EVALUATION OF CELL-WALL MODIFYING ENZYMES FOR IMPROVED REFINING OF PULP FROM TWO *EUCALYPTUS* SPECIES

by

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DECLARATION

I the undersigned hereby declare that the thesis submitted herewith for the degree *Magister Scientae* to the University of the Free State, is my own independent work and that I have not previously submitted the same work for a qualification at another University."

Crystal Steel
November 2010

Luk 1:37 For with God nothing shall be impossible.

CONTENTS

ABBREVIATIONS

μg/ml	micrograms per millilitre
ba	bar angle
bh	bar height
BKP	bleached kraft pulp
bw	bar width
BCP	bleached chemical pulp
CBH	cellobiohydrolase
CBHI	cellobiohydrolase one
CBHII	cellobiohydrolase two
Cellu	cellulase
cm	centimetres
CMC	carboxymethylcellulose
CTMP	chemi-thermo mechanical pulp
D	dried
DHW	dried hardwood
DSW	dried softwood
DNS	dinitrosalicylic acid
EG	endoglucanase
FPU/ml	filter paper units per millilitre
glu	glucose
gw	groove width
g/kg	grams per kilograms
g/l	grams per litre
g/mol	grams per mol
g/m^2	grams per metre square
HW	hardwood
Hemicellu	hemicellulase
Hz	hertz
IU/ml	International units per millilitre
KP	kraft pulp
kN/m	kilo Newton per metre
kWh/t	kilo watt hours per tonne
L	litre
LS	longitudinal section
man	mannose
MD	machine direction
min	minute
mm	millimetre
MP	mechanical pulp
ml/t	millilitres per tonne
n.a.	not applicable
N/cm	Newton per centimetre
N-D	never-dried
nm	nanometre
NR	non reducing

pp	page
R	reducing
Ref	refined
Ref MP	refined mechanical pulp
SW	softwood
TMP	thermo mechanical pulp
TS	transverse section
UBKP	unbleached kraft pulp
UBKP	unbleached kraft pulp
WRV	water retention value
ws/m	watt second per metre
xyl	xylose

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PREFACE

The pulp and paper industry has grown successfully in South Africa, from where large companies such as Sappi and Mondi have expanded into the international arena. It is, therefore, important to make sure that the manufacturing of pulp and paper utilises the latest technology to remain competitive in the global market. One of the areas that received significant interest is the improvement of the refining of wood pulps by using enzyme technologies. This dissertation deals with an investigation of commercial enzymes for application in the pulp and paper industry, with the aim of improving the refining efficiency in paper mills.

Literature research was conducted to understand the properties of wood pulps and the enzymes available to modify different wood pulps as well as the application of cellulases and hemicellulases on softwoods and hardwoods (Chapter 1). Literature showed that the application of cellulases to dried and never-dried *Eucalyptus globulus* pulps gave different results; for example, an endoglucanase caused the tear strength of dried *E. globulus* pulps to increase, whilst it decreased tear strength on the never-dried *E. globulus* pulps (Garcia *et al*., 2002). It was also realised that the application of enzymes to *E. nitens* and *E. grandis* pulps has not previously been investigated.

The literature review indicated that cellulases showed more potential for application than hemicellulases. Chapter 2, therefore, deals with selected commercial cellulases that were characterised in terms of their pH and temperature profiles. The effect of over-dosing or an extended incubation time on fibre properties were investigated. This investigation of the commercial enzymes was designed to select enzymes for fibre modification on pilot scale.

The *E. grandis* and *E. nitens* pulps were of interest to the present study due to the predominant utilisation of these two species in pulp and paper mills in South Africa. Integrated paper mills generally utilise never-dried pulps to manufacture paper, whilst other paper mills tend to use dried pulps. Drying causes an irreversible bonding within the pulp fibres called hornification. Hornification occurs when water is removed from

the fibres, which causes a decrease in the number of hydrogen bonds formed between the fibrils and the water molecules (Abubakr *et al*., 1996). Therefore, dried pulp fibres have a reduced ability to swell and participate in surface bonding than never-dried pulps (Sutjipto *et al*., 2008). In Chapters 3 and 4 an investigation of selected enzymes on the dried and never-dried *E. grandis* and *E. nitens* pulps before refining on pilot-scale is reported. As seen before with *E. globulus* (Garcia *et al*., 2002), the different cellulases had different effects on the dried and never-dried fibres of the two *Eucalyptus* pulps. The cellulases all had potential of either saving on refining energy or improving the strength properties.

An opportunity was presented to investigate an enzyme application on commercial scale and Chapter 5 describes a mill trial conducted on a paper machine that used *E. nitens* pulp in it's furnish. Improvement of the paper-machine profitability required an optimisation in the refining efficiency. Therefore, Novozyme 476 was chosen for application to the *E. nitens* pulp. The successful completion of this trial also concluded the preliminary work on the enzymatic refining of hardwood. Future work will include the permanent application of this enzyme at the paper machine investigated and others.

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CHAPTER 1

LITERATURE REVIEW:

THE INFLUENCE OF REFINING AND CELL-WALL MODIFYING ENYZMES ON DRAINAGE AND PAPER PROPERTIES OF DIFFERENT WOOD PULPS

Sappi Enstra mill, Springs, South Africa.

ABSTRACT

Wood pulps must be refined to produce a specific paper grade and selected pulps are refined according to the nature of their fibres. Softwoods have longer fibres than hardwoods and need larger bars on the refining plates and more energy to refine, whilst dried fibres are easier to refine due to their amorphous cellulose content. Refining uses a large amount of energy and capital input for equipment maintenance and plate replacement. Wood fibres can be pre-treated chemically, mechanically or enzymatically prior to refining to reduce the refining energy on the pulps and/or improve drainage and strength properties. Chemical pre-treatment is used to remove lignin and extractives, thus ensuring efficient exposure of the cellulose and hemicellulose to refining. Mechanical treatment includes pulping and other abrasive devices that are not as efficient as they all require additional energy. Cell-wall modifying enzymes such as the cellulases and hemicellulases can assist by opening up the crystalline and amorphous cellulose and hemicellulose chains in the fibres, thereby making the fibres easier to refine. The application of enzymes on the different pulps requires a similar approach to the refining of different pulps, in that a cell-wall modifying enzyme should be matched to the pulp it is expected to improve.

INTRODUCTION

In the pulp and paper industry, refining forms an integral part in the preparation of papermaking stock, which in turn forms the interface between the pulp mill and the paper machine. Cellulosic pulp goes through two types of refining stages in the pulp and paper mill, the first is associated with fibre separation and the other is developing fibres for stock preparation, the latter being the main focus of this review.

Cellulose forms the framework of wood cell walls in the form of microfibres, whereas hemicelluloses and other carbohydrates form the matrix substances (Sixta, 2006). Cell-wall modifying enzymes have the ability to open up the crystalline and amorphous cellulose and hemicellulose chains in the fibres, thus making the fibres more amenable to refining or can altogether remove the need for refining (Bhardwaj *et al*., 1995; Clark *et al*., 1997; Dienes *et al*., 2004). Commercial cellulases and hemicellulases are characterised and then selected for application on dried and never-dried softwoods and hardwoods prior to beating or refining, which have resulted in energy savings and improvements in drainage and strength properties of pulp and paper (Bhardwaj *et al.*, 1995; Mansfield *et al*., 1996). The cellulases and hemicellulases modify compounds in the cell-wall of the fibres to produce higher quality pulp and paper (Lumme *et al*., 1999). By combining enzyme treatments with a fibre refiner, the development of fibres is more energy efficient and drainage and strength properties of paper are improved (Dickson *et al*., 2000; García *et al*., 2002). A suitable laboratory scale beating device and a selection of cellulases and hemicellulases is required for the investigation of these enzymes on the development of softwood and hardwood pulps for paper production.

The aim of this chapter is to provide a background of information on the wood components, the refining processes in the pulp and paper industry and a review of the work done in the application of the cellulases and hemicellulases on pulps.

WOOD STRUCTURE AND MORPHOLOGY

Cellulose forms the framework of wood cell walls in the form of microfibres, whereas hemicelluloses and other carbohydrates form the matrix substances and lignin is incrusted in the microcapillary regions of the cell wall (Sixta, 2006). The fibrillar structure of the cellulose begins with the elementary fibrils, which are 3.5 to 35 nm in diameter and consist of chains of several thousand cellobiose units (Figure 1-1).

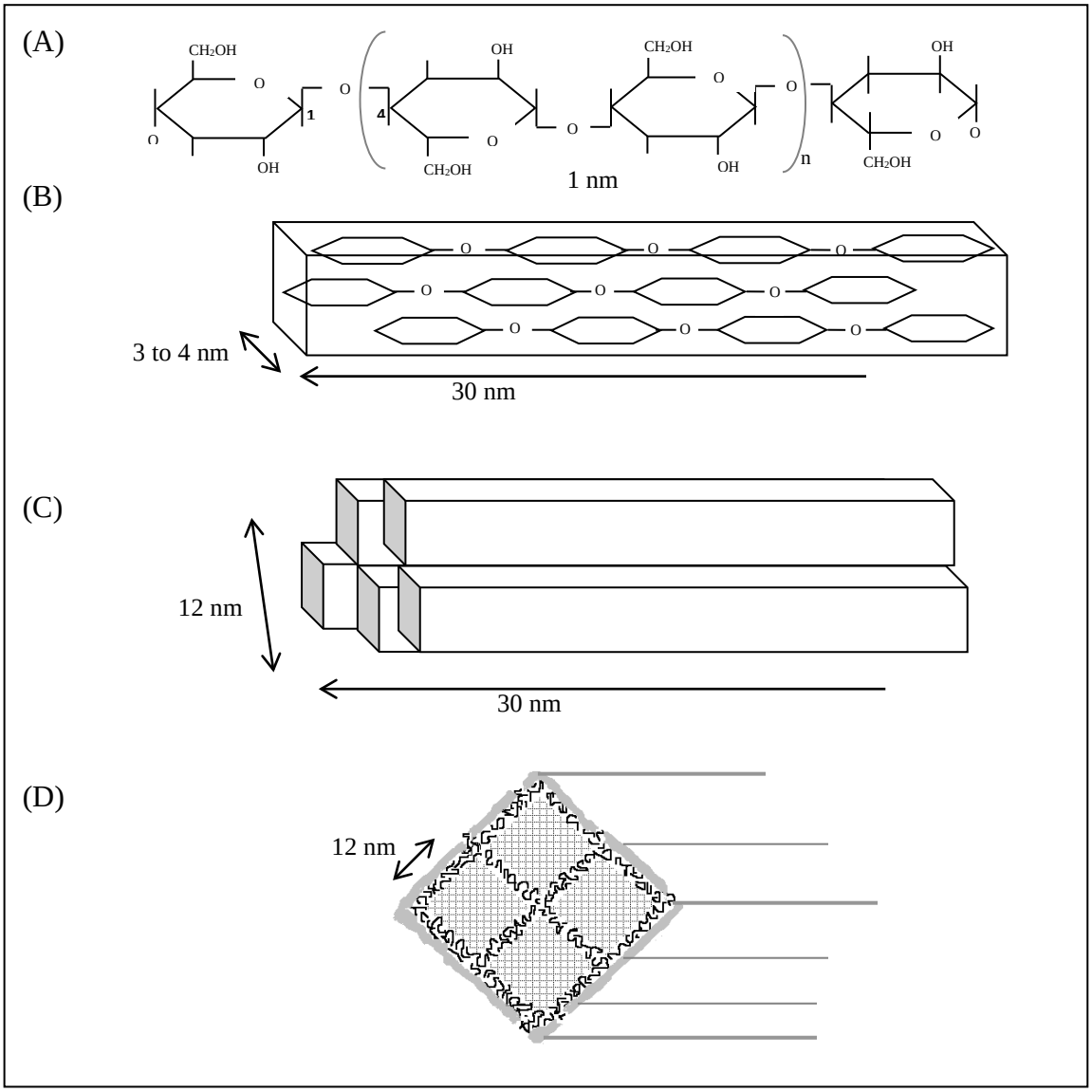

Figure 1-1 Chemical composition of the plant cell wall. (A) The cellulose backbone, cellobiose indicated in brackets and an indication of the length of the unit; (B) the diameter of the elementary fibril with cellulose chains; (C) microfibril dimensions; (D) macrofibril in cross section showing the microfibrils, hemicellulose and lignin matrix. (Adapted from Ramos, 2003).

Microfibrils (10 nm wide and 30 nm long) consist of about 40 to 60 chains (elementary fibrils) that are aligned parallel, linked by hydrogen bonds and surrounded by hemicellulose. Microfibrils then form the subunits of macrofibrils, which also contain lignin and hemicelluloses that have been deposited between the microfibrils (Figure 1-1). Hydrogen bonds are formed between the cellulose, lignin and hemicellulose components forming the basic structure of the cell walls (Uhlig, 1998).

The cell wall of wood consists of several layers, namely the middle lamella, primary wall and the secondary wall (Figure 1-2). The middle lamella functions as a cementing substance between cells and was formed from the division of a cambial initial where a daughter cell becomes separated from the equatorial plane by a thin tangential wall consisting primarily of pectic material. The deposition of hemicellulose and pectin by the wood cell on the middle lamella forms the primary wall. The primary wall consists of a loose aggregation of cellulose microfibrils that are twisted along the axis of the glucan chains and are stabilized by the hydrogen bonds between the chains. When the wood cell wall is near to its final thickness, the stiff secondary wall is deposited in three layers (S1, S2 and S3). S1 is closest to the primary layer, S2 is thicker than S1 and S3 is a thin wall that forms an interface with cytoplasm or the cell lumen in dead cells. S2 is the layer that contributes the most towards the physical and mechanical strength properties (Sixta, 2006). These layers differ from one another concerning their structure and chemical composition (Figure 1-2). The cell axis in which the microfibrils wind, the left (S-helix) or the right (Z-helix) differs between layers. The direction the microfibril winds will cause physical differences in the fibre. Fibres differ in length, thickness, cell wall thickness and vessel content depending on whether they came from a softwood or hardwood (Sjöström, 1993).

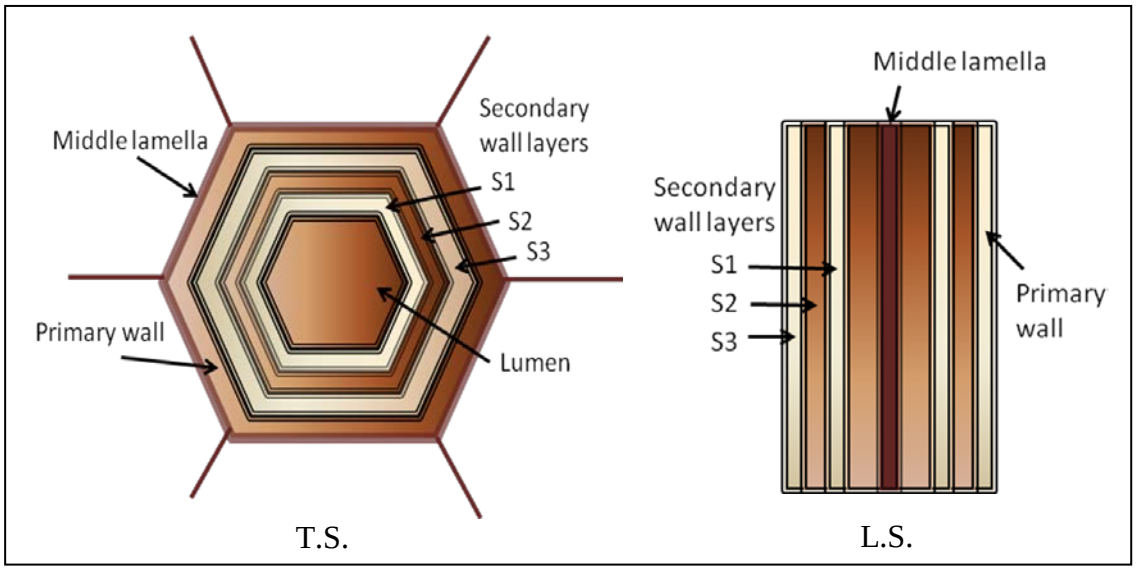

Figure 1-2 Layers of the cell wall in transverse section (T.S.) and longitudinal section (L.S.). The L.S. shows two adjacent cells (Adapted from Karlsson, 2006).

Softwood and hardwood

Both softwoods and the hardwoods provide good sources of fibre for the manufacture of various paper products. Softwood fibres generally have a larger diameter, whilst the hardwoods have fibres that are of a smaller diameter and yield a paper with a smoother texture. Paper products are made from softwood pulp, hardwood pulp and from mixtures hardwood and softwood pulp. Softwoods are evergreens with fibres that are on average about 3.6 mm in length, depending on the source, and can get to about 7.0 mm long. The anatomy of softwoods is characterized by longitudinal fibre tracheids (90 to 95%), ray cells (5 to 10%) and resin cells (0.5 to 1.0%). Softwoods are used for paper requiring higher strength properties because of their fibre length, whilst hardwoods are used for making smoother paper that prints well (Biermann, 1996).

The structure of hardwoods is slightly more complex than the softwood in that they have vessel elements (20 to 55%), fibre tracheids and libriform fibres (36 to 70%), ray cells (6 to 20%) and parenchyma cells (2%). The length of the hardwood fibres is about 0.9 to 1.5 mm, which contributes to a smoother paper of lower strength than paper from softwoods (Biermann, 1996). In the manufacture of higher quality printing papers, it is

desirable to use a large percentage of hardwood pulp because of its superior opacity, ability to reduce apparent density and improved formation (Olson and Lutz III, 1990). The vessel element cells are only found in hardwoods and are not fibrous in nature, and consequently do not add to the strength or quality of the paper. Vessel elements can be bleached and refined and mixed into the final product; However, when the large unbonded vessel elements collect on the surface of the paper they cause problems in printing. Starch and different refining methods have been utilised to try to lessen the number of the vessel elements or circumvent their effect, but the applications have had little improvements on the number and effect of the vessel elements (Olsen and Lutz III, 1990).

Composition of dried and never-dried fibres

Due to environmental concerns about paper waste and fibre shortage, recycling of paper has become routine for many paper mills. Fibres undergo irreversible changes in their structure when they are dried, as they tend to contain more regions that are amorphous and do not swell as well as virgin fibres. Another reason why dried fibres cannot swell is that they are unable to absorb as much water due to the removal of hydrogen bonds in the drying process. The amorphous phase of virgin fibres tend to be located at the microfibril surface and the crystalline phase is most represented at the core (Larsson *et al*., 1997). However, after drying, the amorphous regions may be located closer towards the core region. Hornification describes the irreversible fibre bonding that occurs during drying and refers to those fibres that also resist swelling (Abubakr *et al*., 1996).

WOOD PULP

The main elements of wood, namely cellulose, hemicellulose and lignin, give wood its structural characteristics. Cellulose is the most abundant, forming about 49% of the wood cell wall, whereas hemicellulose and lignin contribute about 20% each to the cell wall, and the remainder 10% of the constituents of the cell are extractives. The

composition is similar for both hardwoods and softwoods, however they differ in the type of hemicelluloses within their structure.

Cellulose

Cellulose is a linear polymer consisting of β-1, 4-glycosidic linked glucopyranose units in a $^{1}C_{4}$ conformation (Figure 1-3). Two linked glucopyranose units form one cellobiose unit, the basic unit of cellulose (Sixta, 2006). The degree of polymerisation (DP) is used to classify cellulose with respect to its length and weight ratio and is defined as the number of repeating units in a chain and a measure of the molecular weight (Uhlig, 1998). For example, native cotton has a DP of about 10 000.

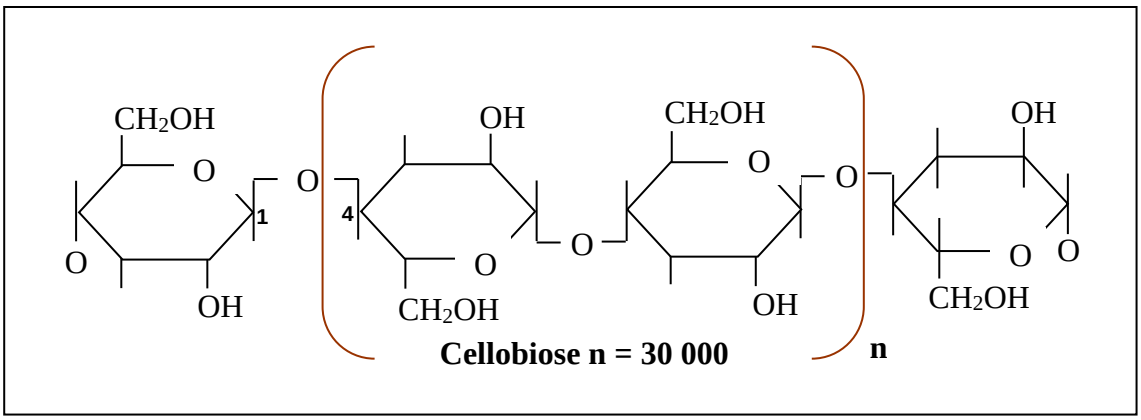

Figure 1-3 Structure of cellulose chain with a cellobiose monomer indicated by the brackets (Adapted from Sjöström, 1993).

The terminal hydroxyl group at C4 is non-reducing, whereas the C1 hydroxyl group has a reducing character. All the glucose residues in the chains run in the same direction and, therefore, all the reducing ends lie at the same ends of the microfibrils. The inherent form of the cellulose polymer appears to be very simple; however, the hydrogen bonds that form within the same cellulose chain or between different chains make cellulose extremely complex. Intramolecular hydrogen bonds formed within the same cellulose chain contribute significantly to chain stiffness and conformation. The extent of the hydrogen bonding in a cellulose chain can be used to differentiate between the crystalline and amorphous cellulose. Intra-molecular hydrogen bonding between the glucan units organises the cellulose chains into crystallite arrangements, which forms

the crystalline cellulose. The region that is much less organized due to fewer intramolecular hydrogen bonds is referred to as the amorphous cellulose (Sixta, 2006).

Hemicellulose

Hemicelluloses provide shape to the fibres and contribute significantly to the strength of paper. They are, however, more soluble than celluloses, which makes them susceptible to chemical degradation and their chemical structure is more unstable than celluloses (Sixta, 2006). Hemicelluloses are heteropolysaccharides, because unlike cellulose, they consist of several sugar moieties. The sugar units of hemicelluloses are the pentoses (xylose and arabinose units), hexoses (glucose and mannose units), hexuronic acids (glucuronic acid) and deoxy-hexoses (rhamnose units). The hemicellulose main chain is usually a hetero-polymer and they are much shorter than cellulose with a DP of 50 to 200 (Sjöström, 1993).

The hemicelluloses found in hardwoods and softwoods differ in their xylan and galactomannan content. Softwoods have a higher proportion of mannose and galactose units than hardwoods, whilst hardwoods have more xylose units and acetyl groups (Sixta, 2006). The hardwood glucomannan chain consists of mannose and glucose units linked by β-(1,4) glucosidic bonds (Figure 1-4). The glucomannan chain of softwoods contains mannose and glucose units with acetyl groups and galactose residues bound to the chain to form *O*-acetylglactoglucomannans (Sixt a, 2006).

Xylan chains are important for maintaining the three dimensional structure of cellulose and the removal of xylan will affect the degree of crystallinity of the cellulose (López Lorenzo *et al*., 2009). The xylan chains in hardwoods are laced at irregular intervals with groups of 4-*O*-methylglucuronic acid with an α-(1,2)-glycosidic linkage at the xylose units. The hydroxyl groups at the carbons C2 and C3 on the xylose units are often substituted with *O*-acetyl groups to give *O*-acetyl-4-*O*-methylglucuronoxylan, which is the main component of hardwoods. The xylans in softwoods have arabinose units instead of the *O*-acetyl groups that are found in the hardwood xylans. The arabinose units are linked by α-(1,3)-glycosidic bonds to the xylose chain to give the softwood xylan; arabino-4-*O*-methylglucuronoxylan (Sixta, 2006).

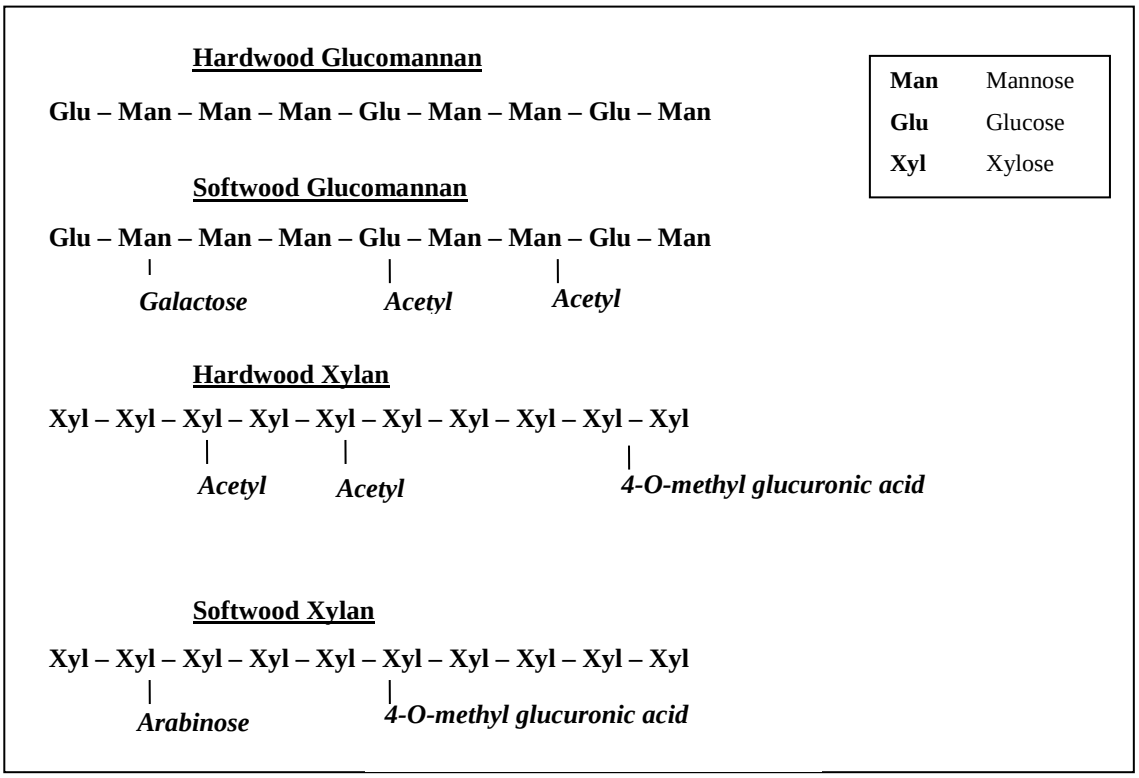

Figure 1-4 Representative structures of glucomannans and xylans in softwoods and hardwoods (Adapted from Sixta, 2006).

Lignin and extractives

Lignin is a complex phenolic polymer that is specialised for water transport and mechanical strength in plants (Fengel and Wegener, 1989). Lignin forms about 20 to 40% of wood and it is distributed unevenly throughout different parts of the tree where it binds the celluloses and hemicelluloses. In softwood branches the lignin is highly concentrated, which functions to protect the cellulose and hemicellulose against enzymatic hydrolysis (Timmel, 1986).

Extractives and ash form about one to eight percent of the total composition of the wood depending on the wood species. Softwoods contain more terpenes than hardwoods, and can be collected in large enough quantities for resale. Triglycerides are also extracted from woods in a Kraft cooking process as black liquor. Ash forms less than 0.5%

weight of the wood and is composed of mainly metallic ions (sodium, potassium, calcium) and the corresponding anions (carbonate, silicate, sulphate) (Biermann, 1996).

FIBRE SEPARATION

There are two types of refining stages in the pulp and paper mill: the first is associated with the fibre separation stage known as pulping and the other with developing fibres for stock preparation, the latter being the main focus of this review. Refining forms an integral part in the preparation of the papermaking stock, which in turn forms the interface between the pulp mill and the paper machine. Refining subjects wood fibres to a mechanical force that develops the fibres into their papermaking properties relative to the final product. Prior to refining, wood fibres have to be separated into their individual fibres via a pulping process which can be achieved mechanically, thermally, chemically or a combination of these treatments (Karlsson, 2006).

Pulping

The method used to separate the fibres influences fibre length and other fibre dimensions. Mechanical pulping utilises increasing levels of energy to physically separate the fibres and uses only water and/or steam (thermo-mechanical) to do so. Mechanical pulps retain the lignin, which interferes with hydrogen bonding and decreases the paper strength (Karlsson, 2006). Mechanical pulps are characterized by high yield, high bulk density, high stiffness and low cost, which suits the production of newsprint and catalogue paper. Chemical pulping usually involves the removal of lignin and hemicelluloses, therefore decreasing the yield. Removal of lignin from these pulps is advantageous for improved strength properties, especially fibre bonding (Biermann, 1996). Mechanical, thermal and chemical pulping can be combined and each process can vary with the type of physical machinery, chemical treatments or temperature manipulation.

Chemical and mechanical pulps go through bleaching if brightness of the pulps needs to be increased. Bleaching of chemical pulps is achieved by the removal of lignin; this,

however, weakens the fibres due to the harshness of the chemicals used. There is a slightly different approach to the bleaching of mechanical pulps as the lignin molecules that absorb light are chemically altered. Chemicals used in the bleaching process include oxygen, peroxide and hypochlorite.

Beating

Papermaking properties were originally achieved using a beating device and this has been used interchangeably with refining to develop fibre properties. Beating refers to a mechanical action of rotating bars opposing a stationary bedplate on a circulating fibre suspension where the individual fibres are oriented perpendicular to the bars (Smook, 1992). Beating involves a batch treatment of pulp at about a six percent consistency and it simulates fibre brushing, which roughens the fibre surface for fibre bond improvement (Biermann, 1996). By the mid 1970s, beating was considered too expensive and slow and so it was phased out and replaced with refiners. Laboratory scale refining still uses beaters such as valley beaters or PFI mills, to simulate the fibre development properties in the mills. However, using beaters to simulate refining cannot be directly compared to commercial scale refining.

REFINING

Refining has been described as the mechanical chafing action of cellulosic fibres between two plates, which contain ridges and grooves designed in such a way to work the stock from the centre of the plates to the outer periphery. The mechanical chafing causes fibrillation of the fibres where the primary cell wall of the fibre is broken, which causes the fibrils of the secondary cell wall to be exposed from the fibre surfaces. Refining develops the fibres in pulp to their optimum paper making properties (Biermann, 1996). It would depend on the mill requirements as to what the optimum properties of paper are.

Mechanism of refining

The grooves, bars and channels of the refiner plates impart rolling, twisting and tensional shear stresses on the fibres. Bending, crushing and a pulling/pushing action

forces are caused by the fibre clumps that are caught between the bar-to-bar surfaces (Smook, 1992). The major effects of refining are divided into primary and secondary effects. Primary effects begin in the early stages of refining where the primary wall is removed and water can pass freely through it. The primary wall cannot swell and the fibre is prevented from swelling, even though the water can pass through easily. In the early stages of refining when the primary cell wall is removed, the secondary wall is exposed and parts of the S1 layer of the secondary wall is removed. Once water penetrates the secondary cell wall the fibres will swell. Removal of the primary wall and some of the S1 layer sets up the fibre for internal and external fibrillation (Levlin and Jousimaa, 1988).

After removal of the primary wall, internal and external fibrillation are the most important effects exerted on the fibres during chemical pulp refining. Internal fibrillation breaks open the crosslinks between micro-fibrils, which occurs when fibres twist whilst being compressed (Wang *et al.,* 2006). Internal fibrillation increases the flexibility and surface areas of the fibres. Increased flexibility results in the cell walls collapsing in, thus forming a more ribbon-like structure, which is better for conformability (Smook, 1992). Internal fibrillation also promotes the straightening of the slack fibre segments in the fibre network during drying, which in turn increases tensile strength and stiffness. Internal fibrillation occurs in the early stages of refining where there is a more compressive action, whereas external fibrillation tends to occur in the latter stages of refining where abrasive action is employed (Wang *et al.,* 2006).

External fibrillation loosens the fibrils and micro-fibrils on the surface of the fibres, also increasing the surface area of the beaten fibres (Smook, 1992). Refining and beating brings about different levels of internal and external fibrillation; beaters generate higher internal fibrillation, whereas refiners impose higher external fibrillation. Internal and external fibrillation can be controlled by different refining actions, depending on the paper properties desired. For high tensile strength, fibres should have a higher internal fibrillation, whereas for improved bonding and light scattering coefficient, external fibrillation is required (Wang *et al.,* 2006).

The secondary effects on pulps during refining are as important as the primary effects. One of these effects is the straightening of the fibres due to tension and fibre swelling. The straightening of the fibres is limited to low consistency pulps and has been shown to increase tensile strength . Other secondary effects include cell wall fracture, fibre stretching and compression, partial solubilisation of hemicellulose into gels and curling of high consistency pulp fibres (Wang *et al.*, 2006).

Variables affecting refining

Energy

Refiner performance can be monitored by the amount of effective energy applied per unit weight of pulp, known as net specific energy, and the rate at which the energy is applied, known as refining intensity. Specific edge load is used to evaluate the refining intensity, which is how intensely the fibres are hit by the plates. Specific edge load is calculated by dividing the rate of the net specific energy by the total length of the bar edges contacting the stock per unit time (Smook, 1992).

The energy transfer from the refiner to the fibres depends on the sharpness of the bar, the width of the bars and grooves and the roughness of the bar surface. There are three phases of energy transfer to the fibres, namely edge-to-edge, edge-to-surface and surface to surface. In the edge-to-edge phase the fibres that are trapped between the bar edges get a hard hit over a short length of fibres. If most of the energy is used in the edge-to-edge phase, the fibres are more likely to undergo severe cutting. The edge-to-surface phase involves the bars of the rotor and stator plates pushing against the fibres and slightly brushing them as they move past each other. The surface-to-surface phase involves the trailing edge of the stator and rotor bars finally clearing each other. If most of the energy is utilised in the last two phases then the fibres are expected to be more fibrillated (Smook, 1992).

Fibre chemistry

Fibre morphology, length, coarseness and the process of pulping and bleaching treatments affect the refining outcome. The chemical composition of the fibre

influences the refining effect, as high lignin content such as unbleached pulps, interferes with swelling, whilst a high content of hemicellulose makes pulps easier to refine. Hemicelluloses are easier to refine as they have a high affinity for water, thereby increasing the swelling and flexibility of the fibre. Some pulps are easier to refine, depending on the process they have gone through. For example, sulphite pulps require less energy than kraft pulps and soda pulps are the easiest to refine (Smook, 1992). Previously dried pulp fibres, especially the secondary fibres, do not absorb water as well as never-dried pulps and are more difficult to refine; that is, they require more energy (Seth, 2001). Never-dried fibres might be more easily beaten, but their drainage resistance and strength properties also develop faster as a function of energy applied. A never-dried pulp may require 20 to 50% less energy than a dried pulp to reach a target tensile strength (Levlin and Jousimaa, 1988).

Refining equipment (Refining plates)

There are two principle types of refiners; namely the conical and the disk refiners. These refiners consist of a rotor (the rotating disk) and a stator (the stationary disk), both of which have metal bars that are mounted perpendicularly to the plates and then rotate in opposite directions. The disk refiners are more popular than the conical refiners, because they operate at higher consistencies, resulting in better fibrillation, have a lower no-load energy, that is the energy not involved in refining, and they are more compact and are easier to maintain (Biermann, 1996).

The refining plates are especially important in achieving a given refining effect on any fibre and they consist of a variety of bars cast onto a base plate (Figure 1-5). The bar size and shape, area of the bar and groove, depth of the groove, material it is made of and the angles of the bars and grooves are all important plate characteristics that influence the refining of pulp. The bar and groove widths used for the longer softwood fibres are wider and for the shorter hardwood fibres they are narrower. The angles on the plates influence the fibre cutting; in general, the higher the angle, the higher the consistency of the pulp that can be refined, whereas the lower angles favour fibre cutting.

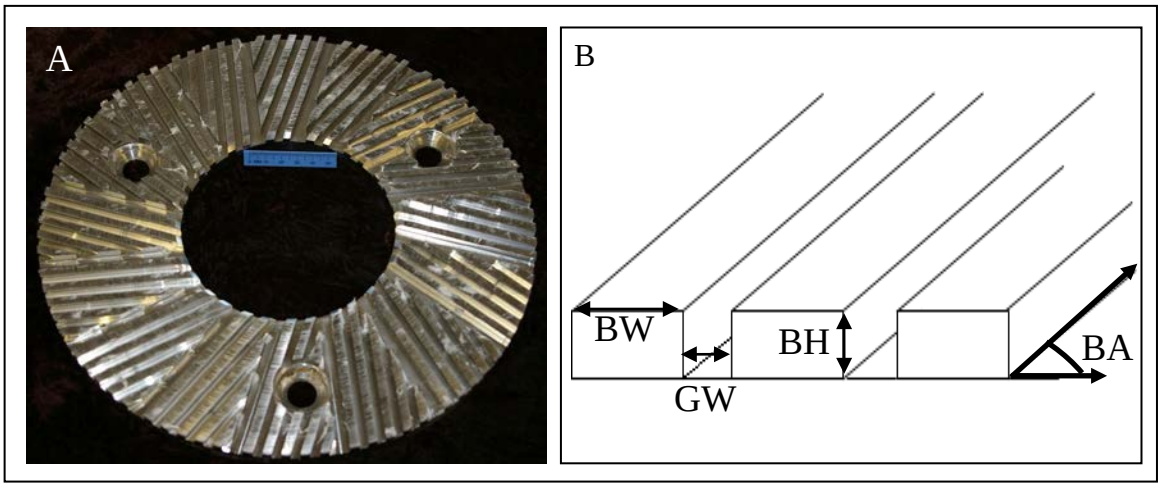

Figure 1-5 An example of the classical hardwood refining plate (A) for the Laboratory Single Disc Refiner (LSDR) and the refiner bars of the hardwood plate (B). The bar angle (BA), bar width (BW), groove width (GW) and bar height (BH) are illustrated. Picture: Pilot-refiner plate at Sappi technology centre, Pretoria.

The patterns on the plates give different effects. A coarse pattern is used with a high intensity action that causes fibre cutting, whereas a finer pattern results in strength development (Smook, 1992). Plates are worn down naturally over time and the abrasion is accelerated by foreign material in the stock, such as wires and sand. Refiner plates and their energy requirements are a huge capital expense in the pulp and paper industry and replacement of a typical plate, usually after 1000 to 1200 h of refining, correlates with the quality deterioration of the paper making properties (Christensen *et al.*, 1994). Temperature and pH affect the lifetime of the plates: heat cracks appear in the bars and grooves, whilst extreme pH media can corrode the plates creating micro-cracks (Smook, 1992). Most refining should be done between three and five percent consistency, as a consistency less than three percent tends to cause undue wear and tear on the plate (Biermann, 1996).

Other variables

Cellulose appears to swell more in alkaline media and, therefore, refines easier than cellulose in acidic media (Smook, 1992). A high consistency pulp tends to undergo

beating because there is more fibre-to-fibre contact, whereas a low consistency pulp will have more fibre-to-metal contact, which can cause more cutting action (Figure 1-6).

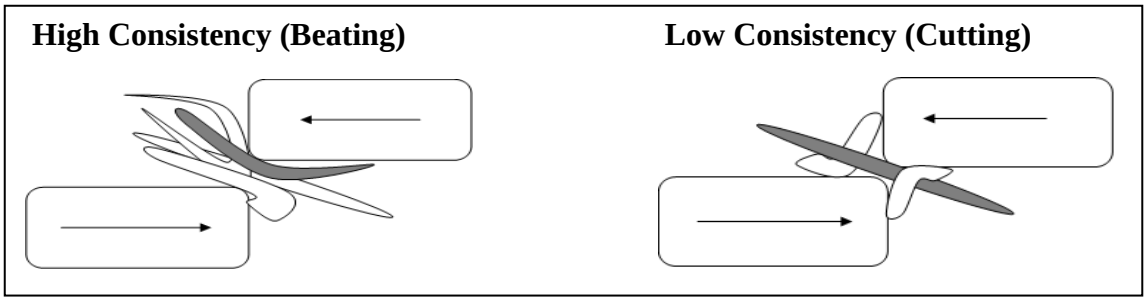

Figure 1-6 The effect of pulp consistency on the action imparted on fibres. A beating effect is imposed on high consistency pulps and a cutting effect is imposed on low consistency pulps.

Pre-treatment of pulp prior to refining makes the pulp fibres more amenable to refining with the intention of saving refining energy, drying energy or improving the paper properties. Abrasive treatment with an ultra-fine friction grinder used to pre-treat high-freeness thermo-mechanical pulp prior to refining improved the refining response of the pulp. The pre-treated pulp was easier to refine that is it required less energy, due to the weakened fibre cell walls (Somboon *et al.*, 2007).

Papermaking properties

Refining brings about changes in fibre properties and the final paper properties. The influence that refining and beating has on pulps and paper properties has been discussed in detail by many authors (Smook, 1992; Biermann, 1996; Seth and Chan, 1999; Kerekes, 2005; Sixta, 2006; Wang *et al*., 2006; Somboon *et al*., 2007). The pulp and paper properties that are directly influenced by refining are discussed in the next paragraphs.

Freeness

The most obvious effect of refining of pulps is the change in the drainage speed of the pulp. The drainage speed of pulp is decreased with an increase in refining; this is because of the build-up in the number of fines and increased internal and external fibrillation (Levlin and Jousimaa, 1988). Most chemical pulps are refined to a target a specific freeness, which is a measure of the drainage ability of the fibres. The standard test in measuring freeness of the fibres in North America is the Canadian Standard Freeness (CSF) and is commonly used to measure the level of refining in the pulp and paper mills. CSF is defined by the number of millilitres of water collected from the side orifice of the standard tester when a pulp suspension drains through a screen plate at a 0.3% consistency at 20°C (Smook, 1992). The other common test for freeness is the Schopper Reigler test, which is similar to the CSF test (Biermann, 1996). The speed of the paper machine depends on the drainage ability of the pulps and if the freeness is too low, the paper machine will run slower and paper production will be less.

Water retention value

The water retention value measures the ability of pulps to take up water and swell. It is intended to simulate the water content after the press section in a paper machine (Karlsson, 2006). The test is carried out by filling a glass filter with a pad of pulp and then fitting it to a centrifuge bottle. The centrifuge is accelerated at 900 g to remove water from the outside surfaces and lumens of the fibre. The remaining water is believed to be associated with submicroscopic pores within the cell wall (Maloney, 2000). The centrifuged fibre pad is weighed, dried at 105°C for 24 h and then reweighed. The water retention value value equals the ratio of the water mass to the dry mass. An unrefined wood cell wall has a swelling ability of 0.2 to 0.5 g/g, where all the water is in the micropores. The macropores are formed from chemical pulping, which dissolves out the lignin and hemicelluloses, leaving behind large gaps in between the microfibrils (Maloney and Paulapuro, 1999). These pores assist with the accessibility of enzymes to the fibre cell walls. The water retention value of a papermaking furnish tends to increase when refining is increased (Hyoung-Jin and Byoung-Muk, 2000) or pH is increased (Hubbe and Pancyzk, 2007). The water retention value tends to decrease when kraft fibres are dried prior to refining and then

repulped. The drying effect on the fibres is known as hornification and it changes the dewatering and bonding properties of the pulps (Maloney, 2000). Hornification could occur from the collapsing of the macropores, and if drying conditions are harsh enough, the micropores will close (Stone and Scallan, 1968). The water retention value test has been used with good repeatability on chemical pulps, whereas with mechanical pulps it has a poor repeatability (Karlsson, 2006). This measurement is important for paper makers as a large amount of water (0.6 to 2.0 g water per g of solids for commercial pulps) is held in the fibre cell wall before it enters the dewatering stage (Stone and Scallan, 1967; 1968).

Tensile strength

This measurement is used primarily for testing the capability of bonding between fibres in pulp and it is defined as the maximum tensile force per unit-width that a test sample can endure (Karlsson, 2006). Tensile strength is increased with refining as it heavily relies on fibre bonding (Biermann, 1996) and is determined by measuring the force required to break a narrow strip of paper, where both the length of the strip and the loading are closely specified (Smook, 1992). At the start of a tensile test, the tensile force acts on the strip of paper, causing the curled fibres to straighten and breaks some of the joints. The strip of paper is now longer and fully strained and close to rupture. The process leading to the rupture of the paper is divided into two steps. The first involves the transfer of the load from the inactive fibres to the active fibres. Secondly, the active fibres are strained until the force needed to break them is exceeded (Page *et al*., 1979). The stretch and rupture are recorded often at the same time (Smook, 1992). Tensile strength can be used as a potential indicator of resistance to web breaking during printing or converting.

Tear strength

Tear strength measurement is a more complex form of stress test than tensile strength as it depends on fibre length, fibre strength, cross section properties, degree of bonding between fibres and the degree of orientation of the fibres in the paper (Karlsson, 2006). Tearing strength is a measure of the energy required to propagate an out of plane tear failure line over a predetermined distance in a sheet of paper (Cowan, 1995). In

general, low levels of beating/refining improve tear strength; however, it will rapidly decline when beating continues due to the reduction in fibre strength (Biermann, 1996). Longer fibres will distribute the stress over a greater area and more fibres and more bonds whereas shorter fibres concentrate stress over a smaller area. Fibre strength is important for tear strength as the energy released on tearing depends on the average load in a fibre, the strength of the fibre and the number of breaking elements, all of which are dependent on fibre strength. Fibre bonding is important in the early stages of tearing as the fibres are pulled out of the network before the actual fibres are broken. Tear strength indicates the papers web runnability, quality of newsprint and characterises the toughness of packaging papers (Kärenlampi, 1996).

Bulk density

The bulk of paper is the mass of all the fibres of the paper divided by the total volume they occupy and it is calculated from calliper, that is thickness, and basis weight: bulk density (cm^3/g) = thickness (mm) x grammage (g/m^2) x 1000. A decrease in bulk density, i.e. an increase in density, makes the sheet smoother, glossier, less opaque, darker and lower in strength. A high bulk density is desirable in absorbent papers, whereas a lower bulk density is good for printing papers. Refining typically decreases the porosity and bulk density of paper (Smook, 1992; Biermann, 1996) and the bulk density of paper cannot be significantly improved even if refining is reduced (Ivan *et al*., 1998).

Porosity

The porosity of a sheet of paper is an indication of the ability of the sheets to accept ink or water or it can be a factor in the vacuum feeding operation on a printing press. The porosity test measures the total horizontal and vertical void spaces in the fibre, and is mostly measured in ml/min. The total void space will include everything that is not cellulose, other added materials or any immobilised water. For a more detailed account of porosity in paper, the measurements, analysis and dispersion effects of relative flow porosity, see Lindsay (1994). Paper is highly porous (~70% air) and it is a critical factor in papers for printing, laminating, filtering, smoking, bags, anti-tarnishing, laminating, labelling etc. There are several methods for determining the air porosity of

paper, but they all incorporate an air resistance method, which is an indirect indicator of the degree of beating, compaction of fibres and the type and amount of fillers. Refining of pulp fibres decreases the porosity of the sheets by increasing fibre bonding from increased fibrillation, which in turn decreases the voids in the paper (Smook, 1992; Biermann, 1996). The porosity of dried fibres is lower than that for never-dried fibres and could be because many of the voids in dried paper are closed off or inaccessible to air flow (Lindsay, 1994).

Fibre strength and fibre bonding

The properties of pulp fibres determine paper strength. Due to the shearing effect of the refiner plates on the fibres the surface area of the fibres is increased when refining is increased, which in turn increases the fibre bonding potential. Bonding is important for the strength properties of paper (Biermann, 1996).

Fibre strength will typically decrease with an increase in refining due to the cutting action of the refiner bars. The loss of fibre strength can be reduced by controlling the consistency of the pulp, refining intensity or enzyme treatment to encourage collapsibility of the fibres rather than fibrillation, which weakens the fibre structure (Biermann, 1996).

Fibre strength and fibre bond is measured using a Zero-span testing technology. Previous evaluations of pulp strength were slower and unreliable; therefore, Zero-span testing technology was introduced into production environments, which could give almost immediate and accurate repeatable results on the fibre bond, fibre strength and fibre length of pulps (Balint, 1999). The Zero-span tensile test measures the average strength of the fibres that are clamped between both jaws of the machine at failure. Fibre strength number (N/cm) is equal to the average of more than ten wet Zero-span tensile testes normalised to 60 g/m^2. The length number represents the average of more than ten re-wet short-span tensile tests divided by the average of more than ten re-wet Zero-span tensile tests. The bonding number (%) is an average of ten dry short span tensile tests divided by the average of ten re-wet short-span tensile test (Cowan, 1994; 1995).

Fibre morphologies

The quality of the distribution and characteristics of the fibres in the pulp governs the quality of the product. Fibre flexibility is important for uniform sheet formation and by post refining a thermo-mechanical pulp, fibre flexibility can be enhanced and a more uniform sheet can be formed (Huber *et al.*, 2008). An increase in the fibre wall thickness and a decrease in fibre perimeter, will increase tear strength. An increase in fibre wall thickness will decrease opacity and the light scattering coefficient, both important parameters for paper making (Braaten, 1997; Lecourt *et al*., 2006). However, one would expect that an increased wall thickness would increase the light scattering and, therefore, increase opacity. A higher burst, tensile energy and stiffness can be achieved with a longer tracheid and a higher cellulose content (Jones, 1999).

Pulp properties have many correlations: for example, more fines will indicate a higher light scattering coefficient or a long fibre length a low fines content. Other correlations include the freeness and brightness of pulp, as an increase in freeness and brightness tends to lower the pulp physical properties. Constant monitoring of freeness and brightness will, therefore give an indication of what the final pulp properties will look like and hopefully give enough time to rectify the problem before too much paper is made of that low grade. An increase in refining energy will lead to an increase in fibre shortening, a decreased brightness and a decreased light scattering, thus degrading the pulp quality (Lecourt *et al.*, 2006).

Refining pulp with enzymes

Natural degradation of cellulosic biomass forms an integral part of the carbon cycle and the enzymes responsible for the hydrolysis of the cellulose fibres have been extensively studied (Morag *et al.*, 1992; Braunstein *et al*., 1994; Clark *et al.*, 1997; Medve *et al*., 1998; Wong and Mansfield, 1999; Schwarz, 2001; Esteghlalian *et al*., 2002; Dienes *et al.*, 2004). Biotechnologists have recognised that cellulolytic enzymes have enormous potential in industry, especially in the pulp and paper industry as it draws heavily on cellulose resources. There have also been a number of reviews of the

cellulose degrading enzymes, especially those of use in the pulp and paper industry (Beguin and Aubert, 1994; Kirk and Jeffries, 1996; Kenealy *et al.*, 2003).

Research has focused on using enzyme technologies, more specifically the cellulases, for fibre modification prior to or in place of refining. However, due to the traditionally high cost of enzymes and the misguided applications in the pulp and paper industry (Mohlin and Pettersson, 2002), these technologies are hampered in gaining their full potential. Modification of the cell wall by enzymatic means is considered an environmentally friendly choice of technology as compared to chemical hydrolysis. Enzymatic hydrolysis is also process specific, works under milder conditions (pH 5 and 50°C) and lowers energy input (Mussatto *et al.*, 2008).

Cell-wall modifying enzymes such as the cellulases and hemicellulases target hydrolysis of the primary and secondary cell walls of the fibre. These enzymes can also save energy in the refining and drying process by improving the fibrillation of the fibres or improving the drainage ability of the pulps (Pommier, 1991; Bhardwaj *et al.*, 1995; Dickson *et al.*, 2000). They can be used to improve the fibre properties and strength properties of the paper or modify bulk density and porosity (Wong *et al.*, 2000).

CELL-WALL MODIFYING ENZYMES

Cell-wall modifying enzymes have the ability to open up the crystalline and amorphous cellulose and hemicelluloses in the fibres, thus making the fibres more amenable to refining. These enzymes could also be applied to a pulp and altogether remove the need for refining. The ideal enzyme treatment should improve the surface area by fibrillation and increase the flexibility of the fibres, whilst maintaining fibre length (López Lorenzo *et al.*, 2009). Cell wall modification from enzymes can improve fibre stability, which refers to the collapsibility of the fibres and the ability of the fibres to stay collapsed. Fibre stability is influenced by the strength properties of the fibres, which are maintained even after rewetting (Strey *et al.*, 2009).

Cellulases

Cellulose is hydrolysed by two types of cellulases, the endocellulases that hydrolyse the internal gylcosidic linkages and the exocellulases that hydrolyse the terminal regions of the cellulose (Teeri, 1997). The endoglucanases form part of the endocellulase family and hydrolyse the amorphic region of the cellulose chain (Figure 1-7).

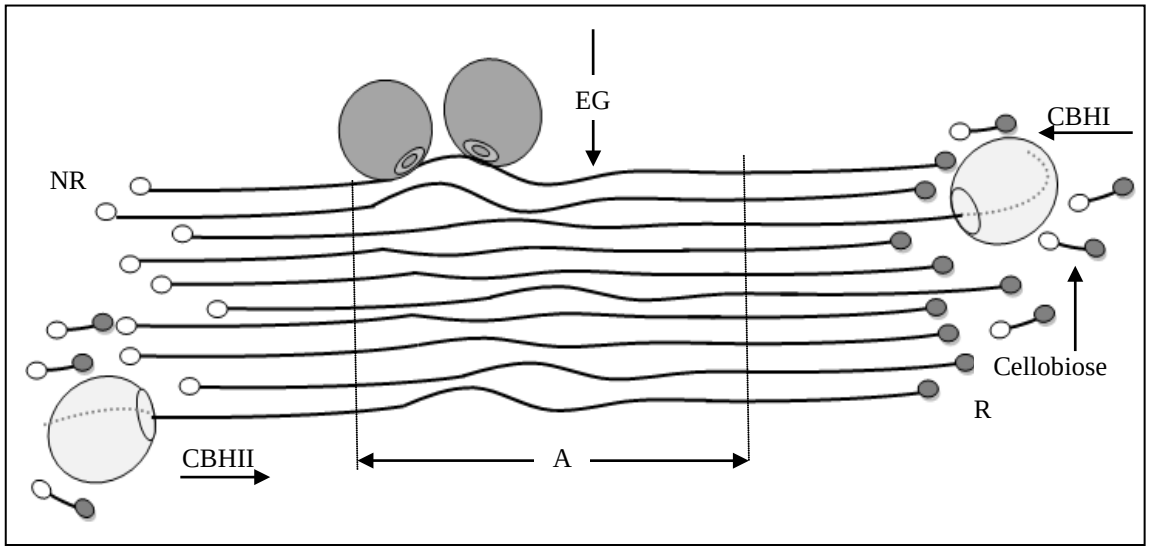

Figure 1-7 A schematic representation of the degradation of crystalline and amorphous cellulose where (A) indicates the amorphous region and the tightly packed crystalline regions are shown either side. Degradation of the substrate occurs through the synergistic action of the endoglucanases (EG) and exoglucanases or cellobiohydrolases (CBH). CBHII cleaves from the non-reducing ends (NR) and CBHI cleaves from the reducing ends (R). (Adapted from Teeri, 1997).

When the cellulases work in synergism, the endoglucanase will initiate the cellulose hydrolysis by opening up the amorphous regions of the cellulose. The cellobiohydrolases are exo-cellulases with hydrolytic specificity for the crystalline regions (Teeri, 1997). Once the amorphous regions have been hydrolysed by the endoglucanase, the cellobiohydrolases can begin hydrolysis of the crystalline regions. There are two types of cellobiohydrolases, cellobiohydrolase (CBH) I and II, where the former is specific for the hydrolysis of the reducing ends of the crystalline cellulose chains, whilst CBH II is responsible for the hydrolysis of the non-reducing ends (Teeri, 1997). Cellobiohydrolases degrades the cellulose into smaller dimers of glucose units

called cellobiose, the main sub-unit of cellulose. The cellobiohydrolase activities are inhibited by the build up of their own products (cellobiose) (Medve *et al*., 1998). Cellobiose is hydrolysed by cellobiase into glucose and in the pulp and paper industry it is important to maintain as much of the yield as possible, so these enzymes (cellobiases) are avoided because of their hydrolytic activity.

Some exocellulases are processive; that is, they bind and hydrolyse the whole chain whilst others are more mobile in that they dissociate after cleaving a cellobiose or glucose unit from the chain and move onto another chain (Barr *et al*., 1996). It is clear than different cellulases differ in their catalytic activity on different regions of the cellulose. Their catalytic domain is the first of two ways that a cellulase can bind to cellulose. The second way is to bind to cellulose using a substrate binding module named a cellulose binding domain or more recently classified as a cellulose binding module (Gilkes *et al*., 1988;Tomme *et al.,* 1988).

Different cellulose binding modules target different sites on the cellulose surface and their function is to deliver the resident catalytic domain to the surface of the cellulose substrate. The cellulose binding module plays a more direct part in the catalytic function of the enzyme as it contributes to the property of processivity: that is, the sequential cleavage of the cellulose chain (Bayer *et al.,* 1998). As mentioned above, different cellulose binding modules target different sites, there are three families of cellulose binding module, I, II and III (Tormo *et al*., 1996). According to Schwartz, cellulose binding module IIIa is associated with the cellulosomal scaffolding and it has a higher affinity for crystalline regions. However, the cellulases of the cellulosome also appear to have an additional cellulose binding module, but a cellulose binding module IIIc, which has higher specificity for amorphous cellulose (Demain *et al.,* 2005). Therefore, it would appear that the cellulosome has within its protein scaffold catalytic specificity for any specific binding sites on the cellulose. Some non-cellulosomal cellulases have an additional cellulose binding module IIIa, which they probably rely on to bind to the amorphous regions of the cellulose.

Endoglucanases are typically not processive (Demain *et al.*, 2005), as they tend to operate by creating "nicks" in the cellulose chain (in the amorphous region specifically) thereby opening up the chain for the action of cellobiohydrolases. If the cellobiohydrolase contain a cellulose binding module IIIa, then it can bind to the amorphous region where the endoglucanase created the free end and act processively, releasing cellobiose molecules as it remains attached to the molecule. The fact that the cellulose binding module provides the cellobiohydrolase the ability to bind to the amorphous cellulose, would explain why Medve *et al*. (1998) discovered that when they combined an endoglucanase and cellobiohydrolase, the two enzymes affected each other negatively as they appeared to compete for the same binding or adsorption sites. There are some endoglucanases that do act processively where they will bind to the polysaccharide and release some oligosaccharides before detaching, sometimes acting more processive than a cellobiohydrolase. The CBHI is not a perfectly processive enzyme as it will cleave off about 5 to 10 cellobiose units at a time before releasing the cellulose chain from its active site (Medve *et al*., 1998). The cellulose binding module of the CBHI is very important for the catalytic activity of the enzyme and if the enzyme is without the cellulose binding module, it has limited overall action on cellulose: that is, the activity would cease much sooner than in the case of a CBHI with a cellulose binding module (Lee and Brown, 1997).

A combination of cellobiohydrolase and endoglucanase with non-competitive binding sites could extensively degrade the amorphous and crystalline regions, thereby causing excessive fibre damage and weakened fibres. Fibres contribute to the strength properties of paper; a fibre weakened through enzymatic treatment will result in a decrease in the tear strength of the paper. It appears that in the application of the cellulose degrading enzymes, mono-component formulations should be used to avoid intensive fibre damage (Pere *et al*., 1996). In the event that a multi-component enzyme mix is used, the selection of enzymes should have similar activities or binding sites or it should be a mix of a cellulase and a hemicellulase to avoid excessive fibre degradation (Pere *et al*., 1996; Medve *et al*., 1998).

The specific activities of the exo-cellulase enzymes on crystalline cellulose can be tested with pure cellulose, such as Avicel, filter paper and cotton. Amorphous cellulose is available in the form of carboxymethylcellulose or acid swollen cellulose, which are suitable for testing the activities of the endo-cellulases (Beguin and Aubert, 1994).

Hemicellulases

The hemicellulases hydrolyse xylans and glucomannans, which are the main components of hemicellulose. Endo-xylanases (1,4-β-D-xylan xylanohydrolases, EC 3.2.1.8) hydrolyse the backbone of xylan comprising xylose groups, whilst endomannanases (1,4-β-D-mannan mannanohydrolase, EC 3.2.1.78) target the backbone of glucomannan (Figure 1-8).

Xylanases have been structurally classified into families where the two major families are the glucosyl hydrolase families; namely Family 10 and 11. Family 10 xylanases occasionally exhibit endocellulase activity; they generally have a higher molecular weight than Family 11, and they occasionally will possess a cellulose-binding domain. Family 11 xylanases are true xylanases; that is, they do not have cellulase activity. The mannanases are too heterogeneous in their biochemistry to be classified into groups (Beily *et al*., 1997). In the application of these hemicellulases to different wood pulps, it should be taken into consideration that softwoods have a higher content of mannans, whereas the hardwoods have a higher content of xylans.

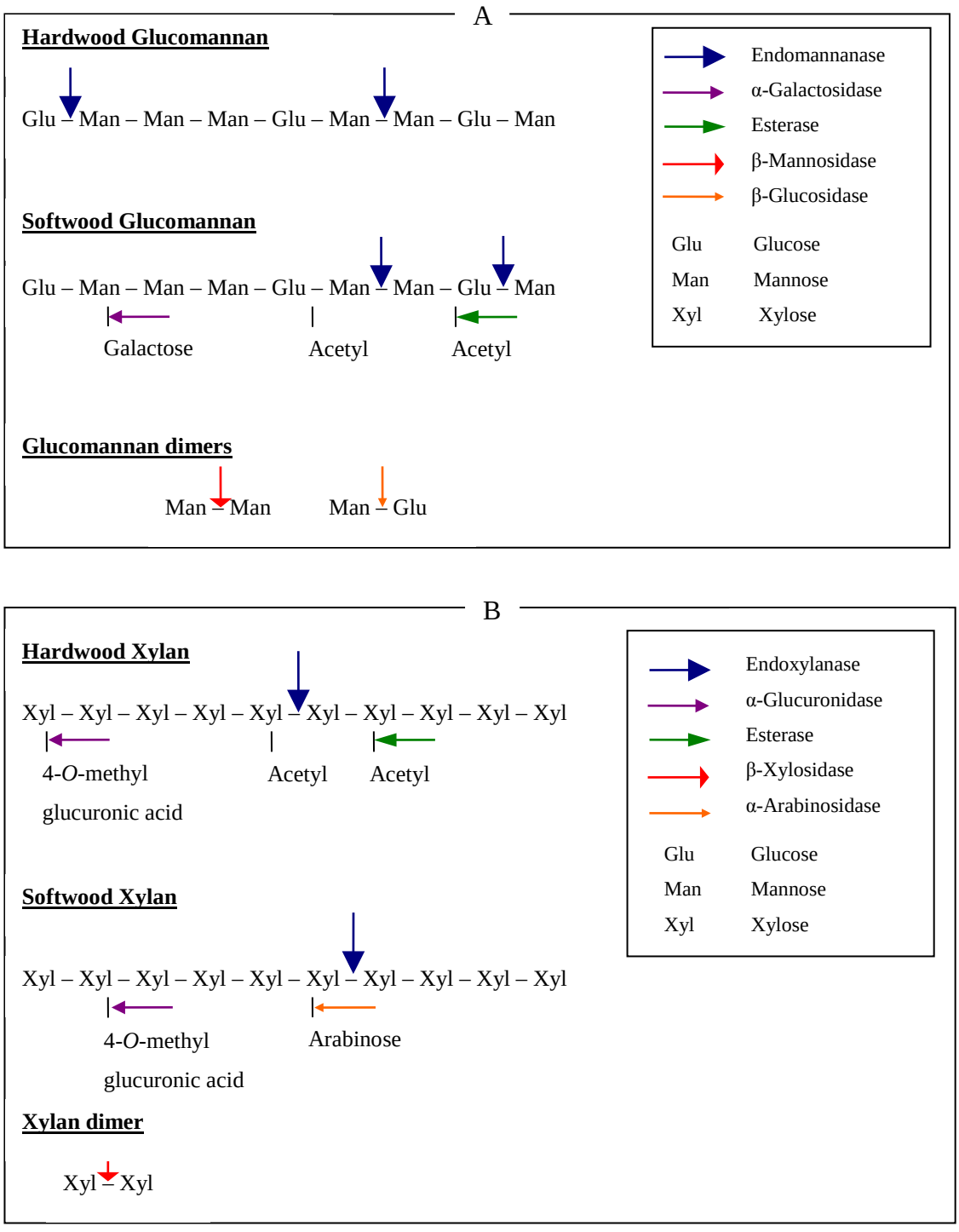

Figure 1-8 Hydrolytic hemicellulase activities on hardwood and softwood hemicelluloses: glucomannan (A) and xylan (B) chains and their sugar components (Adapted from Sixta, 2006).

Other cell-wall modifying enzymes

Pectinases are enzymes that break down the pectic compounds (rhamnogalacturonans, galactans and arabinans) of plant tissues into simpler molecules like galacturonic acids. Pectic compounds comprise a small percentage of the wood structural components (4 to 9%) (Sjöström, 1993) and it is therefore necessary to note the effect of pectinases on pulping or refining of wood pulps. Pectinases have been used in pulping to degrade pectins on the fibres, thus weakening the bond between lignin and cellulose and further refining the pulp before bleaching (Thornton *et al*., 1996). In combination with cellulases, pectinases have also been used to improve the drainage ability of recycled fibres (Olsen *et al*., 2000).

The use of laccase and protease are reported to reduce the energy requirements in mechanical pulping. Laccase is a phenol-oxidative enzyme that appears to modify lignin without depolymerising the lignin or removing it from the pulp (Wong *et al*., 2000). Laccase has had success in improving paper strength properties. However, it needs a mediator such as 10-phenothia-zine-propionic acid or 2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonate (Lund and Felby, 2003) or gallic acid (Chandra *et al*., 2003). These mediators are used to enhance the activity of laccase as this enzyme requires direct contact with the polymer in order to oxidize. Mediators are used to generate long-lived radicals that can oxidize polymers at a distance from the enzyme (Kenealy *et al*., 2003).

A 10% saving in energy consumption was achieved with a protease treatment before the refining of mechanical pulp and the quality of the pulp furnish was also maintained (Mansfield *et al*., 1999). Protease targets an important structural protein that is embedded in the cell wall; the same protein that is present in xylem cell walls during lignification. Bao *et al*. (1992) proposed that if proteins are involved in the differentiation of xylem they could affect the properties of wood. The research of Mansfield *et al*. (1999) seems to confirm the proposal of Bao *et al*. (1992) about the structural significance of the protein in the cell wall, as the removal of the proteins appeared to make the fibres easier to refine, which transposed to energy savings.

Sources of cellulase and hemicellulase

To efficiently break down cellulose, including the insoluble crystalline microfibrils, cellulolytic micro-organisms secrete cellulases and hemicellulases. The cellulases of the fungal and bacterial systems differ in the mechanism used to break down cellulose. Most bacterial cellulases come from a thermophilic or anaerobic species and they are arranged in an efficient energy-conserving multi-enzyme complex, the cellulosome. The most intensively studied bacterium, *Clostridium thermocellum*, secretes cellulosomes that are bound to the surface of the microorganism. The cellulosome consists of a group of cellulases that are bound onto a protein scaffold, which in turn is docked onto the microorganism and has a cellulose binding domain to lock onto the cellulose to bring the enzymes in close proximity to the cellulose (Bayer *et al*., 1998).

Fungi secrete their cellulases into the medium. However, the cellulases are not bound to the fungi and are either free or bound to each other. The cellulases of fungi or bacteria all work in synergy to effectively break down cellulose into molecules such as glucose that are easier to absorb (Bayer *et al*., 1998).

APPLICATIONS OF FIBRE MODIFYING ENZYMES

The history of cellulase and hemicellulase applications dates back to around 1959 when the use of cellulases to facilitate the fibrillation of cotton fibres was patented (Bolaski *et al.*, 1962). Between 1959 and 1988 bleaching and beating of pulps using xylanases dominated most of the cellulase/hemicellulase research. After 1988 research diversified to enhanced drainage and deinking with cellulases and xylanases (Kim *et al*., 1991).

Tables 1-1 to 1-5 provide an overview of the laboratory results published thus far on cell-wall modifying enzymes. There are some factors to take into consideration when viewing the tables, as the results determined in the laboratory may be different on a mill-scale experiment. A common difference between laboratory and mill scale experiments is that most strength tests are done on hand-sheets, which are very different to the sheets formed on the paper machine. The effect of changing enzyme dosage on

pulps can only be compared on a laboratory scale as scaling up the dosage of pulps to industrial scale may give different results. Another factor to consider is that it is difficult to compare enzymes that differ in their international units of activity such as filter paper units or endo-cellulase units. Enzymes should, therefore, be dosed on a specific recommended volume or an equal protein basis (Mansfield *et al*., 1996, Suchy *et al*., 2009). Other factors to note in the table is the pulping process the fibres were exposed to and also from which tree species the fibre originated since chemical fibres are easier for enzymes to hydrolyse and access than mechanical fibres and kraft fibres appear to respond the best to lower doses of cellulases (Mansfield *et al*., 1996). The influence of the enzymes on the pulp will also depend on the incubation time, the pH, temperature and pulp consistency. A pulp consistency of about 2.4 to 4.5% is best for enzyme treatment (Moran, 1996).

The highest electricity and steam consumption in a modern kraft market pulp mill is due to the paper machine (Francis *et al*., 2002), and with energy becoming more expensive, the paper industry needs to make some changes to its operating systems. One of those changes would be to use cell-wall modifying enzymes to save energy in the refining or drying of wood pulps (Table 1-1).

Table 1-1 Changes in refining and drying energy when different pulps were treated with a range of cell-wall modifying enzymes.

Parameters	Change (%)	Enzyme	Fibre	Process	Reference
Refining Energy	-20 to -40	CBH I	*Pinus abies*	Ref MP	1
	-50	Xylanase	N-D *P. radiata*	UBKP	2
	-23	Cellu	HW	BKP	3
	-40 to -70	Cellu	SW	BKP	4
	-10	Protease	*P. radiata*	MP	5
Drying Energy	-20 to -40	Cellu	80% SW, 20% HW	BKP	3

REFERENCES: 1 Pere *et al*. (1996); 2 Dickson *et al*. (2000); 3 Michalopoulos *et al*. (2005); 4 Mohlin and Pettersson (2002); 5 Mansfield *et al*. (1999).

Whilst the protease caused a 10% saving in refining energy on mechanical pulps, a higher saving would probably have been realised on the bleached pulps, as was the case

with a cellulase that can save up to 70% refining energy on the bleached softwood kraft pulps. It has been suggested that the cellulases responsible for the reduction of refining energy for the refining of mechanical pulp are the exo-cellulases such as the cellobiohydrolases (Pere *et al.,* 1996). Investigation of the influence of enzymes on drying energy on a laboratory scale is scarce, possibly due to the assumption that water retention value is a suitable means to simulate drying energy in the mill. However, during a mill trial, a cellulase was applied to bleached softwood and hardwood kraft pulp and by comparing the steam usage, the enzymes saved 40% of drying energy (Michalopoulos *et al*., 2005).

Mono-component cellulases and multi-component cellulases and hemicellulases, in most cases, increase the drainage of a variety of pulps regardless of the process that it came from (Table 1-2). According to Stork *et al*. (1995), use of endoglucanase improves drainage by removing the amorphous regions of the cellulose. Drainage will decrease due to a build up of fines or excessive fibrillation, which was probably the cause of the 24% decrease in drainage of the *Pinus abies* that had been overdosed with CBH I and extensively refined (Table 1-2). It appears that water retention value was increased with treatment of cellulases and the mono-component endoglucanases. The literature appears to lack information on the influence of mono-component hemicellulases on the water retention value of pulps. A combination of a cellulase and hemicellulase decreased the water retention value of a recycled pulp, which suggested that the key to decreasing the water retention value of pulps is using a hemicellulase in the application (Table 1-2). A decreased water retention value reflects the ease by which that pulp will release water and, therefore, the speed of the machine will be increased.

Table 1-2 Changes in the drainage properties when different pulps were treated with a range of cell-wall modifying enzymes.

Parameters	Change (%)	Enzyme	Fibre	Process	Reference
Freeness	21	EG	Recycled sack	n.a.	1
	+10 to -24	CBH I	*Pinus abies*	Ref MP	2
	30	Cellu	DHW & DSW	BKP	3
	+24 to +28	Cellu	Waste	60% KP	4
	7	Cellu & Hemicellu	Recycled sack	n.a.	1
	+25 to +31	Cellu & Hemicellu	Corrugated waste	KP	5
	+15 to +42	Cellu & Xylanase	Douglas fir	MP	6
	+1.4 to +3.2	Cellu & Xylanase	Douglas fir	KP	6
Water retention value	+1.6 to +5	EG	N-D *Eucalyptus globulus*	BKP	7
	+3 to +16	EG	D *E. globulus*	BKP	7
	17	EG	Recycled sack	n.a.	1
	+5 to +21	Cellu	SW	BKP	8
	+5 to +27	Cellu	SW	BKP	8
	+5 to +28	Cellu	D *E. globulus*	BKP	7
	0 to +10	Cellu	N-D *E. globulus*	BKP	7
	13	Cellu	D& N-D *E. globulus*	n.a.	7
	-7	Cellu & Hemicellu	Recycled sack	n.a.	1

REFERENCES: 1 Dienes *et al*. (2004); 2 Pere *et al*. (1996); 3 Abubakr *et al*. (1996); 4 Pala *et al*. (2004); 5 Bhardwaj *et al*. (1995); 6 Mansfield *et al*. (1996); 7 García *et al*. (2002); 8 Mohlin and Pettersson (2002).

Cell-wall modifying enzymes alter the surface of fibres, but the change will depend on the fibre source and enzyme selected. The changes to the fibres will reflect in the pulp and paper properties (Table 1-3). The coarseness of fibres are decreased by a mannanase or a cellulase and hemicellulase mix. However, the endoglucanases, as mono-component or as a multi-component formulation, increase the coarseness of fibres by about 3% (Table 1-3). Coarse fibres will influence the formation and the smoothness of paper; therefore, mannanase should be considered for improvements in formation of paper.

Cellulases and hemicellulases cannot add to the fibre length because of their hydrolytic nature. However, they could preserve the length of the fibres by ensuring that no fibre cutting occurs. The most damage to fibre length occurs with cellulases that are overdosed and the treated pulps are refined at high energy levels with a laboratory beater or a pilot scale refiner (Table 1-3). Apparently, shortening of the fibres has been attributed to the action of endocellulases such as the endoglucanases (Pere *et al.*, 1996). The shorter the fibres, the better the formation of paper (Mohlin and Pettersson, 2002). Treatment of a bleached chemi-thermo-mechanical pulp (CTMP) with cellulases or xylanases does not influence fibre width by more than 1%. However, the pulp was not refined or beaten after enzyme treatment, so the full influence of the enzymes could not be determined (Strey *et al*., 2009).

The efficiency of the cellulases at cleaning up the fines in any furnish is clear in the improved or increased drainage. However, one would expect a greater reduction in the fines content of pulps when treated with these enzymes (Table 1-3). The endoglucanases, mannanases, xylanases and mixed formulations decreased fines content only as much as 5% (Table 1-3). Few researchers measured the fines content, as the equipment is costly to obtain. However, if more results on the fines content of pulps were available, the reduction of fines would probably be substantial if compared to the results tabulated thus far. Fines content increased by 5% with treatment of a mixture of cellulase and hemicellulase, but the increased fines was probably due to overdosing on waste pulps (Dienes *et al*., 2004). Fibrillation is difficult to quantify, however, visual proof of fibrillation is the best way to illustrate the influence of an enzyme on fibres. In the early stages of cellulase research, treatment of cotton fibres with cellulases improved their fibrillation (Bolaski *et al*., 1962) (Table 1-3).

Table 1-3 Changes in the fibre properties when different pulps were treated with a range of cell-wall modifying enzymes.

Parameters	Change (%)	Enzyme	Fibre	Process	Reference
Coarseness	-0.3	EG	N-D Douglas fir	BCP	1
	3	EG	Spruce	CTMP	2
	-10	Mannanase	Spruce	CTMP	2
	3	EG & Mannanase	Spruce	CTMP	2
	-7 to -11	Cellu & Xylanase	Douglas fir	MP	3
	-3 to -10	Cellu & Xylanase	Douglas fir	KP	3
Fibre length	6	EG	Recycled sack	n.a.	4
	0.8	EG	Spruce	CTMP	2
	-2	EG	N-D Douglas fir	BCP	1
	-0.5	CBH I	*Pinus abies*	Ref MP	5
	3	Mannanase	Spruce	CTMP	2
	2	Endoxylanase	Spruce	BTMP	6
	0 to -68	Cellu	SW	Beaten BKP	7
	0 to -40	Cellu	SW	Ref BKP	7
	0.3	EG & Mannanase	Spruce	CTMP	2
	-2	Cellu & Hemicellu	Recycled sack	n.a.	4
Fibre width	-0.1	EG	Spruce	CTMP	2
	0.4	Mannanase	Spruce	CTMP	2
	-0.4	EG & Mannanase	Spruce	CTMP	2
Fines	-5	EG	Recycled sack	n.a.	4
	0	EG	Spruce	CTMP	2
	-0.9	Mannanase	Spruce	CTMP	2
	0.6	Endoxylanase	Spruce	BTMP	6
	0.6	EG & Mannanase	Spruce	CTMP	2
	5	Cellu & Hemicellu	Recycled sack	n.a.	4
Fibrillation	>0	Cellu	Cotton linters	n.a.	8
Flexibility	2	Xylanase	N-D *P. radiata*	UBKP	9

REFERENCES: 1 Mansfield and Dickson (2001); 2 Strey *et al.* (2009); 3 Mansfield *et al.* (1996); 4 Dienes *et al.* (2004); 5 Pere *et al.* (1996); 6 López Lorenzo *et al.* (2009); 7 Mohlin and Pettersson (2002); 8 Bolaski *et al.* (1962); 9 Dickson *et al.* (2000).

In theory it can be assumed that cellulases and hemicellulases could improve the flexibility of fibres by "softening" the walls of the fibres through cellulose and hemicellulose hydrolysis. The xylanase treatment of *Pinus radiata* appeared to improve

the flexibility of the fibres, which would improve the formation of the final sheet (Table 1-3).

Table 1-4 indicates the influence of the enzymes on the strength properties of handsheets. Endoglucanase treatment of pulps generally caused an increase in the burst strength; sometimes the increase was as much as 89% (García *et al*., 2002) (Table 1-4). When CBH I dosage was increased, the burst strength for the pulp increased from -12% to +4%. However, when the dosage of cellulases was increased, the burst strength decreased (Pere *et al*., 1996)(Table 1-4). In general, cellulases and mixtures of cellulases and hemicellulases decreased the burst strength of pulps. Laccase caused a large improvement in burst strength by 24% in unrefined bleached kraft pulps and when combined with a mediator, 4-HPA, it increased the burst strength of thermo-mechanical spruce pulps, but only by 7% (Chandra *et al*., 2003).

When improving the fibrillation of the fibre, enzyme treatment tends to reduce fibre strength, which in turn decreases tear strength. The use of mono-component endoglucanases and cellobiohydrolases, and multi-component cellulases and hemicellulases all reduce tear strength, especially when the dosage of the enzymes are increased (Table 1-4). However, at low dosages and low refining energies, the tear strength can be improved by using endoglucanase and cellulases. Treatment of dried *Eucalyptus* pulps with endoglucanase using a low dosage increased tear strength by 91%, whereas at the same dosage on the never-dried *Eucalyptus* the endoglucanase decreased tear strength by 7%, thereby highlighting the difference an enzyme can have on dried and never-dried pulps. The dried fibres contain more amorphous regions due to the drying process (Konturri and Vuorinen, 2009) and this was why the activity of this endoglucanase on the two pulps was so different. Laccase treatment with and without a mediator increased the tear strength of refined and unrefined kraft pulps and mechanical pulps. However, there appears to be a gap in information regarding the influence of mono-component hemicellulases on the tear strength properties of pulps.

Table 1-4 Changes in the hand-sheet strength properties when different pulps were treated with a range of cell-wall modifying enzymes.

Parameters	Change (%)	Enzyme	Fibre	Process	Reference
Burst index	+6 to +7	EG	N-D Douglas fir	BCP	1
	+38 to -4	EG	N-D *Eucalyptus globulus*	BKP	2
	+89 to +37	EG	D *E. globulus*	BKP	2
	6	EG	Recycled sack	n.a.	3
	-12 to +4	CBH I	*P. abies*	Ref MP	4
	-17	Cellu	DHW & DSW	Unref BKP	5
	0	Cellu	DHW & DSW	Ref BKP	5
	+19 to -3	Cellu	N-D *E. globulus*	BKP	2
	+67 to +3	Cellu	D *E. globulus*	BKP	2
	-11 to -29	Cellu	Waste	60% KP	6
	8	Cellu	80% SW&20%HW	BKP	7
	-2	Laccase	SW linerboard	Ref KP	8
	24	Laccase	SW linerboard	Unref KP	8
	-17 to -52	Cellu & Xylanase	Douglas fir	MP	9
	5	Cellu & Hemicellu	Recycled sack	n.a.	3
	7	Laccase + 4-HPA	Spruce	TMP	10
Tear strength	-7 to -21	EG	N-D *E. globulus*	BKP	2
	+91 to -25	EG	D *E. globulus*	BKP	2
	-26	EG	Recycled sack	n.a.	3
	+6 to -10	CBH I	*P. abies*	Ref MP	4
	+2 to -18	Cellu	N-D *E. globulus*	BKP	2
	+55 to -51	Cellu	D *E. globulus*	BKP	2
	-9 to -27	Cellu	Waste	60% KP	6
	9	Cellu	80% SW&20%HW	BK	7
	12	Laccase	SW	Ref KP	8
	9	Laccase	SW	Unref KP	8
	0 to -20	Cellu & Xylanase	Douglas fir	MP	9
	-12	Cellu & Hemicellu	Recycled sack	n.a.	3
	3	Laccase + 4-HPA	Spruce	TMP	10
Tear-tensile strength	-70	EG	*P. radiate*	UBKP	11
	-40	EG	*P. radiate*	BKP	11
	0	Xylanase	N-D *P. Radiate*	UBKP	12
Tensile strength	+4 to +7	EG	N-D Douglas fir	BCP	1
	-9 to -58	EG	N-D *E. globulus*	BKP	2
	-11 to -88	EG	D *E. globulus*	BKP	2
	2	EG	Recycled sack	n.a.	3
	-9 to +10	CBH I	*P. abies*	Ref MP	4
	-11	Cellu	D HW & SW	Ref BKP	5
	-8 to -55	Cellu	N-D *E. globulus*	BKP	2
	-4 to -93	Cellu	D *E. globulus*	BKP	2
	-7 to -18	Cellu	Waste	60% KP	6
	-0.3	Laccase	SW linerboard	Refined KP	8
	9	Laccase	SW linerboard	Unref KP	8
	-9 to -35	Cellu & Xylanase	Douglas fir	MP	9
	5	Cellu & Hemicellu	Corrugated waste	KP	13
	5	Cellu & Hemicellu	Recycled sack	n.a.	3
	-4	Laccase + 4-HPA	Spruce	TMP	10

REFERENCES: 1 Mansfield and Dickson (2001); 2 García *et al*. (2002); 3 Dienes *et al*. (2004); 4 Pere *et al*. (1996); 5 Abubakr *et al*. (1996); 6 Pala *et al.(* 2004); 7 Michalopoulos *et al*. (2005); 8 Chandra *et al*. (2003); 9 Mansfield *et al*. (1996); 10 Kenealy *et al*. (2003); 11 Clark *et al*. (1997); 12 Dickson *et al*. (2000); 13 Bhardwaj *et al*. (1995).

Concerning the tear-tensile relationship of enzyme treated pulps, it was clear that the endoglucanase treatments of *P. radiata* caused a severe drop in the tear-tensile curve of about 40 to 70% (Clark *et al*., 1997)(Table 1-4). The xylanase treatment did not influence tear or tensile strength of the *P. radiata*, which probably due to the low xylan content of softwoods. It would have been more appropriate to apply a mannanase to the fibres of the *P. Radiata* as there are more mannans in softwoods (Sixta, 2006).

The endoglucanases increased the tensile strength of never-dried bleached chemical pulp by 7% (Mansfield and Dickson, 2001), but decreased the tensile strength of never--dried bleached kraft *E. globulus* pulp by 58% (García *et al*., 2002)(Table 1-4). The differences in the effect of the enzyme on tensile strength could have been due to the pulping process or it could have been attributed to the dosage levels of the endoglucanase. The endoglucanases also tend to influence the tensile strength of never-dried pulps less than dried pulps, as equal dosages of endoglucanases applied to dried and never-dried pulps caused a greater reduction in tensile strength of the dried *Eucalyptus* pulp (García *et al*., 2002)(Table 1-4). It could be likely that the dosage of the endoglucanase to the *Eucalyptus* pulps was exceeded at the lower levels and optimisation of the dosage could result in improvements in the tensile strength. When a higher dose of cellobiohydrolase was combined with refining of mechanical *P. abies* pulp the tensile strength was improved by 10% (Pere *et al*., 1996). Cellulases, on the other hand, regardless of dosage, decreased tensile strength with the worst reduction occurring on the dried pulps, which saw a 93% loss of tensile strength (García *et al*., 2002). Cellulase and hemicellulase combinations have mixed effects on the pulps concerning tensile strength. A decrease in tensile strength was seen on a mechanical pulp, whereas improvements were seen with waste kraft pulps (Table 1-4). The laccase treatments had a relatively small influence on tensile strength; without a mediator the tensile strength increased by 9%, whilst with a mediator there was a 4% decrease (Kenealy *et al*., 2003).

Research into the influence of cellulases and hemicellulases on the brightness of pulps has been scarce, possibly because most of the attention focused on the effect of enzymes on strength and drainage due the surface changes occurring on the fibres. Brightness

was marginally affected by treatment with a cellobiohydrolase. However, the influence of laccases and hemicellulases on brightness should be considered in future research related to enzymes and paper properties (Table 1-5).

Table 1-5 Changes in surface properties of hand-sheets when different pulps were treated with a range of cell-wall modifying enzymes.

Parameters	Change (%)	Enzyme	Fibre	Process	Reference
Brightness	+0.1 to -0.4	CBH I	*P. abies*	Ref MP	1
Bulk density	3	Cellu & Hemicellu	Corrugated waste	KP	2
Curl	-0.7	EG	Spruce	CTMP	3
	-1.4	Mannanase	Spruce	CTMP	3
	-1.4	EG & Mannanase	Spruce	CTMP	3
Porosity	-9 to -58	EG	N-D *E. globulus*	BKP	4
	-11 to -81	EG	D *E. globulus*	BKP	4
	16	EG	Recycled sack	n.a.	5
	-8 to -54	Cellu	N-D *E. globulus*	BKP	4
	-4 to -93	Cellu	D *E. globulus*	BKP	4
	+13 to+175	Cellu & Xylanase	Douglas fir	MP	6
	24	Cellu & Hemicellu	Recycled sack	n.a.	5
Roughness	0 to -1	Cellu & Xylanase	Douglas fir	MP	6

REFERENCES: 1 Pere *et al*. (1996); 2 Bhardwaj *et al*. (1995); 3 Strey *et al*. (2009); 4 García *et al*. (2002); 5 Dienes *et al*. (2004); 6 Mansfield *et al*. (1996).

Curl is rarely reported on despite it having a large influence on paper formation. The endoglucanase and mannanase treatment of spruce decreased curl by a small margin, however, with refining it may be possible that the margin will increase. Cellulase treatment of dried and never-dried *Eucalyptus* pulp reduced porosity, with the greatest decrease seen in the dried pulps (Table 1-5). The porosity decreased with an increase in the number of fines and bonding; therefore, the enzymes created more fines and fibrils on the dried fibres compared to the never-dried fibres. An endoglucanase and a cellulase and hemicellulase mix that were applied to fibre waste increased porosity by 16% and 24%, respectively. The enzyme applied to the waste probably assisted in removing the excess number of fines typically found in waste, thereby increasing the

porosity of the fibres. A cellulase and xylanase mix that has been applied to a mechanical pulp increased porosity by about 175%, suggesting an appropriate selection of dosage and enzyme combination that removed the fines and controlled fibrillation of the fibres to such an extent that porosity was improved (Table 1-5).

CONCLUSIONS

Cell-wall modifying enzymes have the potential to modify the fibres of wood pulps to render them easier to refine thereby, saving refining energy (García *et al*., 2002). In addition these enzymes can also modify the pulp fibres to improve the drainage and drying speed of the pulps, which is advantageous for paper production (Michalopoulos *et al*., 2005). Furthermore, the paper properties, strength and brightness could also be improved by the cell-wall modifying enzymes (Pere *et al*., 1996; Mansfield and Dickson, 2001). Such efficiency of the cellulases on hydrolysis of cellulose provides an opportunity for application in the pulp and paper industry where enzymes could replace an existing technology or become a new technology to improve a process. However, some factors need to be considered in the application of the enzyme, such as the fibre source and the right enzyme for the application. Bleaching or drying influences the fibre morphology, because bleaching removes lignin and extractives, whereas drying modifies the inherent structure with changes in the hydrogen or carboxyl bonds between glucose dimers (Biermann, 1996).

Research on the application of enzymes on fibres has been well investigated on a laboratory scale (Bhardwaj *et al*., 1995; Pere *et al*., 1996; Mansfield *et al*., 1996; Mansfield and Dickson, 2001; García *et al*., 2002; Dienes *et al*., 2004; Strey *et al*., 2009), however, many of these results are never verified in the mill. Application of the enzymes on a larger scale will indicate how reliable laboratory testing is and how different volumes and unpredictable production processes will influence the effect of a cellulase on the pulps. Research needs to broaden to include mill-scale applications, with more focus on optimising the enzyme dosage on the pulps and monitoring the effect of the enzymes on the different paper properties. Water tends to be recycled in a paper mill, so it will be of interest to observe the water lines and determine if the enzymes are re-circulated into the raw stock chests when and if they are washed out of the treated pulps before entering the machine drying section.

Application of cell-wall modifying enzymes to wood pulps will require much background analysis on the pulp source and cell-wall modification that will occur. It is

clear from the previous tables that a specific enzyme applied to the same wood species could have a different influence on the fibres because the wood species have been through different fibre preparation histories (bleaching, refining, and drying). The type of cell-wall modifying enzyme needs to be matched to the fibre source (wood type and process) to achieve a specific refining and/or drainage effect and paper property.

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CHAPTER 2

EVALUATION OF COMMERCIAL CELLULASES FOR MODIFICATION OF PULP FIBRES

Test tubes of standards from enzyme assay and spectrophotometer.

ABSTRACT

Commercial cellulases have been applied in paper making in order to improve drainage, reduce hornification and improve tissue softening. Cellulases can also be applied prior to refining of pulp to improve fibre development and paper quality. In this study commercially available cellulases were characterised using filter paper and carboxymethylcellulose as substrates to determine the activity across a temperature and pH range. The protein content of the formulations was determined to establish a comparative dosing range for the enzymes. Subsequently, the effect of over-dosing and the extended incubation with the enzymes were also determined. The activities of the enzymes were suitable for mill applications at a temperature of 50°C and pH of 4.8. The protein concentration ranged from 13 g/l to 105 g/l for the formulations tested. The pulp yield was at risk of being reduced with an increase in the enzyme dosages as the release of reducing sugars was increased with an increase in dosage. The sugar content of the pulps was increased by application of Ecopulp® Energy, Novozyme 613 and Celluclast 1.5L. Fibre strength and fibre bonding were unaffected by the Ecopulp® Energy and Novozyme 613 treatment, however, Celluclast 1.5L increased fibre bonding and decreased fibre strength. The biggest change was observed in the first 30 min when pulp was incubated with an enzyme preparation; thereafter the strength properties remained unchanged. The most suitable enzyme for application to a paper mill appeared to be Novozyme 476 due to the improved bonding strength and minimal release of reducing sugars.

INTRODUCTION

A number of cellulases have been developed for fibre modification in the pulp and paper industry; however, many of them do not reach the implementation stage. The main reason for some cellulases being unsuitable for implementation is that they have caused reduced pulp yield and decreased fibre strength (Mohlin and Pettersson, 2002), both of which are undesirable in the paper-making process. The aim of the present study was to avoid the negative effects of cellulases by fully evaluating the enzymes before application on paper pulps.

Commercial cellulase formulations are released with product sheets (Novozymes, 2001a; 2001b) and material safety data sheets (AB Enzymes, 2007a; 2007b; Novozymes, 2005a; 2005b), which document some of the required information on the activities of the products. The product sheets sometimes provide information on the optimum pH and temperature ranges of the enzymes, but neglect to include the substrate on which they were tested. These product sheets often do not include information on the recommended dosages and/or the required incubation time for specific fibres. Amongst other details, material safety data sheets also document the protein concentration of the formulations; however, only a range is given. Most paper mills are unable to change process parameters to suit the enzymes, so the enzymes have to be chosen to match the existing parameters of the mill. It is, therefore, necessary to determine the pH and temperature optima as well as substrate specificity of commercial enzymes before application.

The substrate on which the enzyme activity is determined is relevant to the specific application. The activities of the cellobiohydrolases on crystalline cellulose can be tested with pure cellulose, such as Avicel, filter paper or cotton (Teeri, 1997). Amorphous cellulose is available in the form of carboxymethylcellulose or acid-swollen cellulose, which are suitable for testing the activities of an endoglucanase (Beguin and Aubert, 1994). An enzyme that is very active on carboxymethylcellulose is more active on the amorphous regions of cellulose when applied to pulp fibres (Beguin and Aubert, 1994; Demain *et al.*, 2005). The amorphous phase of virgin fibres tends to be located at

the microfibril surface and the crystalline regions mostly occur at the core (Larsson *et al*., 1997). Treatment with an endoglucanase or a cellulase containing a cellulose binding module specific for amorphous regions could assist in opening up the fibre walls and increasing fibrillation. Increased fibrillation can potentially increase the surface area of the fibres, which in turn gives tensile strength to the product; however, a reduction in tear strength can also be expected as damage to the fibre decreases fibre strength. The potential negative consequences to the enzyme treatment require application of the enzymes at optimal dosages. Dosage selection and comparison of formulations require a standard unit of application. The fact that formulations contain different activities and that stability factors are added to these formulations have resulted in enzymes being applied on an equal protein basis (Mansfield *et al*., 1996; Pere *et al*., 1996; Suchy *et al*., 2009).

For successful application, more needs to be known about the properties of the enzymes contained in the formulations than is available through the material safety data sheets and product sheets. In the present study, the protein content, temperature and pH optima, as well as the optimal dosage and the effect of incubation time were determined for the commercial enzyme formulations. This information should make it possible to select the most appropriate enzyme for a specific application.

MATERIALS AND METHODS

Enzymes

A variety of commercial cellulase formulations were obtained from different suppliers (Table 2-1). The tested enzymes included Ecopulp® Energy (AB enzymes, Finland) described as a cellobiohydrolase formulation with some xylanase side activities (Suchy *et al*., 2009). Two formulations, Novozyme 476 and Novozyme 613 (both from Novozymes, Denmark), each contained a mono-component endoglucanase (Novozymes, 2001a; 2001b). The multi-component formulation Celluclast 1.5L (Novozymes, Denmark), contained three endoglucanases, EG I, EG II and EG III, and two cellobiohydrolases, CBH I and CBH II (Zandoná Filho *et al*., 2006).

Table 2-1 The properties of selected enzyme formulations as listed in material safety data sheets and product sheets.

Formulation	Protein (g/l)	Optimal Temperature (°C)	Optimal pH	References
Ecopulp® Energy	10 to 50	n.a.	n.a.	AB Enzymes, 2007a
Novozyme 476	10 to 100	40 to 65	6-9	Novozymes, 2001a; 2005a
Novozyme 613	10 to 100	40 to 60	6-9	Novozymes, 2001b; 2005b
Celluclast 1.5L	100 to 400	n.a.	n.a.	AB Enzymes, 2007b

n.a. = not available

Protein determination

The BCA™ Protein Assay Kit 23225 (Pierce, USA) was used to determine the protein content of the formulations, using bovine serum albumin that was provided with the kit, as the standard. The absorbance of the bicinchoninic acid reagent was read at 562 nm and was linear for the protein concentration range between 20 to 2000 µg/ml.

Enzyme activities

Endoglucanases specifically hydrolyse the amorphous regions of the cellulose chains, and therefore, the amorphous cellulose substrate carboxymethylcellulose (CMC) was used (Beguin and Aubert, 1994). Filter paper mainly consists of crystalline cellulose, which is typically hydrolysed by cellobiohydrolases (Teeri, 1997). The CMC assay tested the endoglucanase activity, whilst the activities of the cellobiohydrolases were determined using a filter paper assay (Mandel, 1969). The CMC and filter paper assays used the dinitrosalicylic acid (DNS) method to quantify the concentration of reducing sugars (Miller, 1959).

The dilution of the enzymes was determined empirically so that the concentration of sugars released gave an absorbance that was less than 0.25 when the activity was tested on CMC and an absorbance of between 0.2 and 0.6 when tested on filter paper (Mandels *et al.*, 1976). The amount of reducing sugars liberated in each reaction was read from a standard curve for glucose concentration. The CMC-ase activity (IU/ml) was calculated as:

$$\text{IU/ml} = \frac{\text{Abs} - b}{m \text{ x } 0.5 \text{ x } 180.16 \text{ x } 30} \text{ x } 1000 \text{ x } D$$

where Abs was absorbance at 550 nm, *b* the intercept of the DNS calibration curve, *m* the slope of the DNS calibration curve, 0.5 the sample volume in ml, 180.16 the molecular weight of glucose in g/mol, 30 the reaction time in minutes and *D* the dilution factor of the enzyme before the assay.

Filter paper activity (FPU/ml) was calculated as:

$$\text{FPU/ml} = \frac{\text{Abs} - b}{m \text{ x } 0.5 \text{ x } 180.16 \text{ x } 60} \text{ x } 1000 \text{ x } D$$

where each term had the same meaning as above, except that the time of incubation was 60 min.

The activities of the enzymes were tested over a temperature range before testing them across a pH range. To determine the activity of the enzymes across a temperature range, a 1% CMC solution or a 1 × 3 cm piece of Whatman No 1 filter paper was suspended in a 0.05 M citrate buffer (pH 4.8). The temperature range was based on the extreme temperatures that may be expected in a paper mill environment (Wolfaardt *et al*., 2007), namely 30 °C to 80 °C, and was tested in 10 °C increments. The enzymes were incubated with the CMC for 30 min and with the filter paper for 60 min at the selected temperatures before addition of the DNS reagent to terminate the reaction, followed by boiling the reaction mixture for 5 min. The sample mixtures were cooled on ice; subsequently the standards, blanks and reaction mixtures were diluted with deionised water. The absorbance of the CMC samples were read immediately using a spectrophotometer (Biomate 5, Thermo Scientific) at 550 nm. The absorbance of the filter paper samples was read after centrifugation of the samples.

The pH range was chosen based on the most extreme range of pH values that was expected at a paper mill, which was between pH 3 and pH 9. To determine the activity

of the enzymes across the pH range, a 1% CMC solution or a 1 × 3 cm piece of Whatman No 1 filter paper was prepared in a 0.1 M Britton-Robinson buffer (Britton and Robinson, 1931) made to specific pH levels. The enzyme activity was then determined as described above.

Dosage optimisation

The effect of overdosing had to be tested on long pulp fibres to allow proper sheet formation regardless of the level of degradation of the fibres. Bleached pine kraft pulp was, therefore, repulped to a consistency of 3.5% and the different enzymes were applied at 0.0, 0.1, 1.0, 10.0 and 100.0 g protein/kg dried pulp to determine the optimal dosage of the enzymes. The range of dosages extended to a thousand fold overdose relative to the recommendation of the suppliers (Novozymes, 2001a; 2001b). The samples were incubated at 50 °C for 30 min with shaking at 5 min intervals. The pH of the pulp was not adjusted but instead used at the pH of tap water, which was at pH 6.9. The pulp was thickened and used to determine fibre strength and fibre bond on a Pulmac Z-span 3000 tester (Pulmac International, USA). Pulp was made to a 0.2% consistency to make nine Pulmac sheets of equal weight. The sheets were then tested in sets of three; two were wet before testing to test fibre strength and one remained dry to test fibre bonding. The amount of reducing sugars in the filtrate was determined as before.

Incubation time

The pine pulp was prepared as above. The enzymes dosage of 1.0 g protein/kg pulp was based on the results obtained with the dosage investigation above. Treated pulps were incubated for 0, 30, 90, 150 or 240 min at 50 °C with shaking every 15 min. The pH of the pulp was not adjusted but instead used at the pH of tap water, which was at pH 6.9. The enzyme-treated pulp samples were then tested for fibre strength and bonding as well as reducing sugars as before.

RESULTS AND DISCUSSION

Protein content

The protein content of the enzyme formulations varied quite substantially from 13 to 105 g/l. The protein content of Ecopulp® Energy was determined to be 77 g/l, which was higher than the range given by the supplier (Table 2-1). The protein content of Novozyme 476 and 613 was 13 and 14 g/l respectively, both of which fitted into the ranges given by the supplier (Table 2-1). Cellulase 1.5L had the highest protein concentration (105 g/l); although this was not that high as the supplier suggested protein values as high as 400 g/l.

Optimum temperature and pH

Ecopulp® Energy and Celluclast 1.5L exhibited the highest activity (> 80% of the maximum) on the CMC between 40 and 60 °C (Figure 2-1). Novozyme 476 had the highest activity across a wide temperature range between 40 and 80°C. The activity of Novozyme 613 on CMC was greater than 80% of the relative activity between 50 and 60 °C (Figure 2-1). The temperature profiles of all the enzymes were not typically bell shaped as expected, however, the curves of Novozyme 476 and Novozyme 613 were very similar to those curves given in the product sheets (Novozymes, 2001a; 2001b).

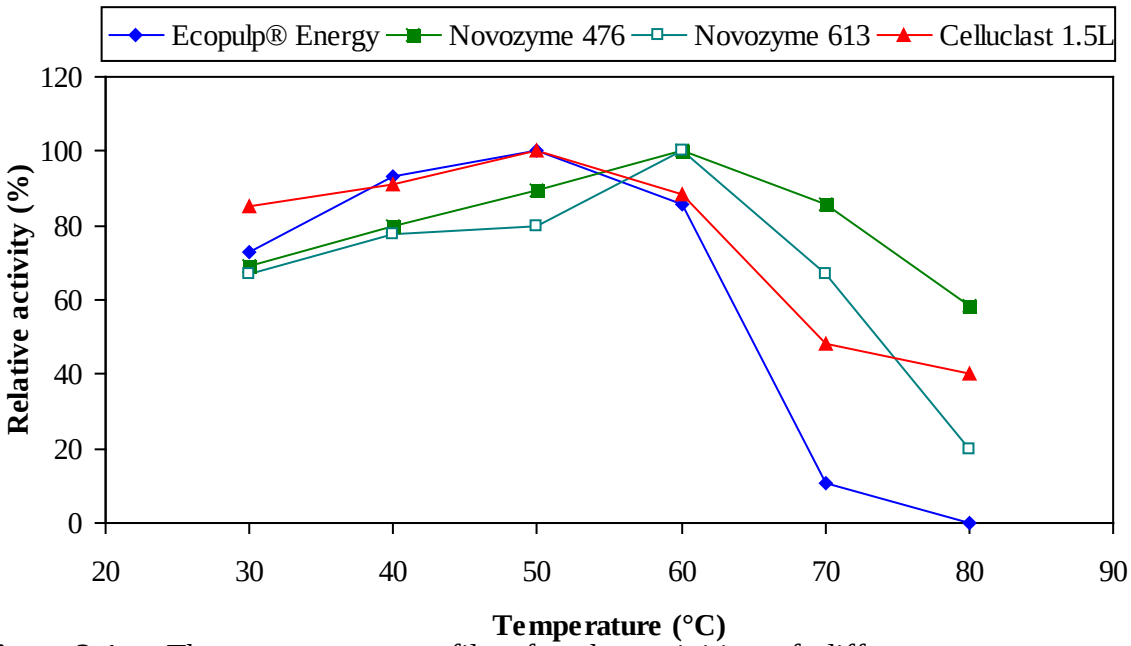

Figure 2-1 The temperature profiles for the activities of different enzymes on carboxymethylcellulose (pH of the experiments were at pH 4.8).

Ecopulp® Energy and Celluclast 1.5L had their highest activity on the CMC between pH 4 and 6 (Figure 2-2). The activity of Novozyme 476 on CMC was highest between pH 5 and 8 and that of Novozyme 613 between pH 3 and 7.

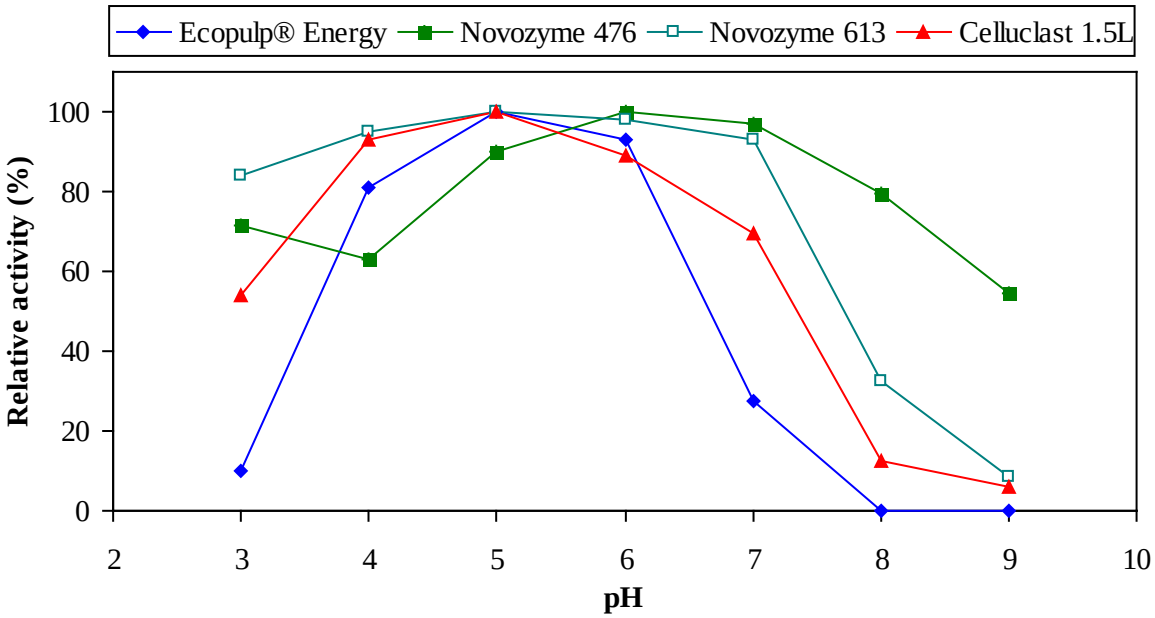

Figure 2-2 The pH profiles of the enzymes using carboxymethylcellulose as substrate the respective optimum temperatures.

The Ecopulp® Energy had highest activity on filter paper at 60 °C (Figure 2-3). Novozyme 476 was more than 80% active on filter paper between 60 and 80 °C, whilst Novozyme 613 had its highest activities between 50 and 60 °C (Figure 2-3). The activity of Celluclast 1.5L was highest between 30 and 60 °C on filter paper.

The Ecopulp® Energy had its highest activity on filter paper at pH 4 and Novozyme 476 being most active between pH 4 and 7 (Figure 2-4). Novozyme 613 had its highest activity between 6 and 9 on filter paper, whilst that of Celluclast 1.5L was between pH 5 and 6 (Figure 2-4).

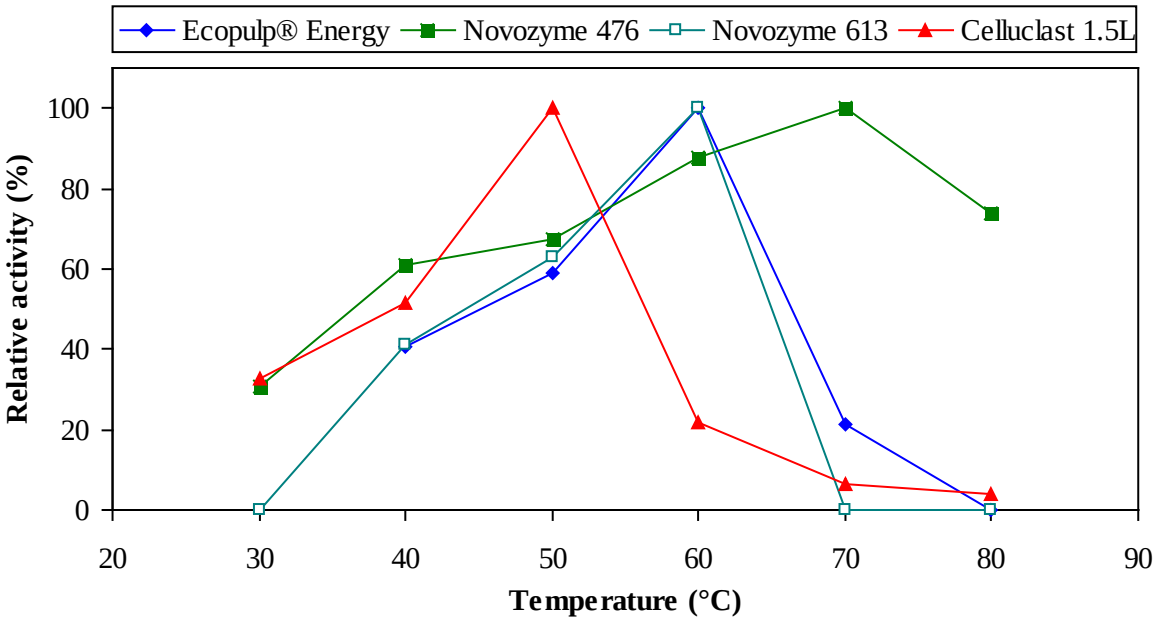

Figure 2-3 The temperature profiles for the activity of different enzymes on filter paper.

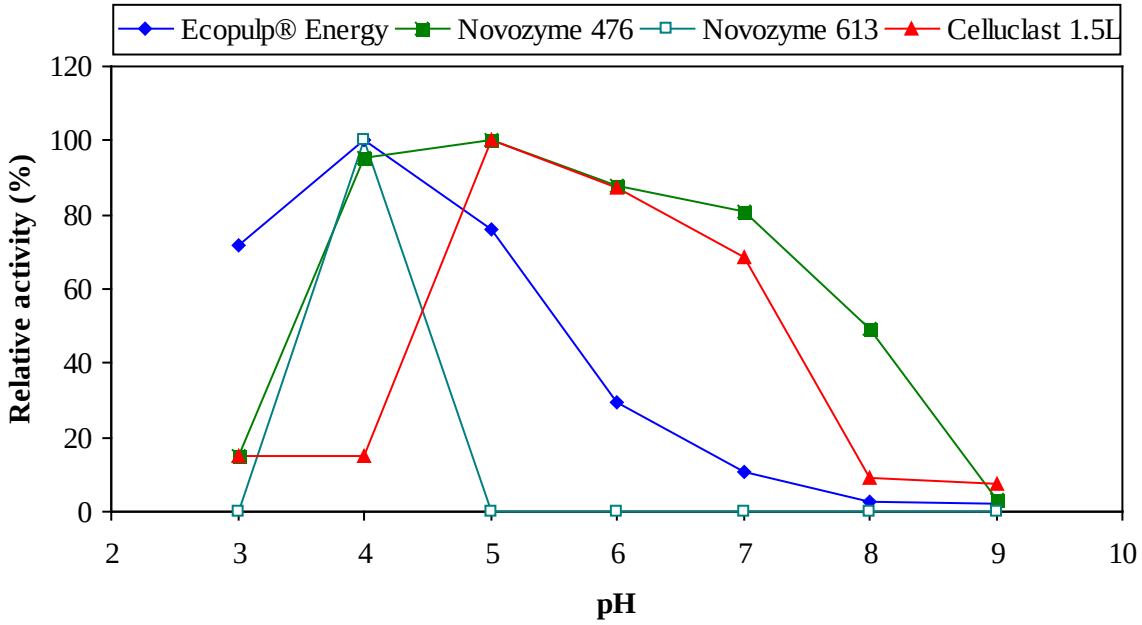

Figure 2-4 The pH profiles of enzymes using filter paper as substrate at the respective optimum temperatures.

The typical conditions expected in a paper mill are temperatures of around 50 °C and pH values between pH 4 and 5. All of the enzymes have more than 80% of activity on

either CMC or filter paper under these conditions. When the specific activities (enzyme activity/mg protein) were compared, it was noted that Novozyme 613 has the highest activity on CMC, with Novozyme 476 having the second highest activity (Table 2-2). Ecopulp® Energy and Celluclast 1.5L had the same level of activity on the CMC. The highest specific enzyme activity on filter paper was obtained with Novozyme 476, which was considerably higher than the activity of the other enzymes on filter paper.

Table 2-2 The specific enzyme activity on CMC and filter paper at 50°C and pH 4.8

Formulation	**Enzyme activity (IU/mg)**	**Enzyme activity(FPU/mg)**
Ecopulp® Energy	0.83	0.12
Novozyme 476	1.47	6.11
Novozyme 613	2.58	0.13
Celluclast 1.5L	0.83	0.25

Enzyme dosages

An increase in released reducing sugars may be an indication of the potential loss of pulp yield and it was, therefore, used as an indicator of fibre degradation. The concentration of reducing ends was increased with an increased dosage of all the enzymes (Figure 2-5).

Ecopulp® Energy, Novozyme 613 and Novozyme 476 applied at a dosage of 10 g protein /kg pulp released similar amounts of reducing sugars. At 100 g/kg, Ecopulp® Energy and Novozyme 613 released almost five times more reducing sugars than what was released at 10 g/kg. Novozyme 476 released the lowest concentration of reducing sugars at a dosage of 100 g/kg. Celluclast 1.5L-treated pulp released the highest concentration of reducing sugars at all dosages. The effect of overdosing with all of the enzymes may, therefore, result in a reduction in pulp yield.

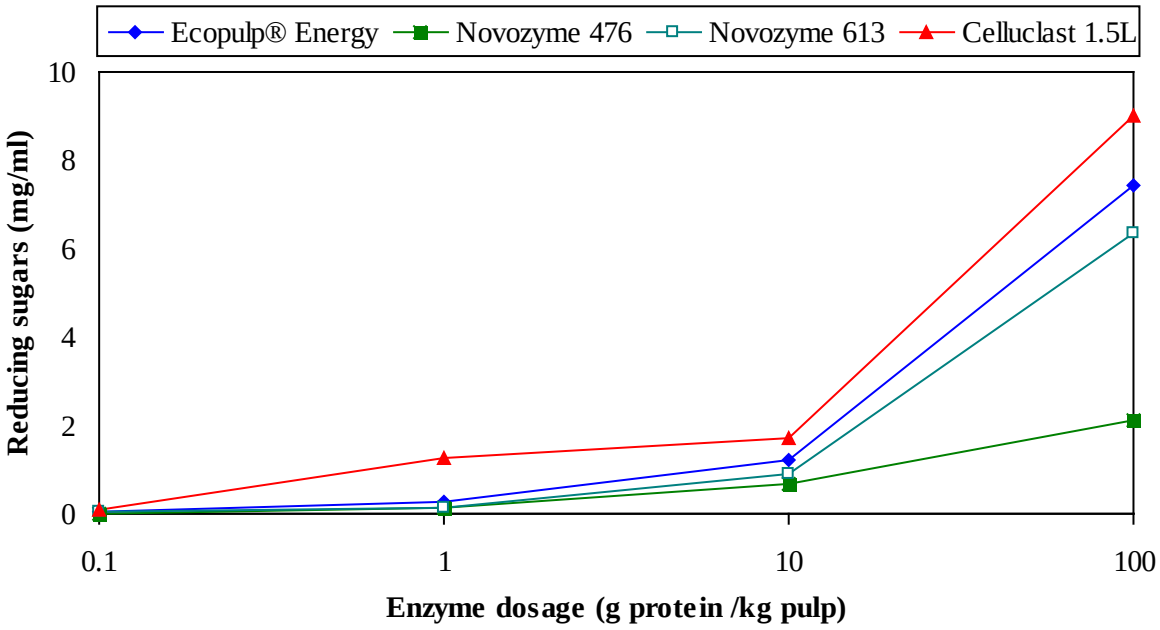

Figure 2-5 The concentration of reducing sugars of the filtrate after using increased dosages of enzyme.

Fibre strength reflects the degree of fibre damage inflicted by the enzymes, refining or both. Treatment with 0.1 g/kg of Ecopulp® Energy did not reduce fibre strength, however, fibre strength gradually decreased with dosages above 1.0 g/kg (Figure 2-6). At a dosage of 100 g/kg, Ecopulp® Energy reduced fibre strength by 60%, which was clearly an overdose. Similarly, the fibre strength at 0.1 g/kg of Novozyme 613 was unaffected but with higher dosages a gradual decrease in fibre strength occurred (Figure 2-6). Novozyme 476 dosed at 0.1 g/kg and higher caused the greatest loss in fibre strength up to and including a dosage of 100 g/kg where there was a 60% reduction in fibre strength. Celluclast 1.5L also gradually reduced fibre strength on increasing the dosage and at a dosage of 10.0 g/kg the fibre strength was reduced by 50%. At a dosage of 100 g/kg of Celluclast 1.5L the pulp was deteriorated to the extent where sheets could not be formed on the Pulmac former. The Celluclast 1.5L also released the most reducing sugar, especially at a dosage of 100 g/kg, therefore, it was no surprise that the fibres were too deteriorated to form Pulmac sheets.

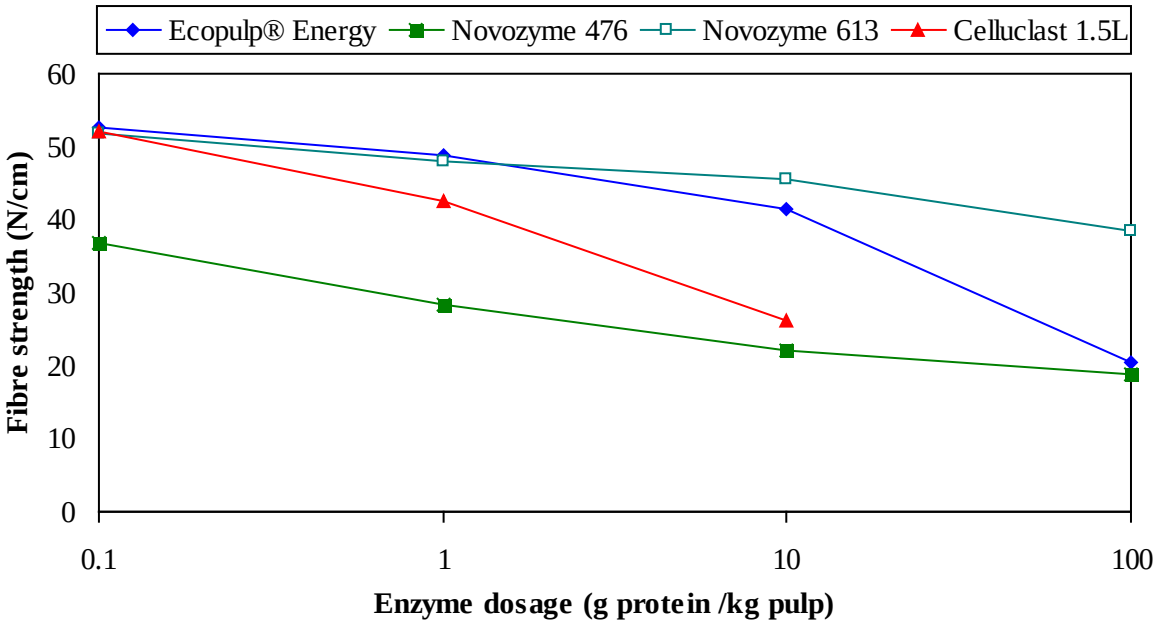

Figure 2-6 Fibre strength of the pulps treated with different dosages of enzymes. (Control 57 N/cm)

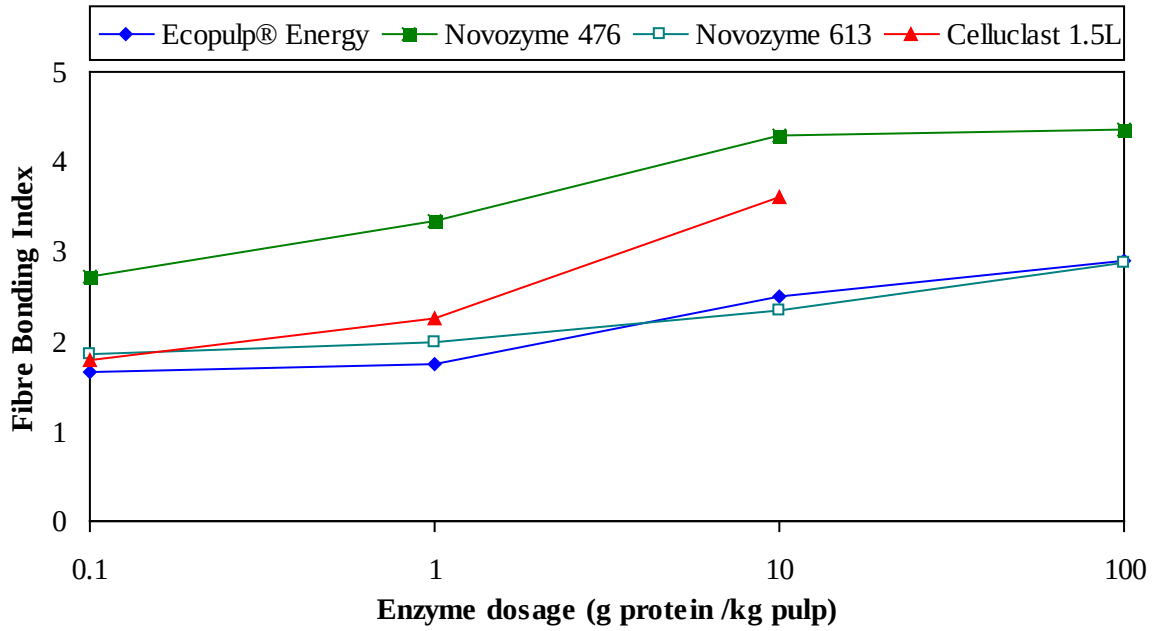

Figure 2-7 Fibre bonding of the pulps treated with different dosages of enzymes. (Control 1.63)

Fibre bonding increased when the fibres were fibrillated or became more collapsible (Strey *et al.*, 2009). In general, as the dosage of the enzymes increased, so did the fibre

bonding increase (Figure 2-7). Fibre bonding increased the most with Novozyme 476, with a 168% increase in fibre bonding at a dosage of 100 g/kg. Treatment with Novozyme 613 and Ecopulp® Energy gradually increased fibre bonding as the dosage was increased, but not as much as Novozyme 476 treatment. Celluclast 1.5L only really increased fibre bonding with a dosage of 10 g/kg (Figure 2-7).

Incubation time

Exposure of fibres to enzymes for an extended period (such as during a machine close down) may cause fibre degradation. Reducing sugars were increased by all of the tested enzymes over the four hour incubation period, indicating some risk of yield loss (Figure 2-8). The Celluclast 1.5L released the most reducing sugars over time, whilst Ecopulp® Energy also released an equal amount of reducing sugars from 120 min onwards (Figure 2-8). Novozyme 476 and 613 both released the lowest amount of reducing sugars over the four hour period.

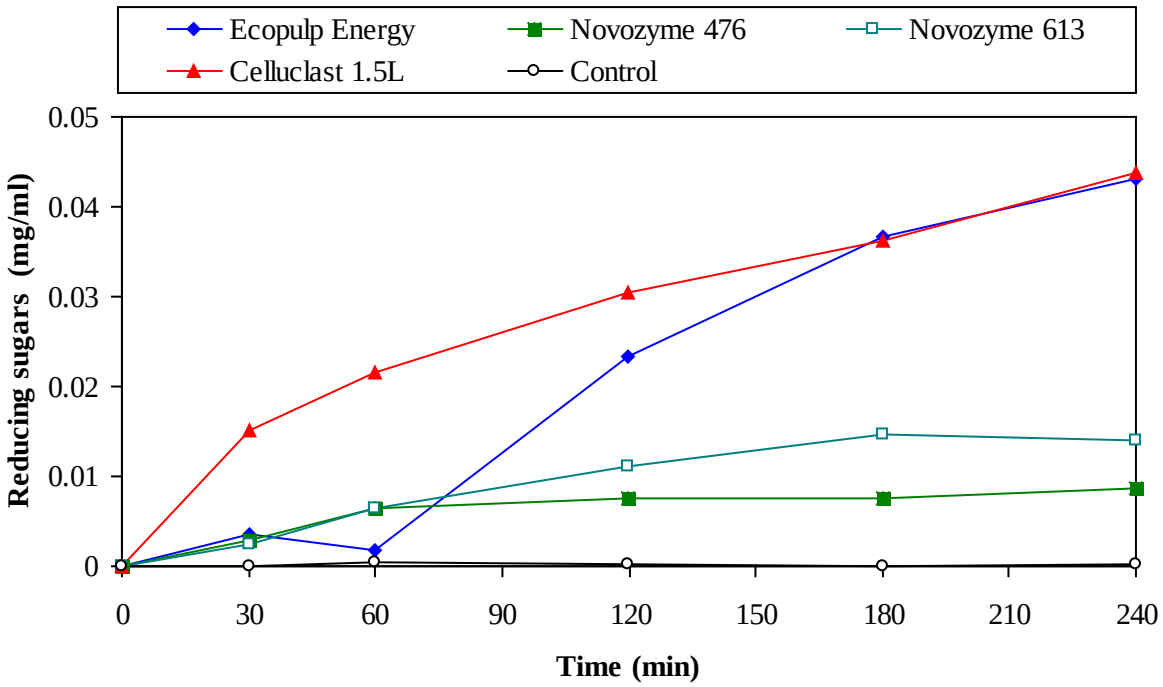

Figure 2-8 Reducing sugars liberated during incubation of 1.0 g/kg enzyme over four hours.

The worst damage of fibre strength was done in the first 30 min of incubation (Figure 2-9). The fibre strength was most reduced by Novozyme 476, whilst Novozyme 613 and Ecopulp® Energy treatment did not reduce fibre strength (Figure 2-9). Celluclast 1.5L also reduced fibre strength; however, the greatest decrease was reached in the first 30 min; thereafter, the fibre strength was maintained during the rest of the incubation period.

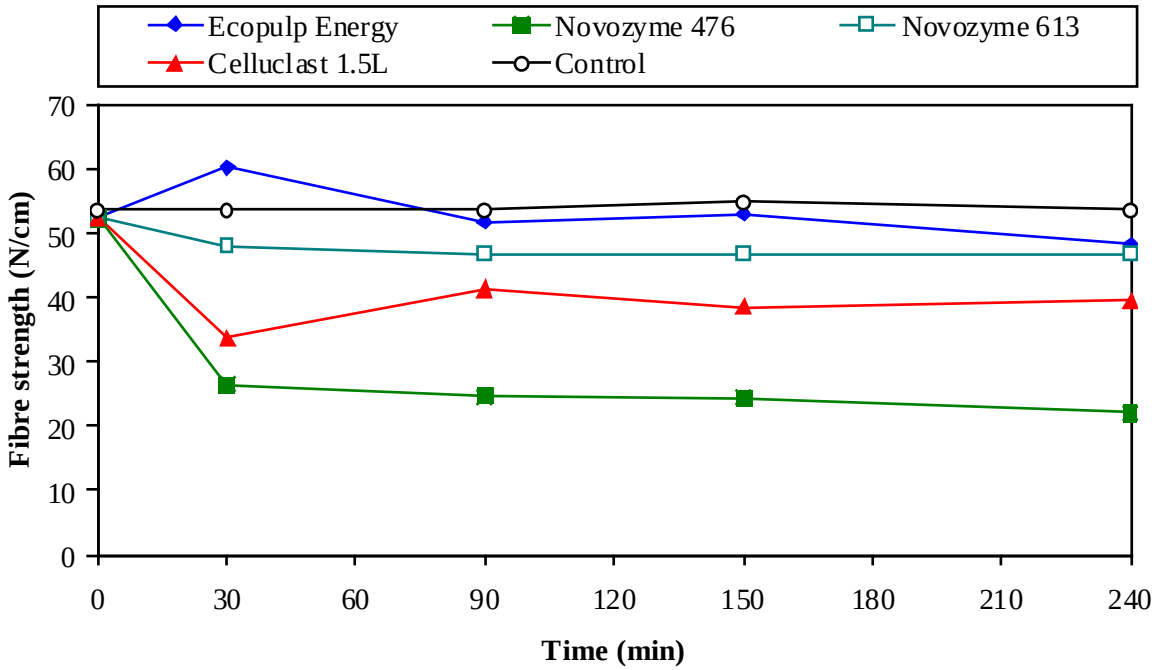

Figure 2-9 Fibre strengths of the pulps treated with the different enzymes at dosages of 1.0 g/kg tested over four hours.

The enzymes that reduced fibre strength increased fibre bonding (Figure 2-10). Enzymatic treatment probably increased fibrillation, which increased the bonding of the fibres. Novozyme 476 increased fibre bond strength the most and the biggest change occurred in the first 30 min. Fibre bonding strength was not affected by treatment with Novozyme 613 and Ecopulp® Energy, and it was slightly increased by the Celluclast 1.5L treatment (Figure 2-10).

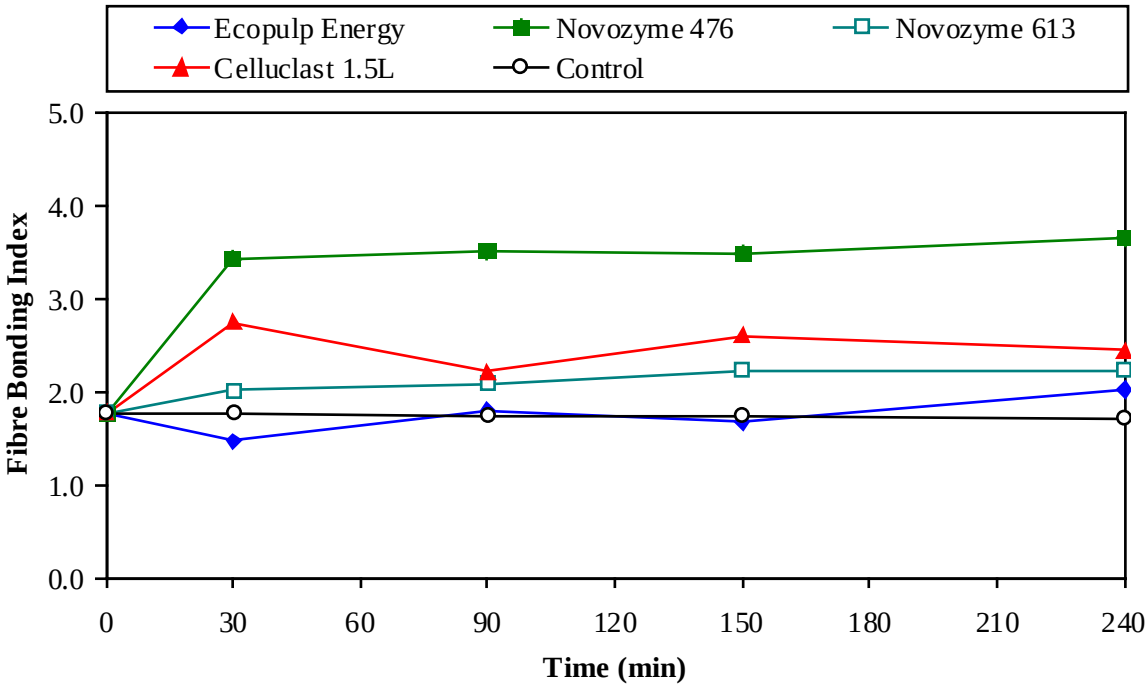

Figure 2-10: Fibre bonding index of the pulps treated with different enzymes at dosages of 1.0 g/kg tested over four hours.

Proposed mechanism for the enzymatic modification of fibres

The enzymes tested here apparently modified the cell walls and predisposed the fibres in different ways. These differences became apparent in the released reducing sugars, fibre strength and fibre bonding. However, interpretation was limited by the fact that the surface morphology of the fibres was not imaged. Within the constraints of the present dataset, the following scenarios for enzymatic action on the fibres are proposed (Figure 2-11).

The Ecopulp® Energy did not appear to improve fibrillation, since no change in bonding was observed (Figure 2-10). Fines were assumed to also have been broken down, as they would have contributed to increased fibre bonding and an increased release of reducing sugars (Figure 2-8). Novozyme 476 was effective in the fibrillation of the fibres (Figure 2-11C), as fibre bonding was the most improved (Figure 2-10) and reduced fibre strength more (Figure 2-9). Fines were assumed to contribute to the fibre bonding increase as well, since minimal release of reducing sugars was evident (Figure

2-8). The pulp sample after Novozyme 613 treatment was assumed to contain fibres with little to no fibrillated fibres and with no fines (Figure 2-11D), as fibre strength and bonding index were unchanged and reducing sugars were increased (Figure 2-8, 2-9 and 2-10). Treatment with Celluclast 1.5L released the most reducing sugars (Figure 2-8) and it also reduced fibre strength and increased fibre bonding (Figure 2-9 and Figure 2-10, respectively). These results suggested that enzyme treatment removed the fines and created small fibrils (Figure 2-11 E).

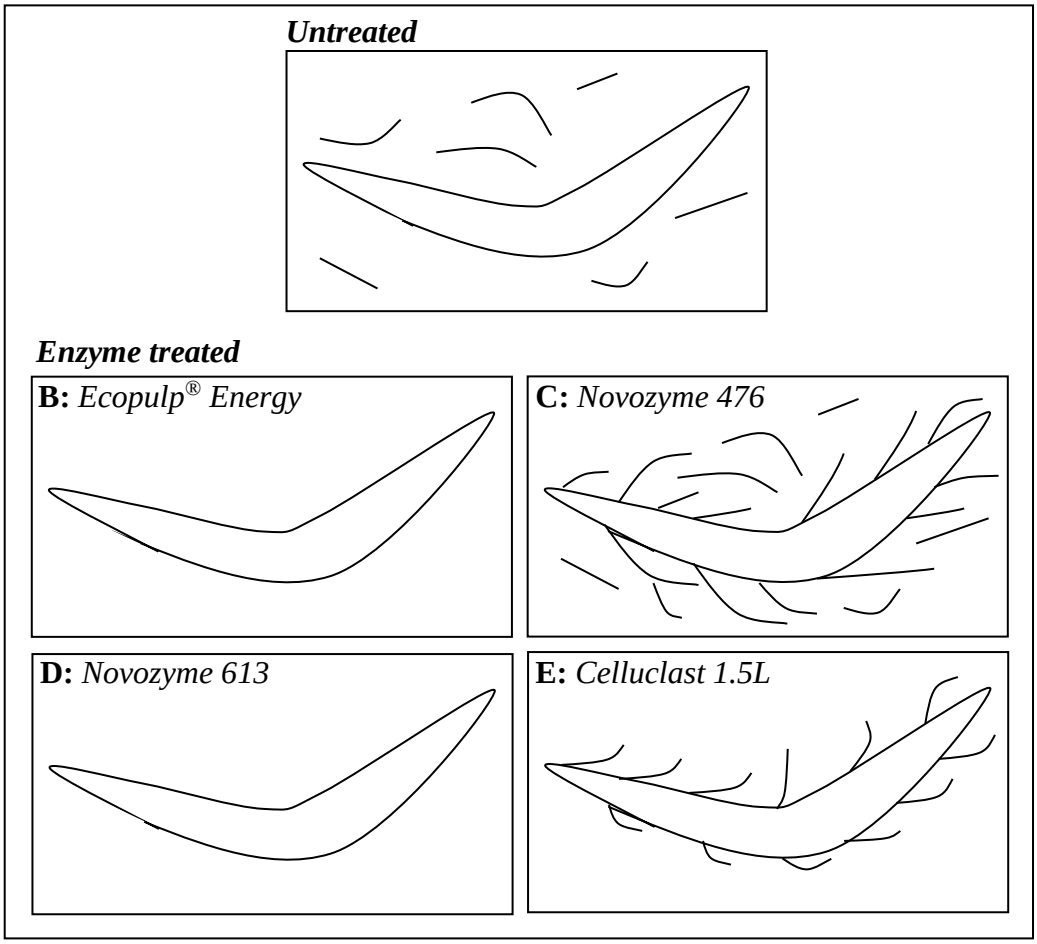

Figure 2-11: Schematic representation of the proposed mechanism for the enzymatic modification of fibres (pulp after four hours incubation with the respective enzymes; B to E).

CONCLUSIONS

The product sheets gave wide ranges of the protein concentration in the different enzyme formulations. The actual protein content of each enzyme sample was determined and used to ensure the enzymes were applied on an equal protein basis, because it was difficult to compare enzymes in a formulation with different activities (Mansfield *et al*., 1996; Suchy *et al*., 2009).

The activity profiles of the enzymes at different pH values and temperatures showed that the enzymes were suitable for mill scale applications. This was true for both filter paper and CMC activities. Pure enzymes usually display bell shaped activity curves, but the temperature and pH profiles did not display a bell shaped curve. However, the curves that were obtained were similar to those displayed in the product sheets. The stabilizers that were added to the formulation were probably assisting the enzymes to have a high activity over a wider range of temperatures. In general pulp flows in industry do not allow for enzymes to be incubated for periods longer than 30 min; therefore, the stability of the enzymes was not tested; instead, the assays were used as an indication of the enzyme stability for at least 30 min.

The over-dosing of enzymes could potentially reduce pulp yield and be uneconomical. Increased enzyme dosage caused the release of reducing sugars and a reduction in fibre strength, both of which were a clear indication that pulp fibres were being degraded. These enzymes should probably be dosed at economical dosages such as 0.1 to 1.0 g protein/kg, which would induce less fibre degradation.

In a paper mill there is always a risk of a machine shut-down and pulp that could remain for extended periods in a chest before being made into paper. Extended incubation with the enzymes showed that the most changes to the surface of the fibre occured in the first 30 min of incubation. The proposed mechanism suggested that the best enzyme for application was Novozyme 476, as it had the potential to improve paper strength properties, such as burst and tensile strength, that are dependent on fibre bonding.

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CHAPTER 3

REFINING OF ENZYME-TREATED *EUCALYPTUS GRANDIS* PULPS ON PILOT SCALE

Pilot-scale single disc refiner (right), re-pulper (middle) and geyser (left) at Sappi Technology Centre, Pretoria.

ABSTRACT

Cellulases have the ability to open up the cellulose polymers in the wood fibres to render the fibres more amenable to refining. It has been shown that dried softwood and *Eucalyptus globulus* pulps respond differently to enzyme treatments than their never-dried counterparts. It was, therefore, likely that dried and never-dried *E. grandis* pulps would respond differently to enzyme treatment. *Eucalyptus grandis* pulps were, therefore, treated with Ecopulp® Energy, Novozyme 476 and Celluclast 1.5L, respectively, and refined on pilot scale. The drainage and paper strength properties were used to determine how different fibres respond to the enzyme applications. Ecopulp® Energy caused the same response in the drainage properties for both dried and never-dried pulps, improved the strength properties and porosity on the dried pulp and bulk density on the never-dried pulp. Novozyme 476 reduced freeness and porosity only on dried pulp, but had a similar influence on the strength properties on both pulps. Celluclast 1.5L caused the same changes to occur on both dried and never-dried pulps. In general the dried fibres responded differently to enzyme treatment than never-dried fibres and enzymes should, therefore, be matched to the specific fibre used. The results also indicated that enzymes can be used to save on refining energy as well as to improve strength properties of paper made from dried and never-dried *E. grandis* pulps.

INTRODUCTION

Refining forms an integral part in the preparation of the papermaking stock, which in turn forms the interface between the pulp mill and the paper machine. The aim of refining is to develop the fibres in pulp to their optimum paper making properties (Biermann, 1996). The mechanical chafing from refining causes fibrillation of the fibres where the outer layers (primary cell wall) of the fibre are broken, causing the fibrils of the secondary cell wall to be freed. Fibrillation causes weaker fibres, thereby reducing tear strength. However, the fibrillation increases the bonding potential of the fibres and consequently the tensile strength was increased. Refining of an enzyme-treated pulp sometimes causes the fibre properties to change drastically, and the progress of change will be different to an untreated pulp (Koskenhely, 2007).

Cell-wall modifying enzymes have the ability to open up the crystalline and amorphous cellulose and hemicellulose polymers in the fibres (Pérez *et al*., 2002), thus making the fibres more amenable to refining or can altogether remove the need for refining (Bhardwaj *et al*., 1995; Clark *et al*., 1997; Dienes *et al*., 2004). These enzymes can, therefore, save energy in the refining and drying process by improving the fibrillation of the fibres or can improve the drainage ability of the pulps (Pommier *et al.*, 1990; Bhardwaj *et al*., 1995; Dickson *et al.*, 2000). They can also be used to improve tear and tensile strength of the papers being manufactured by increasing the fibrillation of the fibres (Mansfield and Dickson, 2001; García *et al.*, 2002). Cellulases also assist in the reduction of bulk density of paper, which translates into a paper that is smoother and glossier as well as darker and lower in strength, typical of papers used for printing (Smook, 1992; Biermann, 1996). As fibrillation increases from enzyme treatment, the spaces between the fibres in the paper are filled and as a consequence the porosity of paper will be reduced (Lindsay, 1994). The cell-wall modifying enzymes act differently on fibres that have been previously dried to those that have never been dried (Abubakr *et al*., 1996; Dickson *et al*., 2000; García *et al*., 2002).

Previously dried pulp fibres, especially the secondary fibres, do not absorb water as well as never-dried pulps and are more difficult to refine. That is, they require more energy

in the refining step. Never-dried fibres consequently fibrillate to a greater extent than dried fibres, which translates to a decrease in refining energy, an increased swelling and bonding area and improved tensile properties (Seth, 2001). Fibres in dried and never-dried pulp differ as a result of hornification, which refers to the reduced ability of pulp fibres to swell and participate in surface bonding (Sutjipto *et al*., 2008). Hornification occurs when water is removed from the fibres that results in a decrease in the number of hydrogen bonds formed between the fibrils and the water molecules. The fibrils then form hydrogen bonds between themselves, known as the "Campbell effect", which is difficult to reverse and results in a more rigid fibre (Campbell, 1959). Hornification can be measured using the water retention value method, which measures the ability of a pulp to absorb or swell with water. Dried pulp has been shown to have a lower water retention values than never-dried pulp (Seth, 2001).

Although some understanding has been gained on the application of cellulases on dried and never-dried softwood kraft pulps (Abubakr *et al*., 1996; Pere *et al*., 1996; Dickson *et al*., 2000), and dried and never-dried *Eucalyptus globulus* pulps (García *et al*., 2002), no reports on the impact of cellulases on dried and never-dried bleached *E. grandis* pulp could be found. The aim of the present study was, therefore, to investigate the response of dried and never-dried *E. grandis* pulp to selected cell-wall modifying enzymes by comparing the strength and drainage properties of the treated pulps after refining.

MATERIALS AND METHODS

Dried and never-dried *Eucalyptus grandis* pulps

Dried *E. grandis* pulp, at a moisture content of 8%, was collected from bales at the dry end of an uptake paper machine. Never-dried *E. grandis* pulp, at a moisture content of 58%, was collected from the press section of the same machine. The pulps were fully bleached including an oxygen stage.

Enzyme application

The pulps were repulped to a consistency of 3.5% for 20 min in 200 L of hot water (50 °C). A sample was taken from the re-pulper before the enzymes were dosed. The respective enzymes; Ecopulp® Energy (AB enzymes), Novozyme 476 (Novozymes) and Celluclast 1.5L (Novozymes) were applied to the pulp at a dosage equal to approximately 0.2 g protein/kg dried pulp. The enzyme treated pulp was mixed continuously in the re-pulper at 45 Hz for 20 min at 50 °C. The pH of the pulp was not changed but instead used at the pH of the dilution water at pH 7.2.

Pilot-scale refining

Pilot refining was done at a low-intensity of 0.3 Ws/m using classical refiner plates with a 2 mm bar width, 2 mm groove width and 4 mm bar height. The refining was carried out in "Hydracycle" mode (circulating the pulp through the refiner) and six samples were collected after an energy input of 0, 24.9, 49.2, 73.1, 96.5 and 119.4 kWh/t, respectively.

Drainage properties

The Canadian Standard Freeness (International standard, 2001) of each sample was determined immediately after refining. The water retention value was also determined on the pulp samples using standard test methods (International standard, 2007). Fibrillation of fibres and increased fines directly impact on the drainage; therefore, freeness and water retention values are good indicators of the refining efficiency. In a typical paper mill, the pulp is refined to a target freeness, which informs the paper makers about drainage. Water retention value was used to indicate the drying ability of the pulp on the paper machine (Karlsson, 2006).

Physical properties

Handsheets were made to a grammage of 75 g/m^2 from the pulp samples using the Rapid Köthen method (International standard, 2004). Tensile strength (International standard, 1992a), tear strength (International standard, 1990), bulk density (International standard, 2005) and porosity (International standard, 1992b) were determined on conditioned (22 °C, 50% humidity, 24h) handsheets using the relevant standard

international test methods. The test for tear strength reflects the changes that are occurring in fibre strength (Kärenlampi, 1996), whilst the test for tensile strength reflects fibre bonding, which in turn indicates the level of fibrillation of the fibres (Wang *et al.*, 2006). Bulk density and porosity indicate the fines generated during the refining process as well as the level of fibrillation (Lindsay, 1994).

Data analysis

The enzyme treatments were compared based on the change in the properties of the refined samples relative to the respective unrefined samples. For the raw data see the Appendices. All pulp properties were plotted against refining energy and a polynomial trend line was fitted to the respective points.

RESULTS AND DISCUSSION

As refining energy was increased, the freeness of all the pulps decreased (Figure 3-1). On the dried pulps, all the enzymes reduced freeness more than the control (Figure 3-1A). The Novozyme 476 reduced freeness less than Ecopulp® Energy and Celluclast 1.5L treatments (Figure 3-1A). On the never-dried pulps, the enzyme treatments reduced the freeness at the same rate as the rate at which the control freeness was reduced with refining. Freeness was probably reduced by the increased fibrillation of the fibres and fines generated; therefore, on the dried pulps the enzymes facilitated the fibrillation and fines generation, whilst on the never-dried pulps the enzymes had no effect on the fibres.

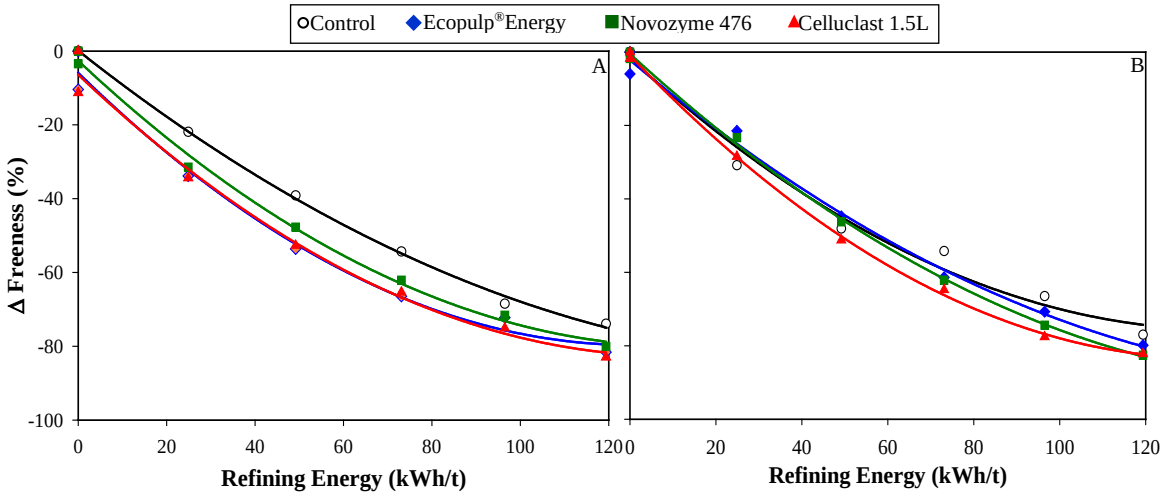

Figure 3-1 Influence of refining energy on the freeness of dried (A) and never-dried (B) *Eucalyptus grandis* pulp after treatment with the different enzymes. (Change in freeness of pulps after incubation and refining is expressed relative to the freeness of the pulp before incubation. For raw data see Appendix 3-1).

The water retention value was increased by all the enzymes applied to the pulps (Figure 3-2). On the dried pulps, Ecopulp® Energy and Celluclast 1.5L increased water retention values by the same percentage and Novozyme 476 increased water retention the most (Figure 3-2A). On the never-dried pulps the enzymes increased the water retention value by the same percentage (Figure 3-2B). These enzymes opened up the fibres to allow water into the fibre so that the water cannot be removed by passive drainage and would, therefore, increase the drying energy as more heat would be used to dry the paper. Similar results were seen with cellulases that were applied to softwood pulps as well as dried and never-dried *E. globulus* pulps (García *et al.*, 2002; Mohlin and Pettersson, 2002; Dienes *et al.*, 2004).

The tensile index increased with increased refining for both pulps (Figure 3-3). On the dried pulps, the Ecopulp® Energy and Celluclast 1.5L increased tensile index more than the control, whilst Novozyme 476 treatment maintained tensile strength equal to the control (Figure 3-3A). On the never-dried pulps, Ecopulp® Energy reduced the tensile

strength, whilst Novozyme 476 and Celluclast 1.5L maintained tensile index equal to the control (Figure 3-3B).

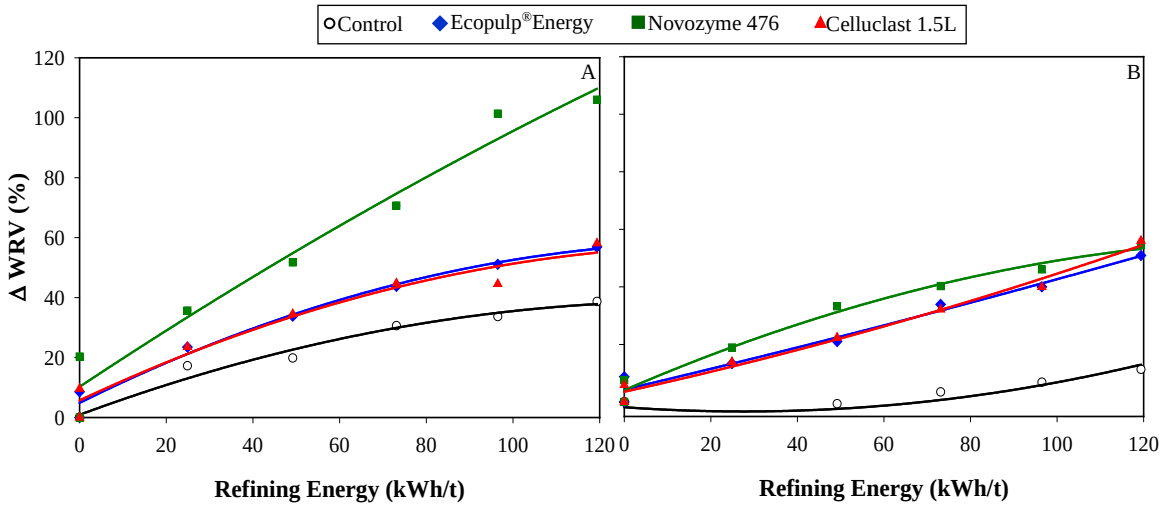

Figure 3-2 Influence of refining energy on the water retention value (WRV) of dried (A) and never-dried (B) *Eucalyptus grandis* pulp after treatment with the different enzymes. (Change in WRV of pulps after incubation and refining is expressed relative to the WRV of the pulp before incubation. For raw data see Appendix 3-2).

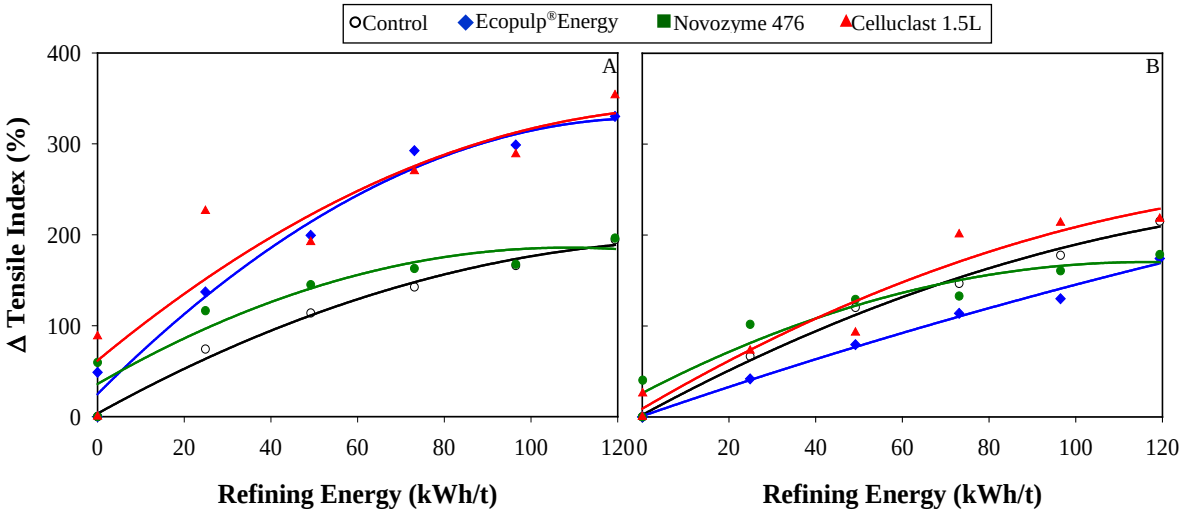

Figure 3-3 Influence of refining energy on the tensile index of of dried (A) and never-dried (B) *Eucalyptus grandis* pulp after treatment with the different enzymes. (Change in tensile index of pulps after incubation and refining is expressed relative to the tensile index of the pulp before incubation. For raw data see Appendix 3-3).

Tensile strength has been reduced by treatments with endoglucanases and cellulases on softwoods as well as dried and never-dried *E. globulus* pulp (García *et al.*, 2002). Despite literature, enzyme treatment of fibres in theory should increase the tensile strength due to the increased bonding; therefore, it could be the effects of overdosing that cause the tensile strength to decrease.

The dried and never-dried pulps responded differently to the enzyme treatments concerning the tear index (Figure 3-4). On the dried pulps, Ecopulp® Energy and Celluclast 1.5L increased the tear index compared to the control, whilst Novozyme 476 reduced the tear index (Figure 3-4). On the never-dried pulps the Novozyme 476 also reduced the tear index and the Ecopulp® Energy and Celluclast 1.5L maintained the tear index equal to the control pulp (Figure 3-4).

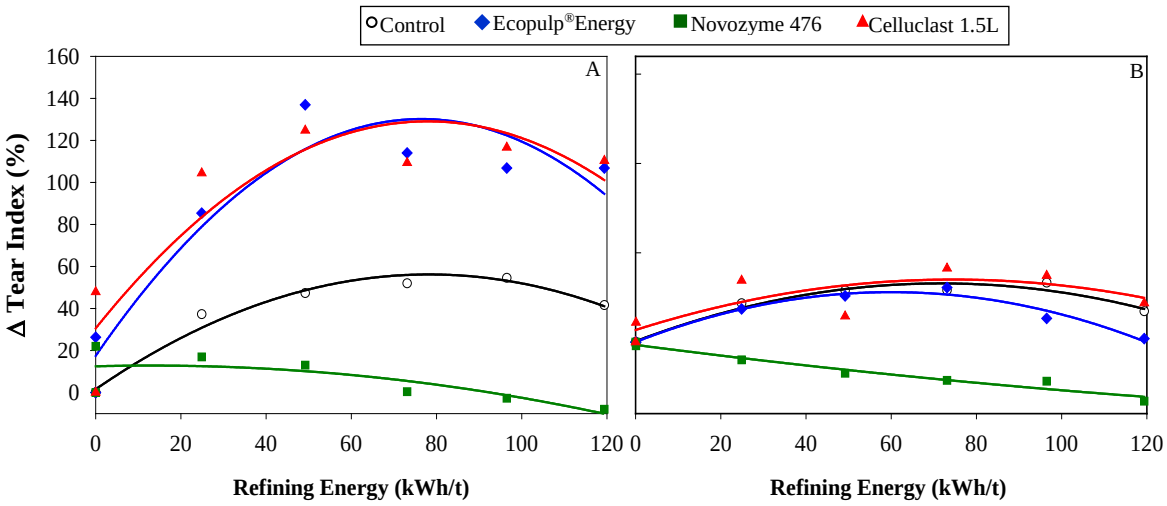

Figure 3-4 Influence of refining energy on the change in tear index of dried (A) and never dried (B) *Eucalyptus grandis* pulp after treatment with the different enzymes. (Change in tear index of pulps after incubation and refining is expressed relative to the tear index of the pulp before incubation. For raw data see Appendix 3-4).

Changes in fibre strength influence the tear strength; therefore, weakened fibres will cause the tear index to decrease. The tear index usually decreased with enzyme treatments (García *et al.*, 2002) and this was confirmed by the treatment with

Novozyme 476 (Figure 3-4). However, the tear strength was also increased in the case of the enzyme-treated dried pulps, which could be due to the dosage differences between the formulations as tear strength has been reported to increase at lower dosages of enzymes (Pere *et al*., 1996; García *et al*., 2002).

In summary, on the dried pulps, the tensile and tear indices were increased by treatment with Ecopulp® Energy and Celluclast 1.5L, whilst Novozyme 476 maintained the tensile strength but reduced the tear strength of the pulps (Figure 3-3 and 3-4). On the never-dried pulps, the tensile strength was barely influenced by the enzyme treatments. However, Novozyme 476 reduced the tear strength (Figure 3-3 and 3-4).

On all the pulps, bulk density was reduced as refining increased (Figure 3-5). On the dried pulps all the enzyme treatments reduced the bulk density more than the control. On the never-dried pulps the Celluclast 1.5L and Novozyme 476 also reduced bulk density. However, Ecopulp®Energy increased the bulk density.

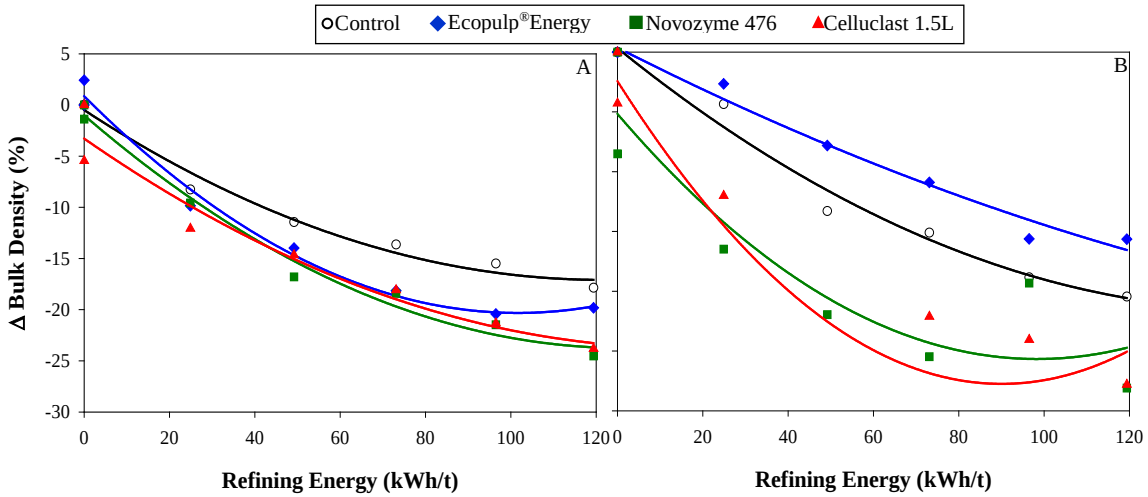

Figure 3-5 Influence of refining energy on the bulk density of dried (A) and never-dried (B) *Eucalyptus grandis* pulp after treatment with the different enzymes. (Change in bulk density of pulps after incubation and refining is expressed relative to the bulk density of the pulp before incubation. For raw data see Appendix 3-5).

The porosity of the pulps decreased with an increase in refining energy (Figure 3-6). On the dried pulps, all the enzymes reduced porosity more than that of the control. On the never-dried pulps, the enzyme treatments maintained the porosity of the pulps equal to that of the control (Figure 3-6). The mechanical refining of pulp typically decreases the porosity and bulk density of paper (Smook, 1992; Biermann, 1996) and this was evident during the refining of the dried and never-dried pulps (Figure 3-5 and 3-6). It has been reported that the use of refining enzymes improved the bulk density and porosity of paper (Bhardwaj *et al*., 1995; Mansfield *et al*., 1996; Wong *et al*., 2000).

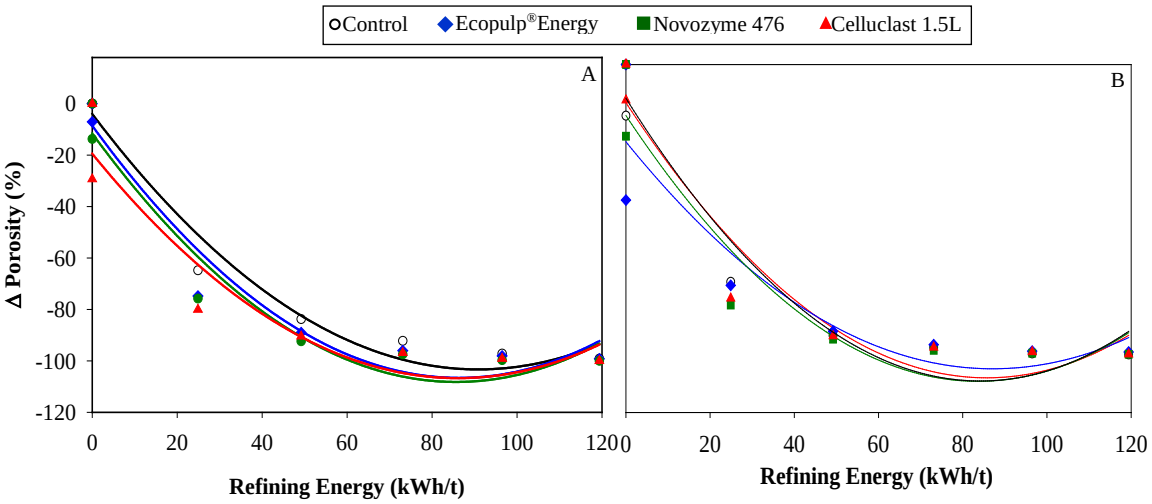

Figure 3-6 Influence of refining energy on the porosity of dried (A) and never-dried (B) *Eucalyptus grandis* pulp after treatment with the different enzymes. (Change in porosity of pulps after incubation and refining is expressed relative to the porosity of the pulp before incubation. For raw data see Appendix 3-6).

CONCLUSIONS

Dried and never-dried fibres responded differently to refining. Although freeness has been shown to increase with enzyme treatments of dried and never-dried fibre types (Bhardwaj *et al*., 1995; Abubakr *et al*., 1996; Mansfield *et al*., 1996; Pere *et al*., 1996; García *et al*., 2002; Mohlin and Pettersson, 2002; Dienes *et al*., 2004; Pala *et al*., 2004), the freeness was only decreased when enzymes were applied to the dried pulps. The tensile and tear indices were also easier to improve on the dried pulps with refining after treatment with Ecopulp® Energy and Celluclast 1.5L. It was clear that after treatment with enzymes, the dried *E. grandis* pulps were easier to refine and developed tear and tensile strength to a greater extent than the never-dried counterparts.

Celluclast 1.5L and Ecopulp® Energy improved the refining of dried *E. grandis* pulps. On the dried pulps, all of the enzymes were able to reduce freeness faster than the control. The selected enzymes have the potential for energy savings in a paper mill as the treated pulps required less energy to achieve the same freeness as an untreated pulp. Celluclast 1.5L and Ecopulp® Energy increased the tensile strength without compromising on tear strength. It was expected that tear strength would decrease as tensile strength increased, as an increased fibre bond translated into a reduced fibre strength when using Celluclast 1.5L and Ecopulp® Energy (Chapter 2). Celluclast 1.5L and Ecopulp® Energy could be used for energy savings as well as improvements in the tear and tensile strength of pulp in the paper mills that utilise dried *E. grandis* pulp.

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APPENDICES

Appendix 3-1: Freeness and change in freeness of untreated enzyme treated dried and never-dried *Eucalyptus grandis* pulps

Sample		Energy (kWh/t)	Freeness (ml/min)		Δ Freeness (%)	
			Dried	Never dried	Dried	Never dried
Control	Repulped	0	552	522	0	0
	Incubated	0	552	522	0	0
	Refined 1	24.9	431	361	-22	-31
	Refined 2	49.2	336	271	-39	-48
	Refined 3	73.1	252	239	-54	-54
	Refined 4	96.5	174	175	-68	-66
	Refined 5	119.4	144	120	-74	-77
Ecopulp® Energy	Repulped	0	576	536	0	0
	Incubated	0	516	504	-10	-6
	Refined 1	24.9	381	421	-34	-21
	Refined 2	49.2	267	296	-54	-45
	Refined 3	73.1	193	207	-66	-61
	Refined 4	96.5	160	157	-72	-71
	Refined 5	119.4	106	108	-82	-80
Novozyme 476	Repulped	0	578	562	0	0
	Incubated	0	558	552	-3	-2
	Refined 1	24.9	396	431	-31	-23
	Refined 2	49.2	302	302	-48	-46
	Refined 3	73.1	219	213	-62	-62
	Refined 4	96.5	164	144	-72	-74
	Refined 5	119.4	115	98	-80	-83
Celluclast 1.5L	Repulped	0	607	562	0	0
	Incubated	0	538	552	-11	-2
	Refined 1	24.9	398	402	-34	-28
	Refined 2	49.2	287	274	-53	-51
	Refined 3	73.1	210	198	-65	-65
	Refined 4	96.5	151	126	-75	-78
	Refined 5	119.4	103	100	-83	-82

Appendix 3-2: Water retention value (WRV) and change in water retention value of untreated and treated dried and never-dried *Eucalyptus grandis* pulp.

Sample		Energy (kWh/t)	WRV (g/g)		Δ WRV (%)	
			Dried	Never dried	Dried	Never dried
Control	Repulped	0	2.56	3.49	0	0
	Incubated	0	2.56	3.49	0	0
	Refined 1	24.9	3.01	3.12	18	-11
	Refined 2	49.2	3.07	3.47	20	-1
	Refined 3	73.1	3.35	3.61	31	3
	Refined 4	96.5	3.43	3.73	34	7
	Refined 5	119.4	3.55	3.89	39	11
Ecopulp Energy	Repulped	0	2.41	2.76	0	0
	Incubated	0	2.62	3.00	9	9
	Refined 1	24.9	2.98	3.13	24	13
	Refined 2	49.2	3.23	3.34	34	21
	Refined 3	73.1	3.46	3.70	44	34
	Refined 4	96.5	3.64	3.87	51	40
	Refined 5	119.4	3.78	4.17	57	51
Novozyme 476	Repulped	0	2.45	2.93	0	0
	Incubated	0	2.95	3.15	20	8
	Refined 1	24.9	3.32	3.48	36	19
	Refined 2	49.2	3.72	3.90	52	33
	Refined 3	73.1	4.18	4.11	71	40
	Refined 4	96.5	4.93	4.28	101	46
	Refined 5	119.4	5.05	4.52	106	54
Celluclast 1.5L	Repulped	0	2.42	2.73	0	0
	Incubated	0	2.65	2.89	10	6
	Refined 1	24.9	2.99	3.11	24	14
	Refined 2	49.2	3.25	3.34	34	22
	Refined 3	73.1	3.50	3.61	45	32
	Refined 4	96.5	3.49	3.82	44	40
	Refined 5	119.4	3.82	4.26	58	56

Appendix 3-3: Tensile index and change in tensile index of untreated and enzyme treated dried and never-dried *Eucalyptus grandis* pulps.

Sample		Energy (kWh/t)	Tensile index (Nm/g)		Δ Tensile index (%)	
			Dried	Never dried	Dried	Never dried
Control	Repulped	0	23.38	27.09	0	0
	Incubated	0	23.38	27.09	0	0
	Refined 1	24.9	40.72	45.11	74	67
	Refined 2	49.2	49.99	59.68	114	120
	Refined 3	73.1	56.71	66.83	143	147
	Refined 4	96.5	62.20	75.22	166	178
	Refined 5	119.4	68.87	85.36	195	215
Ecopulp Energy	Repulped	0	16.06	29.63	0	0
	Incubated	0	23.88	29.63	49	0
	Refined 1	24.9	38.09	42.00	137	42
	Refined 2	49.2	48.08	53.19	199	80
	Refined 3	73.1	63.05	63.38	293	114
	Refined 4	96.5	64.04	68.14	299	130
	Refined 5	119.4	69.08	81.24	330	174
Novozyme 476	Repulped	0	20.85	26.22	0	0
	Incubated	0	33.25	36.76	59	40
	Refined 1	24.9	45.11	52.89	116	102
	Refined 2	49.2	51.07	60.03	145	129
	Refined 3	73.1	54.78	61.03	163	133
	Refined 4	96.5	55.76	68.32	167	161
	Refined 5	119.4	61.75	72.98	196	178
Celluclast 1.5L	Repulped	0	14.96	23.43	0	0
	Incubated	0	28.17	29.44	88	26
	Refined 1	24.9	48.79	40.47	226	73
	Refined 2	49.2	43.67	45.12	192	93
	Refined 3	73.1	55.35	70.44	270	201
	Refined 4	96.5	58.11	73.44	288	213
	Refined 5	119.4	67.83	74.44	353	218

Appendix 3-4: Tear index and change in tear index of untreated and enzyme treated dried and never-dried *Eucalyptus grandis* pulps

Sample		Energy (kWh/t)	Tear index (mNm^2/g)		Δ Tear index (%)	
			Dried	Never dried	Dried	Never dried
Control	Repulped	0	1.64	1.77	0	0
	Incubated	0	1.64	1.77	0	0
	Refined 1	24.9	2.25	2.15	37	21
	Refined 2	49.2	2.42	2.28	48	29
	Refined 3	73.1	2.49	2.28	52	29
	Refined 4	96.5	2.54	2.35	55	33
	Refined 5	119.4	2.32	2.07	41	17
Ecopulp Energy	Repulped	0	1.11	1.84	0	0
	Incubated	0	1.41	1.84	27	0
	Refined 1	24.9	2.06	2.18	86	18
	Refined 2	49.2	2.64	2.31	138	26
	Refined 3	73.1	2.38	2.40	114	30
	Refined 4	96.5	2.30	2.08	107	13
	Refined 5	119.4	2.30	1.87	107	2
Novozyme 476	Repulped	0	1.26	1.60	0	0
	Incubated	0	1.54	1.57	22	-2
	Refined 1	24.9	1.48	1.44	17	-10
	Refined 2	49.2	1.43	1.32	13	-18
	Refined 3	73.1	1.27	1.26	1	-21
	Refined 4	96.5	1.23	1.25	-2	-22
	Refined 5	119.4	1.16	1.07	-8	-33
Celluclast 1.5L	Repulped	0	1.06	1.49	0	0
	Incubated	0	1.57	1.65	48	11
	Refined 1	24.9	2.17	2.00	105	34
	Refined 2	49.2	2.39	1.70	125	14
	Refined 3	73.1	2.22	2.10	109	41
	Refined 4	96.5	2.30	2.04	117	37
	Refined 5	119.4	2.23	1.81	110	21

Appendix 3-5: Bulk density and change in bulk density of untreated and enzyme treated dried and never-dried *Eucalyptus grandis* pulps

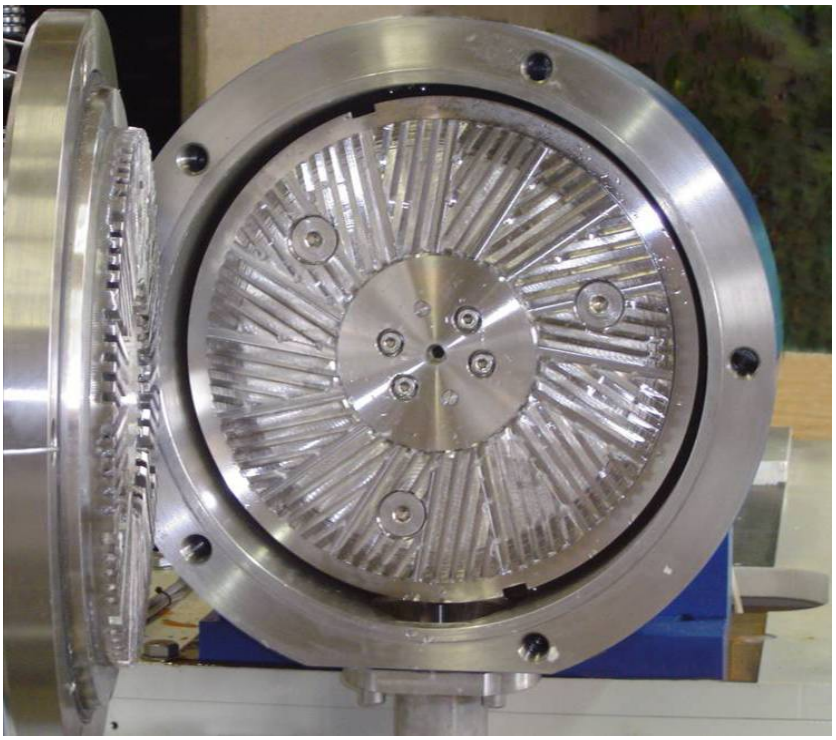
Sample		Energy (kWh/t)	Bulk density (cm^3/g)		Δ Bulk density (%)	
			Dried	Never dried	Dried	Never dried
Control	Repulped	0	1.73	1.65	0	0
	Incubated	0	1.73	1.65	0	0
	Refined 1	24.9	1.59	1.58	-8	-4
	Refined 2	49.2	1.53	1.43	-12	-13
	Refined 3	73.1	1.49	1.4	-14	-15
	Refined 4	96.5	1.46	1.34	-16	-19
	Refined 5	119.4	1.42	1.32	-18	-20
Ecopulp Energy	Repulped	0	1.78	1.61	0	0
	Incubated	0	1.82	1.61	2	0
	Refined 1	24.9	1.60	1.57	-10	-2
	Refined 2	49.2	1.53	1.49	-14	-7
	Refined 3	73.1	1.45	1.44	-19	-11
	Refined 4	96.5	1.41	1.36	-21	-16
	Refined 5	119.4	1.42	1.36	-20	-16
Novozyme 476	Repulped	0	1.74	1.82	0	0
	Incubated	0	1.72	1.67	-1	-8
	Refined 1	24.9	1.58	1.52	-9	-16
	Refined 2	49.2	1.45	1.42	-17	-22
	Refined 3	73.1	1.42	1.36	-18	-25
	Refined 4	96.5	1.37	1.47	-21	-19
	Refined 5	119.4	1.32	1.31	-24	-28
Celluclast 1.5L	Repulped	0	1.82	1.76	0	0
	Incubated	0	1.72	1.68	-5	-5
	Refined 1	24.9	1.60	1.55	-12	-12
	Refined 2	49.2	1.55	1.21	-15	-31
	Refined 3	73.1	1.49	1.37	-18	-22
	Refined 4	96.5	1.43	1.34	-21	-24
	Refined 5	119.4	1.39	1.27	-24	-28

Appendix 3-6: Porosity and change in porosity of untreated and enzyme treated dried and never-dried *Eucalyptus grandis* pulps

Sample		Energy (kWh/t)	Porosity (ml/min)		Δ Porosity (%)	
			Dried	Never dried	Dried	Never dried
Control	Repulped	0	5000	5000	0	0
	Incubated	0	3860	4118	-23	-18
	Refined 1	24.9	1358	1252	-73	-75
	Refined 2	49.2	626	326	-87	-93
	Refined 3	73.1	299	122	-94	-98
	Refined 4	96.5	108	17	-98	-100
	Refined 5	119.4	32	0	-99	-100
Ecopulp Energy	Repulped	0	5000	5000	0	0
	Incubated	0	4647	2661	-73	-55
	Refined 1	24.9	1261	1190	-88	-85
	Refined 2	49.2	550	400	-96	-94
	Refined 3	73.1	198	167	-98	-98
	Refined 4	96.5	97	56	-99	-98
	Refined 5	119.4	38	42	-2	68
Novozyme 476	Repulped	0	4576	4464	0	0
	Incubated	0	3948	3357	-72	-77
	Refined 1	24.9	1106	758	-91	-93
	Refined 2	49.2	346	233	-97	-98
	Refined 3	73.1	116	62	-100	-99
	Refined 4	96.5	11	18	-100	-100
	Refined 5	119.4	0	0	27	36
Celluclast 1.5L	Repulped	0	5000	4571	0	0
	Incubated	0	3534	4004	-72	-78
	Refined 1	24.9	998	882	-87	-93
	Refined 2	49.2	476	287	-96	-97
	Refined 3	73.1	155	108	-99	-99
	Refined 4	96.5	43	36	-100	-100
	Refined 5	119.4	0	1	-100	-100

CHAPTER 4

REFINING OF ENZYME-TREATED *EUCALYPTUS NITENS* PULPS ON PILOT SCALE



Classical hardwood refiner plates with 2-mm bar width, 2-mm groove width and 4-mm bar height for pilot-scale single disc refiner.

ABSTRACT

Dried and never-dried *Eucalyptus grandis* pulps responded differently to enzyme treatments compared to their never-dried counterparts. It was, therefore, likely that dried and never-dried *E. nitens* pulps could respond differently to enzyme treatment as well. *Eucalyptus nitens* pulps were, therefore, treated with Ecopulp® Energy and Novozyme 476, respectively, and refined on pilot scale. The drainage and paper-strength properties were used to determine how different fibres respond to the enzyme applications. The dried and never-dried *E. nitens* fibres responded differently to enzymatic refining: Novozyme 476 was the best enzyme for application on the dried *E. nitens* pulps, because it saved on refining and drying energy due to the decreased freeness and water retention values. Novozyme 476 also improved the physical properties of the pulps where tear and tensile index and porosity were increased and bulk was reduced. This work demonstrated that enzymes must be selected to match the pulps being used to suit product requirements.

INTRODUCTION

Eucalyptus nitens has become a popular choice of hardwood for the pulp and paper industry as it grows fast, is adaptable to the changing environment and it produces fibres of good quality (Wei and Xu, 2003). *Eucalyptus grandis* was traditionally used throughout South Africa as the main source of wood timber; however, as the pulp and paper industry expanded, the demand for wood increased and more plantations were, therefore, required. However, the land available for plantations was at high altitude; therefore, *E. nitens* was grown due to its cold tolerance (Darrow, 1996; Gardner and Swain, 1996).

The morphological and chemical properties of *E. grandis* and *E. nitens* pulps differ and consequently there are large differences between these pulps with regard to refining for specific paper properties. *Eucalyptus nitens* pulps requires less refining energy to reach a given tensile strength than *E. grandis* pulps, which is not only important for energy savings but also important for superior sheet opacity, porosity and bulk density (Cotterill and Macrae, 1997). *Eucalyptus grandis* pulps tend to produce papers that are low in bulk density and porosity, which are suited for the standard coated and uncoated printing and writing papers. The high strength, high opacity, and low porosity of *E. nitens* pulps make them well suited for the better quality coated and uncoated printing, writing and security papers. One of the reasons why the two *Eucalyptus* species are different is because the *E. nitens* pulp fibres are thinner and longer than *E. grandis* pulp fibres (Cotterill and Macrae, 1997). It is likely that the fibres of the *E. nitens* pulp could respond differently to enzyme treatments compared to *E. grandis* pulps due to the difference in their fibre morphology, chemical composition and response to refining.

Pulp fibres that have been dried before because of recycling or storage purposes, refine differently to never-dried fibres (Sutjipto *et al*., 2008). With this in mind, when the same enzymes were applied to the dried and never-dried pulps, there was a very different response of these pulps to refining (Chapter 3). The never-dried *E. grandis* pulps did not refine as easily as the dried pulps after enzyme treatment. Application of enzymes to the dried pulps may have facilitated the swelling ability of the fibres to

refine easier than untreated dried fibres. It was clear that after treatment with the enzymes, the dried *E. grandis* pulps were easier to refine and they developed tear and tensile strength more than their never-dried counter parts (Chapter 3). A reduced bulk density in enzyme treated *E. grandis* resulted in a smoother and glossier paper that was lower in strength, typical of papers used for printing (Smook, 1992; Biermann, 1996). The porosity of the *E. grandis* pulps was unchanged, which was unexpected as the fibres should be more fibrillated and the spaces between the fibres decreased (Lindsay, 1994).

Although a better understanding has been gained on the application of cellulases on dried and never-dried *E. grandis* pulps (Chapter 3), *E. nitens* pulps require a similar investigation as they are becoming the preferred species for paper making. The enzyme Celluclast 1,5L was not included in the present investigation due to its aggressive nature on the fibres and risk of yield loss due to the enzyme treatment (Chapter 2 and 3). The aim of the present study was, therefore, to investigate the response of dried and never-dried *E. nitens* pulp to Ecopulp® Energy and Novozyme 476 by comparing the strength and drainage properties of the treated pulps.

MATERIALS AND METHODS

Dried and never-dried *Eucalyptus nitens* pulps

Dried *E. nitens* pulp, at a moisture content of 6%, was collected from bales at the dry end of an uptake paper machine. Never-dried *E. nitens* pulp, at a moisture content of 60%, was collected from the press section of the same machine. The pulps were fully bleached, including an oxygen stage.

Enzyme application

The pulps were repulped to a consistency of 3.5% for 20 min in 200 L of hot water at 50 °C. A sample was taken from the re-pulper before the enzymes were dosed. The respective enzymes Ecopulp® Energy (AB enzymes, Germany) and Novozyme 476 (Novozymes, Denmark) were applied to the pulp at a dosage equal to approximately 0.2 g protein/kg dried pulp. The enzyme treated pulp was mixed continuously in the

re-pulper at 45 Hz for 20 min at 50 °C. The pH of the pulp was not changed but instead used at the pH of the dilution water at pH 7.2.

Pilot-scale refining

Pilot refining was done at a low-intensity of 0.3 Ws/m using classical refiner plates with a 2 mm bar width, 2 mm groove width and 4 mm bar height specific for hardwood refining. The refining was carried out in "Hydracycle" mode and six samples were collected after energy inputs of 0, 21.5, 42.3, 62.6, 82.2 and 101.3 kWh/t, respectively.

Drainage properties

The Canadian Standard Freeness (International standard, 2001) of each sample was determined immediately after refining. The water retention value was also determined on the pulp samples using standard test methods (International standard, 2007). Fibrillation of fibres and increased fines in the pulps directly impact on the drainage; therefore, freeness and water retention values are good indicators of the refining efficiency. In a typical paper mill the pulp is refined to a target freeness, which informs the paper makers about drainage. The water retention value is used to indicate the drying ability of the pulp on the paper machine (Karlsson, 2006).

Physical properties

Handsheets were made to a grammage of 75 g/m^2 from the pulp samples using the Rapid Köthen method (International standard, 2004). Tensile strength (International standard, 1992a), tear strength (International standard, 1990), bulk density (International standard, 2005) and porosity (International standard, 1992b) were determined on handsheets conditioned at 22 °C, 50% humidity for 24 h, using the relevant standard international test methods. The test for tear strength reflects the changes that are occurring in fibre strength (Kärenlampi, 1996), whilst the test for tensile strength reflects the changes in fibre bonding, which in turn indicates the level of fibrillation (Wang *et al.*, 2006). Bulk density and porosity indicate the fines generated during the refining process as well as the level of fibrillation (Lindsay, 1994).

Data analysis

The enzyme treatments were compared based on the change in the properties of the refined samples relative to the respective unrefined samples. For the raw data see the Appendices. All pulp properties were plotted against refining energy and a polynomial trend line was fitted to the respective data.

RESULTS AND DISCUSSION

As refining energy was increased, the freeness of all the pulp samples decreased at a similar rate (Figure 4-1). Both enzymes reduced freeness more than the control on the dried and never-dried pulps (Figure 4-1). On the dried pulps, Ecopulp® Energy reduced freeness more than Novozyme 476, whilst Novozyme 476 reduced freeness more than Ecopulp® Energy on the never-dried pulps (Figure 4-1).

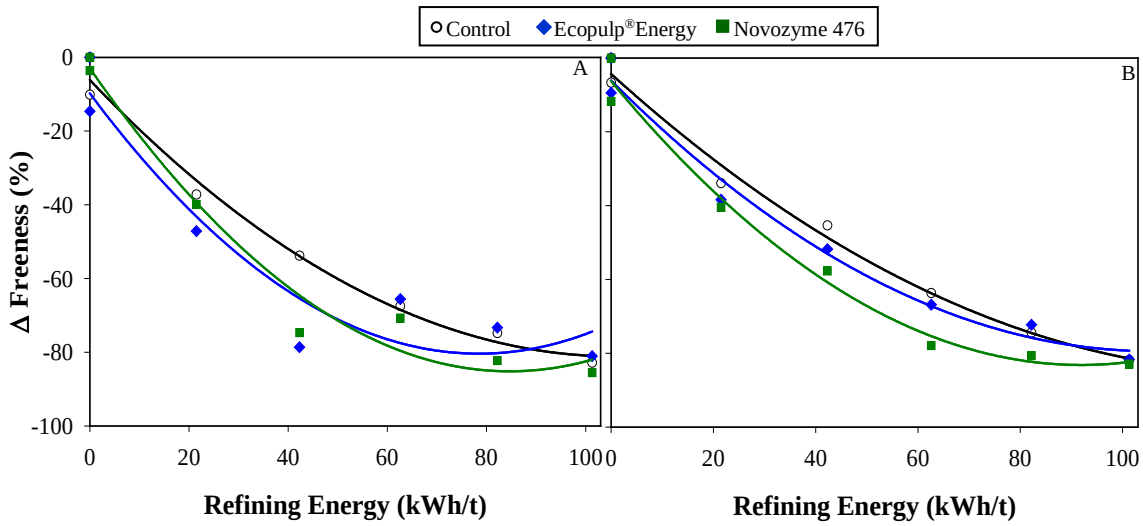


Figure 4-1 Influence of refining energy on the freeness of dried (A) and never-dried (B) *Eucalyptus nitens* pulps after treatment with the different enzymes. (Change in freeness of pulps after incubation and refining is expressed relative to the freeness of the pulp before incubation. For raw data see Appendix 4-1).

The enzymes induced a different response in the water retention values of the dried and never-dried pulps (Figure 4-2). On the dried pulps, Ecopulp® Energy increased the

water retention, whilst Novozyme 476 decreased the water retention of the fibres (Figure 4-2A). However, on the never-dried pulps, Novozyme 476 increased the water retention value, whilst Ecopulp® Energy reduced it (Figure 4-2B). It was clear that the different enzymes acted differently on the dried and never-dried fibres regarding water retention values.

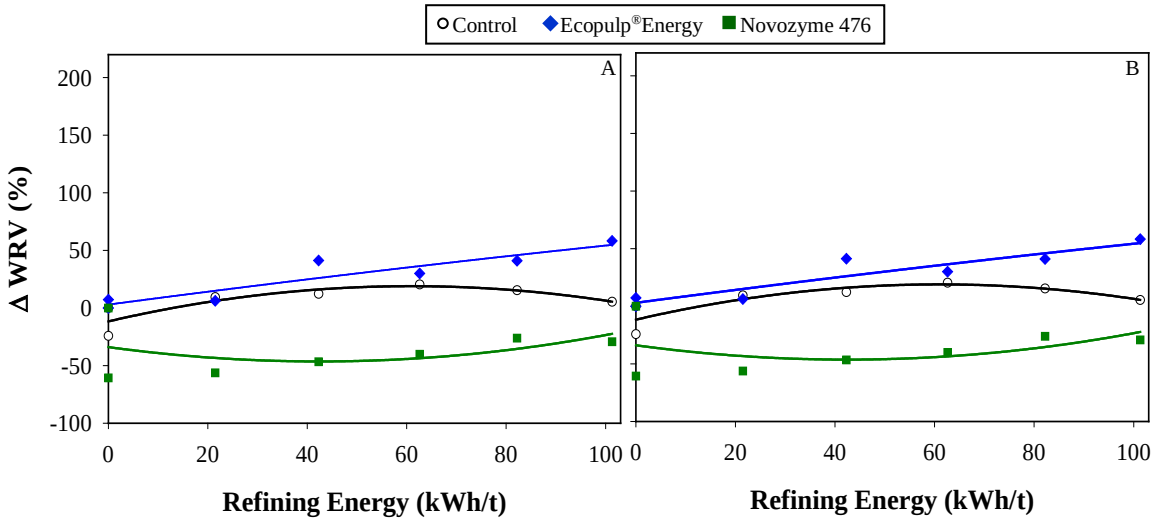


Figure 4-2 Influence of refining energy on the water retention value (WRV) of dried (A) and never-dried (B) *Eucalyptus nitens* pulps after treatment with the different enzymes. (Change in water retention value of pulps after incubation and refining is expressed relative to the water retention value of the pulp before incubation. For raw data see Appendix 4-2).

The tensile index increased with increased refining for both pulps, but not at the same rate (Figure 4-3). On the dried pulps, the Novozyme 476 increased tensile index at a faster rate and more than the control, whilst Ecopulp® Energy treatment maintained tensile strength at levels similar to the control (Figure 4-3A). On the never-dried pulps, the enzymes maintained tensile strength.

The dried and never-dried pulps responded differently to the enzyme treatments regarding the tear index (Figure 4-4). On the dried pulps, Novozyme 476 increased the tear index at a faster rate than the control, but the tear index decreased above a refining energy level of 60 kWh/t. Ecopulp® Energy reduced the tear index but at the same rate as the control pulp. On the never-dried pulps the Ecopulp® Energy and Novozyme 476

reduced the tear index more than the control, where Novozyme 476 reducing tear index the most (Figure 4-4).

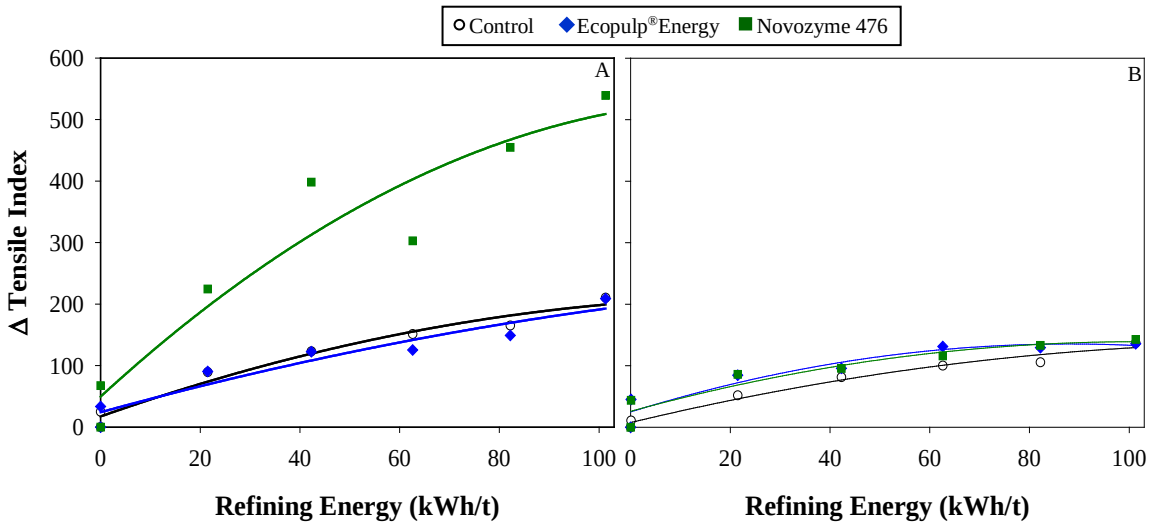


Figure 4-3 Influence of refining energy on the tensile index of dried (A) and never-dried (B) *Eucalyptus nitens* pulp after treatment with the different enzymes. (Change in tensile index of pulps after incubation and refining is expressed relative to the tensile index of the pulp before incubation. For raw data see Appendix 4-3).

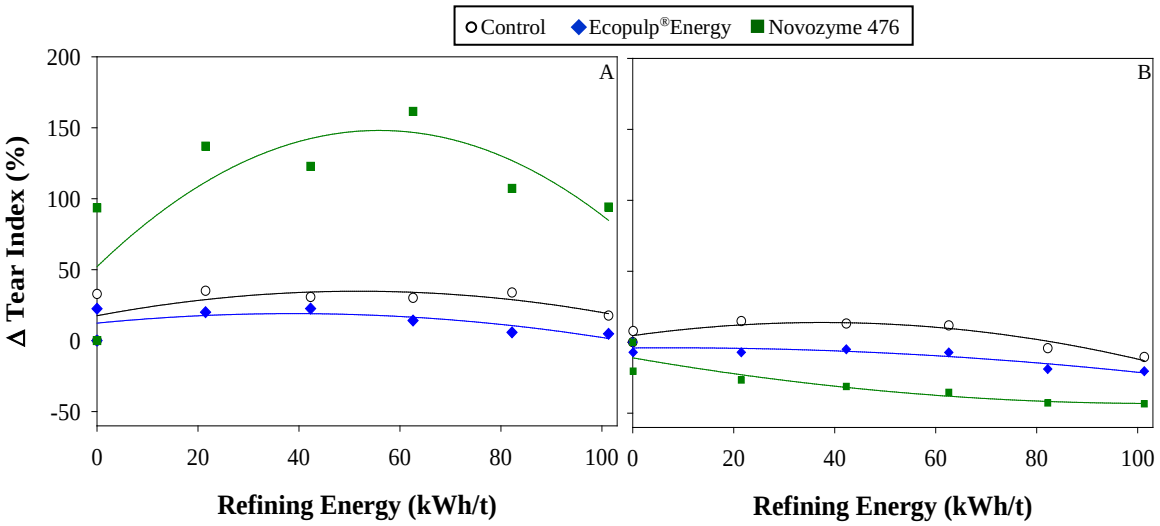


Figure 4-4 Influence of refining energy on the tear strength of dried (A) and never-dried (B) *Eucalyptus nitens* pulp after treatment with the different enzymes (Change in tear index of pulps after incubation and refining is expressed relative to the tear index of the pulp before incubation. For raw data see Appendix 3-1)

On all the pulps, bulk density was reduced as refining increased (Figure 4-5). On the dried pulps, only Novozyme 476 reduced the bulk density more than the control, whilst Ecopulp® Energy maintained it similar to the control. On the never-dried pulps the Novozyme 476 reduced bulk density, but not at the same rate as the dried pulps. Ecopulp®Energy reduced bulk density on the never-dried pulps at the same rate on the dried pulps (Figure 4-5).

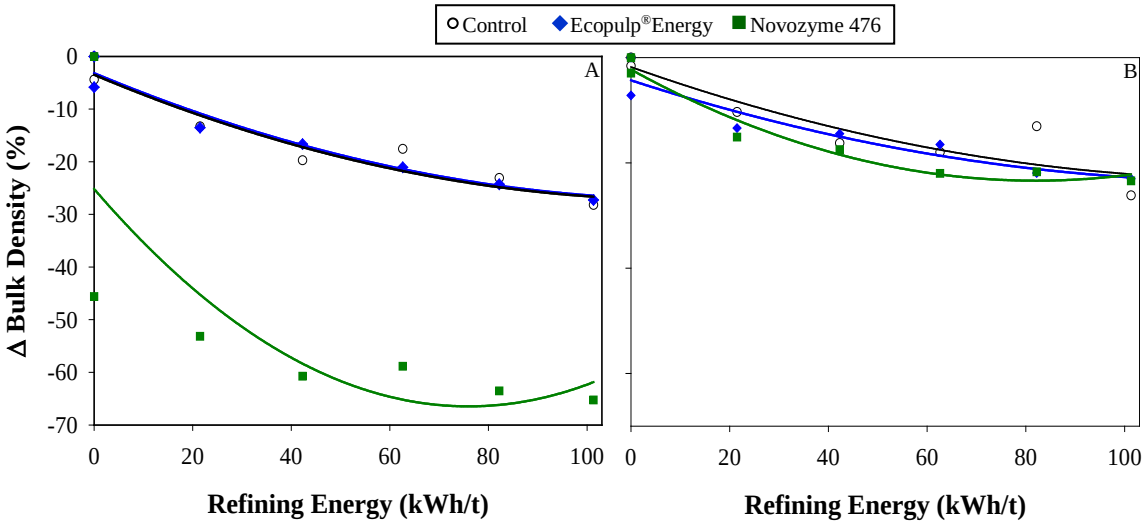


Figure 4-5 Influence of refining energy on the bulk density of dried (A) and never-dried (B) *Eucalyptus nitens* pulp after treatment with the different enzymes. (Change in bulk density of pulps after incubation and refining is expressed relative to the bulk density of the pulp before incubation. For raw data see Appendix 4-5).

The porosity of the pulps decreased with an increase in refining energy (Figure 4-6). In the early stages of refining of the dried pulps, up to 20 kWh/t, Ecopulp®Energy treatment reduced the porosity of the pulps and Novozyme 476 increased the porosity compared to the control (Figure 4-6A). At the remainder of the refining energies, the porosity of the enzyme treated dried pulps were equal to the control. On the never-dried pulps, the enzyme treated pulps reduced the porosity of the pulps more than the control up to refining energy of 40 kWh/t (Figure 4-6B). After 40 kWh/t, the enzyme treated pulps equalled the porosity of the control pulps (Figure 4-6B).

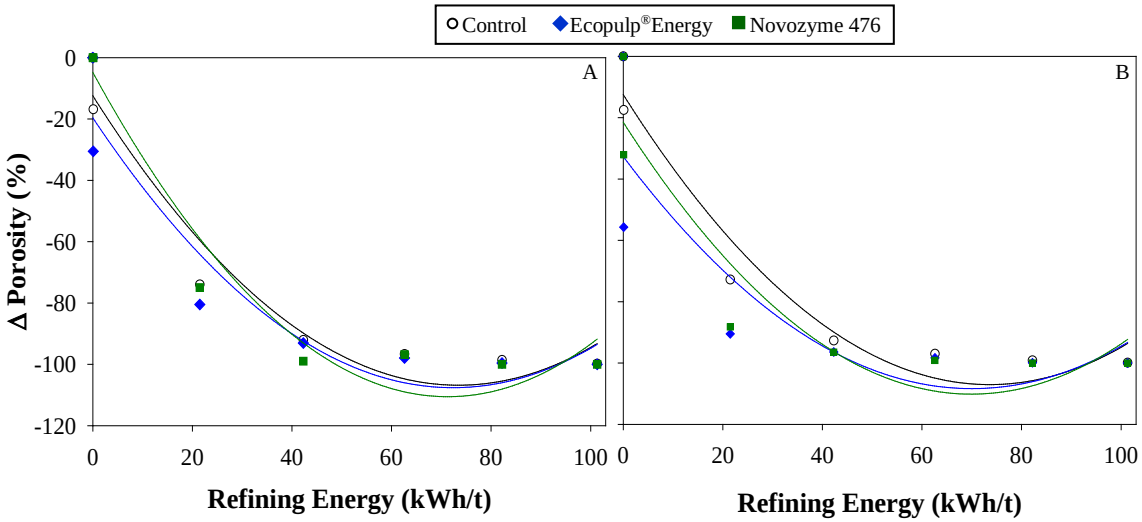


Figure 4-6 Influence of refining energy on the porosity of dried (A) and never-dried (B) *Eucalyptus nitens* pulp after treatment with the different enzymes. (Change in porosity of pulps after incubation and refining is expressed relative to the porosity of the pulp before incubation. For raw data see Appendix 4-6)

CONCLUSIONS

Ideally, the enzyme applications should be replicated to ensure accuracy through repeatability. However, due to the large volume of pulp required for each refining run, it was not realistic or possible to replicate the treatments. The refining curves were generated to give points that had trend lines with good fit ($R^2 = 0.94$).

The dried and never-dried *E. nitens* fibres responded differently to refining, as was also seen with the dried and never-dried *E. grandis* pulps (Chapter 3). The enzyme applications slowed the drainage and drying properties of the pulps by reducing freeness and increasing water retention values, respectively. Novozyme 476 was the best enzyme for application on *E. nitens* pulps as it has the potential to save on refining and drying energy due to the decreased freeness and water retention values, especially on the dried *E. nitens* pulps. Novozyme 476 also improved the physical properties of the dried pulps as tear and tensile index and porosity increased, whereas bulk was reduced. These physical properties are characteristic of high quality coated and uncoated printing and writing papers (Cotterill and Macrae, 1997). Novozyme 476 should be applied to dried *E. nitens* pulps to optimise refining and paper production. While both enzymes could improve the refining and paper properties on the never-dried pulps, they did not improve the properties as much as Novozyme 476 did on the dried pulps.

Ecopulp® Energy and Novozyme 476 acted very differently on the dried pulps and the never-dried pulps in respect of both species investigated. It was clear that application of the enzymes to the never-dried *E. nitens* pulps reduced freeness, whereas on the never-dried *E. grandis* pulps the enzyme applications barely altered freeness (Chapter 3). In general, the drainage and physical properties of the pulps investigated were improved by the selected enzyme applications. There is enormous potential for application of the enzymes on commercial scale to improve the refining efficiency of the *Eucalyptus* pulps.

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APPENDICES

Appendix 4-1 Freeness and change in freeness of untreated enzyme treated dried and never-dried *Eucalyptus nitens* pulps

Sample		Energy (kWh/t)	Freeness (ml/min)		Δ Freeness (%)	
			Dried	Never dried	Dried	Never dried
Control	Repulped	0	572	551	0	0
	Incubated	0	514	514	-10	-7
	Refined 1	24.9	359	364	-37	-34
	Refined 2	49.2	264	301	-54	-45
	Refined 3	73.1	186	200	-67	-64
	Refined 4	96.5	144	141	-75	-74
	Refined 5	119.4	98	100	-83	-82
Ecopulp Energy	Repulped	0	581	552	0	0
	Incubated	0	496	500	-15	-9
	Refined 1	24.9	307	340	-47	-38
	Refined 2	49.2	124	266	-79	-52
	Refined 3	73.1	200	183	-66	-67
	Refined 4	96.5	155	153	-73	-72
	Refined 5	119.4	110	101	-81	-82
Novozyme 476	Repulped	0	619	579	0	0
	Incubated	0	560	511	-10	-12
	Refined 1	24.9	349	345	-44	-40
	Refined 2	49.2	147	245	-76	-58
	Refined 3	73.1	170	128	-73	-78
	Refined 4	96.5	103	112	-83	-81
	Refined 5	119.4	84	99	-86	-83

Appendix 4-2: Water retention value (WRV) and change in water retention value of untreated and treated dried and never-dried *Eucalyptus nitens* pulp.

Sample		Energy (kWh/t)	WRV (g/g)		Δ WRV (%)	
			Dried	Never dried	Dried	Never dried
Control	Repulped	0	3.42	1.82	0	0
	Incubated	0	2.59	1.90	-24	4
	Refined 1	24.9	3.74	2.33	9	28
	Refined 2	49.2	3.83	2.60	12	43
	Refined 3	73.1	4.11	3.25	20	79
	Refined 4	96.5	3.94	3.24	15	78
	Refined 5	119.4	3.60	3.03	5	66
Ecopulp Energy	Repulped	0	2.93	2.00	0	0
	Incubated	0	3.14	2.13	7	6
	Refined 1	24.9	3.12	2.36	6	18
	Refined 2	49.2	4.14	2.57	41	29
	Refined 3	73.1	3.81	2.98	30	49
	Refined 4	96.5	4.13	3.08	41	54
	Refined 5	119.4	4.64	3.56	58	78
Novozyme 476	Repulped	0	3.68	1.77	0	0
	Incubated	0	1.45	2.40	-61	36
	Refined 1	24.9	1.61	3.17	-56	79
	Refined 2	49.2	1.96	3.37	-47	90
	Refined 3	73.1	2.20	2.52	-40	42
	Refined 4	96.5	2.72	3.99	-26	125
	Refined 5	119.4	2.60	5.19	-29	193

Appendix 4-3: Tensile index and change in tensile index of untreated and enzyme treated dried and never-dried *Eucalyptus nitens* pulps

Sample		Energy (kWh/t)	Tensile index (Nm/g)		Δ Tensile index (%)	
			Dried	Never dried	Dried	Never dried
Control	Repulped	0	27.72	39.87	0	0
	Incubated	0	34.60	44.11	25	11
	Refined 1	24.9	52.43	60.49	89	52
	Refined 2	49.2	61.84	72.24	123	81
	Refined 3	73.1	69.63	79.73	151	100
	Refined 4	96.5	73.45	81.91	165	105
	Refined 5	119.4	85.98	94.43	210	137
Ecopulp Energy	Repulped	0	29.28	34.22	0	0
	Incubated	0	39.04	49.66	33	45
	Refined 1	24.9	55.73	63.14	90	85
	Refined 2	49.2	65.11	66.98	122	96
	Refined 3	73.1	65.99	79.09	125	131
	Refined 4	96.5	74.95	78.49	156	129
	Refined 5	119.4	90.4	80.63	209	136
Novozyme 476	Repulped	0	15.00	33.51	0	0
	Incubated	0	25.10	48.13	67	44
	Refined 1	24.9	48.67	62.29	224	86
	Refined 2	49.2	74.74	65.49	398	95
	Refined 3	73.1	60.41	72.35	303	116
	Refined 4	96.5	83.23	78.07	455	133
	Refined 5	119.4	95.88	81.33	539	143

Appendix 4-4: Tear index and change in tear index of untreated and enzyme treated dried and never-dried *Eucalyptus nitens* pulps

Sample		Energy (kWh/t)	Tear index (mNm^2/g)		Δ Tear index (%)	
			Dried	Never dried	Dried	Never dried
Control	Repulped	0	1.71	2.02	0	0
	Incubated	0	2.27	2.18	33	8
	Refined 1	24.9	2.30	2.32	35	15
	Refined 2	49.2	2.23	2.28	30	13
	Refined 3	73.1	2.22	2.25	30	11
	Refined 4	96.5	2.28	1.93	33	-4
	Refined 5	119.4	2	1.81	17	-10
Ecopulp Energy	Repulped	0	1.77	2.07	0	0
	Incubated	0	2.16	1.92	22	-7
	Refined 1	24.9	2.12	1.92	20	-7
	Refined 2	49.2	2.16	1.97	22	-5
	Refined 3	73.1	2.02	1.92	14	-7
	Refined 4	96.5	1.87	1.68	6	-19
	Refined 5	119.4	1.85	1.65	5	-20
Novozyme 476	Repulped	0	0.62	1.92	0	0
	Incubated	0	1.20	1.53	94	-20
	Refined 1	24.9	1.48	1.41	139	-27
	Refined 2	49.2	1.39	1.32	124	-31
	Refined 3	73.1	1.63	1.24	163	-35
	Refined 4	96.5	1.29	1.1	108	-43
	Refined 5	119.4	1.21	1.09	95	-43

Appendix 4-5: Bulk density and change in bulk density of untreated and enzyme treated dried and never-dried *Eucalyptus nitens* pulps

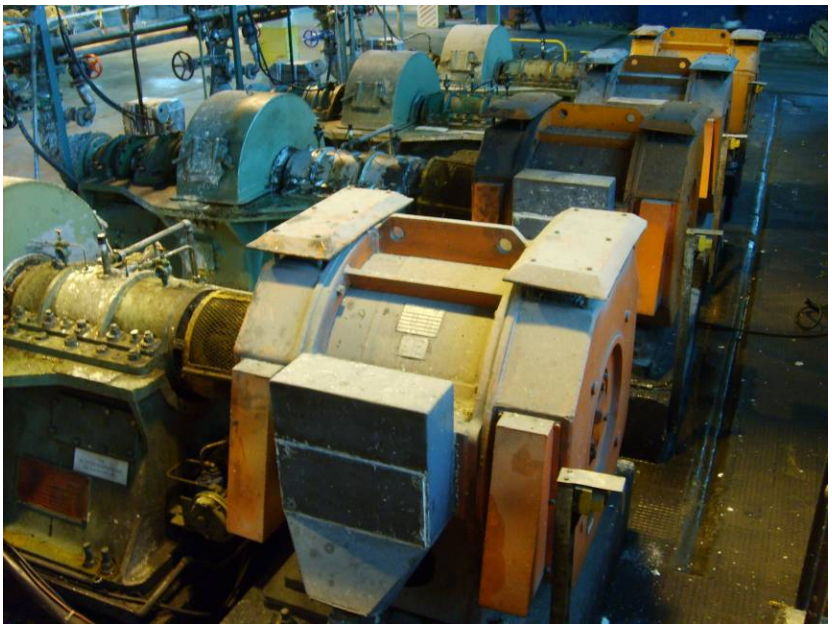
Sample		Energy (kWh/t)	Bulk density (cm^3/g)		Δ Bulk density (%)	
			Dried	Never dried	Dried	Never dried
Control	Repulped	0	1.86	1.75	0	0
	Incubated	0	1.78	1.72	-4	-2
	Refined 1	24.9	1.61	1.57	-13	-10
	Refined 2	49.2	1.49	1.46	-20	-17
	Refined 3	73.1	1.53	1.43	-18	-18
	Refined 4	96.5	1.43	1.52	-23	-13
	Refined 5	119.4	1.33	1.29	-28	-26
Ecopulp Energy	Repulped	0	1.79	1.62	0	0
	Incubated	0	1.69	1.50	-6	-7
	Refined 1	24.9	1.55	1.40	-13	-14
	Refined 2	49.2	1.5	1.38	-16	-15
	Refined 3	73.1	1.42	1.35	-21	-17
	Refined 4	96.5	1.36	1.26	-24	-22
	Refined 5	119.4	1.31	1.24	-27	-23
Novozyme 476	Repulped	0	4.04	1.75	0	0
	Incubated	0	2.2	1.70	-46	-3
	Refined 1	24.9	1.89	1.49	-53	-15
	Refined 2	49.2	1.59	1.45	-61	-17
	Refined 3	73.1	1.66	1.37	-59	-22
	Refined 4	96.5	1.47	1.37	-64	-22
	Refined 5	119.4	1.4	1.34	-65	-23

Appendix 4-6: Porosity and change in porosity of untreated and enzyme treated dried and never-dried *Eucalyptus nitens* pulps

Sample		Energy (kWh/t)	Porosity (ml/min)		Δ Porosity (%)	
			Dried	Never dried	Dried	Never dried
Control	**Repulped**	0	5000	5000	0	0
	Incubated	0	4158	4124	-17	-18
	Refined 1	24.9	1301	1360	-74	-73
	Refined 2	49.2	396	367	-92	-93
	Refined 3	73.1	167	150	-97	-97
	Refined 4	96.5	70	42	-99	-99
	Refined 5	119.4	10	5	-100	-100
Ecopulp Energy	**Repulped**	0	5000	5000	0	0
	Incubated	0	3473	2217	-72	-79
	Refined 1	24.9	976	476	-90	-92
	Refined 2	49.2	348	180	-97	-96
	Refined 3	73.1	104	85	-99	-100
	Refined 4	96.5	22	6	-100	-100
	Refined 5	119.4	0	0	44	126
Novozyme 476	**Repulped**	0	5000	5000	0	0
	Incubated	0	5000	3397	-75	-83
	Refined 1	24.9	1252	592	-99	-95
	Refined 2	49.2	52	179	-97	-99
	Refined 3	73.1	160	44	-100	-100
	Refined 4	96.5	0	0	-100	-100
	Refined 5	119.4	0	0	-100	-100

CHAPTER 5

MILL SCALE EVALUATION OF NOVOZYME 476 TO IMPROVE REFINING EFFICIENCY



Southerland hardwood (front) and softwood refiners (back) at Sappi Enstra mill.

ABSTRACT

On pilot-scale, Novozyme 476 improved the physical properties of *Eucalyptus nitens* pulps and was, therefore, selected for application in a trial on commercial scale. A fine paper machine that uses *E. nitens* pulp was chosen for application of this enzyme to improve the refining efficiency by saving on refining energy and improving tensile strength. The endoglucanase was applied at dosages of 0, 50 and 100 ml/t of pulp. The hardwood refiner was switched off and the refining current of the mixed stock refiner was reduced when the dosage was at 100 ml/t. Despite the reduction in the refining current, the porosity and tear strength was reduced and tensile strength was increased as a result of the enzyme application. The reduction in tear strength was probably due to too much refining energy applied to enzyme treated pulp, thereby damaging the fibres. The damage to the fibres was confirmed by electron microscopy, which showed excessive fibrillation of the fibres treated with the endoglucanase. It was clear that the duration of each treatment must be long enough for changes to be noticed on the paper machine. The endoglucanase successfully improved the refining efficiency of the paper machine and should be considered for application on a permanent basis.

INTRODUCTION

The potential value of Novozyme 476 has been demonstrated on pilot scale to save on refining energy as well as improve the tensile strength of dried *Eucalyptus nitens* pulps (Chapter 4). It has been shown that results from enzyme application on a laboratory scale could be repeated on commercial scale (Abubakr *et al*., 1996, Michalopoulos *et al*., 2005). However, exceptions have been noted. Porosity, for example, was reduced on a laboratory scale (García *et al.*, 2002; Dienes *et al*., 2004), but it remained unchanged on the mill scale (Michalopoulos *et al*., 2005). Longer trials on a mill scale have the benefit that process parameters, such as refining energy and enzyme dosage, could be managed to produce optimal results.

The refining energy has a direct impact on the fibres and influences the drainage of the pulps as well as the physical properties such as tear and tensile strength. Application of enzymes to pulps prior to refining was able to assist the refiner with the fibre development (Abubakr, 1996; Dickson *et al*., 2000; Lecourt *et al*., 2010). However, the disadvantage of enzyme treatment combined with refining is that it can damage the fibres, resulting in reduced tear strength (García *et al*., 2002; Lecourt *et al*., 2010). The reduced tear strength may be avoided by adjusting the refining energy or the enzyme dosage to regulate fibre development. Application of an enzyme on commercial scale requires production parameters to be as stable as possible throughout the trial in order to optimise the refining energy and enzyme dosage. The enzyme dosages can only be optimised on a commercial scale due to the build up of the enzyme in the water systems and recirculation to the treatment vessel.

The operation of a paper machine at a fine-paper mill was not profitable and the production efficiency needed to be improved. This paper machine had three refiners: two for the raw hardwood and the other a mixed-stock refiner for the blended softwood and hardwood pulp. Thus, there was potential for improving the refining efficiency on the hardwood and mixed stock refiners by treating the hardwood with an enzyme before refining. Novozyme 476 was selected for application on a commercial scale based on laboratory analysis (Chapter 2 and 4) and was applied to *E. nitens* pulp at different

dosages. The aim of the trial was to improve the refining efficiency to save on energy and increase the tensile strength. The strategy of this investigation was to apply the enzyme at a very low dosage for about 8 to 12 h. The dosage would then be applied at increased levels until the no further benefits were evident.

MATERIALS AND METHODS

Process flow

The paper machine that was the subject of the present study manufactures uncoated fine paper with a basis weight of 58 g/m^2 and is operated using a mixture of 68% *E. nitens* pulp and 32% pine pulp. The *E. nitens* pulps pass through the hardwood refiners before being mixed with the refined softwood (Figure 5-1). The blended pulp then passes through the mixed-stock refiner before going onto the paper machine.

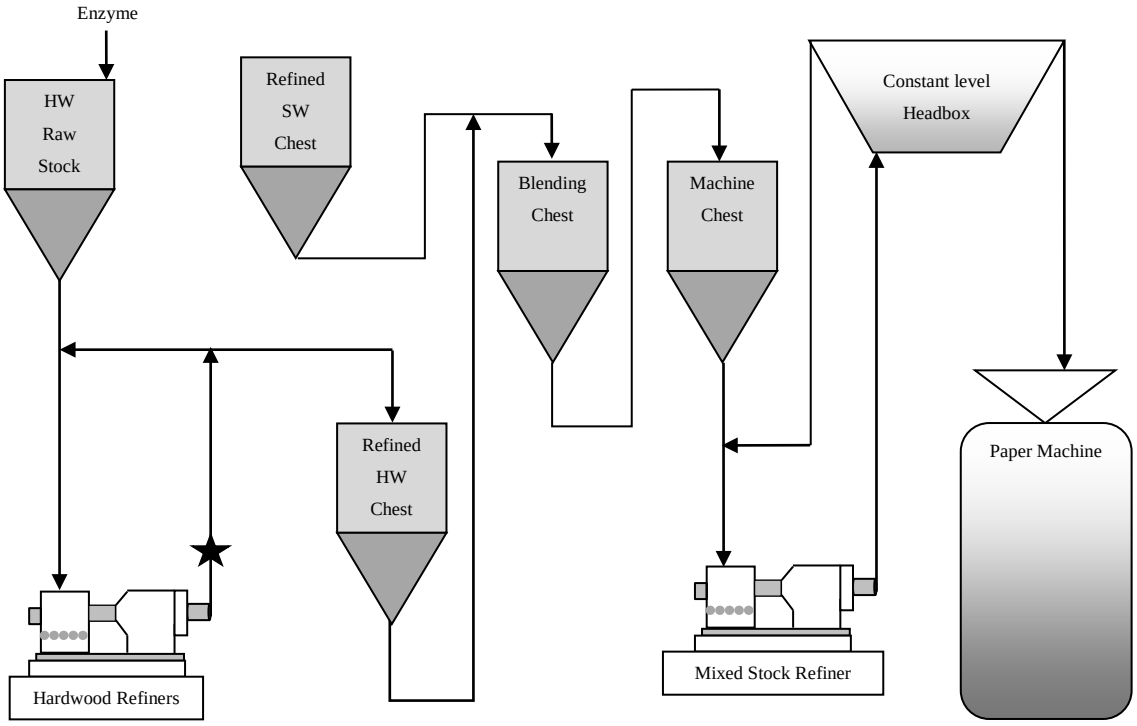


Figure 5-1 Flow diagram of pulp from the stock chests through refiners to the paper machine. HW = hardwood; SW = softwood; star indicate sampling point for scanning electron .microscopy.

Enzymatic treatment

Novozyme 476 (Novozymes, Denmark) was applied to the never-dried *E. nitens* pulp in the hardwood raw-stock chest (Figure 5-1). The enzyme was applied at 0, 50 and 100 ml of formulation per tonne of pulp.

Trial plan

Baseline data were recorded for 10 h before the trial started. Batches of the enzyme sufficient for 8 h of application were diluted to 20% and applied to the pulp with a peristaltic pump. The hardwood pulp was treated with the enzyme for approximately 30 min before reaching the hardwood refiner. The enzyme was first applied at 50 ml/t for 11 h. The dosage was then increased to 100 ml/t as there were no obvious changes at the lower dosage. After 27 h at 100 ml/t it was clear that the enzyme had built up to effective levels in the water system. The dosage was, therefore, reduced to 50 ml/t, which continued for 16 h before stopping the enzyme application. Baseline data was then collected for a further 14 h.

Measurement and analysis

The refining current of the mixed stock refiner was recorded every hour. The refining current of the hardwood refiner was not considered, since it was not used for refining at the time of the trial. Tensile index in the machine direction and cross direction, tear index in the cross direction as well as porosity were determined in the mill laboratory using the standard test procedures. The results were, therefore, delayed due to the tests and changes could only be detected after several hours. The influence of the enzymes on the relevant process parameters and sheet properties was determined by comparing the data sets recorded during the application of each dosage using Student's t-test at 95% confidence. The data sets consisted of the values obtained in the last 8 h of each enzyme dosage.

Scanning electron microscopy

Samples were collected from the outlet of the hardwood refiner (Figure 5-1) and the first sample (control) was collected 2 h before the enzyme application started. The hardwood refiner was not refining, but pulp was still being passed through it to the

mixed stock refiner. The second sample was collected after 40 h when the enzyme was applied at 100 ml/t. The samples were thickened onto filter paper and sent to the Electron Microscope Unit at the University of KwaZulu-Natal, Durban for imaging. The samples were prepared by removing small bunches of fibres that were stuck onto 0.5 cm^2 foil and freeze dried over 24 h. Samples were placed on stubs using double sided tape and gold coated using a Polaron Sputter coater. Samples were viewed using a LEO 1450 scanning electron microscope (Figure 5-7).

RESULTS AND DISCUSSION

Paper machine and refining data

The enzyme application improved the refining efficiency of the paper machine. The mixed stock refining energy was significantly reduced (p = 0.0012) with an enzyme dosage of 50 ml/t (Figure 5-2) and 100 ml/t reduced the refining energy further (p = 0.0049). When the enzyme application was stopped, the refining current returned to the same level it was before the enzyme application. There was an increased refining current after 20 h due to addition of extra softwood to the overall pulp furnish based on a decision by the paper machine staff to recover tear strength. However, the reduced tear strength was likely due to over-refining and the addition of more softwood could have been avoided.

Initially, the tensile strength of the pulps in the cross direction was not influenced by the enzyme at the low dosage. However, tensile strength increased (p = 0.0011) when the enzyme dosage was raised to 100 ml/t (Figure 5-3). Even when the enzyme dosage was reduced, the tensile strength remained high, possibly due to a build up of the enzyme. The tensile strength was still high after 65 h when the enzyme dosage was stopped, which could be attributed to the increased refiner current (Figure 5-2) and re-circulated enzyme.

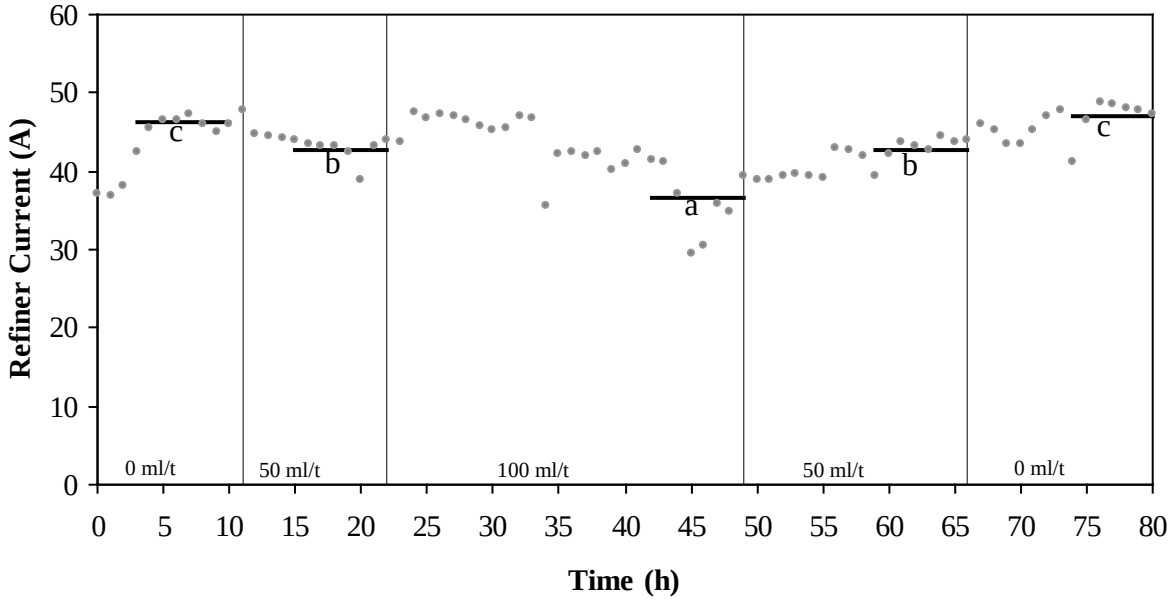


Figure 5-2 The influence of the endoglucanase on the current applied at the mixed stock refiner during the mill trial. Vertical lines indicate changes in enzyme dosage. Horizontal lines indicate mean refiner current during the last 8 h of each treatment. a, b, c: means with same letters do not differ significantly ($p \leq 0.05$).

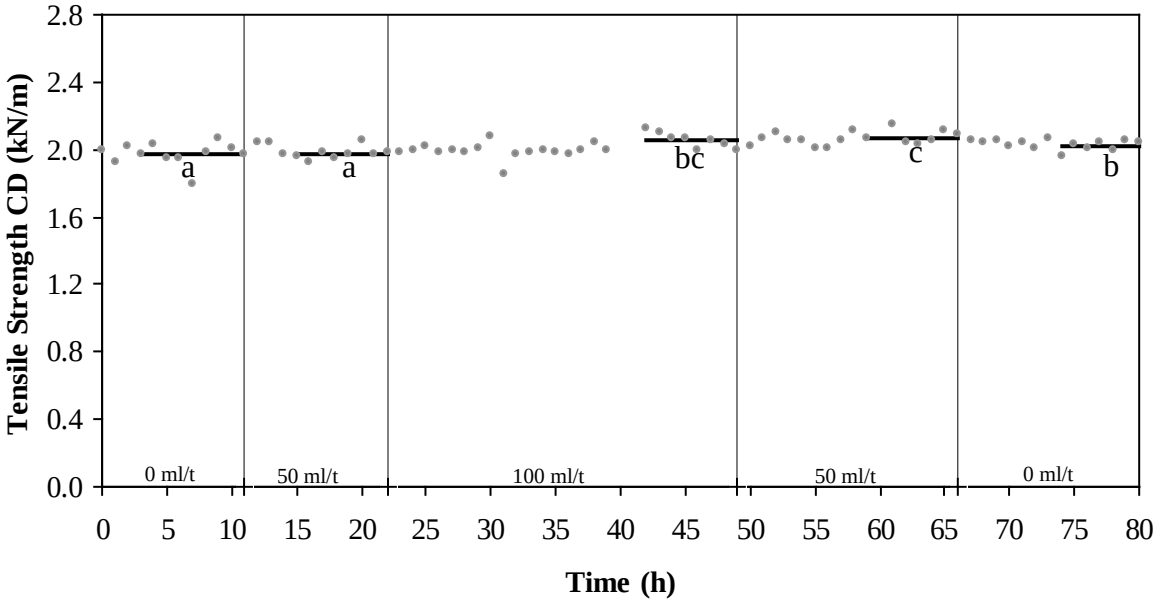


Figure 5-3 **T**he influence of the endoglucanase on the tensile strength in the cross direction (CD) during the mill trial. Vertical lines indicate changes in enzyme dosage. Horizontal lines indicate mean refiner current during the last 8 h of each treatment. a, b, c: means with same letters do not differ significantly ($p \leq 0.05$).

Tensile strength measured in the cross direction depends mostly on the inter-fibre bonding. However, because the fibres are laid down length-wise in the machine direction, the tensile strength in the machine direction will also depend on fibre strength. The tensile strength in the machine direction was, therefore, significantly reduced (p = 0.0050) when the enzyme dosage was started and remained low when the dosage was increased to 100 ml/t (Figure 5-4). After the enzyme dosage was stopped, the tensile strength returned to the same level that it was before the enzyme application started.

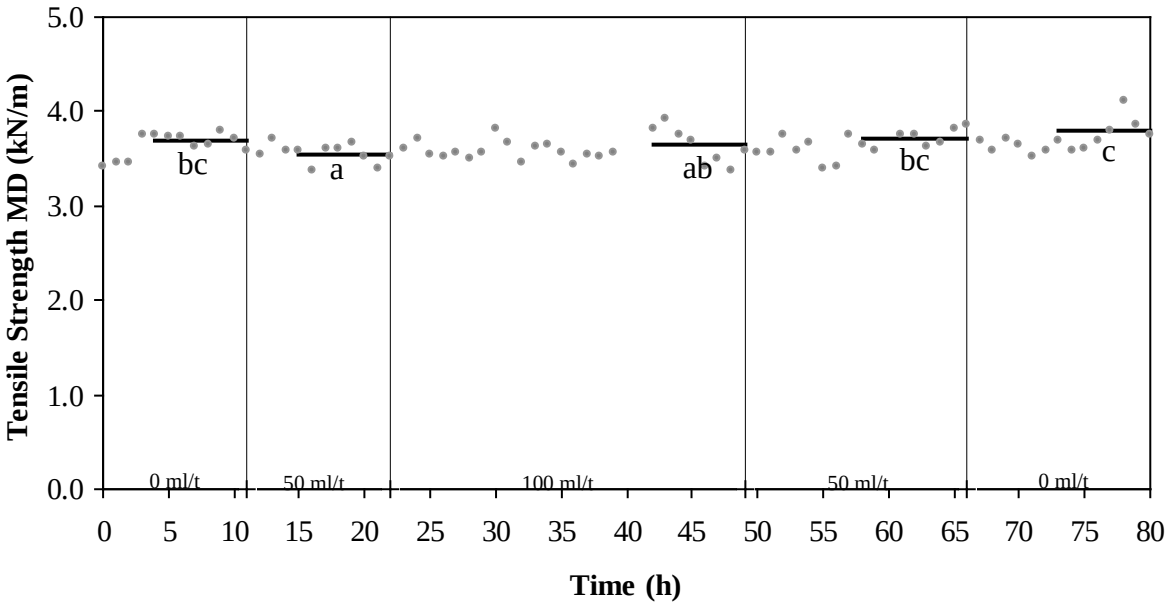


Figure 5-4 The influence of the endoglucanase on the tensile strength in the machine direction (MD) during the mill trial. Vertical lines indicate changes in enzyme dosage. Horizontal lines indicate mean refiner current during the last 8 h of each treatment. a, b, c: means with same letters do not differ significantly ($p \leq 0.05$).

Tear strength was significantly reduced (p = 0.0001) by the higher enzyme dosage and increased when it was terminated (Figure 5-5). The reduced tear strength was due to over-refining of the fibres, which made them weaker and easier to break. The weakened fibres contributed to the reduction in both tensile strength in the machine direction (Figure 5-4) and tear strength (Figure 5-5). The inverse relationship between tear and

tensile strength seen on pilot scale (Chapters 3 and 4) was replicated during the mill trial where an increased tensile strength in the cross direction was accompanied by a reduced tear strength. Tear strength was a parameter that was not as critical as tensile strength and for the paper grade produced, it was more important to focus on tensile improvements than any loss in tear strength.

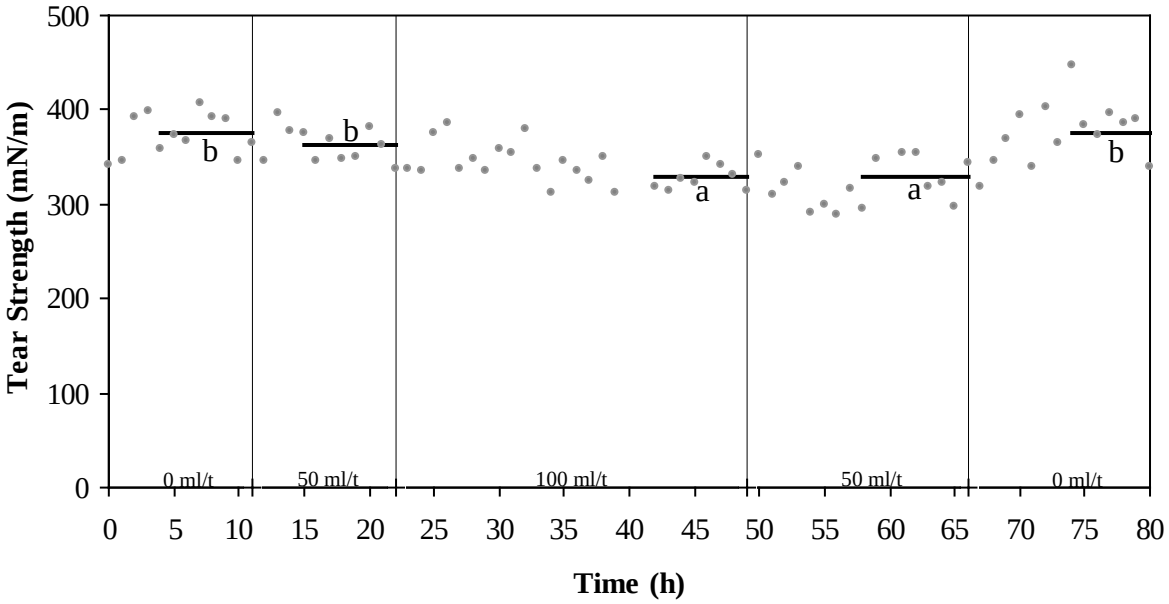


Figure 5-5 The influence of the endoglucanase on the tear strength during the mill trial. Vertical lines indicate changes in enzyme dosage. Horizontal lines indicate mean refiner current during the last 8 h of each treatment. a, b, c: means with same letters do not differ significantly ($p \leq 0.05$).

The target porosity for refining pulp to make the specific product with a grammage of 58 g/m^2, was 1000 ml/min. Before the enzyme application began, the porosity of the pulp was out of the specified range (Figure 5-6). When the enzyme application was started, the porosity was reduced and it remained at approximately 1000 ml/min even when the enzyme application was stopped. The target porosity was then maintained by increasing the refiner current.

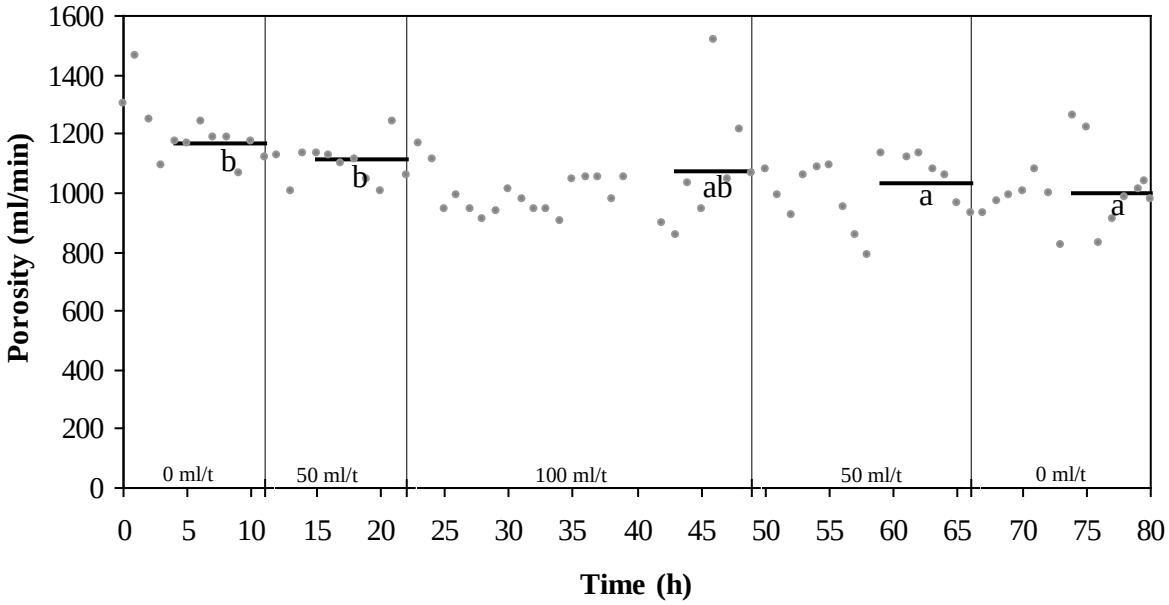


Figure 5-6 The influence of the endoglucanase on the tear strength during the mill trial. Vertical lines indicate changes in enzyme dosage. Horizontal lines indicate mean refiner current during the last 8 h of each treatment. a, b, c: means with same letters do not differ significantly ($p \leq 0.05$).

Surface analysis

The scanning electron micrographs demonstrated the difference in pulps caused by the enzyme (Figure 5-7). The fibres that were exposed to the endoglucanase were clearly more fibrillated than the untreated fibres.

The images also showed extensive fibrillation when the enzyme was applied despite the refining current being at its lowest. The fibrillation may have contributed to a weakened fibre and, therefore, reduced tear strength and tensile strength in the machine direction (Figure 5-5 and 5-4). The images demonstrated the effect of over-refining and that the enzyme dosage could play a role in the excessive fibrillation.

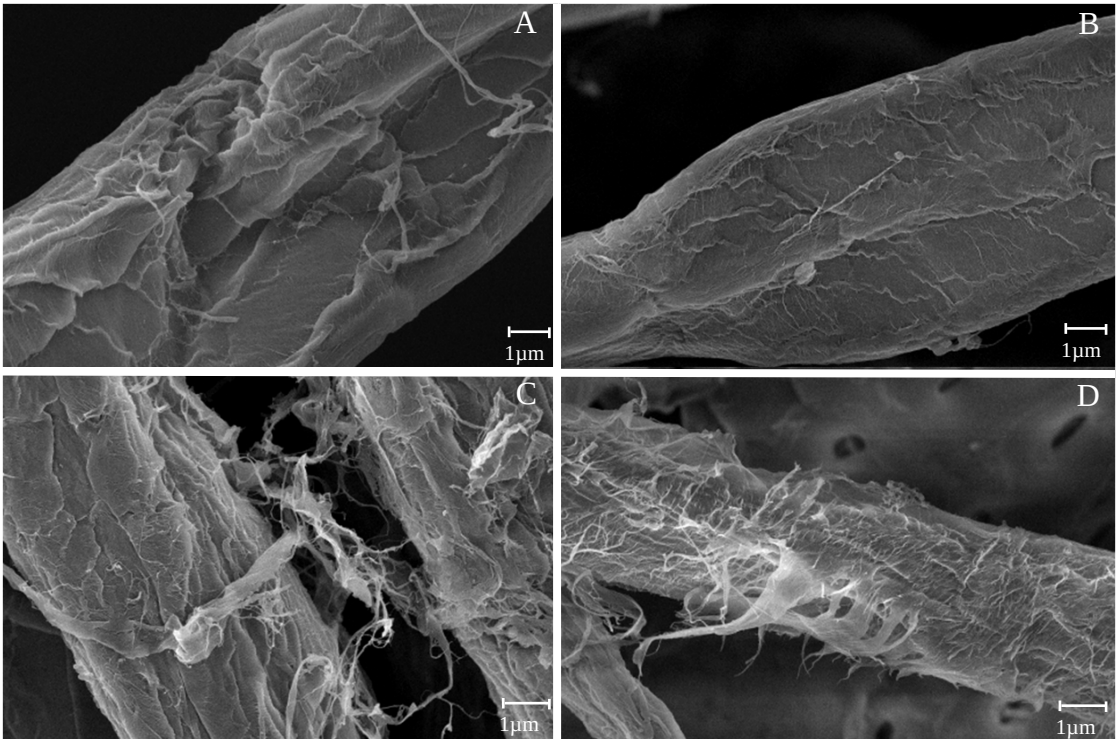


Figure 5-7 Scanning electron micrographs of untreated pulp (A and B) and pulp treated with 100 ml/t of Novozyme 476 (C and D) after refining.

CONCLUSIONS

The refining efficiency was improved by the enzyme application by saving on refining energy as well as improving tensile strength. However, the enzyme dosage has to be optimised in terms of volume and treatment time to ensure efficient fibre development (Bhardwaj *et al*., 1995). It was, therefore, understandable that most of the changes to the tensile and tear strengths and porosity that were observed on the paper machine could be related to the changes in dosing levels of enzyme. The present trial started with a low enzyme dosage that appeared to have little influence on the physical properties. However, after the dosage was doubled, the changes to the paper properties became more obvious. The present trial showed that enzyme dosage has to be optimised on commercial scale and not on laboratory scale, because of recirculation and build up of the residual enzyme in the water system of the paper machine. To account for this build up of enzyme, the enzyme dosage was approximately 100-fold less on commercial scale than on pilot scale. On pilot scale, a high enzyme dosage was applied to ensure a change was observed and to establish the worst possible effect the enzymes could inflict on the fibres (Chapter 3 and 4). Previous work demonstrated that enzyme dosage was more important to the quality of the fibres than the retention time (Dienes *et al.,* 2004). However, during the present study the changes to the paper properties were most notable toward the end of each dosage period. The fact that the changes only occurred towards the end indicate that it took time for the enzyme to build up to effective levels in the system.

The endoglucanase improved the refining efficiency in the present case study by reducing the refining current as well as improving the tensile strength. In addition, the hardwood refiner could also be switched off as the mixed stock refiner was sufficient for the fibre development. Consequently, there would also be a saving in the maintenance costs of hardwood refiner. The savings in refining energy and increased tensile strength corroborated previous results obtained on pilot scale (Chapter 4) and in the literature (Pere *et al*., 1996; Mansfield and Dickson, 2001; García *et al*., 2002; Lecourt *et al.,* 2010). The fibres were damaged through excessive refining, which contributed to reduced tear strength. The potential degree of fibre damage that can

occur with over-refining was notable in the scanning electron micrographs. The reduction in tear strength during the trial was expected, as tear strength had been reduced earlier on pilot-scale experiment with Novozyme 476 on the *Eucalyptus* pulps (Chapters 3 and 4). Optimisation through manipulation of the enzyme dosage and the refiners would allow more control over the drainage and strength properties of the sheet.

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CHAPTER 6

DISCUSSION AND CONCLUSIONS

In the pulp and paper industry, refining forms an integral part in the preparation of papermaking stock, which in turn forms the interface between the pulp mill and the paper machine (Biermann, 1996). The hydrolytic efficiency of cellulases provides an opportunity for modification of the existing technology (Bhardwaj *et al*., 1995; Clark *et al*., 1997; Dienes *et al*., 2004). Application of cellulases have the potential to save on refining energy (García *et al*., 2002) or to modify the pulp fibres to improve the drainage and drying speed of the pulps (Michalopoulos *et al*., 2005). The application of enzymes on fibres has been thoroughly investigated on a laboratory scale (Bhardwaj *et al*., 1995; Pere *et al*., 1996; Mansfield *et al*., 1996; Mansfield and Dickson, 2001; García *et al*., 2002; Dienes *et al*., 2004; Strey *et al*., 2009); however, applications are rarely commercialised. There is a need in the industry to investigate mill-scale applications, with more focus on optimising the enzyme dosage on the pulps and monitoring the effect of the enzymes on the different paper properties. The type of cell-wall modifying enzyme needs to be matched to the fibre source (wood type and process) to achieve a specific refining outcome.

The first challenge in the present study was to characterise some of the commercially available enzymes for their potential as refining enzymes. The protein content of the formulations were determined in order to compare the enzyme treatments on an equal protein basis, since each formulation contained different activities (Mansfield *et al*., 1996, Suchy *et al*., 2009). The protein concentration varied extensively (13 to 105 g/l) indicating the significance of optimising the dosage for each enzyme. The tested enzymes were all suitable for application under industrial conditions (approximately pH 5 and 50 °C) in terms of their filter paper and carboxymethylcellulose degrading activities. Since the incubation time of the assays was an average of 30 min, the results also reflected the stability of the enzymes for this period.

The optimal enzyme dosage under laboratory conditions was determined to be between 0.1 to 1.0 g protein/kg, as it would induce the least amount of fibre degradation. Increased enzyme dosage caused the release of more reducing sugars and a reduction in fibre strength, both of which were a clear indication that pulp fibres were being degraded. The time that enzymes can have an effect on the fibres is important for paper mills where there is always a risk of a machine shut-down and pulp that could remain in a chest for extended periods before being made into paper. The critical period for fibre improvement by the enzymes was 30 min; thereafter the fibres were not notably modified further. A model was proposed for the mechanism by which the different enzymes modified the fibres in the pulps. The model demonstrated the possible fibrillation or fines removal that was caused by each enzyme treatment on the pulps. According to the model, the endoglucanase, Novozyme 476, had the greatest potential to improve tensile strength, which is a paper strength property that depends primarily on fibre bonding.

Following characterisation of the enzymes, Novozyme 476, Ecopulp® Energy and Celluclast 1.5L were applied to dried and never-dried *E. grandis* pulps and then refined on pilot-scale. The dried and never-dried fibres responded differently to refining. After treatment of dried pulps, freeness was reduced more and tensile and tear indices were easier to improve with enzymatic refining than on never-dried pulps. Research has shown that freeness increased with enzyme treatments of both dried and never-dried fibre types (Dienes *et al*., 2004; Pere *et al*., 1996; Abubakr *et al*., 1996; Pala *et al*., 2004; Bhardwaj *et al*., 1995; Mansfield *et al*., 1996; García *et al*., 2002; Mohlin and Pettersson, 2002): However, in the present study, freeness was either maintained or reduced after treatment with the selected enzymes. The freeness was possibly reduced due to the fibrillation caused by the enzymes, thereby slowing down drainage. Celluclast 1.5L and Ecopulp® Energy improved the refining of dried *E. grandis* pulps and appeared to have potential for savings in refining energy, as the treated pulps required less refining to achieve the same freeness as an untreated pulp.

The dried and never-dried *E. nitens* fibres, treated with Novozyme 476 and Ecopulp® Energy, also responded differently to refining. Both enzymes improved the

refining efficiency on the never-dried pulps, but Novozyme 476 outperformed Ecopulp® Energy in savings of refining energy as well as improved tensile strength. There were, however some changes that were similar as both enzyme treatments slowed the drainage and reduced the drying ability of the pulps by reducing freeness and increasing water retention, respectively. The best enzyme for application on the *E. nitens* pulps was the Novozyme 476, as it showed the potential to save on refining and drying energy. This enzyme has the potential to improve the physical properties of high quality coated and uncoated printing and writing papers, because it increased porosity, tear and tensile indices and reduced bulk (Cotterill and Macrae, 1997). There is, therefore, potential for application of the endoglucanase on commercial scale to improve the efficiency of refining the *E. nitens* pulps.

The opportunity to evaluate Novozyme 476 on commercial scale was presented when reduced production costs on a paper machine that uses a large amount of *E. nitens* pulp in its furnish was required. Application of Novozyme 476 saved on refining energy as well as improved tensile strength during the trial. The savings in refining energy and increased tensile strength corroborated previous results obtained on pilot scale (Chapter 4) and in literature (Pere *et al*., 1996; Mansfield and Dickson, 2001; García *et al*., 2002; Lecourt *et al.*, 2010). During the trial, the changes to the paper properties were most notable toward the end of each application, as it took time for the enzyme to build up in the system. This build up of enzyme showed why the dosage of the enzyme has to be optimised on commercial scale and why the dosage of the enzyme was 100-fold less than what was applied on pilot scale. The higher enzyme dosage on pilot scale was required in order to observe all the possible effects of the enzyme on the fibres. Scanning electron micrographs of paper samples collected during the trial confirmed the fibre damage that occurred with over-refining, which, in turn contributed to the reduced tear strength observed. The reduction in tear strength during the trial confirmed the reduction in tear strength observed during pilot-scale experiments with Novozyme 476 on the *Eucalyptus* pulps (Chapter 3 and 4). The enzyme dosage and the refiner current should be manipulated to optimise the fibre development and avoid over-refining. Future work should involve the implementation of Novozyme 476 on a permanent basis at that specific mill and possibly at other fine paper mills.

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SUMMARY

Keywords: endoglucanase, energy, enzymes, *Eucalyptus grandis*, *Eucalyptus nitens*, fibrillation, freeness, fibre modification, pulp fibres, refining, tensile strength.

Refining of pulps requires a large amount of energy, capital input and maintenance costs. Wood fibres can be treated enzymatically prior to refining to reduce the refining energy requirement of the pulps and/or to improve drainage and strength properties. Cell-wall modifying enzymes such as the cellulases and hemicellulases can assist refining by opening up the crystalline and amorphous cellulose and hemicellulose in the fibre walls. This application requires cell-wall modifying enzymes to be matched to the specific pulp.

Characterisation of commercially-available cellulases showed that the activities of the enzymes were suitable for paper mills. The protein content of the enzyme formulations were determined to establish levels for comparative dosing. Using this information the effects of over-dosing and the extended incubation with the enzymes were also determined. The pulp yield and fibre strength appeared to be at risk of being decreased with elevated enzyme dosages, since Ecopulp® Energy, Novozyme 613 and Celluclast 1.5L increased the release of reducing sugars. The critical period for fibre modification by enzyme treatment was the first 30 min of incubation. The most suitable enzyme for application to a paper mill appeared to be Novozyme 476 due to its ability to improve bonding strength with minimal loss in fibre strength.

According to published reports, dried and never-dried *Eucalyptus globulus* pulps responded differently to enzyme treatments and it was, therefore, likely that dried and never-dried *E. grandis* pulps would also respond differently. Ecopulp® Energy, Novozyme 476 and Celluclast 1.5L were applied to *E. grandis* pulps and refined on pilot scale. Overall, the dried *E. grandis* fibres responded differently to the enzyme treatments than never-dried fibres and enzymes should, therefore, be matched to the specific fibre used. It was proved that the enzymes can be used to save on refining

energy usage as well as to improve strength properties of paper made from dried or never-dried *E. grandis* pulps.

Ecopulp® Energy and Novozyme 476 were subsequently applied to the *E. nitens* pulps and refined on pilot scale. All of the enzyme applications had a similar effect on the never-dried pulp, where the properties were not changed as much as on the dried pulp. Novozyme 476 was the most suitable enzyme for application on the dried *E. nitens* pulps, because it saved on refining and drying energy due to the decreased freeness and water retention values. The Novozyme 476 also improved the physical properties of the pulps where tear and tensile indices and porosity were increased and bulk was reduced. This work demonstrated that enzymes must be selected to match the pulps being used and to suit product requirements.

Novozyme 476 was selected for application on a fine-paper machine in order to improve the refining efficiency of *E. nitens* pulp. At the high dosage (100 ml/t) the refining current of the mixed stock refiner was reduced and the porosity and tear strength was decreased and tensile strength was increased. The reduction in tear strength was probably due to too much refining energy applied to enzyme treated pulp, thereby damaging the fibres. The damage to the fibres was confirmed by electron microscopy, showing excessive fibrillation of the fibres treated with the endoglucanase. The endoglucanase successfully improved the refining efficiency of the paper machine and should be considered for application on a permanent basis.

OPSOMMING

Sleutelwoorde: endoglukanase, energie, ensieme, *Eucalyptus grandis*, *Eucalyptus nitens*, fibrillering, pulpvesels, treksterkte, verfyning, veselmodifikasie, vrydreinering.

Die verfyning van pulp verbruik groot hoeveelhede energie asook besteding aan kapitaal en onderhoudskoste. Houtvesels kan ensimaties behandel word voor verfyning om die behoefte aan verfyningsenergie te verminder en/of die dreinerings- en sterkte-eienskappe te verbeter. Ensieme soos sellulase en hemisellulase kan verfyning aanhelp deur die kristallyne en amorfe sellulose en hemisellulose in die veselwande los te maak. Hierdie toepassing vereis dat selwand-modifiserende ensieme vir spesifieke pulptipes geselekteer word.

Die karakterisering van ensieme wat kommersieel beskikbaar is, het getoon dat die aktiwiteit van die ensieme geskik is vir toestande in papiermeulens. Die proteïeninhoud van die formulasies is bepaal om te beslis oor vergelykbare vlakke van ensiemtoediening. Deur hierdie inligting te gebruik, kon die effek van oordosering en verlengde inkubasie met die ensieme ook bepaal word. Dit kom voor of die pulpopbrengs en veselsterkte verlaag word deur hoër ensiemdosisse omdat Ecopulp® Energy, Novozyme 613 en Celluclast 1.5L die vrystelling van reduserende suikers verhoog het. Die kritiese tydperk vir veselmodifikasie met ensiembehandeling was die eerste 30 min van inkubasie. Die mees geskikte ensiem vir toepassing in ’n papiermeule blyk Novozyme 476 te wees as gevolg van sy vermoë on bindingsterkte te verbeter met ’n minimale verlies aan veselsterkte.

Volgens gepubliseerde resultate, reageer gedroogde en ongedroogde *Eucalyptus globulus* pulp verskillend op ensiembehandeling en dit is daarom moontlik dat *E. grandis* pulp ook verskillend kan reageer. *Eucalyptus grandis* pulp is met Ecopulp® Energy, Novozyme 476 en Celluclast 1.5L behandel en op loodsskaal verfyn. Oor die algemeen het die gedroogde *E. grandis* vesels anders op ensiembehandeling gereageer as ongedroogde pulp en ensieme behoort dus vir ‘n spesifieke veseltipe geselekteer te word. Dit is ook bewys dat die ensieme gebruik kan word om verfyningsenergie te

bespaar en sterkte-eienskappe van papier wat van gedroogde en ongedroogde *E. grandis* pulp vervaardig is, te verbeter.

Ecopulp® Energy en Novozyme 476 is vervolgens op *E. nitens* pulp aangewend en op loodsskaal verfyn. Al die ensiembehandelings het ’n soortgelyke effek op ongedroogde pulp gehad, waar die invloed minder was as op gedroogde pulp. Novozyme 476 was die mees geskikte ensiem vir toediening op gedroogde *E. nitens* pulp omdat dit verfynings- en drogingsenergie kan bespaar a.g.v. laer vrydreinering en waterretensie waardes. Die Novozyme 476 het ook die fisiese eienskappe van die pulp verbeter deur skeur- en trekindekse en porositeit te verhoog en digtheid te verlaag. Hierdie werk het gedemonstreer dat ensieme vir die pulp wat gebruik word, geselekteer moet word en ook om te pas by produkvereistes.

Novozyme 476 is gekies vir aanwending by ’n fynpapiermasjien om die doeltreffendheid van die verfyning van *E. nitens* pulp te verbeter. Teen die hoër dosis (100 ml/t) is die elektriese stroom na die gemengde-pulpverfyner verminder terwyl die porositeit en skeursterkte verminder en treksterkte verhoog het. Die verlaging in skeursterkte kan moontlik aan die aanwending van te veel verfyningsenergie aan die ensiembehandelde pulp toegeskryf word wat daardeur die beskadiging van vesels veroorsaak het. Die beskadiging van die vesels is deur elektronmikroskopie bevestig, wat oormatige fibrillering van die vesels wat met endoglukanase behandel is, getoon het. Die endoglukanase het die doeltreffendheid van die verfyning vir die papiermasjien verbeter en die toediening van die ensiem op ’n permanente basis moet oorweeg word.