, 50ml of water was added into the flask to cool down to room temperature. 2 to 4 drops of ferroin indicator was then added and 0.1N ferrous

ammonium sulfate was used to titrate the solution to a purple color. The alkaline solubility could then be calculated by:

$$S(\%) = \frac{(V_2 - V_1) \times N \times 6.85 \times 10}{A \times W}$$

Where V_1 is the titration of the pulp filtrate, ml; V_2 is the blank titration, ml; N is the normality of the ferrous ammonium sulfate solution; A is the volume of the pulp filtrate used in the oxidation, ml; and W is the oven-dry weight of the pulp sample, g.

2.9.3 Water retention value

Fibre swelling was estimated by the water retention value (WRV), which was performed according to the Technical Association of the Pulp and Paper Industry's (TAPPI UM 256). Briefly, 0.5(oven dry weight) of never-dried pulp was suspended in 50ml deionized water and shaken vigorously to break the pulp apart. The pulp slurry was allowed to soak overnight at room temperature and filtered through a 325-mesh screen in a centrifuge cup. The filtrates were recirculated three times to prevent the loss of fines and the resulting pulp pads were centrifuged (900G, 25°C) for 30 minutes. The wet pulp pad after centrifugation were then weighted and dried overnight at 105°C oven and reweight. WRV was calculated as the weight of water retained in the pulp pad after centrifugation divided by the dry weight of the fibres according to the following equation:

$$WRV = \frac{(W_w - W_d)}{W_d}$$

Where W_w is the weight of the wet sample after centrifuging, and W_d is that of the dried pulp.

2.9.4 Simons' staining

Cellulose accessibility to cellulases was estimated by the Simons' stain technique according to the modified procedure by Chandra et al. (2008). Generally, direct orange (DO-Pontamine Fast Orange 6RN) or direct blue (DB-Pontamine Fast Sky Blue 6BX) dyes were obtained from Pylam Products Co. Inc. (Garden City, NY, US) and fractionated by an Amicon filtration system. Approximately 10mg (DW) of never-dried samples were weighed into each of six 2ml polypropylene centrifuge tubes. Each tube received 100 μ l of phosphate buffered saline (PBS, pH 6) followed by the addition of DO or DB dyes solution (10mg/ml), in a series of increasing volumes (25, 50, 75, 100, 150, and 200 μ l). The nanopure water was added to make up the final volume of the samples to 1ml in each tube. The tubes were incubated overnight at 70°C in an orbital shaker at 200rpm. After the incubation, the tubes were centrifuged at 5000rpm for 10 min, and a sample of the supernatant was placed in a cuvette and the absorbance read on a Cary 50 UV-Vis spectrophotometer at 625 (for DB) and 450nm (for DO). The amount of dye adsorbed on the fibre was calculated using the difference in the concentration of the initially added dye and the dye left in the supernatant according to the Beer-lambert law. The extinction coefficients were calculated by preparing standard curves of DO or DB dyes and measuring the slope of their absorbency at 450 and 625 nm, respectively.

2.9.5 Crystallinity index

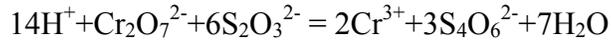
The cellulose crystallinity index was measured by X-ray diffraction. Pulp samples were washed, filtered and freeze-dried before mounting onto a zero-background plate. The data was collected with a Bruker D8-Advance powder X-ray diffractometer. Bruker TOPAS version 4.2 was used to model percent crystallinity and cellulose 1 β was used to model cellulose. The percentage

cellulose crystallinity was calculated as 100 times the crystalline area and the divide the total area, where the total area is equal to the sum of crystalline area and the amorphous area.

2.9.6 Fock's reactivity measurement

The Fock's reactivity test was carried out according to Fock (Fock, 1959). A sample of pulp weighing 0.50 g oven dry basis, was transferred to a 100 ml Erlenmeyer flask with a glass stopcock and a magnet. 50 ml of NaOH (9% w/v) and 1 ml CS₂ were added and the bottle was subsequently sealed with the stopcock. The mixture was stirred continuously with a magnetic stirrer for 4 h before being transferred into a round bottom flask. Deionized water was then added to obtain a total weight of 100 g. The round bottom flask was sealed with a stopper and shaken vigorously to obtain a homogenous solution. Forty milliliters (40 ml) of the solution was centrifuged at 3000 rpm for 10 min, after which 10 ml of the clear liquid phase was transferred to a 100 ml beaker and neutralized with 3 ml sulfuric acid (22% w/v). The mixture was left in the fume hood to de-gas overnight in room temperature. The next day 20 ml of sulfuric acid (68% w/v) was added to the mixture which was allowed to stir with a magnetic stirrer for 1 h. The mixture was then transferred to a round bottom flask where 10 ml of potassium dichromate (K₂Cr₂O₇) (1/6 mol/dm³) was then added. The mixture was allowed boil with reflux for 15 minutes in order to oxidize the cellulose. The mixture was then left to cool until it reached room temperature before being transferred into a 100 ml measuring cylinder and diluted to the 100 ml with deionized water. Forty milliliters (40 ml) was then transferred to a beaker and 5 ml of potassium iodide (KI) (10% w/v) was added. The iodine produced in the beaker was titrated with sodium thiosulfate (Na₂S₂O₃) (0.1 mol/dm³) using starch as an indicator. The reactivity was calculated based on the amount of non-reduced Cr⁶⁺ that remained after the oxidative reaction

that occurred between the potassium dichromate and the cellulose. The total reaction was as follows:



With the resulting calculation:

$$\text{Reacted cellulose (\%)} = \frac{9.62 \times 100 \times M \times (V_1 C_1 - 2.5 \times V_2 C_2)}{6 \times 4 \times Y}$$

Where Y is the weight of sample (g), M is the molecular mass of glucose, $\text{C}_6\text{H}_{10}\text{O}_5$ (162 g/mol), V_1 is the volume of added $\text{K}_2\text{Cr}_2\text{O}_7$ (L), V_2 is the volume of titrated $\text{Na}_2\text{S}_2\text{O}_3$ (L), C_1 is the concentration of $\text{K}_2\text{Cr}_2\text{O}_7$ (mol/L), C_2 is the concentration of $\text{Na}_2\text{S}_2\text{O}_3$ (mol/L), 9.62 is the first dilution to 100 mL and outtake of 10 mL ($10.4 \text{ g} = 100/10.4 = 9.62$) and 2.5 is the second dilution of the sample to 100 mL and outtake of 40 mL = $100/40$.

2.10 Fibre quality analyzer

Fibre dimensions and fines content were determined using a high resolution fibre quality analyzer (FQA) (LDA02. OpTest Equipment, Inc., Hawkesbury, ON, Canada) as described previously (Robertson et al., 1999). Generally, the parameters on FQA were pre-set to measure particles down to 0.07 mm. A diluted suspension of fibres with a frequency of 25-40 events per second was transported through a sheath flow cell where the fibres were oriented and positioned. The images of the fibres were detected by a built-in CCD camera and the fibre length and width were calculated by circular polarized light. Vessels and coarseness were also tested by FQA. Fines are defined as fibre length in between of 0.07 mm to 0.20 mm. The ranges for of fibre length

and fibre width of vessel measured in this study were 0.1-1.5mm and 80-400 μm . 20mg pulps were used for coarseness measurement.

2.11 Carbohydrate Binding Module (CBM) adsorption assay

The relative amount of accessible crystalline cellulose and amorphous cellulose was assessed by two specific carbohydrate binding modules (CBM). CBM 2a has a planar binding face and preferentially adsorbs to crystalline cellulose (Gourlay, et al., 2012). Generally, never-dried samples were mixed with 350 μg CBM and made up to a final volume of 1ml with phosphate buffer (50mM, pH 7.0). Samples were incubated at room temperature for 1 h followed by centrifugation at 18000 $\times\text{g}$ for 10min. The concentration of CBM 2a in the supernatant was determined by measuring the absorbance of the solution at 280nm (Cary 50 UN-Vis spectrophotometer). The extinction coefficients for CBM2a was 27625 M^{-1} (Gourlay, et al., 2012).

2.12 Nitrogen adsorption

The surface area of different pretreated biomass samples was determined by nitrogen adsorption using an 11-point BET procedure (Satterfield, 1991) using an Autosorb-1 surface area analyzer (Quantachrome instruments, FL, USA). The specific surface area and pore volume distribution of differently treated pulps were analyzed by the Barret-Joyner-Halenda (BJH) method.

2.13 Solute exclusion technique

The pore volume of the pulps was measured and compared to the different fibre fractions from Kraft pulp and commercial dissolving pulp in order to see whether the different fractions had an

effect on the pore structure which could then influence the accessibility and reactivity on downstream derivatization. The fibre swelling was determined by the solute exclusion as the fibre saturation point (FSP), which is a term used in wood mechanics and especially wood drying, to denote the point in the drying process at which only water bound in the cell walls and all other free water is removed from cell cavities (Stone and Scallan, 1968; Grönqvist et al., 2014). The water is inaccessible to a dextran with a size of 54nm since the probe is too large to penetrate into the pores of cell walls. The measurement was done by adding 0.8 g (O.D weight) pulp at 20% solid content to a 50ml centrifuge tube. 35ml of 2% dextran solution was added to the tube. The tube was then gently mixed for 1hour in a rotational mixer, and then centrifuged at 3500 RPM for 15min. The dextran supernatant was extracted with a syringe, and expelled through a 0.45 µm syringe filter into a HPLC vial. HPLC was used to test the concentration of dextran. The exact quantity of added pulp was determined after the removal of residual dextran and water. The pore volume, expressed as FSP, was calculated by Eq.

$$FSP = \frac{W_{dex} + W_{water}}{W_{pulp}} - \frac{W_{dex}}{W_{pulp}} \times \frac{C_i}{C_f}$$

Where W_{dex} , W_{water} and W_{pulp} are masses of dextran solution, water in the sample and dry pulp, respectively, and C_i and C_f are initial and final concentrations of the dextran solution.

Microfibril swelling was determined by the above method but with a 3.6nm dextran (T1000 from Pharmacia). The T1000 probe is small enough to be excluded from fibrils. Thus it can be used to determine fibril swelling (Grönqvist et al., 2014). The accessible surface area (ASA) was calculated by Eq.

$$ASA = \frac{FSP - V_{3.6nm}}{0.0116}$$

Where $V_{3.6nm}$ is the pore volume determined with 3.6nm diameter dextran and 0.0116 is a geometric constant based on the assumption of cylindrical pores of 3.6nm diameter.

2.14 Solid state CP/MAS ^{13}C -NMR measurements

All NMR spectra were recorded on dried rewetted pulp fibres. Unbeaten hand sheets were delignified according to Hult et al. (2000) and hydrolyzed for 17h in 2.5M HCl at 100°C in order to remove interfering signals from lignin and hemicellulose. All spectra were recorded on wet samples (moisture content is in between of 40% to 60%). The CP/MAS ^{13}C -NMR spectra were recorded using a Bruker AMX-300 instrument operating at 7.04T. A zirconium oxide rotor was used. The MAS rate was 4-5 kHz. Acquisition was performed with a CP pulse sequence using a 3.5 μs proton 90° pulse, 800 μs contact pulse and a 2.5s delay between repetitions. Glycine was used for the Hartman-Hahn matching procedure and as external standard for the calibration of the chemical shift scale relative to tetramethylsilane. The data point of maximum intensity in the glycine carbonyl line was assigned a chemical shift of 176.03ppm. A method based on non-linear least square fitting of the cellulose C₄ region of the CP/MAS ^{13}C -NMR spectra has been developed in order to quantify the states of order found within cellulose I fibril (Larsson et al., 1995). The method allows the relative amount of crystalline and paracrystalline cellulose, cellulose at inaccessible and accessible fibril surfaces to be determined.

2.15 Pulp fractionation

Pulps were fractionated using a Bauer-Mcnett fibre classifier to collect the R48, R100 and R200 fractions, according to method Tappi T 233cm-95. The fibres are separated on the basis of fibre

length, such that the longest fibres in this study are retained on the 48-mesh screen (R48) and the smallest fines are not retained by any of the screens.

2.16 Paper strength test

Tensile strength of paper was measured according Tappi method T494 om-1, and tear strength was tested based on Tappi method T 414.

3. Results and discussion

3.1 Influence of chemical and enzymatic modification on the conversion of traditional Kraft pulp to dissolving pulp

3.1.1 Background

Earlier work had shown that the use of high loadings of hemicellulases resulted in the removal of only 60% of the total hemicellulose from dissolving pulp which likely due to the less accessible hemicellulose to enzymes after several chemical treatments (Gübitz et al., 1998). Thus, it was hypothesized that hemicellulose accessibility was one of the main factors influencing the ability of xylanases to convert hemicellulose rich Kraft pulp to highly pure dissolving grade pulp cellulose. Therefore, at the beginning of this thesis we evaluated and compared the impact of the accessibility of the residual 20% hemicellulose in Kraft pulp to hemicellulases versus a much smaller dicarboxylic acid which has been reported to biomimetically remove hemicellulose under mild conditions (Lee et al., 2010; 2011; 2013; Qin et al., 2016). It was anticipated that the hemicellulose removal during oxalic acid treatment might surpass that of the xylanase. It was also hypothesized that during the process to make dissolving pulp, the hemicellulose becomes less accessible as it is protected by the complicated cellulose matrix, thereby compromising its removal by using enzymes.

As discussed before, in the case of dissolving pulp production, xylanases can be employed to specifically remove xylan by cleaving the chains to increase their solubility during subsequent alkaline extraction. In related work, xylanases and mannanases were applied with a subsequent alkaline extraction to remove the last vestiges of hemicellulose (4%) from dissolving pulp. These enzyme-alkali treatments could only reduce the hemicellulose content from 3.1 to 1.3%,

suggesting that the hemicellulase enzymes could not access this last portion (approximately 2%) of the residual hemicellulose in the pulp (Gübitz and Saddler, 1998). This is likely due to the inability of the enzymatic treatments to find the 4% hemicellulose in the presence of >90% cellulose.

Compared to xylanases, dicarboxylic acids such as oxalic acid are relatively small (90 g/mol). Oxalic acid has been investigated for its potential to act as a biomimetic agent similar to hydrolytic enzymes due to its two carboxylic acid groups that are hypothesized to act in a similar fashion to the retaining mechanism observed with carbohydrate active enzymes (Figure 9) (Kayser et al., 2013). Previous work assessed the potential of oxalic acid to selectively remove hemicellulose from biomass during biomass pretreatments that aim to improve the accessibility of the biomass to cellulases for subsequent enzymatic hydrolysis of cellulose to glucose (Lee and Jeffries, 2011). It was found that, under mild (80-120°C) conditions, oxalic acid does not show significant hydrolytic action toward cellulose but instead selectively hydrolyzes hemicellulose (Lee et al., 2010). The selectivity of dicarboxylic acids for hemicellulose is likely due to the increased accessibility of the amorphous lower molecular weight hemicellulose compared to the crystalline, linear high molecular weight cellulose (vom Stein et al., 2011). Consequently, the addition of a high concentrations of salt has been shown to facilitate the hydrolysis of crystalline cellulose by oxalic acids, presumably by an increase in cellulose swelling and accessibility caused by a salt-induced disruption of the hydrogen bonds within cellulose (vom Stein et al., 2010). Therefore, we anticipated that oxalic acid would have a greater ability to access the hemicellulose in the pulp compared to the larger xylanase enzymes. In addition, previous work had also shown that dicarboxylic acids have attractive properties including controlled stepwise

acidity, ready biodegradability and a reduced tendency to corrode processing equipment (vom Stein et al., 2011).

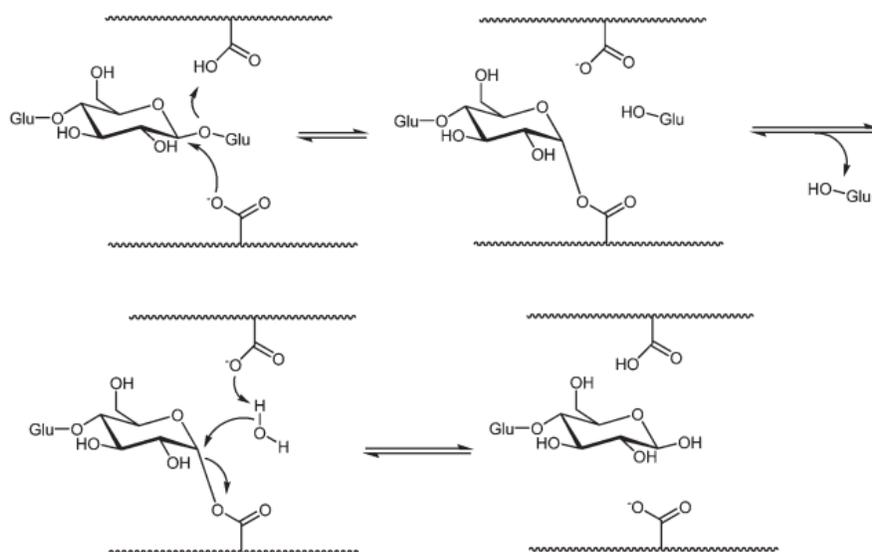


Figure 9: Retaining mechanism for the cooperative action of the two carboxylic acid groups in the active site of cellulases. Oxalic acid has the same two carboxylic acid groups and could mimic the enzymes. One carboxyl group acts as a proton donor and the other carboxyl group could function as a nucleophile to mimic the retaining hydrolysis mechanism of cellulase (McCarter et al., 1994; Kayser et al., 2013).

A small amount of cellulose hydrolysis by oxalic acid may also be desirable when converting a Kraft pulp to a dissolving grade pulp since a secondary goal is to reduce the high degree of polymerization of cellulose found in Kraft pulp to improve the pulp solubility and reactivity during downstream derivatization (Qin et al., 2016; Chen et al., 2016). Therefore, we speculated that, although oxalic acid treatment would be less specific than xylanase enzymes, this lower specificity and smaller size might be exploited to access and remove a greater amount of hemicellulose while also reducing the degree of polymerization of pulp cellulose (Qin et al., 2016; Chen et al., 2016). This “double function” of oxalic acid may be beneficial towards converting conventional Kraft pulp to cellulose-rich dissolving pulp constituted of cellulose

exhibiting a reduced molecular weight. Xylanase and oxalic acid treatments were applied to hardwood Kraft pulp, a substrate which would present both reagents with a significant amount of accessible hemicellulose (>20%) with subsequent alkaline extraction and endoglucanase treatment required to reduce the degree of polymerization of cellulose. For both the chemically and enzymatically modified pulps, the residual hemicellulose content, cellulose intrinsic viscosity, crystallinity, accessibility and reactivity were investigated to compare with a commercial grade dissolving pulp. These tests were employed to determine if purification and modification of the pulp cellulose via the combined actions of hemicellulases and endoglucanases was more effective than using oxalic acid to convert the conventional Kraft pulp to a commercial dissolving grade cellulose feedstock.

3.1.2 Results and discussion

3.1.2.1 The specific activities of the enzymes

At the beginning, the enzyme activity of xylanase and endoglucanase were assayed. The monocomponent endoglucanase (Fibrecare R) and xylanase (Pulpzyme HC 2500) were provided by Novozymes, Franklinton, NC. The cellulolytic activity of endoglucanase was determined by the manufacturer and expressed in Endo Cellulase Units (ECU) on the substrate hydroxyethylcellulose and the activity of Fibrecare R was 4500 ECU/g. The activity of xylanase was measured by Birchwood Xylan Unit (BXU) development by Enzyme Development Corporation which uses commercial birch wood xylan as the substrate to test the xylose release during enzymatic hydrolysis for per unit enzyme in per second, and the activity of Pulpzyme HC 2500 was 1685 BXU/ml.

3.1.2.2 Effect of xylanases and oxalic acid on hemicellulose removal

The initial loadings employed for the xylanase (500BXU/g) and oxalic acid (0.057g/g) treatments were determined using the results of previous studies (Gehmayr and Sixta, 2012; Lu and Mosier, 2007). Previous work had shown that a temperature of $>130^{\circ}\text{C}$ was necessary to facilitate the oxalic acid hydrolysis of the cellulose component of Avicel (vom Stein et al., 2010; Qin et al., 2016). Since amorphous hemicellulose was the main target for removal by oxalic acid and the peeling reactions undergone by the cellulose component during Kraft pulping could potential have increased its susceptibility to acidic hydrolysis (Sjöström, 1981), we employed a lower temperature of 121°C for the oxalic acid treatment. Remarkably, despite their difference in size, it was apparent that both the xylanase and the oxalic acid treatment resulted in similar amounts of hemicellulose removal from the pulp. It was apparent that after both the enzymatic and chemical treatments a significant amount of hemicellulose remained in the pulp ($\sim 17\%$) (Figure 10).

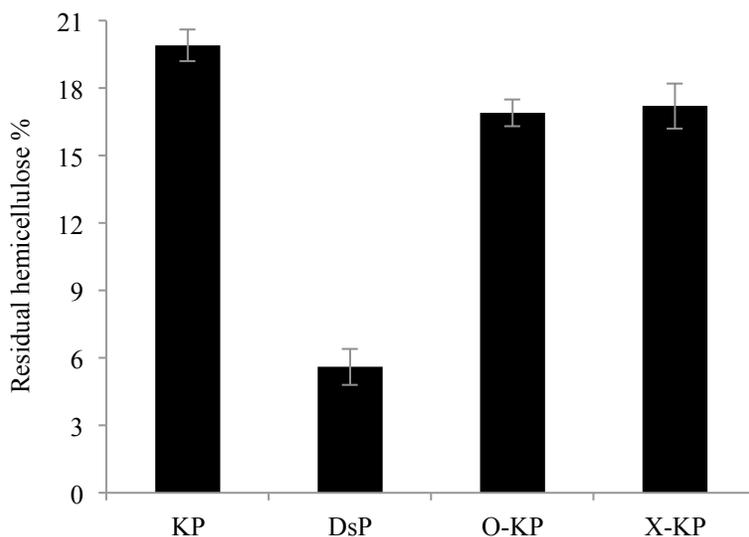


Figure 10: Impact of oxalic acid or xylanase treatment on the hemicellulose content of commercial Kraft pulp. Oxalic acid treatment was performed at a pulp solids loading of 5% and a temperature of 121°C in an autoclave for 1 hour using an oxalic acid loading of 0.054g/g. Xylanase treatment was performed at pulp consistency 5% at a temperature 60°C, 2 hours with a xylanase loading of 500 BXU. KP: Kraft pulp. DsP: Commercial dissolving-grade pulp; O-KP: oxalic acid treated Kraft pulp; X-KP: xylanase treated Kraft pulp.

It was hypothesized that the loading of oxalic acid may be a factor limiting its ability to remove hemicellulose from Kraft pulp. However, it was shown that doubling the oxalic loading from 0.057g/g to 0.113g/g did not enhance hemicellulose removal to a great extent, but instead reduced the pulp yield below 80% (Figure 11). These results indicated the non-specific cleavage of cellulose by oxalic acid when the loading in the reaction was increased. Similar to the oxalic acid treatment, increasing the xylanase dosage on both the Kraft and a commercial dissolving pulp did not provide additional hemicellulose removal (Figure 12). It was apparent that regardless of whether the larger, more specific xylanase enzymes or the smaller less-specific oxalic acid was added to the pulp, there was a limit to the amount of hemicellulose that could be removed. To determine if the hemicellulose could be enzymatically solubilized if cellulose is removed as a structural impediment, xylanases were then added in combination with cellulases to the Kraft pulp which resulted in a full conversion to sugars of the pulp. These results indicated that the hemicellulose was inaccessible to the enzyme treatment regardless of the dosage that was employed, while cellulose loss occurred at higher oxalic acid loadings indicating its lack of specificity compared to the enzymatic treatment (Figure 11).

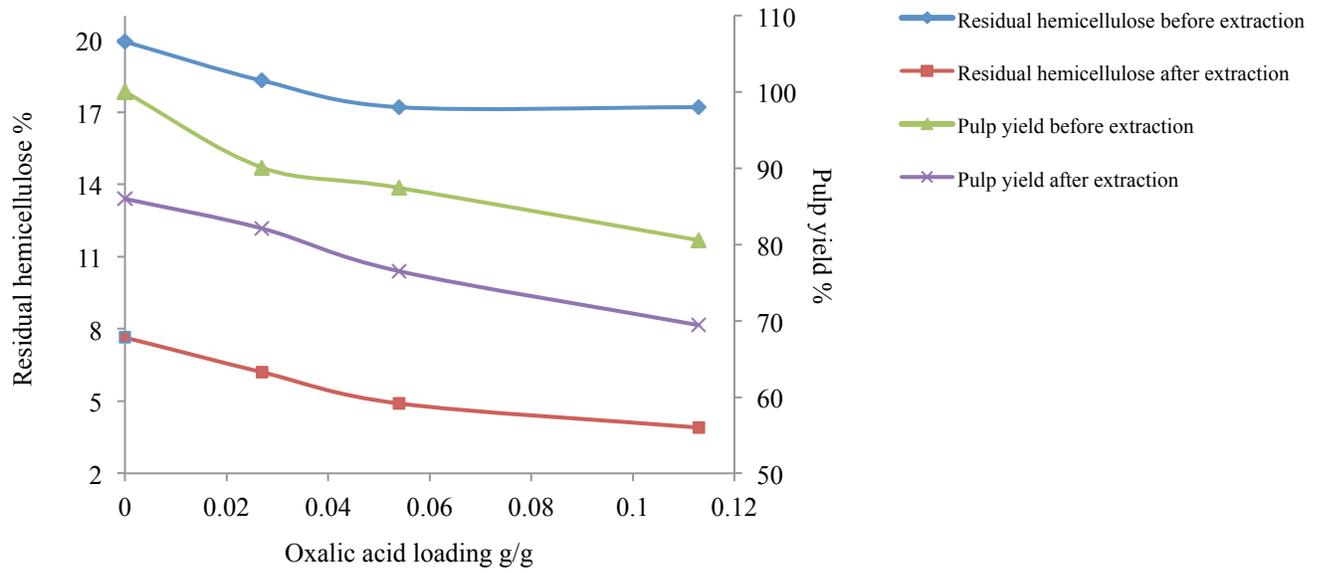


Figure 11: The impact of oxalic acid loading during autoclave treatment on the residual hemicellulose content of hardwood Kraft pulp. Oxalic acid treatment was performed at a pulp consistency of 5%, a temperature of 121°C in an autoclave for 1 hour. As specified, some pulps were subsequently subjected to an alkaline extraction at a pulp consistency of 7% using a 7% NaOH charge at room temperature for 30 min in an orbital shaker rotating at 150rpm.

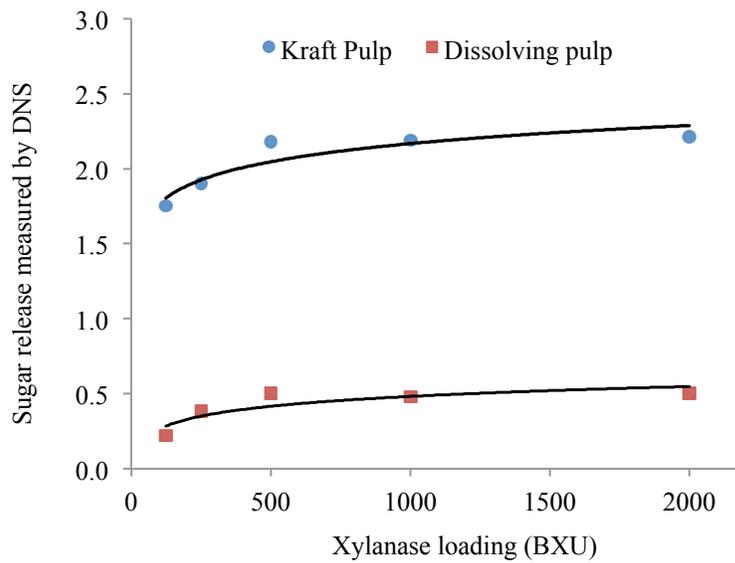


Figure 12: Impact of xylanase loading on xylose release from Kraft pulp and dissolving pulp measured using the dinitrosalicylic acid method. Xylanase hydrolysis was conducted at 60°C, 5% pulp solid loading for 2 hours at a xylanase loading of 500 BXU/g pulp.

3.1.2.3 Application of alkaline extraction to remove hemicellulose

Unlike previous work utilizing enzymes and oxalic acid to remove hemicellulose from substrates for downstream conversion of the sugar to fuels (Scordia et al., 2011; Lee et al., 2011; 2013; Sixta and Schild, 2009), most of the literature using xylanases for hemicellulose removal from pulps has employed a subsequent alkaline extraction to promote the solubilization of the hemicellulose fragments (Sixta and Schild, 2009; Ibarra et al., 2010). The residual hemicellulose after the hemicellulase treatments is enzymatically cleaved but likely remain trapped in the cellulose matrix thus requiring the alkaline extraction. It should be noted that the hemicellulose in Kraft pulp undergo de-branching during the pulping process and likely becomes more closely associated with the linear cellulose/hemicellulose components due to the removal of lignin (Viikari et al., 1994). Therefore, it is likely that the oxalic acid and xylanase treatments solubilized a portion of the lower molecular weight xylan while the remaining hemicellulose required a subsequent alkaline extraction to facilitate its removal. Previous work has shown that paper grade pulp could be converted to dissolving pulp using two successive alkaline extraction steps to decrease pulp xylan content to a level below 5% (Ibarra et al., 2010). Since it appeared that an alkaline extraction was necessary to reduce the hemicellulose content of the Kraft pulp regardless of whether xylanases or oxalic acid were used for the pulp treatment, we then investigated the effect of the combination of either mild oxalic or xylanase treatments with subsequent alkaline extraction. However, as will be discussed below, it has also been shown that increasing the concentration of alkali during the extraction of hemicellulose can compromise downstream cellulose reactivity (Gehmayr and Sixta, 2012). Therefore, we first assessed the effects of the alkaline concentration employed during the alkaline extraction on the effectiveness of hemicellulose removal from the pulp and pulp reactivity.

It has been shown that the treatment of cellulose at an alkaline concentration of >8% can result in the conversion of cellulose I to cellulose II (Gehmayr et al., 2011; Ibarra et al., 2010; Sixta and Schild, 2009). In addition, from a practical standpoint, increased concentrations of alkali utilized during pulp processing also places an additional load on pulp mill recovery processes and thus can be viewed as a bottleneck in the pulp mill operations (Ali and Sreekrishnan, 2001). It was apparent that a NaOH concentration of 7% was ideal for the alkaline extraction, as increasing the concentration from 7 to 8% only resulted in an additional 1% reduction in the hemicellulose content (Figure 13). Therefore, 7% NaOH was chosen as the alkali concentration used for the subsequent pulp treatments in this work to limit the alkali loading and the potential for reduced reactivity as a result of fibril aggregation. This concentration has also been used in previous literature (Köpcke et al., 2010).

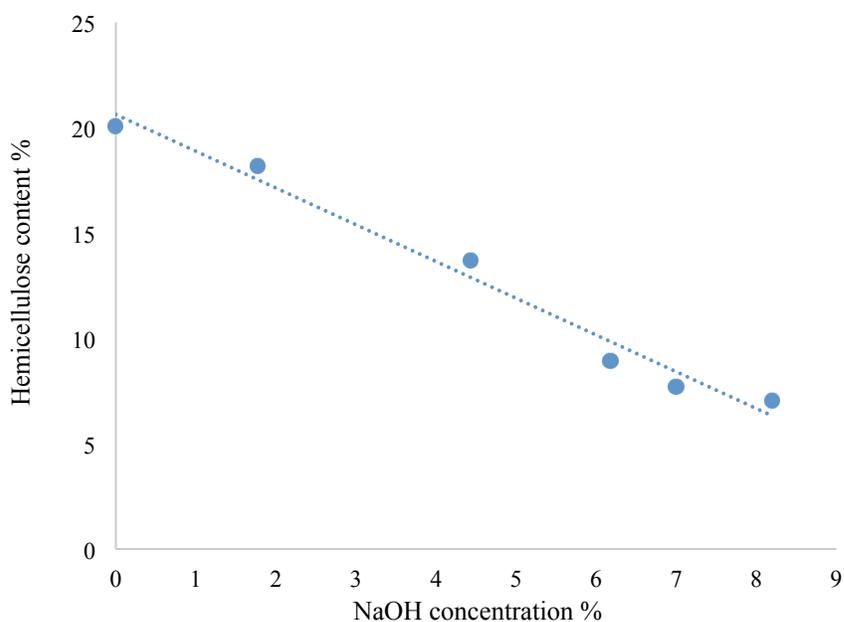


Figure 13: Impact of sodium hydroxide concentration on the residual hemicellulose content of kraft pulp. The alkaline extraction was conducted using a pulp consistency of 7% at room temperature, for 30 minutes.

3.1.2.4 Final characteristics of converted dissolving-grade pulps

Combining either the xylanase or oxalic acid with the subsequent alkaline extraction at 7% NaOH (O-CCE, X-CCE) reduced the hemicellulose content of the conventional Kraft pulp from 19.9% to the range of a typical dissolving-grade pulp (4-6%) (Table 6). Increasing the oxalic acid loading to 0.113g/g with the subsequent alkaline extraction decreased the hemicellulose content to 3.9%. However, the pulp yield decreased to a level below 70%, indicating that the treatment lost specificity as a greater amount of cellulose was being removed compared to hemicellulose (Figure 11). An oxalic acid loading of 0.057g/g (O-CCE) reduced the pulp yield to 76.5%, while decreasing the hemicellulose content by approximately 15%. These results suggested an additional 10% of the cellulose was lost during the oxalic acid treatment of cellulose when compared to the X-CCE sample where the hemicellulose content was reduced by approximately 14% but the pulp yield was 84.1% (Table 6). Therefore, it was apparent that the xylanase treatment was far more specific than the oxalic acid treatment as there was only a slight loss of cellulose when using the xylanase treatment to reduce the hemicellulose content.

Table 6: Characteristics of the different treated pulps. KP: Kraft pulp. DP: Dissolving pulp. O: Oxalic acid (0.057g/g) treated Kraft pulp. O-CCE: 7% sodium hydroxide extracted O. X: Xylanase (500BXU/g) treated Kraft pulp. X-CCE: 7% sodium hydroxide extracted X. X-CCE-EG: EG (45ECU/g) treated X-CCE. CCE: 7% sodium hydroxide extracted Kraft pulp

Substrates	Cellulose (%)	Hemicellulose (%)	Lignin (%)	S18 (%)	S10 (%)	Yield (%)	Specificity (%)	Crystallinity (%)
KP ^a	79.2	19.9	0.9	13.4	14.7	100	-	72
DsP	92.6	5.6	0.5	1.1	1.9	-	-	71
O	82.5	16.9	0.3	14.7	16.6	87.4	37	63
O-CCE	94.8	4.9	0.3	4.1	7.0	76.5	64	72
X	83.3	17.2	0.8	13.9	15.6	96.2	96	68
X-CCE	93.9	5.6	0.5	1.4	2.2	84.1	90	70
X-CCE-EG	94.0	5.7	0.3	3.3	4.8	82.3	91	72
CCE	92.0	7.7	0.3	3.0	4.0	86.0	95	63

^aKP: Kraft pulp. DsP: Dissolving pulp. O: Oxalic acid (0.057g/g) treated Kraft pulp. O-CCE: 7% sodium hydroxide extracted O. X: Xylanase (500BXU/g) treated Kraft pulp. X-CCE: 7% sodium hydroxide extracted X. X-CCE-EG: EG (45ECU/g) treated X-CCE. CCE: 7% sodium hydroxide extracted Kraft pulp

Treatment of the Kraft pulp exclusively with an alkaline extraction (7% NaOH) reduced the hemicellulose content to 7.7%. Therefore, the combination of oxalic acid/alkaline extraction or xylanase/alkaline extraction underwent an additional reduction in hemicellulose content by 2-3%. Similar to the work reported here, previously it was shown that treating conventional Kraft pulp with a combination of xylanase and alkaline extraction could result in a lower hemicellulose content than when applying two stages of alkaline extraction to a conventional Kraft pulp (Köpcke et al., 2010). The additional efficacy of the oxalic acid/xylanase treatment over the

exclusive use of an alkaline extraction is likely due to the reduction in the molecular weight of the hemicellulose during the initial oxalic acid/xylanase treatment to a level where the hemicellulose is solubilized during the subsequent alkaline extraction. It has also been postulated that hemicellulose removal prior to alkaline extraction increases the permeability of the pulp to the sodium hydroxide, resulting in enhanced xylan removal (Viikari et al., 1994).

It has been reported that sodium hydroxide at a concentration of 10% is capable of dissolving hemicellulose and cellulose molecules with molecular weights of less than 25000 (Strunk, 2012). However, water can only solubilize cellulose oligomers with a molecular weight of lower than 2000. Therefore, the oxalic acid or xylanase treatment likely lowers the molecular weight of a portion of the residual hemicellulose in the pulp to an intermediate molecular weight between 2000 and 25000 facilitating its solubility in the subsequent alkaline extraction. It should be noted that since Kraft pulping aims to remove lignin while preserving the integrity of the cellulose and hemicellulose, the residual hemicellulose in Kraft pulp is expected to have a relatively high molecular weight and be less prone to solubilization during alkaline extraction (Dahlman et al., 2003). Therefore, although the initial data suggested that both the xylanase and oxalic acid had only limited accessibility to the pulp hemicellulose (Figure 10), it was apparent that both the xylanase and oxalic acid were able to react with some of the pulp hemicellulose, thereby facilitating hemicellulose removal by subsequent alkaline extraction. However, the action of oxalic acid appeared to be far less specific than xylanase.

The purity of dissolving grade pulps is crucial to both the ability to process the cellulose and the mechanical strength of the resulting cellulose derivatives such as viscose fibres. The presence of impurities such as low molecular weight cellulose and hemicellulose that have a high susceptibility to react with alkali can deteriorate the uniformity and compromises the strength of

the polymer mixture, especially when the resulting cellulose is spun into fibres for textile applications (Strunk, 2012). Pulp purity is related to the molecular weight of the components in pulps and it can be characterized by the alkaline solubility in 10% and 18% sodium hydroxide providing standard S10 and S18 values (Strunk et al., 2013). Typically, the 10% NaOH solubilizes both the hemicellulose and low molecular cellulose (S10) while the 18% NaOH primarily solubilizes the hemicellulose impurities (S18) in the pulp (Strunk, 2012). Therefore, higher S10 and S18 values indicate a higher amount of low molecular fragments that are soluble in sodium hydroxide and thus lower pulp purity. It was apparent that the oxalic acid-treated pulp (O) had a higher alkaline solubility and lower pulp yield relative to the enzyme-treated pulps (Table 6). This can be attributed to the non-specific cleavage of cellulose chains into small fragments during the treatment, resulting in a greater amount of carbohydrate dissolution during the S10 measurement. As well as pulp purity, another critical requirement for dissolving grade pulp is to maximize the reactivity of the three hydroxyl groups on cellulose at C₂/C₃/C₆ with derivatizing reagents to create products such as cellulose xanthate, nitrate and acetate (Sjöström, 1981). The reactivity of a given pulp is heavily influenced by the molecular weight of cellulose and the accessibility of the cellulose hydroxyl groups to derivatizing agents. Typically substrates with low molecular weight cellulose in addition to larger pores and a disrupted cellulose crystal structure exhibit increased reactivity (Strunk et al., 2011).

3.1.2.5 Correlating intrinsic viscosity and accessibility with pulp reactivity of converted dissolving-grade pulp

Previous work has indicated that pulp reactivity is inversely proportional to the intrinsic viscosity, or the average cellulose molecular weight in a given pulp sample (Engström et al., 2006). The decrease in molecular weight of cellulose is also a key processing step in the production of dissolving pulp which is achieved using processes such as the hypochlorite bleaching stage which acts to both remove lignin and impart a slight decrease in the molecular weight of the cellulose (Köpcke et al., 2010). As discussed above, we had anticipated that the non-specific oxalic acid treatment would simultaneously remove hemicellulose while reducing the molecular weight of cellulose. For comparison to the oxalic acid treatment, to reduce the molecular weight of the xylanase treated pulp, a subsequent endoglucanase treatment was applied to the xylanase (X-CCE) treated pulp. Despite the nonspecific nature of the oxalic acid treatment, both of these pulp samples (O-CCE, X-CCE-EG) had remarkably similar levels of intrinsic viscosity as they both were able to reach the intrinsic viscosity range of a commercial dissolving grade pulp. Consequently, the decrease in viscosity as a result of either the oxalic acid or the EG treatment also resulted in an increase in pulp reactivity (Figure 14).

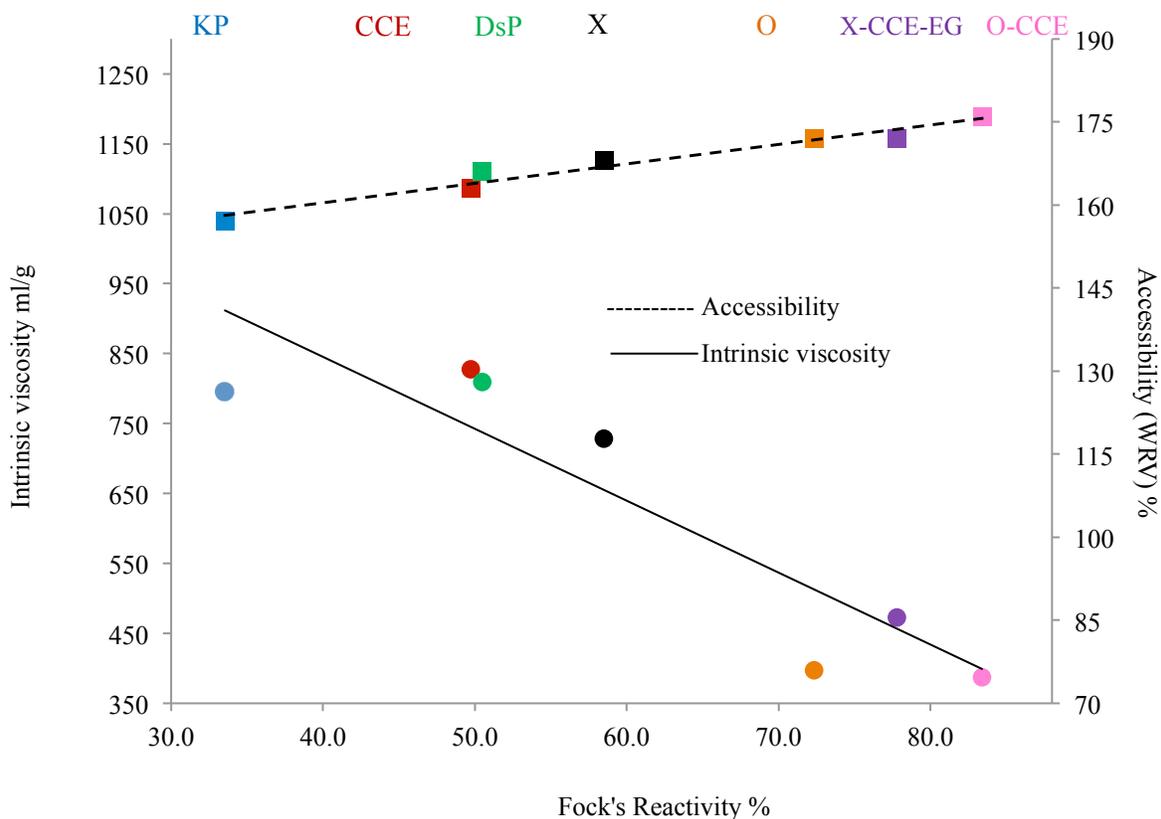


Figure 14: Relationship between cellulose reactivity, intrinsic viscosity and accessibility (WRV) of treated pulps. Oxalic acid treatment was performed at a chemical loading of 0.057 g/g for 1 hour at 121°C using a 5% pulp consistency. Xylanase treatment was performed at an enzyme loading of 500 BXU/g for 2 hours at 60°C using a 5% pulp consistency. The alkaline extraction as performed at a NaOH concentration of 7% for 30min at room temperature using a 7% pulp consistency. The endoglucanase treatment (EG) was performed at an enzyme loading of 45 ECU/g for 2 hours at 50°C using a 5% pulp consistency. KP: Kraft pulp. DsP: Dissolving pulp. O: Oxalic acid treated Kraft pulp. O-CCE: 7% sodium hydroxide extracted O. X: Xylanase treated Kraft pulp. X-CCE: 7% sodium hydroxide extracted X. X-CCE-EG: EG treated X-CCE. CCE: 7% sodium hydroxide extracted Kraft pulp.

Both the decrease in intrinsic viscosity from 796 to 395 ml/g and crystallinity from 72 to 63 % indicated the oxalic acid treatment resulted in the non-specific cleavage of cellulose and deconstruction of recalcitrant cellulose crystal structures (Table 6). These decreases in the cellulose viscosity and crystallinity likely increased the accessibility to the xanthation reagents (carbon disulfide) as there was a 1.5-fold increase in reactivity as compared to the initial Kraft pulp (Figure 14). However, due to the high specificity of xylanase for the removal of

hemicellulose and its limited activity on cellulose, both the intrinsic viscosity and crystallinity of the xylanase treated pulp (X) were barely affected by the xylanase treatment (Table 6). Therefore, endoglucanase treatment was applied after the xylanase treatment in order to decrease the molecular weight of the cellulose and, hopefully, increase its reactivity. Upon EG treatment, it was found that the reactivity of final pulp was 1.3-fold higher than the Kraft pulp. It had been anticipated that the endoglucanase would react with both the amorphous regions between crystalline microfibrils and the less ordered regions of cellulose fibrils located on the surface. This would result in increased fibre swelling and thus accessibility of the pulp towards derivatization reagents (Engström et al., 2006). Compared to the other methods that have been used to explore the accessibility of cellulose to cellulases for subsequent enzymatic degradation, such as Simons' staining (Chandra et al., 2008a) and cellulose binding modules (CBM) (Gourlay et al., 2012), the methods used for evaluating cellulose reactivity must be able to measure accessibility at a smaller scale, as cellulases are typically much larger (30-50kDa) compared to the smaller chemical reagents used for derivatization of cellulose. The water retention value (WRV), which has been reported to characterize the pores within the substrate, has been shown to be an easy method of characterizing cellulose providing results that directly correlate with those measured using more laborious pore volume measurement techniques (Jayme and Roffael, 1970). The WRV has also been shown to be a useful measurement for characterizing the swelling of pulp for subsequent papermaking and pretreated biomass for biological conversion (Luo et al., 2011). However, the current work shows WRV can also be used a good indicator of a dissolving pulp's reactivity. It was apparent that there was a positive correlation in between of pulp reactivity and accessibility ($R^2=0.96$). Since the WRV provides an indication of the ability of fibres to take up water and swell, fibres that have higher WRV are generally those that possess

higher bonding ability with water through the formation of hydrogen bonds at available free hydroxyl groups on cellulose as carbons 2, 3 or 6. Similarly, the one of the definitions of the reactivity of dissolving pulp is the ability of these hydroxyl groups to react with derivatizing reagents such as carbon disulfide. Therefore, the WRV seems to be a good estimate of the ability of hydroxyl groups to bond/react with small reagents due to their increased accessibility to free hydroxyl groups on cellulose.

In the case of the WRV, both low molecular weight cellulose and high hemicellulose content are characteristics that can enhance the water retention value (Gomes et al., 2014). It was shown that after oxalic acid/alkaline extraction and xylanase/EG/alkaline extraction the WRV increased from 157% to >170%. However, the increased WRV was accompanied by a concomitant decrease in the hemicellulose content from 20% to 4-6%, indicating the oxalic acid/alkaline extraction or the xylanase/EG/alkaline extraction likely enhanced the accessibility of the cellulose component of the pulps (Figure 14). Associated with the decrease of cellulose intrinsic viscosity and increase of WRV, a slightly increased cellulose crystallinity index from 68 to 72% was also exhibited after the EG treatment likely due to the hydrolysis of amorphous cellulose which consequently enriched the crystalline regions of cellulose (Sixta and Schild, 2009). As mentioned earlier, after oxalic acid treatment the crystallinity index was decreased. This decrease in crystallinity was likely due to the nonspecific cleavage of cellulose, resulting in more amorphous regions and shorter cellulose molecules.

One of the most crucial elements when comparing the efficiency of chemical and enzymatic approaches to upgrade Kraft pulps to dissolving grade pulps is the effective removal of the hemicellulose with minimal effects on the cellulose molecular weight and reactivity. In the work here we defined specificity as the weight ratio of hemicellulose in the total yield loss caused by

the hydrolysis of both hemicellulose and cellulose. Generally a high specificity was indicated by a low pulp yield loss or high hemicellulose content in the filtrate. It was shown that the xylanase treatment had an approximately 1.6-fold higher specificity toward the removal of hemicellulose than did the oxalic acid. Although the residual hemicellulose content from both of the treated pulps was similar (Table 6), the yield loss after oxalic acid treatment was 2.3-fold higher than the pulp that had undergone the xylanase treatment. Therefore, an additional 10% of the cellulose was likely cleaved and subsequently solubilized during the oxalic acid treatment. These results illustrate the highly specific nature of the enzymatic treatments compared to acid treatments. Xylanase treatment can remove hemicellulose while the subsequent addition of endoglucanases can tailor the molecular weight of the cellulose and enhance its reactivity.

3.1.2.6 Morphology of oxalic acid/enzyme converted dissolving-grade pulps

The high specificity of the enzymatic treatments compared to oxalic acid was also demonstrated by the resulting morphological changes undergone by the pulps after chemical and/or enzymatic treatments. These results indicated that the fibrillation observed with the control Kraft pulp was eliminated during subsequent alkaline extraction, leaving behind a smooth fibre surface (Figure 15). The enzyme treatment with subsequent alkaline extraction exhibited limited changes compared to the pulp that underwent alkaline extraction without enzyme treatment. In contrast, the oxalic acid treated pulp appeared delaminated, which likely increased its accessibility to carbon disulfide during subsequent derivatization reagents. However, as discussed above, the limited specificity of the oxalic acid treatment resulted in significant yield losses, likely due to the damage undergone by cellulose during oxalic acid treatment.

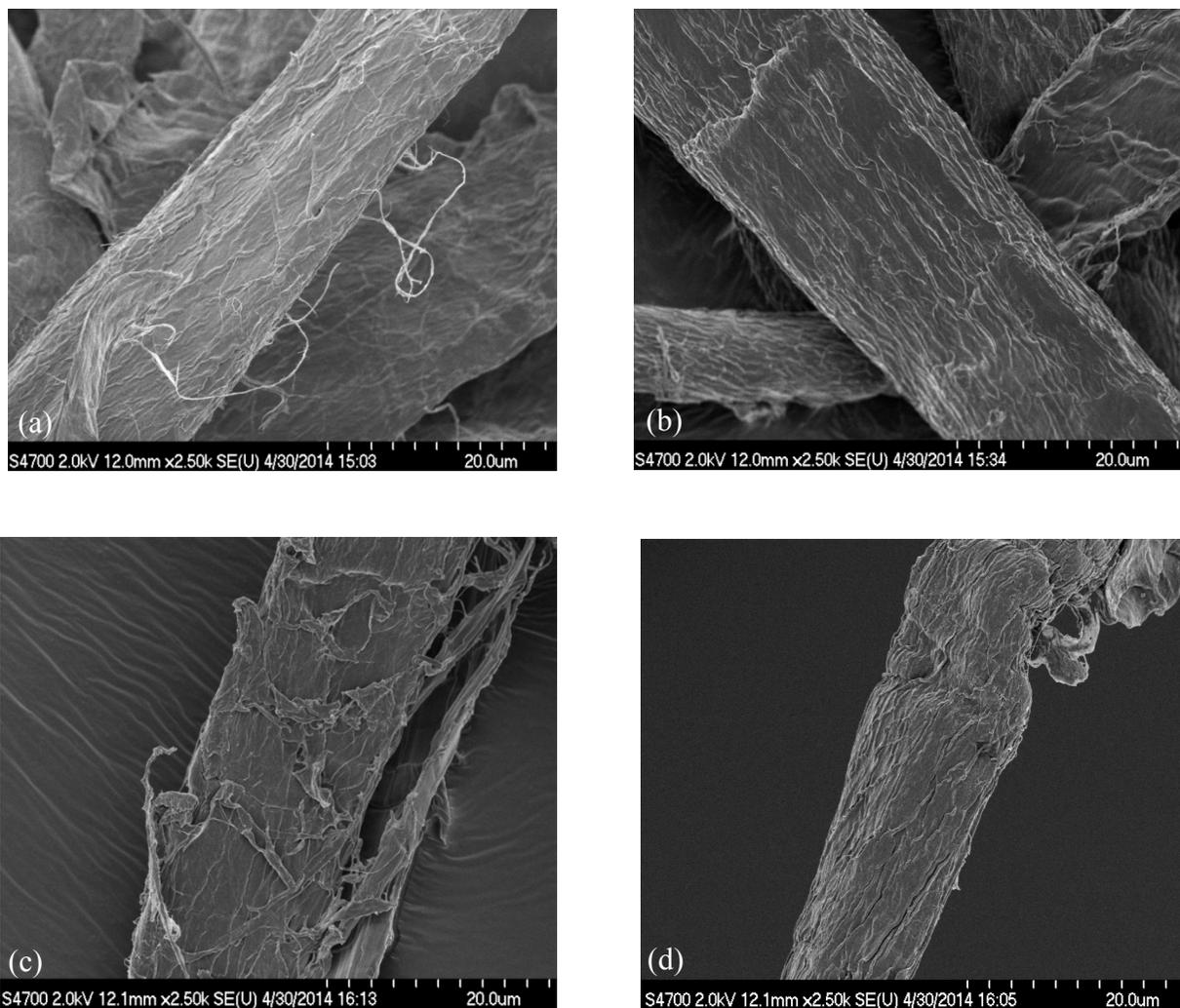


Figure 15: FE-SEM pictures of the different treated pulps (a) Kraft pulp (b) CCE: 7% NaOH extracted Kraft pulp (c) O-CCE: Oxalic acid (0.057g/g) treated Kraft pulp, followed by 7% NaOH extraction (d) X-CCE-EG: 7% NaOH extracted xylanase (500 BXU/g) treated Kraft pulp, followed by EG (45ECU/g) treatment

3.1.3 Conclusions

This work showed that a conventional Kraft pulp can be “upgraded” to dissolving grade pulp through the removal of hemicellulose and the enhancement of cellulose reactivity by using oxalic acid/CCE or xylanase/CCE with subsequent treatment with a mono-component endoglucanase. The accessibility of the hemicellulose and reactivity of cellulose after the CCE appear to be the main obstacles during the conversion process. Oxalic acid was previously reported to be a small,

potentially biomimetic acid, with a high specificity for removing hemicellulose. Therefore, we had treated the Kraft pulp with oxalic acid hoping to remove hemicellulose without compromising cellulose yield but rather cause sufficient damage to cellulose to enhance its reactivity during subsequent derivatization. However, the oxalic acid treatment could only solubilize a small amount of hemicellulose without the aid of an alkaline extraction and also randomly cleaved cellulose, resulting in undesired losses in pulp yield.

3.2 Exploring the role of hemicellulolytic and cellulose modifying enzymes on model substrates

3.2.1 Background

The work in 3.1 established the crucial role that the accessibility of hemicellulose plays in affecting its ease of removal from Kraft pulps. However, the application of hemicellulases to Kraft pulps for bleach boosting and the application of enzymes to reduce the hemicellulose content to convert Kraft to dissolving grade pulps have not been explored. Specifically, the effect of substrate characteristics on the ease of enzymatic action, as well as the specific action of each enzyme on the substrate remains unclear. Although some information on the action of cellulase components, xylanases and their synergy can be obtained from the literature on biochemical conversion of lignocellulosic biomass (Hu et al., 2011; 2013), it should be noted that the degradation of lignocellulose to monomeric sugars for bioconversion is quite a different reaction compared to the enzymatic reaction to modify pulp fibres for bleaching/converting Kraft to dissolving-grade pulp. Especially when considering both the duration of the reaction and enzyme loadings employed. It is likely that the 24-72 hours' reaction time typically employed for biochemical conversion to degrade lignocellulose presents a completely different dynamic than

the 1-2 hours' reaction time typically utilized for treating chemical pulps. The action of Family 10 (GH10) vs. Family 11 (GH11) xylanases on the properties of the xylan substrate is also known to be different (Hu, 2014). It has been shown that the GH10 xylanases are highly active on lower molecular weight xylo-oligosaccharides, thus it is likely that the substrate binding sites for these enzymes would be smaller. Although these enzymes have been shown to be highly specific for xylan, it has been hypothesized that they may also be active on lower molecular weight cellulose (Hu, 2014). Compared to most of the GH10 xylanases that can attack the third xylosidic linkage after a substituted residue, GH11 xylanases usually have a lower molecular weight and have been shown to work more effectively on linear xylan due to the steric hindrance presented by hemicellulosic sugar substituents (Biely et al., 1997; Collines et al., 2005). The work in this chapter was split into multiple sets of model substrates each aiding to answer specific questions including model hemicellulose and cellulose substrates to study the influence of their characteristics to the action of enzymes.

Earlier work had shown the existence of true “xylanases” that react with xylan with high specificity (Wong and Saddler, 1992). However, it was anticipated that the ability of either GH10 or GH11 xylanases to react with a given xylan sample would be dependent on its structural characteristics including its molecular weight and branching. Therefore, we initially added both GH10 and GH11 endo-xylanases with various commercially available xylan preparations to determine their activity including birch xylan, oat spelt xylan, and xylan isolated from hardwood Kraft pulp using alkaline extraction. Initially, we utilized each of these substrates to determine the activity of Multifect (predominantly GH11 xylanase) and HTec (predominantly GH10 xylanase) commercial xylanase preparations. We anticipated that the GH11 xylanase would be more effective on the less branched birch wood xylan, or xylan isolated from the

commercial Kraft pulp, while GH10 xylanase would be more active upon the oat spelt xylan which has more substituents (Viikari et al., 1994). It was hoped that this work would help reveal the specificity of the xylanases and the tendency of various xylanase preparations to deconstruct hemicellulose preparations. For example, it was expected that after the Kraft pulping process, the hemicellulose components would contain minimal branching due to the highly alkaline conditions utilized for the pulping reaction. Thus, it was likely that GH11 xylanases would be more effective in hydrolyzing the residual xylan in the pulp compared to GH10 xylanases.

In addition to the characteristics of the hemicellulose, it is likely that the accessibility of the cellulose itself will govern its ease of modification by enzymatic treatments such as endoglucanases and more importantly its overall reactivity. We anticipated that increased cellulose crystallinity would result in a decrease in cellulose reactivity due to the lower exposure of the hydroxyl groups. However, previous literature has found it challenging to link a given pulps cellulose reactivity to its crystallinity. In the bioconversion area, where the crystallinity of cellulose has been widely studied, there is still no consensus over the influence of the crystallinity of cellulose on enzymatic hydrolyzability of cellulose. Some authors argue that cellulose crystallinity is a key determinant of enzymatic hydrolysis rate (Chen et al., 2007; Hall et al., 2010), while others suggest crystallinity has little effect on hydrolysis (Agarwal et al., 2013; Converse, 1993; Kawakubo et al., 2010; Mansfield et al., 1999). Therefore, the effect of cellulose crystallinity on its reactivity with small chemical derivatization reagents is particularly worthy of investigation as it has received less attention than the literature on enzymatic hydrolysis of cellulose. It has been reported that treating Avicel microcrystalline cellulose with 85% phosphoric acid to produce phosphoric acid swollen cellulose (PASC) results in a complete dissolution of the sample and such treatment was shown to have no impact on the DP of

cellulose if performed under refrigerated conditions (Jeoh et al., 2007; Zhang et al., 2006). Work from Bommarius et al. also showed that PASC at a range of crystallinities could be obtained when varying the concentration of phosphoric acid from 75% to 80% for generating the swollen cellulose (Hall et al., 2010). Therefore, in this work, PASC samples with controlled degrees of crystallinity were produced using Avicel, to determine the impact of crystallinity on cellulose reactivity with derivatization reagents such as carbon disulfide.

Those substrate characteristics that are often involved in the slowdown of the reaction rate include surface area, porosity, the degree of polymerization, crystallinity, and the overall composition of the substrate (Hall et al., 2010). It has also been reported that splitting up fibrillar aggregates, disrupting crystallinity and increasing fibre surface area are required to obtain a highly reactive cellulose pulp (Strunk, 2012). Therefore, since all of the characteristics mentioned above relate to the accessibility of cellulose, the influence of cellulose surface area was also investigated. Mechanical refining has been shown to increase the surface area of cellulose pulps through external fibrillation, internal delamination and fibre cutting, which create certain microfibrils on the surface of fibres, swell and loosen the internal structures of fibres (Kerekes, 2005). It is expected that the normal force during the mechanical refining will enhance surface fibrillation and internal delamination of fibres, which could disrupt the crystalline structure of cellulose microfibrils and create more accessible surface area on pulps to increase available hydroxyl groups (Jones et al., 2013). Therefore, mechanical refining was applied to create pulps with varying surface areas so we could explore its influence on cellulose reactivity. As well as mechanical refining, previous work had indicated that pulp reactivity was inversely proportional to the intrinsic viscosity, or the average cellulose molecular weight of a given pulp sample (Engström, et al., 2006). The decrease in molecular weight of cellulose is also a key

processing step in the production of dissolving pulp which is achieved using processes such as the hypochlorite bleaching stage, which acts to both remove lignin and impart a slight decrease in the molecular weight of the cellulose (Engström, et al., 2006). In the work reported here, the pulp samples with controlled DP were used to study their impact to cellulose reactivity.

The “model” substrates mentioned above should help elucidate the effect of pulp characteristics on the ease of enzymatic action and accessibility to chemical reagents. The important role that the accessibility of hemicellulose plays during the conversion of Kraft pulp to dissolving pulp was assessed by comparing the xylanase hydrolysis results on isolated Kraft xylan. This should represent a xylan substrate which is completely accessible, as opposed to a traditional Kraft pulp, where we have shown in our preliminary experiments that a portion of the less accessible xylan is buried inside the cellulose matrix and could not be hydrolyzed by xylanases unless cellulases were added to the enzyme mixture. Creating the cellulose model substrates with varying crystallinity, DP or surface area should aid our understanding of how the various characteristics of cellulose pulp influence the ability of the hydroxyl groups on cellulose to react with chemicals.

3.2.2 Results and discussion

3.2.2.1 Chemical composition of "model" xylan substrates

Most of the xylan backbones from hardwood consists of at least 70 β -xylopyranose residues with an average degree of polymerization between 150 and 200, linked by β -1, 4-glycosidic bonds (Beg et al., 2001). On average, every tenth xylose residue carries a 4-O-methyl- α -D-glucuronic acid residue attached to xylose at C₂ position (Beg et al., 2001; Teleman et al., 2001). Hardwood xylans are generally highly acetylated to increase their solubility in water. Most of the xylose

residues contain acetyl groups at the C₂ and C₃ positions, and more frequently at the C₃ position than C₂ (Beg et al., 2001). Wood xylan is partially degraded under the alkaline conditions employed during Kraft pulping due to peeling reactions. The acetyl substituents are generally hydrolyzed at the very beginning of the Kraft cook, while the linkage between uronic acid and xylose are resistant to alkaline conditions (Rydholm and Gedda, 1967; Malinen and Sjoström, 1975). Furthermore, a major portion of the 4-O-methyl- α -D-glucuronic acids are then converted into 4-deoxyhex-4-enuronic acid (Hex A) groups during the Kraft cooking process (Buchert et al., 1995; Teleman et al., 1995). In addition, during bleaching with ozone or chlorine dioxide, the 4-deoxyhex-4-enuronic acid groups can be subsequently degraded (Buchert et al., 1995; Vuorinen et al., 1999).

Most of the xylans can be obtained by extracting the starting materials such as wood chips or pulps by using sodium hydroxide or potassium hydroxide. The isolation of xylans from intact wood or pulps also leads to modifications in the structure of the xylans, such as most of the inherent acetyl side groups that decorate the backbone of the hemicellulose macromolecules are readily de-branched when the wood or pulps are subjected to an alkali extraction (Viikari et al., 1994; Sunna and Antranikian, 1997). A higher efficiency of extraction of xylan can be achieved by using higher alkali concentrations (Sjoström and Enström, 1967). However, the xylans isolated by alkaline extraction methods have some impurities such as glucose and lignin due to the covalent bonds formed between the hemicellulose and lignin in the form of LCC's. Low molecular weight cellulose can also be solubilized during the extraction process. In addition, it was found that under certain conditions (such as the considerable removal of xylan side groups, and the extensive removal of water), the high molecular weight xylan has a high potential to

crystallize together (Roelofsen 1954; Nieduszynski and Marchessault, 1972; Chanzy et al., 1979), although, originally, these xylan polysaccharides are not crystalline in situ in the wood cell wall.

Table 7: Chemical composition (%) of various “model” xylan substrates.

Substrates	Cellulose	Hemicellulose	Lignin	Acetyl groups	Me-GlucA^a
Birchwood chips	43.9	28.9	20.2	-	-
Birchwood xylan	1.2	89.8	1.5	0.1	3.2
Kraft pulp (KP)	79.2	19.9	0.9	0	0
Isolated xylan from KP	0.5	98.1	0.0	0	0
Oat spelt	15.5	81.7	2.3	-	4.3

^a**Me-GlucA:** 4-O-Methyl-D-glucuronic acid

The chemical compositions of the different substrates were analyzed after acid hydrolysis under high temperature and pressure (Table 7). The alkaline extraction method was employed. Since most of the acetyl groups are readily removed when xylan is subjected to alkali extraction, most of the xylan substrates contained very low amounts of acetyl groups (Table 7). The main hemicellulosic sugar in birch wood xylan was xylose, with only 3.2% of the sugars derived from glucomannan. A high content of glucose and 4-O-methyl-D-glucuronic acid were detected in the oat spelt xylan. After Kraft pulping, no acetyl groups were detected and the xylan content was 20%. For the isolated xylan from Kraft pulp, there were almost no other components. Xylose accounted for more than 98% over the total weight, which was probably because most of the low

molecular weight cellulose and lignin had been removed during the Kraft pulping and multiple bleaching steps.

3.2.2.2 Xylanase purification, protein loading and specific activity quantification

The isolated substrates in 3.2.2.1 were then used to study the action of xylanases. The full Multifect xylanase preparation (GH11 xylanase) and HTec (GH10 xylanase) xylanase preparation were employed to study their hydrolysis mechanism on xylans. The primary xylanases from Multifect and HTec preparations had been purified during previous work in our laboratory (Hu, 2014). As expected, the major protein within the Multifect xylanase was found to be GH11 endo-xylanase (Figure 16), with this protein constituting more than 80% of the total protein present in the preparation (Hu, 2014). The GH11 xylanase showed a relatively low molecular weight (20kDa) (Figure 16), which corresponded with previous reports (Viikari et al., 1994; Collins et al., 2005). The GH10 endo-xylanase, which had a much larger molecular weight compared to GH11 endo-xylanase, was purified from HTec (Figure 16). Thus, the main protein in the Multifect enzyme preparation had a much smaller size than the xylanase from the HTec enzyme preparation.

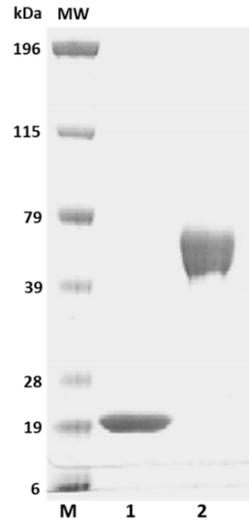


Figure 16: SDS-PAGE of purified enzymes: GH11 endo-xylanase (lane 1), GH10 endo-xylanase (lane 2) and marker (lane M). Proteins were identified by LC-MS/MS. Proteins are named according to their glycoside hydrolase family (Hu, 2014)

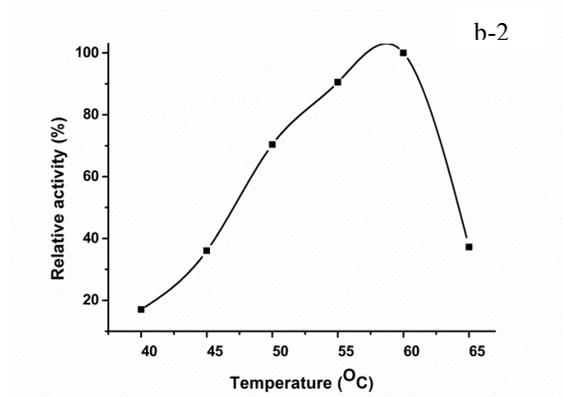
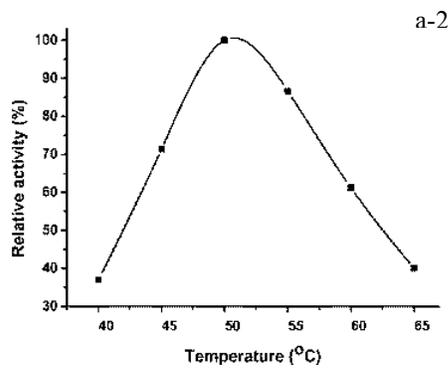
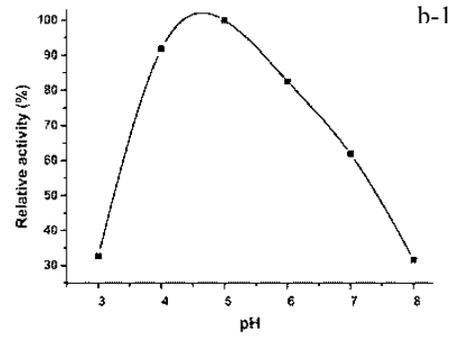
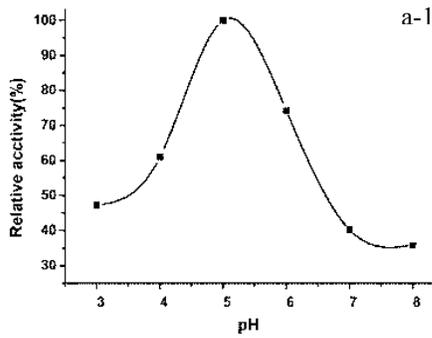


Figure 17: Impact of pH/temperature to the activity of commercial xylanases (a) Multifect xylanase (b) HTec xylanase. Xylanase hydrolysis was applied on birch wood xylan (Lingfeng Long, 2016).

When the optimum hydrolysis conditions such as pH and temperature for both the commercial xylanases preparations were compared, it was found Multifect xylanase preparation had a relatively higher adaptive hydrolysis pH and lower optimum temperature as compared to the HTec xylanase preparation. It was found that more than 90% of the xylanase activity was reserved for Multifect xylanase when the pH was 5-5.5 (Figure 17 a-1), and the optimum pH for the HTec xylanase preparation was lower at pH 4.5-5 (Figure 17 b-1). When a temperature of 60°C was reached (Figure 17 b-2), the HTec xylanase retained nearly all of its activity while the Multifect xylanase retained less than 60% of its activity, indicating the potentially higher thermal stability of the HTec xylanase. This observation was similar to previous reports that GH11 xylanase generally had a pH optimum around 5.0-5.5 (Tenkanen et al., 1992a; Viikari et al., 1994; Collins et al., 2005), while GH10 xylanase had a pH optimum around 4.0-4.5 (Tenkanen et al., 1992a; Viikari et al., 1994; Collins et al., 2005).

Table 8: Protein content (mg/ml), specific activities (U/ml), and xylanase activity on birch wood and oat spelt xylans (U/mg) results of Multifect and HTec xylanases. Protein content and specific activities were applied on commercial xylanases preparations. Activity on birch wood and oat spelt xylans were by purified xylanase.

Xylanases	Protein content	Xylanase activity	Birch wood xylan ^a	Oat spelt xylan ^b
	mg/ml	U/ml	U/mg	U/mg
HTec	31.1	1600	100	160
Multifect	29.1	2600	190	230

^aBirch wood xylan and ^bOat spelt xylan activity were from Hu, 2014

As expected, both of the GH10 and GH11 xylanases were able to hydrolyze the xylan model substrates. Multifect had a slightly higher protein content than HTec commercial xylanase preparation (Table 8). However, the purified xylanase from Multifect (GH11 xylanase) showed a much higher hydrolytic activity on the xylan substrates (190-230 U/mg) as compared to the purified xylanase from HTec (xylanase) (100-160 U/mg). As a result, under the same protein loading, the commercial preparation of Multifect xylanase of which the primary xylanase was GH11 xylanase had a 1.5-fold higher xylanase activity than HTec. The different xylanase activities had also been previously observed by some other researchers (Zhang et al., 2011b). In the work reported below, 10mg/g was the total protein loading employed for both xylanases which is the current standard used in recent research on enzymatic treatment of lignocellulose (Hu et al., 2011; 2013). Due to the higher xylanase activity, smaller size and partial cellulase activity exhibited by Multifect xylanase, this enzyme preparation may be more effective for removing the “hard to access” xylan from Kraft pulps. Multifect xylanase would likely be more effective for hydrolyzing the xylan contained in the model substrates below.

3.2.2.3 Xylanase hydrolysis on xyans from different substrates

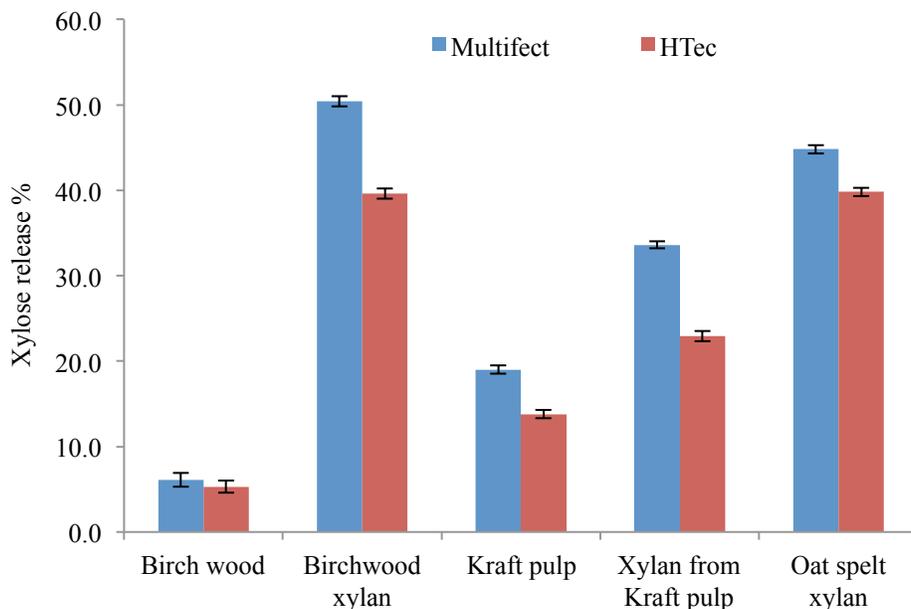


Figure 18: Solubilization of birch/oat spelt xyans with Multifect and HTec commercial xylanase preparations. Xylanase hydrolysis was applied on ground birch wood, isolated birch wood xylan, Kraft pulp, isolated xylan from Kraft pulp and isolated oat spelt xylan. Xylanase hydrolysis was conducted under 50°C, 150rpm shaking, and 5% substrate consistency for 2 hours. 10 mg/g protein loading was used in this work.

Samples from ground birchwood, isolated birchwood xylan, Kraft pulp, isolated xylan from Kraft and isolated oat spelt xylan were hydrolyzed using the two commercial xylanases. The degree of solubilization of all of the enzymatically treated samples was calculated from the detected monomeric carbohydrates after a second acid hydrolysis. This hydrolyzed the oligomeric xylose to monomeric xylose from the carbohydrates solubilized during the xylanase hydrolysis. Less than 10% of the total xylan was solubilized by both xylanases from ground birch wood (Figure 18). It was likely that the low xylan hydrolysis yield was due to the poor accessibility of the xylan in the intact wood powder to xylanases. For example, it has been reported the average pore size of the wood chips was less than one fifth of the cooked pulps, and these small pores are particularly inaccessible to enzymes (Stone et al., 1969; Grethlein, 1985).

In addition, as explained above, in plants and wood, the xylan is either covalently bonded with lignin via LCC or it forms complex hydrogen bonds with cellulose. As a result, the high retention of cellulose and lignin from the wood would physically hinder the interaction of xylanases with xylan. However, in contrast to the fibre-bound substrates, isolated xyans from wood should be solubilized more extensively by xylanases. The Multifect xylanase preparation was more efficient (~1.2-fold higher) than the HTec xylanase during the hydrolysis of the isolated birch wood xylan, reaching a hydrolysis yield of 50% and 40% respectively (Figure 18), indicating the important role that the accessibility of xylan plays for effective xylanase hydrolysis.

After the Kraft cooking and multiple bleaching steps, during which most of the lignin was removed, treatment with both commercial xylanase preparations could barely reach a hydrolysis yield of 20%. The increased accessibility of xylan to xylanases from bleached pulp as compared to ground birch wood was anticipated to be a result of factors such as, an increase in pore size and surface area, the removal of most of the lignin, the decrease in cellulose molecular weight and the relocation of xylan component (Viikari et al., 1994). It has been shown that after the Kraft pulping and bleaching processes, the median pore size of the pulp as compared with the starting wood chips was improved from 10 to 50 Å (Stone and Scallan, 1968), and most of those pores from cooked pulp were accessible to xylanase. The re-precipitation of xylan during cooking and bleaching processes was also regarded as an important factor to enhance its removal by xylanases. In previous work, one of the proposed mechanisms of enzymatic bleaching of Kraft pulp was its action on the re-precipitated xylan relocated on the surface of the cellulose fibres which occurs at the final stages of Kraft pulping after the removal of most side groups from xylan (Kantelinen et al., 1991). It was also found most of the xylan relocated on the surface of the fibres was highly accessible to xylanase (Kantelinen et al., 1993).

As expected, when the xylan was isolated from Kraft pulp, it was hydrolyzed more extensively by both xylanases than the xylan contained in the original pulps. Surprisingly, the overall hydrolysis yield by xylanases on the isolated xylan from Kraft pulp was lower than that from the isolated birch wood xylan, suggesting that, under certain conditions, xylans might crystallize due to the removal of sides groups or long xylan chains after undergoing the aggressive cooking and bleaching conditions which limits subsequent accessibility to the enzymes (Roelofsen 1954; Nieduszynski and Marchessault, 1972; Chanzy et al., 1979).

When an equivalent protein loading was applied to all of the model substrates, the Multifect xylanase preparation was able to access and remove a greater amount of xylan (Figure 18). One probable reason was as discussed above, the primary xylanase in Multifect preparation, GH11 xylanase, had a higher protein activity (1.5 times) than the primary xylanase in the HTec preparation that contains mainly the GH10 xylanase. Another possible reason for the increased effectiveness of the Multifect xylanase was that it has a smaller size than the HTec xylanase preparation (Figure 16), which could facilitate its access to the xylan in the model substrates. In addition, a small amount of cellulase activity was found in the commercial Multifect xylanase preparation. During the hydrolysis, the Multifect xylanase could hydrolyze part of the cellulose component (~3%), further enhancing the accessibility of xylan to xylanase. As well as this potential synergism, when comparing the xylose release resulting from the hydrolysis of oat spelt xylan to birch wood xylan, there was a smaller difference in xylose release with the Multifect xylanase preparation than that from the HTec xylanase preparation. This was probably because oat spelt xylan has a more branched structure which reacted more readily with the HTec containing G10 xylanase which has been reported to work more effectively on the substituted xylan (Viikari et al., 1994).

3.2.2.4 Influence of cellulose crystallinity on reactivity

In addition to the characteristics of the hemicellulose, the influence of the characteristics of cellulose on its reactivity with enzymes and chemicals will impact the behavior of a dissolving pulp. It is likely that the accessibility of the cellulose itself will govern its ease of modification by enzymatic treatments such as endoglucanases and more importantly its overall reactivity. As described earlier, cellulose reactivity is one of the most important parameters which affects the potential utility of dissolving pulps. Cellulose reactivity is highly dependent on the accessibility of its hydroxyl groups and thus the ability of reactants to overcome steric hindrance to reach the hydroxyl groups on cellulose (Sjöström, 1981). Typically, decreases in cellulose crystallinity, increases in accessible surface area, low cellulose molecular weight with high amount of hydroxyls available to derivatization reagents are favorable properties for maximizing cellulose reactivity (Strunk, 2012). Therefore, this work investigated the influence of these factors on cellulose reactivity.

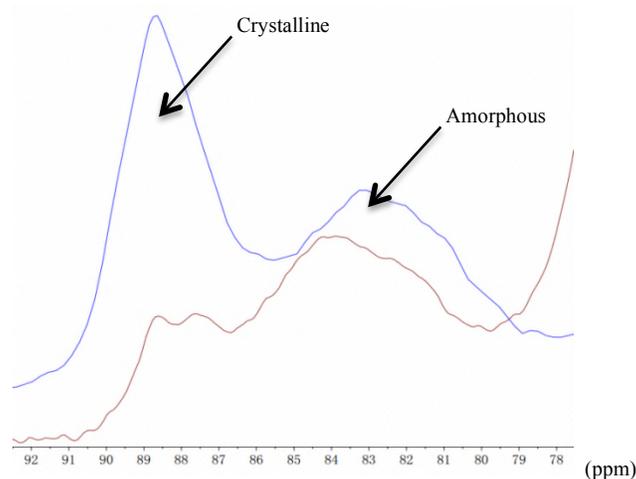


Figure 19: Results of the spectral of the cellulose C₄-region recorded by solid state CP/MAS ¹³C NMR on Avicel microcrystalline cellulose and PASC (78% phosphoric acid swollen cellulose). Blue line was on Avicel, and red line was on the PASC. Phosphoric acid treatment was conducted under very low temperature (~0°C) according to Mélanie Hall and Andreas S. Bommarius (2010).

It has been reported that PASC can be created with varying levels of crystallinity using Avicel as starting material through dissolution in a range of phosphoric acid concentrations (Hall et al., 2010). Since this technique was also reported to have no impact on cellulose molecular weight (Jeoh et al., 2001; Zhang and Lynd, 2005), we used this technique to prepare PASC and explore the impact of crystallinity on cellulose reactivity with derivatization reagents such as carbon disulfide. The spectral fitting of the cellulose C₄-region recorded using this method indicated that the large signal from 86 to 91ppm are a result of the crystalline and para-crystalline cellulose, while the signals from 80 to 86 ppm represent cellulose at amorphous regions including both accessible and inaccessible fibre surfaces (Figure 19) (Duchesne et al., 2001). The crystallinity index is the ratio of the area of crystalline region to the area of the amorphous region. There was a distinct difference between the spectrum of the cellulose from PASC and Avicel (Figure 19). It was apparently that the 78% phosphoric acid swollen cellulose (denoted by a red line) had a smaller crystalline region and a larger amorphous region as compared to the starting Avicel microcrystalline cellulose denoted by the blue line (Figure 19).

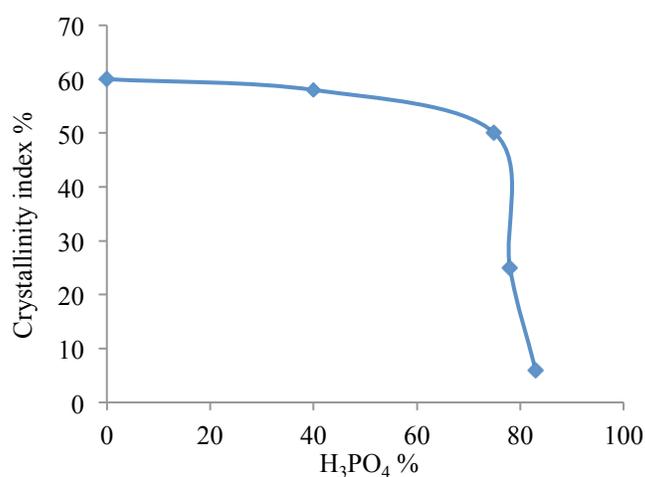


Figure 20: Effect of phosphoric acid concentration on the crystallinity index of Avicel microcrystalline cellulose. The crystallinity index was determined by solid state CP/MAS ¹³C NMR.

The starting cellulose sample had a crystallinity index around 60%. When the concentration of phosphoric acid was lower than 75% during the swelling treatment, there was not a significant change in cellulose crystallinity (Figure 20). However, when the concentration of phosphoric acid exceeded 75%, there was a dramatic drop in cellulose crystallinity, ranging from 50% to almost 0% when the concentration of phosphoric acid was higher than 82% (Figure 20). Similar results were reported showing that cellulose crystallinity index did not change at a concentration of 55-60% phosphoric acid. However, the cellulose crystallinity decreases virtually linearly to 0% at phosphoric acid concentrations ranging from 75 to 80% (Hall et al., 2010). Previous work using phosphoric acid to increase cellulose accessibility of lignocellulosic materials also demonstrated that decreasing the concentration of phosphoric acid below 81% slowed down the enzymatic hydrolysis rate (Moxley et al., 2008). The effect of phosphoric acid during the swelling process was reported not only related with its ability to be dissolved, but also its capacity to disrupt the crystalline structure of cellulose fibres to convert the crystalline regions of cellulose to amorphous. With the increase in phosphoric acid concentration, more crystalline regions from Avicel could be transformed into amorphous cellulose (Hall et al., 2010). In this work, the highly crystalline cellulose and a range of samples with decreasing crystallinity were used to investigate the influence of crystallinity on cellulose reactivity.

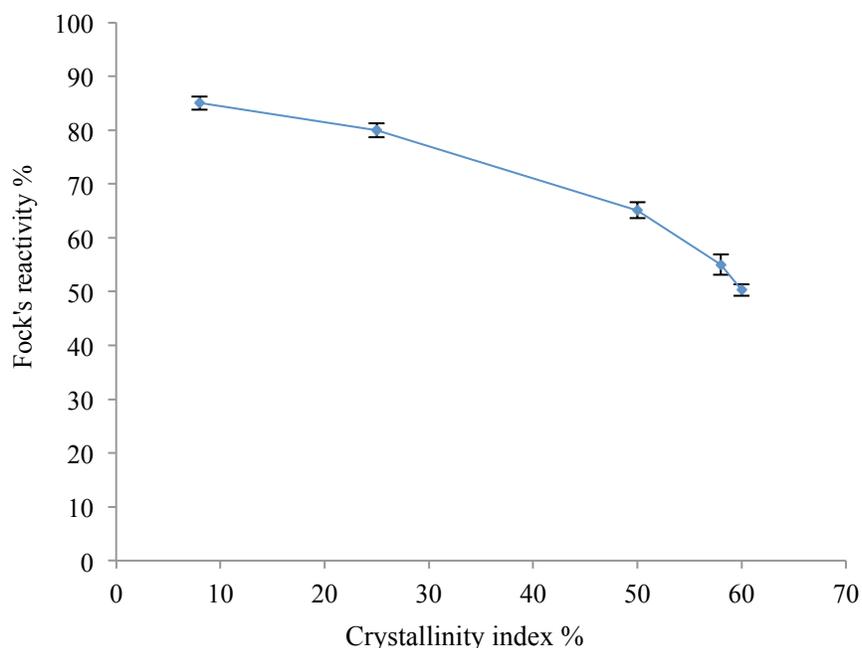


Figure 21: Influence of crystallinity index on Fock's reactivity of cellulose. The crystallinity index was determined by solid state CP/MAS ^{13}C NMR.

In bioconversion where cellulose crystallinity has been widely explored, the crystallinity index has been reported one of the major factors that affect the enzymatic hydrolysis of cellulosic substrates (Chen et al., 2007; Yoshida et al., 2008; Hall et al., 2010). It has been shown cellulose crystallinity could particularly impact the initial hydrolysis rate of cellulose by cellulase enzymes (Yoshida et al., 2008). Various physical or chemical treatments for disrupting the crystalline structure of cellulose could promote the overall hydrolysis of biomass (Yoshida et al., 2008). For dissolving pulp, it has been reported disrupting crystallinity could potentially increase the amount of available hydroxyl groups to increase final pulp reactivity (Strunk, 2012). However, there has to be any studies that yet specially investigate the influence of crystallinity on cellulose reactivity. It was anticipated that the increased cellulose crystallinity may result in a decrease in cellulose reactivity due to a lower exposure of hydroxyl groups available to derivatization

reagents. However, previous literature has found it challenging to link a given pulps cellulose reactivity to its crystallinity. Thus we investigated this relationship by using cellulose “model” substrates. An approximately inversely proportional correlation was found between the crystallinity index and cellulose reactivity (Figure 21). The starting Avicel microcrystalline cellulose had a crystallinity index of approximately 60%. However, when the crystallinity was decreased to 50% after undergoing a 75% phosphoric acid treatment, there was a more dramatic increase in cellulose reactivity compared to the reactivity enhancement observed when the crystallinity was decreased from 50% to 8% after the more aggressive phosphoric acid treatment (Figure 21). One of the possible reasons could be when the highly crystalline cellulose was treated with phosphoric acid at a 75% concentration, although the crystallinity index was only decreased by 10%, the cellulose crystalline regions were highly swollen and became accessible to the small derivatization reagents (CS₂). Therefore, a dramatic increase in cellulose reactivity was obtained using the 75% phosphoric acid concentration. As the phosphoric acid concentration was raised from 75% to 83%, the cellulose crystallinity index dropped from 50% to 8%, and the resulting cellulose reactivity increased from 65% to 85%.

3.2.2.5 Influence of pulp morphological properties on cellulose reactivity

The crucial role that pulp characteristics such as degree of fibre swelling, pore structure and surface area play during the bioconversion process of lignocellulosic pulps has been reported previously (Chandra et al., 2008a; Arantes and Saddler, 2010, 2011; Esteghlalian et al., 2001). For dissolving pulps, the morphological properties of cellulose fibres such as the degree of fibre swelling and surface area can extensively influence their ability to be accessed by reagents

during the downstream derivatization process (Strunk, 2012). Therefore, they are regarded as crucial parameters for cellulose fibres and have received significant attention (Strunk, 2012). Generally, large, wide pores and a high surface area are beneficial attributes for increasing cellulose accessibility and exposing hydroxyl groups. This leads to a highly reactive cellulose pulp (Strunk, 2012). It has been shown that pretreatments such as steam explosion, mechanical refining or alkaline swelling are able to aid in expanding capillaries and increase the surface area of pulps (Yamashiki et al., 1990; Kihlman et al., 2011; 2013; Strunk, 2012). In particular, mechanical refining has been reported to be one of the most effective treatments to expand capillaries, split fibre aggregations to increase the accessible surface of fibril aggregates and disrupt the compact cellulose matrix (Tian et al., 2014; Wu et al., 2014). Therefore, we used this technique to create pulp samples with varying morphological properties such as increased accessible surface area and degree of fibril/fibre swelling to investigate these properties influence on cellulose reactivity.

Table 9: Influence of pulp morphologies on cellulose reactivity after mechanical refining. Refining was conducted on CCE treated Kraft pulp. Fibril/fibre swelling and ASA were obtained by solute exclusion technique on wet samples, and surface area was based on BET analysis of nitrogen absorption isotherm. ASA: accessible surface area; DsP: dissolving-grade pulp

Revolutions	Fibril swelling ml/g	Fibre swelling ml/g	ASA m ² /g	Surface area m ² /g	Reactivity %
0	0.85	1.48	50.9	2.3	31.6
10000	0.90	1.65	64.7	-	38.3
20000	0.92	1.84	70.7	5.0	54.5
Commercial DsP	0.53	0.93	34.5	2.0	62.5

It has been shown that mechanical refining can effectively improve fibril/fibre swelling and the accessible surface area, leading to a dramatic increase in pulp reactivity by more than 70% (Table 9). When a mild mechanical refining (10000 PFI revolutions) was employed, the improvement in cellulose reactivity was not as significant. It is likely that the intensity of the treatment using 10000 PFI revolutions was not sufficient to create fibrillation to make cellulose highly reactive (Table 9). A similar phenomenon has been previously reported when mechanical refining below 20000 PFI revolutions was not able to increase the specific surface area of dissolving pulp fibres as well as their final reactivity (Tian et al., 2014). However, compared to a commercial dissolving-grade pulp, which has a relatively lower surface area and less fibril/fibre swelling, the Kraft pulps that were subjected to mechanical refining still had a lower reactivity (Table 9). It was likely that the main reason for this was that mechanical refining only increased the surface area and degree of fibre swelling but did not decrease the molecular weight of cellulose which aids in cellulose reactivity. Therefore, the refined pulp still had a high cellulose molecular weight compared to the dissolving-grade pulp, which made it less reactive with the derivatization reagents. Therefore, to obtain a pulp with highly reactive cellulose, some degradative treatments such as acid or endoglucanase hydrolysis might be productively employed after mechanical refining to decrease the DP of the cellulose.

3.2.3 Conclusions

To further illustrate the role that hemicellulose accessibility plays during the conversion of Kraft to dissolving-grade pulp, and to gain insights on how pulp characteristics affect the action of enzymes, model xylan substrates isolated from different sources with varying accessibility and structure were employed. Compared with the xylan removed by xylanases from ground wood chips and Kraft pulp, the isolated xylans were far more easily hydrolyzed. These results indicated

that a limiting factor in the enzymatic removal of xylan from pulp using xylanases was its accessibility. In addition, crystallinity, surface area and degree of fibre swelling were also investigated to elucidate their influence on the final cellulose reactivity. An inverse correlation was found between cellulose crystallinity index and reactivity, indicating that it is favorable to decrease cellulose crystallinity to improve reactivity. It was also shown that the pulps with high surface area and degree of fibre swelling that were generated using mechanical refining were highly reactive during subsequent cellulose derivatization.

3.3 The influence of prehydrolysis and cold caustic extraction on the purity, accessibility and reactivity of dissolving-grade pulp

3.3.1 Background

The previous chapter showed that substrate characteristics such as the accessibility of hemicellulose and the crystallinity/DP/accessible surface area of cellulose influenced the reactivity of enzymes and chemicals with the fibres (Engström et al., 2006; Jeoh et al., 2007; Yoshida et al., 2008; Agarwal et al., 2013). Therefore, it can be anticipated that, in the conversion of Kraft pulp to dissolving-grade pulp, the application of either a PHK or CCE treatment to remove hemicellulose, should result in pulps that vary in properties such as pore size, accessible surface area and microfibril aggregation. Although the prehydrolysis approach is effective in achieving hemicellulose removal, it presents a bottleneck for pulp production. It also increases the strain on the chemical recovery systems and results in the need for pH swings when going from the acidic prehydrolysis conditions to highly alkaline Kraft pulping process (Testova et al., 2014; Luo et al., 2011; Borrega and Sixta, 2013; Borrega et al., 2013).

As an alternative to a prehydrolysis step that removes hemicellulose prior to Kraft pulping, a CCE sequence at room temperature can be applied after the pulping process to reduce the hemicellulose content of the pulp (Engström, et al., 2006; Köpcke et al., 2010). Unlike pre-a hydrolysis process which cooks the chips under acidic conditions, CCE is performed under alkaline conditions at room temperature, facilitating chemical recovery in the Kraft mill. It does not require the extra digester capacity prior to pulping thereby debottlenecking the hemicellulose removal process. However, it has been shown that the removal of hemicellulose during CCE compromises downstream cellulose reactivity (Gehmayr and Sixta, 2012; Gehmayr et al., 2012). Although the factors that allow prehydrolysis to retain cellulose reactivity remain unclear, a possible reason for the decreased reactivity after CCE is the removal of the residual hemicellulose and low molecular weight cellulose that act as “spacers” between cellulose microfibrils to prevent cellulose “fibril aggregation” (Gehmayr et al., 2012). Thus, the cellulose aggregation that occurs upon the solubilization of the lower molecular weight cellulose and hemicellulose components during CCE might limit the accessibility of cellulose to chemicals during downstream derivatization (Figure 22). Cellulose fibril aggregation has been shown to manifest itself through increases in the microfibril size and a more compact crystal structure (Duchesne et al., 2001; Hult et al., 2001). Another potential reason for the compromised reactivity after the application of CCE, might be the alkali induced conversion of cellulose I to cellulose II which is not desired for a reactive cellulose feedstock. Therefore, post-treatments are likely to be required for a dissolving pulp obtained from a CCE process to improve the final cellulose reactivity. However, it may be possible to increase the reactivity of cellulose via treatments that either decrease cellulose degree of polymerization, such as chemical or enzymatic

hydrolysis, or create increased surface area through mechanical action such on the cellulose (Strunk, 2012).

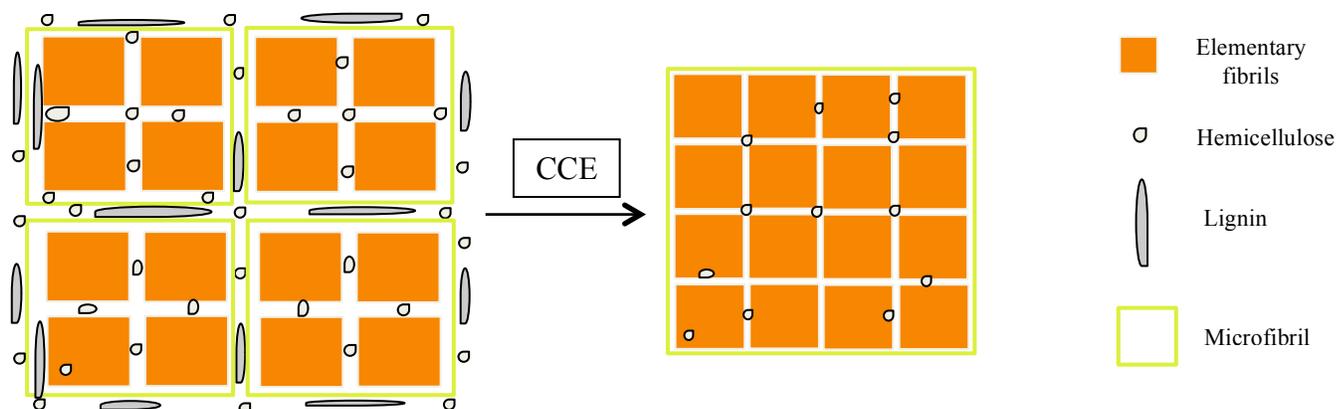


Figure 22: Schemes about cellulose microfibril aggregation after alkaline extraction

Previous work by Kamide and later by Kihlman showed that the high shear decompression undergone by cellulose during steam explosion treatment resulted in an increase in dissolving pulp cellulose reactivity during subsequent derivatization (Yamashiki et al., 1990; Kihlman et al., 2011; 2013). Similarly, mechanical refining, which increases the surface area of pulps through imparting fibrillation has also been shown to result in an increase in cellulose reactivity, presumably via increased pore size, surface area and swelling (Tian et al., 2014). Chemical or enzymatic treatments such as the use of acid or endoglucanases to cleave cellulose have been used for increasing cellulose reactivity by decreasing the cellulose DP (Engström et al., 2006). However, the use of acid to reduce cellulose DP and remove hemicellulose has been shown to compromise yield and increase cellulose polydispersity when compared to the specific action of enzymes (Engström et al., 2006). Research concerning fibre “dislocations” has been investigated in pulp and paper due to the detrimental effects of fibre “curl” and dislocations on mechanical strength of fibres and paper strength as well (Page et al., 1985). These dislocations or axial

micro-compressions associated with fibre “kinks” and “curl” have been shown to be areas of the cellulose containing less ordered molecular chains that are highly susceptible to enzymes and chemicals and thus may be areas of highly reactive cellulose (Thygesen et al., 2011; Hidayat et al., 2012). Since dissolving grade cellulose is not used as a papermaking furnish it may be possible to intentionally induce kinks and curls into a dissolving pulp furnish by applying physical action at high pulp consistency (Zeng et al., 2012) as a means to increase cellulose reactivity.

It is evident that the application of either a prehydrolysis or CCE result in pulps that vary in properties such as pore size, accessible surface area and microfibril aggregation that profoundly impact cellulose accessibility and reactivity (Gehmayr et al., 2011; 2012; Gehmayr and Sixta, 2012). Previous work has yet to differentiate effects of removing hemicellulose by either the PHK or CCE on the structure and accessibility of the resulting dissolving pulp cellulose as well as a comprehensive assessment of techniques that can increase cellulose reactivity via enhancing accessible cellulose surface area (Gehmayr et al., 2011; 2012). To gain insights into the factors that might influence the reactivity of PHK and CCE treated cellulose, we compared the properties of commercial dissolving grade cellulose produced through prehydrolysis to another Kraft pulp upgraded to dissolving grade cellulose using the CCE process. Fibre properties related to cellulose accessibility such as pore size and accessible surface area as well as cellulose molecular properties including crystallinity, DP and fibril aggregate size were assessed for their relative influence on cellulose reactivity of both pulps. “Post-treatments” such as mechanical refining, steam explosion, induction of fibre kink and curl, sulfuric acid hydrolysis and endoglucanase hydrolysis were also applied to determine if increasing cellulose surface area

could recover the reactivity lost during the production of dissolving pulp cellulose using a CCE step.

3.3.2 Results and discussion

3.3.2.1 Influence of PHK and CCE on the purity and reactivity of dissolving pulps

Both the CEE and PHK processes have been shown to remove sufficient amounts of hemicellulose to allow the conversion of conventional Kraft pulp to dissolving pulp (Testova et al., 2014; Borrega et al., 2013; Borrega and Sixta, 2013; Gehmayr et al., 2011; 2012; Köpcke et al., 2010). However, unlike the PHK process that is applied to chips prior to pulping, applying a CCE to Kraft pulp results in a decrease in cellulose reactivity (Gehmayr and Sixta, 2012; Krässig, 1984; Sears et al., 1982; Schild and Sixta, 2011). To assess the possible reasons for the decrease in reactivity undergone by Kraft pulp during the CCE process, a commercial dissolving cellulose pulp produced using the PHK process was used as a control sample to compare to a Kraft pulp that was subjected to CCE at varying levels of NaOH concentration.

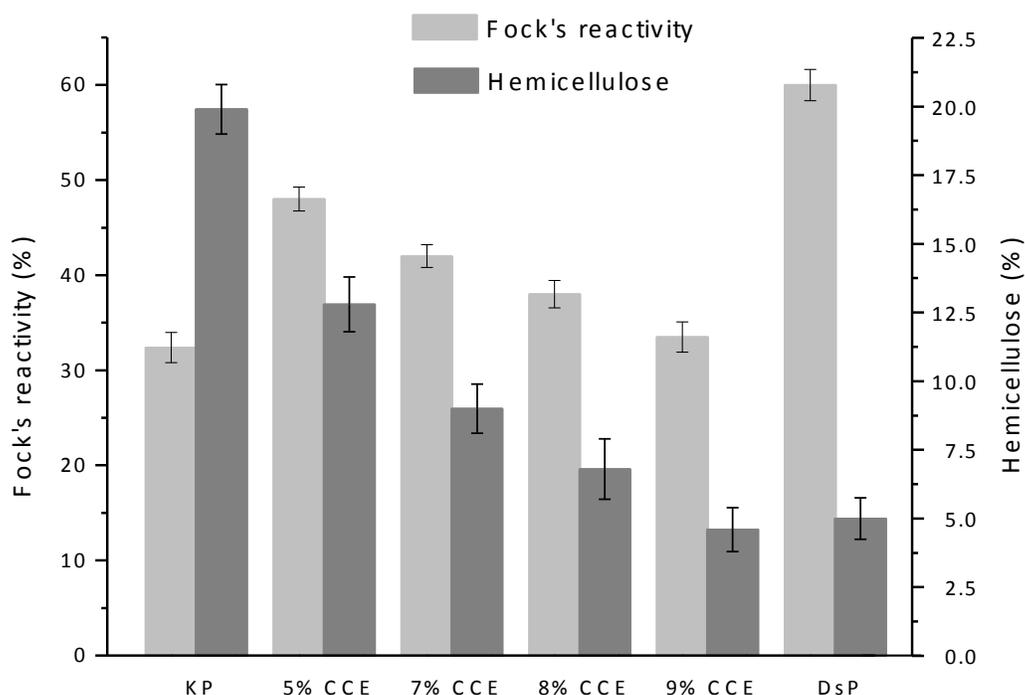


Figure 23: Residual hemicellulose content and Fock's reactivity of different alkaline extracted pulps. KP: Kraft pulp (Dried). 5/7/8/9% CCE: 5%/7%/8%/9% NaOH extracted Kraft pulp. DsP: Commercial dissolving pulp obtained from prehydrolysis process (undried).

When the concentration of NaOH was raised during the CCE step, the residual hemicellulose in the cellulose pulp was reduced (Figure 23). The concentration of 9% (w/w) NaOH could reduce the final hemicellulose content from 20% down to the range of dissolving-grade pulp. However, it was apparent that the reactivity was compromised as the Fock's reactivity was only 34% compared to the commercial dissolving pulp (DsP) that was 60% reactive to derivative reagents (Figure 23). There was a compromise between hemicellulose removal and the decrease in cellulose reactivity as the concentration of NaOH used for CCE was decreased as a 5% NaOH concentration only solubilized 40% of total Kraft pulp hemicellulose, but the reactivity was improved from 33.5% to 50.3%. However, this level of reactivity was still lower than the

commercial dissolving pulp cellulose (Figure 23). The presence of increasing amounts of hemicellulose in Kraft pulp can increase pulp reactivity as the amorphous hemicellulose is more accessible to derivatization reagents such as CS₂ during reactivity measurements. However, the hemicellulose also consumes alkali that would otherwise be used to ionize the hydroxyl groups of less accessible portions of cellulose for subsequent xanthation (Strunk, 2012). It has been hypothesized that, although the hemicellulose is more reactive, the decreased overall ionization of cellulose in the presence of hemicellulose results in a decreased overall cellulose reactivity for Kraft pulp that contains increasing amounts of hemicellulose (>12%). However, when the CCE decreases the hemicellulose content below 10%, the cellulose reactivity is compromised, likely due to aggregation of the cellulose after the solubilization of the small molecular weight hemicellulose (Gehmayr and Sixta, 2012; Duchesne et al., 2001). In contrast, the dissolving grade pulps produced using a PHK process typically retains some of the low molecular weight hemicellulose since alkaline conditions employed during subsequent Kraft pulping are aimed at solubilizing the lignin component while retaining as much of the carbohydrate components as possible (Gehmayr et al., 2012).

3.3.2.2 Influence of PHK and CCE on the fibre surface morphology of dissolving pulps

Table 10: Macroscopic properties of differently treated pulps. KP: Kraft pulp. DsP: Dissolving pulp made from PHK process. CCE KP: 9 % sodium hydroxide extracted Kraft pulp. ASA: Accessible surface area.

Pulps	Orange dye adsorption mg/g	CBM 2a adsorption mg/g	Fibril swelling ml/g	Fibre swelling ml/g	ASA m²/g	Fibre length mm	Specific surface area m²/g
KP	283	31.2	0.81	1.28	40.5	0.704	2.97
DsP	295	36.5	0.53	0.93	34.5	0.546	2.04
CCE KP	246	45.3	0.89	1.48	50.9	0.641	2.33

To assess the main reasons that result in a poor reactivity by CCE to dissolving pulps compared with PHK process, some macroscopic properties such as accessibility of cellulose to direct orange dye and purified CBM, fibre/fibril swelling and accessible surface area by solute exclusion, fibre length and specific surface area by BET analysis were studied. It was apparent that pulps treated by the CCE process contained a higher proportion of crystalline cellulose as there was an increase in the adsorption for CBM2a which has a higher affinity for crystalline cellulose (Table 10). There was an increase in CBM 2a adsorption, which was mainly because CBM 2a has high affinity with cellulose at crystalline region (Gourlay et al., 2012), indicating there was high ratio of crystalline cellulose on dissolving pulp made from CCE process. However, the CCE increased the fibre swelling as measured by solute exclusion as well as the accessible surface area of the wet pulp likely by extracting the small molecular weight components and enlarging pores. However, there was a decrease on specific surface area after drying the CCE treated pulp when the dry samples were measured using the BET method (Table 10). These results suggest that the CCE can enhance accessibility of the pulp cellulose if the pulp

remains wet, but the increased accessibility leads to a greater amount of collapse of the cellulose structure and reduced accessibility upon drying (Gehmayr and Sixta, 2012).

3.3.2.3 Cellulose microfibril and fibril aggregate size estimated by CP/MAS ^{13}C -NMR

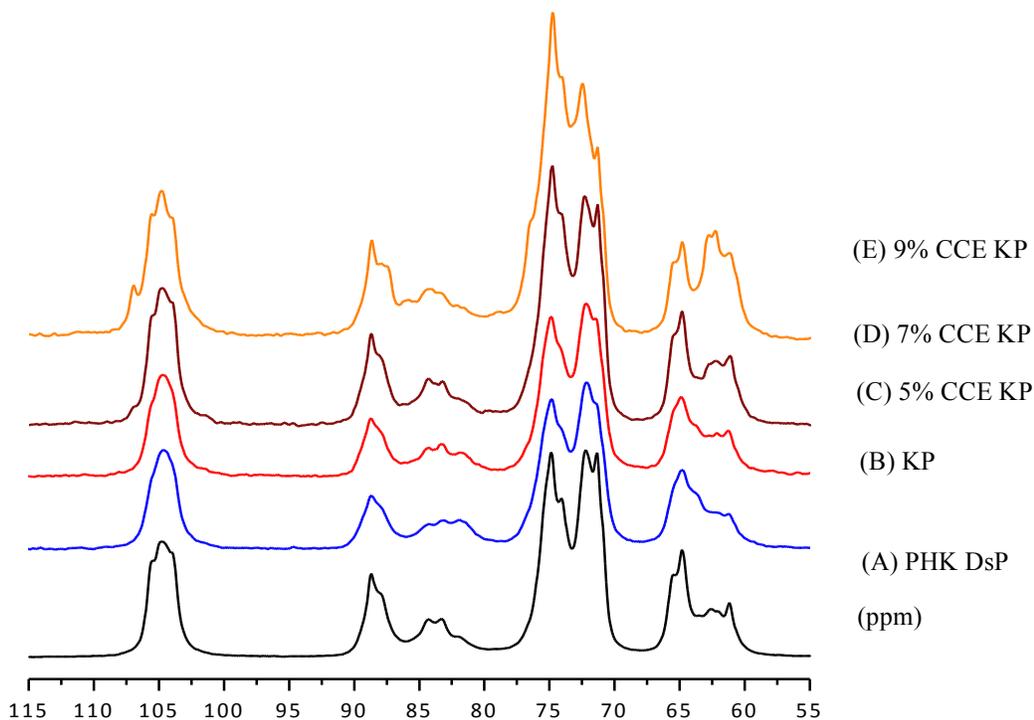


Figure 24: CP/MAS ^{13}C NMR spectra of different treated pulps. (A) Undried dissolving pulp obtained by prehydrolysis process; (B) Kraft pulp; (C) 5% NaOH extracted KP; (D) 7% NaOH extracted KP; (E) 9% NaOH extracted KP.

Changes to the supramolecular structure of the various treated pulp samples were detected using CP/MAS ^{13}C -NMR. The method allows for observation of the surface fibril aggregates (Hult et al., 2001; Zhang et al., 2006). The average fibril and fibril aggregate width could also be estimated by spectral fitting of the experimental NMR spectra (Larsson et al., 1997; Hult et al., 2001). It was apparent that the signal at 81.7ppm, which represented accessible hemicellulose

almost disappeared upon dissolution of approximately 80% of the xylan from the Kraft pulp using a CCE at an alkaline concentration of 9% (Teleman et al., 2011) (Figure 24 E). As the alkaline concentration was increased from 5 to 9%, the signal at 83.5ppm that has been shown to represent inaccessible xylan (Teleman et al., 2011), became broader, indicating that the residual xylan likely became aggregated with the cellulose microfibrils due to the alkali-induced removal of accessible hemicellulose (Figure 24: B-E) (Teleman et al., 2001). However, compared to the pulp treated using a CCE at a 9% alkaline concentration, the resonance at 83.5ppm on dissolving pulp made from PHK process was as narrow as Kraft pulp, demonstrating that even though the pulps made using the PHK and CCE processes had similar levels of residual hemicellulose, the resulting accessibility of the residual hemicellulose was quite different. Compared to the residual hemicellulose in the cellulose rich pulp made using the CCE process, the hemicellulose components of the pulp produced using the PHK process were far more accessible to chemicals likely due to a lower amount of aggregation between hemicellulose and the cellulose microfibrils. These results are supported by our previous work that showed that a 9% CCE removed more than 50% of the hemicellulose from dissolving grade pulp produced using a PHK process. It was apparent that although both the PHK and CCE pulps have similar hemicellulose content, the character of the residual hemicellulose differed. These differences may be attributed to the cleavage of acetate esters that comprise the side chains of hemicellulose that occurs during the prehydrolysis process (Sjöström, 1981). This release of acetic acid from the hemicellulose components that occurs during the prehydrolysis process promotes the hydrolytic cleavage of β -1,4-glycosidic bonds and finally solubilizes approximately 70% of the hemicellulose component (Testova et al., 2014). The hemicellulose that remain in the pulp likely undergoes a reduction in molecular weight and branching during the prehydrolysis process, but likely plays a role as a

“spacer” between cellulose microfibrils, thereby reducing the amount of aggregation that occurs between cellulose microfibrils during subsequent Kraft pulping (Gehmayr et al., 2012).

However, during the CCE stages, which is applied after the pulping process, the sodium hydroxide acts as a strong swelling reagent, resulting in the solubilization of the residual hemicellulose. This had likely undergone deacetylation, debranching and a reduction in molecular weight during the pulping process due to peeling and chain cleavage reactions (Sjöström, 1981; Strunk, 2012). The remaining hemicellulose after the CCE is likely linear and with a higher molecular weight (Gehmayr et al., 2012), thus it can hydrogen bond with cellulose without contributing towards the decrease in aggregation between cellulose microfibrils.

In the case of the 9% CCE treated Kraft pulp, the CP/MAS ^{13}C NMR spectrum showed a distinct signal at 107ppm, originating from cellulose II (Figure 24 E) (Teleman et al., 2001). The same peak was slightly discernible with the 7% CCE treated pulp, indicating a portion of the cellulose underwent mercerization by the alkaline treatment during CCE, especially when the NaOH concentration exceeded 7%. It was previously reported that, when the concentration of NaOH solution during CCE treatment exceeded 8%, the initial cellulose I on Kraft pulps was gradually modified to cellulose II (Gehmayr and Sixta, 2012). It was also shown that performing an alkaline extraction using an alkaline concentration of 9% alkaline could convert 13% of the cellulose in Kraft pulp to cellulose II (Gehmayr and Sixta, 2012). Due to the configuration change of the O_6 on Cellulose II, (an irreversible conversion from trans-gauche structure on cellulose I to gauche-trans orientation), there was an increased number of inter- and intra-planar hydrogen bonds after drying, resulting in a decreased surface area and less availability of hydroxyl groups and thus less reactive pulps (Gehmayr and Sixta, 2012).

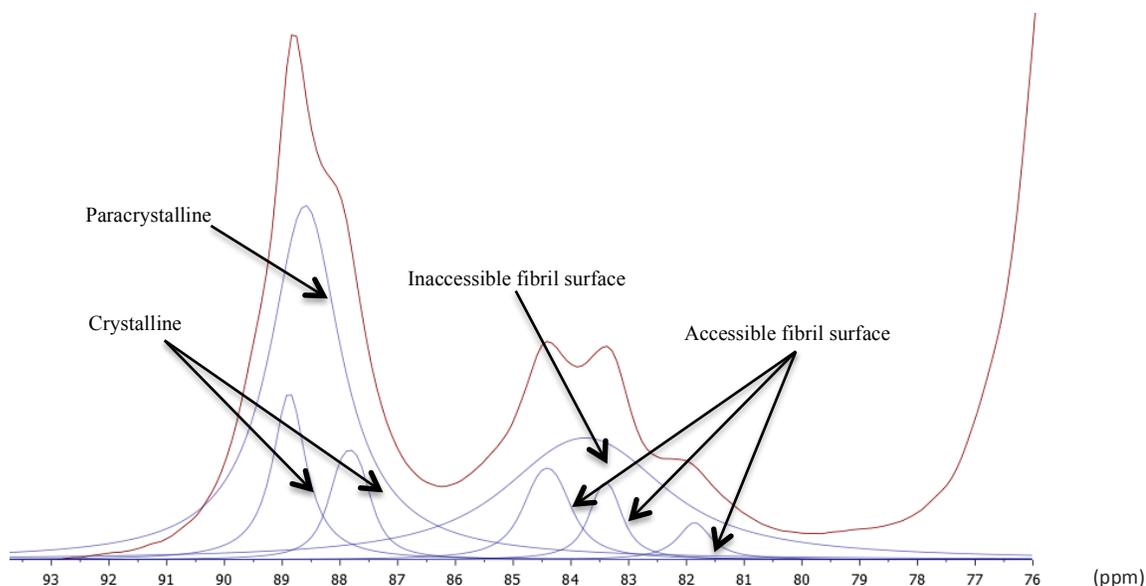


Figure 25: Results of the spectral fitting of the cellulose C₄-region recorded on dissolving pulp made using the prehydrolysis Kraft process. The black lines represent the experimental spectra. The fitted lines are shown in blue color.

Table 11: Fibril size and fibril aggregate size of different treated pulps. Quantifications were made by spectral fitting of the cellulose C₄ region of the CP/MAS ¹³C-NMR spectra. KP: Kraft pulp. DsP: Dissolving pulp. 4.5/6.5/9% CCE KP: 4.5/6.5/9% sodium hydroxide extracted Kraft pulp

Substrates	KP	DsP	5% CCE KP	7% CCE KP	9% CCE KP
Hemicellulose %	19.9	5.0	12.8	8.9	4.6
Crystalline cellulose %	21.6	18.6	20.3	19.1	18.1
Paracrystalline cellulose %	33.1	38.3	35.5	38.2	38.8
Inaccessible fibril surface %	32.0	34.9	33.7	33.9	35.2
Accessible fibril surface %	13.3	8.2	10.5	8.8	7.9
Fibril size (nm)	4.4	4.6	4.5	4.6	4.6
Fibril aggregate size (nm)	16.7	27.2	21.1	25.3	28.3

To gain further insight into the impact of PHK and CCE on the supramolecular properties of cellulose such as crystallinity, accessible/inaccessible fibre surface, size of fibril and fibril aggregate of dissolving pulps, cellulose was isolated and detected by CP/MAS ^{13}C -NMR. The results of spectral fitting of the cellulose C_4 -region recorded using this method indicate that the large signal from 86 to 91ppm (Figure 25, denoted in red) are a result of the crystalline and paracrystalline cellulose, while the signals from 80 to 86 ppm represent cellulose at amorphous regions including both accessible and inaccessible fibre surfaces (Teleman et al., 2001; Hult et al., 2001). Generally, a higher proportion of amorphous cellulose, especially accessible fibre surfaces, is desired for pulps to maintain high accessibility and reactivity during subsequent derivatization (Strunk, 2012). The results indicated that the proportion of crystalline cellulose (paracrystalline cellulose included) increased upon the removal of hemicellulose while the amount of accessible fibril surface decreased from 13.3 to 7.9%, resulting in a 70% increase in fibre aggregate size. This suggested the cellulose microfibrils aggregated due to the removal of hemicellulose (Table 11). The fibril sizes of the pulps treated under the various CCE conditions exhibited fibril aggregate sizes within a narrow range from 4.4 to 4.6nm. This was likely due to the increasing inaccessibility of elementary fibrils, consisting of pure and highly ordered cellulose, towards chemicals or enzymes. Compared to the dissolving pulps produced using CCE, the pulps produced using the PHK method had higher accessible surface areas and smaller fibre aggregate sizes (Table 11). The dissolving pulps obtained from the PHK and CCE methods of hemicellulose removal both had similar hemicellulose content (Figure 23). However, the PHK treated pulp had a more accessible surface area and smaller fibre aggregate size, indicating the residual hemicellulose on PHK treated pulp did not aggregate with cellulose and the relatively low molecular weight hemicellulose might act as “spacers” between the cellulose microfibrils,

preventing cellulose fibril aggregation. In comparison, the residual hemicellulose from the pulp obtained from CCE process had relatively higher molecular weight and linear structure (Gehmayr et al., 2012) and thus high potential to assemble together with the complex cellulose matrix. This could be one of the important reasons that PHK treated pulp had a higher reactivity towards derivatization reagents (CS₂) than did the CCE treated dissolving pulp.

However, unlike pre-hydrolysis, CCE is performed under alkaline conditions facilitating chemical recovery in the Kraft mill and does not require the extra digester prior to pulping. The mild CCE reaction conditions also preserved the integrity of cellulose, thereby decreasing sacrifices to pulp yield. The CCE approach is still regarded as an attractive treatment for the production of dissolving-grade pulps except that it lowers cellulose reactivity due to the reasons discussed above. As a result, post-treatments were required to enhance the accessibility and reactivity of dissolving pulp treated with a CCE step to downstream derivative chemicals. The purpose of activation is to open up the capillaries and pores, disrupt the complex crystalline cellulose matrix, shorten cellulose DP and break down the existing inner- or intramolecular hydrogen bonding, thus more hydroxyl groups are available and accessible for derivatization (Strunk, 2012). Widely used post-treatment methods include degradative treatments such as chemical or enzymatic hydrolysis, mechanical treatments such as PFI refining or steam explosion, or combination thereof (Strunk, 2012; Tian et al., 2014; Wu et al., 2014; Östberg et al., 2012; Köpcke et al., 2010)

3.3.2.4 Post-treatments to enhance the reactivity of dissolving pulp obtained from CCE process

Previous work by Kihlman showed that steam explosion resulted in an increased solubility of dissolving pulp cellulose in NaOH/urea/thiourea (Kihlman et al., 2011; 2012; 2013). The probable reasons for this effect could be that the release of high pressure increased the surface area of dissolving pulp and promoted fibre separation due to the fragmentation caused by the mechanical shearing force. Thus, the accessibility of cellulose to the solvents (NaOH/urea/thiourea) was increased (Kihlman et al., 2011; 2012; 2013). In addition, the decrease in cellulose molecular weight may also occur under the high steam explosion severity which could enhance the reactivity of cellulose pulp (Kihlman et al., 2012). Similarly, mechanical refining increases the surface area of cellulose pulps through external fibrillation, internal delamination and fibre cutting, which creates certain microfibrils on the surface of fibres, swells and loosens the internal structures of the fibres (Kerekes, 2005; Tian et al., 2014; Wu et al., 2014). It was expected that the normal forces during the mechanical refining allowed for surface fibrillation and internal delamination of fibres. This could disrupt the crystalline structure of cellulose microfibrils and create more accessible surface area on pulps (Jones et al., 2013). Chemical or enzymatic treatments such as the use of acid or endoglucanases to cleave cellulose chains have been used to increase cellulose reactivity by decreasing the DP of the cellulose (Östberg et al., 2012; Köpcke et al., 2010).

Research concerning fibre “dislocations” has long been investigated in pulp and paper making industry due to the detrimental effects of fibre “curl” and dislocations on mechanical strength of final fibres and paper strength (Page et al., 1985). The dislocation or axial micro-compressions associated with fibre “curl” and “kinks” occur in the regions of cellulose fibres composed of less

ordered cellulose. Due to a less crystal structure, dislocation regions are highly susceptible to enzymes and chemicals and thus they are regarded as the weak points of the whole cellulose fibres (Thygesen et al., 2011; Hidayat et al., 2012). The curl and kinks regions maybe also the areas of highly reactive cellulose. Since dissolving-grade pulp is not used as a papermaking furnish, it may be possible to intentionally induce “curls” and “kinks” into a dissolving pulp furnish by applying physical actions such as shearing at a high pulp consistency (Zeng et al., 2012) as a means to increase cellulose pulp reactivity. In the work reported here, the “curl” and “kinks” were induced to the dissolving pulps obtained after CCE treatment combined with endoglucanase treatment as one of the post-treatment methods to enhance pulp reactivity to derivatization reagents. It has been previously reported that the increased presence of the fibre dislocation correlates with the decrease in the wet zero-span value of treated samples (Suchy et al., 2008). As a result, we used the wet zero-span value as a measurement for success in inducing curl and kinks to the pulp. The curl and kink index, kink angle number increased and wet zero-span strength of single fibre decreased, all indicated that curls and kinks were successfully induced to a dissolving pulp treated by CCE after shearing the pulp at 80% pulp in a Kitchen aid stand mixer (Table 12).

Table 12: Curls and kinks results on different treated pulps

Substrates	Fines %	Curl index	Kink index	Kink angles °	Wet zero span strength N/cm
KP	27.4	0.11	2.20	33.3	156.8
CCE KP	16.4	0.20	2.93	44.4	134.0
Curl CCE KP	18.6	0.25	3.32	51.7	118.5
PFI CCE KP	19.6	0.19	2.75	42.2	137.2

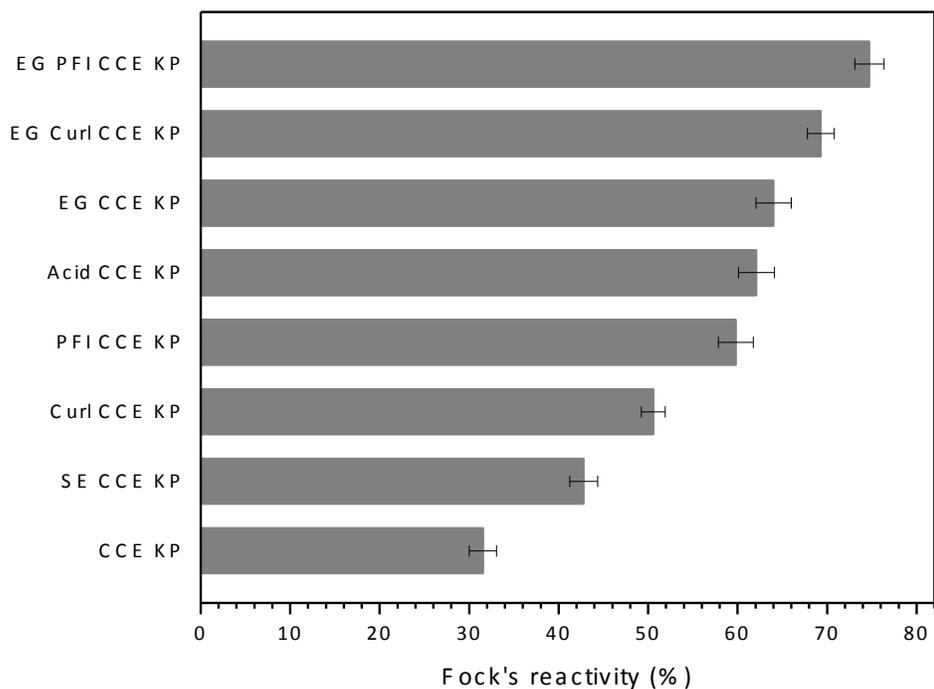


Figure 26: Fock's reactivity of different post-treated dissolving pulps

After curls and kinks were induced to the pulp, endoglucanase treatments were also applied as a means to further improve pulp reactivity. All post-treatments markedly improved the pulp reactivity ranging from 35% to 135% (Figure 26). Degradative treatments such as endoglucanase and sulfuric acid hydrolysis doubled the pulp reactivity of a dissolving pulp by decreasing cellulose DP (Table 13). It has been suggested that endoglucanases can attack both the amorphous regions between crystalline microfibrils and the less ordered regions of cellulose fibrils located on the surface, resulting in increased fibre swelling and thus accessibility of the pulp towards derivatization reagents. An alternative suggestion is that, after alkaline extraction, cellulose II is formed in pulps and this more accessible structure has a higher swelling capacity and susceptibility to attack by endoglucanases than native cellulose, resulting in a high pulp

reactivity (Gehmayr and Sixta, 2012). Compared to endoglucanase treatment, acid hydrolysis excessively lowered the pulp yield due to the non-specificity of acid to cellulose. Inducing curl and kinks on pulps enhanced the reactivity by more than 60%, and the following endoglucanase treatment further increased the reactivity of CCE-made dissolving pulp from 31.6% to 70.3% due to the high accessibility of curled regions on cellulose to enzymes. This was much higher than undried commercial dissolving pulp made from the PHK process (63.1%). Steam explosion slightly decreased the molecular weight of the cellulose and increased fibre swelling and accessible surface area. However, the improvement to cellulose reactivity was insignificant compared to that achieved with mechanical refining. Mechanical refining has been shown to be one of the most effective treatments to expand capillaries or pores, split the fibre aggregates to increase the accessible surface of fibril aggregates and disrupt the compact cellulose matrix (Tian et al., 2014; Wu et al., 2014). The total pore volume and accessible surface area remained similar after alkaline extraction of the Kraft pulp. After a PFI mechanical refining, there was a more than 50% and 60% improvements in total volume and accessible surface area respectively (Figure 27). It is likely alkaline extraction removed the less ordered overlying primary cell wall (Duchesne et al., 2001). The following mechanical refining disrupted the highly ordered cellulose microfibrils to increase fibrillation. This made the pulp highly accessible to derivatization reagents which dramatically improved the reactivity from 31.6% to 54.5% (Figure 26). After PFI refining, combined with endoglucanase treatment (which could hydrolytically cleave cellulose from the amorphous regions), the cellulose molecular weight was further decreased, resulting in the highest cellulose reactivity improvement of all of the post-treatments (Figure 26).

Table 13: Various post-treatments on KP obtained from CCE process to enhance end product properties. Steam explosion: 170°C, 5 min; Acid: 0.4% sulfuric acid hydrolysis, 5% pulp consistency, 121°C, 1 hour; PFI: 20K revolutions PFI refining; EG: 450 ECU/g Fibrecare R hydrolysis, 5% pulp consistency, 50°C, 2 hours; Curl: Kitchenaid stand mixer, 80% pulp consistency, 30 minutes. ASA: Accessible surface area

Substrates	Intrinsic viscosity ml/g	Orange dye adsorption mg/g	Fibril swelling ml/g	Fibre swelling ml/g	ASA m²/g
CCE KP	856	246	0.89	1.48	50.9
Steam CCE KP	728	261	0.85	1.52	57.8
Acid CCE KP	350	302	0.80	1.59	68.1
PFI CCE KP	830	327	0.92	1.64	70.7
EG CCE KP	425	290	0.85	1.59	66.3
Curl CCE KP	836	270	-	-	-
EG Curl CCE KP	415	295	-	-	-
EG PFI CCE KP	403	340	-	-	-
DsP	494	290	0.85	1.59	66.3

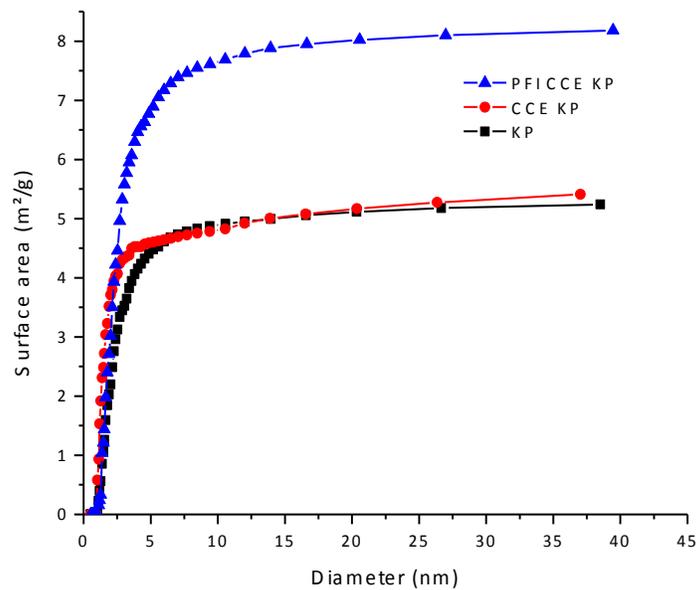
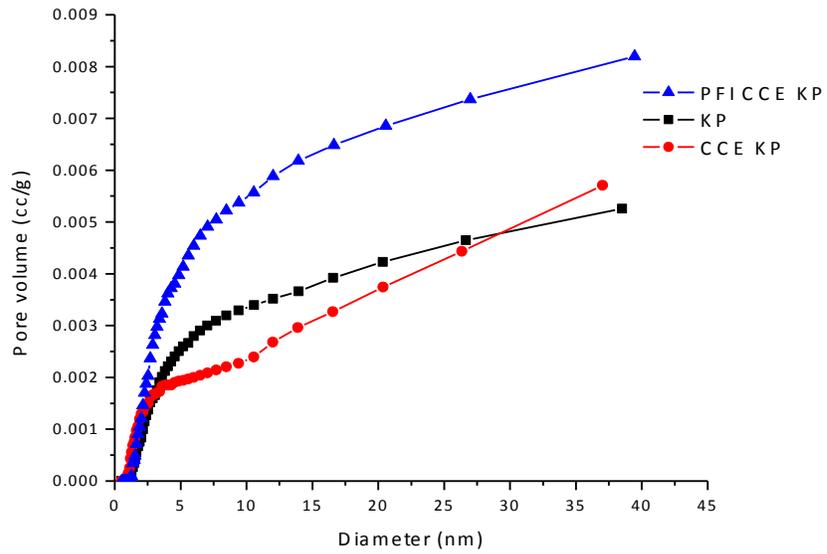
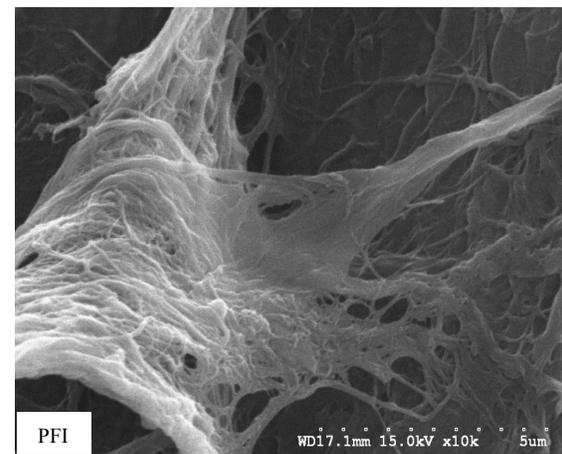
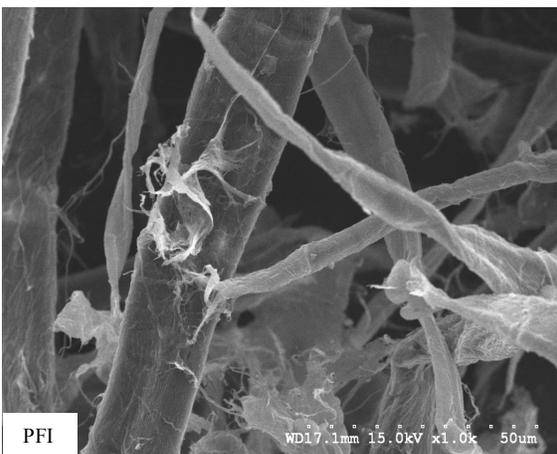
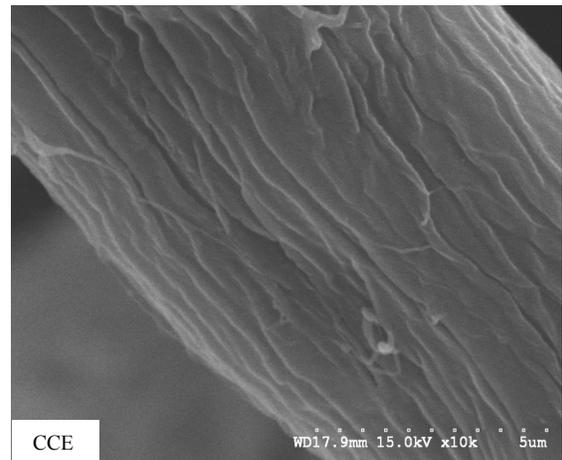
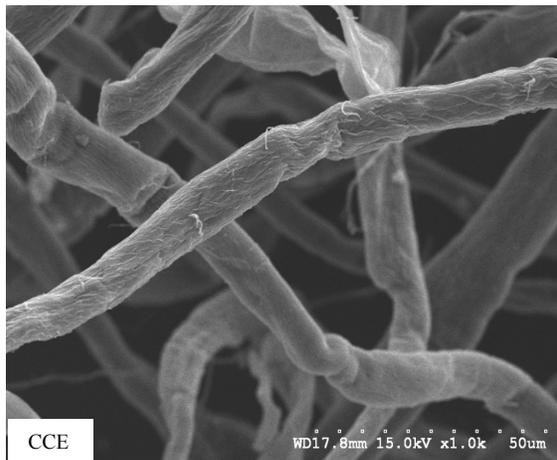
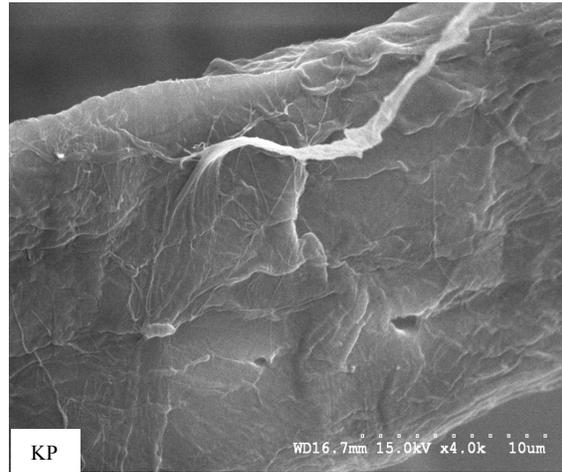
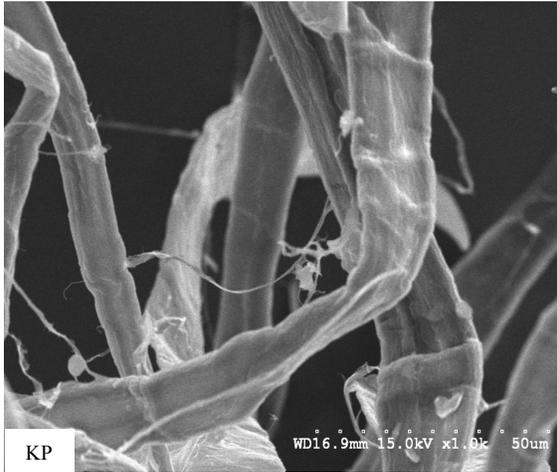


Figure 27: BJH cumulative pore volume/adsorption surface area of Kraft pulp (KP), 7% NaOH extracted Kraft pulp (CCE KP) and PFI mechanical refined CCE KP (PFI CCE KP). The BET results showed the total pore volume and accessible surface area remained similar after alkaline extraction on Kraft pulp, but after PFI mechanical refining, there was a more than 50% and 60% improvement in total volume and accessible surface area respectively.

3.3.2.5 FE-SEM micrographs



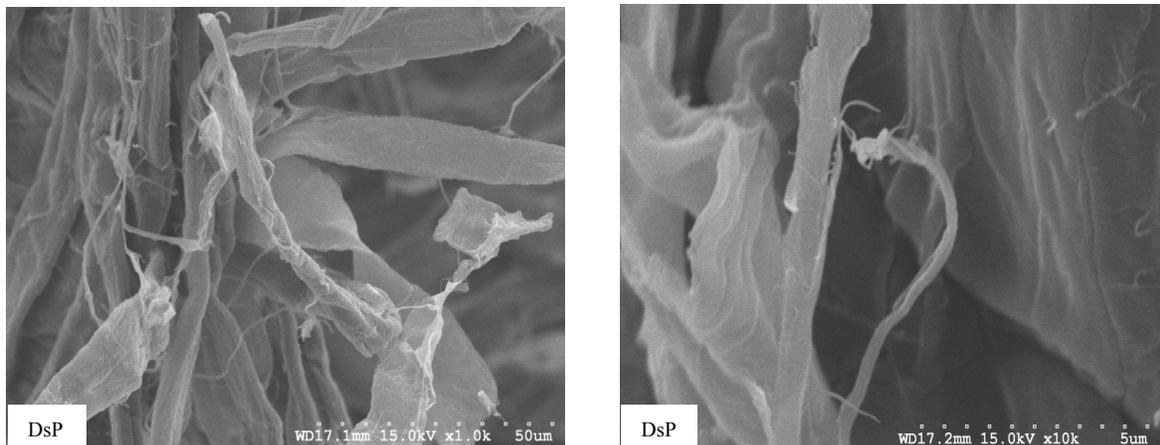


Figure 28: FE-SEM micrographs showing the microscopic structure of KP (Kraft pulp), CCE KP (9% NaOH extracted Kraft pulp), PFI (mechanical refined CCE KP) and DsP (Dissolving pulp obtained from prehydrolysis process)

The large amount of fibrillation created by mechanical refining was also demonstrated by the resulting morphological changes undergone by the pulps. It appeared that the initial fibrillation of the control Kraft pulp was dissolved in the alkali during the subsequent alkaline extraction leaving behind a smoother, flattened and more compact fibre surface structure (Figure 28). These results indicated the compaction of the fibre wall occurred during CCE by allowing cellulosic fibrils to associate more closely into larger fibril aggregate. It was apparent that the mechanical refining step disrupted this compact association and created a large amount of fibrillation on the fibre which likely increased its accessibility to carbon disulfide during subsequent derivatization. As discussed earlier, the creation of fibrillation also increased the accessibility of cellulose fibres to endoglucanase which could decrease the molecular weight of cellulose and dramatically enhance the final pulp reactivity.

3.3.3 Conclusion:

Either a PHK or CCE approach seems to be able to help convert a traditional Kraft pulp to a dissolving-grade pulp. The use of either a PHK or CCE approach results in pulps that vary in properties such as pore size, accessible surface area and microfibril aggregation, etc., that profoundly impact cellulose accessibility and reactivity. To gain a better insight into the factors that might influence the reactivity of PHK and CCE treated cellulose, we compared the properties of commercial dissolving grade cellulose produced through prehydrolysis to another Kraft pulp upgraded to dissolving grade cellulose using a CCE approach. Fibre properties related to cellulose accessibility such as pore size and accessible surface area as well as cellulose molecular properties including crystallinity, DP and fibril aggregate size were assessed for their relative influence on the cellulose reactivity of both pulps. In contrast to the acidic PHK process, CCE is performed under alkaline conditions, thus facilitating chemical recovery in the Kraft mill. Thus it will not require extra digester capacity prior to pulping, thereby debottlenecking the hemicellulose removal process. However, CCE treatments reduced the final pulp reactivity. As a result, various "post-treatments" such as mechanical refining, steam explosion, induction of fibre kink and curl, sulfuric acid hydrolysis and endoglucanase hydrolysis were applied to determine if increasing cellulose surface area could recover the reactivity lost during the production of dissolving pulp cellulose. All of the post-treatments effectively increased pulp reactivity with mechanical refining combined with endoglucanase treatment having the greatest effect, increasing reactivity from 32% to 75%.

3.4 Assessing the influence of varying prehydrolysis and pulping conditions on the action of enzymatic hydrolysis and the removal of hemicellulose on increasing cellulose reactivity

3.4.1 Background

It was apparent that the nature of the pulp substrate at the various points in the process during the conversion of a Kraft to dissolving grade pulp (PHK vs. CCE) influenced the ease of enzymatic removal of hemicellulose. As mentioned earlier, the prehydrolysis and CCE steps resulted in changes in both the location and structure of the hemicellulose and cellulose and the overall accessibility of the substrate to enzymes (Gehmayr et al., 2011; 2012; Gehmayr and Sixta, 2012; Schild et al., 2011). In the previous chapter we utilized the pulps from prehydrolysis or CCE to determine the optimum pulp characteristics that facilitate enzymatic hydrolysis of the hemicellulose and cellulose reactivity. We showed that the upstream prehydrolysis and Kraft pulping conditions played a significant role in affecting the characteristics of the resulting bleached pulp (Wollboldt et al., 2010; Wan et al., 2010; Testova et al., 2014; Luo et al., 2011; Schild et al., 2011). The effects of prehydrolysis/pulping include the prehydrolysis conditions affecting the distribution/amount of hemicellulose, the DP and accessibility of cellulose and the ease of removal of the lignin component (Testova et al., 2014; Luo et al., 2011; Borrega and Sixta, 2013; Borrega et al., 2013; Germgård, 2012). Therefore, in this work, both the prehydrolysis and pulping conditions were altered to try to create substrates that could be used to gain further insight into the characteristics that improve their susceptibility to enzymatic hemicellulose hydrolysis and cellulose modification by endoglucanases. We hoped that these studies would also help us determine if certain prehydrolysis or pulping conditions could be

“tuned” to increase hemicellulose and cellulose reactivity, using enzymes and chemicals, during subsequent cellulose derivatization.

As mentioned earlier, the hemicellulose on the fibre surface was anticipated to have the greatest influence on the ease of enzymatic hydrolysis (Viikari et al., 1994; Kim et al., 2012). For example, when employing different prehydrolysis conditions, the use of acid results in a greater amount of hemicellulose being solubilized and a reduction in cellulose DP (Li et al., 2010). It is likely that the increased amount of hemicellulose solubilized under acidic conditions could also reduce the amount of hemicellulose at the fibre surface, thus potentially decreasing the activity of hemicellulases on the pulp. However, it could also be possible that the acidic deconstruction of the cellulose could increase the accessibility of the hemicellulose to the hemicellulase treatments. Therefore, initial work focused on using three different prehydrolysis conditions employing water, acid and without a prehydrolysis step. With the high hemicellulose content within the starting wood chips, it was anticipated that the resulting brown stock pulp without prehydrolysis would likely be the most amenable to hemicellulose removal. This is a result of the propensity for hemicellulose to redeposit on the fibre surface during the later stages of a typical Kraft pulping process. This inability to remove the hemicellulose from brown stock pulp could be attributed to the increased linear nature of the hemicellulose after the highly alkaline pulping process as a result of hemicellulose debranching (Sjöström, 1981). Consequently, the increased linearity of hemicellulose may result in an increased association with linear cellulose molecules that could also reduce the accessibility of the residual hemicellulose. In the work reported here, prehydrolysis under different conditions was employed to make dissolving pulps to elucidate the influence of the prehydrolysis conditions on the structure/location/accessibility of hemicellulose and cellulose. In order to evaluate the properties of the bleached versions of these pulps produced

by varying the prehydrolysis conditions, they were delignified at room temperature using a three-step chlorite delignification (Figure 29).

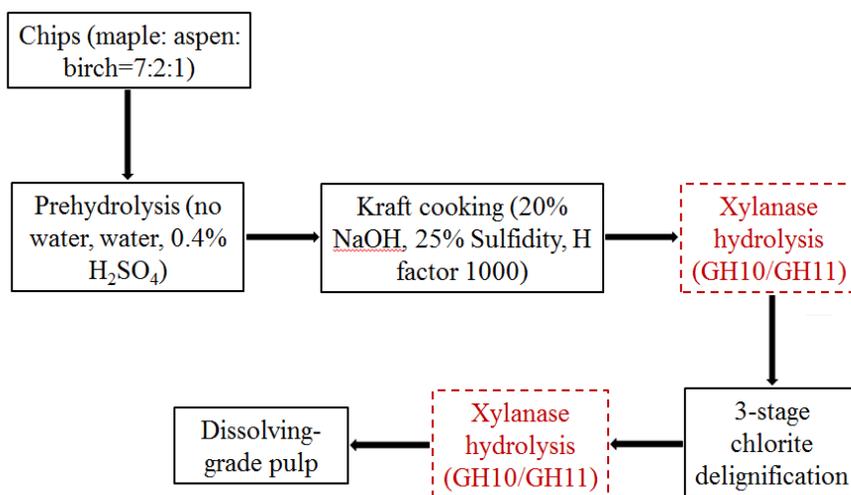


Figure 29: Flow chart of the impact of varying prehydrolysis on the characteristics of substrates such as the accessibility of hemicellulose to enzymes or the reactivity of cellulose to derivatization reagents. Sulfuric acid, autocatalysis and without prehydrolysis were conducted to generate pulps varying in their different characteristics such as the accessibility of hemicellulose and cellulose molecular weight with subsequent Kraft pulping. Xylanase hydrolysis (Multifect and HTec commercial xylanase preparations) was employed before and after delignification steps.

After investigating varying prehydrolysis conditions, we next focused on altering the Kraft pulping conditions to create substrates that would provide further insights into the properties that could improve the susceptibility of Kraft pulp to hydrolysis by hemicellulases. Changing the hemicellulose profile on the pulps was accomplished by altering the alkalinity in the Kraft cook. Previous work had shown that a greater amount of hemicellulose would precipitate at the surface of the pulp at the conclusion of a Kraft cooking process when a reduced alkaline loading was employed (Pinto et al., 2005; Kantelinen et al., 1991). Therefore, we varied the alkalinity to generate substrates enriched in xylan at the fibre surface to determine if these substrates with increased accessible xylan were more prone to xylan removal using xylanases. The pulps were

produced using Kraft pulping at high (24% NaOH on chips), low (16% NaOH on chips) and medium alkalinity (20% NaOH on chips) using unprehydrolyzed chips with subsequent bleaching using the chlorite delignification technique. The pulps were subsequently tested for their susceptibility to xylanase treatment (Figure 30). During the conversion process, a mild CCE (7% NaOH) treatment was employed after delignification to remove the extra hemicellulose to control the final hemicellulose content within the range of a dissolving-grade pulp (Gehmayr et al., 2011; Gehmayr and Sixta, 2011). Due to potential variations in the hemicellulose accessibility of the different pulps after each process, the influence of xylanase addition was assessed at specific addition points, such as before and after delignification, or after CCE treatment, to compare the different accessibility of xylan to xylanases. After CCE treatment, the pulp was subjected to an endoglucanase treatment to lower the cellulose molecular weight and to, hopefully, enhance the reactivity of the resulting dissolving grade pulp (Östberg et al., 2012; 2013).

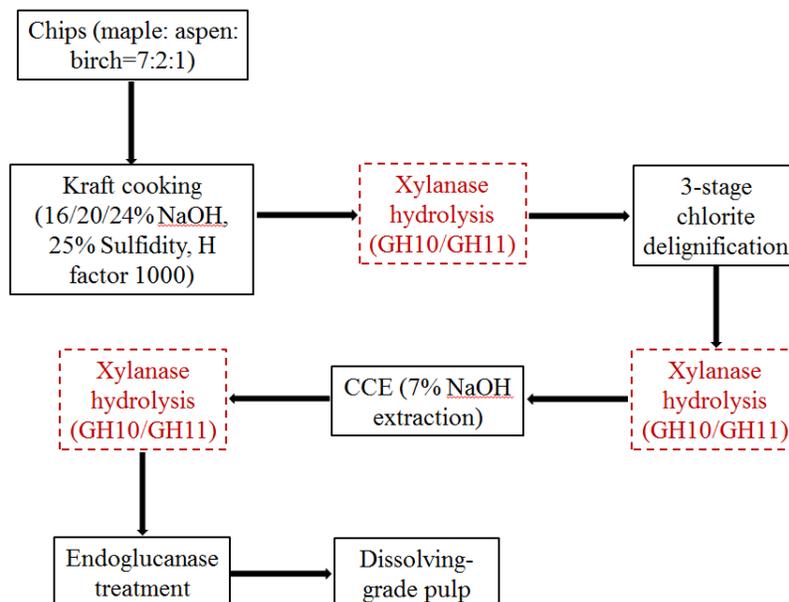


Figure 30: Flow chart of the impact of varying Kraft cooking conditions on the characteristics of substrates such as the accessibility of hemicellulose to enzymes or the reactivity of cellulose to derivatization reagents. 24%, 20% and 16% of NaOH were employed to generate pulps varying in their different characteristics such as the accessibility of hemicellulose and cellulose molecular weight. Multifect commercial xylanase preparation of which the primary xylanase was from GH10, and HTec commercial xylanase preparation of which the primary xylanase was from GH11 were employed before/after the delignification steps, and after CCE treatment.

3.4.2 Results and discussion

3.4.2.1 Chemical compositional analysis from prehydrolysis liquor

As mentioned earlier the, PHK sequence has been shown to be an effective approach to remove hemicellulose and retain cellulose reactivity during the production of dissolving pulp. The advantages of the PHK process are good chemical recovery while producing pulps with a narrow molar mass distribution and high yield after alkaline treatments (Testova et al., 2014). During prehydrolysis, the formation of acetic acid from acetyl groups bound to hemicellulose promotes the hydrolytic cleavage of glycosidic bonds and the subsequent solubilization of carbohydrates, particularly the amorphous and low molecular weight hemicellulose (Sjöström, 1981; Testova et

al., 2014). It has been shown that during the prehydrolysis step, more than 60% of hemicellulose could be solubilized from the starting chips (Luo et al., 2011; Testova et al., 2014; Borrega and Sixta, 2013; Borrega et al., 2013). In addition, the prehydrolysis step imparts increased permeability to the wood chips, which facilitates the penetration of the alkaline pulping chemicals into the resulting wood chips thereby accelerating delignification during subsequent Kraft pulping (Testova et al., 2014). It has been reported that up to 90% of the initial xylan was removed during the autohydrolysis of eucalyptus while the removal of cellulose was negligible (Garrote and Parajó, 2002; Garrote et al., 2003). The prehydrolysis conditions such as temperature, time and the addition of catalysts such as acid have been shown to affect the sugar composition significantly (Li et al., 2010). A systematic study was carried out to analyze the dissolution profile of the main wood components from hardwood chips during the prehydrolysis process with a temperature ranging from 130°C to 170°C (Tunc and van Heiningen, 2008). It was found that when the temperature was higher than 150°C, oligomeric xylose was the predominant component in the hydrolysate, and a small amount of furfural and HMF could also be found from the hydrolysate at the temperature higher than 160°C (Tunc and van Heiningen, 2008). It was also found that at a temperature higher than 160°C, most of the arabinan and galactan could be solubilized from the wood (Tunc and van Heiningen, 2008).

The high temperature (usually over 170°C) or the addition of high concentrations of acid might be problematic. For example, although the use of a prehydrolysis step can remove hemicellulose, it has also been shown that it posed challenges for the subsequent pulping stage with regard to extractives accumulation and pH swings from the acidic pH utilized in prehydrolysis to the alkaline pH in Kraft pulping (Liu et al., 2011). Prehydrolysis also poses some challenges as it increases the requirement for digester capacity and the final pulp yield is generally less than 35%,

indicating a high amount of chemicals, lignin and hemicellulose need to be sent to the Kraft recovery boiler which has been shown to one of the main bottlenecks in the overall PHK process. In addition, during prehydrolysis as the cooking temperature and time are raised to improve hemicellulose removal, the lignin might undergo condensation, thus compromising the ability of subsequent Kraft pulping to remove lignin (Germgård, 2012). The acidic conditions employed to remove hemicellulose during prehydrolysis also present challenges toward subsequent alkaline Kraft pulping necessitating significant washing prior to pulping (Luo et al., 2014). Consequently, a moderate prehydrolysis condition was applied at a maximum temperature of 170°C with a 90-minutes residence time. In the case of the acid prehydrolysis, 0.4% of sulfuric acid (on chips) was employed.

Table 14: Sugar analysis of prehydrolysis liquors from different conditions (based on wood, %). Water PHK was the autocatalyzed prehydrolysis. Acid PHK was the 0.4% sulfuric catalyzed prehydrolysis.

Sugar		Water PHK hydrolysate %	Acid PHK hydrolysate %
Arabinose	Oligomer	0.02	0.06
	Monomer	0.53	0.63
Galactose	Oligomer	0.29	0.58
	Monomer	0.05	0.49
Glucose	Oligomer	1.22	1.42
	Monomer	0.31	0.62
Xylose	Oligomer	11.13	9.64
	Monomer	2.19	4.83
Mannose	Oligomer	1.73	1.96
	Monomer	0.22	0.44
Yield %		79.82	73.22

The water PHK and Acid PHK processes solubilized 62% and 72% of total hemicellulose from the starting chips respectively (Table 14). At 170°C, the total hemicellulose hydrolyzed by Water PHK were 16.2% (based on the starting wood) with the majority in the oligomeric form, which

was 5 times higher than the monomers. The total hemicellulose sugars removed by Acid PHK were 18.6% (based on starting wood) with a relatively high ratio of monomeric sugars (Table 14). Similar observations of increasing monomers were made when a more severe prehydrolysis has employed, such as when the prehydrolysis time was extended from 90 to 120 minutes (Li et al., 2010). Due to the addition of acid, the Acid PHK process had a greater amount of monomers and less oligomers compared to the Water PHK. This could be due to the severe acidic cooking conditions converting the xylose from oligomers to monomers. A small amount of glucose (<2% of total wood chips) was released during both processes, indicating only a minor loss of cellulose occurred. Additionally, the yield of chips obtained after Water PHK was more than 6.5% greater than that from the Acid PHK process indicating the increased hemicellulose removal when acid was added to the PHK. As expected, and previously reported, increasing prehydrolysis intensity by increasing temperature, residence time or adding an acid catalyst resulted in pulps of higher purity but lower cellulose yield and degree of polymerization (Testova et al., 2014).

3.4.2.2 Chemical composition analysis on substrates after different treatments

Table 15: Chemical composition and yield analysis of pulps made from different prehydrolysis process after going through different chemical/enzymatic treatments. Water PHK was the autocatalyzed prehydrolysis. Acid PHK was the 0.4% sulfuric acid catalyzed prehydrolysis. Multifect/HTec xylanases used in this work were the commercial xylanase preparations.

Substrate	Cellulose %	Hemicellulose %	Lignin %	Yield %
Starting pulp	44.5	28.6	23.8	100.0
<i>Water PHK</i>				79.8
Kraft pulping	94.7	4.8	2.3	43.9
Multifect/HTec xylanase	-	4.4/4.2	-	-
Delignification	94.2	4.3	0.8	37.5
Multifect/HTec xylanase	-	3.8/3.7	-	-
<i>Acid PHK</i>				73.2
Kraft pulping	94.3	4.1	3.0	40.3
Multifect/HTec xylanase	-	3.8/3.7	-	-
Delignification	95.6	3.9	0.7	32.7
Multifect/HTec xylanase	-	3.6/3.5	-	-

It was hypothesized that the increased amount of hemicellulose solubilization under acidic conditions could also reduce the amount of hemicellulose reprecipitated at the fibre surface, thus potentially decreasing the activity of hemicellulases on the pulps. Surprisingly, in our work, the two processes generated the pulps with similar content of residual hemicellulose (Table 15), and the Acid PHK process only resulted in a slightly lower amount of hemicellulose (around 10% more hemicellulose removal) on final dissolving pulp compared to Water PHK. One of the reasons might be, as discussed previously, the formation of acetic acid during water prehydrolysis from acetyl groups from hemicellulose promotes the hydrolytic cleavage of glycosidic bonds and aid in the subsequent solubilization of carbohydrates, particularly the amorphous and low molecular weight hemicellulose (Testova et al., 2014). The amount of released acetic acid might be capable of hydrolyzing most of the accessible hemicellulose even without the addition of sulfuric acid, since the final pH of the hydrolysate was lower than 3. In addition, xylanase hydrolysis did not have a significant impact on removing xylan from the pulps obtained PHK probably due to the poor accessibility of xylan after prehydrolysis and the Kraft cooking processes. Overall, the hemicellulose content from both pulps was as low as a dissolving-grade pulp which was less than 5% (Gehmayr et al., 2010).

Previous research has hypothesized that the lignin could undergo condensation under acidic conditions that might potentially compromise its removal during subsequent Kraft pulping (Luo et al., 2014), while others thought the prehydrolysis could enhance lignin removal due to the dissolution of hemicellulose which made the substrates more porous and permeable to alkaline pulping reagents (Testova et al., 2014). In our work, the difference in lignin content after the two prehydrolysis and Kraft cooking processes was almost negligible, indicating lignin condensation was not a problem during our PHK processes. Interestingly, as discussed before, the glucose

release (Table 14) and total hemicellulose removal (Table 15) were identical for the pulps obtained from those two prehydrolysis processes, but acid hydrolysis had an approximately 5% lower final dissolving pulp yield (Acid PHK) than the water prehydrolysis process (Water PHK) (Table 15). One of the possible reasons could be the addition of the small amount of sulfuric acid was capable of reducing the molecular weight of the pulp cellulose and hemicellulose, rather than solubilizing additional cellulose and hemicellulose. Consequently, these fragments with reduced molecular weight were likely more prone to dissolution during subsequent Kraft pulping.

After investigating varying prehydrolysis conditions, the next set of experiments focused on altering the Kraft pulping conditions to create substrates that potentially had a higher amount of accessible hemicellulose available for hydrolysis by hemicellulases. Altering the hemicellulose profile in the cook was achieved by varying the alkalinity in the Kraft process. Previous work had shown that a greater amount of hemicellulose would precipitate at the surface of the pulp at the conclusion of a Kraft cooking process when a reduced alkaline loading is employed (Sixta, 2006). It has also been reported at the beginning of bulk delignification, a high concentration of sodium hydroxide degrades hemicellulose, predominantly xylan (Sixta, 2006). During the final cooking stage, the re-precipitation of xylan onto the out layer of fibres is limited due to the high sodium hydroxide concentration (Sixta, 2006). Therefore, decreasing the alkalinity could generate substrates that are enriched in xylan at the fibre surface.

Table 16: Chemical composition and yield analysis on pulps after going through different chemical/enzymatic treatments. Low/Med/High Alk, Kraft cooking process with low (16% NaOH), medium (20% NaOH) and high (24% NaOH) alkalinity. Multifect/HTec xylanases used in this work were the commercial xylanase preparations. Delignification was a 3-step chlorite delignification to remove most of the lignin from the pulps.

Substrate	Cellulose %	Hemicellulose %	Lignin %	Yield %
Starting pulp	44.5	28.6	23.8	100
<i>Low Alk</i>				
Kraft pulping	71.5	22.0	7.1	60.1
Multifect/HTec xylanase	-	13.9/11.1	-	-
Delignification	73.3	25.3	1.2	28.7
Multifect/HTec xylanase	-	14.9/11.4	-	-
CCE (7% NaOH)	94.5	4.5	0.8	22.4
Multifect/HTec xylanase	-	3.3/2.8	-	-
<i>Med Alk</i>				
Kraft pulping	73.6	22.1	3.7	55.8
Multifect/HTec xylanase	-	14.6/11.6	-	-
Delignification	74.9	22.5	0.9	47.2

Substrate	Cellulose %	Hemicellulose %	Lignin %	Yield %
Multifect/HTec xylanase	-	13.9/10.4	-	-
CCE (7% NaOH)	95.2	4.2	0.6	37.4
Multifect/HTec xylanase	-	3.2/2.8	-	-
<i>High Alk</i>				
Kraft pulping	74.1	21.0	3.0	52.4
Multifect/HTec xylanase	-	14.5/11.6	-	-
Delignification	76.2	20.1	0.9	45.3
Multifect/HTec xylanase	-	13.3/10.3	-	-
CCE (7% NaOH)	95.3	4.2	0.4	36.0
Multifect/HTec xylanase	-	3.3/3.0	-	-

The low alkalinity (16% NaOH) pulping experiment (Low Alk) resulted in approximately a 10% higher unscreened pulp yield than the high alkalinity (24% NaOH) experiment (High Alk). This was a similar trend to previous work where the high loading of alkali during Kraft cooking process lowered the final pulp yield (Sixta, 2006). However, it was found that the yield of the pulp obtained from the low alkalinity cooking process dropped dramatically after the screening step (Table 16), which was a result of the low dosage of sodium hydroxide removing less lignin, therefore creating a greater amount of “rejects” during the screening process. Kinetic studies

have also demonstrated that the rate of delignification in the initial phase and bulk delignification of Kraft pulping is independent of the alkali concentration (Sixta, 2006). It has been shown that an increase in alkali charge accelerates the delignification rate and the transition from bulk to final delignification phase, resulting in a lower lignin content (Sixta, 2006). The difference in lignin content (>4%) from Low Alk and High Alk supports the lower delignification when a lower alkalinity was used (Table 16). As a result, there was a high amount of “rejects” remaining in the pulp from the low alkalinity cooking process which compromised the yield of the final dissolving pulp (22.4%).

It was also noted that the residual hemicellulose content of 16% NaOH cooked pulp (Low Alk) was more than 5% higher than that of 24% NaOH cooked pulp (High Alk) after going through the delignification steps (Table 16). This result supports the previous hypothesis that using a lower alkalinity (16% NaOH) results in an increased amount of hemicellulose that re-precipitates on the surface of the Kraft fibres. The pulps made using the three levels of alkalinity all had a high retention of hemicellulose (>20%) because they did not undergo a prehydrolysis step prior to pulping. The CCE process, which has been reported to effectively remove most of the hemicellulose and part of low molecular weight cellulose to obtain homogeneous and purified cellulose feedstock, was therefore applied to these pulps to upgrade them to dissolving-grade pulps (Gehmayr et al., 2011; 2012; Gehmayr and Sixta, 2012). The treatment of the Kraft pulps, which had more than 20% of hemicellulose with a moderate alkaline extraction (7% NaOH) after a xylanase hydrolysis, reduced the hemicellulose content to a range lower than 5% (Table 16). This pulp could meet the requirement for a dissolving-grade pulp (Sixta, 2006). It was apparent that the Low Alk pulp had a greater amount of hemicellulose removal resulting from the combination of xylanase and CCE treatments (Table 16). This indicated that the low dosage of

alkaline during Kraft pulping could enhance the accessibility of hemicellulose to chemical/enzymatic reagents.

3.4.2.3 Influence of different PHK conditions on fibre length/width and fines content

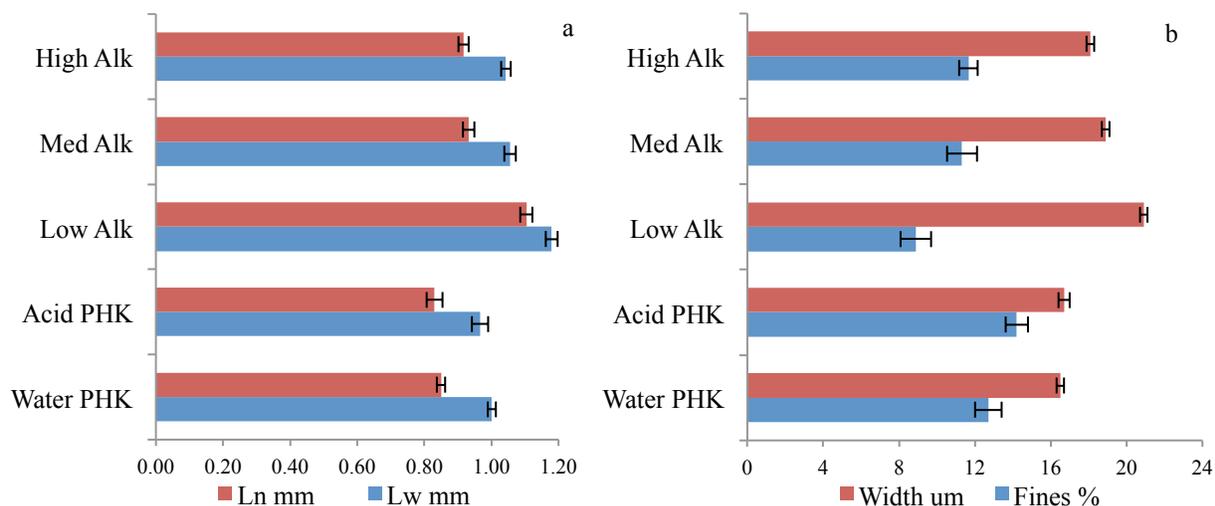


Figure 31: Fibre length, width and fines analysis by Fibre Quality Analyzer (FQA) on different pulps. Water PHK was the autocatalyzed prehydrolysis. Acid PHK was the 0.4% sulfuric acid catalyzed prehydrolysis. Low/Med/High Alk: Pulps obtained from Kraft cooking process with low (16% NaOH), medium (20% NaOH) and high (24% NaOH) alkalinity. All the measurements were employed on the pulps that had been gone through all the conversion processes and became dissolving-grade pulps. Fines are defined as fibre length in between of 0.07mm to 0.20mm. L_w : weight-weighted average fibre length. L_n : length-weighted average fibre length.

It was found that the pulps obtained by prehydrolysis (Water/Acid PHK) had relatively shorter fibre lengths and widths compared with the pulps obtained from the CCE process (Low/Med/High Alk) (Figure 31). This reduced length and width were likely because the acid, formed by the release of acetyl groups that were attached to the backbone of the hemicellulose macromolecules, randomly cleaved cellulose and decreased the molecular weight of the cellulose (Suchy et al., 2009; Hidayat et al., 2012; Thygesen et al., 2011). However, during the CCE process, the alkaline only solubilized the small molecular weight cellulose and hemicellulose components without randomly cleaving the cellulose as the acid does on cellulose. Additionally,

alkaline is a strong swelling reagent that can expand the capillaries and enlarge the pore volume of the substrates. Thus the cellulose could be “activated” by the swelling of the fibres and the breakage of intra- and intermolecular hydrogen bonds in and between the cellulose by caustic lye (Sjöström, 1981), likely increasing the final fibre length and width. When comparing the size of the three pulps without going through a prehydrolysis process, the high loading of alkalinity (High Alk) reduced the fibre length by around 20% (L_n) and width by approximately 15% than the low alkalinity cooked pulp (Low Alk). The reduced fibre length/width caused by a high loading of alkali implied a more aggressive peeling reaction occurred on the cellulose under a higher loading of sodium hydroxide cooking process. In addition, the lowest content of fines was achieved on the low alkalinity cooked pulp (Low Alk) among all of those five pulps. The possible reason could be that the less severe cooking conditions resulted in minor damage to the cellulose fibres, meaning that fewer fines were produced. Subsequently, the fines were solubilized during the subsequent CCE process due to the low molecular weight of those fines.

3.4.2.4 Influence of different PHK conditions on the accessibility of pulps

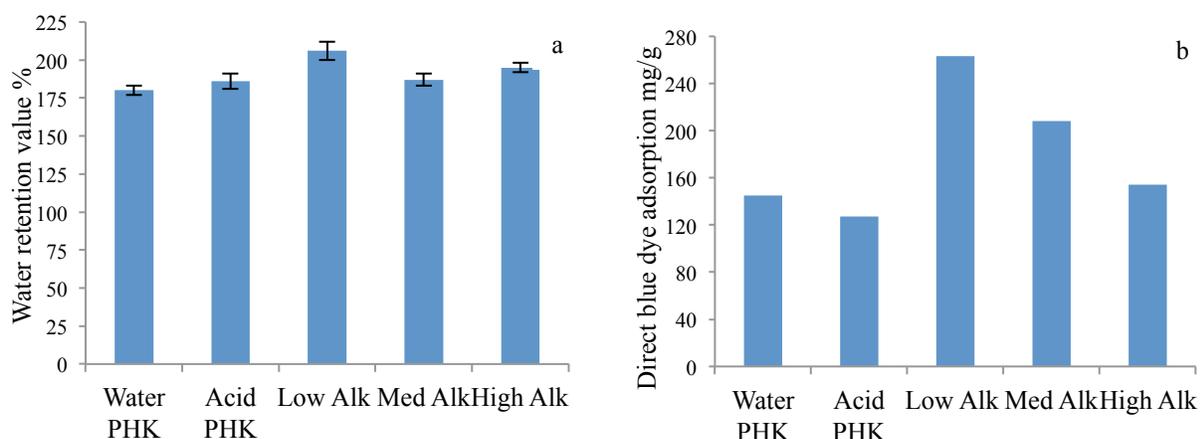


Figure 32: Accessibility of pulps varying in their prehydrolysis and pulping conditions to water (a) and direct blue dye (b). Water PHK was the autocatalyzed prehydrolysis. Acid PHK was the 0.4% sulfuric acid catalyzed prehydrolysis. Low/Med/High Alk: Pulps obtained from Kraft cooking process with low (16% NaOH), medium (20% NaOH) and high (24% NaOH) alkalinity. All the measurements were employed on the pulps that had been gone through all the conversion processes and became dissolving-grade pulps.

To assess the influence of different prehydrolysis/pulping conditions on the accessibility of pulps to enzymes for fibre modification, WRV and Simons' staining approaches were applied. When measuring the WRV of the pulps, it was found that there was no significant difference between the samples with the exception that the pulp cooked under a low alkalinity (Low Alk) had a slightly higher WRV than the other pulps (Figure 32 a). Simons' staining is an effective measurement for estimate the overall accessible surface area of pulps and pretreated biomass pulps (Yu and Atalla, 1998; Chandra et al., 2008a). Generally the small molecular direct blue dye could penetrate into both the large pores and the small pores of cellulosic fibres and bind with cellulose (Chandra et al., 2008a). Of the five pulps, the pulps that had not gone through a prehydrolysis step (Low/Med/High Alk) had a much higher direct blue dye adsorption than did the pulps obtained when using a prehydrolysis step. In addition, the low alkalinity cooked pulp

(Low Alk) had the highest direct blue dye adsorption than other pulps (Figure 32 b), indicating the higher accessibility of the pulps cooked under a lower loading of alkali condition.

3.4.2.5 Xylanase hydrolysis of xylans from different pulps

As discussed throughout the thesis, aggressive chemical treatments are currently used to convert Kraft pulps to dissolving-grade pulps. As a result, several studies have been focused on investigating the applicability of specific enzymes such as xylanases, such as Multifect and HTec, and endoglucanases during the conversion process to dissolving pulp, aiming to decrease the usage of chemicals and improve pulp yield (Köpcke et al., 2010; Gehmayr et al., 2011; 2012). It has been previously shown that the hemicellulose components of pulps have different structure and accessibility after going through different treatments, which would profoundly impact the interactions of xylans with xylanases (Sjöström, 1981; Wong and Saddler, 1992; Kim et al., 2000). For example, it has been reported that during the conversion of Kraft pulp to dissolving pulp that, after multiple delignification and bleaching steps, the xylan originally re-precipitated at the outer layers of fibres was de-branched and trapped within cellulose which limiting its accessibility to xylanases (Wong and Saddler, 1992; Kim et al., 2000). Therefore, we next carried out xylanase hydrolysis at three different additional points, before/after bleaching, and after the CCE process. This was done to assess the various structure and accessibility issues that the xylan might encounter after going through these different treatments (Figure 31). In addition, xylanase hydrolysis on the pulps cooked under different alkaline conditions was also compared since it was anticipated that the different alkalinity conditions employed during the pulping process could generate substrates with varying accessibility of hemicellulose to Multifect and HTec xylanases.

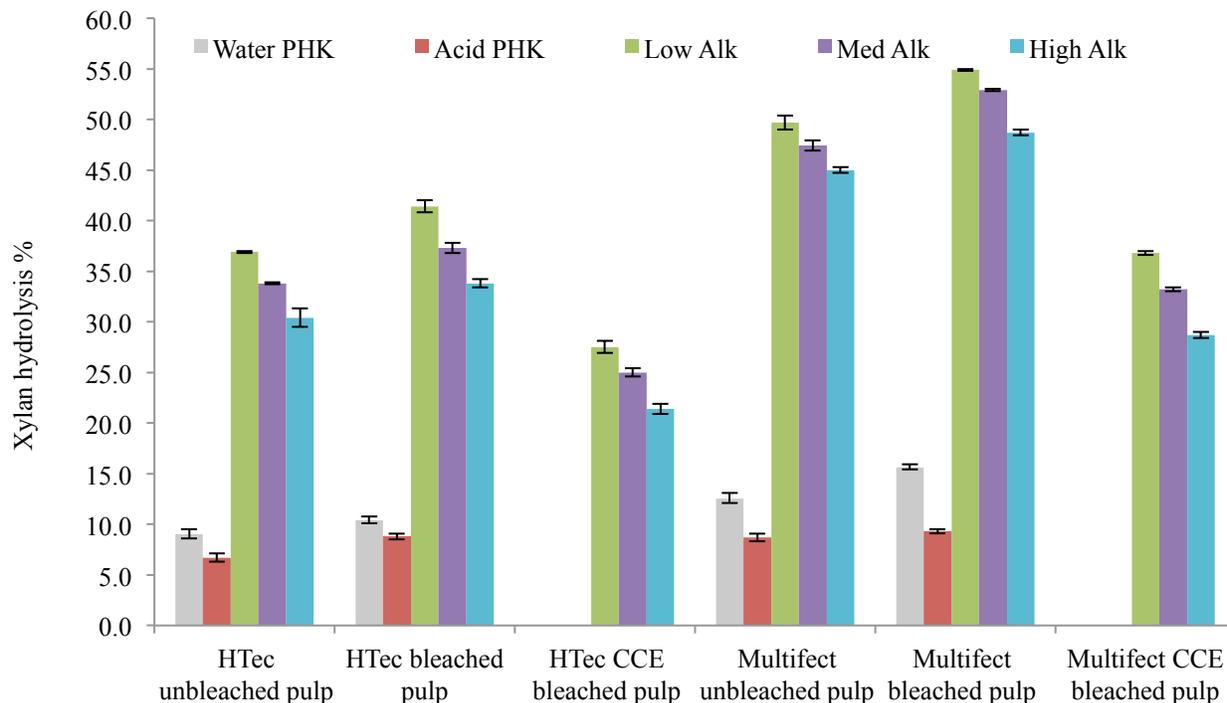


Figure 33: Xylan removal from different substrates by Multifect and HTec xylanases. Xylanase hydrolysis was conducted according to the different additional points shown in Figure 29 and Figure 30. Water PHK was the autocatalyzed prehydrolysis. Acid PHK was the 0.4% sulfuric acid catalyzed prehydrolysis. Low/Med/High Alk: Pulps obtained from Kraft cooking process with low (16% NaOH), medium (20% NaOH) and high (24% NaOH) alkalinity. Multifect/HTec xylanases used in this work were the commercial xylanase preparations.

When the two enzyme preparations were applied to the pulp at an equivalent protein loading, it was found the Multifect xylanase was able to access and remove a greater amount of xylan (Figure 33) while hydrolyzing a lower amount of cellulose (~3-4%). As described earlier, Multifect xylanase also contains cellulase activity, which could further enhance the accessibility of xylan to xylanase due to the synergism between xylanase and cellulase enzymes. An analogy can be made from bioconversion research where it has been shown that supplementing xylanases into cellulose cocktails dramatically increased the hydrolytic performance of “cellulase mixtures”

when adding to pretreated lignocellulosic substrates (Kumar and Wyman, 2009b, Várnai et al., 2010; 2011; Hu, et al., 2013). In addition, another possible reason could be, as shown in Section 3.2, that the GH11 xylanase has a much smaller size than the GH10 xylanase (Collins et al., 2005). The smaller sized GH11 xylanase had a higher potential to better access and hydrolyze the xylan from pulps compared to GH10 xylanase, leading to a higher xylan removal result.

It was expected that the unbleached pulps would have a higher accessibility to xylanases than the bleached pulps due to the high amount of re-precipitated xylan on the surface of the fibres at the conclusion of the cooking process (Sixta, 2006). However, our work showed the bleached pulps had a slightly higher xylan removal (around 5%) by both HTec and Multifect xylanases than that from unbleached pulps, indicating that lignin might play a negative role in the ease of hydrolysis of xylan by xylanase from the unbleached pulp. In bioconversion area, the lignin mainly posed a physical barrier or caused non-productive binding with cellulases, thereby reducing the enzymes available for subsequent hydrolysis process (Tu et al., 2007; Kumar et al., 2012). The initial work by our group showed only a 60% of total hemicellulose could be solubilized by hemicellulases from dissolving pulp (Gübitz et al., 1997). It was hypothesized that the remaining hemicellulose after the enzymatic treatments was linked with lignin through LCC that were inaccessible to hemicellulases (Gübitz et al., 1998; Wong et al., 1997). Later work by our group also demonstrated that the existence of lignin or LCC could inhibit xylanase activity (Berlin et al., 2006). In addition, the xylans from the pulps that had gone through CCE treatments had the lowest accessibility to xylanases as there was an approximate 15% lower xylose release than the pulps that were not subjected to CCE (Figure 33).

The results also showed the pulp obtained under a low alkalinity during the pulping process (Low Alk) possessed the highest xylan removal result by xylanases no matter at which point that

the xylanases were added (Figure 33). This result further approved the hypothesis that a greater amount of accessible hemicellulose would precipitate at the surface of the pulp at the conclusion of a Kraft pulping when a reduced alkaline loading was employed (Pinto et al., 2005; Kantelinen et al., 1991). In addition, the highest accessibility to direct blue dye on Low Alk (Figure 32) could also support the hypothesis that a lower loading of sodium hydroxide during pulping could increase the accessibility of xylans on pulps and thus their xylan removal by xylanases (Figure 33).

3.4.2.6 Influence of different PHK conditions on the reactivity of pulps

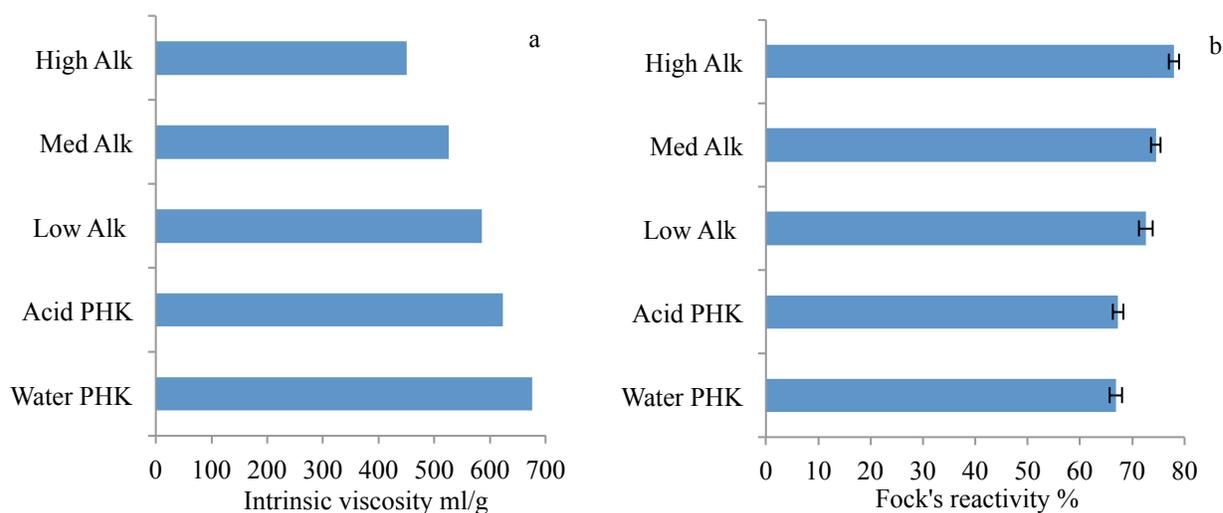


Figure 34: Intrinsic viscosity (a) and Fock's reactivity (b) of different pulps. Water PHK was the autocatalyzed prehydrolysis. Acid PHK was the 0.4% sulfuric acid catalyzed prehydrolysis. Low/Med/High Alk: Pulps obtained from Kraft cooking process with low (16% NaOH), medium (20% NaOH) and high (24% NaOH) alkalinity. All the measurements were employed on the pulps that had undergone through all the conversion process and became dissolving-grade pulps.

When employing different prehydrolysis conditions, the use of acid results in a reduction in cellulose molecular weight. The pulps that have undergone a CCE process (Low/Med/High Alk) had high molecular weight and poor pulp reactivity. This may be because the CCE has been

previously shown to increase the aggregation between cellulose microfibrils and enrich high molecular weight cellulose due to the removal of low molecular weight components (Gehmayr et al., 2011). As a result, it was necessary to employ a subsequent endoglucanase treatment to the pulps obtained by a CCE process to enhance final pulp reactivity. It was found there was an approximately 10% decrease in intrinsic viscosity of the pulp obtained after the addition of small amount of sulfuric acid (Acid PHK) compared with the pulp obtained by the water prehydrolysis process (auto-catalyzed) (Figure 34 a). All pulps that underwent endoglucanase treatment had a relatively lower intrinsic viscosity than pulps that had undergone a prehydrolysis process without the endoglucanase treatment (Water/Acid PHK). Overall, the intrinsic viscosity of those pulps had similar trend with their reactivity results, and the pulps undergone EG treatment possessed lower intrinsic viscosity and high cellulose reactivity (Figure 34). In addition, high alkalinity decreased cellulose intrinsic viscosity more aggressively likely due to a greater amount of peeling reactions under the high loading of alkali, which resulted in a final intrinsic viscosity more than 20% lower than the low alkalinity cooked pulp (Low Alk).

All pulps had remarkably high levels of reactivity comparable to the reactivity of a commercial dissolving-grade pulp (Figure 34 b). Compared to the water prehydrolyzed pulp (Water PHK), the pulp produced using a high alkalinity (High Alk) had an approximately 15% higher reactivity (Figure 34 b). The result was in correspondence with the previous report that the reactivity of a given pulp is heavily influenced by the molecular weight of cellulose and the accessibility of the cellulose hydroxyl groups to derivatization reagents (Engström et al., 2006). Typically substrates with lower molecular weight cellulose in addition to larger pores and a disrupted cellulose crystal structure exhibit increased reactivity (Peter Strunk, 2012). Additionally, cellulose accessibility also plays a crucial role during the actual derivatization process (Engström, et al., 2006; Köpcke

et al., 2010; Östberg et al., 2011; 2012). Those could explain why the pulps undergone CCE and endoglucanase treatments (Low/Med/High Alk), (having a higher accessibility (Figure 33) and lower intrinsic viscosity (Figure 34 a), exhibited higher cellulose reactivity than pulps obtained from prehydrolysis process but no endoglucanase treatment (Water/Acid PHK). In addition, previous work had indicated that pulp reactivity was inversely proportional to intrinsic viscosity, or the average cellulose molecular weight in a given pulp sample (Engström, et al., 2006). Interestingly, in this work when plotting the intrinsic viscosity of the five pulps against their reactivity showed an inversely proportional relationship (Figure 35). These results could indicate that a lower intrinsic viscosity was a beneficial attribute to obtain higher cellulose reactivity for dissolving-grade pulps.

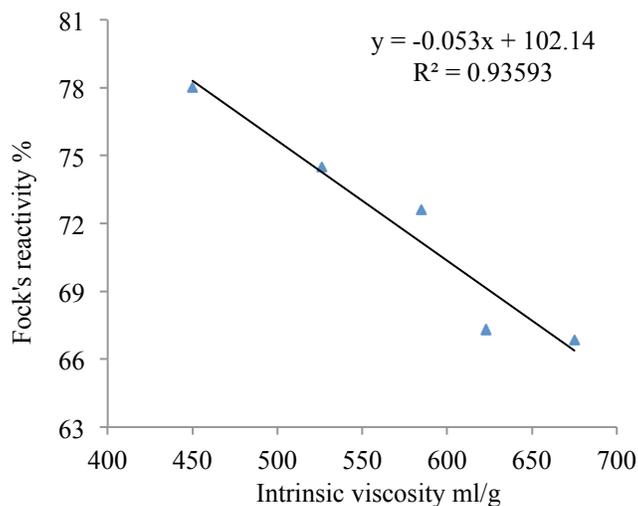


Figure 35: Relationship between cellulose reactivity and intrinsic viscosity. Pulp reactivity is inversely proportional to intrinsic viscosity, or the average cellulose molecular weight.

3.4.3 Conclusions

Prehydrolysis and pulping conditions could be altered to gain further insights into the characteristics that improve pulps susceptibility to enzymatic hemicellulose hydrolysis and

cellulose modification. In this work, in order to create the substrates with varying amounts of hemicellulose, DP and the accessibility of cellulose, the prehydrolysis conditions and alkali loading during Kraft cooking were altered. More than 60% of the totally hemicellulose could be removed by autocatalyzed prehydrolysis without losses in cellulose yield. The addition of a small amount of sulfuric acid (0.4%) to the prehydrolysis removed an additional 10% of the hemicellulose. However more of the cellulose was degraded and it had a lower intrinsic viscosity. Applying different alkalinity during Kraft pulping produced pulps with different amounts of accessible hemicellulose on fibre surface (~5%) and different cellulose molecular weights. It was also shown that lowering the addition of sodium hydroxide during the pulping process could result in a pulp with more accessible xylan to xylanase hydrolysis. In addition, increasing the alkali loading reduced the molecular weight of cellulose and pulp yield but increased the cellulose reactivity, likely due to the more severe peeling reactions occurred on cellulose component.

3.5 Upgrading hardwood Kraft pulps to dissolving-grade pulps: The importance of fibre size for pulp purity, accessibility and reactivity

3.5.1 Background

Earlier work had elucidated that pulp properties such as hemicellulose/cellulose accessibility, fibre morphological properties and the aggregation of cellulose microfibrils all influenced the ability of enzymes to facilitate the conversion of hardwood Kraft to dissolving pulps. Since these factors vary among different pulp furnishes, it is also likely that the heterogeneity among different cell types in hardwood would present these variations in substrate characteristics among the vessel elements, fibres, tracheid and longitudinal parenchyma, which also vary in their size.

Therefore, the properties of individual size fractions of fibres may also play a role in affecting the susceptibility of Kraft pulps to chemo-enzymatic conversion to dissolving pulps. For example, previous work on xylanase pre-bleaching of Kraft pulp fibre fractions separated using a Bauer Mcnett fibre classifier has shown that the short fibres and primary fines fraction were heavily enriched in hemicellulose and lignin. It was shown that these variations in chemical composition played a crucial role in the bleach-boosting effect of xylanase treatments (Mansfield et al., 1996). The results showed there was a greater increase in pulp brightness obtained from the shorter fibre fraction which may be more related to a reduction in their higher lignin content after a xylanase treatment (Mansfield et al., 1996). It has also been shown that pulp size fractions had varying accessibility towards enzymatic hydrolysis during bioconversion (Jackson et al., 1993; Mooney et al., 1999). One of the reasons was that the separated fibre fractions, especially in the case of hardwood, consist of different fibre cell types that vary in their chemical compositions and morphologies such as pore structure, accessibility and surface area, thus may vary in their susceptibility to downstream chemical or enzymatic treatments.

In hardwoods, the short fibre fraction generally includes vessel elements, ray parenchyma cells and short tracheid and fibres (Lindström, 1978). Vessels are the widest and shortest cells in hardwood. With their thin walls, large cell cavities and the existence of perforations in the adjacent vessel elements generating a continuous conductive passageway, they are well adapted for water conduction in trees (Hoadley, 1990). It has been reported that vessel elements in hardwood are rich in lignin, especially guaiacyl type lignin (Higuchi, 1985), which likely restricts the swelling of cellulose fibres and limits the enzymatic hydrolysis of cellulose (Ramos et al., 1992). Tracheids are usually imperforated (Higuchi, 1985). The cell wall in tracheid is also thin, with pits on the side wall. Fibres, which account for 40-90% of total weight of the cells,

have thickened walls and a small diameter. Parenchyma cells are rich in hemicellulose as it has been reported that 80% of all of the polysaccharides present consist of xylan (Higuchi, 1985). As a result of these specific properties, each of these pulp size fractions may be more suitable for particular end product applications. Generally the high-grade fraction (typically considered the long fibres) could be used to produce the high-quality paper, ideally at a quality similar to the original fibre furnish. The short fraction could be used to produce a lower-grade paper (Scott and Abubakr, 1994), or be used for other non-paper but valued purposes, such as bioethanol or dissolving-grade pulps, a chemical feedstock not affected by fibre length. In the work reported here it was projected that the long Kraft pulp size fraction might have suitable properties for the production of traditional paper products, while short fibre fraction might be an optimal pulp furnish for the chemo-enzymatic conversion of Kraft pulp to dissolving-grade pulps.

Previous work indicated that the short fibre fraction was preferentially attacked by enzymes. For example, it has been suggested that fines collected in the short fibre fraction are the main targets for hydrolytic enzymes to preferentially attack due to their high specific surface area and accessibility (Jackson et al. 1993). It has also been shown that the short fibres and fines have increased specific surface area, so they could be hydrolyzed by cellulases at a faster rate and more completely than the long fraction (Mooney et al., 1999). Therefore, in this work we anticipated that the shorter fibre fraction would be more prone to hydrolysis by xylanases and endoglucanases, thereby enhancing xylan removal and cellulose reactivity when converting the short fibre fraction to a dissolving grade pulp. The objective of this part of the thesis was to investigate the influence of fibre size on the susceptibility of enzymes (xylanases and endoglucanase) and the potential to upgrade hardwood Kraft pulps to dissolving pulp grades. A hardwood Kraft pulp was fractionated using a Bauer-Mcnett fibre fractionator. The resulting

fibre fractions were characterized for their chemical composition, surface area, pore structure, accessibility, fibre length/width, and the degree of polymerization of their carbohydrate components. The fibres were then subjected to enzymatic treatments to assess their susceptibility to hemicellulose removal using the combination of xylanase treatment with a subsequent CCE and reactivity enhancement using endoglucanases.

3.5.2 Results and discussion

3.5.2.1 Applications of different fractions from unbleached hardwood Kraft pulps

It was expected that the chemical composition and/or morphological properties of fibres would play an important role in determining the pulps susceptibility to treatment by enzymes/chemicals. This includes xylan removal via xylanase, cellulose molecular weight adjustment by endoglucanase or acid, or the cellulose pulp reactivity to derivatization reagents. Thus, the effect of chemical composition and pulp morphology on their accessibility to xylanase for removing xylan and cellulose reactivity to chemicals were studied.

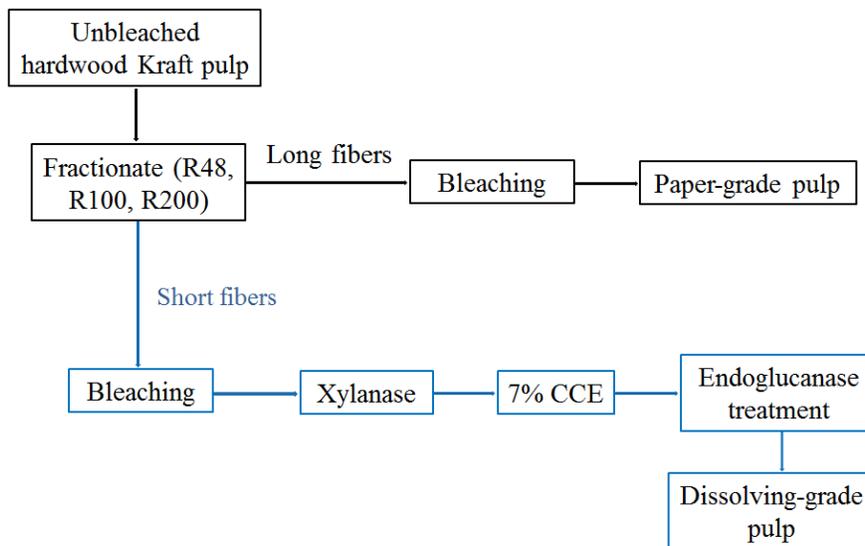


Figure 36: Schematic processing of various applications of different fractions from unbleached hardwood Kraft pulps

Unbleached hardwood Kraft pulps were fractionated and the pulp fibres were separated into two portions based on their fibre size. The long fibre fraction included the R48 and R100 which meant the pulp fraction that was retained on the 48-mesh screen and the 100-mesh screen. The short fibre fraction included the fibres that retained on the 200-mesh screen (R200) (Figure 36). After the multiple chlorite delignification steps, the long fibre fraction was used to make paper products. The short fraction (R200) went through bleaching, xylanase hydrolysis, CCE and endoglucanase treatment to upgrade to dissolving-grade pulp.

3.5.2.2 Chemical composition of pulp fibre fractions

Table 17: Chemical composition of isolated fibre fractions from hardwood Kraft pulp. KP: Kraft pulp. Commercial DsP: Commercial dissolving pulp. Unbleached R48/100/200: Unbleached fibre fractions retained on 48/100/200 meshes of Bauer-Mcnett fibre classifier. Upgraded R48/100/200: Unbleached fibre fractions went through all of the treatments (bleaching, xylanase, CCE, and endoglucanase treatment) resulting in dissolving-grade pulp fractions. Mass ratio was the quantity of different unbleached fractions after fractionation. There was about 7.2% total fines loss during the fractionation process.

Substrates	Cellulose %	Hemicellulose %	Lignin %	Intrinsic viscosity ml/g	Mass ratio %
Unbleached KP	72.0	22.4	5.0	-	100
Unbleached R48	77.5	20.9	4.3	-	62.3
Unbleached R100	75.3	22.4	4.7	-	23.6
Unbleached R200	72.5	23.5	5.7	-	6.9
Upgraded KP	93.5	5.0	0.7	536	-
Upgraded R48	93.7	5.3	0.7	581	-
Upgraded R100	94.5	4.8	0.8	520	-
Upgraded R200	95.2	4.1	0.8	479	-
Commercial DsP	92.6	5.6	0.5	494	-

Compared to softwood pulp, hardwood Kraft pulp has a shorter overall fibre length. In the case of softwood, it has been shown that more than 50% of the total fibres were retained on the 14 mesh screen (Mansfield et al., 1996), while for hardwood only the R48, R100 and R200 fractions could be collected in sufficient quantities to allow an effective evaluation of their properties

during the fractionation process (Table 17). The initial calculation of the amount of fibres from the unfractionated pulp indicated that the majority of the Kraft pulp was composed of long fibres since more than 60% of the fibres from the original pulp were retained on 48-mesh screen (R48). Approximately 7.2% of the pulp could not be accounted for by the summation of the fibres fractions retained by the R48 to the R200 screens (Table 17). It was expected that for hardwood Kraft pulp, the long fibre fraction would mainly consist of fibres while more of the vessel elements and longitudinal parenchyma were present in the short fraction fibres.

Chemical compositional analysis revealed that, compared with the long fibre fraction, the short fibre fraction was rich in both hemicellulose and lignin (Table 17). This was most likely because the short fibre fraction had a high content of vessel elements and longitudinal parenchyma (Lindstrom, 1978). It has been previously reported that longitudinal parenchyma cells were rich in hemicellulose (Higuchi, 1985). Similarly it has also been reported that for softwood Kraft pulp, the short fibre fraction had a higher hemicellulose content than the long fibre fraction because the short fraction had a high content of short and narrow parenchyma (Li et al., 2015). However, after upgrading to dissolving-grade pulps by going through the xylanase treatment, CCE and endoglucanase treatment (Figure 36), each fibre fraction had a similar hemicellulose content as the chemical and enzymatic treatments during the conversion process could remove most of the hemicellulose from the starting pulps (Table 17).

After being upgraded to dissolving-grade pulps, it was evident that the cellulose from the short fibre fraction had a lower intrinsic viscosity (479 ml/g) than the long fibre fraction (581 ml/g) (Table 17). The short fibre fraction had a similar intrinsic viscosity to commercial dissolving pulp (494 ml/g). One of the reasons could be that, as discussed above, the short fraction contained a greater amount of vessel elements. Vessel elements are known to possess thin cell

walls, large diameters and perforations (Hoadley, 1990) which could result in generating a high accessibility. However, fibres have very thick cell walls, with limited pitting which would decrease their accessibility to reagents. Therefore, the short fraction was more susceptible to the chemical or enzymatic reagents when converting the Kraft to dissolving pulp. Another possible reason could be that the short fraction also had a high content of short fibre fragments caused by the severe cooking conditions during the pulping process and those fibre fragments possessed a lower cellulose molecular weight (Lapierre et al., 2006). Therefore, the short fraction was more accessible to chemical or enzymatic treatments during the conversion process.

Compared to the long fibre fraction, the short fibre fraction had a higher content of hemicellulose and a lower cellulose molecular weight likely due to the presence of different fibre cells such as vessel elements and longitudinal parenchyma (Table 17). Since the different fibre cells possess varying morphologies such as fibre length/width/coarseness, pore structure and surface area, in the following section those morphological properties of fibres from each fraction were investigated and compared. It was anticipated that compared to the long fibre fraction, the short fibre fraction would have a smaller fibre diameter, higher surface area and accessibility due to the high content of vessel elements, short fibres and fines.

3.5.2.3 Fibre morphological properties of the different pulp fractions

As discussed in 3.5.2.2, approximately 7% of the fines fraction was lost during the fractionation process (Table 17). After being converted to dissolving-grade pulps, the unfractionated pulp had a fines content of 26%, which was much higher than each fibre fraction (Table 18). The fibre length of the long fibre fraction (1.34mm) was more than 2 times higher than the short fibre fraction (0.61mm), which was shorter than the commercial dissolving-grade pulp (0.96mm).

When comparing the fines content of all the fractionated pulps, it was found that the short fraction had a higher fines content (almost 3 times higher) than the long fraction. These results indicated that some of the fibre fragments might have been produced from the severe cooking conditions that shortened the fibre length during pulping process and these fragments were collected in the short fibre fraction.

Table 18: Fibre dimensions, fines content, coarseness and vessel results measured by FQA. KP: Kraft pulp. Commercial DsP: Commercial dissolving pulp. Upgraded R48/100/200: Fibres retained on 48/100/200 meshes of Bauer-Mcnett fibre classifier, they were all dissolving-grade pulps upgraded from the different unbleached Kraft pulp fractions. Fines were defined as fibre length in between of 0.07mm to 0.20mm. Fibre length was the length weighted average fibre length. Vessel: The ranges for of fibre length and fibre width of vessel measured in this study were 0.1-1.5mm and 80-400 μm .

Substrates	Fines %	Length mm	Width μm	Coarseness mg/m	Vessel m
Upgraded KP	25.9	0.96	22.1	0.102	11.2
Upgraded R48	3.2	1.34	23.3	0.163	7.4
Upgraded R100	3.6	0.94	21.6	0.094	8.9
Upgraded R200	9.7	0.61	19.8	0.064	9.4
Commercial DsP	12.2	0.96	20.9	-	-

Fibre coarseness is defined as the weight per unit length of fibre expressed as milligrams per 100 meters of fibre length (Mooney et al., 1999). Generally, for the same cells, the higher coarseness can be used to characterize the thickness of the cell walls since a thicker cell wall would indicate an increased weight. However, when comparing the different types of cells, coarseness may not be related to the thickness of cell wall. It could be possible that a wood cell with a large diameter but thin cell wall, such as vessel element, has the same or higher coarseness than the wood cell

with a much smaller diameter but thicker cell wall, such as fibre cell. However, since vessel elements comprise only a small proportion of the overall population of wood cells, most of the cells in each pulp fraction are still fibres, which account for 40-90% by weight of the total wood. It was found that the coarseness of the long fibre fraction (0.163 mg/m) was 2.5-fold higher than the short fibre fraction (0.064 mg/m) (Table 18). It is likely that during the cooking process, some of the wood fibres were excessively treated (Lapierre et al., 2006). As a consequence, those fibre fragments had thinner cell walls were shorter so they were isolated with the short fibre fraction.

In addition to fibre length and coarseness, the content of vessels was also measured using the FQA. In the results reported here, the ranges set on FQA for the vessel length and width were 0.1-1.5mm and 80-400 μm . As anticipated, the short fibre fraction had higher content of vessel elements (9.4 m) than the long fibre fraction (7.4 m) (Table 18). Since the average fibre length from short fibre fraction was much lower than the long fibre fraction, the difference in the amount of vessels from two fractions was very apparent. It has been reported that vessels are the widest and shortest cells in hardwood. With their thin walls and large cell cavities, and the existence of perforations in the adjacent vessel element generating a continuous conductive passageway, they are well adapted to water conduction within trees (Hoadley, 1990). Vessels were expected to have a high susceptibility to downstream chemical or enzymatic treatments. Therefore, the amount of vessels in the pulp fraction was expected to play a crucial role during process of converting a Kraft pulp to a dissolving-grade pulp.

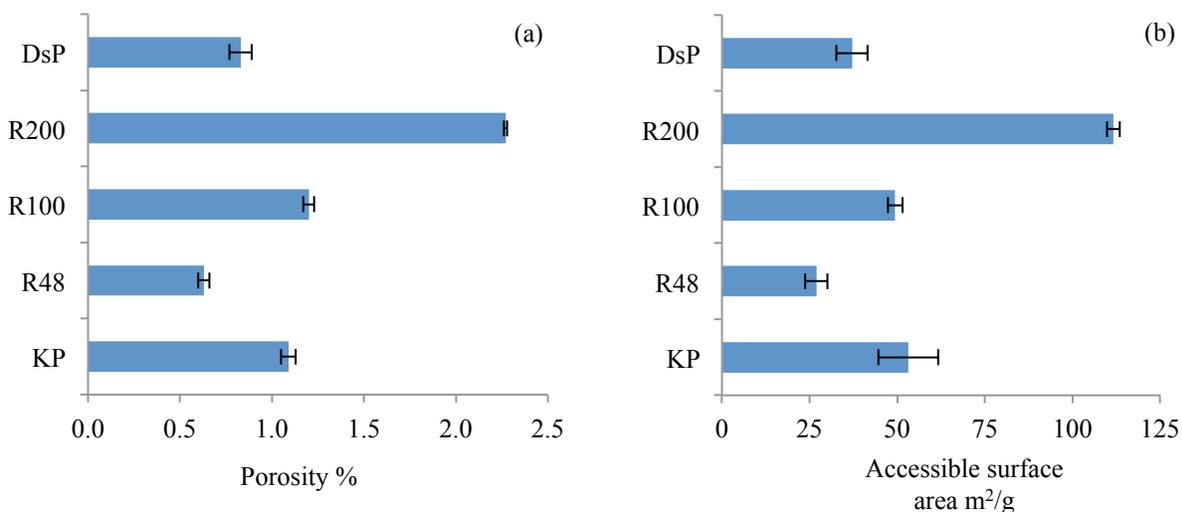


Figure 37: Porosity (a) and accessible surface area (b) of fibre fractions measured by solute exclusion technique. KP: Kraft pulp. DsP: Commercial dissolving pulp obtained from PHK process. R48/100/200: Fibres retained on 48/100/200-mesh screens of Bauer-Mcnett fibre classifier. All the measurements were on the pulp fractions that had gone through all the conversion process to dissolving-grade pulps. Solute exclusion was used to investigate wet pulps as it is an accurate method to analyze the pore structure of pulps.

It had been previously shown that the pore structure and surface area of the fibres played an important role in affecting the ability of enzymes or chemicals to remove lignin or hemicellulose (Kerr and Goring 1975; Chandra et al., 2008a). Generally the pulp fibres with larger pores had a higher delignification rate or higher hemicellulose removal than those with smaller pores (Kerr and Goring 1975). Previous work has shown that the accessible surface area was one of the most crucial factors that determines the ease of enzymatic hydrolysis of lignocellulosic substrates (Wong et al., 1988; Chandra et al., 2007; Mansfield et al., 1999). It was also found that large pores on fibres could increase their accessibility to cellulases (Grethlein, 1985; Chandra et al., 2007; Mooney et al., 1998; Meng and Ragauskas, 2014). Thus, it is reasonable to surmise that smaller sized pores with poor accessibility to reagents may hinder their removal of hemicellulose, while large pores which are highly accessible to reagents are a beneficial attribute for hemicellulose removal and cellulose reactivity (Li et al., 2015). It was apparent that both the

porosity and accessible surface area of the short fibre fraction were much higher than the commercial dissolving pulp (Figure 37). The average porosity of the short fibre fraction (2.27 ml/g) was 3.5 times higher than the long fibre fraction (0.63ml/g). It was found the accessible surface area of the short fibre fraction was 4-fold higher than the long fibre fraction (Figure 37). The high volume of pores and the increased accessible surface area possessed by the short fibre fraction indicated a high accessibility to downstream derivatization reagents, which was one of the most important requirements for dissolving-grade pulps.

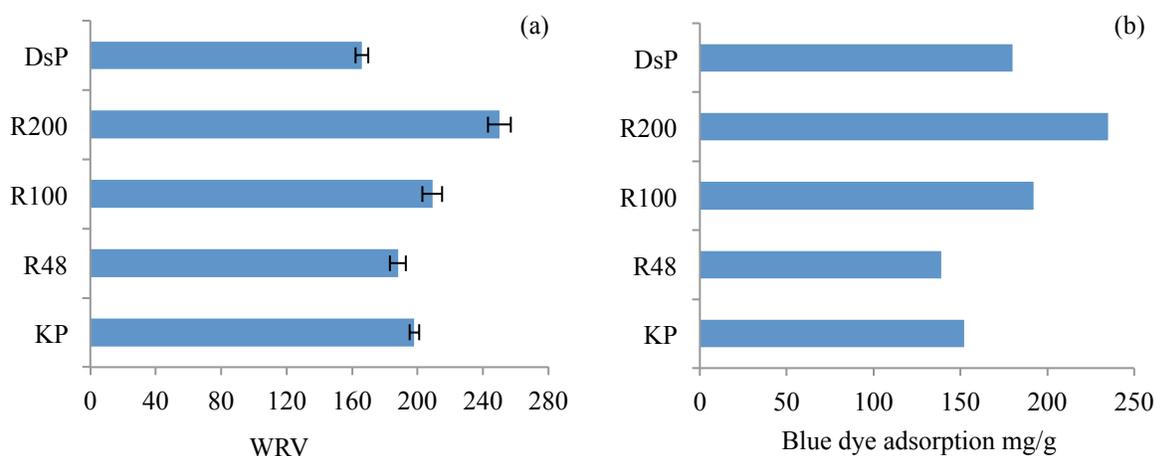


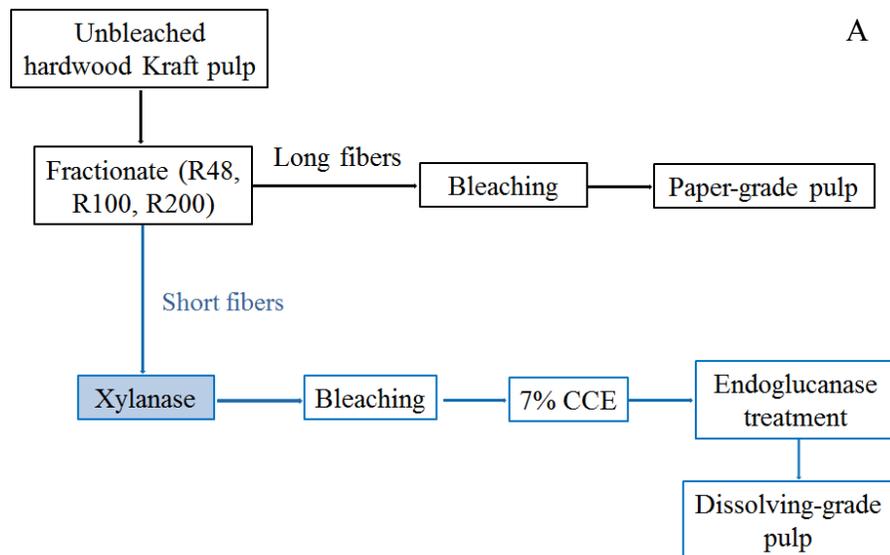
Figure 38: Accessibility of pulp fractions to water (a) and direct blue dye (b). KP: Kraft pulp. DsP: Commercial dissolving pulp made from PHK process. R48/100/200: Fibres retained on 48/100/200-mesh screens of Bauer-Mcnett fibre classifier. WRV: Water retention value. All the measurements were on the pulp fractions that had been gone through all the conversion process to dissolving-grade pulps.

As described earlier, the water retention value (WRV) has been used to quantify the pores within the substrate (Jayme and Roffael, 1970). The WRV has also been shown to be a useful technique to characterize the swelling of pulps for subsequent papermaking and pretreated biomass for biological conversion (Luo et al., 2011). In addition, Simons' staining has also been used to assess the accessibility of cellulose to cellulases for subsequent enzymatic hydrolysis of the cellulose for use of the sugars as a fermentation feedstock (Chandra et al., 2008). Both the WRV

and Simons' staining results showed the short fibre fraction had a higher accessibility to both the small molecule (water) and the larger molecule (direct blue dye) than the long fibre fraction (Figure 38).

3.5.2.4 Xylanase hydrolysis on xylans from different fibre fractions

As discussed previously, we wanted to assess the different fibre fractions susceptibility to downstream chemical or enzymatic treatments resulting from their differences in chemical composition and morphological properties. Previous work had indicated the response to the xylanase treatments exhibited by all pulp fractions was not uniform, and that fibre composition played a key role in the effectiveness of xylanase treatments as a bleaching boosting approach (Mansfield et al., 1996). It was likely that the vessel elements, parenchyma and fines obtained mainly in the short fibre fraction had a high content of accessible hemicellulose thereby reacting with xylanases more susceptible. Therefore, subsequent work assessed the xylanase hydrolysis on xylans from the two fibre fractions.



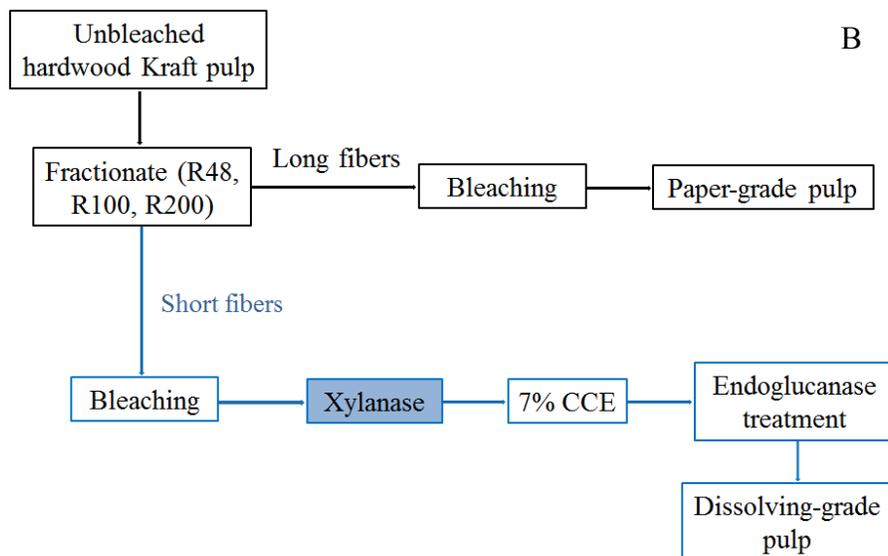


Figure 39: Xylanase hydrolysis on unbleached short fraction pulp; (A) before bleaching (B) after bleaching

The Multifect and HTec commercial xylanase preparations were the two main xylanases that were used. Xylanase hydrolysis was conducted before and after bleaching since it has been reported that the multiple delignification and bleaching steps could change the accessibility of xylan (Wong and Saddler, 1992; Kim et al., 2000). In addition, it was anticipated that the xylanases will be more effective on the substrates with more accessible xylan as shown by the xylanase action on the model substrates in section 3.2. The short fibre fraction, which was enriched in vessel elements and fibre fines, was shown to exhibit higher hemicellulose accessibility than the long fibre fraction. Therefore, this section investigated the xylanase hydrolysis on different fibre fractions taken from various points such as before bleaching (Figure 39-1) and after bleaching (Figure 39-2) in a Kraft process.

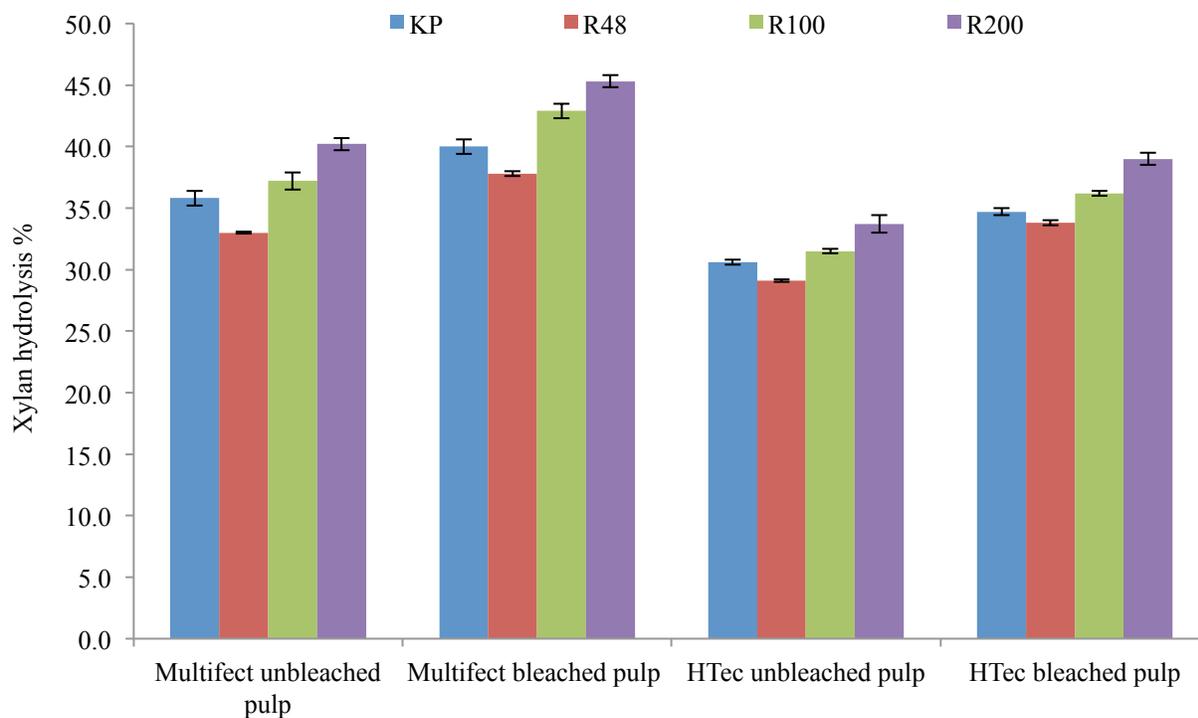


Figure 40: Xylan hydrolysis by xylanases from different fibre fractions. KP: Kraft pulp. R48/100/200: Fibres retained on 48/100/200-mesh screens of Bauer-Mcnett fibre classifier. Multifect and HTec xylanases used in this work were the commercial xylanase preparations. Xylanase hydrolysis was conducted under 50°C, 5% solid loading in sodium acetate buffer (50mM, pH 4.8), and the protein loading was 10mg/g.

It was apparent that, the short fibre fraction was more amenable to hydrolysis by xylanases than the long fibre fraction by approximately 5% to 10% (Figure 40). On bleached pulps the xylan hydrolysis from the short fibre fraction by xylanases was much higher compared to the hydrolysis result on the long fibre fraction (Figure 40). One of the reasons was that as illustrated in 3.5.2.3, compared to the long fibre fraction, the short fraction had a higher porosity and more accessible surface area (Figure 37), generating a higher accessibility for xylanases during the hydrolysis process. In addition, the short fibre fraction, which was enriched in vessel elements, was shown to exhibit a higher amount of accessible hemicellulose. Therefore, these fibres were shown to be more amenable to xylanase hydrolysis.

Surprisingly, when the xylan hydrolysis results from the pulps before and after bleaching were compared their xylan removal results by xylanases, the bleached pulp had a slightly higher (approximately 5%) xylan removal (Figure 40). It has been previously reported that xylanases could remove more xylan from brown stock than the bleached pulp due to the high amount of accessible xylan re-precipitated on the outside of fibres of the unbleached brown stock pulp (Christov and Prior, 1993). However, the different result from our work indicated that lignin might be implicated in playing a role in affecting the ease of hydrolysis of the xylan by xylanase from the brown stock. Although the Kraft brown stock is expected to have a relatively low lignin content in the range of 4-6% (Table 17), lignin contents in this range have been shown to have a negative impact on the ease of hydrolysis cellulose by cellulases (Tu et al., 2007). In bioconversion research, lignin inhibits the action of cellulases by posing a physical barrier or causing non-productive binding of cellulase enzymes, thereby reducing the enzymes available for subsequent enzymatic hydrolysis (Kumar et al., 2012).

3.5.2.5 Hemicellulose removal from different fibre fractions by CCE

We had previously reported that the CCE sequence effectively remove most of the hemicellulose and part of the low molecular weight cellulose to generate a homogeneous and purified cellulose feedstock. The effect of CCE on the hemicellulose removal from different fibre fractions was next studied. Since our previous work in Section 3.1 showed that 7% NaOH employed during CCE could solubilize a high amount of hemicellulose without compromising cellulose reactivity significantly, it was also applied to the fibre fractions.

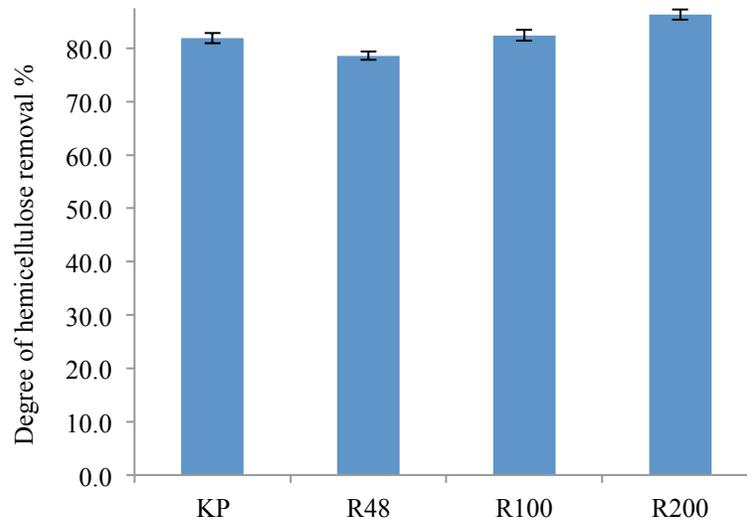


Figure 41: Degree of hemicellulose removal during CCE. CCE was conducted at 7% pulp consistency, 7% NaOH (w/v), room temperature for 30 minutes. CCE: Cold Caustic Extraction. R48/100/200: Fibres retained on 48/100/200-mesh screens of Bauer-Mcnett fibre classifier. CCE was conducted on the pulps that went through the delignification and xylanase hydrolysis steps.

The degree of hemicellulose removal during CCE was defined as the ratio of the amount of hemicellulose removed after an alkali extraction to the total amount of hemicellulose from the starting pulps. The degree of hemicellulose removal can indicate the accessibility of the hemicellulose contained in the different fibre fractions to the sodium hydroxide, and the content of small molecular weight carbohydrates (Li et al., 2015; Strunk, 2012). In this work, treatment of the Kraft pulp with an alkaline extraction (7% NaOH) after a xylanase hydrolysis reduced the hemicellulose content to a level lower than 5.5% (Table 17). It was found that there was a relatively higher degree of hemicellulose removal from the short fibre fraction than the long fibre fraction (~8%) (Figure 41), indicating a higher accessibility of the hemicellulose or a high content of low molecular weight carbohydrates from short fibre fraction than the long fibre fraction. One of the possible reasons could be that the short fibre fraction possessed a larger proportion of fines and low molecular weight cellulose caused by being treated more severely

during the pulping process (Table 17), leading to a higher solubility in the CCE. In addition, the high porosity and accessible surface area (Figure 37) possessed by the short fibre fraction further enhanced its accessibility to the alkali.

3.5.2.6 The reactivity of cellulose in the fibre fractions

Pulp reactivity has been regarded as one of the most critical parameters affecting the subsequent utility of a given dissolving pulp sample. Due to the high specificity of xylanase for the removal of xylan and its limited activity on cellulose, the intrinsic viscosity of the pulp, which has been indicated to be inversely proportional to pulp reactivity in a given pulp sample (Engström, et al., 2006), was barely affected by the xylanase treatment. As discussed in previous chapters, CCE could remove the low molecular weight components which have been reported to act as “spacers” between of cellulose microfibrils to prevent fibril aggregation, leaving the final pulp with high molecular weight and poor cellulose reactivity (Gehmayr et al., 2012). Therefore, to enhance final pulp reactivity, endoglucanase treatment was applied after the xylanase and CCE treatments to decrease the molecular weight of cellulose. Our previous work showed that the reactivity increased by 1.3 fold after an endoglucanase treatment (Section 3.1).

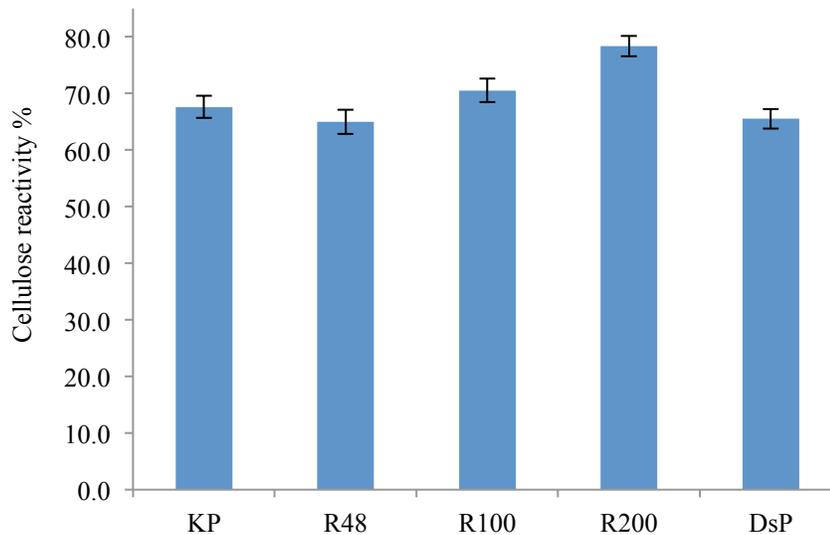


Figure 42: Fock's reactivity of different fibre fractions. KP: Kraft pulp. DsP: Commercial dissolving pulp. R48/100/200: Fibres retained on 48/100/200-mesh screens of Bauer-Mcnett fibre classifier. Endoglucanase was applied after CCE treatment.

After an endoglucanase treatment to upgrade both fibre fractions to dissolving-grade pulps, they both had remarkably high levels of reactivity, reaching the range of the reactivity of a commercial dissolving-grade pulp. Compared with the long fibre fraction, the short fibre fraction had more than 13% higher cellulose reactivity (Figure 42). It was evident that the short fibre fraction had a high porosity and surface area compared to the long fibre fraction (Figure 38). As a result, it is likely that the hydroxyl groups on cellulose at $C_2/C_3/C_6$ from the short fibre fraction were more accessible to xanthation during the Fock's reactivity test (Figure 42). It was apparent that short fibre fraction was superior to the long fibre fraction and could potentially provide a pulp furnish that could be used for the production of dissolving-grade pulps. In addition, it was found there was an inversely relationship between the intrinsic viscosity of different fibre fractions and their reactivity (Figure 43). This indicated that a lower intrinsic viscosity was likely a beneficial attribute to help produce a pulp with high cellulose reactivity.

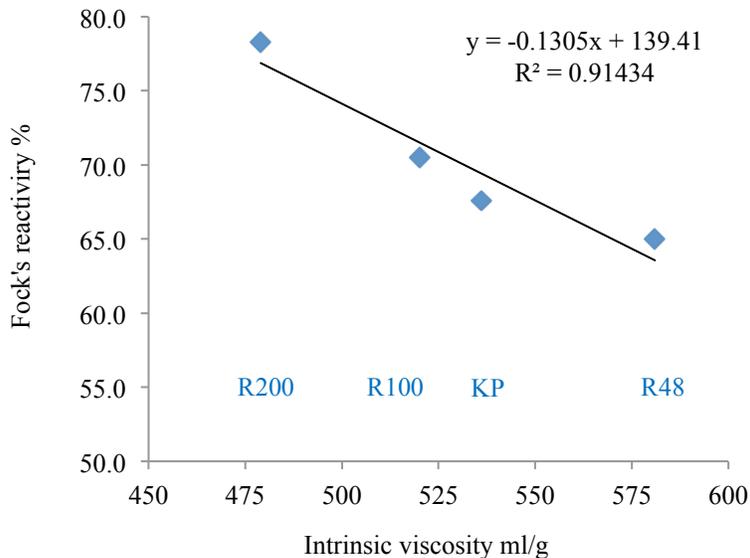


Figure 43: Relationship between cellulose intrinsic viscosity and reactivity (Fock's method). KP: Kraft pulp. DsP: Commercial dissolving pulp. R48/100/200: Fibres retained on 48/100/200-mesh screens of Bauer-Mcnett fibre classifier. Endoglucanase was applied after CCE treatment.

3.5.2.7 Microscopy of different pulp fractions

Most of the wood cells from long fibre fraction were fibres (R48), which had a small diameter, dark color and were slender and had tapered ends. Part of vessel elements and tracheid were found in R100, and the shortest fraction (R200) had the highest content of vessel elements (Figure 44). It was apparent that the vessels had a much larger diameter than fibres (Figure 44). Pores have been reported to vary in size according to vessel diameter from a minimum of 50 to 60 micrometers (Hoadley, 1990). The existence of perforations in the vessel elements was apparent (Figure 44), and this would provide a continuous conductive passageway between vessels (Hoadley, 1990) and enhanced access to enzymatic/chemical modification during the conversion of Kraft pulps to dissolving-grade pulps (Figure 40 and 41).

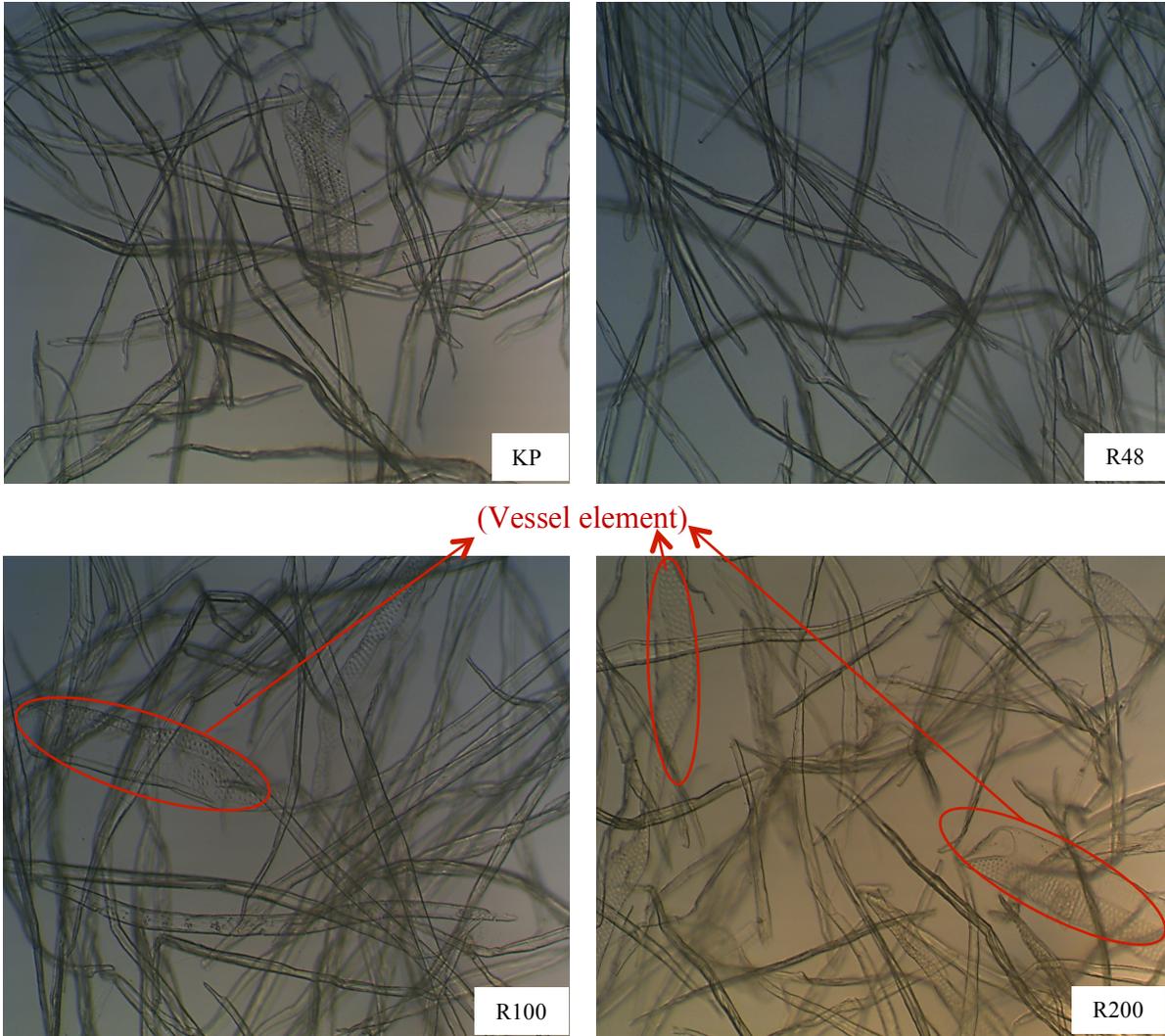


Figure 44: Microscopy of different pulp fractions. KP: Kraft pulp. DsP: Commercial dissolving pulp. R48/100/200: Fibres retained on 48/100/200-mesh screens of Bauer-Mcnett fibre classifier.

3.5.2.8 The impact of removing the fines fraction on pulp mechanical strength

Table 19: Impact of short fibre fraction on the mechanical strength (tensile and tear index) of paper. There was an around 10% decrease in tensile index due to the removal of short fibre fraction.

Pulps	Stretch %	Tensile strength (kN/m)	Tensile index (kNm/kg)	Tear index (mNM²/g)
KP	1.2	2.4	39.3	0.004
KP no R200	1.0	2.0	35.4	0.003

The characteristics of chemical pulp fines and their role in papermaking have been widely studied (Aaltio, 1962; Lobben 1977; Retulainen et al. 1993; Retulainen and Nieminen 1996; Xu and Pelton 2005; Taipale et al., 2010), showing the addition of fines to a pulp suspension increased the final paper strength properties. One hypothesis in this work was fines or short fibre fraction could be ideal pulp furnish to produce a dissolving-grade pulp, while the long fibre fraction could be mainly used to make traditional paper products. One potential concern is after the removal of the short fibre fraction, which has been shown to have a high content of fines that are beneficial to increase the paper strength is whether the mechanical strength of the final paper products would be sufficient for the production of traditional paper products. Therefore, in the last part of this work, the impact of the removal of the R200 fibre fraction on the resulting tensile and tear index of the paper sheets made from the Kraft pulp brown stock was investigated. It was found that the tensile strength of the hand-sheets made from the long fibre fraction after removing the R200 fibres was 10% lower than the tensile strength of the hand-sheets from the unfractionated Kraft fibres. The results further demonstrated that the long fibre fraction had

acceptable properties for the production of paper products if the loss in tensile could be tolerated then a potential addition product stream could be produced using the short fibre fraction.

3.5.3 Conclusions

A sequential treatment involving pulp fractionation was assessed. Xylanase hydrolysis and a mild CCE (removing hemicellulose from the short fibre fraction of an unbleached hardwood Kraft pulp) followed by subsequent endoglucanase treatment (to decrease the molecular weight of cellulose) was shown to enhance the reactivity of pulps. The separated fibre fractions consist of various fibre types that vary in their chemical composition and morphologies. Thus they vary in their susceptibility to downstream chemical or enzymatic treatments. Subsequent fractionation showed that the short fibres had a higher hemicellulose and lignin content than did the long fibres. Overall, the short fibre fraction exhibited a higher porosity, accessible surface area and accessibility due to the higher content of vessel elements and fines. These attributes have been shown to be beneficial for facilitating xylan removal by xylanases, hemicellulose removal by CCE and reactivity enhancement by endoglucanases. Consequently, the short fibre fraction was superior to the long fibre fraction and thus a potential pulp furnish for the production of dissolving-grade pulps due to the high content of vessel elements and fines with a higher accessibility of hemicellulose and cellulose.

4. Conclusions and future work

4.1 Conclusions

The overall goal of this work was to use enzymes to reduce the chemical intensive and non-specific nature of the current processes used to produce dissolving pulp from hardwoods. The working hypothesis was that the accessibility of the hemicellulose and cellulose was one of the most important parameters during the chemo-enzymatic conversion of Kraft pulp to dissolving pulp. More specifically, it was shown that the accessibility of hemicellulose affected its ease of removal by enzymes while the reactivity of the dissolving pulp was influenced by the accessibility of the cellulose component. It was shown that the purity and reactivity could be enhanced by altering the upstream pre-hydrolysis/pulping/CCE conditions. Processing conditions that maximized hemicellulose accessibility and limited or reversed cellulose micro-fibril aggregation facilitated the ability of xylanases and endoglucanases to aid in the conversion of Kraft to dissolving pulps using milder processing conditions.

The initial work compared a small biomimetic dicarboxylic acid (oxalic acid) to xylanases for their ability to access and remove xylan from both a Kraft and commercial dissolving pulp. The smaller oxalic acid was expected to access and remove a greater amount of hemicellulose compared to the xylanase enzymes, while the paper grade Kraft pulp was expected to provide a higher proportion of accessible xylan compared to the dissolving grade pulp. As anticipated, compared to oxalic acid, enzymes such as xylanase and endoglucanase removed hemicellulose and boosted cellulose reactivity with greater specificity than oxalic acid while the paper grade Kraft pulp underwent a greater amount of hemicellulose removal. However, both xylanase and oxalic acid treatment resulted in insufficient xylan removal, which indicated a limitation in the

accessibility of hemicellulose in Kraft pulps. This hemicellulose accessibility was investigated in greater details by modifying the upstream treatment/pulping processes.

This effect of pulp characteristics on the ease of chemical/enzymatic action was further demonstrated by subsequent work on model substrates. Model substrates consisted of both commercially available xylan preparations and laboratory isolated xylan from wood chips and Kraft pulp. The ability of xylanases to hydrolyze these xylan preparations was compared to the enzymatic hydrolysis of xylan contained in pulp and wood samples. It was apparent that the xylan preparations was more accessible to the enzymes compared to the xylan contained in the pulp and wood as the enzymes could hydrolyze the xylan preparations to a greater extent. These results supported the results of the first chapter, indicating that accessibility of the hemicellulose components was one of the most important parameters affecting the enzymatic conversion of Kraft pulp to dissolving pulp. In addition, model cellulose substrates with varying properties such as crystallinity, surface area and degree of fibre swelling were also investigated to elucidate their influence on final cellulose reactivity. It was shown that a decrease in cellulose crystallinity improved its reactivity. It was also shown that pulps with higher surface areas enhanced fibre swelling generated using mechanical refining were highly reactive during subsequent cellulose derivatization. The results indicated lower crystallinity, higher surface areas and degree of fibre swelling, attributes that all relate to an increase in cellulose accessibility were beneficial attributes for obtaining a more reactive dissolving pulp feedstock.

Based on the results above, subsequent work investigated the influence of the characteristics of pulps that underwent hemicellulose removal using a pre-hydrolysis process (PHK) to those where the hemicellulose was removed using a cold caustic extraction (CCE). It was found that CCE compromised hemicellulose/cellulose accessibility and enzymatic digestibility due to the

solubilization of the highly accessible portions of hemicellulose and low molecular weight cellulose. It was apparent that, in addition to leaving behind the more inaccessible cellulose and hemicellulose, the low molecular weight cellulose and accessible hemicellulose also acted as “spacers” between cellulose microfibrils to prevent fibril aggregation. Therefore, the solubilization of the easily accessible hemicellulose resulted in the collapse of the cellulose structure which compromised its subsequent reactivity. However, applying post-treatments to the CCE pulp, such as mechanical refining, induction of curl, sulfuric acid hydrolysis and endoglucanase treatment effectively increased pulp reactivity. In particular, mechanical refining combined with endoglucanase treatment increased the pulp reactivity from 32 to 75% when applied to a pulp that had already undergone the CCE treatment to remove the hemicellulose components. These results were mainly because mechanical refining could effectively increase the surface area and swelling of fibres thereby increasing their accessibility to both chemical reagents and to endoglucanase treatments.

From the previous chapters, the underlying theme was that the characteristics of the substrates at the various points in the process (PHK, CCE, pulping and bleaching) in the conversion of a Kraft to dissolving pulp would determine substrate factors that facilitate the ease of enzymatic hemicellulose removal. It was also known that prehydrolysis and pulping conditions could be altered to create substrates varying in the distribution/amount of hemicellulose, the DP and accessibility of cellulose and the ease of removal of the lignin component to investigate their susceptibility to enzymes. Therefore, we used three different prehydrolysis conditions and pulping conditions to create pulps that could be used to gain further insight into the characteristics that improve their susceptibility to enzymatic hemicellulose hydrolysis and cellulose modification by endoglucanases. Our results showed that approximately 60% of the

total hemicellulose was removed by prehydrolysis without losses in cellulose yield. The addition of a small amount of sulfuric acid (0.4%) removed an additional 10% of the hemicellulose but compromised the integrity of the cellulose. Varying the alkalinity during Kraft pulping generated the pulps with different cellulose molecular weight and increased the amount of accessible hemicellulose on the fibre surface. It was also shown that lowering the addition of sodium hydroxide during the pulping process could obtain a pulp with more accessible xylan to xylanase hydrolysis. In addition, increasing the alkali loading reduced the molecular weight of cellulose which increased cellulose reactivity and pulp yield likely due to the more severe peeling reactions that occurred on the cellulose component. This work further demonstrated that the accessibility of hemicellulose and cellulose reactivity could be altered by varying the upstream prehydrolysis and pulping conditions.

Throughout the thesis it was shown that the accessibility of hemicellulose and cellulose reactivity could be enhanced during the conversion of hardwood Kraft pulps to dissolving pulps by altering the upstream prehydrolysis/pulping/CCE conditions. These conditions were shown to be a result of the fibre properties imparted by each treatment. Hardwood fibres themselves are also highly heterogeneous in their cell types, chemical composition and morphology. The short vessel fibres and fibre fines have been reported to possess high porosity and surface area which were anticipated to be beneficial attributes for the production of dissolving pulps. Indeed, the short fibre fraction, which was enriched in vessel elements, was shown to exhibit higher hemicellulose accessibility/reduced cellulose molecular weight and higher porosity. Therefore, these fibres were shown to be more amenable to enzymatic conversion to dissolving pulp with high cellulose purity and reactivity. The long fibre fraction could be able to make traditional paper products if

the 10% of the loss in tensile strength of the hand-sheet made from the long fraction could be tolerated.

In summary, we elucidated the various pulp properties, such as the accessibility of hemicellulose/cellulose, cellulose microfibril aggregation, and the surface area/pore structure/size of fibres that influence the ability of enzymes to facilitate the conversion of hardwood Kraft to dissolving pulps. The work demonstrated that enzymes such as xylanases and endoglucanases could specifically modify the fibre properties of a conventional kraft pulp to provide a dissolving pulp feedstock. The enzymatic modification process was highly influenced by the pulp properties mentioned above.

4.2 Future work

4.2.1 Improvements and proper quantification of the reactivity of cellulose hydroxyl groups in dissolving pulps

A key issue with the study of dissolving pulps is the development of effective tests for the quantification of accessible hydroxyl groups at C₂, C₃ and C₆, which could be regarded as one of the most critical parameters affecting the subsequent utility of a given pulp sample (Strunk, 2012). It is essential that the methods of analysis including the preparation of the pulp, and the capabilities of the methods are understood when investigating the reactivity of cellulose. It has been reported that the reactivity of cellulose during derivatization is determined by the structure and morphology of the cellulose (Strunk et al., 2011; 2012). There have not yet to be studies that compare the various derivatization techniques for their ability to estimate the “reactivity” of cellulose hydroxyl groups. For example, if the derivatization of cellulose is carried out under

acidic conditions such as during nitration and acetylation, the cellulose may be far less swollen and accessible than reactions such as xanthation performed under alkaline conditions. In addition, since viscose is the main product currently being made using dissolving pulp, most studies aiming to quantify cellulose reactivity have employed the Fock's test, which can be regarded as tedious and challenging and employ toxic chemicals such as carbon disulfide. As a result, it will be very interesting to improve and properly quantify the reactivity of cellulose pulp.

4.2.2 Employing steam explosion with acid catalyst to replace prehydrolysis step to produce dissolving pulp

Steam explosion has been reported as an effective approach to remove hemicellulose which is similar with prehydrolysis, one of the primary approach to remove hemicellulose during the production of dissolving pulp. However, steam explosion consumes less time and efforts compared to prehydrolysis, so it could be a potential approach to replace prehydrolysis to make dissolving-grade pulp. One of the attractive aspects of using the steam pretreatment process to potentially produce dissolving-grade pulps is that it allows the selective solubilization of the hemicellulose components. After it is combined with an acidic catalyst such as sulfuric acid, this process could fractionate the lignocellulosic biomass efficiently and improve its downstream enzymatic hydrolysis. Steam pretreatment has also been shown to impart changes to the cellulose structure including the reduction of cellulose DP, and a general increase in the accessibility of cellulose to downstream derivatization reagents (Chandra et al, 2007; Arantes and Saddler, 2011). It has also been shown that the reactivity of a dissolving-grade pulp was significantly enhanced after going through a steam explosion process (Tikkanen, 2014). As a result, steam explosion could be potentially used to make dissolving-grade pulp.

4.2.3 The influence of mechanical refining on xylan removal by xylanase and cellulose reactivity improvement

Mechanical refining has been shown to be an effective approach to improve cellulose reactivity by inducing fibrillations and surface area on cellulose (Tian et al., 2014; Wu et al, 2014). It has been reported as one of the most effective treatments to expand capillaries or pores, split the fibre aggregations to increase the accessible surface of fibril aggregates and disrupt the compact cellulose matrix (Tian et al., 2014; Wu et al, 2014). Except for improving cellulose reactivity, mechanical refining could also be an effective way to enhance the xylan removal by xylanase if we apply the xylanase hydrolysis on a pulp that has gone through a mechanical refining process (Figure 45).

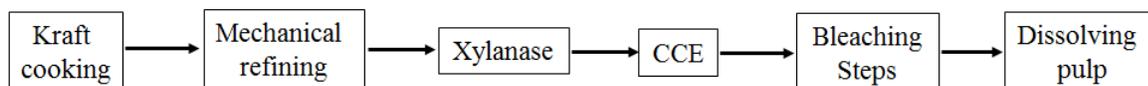


Figure 45: Mechanical refining to enhance xylan removal by xylanase

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