

Modification of dissolving wood pulp using endoglucanase

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Abstract

Dissolving wood pulp, a final product of the pulping and bleaching processes, with a cellulose content greater than 90%, has many applications. Each application requires specific properties of the dissolving wood pulp and to achieve these properties, alteration is required. Various techniques exist to modify cellulose, such as chemical, mechanical and enzymatic modification. Enzymatic modification has the benefits of reduced energy input, reduced chemical consumption during bleaching and viscose production, reduced environmental influence and a high specificity towards cellulose molecules.

The aim of this study was to improve the properties (reactivity and viscosity) of dissolving wood pulp (cellulose) by treatment with endoglucanase enzymes. Therefore, the objectives of this study were to investigate the effects of two different formulations of endoglucanases, using a range of enzyme concentrations and incubation times, on pulp viscosity and reactivity and to integrate the enzyme treatments with a bleaching sequence. This study comprised two experimental phases. In the first phase, bench-scale tests were conducted on semi-bleached pulp to determine the best enzyme formulation to use. In the second phase, the enzyme treatments were integrated with a bleaching sequence to produce fully bleached pulp.

The bench-scale tests showed that endoglucanase could successfully modify pulp properties. The pulp viscosity could be reduced by 50 to 100 units, with relatively low enzyme dosages (<250 mg/kg), without modifying the alkali solubility (S10) of the pulp ($\pm 5.5\%$). Integration of the enzyme treatment with a bleaching sequence had several benefits. The treatment reduced chemical charges of pulp for ozone (40%) and peroxide (>63%), improved brightness, and resulted in economic benefits (>30% savings).

These results show that endoglucanases can successfully reduce pulp viscosity without causing excessive damage to the cellulose. Integration of the enzyme with a bleaching sequence is straightforward and can lead to reduced chemical consumption and increased cost savings. In future studies, complete enzyme assays should be conducted to understand the limitations of the enzyme and the brightness boosting effect should be investigated for further benefits of the enzyme.

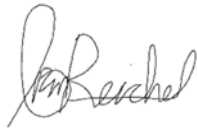
Declaration of authorship

I declare that this report is a presentation of my own original work.

Whenever contributions of others are involved, every effort was made to indicate this clearly, with due reference to the literature.

No part of this work has been submitted in the past, or is being submitted, for a degree or examination at any other university or course.

Signed on this, 14th day of December 2020 in Potchefstroom.



INITIALS AND SURNAME

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Chapter 1: Introduction

1.1 Background and motivation

Cellulose is one of the most abundant polymers on earth, since approximately 40% to 45% of dry wood matter contain cellulose. Cellulose is found within the plant cell walls of higher plants and is associated with other major cell wall components such as lignin and hemicellulose [1 p.4]. The cellulose is bound to the lignin by the hemicellulose in the plant cell walls and must be liberated from the lignin and hemicellulose by pulping processes to produce dissolving wood pulp (DWP). DWP has a cellulose content of more than 90% and is used in the production of many commercial products [2 p.10].

Products of cellulose include viscose and cellophane, along with cellulose esters and cellulose ethers [1 pp.204-229], [2 p.7]. Applications of cellulose even exist in the field of ion-exchange technology [1 pp.222-224]. Viscose production (from DWP) accounted for 69% of the total DWP production (4.9 million ton) in 2010 [3].

Various processes exist to produce DWP, including the pre-hydrolysis kraft (PHK) process, the sulphite process and its variations such as the neutral-sulphite process and alkaline-sulphite process, [2 pp. 8-14]. The sulphite process is considered to be the leading process for DWP manufacturing, accounting for approximately 65% of the total DWP annual production [4]. The preference for this process is due to the higher reactivity, higher pulp yield, and better bleachability of the sulphite pulp compared to the PHK-produced pulp [2 p.6].

Bleaching is an important aspect in producing DWP, as bleaching not only ensures that the correct brightness is achieved by the removal of residual lignin, but also improves the cleanliness of the pulp [1 p.165]. Other DWP properties, including viscosity, are also controlled by the bleaching of the pulp by using bleaching technology such as multistage bleaching wherein various subsequent bleaching agents are employed to alter the properties of DWP [5 p.245-246].

Alteration of the properties of DWP can be achieved by the modification of cellulose through various methods such as chemical modification, mechanical modification and enzymatic modification. Mechanical modification is often used in conjunction with other modification methods. Chemical modification, such as grafting, involves the addition of polymers to the cellulose chain, but the grafting technique is greatly influenced by the physical structure of cellulose [1 p.220]. Chemical modification by means of oxidation of the cellulose chain is termed oxidative modification but has a low selectivity [6]. In contrast, enzymatic modification

has the benefits of reduced energy input, reduced environmental influence and a high specificity towards the cellulose molecules in the microfibrils.

Enzymes can be classified into families and members. Cellulase is classified into the glycoside hydrolase family (and then into the appropriate members depending on their characteristics), the group of enzymes capable of hydrolysing glycosidic bonds. This specific group of cellulase enzymes comprises various enzymes which include endoglucanase (EG) and cellobiohydrolase (CBH), each with their own unique functionality and purpose in cellulose degradation for modification of DWP [7].

The modification of DWP by enzymatic treatment not only alters DWP's properties such as viscosity and reactivity, but also reduces chemical consumption during the hypochlorite bleaching stage [8], and during carbon disulphide (CS_2) viscose production [9]. Due to the potential reduction in chemical consumption, a reduced waste stream is also possible. A reduced chemical consumption and reduced waste production would provide economic benefits [9].

1.2 Problem statement and hypothesis

The improvement of DWP's properties could lead to a reduction in the use of chemicals in subsequent processes such as hypochlorite bleaching and CS_2 viscose production. This reduction could result in an economic benefit from smaller waste stream production and a reduced environmental effect.

1.3 Aim and objectives

The aim of this study is to improve the properties of PHK-produced DWP through the application of EG.

The following objectives are formulated to meet the aim of the study:

1. Determine the effect of bench-scale EG treatment using two commercial enzyme formulations on the properties of semi-bleached DWP.
2. Determine the pilot-scale bleaching response of fully bleached DWP to EG treatment.

1.4 Scope of the investigation

This dissertation consists of:

- An introduction (Chapter 1):
 - o The background and motivation, the aim and objectives, and the scope are discussed in this chapter.

- A literature survey (Chapter 2):
 - o The title of this chapter is '*Literature review of the application of cellulase for the modification of dissolving wood pulp*'. In this chapter the basic properties of cellulose are discussed, followed by the methods which are used to characterise pulp. The different modification techniques are then briefly discussed followed by an in-depth investigation of recent studies where EGs were used to modify pulp properties.
- Experimental phase one (Chapter 3):
 - o The title of this chapter is '*The response of semi-bleached dissolving wood pulp to endoglucanase treatment*'. In this chapter a high-level enzyme assay was used to quickly characterise the enzymes. The enzymes were then applied at bench-scale to semi-bleached DWP and afterwards the properties were measured. The properties measured were viscosity, alkali solubility (S10 and S18), crystallinity, Berger whiteness index (BWI) and reactivity. Not all samples were tested for reactivity due to the time that would be required for reactivity testing.
- Experimental phase two (Chapter 4):
 - o This chapter has the title '*The bleaching response of cellulase-treated dissolving pulp*'. In this chapter the findings of the previous chapter were used to incorporate the enzyme treatments with a bleaching sequence to produce fully bleached DWP. The viscosity and Kappa numbers were measured at each stage, whereas the brightness, alkali solubility, copper numbers and reactivity were only measured after the pulp was bleached.
- Conclusions and recommendations (Chapter 5):
 - o The general conclusions and recommendations are discussed in this chapter to bring the entire dissertation to a close.

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Chapter 2: Literature review – application of cellulase for the modification of cellulose

2.1 Introduction

Viscose production (from DWP) accounted for 69% of the total DWP production (4.9 million tons) in 2010 [1] and according to Deshpande [2 p.21], there is a growing market for DWP and viscose production (Figure 2-1).

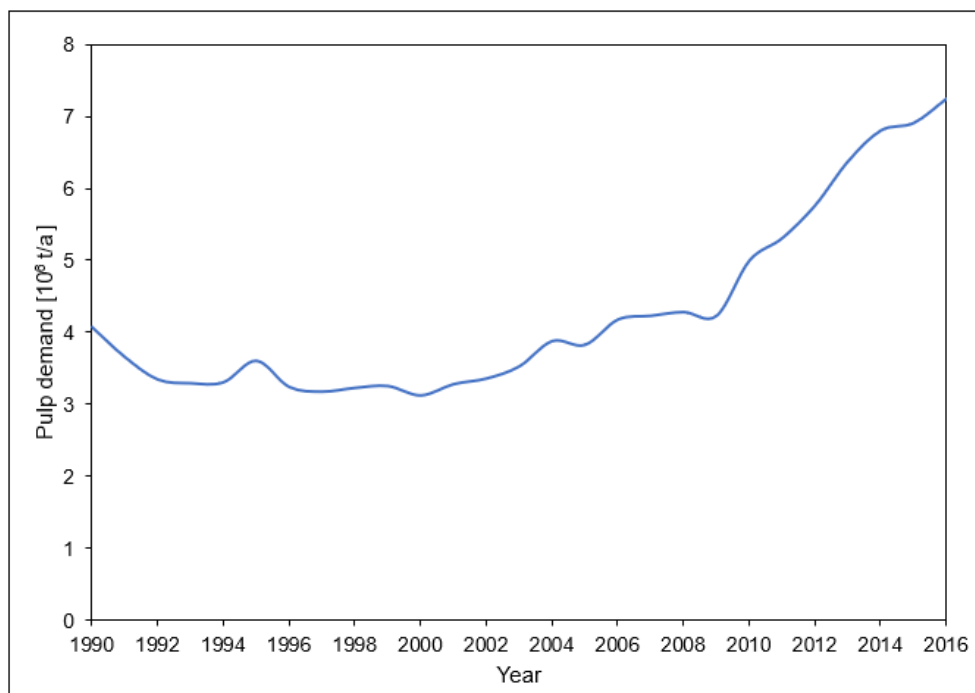


Figure 2-1: Global dissolving wood pulp demand from 1990 to 2016 (adapted from Deshpande [2 p.21])

DWP properties, including viscosity, are controlled during the bleaching of the pulp by using technology such as multistage bleaching wherein various bleaching agents are employed [3 pp.245-246].

DWP could also be modified using other methods: mechanical modification is often used in conjunction with other modification methods. Chemical modification, done through oxidation of the cellulose chain, is termed oxidative modification, but this method has a low selectivity [4].

The modification of DWP by enzymatic treatment reduces chemical consumption during the hypochlorite bleaching stage [5] and during CS_2 viscose production [6]. Due to the reduced

chemical consumption, a reduced waste stream is also produced, with obvious economic benefits [6]. The aim of the present literature study is to review the potential of cellulase to be applied in the modification of DWP.

2.2 Cellulose

Woody material contains approximately 40% to 45% cellulose, which makes cellulose one of the most abundant bio-renewable polymers on earth [7]. Cellulose is a linear homopolymer, consisting of D-glucopyranose units, linked with β -1,4-glycosidic bonds (Figure 2-2). These D-glucopyranose units are known as anhydrous glucose units (AGU) [7 pp.24-25]. Cellobiose (consisting of two linked AGUs) is considered to be the basic unit of cellulose and, consequently, cellulose can be viewed as an isostatic polymer of cellobiose. The AGU contains hydroxyl groups at the carbon positions 2, 3 and 6, which are capable of all primary and secondary alcohol reactions and play a major role in the nature and structure of cellulose [7 pp.24-25].

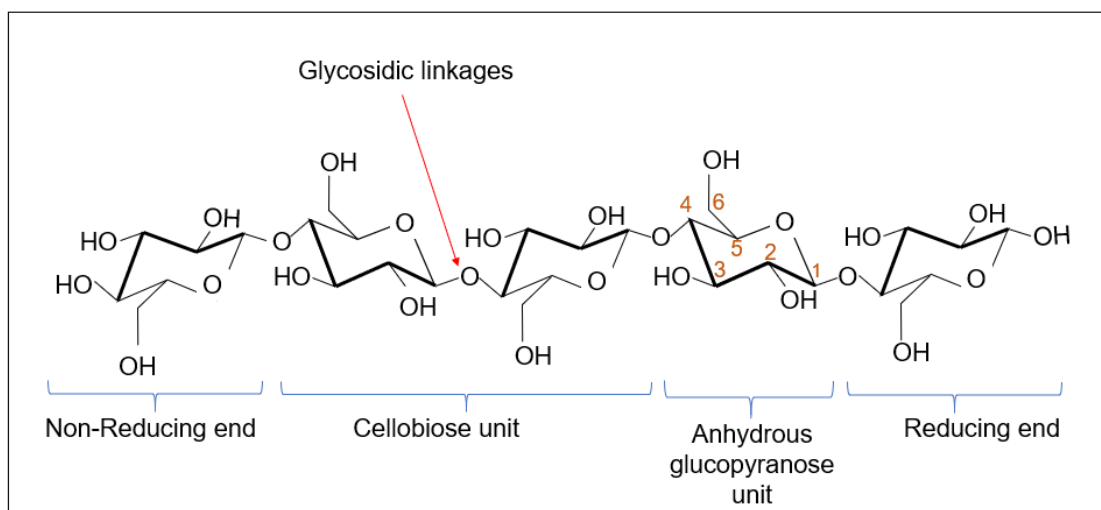


Figure 2-2: Molecular structure of cellulose

The molecular structure of cellulose does appear simple; however, the supramolecular structure allows for various types of hydrogen bonds to form due to the presence of hydroxyl groups (Figure 2-3). These hydrogen bonds can appear either in the same cellulose chain (intramolecular) or between different cellulose chains (intermolecular). Due to the intermolecular hydrogen bonds, aggregation of parallel cellulose chains occurs to form microfibrils. Microfibrils have a crystalline nature, however they are comprised both amorphous and crystalline regions [7 p.25].

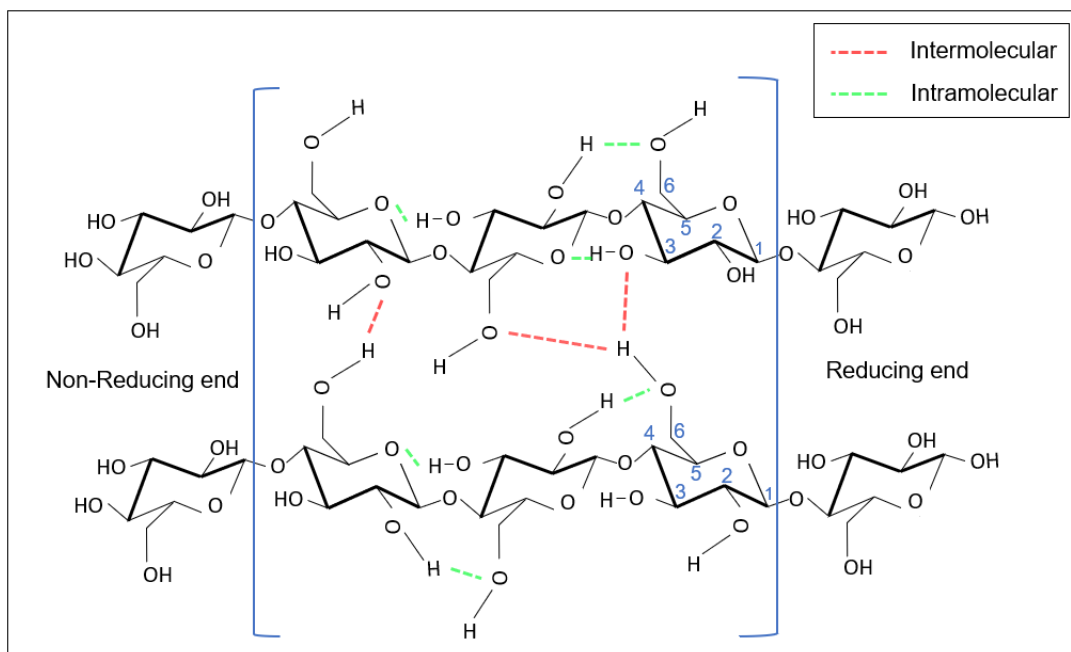


Figure 2-3: Illustration of two parallel cellulose chains forming intermolecular and intramolecular bonds (adapted from [7 p.25])

The crystalline structure of cellulose has been interpreted in different ways (Figure 2-4). Larrson *et al.* [8] described the nature of cellulose as having the amorphous cellulose located at the surface of the microfibril, while the core of the microfibril consists of crystalline cellulose. Conversely, Mosier *et al.* [9] described the nature of cellulose as having regions of high crystallinity that are separated by amorphous regions. However, it is possible that the crystalline structure of cellulose is a combination of these two interpretations, with amorphous cellulose on the surface of the microfibril and crystalline cellulose at the core of the microfibril with amorphous regions in between.

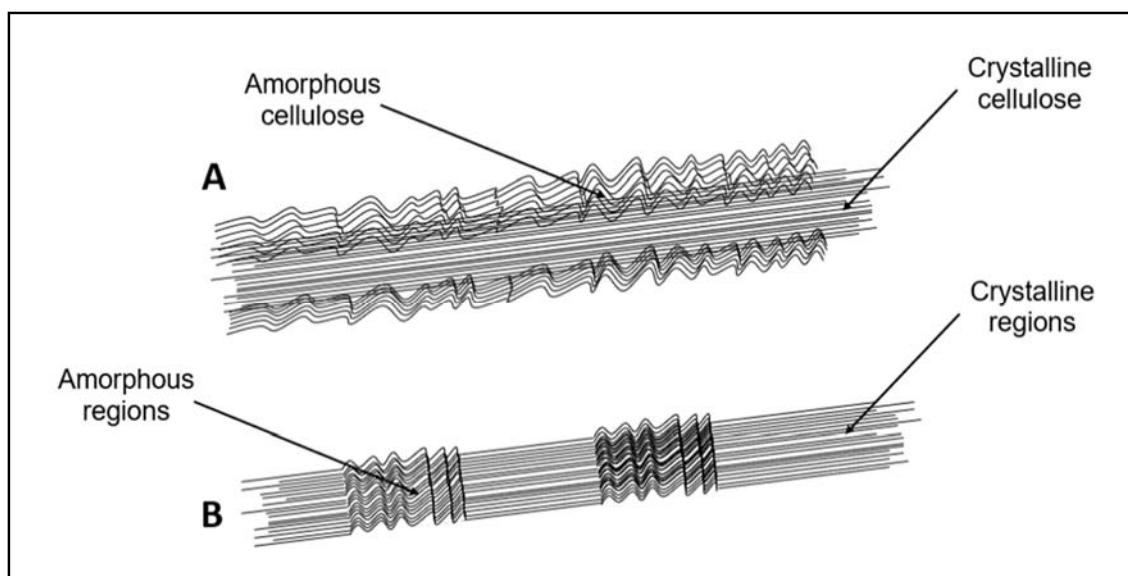


Figure 2-4: Different interpretations of the structure of cellulose (with **A** illustrating the interpretation according to Larsson *et al.* [8], while **B** illustrates the interpretation according to Mosier *et al.* [9]; adapted from [10 p.8])

Due to the amorphous and crystalline regions, cellulose can be described in terms of its degree of crystallinity, i.e. how much of the cellulose fibre is consisting of crystalline regions. Cellulose in DWP typically has a degree of crystallinity ranging from 40% to 60%, with the degree of crystallinity of PHK-produced DWP being greater than that of sulphite-produced DWP. The fact that sulphite-produced DWP has a lower crystallinity with a higher reactivity than PHK-produced DWP has led many to believe that crystallinity negatively influences the reactivity of DWP. However, studies have shown that crystallinity could positively influence the reactivity of pulp. For example, Helmy [11] reported that the crystalline regions are protected by the more susceptible amorphous regions, and therefore, lowering the reactivity. Similarly, Yamane *et al.* [12] concluded that crystallinity leads to a higher density of hydroxyl groups and, therefore, a higher reactivity.

Aside from the degree of crystallinity, the crystal structure of cellulose can be described in terms of unit cells. The unit cell is the basic repeating molecules (cellobiose) that make up the crystal structure. From the unit cell, lattice parameters (a , b , c and α , β , γ) can be calculated and are used to classify the unit cell type. In the case of cellulose, seven different crystalline structures (I_α , I_β , II , III_I , III_{II} , IV_I and IV_{II}) exist and the basic differences between cellulose I , II , III and IV are illustrated in **Figure 2-6**. The crystalline nature of cellulose is a macromolecular property and is determined by the hydroxyl groups via the inter- and intramolecular bonds that are formed; while there are many different unit cells in crystallography, the unit cells of interest in this study are the monoclinic and triclinic unit cells (Figure 2-5). The lattice parameter lengths (a , b and c) are all unequal in both the triclinic and

monoclinic structures. The angles of the lattice parameters (α , β and γ) in the triclinic structure are all unequal, while two of the angles are square in the monoclinic structure. The crystalline structure of cellulose I_β is of the monoclinic unit cell type (Figure 2-7), while cellulose I_α is of the triclinic unit cell type (Figure 2-8) [13]. The crystalline structure of cellulose I_β is more stable and more amorphous than the crystalline lattice structure of cellulose I_α . These amorphous and crystalline regions play an important part in enzymatic hydrolysis as the amorphous regions are more readily hydrolysed than the crystalline regions [14]. Whereas cellulose I is native, cellulose II, III and IV, all with different crystalline properties and applications, could be obtained following chemical treatment of cellulose I [15 p.57].

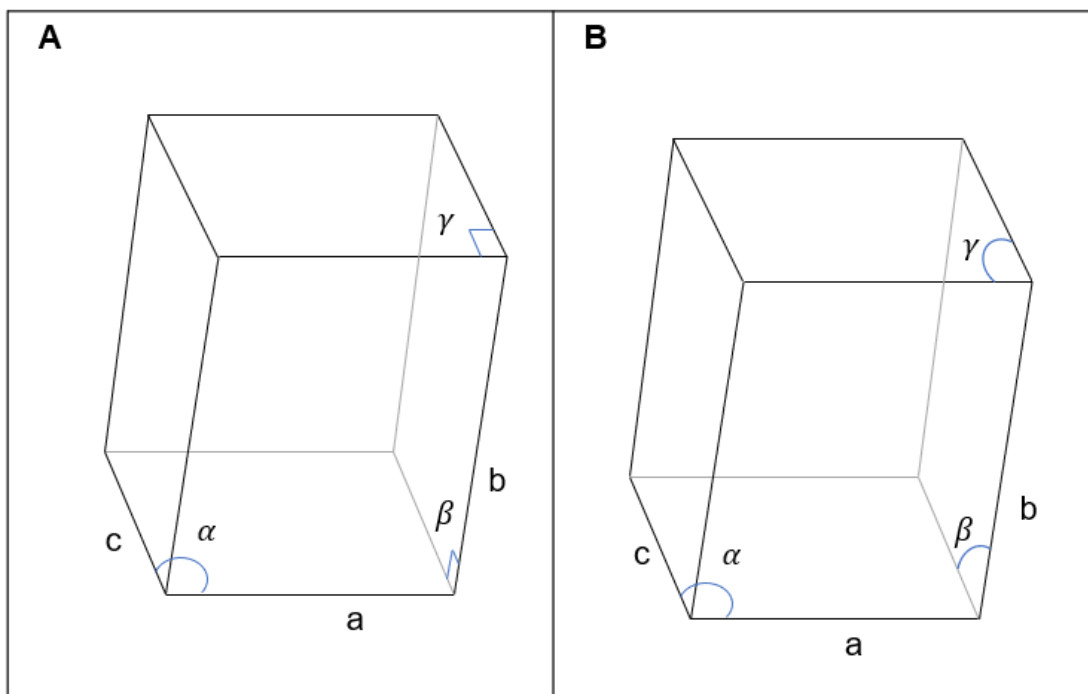


Figure 2-5: Illustration of the monoclinic (A) and triclinic (B) unit cells

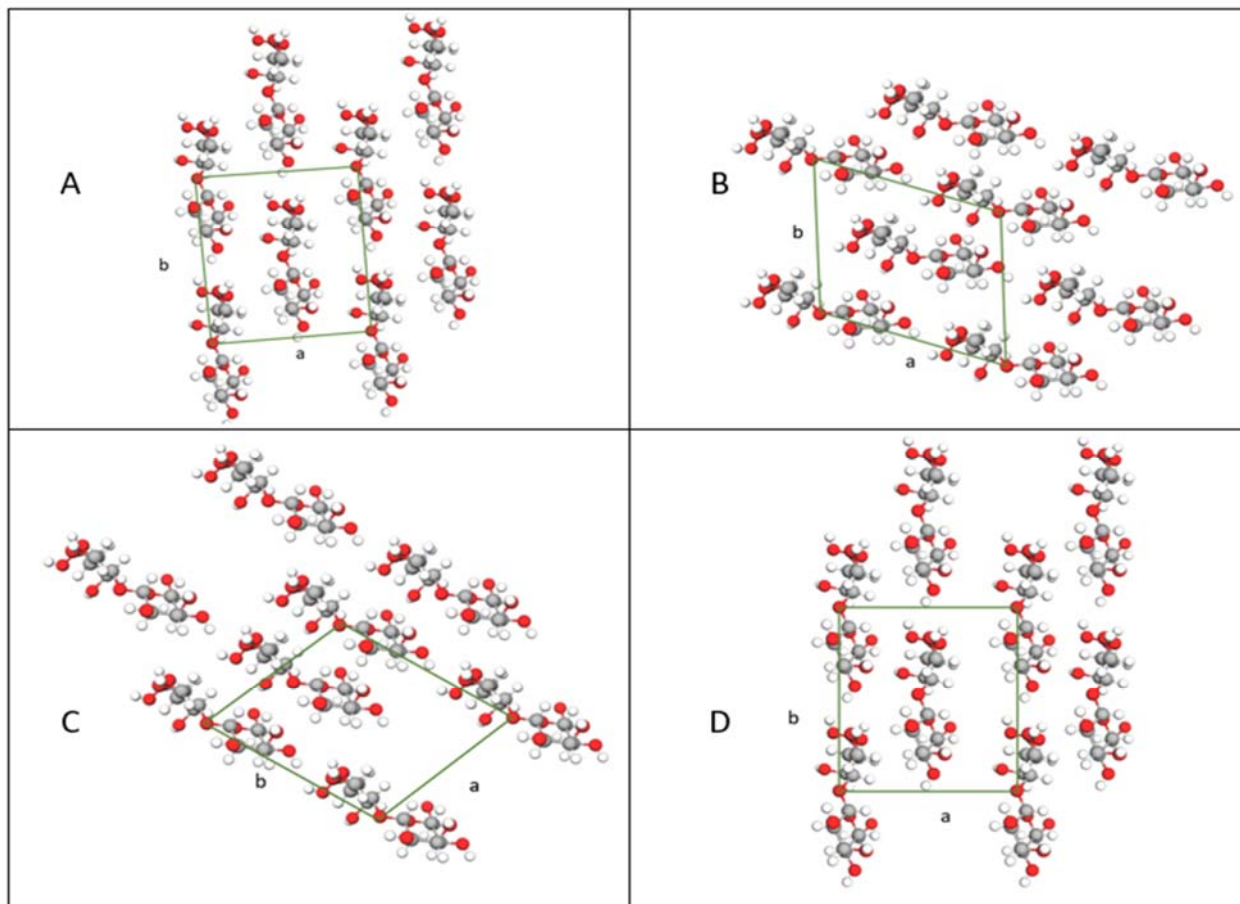


Figure 2-6: The main differences between the various cellulose structures. A illustrates the cellulose I structure, B illustrates the Cellulose II structure, C illustrates the cellulose III structure, D illustrates the cellulose IV structure. The lattice parameters lengths, a and b , are included in the illustration for better understanding of the crystal structure. Adapted from [16].

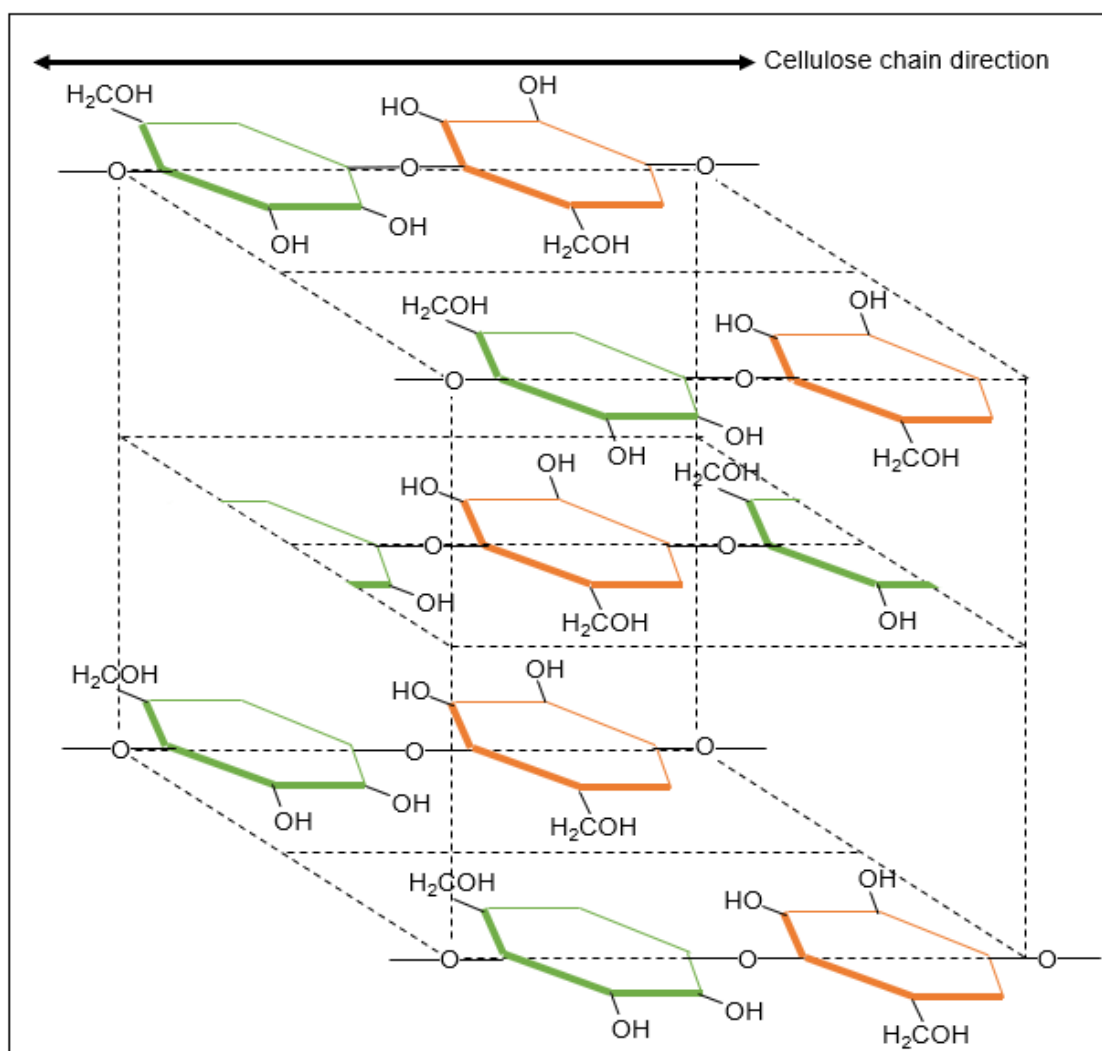


Figure 2-7: Illustration of the monoclinic unit cell of cellulose (the glucose units highlighted in orange all have the α configuration, while the glucose units highlighted in green all have the β configuration; adapted from [13])

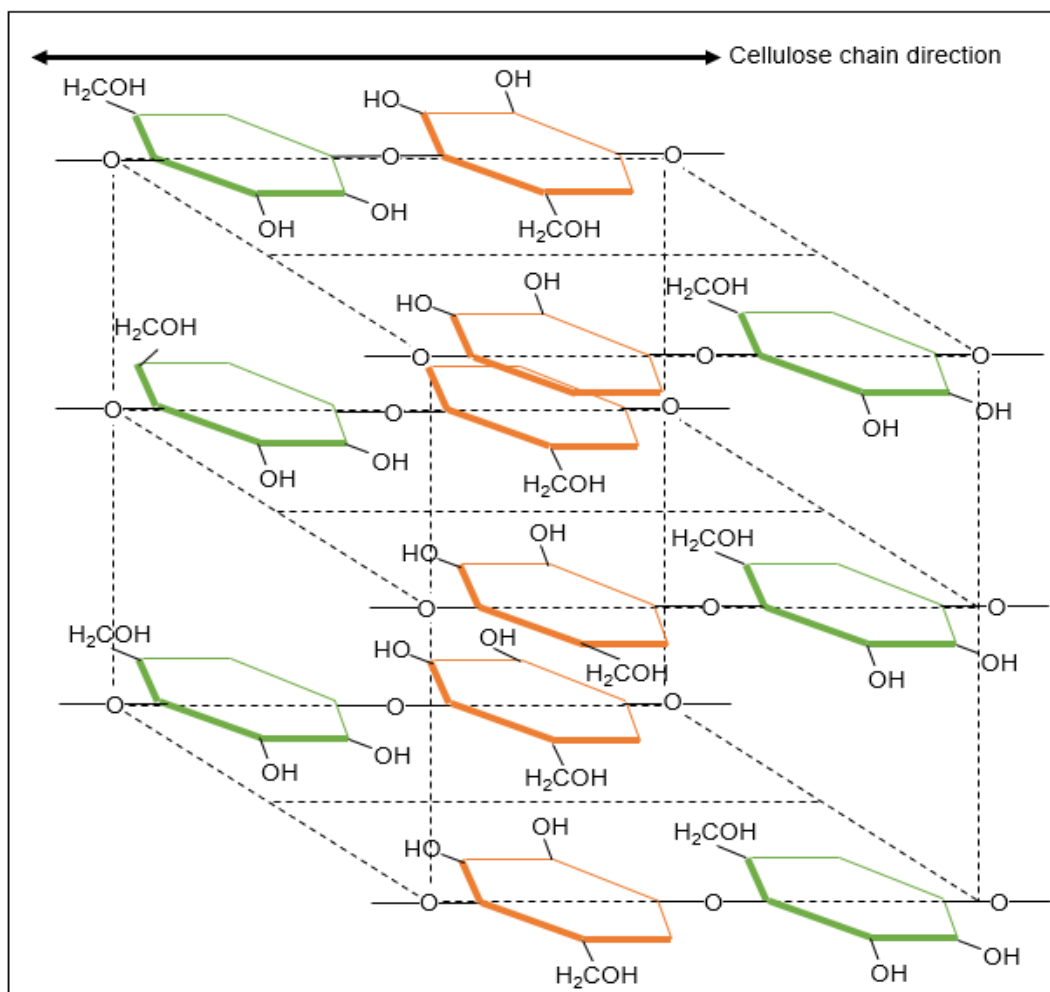


Figure 2-8: Illustration of the triclinic unit cell of cellulose (the glucose units highlighted in orange all have the alpha configuration, while the glucose units highlighted in green all have the beta configuration; adapted from [13])

The hydrophobic and hydrophilic properties of cellulose at the macromolecular level are provided by the hydroxyl groups [17]. The amphiphilic nature of cellulose can be ascribed to hydroxyl groups in specific positions of the AGU ring (Figure 2-9). The hydroxyl groups located at the equatorial positions (carbon positions 2, 3 and 6) of the AGU ring are polar and can form hydrogen bonds, resulting in a hydrophilic surface on the sides of the cellulose chain. The hydrogen atoms or aliphatic protons located at the carbon positions 1 to 6 (in the axial positions) of the AGU rings form Van der Waals bonds (C–H), resulting in the hydrophobic surface on the top and bottom of the cellulose chain [17].

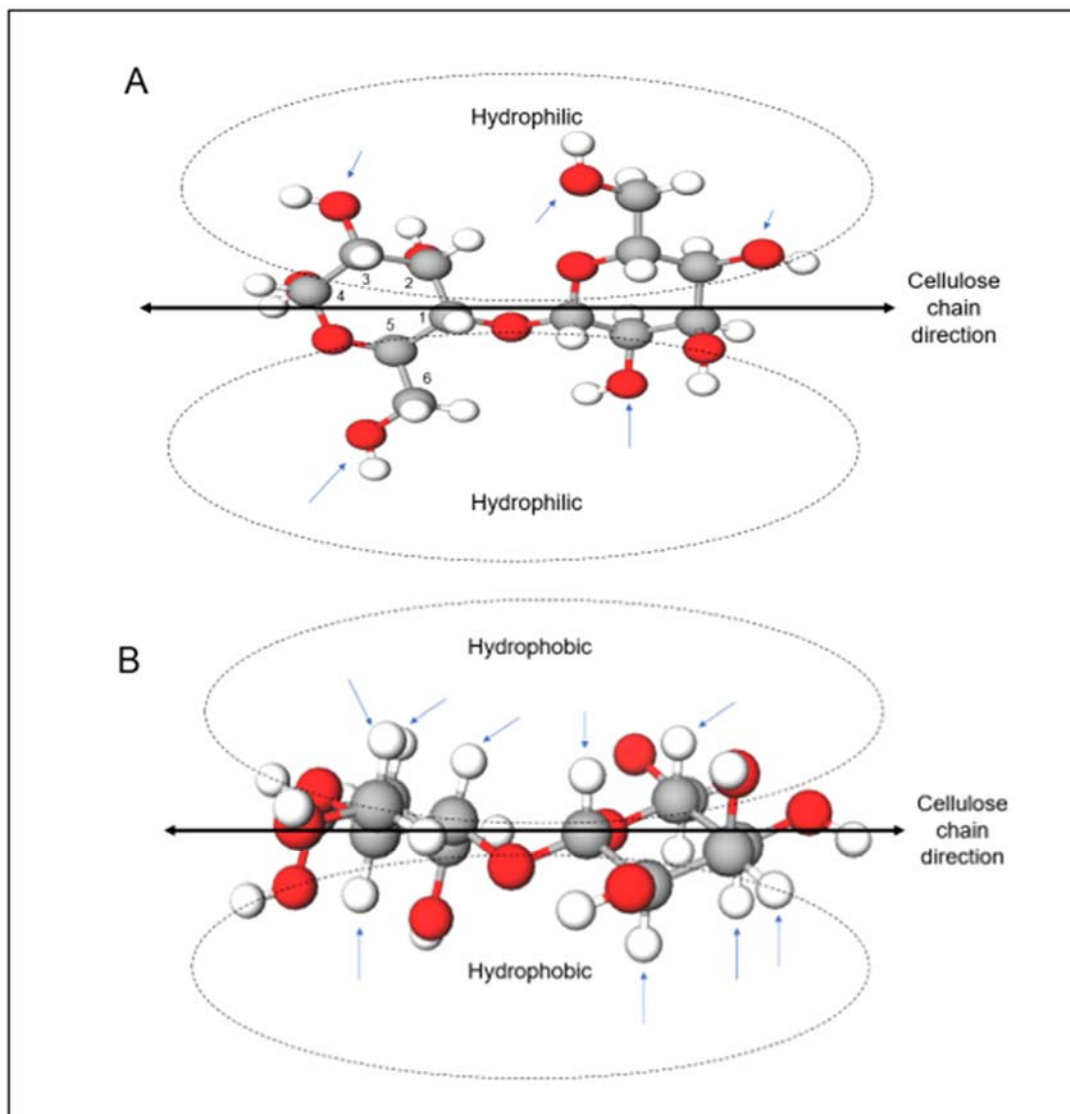


Figure 2-9: Side view of the cellobiose model (A) and the same model rotated through 90° (B) to illustrate the amphiphilic nature of cellulose (the hydrophobic and hydrophilic regions are demarcated by the dotted ellipse; oxygen is represented by red, while carbon is represented by grey; aliphatic protons [hydrogen] are represented by white; adapted from [18])

The applications of cellulose have been reviewed in [19] along with the unique properties that make them suitable for their applications. For example, the crystalline regions of cellulose allow for the manufacture of microcrystalline cellulose (MCC), which is insoluble in water, ethanol and diluted mineral acids. This insolubility makes it an excellent anti-caking agent and stabiliser. The amphiphilic nature of hydroxypropyl cellulose (HPC) makes it soluble in water below 45°C. This solubility makes it an excellent thickener and food additive. Similarly, the hydrophilic regions of cellulose, allow for the application of the methylcellulose derivative to be utilised as a thickener and emulsifier due to its cold-water solubility.

2.3 Characterisation of dissolving wood pulp

The characterisation of DWP is of extreme importance as the properties of DWP greatly influence its processability. This includes bleaching and further downstream processes, such as viscose production. During bleaching, the Kappa number is of great significance, relating to delignification and brightness. The pulp brightness influences the appearance of the final product and consequently customer satisfaction. Viscosity is controlled in the bleaching sequence and plays a major role in viscose production [3 pp.245-246].

The Kappa number is an indication of the lignin content of the pulp and is determined by using an oxidative reaction between a potassium permanganate solution and the lignin in the pulp sample [20]. According to [21 p.1061], for fully bleached pulp, used to produce viscose products, the Kappa number should be approximately 0.06 for hardwood sulphite-produced pulp and 0.07 for hardwood PHK-produced pulp.

The brightness of the pulp is influenced by the presence of lignin and other chromophore components and, therefore, related to the Kappa number. Similarly, DWP used for viscose products should have a brightness of 93% for hardwood sulphite-produced pulp and 89% for hardwood PHK-produced pulp [21 p.1061]. According to [22], brightness, or more accurately whiteness, is the measurement of the reflective properties of an object and can differ from one observer to another. Due to this variability, different formulations of whiteness have been established, such as Berger, Hunter and Stensby whiteness. Berger whiteness has been used since the early 1960s and, aside from ISO brightness, is the most common method used for whiteness determination. An important difference between ISO brightness and the other whiteness measurements is that the ISO brightness measures the blue reflectance of the pulp sample, while whiteness measures the reflectance across the entire light spectrum [23]. The Berger whiteness can be determined with any optical analysis machine that has the capability to provide data on tristimulus functions. Tristimulus functions provide data on the reflectance of the red, blue and green colours of an object in the wavelength range of 400 nm to 700 nm. The tristimulus data is then used in one of the whiteness standards mentioned above to calculate the whiteness and express it as a percentage [22].

The pulp viscosity is an indication of the average degree of polymerisation according to the scope of the ISO standard [24]. Pulp viscosity can be determined by using a capillary viscometer and a pulp sample dissolved in a cupriethylenediamine (CED) solution, with the limitation that the pulp should only contain components soluble in the CED solution. Pulp viscosity is often determined from unbleached pulp and after each bleaching stage in a bleaching sequence [3 pp.248-249]. The pulp viscosity should be in the range of 450 ml/g to 500 ml/g for fully bleached pulp [21 p.1061].

The degree of polymerisation (DP) as described in [25 pp.685-686] is the number of monomeric units in a single polymer chain. Many different techniques exist to determine the DP of the pulp [25 p.686]; however, the most widely used technique is gel permeation chromatography (GPC), which separates molecules based on their size. The instrument used for this analysis has a column packed with porous beads and, when a sample is analysed, the shorter polymer chains are retained longer in the pores of the beads than the longer polymer chains. The molecular weight and the molecular-weight distribution (MWD) of the polymer can be determined using GPC. The MWD of a polymer is the index of non-uniformity of the DP in a sample (Figure 2-10). The more non-uniform the MWD of a sample is, the greater the difficulty in processability; therefore, cellulose should preferably have a more uniform MWD and relatively lower DP. Cellulose with a high DP is more resistant to alkali solubility than cellulose chains with a lower DP [26 pp.1229-1230].

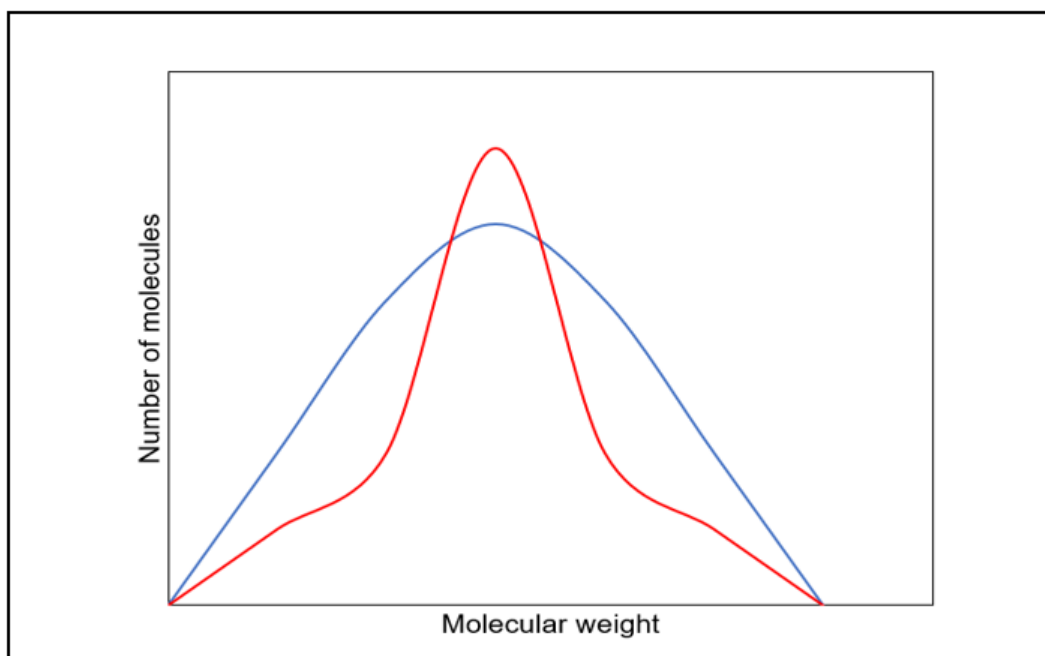


Figure 2-10: General molecular-weight distribution graph (blue illustrates a broad MWD, while red illustrates a narrow MWD)

The alkali solubility of pulp is determined with sodium hydroxide (NaOH) solutions of different concentrations [27]. A less concentrated solution, commonly a 10% solution, dissolves both hemicellulose and degraded cellulose, while a more concentrated solution, commonly an 18% solution, only dissolves the hemicellulose. By using a titrimetric method, the alkali solubility for a 10% solution and 18% solution can be determined respectively, often referred to as the S10 and S18 of the pulp. By calculating the difference between the S10 and the S18 values the amount of degraded cellulose can be determined [27]. The solubility of hemicellulose and

cellulose are dependent upon their monomer components, branching and DP, where the cellulose tends to be more resistant to alkali than hemicellulose. The alkali solubility for the S10 and the S18 should be approximately 10% and 5% respectively for viscose products [21 p.1061].

The degree of crystallinity can be determined by X-ray diffraction (XRD) analysis [28 pp.1259-1261]. The pulp sample is irradiated by filtered and collimated (parallel) X-ray beams with a known wavelength. The beams are scattered when passing through the sample and this intensity, referred to as scattered intensity, is measured as a function of the diffraction angle. The scattered intensity measured is plotted against the diffraction angle. This plot is referred to as a diffractogram, with the crystalline regions characterised by narrow peaks, while amorphous regions are characterised by broader peaks. These narrow peaks and broader peaks are then separated from each other using numerical software and once separated, the degree of crystallinity can be calculated by taking the ratio of the area of the crystalline peaks to the combined crystalline and amorphous peaks [28 p.1260].

The reactivity of the pulp can be determined using Fock's method, which was later modified by [29]. This method is a small batch scale version of the viscose process. Cellulose (fully bleached pulp) is mixed with a NaOH and CS₂ solution. After the cellulose dissolves in this solution it is allowed to react with sulphuric acid. The unreacted pulp precipitates while the reacted pulp remains in solution. The reacted pulp is separated from the unreacted pulp and a titrimetric method is used to determine the pulp reactivity [29].

2.4 Modification of cellulose

According to [30], several approaches can be adopted for cellulose modification. These adopted strategies all have a single objective, namely, to improve the accessibility of cellulose to chemicals. These methods involve breaking or removing the primary fibre wall, expanding or opening of the fibre voids or pores and separating fibre aggregations. The accessibility could also be increased through disruption of the crystalline regions and the shortening of cellulose chains. The first three approaches are commonly related to mechanical modification techniques, while the last two approaches are more commonly associated with chemical and enzymatic modification techniques.

2.4.1 Chemical modification of cellulose

Typical chemical modification can be achieved by the alteration of the hydroxyl groups in the cellulose chain or through nucleophilic displacement reactions of the carbon atoms of the AGU [31]. This result can be obtained through chemical grafting or the oxidation of cellulose. During chemical grafting, the reactions occur between the functional groups of the cellulose. The technique of grafting allows for the introduction of new physical and chemical properties to cellulose through the addition of polymers to the cellulose chain; however, the technique is greatly influenced by the physical structure of cellulose [32 p.755]. The various grafting techniques are described in detail in [15 pp219-222]. According to [4], oxidative modification is a broad term related to the oxidation techniques for cellulose modification using agents such as ozone, sodium hypochlorite and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO).

Ozone, with the aid of sodium borohydride, could be used to control the viscosity of pulp [3 p.232]. The reaction with ozone produces carbonyl groups along the cellulose chain, resulting in cellulose chain scission and ultimately a reduction in viscosity of the pulp. When the cellulose is then treated with sodium borohydride in an alkaline environment, the carbonyl groups are reduced back to the original alcohol groups to leave the viscosity unchanged. A limitation of ozone is that the ozone preferentially reacts with lignin when the content thereof is high in the pulp.

Due to the limitation of ozone, it is not the preferred choice for viscosity control, but rather sodium hypochlorite [33 p.11]. The viscosity of cellulose is controlled via the alteration of the DP. Hypochlorite attacks the cellulose resulting in oxidation of the carbonyl groups at carbon positions 2, 3 and 6. The carbonyl groups are oxidised to carboxyl groups, thereby reducing the DP through cellulose chain scission [15 p.189-191]. The reaction rate of hypochlorite oxidation is dependent on pH and temperature. Oxidation with ozone and sodium hypochlorite both display a low selectivity, which is unfavourable [4].

2.4.2 Mechanical modification of cellulose

In contrast to chemical modification, mechanical modification is seldom used on its own, but rather in conjunction with another modification technique such as enzymatic modification. According to a review by [34], mechanical modification causes morphological changes in the cellulose fibres that lead to increased specific surface areas. These morphological changes can either be on the surface of the fibre (external) or inside the fibre (internal). During external fibrillation, strands fray or peel away from the surface, while the intermolecular bonds are broken during internal fibrillation to cause pore formation and widening. By controlling the mechanical conditions such as the number of revolutions, rotational speed and intensity, the extent of fibrillation may be controlled [34].

Common mechanical modification techniques include the use of a *Papirindustriens Forskningsinstitutt* (PFI) mill or grinder. Typically, grinders 'cut' the fibres and thus create more surface area, while a PFI mill beats the pulp leading to pore formation and widening. All mechanical treatments are energy-intensive and, therefore, only employed on a small scale [34].

Sonication is an alternative method available for mechanical modification. Sonication utilises ultrasonic sound for the 'development' of cellulose fibres [31]. Ultrasound waves are generated at a frequency higher than 20 kHz and is subdividable into power ultrasound and diagnostic ultrasound. From an energy point of view, only power ultrasound is suitable for biomass treatment. Ultrasound treatment has the ability to cut the fibres, reducing fibre length and crystallinity [35]. A major drawback of sonication is that heat is generated which reduces the efficiency of sonication treatment [36]. For laboratory-scale sonication, four types of equipment are available, namely the whistle reactor, cleaning bath, probe, and cup horn, of which the probe and cup horn are the most suitable for polymer processing. In contrast, industrial scale equipment is more difficult to use owing to the treatment volumes that need to be considered. For example, some processes are more suited for a whistle reactor in a large-scale setup than it was during laboratory testing. Similar equipment as in laboratory-scale sonication is available for industrial-scale sonication, such as whistle systems and low and high intensity systems. The whistle system is similar to the whistle reactor, whereas the low intensity system is similar to the cleaning bath; the high intensity system consists of flow cells with probes. Sonication and the available equipment and application are fully elaborated on in [36].

2.4.3 Enzymatic modification of cellulose

Traditionally only mechanical and chemical modification was employed in the pulp and paper industry, and it was not until the late 1980s that enzymes were introduced [37]. Enzymatic modification entails the use of enzymes to modify cellulose properties [38]. Cellulase can be classified by either its substrate specificities or by its structural similarities. However, the classification of enzymes (in general) according to their substrate specificities is difficult due to the overlapping of enzyme activities. The newer system classifies enzymes into members and families and is, therefore, preferred. This new classification system classifies enzymes according to similarities of the amino acid sequence of the catalytic domain (CD) [38]. Cellulase is classified into the family of enzymes known as the glycosidic hydrolase family, i.e. those enzymes capable of hydrolysing glycosidic bonds.

According to [35], cellulase is a modular enzyme that consists of structurally and functionally independent units that are folded or intertwined. These independent units form not only the entire enzyme but also the active sites of the enzyme. The active site of an enzyme always consists of a CD and in most cases a carbohydrate-binding module (CBM). The CBM of the enzyme allows for the enzyme to bind to the cellulose, to increase the effective concentration of the enzyme and the time the enzyme spends in the proximity of the substrate [39]. The CD is linked to a CBM by a linker peptide (Figure 2-11). In some cases, a cellulase molecule only consists of a CD and is called a core enzyme. In contrast, full-length enzymes are in possession of both a CD and CBM and appear to have a greater affinity towards cellulose, compared to core enzymes [40].

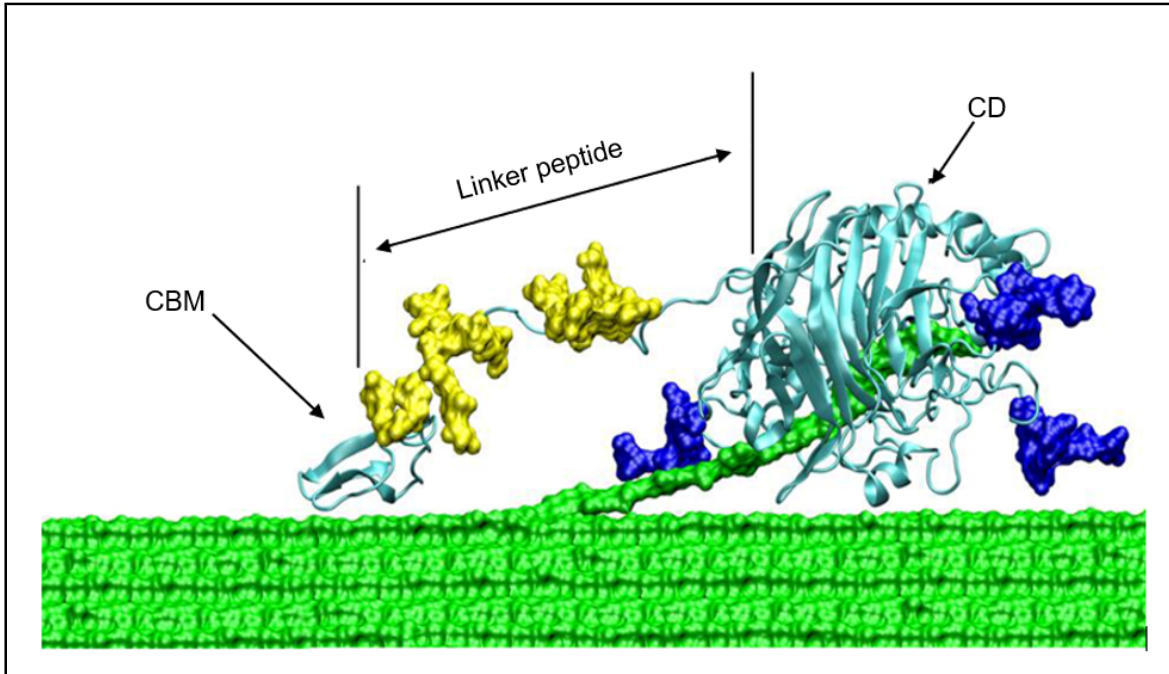


Figure 2-11: Cellulase catalytic domain and carbohydrate-binding module during cellulose degradation (taken from [41])

Cellulase is responsible for cellulose degradation (Figure 2-12) and can be separated into three major subclasses. These subclasses are EG, exoglucanase or CBH and β -glucosidase (β -G) [42]. EG selectively hydrolyses the amorphous regions of cellulose by hydrolysing cellulose within the cellulose chains, known as endo-mode hydrolysis. In contrast, CBH can hydrolyse the crystalline regions of cellulose, from either the reducing or non-reducing end (CBH I and CBH II, respectively). The products of EG and CBH are cellobiose and D-glucose which are hydrolysed by β -G.

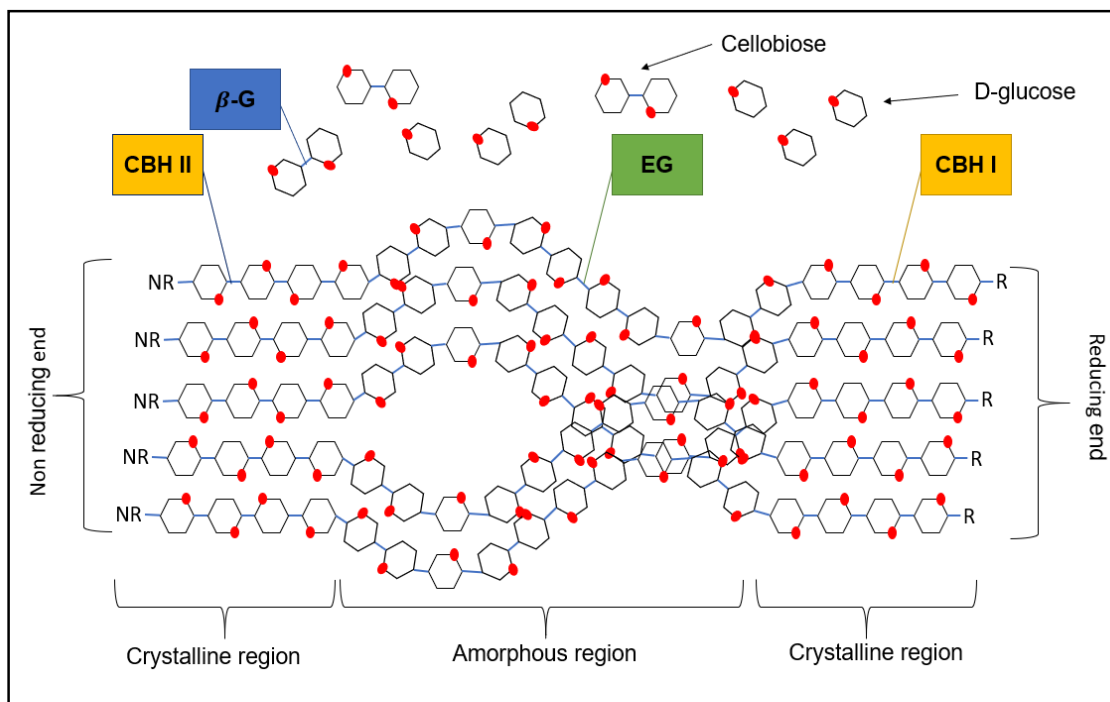


Figure 2-12: Classic model for cellulose degradation by cellulases (with EG hydrolysing the amorphous regions and CBH hydrolysing from the reducing and non-reducing ends, respectively [CHB I and CBH II]; and β -G hydrolysing the cellobiose products of EG, CBH I and CHB II; adapted from [43])

The hydrolysis of cellulose occurs at the active site of an enzyme, and the features thereof play a vital part in cellulose degradation. The active site of EG is cleft- or groove-shaped (Figure 2-13). This unique structural feature allows the enzyme to bind itself to the cellulose chain and randomly break the β -1,4-bonds of the cellulose to produce cellobiose and cellodextrins as products, amongst others [35, 44]. Product inhibition through cellobiose and glucose has been reported by [45], and cellobiose appears to have a greater inhibitory effect than glucose, but the type of inhibition is still poorly characterised [46]. While cellobiose and glucose units inhibit EG, the new ends produced by EG are susceptible to hydrolysis by CBH [47].

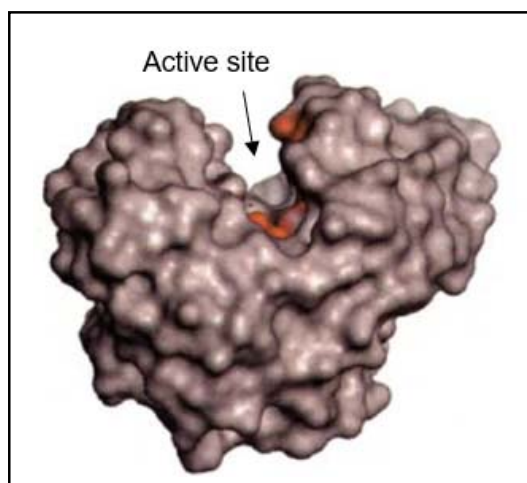


Figure 2-13: The cleft- or groove-shaped active site of the catalytic domain of endoglucanase (taken from [35])

The action of EG produces reducing and non-reducing ends, both of which are susceptible to CBH degradation [42]. The active site of CBH is tunnel-shaped (Figure 2-14), and this unique structural feature allows this enzyme to remain bound to the cellulose, while releasing the products of hydrolysis. The key structural differences between CBH I and CBH II are the number of surface loops and the length of the active-site tunnel. In CBH I the tunnel length is approximately 5 nm, corresponding to four surface loops. The tunnel length for CBH II is approximately 2 nm and this corresponds to two surface loops. The products released by both CBH I and CBH II include cellobiose and glucose, and these products also show an inhibitory effect on CBH.

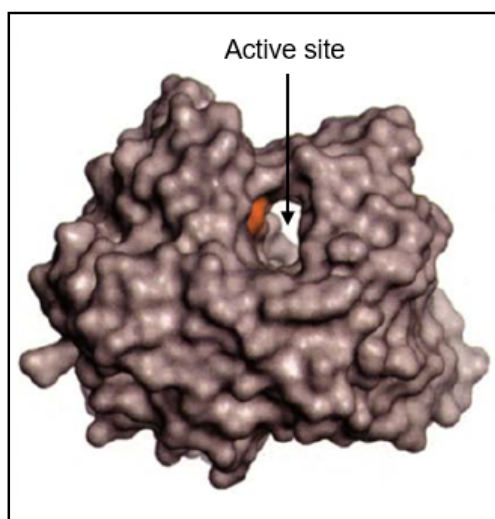


Figure 2-14: The tunnel-shaped active site of the catalytic domain of cellobiohydrolase (taken from [35])

In contrast to CBH and EG, which are inhibited by cellobiose, β -G can degrade cellobiose. The active site of β -G does not contain a CBM, but only a CD that is in the shape of a pocket (Figure 2-15). A unique feature of this active site is that β -G can bind to the non-reducing glucose unit of cellobiose and split the glucose units.

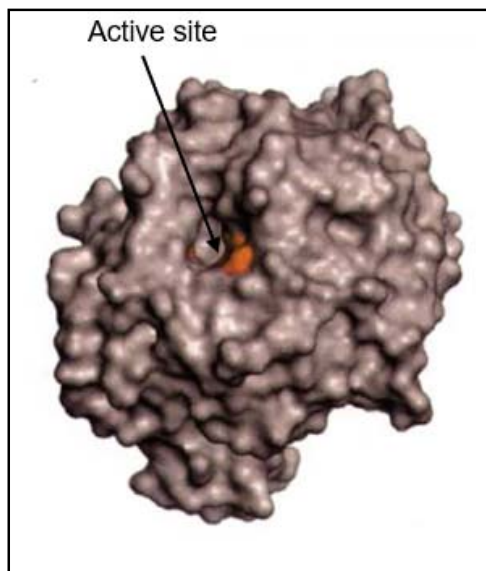


Figure 2-15: The pocket-shaped active site of the catalytic domain of β -glucosidase (taken from [35])

The hydrolysis reaction of cellulose occurs at the active site of the CD of the enzyme. The hydrolysis reaction can proceed by one of two mechanisms, namely the inverting mechanism or the retaining mechanism [35]. For both the inverting and retaining mechanisms, two catalytic residues are required from the enzyme, namely a base (nucleophile) and an acid (proton donor). The inverting and retaining enzymes differ in the distance between the catalytic residues. Inverting enzymes have approximately 1 nm distance between the residues, while for retaining enzymes a distance of 0.55 nm was recorded. Another difference between the two is that glycosylation (glycosidic bond formation) cannot be performed by inverting enzymes, while the retaining enzymes can perform glycosylation [35].

The inverting mechanism refers to the inversion of the anomeric C₁ carbon of a glucose molecule in the sugar product from the β configuration to the α configuration [35]. The inverting mechanism proceeds via a single nucleophilic displacement reaction, where the charged environment of the catalytic site is utilised for the inversion reaction (Figure 2-16). This inversion reaction is initiated by an amino acid donating a proton, while a water molecule is activated to act as a nucleophile. After the nucleophilic attack, the reaction ends, with the anomeric carbon inverted to the α configuration.

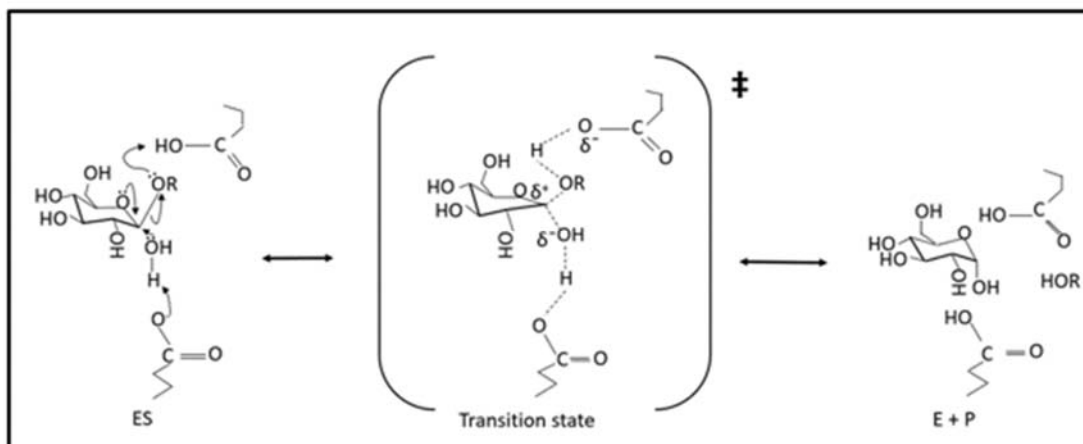


Figure 2-16: Illustration of the inverting mechanism steps in enzymatic hydrolysis (adapted from [48])

In contrast, the anomeric carbon retains its configuration after the two-step hydrolysis reaction, due to the retaining mechanism (Figure 2-17). Two-step hydrolysis is also known as a double displacement hydrolysis reaction, wherein the glycosyl bond is attacked by a charged amino acid during the first step. This nucleophilic attack by the amino acid forms a covalent-bond intermediate, followed by recharging of the proton donor. After the proton donor has been recharged, the hydrolysis product is freed by a water molecule [35].

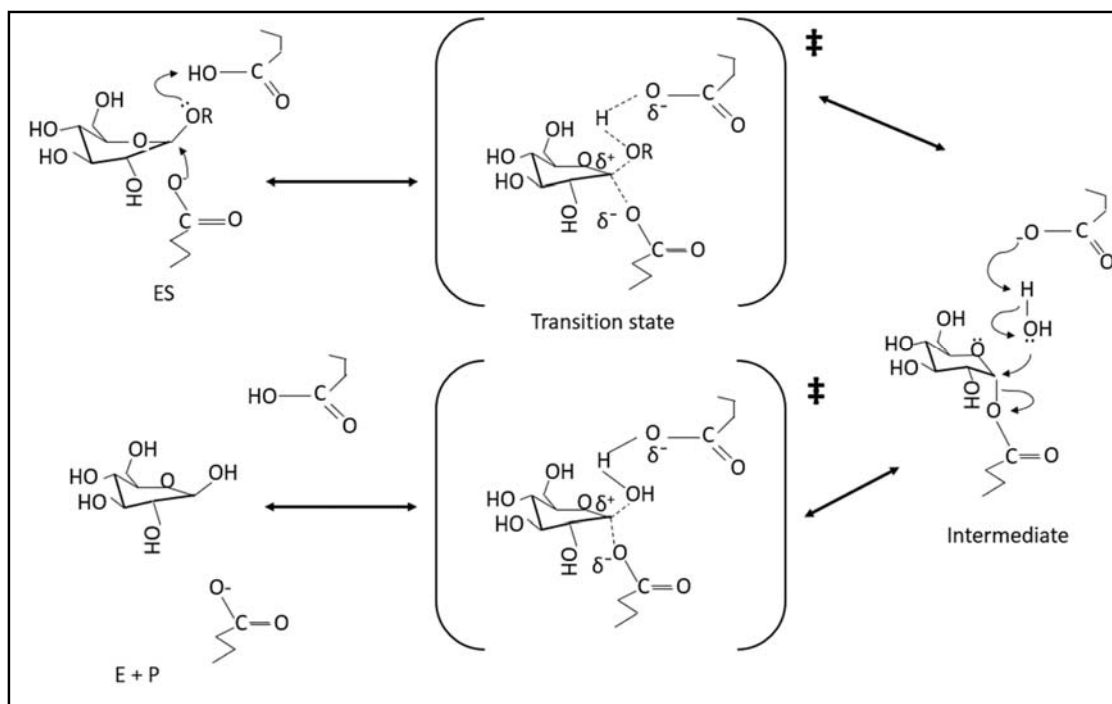


Figure 2-17: Illustration of the retaining mechanism steps in enzymatic hydrolysis (adapted from [48])

EG can follow either the inverting or retaining mechanism, depending on the origin of the enzyme. For example, EG from *Humicola insolens* can follow the inverting mechanism, while EG from *Plasmodium brasilianum* follows the retaining mechanism [10 p.49]. In contrast, CBH I will always follow the retaining mechanism, while CBH II will always follow the inverting mechanism, regardless of their origin [47].

Globally, cellulase is one of the commercially most produced enzymes [49]. Purified EG, CBH I and CBH II are commercially produced from *Trichoderma reesei*. Purified β -G and some EG are produced from *Aspergillus niger* [42]. According to a survey by [50], cellulase was also commercially produced from *Humicola insolens*, but more recent information is not available regarding commercial enzyme production.

2.5 Applications of enzymatic modification of dissolving wood pulp

The reactivity of pulp plays a major role in downstream processability and influences the chemical consumption and waste-stream production, for example, as during the xanthation reaction of viscose production. The improvement of pulp reactivity through enzymatic modification of cellulose has been the subject of several studies (Table 2-1). Most of the studies used a semi-bleached sulphite-produced pulp from softwood blend and the reactivity was determined through Fock's method. The enzymatic treatments increased the reactivity of the pulp ranging from 13% to 25%. The minor change in pulp reactivity (13%) for the study by [6] might be due to dilution of the enzymes at the extremely low pulp consistency (1%) at which the treatments were conducted. In contrast, the study by [51] showed a remarkably large increase in pulp reactivity, which may be due to the mechanical treatments combined with the enzymatic treatments. The PHK-produced pulp showed a notable increase in reactivity as, generally, PHK-produced pulp has a low reactivity [52 p.395].

Table 2-1: Studies of reactivity improvement of pulps treated with endoglucanase, as recorded with Fock's reactivity test

Pulping process	Raw material	Bleaching	Change in reactivity	References
Sulphite	Softwood blend	Semi	+13%	[6]
Sulphite	Softwood blend	Semi	+25%	[51]
Sulphite	Softwood blend	Semi	+15%	[53]
Unknown	Hardwood	Semi	+20%	[53]
PHK	Unknown	Fully	+25%	[54]

It is generally accepted that cellulose crystallinity negatively influences the reactivity of pulp [55]. However, in some studies it has been found that higher crystallinity could positively influence the reactivity of pulp. It was suggested by [11] that the amorphous regions protect the crystalline regions from chemical attack. In another study by [12], it was concluded that a higher degree of crystallinity leads to a higher average density of hydroxyl groups. The hydroxyl groups are the reactive sites of cellulose and a higher density thereof means more reactive sites available for reaction.

Viscosity with the corresponding DP and uniform MWD of pulp are key parameters for good quality DWP [21 p.1034]. A few studies investigated the changes in viscosity, DP and MWD of cellulose treated with cellulases (Table 2-2). Most of the pulp used in these studies were softwood semi-bleached sulphite-produced pulp. A significant reduction in viscosity was observed for all the pulps; however, the effect was more pronounced in softwood than in hardwood pulps as reported by [53]. All the studies used a commercial EG for the enzymatic treatments and a capillary viscometer to determine the viscosities of the pulps. The DP was

measured by either GPC or inferred from viscosity. The magnitude of the changes in viscosities may be due to the different starting viscosities of the pulps. The MWD was also determined through GPC and it was found that, after enzymatic treatment, all of the pulps showed a more uniform MWD, with less high molecular-weight substances present than in the control pulp.

Table 2-2: Viscosity, degree of polymerisation and molecular-weight distribution improvement of endoglucanase treated pulps

Pulping process	Raw material	Bleaching	Change in viscosity	Change in DP	Change in MWD	References
Sulphite	Softwood blend	Semi	-33%	-33%	Unknown	[6]
Sulphite	Softwood blend	Semi	-33%	Not determined	More uniform	[51]
Sulphite	Softwood blend	Semi	-100 units	-37%	More uniform	[53]
Sulphite	Unknown	Fully	Not determined	Not determined	More uniform	[56]
PHK	Unknown	Fully	-36%	Not determined	Not determined	[54]
PHK	Softwood	Fully	Not determined	-18%	Not determined	[57]
Unknown	Hardwood	Semi	-30 units	-8%	More uniform	[53]
Unknown	Hardwood	Unknown	Not determined	Approximately 27%	Not determined	[58]
Unknown	Softwood	Unknown	Not determined	Approximately 27%	Not determined	[58]

The alkali solubilities of pulp increased with enzymatic treatments (Table 2-3) and according to [58], this increase was due to the decrease in DP, since low molecular-weight cellulose tends to be less alkali resistant than high molecular-weight cellulose. All the studies used a commercial EG and the alkali solubility was determined by the ISO method, except for [58], which used a modified method based on centrifugation. According to [50], alkali solubility is an indirect measurement of cellulose accessibility and reactivity and, therefore, a pulp with higher alkali solubility is preferred.

Table 2-3: Alkali solubility characteristics of endoglucanase treated pulps

Pulping process	Raw material	Bleaching	Alkali solution strength	Change in alkali solubility ($\leq 10\%$ solution)	References
PHK	Unknown	Fully	10% NaOH	+21%	[54]
			18% NaOH	+16%	[54]
Unknown	Hardwood	Unknown	9% NaOH	+21%	[58]
Unknown	Softwood	Unknown	9% NaOH	+37%	[58]

In general, the enzymatic treatments were conducted at the optimum conditions for the enzymes; further information regarding specific studies are summarised in Table 2-4. These optimum conditions are a pH of 7 and an incubation temperature of 50°C. The enzyme dosage varied, depending on the activity of the enzymes. The activity of the enzymes was measured through the cellulase analytical method which reports the activity of the enzyme in EG units (ECU). The ECU activity is defined as the amount of enzyme producing one nanomole of reducing sugars as glucose in one second. In these studies, a maximum dosage of 30 ECU per gram of dry pulp was used for the enzymatic treatments.

Table 2-4: Conditions for enzymatic treatment of pulps

References	Incubation time	Consistency	Mechanical effects	
			Stirring	Kneading
[6]	22.5 hr	1%	x	
[51]	10, 30 & 90 minutes	3%		x
[58]	Varing incubation time up to 24 hr	Unknown	x	
[56]	2 hr	Unknown		
[53]	10, 30 & 90 minutes	3%		x
[57]	1 hr	3%		x
[54]	90 minutes	10%		

2.6 Concluding remarks

Cellulose is a homopolymer with hydroxyl groups at various carbon positions that provide many different properties to cellulose. These properties include crystallinity, and hydrophobic and hydrophilic properties. These properties of cellulose allow for the many different applications of cellulose, for example, as anti-caking agents and food additives.

Pulp characterisation is an important aspect, as the properties of DWP greatly influence its processability. Pulp characterisation methods include the determination of the Kappa number, brightness, viscosity, DP, MWD, degree of crystallinity, and pulp reactivity. The Kappa number is an indication of the lignin content of the DWP which influences the brightness. Viscosity is determined as the intrinsic viscosity of the cellulose and relates to the DP of the cellulose and the MWD of the pulp. The DP and MWD of the pulp influence downstream processability of the pulp. The alkali solubility of the pulp is an indication of the degradation of cellulose and is influenced by the DP of the cellulose. The degree of crystallinity is determined by XRD and influences the processability of the pulp, but the exact influences are not fully characterised. The reactivity as determined by Fock's method is an indication of the amount of CS₂ required to dissolve the cellulose during viscose production.

Various methods of modification of cellulose exist to improve the properties of cellulose. Such methods include chemical modification, mechanical modification and enzymatic modification. In chemical modification, specifically oxidative modification, the major drawback is the low selectivity and the control conditions required to avoid excessive cellulose oxidation during ozone bleaching. Mechanical modification is energy-intensive and seldom used as a stand-alone technique. Mechanical modification techniques include grinding, refining and sonication, of which sonication is the least energy-intensive. Enzymatic modification requires little energy input, is highly selective towards cellulose and, therefore, shows promising results for cellulose modification.

Cellulase is a system of enzymes that includes EG, CBH and β -G. Cellulase can hydrolyse cellulose through either the inverting or retaining hydrolysis mechanism, depending on the spatial effects of the enzyme. EG with its unique ability to hydrolyse the amorphous regions of cellulose is the preferred enzyme to improve the properties of cellulose.

The advantages associated with the treatment of DWP pulp with EGs include reduced chemical consumption such as of hypochlorite during bleaching [54] and of CS₂ in the viscose production process [6]. This reduction in chemical consumption allows for lower economic costs and a reduced waste stream. The costs are lower due to a reduction of both chemical consumption during production and of waste disposal [6].

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Chapter 3: The response of semi-bleached dissolving wood pulp to endoglucanase treatment

3.1 Introduction

The demand for DWP or high-purity cellulose has increased in recent years due to its applications in the textile industry, amongst others [1]. DWP is used as the primary feedstock in many processes to produce cellulose derivatives and other cellulose-based products such as MCC and nanocrystalline cellulose [2].

DWP can be produced by many different processes, such as the PHK process or acid sulphite pulping, and is then further purified through bleaching. During the bleaching process, several properties are measured, and these properties govern certain aspects of the bleaching process. Amongst the parameters routinely measured, are the lignin content (Kappa number), whiteness, viscosity and alkali solubility, of which viscosity is probably the most important [2]. In some instances, crystallinity and reactivity are also measured, with reactivity being more important [3]. The chemical charge can control the viscosity during certain bleaching stages, such as the hypochlorite (H) or ozone (Z) stages, but the reactivity is a more complex parameter to control. The reactivity, according to [3 p.1060], is influenced by all three physical structure levels, namely the molecular, supramolecular (such as the crystallinity) and fibril levels, and the chemical interaction with the reagent.

It has been reported that the use of certain enzymes, specifically cellulase, during the bleaching process could improve pulp properties such as viscosity, alkali solubility and reactivity [2], [4–6]. Cellulase consists of several enzymes, including EG, CBH and β -G, each with a unique structure and function. EG can hydrolyse the cellulose chains from within to produce shorter cellulose chains, and the product of this enzymatic reaction is, amongst others, cellobiose. It is known that cellobiose inhibits EG activity [7]. The CBH enzymes hydrolyse the cellulose from either the reducing end (through CBH I) or non-reducing end (through CBH II), and the product of this enzymatic reaction is also cellobiose. The β -G breaks the cellobiose further down into glucose [8]. EG was reported to be the most effective enzyme to reduce viscosity and increase reactivity through the cleaving of the glycosidic linkages in the amorphous regions of the cellulose polymer chain [9], [10].

In this study, a high-level enzyme assay was conducted to clarify some differences between the two commercial enzymes obtained for the experiments. Furthermore, the effect of EG treatment on the pulp properties of semi-bleached PHK-produced DWP treated with the assayed enzymes was investigated. The properties determined were viscosity, alkali solubility,

crystallinity, BWI, and reactivity. The aim was to achieve a viscosity drop of between 50 and 100 units, without increasing the amount of degraded cellulose and to determine if there was an increase in the pulp reactivity. The BWI was measured to determine if any visual changes occurred after treatments, and crystallinity was measured to observe structural changes, as changes in crystallinity after enzymatic treatments were previously reported [9].

3.2 Materials and methods

3.2.1 Enzyme characterisation

A high-level assessment of two commercially available enzyme formulations (Table 3-1), was conducted using a modified filter paper assay (FPA) as described by Chu *et al.* [11]. The high-level assessment provided information regarding the activities of the two enzyme formulations, and also how optimum conditions (pH and temperature) of the enzymes differed from the mill operating conditions. The enzyme activities were not converted to the Standard International units (ECU), instead the activities were directly related to the amount of product released during the enzymatic reaction.

Table 3-1: Supplier information and optimum ranges for the commercial endoglucanases obtained from two suppliers

Enzyme formulation	Supplier	Temp. (°C)	pH range	Reference
Ecopulp R	AB enzymes (Darmstadt, Germany)	30 to 65	4.5 to 7.5	[12]
NS 51179	Novozymes (Bagsværd, Denmark)	50 to 70	5 to 7	[13]

The enzyme assay was conducted in test tubes containing 50 mg Whatman Grade 1 filter paper (6 x 1 cm strips) and 1 ml Britton-Robinson (BR) pH buffer solution [14] instead of a citrate buffer. The BR buffer was used because of its wide range of pH adjustability (pH of 2 up to pH of 11). The reaction mixtures were pre-incubated for 5 min in a water bath at a specified temperature. After the pre-incubation, 0.5 ml of the 5 mg/ml enzyme dilution (diluted in the BR buffer) was added to the test tube, and the mixture was left to react for 10 min at the respective temperature. An enzyme blank (the 0.5 ml enzyme was replaced with 0.5 ml BR solution) and a substrate control (no filter paper) were also incubated for 10 min. Unlike the assay described by Chu *et al.* [11], the enzymatic reaction was not stopped by adding 3,5-dinitrosalicylic acid (DNS) but by placing the test tubes in boiling water for 10 min. This was done due to the unavailability of DNS.

The reaction mixtures were then decanted into 1.5 ml Eppendorf tubes and centrifuged using an Eppendorf 5804R centrifuge (Eppendorf AG, Germany) at 4°C and 10 000 rpm for 5 min.

The supernatant was pipetted into clean Eppendorf tubes and stored at -18°C until a high-performance liquid chromatography (HPLC) analysis could be conducted.

The effect of temperature on enzyme performance was first tested in triplicate at a pH of 6 for different temperatures ranging from 45°C to 65°C, in 5°C increments. After the temperature that resulted in the best performance was identified, the effect of pH on enzyme performance was tested (in triplicate) at the optimum temperature at different pH values (3, 4, 5, 6, 7 and 8).

The HPLC analysis [15] was configured to identify the amount of cellobiose and glucose in the supernatants. A standard calibration curve was prepared for using analytical grade glucose and cellobiose (obtained from Sigma-Aldrich). The glucose and cellobiose content in the supernatants were determined by high-performance anion exchange chromatography (HPAEC) with pulsed amperometric detection (PAD). The separation of the sugars was done on a Dionex Ultimate 3000 system (Thermo Fisher Scientific) with a CarboPac® PA1 column (250 x 4 mm; Thermo Fisher Scientific) in combination with a CarboPac® PA1 guard column (50 x 4 mm; Thermo Fisher Scientific). Samples were kept at 5°C until injection (20 µl) and analysis was carried out at 30°C. Constituents were separated using HPLC-grade water as eluent at a flow rate of 1.0 ml/min for 30 min. NaOH (132 mM) was added post-column (1.0 ml/min) as a supporting electrolyte for the detector. The PAD settings were $E_1 = 200$ mV, $E_2 = -1000$ mV, $E_3 = 600$ mV and $E_4 = -100$ mV for 500, 10, 10 and 40 milliseconds, respectively at a sensitivity of 100 nC. The delay time was set at 360 ms at a guard cell potential of 0.0 mV. The detector output was analysed using Chromeleon® version 7.2 software (Thermo Fisher Scientific). The glucose and cellobiose were quantified by integrating the peak areas. There was no statistical evaluation required for the purposes of the high-level assay.

3.2.2 Enzyme treatments

The pulp was produced from a *Eucalyptus* wood species using a PHK-pulping process, followed by delignification using an oxygen stage. After delignification, the pulp was washed proprietary by the mill. The pulp was diluted to a 10% consistency with filtrate from the next stage (chlorine dioxide) at a pH of 6 and then treated with two commercial EGs obtained from different manufacturers (Table 3-1).

In the first experiment, the enzymatic treatments of the pulp were conducted on the equivalent of 50 g dry pulp in heavy-duty plastic bags. Treated samples were incubated in a water bath at 60°C for different periods of time. The treatments consisted of nine different enzyme dosages (0, 10, 30, 50, 100, 150, 300, 500 and 1000 mg/kg dry pulp), each incubated for 1, 2, 3, 4, 6 and 24 h. The enzymatic reactions were stopped by first adding 2 L of boiling

water to the treated pulps and then washing the pulps twice with 1 L of boiling water in a Büchner funnel (240 mm diameter) with filter paper (Munktell Grade 1288, obtained from Ahlstrom-Munksjö, Helsinki, Finland). The thickened pulp samples were then sealed in plastic bags and kept at 4°C until hand sheets were made.

Hand sheets were made according to the ISO 5269-2:2004 standard [16] by disintegrating the pulp in deionised water and sheets were formed using a Rapid-Köthen sheet former. Hand sheets were tested in-house for viscosity, alkali solubility and crystallinity and selected sheets were also tested for Kappa number.

3.2.3 Hand sheet analysis

3.2.3.1 Viscosity and alkali solubility

The viscosity was determined through a capillary viscometer according to the ISO 5351:2010 standard [17]. The pulp was dissolved in a CED solution and then passed through a capillary viscometer, while the efflux time was recorded. Similarly, the alkali solubility of the pulp was determined according to the ISO 692:1982 standard [18], where the pulp was dissolved in a standardised 10% or 18% NaOH solution, followed by iodometric titration to determine the amount of degraded cellulose and hemicellulose (S10) or only hemicellulose (S18).

3.2.3.2 Degree of crystallinity

The degree of crystallinity was determined through XRD. The pulp samples had an average moisture content of 5%, and approximately 2.5 g were fluffed and placed on a silicon disc to eliminate background noise. The samples were then loaded into the XRD instrument (PANalytical X'Pert Pro) onto a spinner stage and scanned from 4° to 110° (known as 2 Θ) using X-rays. The X-rays were generated with a cobalt X-ray tube, and the diffractogram produced using an X'Celerator detector. The diffractogram was then analysed through a peak finding method, and the degree of crystallinity was calculated as described by Rogovina *et al.* [19].

3.2.3.3 Kappa number determination

The Kappa number of the pulp was determined by the Sappi Technology Centre (Pretoria, South Africa) through a procedure based on the French method (FDT-12-019) for Kappa number determination. In this method a pulp sample is subjected to a standardised potassium permanganate (KMnO₄) solution, where the pulp components (mainly lignin and hemicellulose) react with this standardised solution. Later a standardised potassium iodine (KI) solution is added to the reaction mixture and the iodine that was liberated from the reaction is titrated with a standardised sodium thiosulfate (Na₂S₂O₃) solution. The kappa number is then determined through calculations using the volume of sodium thiosulfate.

3.2.3.4 Reactivity

The reactivity of the pulp was determined by Sappi's Saiccor Mill (Umkomaas, South Africa) using a quick reactivity measurement developed in-house, similar to Fock's reactivity test. The pulp was dissolved in a 18% NaOH solution, followed by the addition of CS₂. The reaction of the cellulose fibres with the CS₂ allows the cellulose to dissolve in the NaOH solution (to produce the viscose dope), while the unreactive cellulose, the cellulose which did not xanthate well, remains undissolved. The dope was separated from the undissolved fibres through a centrifuge and then discarded, while the undissolved fibres were weighed and then inferred as a mass fraction of the total mass used for the test. The reactivity was indirectly proportional to the amount of undissolved fibres, i.e. the less undissolved fibres remaining after testing, the greater the reactivity of the pulp.

3.2.3.5 Brightness

Brightness sheets were made according to the ISO 3688:1999 standard [20] using the funnel procedure, without adjusting the pH as this was not required to determine the BWI. The funnel had a diameter of 150 mm, and Whatman (Grade 4) filter paper was used. The brightness sheets were conditioned at 32°C and a 12.5% relative humidity for 24 h in a conditioning room with a heating, ventilation, and air conditioning (HVAC) system. The whiteness was measured using a Technidyne Colour Touch 2 instrument with a D65 illuminant at a 10° angle for the observer. The BWI was then calculated from the red (X), green (Y) and blue (Z) wavebands.

3.2.4 Data analysis

3.2.4.1 Comparison of enzyme performance

For the first experiment, the results were subjected to a two-way Analysis of Variance (ANOVA) and the effects visualised using surface plots made with the MATLAB R2017b software with its curve fitting toolbox.

3.2.3.7 Second experiment and data analysis

A second experiment was then conducted based on the results of the first experiment. One enzyme (NS 51179) was selected and used to dose a smaller amount of dry pulp (30 g) and each treatment was replicated three times. The enzymatic treatments for this experiment consisted of enzyme dosages in increments of 50 mg/kg dry pulp from 0 to 300 mg/kg dry pulp and incubated for 1 h or 2 h. The enzyme reactions were stopped as described previously. Hand sheets were made and tested as before, except for the Kappa number as this was not of further interest. The data were subjected to a two-way ANOVA and means of the different treatments were tested for significant differences with Tukey's test at a 95% level of confidence.

3.2.3.8 Third experiment and data analysis

To compare the effectiveness of property alteration of the two enzyme formulations, a third experiment was conducted (with the Ecopulp R enzyme) similarly to the second experiment, but with 50 g instead of 30 g equivalent dry pulp. The same pulp as before was obtained from the mill, but it had a pH of 4 and, therefore, the pH was adjusted using a 10% NaOH solution to a pH of 6. The data were tested for significant differences, as described previously. Due to practical considerations and positive indications from the statistical analysis of the two replicated experiments, only selected samples were sent for reactivity testing.

3.3 Results and discussion

3.3.1 Enzyme characterisation: effect of temperature

The optimum temperature for the two commercial enzymes was determined and the effect of temperature on the activity of the two commercial enzymes are given in Figure 3-1 below.

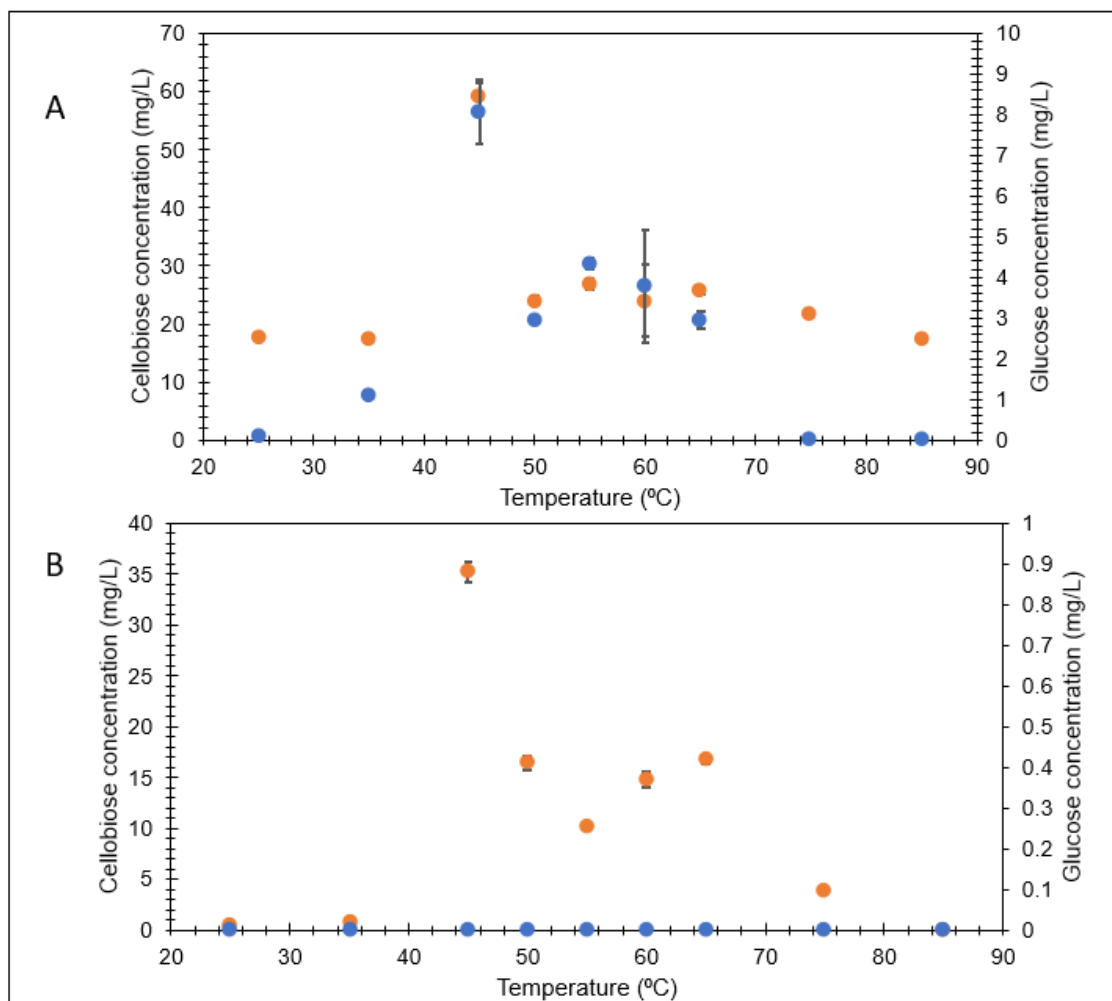


Figure 3-1: Effect of temperature for endoglucanase and β -glucosidase activities (represented as cellobiose [orange] and glucose [blue] concentrations, respectively in the Ecopulp R formulation [A] and NS 51179 formulation [B])

As expected for EG activity, treatment of the filter paper with the two commercial enzymes mainly resulted in the release of cellobiose, which is the main product of EG activity [7]. Both formulations showed a peak cellobiose concentration at 45°C (Figure 3-1). However, the concentration of cellobiose was higher when the Ecopulp R (59.1 mg/L cellobiose) was used, compared to the NS 51179 formulation (35.2 mg/L cellobiose). The Ecopulp R also resulted in the release of higher cellobiose concentrations at all the other tested temperatures compared to NS 51179. Zhang and Zhang [8] observed that some formulations of EG did not

have any significant effect on the pulp properties and this is directly related to the enzyme activities. Glucose was also present in the supernatant of the Ecopulp R, but not in the NS 51179. The presence of glucose indicated the presence of β -G activity in the formulation [8]. The Ecopulp R enzyme displayed a much lower activity when the temperature was increased to 50°C. Smaller decreases in activity were observed for higher temperatures. In contrast to the Ecopulp R, the NS 51179 had a noticeably smaller decrease in activity when the temperature increased from 45°C to 50°C. Similar to the Ecopulp R, the activity did not change much at temperatures beyond 50°C. As the enzyme deactivated beyond 50°C and achieved its highest cellobiose concentration at 45°C, it indicates that the optimum temperature was also 45°C.

3.3.2 Enzyme characterisation: effect of pH

After the optimum temperature was determined, a pH-profile for the enzyme was determined and is given in Figure 3-2 below.

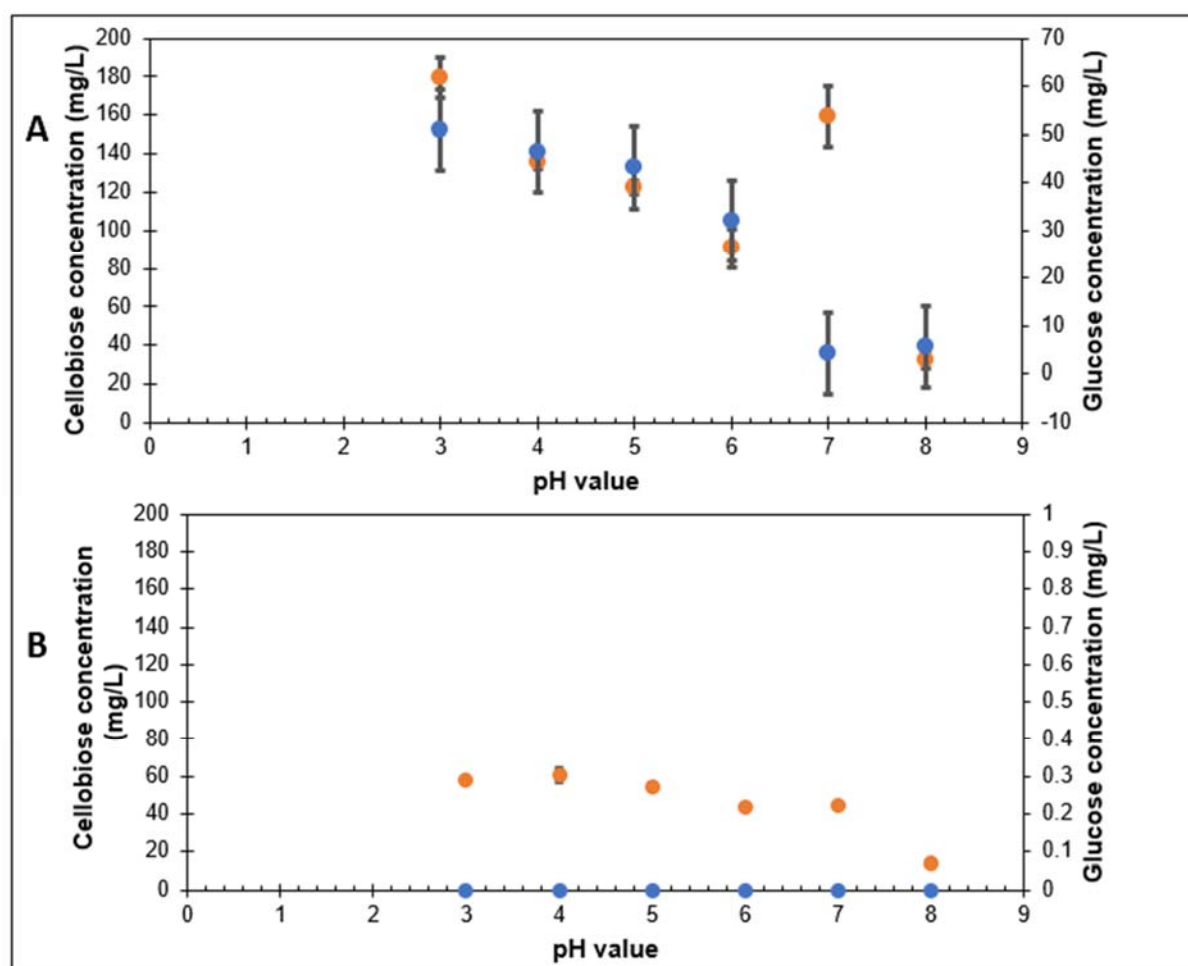


Figure 3-2: pH-profiles for endoglucanase and β -glucosidase activities (represented as cellobiose (orange) and glucose (blue) concentrations respectively in the Ecopulp R

formulation **[A]** and NS 51179 formulation **[B]**; each data point is the average of the three replicates, with the standard error calculated from the standard deviation between the data points)

The pH-profile for the Ecopulp R formulation displayed two optimum peaks for EG activity (as indicated by cellobiose concentration) at a pH of 3 (179.7 mg/L) and a pH of 7 (159.2 mg/L) as compared to the concentrations at the other pH values (Figure 3-2). This could be an indication of the presence of iso-enzymes, i.e. enzymes that catalyse the same chemical reaction but with a different physical structure. It can also indicate partial deactivation of the enzyme where the CBM is destroyed, decreasing the activity until it is fully destroyed, and activity can resume through the CD without the CBM. In another study by Vanessa et al. [21], the presence of isoenzymes was observed for endoglucanase. The presence of glucose was again detected in the Ecopulp R, with only one peak at a pH of 3 (50.9 mg/L), indicating β -G activity.

In contrast to the pH-profile of the Ecopulp R enzyme, the pH-profile of the NS 51179 enzyme displayed optimum EG activity at a single pH value (a pH of 4) as indicated by cellobiose concentration of 60.7 mg/L, with no glucose present. The EG activity of the formulation between a pH of 3 to a pH of 6 remained similar, with only a relatively large drop in activity from a pH of 7 to a pH of 8. Overall, the Ecopulp R displayed higher EG activity at all the tested pH values than the NS 51179, as indicated by the higher cellobiose concentrations.

As the enzyme will be applied at the mill, the operating temperature of the mill should be considered, which is at 60°C and is higher than the optimum temperature determined for both enzymes. Therefore, the enzyme would experience some loss of activity and this loss was calculated to be approximately 59% and 35% of activity for the Ecopulp R and NS 51179 formulations, respectively. Furthermore, the mill operates at a pH of 6, which is higher than the optimum determined for the enzymes and this should be considered as activity losses will occur. The activity losses at a pH of 6 was calculated to be approximately 50% and 10% for Ecopulp R and NS 51179, respectively. Activity losses should be avoided because more enzymes must then be used to make up for the activity losses experienced or alternatively the incubation time should increase, provided that the thermostability of the enzymes remains constant.

3.4 Enzymatic treatments

During the first set of experiments, pulps were treated with the two commercial enzymes, and the effect of the enzymes on pulp properties was evaluated. A two-way ANOVA was conducted on these results to confirm any significance.

3.4.1 Effect on viscosity

The effects of enzyme dosage and incubation time on the pulp viscosity was visualised through a surface plot and is given in Figure 3-3 below. The data had a mean (μ) of 652 ml/g and a standard deviation (σ)=93 ml/g.

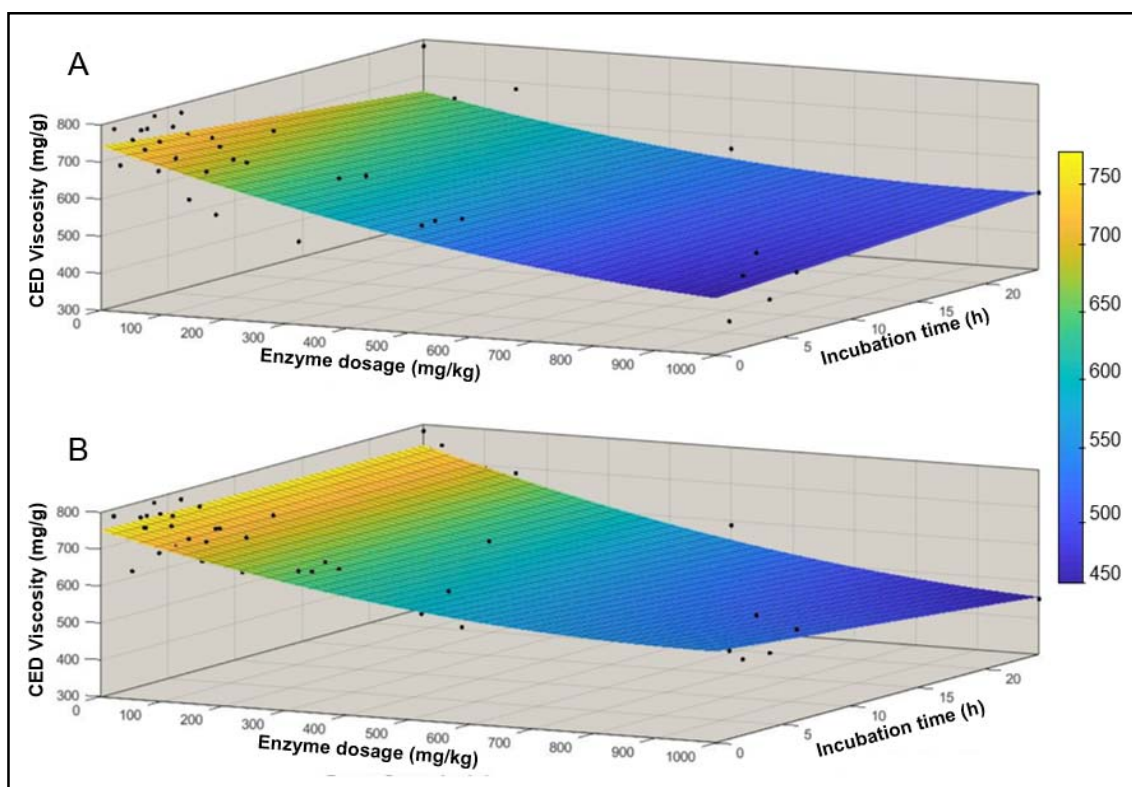


Figure 3-3: Effect of enzyme treatment on the viscosity of pulp after enzymatic treatment (with the Ecopulp R formulation [A] or NS 51179 formulation [B] at different dosages and different incubation times)

Both enzyme formulations reduced the viscosity of the pulp and Ecopulp R had a larger effect on the viscosity of the pulp than NS 51179. The longer the incubation time for an enzyme dosage, the greater the reduction in viscosity that was observed for the Ecopulp R, but for the NS 51179 this phenomenon was only observed at high enzyme dosages. One possible explanation for the difference in viscosity reduction for the two treated pulps was the difference in the activity of the two enzymes used, with the Ecopulp R enzyme having a higher activity than the NS 51179 enzyme, meaning more glycosidic linkages in the cellulose chains were

cut by the enzyme with, consequently, a greater reduction in viscosity. The significance for the main factors (enzyme dosage and incubation time) were tested through a two-way ANOVA without replication and the results are given in Table 3-2 below.

Table 3-2: The p-values for viscosity obtained from a two-way ANOVA without replication for the two enzymes

Formulation	Factor	p-value
Ecopulp R	Enzyme dosage	<0.001
	Incubation time	0.148
NS 51179	Enzyme dosage	<0.001
	Incubation time	0.880

There was a significant effect on the pulp viscosity due to the increase in enzyme dosage; however, there was no significant effect on the pulp viscosity due to the incubation time which could also be seen in the surface plot where the slope in the time axis was fairly low. The reason that incubation time did not affect the pulp viscosity may be due to the impurities present within the pulp such as the residual hemicellulose and lignin. Lignin consists mainly of phenolic components and these are known to cause inhibition towards cellulase [22].

3.4.2 Effect on alkali solubility (S10 value)

The effect the enzymatic treatments had on the alkali solubility, specifically the S10 value of the pulp is visualised in a surface plot in Figure 3-4 below. The data had a mean (μ) of 5.94 % and a standard deviation (σ)=0.98 %

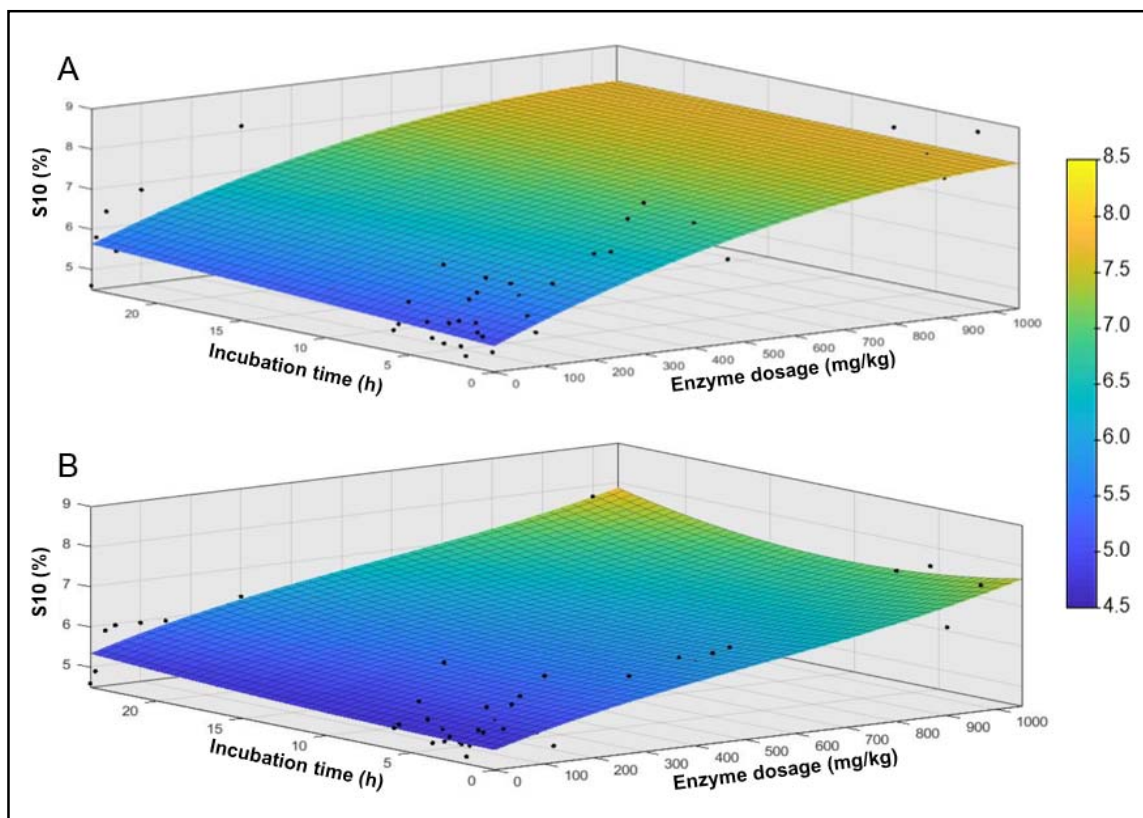


Figure 3-4: Surface plots of the S10 values of the pulp after enzymatic treatment (with the Ecopulp R formulation [A] or NS 51179 formulation [B] at different dosages and different incubation times)

The alkali solubility, specifically the S10 values, increased for both enzyme formulations but the increase was higher for the Ecopulp R than for NS 51179. The incubation time did not appear to influence the S10 values. The reason for the increase in S10 values is due to the random cleavage of the glycosidic linkages in the cellulose chains. The random cutting of the chains produces both long and short cellulose chains and the short cellulose chains are dissolved by the 10% NaOH solution as seen in the surface plots above. The higher increase of the S10 values for the Ecopulp R-treated pulp can again be ascribed to the higher activity of the Ecopulp R formulation. It should be noted that as the viscosity decreased there was an increase in the S10 value, which can be explained by the random cutting of the cellulose chains. Viscosity is an indication of the DP and as the viscosity decreased, the DP also decreased. It can therefore be said that the random cutting effect can produce short cellulose chains (lower DP) which is dissolvable by a 10% NaOH solution. The significance for the main factors was tested through a two-way ANOVA without replication and the results are given in

Table 3-3: The p-values for S10 values obtained from a two-way ANOVA without replication for the two enzymes

Formulation	Factor	p-value
Ecopulp R	Enzyme dosage	<0.001
	Incubation time	0.908
NS 51179	Enzyme dosage	<0.001
	Incubation time	0.144

Similar to the viscosity results, significant effects from the enzyme dosage were only seen for the S10 values which can again be observed in the surface plot where the slope in the time axis was low. As incubation time had no effect on the pulp viscosity it correspondingly also did not influence the S10 values of the pulp.

3.4.3 Effect on crystallinity

The effect of the enzymatic treatment on the crystallinity of the pulp was visualised through a surface plot and is given in Figure 3-5 below. The data had a mean (μ) of 80.3 % and a standard deviation (σ)=0.7 %

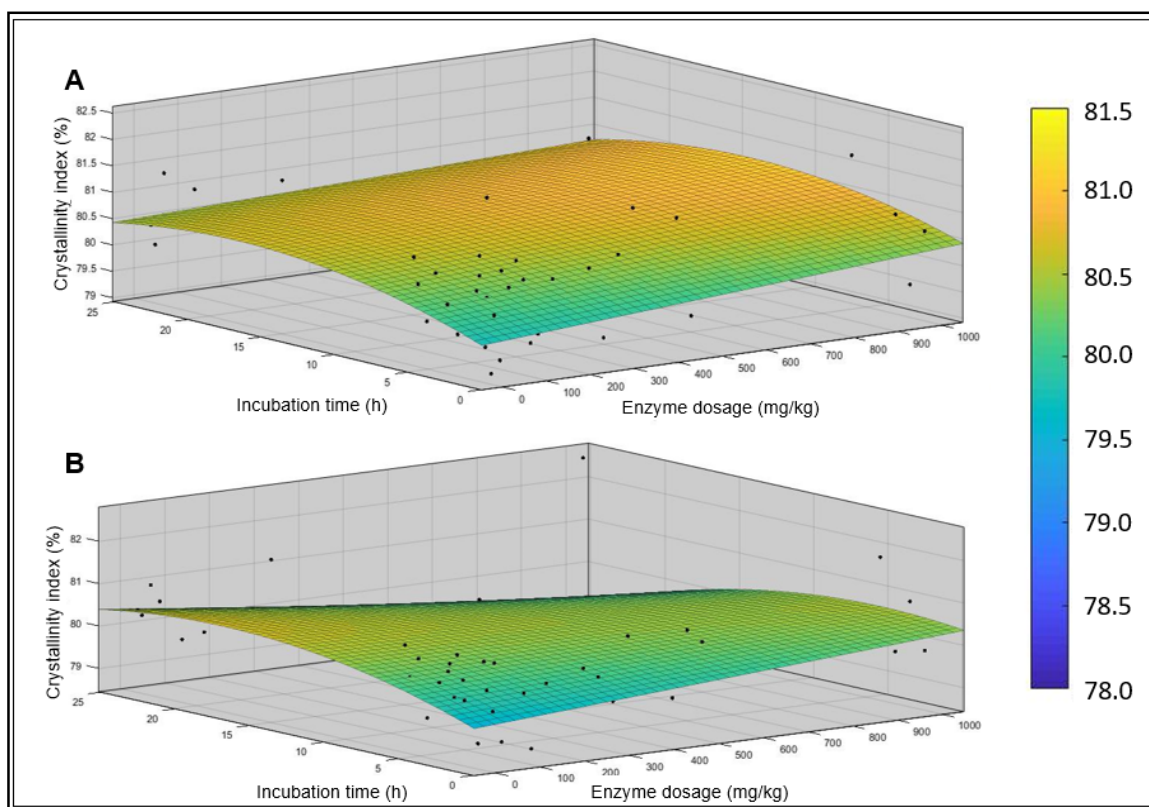


Figure 3-5: Effect of enzyme dosage on the crystallinity of the pulp after enzymatic treatment (with the Ecopulp R formulation [A] or NS 51179 formulation [B] at different dosages and different incubation times)

The degree of crystallinity increased with enzyme dosage but decreased with longer incubation times. This increase and decrease seen in the surface response plot may point to an interaction between the enzyme dosage and the incubation time of the treatments. The crystallinity of both the pulps appeared to be, on average, the same, with the crystallinity of A ranging from 79% to 81.5%, while for B the crystallinity ranged from 78% to 81.8%. The effect of crystallinity on cellulose reactivity is not agreed upon in literature. For example, Helmy [23] and Yamane *et al.* [24] concluded that a higher crystallinity led to a higher reactivity because the amorphous regions protected the crystalline regions of the cellulose which contained a higher density of hydroxyl groups. A higher density of hydroxyl groups meant a higher reactivity. The significance for the main factors were tested through a two-way ANOVA without replication and the results are given in Table 3-4 below.

Table 3-4: The p-values for the crystallinity obtained from a two-way ANOVA without replication for the two enzymes

Formulation	Factor	p-value
Ecopulp R	Enzyme dosage	0.60
	Incubation time	0.22
NS 51179	Enzyme dosage	0.24
	Incubation time	0.38

There was no significant change in the crystallinity due to either the enzyme dosage or the incubation time. Unfortunately, the interaction between the two main factors could not be determined without replicated results (see section 3.4.7). The reason for this is linked to literature which states that EG preferentially attacks the amorphous regions rather than the crystalline regions, which effectively increases the crystallinity, but thermal degradation of the crystalline regions of cellulose chains again decreases the crystallinity [25].

3.4.4 Viscosity reduction versus alkali solubility (S10 values)

One of the main desired properties of a good quality, fully bleached DWP is to have a low alkali solubility of approximately 10% for S10 and 5% for S18, with an approximate viscosity of 500 ml/g [3 p.1062]. To achieve these properties, a large change in viscosity (50 ml/g to 100 ml/g) with a small change in alkali solubility (S10) should be targeted with the enzymatic treatments. The change in viscosity versus the S10 values is given in Figure 3-6 below.

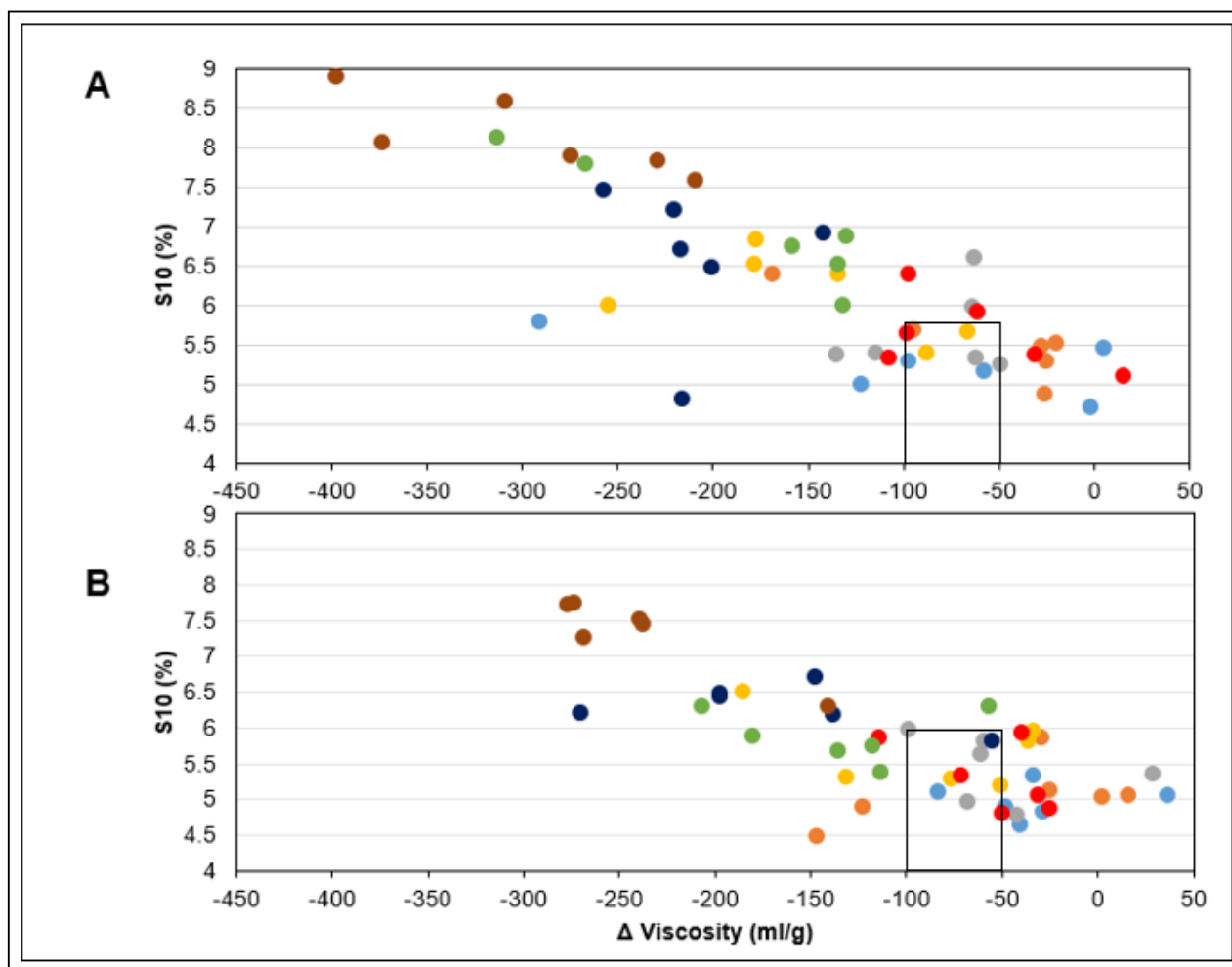


Figure 3-6: Scatter plots showing the relationship between the change in viscosity and the corresponding alkali solubility (S10) for the Ecopulp R formulation (A) and the NS 51179 formulation (B) at different enzyme dosages (the light blue dots represent the 10 mg/kg dosages, while the dark orange dots represent the 30 mg/kg dosages; the grey dots represent the 50 mg/kg dosage and the yellow dots represent the 100 mg/kg dosages; the red and green dots represent the 150 mg/kg and 300 mg/kg dosages respectively; the dark blue dots represent the 500 mg/kg dosage and the brown dots represent the 1000 mg/kg dosages; the black outlined square indicates the viscosity reduction range and S10 value range targeted)

The plot shows, firstly, that there exists a linear relationship between the reduction in viscosity and the increase in S10 values. Short cellulose chains are also produced due to the random cleavage of the cellulose, resulting in the reduction in viscosity. The short chains are dissolvable by the 10% NaOH solution and therefore an increase in the S10 value is observed.

The plot shows, secondly, that the desired viscosity change with little alkali solubility increase could be achieved with enzyme dosages of up to 150 mg/kg. Replicated experiments were further used to statistically prove the significances of the desired change in viscosity and increase in alkali solubility (S10) (see following sections).

3.4.5 Effect on Kappa number

The Kappa number of selected samples were determined by the Sappi Technology Centre (Pretoria, South Africa) through the modified French method for Kappa number determination (see section 3.2.2). The samples selected for Kappa number determination included high enzyme dosages and long incubation times and was determined to be 2.9 for all selected samples. Because the Kappa number did not change, it was not tested during the second and third sets of experiments.

3.4.6 Effect on Berger whiteness index

The effect of enzymatic treatment on the BWI is given in Figure 3-7 below. The data had a mean (μ) of 19.7 % and a standard deviation (σ)=0.8 %

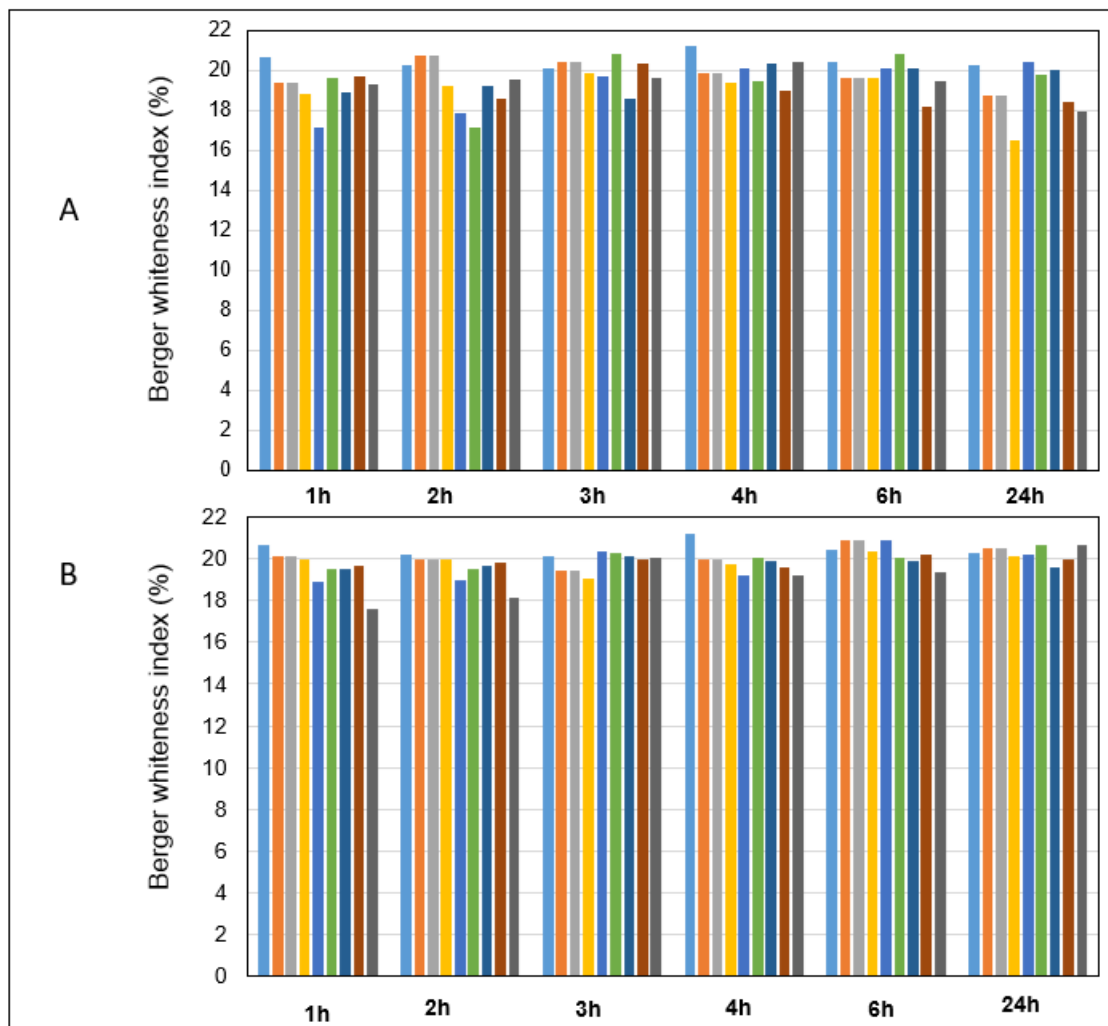


Figure 3-7: The Berger whiteness index for each pulp after enzymatic treatment for Ecopulp R (A) and NS 51179 (B) (the light blue bars represent the 0 mg/kg dosages and the orange bars represent the 1 mg/kg dosages; the grey and yellow bars represent the 30 mg/kg

and 50 mg/kg dosages respectively, while the dark blue and green bars represent the 100 mg/kg and 150 mg/kg dosages, respectively; the purple bars represent the 300 mg/kg dosage, while the brown bars represent the 500 mg/kg dosage; the dark grey bars represent the 1000 mg/kg dosage)

On average the enzymatic treatments did alter the BWI and the reason for this is unclear since the enzymes do not act on the colour robbing components (hemicellulose and lignin). Theoretically, the BWI should have remained unchanged. As the pulp was not fully bleached yet, the BWI was very low (in the 20% range). However, at this stage of the bleaching sequence, the BWI was not of much concern as there were multiple opportunities to address the BWI before the pulp was fully bleached. The fully bleached pulp should reach a BWI greater than 78% or greater than 90% ISO brightness [3 p.1062].

Upon further inspection of the BWI data, the enzymatic treatments did reduce the BWI of the pulp and the change in BWI is given in Figure 3-8 below.

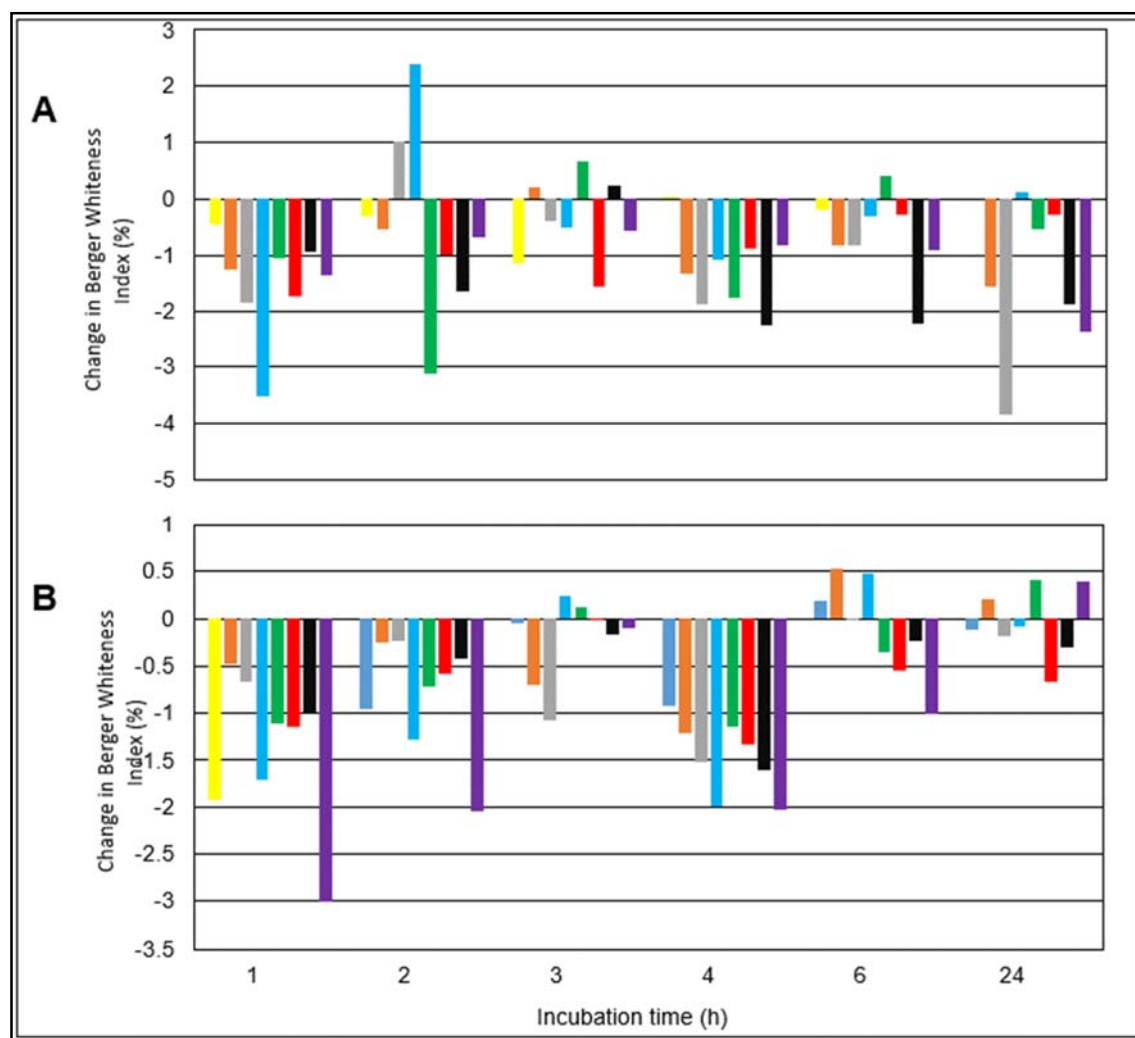


Figure 3-8: Change in the Berger whiteness index of the pulp after enzymatic treatment with the Ecopulp R formulation (A) or the NS 51179 formulation (B) at different dosages and for different incubation times (the change in the BWI was calculated relative to the control incubated for the same amount of time; the yellow bars represent the 10 mg/kg dosages, while the orange bars represent the 30 mg/kg; the grey and light blue bars represent the 50 mg/kg and 100 mg/kg dosages, respectively; the green bars represent the 150 mg/kg dosages, while the red bars represent the 300 mg/kg dosages; the black and purple bars represent the 500 mg/kg and 1000 mg/kg dosages, respectively)

Some of the treatments appeared to have increased the BWI but there was no clear reason for these increases or decreases. In some treatments the Ecopulp R formulation reduced the BWI more than the NS 51179 formulation, and at longer incubation times (6 h and 24 h) the NS 51179 did not reduce the BWI as much as during shorter incubation times. However, as stated previously, the BWI was not a major concern at this point as there were still several opportunities to address this before the pulp was fully bleached. The significance for the main factors were tested through a two-way ANOVA without replication and the results are given in Table 3-5 below.

Table 3-5: The p-values for the Berger whiteness index obtained from a two-way ANOVA without replication for the two enzymes

Formulation	Factor	p-value
Ecopulp R	Enzyme dosage	0.082
	Incubation time	0.121
NS 51179	Enzyme dosage	0.019
	Incubation time	0.003

The treatment of the pulp with the Ecopulp R formulation did not have a significant effect on the BWI of the pulp, while the treatment of the pulp with the NS 51179 enzyme did. For the NS 51179-treated pulp both enzyme dosage and incubation time influenced the BWI of the pulp but the reason for this is not yet clear. One possible explanation for this could be that as the enzyme cut the cellulose chains, some hemicellulose or lignin was liberated from the cellulose matrix and was washed out during the enzyme deactivation after the pulp treatments. In a study by Ladeira Azar [26], they found that some lignin compounds could be liberated from cellulose-hemicellulose-lignin matrix, though their study focussed more on heat treatment of lignocellulose material than enzymatic treatment. In another study by Baig [27], it was found that cellulase could indeed liberate some lignin compounds from the polymer matrix.

3.4.7 Second experiment: NS 51179 replicated set

The p-values for the enzyme dosage factor, incubation time factor, and interaction between these two factors are given in

Table 3-6 below.

Table 3-6: The p-values from the two-way ANOVA for the replicated set of experiments for NS 51179 (the p-values are given for the enzyme dosage factor, incubation time factor and the interaction between these factors; the confidence level $[\alpha]$ was chosen as 0.05)

Property	Factor	p-value
Viscosity	Enzyme dosage	0.30
	Incubation time	0.28
	Interaction	0.41
S10	Enzyme dosage	0.35
	Incubation time	0.06
	Interaction	1.00
S18	Enzyme dosage	0.54
	Incubation time	0.72
	Interaction	0.17
Degraded cellulose	Enzyme dosage	0.52
	Incubation time	0.11
	Interaction	0.80
Berger whiteness index	Enzyme dosage	0.93
	Incubation time	0.40
	Interaction	0.95
Crystallinity	Enzyme dosage	0.56
	Incubation time	0.10
	Interaction	0.26

Based on the p-values, neither the enzyme dosage nor incubation time had an impact on the pulp viscosity, S10 values, S18 values, degraded cellulose, BWI or crystallinity. There was likewise also no significant interaction between these factors for each of the pulp properties.

The average values for each pulp property are given in Table 3-7 below. Each average was calculated from the three replicates for the given enzyme dosage and incubation time with the error calculated from the standard deviation for the average.

Table 3-7: Properties of the dissolving wood pulp after enzymatic treatment with the NS 51179 formulation

Enzyme dosage (mg/kg)	Incubation time(h)	Viscosity (ml/g)	S10 (%)	S18 (%)	Degraded cellulose (%)	Berger whiteness index (%)	Crystallinity (%)
0	1	709±18	5.5±0.1	3.4±0.2	2.1±0.1	21.1±0.8	73.0±0.3
	2	709±23	5.3±0.2	3.5±0.1	1.9±0.3	20.0±1.3	72.6±0.6
50	1	718±28	5.6±0.2	3.3±0.1	2.3±0.1	2.1±0.4	71.3±1.4
	2	747±35	5.5±0.4	3.5±0.1	2.0±0.4	21.2±0.4	72.0±0.6
100	1	706±21	5.5±0.2	3.4±0.2	2.1±0.2	21.0±1.2	73.0±0.6
	2	736±23	5.4±0.2	3.4±0.2	2.0±0.3	20.8±0.4	71.2±1.3
150	1	731±17	5.7±0.1	3.3±0.1	2.4±0.1	20.7±0.7	73.4±0.6
	2	705±36	5.5±0.3	3.5±0.1	2.1±0.2	20.1±1.8	71.1±0.6
200	1	707±20	5.72±0.3	3.3±0.1	2.3±0.4	20.3±1.0	73.1±1.4
	2	733±25	5.4±0.1	3.3±0.1	2.1±0.2	20.3±1.4	72.1±1.0
250	1	684±4	5.8±0.2	3.6±0.0	2.2±0.2	20.5±0.6	71.5±0.8
	2	711±17	5.6±0.3	3.4±0.1	2.2±0.4	20.6±1.4	71.9±1.5
300	1	706±20	5.8±0.4	3.6±0.1	2.3±0.3	20.8±0.4	72.5±0.9
	2	688±20	5.7±0.1	3.3±0.1	2.4±0.1	20.0±1.3	71.9±1.1

The viscosity of the pulp was not reduced statistically and the reason, therefore, may be attributed to the initial viscosity being low. Furthermore, since the viscosity was not altered, the S10 and S18 values also did not change significantly, and with the degraded cellulose being dependant on the S10 and S18 values, it did not change either. The BWI did not change significantly and this is a positive result because it implies that the need for higher chemical charges further down the bleaching sequences would not be required to brighten the pulp to the desired level. The crystallinity remained unchanged and, as stated previously, may be attributed to thermal degradation due to prolonged exposure to the heat [25].

3.4.8 Third experiment: Ecopulp R replicated set

The p-values for the enzyme dosage factor, incubation time factor and interaction between these two factors are given in Table 3-8 below.

Table 3-8: The p-values from the two-way ANOVA for the replicated set of experiments for Ecopulp R (the p-values are given for the enzyme dosage factor, incubation time factor and the interaction between these factors; the confidence level [α] was chosen as 0.05)

Property	Factor	p-value
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Viscosity	Enzyme dosage	<0.001
	Incubation time	<0.001
	Interaction	0.051
S10	Enzyme dosage	0.353
	Incubation time	0.063
	Interaction	0.996
S18	Enzyme dosage	0.070
	Incubation time	0.116
	Interaction	0.189
Degraded cellulose	Enzyme dosage	<0.001
	Incubation time	0.305
	Interaction	0.055
Berger whiteness index	Enzyme dosage	0.843
	Incubation time	0.005
	Interaction	0.350
Crystallinity	Enzyme dosage	0.727
	Incubation time	0.964
	Interaction	0.047

Based on the p-values, there was a significant difference between the viscosities for both the enzyme dosage factor and the incubation time factor; however, there was no significant interaction between the two factors. Conversely, there was no significant change between the S10 and S18 values for either the enzyme dosage factor or incubation time factor and this is a positive result as the viscosity needs to be reduced without increasing the S10 value too much. There was, however, a significant change in the degraded cellulose for the enzyme dosage factor despite there being no statistical change in the S10 or S18 values; this should be considered when moving forward to the pilot phase. There was only a significant change in the BWI for the incubation time factor and again the reason for this is unclear, however, a possible explanation for this might be the formation of colour robbing components due to the longer incubation time. There was no significant change in the crystallinity due to either the enzyme dosage factor or incubation time factor; there was, however, a significant interaction between these two factors. The interaction supports the statement made earlier regarding crystallinity, where although the enzyme increases crystallinity by preferentially attacking the amorphous parts of the cellulose, the exposure to heat can cause thermal degradation, reducing the crystallinity again. It should be noted that Zhang and Zhang [8] made some similar observation where different formulations of EG did not have any significant effect on the pulp properties, as in the case with the NS 51179 formation and the Ecopulp R formulation in this study.

The average values for each pulp property are given in Table 3-9 below. Each average was calculated from the three replicates for the given enzyme dosage and incubation time with the error calculated from the standard deviation for the average.

Table 3-9: Properties of the dissolving wood pulp after enzymatic treatment with the Ecopulp R formulation

Enzyme dosage (mg/kg)	Incubation time(h)	Viscosity (ml/g)	S10 (%)	S18 (%)	Degraded cellulose (%)	Berger whiteness index (%)	Crystallinity (%)
0	1	765±14	5.5±0.1	3.3±0.2	2.0±0.2	13.5±0.4	74.3±0.2
	2	761±6	5.3±0.2	3.5±0.1	1.9±0.3	12.8±0.7	76.1±0.6
50	1	758±6	5.6±0.2	3.5±0.1	1.7±0.1	13.5±0.4	75.0±1.3
	2	763±15	5.5±0.4	3.3±0.2	2.4±0.1	12.6±0.7	76.6±0.8
100	1	760±10	5.5±0.2	3.4±0.2	1.9±0.2	13.8±0.2	76.9±0.4
	2	755±18	5.4±0.2	3.6±0.1	1.8±0.1	12.6±0.5	75.7±0.6
150	1	755±2	5.7±0.1	3.3±0.3	2.2±0.1	13.1±0.2	78.0±2.9
	2	717±9	5.5±0.3	3.4±0.2	2.3±0.1	13.2±0.7	75.2±1.0
200	1	746±16	5.7±0.3	3.3±0.1	2.1±0.2	13.6±0.2	75.7±0.5
	2	726±26	5.4±0.1	3.4±0.1	2.1±0.1	13.4±0.8	77.0±1.1
250	1	734±5	5.8±0.2	3.3±0.0	2.3±0.1	14.0±0.2	75.7±0.8
	2	681±7	5.6±0.3	3.6±0.1	2.2±0.3	12.6±0.5	75.9±0.5
300	1	733±12	5.8±0.4	3.1±0.1	2.6±0.2	13.0±0.3	76.5±0.5
	2	709±17	5.7±0.1	3.4±0.3	2.6±0.2	12.9±0.8	75.7±0.8

In contrast to NS 51179 replicated experiment, there was a significant difference between the enzyme dosages and incubation time for the viscosity of the pulp and the reason for this may be the higher initial viscosity of the pulp compared to the previous set of experiments. This suggests that the enzyme is sensitive to the initial viscosity, and, therefore, the lower the initial viscosity the lower the reduction in viscosity. In a study by Ballesteros [28], it was observed that hydrolysis (and consequent viscosity reduced) is influenced by the initial viscosity; however, it should be noted that their study focused on cellulose hydrolysis for ethanol production. The greatest viscosity drop was observed for the 250 mg/kg dosage and 2 h incubation time compared to the control incubated for both 1 h and 2 h. This is quite unexpected as, theoretically, based on the first set of experiments the greatest drop should have been seen for the 300 mg/kg dosage and 2h incubation time; however, this may suggest that at higher dosages the enzyme may experience some sort of inhibition, whether it be product or substrate inhibition.

The S10 values did not increase with an increase in the enzyme dosage or incubation time which is a positive result as the viscosity needs to be reduced without increasing the S10 value; however, the S10 value measured alone is not enough as the S10 value is an indication of degraded cellulose. Therefore, measuring the S18 value and then inferring the degraded cellulose from that would provide a true value for the amount of degraded cellulose, which is the value that does not need to be increased. The S18 values did not change either with the increase in enzyme dosage or incubation time.

In contrast, the degraded cellulose did increase with an increase in enzyme dosage but not with incubation time and this may be ascribed to the reduction in viscosity of the pulp. The

greatest increase in degraded cellulose was observed for the 300 mg/kg dosage for both a 1h and 2h incubation time compared to the control pulp incubated for the same time. It is reported by [2] that the semi-bleached pulp, treated with EG and then further bleached, showed an increase in the alkali solubility (S10 and S18) and consequently, the amount of degraded cellulose.

The BWI did not change with a change in the enzyme dosage; however, it did change with an increase in the incubation time and this suggests that the enzyme does not affect the BWI but rather the heat generated during the treatment. It may be possible that the prolonged exposure to the heat could allow some colour robbing components to form and these components would originate from either the lignin or hemicellulose constituents.

Lastly, the crystallinity remained unchanged despite an increase in enzyme dosage and incubation time and as stated previously this may be caused by thermal degradation due to the exposure to heat [25]. There was, however, a significant interaction between the enzyme dosage and incubation time for crystallinity; the interaction effects plot can be seen in Figure 3-9 below.

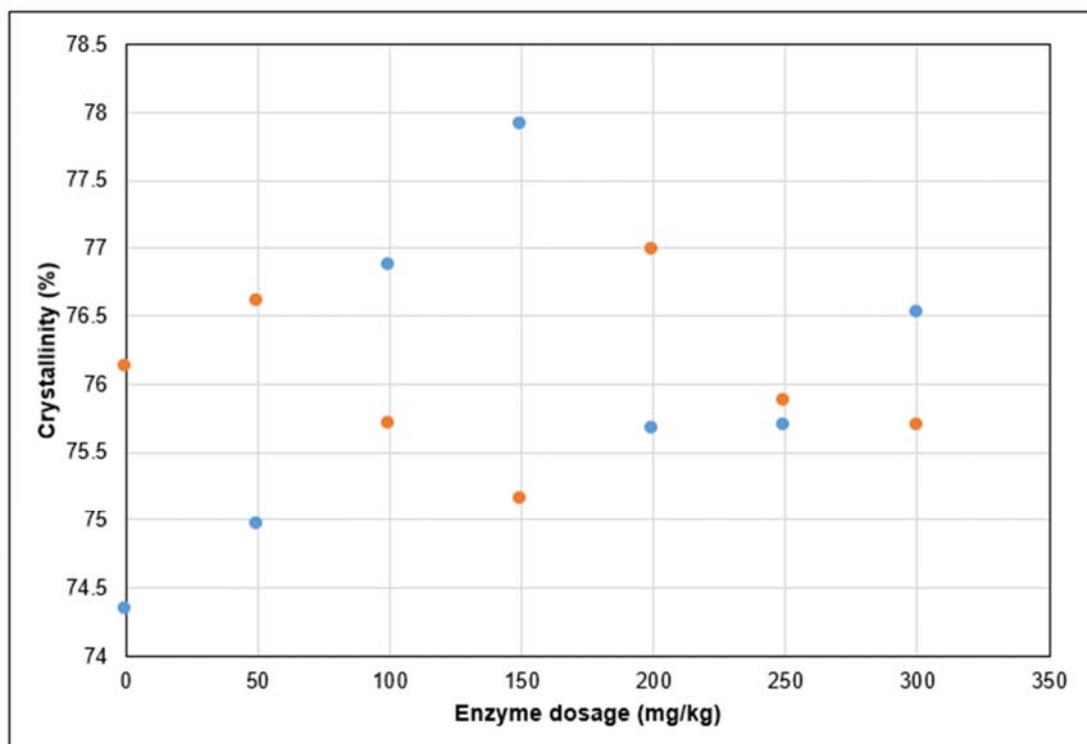


Figure 3-9: Interaction effects plot of the enzyme dosage and incubation time factors for crystallinity (each data point is the average of the three replicates at the given enzyme

dosage and incubation time; the orange dots represent the 2h incubation time, while the blue dots represent the 1h incubation time)

The results agree with the literature, where only significant increases in crystallinity were seen from 5% to 10% [9]. Overall, for the 1 h incubation time, the crystallinity increased, whereas for the 2 h incubation time the crystallinity decreased. A possible explanation for the interaction is that while the enzyme removes the amorphous part of the cellulose (effectively increasing the crystallinity), the heat would, after prolonged exposure, also cleave some of the glycosidic linkages. The cleavage of the glycosidic linkages due to thermal degradation, leads to a decrease in crystallinity [25]. In another study by Kruer-Zerhusen *et al.* [29], it was reported that when using Fourier-Transform Infrared (FTIR) analysis during cellulose digestion with cellulase there was no significant change in the crystallinity or hydrogen bond intensity, meaning that there was no increase in the intermolecular hydrogen bonds.

The enzyme dosages were also compared statistically, using Tukey's comparison test, and it showed that only the high enzyme dosages differed significantly from the control treatments. For viscosity, only the 250 mg/kg and 300 mg/kg enzyme dosages differed from the control treatments, while only the 300 mg/kg dosage seemed to have had a significant effect on the amount of degraded cellulose. Due to the results from Tukey's test and practical considerations concerning time, only selected samples from the third experiment (the Ecopulp R formulation) was sent for reactivity testing. The samples sent for reactivity testing were both the 1 h and 2 h incubation times for the control samples, and for the 100 mg/kg, 250 mg/kg and 300 mg/kg dosage samples. The p-values for the two-way ANOVA for the reactivity is given in Table 3-10 below.

Table 3-10: The p-values for the reactivity of the selected samples from the Ecopulp R set of replicated experiments

Property	Factor	p-value
Reactivity	Enzyme dosage	0.27
	Incubation time	0.57
	Interaction	0.22

For the reactivity, there was no significant difference between different enzyme dosages or the two incubation times. The reactivity was tested on semi-bleached pulp and it is important to note that the reactivity test was not optimised for semi-bleached pulp. Currently, it is unclear if any observed reactivity changes of the semi-bleached pulp would reflect in the final fully bleached pulp. The reactivity of the selected samples is given in Figure 3-10 below.

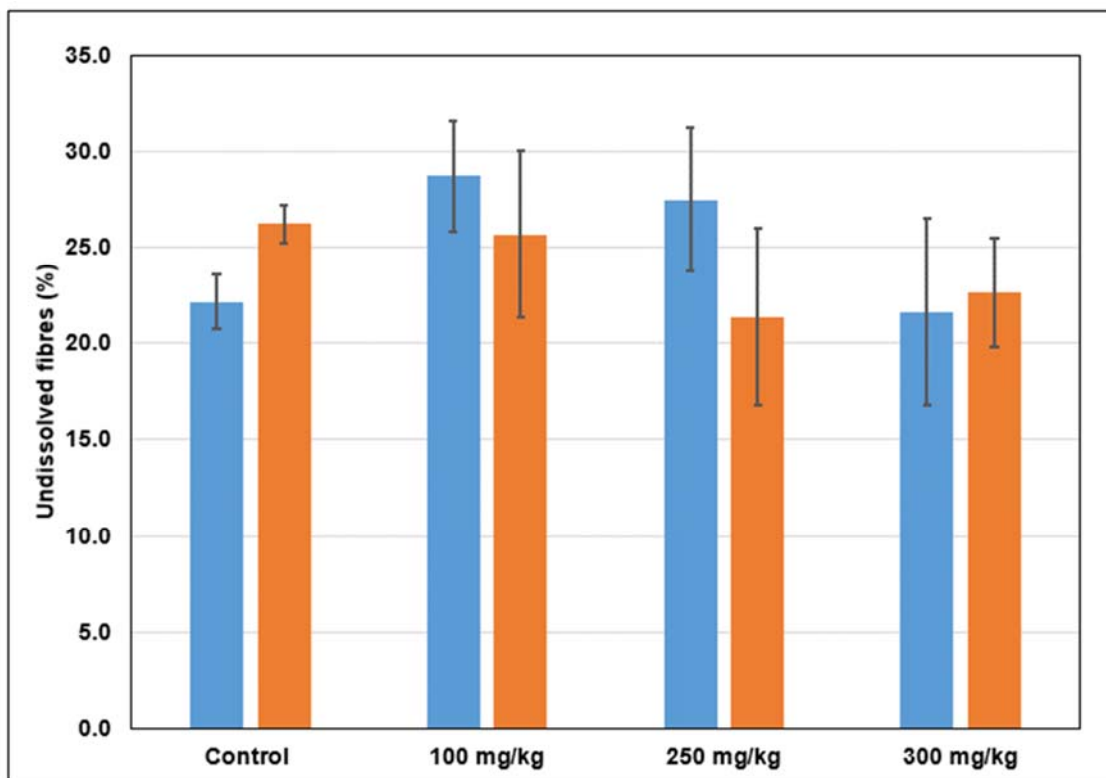


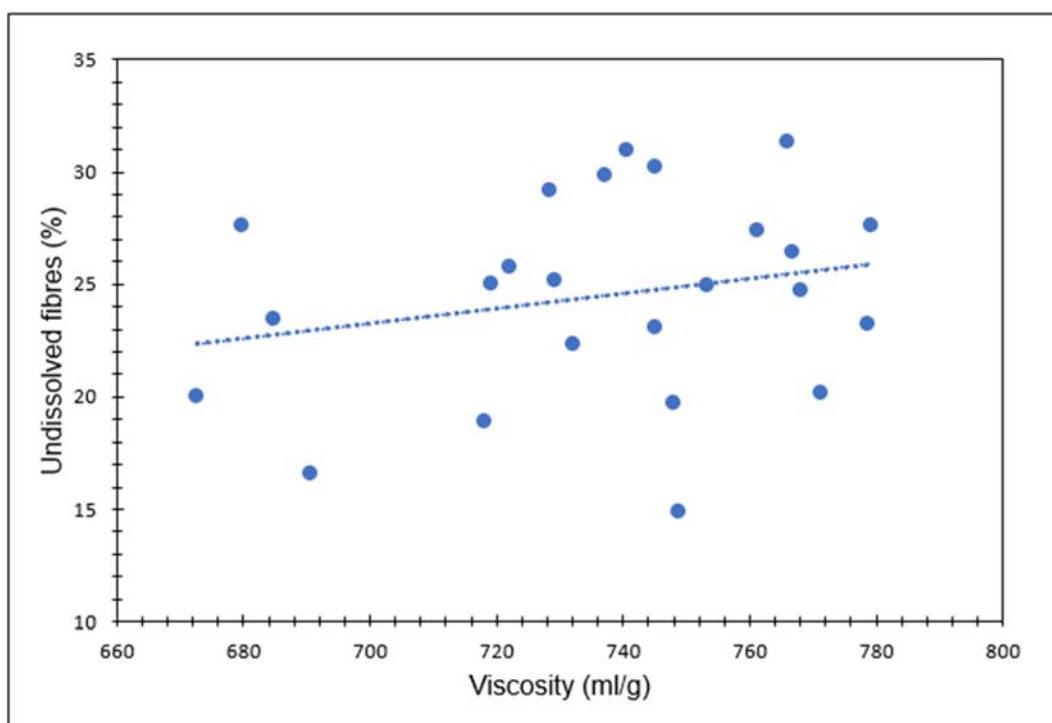
Figure 3-10: The reactivity of the selected samples of the third experimental set (the blue bars represent the 1h incubation time and the orange bars represent the 2h incubation time; the less undissolved fibres there are, the higher the reactivity; the error was calculated from the standard deviation of the three replicates for the given incubation time and enzyme dosage)

The amount of undissolved fibres for the control pulp was 22.1% and 26.2% for the 1h and 2h incubation times, respectively. However, there was no statistically significant difference (p -value > 0.05). Furthermore, it was suspected that the least amount of undissolved fibres would be observed for the highest enzyme dosage and longest incubation time; however, this was not the case and the least amount was observed for the 250 mg/kg dosage and 2 h incubation time. As mentioned earlier, the method was not optimised for semi-bleached pulp; however, perhaps this phenomenon could be explained by the lower viscosity of the 250 mg/kg dosage and 2 h incubation time (681 ml/g) compared to the 300 mg/kg dosage and 2 h incubation time (709 ml/g). To confirm this another two-way ANOVA was conducted to determine if any relationship exists between the independent variables of viscosity, S10 and S18 values, the amount of degraded cellulose, BWI, crystallinity, and reactivity. The results are given in Table 3-11 below.

Table 3-11: The p-values obtained from a two-way ANOVA to determine any relationship between the independent variables and the reactivity

Independent variable	p-value
Viscosity (ml/g)	<0.001
S10 (%)	0.334
S18 (%)	0.222
Degraded cellulose (%)	0.403
Berger whiteness index (%)	0.262
Crystallinity (%)	0.227

It would appear that the reactivity of semi-bleached pulp was only influenced by the viscosity of the pulp, whereas it is stated in literature that viscosity is not the only factor to influence the reactivity of pulp; however, this statement was made for fully bleached pulp [6]. The relationship between the drop in viscosity and reactivity is given in Figure 3-11 below.

**Figure 3-11: The relationship between the drop in viscosity and the increase in reactivity** (the drop in viscosity was calculated from the control pulp for the corresponding incubation time)

Although the correlation between the reactivity and viscosity was not a strong correlation, as can be seen in the graph above, the general idea that can be drawn from this was that as the viscosity decreased the reactivity seemed to have increased because less undissolved fibres remained.

3.5 Conclusions and recommendations

The Ecopulp R enzyme displayed higher EG activity than the NS 51179 enzyme at all tested temperatures and pH values. The presence of two optimum pH peaks for the EG activity of Ecopulp R indicated the possible presence of iso-enzymes or partial deactivation of the enzymes but would have to be confirmed during further testing. The operating temperature and pH of the mill differ from the optimum temperature and pH determined in the FPA experiments. It is therefore recommended that a complete assay at all temperature and pH values should be conducted to fully understand the limitations of each enzyme. The higher activity observed in the Ecopulp R could mean that a greater viscosity reduction would be seen and that fewer enzymes could be used compared to the NS 51179 enzyme.

While both enzyme formulations reduced the viscosity of the pulp with an increase in enzyme dosage, the Ecopulp R formulation had a more pronounced effect on the viscosity of the pulp than the NS 51179 formulation. Similarly, as the enzyme dosages increased, the alkali solubility (S10) of the pulp increased; however, the rate of the increase in alkali solubility was similar for both formulations. The crystallinity increased for both enzyme formulations, but the Ecopulp R did result in a higher crystallinity than the NS 51179. However, based on the results of the ANOVA, there were no significant changes in the crystallinity of the pulp. In general, the enzymatic treatments reduced the BWI of the pulp; however, the effect was more pronounced with the NS 51179 enzyme, based on the results of the ANOVA.

Despite the positive results obtained during the first experiment, which included high dosages and long incubation times, the NS 51179 formulation failed to significantly alter the properties of the pulp in the second experiment. The NS 51179 replicated experiment had no significant effect on the pulp properties regardless of the different enzyme dosages or incubation times. A similar effect was observed by Zhang and Zhang [8] where some formulations of EG did not have any significant effect on the pulp properties. According to these authors, the viscosity was left unaltered because the formulations were of a purely mono-component EG that has a lower activity towards the crystalline regions of cellulose.

The Ecopulp R formulation succeeded in reducing the viscosity when using an enzyme dosage of 250 mg/kg or 300 mg/kg, while the other properties such as the S10 and S18 remained unchanged. The degraded cellulose increased when a high enzyme dosage of 300 mg/kg was used. It is possible with an enzyme dosage of 250 mg/kg to obtain the desired viscosity drop (50 ml/g to 100 ml/g), without changing the alkali solubility (and degraded cellulose). The change in crystallinity was unclear since there was an interaction between the enzyme dosage and incubation time. The reactivity did not significantly increase during the enzymatic treatments; however, there does appear to be a relationship between the drop in viscosity and

reactivity. The greater the drop in viscosity the less undissolved fibres remained in the reactivity test and therefore the reactivity increased. It should be noted that the reactivity was not tested on fully bleached pulp. While these enzymes could be used for either viscosity control purposes or reactivity increase [9], [10], it currently only shows an aptitude towards viscosity control. To further assess whether the reactivity could be increased the pulp will have to be processed until fully bleached and the reactivity tested again. Alternatively, the pulp could also be treated after it has been fully bleached and then the reactivity could be tested again.

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Chapter 4: The bleaching response of cellulase-treated dissolving wood pulp

4.1 Introduction

The chemical pulping process produces pulp of quality that is unsatisfactory for most applications. This pulp is therefore further purified during a bleaching process that consists of several stages to remove impurities such as lignin and hemicellulose from DWP [1]. During the bleaching stages, different chemical agents are used, and each bleaching agent has a specific function or goal [2 pp.165-203]. Some chemical agents are used mainly for brightening the pulp such as chlorine dioxide (referred to as the D-stage) [3 p.756], while other agents such as ozone (Z-stage) are mainly used for viscosity control purposes [4]. While ozone is mainly used for viscosity control when the lignin content is low, it also provides some whitening effect. In contrast, (hydrogen) peroxide (P-stage or E_p-stage) is mainly used for the brightening effects it provides during the later stages of bleaching, but it can cause some cellulose degradation (viscosity reduction) [3 p.853].

Enzymes are often used in the pulp and paper industry during the bleaching process with one of the key objectives being fibre modification [1]. Typical enzymes used during a bleaching sequence is xylanase and less commonly, cellulase [1], [5]. Cellulase consists of EG, CBH I, CBH II and β -G, and each enzyme serves a specific function [6]. EG can cut the cellulose chains from within and preferentially attacks the amorphous regions of the cellulose, while CBH preferentially attacks the crystalline regions of cellulose. β -G acts mainly as a support enzyme and further degrades the products released by EG and CBH. In several studies, the effect of EG treatment was investigated for fibre modification with the focus mainly on viscosity control and reactivity improvement [6-9].

Fully bleached DWP has a high cellulose purity of more than 90% alpha-cellulose, with a low lignin content (a Kappa number of less than one) and an alkali solubility of approximately 8% and 4% when treated with 10% and 18% NaOH, respectively [10 pp.1022-1062]. Furthermore, the fully bleached pulp must have an intrinsic viscosity of approximately 500 ml/g (CED) for viscose applications and a copper number of approximately 0.3. The ISO brightness should be at least 89%.

In this study, the potential use of commercial EGs in a pre-bleaching treatment was investigated. The aim of this study was to produce fully bleached pulp with properties similar to commercially produced DWP with the aid of the EGs. The enzymes were applied at bench-scale in a setup similar to a storage tower and the pulp was then passed through the remainder

of the bleaching sequence. The Kappa number and viscosity were determined after each bleaching stage, while the ISO brightness and BWI along with the alkali solubility, copper number and reactivity were determined for the fully bleached pulp. Other selected samples were also sent for reactivity measurement. Furthermore, a high-level cost impact analysis was conducted to determine any potential savings.

4.2 Materials and methods

4.2.1 Preliminary investigation of the effect on pulp viscosity

Due to the unavailability of the previously tested *Eucalyptus* pulp, pulp produced from a different *Eucalyptus* wood species was used during this part of the study. It should be noted that pulps from both species are used to produce DWP products with similar properties. The pulp was produced using the PHK-pulping process followed by delignification in an oxygen stage and then passed through two wash presses. As mentioned in the previous chapter, the delignification and washing were done according to a proprietary process. The pulp had a consistency of 9% and a pH of 6 and was treated with two commercially available EGs obtained from different suppliers (Table 1). Before proceeding with the pilot phase bleaching, a small bench-scale experiment was designed based on the indications of the previous experiments, to evaluate the ability of the enzymes to reduce the pulp viscosity by 50 units to 100 units.

Table 4-1: Supplier information and optimum ranges for the commercially available endoglucanases obtained from two suppliers

Enzyme formulation	Supplier	Temperature range	pH range	Reference
Ecopulp R	AB Enzymes (Darmstadt, Germany)	30 to 65°C	4.5 to 7.5	[11]
NS 51179	Novozymes (Bagsværd, Denmark)	50 to 70°C	5 to 7	[12]

The equivalent of 30 g oven-dry pulp in a plastic bag was placed in a water bath at 60°C and dosed with 250 mg/kg of either the NS 51179 formulation or the Ecopulp R formulation. The pulp was incubated for 2 h and the enzymatic reaction stopped by adding 2.0 L of boiling water to the pulp in a bucket. The pulp was then added to a Büchner funnel (240 mm) with filter paper (Munktell Grade 1288), followed by washing twice with 1.0 L of boiling water and thickened into a cake. Each treatment and the control (no enzyme dosing) were conducted in three replicates.

4.2.2 Hand sheet formation for analysis

Hand sheets were then made according to the ISO 5269-2:2004 standard [13], where the pulp was disintegrated in deionised water and sheets formed using a Rapid-Köthen sheet former. The viscosity was determined with a capillary viscometer according to the ISO 5351:2010 standard [14] using a CED solution. The data was subjected to an ANOVA to determine statistical significance.

4.2.3 Pilot-scale bleaching

Prior to the bleaching at pilot-scale, the pulp obtained from the mill was centrifuged (pilot centrifuge) in a Rousselet Robatel SA40Vx no 40588 (Annonay, France) at 2000 rpm for 5 min. The filtrate was captured and stored in 20 L buckets and was used to adjust consistency of the pulp for the enzyme treatment stage. The consistency of the centrifuged pulp was determined using an Ohaus MB25 moisture balance (Shanghai, China) and the pulp stored at 4°C until bleaching commenced. For all the bleaching stages the pH was measured afterwards, except for the Z-stage as the pulp was bleached at high consistency and no pH adjustment was required.

The bleaching on pilot-scale was done on a pilot reactor system that was purchased from Primozone (Löddeköpinge, Sweden) and modified by CRS Reactor Engineering (Stenkullen, Sweden). The modified system consisted of an ozone generator with an accompanying reactor vessel and an additional reactor vessel that could handle other bleaching agents including chlorine dioxide (ClO_2), peroxide and hypochlorite. The pilot reactors required approximately 2 kg dry pulp and the bleaching sequence used was similar to that at a mill (-D₀-Z/E_{op}-D1). The enzyme treatment was performed prior to the D₀-stage at 60°C, a pH of 6 and a 10% consistency. The enzymes were first diluted in 500 ml deionised water, with the pH adjusted to a pH of 6 using a BR buffer (buffer at pH 6). Once the pulp was at temperature, the enzymes were added and mixed into the pulp through stirring at 120 rpm for 5 min. After the 5 min the mixing was stopped, and the pulp kept in the reactor for 2 h, after which the enzymatic reaction was stopped by adding 300 ml of a 2.5 M NaOH solution and mixing the solution into the pulp for 2 min at 120 rpm. The pulp was transferred from the reactor into a large container using one reactor volume of water. The pulp was then transferred to the pilot centrifuge and centrifuged for 5 min at 2000 rpm, followed by another centrifuged cycle with one reactor volume of water at 2000 rpm for 5 min. The pulp was then centrifuged for a third time with 20 L of water for 10 min at 2000 rpm. Thereafter, the pulp was transferred to a plastic bag, crumbed and the consistency determined using a moisture balance. Hand sheets were made as before, with 40 g pulp for Kappa number (lignin content) determination and viscosity testing.

The Kappa number of the pulp was determined by Sappi Technology Centre (Pretoria, South Africa) through a method based on the French method for Kappa number determination (FDT-12-019).

The viscosities of the two enzyme-treated pulps were determined before continuing with the bleaching sequence, using the same method as mentioned before. It should be noted that due to the pilot-scale nature of the study, bleaching could not be replicated, and therefore no statistical analysis could be performed on the data. However, in some instances, reference

will be made to practical significance, which is defined as a difference large enough to be meaningful in real life.

Due to concerns regarding the differences between the bench-top treatment method and the pilot reactor treatment method, a second pilot reactor experiment was conducted. In the second experiment the enzyme dosage was reduced from 250 mg/kg to 100 mg/kg for both enzymes based on the viscosity results of the first experiment. In a third experiment, the dosage was further reduced to 70 mg/kg for the Ecopulp R formulation due to the results obtained from the second experiment.

The pulp was then bleached according to mill standards for the D₀-stage; therefore, the relevant conditions and dosages could not be disclosed. The pulp was removed from the reactor and centrifuged as before, twice with one reactor volume water for 5 min and once with 20 L of water for 10 min. The pulp was then transferred to a plastic bag, after which the pulp was crumbed, and the consistency determined using the moisture balance. Hand sheets were made from 40 g of pulp to determine the Kappa number and viscosity.

Prior to the Z-stage, the chemical charge for ozone was determined through small bench-scale experiments, where 50 g equivalent oven-dry pulp was treated with different ozone charges at approximately 35% consistency. The ozone was generated from oxygen through a PCI ozone and control systems model G-2 generator (California, United States of America). The charges ranged from 0.10% to 0.30%, after which the pulp was washed, centrifuged (laboratory centrifuge) in a Rousselet Robatel SC70Vx no 71136 (Annonay, France) at 2000 rpm for 5 min. Hand sheets were then made to determine the viscosities. A calibration curve of viscosity vs charge was constructed to determine the required ozone charge for pilot-scale bleaching. The pilot-scale ozone bleaching was done with a charge of 0.6 kg/t for the NS 51179-treated pulp and 0.7 kg/t for the control pulp and Ecopulp R-treated pulp. The pilot-scale bleaching was done at the same consistency as for the bench-top experiments and the pulp was afterwards removed from the reactor using two reactor volumes of water. The pulp was then centrifuged in the pilot centrifuge as before and the consistency was determined using the same moisture balance. Hand sheets were made from 40 g pulp for Kappa number determination and viscosity testing.

4.2.4 Bench-top scale bleaching

Due to practical considerations, it was decided to switch from pilot-scale bleaching to bench-top bleaching; however, the aim was now to produce the equivalent of 260 g oven-dry fully bleached pulp instead of 2 kg. Similar to the Z-stage, the E_{op}-stage had to be calibrated with a viscosity vs peroxide charge curve to determine the peroxide charge needed for the E_p-

stage. The equivalent of 5 g oven-dry pulp at 10% consistency was placed in plastic bags with a NaOH charge that was determined using undisclosed mill standards. The pulp was then charged with 0.25%, 0.50%, 0.75% or 1.00% hydrogen peroxide (H_2O_2) and incubated at a specific temperature for a predetermined retention time before being removed. The pulp was then washed five times with 2 L of deionised water and thickened in a Büchner funnel (150 mm diameter) over filter paper (Whatman Grade 4). Hand sheets were made from the pulp and tested for viscosity. The charge was then determined from the viscosity vs charge curve and the pulp bleached with corresponding peroxide charge. The equivalent of 300 g oven-dry pulp, at 10% consistency was placed in plastic bottles and charged with NaOH (similar as before) and the corresponding peroxide. Thereafter, the bottles were placed in a water bath at the specific temperature and retention time as before. The pulp was then filtered using a Büchner funnel (240 mm diameter) with filter paper (Munktell Grade 1288) to obtain the filtrate to measure the pH. The pulp was washed five times with 2 L of deionised water and thickened in the same Büchner funnel with the same filter paper. The thickened pulp was centrifuged in the laboratory centrifuge at 2000 rpm for 5 min, and then hand sheets for viscosity testing and Kappa number determination were made from 40 g pulp.

For the final D-stage (D_1 -stage), the equivalent to 260 g oven-dry pulp was placed in plastic bottles and bleached according to the mill standards for this stage. The pulp was bleached for a predetermined retention time and at a specific temperature before being removed and filtered in the 240 mm Büchner funnel to obtain the required filtrate for pH measurement. The pulp was then washed five times with 2 L deionised water and then centrifuged in the laboratory centrifuge for 5 mins at 2000 rpm. Hand sheets were then made from 80 g pulp for viscosity testing, Kappa number determination, alkali solubility determination, reactivity testing and copper number determination. Additionally, brightness hand sheets were made according to the ISO 3688:1999 standard and tested for ISO brightness (with the ISO 2740-1 standard) and BWI. The alkali solubility was determined according to the ISO 692:1982 standard [15]. Due to practical considerations, six samples were selected and sent for reactivity testing at Sappi's Saiccor Mill using a quick reactivity method, developed in-house, similar to Fock's reactivity test. One sample each was selected prior to the enzyme treatment, from each enzyme treatment and from each of the three fully bleached pulps. The copper number, which is an indication of the amount of damage done by the bleaching agents to the cellulose, was determined by Sappi Technology Centre (Pretoria, South Africa) according to the TAPPI Test Method T430 cm-09 [16].

4.2.5 Cost evaluation

Due to the potential of chemical reduction in the Z-stage and the E_p-stage, there consequently exists a potential for cost savings and, therefore, a simple costs analysis was conducted. The enzyme costs were estimated using the data from Liu *et al.* [17], where five enzyme cost cases along with data obtained from a mill were investigated (Table 4-2). It should be noted that although the enzymes were also cellulase enzymes, the application was for biofuel production.

Liu *et al.* [17] considered the following cases where in the first case, it was assumed that the enzyme proteins cost the same as soy proteins, which is the cheapest protein available. In the second case it was assumed that the price would be the same as that estimated by another source, while in the third case it was assumed that the price would be equal to a statement by Novozymes. For the fourth case, it was assumed that the price would be equal to an estimation made by another source for fungal cellulase, and in the fifth case it was assumed the price would be equal to cellulase in the Chinese enzyme market.

The costs were first converted to South African Rand, using the average exchange rate for the same year, followed by the adjustment of the price up to the year 2020 using the relevant inflation rates. The exchange rates were obtained from [18], while the inflation rate was obtained from [19]. The enzyme cost was expressed in terms of cost per ton of dry pulp using the relevant dosage and each enzyme case was investigated individually to fully explore the spectrum of the potential costs. Other chemical prices were obtained from the mill and were also expressed in terms of cost per ton dry pulp. The costs obtained from the mill were adjusted to the chemical charges for the control pulp and the enzyme-treated pulps, followed by finding the total cost of production for the control pulp and enzyme-treated pulps. It should be noted that due to the confidentiality of the data, the costs were normalised and expressed in terms of percentages. The cost savings were then calculated relative to the control pulp; therefore, it was assumed that chemical and enzyme costs would be 100% and 0% respectively for the control pulp compared to the enzyme-treated pulps.

Table 4-2: Enzyme cost estimations obtained from literature

Case	Cost (\$/kg)	Year	Reference
1	1.25	2011	[17]
2	5.07	2007	[17]
3	6.27	2010	[17]
4	10.14	2007	[17]
5	23.3	2013	[17]

4.3 Results and discussions

4.3.1 Preliminary investigation

The validity of the enzyme dosage for the different wood species was determined through a single factor ANOVA and the results are presented in Table 4-3 below.

Table 4-3: Bench-scale experiment results (the p-value was back-calculated from the F-statistic)

Enzyme formulation	Enzyme dosage (mg/kg)	Viscosity (ml/g)	p-value
N/A	0	722	N/A
Ecopulp R	250	645	0.037
NS 51179	250	672	0.033

The reduction in viscosity was statistically significant with the p-value below the significance level ($p \leq 0.05$). The drop in viscosity for the NS 51179-treated pulp was 50 units and for the Ecopulp R-treated pulp, 77 units. The aim to reduce the pulp viscosity by 50 units to 100 units was achieved using the 250 mg/kg enzyme dosage for the new wood species; therefore, the 250 mg/kg dosage would be used in the upcoming pilot phase.

4.3.2 Pilot-scale bleaching – enzyme stage

The results of treating the pulp with the enzymes at pilot-scale are given in Table 4-4 below.

Table 4-4: Data for the enzyme stage at pilot-scale

Enzyme dosage (mg/kg)	Enzyme formulation	Viscosity (ml/g)	Kappa number (ml)	Initial pH	Final pH after enzyme deactivation	Yield (%)
250	Ecopulp R	506	N/A	5.82	10.89	99.6
250	NS 51179	587	N/A	5.58	10.69	99.4
0	Control	722	3.05	N/A	N/A	N/A
100	Ecopulp R	568	N/A	7.00	11.53	99.5
100	NS 51179	633	2.98	6.80	11.09	99.4
70	Ecopulp R	622	2.97	6.11	11.61	99.5

The pulp viscosity was reduced by 216 units and 135 units for Ecopulp R and NS 51179, respectively. The reason for the large drop in viscosity could be due to greater mobility of the enzymes caused by the method of mixing in the pilot reactor. During bench-scale experiments the enzymes were added using a micropipette, the plastic bag sealed and then kneaded by hand to obtain mixing; however, in the pilot reactor an agitator was used to perform the mixing. The agitator was only used for initial mixing, but still provided improved mobility for the enzymes compared to hand kneading. The large drop in viscosity was not acceptable and an educated guess was made to lower the enzyme dosage to 100 mg/kg dosage and rerun the

enzyme treatment. At a 100 mg/kg dosage, the NS 51179 reduced the pulp viscosity by 89 units, while the Ecopulp R reduced the pulp viscosity by 154 units. The reduction in viscosity when treated with NS 51179 was considered to be acceptable; however, the Ecopulp R, at a notably lower dosage, reduced the pulp viscosity by 50 units more than the acceptable amount. The Ecopulp R dosage was reduced to 70 mg/kg and the enzyme treatment repeated. The viscosity reduction obtained by this dosage was 100 units, which was at the upper limit of the acceptable range. The final viscosity prior to the first ClO_2 was 722 ml/g, 633 ml/g and 622 ml/g for the control pulp, NS 51179-treated pulp and Ecopulp R-treated pulp, respectively. The Kappa numbers were 3.05 ml, 2.98 ml and 2.97 ml for the control pulp, NS 51179- and the Ecopulp R-treated pulps, respectively and were considered as similar. The small variation in the Kappa number agreed with results from the previous bench-top experiments where the Kappa number also remained unchanged. The yield of the enzyme stage was 99.4% and 99.5% for the NS 51179 and Ecopulp R, respectively (Table 4-4), which is similar to the yield obtained by Engström *et al.* [20] and Henriksson *et al.* [9].

4.3.3 Pilot-scale bleaching – chlorine dioxide stage

After the enzymatic treatment stage, the pulp was bleached with ClO_2 and the results of this stage are given in Table 4-5 below.

Table 4-5: Data for the first chlorine dioxide (ClO_2) stage at pilot-scale

Enzyme formulation	Viscosity (ml/g)	Kappa number (ml)	Final pH	Yield (%)
Control	692	1.04	2.40	98.7
Ecopulp R	584	1.09	2.12	99.0
NS 51179	608	0.96	2.13	98.8

Although ClO_2 is not utilised as a chemical for viscosity control, a drop of 38 units, 25 units and 30 units was observed for the Ecopulp R-treated pulp, NS 51179-treated pulp and the control pulp, respectively. The viscosity drops were not considered as practically significant; however, the control pulp did have a noticeably higher viscosity after the ClO_2 stage compared to the two enzyme-treated pulps. The control pulp differed by 84 units from the NS 51179-treated pulp and 108 units from the Ecopulp R-treated pulp. The Kappa number of all three pulps were similar, but the Kappa number of the NS 51179 pulp was the lowest (0.96 ml). There was a drop in the Kappa number from an average of 3 ml for the enzyme stage to an average of 1.03 ml for the D_0 -stage, which resulted in a noticeable increase in visible whiteness of the pulp from a light brownish colour to a creamy white colour. The yield was as expected to be close to 99% for all three pulps and compared very well to mill yields.

4.3.5 Pilot-scale bleaching – ozone stage

Ozone is generally considered to be a viscosity controlling agent, with some whitening effect and a major effect in preventing a drop in viscosity. A calibration curve was generated for each pulp to determine the ozone charge required for bleaching. The results of the calibration curves are presented in Figure 4-1 below.

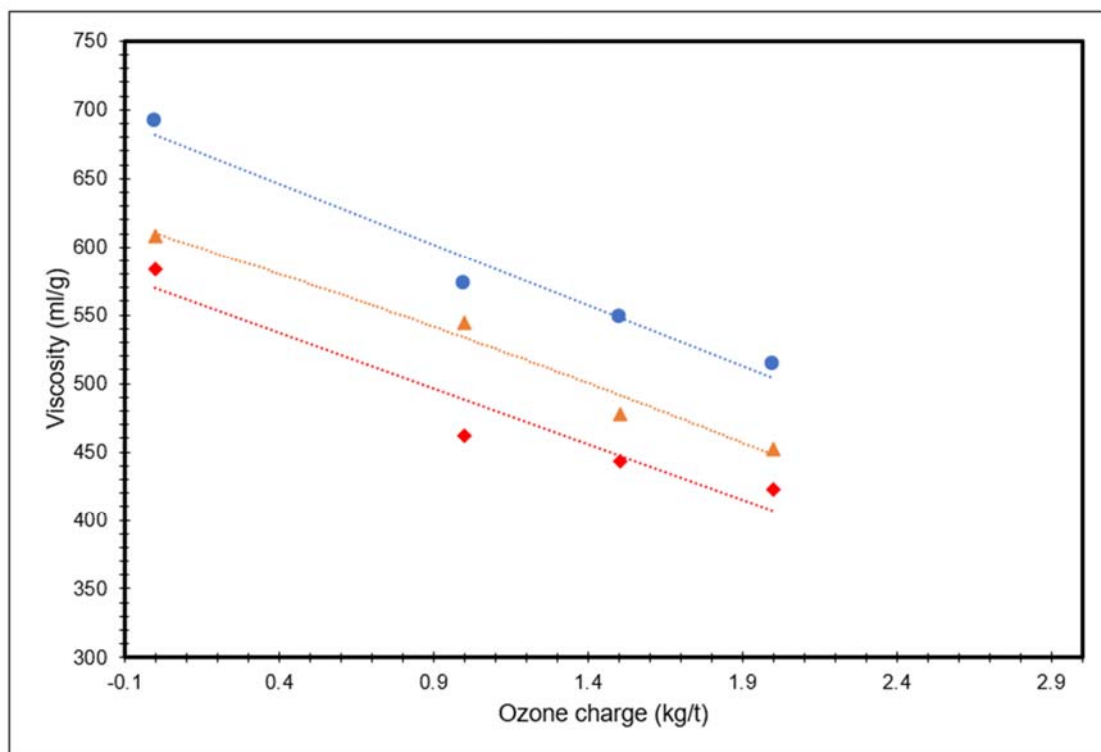


Figure 4-1: Calibration curves to determine ozone charges for the control pulp and the two enzyme-treated pulps (the control pulp is represented by the blue circles, the Ecopulp R-treated pulp is represented by the orange triangles and the NS 51179-treated pulp is represented by the red diamonds; the coefficient of determination (R^2) for the control pulp was 0.97, while for the Ecopulp R-treated pulp it was 0.97; the NS 51179-treated pulp had a R^2 value of 0.93)

To avoid any further unwanted degradation of the cellulose and ending up with a final product that is outside of the market specifications, the viscosity (at this stage) should preferably not drop below 500 ml/g. Based on the slopes of the calibration curves, the viscosity of the control pulp seemed to have dropped more rapidly, with a slope (m) of -89.1 units, compared to the slope of the NS 51179-treated pulp ($m = -81.2$ units). However, the viscosity of the Ecopulp R-treated pulp appeared to flatten out as the ozone charge increased, while the viscosity of the NS 51179-treated pulp seemed to decrease linearly. The reason for this is unclear, but it would be worthwhile to investigate this phenomenon further to fully understand the effect of the enzyme treatment on the bleaching sequence. Based on the calibration curve an ozone

charge of 0.5 kg/t for both the control pulp and the Ecopulp R-treated pulp was selected, while a charge of 0.3 kg/t for NS 51179-treated pulp was used. The charge difference between the control pulp and the NS 51179-treated pulp, indicated a potential for chemical savings. The chemical saving was calculated relative to the charge of the control pulp and was found to be 40%. Similar statements were made regarding the potential chemical savings and economic benefits by Henriksson *et al.* [9]. After the ozone charges were determined, the pulp was bleached at pilot-scale and the results are given in Table 4-6 below.

Table 4-6: Data for the ozone stage at pilot-scale

Enzyme formulation	Chemical charge aim (%)	Chemical reduction (%)	Chemical charge actual (%)	Viscosity (ml/g)	Kappa number (ml)	pH	Yield (%)
Control (no enzyme)	0.05	N/A	0.07	617	0.92	N/A	96.0
Ecopulp R	0.05	0.00	0.07	537	0.73	N/A	97.6
NS 51179	0.03	0.57	0.06	526	0.73	N/A	96.4

Due to equipment limitations of the pilot reactor, the control pulp and Ecopulp R-treated pulp was charged with 0.7 kg/t ozone, while the NS 51179-treated pulp was charged with 0.6 kg/t ozone. The resulting viscosities of the charges were 617 ml/g, 537 ml/g and 526 ml/g for the control pulp, Ecopulp R-treated pulp and NS 51179-treated pulp, respectively. In general, the enzyme-treated pulps had a lower lignin content (Kappa number) than the control pulp. The Kappa numbers of the two enzyme-treated pulps were identical (0.73 ml), despite the lower ozone charge for the NS 51179-treated pulp. Similarly, the Kappa number of the control pulp (0.92 ml) was higher than the Ecopulp R-treated pulp, even though the charges were identical, and the Ecopulp R-treated pulp had a higher starting Kappa number from the previous stage.

The reason for the higher Kappa number of the control pulp is unclear at this stage, but it is suspected that the enzyme treatment may have loosened some of the lignin and hemicellulose entangled between the cellulose and consequently they could be more easily removed.

The yield from the ozone stage was greater for the two enzyme-treated pulps compared to the control pulp, but the yields were not notably higher or lower than what was normally expected from the ozone stage [21].

4.3.6 Bench-top bleaching – peroxide stage

While peroxide is mainly employed for its effect on brightness, peroxide can directly attack the cellulose. To avoid significant degradation of the cellulose, a calibration curve of peroxide charge vs viscosity was required, which is given in Figure 4-2 below.

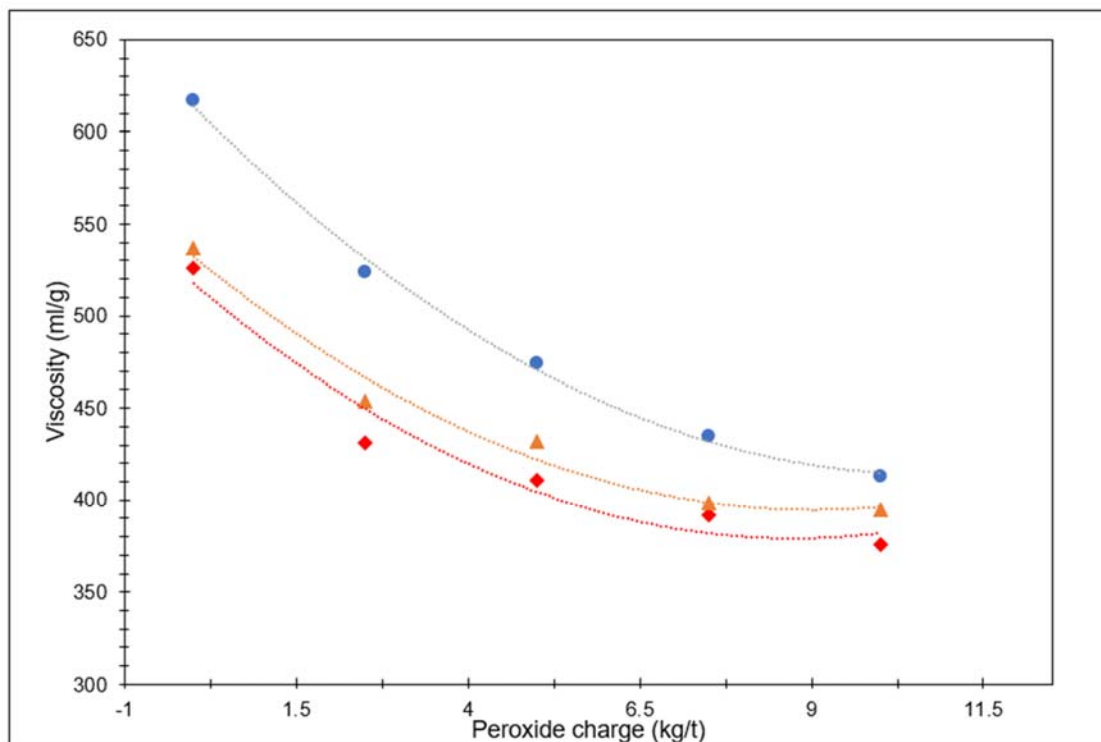


Figure 4-2: Calibration curves to determine peroxide charge for control pulp and the two enzyme-treated pulps (the control pulp is represented by the blue circles, the Ecopulp R-treated pulp is represented by the orange triangles and the NS 51179-treated pulp represented by the red diamonds; the coefficient of determination [R^2] for the control pulp was 0.99, while for the Ecopulp R-treated pulp it was 0.98; the NS 51179-treated pulp had a R^2 value of 0.96)

The viscosities of both the enzyme-treated pulps and the control pulp seemed to decrease and then flatten off slowly, despite the initial lower viscosity of the NS 51179-treated pulp (Figure 4-2). In all three cases, there was an initial rapid decrease in the viscosity followed by a slower decrease in viscosity. The viscosity after the E_p -stage should preferably not drop below 475 ml/g. Based on the calibration curve it was decided to use peroxide charges of 0.20%, 0.15% and 0.55% for the Ecopulp R-treated pulp, NS 51179-treated pulp and control pulp, respectively. Due to the already low viscosities of the enzyme-treated pulps, extremely little peroxide could be used in the E_p -stage; however, for the control pulp, which had a higher viscosity, more peroxide could be used. Similar to the ozone stage where there was a potential for chemical and cost savings, here at the peroxide stage there also existed a potential for savings. The chemical savings were also calculated relative to the control pulp and were found

to be 73% and 96% for the NS 51179-treated pulp and Ecopulp R-treated pulp, respectively. The results of the E_p-stage bleaching are given in Table 4-7 below.

Table 4-7: Data for peroxide stage at bench-scale

Chemical charge (%)	Enzyme formulation	Viscosity (ml/g)	Kappa number (ml)	Final pH	Yield (%)
0.55	Control (no enzyme)	471	0.43	11.55	98.6
0.20	Ecopulp R	512	0.53	11.56	98.7
0.15	NS 51179	497	0.59	11.55	98.8

After the E_p-stage, the viscosities dropped by 25 units, 29 units and 147 units to 512 ml/g, 497 ml/g and 471 ml/g for Ecopulp R-treated pulp, NS 51179-treated pulp and the control pulp, respectively. The large drop in viscosity for the control pulp can be ascribed to the high chemical charge of peroxide compared to the two enzyme-treated pulps. In general, the Kappa number dropped from an average of 0.79 ml in the Z-stage to an average of 0.52 ml in the E_p-stage. Similar to the viscosity drop, the control pulp exhibited a high drop in the Kappa number, from 0.92 ml to 0.43 ml, which can be attributed to the higher peroxide charge compared to the two enzyme-treated pulps. The Kappa number of the NS 51179-treated pulp was slightly higher (0.59 ml) compared to the Ecopulp R-treated pulp (0.53 ml); however, it is unclear why the NS 51179-treated pulp had a lower drop in lignin content, despite the higher chemical charge compared to the Ecopulp R-treated pulp. A possible explanation for this might be that the enzymes could also attack some of the glycosidic linkages in the hemicellulose thereby liberating some lignin from the cellulose-hemicellulose and lignin matrix. The differences between the Kappa number of the three pulps were not considered to be notable. The yields for all three pulps were similar at approximately 98.7% and was similar to what can be expected at the mill.

4.3.7 Bench-top bleaching – final chlorine dioxide stage

After the E_p-stage, the pulp was bleached with a final ClO₂ stage and the results are given in Table 4-8 below.

Table 4-8: Data for the final chlorine dioxide stage at bench-scale

Enzyme formulation	Viscosity (ml/g)	Kappa number (ml)	Copper number (%)	Final pH	Yield (%)
Control (no enzymes)	475	0.29	0.44	3.13	98.8
Ecopulp R	508	0.53	0.49	2.88	98.8
NS 51179	500	0.46	0.54	2.85	99.0

In the final D-stage, with a low chemical charge the viscosities did not change particularly. The final pulp viscosities were within the acceptable viscosity range according to the general standards provided by [10 pp.1061-1062] (450 ml/g to approximately 500 ml/g) and that of the mill. The viscosities were 475 ml/g, 508 ml/g and 500 ml/g for the control pulp, Ecopulp R-treated pulp and NS 51179-treated pulp, respectively. The Kappa number did not change notably from the peroxide stage (average of 0.52 ml) compared to the D₁-stage (average of 0.49 ml) and was close to the general standards provided by [10 pp.1061-1062] of 0.3 ml. It should be noted that the general standard of [10 pp.1061-1062] was obtained from total chlorine free (TCF) bleaching sequences. The NS 51179-treated pulp had a higher Kappa number compared to the control pulp and Ecopulp R-treated pulp, while the control pulp had the lowest Kappa number. The overall yields for all three pulps were again similar, and were 93.8%, 92.6% and 92.3% for the Ecopulp R-treated pulp, NS 51179-treated pulp and control pulp, respectively; and the similarity indicates that there were no substantial losses due to the enzyme treatment.

4.3.8 Fully bleached pulp properties

After the pulp has been fully bleached, the copper number and alkali solubility of the pulp were determined, and the results are given in Table 4-9 below.

Table 4-9: The copper number and alkali solubility of the fully bleached pulp

Pulp	Copper number (%)	S10(%)	S18(%)
Control (no enzyme)	0.44	6.1	2.6
Ecopulp R	0.49	6.3	3.0
NS 51179	0.54	6.7	2.9

The copper number for the three pulps were similar and close to the general standard provided by [10 pp.1061-1062], which is 0.4% (Table 4-9). It should, however, be noted that [10 pp.1061-1062] obtained the information from TCF bleaching sequences and not elemental chlorine free (ECF) bleaching sequences. The NS 51179-treated pulp did appear to have a slightly higher copper number compared to the other two pulps. The S10 values of the two enzyme-treated pulps were slightly higher compared to the control pulp, but the differences were not notable. The S18 values were also similar for the three pulps; however, the two enzyme-treated pulps did have slightly higher S18 values. The ISO brightness and BWI of the fully bleached pulp were also determined, and the results are given in Figure 4-3 below.

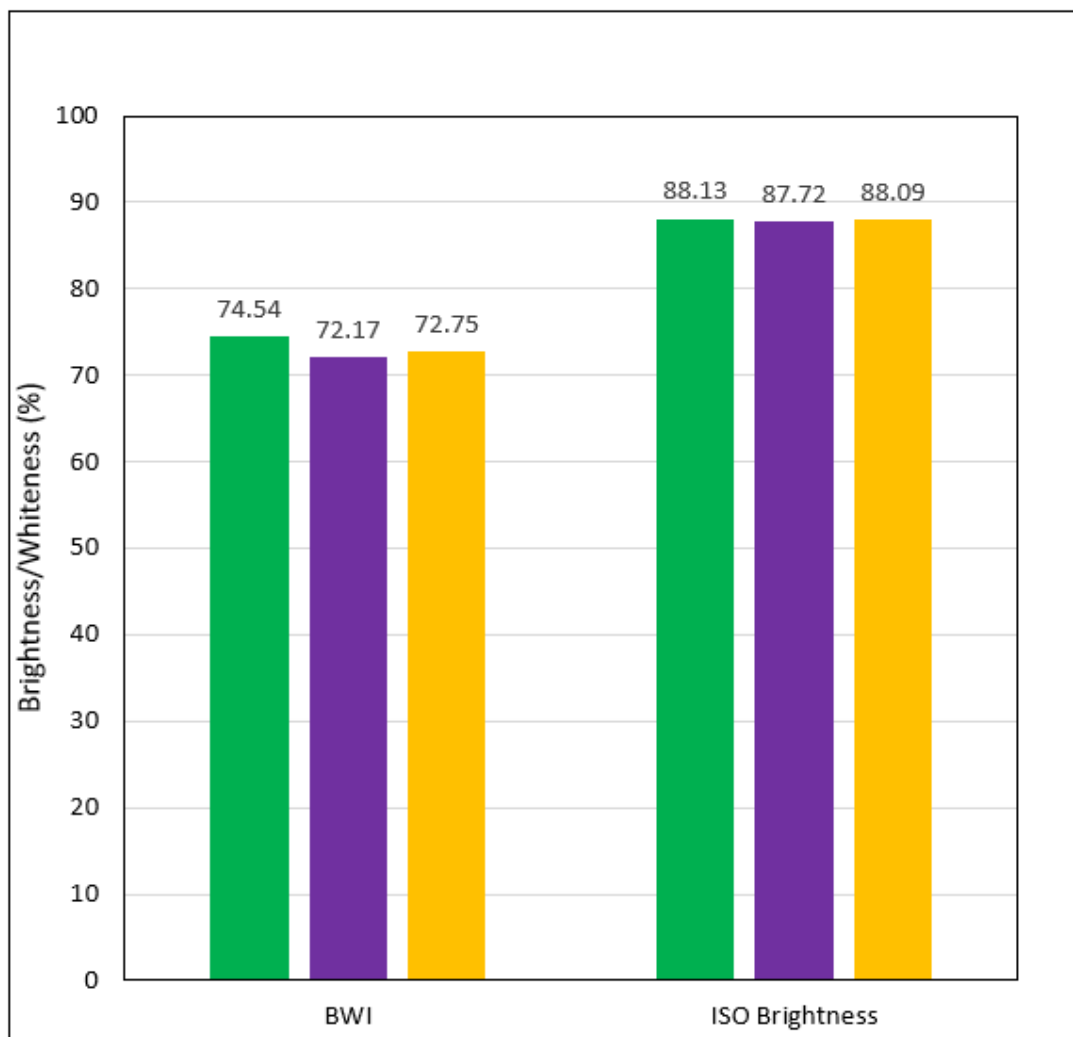


Figure 4-3: The BWI and ISO brightness of the three pulps (the measured values are the average of four measurements on the hand sheet; the control pulp is represented by green, while the NS 51179-treated pulp is represented by purple and the Ecopulp R-treated pulp is represented by yellow)

The brightness or whiteness of the pulp is an important aesthetic parameter for the customer and should therefore be routinely measured. One of the concerns regarding the brightness and whiteness of the two enzyme-treated pulps was that the brightness and whiteness would not be within acceptable range, as peroxide is generally used to control brightness and viscosity. Surprisingly, the ISO brightness (Figure 4-3) of all three pulps was similar and was approximately 88%, which was very close to the 89% general standard provided by [10 pp.1061-1062]. Both enzyme-treated pulps had a lower ISO brightness compared to the control pulp, but the differences were not notable and the lower ISO brightness may be attributed to the lower peroxide charges and the slightly higher Kappa number and S18 values in the enzyme-treated pulps. Similar results were observed by Quintana *et al.* [21].

Similarly, the BWI of the three pulps were similar; however, the two enzyme-treated pulps did have a slightly lower BWI compared to the control pulp that can again be attributed to the slightly higher Kappa numbers and S18 values of the two enzyme-treated pulps.

The reason for the high ISO brightness and BWI of the two enzyme-treated pulps compared to the control pulp is unclear, but as suggested by Henriksson *et al.* [9], and Cao and Tan [22], the use of enzymes could boost the bleaching efficiency of the peroxide stage to improve the brightness. However, in the case of both Henriksson *et al.* [9], and Cao and Tan [22], a laccase and EG enzyme were used. Further investigation is recommended of the effects of the enzyme treatments on the bleachability of the pulp to improve brightness.

In some instances, it is required that the pulp should be able to retain its brightness or whiteness over a long period of time, in which case the reversion of brightness and whiteness must be measured. The results of the reversion are given in Figure 4-4 below.

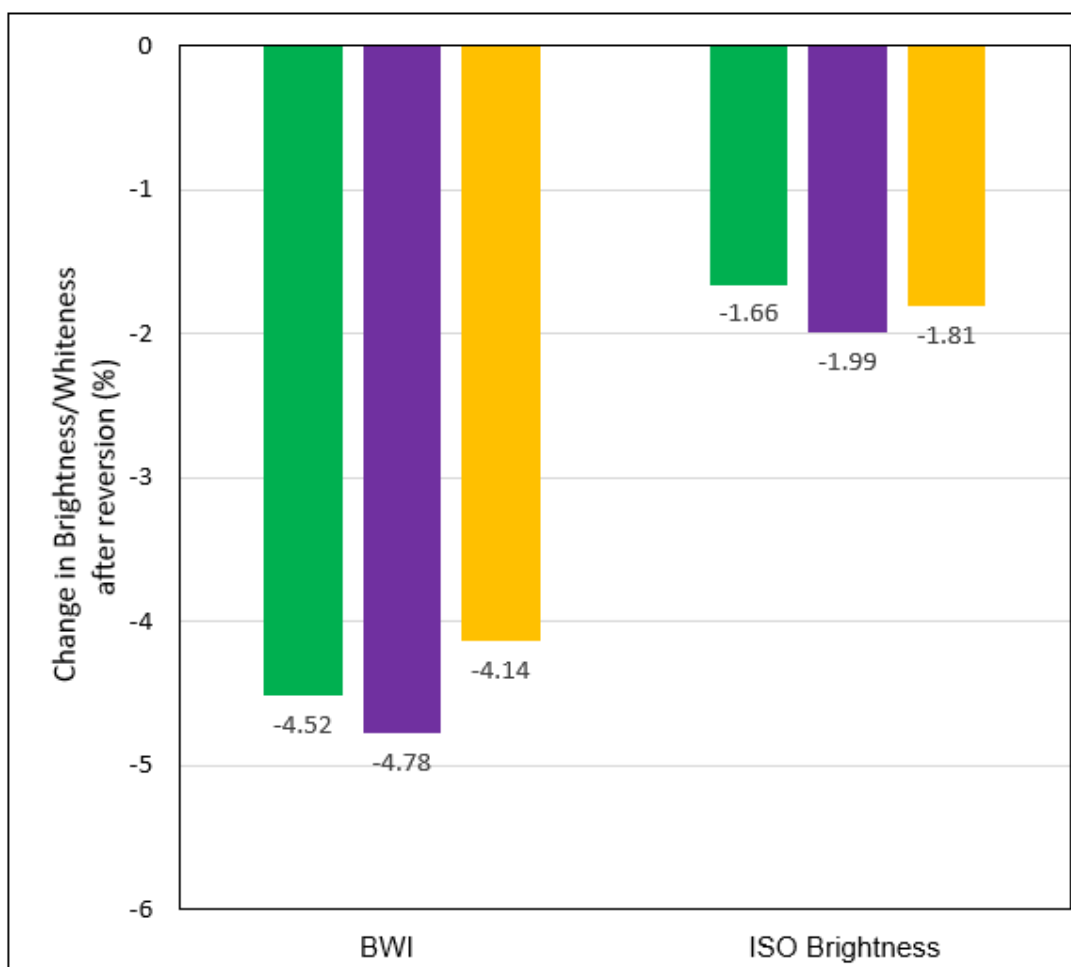


Figure 4-4: The change in BWI and ISO brightness of the two enzyme-treated pulps and the control pulp (the measurement is the average of four measurements on the hand sheets)

and each change is calculated relative to the corresponding BWI or ISO brightness before reversion; the control pulp is represented by green, while the NS 51179-treated pulp is represented by purple and the Ecopulp R-treated pulp is represented by yellow)

Both the control pulp and enzyme-treated pulps showed similar reversion for the BWI and the ISO brightness, with the change in the BWI being greater than the change in the ISO brightness. The reason for the greater drop in BWI is due to the equation used to infer the BWI, which has a greater factor associated with the Z value (blue reflectance) of the tristimulus colour map.

As previously mentioned, the enzymes could either be used for viscosity control or reactivity improvement and from the previous results, it appeared that the enzymes had the ability to influence viscosity. To investigate if the enzymes did increase the reactivity despite being used at the beginning of the bleaching sequence, samples taken after the enzyme treatment and after full bleaching were sent for quick reactivity testing. The results of the quick reactivity testing are given in Figure 4-5 below.

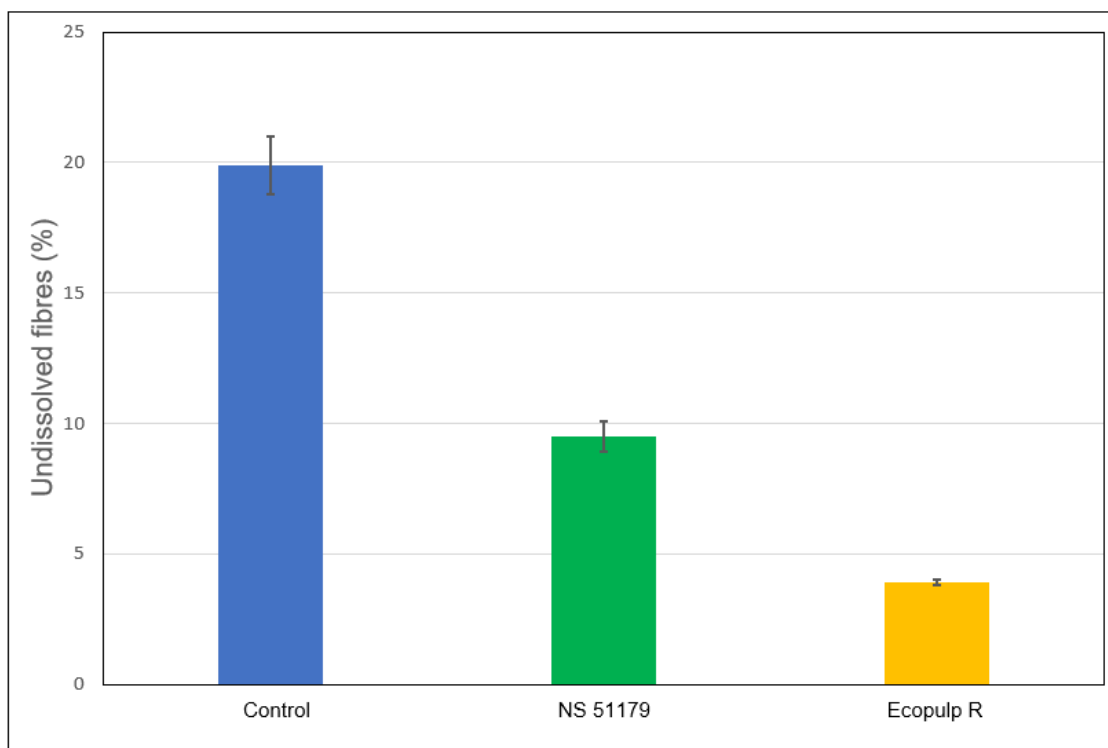


Figure 4-5: The amount of undissolved fibres for the control pulp (blue), NS 51179-treated pulp (green) and Ecopulp R-treated pulp (yellow) in the post-oxygen stage (the lower the amount of undissolved fibres the more reactive the pulp; the error was calculated from duplicate measurements for each pulp)

It should be noted that the quick reactivity method was not optimised for semi-bleached pulp and therefore, any interactions that the residual lignin and hemicellulose may have had was unclear. The reactivity is indirectly proportional to the amount of undissolved fibres. The enzyme-treated pulp in the post-oxygen stage, showed a remarkable difference in undissolved fibres compared to the control pulp (19.9%), with the Ecopulp R-treated pulp (3.9%) having the least amount of undissolved fibre. The NS 51179-treated pulp (9.5%) only contained half the amount of undissolved fibres compared to the control pulp, while the Ecopulp R-treated pulp contained approximately five times less undissolved fibres than the control. The differences in the amount of undissolved fibres were both practically and statistically significant (based on the error of the measurements). This was a positive indication that despite the enzymes being used at the beginning of the bleaching sequences, there may be reactivity benefits once the pulp has been fully bleached.

To see if the reactivity benefits were retained in the fully bleached pulp, the reactivity was tested again with the fully bleached samples and these results are given in Figure 4-6 below.

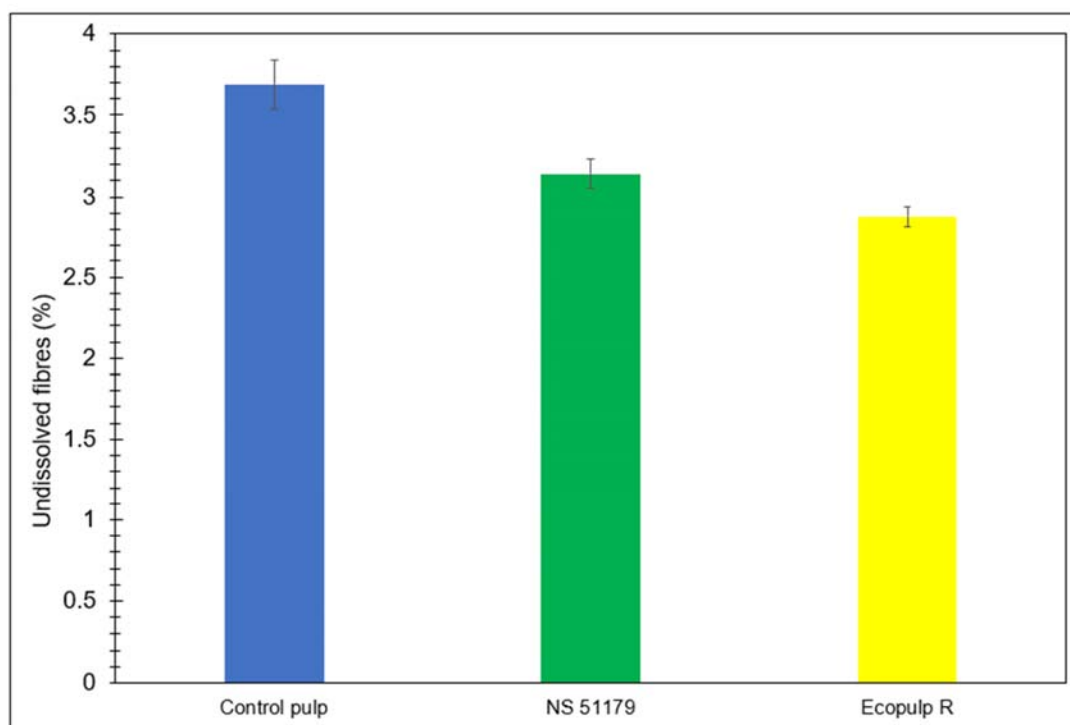


Figure 4-6: The amount of undissolved fibres for the control pulp (blue), NS 51179-treated pulp (green) and Ecopulp R-treated pulp (yellow) after being fully bleached (the lower the amount of undissolved fibres the more reactive the pulp; the error was calculated from duplicate measurements for each pulp)

Although the reactivity of the enzyme-treated pulps did statistically differ from the control pulp based on the error of the measurement, the differences were not practically significant (Figure 4-6). The NS 51179-treated pulp had the lowest reactivity of the three pulps and the similarity of the amount of undissolved fibres between the three pulps indicated that the reactivity boost received in the beginning of the bleaching sequences was not retained. It may therefore be suggested that a so-called polishing step of the pulp after the pulp had been fully bleached would be required for reactivity improvement [8, 9,12,20].

4.3.9 Cost evaluation

4.3.9.1 Adjusted prices

Due to the chemical savings in the Z-stage and E_p-stage, it should follow that there would be economic benefits from the use of the enzymes. The enzyme cost estimations from literature and from the mill, followed by the adjustment for the South African currency and the current year resulted in a wide spectrum of potential prices for the cellulase enzymes. The potential prices are given in Table 4-10 below.

Table 4-10: The adjusted price of enzyme cost estimation (the price was adjusted for South Africa and for the year 2020 using the exchange rate for the same year and then adjusted for the year 2020 using inflation rates)

Case study	Definition	Reference	Adjusted price (R/kg enzyme)
1	Soy protein equivalent	[17]	11.24
2	Literature estimated price	[17]	14.33
3	Novozymes statement	[17]	74.40
4	Fungal cellulase, literature estimate	[17]	145.26
5	Chinese enzyme market	[17]	310.65

The cheapest price was found in the first enzyme case study with an estimation of only R11.24 per kg of enzyme, while the most expensive price was that of the data obtained from the Chinese enzyme market (R310.26 per kg enzyme). The enzyme costs would be more closely related to the price of the Chinese enzyme market because I could not obtain prices from the suppliers.

4.3.9.2 Cost savings

The costs of using the enzyme in the bleaching sequence for each case study was calculated and the results are given in Table 4-11 below.

Table 4-11: The costs for peroxide and ozone as a percentage of the cost of the control pulp (the total cost without the enzyme and the total cost with the enzyme are also shown to underline the influence of the enzyme costs; the cost saving was calculated relative to the control pulp in each case)

Case study	Enzyme-treated pulp	Peroxide costs (%)	Ozone costs (%)	Total costs without the enzyme (%)	Total costs with the enzyme (%)
-	Control pulp	100.0	100.0	100.0	100.0
Case 1	NS 51179 pulp	27.3	60.0	35.4	36.6
	Ecopulp R pulp	3.6	100.0	27.5	28.3
Case 2	NS 51179 pulp	27.3	60.0	35.4	36.9
	Ecopulp R pulp	3.6	100.0	27.5	28.6
Case 3	NS 51179 pulp	27.3	60.0	35.4	43.5
	Ecopulp R pulp	3.6	100.0	27.5	33.2
Case 4	NS 51179 pulp	27.3	60.0	35.4	51.3
	Ecopulp R pulp	3.6	100.0	27.5	38.6
Case 5	NS 51179 pulp	27.3	60.0	35.4	69.4
	Ecopulp R pulp	3.6	100.0	27.5	51.3

The greatest savings were observed for the peroxide costs, with the cost for peroxide as low as 3.6% for the Ecopulp R-treated pulp compared to the cost for the control pulp (Table 4-11). The massive saving in peroxide is due to not only the high cost of peroxide compared to ozone, but also to the low charge of peroxide that was used. In contrast, the cost of ozone dropped by 40% to 60% compared to cost of the control pulp. The reduced cost was only calculated for the NS 51179 enzyme, as there was no reduction in the ozone charge for the Ecopulp R-treated pulp. The total cost with and without the enzyme is similar for both case study 1 and case study 2 and this is due to the similar cost of the enzyme for the two case studies (R11.24 per kg enzyme and R14.33 per kg enzyme respectively). From case study 3 and onward, the total cost with the enzyme increases drastically and this can be attributed to the higher costs of the enzymes in the respective case studies. The highest total cost with the enzyme was calculated for the NS 51179 enzyme in case study 5 (69.4%) followed by the total cost with the enzyme for the Ecopulp R enzyme in case study 5 (51.3%). The reason for the NS 51179 enzyme cases being the more expensive can be attributed to the higher charge required during operation compared to the Ecopulp R enzyme. The total cost with the Ecopulp R enzyme was the greatest in the fifth case study, being only 12.7% higher compared to the fourth case study.

The costs savings due to the use of the enzymes are visualised in Figure 4-7 below.

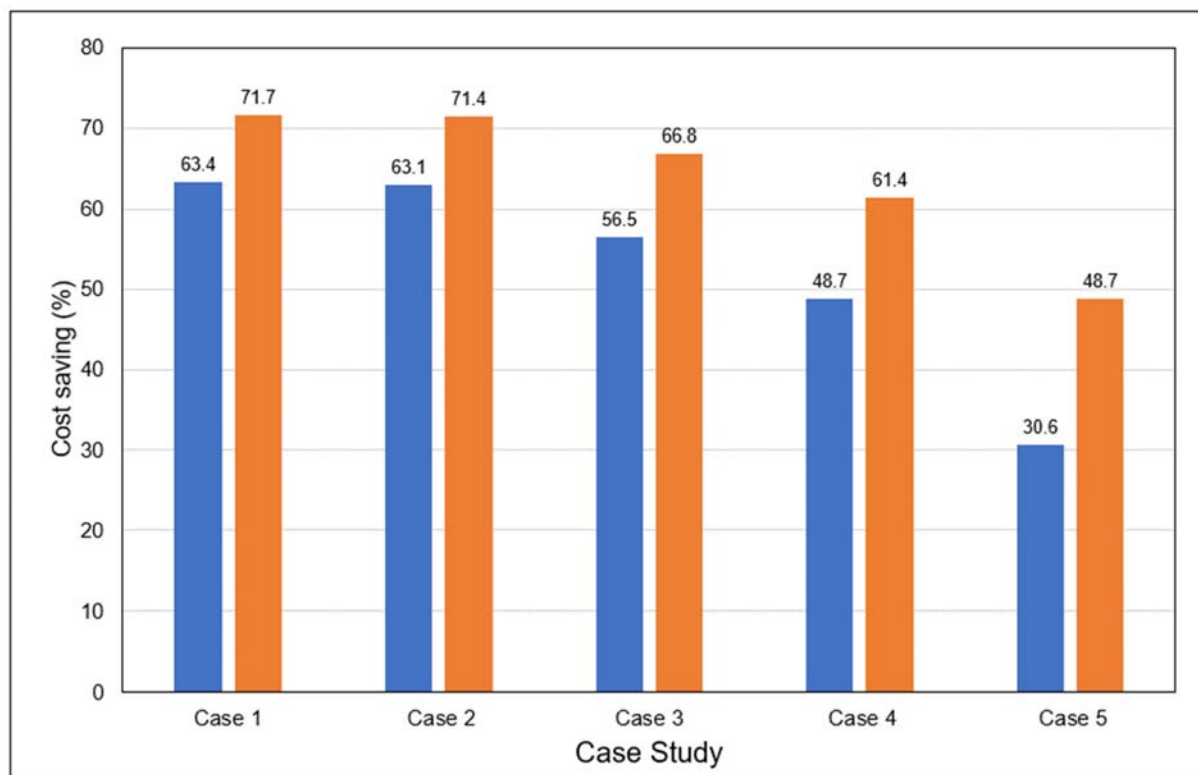


Figure 4-7: Bar chart showing the cost savings associated with the NS 51179 enzyme (blue) and Ecopulp R (orange) for each enzyme cost case study (in the first case study, enzyme protein was assumed to cost the same as soy protein, and in the second case study, the enzyme cost was estimated based on a literature source; in the third case study the cost estimate was based on a statement made by Novozymes, while in the fourth case study the estimate was made for fungal produced cellulase; in the fifth case study, the costs were that of prices obtained from the Chinese enzyme market, while in the sixth case study the price was obtained from a mill)

The greatest cost saving was observed with the Ecopulp R enzyme for all the case studies (Figure 4-7), with the greatest difference between the Ecopulp R and NS 51179 seen in the fifth case study (18.1%). The lower cost savings calculated for the NS 51179 enzyme can be attributed to the higher charge required as mentioned before. Despite the great cost savings calculated for case studies 1 to 4, these were unrealistically low costs for enzymes and therefore, the savings calculated from the fifth case study are more likely to be closer to the actual savings that would be observed. It should be noted that these savings do not include changes in other chemical charges, such as additives, and as this study only focused on the active chemicals and no other chemicals, it is impossible to calculate real world savings.

4.4 Conclusions and recommendations

The enzymatic treatments were successful at reducing the viscosity of the semi-bleached pulp despite the use of a different *Eucalyptus* wood species. In agreement with the previous bench-top experiments, the Ecopulp R enzyme caused a greater reduction in viscosity compared to the NS 51179 enzyme. In future studies, the effect the enzymes had on pulp viscosity could be investigated for different wood species. Additionally, the dosage could be optimised for single species pulp and mixed species pulp.

Using EGs reduced the viscosity to such an extent that the ozone charge was reduced which is definitely a potential for chemical savings; however, the commercial production of ozone is fairly cheap [3 pp. 777-778]. The chemical reduction was only applicable to the NS 51179-treated pulp, despite the viscosity reduction of the enzyme being lower than with the Ecopulp R-treated pulp. Therefore, further investigation is recommended of the effects of the enzyme treatments on the ozone stage to fully understand how the reduced viscosity of the pulp would influence the ozone charge and other interactions such as delignification.

Similar to the ozone stage, there was an even greater reduction in chemical charge for the peroxide stage and, therefore, the potential for chemical savings and economic benefits were also elevated. Added to the economic benefits are the high costs associated with peroxide compared to ozone [3 pp.849,862,877]. The chemical savings were more pronounced with the Ecopulp R-treated pulp than with the NS 51179-treated pulp. Despite the low charge of peroxide, the ISO brightness of the pulp was on par with the control pulp and that of commercially produced DWP. Further investigation is, therefore, recommended of the effect the enzymatic treatments had on the peroxide stage to fully understand how the enzymes influenced the pulp to achieve such brightness levels despite the low peroxide charge.

Despite the similarity of the Kappa numbers for all three pulps in each stage, there appears to be some boosting effect associated with the use of EG, especially in the Z and E_p-stages. The yield of each stage and the overall yield of each pulp agreed with that of commonly achieved yields [23]. Furthermore, the copper number and S10 values of the enzyme-treated pulps were within specifications and suggest that the enzyme did not cause excessive damage to the cellulose. The ISO brightness and BWI also compared well with the specifications, despite the lower chemical charges of peroxide and suggested a boosting effect associated with the enzymes. The reactivity of the post-oxygen pulps compared to the fully bleached pulp suggested that there was no retention of the reactivity benefits gained before bleaching. Therefore, it is suggested to apply an extremely small dosage of the enzyme to the pulp after the pulp has been fully bleached to regain the reactivity improvement.

The use of the enzymes resulted not only in chemical reduction for ozone and peroxide, but also in an overall cost reduction for producing fully bleached pulp, with greater substantiated savings for the Ecopulp R enzyme as calculated from literature and mill data.

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Chapter 5: Conclusions and recommendations

5.1 Conclusion

Based on the high-level enzyme assay, the Ecopulp R enzyme displayed a higher activity than the NS 51179 enzyme, with the possibility of iso-enzymes present in the Ecopulp R formulation. Furthermore, the higher activity of the Ecopulp R enzyme meant better property modification as was seen in subsequent experiments.

In the bench-top experiment, with the wide range of enzyme dosages and long incubation times, both formulations reduced the pulp viscosity, and it was found that the change in viscosity was directly proportional to the enzyme dosage. Furthermore, the alkali solubility, specifically the S10 solubility, was increased by both formulations and it was found that the increase in alkali solubility was also directly proportional to the enzyme dosage.

The ANOVA results regarding the crystallinity showed that the crystallinity of the pulp was not affected by the enzymes. The BWI was only affected by the NS 51179 enzyme, but not the Ecopulp R enzyme, and the reason for this was unclear.

During the replicated experiment with low enzyme dosages and short incubation times with the NS 51179 enzyme, the enzyme failed to modify any of the pulp properties and this might be due to the mono-component EG having a greater affinity towards the amorphous regions of the cellulose. In contrast, the replicated experiment with the Ecopulp R enzyme under the same conditions showed successful reduction in pulp viscosity at the high-end of the enzyme dosages, while the alkali solubility (S10 and S18) remained unchanged. Furthermore, the degraded cellulose only became significant at the highest dosage (300 mg/kg). Although the crystallinity was not affected by either the enzyme dosage or incubation time, the interaction effects were significant.

During the pilot phase of the study, the enzymes were tested on a different wood species and successfully reduced viscosity. This suggests that the enzymes are versatile regarding wood species. The use of the enzyme during the bleaching process showed that there was a potential for chemical savings due to the reduction in both ozone and peroxide charges. The reduction in ozone charge, based on the viscosity drop, was only applicable to the NS 51179 enzyme and not the Ecopulp R enzyme. A reduction in peroxide usage, based on the viscosity drop, was observed in the application of both enzymes, but was more significant for the Ecopulp R-treated pulp. It was observed that a high ISO brightness and BWI index was still obtained for the pulp, despite the low peroxide charge. Furthermore, due to these chemical reductions there is a potential for economic benefits. The Kappa numbers of the pulps at the

ozone and peroxide stages, suggested that there was some boosting effect associated with the use of EG, regarding the removal of lignin.

The overall yields agreed with commonly achieved yields, and the copper numbers and S10 values were within specification, suggesting that the enzymes, when incorporated into a bleaching process do not excessively damage the cellulose. Furthermore, the reactivity of the post-oxygen pulps compared to the fully bleached pulps suggested that there was no retention of the reactivity benefits gained before bleaching.

5.2 Recommendations

The operating conditions of the mill differed from the optimum conditions of the enzymes as determined through the assay. Therefore, a full analysis of the enzymes to entirely understand the limitations of the enzymes is recommended.

As was seen during the incorporation of the enzymes in a bleaching sequence, there were major brightness benefits. To take full advantage of the enzymes in the bleaching process, an investigation of the effect the enzymes have on pulp brightness is recommended.

The reactivity was not improved through the treatment with the enzymes, mainly because the pulp still contained residual lignin.

The enzymes were observed to be versatile in reducing the viscosity of DWP obtained from different wood species. However, further studies on different wood species could confirm this, and optimise the dosage for different wood species.

The reduction in ozone, based on the viscosity drop, was only applicable to the NS 51179 enzyme and therefore a further investigation of the effects of the enzymes on the ozone stage is recommended to further understand the influence of the enzymes on other bleaching agents.

Additionally, an exciting observation was made regarding the ISO brightness and BWI of the fully bleached pulp. Despite the low peroxide charge, the pulp still attained a high ISO brightness and BWI. Therefore, a further investigation of the effects of the enzymatic treatments on the peroxide stage is recommended to fully comprehend the influence of the enzymes on the pulp to attain such brightness levels.

Appendix

Raw data of the high-level enzyme assay

Table 1: Raw data of the peak areas of the enzyme assay for temperature profile (the data below are for all un-replicated data points)

Sample name	Temperature (°C)	Peak areas	
		Glucose concentration (mg/l)	Cellobiose concentration (mg/l)
Ecopulp R R1	25	1.50	17.67
Ecopulp R Blank	25	0.66	0.00
Ecopulp R Control	25	0.72	0.00
NS 51179 R1	25	0.66	4.45
NS 51179 Blank	25	0.64	0.00
NS 51179 Control	25	0.53	4.16
Ecopulp R R1	35	2.29	17.28
Ecopulp R Blank	35	0.50	0.00
Ecopulp R Control	35	0.69	0.00
NS 51179 R1	35	0.64	4.99
NS 51179 Blank	35	0.55	0.00
NS 51179 Control	35	0.43	4.25
Ecopulp R R1	45	5.35	30.43
Ecopulp R Blank	45	0.48	0.00
Ecopulp R Control	45	1.00	0.00
NS 51179 R1	45	0.78	38.84
NS 51179 Blank	45	0.63	0.00
NS 51179 Control	45	0.54	0.00
Ecopulp R R1	55	3.44	19.82
Ecopulp R Blank	55	0.70	0.00
Ecopulp R Control	55	0.68	0.00
NS 51179 R1	55	0.67	9.61
NS 51179 Blank	55	0.58	0.00
NS 51179 Control	55	0.46	0.00
Ecopulp R R1	65	6.06	31.85
Ecopulp R Blank	65	0.76	0.00
Ecopulp R Control	65	1.00	0.00
NS 51179 R1	65	0.83	35.39
NS 51179 Blank	65	0.60	0.00
NS 51179 Control	65	0.56	0.00
Ecopulp R R1	75	1.11	21.57
Ecopulp R Blank	75	0.60	0.00

Ecopulp R Control	75	0.70	0.00
NS 51179 R1	75	0.64	3.66
NS 51179 Blank	75	0.60	0.00
NS 51179 Control	75	0.46	0.00
Ecopulp R R1	85	1.12	17.43
Ecopulp R Blank	85	1.17	0.00
Ecopulp R Control	85	1.07	0.00
NS 51179 R1	85	0.58	0.00
NS 51179 Blank	85	0.65	0.00
NS 51179 Control	85	0.55	0.00

Table 2: Raw data of the temperature profile for the enzyme assays (R1 represents the first replicate, R2 represents the second replicate, while R3 represents the third replicate)

Sample name	Temperature (°C)	Peak areas	
		Glucose concentration (mg/l)	Cellobiose concentration (mg/l)
Ecopulp R R1	45	7.55	54.78
Ecopulp R R2	45	7.48	61.46
Ecopulp R R3	45	9.08	61.09
Ecopulp R Blank	45	1.07	Below detection limit
Ecopulp R Control	45	1.53	9.98
NS 51179 R1	45	1.37	35.15
NS 51179 R2	45	Below detection limit	36.37
NS 51179 R3	45	Below detection limit	34.08
NS 51179 Blank	45	1.49	Below detection limit
NS 51179 Control	45	Below detection limit	10.11
Ecopulp R R1	50	2.96	24.41
Ecopulp R Control	50	2.99	Below detection limit
Ecopulp R Blank	50	Below detection limit	Below detection limit
Ecopulp R R3	50	2.87	23.11
Ecopulp R R2	50	2.99	24.72
NS 51179 Blank	50	Below detection limit	Below detection limit
NS 51179 Control	50	Below detection limit	Below detection limit

NS 51179 R3	50	Below detection limit	16.29
NS 51179 R2	50	Below detection limit	17.26
NS 51179 R1	50	Below detection limit	15.62
NS 51179 R1	55	Below detection limit	10.07
NS 51179 R2	55	Below detection limit	10.35
NS 51179 R3	55	Below detection limit	9.73
NS 51179 Control	55	Below detection limit	Below detection limit
NS 51179 Blank	55	Below detection limit	4.45
Ecopulp R R1	55	4.52	27.18
Ecopulp R R2	55	4.21	25.61
Ecopulp R R3	55	4.27	27.92
Ecopulp R Control	55	1.15	Below detection limit
Ecopulp R Blank	55	Below detection limit	Below detection limit
Ecopulp R R1	60	1.92	16.55
Ecopulp R R2	60	5.21	23.85
Ecopulp R Control	60	1.46	Below detection limit
Ecopulp R Blank	60	Below detection limit	Below detection limit
Ecopulp R R3	60	4.23	31.82
NS 51179 R1	60	Below detection limit	15.43
NS 51179 R2	60	Below detection limit	13.73
NS 51179 R3	60	Below detection limit	15.22
NS 51179 Control	60	Below detection limit	9.04
NS 51179 Blank	60	1.03	Below detection limit
Ecopulp R Control	65	3.12	Below detection limit
Ecopulp R Blank	65	Below detection limit	Below detection limit
Ecopulp R R3	65	3.14	25.03
Ecopulp R R2	65	3.08	26.35
Ecopulp R R1	65	2.66	25.33

NS 51179 Control	65	Below detection limit	Below detection limit
NS 51179 Blank	65	Below detection limit	Below detection limit
NS 51179 R3	65	Below detection limit	17.01
NS 51179 R2	65	Below detection limit	16.33
NS 51179 R1	65	Below detection limit	16.61

Table 3: Data of the peak areas for the enzyme assay of the pH-profile (R1 refers to the first replicate, R2 refers to the second replicate and R3 to the third replicate)

Sample name	pH	Peak areas	
		Glucose concentration (mg/l)	Cellobiose concentration (mg/l)
NS 51179 R1	3	Below detection limit	59.53
NS 51179 R2	3	Below detection limit	58.00
NS 51179 R3	3	Below detection limit	57.79
NS 51179 Control	3	Below detection limit	Below detection limit
NS 51179 Blank	3	Below detection limit	Below detection limit
Ecopulp R R1	3	53.00	189.46
Ecopulp R R2	3	52.00	184.95
Ecopulp R R3	3	52.42	164.75
Ecopulp R Control	3	1.59	Below detection limit
Ecopulp R Blank	3	Below detection limit	Below detection limit
NS 51179 R1	4	Below detection limit	55.84
NS 51179 R2	4	Below detection limit	61.35
NS 51179 R3	4	Below detection limit	64.53
NS 51179 Control	4	Below detection limit	Below detection limit
NS 51179 Blank	4	Below detection limit	Below detection limit
Ecopulp R R1	4	50.63	138.20
Ecopulp R R2	4	47.87	136.75
Ecopulp R R3	4	47.29	131.13

Ecopulp R Control	4	2.32	Below detection limit
Ecopulp R Blank	4	0	Below detection limit
NS 51179 R1	5	Below detection limit	53.34
NS 51179 R2	5	Below detection limit	57.01
NS 51179 R3	5	Below detection limit	53.23
NS 51179 Control	5	Below detection limit	Below detection limit
NS 51179 Blank	5	Below detection limit	Below detection limit
Ecopulp R R1	5	42.96	117.2514909
Ecopulp R R2	5	44.68	126.524201
Ecopulp R R3	5	46.32	124.0045696
Ecopulp R Control	5	1.57	Below detection limit
Ecopulp R Blank	5	Below detection limit	Below detection limit
NS 51179 R1	6	Below detection limit	43.29
NS 51179 R2	6	Below detection limit	44.70
NS 51179 R3	6	Below detection limit	43.70
NS 51179 Control	6	Below detection limit	Below detection limit
NS 51179 Blank	6	Below detection limit	Below detection limit
Ecopulp R R1	6	29.93	84.75
Ecopulp R R2	6	31.31	82.26
Ecopulp R R3	6	40.62	105.08
Ecopulp R Control	6	1.83	Below detection limit
Ecopulp R Blank	6	Below detection limit	Below detection limit
NS 51179 R1	7	Below detection limit	45.67
NS 51179 R2	7	Below detection limit	43.68
NS 51179 R3	7	Below detection limit	44.71
NS 51179 Control	7	Below detection limit	Below detection limit

NS 51179 Blank	7	Below detection limit	Below detection limit
Ecopulp R R1	7	4.67	170.97
Ecopulp R R2	7	4.55	137.30
Ecopulp R R3	7	4.25	169.44
Ecopulp R Control	7	Below detection limit	Below detection limit
Ecopulp R Blank	7	0.15	Below detection limit
NS 51179 R1	8	Below detection limit	15.86
NS 51179 R2	8	Below detection limit	12.82
NS 51179 R3	8	Below detection limit	13.49
NS 51179 Control	8	Below detection limit	Below detection limit
NS 51179 Blank	8	Below detection limit	Below detection limit
Ecopulp R R1	8	5.85	28.23
Ecopulp R R2	8	9.63	37.86
Ecopulp R R3	8	6.64	30.18
Ecopulp R Control	8	1.78	Below detection limit
Ecopulp R Blank	8	Below detection limit	Below detection limit

Data of the first experiment

Table 4: Data of the Ecopulp R enzyme for the first un-replicated experiment with high dosages and long incubation times

Enzyme formulation	Enzyme dosage (mg/kg)	Incubation time (h)	Viscosity (ml/g)	Berger whiteness index (%)	S10 (%)	Crystallinity (%)
Control	0	1	779	20.6	5.4	82.4
Control	0	2	725	20.2	5.0	80.0
Control	0	3	756	20.1	5.0	79.7
Control	0	4	785	21.2	5.4	80.0
Control	0	6	775	20.4	5.0	81.0
Control	0	24	781	20.3	4.6	80.4
Ecopulp R	10	1	682	20.1	5.3	79.1
Ecopulp R	10	2	723	19.8	4.7	80.6
Ecopulp R	10	3	762	19.8	5.5	79.9
Ecopulp R	10	4	663	20.1	5.0	80.2
Ecopulp R	10	6	717	20.4	5.2	80.5
Ecopulp R	10	24	490	20.1	5.8	80.0
Ecopulp R	30	1	753	19.4	4.9	79.3
Ecopulp R	30	2	705	20.8	5.5	79.5
Ecopulp R	30	3	729	20.4	5.5	80.0
Ecopulp R	30	4	760	19.9	5.3	79.8
Ecopulp R	30	6	681	19.6	5.7	79.7
Ecopulp R	30	24	612	18.7	6.4	81.3
Ecopulp R	50	1	730	18.8	5.3	80.7
Ecopulp R	50	2	662	19.2	6.6	80.1
Ecopulp R	50	3	693	19.8	6.0	81.2
Ecopulp R	50	4	671	19.3	5.4	80.1
Ecopulp R	50	6	713	19.6	5.3	80.6
Ecopulp R	50	24	646	16.5	5.4	80.3
Ecopulp R	100	1	712	17.1	5.7	79.6
Ecopulp R	100	2	591	17.8	6.4	81.1
Ecopulp R	100	3	669	19.7	5.4	80.8
Ecopulp R	100	4	531	20.1	6.0	80.2
Ecopulp R	100	6	597	20.1	6.5	80.0

Ecopulp R	100	24	604	20.4	6.8	81.0
Ecopulp R	150	1	682	19.6	6.4	80.7
Ecopulp R	150	2	740	17.1	5.1	79.6
Ecopulp R	150	3	695	20.8	5.9	80.6
Ecopulp R	150	4	678	19.4	5.3	80.0
Ecopulp R	150	6	744	20.8	5.4	80.5
Ecopulp R	150	24	683	19.8	5.6	80.4
Ecopulp R	300	1	512	18.9	7.8	81.0
Ecopulp R	300	2	595	19.2	6.9	79.4
Ecopulp R	300	3	598	18.6	6.7	80.6
Ecopulp R	300	4	654	20.3	6.0	79.8
Ecopulp R	300	6	641	20.1	6.5	79.4
Ecopulp R	300	24	468	20.0	8.1	80.9
Ecopulp R	500	1	579	19.7	6.5	80.1
Ecopulp R	500	2	583	18.6	6.9	79.6
Ecopulp R	500	3	536	20.4	7.2	81.3
Ecopulp R	500	4	569	18.9	6.7	80.2
Ecopulp R	500	6	518	18.2	7.5	81.3
Ecopulp R	500	24	565	18.4	4.8	79.9
Ecopulp R	1000	1	382	19.3	8.9	80.6
Ecopulp R	1000	2	496	19.5	7.8	79.6
Ecopulp R	1000	3	547	19.6	7.6	80.8
Ecopulp R	1000	4	412	20.4	8.1	80.5
Ecopulp R	1000	6	466	19.5	8.6	81.7
Ecopulp R	1000	24	507	17.9	7.9	80.9

Table 5: Data of the NS 51179 enzyme for the first un-replicated experiment with high dosages and long incubation times

Enzyme formulation	Enzyme dosage (mg/kg)	Incubation time (h)	Viscosity (ml/g)	Berger whiteness index (%)	S10 (%)	Crystallinity (%)
Control	0	1	779	20.6	5.4	82.4
Control	0	2	725	20.2	5.0	80.0
Control	0	3	756	20.1	5.0	79.7
Control	0	4	785	21.2	5.4	80.0
Control	0	6	775	20.4	5.0	81.0

Control	0	24	781	20.3	4.6	80.4
Ns 51179	10	1	741	18.7	5.3	81.0
Ns 51179	10	2	738	19.3	4.7	79.4
Ns 51179	10	3	762	20.1	5.1	80.0
Ns 51179	10	4	757	20.3	4.8	80.2
Ns 51179	10	6	673	20.6	5.1	80.2
Ns 51179	10	24	733	20.2	4.9	80.3
Ns 51179	30	1	634	20.1	4.5	79.8
Ns 51179	30	2	741	20.0	5.1	78.9
Ns 51179	30	3	663	19.4	4.9	80.3
Ns 51179	30	4	754	20.0	5.1	80.5
Ns 51179	30	6	759	20.9	5.0	80.6
Ns 51179	30	24	746	20.5	5.9	80.9
Ns 51179	50	1	754	20.0	5.4	79.0
Ns 51179	50	2	720	20.0	5.8	80.2
Ns 51179	50	3	739	19.0	4.8	79.4
Ns 51179	50	4	707	19.7	5.0	80.8
Ns 51179	50	6	695	20.4	5.6	79.2
Ns 51179	50	24	687	20.1	6.0	80.5
Ns 51179	100	1	709	18.9	5.3	80.1
Ns 51179	100	2	720	18.9	5.8	79.7
Ns 51179	100	3	650	20.4	5.3	80.7
Ns 51179	100	4	728	19.2	5.2	80.0
Ns 51179	100	6	590	20.9	6.5	80.4
Ns 51179	100	24	691	20.2	6.0	79.6
Ns 51179	150	1	729	19.5	4.8	80.3
Ns 51179	150	2	755	19.5	5.0	78.6
Ns 51179	150	3	642	20.3	5.9	79.9
Ns 51179	150	4	710	20.1	5.3	79.9
Ns 51179	150	6	750	20.0	4.9	80.0
Ns 51179	150	24	685	20.7	5.9	79.7
Ns 51179	300	1	668	19.5	6.3	79.6
Ns 51179	300	2	657	19.6	5.7	80.1
Ns 51179	300	3	672	20.1	5.4	80.3
Ns 51179	300	4	643	19.9	5.7	79.8
Ns 51179	300	6	601	19.9	5.9	80.1
Ns 51179	300	24	550	19.6	6.3	81.2
Ns 51179	500	1	577	19.6	6.7	80.8
Ns 51179	500	2	578	19.8	6.5	81.0
Ns 51179	500	3	619	20.0	6.2	79.3
Ns 51179	500	4	511	19.6	6.2	79.9
Ns 51179	500	6	724	20.2	5.8	80.5
Ns 51179	500	24	588	20.0	6.4	79.6
Ns 51179	1000	1	540	17.6	7.5	79.9

Ns 51179	1000	2	507	18.2	7.3	80.9
Ns 51179	1000	3	616	20.0	6.3	79.7
Ns 51179	1000	4	504	19.2	7.7	81.8
Ns 51179	1000	6	548	19.4	7.4	80.4
Ns 51179	1000	24	451	20.7	7.7	82.6

Data of the second experiment

Table 6: Data of the NS 51179 enzyme for replicated experiment, with low dosages and short incubation times

Enzyme dosage (mg/kg)	Incubation time (h)	Berger whiteness index (%)	Viscosity (ml/g)	S10 (%)	S18 (%)	Degraded cellulose (%)	Crystallinity (%)
50	1	21.4	706	5.8	3.4	2.4	70.2
100	1	22.6	735	5.3	3.2	2.2	72.4
150	1	21.7	754	5.7	3.2	2.5	73.3
200	1	21.5	714	5.9	3.2	2.8	74.8
250	1	21.3	690	6.0	3.6	2.4	73.5
300	1	21.4	699	6.3	3.7	2.6	70.3
0	1	22.3	683	5.7	3.7	2.0	73.4
50	2	21.7	717	5.9	3.5	2.4	72.9
100	2	20.6	713	5.5	3.7	1.9	72.5
150	2	21.0	654	5.9	3.6	2.3	71.4
200	2	21.1	740	5.2	3.2	2.0	72.6
250	2	21.4	724	5.7	3.2	2.6	74.3
300	2	21.6	714	5.7	3.3	2.4	73.4
0	2	21.1	731	5.4	3.5	1.9	72.3
50	1	20.7	757	5.4	3.1	2.2	73.3
100	1	20.2	699	5.4	3.6	1.9	73.0
150	1	20.2	727	5.5	3.3	2.2	72.7
200	1	20.1	680	5.3	3.4	1.9	72.7
250	1	20.2	683	5.5	3.5	1.9	71.6
300	1	20.5	685	5.4	3.5	1.9	72.2
0	1	20.7	723	5.4	3.2	2.2	73.0
50	2	20.8	797	5.4	3.3	2.1	71.7
100	2	20.4	767	5.5	3.2	2.4	69.5
150	2	17.6	732	5.4	3.3	2.1	70.2
200	2	18.4	759	5.5	3.2	2.3	73.0
250	2	18.6	687	6.0	3.5	2.5	72.6
300	2	18.5	665	5.9	3.3	2.6	71.1
0	2	18.1	677	5.6	3.4	2.2	73.5
50	1	20.5	691	5.7	3.4	2.4	70.3
100	1	20.2	685	5.7	3.3	2.4	73.8
150	1	20.1	713	5.8	3.3	2.5	74.2
200	1	19.1	729	5.8	3.5	2.3	71.6
300	2	20.0		5.6	3.4	2.2	71.2
300	1	20.6	733	5.8	3.6	2.2	72.0
0	1	20.5		5.5	3.5	2.0	72.7
50	2	21.1	728	5.1	3.6	1.5	71.5
100	2	21.3	729	5.1	3.4	1.7	71.8
150	2	21.5	729	5.2	3.5	1.7	71.5
200	2	21.4	700	5.3	3.4	1.9	70.7
250	2	21.8	723	5.1	3.4	1.7	70.6

Data of the third experiment

Table 7: Data of the Ecopulp R enzyme for the replicated experiment, with low dosages and short incubation times

Enzyme dosage (mg/kg)	Incubation time (h)	Viscosity (ml/g)	Berger whiteness index (%)	S10 (%)	S18 (%)	Degraded cellulose (%)	Crystallinity (%)
200	2	748	14.4	5.7	3.5	2.2	78.5
0	1	779	13.0	5.3	3.6	1.8	74.2
100	2	779	11.8	5.4	3.6	1.9	76.2
250	2	680	12.4	6.3	3.7	2.5	75.2
150	1	753	13.0	5.9	3.6	2.2	82.0
0	2	753	12.1	5.7	3.4	2.3	76.5
50	2	745	13.3	5.6	3.0	2.6	77.8
300	1	749	12.8	5.5	3.1	2.4	77.3
0	1	771	13.7	5.4	3.2	2.3	74.6
150	1	756	13.0	5.4	3.0	2.4	75.2
150	2	719	13.2	5.4	3.2	2.2	76.5
100	2	737	13.3	5.4	3.7	1.7	76.0
300	2	722	13.7	5.6	2.8	2.8	75.3
150	1	757	13.3	5.3	3.2	2.1	76.6
100	1	766	13.5	5.3	3.4	1.9	77.3
250	1	741	14.3	5.4	3.3	2.2	76.1
50	2	762	11.6	5.6	3.3	2.3	76.0
50	1	765	13.0	5.4	3.6	1.8	73.3
0	2	761	13.7	5.2	3.6	1.7	75.3
50	2	781	13.0	5.7	3.5	2.2	76.1
200	1	757	13.3	5.5	3.2	2.2	75.0
200	1	757	13.8	5.6	3.3	2.3	75.7
300	1	719	13.4	6.0	3.2	2.8	76.2
300	1	729	12.7	5.6	3.1	2.4	76.1
100	1	768	13.9	5.4	3.3	2.1	76.9
200	2	740	13.5	5.5	3.3	2.2	76.6
150	2	705	13.2	6.0	3.6	2.3	74.0
250	1	732	13.7	5.8	3.3	2.5	74.5
300	2	685	13.3	5.9	3.3	2.6	75.0
150	2	726	14.0	5.5	3.3	2.2	75.0
0	2	767	12.6	5.2	3.5	1.7	76.6
100	1	745	13.9	5.3	3.7	1.6	76.4
200	1	723	13.6	5.2	3.4	1.8	76.3
100	2	748	12.3	5.4	3.5	1.8	74.9
50	1	758	13.8	5.0	3.5	1.5	76.5
250	1	729	13.9	5.5	3.2	2.3	76.4
0	1	745	13.6	5.2	3.1	2.1	74.1
250	2	673	12.2	5.9	3.6	2.4	76.2
300	2	718	11.7	5.8	3.5	2.3	76.8
50	1	750	13.9	5.2	3.5	1.7	75.1

200	2	690	12.4	5.4	3.5	1.9	75.8
250	2	691	13.3	4.9	3.5	1.4	76.2

Data for the pilot-scale phase

Table 8: Enzyme treatment stage

Bleach sequence	X	X
Mass pulp _{OD} (g)	2000.0	2000.0
Consistency	9.00	9.00
Total weight	22222.2	22222.2
Mass pulp _{wet} (g)	6589.8	6945.7
Water (ml)	15632.4	15276.6
Enzyme name	Ecopulp R	NS 51179
Temperature (°C)	60.0	60.0
Time (minutes)	120	120
Mass out (g) / O.D.	6636.1	6456.9
Consistency (%)	30.00	30.79
Final pH before deactivation	6.11	6.80
Final pH after deactivation	11.61	11.09
Mass bag / no tag (g)	95.2	95.2
Mass bag and pulp (g)	6731.3	6552.1
Mass pulp (g)	6636.1	6456.9
Yield (%)	99.5	99.4
NOTE:	300ml NaOH was added for enzyme deactivation	

Table 9: Data for the first chlorine dioxide (D₀) stage

Bleaching Sequence	D ₀	D ₀	D ₀
Enzyme Name	Control pulp	Ecopulp R	NS 51179
Mass pulp _{OD} (g)	1900.0	1800.0	1800.0
Consistency	10.0	10.0	10.0
Total Weight	19000.0	18000.0	18000.0
Mass pulp _{wet} (g)	5830.0	6000.0	5846.1
Mass Out (g) / O.D.	5976.6	5844.4	5771.5
Consistency (%)	31.37	30.49	30.80
pH Adjustment			
pH Adjustment Target	2.0-2.5	2.0-2.5	2.0-2.5

Mass Bag / no tag (g)	95.2	95.2	95.2
Mass Bag and pulp (g)	6071.8	5939.6	5866.7
Mass pulp (g)	5976.6	5844.4	5771.5
Yield (%)	98.7	99.0	98.8

Table 10: Data for the ozone (Z) stage calibration

Bleach sequence	Z (high consistency)								
Enzyme name	Control pulp			Ecopulp R			NS 51179		
Mass pulp _{od} (g)	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Consistency	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00
Total Weight	142.9	142.9	142.9	142.9	142.9	142.9	142.9	142.9	142.9
Mass pulp _{wet} (g)	159.4	159.4	159.4	164.0	164.0	164.0	162.3	162.3	162.3
Chemical charge (%):									
Ozone / Aimed (%)	0.10	0.20	0.30	0.10	0.20	0.30	0.10	0.20	0.30
Ozone / Actual (%)	0.09	0.20	0.26	0.09	0.19	0.27	0.09	0.19	0.27
Ozone (Aimed) / g	0.05	0.10	0.15	0.05	0.10	0.15	0.05	0.10	0.15
Ozone (Actual) / g	0.047	0.10	0.13	0.05	0.10	0.14	0.05	0.09	0.14
Temperature (°C)	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0

Time (Minutes)	±10.0	±10.0	±10.0	±10.0	±10.0	±10.0	±10.0	±10.0	±10.0
Ozone charge / Actual (kg/t)	0.9	2.0	2.6	0.9	1.9	2.7	0.9	1.9	2.7
Ozone charge / Aimed (kg/t)	1.0	2.0	3.0	1.0	2.0	3.0	1.0	2.0	3.0
Viscosity / CED (ml/g)	573	548	514	544	478	453	463	443	423

Table 11: Data for the ozone (Z) stage at pilot-scale

Bleaching sequence	Z (high consistency)		
Enzyme name	Control pulp	Ecopulp R	NS 51179
Mass pulp_{OD} (g)	1500.0	1600.0	1600.0
Consistency	35.00	35.00	35.00
Total Weight	4285.7	4571.4	4571.4
Mass pulp_{Wet} (g)	4781.6	5247.6	5194.8
Chemical charge (%):			
Ozone / Aimed (%)	0.05	0.05	0.03
Ozone / Actual (%)	0.07	0.07	0.06
Ozone (Aimed) / g	0.75	0.80	0.48
Ozone (Actual) / g	1.10	1.10	0.90
Water (ml)	-495.9	-676.2	-623.4
Temperature (°C)	<25.0	<25.0	<25.0
Time (Minutes)	±10.0	±10.0	±10.0
Mass Out (g) / O.D.	5084.3	5114.1	5086.1
Consistency (%)	28.32	30.55	30.34
Mass Bag / no tag (g)	95.2	95.2	95.2
Mass Bag and pulp (g)	5179.5	5209.3	5181.3
Mass pulp (g)	5084.3	5114.1	5086.1
Yield %	96.0	97.6	96.4

Table 12: Data for the peroxide (E_p) stage calibration

Enzyme name	Control pulp				Ecopulp R				NS 51179			
Mass pulp _{OD} (g)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Consistency	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Total weight	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Mass pulp _{Wet} (g)	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Chemical charge (%):												
NaOH	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05
H ₂ O ₂	0.25	0.50	0.75	1.00	0.25	0.50	0.75	1.00	0.25	0.50	0.75	1.00
Chemical concentration (g/l):												
NaOH	109.59	109.59	109.59	109.59	109.59	109.59	109.59	109.59	109.59	109.59	109.59	109.59
H ₂ O ₂	29.93	29.93	29.93	29.93	29.93	29.93	29.93	29.93	29.93	29.93	29.93	29.93
Volume charge (ml)												
NaOH	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
H ₂ O ₂	0.42	0.84	1.25	1.67	0.42	0.84	1.25	1.67	0.42	0.84	1.25	1.67
Water	-0.9	-1.3	-1.7	-2.1	-0.9	-1.3	-1.7	-2.1	-0.9	-1.3	-1.7	-2.1
Temperature (°C)	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0
Time (minutes)	60	60	60	60	60	60	60	60	60	60	60	60

Table 13: Data for the peroxide (E_p) stage at bench-scale

Bleach sequence	EP		
Enzyme name	Control pulp	Ecopulp R	NS 51179
Mass pulp _{OD} (g)	100.00	100.00	100.00
Consistency	10.00	10.00	10.00
Total Weight	1000.00	1000.00	1000.00
Mass pulp _{Wet} (g)	353.11	327.33	329.60
Chemical charge (%):			
H ₂ O ₂	0.55	0.02	0.15
Chemical concentration (g/l):			
H ₂ O ₂	27.38	27.38	27.38
Volume charge (ml)			
H ₂ O ₂	20.09	0.73	5.48
Water	617.22	662.36	655.34
Temperature (°C)	80.00	80.00	80.00
Time (Minutes)	60.00	60.00	60.00
Mass Out (g) / O.D.	807.40	866.60	776.70
Consistency (%)	36.65	34.18	38.17
Final pH	11.55	11.56	11.55
Mass Bag / no tag (g)	95.20	59.60	59.60
Mass Bag and pulp (g)	902.60	926.20	836.30
Mass pulp (g)	807.40	866.60	776.70
Yield %	98.64	98.73	98.82
Done three times for each enzyme and the control pulp			

Table 14: Data for the second chlorine dioxide (D₁) stage at bench-scale

Bleach sequence	D ₁		
Enzyme name	Control pulp	Ecopulp R	NS 51179
Mass pulp _{OD} (g)	130.00	130.00	130.00
Consistency	10.00	10.00	10.00
Total Weight	1300.00	1300.00	1300.00
Mass pulp _{Wet} (g)	354.71	380.34	340.58
Chemical charge (%):			
Mass Out (g) / O.D.	743.10	724.00	692.00
Consistency (%)	34.58	35.47	37.18
Mass Bag / no tag (g)	59.60	59.60	59.60
Mass Bag and pulp (g)	802.70	783.60	751.60
Mass pulp (g)	743.10	724.00	692.00
Yield %	98.83	98.77	98.96
Done twice for each pulp to obtain a final pulp mass of 260 g dry pulp			

Cost evaluation data

Table 15: Enzyme costs obtained from literature

Case	Price	Price unit	Year	Exchange rate for the same year	Price corrected for South African currency (R/kg)
1	\$1.25	\$/kg	2011	7.26	9.07
2	\$5.07	\$/kg	2007	7.11	36.05
3	\$6.27	\$/kg	2010	7.30	45.77
4	\$10.14	\$/kg	2007	7.11	72.10
5	\$23.3	\$/kg	2013	9.62	224.15

Table 16: Enzyme costs adjusted for inflation from the year the costs were reported

Year	Inflation rate	Enzyme case 1	Enzyme case 2	Enzyme case 3	Enzyme case 4	Enzyme case 5
2007	0.0715	N/A	R 7.11	N/A	R 72.10	N/A
2008	0.1099	N/A	R 7.89	N/A	R 80.02	N/A
2009	0.0712	N/A	R 8.45	N/A	R 85.72	N/A
2010	0.0426	N/A	R 8.81	R 45.77	R 89.37	N/A
2011	0.0499	R 7.26	R 9.25	R 48.05	R 93.83	N/A
2012	0.0562	R 7.67	R 9.77	R 50.76	R 99.10	N/A
2013	0.0576	R 8.11	R 10.34	R 53.68	R 104.81	R 224.15
2014	0.0609	R 8.60	R 10.97	R 56.95	R 111.19	R 237.80
2015	0.0458	R 8.99	R 11.47	R 59.56	R 116.28	R 248.69
2016	0.0634	R 9.56	R 12.19	R 63.33	R 123.66	R 264.45
2017	0.0527	R 10.07	R 12.84	R 66.67	R 130.17	R 278.39
2018	0.0462	R 10.53	R 13.43	R 69.75	R 136.19	R 291.25
2019	0.0413	R 10.97	R 13.99	R 72.63	R 141.81	R 303.28
2020	0.0243	R 11.24	R 14.33	R 74.40	R 145.26	R 310.65