



The effect of cellobiohydrolase I on low consistency refining and handsheet properties

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ABSTRACT

Papermaking is an energy-intensive process, with mechanical pulp refining accounting for 30% of the total electrical energy consumption during papermaking. Presently, with significant economic and environmental concerns, improving energy efficiency and minimising the environmental impact of paper manufacturing is critical. Biotechnological treatments of pulp with enzymes have demonstrated a high potential for lowering energy consumption and greenhouse gas emissions. The benefits of enzymes include energy savings, environmentally friendly treatment, and improvement in fibre and pulp quality, while requiring only modest adjustments to the existing industrial processes. At present, the most investigated enzymes for fibre modification are cellulases. This research aimed to investigate the effect of the cellulase, cellobiohydrolase I (CBH I) as a refining enzyme. Three structurally different pulps were investigated: a bleached hardwood kraft pulp (BHKP), a fully bleached softwood (FBSW) pulp, and an unbleached softwood (UBSW) pulp. The research project was divided into three phases.

In the first phase, CBH I was characterised by determining the effect of temperature and pH on CBH I activity using a filter paper assay. The thermostability of CBH I was then analysed, using the optimal temperature and pH. In the second phase, the use of CBH I as a pre-treatment on the pulp before refining was investigated using the bijective diagram technique. A 12" single-disc pilot refiner was used to simulate industrial conditions. The effect of varying the Specific Edge Load (SEL) and refiner plate design for the CBH I treated pulp was evaluated against the reference pulp with CBH I (REF CBH I). The fibre morphology, handsheet properties, and energy consumption were evaluated at a specific desired Canadian Standard Freeness (CSF) for each pulp. In the third phase, the refining data obtained in the second phase was modelled using customised refining software to determine the optimum refining parameters required for the best handsheet strength properties with minimum energy consumption. The software-generated optimum refining parameters were validated by refining each pulp three times under the specific conditions. Statistical analysis was used to test the repeatability of the three runs for each pulp.

The first phase showed that CBH I is a thermophile enzyme with an optimum temperature of 55 °C. The enzyme works best in acidic conditions, with an optimum pH of 4. Furthermore, CBH I is thermostable, with 49% of its activity retained at the end of a 3 h incubation.

In the second phase, CBH I pre-treatment resulted in energy savings of 33%, 10%, and 22% for BHKP, FBSW, and UBSW, respectively. All three treated pulps responded better to low intensity (SEL-). In contrast, increasing the refining intensity (SEL+) deteriorated the pulp

quality for all three pulps. Changing the refiner plate design led to a different result for each pulp. For the BHKP, the GC run (plate design with smaller bar width and grooves) led to the highest strength development, but also required the most refining energy. Whereas the GA run (plate design with a bigger bar angle) led to more fibre cutting than fibrillation. For the FBSW, both the GC and GA plate designs improved the fibre development. On the contrary, the GA and GC plates for the UBSW resulted in poor fibre development.

In the third phase, the optimum plate designs generated by the model for the BHKP, FBSW, and UBSW were REF, GA, and REF plate designs, respectively. For all three pulps, one-way ANOVA analysis showed a statistically insignificant difference between the pulp properties of the three runs conducted for each pulp.

In conclusion, the CBH I enzyme has the potential to transform the refining process towards a more environmentally friendly, cost-effective, and efficient operation compared to the conventional method.

Keywords: Cellobiohydrolase I, enzymatic refining, pulp, handsheet properties, cellulase, fibre morphology, Canadian Standard Freeness

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NOMENCLATURE

Units		Abbreviations	
%	Percentage	°SR	Schopper-Reigler
”	Inch	ANOVA	Analysis of variance
°	Degrees	BHKP	Bleached hardwood kraft pulp
°C	Degrees Celsius	C	Consistency
cm ³ /g	Centimetre cubed per gram	CBH	Cellobiohydrolase
g	Gram	CEL	Cutting-edge length
g/m ²	Gram per metre squared	CMT	Corrugating Medium Test
h	Hour	CSF	Canadian Standard Freeness
Hz	Hertz	EG	Endoglucanase
IU/mL	International Unit per millilitres	F	Volumetric flowrate
J/m ²	Joule per metre squared	FBSW	Fully bleached softwood
km	Kilometre	FPA	Filter paper assay
km/s	Kilometre per second	GA	Grinding angle
kN/m	Kilo newton per metre	GC	Grinding code
kPa.m ² /g	Kilopascal metre squared per gram	HPLC	High-performance liquid chromatography
kW	Kilowatt	HW	Hardwood
kWh/t	Kilowatt hour per ton	ISO	International Standard Organisation
L	Litre	$\dot{m}$	Mass flow rate
M	Molarity	n	Rotational speed
m ³ /h	Cubic metre per hour	P _{net}	Net power
mg	Milligram	P _{noload}	No-load power
mg/m	Milligram per metre	P _{tot}	Total power
mg/mL	Milligram per millilitre	L&W FT+	Lorentzen & Wettre Fiber Tester Plus
min	Minute	L _s	Cutting speed of bars
mL	Millilitre	REF	Reference
mL/min	Millilitre per minute	SCT	Short-span Compressive Test

Units		Abbreviations	
mM	Millimolar	SEL	Specific Edge Load
mN.m ² /g	Millinewton metre squared per gram	SEM	Scanning electron microscope
nkat/mL	Nanokatal per millilitre	SRE	Specific refining energy
Nm/g	Newton metres per gram	SSL	Specific Surface Load
rev/s	Revolution per second	SW	Softwood
t/h	Ton per hour	T. reesei	Trichoderma reesei
μL	Microlitre	UBSW	Unbleached softwood
μm/mol	Micrometre per mole	Z _r , Z _s	Number of bars for rotor, stator

CHAPTER 1: INTRODUCTION

In this chapter, a synopsis of the research project is provided. Firstly, in Section 1.1, the background and motivation behind the study are presented by introducing the refining concept, the importance of enzymes, particularly cellulase, in refining, and what has been reported on cellulase refining thus far. Secondly, the problem statement is formulated in Section 1.2 to illustrate the current primary problem faced by the pulp and paper industry with regard to the refining process. Thirdly, the aim and objectives of the research project are defined in Section 1.3. Lastly, Section 1.4 concludes Chapter 1 by providing an overview and brief description of all the chapters in the dissertation.

1.1 Background and motivation

Papermaking is an intricate process with the objective of producing paper sheets with maximum strength from fibres of different sources with minimum cost invested. Paper production from woody biomass is still the most prevalent process. The wood fibres can either be hardwood (HW) or softwood (SW). After pulping, these fibres do not have sufficient bonding ability to form a high-quality paper (Lecourt *et al.*, 2010b; Pathak *et al.*, 2016). It has been reported that the paper produced from this pulp is bulky, has low strength, has a rough surface, and is inappropriate for most paper grades. The fibres of a high-quality paper must be matted into a uniform, smooth surface and form strong inter-fibre bonds. This can only be accomplished if the pulp after pulping, is subjected to a mechanical treatment known as refining (Bajpai, 2005).

Refining is a mechanical treatment that alters the morphology of the fibres to increase the fibre bonding capability and develop the desired final paper properties (Singh & Bhardwaj, 2010). During refining, the fibres are forced to pass through a narrow gap between counter-rotating refiner plates, known as a rotor and a stator. These plates subject the fibres as they pass through the narrow gap, to repeated compression and shearing forces due to the bars and grooves on the plates' surfaces. As a result of these forces, the fibre wall is steadily delaminated. The primary fibre wall (P) is initially peeled off, followed by the first secondary wall (S1), in order to expose the inner secondary cellulose-rich layer (S2) (Cuberos-Martinez & Park, 2012; Torres *et al.*, 2012).

Consequently, the forces experienced by the fibres during refining induce the following refining effects: external and internal fibrillation, fibre cutting, fibre straightening, and fines formation (Gharehkhania *et al.*, 2015; Motamedian *et al.*, 2019). Among these effects, the primary beneficial ones are both the fibrillation effects. Internal fibrillation, also referred to as swelling,

is believed to be the most essential refining effect by several researchers (Bajpai, 2005; Gharehkhania *et al.*, 2015; Wahren, 1983). Internal fibrillation is caused by the breakdown of hydrogen bonds between micro-fibrils. This leads to the adsorption of water by the fibres, thus the fibres 'swell'. The swelling makes the fibres softer, enhancing the flexibility and collapsibility of the fibres. Thus, the fibres become more conformable to other fibres, strengthening the inter-fibre bonding (Motamedian *et al.*, 2019; Przybysz *et al.*, 2020). External fibrillation is a phenomenon whereby the fibrils on the S2 layer unravel but remain attached to the fibre wall, giving the surface a hairy-like appearance. These fibrils increase the surface area, facilitate the bonding between the fibres, and improve the cohesiveness of the final product (Bajpai, 2005; Ek *et al.*, 2009a).

However, the refining process is an extremely energy-intensive treatment. Refining accounts for approximately 30% of the total electrical energy consumption of the paper manufacturing process (Dien *et al.*, 2014; Lecourt *et al.*, 2010a), which corresponds to approximately 18 to 25% of the total manufacturing costs (Pathak *et al.*, 2016). Presently, with energy being an expensive commodity, improving energy efficiency is a primary concern for the pulp and paper sector. Furthermore, with rigorous environmental rules mandating more sustainable, eco-friendly operations, minimising effluents created by fossil fuel burning is critical for long-term growth. Thus, papermakers had to develop energy-saving strategies. Alternate refining techniques such as ultrasound, cavitation, steam explosion, and freezing have not resulted in substantial energy savings. Other various approaches necessitated significant investments. These solutions included changing the refining plate pattern and the pulp consistency, increasing the flow rate through the refiner, or completely revamping the refining strategy. Consequently, a cost-efficient and simpler method had to be developed. The approach should only necessitate minimal modifications to the refining process and should not interfere with wet-end chemistry. The use of enzymes appeared to be a potential alternative (Lecourt *et al.*, 2010b; Lin *et al.*, 2018; Przybysz Buzala *et al.*, 2016; Zollner-Croll & Badakh, 2016).

Enzymes are increasingly being used in mechanical pulp refining because of their mild effect on pulp fibres, as well as the fact that they are target specific, eco-friendly, and cost-effective (Tripathi *et al.*, 2008). Furthermore, the application of enzymes has gained popularity due to the minor adjustments required to the existing industrial processes and decreasing vessel picking in tropical hardwood pulps (Znidarsic-Plazl *et al.*, 2009). Enzymes allow for energy savings without compromising the pulp strength properties (Bajpai *et al.*, 2006; Gil *et al.*, 2009).

Xylanases, cellulases, laccases, pectinases, and manganese peroxidase are some of the enzymes typically employed to assist the refining process (Hyoung-Jin *et al.*, 2006; Torres *et*

et al., 2012). Among these enzymes, cellulases are the most studied enzymes for fibre modification, because they act directly on the cellulose-rich layer (S2) (Pathak *et al.*, 2016). Cellulases are a group of enzymes that act synergistically to cleave the β -1,4 glycosidic linkages of a cellulose chain to its monomeric sugars. Cellulases can be categorized into three main types, namely endoglucanase (EG), cellobiohydrolase (CBH), and β -glucosidase (Jayasekara & Ratnayake, 2019; Juturu & Wu, 2014). Firstly, EG cleaves the amorphous area of the cellulose chain at random, causing the chain length to rapidly decrease and the amount of free reducing groups to gradually grow. Secondly, CBH produces cellobiose, glucose, and occasionally cellotriose by cleaving the reducing and non-reducing ends of the cellulose chain. Lastly, β -glucosidase cleaves the cellobiose generated by EG and CBH to yield glucose. The removal of accumulated cellobiose by β -glucosidase is an essential step in enzymatic hydrolysis, as it reduces end-product inhibition (Mboowa, 2019; Zhai, 2017).

Numerous studies on cellulase-assisted refining for energy consumption reduction and improvement of fibre and handsheet properties have been conducted; the results obtained vary depending on the pulp and refining parameters used (Gil *et al.*, 2009; Gupta *et al.*, 2015; Lecourt *et al.*, 2010b; Liu & Hu, 2012; Singh *et al.*, 2015; Zhang *et al.*, 2013). Out of the three cellulases, the cellulase CBH I from *Trichoderma reesei* has been the one attributed to the reduction in refining energy consumption. This has been attributed to the enzyme's ability to selectively hydrolyse the abundant region of cellulose, *i.e.*, crystalline cellulose (Mohlin & Pettersson, 2002; Pathak *et al.*, 2016; Pere *et al.*, 1996; Singh & Bhardwaj, 2010; Tian *et al.*, 2017).

Even though the benefits of implementing cellulase on both the economy and environmental impact of the pulp and paper sector are well documented, the precise mechanism of how the enzyme interacts with the fibres and affects the handsheet properties is still not understood. Furthermore, the adverse aspects of enzymatic hydrolysis on fibres may restrict the implementation of this sustainable technology. Excessive enzyme treatment might degrade the fibre's intrinsic strength and deteriorate the strength properties of the pulp (Znidarsic-Plazl *et al.*, 2009). Thus, additional research in this field is still required.

1.2 Problem statement

The pulp and paper industry is ranked fourth in terms of energy usage, with mechanical refining accounting for 30% of the electrical energy used in paper production (Dien *et al.*, 2014). As a result, mechanical pulp refining accounts for approximately 18 to 25% of the total manufacturing costs (Pathak *et al.*, 2017). The challenge for papermakers is to find alternative ways to improve fibre development with reduced energy consumption and minimal investment.

Biotechnological treatments of pulp with enzymes have been identified as a possible cost-effective solution. The use of cellulase enzymes for fibre modification has grown over the years and has the potential to reduce energy consumption without affecting paper quality (Lecourt *et al.*, 2010b). However, the precise mechanism of how the enzymes interact with the fibres and affect the handsheet properties is still not understood, necessitating further research in this area.

1.3 Research aim and objectives

The research aim and objectives were formulated in Sections 1.3.1 and 1.3.2 based on the background and motivation to provide the reader with a clear understanding of the project's overall goal.

1.3.1 Aim

The purpose of this research project was to investigate the effect of cellobiohydrolase I (CBH I) as a refining enzyme.

1.3.2 Objectives

The aim of the project was investigated by carrying out the following objectives:

- To functionally characterise CBH I by determining the optimum temperature and pH conditions and thermostability, using the filter paper assay.
- To determine the effect of CBH I on the Canadian Standard Freeness (CSF), fibre morphology and refining energy.
- To determine the effect of CBH I on the handsheet strength, structural and optical properties.
- To optimise CBH I assisted refining parameters based on computational modelling and pilot refining data.

1.4 Dissertation outline

Chapter 1 – Introduction: This chapter provides the project summary by explaining the context and motivation for the research study. Furthermore, the problem statement, aim, and objectives of the research project are presented to the reader to demonstrate the study's goal.

Chapter 2 – Literature review: This chapter establishes familiarity with the primary concepts of the research project. The chapter begins by providing an overview of the papermaking process to give the reader a better understanding of where and how the refining process fits into the papermaking process. Thereafter, the refining process is reviewed in detail. This is

accomplished by initially explaining the refining mechanism and theory used in this research project. Thereafter, the impact that refining has on the fibre morphology and the variables that affect the refining outcome. Lastly, the impact that the change in the fibre morphology, *i.e.*, refining process has on the handsheet properties. A detailed overview of the fibre morphology and refining differences between hardwood and softwood pulps is then presented. This section is followed by a thorough examination of enzymes, specifically cellulase, and the use of cellulase in the pulp and paper industry. The chapter concludes by examining the future prospect of enzymes in refining.

Chapter 3 – Methods and materials: This chapter describes the raw materials and research methodologies employed in this study. The research methods are divided into three phases. The approach used to characterise the enzyme CBH I is described in Phase 1. Phase 2 details the entire refining process and the parameters employed during the bijective studies' refining runs on the pilot refiner for the three pulps under consideration in this project. Furthermore, the procedures utilised to evaluate the degree of refining (CSF), fibre morphology, and handsheet properties are discussed. Phase 3 describes how the optimal refining parameters for each pulp were determined using customised bijective refining software. Furthermore, how the pilot refiner was used to validate these optimum refining parameters. The hypotheses and assumptions for the one-way ANOVA statistical approach for the three optimum runs for each pulp are presented. The chapter concludes with a description of the method used to obtain the micrographs of the fibre development using a Scanning Electron Microscope (SEM).

Chapter 4 – CBH I characterisation: This chapter presents and analyses the results obtained during phase 1, *i.e.*, the effect of temperature and pH on CBH I activity and the thermostability of the enzyme. The results from this phase determine whether CBH I is suitable to be used in the pulp and paper industry.

Chapter 5 – Bijective studies on the pilot refiner: This chapter discusses the results obtained during the second phase of this research project. The results of the effect of CBH I pre-treatment prior to refining as well as the impact of varying Specific Edge Load (SEL) and the refiner plate design are evaluated. This is accomplished by analysing the energy consumption, fibre morphology, and handsheet properties result at a specific CSF of interest for each pulp.

Chapter 6 – Optimisation of refining parameters: This chapter presents the results from phase 3 of the research study. The optimum results generated by the software are analysed. Furthermore, the results obtained from pilot refining utilising the optimum parameters are analysed. These results are then compared with the results of the bijective runs from phase 2

to determine whether the optimum run presents the best compromise of the pulp properties and refining energy. The results from the one-way ANOVA statistical method are then presented and analysed. Lastly, the SEM micrographs of the fibre morphology of the optimum run and two runs with and without CBH I are presented.

Chapter 7 – Conclusions and recommendations: This chapter provides a summary and reflection of the project's key findings. In addition, recommendations for future work based on the results of the three phases are stated in this chapter.

CHAPTER 2: LITERATURE REVIEW

In this chapter, a literature review that is pertinent to the research project is provided in depth. The chapter begins with the papermaking process overview in section 2.1, to give the reader a better understanding of the paper production process and where the refining stage fits into the overall process. Section 2.2 goes over the refining process in detail. Under this section, a description of the refining mechanism together with the refining theory used in this study is provided. This is followed by the five primary refining effects and the variables that affect these refining effects. Lastly, the impact that the refining outcome has on the handsheet properties is provided. Section 2.3 presents the differences between the two types of pulps used in this study and the refining requirements of the pulps. section 2.4 presents a detailed discussion of enzymes, particularly cellulase in the pulp and paper industry. Section 2.5 concludes the chapter by looking at the future prospect of enzymes in refining.

2.1 Papermaking process overview

The pulp and paper industry is very diversified, using several varieties of raw materials to manufacture many different types of paper and packaging products through various methods in mills of all sizes (Bajpai, 2010). Even though a broad range of products exist, nearly all of them follow a basic procedure, depending on the raw material provided. The raw material can originate either from wood, non-wood material, or recycled material (Torres *et al.*, 2012).

However, paper manufactured from woody biomass is still the most prevalent process and follows the primary steps shown in Figure 2-1. The initial steps include wood log debarking and chipping. Debarking is a procedure in which the outer layer of the wood log (bark) is removed. This is followed by chipping, which is a mechanism that breaks down the woody content into small chips to have a higher surface area during pulping (Pathak *et al.*, 2016). The chips are then screened before pulping to achieve a uniform size distribution. The undersized chips (pin chips and fines) are discarded and utilised as fuel. The overly thick chips go to a re-chipper and are decreased to acceptable chip size fractions (Ek *et al.*, 2009a).

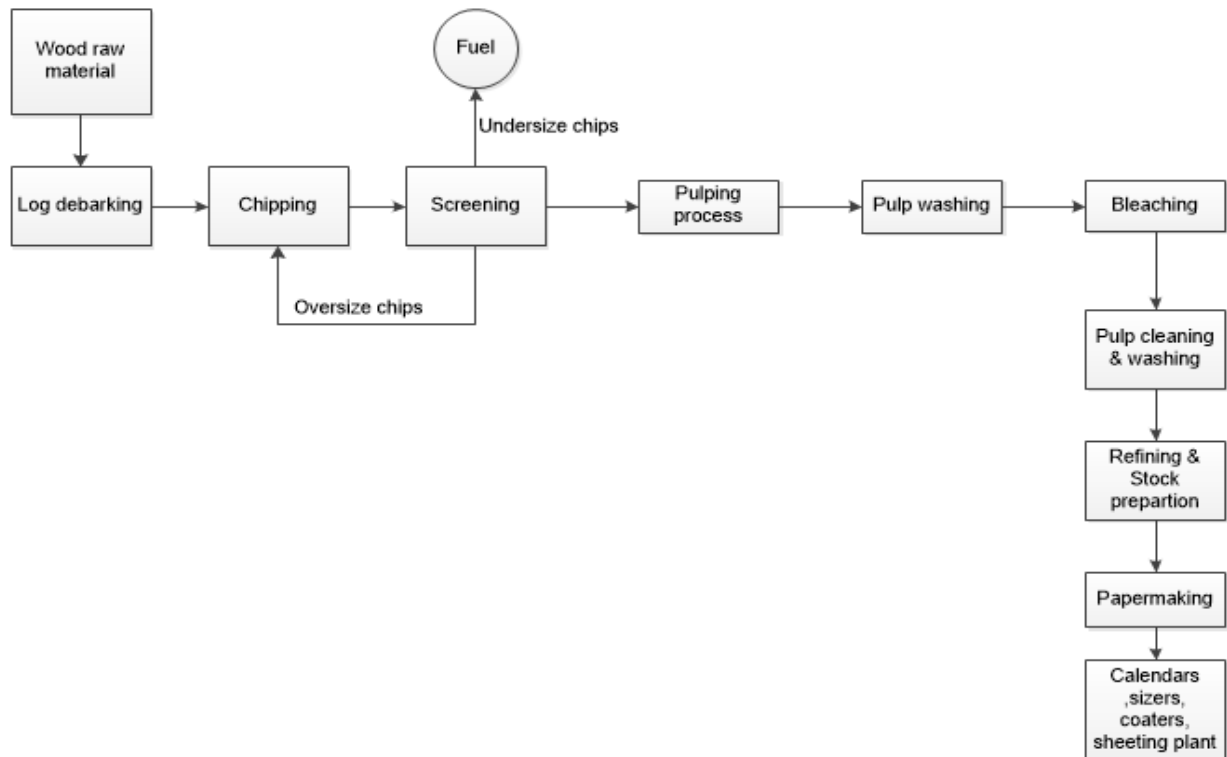


Figure 2-1: Basic flowchart of the papermaking process (adapted and modified from Pathak *et al.* (2017))

The screened wood chips are then sent to the pulping process, which transforms the wood chips into fibrous material (pulp) by extracting most of the lignin found in the wood. Consequently, the pulp obtained is rich in cellulose, which is the main component required for papermaking. The pulping process can be conducted using various methods, such as mechanical pulping, chemical pulping, or a combination of the two processes. Mechanical pulping uses substantial amounts of electrical and mechanical energy to separate the fibres. In contrast, chemical pulping separates the wood fibres by cooking the wood chips at extreme temperatures and pressures using chemicals (Biermann, 1996; Popa, 2013).

After pulping, the pulp is washed to remove any impurities as well as the spent liquor. Pulp washers, known as brown stock washers for kraft pulp and red stock washers for sulfite pulp, are used to remove residual spent cooking liquor from chemical pulping. Excess cooking liquor present in the pulp increases bleaching chemical consumption. Therefore, efficient washing is vital to maximise the return of cooking liquor to chemical recovery and to reduce carryover of cooking liquor (known as washing loss) into the bleach plant (Bajpai, 2010; Ek *et al.*, 2009a).

Pulps obtained from most pulping processes are too dark due to the presence of residual lignin to be used for most paper products without going through a bleaching process. Consequently, the pulp is first bleached to increase the brightness of the pulp as well as to remove any residual lignin, that would cause a yellow discolouration of the paper over time (Bajpai, 2010). Numerous bleaching agents such as chlorine dioxide, oxygen, hydrogen peroxide, and ozone may be implemented, depending on the raw material and pulping process used, and the targeted final brightness. However, owing to environmental issues, alternative bleaching agents such as xylanases enzymes are being used more frequently (Bajpai, 2016).

After bleaching, the pulp is further cleaned and washed to remove bleaching chemicals and solubilised pulp components (Biermann, 1996). Thereafter, the pulp is refined by passing it through a narrow gap between a set of rotating and stationary refiner plates that have metal bars and grooves fitted on them. Refining gives the pulp fibres a variable degree of fibrillation. This is advantageous for paper manufacturers because fibrillated fibres increase the surface area and facilitate inter-fibre bonding, allowing the fibres to bind more tightly with adjacent fibres, leading to a stronger final product (Ghosh *et al.*, 2018).

Depending on the final product, various inorganic fillers (clay, calcium carbonate, and titanium dioxide) and sizing agents are added to the refined pulp solution preceding papermaking, which is referred to as stock preparation. Additional process units such as calendars, sizers, coaters, sheeting plants, and roll wrapping stations may be required after papermaking to meet the desired product specifications (Pathak *et al.*, 2016).

2.2 Refining

The refining stage is regarded as one of the most important unit operations in the papermaking process, as this process essentially ensures that the final products are made according to customer specifications. Any changes made during the refining stage cannot be amended anywhere downstream (Bajpai, 2005; Clark, 1985; Finn, 1991; Lumiainen, 1998; Mansfield, 1997; Roberts, 1996; Singh & Bhardwaj, 2010).

Paper produced from unrefined pulp has been observed to have a rough surface, bulkiness, and low strength. In a high-quality paper, the fibres must form strong inter-fibre bonds and be matted into a uniform sheet. Refining is beneficial as it provides the fibre development necessary for a high-quality paper, changing the undesirable characteristics (Bajpai, 2005). In addition, the refining process greatly affects the runnability of the paper machine, thus affecting productivity (Roberts, 1996).

Refining is a mechanical treatment process that separates and modifies fibres by applying shear and compression forces. The resulting fibres are capable of interacting with each other to create a strong fibre network. Refining is essential as it allows the papermaker to modify the fibre morphology to produce the desired paper properties (Singh & Bhardwaj, 2010).

There are two types of refining, namely, high consistency (HC) refining and low consistency (LC) refining (Biermann, 1996; Stander, 2015), which describe pulp as having a low or high moisture content. In the HC refining process, the pulp suspension is fed into the refiner at a range of 30 to 35% consistency using a screw feeder (Lumiainen, 1998). This process is conducted in an atmospheric discharge refiner. HC refining is advantageous to extensible paper grades where high tension energy absorption is required (Gurnagul *et al.*, 2006). In addition, it results in less fibre cutting and damage, preserving the fibre length and leaving the fibres with micro-compression, which enhances the extensibility of the paper. The disadvantage of the HC refining process is that it preserves long fibres and creates a high quantity of shives, kinks, and curls (Bajpai, 2005).

In the LC refining process, the pulp suspension is fed into the refiner at a consistency range of about 2 to 6% using a pump. In contrast to HC refining, the LC process is beneficial in removing shives, kinks, and curls that are typically formed during HC refining (Harirforoush, 2018). As a result, HC refining is commonly followed by two to three stages of LC refining, and the input energy depends on the end product desired. The disadvantage of LC refining is the high quantity of fines generated (Gurnagul *et al.*, 2006; Lumiainen, 1998).

2.2.1 Refining mechanism

Refining consists of two stages, namely, fibre separation and fibre development (Manorma, 2015; Torres *et al.*, 2012). In the first stage, the fibres are separated into individual fibres by using counter-rotating discs. The second stage gives rise to fibrillation and increased flexibility of the fibres to enhance the fibre characteristics (Torres *et al.*, 2012).

The fibres are forced to pass through a narrow gap between two plates, *i.e.*, a rotor and a stator, during refining (Figure 2-2 (a)). The fibres undergo three phases, namely an edge-to-edge phase, an edge-to-surface phase, and a surface-to-surface phase, as they pass through the narrow gap between the stator and the rotor (Figure 2-2 (b)) (Nugroho, 2012; Smook, 1992). The first phase (section A) is the edge-to-edge phase, where the rotor bars' leading edges intersect with the stator bars' leading edges. The fibres are subjected to a significant refining impact during this phase, and the majority of the water in the fibre is compressed. The second and third phases (Sections B & C) are where the majority of the refining takes place. During these phases, the plates' leading edges slide along the fibres and press the fibres

against the flat bar surface. This process carries on until the leading bar edge contacts the trailing bar edge. The mechanism described above only covers a single refining impact; the entire length of the impact is determined by the width of the bars (Anand *et al.*, 2016; Lumiainen, 1998; Nugroho, 2012).

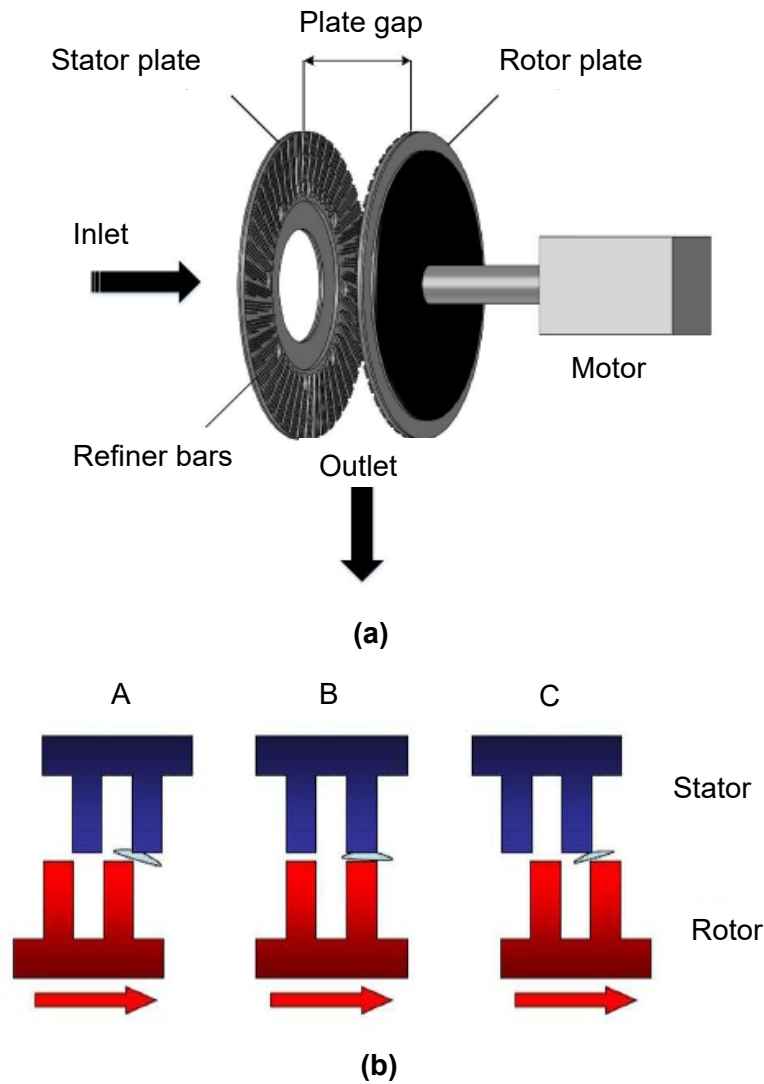


Figure 2-2: (a) Illustration of a single-disc refiner set up. Demonstrating the rotor and stator plates with the refiners' bars, the refiner's motor and the inlet and outlet of the refiner. (Harirforoush, 2018) (b) Illustration of the three stages that pulp fibres undergo during the refining treatment (Nugroho, 2012)

The design of the refining plates (rotor and stator) is crucial, as it determines the type of refining action. The grooves on the plates have two primary purposes, firstly, to carry the fibres through the refiner; and secondly, to provide the bar edges and surfaces required for refining (Rampersadh, 2005). The mechanical refining action is created by the bar crossings on the plates, which generate compression and shear forces that alter the morphology of the fibres (Figure 2-3) (Elahimehr, 2014). These forces gently delaminate and peel the fibre cell wall.

During the refining process, the outer primary layer (P) and the first outer layer of the secondary layer (S1) are peeled off, exposing the cellulose-rich layer of the secondary wall (S2) of the fibres (Figure 2-4). This exposed cellulose-rich layer provides more hydrolysed groups, which facilitate the refining effects that are essential to produce good quality paper (Pathak *et al.*, 2016).

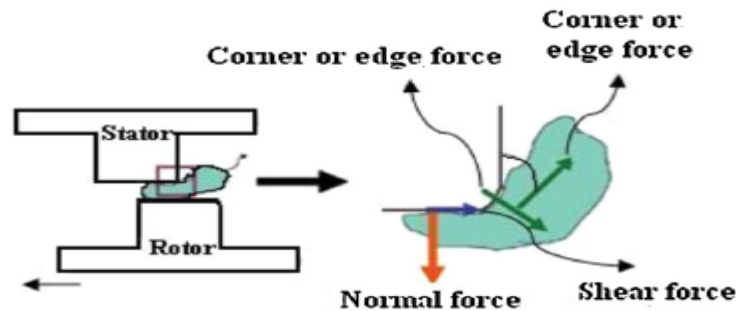


Figure 2-3:The forces created by the bar crossings on the refining plates that act on the fibres during the refining mechanical action. These forces include normal force, shear force and corner or edge force (Anand *et al.*, 2016)

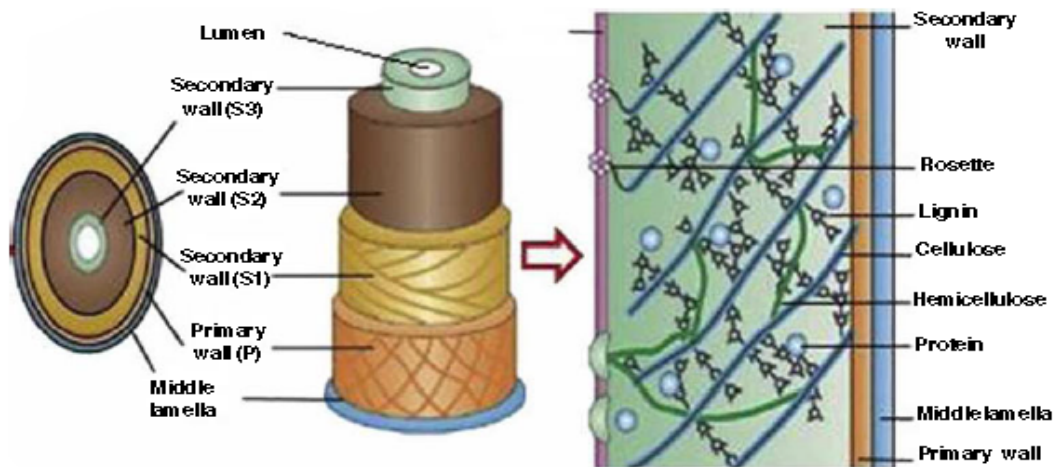


Figure 2-4: Illustration of the different layers in a wood fibre, namely the primary cell wall layer (P), the outer layer of the secondary wall (S1), the cellulose-rich layer (S2), and the inner layer of the secondary wall (S3) (Torres *et al.*, 2012).

2.2.2 Specific edge load refining theory

There are three commonly used refining theories, namely, the Specific Edge Load (SEL) theory, the Specific Surface Load (SSL) theory, and the C-factor theory (Bajpai, 2005; Gharehkhania *et al.*, 2015; Lumiainen, 1998). The purpose of a refining theory is to predict changes in the pulp properties resulting from established refining conditions. The primary

characteristic of the refining theories is that they operate regardless of the type and size of the refiners (Bajpai, 2005; Gharehkhania *et al.*, 2015). This study applied the SEL theory, so this section describes the basis and derivation of the SEL theory.

The SEL theory is one of the most widely known and commonly applied refining theories (Kerekes, 2011; Lumiainen, 1998). This theory can be described as a two-parameter pulp characterisation consisting of Specific Edge Load (SEL) and Specific Refining Energy (SRE) parameters. The SEL parameter is the quantity of energy expended per unit edge length of a bar crossing and is strictly presented as the refiner's intensity, which means that it represents the energy consumed at bar crossings without regard to how the energy is distributed. SEL can be expressed mathematically in Ws/m or J/m using Equation (2-1) (Kerekes, 2011; Kerekes & Senger, 2006).

$$SEL = \frac{P_{net}}{L_s}, \quad P_{net} = P_{tot} - P_{noload} \quad (2-1)$$

Where: P_{net} (kW) is the net power consumed to change the fibre properties; P_{tot} is the total power consumed during refining; and P_{noload} is the no-load power, which is the energy required for the refiner rotor to run. At this stage, no energy is imparted to the pulp fibres.

The no-load power does not contribute to pulp refining and is solely consumed in order to overcome losses such as the loss due to shaft and bearing friction, the energy needed to rotate the rotor close to the stator in the pulp suspension, and the energy needed by the refiner when pumping the pulp suspension from the inlet to the outlet of the refiner. The other parameter used in Equation (2-1) is the cutting speed of the bars (L_s) in km/s, which can be expressed by Equation (2-2) (Gharehkhania *et al.*, 2015; Lumiainen, 1998).

$$L_s = CEL \times n = Z_r \times Z_s \times l \times n \quad (2-2)$$

Where: CEL is the cutting-edge length (km/rev), and n is the rotational speed (rev/s). The CEL is calculated by multiplying the number of the rotor and stator bars (Z_r and Z_s) with their average length (l) in km. The SEL typically used for hardwoods is 0.2 to 1.0 Ws/m and 1.5 to 3 Ws/m for softwoods (Nugroho, 2012).

Another parameter used to describe the SEL theory is Specific Refining Energy (SRE). The SRE is used to measure the amount of energy imparted by the refiner to the fibres, which can be calculated by dividing the net power with the fibre mass flow rate (Gharehkhania *et al.*, 2015). The SRE is directly proportional to the level of refining. At a constant refining speed, pulp consistency, and flow rate, the SRE and refining intensity are only affected by the net power, resulting in a linear correlation between SRE and refining intensity. Therefore, in this

context, the SRE and refining intensity have a direct proportional relationship (Nugroho, 2012). The SRE is calculated in kWh/t using Equation (2-3), where $\dot{m}$ is the mass flow rate in t/h. The mass flow rate is the product of the volumetric flow rate (F) in m³/h and consistency (C) in %.

$$SRE = \frac{P_{net}}{\dot{m}} = \frac{P_{net}}{F \times C} \quad (2-3)$$

From its derivation, the SEL theory measures the net energy consumed per unit length of bar crossings. It only states the quantity of the net energy transferred by a bar edge crossing to the fibres and does not indicate how the net energy was received by the fibres. The theory does not acknowledge that many other essential factors affect the outcome of the refining process. It fails to account for factors such as the net energy during one-pass, refining consistency, width of bars, stapled fibres on bar edges, gap clearance and condition of refiner fillings. The theory simply takes into account the length of the bars and presumes that the aforementioned factors do not affect the refining outcome. Hence, the theory is widely used, due to its simplicity and readily available factors (Bajpai, 2005; Lumiainen, 1998).

2.2.3 The effect of refining on the fibre morphology

The refining mechanical forces created by the bar crossings on the plates induce substantial modifications to the fibre structure. As a result, there are five primary effects: external (macro) fibrillation, internal fibrillation, fibre cutting, fibre straightening, and fines formation (Gharehkhania *et al.*, 2015; Heymer, 2009; Loijas, 2010).

External fibrillation is a phenomenon that occurs when the refining forces act on the fibre surface and results in the fibrils on the fibre wall becoming loose but remaining attached to the fibre wall (Ek *et al.*, 2009a; Wang *et al.*, 2007). External fibrillation gives the fibre surface a hairy-like appearance, consequently increasing the surface area of the fibres. The increased surface area enables an improved bonding between the fibres (Popa, 2013).

Internal fibrillation, also known as swelling, has been widely researched as it is assumed by some investigators to be the most significant refining (Bajpai, 2005; Gharehkhania *et al.*, 2015). Internal fibrillation occurs when the P and S1 layers of fibres are removed, resulting in the breakage of fibril hydrogen bonds. This increases the hydration of the fibres, giving rise to softer and more flexible fibres (fibres swell) (Ek *et al.*, 2009a; Motamedian *et al.*, 2019). The fibrillation effect is shown in Figure 2-5.

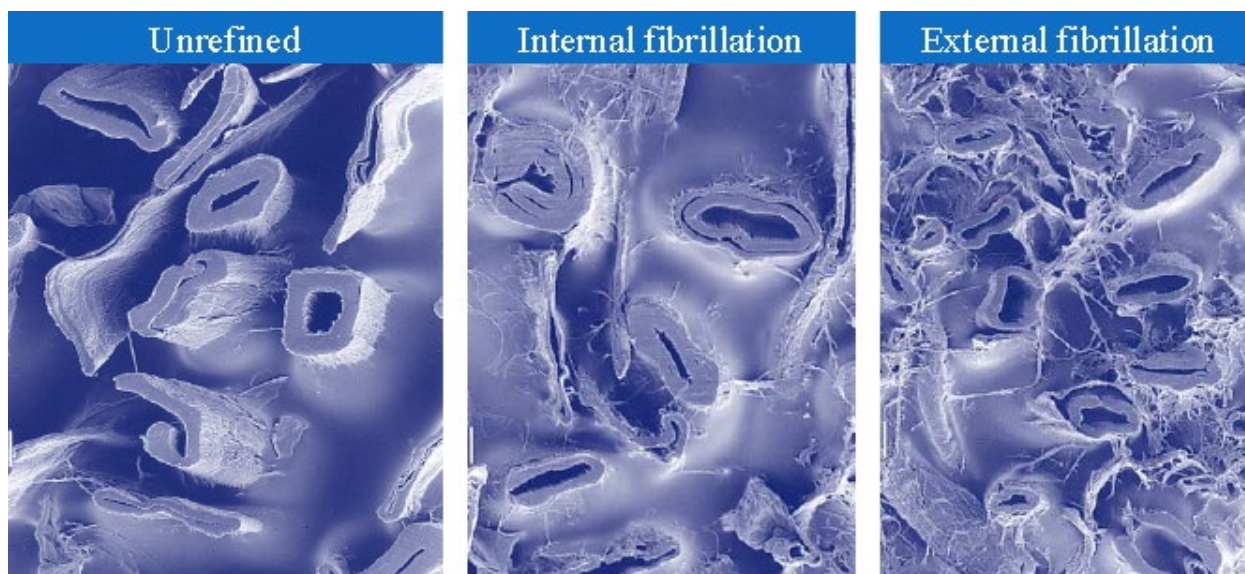


Figure 2-5: Micrographs illustrating the internal and external fibrillation effect on pulp fibres due to refining compared to unrefined fibres (Palmer, 2009).

The third effect of refining is fibre cutting, which leads to a reduction in fibre length and is generally considered to be a negative effect of refining. The fibre length decreases as the refining energy increases. Fibre cutting is caused by tensile or shear forces during refining that are above the capacity that the fibre can endure. With no way to avoid the load, the fibre ends up being cut. As a result, sheet strength properties are negatively affected (Ek *et al.*, 2009a). In a limited number of applications, fibre cutting is the desired result in order to enhance fibre formation by reducing the crowding number. In this case, fibre cutting increases the formation of the sheet tremendously because of a reduction in the crowding number, which successively lowers the flocculation tendency and small floc sizes, leading to a uniform and smooth paper (Bajpai, 2005).

The fourth effect of refining is fibre straightening. Frequently, most of the pulp from the pulp mills are curly, kinked, and crimped (Nugroho, 2012). Fibre straightening plays a significant role in paper properties. The stretching of fibres enhances the load-carrying ability and the stress distribution in the fibre network. In addition, it predominantly improves the elastic modulus and the tensile strength of a paper (Heymer, 2009).

The last effect of refining is the generation of fines, which is the result of the cutting action caused by the compression and shear forces. Fines are defined as the fraction of the pulp fibres that have a length of under 0.20 mm (Axelsson, 2009). There are two types of fines that exist in pulp: primary and secondary fines. Primary fines are present in unrefined pulp and originate from the ray and parenchyma cells present in the tree's cellular structure, whereas secondary fines are produced during refining and are a result of external fibrillation and/or fibre

shortening (Mandlez *et al.*, 2020; Pokhrel, 2010). Fines have a positive effect on pulp strength because they have a high surface area and thus improve fibre bonding (Mandlez *et al.*, 2020).

A negative consequence of fines formation is the significant reduction in the freeness of pulp. Freeness is a measurement of the quantity of water in a pulp suspension that can flow through a mesh screen. Since the de-watering of pulp is dependent on fibrillation and fines formation, freeness is used to measure the effectiveness and degree of refining (Manorma, 2015).

The two main methods used to measure freeness are Canadian Standard Freeness (CSF) and Schopper-Riegler freeness ($^{\circ}\text{SR}$) (Ek *et al.*, 2009a; Sixta, 2006). Both methods use a similar type of equipment set up to measure the drainage rate of water in a pulp suspension (Figure 2-6). To perform a freeness test, the pulp suspension is poured into the cylindrical chamber and allowed to flow through the mesh screen and the funnel. The water is drained from the side and the bottom orifices, and the amount obtained from the side orifice is the freeness value (Gharehkhania *et al.*, 2015). The differences between the CSF and $^{\circ}\text{SR}$ tests include the consistency required for the tests (the CSF method uses a consistency of 3 g/L while the $^{\circ}\text{SR}$ method uses a consistency of 2 g/L) and the use of a wire screen in the $^{\circ}\text{SR}$ apparatus instead of the calibrated screen plate used in the CSF apparatus. In addition, the methods are inversely proportional to one another, where a low CSF indicates a high $^{\circ}\text{SR}$ as the degree of refining increases (Sixta, 2006).

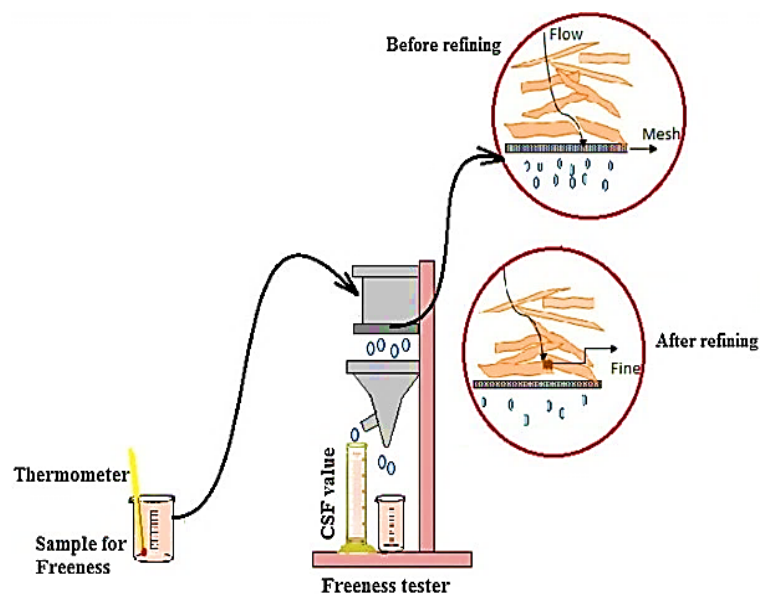


Figure 2-6: Experimental setup used to measure the freeness of pulp (Gharehkhania *et al.*, 2015)

2.2.4 Variables that affect the refining process

There are numerous factors that influence the refining performance, hence the refining effects. These factors may be divided into three main categories, namely, raw materials, equipment characteristics, and process variables, as shown in Table 2-1. The first factor raw materials include the types of wood species *i.e.*, hardwood or softwood, and whether the wood species underwent bleaching or not. Secondly, equipment characteristics include the type of refiner used and the material used to construct the refiner. Lastly, process variables include the temperature, pH, energy, and enzyme treatment used during refining (Bajpai, 2005; Rampersadh, 2005; Steel, 2010)

Table 2-1: Three factors that affect the refining process: raw materials, equipment characteristics and the process variables.

Raw materials	Equipment characteristics	Process variables
Wood species	Depth of grooves	Applied energy
Chemical composition	Bar width and shape	pH
Pulping method	Bar angles	Temperature
Fibre coarseness	Wear patterns	Consistency
Bleaching treatment	Area of bars and grooves	Pre-treatments
Fibre length distribution	Speed of rotation	Additives
Earlywood/latewood ratio	Material of construction	Production rate

2.2.4.1 Effect of raw materials

Depending on the raw material, pulps have different fibre morphology characteristics, and the nature of the pulp significantly influences the refining requirements, thus the refining outcome (Bajpai, 2005; Biermann, 1996). There are numerous variables that contribute to the heterogeneity of wood pulps, which include environmental conditions and genetic factors. The environmental conditions are determined by the climate, the altitude of the plantations, and the condition of the soil, to name a few. Environmental conditions are known to affect the tree species; thus the pulp and paper properties can be assumed to be equally impacted (Rampersadh, 2005).

Pulp fibres that have a high lignin content, such as unbleached pulps, disrupt the swelling process during internal fibrillation. Pulps with a high hemicellulose content are easier to refine because hemicellulose has a high affinity for water, which increases swelling and flexibility of the fibre, therefore improving internal fibrillation. Some pulps are easier to refine depending on the cooking process they have undergone. Sulphite pulps have fewer energy requirements compared to kraft pulps, while soda pulps are the easiest to refine (Bajpai, 2005; Smook, 1992).

2.2.4.2 Effect of equipment characteristics

There are two common types of refiners used in the industry, namely conical and disc refiners (Figure 2-7) (Przybysz Buzala *et al.*, 2016; Steel, 2010). Conical refiners are known for their cutting effect and are more appropriate when plates with narrow bars are used and result in better fibrillation (Lumiainen, 1998). Disc refiners are divided into three categories, namely, single-disc, double-disc, and twin-disc, and are used based on the conditions of the process (Elahimehr, 2014; Sixta, 2006).

Single-disc refiners contain a single rotating disc and are mostly used in high consistency refining. Single-disc refiners were one of the first refiners to be installed and are mostly used in mechanical pulping. In addition, they are suitable for small production and reduced capital costs (Bajpai, 2005; Elahimehr, 2014). Double-disc refiners are the most common and contain two counter-rotating discs. These refiners require less labour, less area and are more efficient compared to the other types of refiners (Anand *et al.*, 2016; Sixta, 2006). Lastly, twin-disc refiners contain three discs, one rotating disc at the centre and two stationary outer discs (Sixta, 2006). Twin-discs are appropriate for low refining energy and high production since they have a large surface area. However, twin-disc refiners are known to have some operational problems (Elahimehr, 2014). Disc refiners are preferred over conical refiners in the industry since they function at higher consistencies, resulting in improved fibrillation. In addition, disc refiners offer minimal no-load power, they are more compact and simpler to maintain (Biermann, 1996).

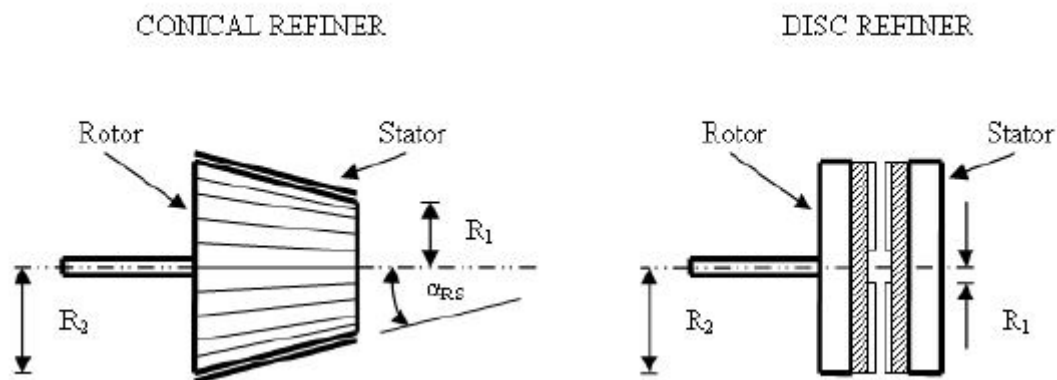


Figure 2-7: A schematic diagram showing the configuration of a conical and disc refiners. Where R_1 and R_2 represent the inner and outer radii of the refining zone, respectively and α_{RS} indicates the angle between the rotor and stator (Heymer *et al.*, 2011)

Conical and disc refiners both contain refiner plates (stator and rotor), which are fitted with metal bars and grooves. The size and shape of the bars, the area, angle, and depth of the

grooves (Figure 2-8), and the material of construction are all essential characteristics of plate design that affect the refining outcome. The plate design controls the strength development of the pulp. Plates with narrow bars offer low-intensity refining compared to plates with broader bars, resulting in less fibre cutting and more strength development. The angles on plates determine the fibre cutting effect, where a higher angle allows for a high consistency (less fibre cutting) refining process, while low angles promote fibre cutting (Rampersadh, 2005; Smook, 1992; Steel, 2010).

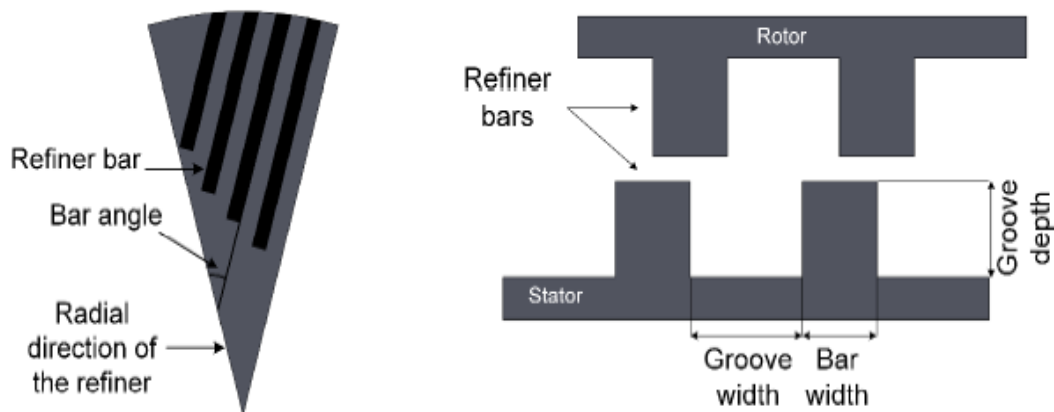


Figure 2-8: A schematic diagram illustrating refiner plates segments, namely, bar angle (left), bar width, groove width and groove depth (right) (Harirforoush, 2018)

2.2.4.3 Effect of process variables

Process variables such as refining energy, refining intensity, pulp consistency, pH, temperature, pressure, flow rate, additives, and pre-treatments also influence the refining process (Bajpai, 2005; Lumiainen, 1998). High pH levels (greater than 7) facilitate the refining process. The high alkalinity enables the fibres to absorb more water, thus improving swelling. This fundamentally increases the sheet strength properties and the sheet bulk. Refining in acidic conditions usually results in more fibre cutting and the generation of fines (Rampersadh, 2005).

Pre-treatment before refining facilitates the refining of pulp fibres by weakening the fibre cell wall, thereby enabling savings on refining and drying energy as well as enhancing paper properties (Somboon *et al.*, 2007). In general, high consistency (lower moisture content) pulp provides better refining since there is more fibre-to-fibre contact, which results in less fibre cutting, compared to low consistency pulp, which has more fibre-to-metal contact. High consistency refining also increases the homogeneity of refining. Additionally, pulps refined at

high consistency were observed to have more elastic and more internal bonded area, indicating greater fibre collapse (Rampersadh, 2005; Smook, 1992; Steel, 2010).

Other process variables that determine the refining outcome include specific refining energy and refining intensity. The energy applied to the fibres is dependent on the bar's sharpness, the roughness of the bar surface, and the width of the grooves and bars (Steel, 2010). The refining intensity increases as specific refining energy increases. However, it is also dependent on the pulp resident time and segment design (Nelsson, 2011). As stated previously (Section 2.2.1), fibres are developed in three phases (edge-to-edge, edge-to-surface, and surface-to-surface phases) during refining. The energy split between the phases depends on the plate design, while the refining outcome itself is dependent on the energy distribution between phases. If majority of the energy is consumed in the edge-to-edge phase, more fibre cutting action will occur. However, if majority of the energy is used in the last two phases, more fibre fibrillation will occur (Smook, 1992).

2.2.5 The effect of refining on handsheet properties

Refining induces structural changes in the fibres, which in turn affect the handsheet properties (Pokhrel, 2010; Popa, 2013). Handsheets are circular paper sheets made by a laboratory handsheet former and are used to test the strength, structural, and optical properties of the sheet (Biermann, 1996). The testing of handsheet properties is essential to distinguish the pulp and evaluate it in comparison with other pulps. In pulp mills, handsheets are used for quality control and to deliver a certain product quality to a customer. The testing of handsheet properties also provides a tool to enhance existing products and develop new products.

Numerous handsheet tests are available; however, the standard tests set up by the International Standardisation Organisation (ISO) are officially recognised (Ek *et al.*, 2009a). Handsheet properties can be divided into three main categories, namely, strength properties, structural properties, and optical properties (Ek *et al.*, 2009a; Nugroho, 2012). The following section will describe these handsheet properties and how they are affected by refining.

2.2.5.1 Handsheet strength properties

The correct strength properties of a particular pulp are an important requirement for many paper grades. This is because the pulp fibres must be strong enough to withstand any force encountered during the papermaking process, such as pressing and drying on the paper machine or the printing process afterwards. Several methods exist to determine the strength properties of a particular pulp. These methods use forces such as pull, tear, compression, burst, and even deformation of the sheet. These strength forces are tested using handsheets

with a specific grammage, typically 60 g/m², depending on the desired paper product (Ek *et al.*, 2009a). The properties of strength typically tested include tensile strength, tear strength, and burst strength.

Tensile strength is one of the fundamental strength properties tested on handsheets in the pulp and paper sector. Papers will be exposed to tensile forces throughout both the production and product application processes (Ek *et al.*, 2009a). The tensile strength property indicates the strength that a paper can endure when pulled in opposite directions without breaking, and is expressed in N/m. Tensile strength is divided by its basis weight (g/m²) to report it as a tensile index (Nm/g) (Elahimehr, 2014).

Tensile strength is usually tested by securing a strip of the handsheet between two clamps and pulling the paper apart. During this testing process, the paper strip is stretched until it can no longer endure the force and breaks. When the tensile force is applied, the inter-fibre bonds and the fibre itself stretch. When the paper strip breaks, some fibres are fractured while others are pulled out of the fibre network (Karlsson, 2010). Tensile strength is dependent on the inter-fibre bonds, the strength of the fibres, and the conditions used to make the handsheet (Fisevora *et al.*, 2009; Welch, 1999).

Even though refining may decrease the length and strength of the fibres due to the cutting effect, the dominating effect on tensile strength is the inter-fibre bonding (Budhram, 2005; Karlsson, 2010). Tensile strength will increase as the bonding strength of fibres increases (Welch, 1999). Thus, tensile strength will keep increasing as the refining energy is increased until a plateau is reached. Pulps are analysed based on the amount of refining energy they need to achieve a particular tensile strength. The lower the amount of refining energy that a pulp needs to reach the desired tensile strength, the more attractive a pulp is (Ek *et al.*, 2009a). Tensile strength has been shown to have a direct correlation to burst strength (Budhram, 2005).

Burst strength is defined as the amount of hydrostatic pressure required to break a piece of paper and can be expressed in psi or kPa. This pressure can be divided by the basis weight of the handsheet to report the burst strength as a burst index (kPa.m²/g) (Biermann, 1996). Burst strength is an essential property to measure for wrapping papers and boxboards, where the paper is exposed to the same stress that is exerted in the burst strength test (Budhram, 2005).

Tear strength is defined as the energy required to increase an initial cut in a handsheet and is expressed in mN (Elahimehr, 2014). Tear strength is an important property since it provides

information about the paper's capability to endure cracks. Excellent tear strength is essential for various paper products as it improves the runnability of the paper machine, the toughness of packaging papers, as well as the quality of newsprint papers (Ek *et al.*, 2009a; Steel, 2010).

In comparison to tensile strength, tear strength is more difficult to measure because it is dependent on many variables such as fibre length, fibre strength, degree of inter-fibre bonding, cross-section properties, and degree of orientation in the handsheet (Steel, 2010). There are different modes used to measure tearing (Figure 2-9), but the most common is the Elmendorf tear resistance tester, which uses tearing mode III (mode III was the method used in this study) (Ek *et al.*, 2009a). In this mode, a tear is made in the paper strip. A swinging pendulum completes the tearing of the paper strip, and the tear force needed to further tear the paper is measured (Karlsson, 2010). The tear index is calculated by dividing the tear strength by the basis weight (g/m^2) of a handsheet and is expressed in mNm^2/g (Elahimehr, 2014). In general, the tear strength of paper will increase with a low degree of refining. However, as the refining energy increases, the tear strength will decrease rapidly due to the decrease in the individual fibre length and strength (Biermann, 1996).

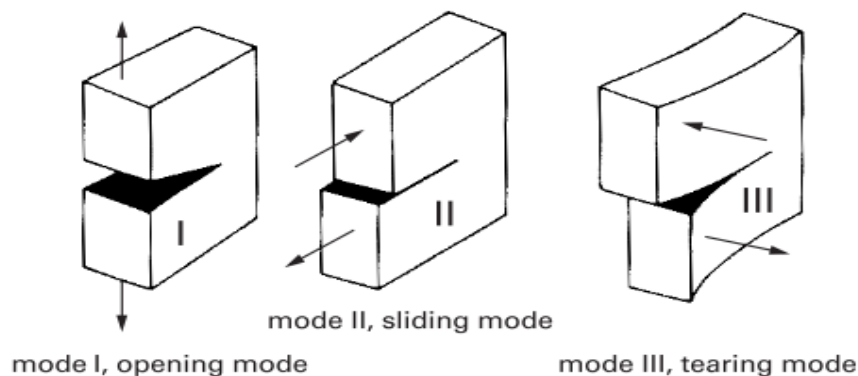


Figure 2-9: Different modes used to measure the tearing force (Ek *et al.*, 2009a)

Unbleached pulps are used in packaging materials (paperboard and cardboard) and the production of corrugated media (fluting) for paperboard. For packaging material, compression strength is crucial because boxes and cartons are frequently stacked on top of one another, resulting in compressional forces. As a result, boxes must be rigid and not crumble during storage and transportation (Ek *et al.*, 2009a; Šarčević *et al.*, 2016).

Furthermore, the compression strength of fluting papers is the most essential attribute because the material passes through multiple rollers and belts during the corrugated board manufacturing process, which compresses the material to varying degrees. This also occurs

when corrugated board sheets are scored, die-cut, and printed (Füst & Gerards, 2016). All the aforementioned factors lead to the importance of testing for compression strength in handsheets of unbleached pulp.

Two types of compression strength tests are typically performed, namely the Corrugating Medium Test (CMT) and the Short-span Compressive Test (SCT). CMT, expressed in N, is a measure of the resistance to the flute being crushed after it has been formed by the corrugator (Bocianowski *et al.*, 2012). This method entails hot corrugating a strip of the handsheet in a laboratory fluter at $177 \pm 8^\circ\text{C}$. The corrugated strip is inserted into a corrugated rack, covered with a comb to ensure the correct geometry, and then affixed with a self-adhesive, pressure-sensitive tape (Figure 2-10) (Füst & Gerards, 2016).

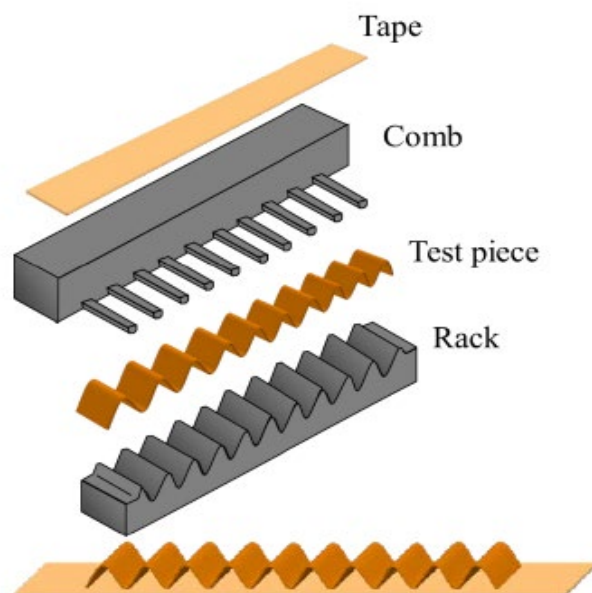


Figure 2-10: Corrugating Medium Test preparation accessories (tape, comb, rack) (Füst & Gerards, 2016)

Following the careful removal of the specimen from the comb-rack accessories, the fluted test strip can be tested in the laboratory crusher after 30 min of conditioning at 23°C and 50% relative humidity (Selebalo, 2019). The fluted strip goes through various degrees of buckling and crushing during compression. The maximum force preceding total collapse is the CMT value (ISO, 2011a).

The SCT (kN/m) is widely used to measure the edgewise compression strength of a paperboard. As shown in Figure 2-11, a handsheet test strip with dimensions of $15 \times 60 \text{ mm}^2$ (width $\times$ length) is compressed in the length direction by two clamps, 0.7 mm apart. The

clamps are moved using a force-controlled program, and the force is recorded. As a result, buckling is avoided, and the compressive properties and strength of the paper can be assessed (Brandberg & Kulachenko, 2020).

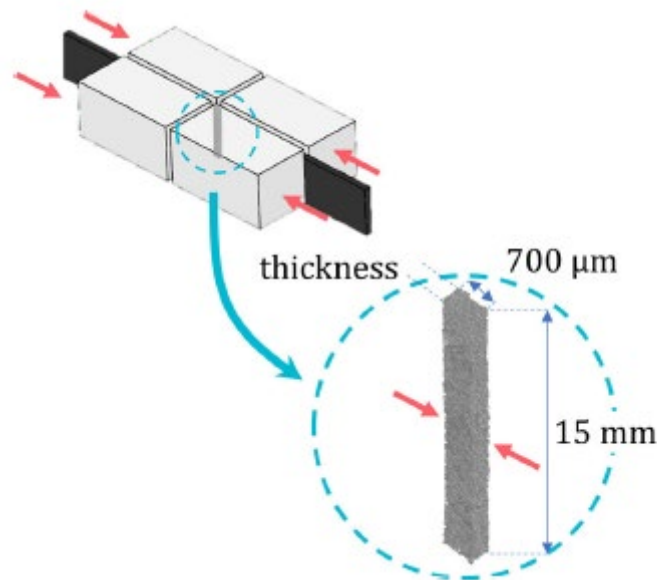


Figure 2-11: Short-span Compressive Test principle (Brandberg & Kulachenko, 2020)

Treatments that enhance the surface bonding area and fibre compressive strength, such as refining, improve the SCT and CMT. Refining straightens fibres, which improves not only the stress distribution under compressive strength but also the axial compressive strength of the fibre (Ju *et al.*, 2005). Both tests are commonly tested at a higher grammage (120 g/m² in this study) because, according to Ju *et al.* (2005), the compressive strength increases with sheet grammage to a maximum of 120 g/m² and then sharply decreases at grammages higher than that.

2.2.5.2 Handsheet structural properties

Bulk, density, and porosity are some of the primary structural properties that are used to evaluate the quality of a handsheet to evaluate refining. Sheet density (kg/m³) is defined as the basis weight of a handsheet, divided by its thickness. Bulk (m³/kg) is defined as the inverse of density (Elahimehr, 2014). These two structural properties are essential to paper manufacturers since, they affect other handsheet properties. In general, a high density (low bulk) demonstrates good fibre bonding and conformability, a smooth surface, low opacity, and is good for printing papers. Bulky papers are appealing to papermakers for specific purposes because they are more adsorbent and opaquer. The sheet density increases as the degree of refining increases. This is because refined fibres are more flexible and bond easily to other

fibres. As a result, the sheet has a larger bonded area and more densely packed fibres. (Ek *et al.*, 2009a; Steel, 2010).

The porosity of paper is defined as the voids in the sheet not occupied by fibres, also known as the volume of air in the sheet (Ek *et al.*, 2009a). Porosity, commonly measured in mL/min, is tested by measuring the total vertical and horizontal voids in the fibre network. The paper consists of approximately 70% air and is highly porous. Consequently, porosity is an essential factor in paper for printing, labelling, bags, laminating, and filtering. There are numerous methods used to measure the porosity of a paper, and they all incorporate the air resistance method, which indirectly indicates the level of refining, type and amount of fillers, and compaction of fibres (Smook, 1992; Steel, 2010). Refining and porosity have an inversely proportional relationship, due to the increase in inter-fibre bonding as the degree of refining increases, which results in a decrease in the number of voids in a handsheet (Biermann, 1996).

2.2.5.3 Handsheet optical properties

Optical properties typically measured include brightness, opacity, light absorption, as well as the light scattering coefficient. These properties allow the papermaker to quantify the readability and printability of a paper (Ek *et al.*, 2009a). Optical properties are reduced as the level of refining increases (Loijas, 2010). Opacity was the optical property evaluated during this study. Opacity (%) is defined as a measure of how much light is prevented from passing through a paper sheet. An ideal opaque paper has 100% opacity and is impermeable to the movement of all visible light. Opacity is essential in printing and book papers since a high opacity in printing enables one to read the front side of a page without seeing the printing on the backside of a page. The opacity of a paper is affected by the thickness, degree of bleaching, and amount of filler (Biermann, 1996; Elahimehr, 2014).

2.3 Hardwoods and softwoods pulp fibres

The type of wood used to manufacture paper and packaging products can be categorised into two primary groups, namely, angiosperms (hardwoods) and gymnosperms (softwoods) (Biermann, 1996; Ek *et al.*, 2009b; Roberts, 1996). The primary difference between hardwoods (HW) and softwoods (SW) is in their wood structure and the type of wood cells that they produce (Nel, 2013; Sixta, 2006).

2.3.1 Differences between hardwood and softwood fibre morphology

Hardwoods have a complex wood anatomy. The primary cell types present in hardwoods are vessel elements, libriform fibres, ray cells, fibre tracheids, and parenchyma cells (Biermann,

1996). The function of the vessel elements is to transmit fluid across the xylem and construct end-to-end connections through structures called perforation plates. Libriform fibres occupy up to 75% of the wood volume and act as a support for the tree. The hardwood fibre tracheids' primary function is water transportation (Antonides, 2000; Sixta, 2006).

In contrast to hardwoods, softwoods have a simple wood anatomy consisting mainly of two types of cells, namely, longitudinal tracheids, which occupy 90 to 95% of the wood volume, and parenchyma cells, which make up the rest of the wood volume (Biermann, 1996; Pokhrel, 2010). The function of the longitudinal tracheids is to transport water and act as structural support. The parenchyma cells are beneficial since they store various extractives, such as starch, oils, fats, polyphenols, and inorganics such as potassium (K), manganese (Mn), magnesium (Mg), silicon (Si) and calcium (Ca) (Antonides, 2000; Nel, 2013).

Another difference besides the cell types present in HW and SW pulps is the difference in their fibre lengths, which has an impact on the refining process and the final product's properties (Pokhrel, 2010; Roberts, 1996). Hardwoods are known as short and thin fibres with a fibre length range of 0.7 to 2 mm, whereas softwood fibres are longer and thicker with a fibre length range of 3.1 to 3.4 mm. Some softwood fibres, such as *Sequoia*, can grow up to 7 mm long (Ek *et al.*, 2009b). The benefit of the smaller hardwood fibres is that they easily provide uniform sheet formation, thus they are typically employed when the papermaker requires a paper that has a smooth printing surface and high opacity. Softwoods are mostly utilised when strength properties are essential for a product (Popa, 2013).

In the pulp and paper industry, a blend of both hardwood and softwood pulp is typically used since many paper products require both the smooth printing surface provided by HW and the strength offered by SW pulp fibres (Ek *et al.*, 2009b; Roberts, 1996). The difference between HW and SW pulp fibres is also attributed to the different environmental and climatic conditions that the trees grow in (Pokhrel, 2010). Although there are numerous differences in the structure of HW and SW pulp fibres, the fact remains that both must first undergo mechanical treatment (refining) to change their chemical and physical properties, in order to be able to produce the paper product desired by the papermaker (Rio, 2012).

2.3.2 Refining of hardwood and softwood pulps

Softwood and hardwood pulp fibres react differently to the refining process and therefore, require different refining conditions due to their fibre morphological differences (Bajpai, 2005; Finn, 1991). The SW and HW pulp fibres require different refining energies, refining intensities, and plate designs. Due to the long and thick fibres and the high refining resistance of SW, these pulps require high refining energy and larger bar widths. In addition, SW requires high

refining intensity. However, too high an intensity greatly decreases the fibre length and affects the freeness obtained.

In contrast, hardwoods require low refining energy with low intensity and small bar widths due to their thin fibres. The low refining intensity requirement for hardwoods is sufficient to develop the pulp fibres, although too low a refining intensity results in an increase in the total energy consumed. A refining intensity that is too high in hardwoods damages the fibre flocs on the bar edges, and the fibres return to the grooves without being refined. Additionally, the fibre shortening effect is increased. (Bajpai, 2005; Lumiainen, 1998; Nugroho, 2012).

Irrespective of the pulp fibres used during refining, the refining process is a high energy-consuming process. In order to counteract this, enzymes as an environmentally friendly alternative have been introduced to the pulp and paper industry, not only to enable energy savings but to improve fibre development (Rio, 2012).

2.4 Enzymes

Enzymes are complex protein molecules that accelerate the chemical reaction in living organisms (Bhatia, 2018). As bio-catalysts, enzymes are required in very small quantities and speed up the chemical reactions they are involved in, without being consumed (Robinson, 2015). The activity of an enzyme depends on the reaction time, presence of inhibitors, activation energy, substrate concentration and type, as well as chemical and physical environment, *i.e.*, temperature, pH. (Singh & Bhardwaj, 2010).

Enzymes are extremely specific both in the reactions they catalyse and in the choice of substrates (Ek *et al.*, 2009b). An enzyme (E) has an active site, where the substrate (S) binds to form an enzyme-substrate complex (ES). At the active site, the reaction continues to form a particular product (P), which is still attached to the enzyme-product complex (EP). Thereafter, the product is released, and the enzyme can participate in another catalysis reaction as shown in Figure 2-12(Singh & Bhardwaj, 2010).

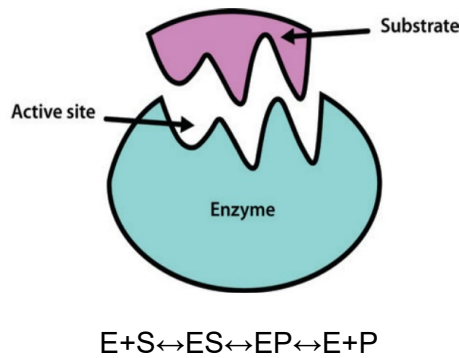


Figure 2-12: A illustration of an enzyme (E) with an active site, where the substrate (S) binds to form an enzyme-substrate complex (ES). At the active site, the reaction continues to form a particular product (P), which is still attached to the enzyme-product complex (EP). (Robinson, 2015).

Enzymes are used globally in various industries such as textiles, food, brewery and wine, pulp and paper, and agriculture (Bhatia, 2018). In the pulp and paper industry, enzymes are beneficial in reducing the use of harsh chemicals and high energy consumption. However, caution must be applied when working with enzymes. As they are proteins, enzymes can easily be denatured and deactivated (Zollner-Croll & Badakh, 2016).

Within the pulp and paper sector, various enzymes are commercially available for numerous applications. The four most commonly used enzymes include cellulases, lipases, laccases, and xylanases (Demuner *et al.*, 2011). Cellulases are used in fibre modification, lipases for pitch control, xylanases for bleach boosting, and laccases for delignification enhancement (Pathak *et al.*, 2016). Other enzymes used in the industry include proteases used for slime control, esterases used for stickies and pitch control, and amylases used for starch modification (Tripathi *et al.*, 2008).

This study's primary focus was cellulase enzymes. Cellulases are preferred due to their potential applications across various industries. Moreover, according to recent global cellulase analysis reports, the demand for cellulase is growing exponentially. Thus, more studies on these enzymes are needed to produce the scientific knowledge needed to meet the increasing demand for cellulases (Jayasekara & Ratnayake, 2019).

2.4.1 Overview of cellulases

Cellulose is a linear β -1-4-glycosidic-bonded, homopolymer of D-glucopyranose units (Rio, 2012). Cellulose molecules have a strong propensity to aggregate due to intra- and intermolecular hydrogen bonding. This aggregation of cellulose molecules results in the formation of ribbon-like structures known as microfibrils, which form either highly ordered (crystalline) or less ordered (amorphous) regions in the molecule. Microfibrils are then aggregated into fibrils, which are then aggregated into cellulose fibres. Hydrogen bonding generates a compact fibre structure that gives cellulose its material qualities, such as high tensile strength and insolubility in most solvents (Rio, 2012; Sjöström & Alen, 1999).

In wood, cellulose is the most abundant compound. However, even though it accounts for 40 to 60% of the plant's cell wall, it cannot be degraded easily (Bayer *et al.*, 1998; Liu *et al.*, 2011; Mosier *et al.*, 1999). Fungi, bacteria, plants, protists, other microorganisms, and a variety of invertebrates use cellulose as their primary carbon source. Therefore, these organisms have an enzymatic system that enables them to readily degrade cellulose (Singh *et al.*, 2016). These organisms have also developed a multiplex group of enzymes that can degrade cellulose to liberate the monomeric sugars (Bayer *et al.*, 1998).

The enzymes that catalyse the hydrolysis of cellulose are termed cellulases (Mosier *et al.*, 1999). Cellulases are a group of enzymes that act synergistically to cleave the β -1-4-glycosidic linkages of the cellulose chain to its monomeric sugars (Ahmed & Bibi, 2018). Cellulases are produced by various fungi and bacteria such as *Trichoderma reesei*, *Phanaerochate chrysosrium*, *Pencillium funiculosum*, *Aspergillus niger*, *Acidothermus cellulolyticus*, *Clostridium*, and *Cellulomonas* (Singh & Bhardwaj, 2010; Singh *et al.*, 2016). Among these cellulase producers, *T. reesei* is the most studied due to its ability to produce a significant amount of sugars from biomass through the hydrolysis process, resulting in a high cellulose-degrading activity (Jeoh *et al.*, 2008; Singh *et al.*, 2016).

There are three types of cellulases, namely, endoglucanase (EG), exoglucanase/cellobiohydrolase (CBH), and β -glucosidase (Juturu & Wu, 2014; Menendez *et al.*, 2015; Zhang & Zhang, 2013). The combination of these three primary forms of cellulases ensures complete hydrolysis of the cellulose chain (Figure 2-13) (Torres *et al.*, 2012).

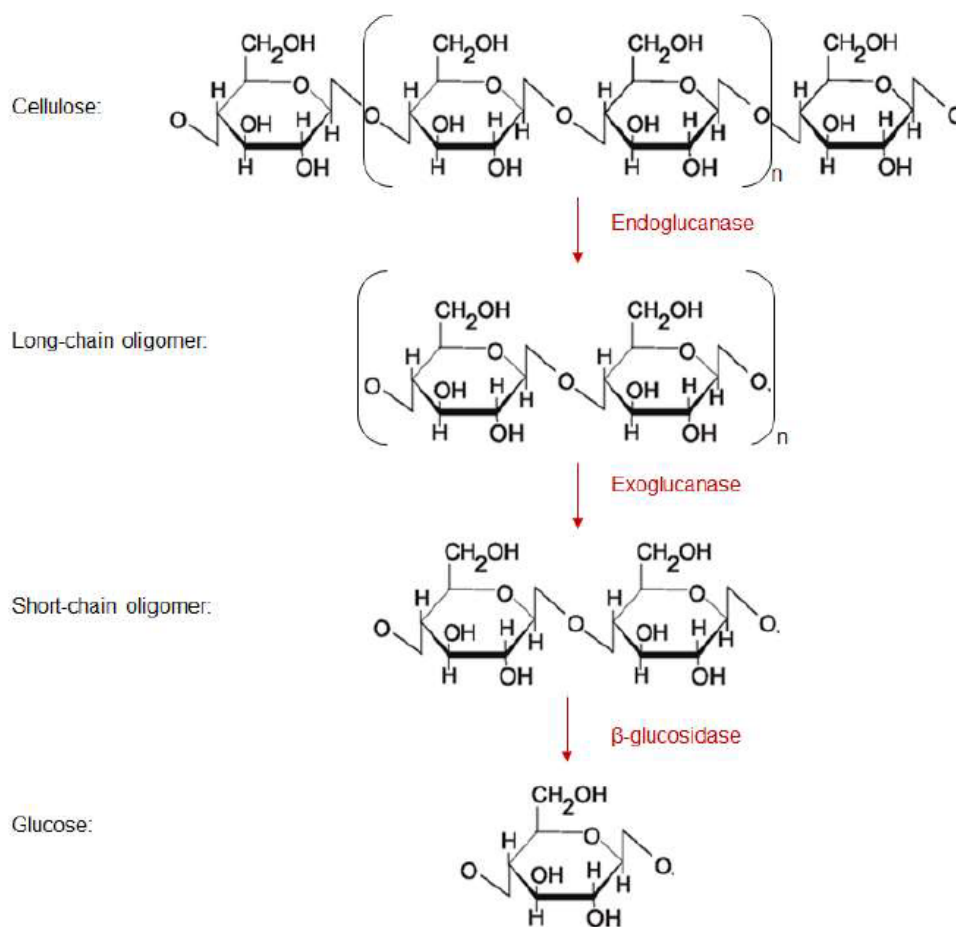


Figure 2-13: Hydrolytic process of cellulases on the cellulose chain Endoglucanase cleaves the amorphous region of the cellulose chain, reducing the chain length to produce cellobiose. Exoglucanase/cellobiohydrolase cleaves the crystalline region of cellulose from reducing and non-reducing ends, to produce cellobiose. β -Glucosidase cleaves the cellobiose produced by endoglucanase and cellobiohydrolase to produce the end product glucose (Juturu & Wu, 2014)

Firstly, endoglucanases randomly cleave the amorphous region of the cellulose chain, causing a substantial reduction in the length of the chain and a gradual increment in the number of free reducing groups (Torres *et al.*, 2012). Since EGs cleave the amorphous region, their activities are measured using soluble cellulose substrates such as carboxymethyl cellulose (CMC) or cellulose substrates substituted with dyes (chromogenic CMC) (Zhai, 2017).

The *T. reesei* cellulolytic system has at least five EGs, namely, EG I, EG II, EG III, EG IV, and EG V. Among these endoglucanases, EG I and EG II, also referred to as Cel7B and CEL5A, respectively, are predominant, accounting for more than 80% of the EG activities. The EG I enzyme is the primary endoglucanase responsible for 6 to 10% of the total protein produced by *T. reesei*, whereas EG II accounts for 1 to 10%. Even though EG II accounts for a smaller

percentage of the total protein produced, it has been shown that it decreases the degree of polymerisation of cellulose chains more efficiently compared to EG I (Mboowa, 2019).

Secondly, CBHs cleave the reducing and non-reducing ends of the cellulose chain to produce cellobiose, glucose, and sometimes cellotriose. Cellobiohydrolases are substrate-specific and their activity is directly proportional to the length of the cellulose polymer chain (Mboowa, 2019). The activity of CBHs is measured using crystalline cellulose as a substrate, e.g. filter paper, avicel, or cotton (Mansfield, 1997).

Cellobiohydrolases can be subdivided into two main categories, namely, CBH I and CBH II, also referred to as Cel7A and Cel6A, respectively, according to their amino acid sequence similarities (Annamalai *et al.*, 2016; Zhai, 2017). Initial reports suggested that both CBH I and CBH II act on the non-reducing ends of the cellulose chain (Teeri *et al.*, 1998). However, recent studies have shown that CBH I acts on the reducing end while CBH II acts on the non-reducing end of the cellulose chain during enzymatic hydrolysis (Mboowa, 2019). Cellobiohydrolase I and CBH II are processive enzymes that cleave cellulose chains consecutively without detaching from the cellulose chain. In comparison to other cellulases, CBH I is the most excreted enzyme and accounts for 60% of the total proteins produced by *T. reesei*, while CBH II accounts for 20% (Rio, 2012).

It has been shown that excess glucose and cellobiose are inhibitory to both CBHs and EGs (Kumar *et al.*, 2011). Consequently, the only way to overcome this inhibition is to add β -glucosidase to the cellulase cocktail. Thus, lastly, β -glucosidases cleave the cellobiose produced by EG and CBH to produce the end-product glucose. The removal of the accumulated cellobiose is a significant step in enzymatic hydrolysis as it helps to minimise end-product inhibition (Zhai, 2017). In addition to hydrolysing cellobiose to glucose, β -glucosidase is essential in trans-glycosylation, ensuring that glucose retains its β -conformation. β -glucosidase has a low substrate specificity, and its activity grows as the substrate concentration declines (Mboowa, 2019). In general, β -glucosidase is responsible for controlling the reaction's overall speed, which has a significant impact on the enzymatic degradation of cellulose (Alves-Prado *et al.*, 2011).

There are two types of catalytic mechanisms that hydrolyse the anomeric configuration of cellulose, known as the retention mechanism and the inversion mechanism (Barati & Amiri, 2015; Ek *et al.*, 2009b; Morana *et al.*, 2011). Cellulases cleave the glucosidic bonds through acid-base catalysis. The two catalytic residues of the enzyme, namely a general acid and a nucleophile/base, conduct the hydrolysis. The catalytic mechanisms are dependent on the spatial position of these two catalytic residues. In the inverting mechanism, after the single

nucleophilic displacement hydrolysis, cellulase inverts the structure of the anomeric C configuration. The retaining mechanism retains the same anomeric C configuration. The mechanism carries the glucosidic bond even after a double-displacement hydrolysis with two main glycosylation or deglycosylation stages (Jayasekara & Ratnayake, 2019; Morana *et al.*, 2011; Zhang & Zhang, 2013).

2.4.2 Application of cellulase in the pulp and paper industry

Cellulases have been used in numerous manufacturing applications for several decades, securing the third position among the annual sales of enzymes globally and predicted to overtake protease in the near future (Ahmed & Bibi, 2018). Cellulases are used for applications such as animal feed, food, pharmaceutical formulations, pulp and paper, textiles, agriculture, and detergent preparations (Menendez *et al.*, 2015; Singh *et al.*, 2016). In the pulp and paper industry, the focus is on how cellulases can enhance the final product's properties, whereas other industries have focused on how cellulases can be used to degrade cellulose (Mansfield, 1997). The application of cellulase in the pulp and paper sector started in the 1980s and is still growing, now also branching into areas such as the deinking process, refining (fibre modification), pulping, bleaching, dewatering improvement, and vessel picking reduction (Jayasekara & Ratnayake, 2019; Kuhad *et al.*, 2011; Singh *et al.*, 2016).

Cellulase alone or used in conjunction with xylanase is advantageous during the deinking of various forms of wastepaper. In most applications, the use of a cellulase and hemicellulase mixture has been proposed to release the ink from the fibre surface by partial hydrolysis of the carbohydrate molecule (Jayasekara & Ratnayake, 2019). Studies have shown that the use of cellulase during deinking results in an increase in process efficiency.

Out of the three cellulase types, EG was found to be more effective at removing ink from several waste papers compared to a cellulase cocktail (Pathak *et al.*, 2016). The primary benefits of enzymatic deinking include higher ISO brightness, improved bleachability, improved strength properties, shorter dewatering time, and reduced fine particles. In addition, enzymatic deinking using cellulase is beneficial in decreasing the use of alkali and prevents the paper from turning yellow (Bajpai, 2015; Kuhad *et al.*, 2011). Overall, enzymatic deinking has become attractive to papermakers compared to the traditional chemical deinking process because of its high process efficiency and minimal environmental concerns (Kumar & Rani, 2019).

The use of cellulase to enhance dewatering has been explored by several mills to improve the production rate (Singh *et al.*, 2016). Improved dewatering during sheet formation and at the press section reduces the time spent in the drying section and/or increases the paper

production capacity. A reduction in the time spent in the drying section decreases the energy consumption, thus minimising the investment cost (Pathak *et al.*, 2016).

Poor water drainage from pulp is due to the higher relative surface area of secondary fines compared to primary fines. Therefore, enzymes improve dewatering by indirectly removing the fines and microfibrils from the fibre surface (Kuhad *et al.*, 2011). In comparison to other enzymes, cellulases result in the largest drainage improvement.

Mixtures of cellulase and hemicellulase have also demonstrated an increase in freeness, while hemicellulase alone showed no effect. Having observed that cellulase was the main reason for improvements in freeness, the individual cellulases were then studied to evaluate their individual effects on water drainage. Endoglucanase showed better improvement compared to CBH (Kenealy & Jeffries, 2003; Mansfield, 1997; Pala *et al.*, 2009).

Cellulase enzymes have also been effective in reducing vessel picking in papers produced from hardwood pulps. Some researchers have shown that treating hardwood fibres with cellulase enzymes four hours before refining can help reduce picking in hardwood papers by 85%. Simultaneously, paper characteristics such as smoothness, tensile index, and drainage were improved by the treatment (Imran *et al.*, 2019; Mansfield, 1997).

Cellulases are also used in refining and pulping to save energy costs, improve fibre development, and enhance paper strength properties (Kuhad *et al.*, 2011). Although cellulase improves fibrillation, the challenge facing papermakers is to achieve the desired fibre fibrillation without damaging the fibres, since overdosing cellulase can negatively affect fibre strength properties (Mansfield, 1997). Fibres that are treated with cellulase are easily refined. However, there is a trade-off between the paper's characteristics and the refining energy. Therefore, the papermaker can decide whether to alter the paper characteristics and maintain a constant refining energy level or to reduce the refining energy and maintain the paper characteristics (Loosvelt, 2009).

Cellulases can be added pre or post refining, as depicted in Figure 2-14. Adding the enzyme before refining is beneficial in reducing the refining energy consumption and the refining time, while adding the enzyme after refining can improve the freeness of the pulp. An enhancement in freeness decreases the drying time in the drying section, hence improving the runnability and speed of the paper machine.

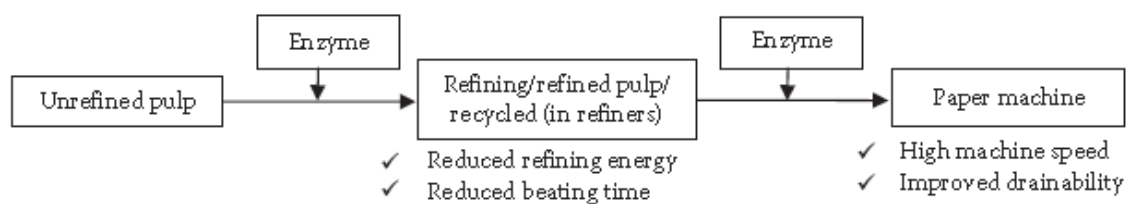


Figure 2-14: Schematic highlighting the benefits of enzymes pre and post refining. Pre-refining beneficial for reducing refining energy consumption. Post-refining beneficial for improving drainability and increasing machine speed. (Pathak *et al.*, 2016)

Process conditions such as temperature, pH, reaction, consistency, and enzyme dosage must be optimised before refining (Pathak *et al.*, 2016; Singh & Bhardwaj, 2010). Cellulase-assisted refining has been conducted and reported by numerous researchers (Bajpai *et al.*, 2006; Garcia *et al.*, 2002; Lecourt *et al.*, 2010b; Mohlin & Pettersson, 2002; Przybysz Buzala *et al.*, 2016; Znidarsic-Plazl *et al.*, 2009). Out of the three mono-components of cellulases, CBH I from *T. reesei* has been reported to be beneficial in reducing energy consumption due to its ability to selectively hydrolyse the most abundant part of cellulose, *i.e.*, crystalline cellulose. In addition, CBH I has been shown to improve fibre development (Pathak *et al.*, 2016).

2.5 Future prospect of enzymes in refining

Enzymes as industrial bio-catalysts provide several benefits over conventional methods, with particular reference to sustainability and process efficiency (Chapman *et al.*, 2018). However, the application of enzymes in the pulp and paper industry is still limited. The primary barriers to enzymatic process implementation are a lack of technical knowledge about enzymatic methods and traditional beliefs among producers, suppliers, and governments regarding the adaptation and acceptance of innovations. To overcome this challenge, it is essential to raise awareness of the potential benefits of enzyme technologies in the pulp and paper industry (Skals *et al.*, 2008).

Another constraint affecting the use of enzymes is the limited operating range of temperature, pH, and storage time associated with their application in industrial processes. Consequently, these operating conditions must be strictly regulated to preserve the optimal enzymatic activity. Furthermore, enhancements in the stability of thermostable and alkali-stable enzymes to withstand harsh industrial conditions are still required (Chapman *et al.*, 2018; Pathak *et al.*, 2016).

Presently, enzymes are only applied in specific mill applications in the pulp and paper industry. One of these applications is refining (Pathak *et al.*, 2016). Refining enzymes currently used include cellulase, xylanases, laccases, amylases, proteases, and pectinases (Manorma, 2015; Torres *et al.*, 2012). The use of these enzymes has been observed to lower energy costs while also providing other advantages such as increased paper machine speed, decreased steam requirements, improved paper strength properties, and improved paper formation (Bajpai, 2005; Singh & Bhardwaj, 2010).

Enzyme-assisted refining is cost-effective, leads to a reduction in the quantity of chemicals and energy consumed, is simple to incorporate into existing manufacturing processes, and does not disrupt daily operations. Enzymes are anticipated to be advantageous to mills that are constrained by refining capacity. Enzymatic refining should result in a decrease in maintenance costs or allow the mill to produce more paper without increasing its capacity.

Modern biotechnology tools are progressing at an incredible speed, resulting in the development of new, more efficient enzymes. Academic and industrial institutions are conducting the necessary research, and recently designed techniques are expected to substantially decrease the cost of enzyme production, while also improving the properties of these enzymes (Bajpai, 2005; Pathak *et al.*, 2016; Tripathi *et al.*, 2008). Overall, the use of eco-friendly refining enzymes in the refining process has the strong potential to ameliorate the economics of the paper manufacturing process and simultaneously reduce its detrimental environmental effects.

CHAPTER 3: MATERIALS AND METHODS

This chapter describes the materials and methods used in this study. The chapter starts with Section 3.1, which describes the enzymes and the pulps used in the study. Section 3.2 is divided into three parts and presents the experimental methods of the study, namely: CBH I characterisation (Phase 1), pilot refining bijective studies (Phase 2) and finally, optimisation of refining parameters (Phase 3).

3.1 Raw materials

3.1.1 Enzyme

A non-commercial enzyme cellobiohydrolase I (CBH I) (EC 3.2.1.176), was supplied by AB Enzymes (Darmstadt, Germany).

3.1.2 Pulps

The following pulps, supplied as flat sheets (bleached pulps) and noodle pulp (unbleached pulp), were used for the bijective study: These included Santa Fe pulp, which is a bleached hardwood kraft pulp (BHKP) made from eucalyptus wood, was supplied by CMPC (Chile). Södra Black pulp, which is a fully bleached softwood (FBSW) pulp made from juvenile spruce and pine trees, was supplied by Södra (Sweden). Lastly, unbleached softwood (UBSW) pulp was supplied by Sappi Ngodwana Mill (South Africa).

3.2 Methods

PHASE 1

3.2.1 CBH I characterisation

Cellobiohydrolase I activity was determined using a modified filter paper assay (Chu *et al.*, 2012). As a substrate, Whatman No.1 filter paper strips (6 x 1 cm) of approximately 50 mg were utilised. A 0.1 M Britton-Robinson buffer solution was prepared by taking 100 mL of stock solution and adjusting the pH to 5 with 0.5 M NaOH, whereafter the solution was made up to 200 mL in a Schott bottle and stored at 4 °C until use. The stock solution was composed of 12 mL of 0.2 M acetic acid, 12.4 mL of 0.2 M boric acid, and 19.6 mL of 0.2 M phosphoric acid in 1 L of ultrapure water.

To investigate the effect of temperature on the CBH I activity, enzyme assays were conducted between 25 and 85 °C, with an incremental increase of 10 °C between temperatures. The pH was adjusted to 5.0 for all seven assays. After the optimum temperature was determined, the CBH I activity was then analysed at a pH range of 2 to 9, with an incremental increase of 1.

For all assays conducted, a 1 mg/mL enzyme solution was prepared by diluting 50 μ L of CBH I in 50 mL of 0.1 M Britton-Robinson buffer. The enzyme solution and the substrate were incubated for 5 min, and thereafter the enzymatic hydrolysis was allowed to take place for 10 min. Afterwards, the reaction was stopped by incubating the samples in boiling water (100 °C) for 10 min. The hydrolysate was immediately cooled and centrifuged using an Eppendorf 5804R centrifuge (Germany) for 5 min at 4°C and 166.7 Hz. The supernatant was then used to quantify the monomeric and dimeric sugars (glucose and cellobiose) released during the assay. The enzymatic release of the reducing sugars is an indirect measure of enzyme activity.

The supernatant of each sample was stored at 4°C until a high-performance liquid chromatography (HPLC) analysis was conducted. Prior to HPLC analysis, the hydrolysates were diluted with ultrapure water (1:9). Together with prepared glucose and cellobiose standards (Sigma Aldrich, USA), the sugar content of all samples was measured by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC PAD) and post-column addition using a Dionex Ultimate 3000 system (Thermo Scientific). The separation of the sugars was achieved on a Dionex CarboPac™ PA1 Bio LC™, 50 x 4 mm (Thermo Scientific) guard column in combination with a Dionex CarboPac™ PA1 Bio LC™, 250 x 4 mm (Thermo Scientific) analytical column. Samples were kept in the auto-sampler trays at 5°C until injection. Samples were injected full loop (20 μ L) on the column (35°C) and sugars were separated with HPLC-grade water at a flow rate of 1.0 mL/min. The injection period for each sample was 70 min. Sodium hydroxide (NaOH, 132 mM) was added post-column at a flow rate of 1.0 mL/min prior to detection. Before and after each batch, a wash was performed to regenerate the column and remove impurities, as well as an equilibrium wash with ultrapure water mobile phase to equilibrate the system after injection.

The thermostability profile of CBH I was determined at the optimum temperature and pH conditions over a 3 h incubation period. A sample was withdrawn every 30 min and analysed for residual activity using the filter paper assay, described above. The reducing sugars released were also measured using HPLC analysis, as described above. The activity of the enzyme for all assays was afterwards calculated in International Units (IU), where 1 IU is the number of micromoles of cellobiose/glucose produced per second of the enzymatic hydrolysis. Each enzyme assay was replicated three times with appropriate controls and blanks, and the average was used for data analysis.

PHASE 2

3.2.2 Bijective studies on pilot refiner

The bijective studies of the selected pulps was carried out using a pilot-scale refiner instead of a laboratory-scale PFI mill to better simulate industrial mill conditions (Lecourt *et al.*, 2010b). A bijective diagram technique was used to carry out the refining. This is a technique for providing detailed graphical information on how the pulp and fibre properties would react under a variety of refining energies and specific edge loads (SEL) with different refiner plate designs.

The pilot-scale refining unit (Figure 3-1) comprises of a geyser, pump, sampling carousel, re-pulper with an impeller at the bottom, 12" single-disc refiner with a stator and rotor plates (Figure 3-2), a waste pulp tank, and an electrical switchboard.



Figure 3-1: Image of the pilot refiner unit at the Sappi Technology Centre. The pilot refining unit comprises of a geyser, pump, sampling carousel, re-pulper with an impeller at the bottom, 12" single-disc refiner with a stator and rotor plates, a waste pulp tank, and an electrical switchboard.

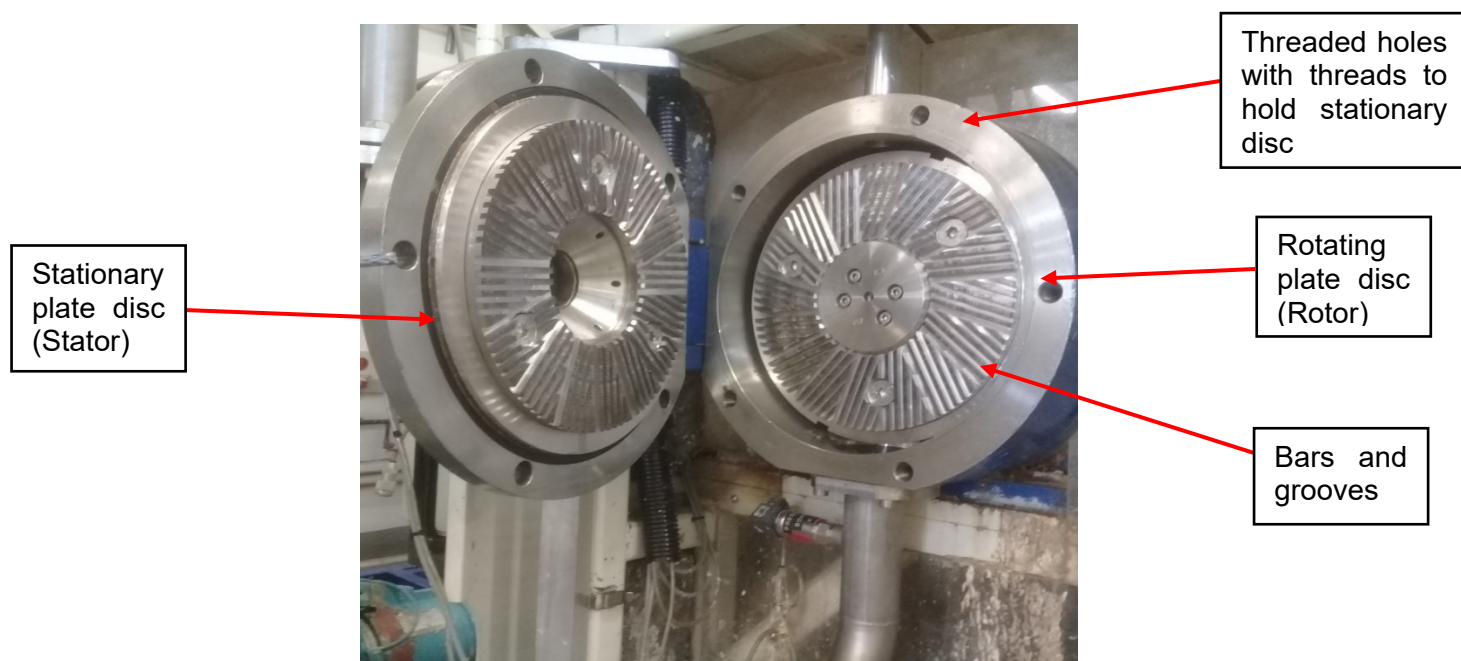


Figure 3-2: Rotor and stator plates of the 12-inch single-disc pilot refiner. The refiner plates have metal bars and grooves mounted on them. The bars and grooves carry the fibres through the refiner and transfer energy to the pulp fibres. The refiner has threaded holes to hold the stator refiner plate stationary.

Refining was carried out at a consistency of 3.5% in hydra-cycle mode, i.e., by circulating the pulp through the refining unit as indicated by the arrows in the process flow diagram in Figure 3-3. For each pulp, five refining runs were conducted with the addition of the enzyme, CBH I. Table 3-1 shows the refining conditions and plate designs used for each pulp. A control sample in the absence of the enzyme was conducted using the reference refining conditions. The various pulp samples (Section 3.1.2) were re-pulped for 30 min at 50 °C and 25 Hz. The enzyme CBH I was introduced at a dosing rate of 1 mL/kg, whereafter the pulp was further re-pulped for 30 min before refining commenced.

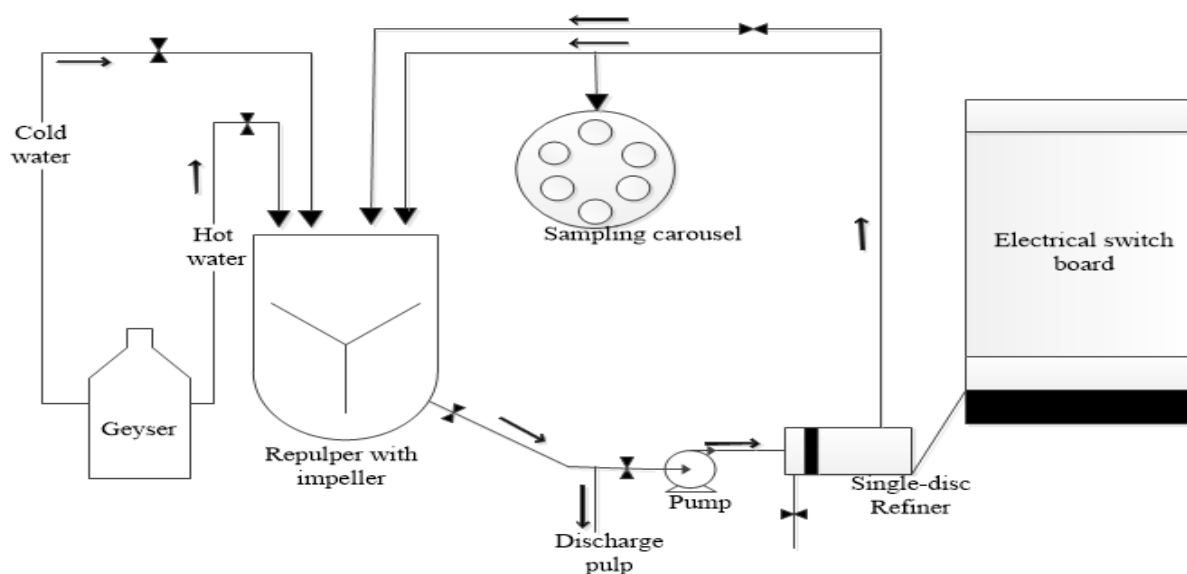


Figure 3-3: Simplified process flow diagram of the pilot refiner system. The refiner system comprises of a geyser, pump, sampling carousel, re-pulper with an impeller at the bottom, 12" single-disc refiner with a stator and rotor plates, a waste pulp tank and an electrical switchboard

Seven samples were collected per refining run. The first two samples were collected during re-pulping (at 5 and 30 min, respectively, at 0 kWh/t) while the remaining five samples were collected at increasing specific refining energies (SRE) specific to each pulp, as shown in Table 3-1. The SRE range for each pulp type was generated by a customised refining software program integrated with the pilot refiner system and supplied by Matech (France) and was based on the fibre morphology of each pulp.

Flow meters, flow control valves, temperature sensors, and a pressure controller, all connected to a programmable logic controller, were used to run the refining process automatically based on pre-defined set points. A customised refining software program maintained a stable control of all the process variables. The inlet pressure was maintained at 1.6 bar and the outlet water flow at 8 m³/h to ensure reproducibility during all refining runs.

Table 3-1: Refining process conditions and plate designs used during the pilot refining of each pulp. The refining process conditions namely, bar code and angle, SEL and specific refining energy were generated by a customised refining software program integrated with the pilot refiner system and supplied by Matech (France) and was based on the fibre morphology of each pulp.

Refiner runs	Bar code* (mm)	Bar angle** (°)	SEL (Ws/m)	Specific refining energy (kWh/t)					
				1	2	3	4	5	6
Bleached hardwood kraft pulp (BHKP)									
Reference (REF)	3/3/6	10	0.7	0	35.3	69.3	102.1	133.6	164
High intensity (SEL+)	3/3/6	10	1.0	0	47.2	92.8	136.7	178.9	219.4
Low intensity (SEL-)	3/3/6	10	0.4	0	33.9	67.0	99.3	130.7	161.3
High bar angle (GA)	3/3/6	25	0.7	0	35.3	69.3	102.1	133.6	164.0
Smaller bars and grooves (GC)	2/2/6	10	0.3	0	40.2	80.2	118.3	145.5	190.3
Fully bleached softwood (FBSW)									
Reference (REF)	5/5/6	10	1.2	0	44.3	87.9	130.8	172.9	214.3
High intensity (SEL+)	5/5/6	10	1.6	0	46.4	91.8	136.2	179.0	221.9
Low intensity (SEL-)	5/5/6	10	0.8	0	48.0	95.6	142.6	189.2	235.3
High bar angle (GA)	5/5/6	25	1.2	0	51.4	102.0	151.8	201.0	249.4
Smaller bars and grooves (GC)	3/3/6	10	0.4	0	50.9	100.9	150.0	198.3	245.7
Unbleached softwood (UBSW)									
Reference (REF)	5/5/6	10	1.2	0	44.3	87.9	130.8	172.9	214.3
High intensity (SEL+)	5/5/6	10	1.6	0	45.2	91.3	135.3	178.3	220.0
Low intensity (SEL-)	5/5/6	10	0.8	0	47.0	95.0	142.0	189.0	235.0
High bar angle (GA)	5/5/6	25	1.2	0	44.3	87.9	130.8	172.9	214.3
Smaller bars and grooves (GC)	3/3/6	10	0.4	0	50.0	101.0	150.0	199.0	247.0

*Bar width/groove width/groove depth = bar code of a refiner plate.

**Sum of rotor and stator bar angles. Bar angle is described as an angle formed by the first bar and the right edge of a single cluster of bars (Liu *et al.*, 2020).

3.2.3 Pulp quality evaluation

3.2.3.1 Measurement of the Canadian Standard Freeness

The consistencies (refer to Annexure A.1) and Canadian Standard Freeness (CSF) for each sample were determined immediately after refining as described in Tappi Test Method

T222 cm-09 (TAPPI, 2009). The method is devised to determine the rate at which a diluted pulp suspension (3 g in 1 L of water) can be drained. The method is suitable for both bleached and unbleached pulps. A step-by-step procedure is provided in Annexure A.2.

3.2.3.2 Handsheet formation and testing

Following the testing of the CSF, several paper handsheets per sample were formed using a standard laboratory Rapid-Köthen handsheet former. The handsheets were formed as described by ISO 5269-2 (ISO, 2004) (refer to Annexure B.1). Handsheets with grammages of 75 g/m² and 120 g/m² were made for testing. The 120 g/m² handsheets were used to test the Short-span Compressive Test (SCT) and the Corrugating Medium Test (CMT) of the unbleached softwood pulp. The handsheet properties were determined using the International Standard Organisation (ISO) test methods. For fully bleached pulps, the handsheet properties that were tested included tensile, tear, burst, bulk, porosity, and opacity. For the unbleached pulp, the same properties were tested, in addition to SCT and CMT. Table 3-2 below shows the ISO test method used to test each property. Annexure B.2 provides the full step-by-step procedures of all the test methods used in this study.

Table 3-2: ISO test methods used to test each handsheet property

Handsheet property	ISO test method	References
Tensile strength	ISO 1924-2	(ISO, 2008c)
Tear strength	ISO 1974	(ISO, 2012a)
Burst strength	ISO 2758	(ISO, 2014)
Thickness	ISO 534	(ISO, 2011b)
Grammage	ISO 536	(ISO, 2012b)
Moisture	ISO 534	(ISO, 2012c)
Opacity	ISO 2471	(ISO, 2008b)
Porosity	ISO 5636-3	(ISO, 2013)
Short-span Compressive Test	ISO 9895	(ISO, 2008a)
Corrugating Medium Test	ISO 7263	(ISO, 2011a)

3.2.3.3 Fibre morphology analysis

For all pulps tested, each of the seven samples collected per refining run were analysed using a Lorentzen & Wettre Fiber Tester Plus (L&W FT+) (ABB, Finland) to quantify any morphological changes to the fibre properties (fibre length, deformations, fibrillation, coarseness, cell wall thickness, and fines). The L&W FT+ uses 1 g bone-dry pulp and consists

of a digital camera and a software package that can analyse the fibre morphological properties of the pulp suspension. To determine the amount of pulp required for a 1 g bone dry suspension Equation (3-1) below was used:

$$\text{Weight required (g)} = \frac{1g \times 100}{\text{Sample consistency}} \quad (3-1)$$

After accurately weighing the amount of pulp required to two decimal places. The pulp was transferred to a 1 L beaker and 300 mL of water was added. A handheld blender was then used to mix the pulp and water. The mixed pulp suspension was then transferred to a 1 L measuring cylinder and 700 mL of water was added to make up 1 L. Thereafter, the pulp suspension was transferred back to a 1 L beaker. The pulp suspension was then used to weight three 100 g samples in 500 mL glass beakers, to obtain three repeats containing 1 g of bone-dry pulp. The average value of the three repeats was used for analysis.

PHASE 3

3.2.4 Computational modelling

Process variables and fibre properties obtained during the pilot refining process, including the fibre morphology and handsheet properties data, were modelled using a bijective study software modelling program (Matech, France). The software uses various regression methods to construct models based on the actual data generated by the pilot refiner runs. The results from the software were analysed to establish optimal refining conditions at the desired CSF of 350 mL for the bleached pulps and 550 mL for the unbleached pulp. Three refiner plate designs were modelled, including the reference plate design (REF), a plate design with a larger bar angle (GA), and a plate design with smaller bar widths (GC).

3.2.5 Optimisation of pilot refining parameters

In order to investigate the repeatability and reliability of the optimal refining conditions produced by the software package, each pulp was refined three times under the optimum conditions (as described in Section 3.2.2). After refining, the pulp properties of the samples were examined again (as described in Section 3.2.3) and compared with the pulp properties generated by the software.

The one-way analysis of variance (ANOVA) was used to determine whether there was a significant variation in the pulp properties between the three optimum pilot refiner runs. A one-way ANOVA statistical method determines whether there is a variance between the means of three or more groups. An alpha level (α) of 0.05 was used for all tests.

The following hypotheses were applied: The null hypothesis states that the group means are statistically equal, *i.e.*, there are no significant differences between the group means. The alternate hypothesis states that at least one mean is statistically different.

The one-way ANOVA statistical analysis was performed under the following assumptions:

1. Normality: each sample is drawn from a population with a normal distribution.
2. Sample independence: each sample has been drawn independently of the other samples.
3. The data type is continuous.

3.2.6 Scanning electron micrographs analysis

The surface morphologies of the optimised samples and the reference runs with and without CBH I, were analysed using a benchtop Scanning Electron Microscope (SEM) (Phenom, The Netherlands). Samples were prepared by mounting them onto metal stubs with double-sided carbon adhesive tape. Each sample was cleaned using compressed air to remove loose particles and other contaminants. The samples were visualised using a charge-reduction sample holder, an acceleration voltage of 10 kV, and a pre-set beam intensity ("point") at a magnification of 1300X and a working distance of 50 μm .

CHAPTER 4: CBH I CHARACTERISATION

In this chapter, the results obtained from phase 1 (CBH I characterisation) were analysed and discussed. section 4.1 begins the chapter with an introduction to enzyme characterisation and its significance. This is followed by the results obtained from phase 1 and what these results signify, especially for the pulp and paper industry, sections 4.1.1 and 4.1.2. Raw data obtained during phase 1 is provided in Annexure C.

4.1 Analysis of CBH I characterisation data

Enzyme characterisation involves the determination of an enzyme's activity by analysing alterations in the activity at various temperatures and pH values (Marais, 2008). Characterising an enzyme is essential to assessing the viability of that enzyme and for the advancement of new enzymes. Since the 1950s, numerous enzyme assay techniques have been reported, and the filter paper assay (FPA) has been the most extensively used of these techniques to measure cellulase activities (Chu *et al.*, 2012). The FPA is advantageous because it uses a widely available substrate, namely, Whatman No.1 filter paper (Mboowa, 2019). However, the disadvantage of this method is that it requires a substantial amount of time. Hence, to improve the effectiveness and quality of the cellulase enzyme assay, a simple method is still needed (Chu *et al.*, 2012). In addition, filter paper characteristics, such as its high cellulose content and dry nature, compromise the ability of the FPA to predict how a realistic biomass substrate would be hydrolysed by an enzyme (Mboowa, 2019). In this study, the activity of cellobiohydrolase I (CBH I) was studied using a modified FPA and the resulting reducing sugars, *i.e.*, cellobiose and glucose, were measured using high-performance liquid chromatography (HPLC).

4.1.1 Results

The characterisation of CBH I was conducted by first determining the optimum temperature and then determining the optimum pH of the enzyme. The thermostability of CBH I was then determined using the optimum conditions. The hydrolytic activity of CBH I was measured using HPLC.

Cellobiohydrolase I is a cellulase enzyme that hydrolyses the crystalline region of the cellulose chain from both the reducing and non-reducing ends, producing mainly cellobiose (Annamalai *et al.*, 2016). However, both cellobiose and glucose were detected by HPLC for all assays (as shown in Figure C-1, Figure C-2 and Figure C-3 in Annexure C), which indicates the activity of another enzyme, namely, β -glucosidase. β -Glucosidase is a cellulase enzyme that cleaves the cellobiose produced by CBHs to produce glucose. The addition of β -glucosidase to a

cellulase cocktail is not unconventional as it is essential in preventing product inhibition, thus improving the overall rate of enzymatic hydrolysis (Zhai, 2017). It must be noted that the activities of CBH I and β -glucosidase and their combined activity were indicated Figure 4-1, Figure 4-2 and Figure 4-3. However, due to the focus of this study only the CBH I activity was discussed.

The activity of CBH I was assayed over a temperature range of 25 to 85 °C at a constant pH of 5. Figure 4-1 shows that the optimum temperature for CBH I was 55 °C, with a maximum total activity of 266 IU/mL. Enzymes that are optimally active at a temperature range of 50 to 80 °C are classified as thermophilic enzymes (Vieille & Zeikus, 2001). The obtained optimum temperature for CBH I indicated that CBH I is a thermophile.

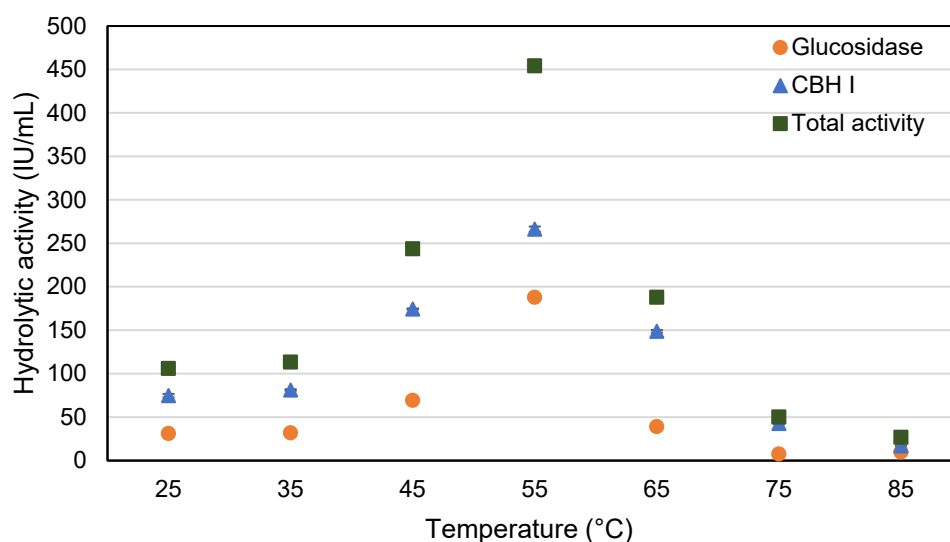


Figure 4-1: Graphic representation of the temperature profiles of CBH I and β -glucosidase activities, as well as the combined activities of the two enzymes. The activities of CBH I and β -glucosidase were calculated from the concentration of cellobiose and glucose that was released, respectively. Errors bars represents the relative standard deviation of the three measurements.

The effect of pH on CBH I activity was subsequently investigated over a range of pH 2 to 9, using the determined optimum temperature of 55 °C. The pH profile (Figure 4-2) shows that the optimum pH of CBH I was 4. Therefore, the enzyme performed best under acidic conditions. The maximum total activity achieved at the optimum pH was 577 IU/mL.

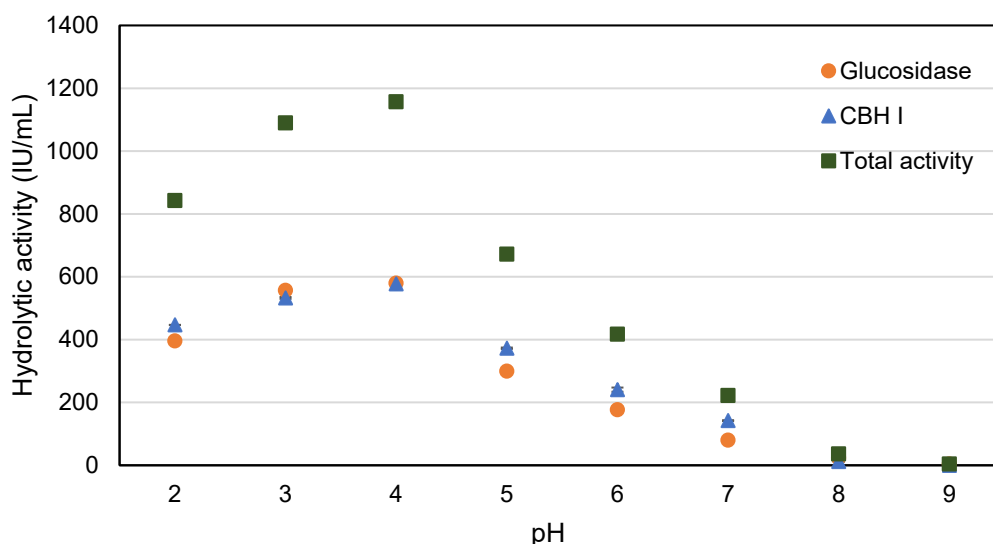


Figure 4-2: Graphic representation of the pH profiles of CBH I and β -glucosidase activities, as well as the combined activities of the two enzymes. The activities of CBH I and β -glucosidase were calculated from the concentration of cellobiose and glucose that was released, respectively. Errors bars represents the relative standard deviation of the three measurements.

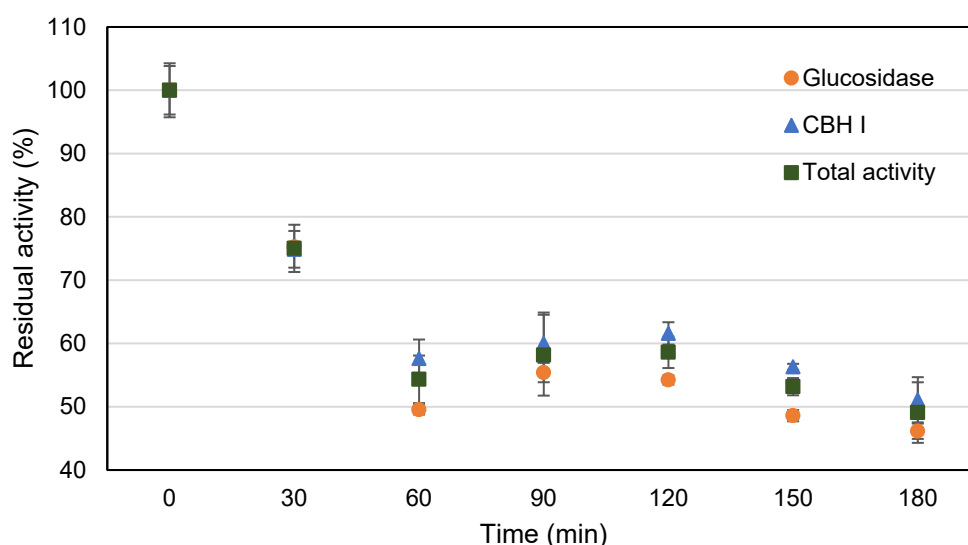


Figure 4-3: Graphic representation of the thermostability profiles of CBH I and β -glucosidase activities, as well as the combined activities of the two enzymes. The residual activities of CBH I and β -glucosidase were calculated from the concentration of cellobiose and glucose that was released, respectively. Errors bars represents the relative standard deviation of the three measurements.

The thermostability profile of CBH I was obtained using the optimum temperature and pH. The enzyme was incubated for 3 h, and samples were withdrawn every 30 min, and the residual activity was determined. Figure 4-3 shows that approximately 43% of the enzyme's activity was lost after 1 h. However, as time progressed, the residual activity approached stability, and

the enzyme retained 51% of its activity at the end of the 3 h incubation period. This indicated that CBH I is a thermostable enzyme.

4.1.2 Discussion

The results obtained in phase 1 correlate with observations by Turner *et al.* (2007) that thermophilic enzymes have a high probability of being thermostable. Thermostable enzymes are advantageous because they can withstand temperatures that would normally denature mesophilic enzymes (Danson *et al.*, 1996). In addition, thermostable and thermophilic enzymes can withstand harsh industrial environments due to their high thermal stability. This is significant since there is no need for cooling and thus productivity is improved, in contrast to mesophiles and some synthetic enzymes.

Furthermore, thermophilic enzymes decrease the possibility of contamination by other enzymes as the temperature rises. This results in less stringent sterility requirements compared to when mesophilic enzymes are used. Altogether, this results in reduced production and labour costs, thus increasing profit (Kumwenda *et al.*, 2013; Lasa & Berenguer, 1993).

However, because of the activity-stability trade-off, thermophilic enzymes can be extremely active at elevated temperatures at the cost of decreased stability. Therefore, when it comes to determining the suitability as an enzyme for industrial applications, operation and stability criteria become complementary to each other, since none of these will be very feasible on its own (Saqib & Siddiqui, 2018). This clearly illustrates the need to correctly measure an enzyme's thermostability along with its behaviour to evaluate its suitability for a specific process application. Nonetheless, CBH I being thermophilic and thermostable showed that it has the potential to be used in industrial applications. As a result, the enzyme was subsequently investigated for refining application in phase 2 of this study.

CHAPTER 5: BIJECTIVE STUDIES ON THE PILOT REFINER

In this chapter, the results from phase 2 (bijjective studies on the pilot refiner) are analysed. In this phase, the effect of CBH I pre-treatment before refining was investigated using the bijjective diagram technique. This is a technique used to give detailed graphical information of how the pulp and fibre properties would react under a wide range of refining energies and specific edge loads (SEL) with various refiner plate designs. The refining runs were conducted using a 12" single-disc pilot refiner. To ascertain the effect of CBH I pre-treatment, three morphologically different pulps were analysed, namely a bleached hardwood kraft pulp (BHKP) in section 5.1, a fully bleached softwood (FBSW) pulp in section 5.2, and an unbleached softwood (UBSW) pulp in section 5.3. section 5.4 concludes the chapter with a discussion and summary of the key results from phase 2. All the results were analysed at a specific desired Canadian Standard Freeness (CSF) for each pulp. The lines connecting the points were intended to facilitate distinguishing between the refining runs and do not represent a model. Raw data obtained during phase 2 is provided in Annexure D.

5.1 Pilot refining bijjective study for the BHKP

5.1.1 Effect of CBH I pre-treatment and refining on the CSF drop

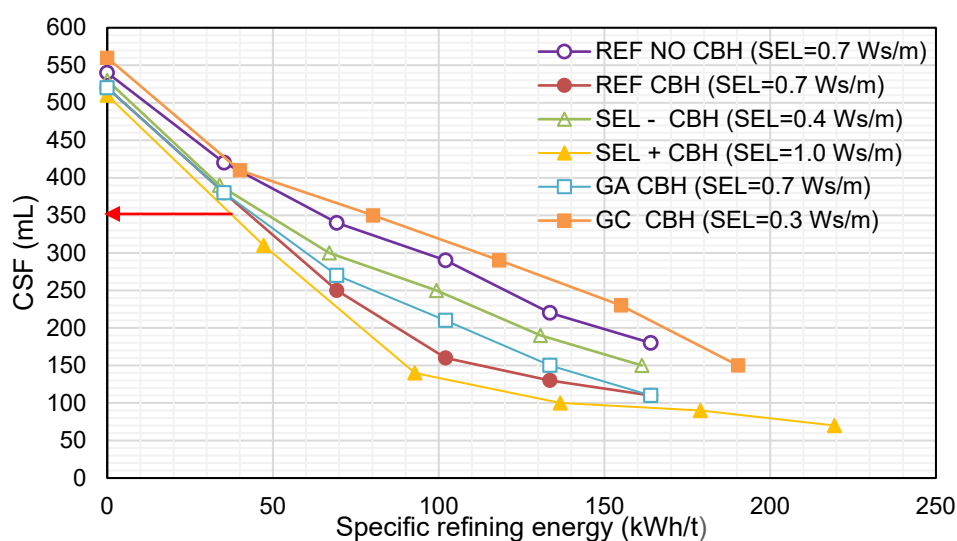


Figure 5-1: Effect of CBH I pre-treatment and specific refining energy on the CSF for the BHKP. All samples except the reference (REF NO CBH) were pre-treated with CBH I.

Table 5-1: Specific refining energy (kWh/t) measured at a CSF of 350 mL for a BHKP that was mechanical refined using different refiner settings. All samples except the reference (REF NO CBH) were pre-treated with CBH I. The change (%) between the reference and the treated samples was also calculated.

Run	Specific refining energy	
	kWh/t	*Change from REF (%)
REF NO CBH (SEL = 0.7 Ws/m)	69.0	NA
REF CBH (SEL = 0.7 Ws/m)	46.0	-33.3
SEL+ CBH (SEL = 1.0 Ws/m)	45.0	-2.2
SEL- CBH (SEL = 0.4 Ws/m)	56.0	+21.7
GC CBH (SEL = 0.3 Ws/m)	86.5	+88.0
GA CBH (SEL = 0.7 Ws/m)	50.5	+9.8
* = change (%) for REF CBH calculated based on change from REF NO CBH, while for all the other runs the change (%) is calculated based on change from REF CBH		

The CSF was determined immediately after refining and the results are shown in Figure 5-1. The CSF of interest for the BHKP was 350 mL, and the specific refining energy required to reach the targeted CSF for each refining run is shown in Table 5-1. As shown in Figure 5-1, the CSF and specific refining energy have an inversely proportional relationship, irrespective of the variation of the refining parameters. This was expected and correlates with literature (Rampersadh, 2005; Steel, 2010). As the refining energy increased, the fibres received a higher refining treatment. This reduced the fibre wall thickness, leading to an increase in fibre collapsibility and flexibility. The thin-walled flexible fibres were more closely packed together compared to unrefined/low-energy refined fibres, thus reducing the CSF. The increase in the refining primary effects (discussed in CHAPTER 2 in section 2.2.3) made it more difficult to de-water the pulp (Ek *et al.*, 2009a; Rampersadh, 2005).

Comparing the two REF runs, the addition of CBH I led to a faster CSF drop. This was advantageous as it indicated that under the same refining conditions, treating the pulp with CBH I prior to refining allowed a certain CSF to be reached at lower refining energy. Energy consumption to reach the desired CSF was reduced by 33.3%. The faster CSF drop for the treated reference run was due to the CBH I pre-treatment softening the fibre surface, hence

increasing the refining effects such as fines, fibrillation, and fibre cutting relative to the untreated reference pulp.

Based on the previous outcome, the effect of specific edge load (SEL) on the pulp fibres during CBH I assisted refining was investigated. The SEL+ and SEL- runs had the same plate design as the REF runs, and the difference was the SEL applied to the fibres. As previously stated in CHAPTER 2, section 2.2.2, SEL is the quantity of energy expended per unit edge length of a bar crossing and is strictly presented as the refining intensity (Gharehkhania *et al.*, 2015). The CSF drop increased as the SEL applied to the fibres increased (Figure 5-1). The high intensity (SEL+) run resulted in a faster CSF drop compared to the REF CBH I run, while the SEL- run had the opposite effect due to its low intensity. When compared to the REF CBH I run, SEL+ required 2.2% less energy input, while SEL- required 21.7% more refining energy input to achieve the desired CSF.

A higher SEL indicated that the fibres received a higher intensity per refining impact. Therefore, the fibres were subjected to intense shear and compression forces between the refiner plate bars. As a result, the outer fibre walls were easily delaminated, resulting in faster refining effects (fines, fibre cutting, fibrillation), which led to a faster CSF drop, as observed for the SEL+ run.

Lastly, the effect of changing the design of the refiner plates was investigated. The design of the bar, groove, and angle pattern on the refiner plates determines the amount of refining energy applied to the fibres. Consequently, the refiner plate design and selection are essential factors that affect the refining outcome (pulp quality) (Guo *et al.*, 2020; Liu *et al.*, 2020). To investigate the influence of changing the bar angle and widths, the GA plate design had a bigger bar angle compared to the REF plate design, while the GC plate design had smaller bar widths.

As seen in Figure 5-1, the GA run led to a slower CSF drop when compared to the REF CBH I run. As a result, 9.8% more refining energy was required to reach the targeted CSF. An increase in the bar angle created a stronger pumping capacity, thus the fibres were forced through the refiner at a faster rate. As a result, a larger bar angle induced a smaller fibre residence time and a higher yield, altogether resulting in poor pulp quality (slow CSF drop) (Liu *et al.*, 2020).

The GC plate design required 88.0% more energy compared to the REF CBH I run. The GC refining run was performed at the same refining power as that of the REF refining run. However, this power was more distributed due to the increase in the number of bars per unit

area in the GC plate design, resulting in a much lower SEL. Furthermore, the length of the refining impact depends on the width of the bar. Therefore, the smaller the bar width (as is the case for GC plate design), the less time the fibre flocs spent compressed between the rotor and stator bars, thus received little refining impact (Anand *et al.*, 2016; Elahimehr, 2014). Presumably, the combination of the aforementioned factors led to the GC run having the slowest CSF drop compared to all the other five refining runs. Nonetheless, to better understand the degree of refining of the six bijective refining runs for the BHKP, the changes in the fibre morphology were analysed.

5.1.2 Effect of CBH I pre-treatment and refining on the fibre morphology

The following fibre morphology changes were analysed namely, macro-fibrillation, fines content produced and the fibre cutting effect. The macro-fibrillation increased as the degree of refining increased (as the CSF decreased), shown in Figure 5-2 (a). As the degree of refining continued, fines generation increased, shown in Figure 5-2 (b), and the average length of the fibres decreased, shown in Figure 5-2 (c). The average fibre length was used to measure the degree of the fibre cutting effect. As the fibre length decreased, the degree of fibre cutting increased. The fibre properties at the desired CSF of 350 mL are shown in Table 5-2.

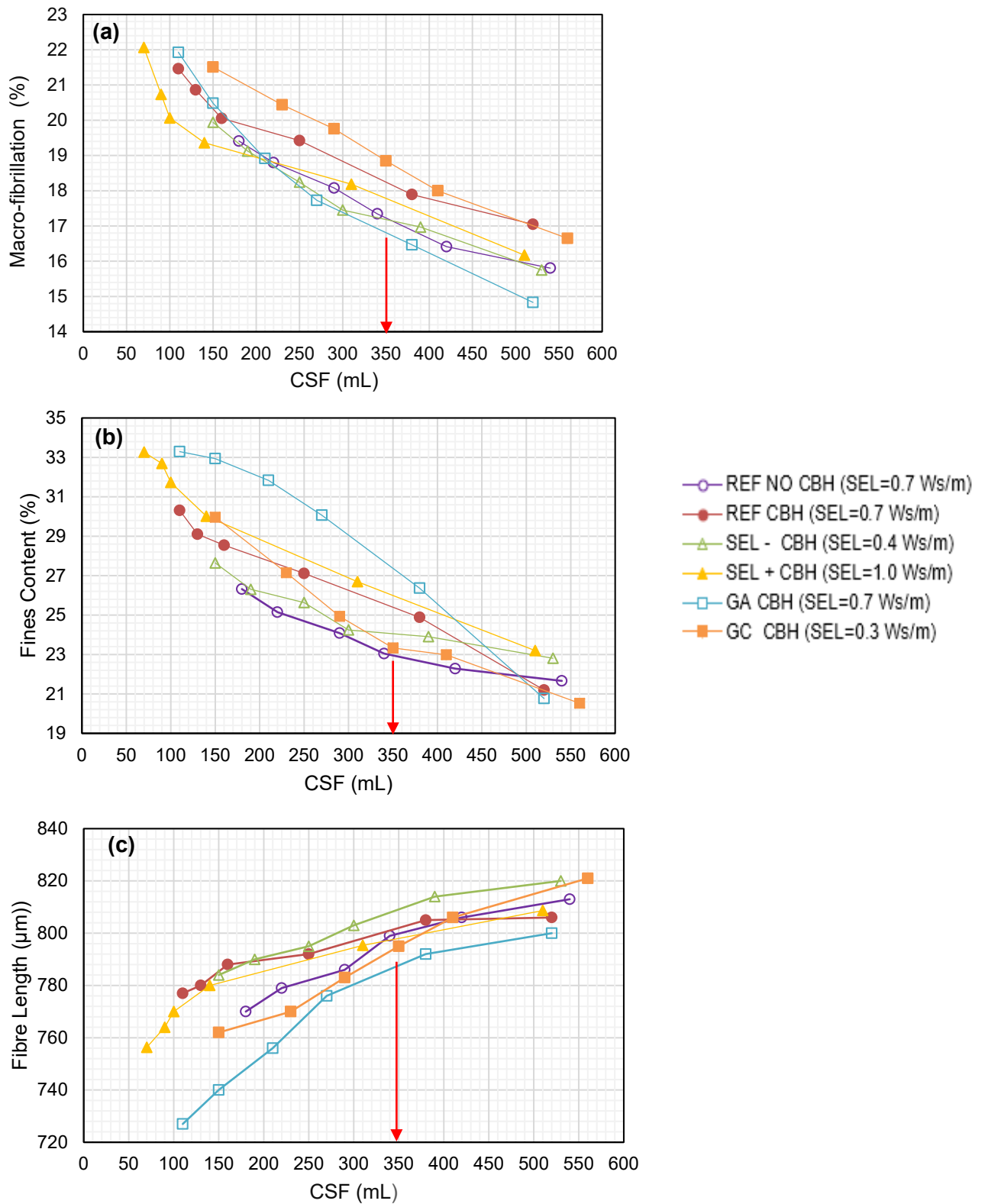


Figure 5-2: Effect of CBH I pre-treatment and mechanical refining on (a) macro-fibrillation (b) fines content and (c) fibre length for the BHKP relative to the CSF. All samples except the reference (REF NO CBH) were pre-treated with CBH I.

Table 5-2: Macro-fibrillation (%), fines content (%) and fibre length (µm) measured at a CSF of 350 mL for a BHKP that was mechanical refined using different refiner settings. All samples except the reference (REF NO CBH) were pre-treated with CBH I. The change (%) between the reference and the treated samples was also calculated.

Run	Macro-fibrillation		Fines content		Fibre length	
	%	*Change from REF (%)	%	*Change from REF (%)	µm	*Change from REF (%)
REF NO CBH (SEL = 0.7 Ws/m)	17.1	NA	23.0	NA	800	NA
REF CBH (SEL = 0.7 Ws/m)	18.2	+6.4	25.2	+9.6	803	+0.4
SEL+ CBH (SEL = 1.0 Ws/m)	17.1	-6.0	25.5	+1.2	795	-1.0
SEL- CBH (SEL = 0.4 Ws/m)	17.0	-6.6	23.3	-7.5	812	+1.1
GC CBH (SEL = 0.3 Ws/m)	18.4	+1.1	23.0	-8.7	808	+0.4
GA CBH (SEL = 0.7 Ws/m)	16.8	-7.7	27.2	+7.4	788	-1.9
* = change (%) for REF CBH calculated based on change from REF NO CBH, while for all the other runs the change (%) is calculated based on change from REF CBH						
Values were obtained using a Microsoft Excel trendline. A trendline with an R-squared value at or near 1 was chosen for computing a specific fibre property at the targeted 350 mL CSF.						

As shown in Figure 5-2 (a-c), the CBH I charge increased both the macro-fibrillation and fines content by 6.4% and 9.6%, respectively, compared to the untreated reference pulp, at the desired CSF of 350 mL. Although the addition of CBH I pre-treatment led to higher fibrillation and fines content, the fibre cutting effect observed remained relatively the same between the two REF runs (0.4% difference). The increased fibrillation and fines content results are in accordance with the CSF drop observed in section 5.1.1.

The alteration of SEL resulted in the macro-fibrillation decreasing for both SEL- and SEL+ by 6.6% and 6.0%, respectively, at the targeted CSF compared to the REF CBH I run (Table 5-2). In the case of SEL-, this may be due to the low intensity that was applied to the fibres, which led to a reduction in the quantity of fines content present and the fibre cutting effect, by 7.5% and 1.1%, respectively, as shown in Table 5-2. Whereas in the case of SEL+ the macro-fibrillation may have been limited due to the fact that hardwood fibres are short, thin, and have low refining resistance (Bajpai, 2005; Lumiainen, 1998), resulting in the fibres being cut rather than being fibrillated at the high intensity. This is substantiated by the 1.0% higher fibre cutting effect (low fibre length) observed for SEL+ compared to the REF CBH I run (Table 5-2).

(Figure 5-2 (c)). Even though the SEL+ run achieved the desired CSF faster with lower refining energy consumption, the run had a slightly 1.2% higher quantity of fines generated compared to the REF CBH I run. The fibre morphology results for both SEL+ and SEL- are consistent with the CSF drop observed for SEL+ and SEL- in comparison to the REF CBH I run.

The GC run resulted in the macro-fibrillation remaining relatively the same at 18% compared to the REF CBH I run. Even though the macro-fibrillation was similar, the GC run had a lower quantity of fines content at the desired CSF. There was an insignificant difference in the fibre cutting effect measured (0.4% difference) between the REF CBH I and GC runs. The GA run resulted in an 7.7% lower macro-fibrillation compared to the REF CBH I run. The GA run resulted in a 1.9% higher fibre cutting effect, which led to the higher fines content of 7.4% at the targeted CSF.

5.1.3 Effect of CBH I pre-treatment and refining on the handsheet properties

The changes in the fibre morphology have a significant impact on the final handsheet properties. As a result, several handsheet strength, optical, and structural properties were measured in this study.

5.1.3.1 Handsheet strength properties

Tensile, burst, and tear strength developments were investigated for the BHKP, and the results are depicted in Figure 5-3 (a), Figure 5-3 (b) and Figure 5-3 (c), respectively. The handsheet strength properties at the desired 350 mL CSF are shown in Table 5-3. As shown in Figure 5-3 (a), the tensile strength increased as the CSF decreased. Tensile strength is determined by inter-fibre bonds, which is the principal element, and fibre strength, which plays a marginal role. The inter-fibre bonding between the fibres was determined by the macro-fibrillation generated through refining, which increased as the CSF decreased. The macro-fibrillation increased the surface area, ultimately increasing the bonding sites, *i.e.*, bonding capacity between fibres, improving the tensile strength (Ghosh, 2006; Kang & Paulapuro, 2006; Rampersadh, 2005). The inverse relationship between burst strength development and CSF is depicted in Figure 5-3 (b). Burst strength development has been reported to be affected by the same factors as tensile strength development (Ghosh, 2006; Rampersadh, 2005).

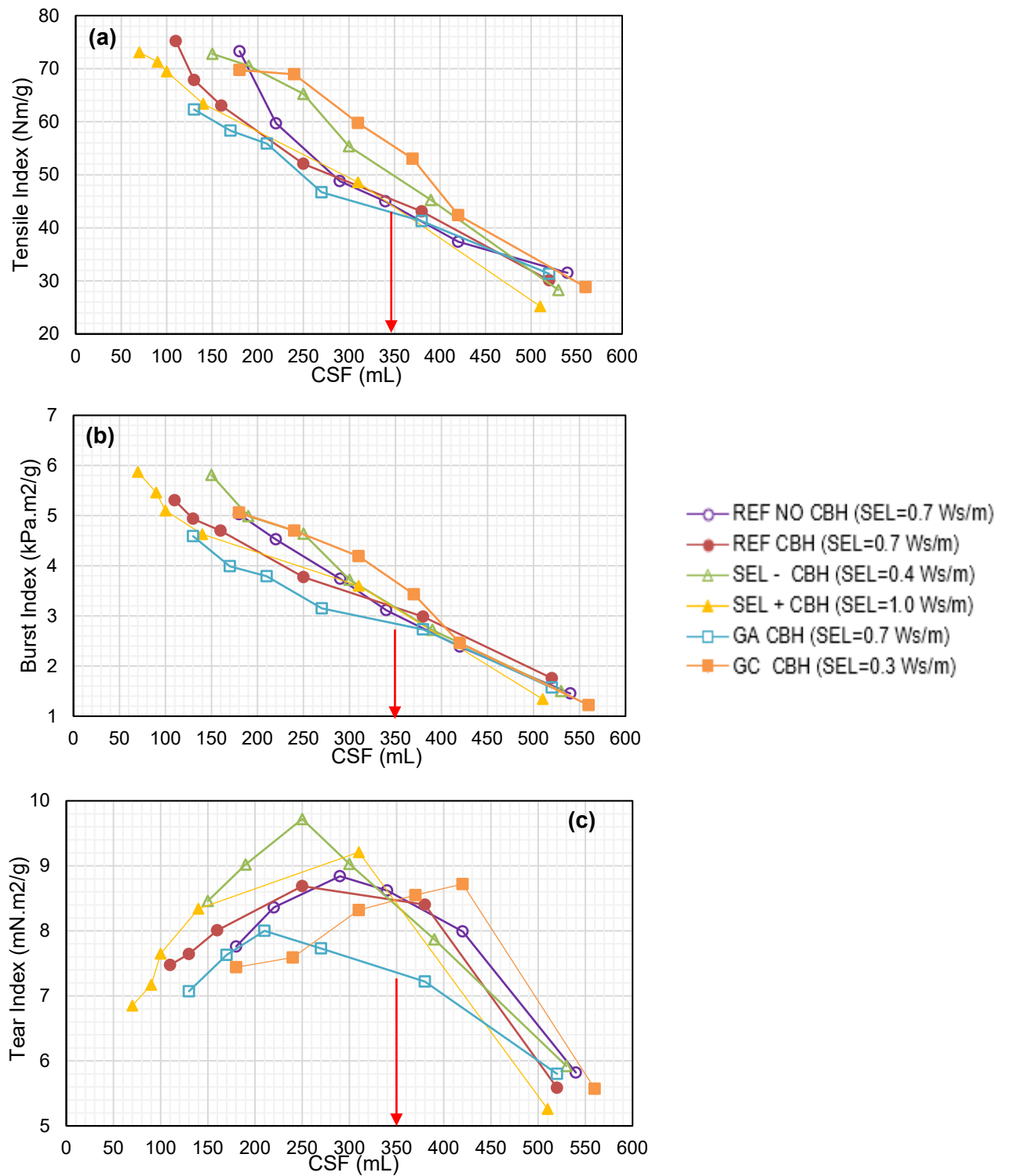


Figure 5-3: Effect of CBH I pre-treatment and mechanical refining on(a) tensile index , (b) burst index and (c) tear index for the BHKP relative to the CSF. All samples except the reference (REF NO CBH) were pre-treated with CBH I.

Table 5-3: Tensile index (Nm/g), burst index (kPa.m²/g) and tear index (mN.m²/g) measured at a CSF of 350 mL for a BHKP that was mechanical refined using different refiner settings. All samples except the reference (REF NO CBH) were pre-treated with CBH I. The change (%) between the reference and the treated samples was also calculated.

Run	Tensile Index		Burst Index		Tear Index	
	Nm/g	*Change from REF (%)	kPa.m ² /g	*Change from REF (%)	mN.m ² /g	*Change from REF (%)
REF NO CBH (SEL = 0.7 Ws/m)	40.9	NA	3.0	NA	9.0	NA
REF CBH (SEL = 0.7 Ws/m)	44.3	+8.3	3.2	+6.7	8.5	-5.6
SEL+ CBH (SEL = 1.0 Ws/m)	42.6	-3.8	2.9	-9.4	8.3	-2.4
SEL- CBH (SEL = 0.4 Ws/m)	51.1	+16.3	3.4	+6.3	9.2	+8.2
GC CBH (SEL = 0.3 Ws/m)	54.4	+22.8	3.8	+18.8	8.9	+4.7
GA CBH (SEL = 0.7 Ws/m)	42.4	-4.7	2.8	-12.5	7.4	-12.9
* = change (%) for REF CBH calculated based on change from REF NO CBH, while for all the other runs the change (%) is calculated based on change from REF CBH						
Values were obtained using a Microsoft Excel trendline. A trendline with an R-squared value at or near 1 was chosen for computing a specific handsheet property at the targeted 350 mL CSF.						

Thus, comparing the two REF runs, the improvement of the macro-fibrillation due to the CBH I pre-treatment led to an increase of 8.3% and 6.7% in the tensile and burst strength development, respectively. Increasing SEL deteriorated the development of tensile and burst strengths by 3.8% and 9.4%, respectively. This was in accordance with the results observed for macro-fibrillation in section 5.1.2. The high intensity of the SEL+ run combined with the enzyme treatment was too harsh for the thin and short hardwood fibres. Hardwood pulps have low refining resistance; thus, a combination of high intensity and CBH I pre-treatment led to excessive refining, inflicting damage on the individual fibres, and deteriorating the pulp strength quality.

The above statements were substantiated by the low intensity run (SEL-), which improved the tensile and burst strength developments by 16.3% and 6.3%, respectively, compared to the REF CBH I run. This indicated that irrespective of the lower micro-fibrillation observed for SEL, the BHKP responded better to low intensity, presumably due to the preservation of the fibre

intrinsic strength. This is further substantiated by the GC run, which had the lowest refining intensity. The GC refining run improved the tensile and burst strength development by 22.8% and 18.8%, respectively, compared to the REF CBH I run. It should be noted, however, that both SEL- and GC required more refining energy to achieve the desired CSF compared to the REF CBH I run. The GA run, which had more fibre cutting than fibrillation, resulted in 4.7% and 12.5% lower tensile and burst strengths, respectively, compared to the REF CBH I run.

As shown in Figure 5-3 (c), tear strength development slightly increased during low refining energy (high CSF), and sharply decreased as the refining continued. According to literature, tear strength is influenced by several factors; however, the dominant ones are fibre length and inter-fibre bonding. As a result, tear strength improves at the beginning of refining due to an improvement in the inter-fibre bonding and sharply decreases as the refining continues due to the fibre cutting effect, which decreases the fibre length and strength (Budhram, 2005).

Even though there was not a significant difference in the average fibre length between the two REF runs. The CBH I pre-treatment resulted in a 5.6% tear loss at the targeted CSF. The low intensity run, SEL-, increased the tear strength by 8.2% compared to the REF CBH I run, due to the low fibre cutting effect observed, resulting in longer fibres compared to the REF CBH I run. Longer fibres are thought to have higher tear strength because they transmit stress over a bigger area, more fibres, and more bonds compared to shorter fibres. Whereas with shorter fibres, stress experienced is concentrated over a smaller region, thus decreasing tear strength (Budhram, 2005; Rampersadh, 2005; Wimmer *et al.*, 2002).

A further attribute to the high tear strength may be due to the low refining intensity applied to the fibres. This can be seen on the GC run which had a 4.7% higher tear strength in comparison to the REF CBH I run, although both the refining runs had relatively the same fibre cutting effect. This further confirmed that the hardwood short and thin fibres, regardless of refiner plate design, developed better in milder, gentler refining treatments that preserved the fibre's intrinsic strength.

The aforementioned statement was substantiated by examining the moderate/higher intensity runs (GA & SEL+), although they had different plate designs, presumably due to the refining intensity applied, both refining runs induced a lower tear strength compared to the REF CBH I run, as seen in Table 5-3. In addition, both the refining runs had a higher fibre cutting effect compared to the REF CBH I run.

5.1.3.2 Handsheet structural properties

Porosity and bulk specific volume were the structural properties analysed for this research project, and the results are shown in Figure 5-4 (a) and Figure 5-4 (b), respectively. As seen in Figure (a and b), both porosity and bulk have a directly proportional relationship with the CSF. As the level of refining increased (reduction in CSF), the fibre wall layers were delaminated, hence the fibre wall thickness decreased, reducing bulk. This increased the conformability and flexibility of the fibres, improving the bonding ability between fibres. As a result, the fibres are packed closer together, increasing the bonding ability and decreasing porosity. Furthermore, porosity is also decreased by the fines generated during refining, which fill any voids in the fibre network (Cui *et al.*, 2015; Rampersadh, 2005). The structural properties for the BHKP at the desired 350 mL are shown in Table 5-4.

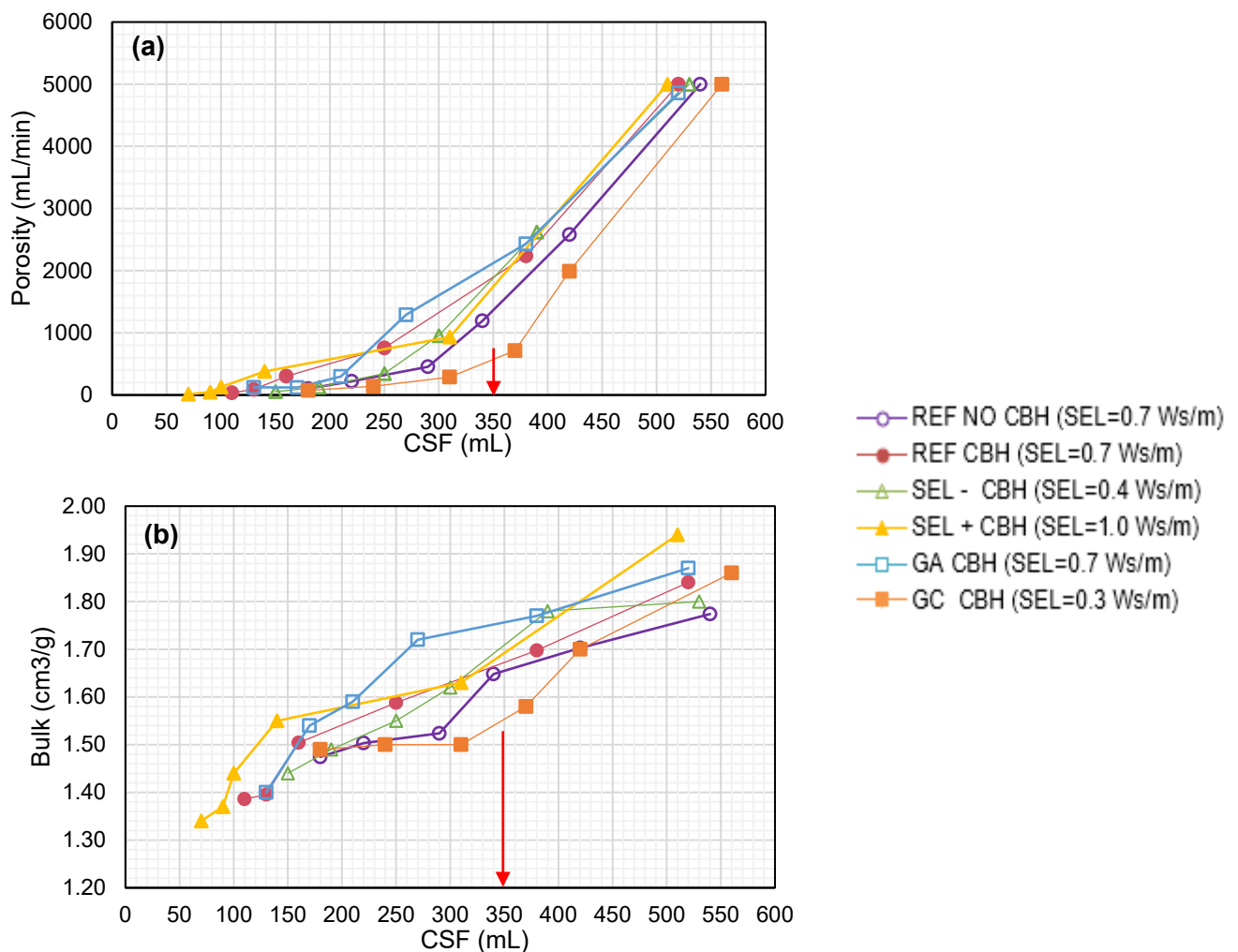


Figure 5-4: Effect of CH I pre-treatment and mechanical refining on (a) porosity and (b) bulk specific volume for the BHKP relative to the CSF. All samples except the reference (REF NO CBH) were pre-treated with CBH I.

Table 5-4: Porosity (mL/min) and bulk (cm³/g) measured at a CSF of 350 mL for a BHKP that was mechanical refined using different refiner settings. All samples except the reference (REF NO CBH) were pre-treated with CBH I. The change (%) between the reference and the treated samples was also calculated.

Run	Porosity		Bulk	
	mL/min	*Change from REF (%)	cm ³ /g	*Change from REF (%)
REF NO CBH (SEL = 0.7 Ws/m)	1534	NA	1.65	NA
REF CBH (SEL = 0.7 Ws/m)	1853	+20.8	1.66	+0.6
SEL+ CBH (SEL = 1.0 Ws/m)	1655	-10.6	1.70	+2.4
SEL- CBH (SEL = 0.4 Ws/m)	1914	+3.3	1.72	+3.6
GC CBH (SEL = 0.3 Ws/m)	726	-60.8	1.62	-2.4
GA CBH (SEL = 0.7 Ws/m)	2031	+9.6	1.73	+4.2
* = change (%) for REF CBH calculated based on change from REF NO CBH, while for all the other runs the change (%) is calculated based on change from REF CBH				
Values were obtained using a trend line. A trendline with an R-squared value at or near 1 was chosen for computing a specific handsheet property at the targeted 350 mL CSF.				

Comparing the two REF runs, the introduction of CBH I during the repulping stage increased the porosity by 20.8% and the bulk remained relatively the same at the desired CSF. Although the REF CBH I run achieved the desired with a less refining energy, it had the same bulk as the untreated reference pulp. The indicated that CBH I facilitated the delamination of the fibre wall layers, and a similar bulk was achieved with lower refining energy, while simultaneously improving the macro-fibrillation. The increase in porosity indicated that the higher fines content observed for the REF CBH I run compared to the untreated reference run had an insignificant impact on porosity. The increase in porosity might have also been due to the enzyme hydrolysing cellulosic hydrophilic debris such as fines.

The poor fibrillation and higher fines content measured for SEL+ decreased the porosity by 10.4% and increased the bulk 2.5% compared to the REF CBH I run. The low fines content and macro-fibrillation measured for SEL- increased both bulk and porosity by 3.3% and 3.6%, respectively, at the desired CSF (Table 5-4). These results were in accordance with the CSF drop observed for SEL+ and SEL- runs in comparison to the REF CBH I run.

The GA run increased both the bulk and porosity by 9.6% and 4.2%, respectively. Presumably, the bulk was higher due to the low macro-fibrillation (indicating low bonding ability) measured for the run. The GA run had higher fines content, but also had a higher porosity. This might be attributed to the fact that porosity is influenced by both fines content and bonding ability. The low bonding ability (high porosity) for the GA run provides a possible explanation for the CSF of the GA run lying above the REF CBH I run. The GC run, which had a higher bonding ability (as seen from the better tensile and burst strengths) compared to the REF CBH I run, induced a lower bulk and porosity (by 60.8% and 2.4%, respectively) compared to the REF CBH I run at the targeted CSF.

5.1.3.3 Handsheet optical properties

Opacity was the optical property measured in this study, mainly because opacity is essential in printing and book papers. A high opacity allows one to read the front side of a paper without being distracted by the printing on the back side of the page (Biermann, 1996). The relationship between the opacity and the CSF is shown in Figure 5-5. The opacity at the CSF of interest of 350 mL is shown in Table 5-5. As shown in Figure 5-5, the opacity decreased as the degree of refining increased (low CSF). The opacity of the two REF runs remained relatively the same (from 80% to 79.9%) at the 350 mL CSF. The alteration of SEL and the refiner plate design also induced insignificant differences in the opacity, as all runs had an opacity of 80% to 81% at the desired CSF (Table 5-5). This suggested that neither the enzyme treatment nor changing the refining parameters during enzymatic refining of the BHKP had a significant impact on the opacity.

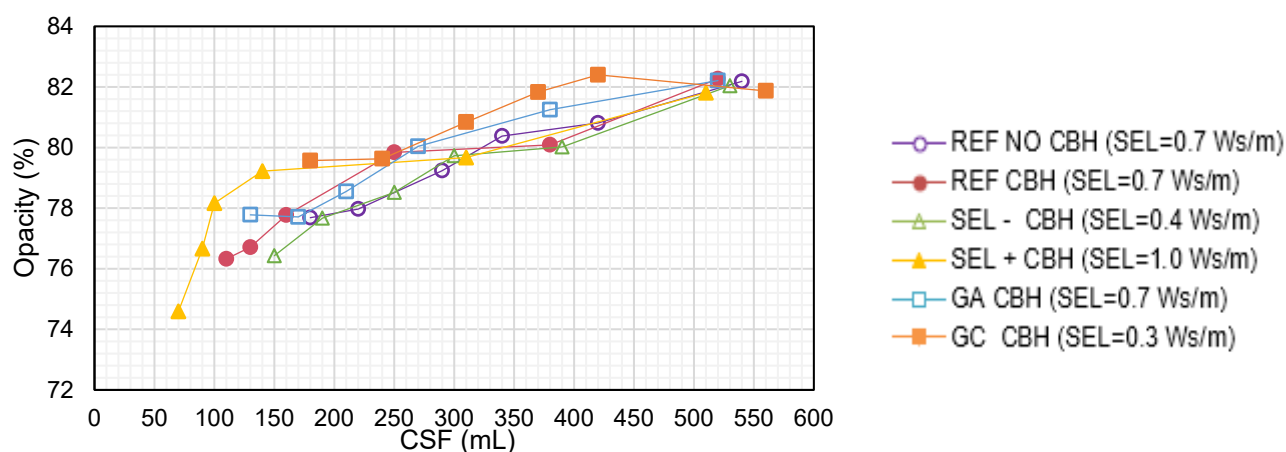


Figure 5-5: Effect of CBH I pre-treatment and mechanical refining on opacity for the BHKP relative to the CSF. All samples except the reference (REF NO CBH) were pre-treated with CBH I.

Table 5-5: Opacity (%) measured at a CSF of 350 mL for a BHKP that was mechanical refined using different refiner settings. All samples except the reference (REF NO CBH) were pre-treated with CBH I. The change (%) between the reference and the treated samples was also calculated.

Run	Opacity	
	%	Change from REF (%)
REF NO CBH (SEL = 0.7 Ws/m)	80.0	NA
*REF CBH (SEL = 0.7 Ws/m)	79.9	-0.1
SEL+ CBH (SEL = 1.0 Ws/m)	80.0	+0.1
SEL- CBH (SEL = 0.4 Ws/m)	79.9	0
GC CBH (SEL = 0.3 Ws/m)	81.6	+2.1
GA CBH (SEL = 0.7 Ws/m)	81.1	+1.5
* = change (%) for REF CBH calculated based on change from REF NO CBH, while for all the other runs the change (%) is calculated based on change from REF CBH		
Values were obtained using a trend line. A trendline with an R-squared value at or near 1 was chosen for computing a specific handsheet property at the targeted 350 mL CSF.		

5.1.4 Recommended bijective refining run for the BHKP

Hardwood pulp is used in refining to give the final product optical and surface properties. The bijective results for the BHKP indicated that the use of CBH I improved the strength properties without negatively affecting the optical properties. Overall, the BHKP responded best to low intensity refining treatment, irrespective of the refiner plate design. Thus, the SEL- and GC runs would be recommended for the refining of the BHKP.

5.2 Pilot Refining bijective study for the FBSW pulp

5.2.1 Effect of CBH I pre-treatment and refining on the CSF drop

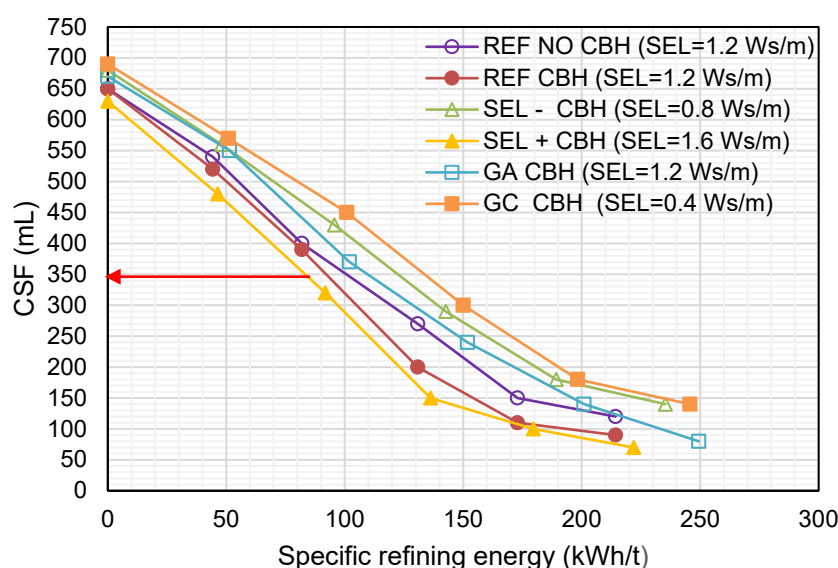


Figure 5-6: Effect of CBH I pre-treatment and the specific refining energy on the CSF for the FBSW pulp. All samples except the reference (REF NO CBH) were pre-treated with CBH I.

Table 5-6: Specific refining energy (kWh/t) measured at a CSF of 350 mL for a FBSW pulp that was mechanical refined using different refiner settings. All samples except the reference (REF NO CBH) were pre-treated with CBH I. The change (%) between the reference and the treated samples was also calculated.

Run	Specific refining energy	
	kWh/t	*Change from REF (%)
REF NO CBH (SEL = 1.2 Ws/m)	109.0	NA
REF CBH (SEL = 1.2 Ws/m)	98.0	-10.1
SEL+ CBH (SEL = 1.6 Ws/m)	85.0	-13.3
SEL- CBH (SEL = 0.8 Ws/m)	122.0	-24.5
GC CBH (SEL = 0.4 Ws/m)	126.8	+29.4
GA CBH (SEL = 1.2 Ws/m)	117.0	+19.4

* = change (%) for REF CBH calculated based on change from REF NO CBH, while for all the other runs the change (%) is calculated based on change from REF CBH

The CSF was determined immediately after refining and the results are shown in Figure 5-6. The CSF of interest for the FBSW was 350 mL. The specific refining energy for the desired CSF are shown in Table 5-6. As seen in Figure 5-6, an inversely proportional relationship was observed between the CSF and the specific refining energy. The reason for this correlation has been explained in section 5.1.1. Comparing the two REF runs, the addition of the CBH I pre-treatment accelerated the CSF drop. As a result, less refining energy was required to reach the desired CSF, resulting in a 10.1% energy savings. The alteration of the SEL and refining plate design during CBH I assisted refining was subsequently investigated. The alteration of these refining parameters proved to have a significant impact on the fibres, hence the CSF. The SEL+ run resulted in a faster CSF drop, enabling 13.3% energy savings, whereas the SEL- run had a slower CSF drop, requiring 24.5% more refining energy. The GA & GC refining runs led to more energy consumption (19.4% and 29.4%, respectively) to reach the desired CSF (Table 5-6), with the GC run requiring the most energy compared to all the refining runs. Explanations for these trends observed for these runs have been explained in section 5.1.1. However, the CSF is affected by the changes in the fibre morphology. Thus, subsequently, the changes in the fibre morphology were examined to better understand the degree of refining for each run.

5.2.2 Effect of CBH I pre-treatment and refining on the fibre morphology

The following FBSW fibre morphology changes were investigated: macro-fibrillation, fines content, and fibre length, with the results shown in Figure 5-7 (a), Figure 5-7 (b) and Figure 5-7 (c), respectively. As shown in Figure 5-7 (a), macro-fibrillation increased as the CSF decreased (level of refining increased). Figure 5-7 (b) depicts that the amount of fines generated increased for each run as the level of refining increased. Figure 5-7 (c) shows that as the fibre cutting effect increased as the CSF decreased. The fibre properties at the desired CSF of 350 mL are shown in Table 5-7.

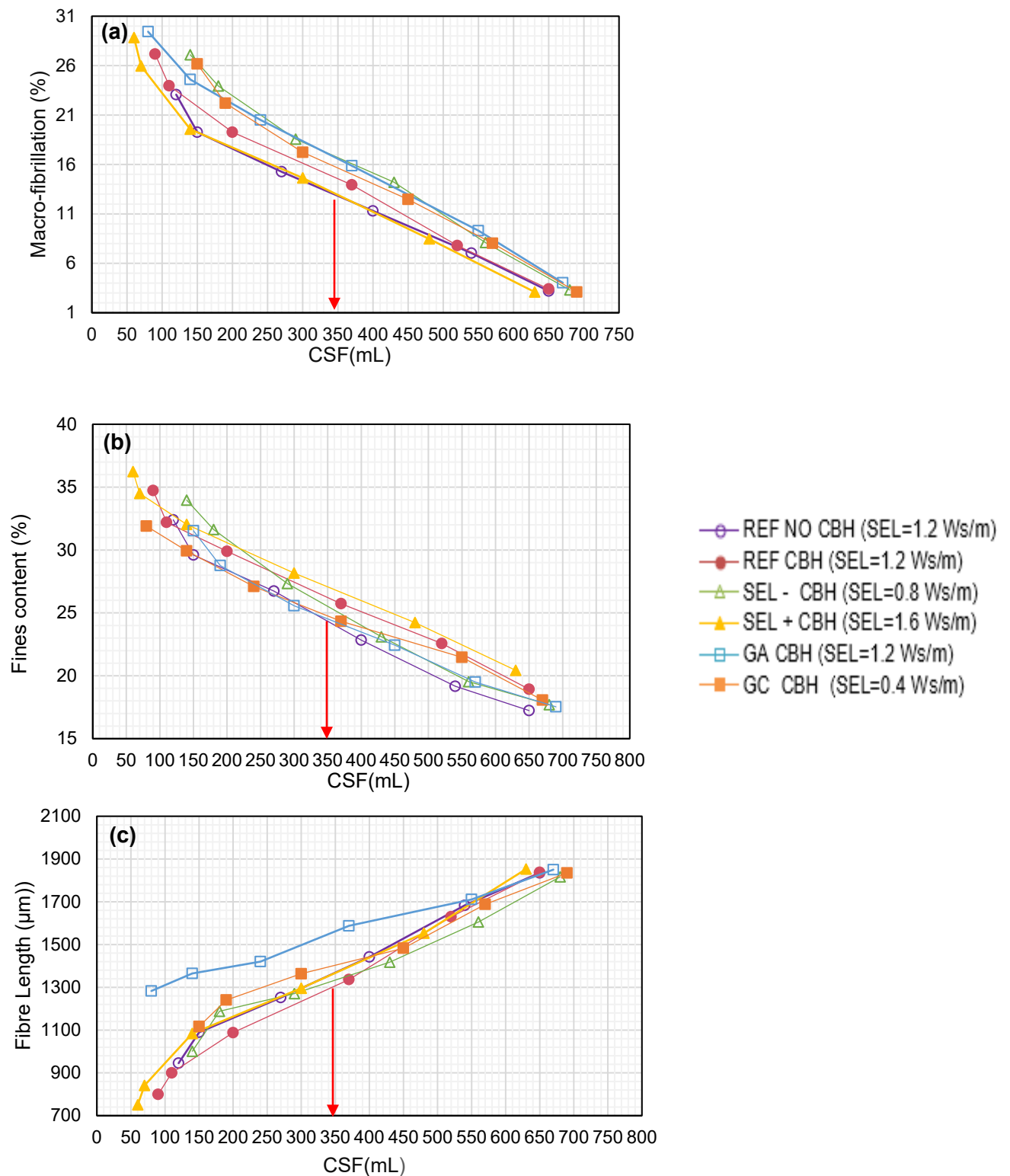


Figure 5-7: Effect of CBH I pre-treatment and mechanical refining on (a) macro-fibrillation (b) fines content and (c) fibre length for the FBSW pulp relative to the CSF. All samples except the reference (REF NO CBH) were pre-treated with CBH I.

Table 5-7: Macro-fibrillation (%), fines content (%) and fibre length (μm) measured at a CSF of 350 mL for a FBSW pulp that was mechanical refined using different refiner settings. All samples except the reference (REF NO CBH) were pre-treated with CBH I. The change (%) between the reference and the treated samples was also calculated.

Run	Macro-fibrillation		Fines content		Fibre length	
	%	*Change from REF (%)	%	*Change from REF (%)	μm	*Change from REF (%)
REF NO CBH (SEL = 1.2 Ws/m)	12.8	NA	24.7	NA	1367	NA
REF CBH (SEL = 1.2 Ws/m)	14.5	+13.2	25.6	+3.6	1307	-4.4
SEL+ CBH (SEL = 1.6 Ws/m)	13.3	-8.3	26.4	+3.1	1317	+0.8
SEL- CBH (SEL = 0.8 Ws/m)	16.4	+13.1	24.5	+4.3	1343	+2.8
GC CBH (SEL = 0.4 Ws/m)	15.8	+8.9	24.6	-3.9	1408	+7.9
GA CBH (SEL = 1.2 Ws/m)	15.6	+7.6	24.8	-3.1	1544	+18.1
* = change (%) for REF CBH calculated based on change from REF NO CBH, while for all the other runs the change (%) is calculated based on change from REF CBH						
Values were obtained using a Microsoft Excel trendline. A trendline with an R-squared value at or near 1 was chosen for computing a specific fibre property at the targeted 350 mL CSF.						

Comparing the two REF runs, as shown in Figure 5-7 (a-c), the introduction of CBH I during the repulping stage improved the macro-fibrillation by 13.2%. Consequently, this led to a slight increase in the fines content present by 3.6%. Furthermore, the fibre cutting effect increased by 4.4% at the targeted CSF. The changes in fibre morphology results for the two REF runs are in accordance with the CSF drop observed in section 5.2.1. Increasing SEL relative to the REF CBH I run had a negative effect, and the macro-fibrillation decreased by 8%. Furthermore, 3.1% higher fines content were measured for the SEL+ run, whereas the percentage change in fibre cutting effect remained relatively the same (0.8% difference) (Table 5-7). This suggested that the high intensity treatment combined with the enzyme treatment was not compatible with the FBSW fibres, resulting in poor fibre development. Decreasing SEL had a positive effect, and an improvement of 13.1% in macro-fibrillation was measured. In addition, a slightly lower fibre cutting effect and quantity of fines present (2.8% and 4.3%, respectively) compared to the REF CBH I run was measured. The results indicated that the SEL- run experienced more fibrillation than cutting, presumably due to the low SEL applied to

the fibres. The fibre morphology results for the SEL runs are consistent with the CSF drop observed relative to the treated reference run.

The alteration of the refiner plate designs (GC & GA) improved the macro-fibrillation (by 8.9% and 7.6%, respectively) in comparison to the REF CBH I run. Changing the plate design to smaller bar widths decreased the fines content by 3.9%. Similarly, the GA run, compared to the REF CBH I run, slightly decreased the fines content by 3.1% at the targeted CSF of 350 mL. The low fines content results measured for GC and GA are consistent with the low fibre cutting effect (7.9% and 18.1%, respectively) measured compared to the REF CBH I run. The fibre morphology results are also consistent with the slower CSF drop observed for GC and GA relative to the REF CBH I run.

5.2.3 Effect of CBH I pre-treatment and refining on the handsheet properties

The changes in the fibre morphology affect the final handsheet properties. Therefore, handsheet strength, structural and optical properties were measured.

5.2.3.1 Handsheet strength properties

The handsheet strength properties analysed for the FBSW were tensile strength, burst strength, and tear strength, and the results are shown in Figure 5-8 (a), Figure 5-8 (b) and Figure 5-8 (c), respectively. Table 5-8 shows the handsheet strength properties of the FBSW pulp at the desired CSF of 350 mL. As shown in Figure 5-8 (a and b) both the tensile and burst strength developments increased as the CSF decreased. Burst strength development has been reported to occur in a comparable manner as the tensile strength development (Rampersadh, 2005). The same pattern between tensile and burst strengths was observed for the FBSW. As previously stated, inter-fibre bonding influences both tensile and burst strength developments, which is influenced by macro-fibrillation.

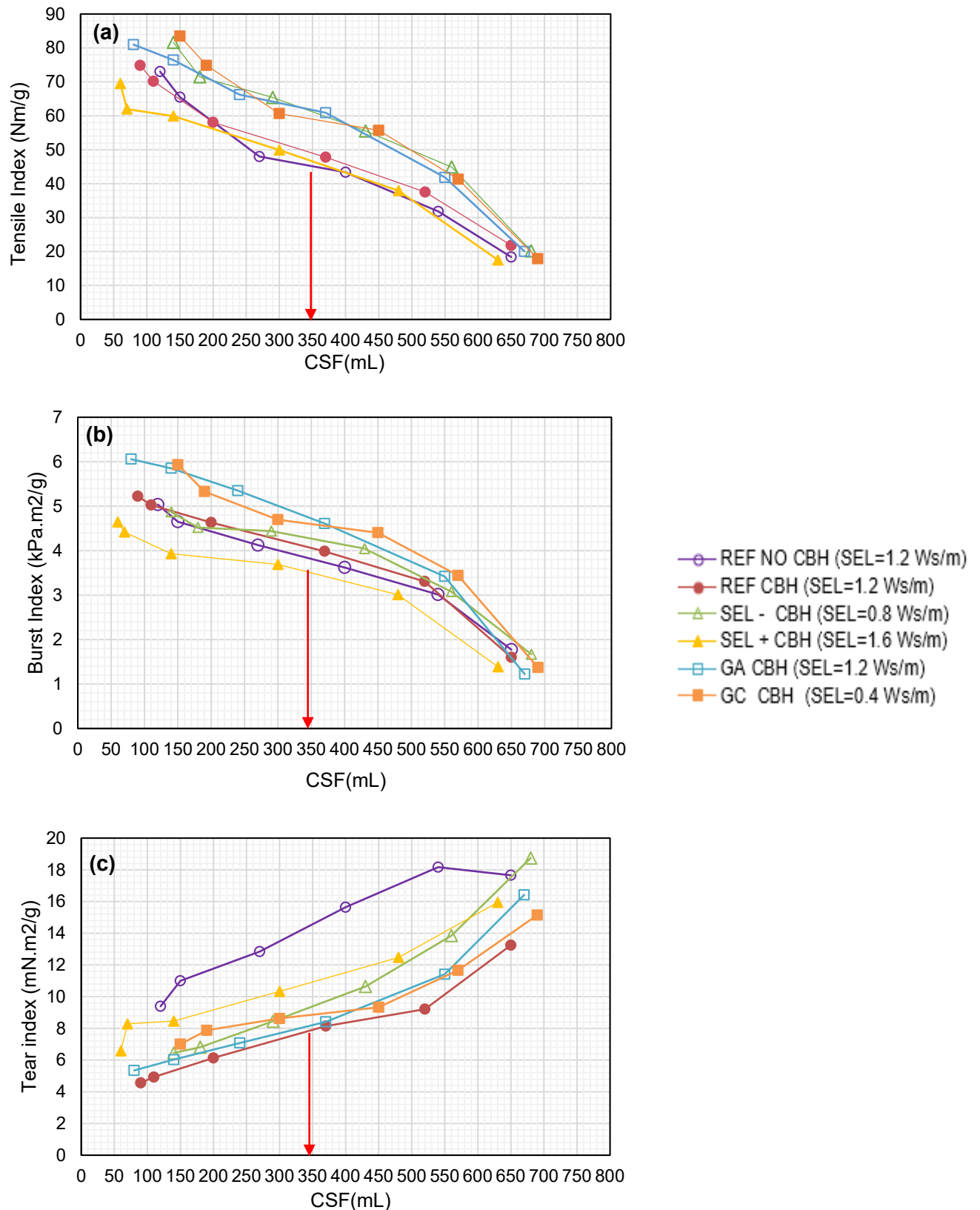


Figure 5-8: Effect of CBH I pre-treatment and mechanical refining on (a) tensile index, (b) burst index and (c) tear index for the FBSW pulp relative to the CSF. All samples except the reference (REF NO CBH) were pre-treated with CBH I.

Table 5-8: Tensile index (Nm/g), burst index (kPa.m²/g) and tear index (mN.m²/g) measured at a CSF of 350 mL for a FBSW pulp that was mechanical refined using different refiner settings. All samples except the reference (REF NO CBH) were pre-treated with CBH I. The change (%) between the reference and the treated samples was also calculated.

Run	Tensile Index		Burst Index		Tear Index	
	Nm/g	*Change from REF (%)	kPa.m ² /g	*Change from REF (%)	mN.m ² /g	*Change from REF (%)
REF NO CBH (SEL = 1.2 Ws/m)	48.1	NA	3.9	NA	14.0	NA
REF CBH (SEL = 1.2 Ws/m)	51.0	+6.0	4.1	+5.1	8.1	-42.0
SEL+ CBH (SEL = 1.6 Ws/m)	46.0	-9.0	3.5	-14.6	10.8	-33.3
SEL- CBH (SEL = 0.8 Ws/m)	60.5	+18.6	4.6	+12.2	10.2	25.9
GC CBH (SEL = 0.4 Ws/m)	62.4	+22.4	4.9	19.5	9.4	16.0
GA CBH (SEL = 1.2 Ws/m)	61.0	+19.6	5.0	22.0	9.3	14.6
* = change (%) for REF CBH calculated based on change from REF NO CBH, while for all the other runs the change (%) is calculated based on change from REF CBH						
Values were obtained using a Microsoft Excel trendline. A trendline with an R-squared value at or near 1 was chosen for computing a specific handsheet property at the targeted 350 mL CSF.						

Comparing the two REF runs, the improvement in macro-fibrillation due to CBH I pre-treatment led to an improvement in both the tensile strength and burst strength developments by 6.0% and 5.1%, respectively, at the targeted CSF. Increasing SEL resulted in a reduction in both the tensile and burst strength developments (by 9.0% and 14.6%, respectively) compared to the REF CBH I run. In contrast, decreasing SEL had the opposite effect, and the tensile and burst strengths increased by 18.6% and 12.2%, respectively. The SEL runs correlate with the macro-fibrillation observed for both runs in section 5.2.2. In comparison to the REF CBH I run, both GA and GC achieved higher tensile (19.6% and 22.4%, respectively) and burst strength (22.0% and 19.5%, respectively) developments, as shown in Table 5-8. This is presumably attributed to the improved fibrillation and low fibre cutting effect induced by both runs, resulting in stronger inter-fibre bonds.

Figure 5-8 (c) shows that the tear strength and the CSF for the FBSW had a direct correlation. As previously mentioned in section 5.1.3, fibre length is the predominant factor influencing the tear resistance of a sheet. The addition of CBH I pre-treatment led to an increase in the fibre

cutting effect. This led to a decrease in the tear strength of 42.0% for the REF CBH I run compared to the untreated reference run.

The remaining refining runs (SEL-, SEL+, GA, GC) had a higher tear strength compared to the REF CBH I run, but lower compared to the untreated reference run (Table 5-8). This was attributed to the low fibre cutting effect experienced by the refining runs compared to the REF CBH I run but a higher fibre cutting effect compared to the untreated reference run.

5.2.3.2 Handsheet structural properties

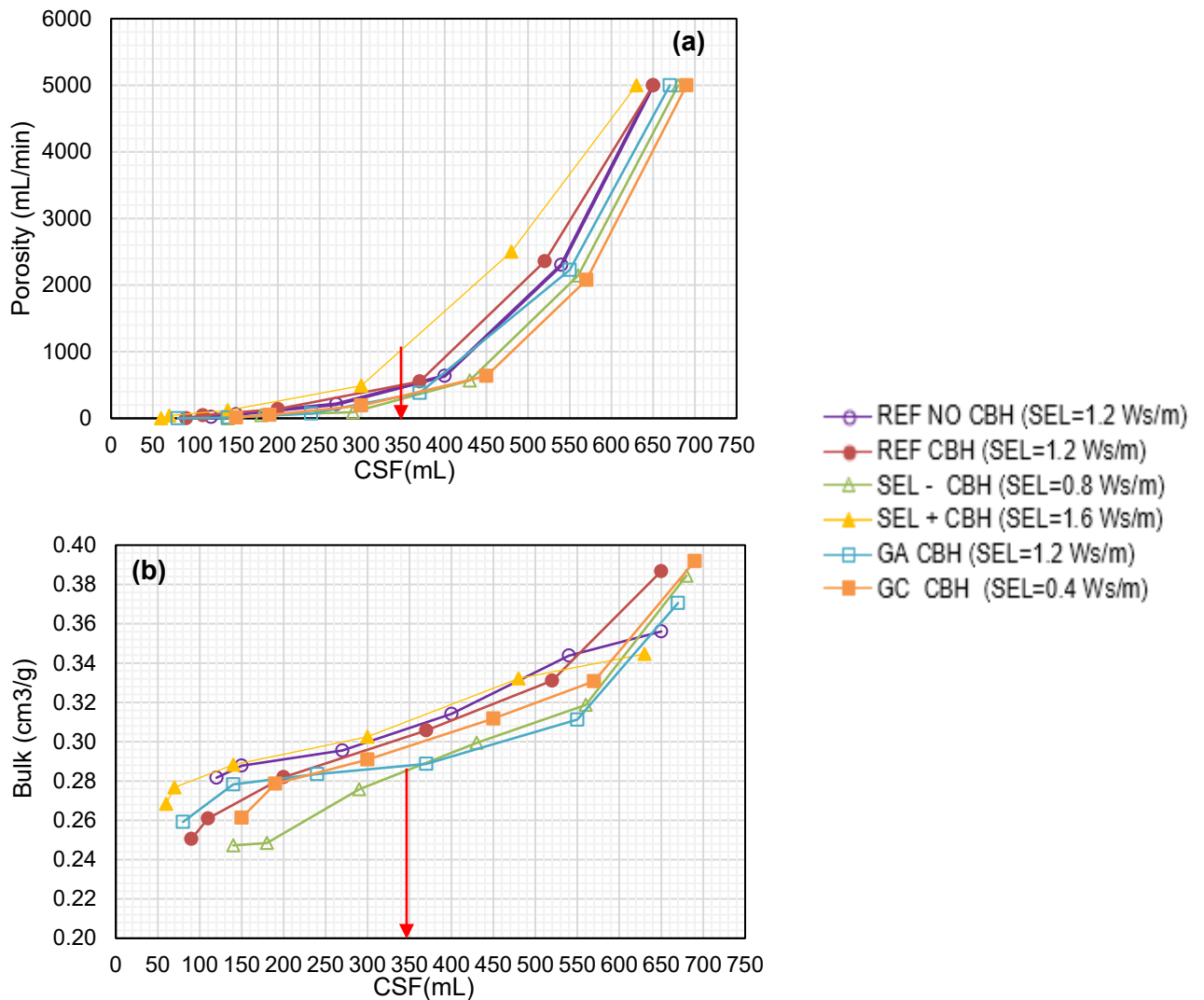


Figure 5-9: Effect of CBH I pre-treatment and mechanical refining on (a) porosity and (b) bulk specific volume for the FBSW pulp relative to the CSF. All samples except the reference (REF NO CBH) were pre-treated with CBH I.

Table 5-9: Porosity (mL/min) and bulk (cm³/g) measured at a CSF of 350 mL for a FBSW pulp that was mechanical refined using different refiner settings. All samples except the reference (REF NO CBH) were pre-treated with CBH I. The change (%) between the reference and the treated samples was also calculated.

Run	Porosity		Bulk	
	mL/min	*Change from REF (%)	cm ³ /g	*Change from REF (%)
REF NO CBH (SEL = 1.2 Ws/m)	472	NA	0.31	NA
REF CBH (SEL = 1.2 Ws/m)	292	-38.1	0.30	-3.3
SEL+ CBH (SEL = 1.6 Ws/m)	970	+232.1	0.31	+3.3
SEL- CBH (SEL = 0.8 Ws/m)	232	-20.5	0.29	-3.3
GC CBH (SEL = 0.4 Ws/m)	200	-31.5	0.29	-3.3
GA CBH (SEL = 1.2 Ws/m)	272	-6.8	0.29	-3.3
* = change (%) for REF CBH calculated based on change from REF NO CBH, while for all the other runs the change (%) is calculated based on change from REF CBH				
Values were obtained using a Microsoft Excel trendline. A trendline with an R-squared value at or near 1 was chosen for computing a specific handsheet property at the targeted 350 mL CSF.				

Porosity and bulk specific volume were the structural properties analysed for the FBSW and the results are shown in Figure 5-9 (a) and Figure 5-9 (b), respectively. The porosity and bulk values for each run at the desired CSF are shown in Table 5-9. As seen in Figure 5-9 (a and b) both porosity and bulk decreased as the degree of refining increased. The reason for this trend has been given in section 5.1.3. Comparing the two REF runs, the CBH I pre-treatment decreased the bulkiness and porosity of the fibres by 3.3% and 38.1%, respectively, at the CSF of 350 mL.

The SEL- run decreased the bulkiness and porosity by 3.3% and 20.7%, respectively. Whereas the SEL+ run increased both properties compared to the REF CBH I run, as seen in Table 5-9. This was consistent with the macro-fibrillation results observed for the SEL runs in section 5.2.2. The GA run resulted in a reduced bulk and porosity by 3.3% and 6.8%, respectively, compared to the REF CBH I run. Similar observations were also made for the GC run and the bulk and porosity decreased by 3.3% and 31.5%, respectively. This was

attributed to the high macro-fibrillation measured for both GA and GC, which decreased the fibre wall thickness and increased the bonding ability of the fibres.

5.2.3.3 Handsheet optical properties

Opacity was the optical property measured in this study, and the results relative to the CSF are shown in Figure 5-10. Table 5-10 shows the optical property values at the desired CSF of 350 mL for the FBSW pulp. As shown in Figure 5-10, the opacity of the FBSW decreased as the CSF decreased. Comparing the two REF runs, the CBH I charge decreased the opacity by 3.9% at the desired CSF. Varying SEL and changing the refiner plate design led to a similar opacity as the REF CBH I run (74-72%) (Table 5-10). The results indicated that the addition of the enzyme treatment had a significant impact on the opacity, while the alteration of SEL and refiner plate designs had little/no significant impact on the opacity.

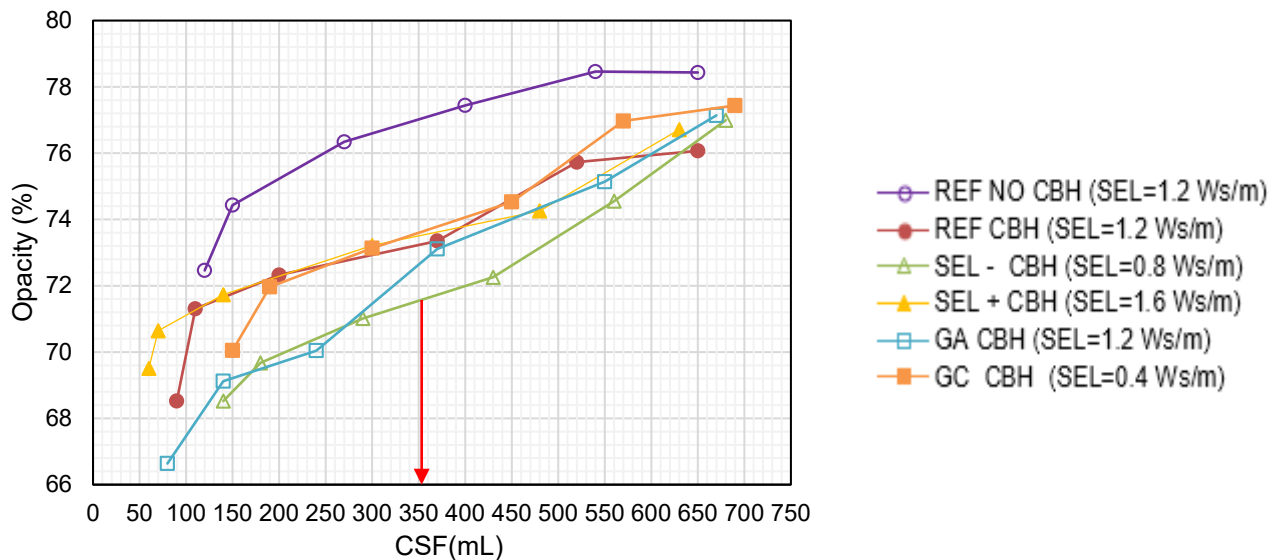


Figure 5-10: Effect of CBH I pre-treatment and mechanical refining on the opacity for the FBSW pulp relative to the CSF. All samples except the reference (REF NO CBH) were pre-treated with CBH I.

Table 5-10: Opacity (%) measured at a CSF of 350 mL for a FBSW pulp that was mechanical refined using different refiner settings. All samples except the reference (REF NO CBH) were pre-treated with CBH I. The change (%) between the reference and the treated samples was also calculated.

Run	Opacity	
	%	*Change from REF (%)
REF NO CBH (SEL = 1.2 Ws/m)	76	NA
REF CBH (SEL = 1.2 Ws/m)	73	-3.9
SEL+ CBH (SEL = 1.6 Ws/m)	74	+1.4
SEL- CBH (SEL = 0.8 Ws/m)	72	-1.4
GC CBH (SEL = 0.4 Ws/m)	74	+1.4
GA CBH (SEL = 1.2 Ws/m)	72	-1.4
* = change (%) for REF CBH calculated based on change from REF NO CBH, while for all the other runs the change (%) is calculated based on change from REF CBH		
Values were obtained using a Microsoft Excel trendline. A trendline with an R-squared value at or near 1 was chosen for computing a specific handsheet property at the targeted 350 mL CSF.		

5.2.4 Recommended bijective refining run for the FBSW pulp

Softwood pulps are primarily used in refining to provide the required strength properties to the final product. The use of CBH I improved the fibre development, which led to an improvement in the FBSW strength properties. However, the REF CBH I run had a higher tear strength of compared to the other refining runs. Consequently, the GA and GC runs would be recommended for FBSW refining as the runs showed the best macro-fibrillation with a low fibre cutting effect. This improved both the tensile and tear strength.

5.3 Pilot refining bijective study for the UBSW pulp

5.3.1 Effect of CBH I pre-treatment and refining on the CSF drop

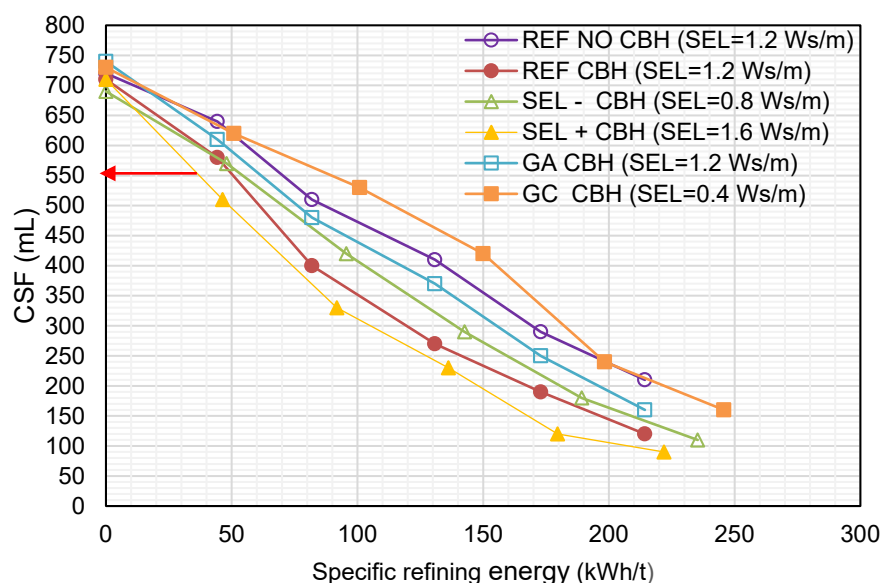


Figure 5-11: Effect of CBH I pre-treatment and the specific refining energy relative to the CSF for the UBSW pulp. All samples except the reference (REF NO CBH) were pre-treated with CBH I.

Table 5-11: Specific refining energy (kWh/t) measured at a CSF of 550 mL for an UBSW pulp that was mechanical refined using different refiner settings. All samples except the reference (REF NO CBH) were pre-treated with CBH I. The change (%) between the reference and the treated samples was also calculated.

Run	Specific refining energy	
	kWh/t	*Change from REF (%)
REF NO CBH (SEL = 1.2 Ws/m)	68.2	NA
REF CBH (SEL = 1.2 Ws/m)	48.2	-29.3
SEL+ CBH (SEL = 1.6 Ws/m)	35.8	-25.7
SEL- CBH (SEL = 0.8 Ws/m)	51.2	+6.2
GC CBH (SEL = 0.4 Ws/m)	82.8	+71.8
GA CBH (SEL = 1.2 Ws/m)	65.2	+35.3
* = change (%) for REF CBH calculated based on change from REF NO CBH, while for all the other runs the change (%) is calculated based on change from REF CBH		

The CSF was determined immediately after refining and the results are shown in Figure 5-11. The CSF of interest for the UBSW pulp was 550 mL. The specific refining energy at the desired CSF of 550 mL for each run is shown in Table 5-11. As seen in Figure 5-11, the CSF decreased as the refining energy transferred to the fibres increased. As previously stated in section 5.1.1, this is expected due to the effects of refining. In comparison to the untreated reference run, the CBH I charge accelerated the CSF drop, allowing the desired CSF to be reached at lower refining energy, enabling energy savings of 29.3%.

The effect of the refining parameter, SEL, on the CSF drop was subsequently investigated. When compared to the REF CBH I run, a higher SEL resulted in a faster CSF drop and a 25.7% lower refining energy demand to reach the desired CSF. A lower SEL resulted in a slower CSF drop and a 6.2% greater refining energy requirement. Changing the refiner plate design to a larger bar angle required 35.3% more refining energy than the REF CBH I run, but less refining energy than the untreated reference run to achieve the desired CSF. On the contrary, the GC run required the highest refining energy (71.8% higher compared to REF CBH I run) to obtain the targeted CSF, which could be attributable to the low SEL of the plate design. The reasons for the observed trends for the CSF have been explained in section 5.1.1. Nevertheless, to better understand the changes in the CSF drop, the changes in the fibre morphology were analysed.

5.3.2 Effect of CBH I pre-treatment and refining on the fibre morphology

Three primary fibre morphology changes were analysed for the UBSW pulp, namely, the macro-fibrillation, fines content, and the fibre length (an indication of the fibre cutting effect); the results are shown in Figure 5-12 (a), Figure 5-12 (b) and Figure 5-12 (c), respectively. As illustrated in Figure 5-12 (a and b), the macro-fibrillation and fines content increased as the CSF dropped. The fibre cutting effect increased as the CSF dropped, as shown in Figure 5-12 (c). The fibre properties obtained at the desired CSF of 350 mL are shown in Table 5-12.

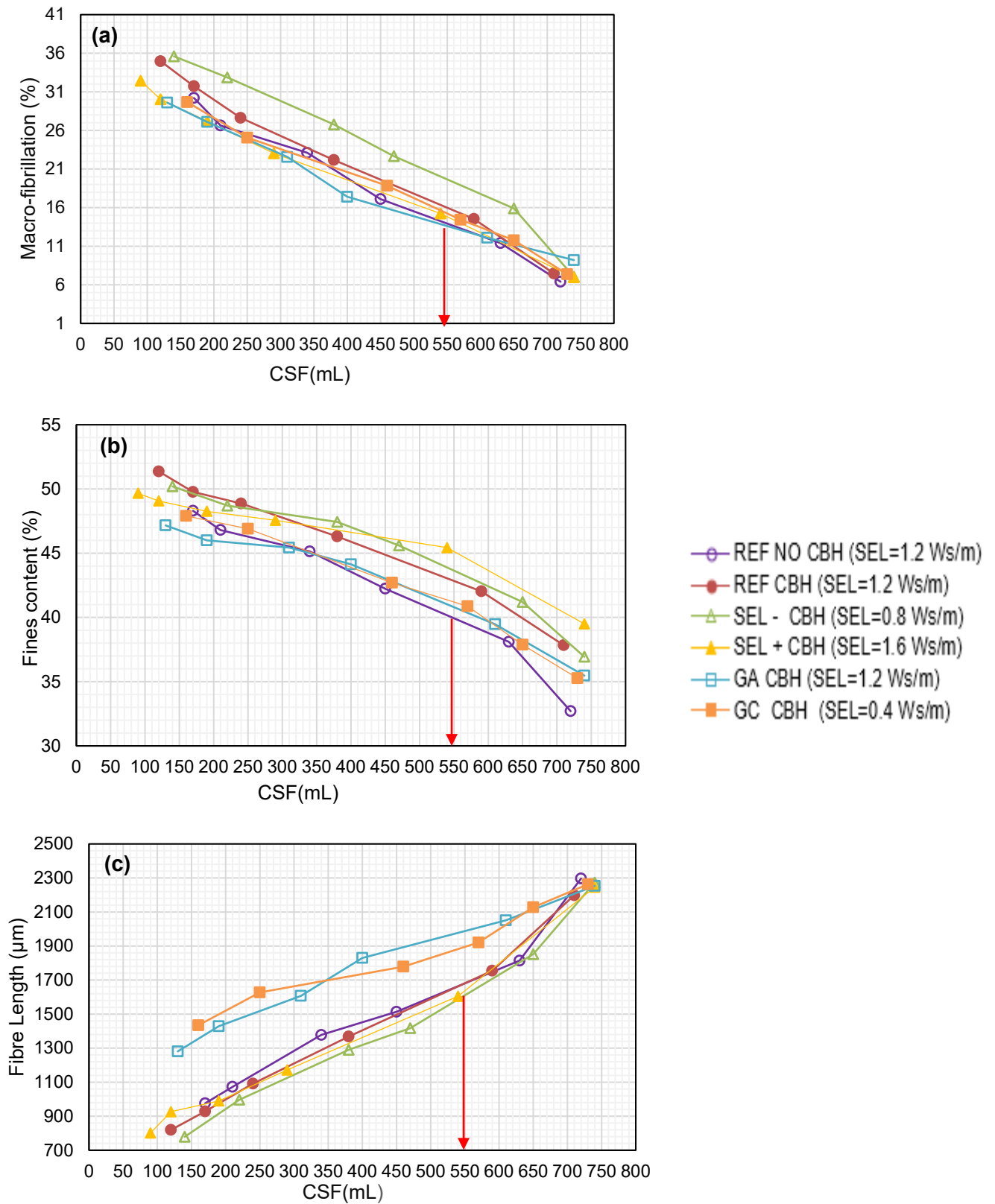


Figure 5-12: Effect of CBH I pre-treatment and mechanical refining on (a) macro-fibrillation, (b) fines content and (c) fibre length for the UBSW pulp relative to the CSF. All samples except the reference (REF NO CBH) were pre-treated with CBH I.

Table 5-12: Macro-fibrillation (%), fines content (%) and fibre length (μm) measured at a CSF of 550 mL for an UBSW pulp that was mechanical refined using different refiner settings. All samples except the reference (REF NO CBH) were pre-treated with CBH I. The change (%) between the reference and the treated samples was also calculated.

Run	Macro-fibrillation		Fines content		Fibre length	
	%	*Change from REF (%)	%	*Change from REF (%)	μm	*Change from REF (%)
REF NO CBH (SEL = 1.2 Ws/m)	13.2	NA	39.0	NA	1543	NA
REF CBH (SEL = 1.2 Ws/m)	14.6	+10.6	43.0	+10.3	1768	+14.6
SEL+ CBH (SEL = 1.6 Ws/m)	13.7	-6.2	45.0	+4.6	1684	-4.8
SEL- CBH (SEL = 0.8 Ws/m)	18.5	+26.7	44.0	+2.3	1510	-14.6
GC CBH (SEL = 0.4 Ws/m)	15.0	+2.7	42.0	-2.3	1917	+8.4
GA CBH (SEL = 1.2 Ws/m)	14.0	-4.1	41.0	-4.7	2020	+14.2
* = change (%) for REF CBH calculated based on change from REF NO CBH, while for all the other runs the change (%) is calculated based on change from REF CBH						
Values were obtained using a Microsoft Excel trendline. A trendline with an R-squared value at or near 1 was chosen for computing a specific fibre property at the targeted 550 mL CSF.						

As shown in Figure 5-12 (a-c), the CBH I charge improved macro-fibrillation by 10.6%, compared to the untreated reference run. Furthermore, the fines content increased from 10.3% at the targeted CSF. Even though the REF CBH I run had higher fines content, it also had a 14.6% lower fibre cutting effect at the desired CSF (Table 5-12). Thus, the higher fines content was due to the higher macro-fibrillation and not fibre cutting. These results implied that keeping the refining conditions constant and introducing the CBH I charge for the UBSW preserved the fibre length, therefore, reducing the fibre cutting effect.

Increasing the SEL decreased the macro-fibrillation by 6.2%. On the contrary, reducing SEL significantly improved the macro-fibrillation by 26.7%. This indicated that the UBSW fibres are compatible with moderate to low refining intensity treatment. The SEL+ and SEL- runs both induced a higher fibre cutting effect (by 4.6% and 2.3%, respectively) compared to the REF CBH I run. Consequently, this induced a higher quantity of fines present at the targeted CSF, as seen in Table 5-12. The results for the SEL+ run are consistent with what has been reported in literature, *i.e.*, a high intensity induces a higher fibre cutting effect (Lecourt *et al.*, 2010b). For the SEL- run, in general, a low-intensity run produces a low fibre cutting effect; however,

the opposite was observed for the UBSW. This might be due to the significant fibrillation observed and the amount of energy consumed by the SEL- run to reach the desired CSF.

The GA led to a decrease in macro-fibrillation of 4.1%, whereas the GC run increased macro-fibrillation by 2.7%, respectively, compared to the REF CBH I run. The refining runs also had a reduced amount of fines present at the desired CSF. Furthermore, the GA and GC (4.7% and 2.3%, respectively) runs resulted in a lower fibre cutting effect compared to all the other runs at the selected CSF of 550 mL (Table 5-12). The fibre morphology results for GA and GC indicated that the UBSW fibres are not compatible with increasing the bar angle and/or decreasing the bar width in the refiner plate design.

5.3.3 Effect of CBH I pre-treatment and refining on the handsheet properties

The changes in fibre morphology affect the final handsheet properties. Consequently, the handsheet strength, structural and optical properties were measured.

5.3.3.1 Handsheet strength properties

The essential strength property in unbleached pulps is compressive strength, also known as stacking strength (Bocianowski *et al.*, 2012; Ju *et al.*, 2005). As previously mentioned in CHAPTER 2, section 2.2.5, unbleached pulps are used in packaging materials, such as boxes and cartons. Therefore, the testing of compressive strength is essential as boxes and cartons are frequently stacked on top of one another, resulting in compressional forces. Thus, packages need to be rigid and not crumble under compressive stress during storage and transportation of the boxes (Ek *et al.*, 2009a). As a result, in this study, the Short-span Compressive Test (SCT) and Corrugating Medium Test (CMT) were analysed for the UBSW. The results for these two properties relative to the CSF are shown in Figure 5-13 (a and b). The SCT and CMT values at the desired CSF are shown in Table 5-13.

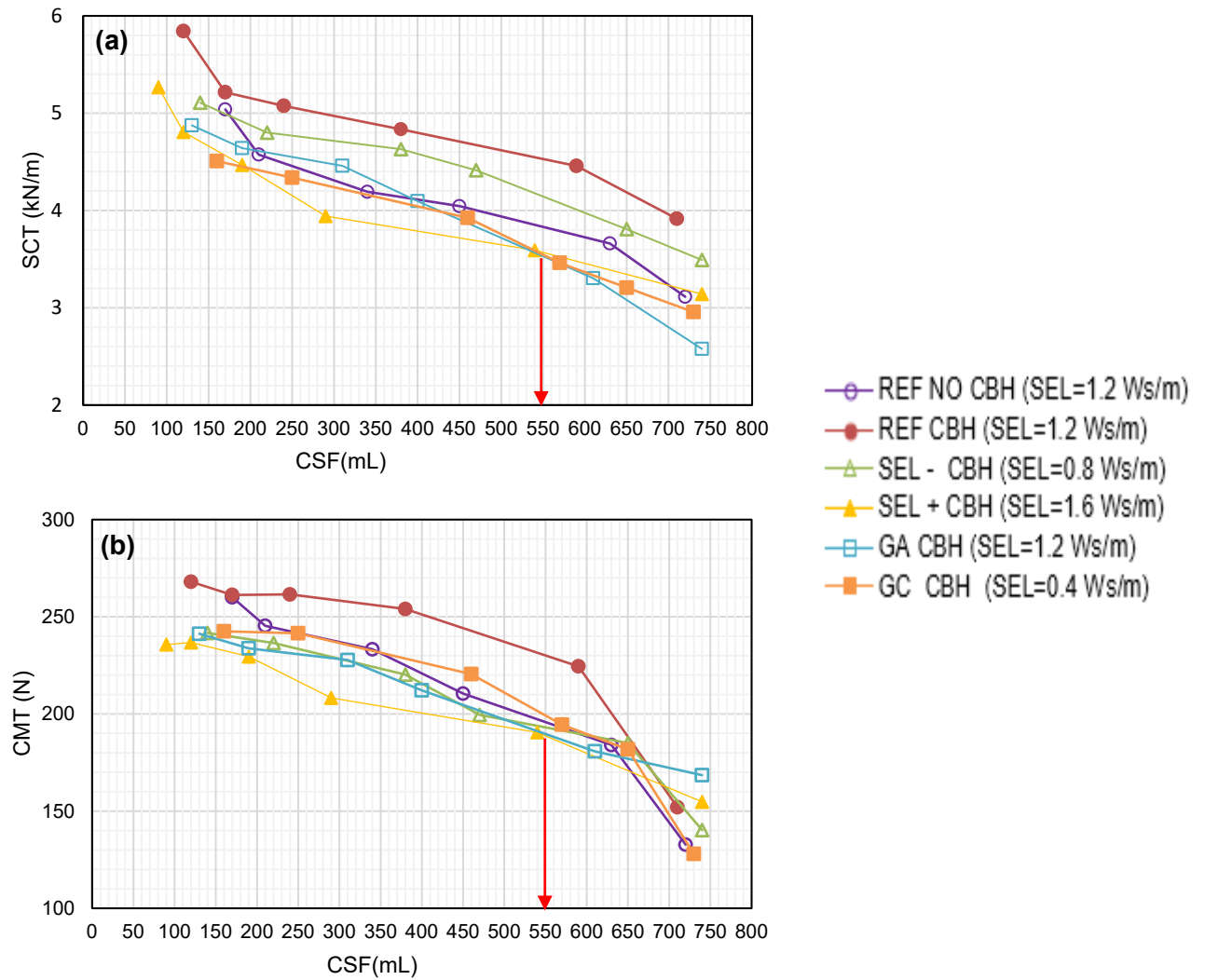


Figure 5-13: Effect of CBH I pre-treatment and mechanical refining on (a) SCT and (b) CMT for the UBSW pulp relative to the CSF. All samples except the reference (REF NO CBH) were pre-treated with CBH I.

Table 5-13: SCT (kN/m) and CMT (N) measured at a CSF of 550 mL for an UBSW pulp that was mechanical refined using different refiner settings. All samples except the reference (REF NO CBH) were pre-treated with CBH I. The change (%) between the reference and the treated samples was also calculated.

Run	SCT		CMT	
	kN/m	*Change from REF (%)	N	*Change from REF (%)
REF NO CBH (SEL = 1.2 Ws/m)	3.8	NA	187	NA
REF CBH (SEL = 1.2 Ws/m)	4.3	+13.2	226	+20.8
SEL+ CBH (SEL = 1.6 Ws/m)	3.6	-16.3	182	-19.5
SEL- CBH (SEL = 0.8 Ws/m)	4.1	-4.7	185	-18.1
GC CBH (SEL = 0.4 Ws/m)	3.5	-18.6	193	-14.6
GA CBH (SEL = 1.2 Ws/m)	3.5	-18.6	192	-15.0
* = change (%) for REF CBH calculated based on change from REF NO CBH, while for all the other runs the change (%) is calculated based on change from REF CBH				
Values were obtained using a Microsoft Excel trendline. A trendline with an R-squared value at or near 1 was chosen for computing a specific handsheet property at the targeted 550 mL CSF.				

As shown in Figure 5-13 (a and b), SCT and CMT increased as the CSF decreased. Refining increased the SCT and CMT of a sheet as a result of an increase in inter-fibre bonding. Furthermore, refining straightened the fibres, which reduced the stress distribution of the fibres (Ju *et al.*, 2005). Comparing the two REF runs, the CBH I pre-treatment improved SCT and CMT (by 13.2% and 20.8%, respectively) at the CSF of interest. The SEL-, SEL+, GC, and GA runs all had lower SCT and CMT compared to the REF CBH I run as seen in Table 5-13. The REF CBH I run showed higher compressive strength due to both the macro-fibrillation and low fibre cutting effect experienced by the run. The SEL- run might have induced a higher macro-fibrillation, however, it also induced a high fibre cutting effect, resulting in shorter fibres compared to the REF CBH I run fibres. Macro-fibrillation and the length of the fibres are both essential factors in compressive strength. Fibrillation increases the surface area, improving the fibre-fibre bonding, whereas the longer fibres are able to distribute stress over a larger area compared to shorter fibres. Tensile, burst, and tear strength developments for the UBSW were also measured, and the results are shown in Figure 5-14 (a), Figure 5-14 (b), and

Figure 5-14 (c), respectively. Table 5-13 shows the handsheet strength properties for each run at the desired 550 mL CSF.

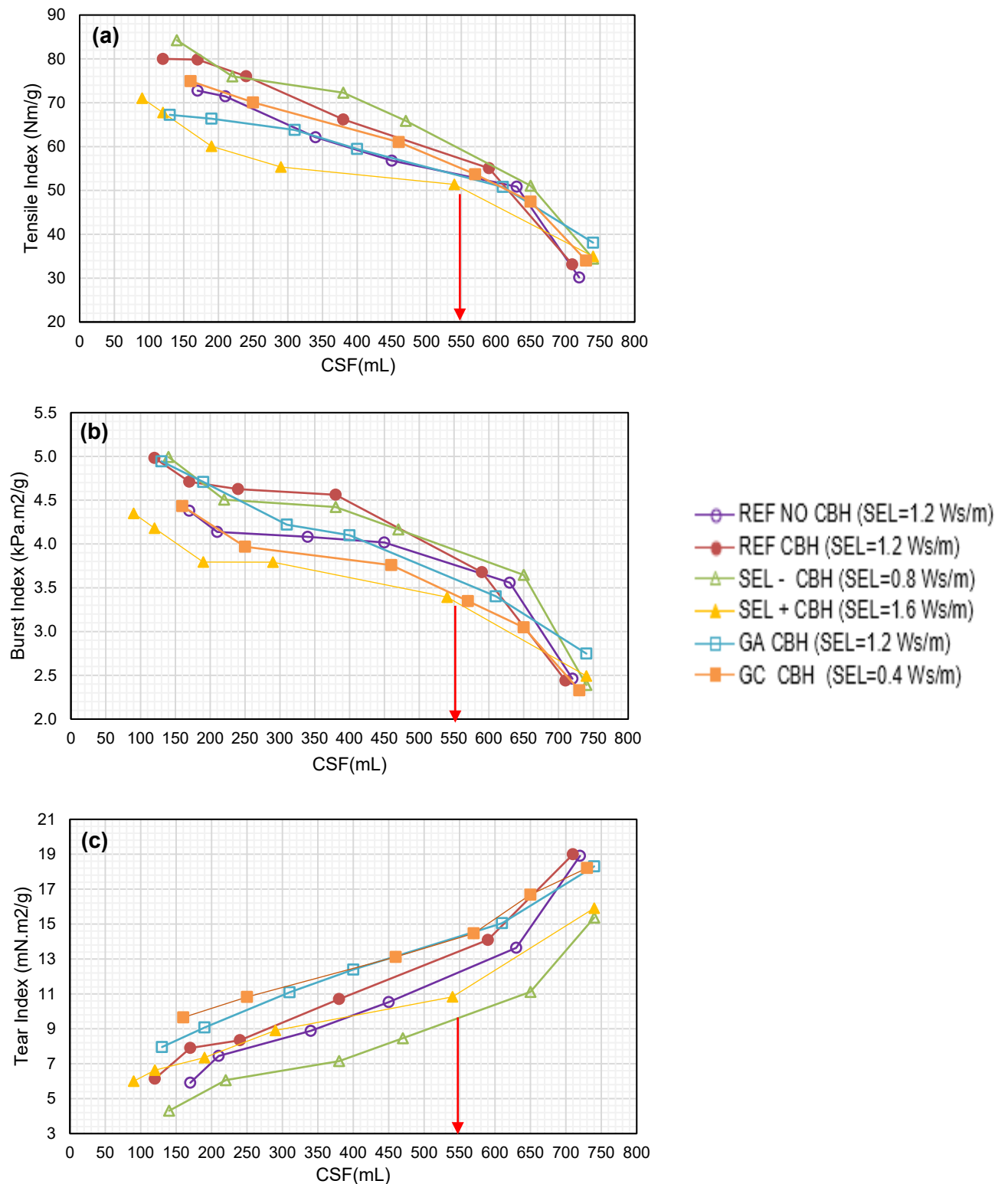


Figure 5-14: Effect of CBH I pre-treatment and mechanical refining on (a) tensile index, (b) burst index and (c) tear index for the UBSW pulp relative to the CSF. All samples except the reference (REF NO CBH) were pre-treated with CBH I.

Table 5-14: Tensile index (Nm/g), burst index (kPa.m2/g) and tear index (mN.m2/g) measured at a CSF of 550 mL for an UBSW pulp that was mechanical refined using different refiner settings. All samples except the reference (REF NO CBH) were pre-treated with CBH I. The change (%) between the reference and the treated samples was also calculated.

Run	Tensile Index		Burst Index		Tear Index	
	Nm/g	*Change from REF (%)	kPa.m2/g	*Change from REF (%)	mN.m2/g	*Change from REF (%)
REF NO CBH (SEL = 1.2 Ws/m)	50.2	NA	3.4	NA	13.6	NA
REF CBH (SEL = 1.2 Ws/m)	52.7	+5.0	3.6	+5.9	14.6	+7.4
SEL+ CBH (SEL = 1.6 Ws/m)	46.0	-12.7	3.5	-2.8	12.6	-13.7
SEL- CBH (SEL = 0.8 Ws/m)	55.5	+5.3	3.8	+5.6	10.6	-27.4
GC CBH (SEL = 0.4 Ws/m)	51.1	-3.0	3.5	-2.8	14.8	+1.4
GA CBH (SEL = 1.2 Ws/m)	50.6	-4.0	3.4	-5.6	14.8	+1.4
* = change (%) for REF CBH calculated based on change from REF NO CBH, while for all the other runs the change (%) is calculated based on change from REF CBH						
Values were obtained using a Microsoft Excel trendline. A trendline with an R-squared value at or near 1 was chosen for computing a specific handsheet property at the targeted 550 mL CSF.						

As shown in Figure 5-14 (a and b), tensile and burst strength developments improved for the REF CBH I run (by 5.0% and 5.9, respectively) in comparison to the untreated reference run. This was due to the CBH I pre-treatment which improved the macro-fibrillation. Higher SEL had a negative effect on the UBSW, due to the low macro-fibrillation observed, decreasing the tensile and burst strength by 12.7% and 2.8%, respectively, compared to the REF CBH I run. In contrast, a reduction in SEL increased both tensile and burst strengths by 5.3% and 5.6%, respectively, compared to the REF CBH I run, presumably due to the high macro-fibrillation observed for the SEL- run at the targeted CSF. The GA plate design showed a decrease in both the tensile and the burst strength developments by 4.0% and 5.6%, respectively, in comparison to the REF CBH I run. Similar results were measured for the GC plate design and the tensile and burst strengths decreased by 3.0% to 3.5%, respectively. Both GC and GA runs results for tensile and burst strength development are consistent with the observed poor fibre development in section 5.3.2.

Figure 5-14 (c) shows the relationship between the tear strength and the CSF. Tear strength is typically reduced by enzymatic refining when compared to an untreated pulp, as previously observed for the BHKP and FBSW. However, the opposite was observed for the UBSW, whereby the tear strength slightly improved at the targeted CSF by 7.4%. Presumably, the similar fibre cutting effect experienced by the REF CBH I run combined with the enzyme treatment improved the tear strength. Due to the high fibre cutting effect, the SEL- and SEL+ runs had lower tear strength (27.4% and 13.7%, respectively) at the desired CSF compared to the REF CBH I run. In contrast, GA and GC runs had a 1.4% higher tear strength compared to the REF CBH I run at the desired CSF. The results for GA and GC are in accordance with the lower fibre cutting effect relative to all the refining runs.

5.3.3.2 Handsheet structural properties

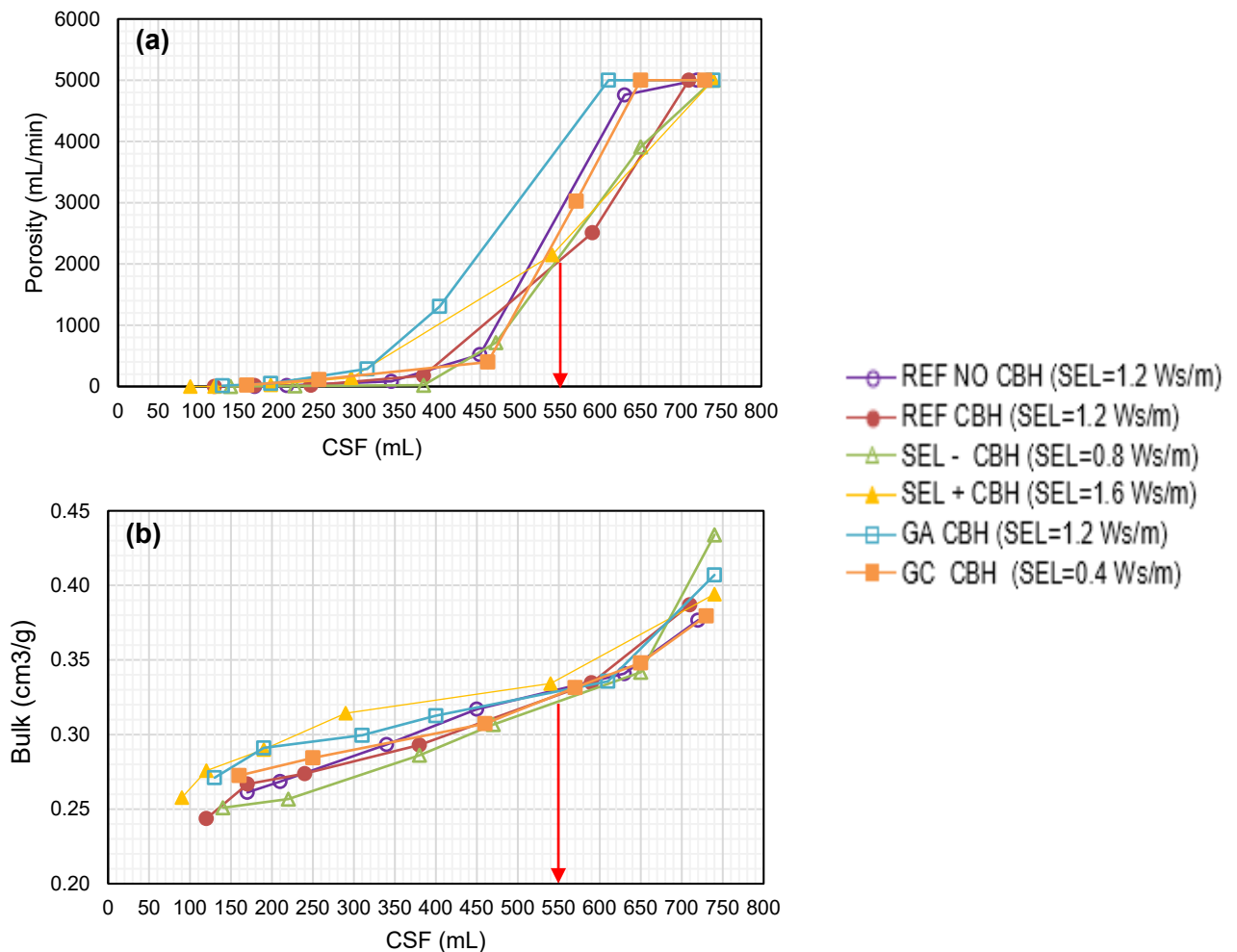


Figure 5-15: Effect of CBH I pre-treatment and mechanical refining on (a) porosity and (b) bulk specific volume for the UBSW pulp relative to the CSF. All samples except the reference (REF NO CBH) were pre-treated with CBH I.

Table 5-15: Porosity (mL/min) and bulk (cm³/g) measured at a CSF of 550 mL for an UBSW pulp that was mechanical refined using different refiner settings. All samples except the reference (REF NO CBH) were pre-treated with CBH I. The change (%) between the reference and the treated samples was also calculated.

Run	Porosity		Bulk	
	mL/min	*Change from REF (%)	cm ³ /g	*Change from REF (%)
REF NO CBH (SEL = 1.2 Ws/m)	3043	NA	0.36	NA
REF CBH (SEL = 1.2 Ws/m)	2736	-9.8	0.33	-8.3
SEL+ CBH (SEL = 1.6 Ws/m)	2776	+1.5	0.35	+6.0
SEL- CBH (SEL = 0.8 Ws/m)	2623	-4.1	0.32	-3.1
GC CBH (SEL = 0.4 Ws/m)	3036	+11.0	0.34	+3.0
GA CBH (SEL = 1.2 Ws/m)	3423	+25.1	0.34	+3.0
* = change (%) for REF CBH calculated based on change from REF NO CBH, while for all the other runs the change (%) is calculated based on change from REF CBH				
Values were obtained using a Microsoft Excel trendline. A trendline with an R-squared value at or near 1 was chosen for computing a specific handsheet property at the targeted 550 mL CSF.				

The structural properties, namely, porosity and bulk specific volume were analysed for the UBSW, and the results are shown in Figure 5-15 (a and b). The structural properties at the CSF of interest for the UBSW pulp are shown in Table 5-14. Figure 5-15 (a and b) shows that both porosity and bulk have a direct correlation with the CSF. The CBH I charge decreased both the bulk and porosity (by 9.8% and 8.3%, respectively) due to the increase in the fibre bonding ability compared to the untreated reference run. The SEL+ run had a 1.5% slightly higher bulk and 6.0% lower porosity compared to the REF CBH I run. The SEL+ results are consistent with the higher fines content (which decreased the porosity) and lower macro-fibrillation (resulting in higher bulk) observed in section 5.3.2. The SEL- run had a reduction in porosity and bulk by 4.1% and 3.1%, respectively compared to the REF CBH I run. This is also consistent with the fines and macro-fibrillation observed for the SEL- run. An increase was observed for the bulk and porosity properties for both the GA (3.0% and 25.1%, respectively) and GC (3.0% and 11.0%, respectively) runs compared to the REF CBH I run, as seen in Table 5-14. The higher bulk and porosity for both runs were consistent with the CSF drop observed for the GA and GC runs, which indicated poor fibre development.

5.3.4 Recommended bijective refining run for the UBSW pulp

As previously mentioned, unbleached pulps are mostly used in packaging materials, thus compressive strength is crucial. Taking this into account, the REF CBH I run improved the fibre development, which led to the highest CMT and SCT compared to all the other refining runs. Therefore, the REF CBH I refining run would be recommended to refine the UBSW pulp.

5.4 Refining bijective studies discussion and concluding remarks

Refining is an important aspect in the production of papermaking stock, which serves as an interlink between the pulp mill and the paper machine. The hydrolytic efficiency of enzymes such as cellulase creates opportunities to counteract the challenges associated with refining while simultaneously improving the process efficiency (Biermann, 1996; Steel, 2010). In this phase, the effect of CBH I pre-treatment was analysed under various refining intensities (SEL) and refiner plate designs. Various pulps that have different fibre morphological properties were used.

In all the pulps, the enzyme-treated reference run induced a faster CSF drop compared to the untreated reference run. This suggested that when the pulp is pre-treated with CBH I, less refining energy was required to reach a specific refining degree. These results were consistent with other research studies that have investigated the potential for energy savings as a result of cellulase treatment prior to refining (Bajpai *et al.*, 2006; Gil *et al.*, 2009; Hyoun-Jin *et al.*, 2006; Lecourt *et al.*, 2010a; Zhang *et al.*, 2013; Znidarsic-Plazl *et al.*, 2009).

Apart from energy savings, CBH I pre-treatment facilitated the delamination of the fibre wall, reducing the bulkiness of the fibre network at the desired CSF. Furthermore, the enzyme-treated reference run also improved the macro-fibrillation and increased the fines generated. Similar findings have been reported in literature studies (Cui *et al.*, 2016; Lin *et al.*, 2018; Tian *et al.*, 2017). This was mostly due to the CBH I enzyme hydrolysing the crystalline region of the cellulose chain. The hydrolytic reaction degenerated the fibre structure, increasing the accessibility of the cellulose-rich layer (S2 layer) during refining. As a result, it amplified the effects of mechanical refining, resulting in more fibrillation, fibre cutting, and fines formation (Znidarsic-Plazl *et al.*, 2009). However, in this study, different fibre cutting effects were observed for each pulp. The fibre cutting effect increased for the FBSW, while for the BHKP the difference was insignificant. The UBSW had the same fibre length as the untreated reference run at the desired CSF. This difference in the fibre cutting effect was presumably attributed to the difference in the fibre morphology properties of each pulp.

The improved macro-fibrillation, in conjunction with the reduction of bulky fibres, enhanced the inter-fibre bonding, thus improving the tensile and burst strengths. This increase in pulp tensile and burst strength after treatment with cellulases is consistent with results found by other researchers for cellulase-assisted refining (Cui *et al.*, 2015; Gil *et al.*, 2009; Lecourt *et al.*, 2010b; Tripathi *et al.*, 2008; Wang *et al.*, 2007; Yoo & Hsieh, 2010). The increase in the fines generated for the enzyme-treated reference run also contributed to the increase in the tensile and burst strength developments. According to Motamedian *et al.* (2019), who investigated the impact of fines on the strength properties, concluded that the addition of fines greatly improved the strength of a sheet due to two mechanisms. Firstly, fines enhanced the inter-fibre bonding by increasing the surface area, fibre swelling as well as chemical compatibility. Thus, fines can fill any voids between the bonded fibres that would otherwise not be bonded if fines were not present. Secondly, fines were presumed to lower stress concentrations in bonded areas, resulting in more uniform stress distributions (Mandlez *et al.*, 2020; Motamedian *et al.*, 2019).

The increase in the fibre cutting effect experienced by the FBSW and BHKP negatively affected the tear strength. Consequently, relative to the untreated pulp, the enzyme-treated reference runs resulted in a 42% and 7% loss for the FBSW and BHKP, respectively. The reduction in the tear strength for the FBSW and BHKP was consistent with what has been reported in literature studies for enzymatic refining (Przybysz Buzala *et al.*, 2016; Wimmer *et al.*, 2002; Zhang *et al.*, 2013). The opposite was observed for the UBSW, and the tear strength improved by 6% compared to the untreated reference run. Although this is not conventional for an enzyme-treated pulp, Znidarsic-Plazl *et al.* (2009) reported similar observations on a softwood pulp treated with a cellulase enzyme. According to Znidarsic-Plazl *et al.* (2009), this action can be explained by the enzyme treatment softening the fibre surface, facilitating the fibrillation effect during refining. As a result, increased fibrillation led to better inter-fibre bonding and, as a result, improved tear resistance.

In comparison to the untreated reference run, the opacity of the bleached pulps was not negatively affected by the CBH I pre-treatment. This indicates that the enzyme treatment can improve the strength properties while maintaining the opacity of a sheet. High opacity is advantageous in printing and book papers (Biermann, 1996).

Investigation of varying SEL during CBH I assisted refining led to notable results. The high refining intensity run (SEL+) was incompatible with all the pulps, resulting in pulp quality deterioration. In contrast, the pulps responded better to the low refining intensity run (SEL-). Similar findings were reported by Lecourt *et al.* (2010b), who investigated the effect of varying SEL during cellulase-assisted refining. According to Lecourt *et al.* (2010b), the low-intensity

treatment results in fibre strength conservation, therefore, improving the strength properties. Whereas the high-intensity treatment combined with the enzyme treatment was too harsh on the fibres and decreases the fibre intrinsic strength. However, the disadvantage of the low-intensity treatments is the high refining energy required to reach the desired CSF. According to Lecourt *et al.* (2010b), this is counteracted by a longer refiner plate life, which is an indirect economic gain in the long run.

In comparison to the enzyme-treated reference run, the investigation of varying the refiner plate design during CBH I assisted refining led to different findings for each pulp. BHKP showed positive results for the GC run, while the GA run resulted in more fibre cutting instead of fibrillation. For the FBSW, both GC and GA runs enhanced the fibre development, leading to an improvement in the handsheet strength properties. On the contrary, both GC and GA runs led to poor fibre development for the UBSW. According to the author's knowledge, there is currently no study reporting on the effect of changing refiner plate designs (increasing bar angle/ decreasing bar widths) during cellulase-assisted refining. Therefore, the results were not comparable to those in literature studies.

The predicted difficulties that may obstruct the implementation of CBH I at an industrial level are that the operating conditions for the enzyme, *i.e.*, temperature and pH, must be strictly regulated. This is related to the fact that enzymes are quickly denatured, even with a minor temperature rise. Furthermore, enzymes are susceptible to contamination with toxic substances, which might have a negative impact on the reaction. This can result in additional energy being consumed, pulp being wasted owing to contamination, resulting in reduced production levels.

The results from this phase showed that the utilisation of CBH I as a refining enzyme has the potential to alleviate the energy/manufacturing costs associated with refining without having a detrimental effect on fibre development. However, optimal refining conditions will need to be determined to optimise the benefits of the enzyme pre-treatment. Thus, in the next phase, the bijective refining data was modelled using customised refining software, with the objective of obtaining the optimal refining parameters at the desired CSF for each pulp.

CHAPTER 6: OPTIMISATION OF REFINING PARAMETERS

Optimisation of the refining parameters is indispensable to ensure optimum paper quality with minimum energy consumption at the desired Canadian Standard Freeness (CSF). Consequently, in phase 3, the CBH I pilot refining data, *i.e.*, CSF, fibre morphology, and handsheet properties obtained during phase 2 for each pulp, was modelled using a customised refining bijective modelling software to optimise CBH I assisted refining. Subsequently, the chosen optimum refining parameters for each pulp were validated by refining the pulp under the optimal conditions in triplicate. In this chapter, the results from this phase are analysed. The chapter starts off in section 6.1 with the analysis of the optimisation results for the BHKP. This is followed by the analysis of the optimisation results for the FBSW and UBSW in sections 6.2 and 6.3, respectively. The chapter concludes in section 6.4 by analysing the scanning electron micrographs of each pulp for the following runs: REF NO CBH I, REF CBH I, and Optimum run. Raw data obtained during this phase is provided in Annexure E.

6.1 BHKP optimisation

A customised refining bijective modelling software was used to model the three different refiner plate designs for the BHKP, namely, REF (3/3/6 mm, 10°), GC (2/2/6 mm, 10°) and GA (3/3/6 mm, 25°). Tensile and tear strengths were then obtained for the BHKP for each plate design at the targeted CSF of 350 mL.

6.1.1 BHKP computational modelling

The models for the three different refiner plate designs at several specific refining energies across different SEL are shown in Figure 6-1. As the targeted CSF was 350 mL, the CSF window of interest was between 300 mL and 400 mL. As shown in Figure 6-1, examining the CSF window of interest, the GC plate design was predicted by the software to produce the highest tensile development, while the GA plate design was predicted to produce the lowest tensile strength development. These results were consistent with the conclusion drawn in phase 2 for the BHKP. The optimum conditions for each plate design were then analysed at the desired CSF of 350 mL, as shown in Table 6-1.

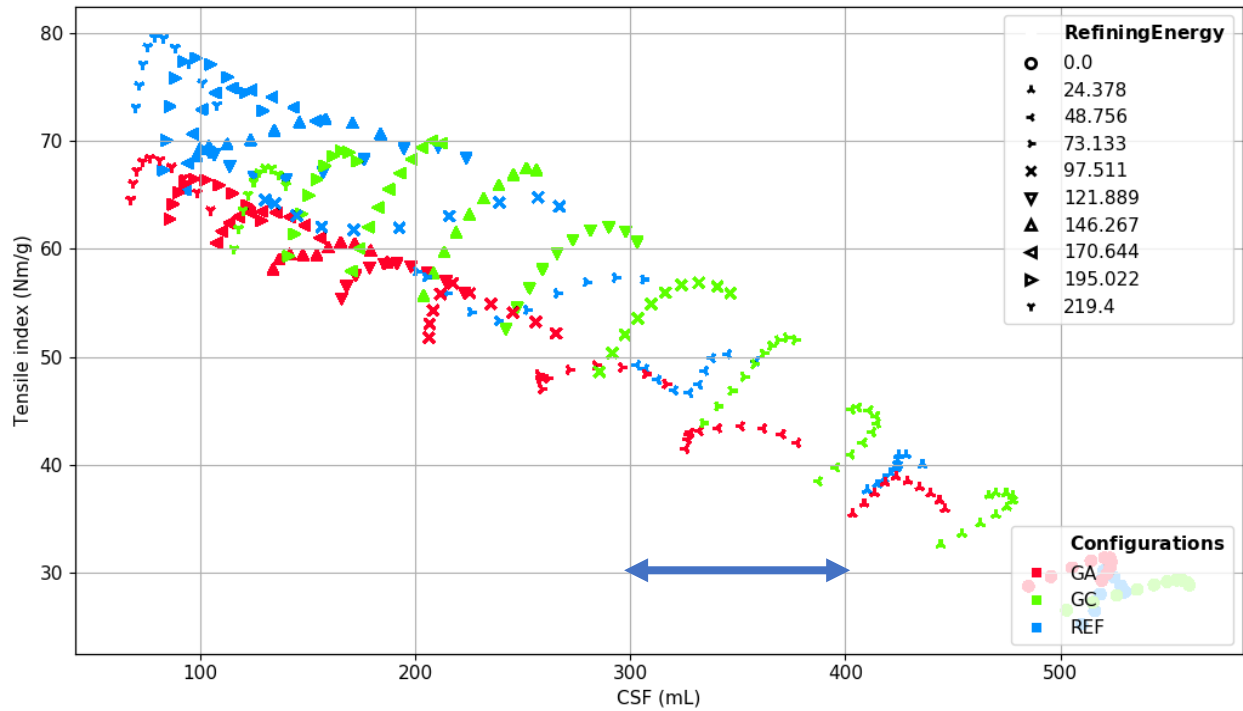


Figure 6-1: Relationship between tensile index and CSF of the BHKP modelling for the three different plate designs

As seen from Table 6-1, the GC plate design was predicted to achieve the highest tensile development of 53.72 Nm/g at the desired CSF. However, also required the highest refining energy (83.5 kWh/t) compared to the other two plate designs. The GA plate design had a lower refining energy requirement compared to the GC plate design; however, the bigger bar angle in the plate design produced the lowest tensile and tear strength development at the desired CSF. The REF plate design required the lowest refining energy requirement (48.5 kWh/t) to reach the targeted CSF. Considering the energy requirement, the tear strength loss, and tensile strength development, the REF plate design presented the best compromise and was chosen to be the optimum plate design to refine the BHKP

Table 6-1: Optimum conditions for the three different plate designs for the BHKP at the targeted CSF of 350 mL

Plate designs (bar code, bar angle)	REF (3/3/6 mm,10°)	GC (2/2/6 mm,10°)	GA (3/3/6 mm,25°)
Refining energy (kWh/t)	45.80	83.50	50.50
SEL (Wm/s)	0.40	0.50	0.50
Tensile Index (Nm/g)	49.38	53.72	43.91
Tear Index (mN.m ² /g)	8.35	8.38	7.45
CSF (mL)	350	350	350

6.1.2 Analysis of BHKP pilot refiner optimisation results

To ascertain that the optimum conditions for the REF plate design generated by the modelling software were reliable, the BHKP was refined using the optimum conditions. The refining run under the optimal conditions was repeated three times for statistical purposes and repeatability, the results are shown in Table 6-2. Afterwards, the average of the three optimum runs was calculated and compared with the six bijective runs conducted in phase 2 for the BHKP, and the results are shown in Table 6-3.

Table 6-2: Pulp properties of the three runs for the BHKP obtained using the optimum conditions generated by the customised bijective modelling software

	Modelling software optimum refining conditions	Run 1	Run 2	Run 3	Standard deviation
Refining energy (kWh/t)	45.8	45.8	45.8	45.8	NA
SEL (Ws/m)	0.4	0.4	0.4	0.4	NA
Tensile Index (Nm/g)	49.38	46.44	43.21	49.39	2.15
Tear Index (mN.m ² /g)	8.35	8.03	9.75	7.98	1.00
CSF (mL)	350	340	370	330	20.82

Table 6-3: Pulp properties of the optimum run and the refining bijective study runs for the BHKP

	Optimum Run	REF NO CBH I	REF CBH I	SEL-	SEL+	GA	GC
Refining energy (kWh/t)	45.8	75.7	50.7	49.7	61.7	55.6	95.6
SEL (Ws/m)	0.4	0.7	0.7	0.4	1.0	0.7	0.3
Tensile Index (Nm/g)	47.18	40.99	42.78	50.38	42.78	43.99	53.20
Tear Index (mN.m ² /g)	8.59	7.84	7.33	7.96	7.02	6.97	7.69
CSF (mL)	347	350	350	350	350	350	350

As shown in Table 6-2, the pulp properties obtained for three optimum runs conducted for the BHKP differ from each other. However, one-way ANOVA at a 95% confidence level indicated that the difference was statistically insignificant between the pulp properties of the three optimum runs. Consequently, the null hypothesis was accepted. This indicated that over a period of time, the pulp properties obtained under the same refining energy and SEL were consistent, and the fluctuations were negligible. Therefore, the customised bijective modelling software's optimum refining energy and SEL were valid and reliable.

As shown in Table 6-3, the optimum run required the lowest refining energy to achieve the desired CSF. For the BHKP, the optimum run was essentially the SEL- run as both runs had the same refiner plate design and SEL, hence the similar tensile strength achieved by both runs. The GC run showed the highest tensile strength as predicted by the modelling software, but also had the highest refining energy requirement. When compared to the untreated run, the optimum run achieved higher tensile strength development at lower refining energy and SEL. This indicates that the CBH I pre-treatment facilitated the partial removal of the fibre wall and swelling of the fibres. As a result, the fibres became more amenable to refining and required less refining energy to reach the desired CSF, without having a detrimental effect on the strength properties. In addition, the low SEL and refining energy were beneficial in preserving the fibre length and strength, as indicated by the higher tear index achieved by the optimum run.

6.2 FBSW optimisation

A customised refining bijective modelling software was used to model the three different refiner plate designs for the FBSW. The plate designs were as follows: REF (5/5/6 mm, 10°), GC (3/3/6 mm, 10°) and GA (5/5/6 mm, 25°). Tensile and tear strengths were then obtained for the FBSW for each plate design at the targeted CSF of 350 mL.

6.2.1 FBSW computational modelling

The models for the three different refiner plate designs at several refining energies across different SEL are shown in Figure 6-2. The freeness window of interest was between 300 mL and 400 mL for the FBSW, as the desired CSF was 350 mL. Thereafter, the pulp properties at the desired CSF were obtained. The results are shown in Table 6-4.

As shown in Figure 6-2, at the CSF window of interest, the GA refiner configuration was predicted by the software to give a slightly higher tensile development compared to the REF and GC refiner plate designs. The results are in accordance with the conclusion drawn in phase 2 for the FBSW.

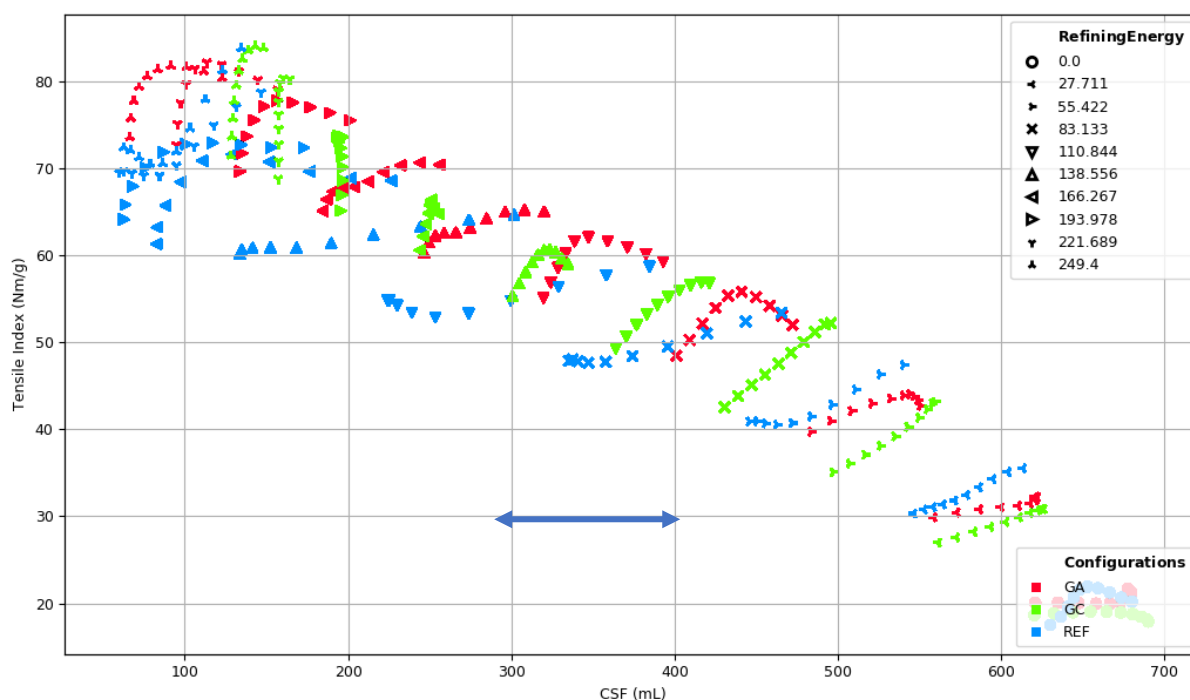


Figure 6-2: Relationship between tensile index and CSF of the FBSW modelling for the three different plate design

As shown in Table 6-4, at the 350 mL CSF, the GA plate design was predicted to attain a slightly higher tensile development at a slightly lower refining energy requirement, compared to the REF plate design. In contrast, the GC plate design was predicted to require the highest refining energy to achieve the lowest tensile strength. Considering the energy requirement and tensile strength development, the GA plate design was chosen as the optimum refiner plate for the FBSW.

Table 6-4: Optimum conditions for the three different plate designs for the FBSW pulp at the targeted CSF of 350 mL

Plate designs (bar code, bar angle)	REF (5/5/6 mm,10°)	GC (3/3/6 mm,10°)	GA (5/5/6 mm,25°)
Refining energy (kWh/t)	122.2	129.1	121
SEL (Wm/s)	0.8	0.7	0.9
Tensile Index (Nm/g)	61.21	59.05	62.93
Tear Index (mN.m ² /g)	9.35	7.52	9.00
CSF (mL)	350	350	350

6.2.2 Analysis of FBSW pilot refiner optimisation results

To ascertain that the optimum conditions for the GA plate design generated by the modelling software were reliable, the FBSW pulp was refined under the optimal refining energy and SEL. The run under the optimal conditions was repeated three times for statistical purposes and repeatability. The results are shown in Table 6-5. Subsequently, the average of the three optimum runs was calculated and compared with the six bijective runs conducted in phase 2 for the FBSW, as shown in Table 6-6.

Table 6-5: Pulp properties of the three runs for FBSW obtained using optimum conditions generated by the customised bijective modelling software

	Modelling software optimum refining conditions	Run 1	Run 2	Run 3	Standard deviation
Refining energy (kWh/t)	121	121	121	121	NA
SEL (Ws/m)	0.88	0.88	0.88	0.88	NA
Tensile Index (Nm/g)	62.93	62.82	61.13	61.13	0.98
Tear Index (mN.m ² /g)	9.00	8.87	9.72	9.92	0.56
CSF (mL)	350	380	400	430	25.17

Table 6-6: Pulp properties of the optimum run and bijective study refining runs for the FBSW pulp

	Optimum Run	REF CBH I	NO CBH I	REF CBH I	SEL-	SEL+	GA	GC
Refining energy (kWh/t)	121.00	109.23	98.18	130.64	88.60	122.63	141.95	
SEL (Ws/m)	0.88	1.20	1.20	0.80	1.60	1.20	0.40	
Tensile (Nm/g)	61.69	48.12	51.13	63.78	46.86	60.98	62.92	
Tear (mN.m ² /g)	9.50	14.04	8.08	10.18	11.34	9.26	9.39	
CSF (mL)	403	350	350	350	350	350	350	

As shown in Table 6-5, the pulp properties obtained for the three runs are different from each other. Using the one-way ANOVA method it was determined that there was no statistically significant difference between the pulp properties of the three runs at a 95% confidence level. In this case, the null hypothesis was accepted. The one-way ANOVA results imply that over a period of time, the variation in the pulp properties under the same refining energy and SEL was insignificant. Thus, the customised bijective modelling software's optimum refining energy and SEL were valid and reliable.

Table 6-6 shows the refining conditions of the average of the three optimum runs compared with the six bijective runs from phase 2 for the FBSW. As previously stated, the GA plate design was chosen as the optimum refiner plate design. Compared to the GA run, the optimum run had a lower SEL and achieved better tensile and tear development at a slightly lower refining energy. The better tear development might have been due to the lower intensity, which preserved the fibre's intrinsic strength. Furthermore, FBSW appears to be compatible with low/moderate intensity (milder) refining treatments, as evidenced by the SEL- and GC runs, which achieved the highest tensile strength development but required the most energy to achieve the desired CSF.

6.3 UBSW optimisation

Three different refiner plate designs, namely, REF (5/5/6 mm, 10°), GC (3/3/6 mm, 10°) and GA (5/5/6 mm, 25°) were modelled for the UBSW using a customised refining bijective software. Short-span Compressive Test (SCT) and Corrugating Medium Test (CMT) strengths were then obtained for each plate design at the desired CSF of 550 mL for the UBSW.

6.3.1 UBSW computational modelling

The models for the three different refiner plate designs at several refining energies across different SEL are shown in Figure 6-3. The freeness window of interest was between 500 mL and 600 mL for the UBSW, as the desired CSF was 550 mL.

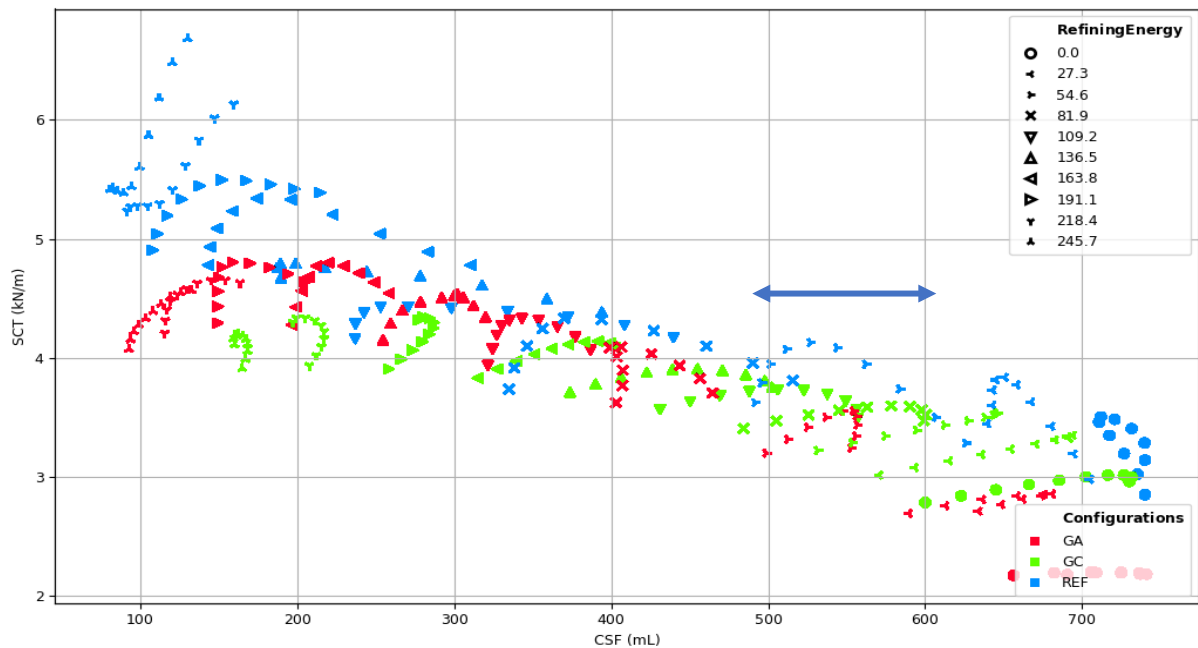


Figure 6-3: Relationship between SCT and CSF of the UBSW pulp modelling for the three different plate design

As shown in Figure 6-3, at the freeness window of interest, 500-600 mL, the REF plate design exhibits the highest SCT strength development in comparison to the GC and GA plated designs. These results are congruent with the outcomes observed for the UBSW pulp in phase 2. The optimum conditions for each plate design were then analysed at the desired CSF of 550 mL, as shown in Table 6-7.

Table 6-7: Optimum conditions for the three different plate designs for the UBSW pulp at the targeted CSF index of 550 mL

Plate designs (bar code, bar angle)	REF (5/5/6 mm,10°)	GC (3/3/6 mm,10°)	GA (5/5/6 mm,25°)
Refining energy (kWh/t)	52	92	55
SEL (Wm/s)	1.2	0.8	1.2
SCT (kN/m)	4.09	3.64	3.57
CMT (N)	233.5	205.2	188.8
CSF (mL)	550	550	550

At a specific CSF of 550 mL, Table 6-7 shows that the REF plate design was predicted to achieve the highest SCT and CMT. In contrast, the GA plate design exhibited the lowest compressive strength development. Whereas the GC run had the highest energy consumption to achieve the desired CSF. Consequently, considering the energy requirement and the SCT and CMT strength developments, the REF plate design was chosen as the optimum plate design for the UBSW.

6.3.2 Analysis of UBSW pilot refiner optimisation results

To ensure the reliability of the optimum conditions generated by the modelling software for the REF plate design for the UBSW. The UBSW pulp was refined three times using the REF plate design's optimum refining energy and SEL. The run under the optimal conditions was repeated three times for statistical purposes and repeatability. The results are shown in Table 6-8. Thereafter, the average of the three optimum runs was calculated and compared with the six bijective runs conducted in phase 2 for the UBSW, as shown in Table 6-9.

Table 6-8: Pulp properties of the three runs for the UBSW pulp obtained using optimum conditions generated by the customised bijective modelling software

	Modelling software optimum refining conditions	Run 1	Run 2	Run 3	Standard deviation
Refining energy (kWh/t)	52	52	52	52	NA
SEL (Ws/m)	1.2	1.2	1.2	1.2	NA
SCT (kN/m)	4.09	4.06	3.92	3.95	0.07
CMT (N)	234	227	222	225	2.53
CSF (mL)	550	540	550	580	25.17

Table 6-9: Pulp properties of the optimum run and bijective study runs for the UBSW pulp

	Optimum Run	REF CBH I	NO CBH I	REF CBH I	SEL-	SEL+	GA	GC
Refining energy (kWh/t)	52	60.93	47.44	74.99	44.62	56.48	92.24	
SEL (Ws/m)	1.2	1.2	1.2	0.8	1.6	1.2	0.4	
SCT (kN/m)	3.98	3.78	3.99	3.70	3.56	3.56	3.62	
CMT (N)	224.85	187.68	205.91	202.74	186.60	185.02	181.05	
CSF (mL)	553	550	550	550	550	550	550	

As shown in Table 6-8, there was a difference in the pulp properties obtained between the three runs. Utilising one-way ANOVA analysis at a confidence level of 95%, the difference between the three runs was rendered statistically insignificant. As a result, the null hypothesis was accepted. The one-way ANOVA findings show that the fluctuation in pulp characteristics over a period time under the same refining energy and SEL was negligible. As a result, the SEL and optimum refining energy generated by the customised refining bijective modelling software were valid and acceptable.

The average of the optimum three runs was compared with the six runs from phase 2 at the targeted CSF, as shown in Table 6-9. In the case of the UBSW pulp, the optimum run was the REF CBH I run with the same plate design and SEL, thus a similar SCT was achieved by both runs. In comparison to the untreated run, the optimum run achieved a higher SCT at relatively lower energy. This might be attributed to the CBH I added during the repulping stage, inducing delamination, and swelling of the fibres, thus facilitating the refining effects. Compared to the remaining refining runs, the optimum run achieved a higher SCT and CMT at the desired CSF, validating the reliability of the computational modelling software used for this study.

6.4 Analysis of scanning electron micrographs

As mentioned earlier, refining induces structural changes in the pulp fibres. The primary changes are internal fibrillation (swelling) and external (macro) fibrillation. Macro-fibrillation is essential as it increases the surface area and strengthens the inter-fibre bonding. Whereas internal fibrillation hydrates the fibres (fibres swell), this increases the conformability of the fibres and enables the fibres to bond easily with each other (Ek *et al.*, 2009a). In order to determine if there was a noticeable and significant change in the surface morphology between the enzyme-treated, untreated, and optimum runs. The images of the fibre network for the REF NO CBH I, REF CBH I and Optimum runs for each pulp were taken using a Scanning Electron Microscope (SEM) at a 1300X magnification. The REF runs were taken at the same energy to see the impact of only the CBH I charge on the fibres. The scanning electron micrographs for BHKP, FBSW, and UBSW are shown in Figure 6-4, Figure 6-5, and Figure 6-6 respectively.

Figure 6-4 shows that there was a visible, significant difference between the fibre development of the untreated run and the REF CBH I run. The REF NO CBH I run fibre network was more porous and bulky compared to the REF CBH I run, which showed a reduction in both porosity and bulkiness, resulting in the fibres being packed closer together. This indicates that the CBH I pre-treatment enhanced the fibre swelling process during repulping, facilitating the flexibility and collapsibility of the fibres. The macro-fibrillation was more pronounced in the optimum run surface morphology image compared to both the REF runs.

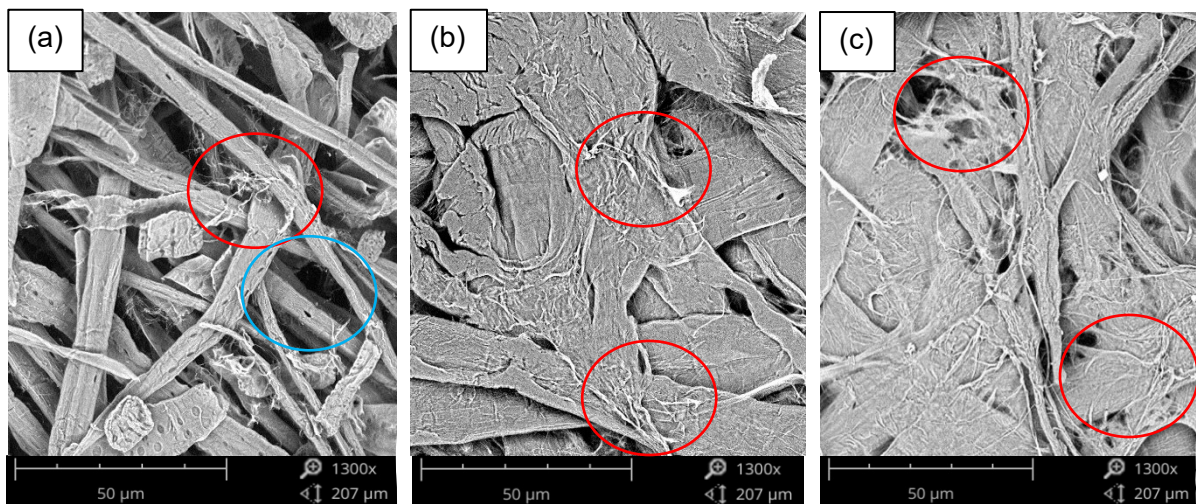


Figure 6-4: BHKP scanning electron micrographs for (a) REF NO CBH I (b) REF CBH I and (c) Optimum run. Macro-fibrillation identified by the red circle and porosity by blue circle. A 1300X magnification and a working distance of 50 µm was used for each refining run.

As shown in Figure 6-5, the optimum run had slightly more fibrillated and denser fibres compared to the REF CBH I run. This was mainly attributed to the GA plate design that was chosen for the optimum run, which led to better strength development, as previously observed in section 6.2.2 with the results from Table 6-6. A small difference was observed in the macro-fibrillation between the REF runs, however, there was no significant visible difference in the bulkiness and porosity of the fibres.

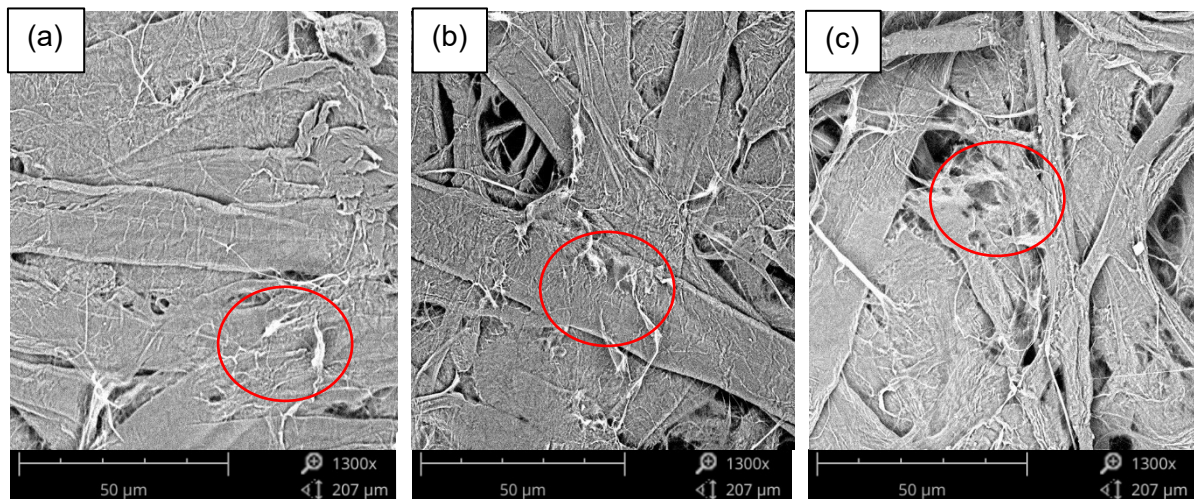


Figure 6-5: FBSW scanning electron micrographs for (a) REF NO CBH I (b) REF CBH I and (c) Optimum run. Macro-fibrillation identified by the red circle. A 1300X magnification and a working distance of 50 μm was used for each refining run.

As shown in Figure 6-6, the impact of CBH I pre-treatment was visible in the UBSW pulp. The REF CBH I run image shows both improved macro-fibrillation and sheet density (less bulky fibres) compared to the untreated run. In contrast, both the REF CBH I run and the optimum run showed no significant difference in the macro-fibrillation and bulkiness. This was expected as the optimum run for the UBSW pulp was essentially the REF CBH I run, as previously observed in Section 6.3.2 with the results from Table 6-9

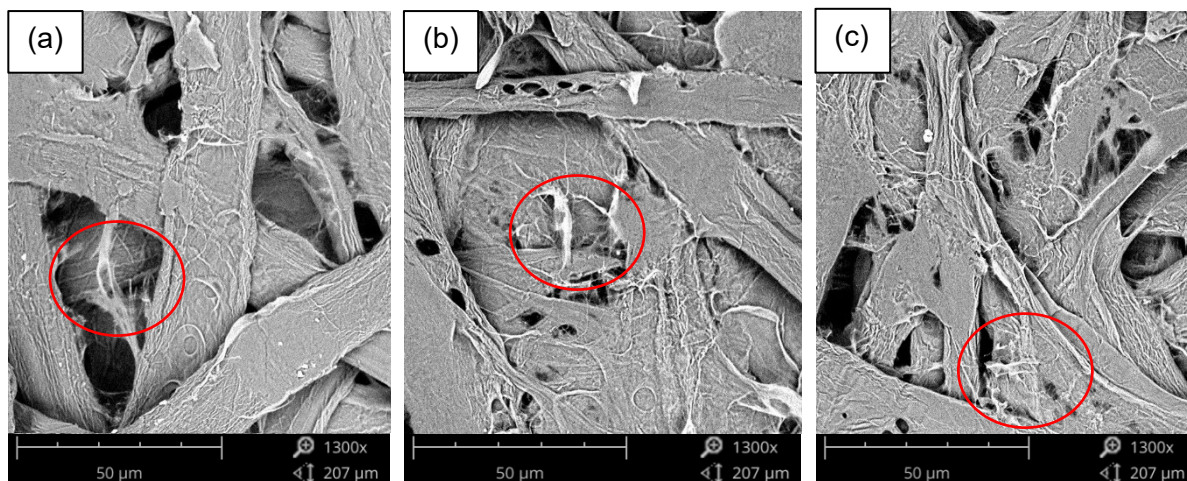


Figure 6-6: UBSW scanning electron micrographs for (a) REF NO CBH I (b) REF CBH I and (c) Optimum run. Macro-fibrillation identified by the red circle. A 1300X magnification and a working distance of 50 μm was used for each refining run.

CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

This chapter starts off in section 7.1 with a summary of the primary outcomes from the CBH I characterisation (Chapter 4), bijective studies on pilot refiner (Chapter 5) and optimisation on refining parameters (Chapter 6) phases. In section 7.2 the chapter concludes by recommending possible future studies on enzymatic refining for the CBH I enzyme based on the conclusions.

7.1 Conclusions

The first phase of this study indicated that CBH I was a thermophile (optimum temperature of 55 °C) and works best in acidic conditions with an optimum pH of 4. In addition, CBH I proved to be thermostable, with 49% of its activity retained after a 3 h incubation period. As a consequence, phase 1 successfully demonstrated that CBH I has the potential to be used for industrial applications.

In the second phase, the effect of CBH I as a refining enzyme using the bijective diagram technique was investigated. For all three pulps, comparing the two REF runs, the introduction of CBH I prior to refining decreased the refining energy demand, resulting in a faster CSF drop. In addition, CBH I pre-treatment led to an improvement in macro-fibrillation, improving the tensile and burst strength developments. However, the bleached pulps experienced significant tear loss. In contrast, with the unbleached pulp, the fibre length was preserved, resulting in an improvement in the tear strength development.

For all the pulps, the high intensity run (SEL+) deteriorated the pulp quality. This indicates that high-intensity combined with the enzyme treatment decreases the fibre's intrinsic strength. The pulps reacted better to the lower-intensity run (SEL-); this type of treatment resulted in fibre strength conservation. In addition, the low-intensity treatment combined with the enzyme pre-treatment improved the macro-fibrillation compared to the REF runs. The disadvantage of low-intensity treatments is the higher energy required to reach a certain CSF. However, even though the energy savings are not significant, a longer refiner plate life is an indirect economic gain for this type of treatment.

The variation of the refiner plate designs led to different findings for each pulp. BHKP showed positive results for the GC run, while the GA run induced more fibre cutting instead of fibrillation. For the FBSW pulp, both GA and GC runs enhanced the fibre development, leading to an improvement in the strength handsheet properties relative to the enzyme-treated reference run. In contrast, for the UBSW pulp, both GC and GA runs led to poor fibre development, decreasing the pulp quality.

Results from the second phase denoted that CBH I pre-treatment alone is insufficient; selecting the correct SEL and refiner plate design is paramount. Thus, in the third phase, the refining process parameters for each pulp were optimised using a customised bijective refining software. The refining data was modelled using three different plate designs across various SEL and refining energies. The BHKP and UBSW both had the REF plate design as the optimum refiner plate. Whereas for the FBSW, the GA plate design was chosen as the optimum plate design. One-way ANOVA analysis confirmed that the difference in pulp properties obtained using the optimal conditions is statistically insignificant at a 95% confidence level.

The surface morphology difference between the optimum run, untreated and treated reference runs was confirmed by microscopic examinations with a Scanning Electron Microscope. The scanning electron micrographs revealed that the enzyme-treated reference fibre network had better fibrillation and low bulk, which led to improved strength properties compared to the untreated reference run.

Overall, considering all the data, the CBH I enzyme has the potential to transform the refining process towards a more environmentally friendly, cost-effective, and efficient operation compared to the conventional method. However, more research is still required before the enzyme can be made commercially available to the pulp and paper industry. Consequently, the next section presents recommendations for future studies that might be done to advance the research of CBH I as a refining enzyme.

7.2 Recommendations for future work

In phase 1 the CBH I enzyme demonstrated the potential to be used in industrial applications by being thermophilic and thermostable. Furthermore, in phase 2, CBH I pre-treatment reduced energy consumption while improving fibre development. However, the results obtained in the pilot-scale refiner do not necessarily guarantee that the exact results will be obtained in the mill-scale refiner. Thus, it is recommended to investigate the mono-component CBH I as a refining enzyme in a mill-scale setting.

To justify investigated CBH I assisted refining level at a mill-scale. The energy cost reduction was calculated taking into account the enzyme cost. This was done using the results from Phase 2 of this study.

Energy cost reduction

Paper machine produces 100 000 t pa (20% filler)

Raw material consist of 80% BHKP and 20% FBSW

Energy cost is R1.11/kWh

Utilising the information above, the cost savings can be calculated as follows

BHKP

Savings = 1600 MWh pa = R1.78 M pa

FBSW

Savings = 176 MWh pa = R0.20 M pa

Taking into account the enzyme cost is R 1.24 M pa @ 30 ppm

Net savings: R0.73 M pa

It has been reported in several studies that cellulase pre-treatment results in energy savings and improved strength properties. Whereas cellulase post-treatment results in an increase in the CSF while also improving the strength properties. The increase in CSF is attributed to the “peeling effect”, whereby cellulase partially hydrolyses cellulosic debris with high water affinity (Bajpai *et al.*, 2006; Pala *et al.*, 2009; Tripathi *et al.*, 2008). Therefore, it is recommended that CBH I treatment post refining be investigated. In addition, some applications may require both refining energy reduction and an increase in the CSF. Thus, an investigation into a two-stage enzyme treatment might prove beneficial.

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ANNEXURE A: PILOT REFINING PULP CONSISTENCY AND CSF

A.1 Procedure for pulp consistency determination

Before determining the pulp freeness, the pulp consistency for each sample is calculated using the below laboratory method.

1. In a clean 500 mL plastic beaker, accurately weigh about 100 g of the wet sample of the pulp and record the mass as W_1 .
2. Add tap water into the beaker and fill it up to the 400 mL mark to make it easier to transfer.
3. Form a handsheet following the handsheet making procedure (described in Annexure B.1)
4. Place the dried handsheet in the desiccator to bring it to ambient temperature.
5. Weigh the cooled handsheet and record the mass as W_2 .
6. Use the recorded mass (W_1, W_2) to calculate the pulp consistency using Equation A-1.

$$\text{Consistency} = \frac{W_2}{W_1} \times 100 \quad (\text{A-1})$$

The pulp consistency is then used to calculate the amount of pulp suspension that should be used to determine the pulp freeness.

$$\text{CSF pulp} = \frac{30 \text{ grams}}{\text{Consistency}} \text{ (for 10 L volume)} \quad (\text{A-2})$$

Divide the CSF pulp obtained from Equation A-2 by 4 to obtain the mass needed for a 2.5 L. The volume is reduced to 2.5 L because only 1 L is needed for determining the pulp freeness of one sample.

A.2 Procedure for pulp CSF determination

The freeness of pulp is a measurement of how quickly a dilute pulp suspension (3 g of pulp in 1 L of water) may be drained. The TAPPI standard method T 221 cm-09 was used in this study to determine the pulp freeness. The procedure for the method is described below, assuming that the CSF pulp has been obtained for each sample and diluted to a volume of 2.5 L as described in Annexure A.1.

1. Clean the freeness tester thoroughly with filtered water.

2. Place the drainage chamber on the higher supporting bracket, closing the bottom lid and opening the top lid and air-cock.
3. Set up a container to collect the discharge from the bottom orifice and a graduated freeness cylinder to accept the discharge from the side orifice.
4. To achieve a uniform solution, thoroughly agitate the stock in the 2.5 L, then properly measure 1000 mL into a clean 1 L cylinder.
5. Cautiously yet swiftly pour the stock into the drainage chamber.
6. Close the drainage chamber's top lid and air-cock. Open the bottom lid. After roughly 5 s have passed since the stock has been added, fully open the air-cock in one motion.
7. When the side discharge has stopped, test the temperature of the filtrate using a thermometer and note the volume discharged from the side orifice in millilitres (freeness) to the nearest 10 mL.
8. In a large enough plastic beaker, combine the pulp from the chamber with the discharges from the side and bottom orifices. Make certain that no fibres are left behind.
9. Create one handsheet with the entire contents of the beaker.
10. Calculate the consistency using Equation A-1.
11. Using the correction tables provided by the TAPPI standard technique, calculate the corrected freeness based on the measured temperature and consistency.
12. To the closest 10 mL, round the corrected freeness result.

ANNEXURE B: HANDSHEET FORMATION AND HANDSHEET TESTING

B.1 Procedure for handsheet formation

This procedure describes a method for the preparation of laboratory handsheets using a Rapid-Köthen sheet former for the subsequent testing of handsheet properties described in this study. It is based on ISO standard method ISO 5269-2 (Preparation of laboratory sheets for physical testing – Part 2: Rapid-Köthen method) and TAPPI standard method T 205-sp (Forming handsheets for physical tests of pulp). These methods are suitable for grammages ranging from 60 to 300 g/m². Since the samples in this study were refined, soaking and disintegration of the pulp was not required. Thus, the procedure was as follows:

1. Determine the pulp consistency according to the method described in Annexure A.1 for each sample.
2. Determine the amount of pulp to give a specific final concentration with water in a 10 L volume.
3. Agitate the 10 L bucket contents to ensure homogeneous suspension.
4. Calculate the volume of pulp suspension required to give a specific grammage handsheet.
5. Measure the volume obtained in step 4 of pulp suspension with a measuring cylinder and swiftly transfer the content of the measuring cylinder into a clean, 1000 mL beaker.
6. To begin the handsheet formation cycle, press the start button on the Rapid-Köthen sheet former machine.
7. Add the pulp suspension as soon as the water level in the stock container reaches 3 to 4 L.
8. Open the stock container once the cycle is finished.
9. Carefully place two blotters onto the handsheet without applying pressure.
10. Place the steel plate on top of the two blotters without applying pressure.
11. Roll the roller back and forth over the sheet over a 2 s period without applying pressure, once in each of two directions at right angles to each other
12. Remove the steel plate.
13. Cautiously remove the blotters with the handsheet from the sheet-forming screen .
14. Label the handsheet as required with a pencil and cover it with one blotter.
15. Place the stack of handsheets and blotters into a presser (a maximum of 10 handsheets is allowed at a time).

16. During a period of 30 s, gradually increase the pressure to 400 kPa and maintain it for 5 min.
17. Remove the stack from the press and separate the blotters from the handsheets.
18. Ensure that the dryer's temperature is $93\pm4^{\circ}\text{C}$ and place one handsheet at a time in the dryer.
19. Close the dryer, press the "Start" button and press the lid down.
20. Dry the handsheet for 7 min (When the timer runs out, an alarm will sound).
21. Remove the handsheet from the dryer and weigh it immediately using a calibrated and verified laboratory balance.
22. Ensure that the weight of the handsheet is within the specified range for that targeted grammage.
23. A minimum of six handsheets are required per refined sample for handsheet testing.

B.2 Procedure for handsheet testing

All the handsheets were conditioned for 24 h at 23°C and 50% relative humidity prior to testing. The procedure for each handsheet property is described below.

For all the handsheet properties, ensure that the test pieces are prepared in the atmospheric conditions used during the conditioning of the handsheets. In addition, before testing ensure all the machines are verified and calibrated.

Tensile strength – ISO method 1924-2

1. From the centre of the handsheet cut out test strips with a width of $15\text{ mm} \pm 0.1\text{ mm}$ and long enough to fit into the clamps.
2. Modify the clamps to the required initial test length and insert the test strip between the clamps.
3. Align the test strip so that it is parallel to the direction of application of the tensile force.
4. Secure the clamps and begin the test, until the test strip breaks into two pieces.
5. Record the measured tensile force.

Bursting strength – ISO method 2758

1. Adjust the clamping mechanism to provide enough clamping pressure to prevent the handsheet from slipping out of the clamps.
2. Raise the clamps and place the handsheet in a position that allows the entire clamping area to be used.
3. Press the start button and test the handsheet with the full clamping pressure.
4. The measured pressure will be indicated in kilopascal.

Tearing resistance – ISO method 1974

1. From the handsheets, cut out rectangular test pieces with a width of $50 \text{ mm} \pm 2 \text{ mm}$, after the initial cut, the width must be $43 \text{ mm} \pm 0.5 \text{ mm}$.
2. Ensure that the calibrated mean values are between 20% and 80% of the maximum scale reading.
3. Insert the test piece (consisting of six rectangular sheets) between the two clamps and secure the clamps.
4. Make a slit in the sheets (to produce the initial cut) using the knife.
5. Firmly depress and retain the pendulum release mechanism. If the pointer is fitted, gently catch the pendulum on its return swing with your hand without changing the position of the pointer.
6. Record the tear resistance reading.

Porosity – ISO method 5636-3

1. Ensure test pieces do not have holes, wrinkles, and any other defects.
2. Change the switch to 'open', insert the test piece in the measuring gap, then change the switch to close.
3. Press the start button and record the air flow measured in mL/min.

Opacity – ISO method 2471

1. Remove the test-piece pad's protective sheets.
2. Measure the intrinsic luminance factor R_{∞} using a pad of sheets, with the sheet of interest at the top.

3. Repeat until five measurements of R_{∞} have been obtained for each sample.
4. Measure the luminance factor R_0 , using a single sheet.
5. Repeat until five measurements of R_0 have been obtained for each sample
6. Opacity automatically calculated using the luminance factors as follows $\frac{100 \times R_0}{R_{\infty}}$.

Grammage – ISO method 536

1. Cut test pieces with a length and width of 200 mm and 250 mm from at least five samples.
2. Weigh each test piece and calculate the area of each test piece.
3. Obtain the grammage using Equation (A-3).

$$\text{Grammage} = \frac{m}{A} \times 10^6 \quad (\text{A-3})$$

Moisture Content – ISO method 287

1. Weigh the test pieces, record the mass, m_0 .
2. Dry the test pieces in an oven at a temperature of $105^\circ\text{C} \pm 2^\circ\text{C}$ for 2 h.
3. Immediately weigh the test samples after 2 h, record the mass, m_1 .
4. Calculate the moisture content using Equation (A-4).

$$\text{Moisture content} = \frac{m_0 - m_1}{m_0} \times 100 \quad (\text{A-4})$$

Bulk thickness – ISO method 534

1. Ensure that the micro-meter is verified and calibrated before testing.
2. Place the test piece between the open pressure faces of the micro-meter.
3. Allow the pressure face to hold the test piece, by permitting the pressure face to move gradually and gently towards the anvil at a velocity less than 3 mm/s, so that any punching effect is prevented.
4. At the end of 1 s to 2 s dwell duration, record the thickness of the micro-meter reading.

Corrugating Medium Test – ISO method 7263

1. Using the centre of the handsheet cut out test strips with a of $12.7 \text{ mm} \pm 0.1 \text{ mm}$ and a length range of 150 mm to 160 mm.

2. Ensure the corrugating rolls temperature is $175\text{ }^{\circ}\text{C} \pm 8\text{ }^{\circ}\text{C}$, thereafter, insert the test strip into the corrugating rolls with its length perpendicular to the nip.
3. Immediately after the corrugated test strip emerges from the corrugating rolls, position it on the rack.
4. Place the comb over the corrugated test strip on the rack, ensuring that it is firmly pressed down on the valleys of the rack.
5. Afterwards attach a piece of adhesive tape to the corrugated test strip. The adhesive tape must be long enough that it is in contact with the ends of the corrugated test strip.
6. Recondition the taped corrugated test strips for 30 min to 35 min.
7. After reconditioning conduct the test by placing the taped corrugated strip under at the centre of the lower platen of the crush tester
8. Begin the compression and record the automatic reading to the nearest 5 N.

Short-span Compressive Test – ISO method 9895

1. Cut test strips with a length of 70 mm and width of $15\text{ mm} \pm 0.1\text{ mm}$ from centre region of the handsheet.
2. Place the test piece in the jaws of the compressive tester.
3. Press the start button and set the jaws in motion.
4. Record the maximum short-span compressive force indicated.

ANNEXURE C: CBH I CHARACTERISATION

C.1 The effect of temperature on CBH I activity

To determine the effect of temperature on CBH I activity a modified filter paper assay was conducted at a temperature range of 25 to 85 °C and a constant pH of 5. The raw data generated by HPLC is shown in Table C-1. Control represented a sample with no substrate (filter paper). Blank represented a sample that did not contain the enzyme.

Table C-1: Glucose and cellobiose concentrations as detected by HPLC at the specified temperature range and pH of 5

Temperature (°C)	Glucose (mg/mL)						Average -control	Standard deviation
	1	2	3	Control	Blank	Average		
25	1.69	3.71	2.47	0.60	0.00	2.62	2.02	1.02
35	3.00	2.27	2.69	0.59	0.11	2.66	2.07	0.37
45	3.77	4.20	3.92	1.47	0.14	3.96	2.49	0.22
55	4.12	5.48	5.27	0.78	0.15	4.96	4.18	0.73
65	2.46	1.66	1.69	0.40	0.00	1.93	1.54	0.45
75	1.50	0.86	0.92	0.61	0.10	1.09	0.48	0.36
85	0.58	0.77	0.96	0.13	0.00	0.77	0.63	0.19
Temperature (°C)	Cellobiose (mg/mL)						Average -control	Standard deviation
	1	2	3	Control	Blank	Average		
25	9.35	11.14	7.19	0.00	0.00	9.23	9.23	1.98
35	10.08	9.16	10.85	0.00	0.00	10.03	10.03	0.85
45	18.57	19.10	17.93	0.00	0.00	18.53	18.53	0.59
55	22.45	28.08	27.08	0.00	0.00	25.87	25.87	3.01
65	19.95	16.81	18.32	0.00	0.00	18.36	18.36	1.57
75	5.71	5.02	5.06	0.00	0.00	5.26	5.26	0.39
85	1.82	2.28	2.17	0.00	0.00	2.09	2.09	0.24

The data was plotted (Figure C-1) and the standard deviation was used to calculate the custom vertical error bars in Microsoft Excel.

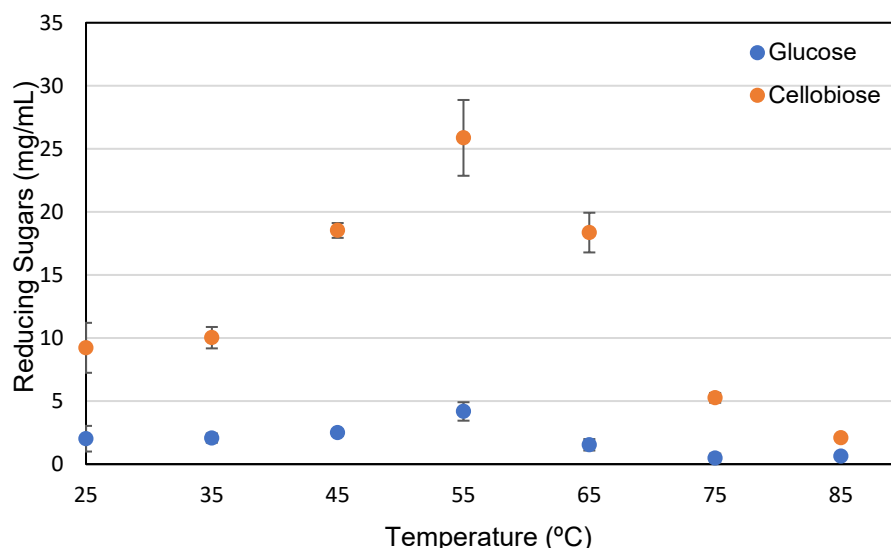


Figure C-1: The effect of temperature on CBH I reducing sugars concentration at pH of 5

The data obtained from HPLC is in mg/mL (Figure C-1). To determine the activity, a conversion was performed to the standard concentration units of IU/mL using Microsoft Excel, the results are in Table C-2.

Table C-2: Conversion of the glucose and cellobiose concentration from mg/L to IU/mL

Glucose					
Temperature (°C)	mg/mL	umol/mL	umol/mL/s	nkat/mL x DF	IU/mL
25	2.01933	1.12088	0.00187	1.86813	31.14179
35	2.06933	1.14863	0.00191	1.91439	31.91289
45	2.49233	1.38343	0.00231	2.30572	38.43632
55	4.18000	2.32021	0.00387	3.86702	64.46321
65	1.53567	0.85241	0.00142	1.42068	23.68277
75	0.48367	0.26847	0.00045	0.44745	7.45902
85	0.63367	0.35173	0.00059	0.58622	9.77229
Cellobiose					
25	9.22567	2.68993	0.00448	4.48322	74.73533
35	10.02933	2.92426	0.00487	4.87377	81.24568
45	18.53233	5.40349	0.00901	9.00581	150.12683
55	25.87200	7.54352	0.01257	12.57253	209.58405
65	18.36167	5.35372	0.00892	8.92287	148.74429
75	5.26167	1.53415	0.00256	2.55691	42.62374
85	2.08667	0.60841	0.00101	1.01402	16.90368

The individual activities of glucose and cellobiose in UI/mL were then combined to give the total activity of CBH I. The results are shown in Table C-3.

Table C-3: The effect of temperature on the total activity of CBH I at pH of 5

Temperature (°C)	Total activity (IU/mL)	Standard deviation
25	105.88	3.00
35	113.16	1.22
45	188.56	0.81
55	454.05	3.74
65	172.43	2.03
75	50.08	0.74
85	26.68	0.43

C.2 The effect of pH on CBH I activity

In the previous data, the optimum temperature was determined to be 55 °C. This temperature was used to test the effect of pH and determine the optimum pH. A modified filter paper assay was used at a pH range of 2 to 9. The raw data generated by HPLC is shown in Table C-4.

Table C-4: Glucose and cellobiose concentrations as detected by HPLC at the specified pH range and optimum temperature

Glucose (mg/mL)								
pH	1	2	3	Control	Blank	Average	Average-control	Standard deviation
2	26.100	23.5	25.200	0.000	0.000	25.650	25.650	0.636
3	35.400	34.800	38.100	0.000	0.000	36.100	36.100	1.758
4	37.300	38.600	36.800	0.000	0.000	37.567	37.567	0.929
5	22.500	19.200	20.700	1.400	0.000	20.800	19.400	1.652
6	10.200	14.400	13.300	1.200	0.000	12.633	11.433	2.178
7	6.200	5.900	6.700	1.100	0.000	6.267	5.167	0.404
8	2.200	3.300	2.700	1.200	0.000	2.733	1.533	0.551
9	1.100	1.100	1.600	1.000	0.000	1.267	0.267	0.289
Cellobiose (mg/mL)								
2	54.900	55.200	59.1	0.000	0.000	55.050	55.050	0.212
3	62.300	67.600	67.000	0.000	0.000	65.633	65.633	2.902
4	70.600	68.900	73.900	0.000	0.000	71.133	71.133	2.542
5	48.200	43.700	45.800	0.000	0.000	45.900	45.900	2.252
6	22.000	33.900	33.100	0.000	0.000	29.667	29.667	6.652
7	18.400	16.700	17.500	0.000	0.000	17.533	17.533	0.850
8	2.100	1.300	1.100	0.000	0.000	1.500	1.500	0.529
9	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

The results were plotted as shown in Figure C-2. The standard deviation was used to calculate the vertical error bars on Microsoft Excel.

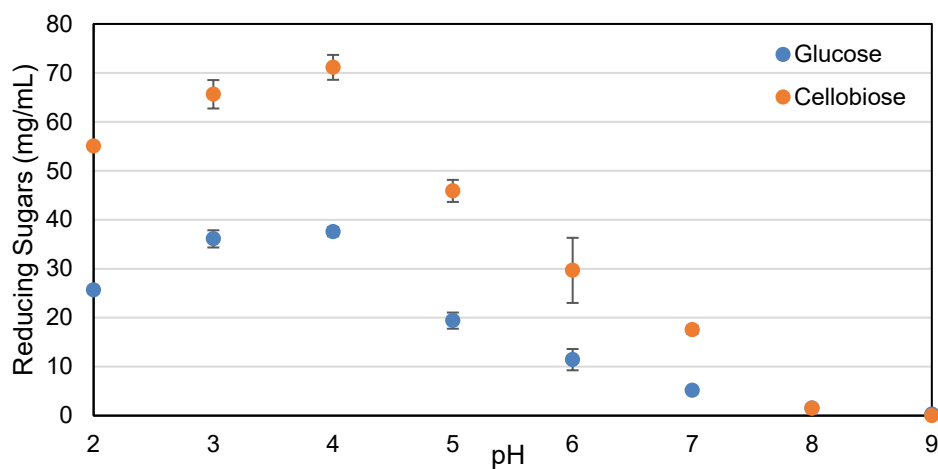


Figure C-2: The effect of pH on CBH I reducing sugars concentration at the optimum temperature

The concentration of glucose and cellobiose was then converted to the standard units of IU/mL from mg/mL to calculate the CBH I activity (Table C-5).

Table C-5: Conversion of the glucose and cellobiose concentration from mg/L to IU/mL

Glucose					
pH	mg/mL	umol/mL	umol/mL/s	nkat/mL x DF	IU/mL
2	25.65000	14.23766	0.02373	23.72943	395.56967
3	36.10000	20.03819	0.03340	33.39698	556.72769
4	37.56667	20.85230	0.03475	34.75383	579.34636
5	19.40000	10.76845	0.01795	17.94741	299.18330
6	11.43333	6.34635	0.01058	10.57725	176.32280
7	5.16667	2.86788	0.00478	4.77981	79.67940
8	1.53333	0.85111	0.00142	1.41852	23.64679
9	0.26667	0.14802	0.00025	0.24670	4.11249
Cellobiose					
2	55.05000	16.08252	0.02680	26.80421	446.82615
3	65.63333	19.17438	0.03196	31.95730	532.72824
4	71.13333	20.78117	0.03464	34.63529	577.37027
5	45.90000	13.40941	0.02235	22.34901	372.55804
6	29.66667	8.66694	0.01444	14.44490	240.79641
7	17.53333	5.12226	0.00854	8.53710	142.31338
8	1.50000	0.43822	0.00073	0.73036	12.17510
9	0.00000	0.00000	0.00000	0.00000	0.00000

The individual concentration of glucose and cellobiose were then combined to yield the total activity of the CBH I activity (Table C-6).

Table C-6: The effect of pH on the total activity of CBH I at the optimum temperature

pH	Total activity (IU/mL)	Standard deviation
2	842.40	0.85
3	1089.46	4.66
4	1156.72	3.47
5	671.74	3.90
6	417.12	8.83
7	221.99	1.25
8	35.82	1.08
9	4.11	0.29

C.3 Thermostability profile for CBH I

The optimum temperature and pH obtained were used to determine the thermostability of the CBH I enzyme. The enzyme was withdrawn every 30 min over a 3 h incubation period. The raw data generated by HPLC is shown in Table C-7. The data was then used to calculate the percentage of the residual sugars (glucose and cellobiose).

Table C-7: Glucose and cellobiose concentrations as detected by HPLC at the optimum temperature and pH

Time (min)	Glucose (mg/mL)						Average- control	% Residual sugar	Standard deviation
	1	2	3	Control	Blank	Average			
0	29.80	30.60	30.50	1.90	0.00	30.30	28.40	100.00	0.44
30	23.00	21.80	21.40	0.70	0.00	22.07	21.37	75.23	0.83
60	16.30	16.30	15.00	1.80	0.00	15.87	14.07	49.53	0.75
90	18.50	17.90	15.60	1.60	0.00	17.33	15.73	55.40	1.53
120	16.70	17.70	18.10	2.10	0.00	17.50	15.40	54.23	0.72
150	15.50	15.10	16.80	2.00	0.00	15.80	13.80	48.59	0.89
180	14.20	15.70	16.60	2.40	0.00	15.50	13.10	46.13	1.21
	Cellobiose (mg/mL)								
	1	2	3	Control	Blank	Average			
0	74.40	80.60	81.40	0.00	0.00	78.80	78.80	100.00	3.83
30	61.90	59.00	56.10	0.00	0.00	59.00	59.00	74.87	2.90
60	46.50	47.70	42.00	0.00	0.00	45.40	45.40	57.61	3.00
90	51.00	49.10	41.80	0.00	0.00	47.30	47.30	60.03	4.86
120	46.50	49.50	49.60	0.00	0.00	48.53	48.53	61.59	1.76
150	44.20	44.00	44.90	0.00	0.00	44.37	44.37	56.30	0.47
180	39.00	37.50	44.30	0.00	0.00	40.27	40.27	51.10	3.57

The percentage of the residual sugars was plotted, and the standard deviation was used to calculate the vertical error bars Figure C-3.

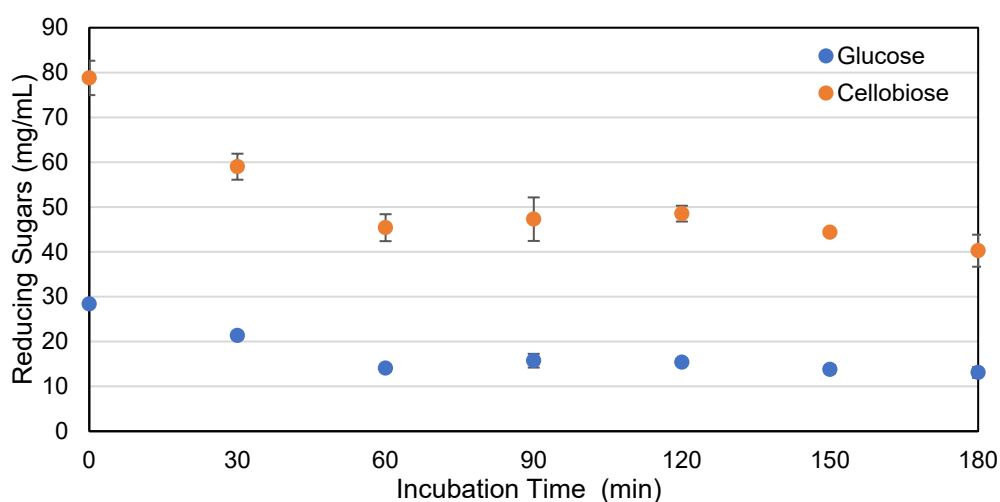


Figure C-3: The effect thermostability of CBH I at the optimum temperature and pH

To determine the residual activity, the sugars' concentration was then converted from mg/mL to IU/mL using Microsoft Excel (Table C-8). The individual residual activity of glucose and cellobiose was then calculated.

Table C-8: Conversion of the glucose and cellobiose concentration from mg/L to IU/mL

Glucose						
Time (min)	mg/mL	$\mu\text{mol/mL}$	$\mu\text{mol/mL/s}$	nkat/mL x DF	IU/mL	Residual activity
0	28.400	15.7641	0.0263	26.2735	437.9797	100.0000
30	21.367	11.8601	0.0198	19.7668	329.5129	75.2347
60	14.067	7.8080	0.0130	13.0134	216.9336	49.5305
90	15.733	8.7332	0.0146	14.5553	242.6366	55.3991
120	15.400	8.5481	0.0142	14.2469	237.4960	54.2254
150	13.800	7.6600	0.0128	12.7667	212.8211	48.5915
180	13.100	7.2715	0.0121	12.1191	202.0258	46.1268
Cellobiose						
0	78.800	23.0209	0.0384	38.3682	639.5986	100.0000
30	59.000	17.2365	0.0287	28.7275	478.8872	74.8731
60	45.400	13.2633	0.0221	22.1056	368.4997	57.6142
90	47.300	13.8184	0.0230	23.0307	383.9215	60.0254
120	48.533	14.1787	0.0236	23.6312	393.9321	61.5905
150	44.367	12.9615	0.0216	21.6024	360.1124	56.3029
180	40.267	11.7637	0.0196	19.6061	326.8338	51.0998

The individual activities of glucose and cellobiose were combined to yield the total activity and total residual activity of CBH I, results shown in Table C-9.

Table C-9: The total activity and total residual activity of CBH I at the optimum temperature and pH

Time (min)	Total activity	Total Residual activity	Standard deviation
0	1077.58	100.00	4.27
30	808.40	75.02	3.73
60	585.43	54.33	3.76
90	626.56	58.15	6.39
120	631.43	58.60	2.48
150	572.93	53.17	1.36
180	528.86	49.08	4.79

ANNEXURE D: BIJECTIVE STUDIES ON THE PILOT REFINER

This section shows the raw data obtained for the bijective refining runs conducted on the 12" pilot refiner for each pulp during phase 2.

D.1 CSF raw data

The CSF obtained for each pulp at the specified refining energies for the six bijective refining runs is shown in Table D-1, Table D-2 and Table D-3. The Canadian Standard Freeness method was used for all pulps to measure the CSF. The numbers 0 to 6 indicate the seven samples collected per refining run at the specified refining energy.

Table D-1: Refining energy with the corresponding CSF obtained for the BHKP

REF NO CBH I							
	0	1	2	3	4	5	6
Refining Energy (kWh/t)	0	0	35.3	69.3	102.1	133.6	164.0
CSF (mL)	560	540	420	340	290	220	180
REF CBH I							
Refining Energy (kWh/t)	0	0	35.3	69.3	102.1	133.6	164
CSF (mL)	520	520	380	250	160	130	110
SEL- CBH I							
Refining Energy (kWh/t)	0	0	33.9	67.0	99.3	130.7	161.3
CSF (mL)	560	530	390	300	250	190	150
SEL+ CBH I							
Refining Energy (kWh/t)	0	0	47.2	92.8	136.7	178.9	219.4
CSF (mL)	520	510	310	140	100	90	70
GA CBH I							
Refining Energy (kWh/t)	0	0	35.3	69.3	102.1	133.6	164
CSF (mL)	550	520	380	270	210	150	110
GC CBH I							
Refining Energy (kWh/t)	0	0	40	80.2	118.3	155	190.3
CSF (mL)	560	560	410	350	290	230	150

Table D-2: Refining energy with the corresponding CSF obtained for the FBSW pulp

REF NO CBH I							
	0	1	2	3	4	5	6
Refining Energy (kWh/t)	0	0	44.30	81.90	130.80	172.90	214.30
CSF (mL)	700	650	540	400	270	150	120
REF CBH I							
Refining Energy (kWh/t)	0	0	44.30	81.90	130.80	172.90	214.30
CSF (mL)	690	650	520	390	200	110	90
SEL- CBH I							
Refining Energy (kWh/t)	0	0	48	95.6	142.6	189.2	235.3
CSF (mL)	700	680	560	430	290	180	140
SEL+ CBH I							
Refining Energy (kWh/t)	0	0	46.40	91.80	136.20	179.60	221.90
CSF (mL)	680	630	480	320	150	100	70
GA CBH I							
Refining Energy (kWh/t)	0	0	51.4	102	151.8	201	249.4
CSF (mL)	680	670	550	370	240	140	80
GC CBH I							
Refining Energy (kWh/t)	0	0	50.9	100.9	150	198.3	245.7
CSF (mL)	690	690	570	450	300	180	140

Table D-3: Refining energy with the corresponding CSF obtained for the UBSW pulp

REF NO CBH I							
	0	1	2	3	4	5	6
Refining Energy (kWh/t)	0	0	44.30	81.90	130.80	172.90	214.30
CSF (mL)	750	720	630	450	340	210	170
REF CBH I							
Refining Energy (kWh/t)	0	0	44.30	81.90	130.80	172.90	214.30
CSF (mL)	760	710	590	380	240	170	120
SEL- CBH I							
Refining Energy (kWh/t)	0	0	48	95.6	142.6	189.2	235.3
CSF (mL)	740	740	650	470	380	220	140
SEL+ CBH I							
Refining Energy (kWh/t)	0	0	46.40	91.80	136.20	179.60	221.90
CSF (mL)	740	740	540	290	190	120	90
GA CBH I							
Refining Energy (kWh/t)	0	0	44.30	81.90	130.80	172.90	214.30
CSF (mL)	740	740	610	400	310	190	130
GC CBH I							
Refining Energy (kWh/t)	0	0	50.9	100.9	150	198.3	245.7
CSF (mL)	730	730	650	570	460	250	160

D.2 Handsheet and fibre properties raw data

The Lorentzen & Wettre Fiber Tester Plus (L&W FT+) was used to determine the fibre morphology for each refining run. The raw data generated with the software package is shown in Table D-4, Table D-5, and Table D-6.

The change in the fibre morphology affects the handsheet properties. The raw data of the tested handsheet properties for all refining runs conducted is shown in Table D-7, Table D-8, and Table D-9.

Table D-4: Fibre morphology results obtained for the six bijective refining runs conducted for the BHKP

REF NO CBH I							
Fibre morphology	0	1	2	3	4	5	6
Mean length (µm)	816	813.	806	799.	786	779.	770.
Mean width (µm)	16.53	16.40	16.57	16.80	17.00	17.47	17.53
Macro-fibrillation index (%)	15.54	15.30	15.91	16.85	17.58	18.30	18.91
Mean fines (%)	21.24	21.66	22.28	23.05	24.08	25.15	26.32
Coarseness (mg/m)	0.06	0.07	0.06	0.07	0.07	0.07	0.07
Mean kink index (%)	41.74	41.51	36.67	34.50	34.27	32.07	32.73
REF CBH I							
Mean length (µm)	809	806	805	792	788	780	777
Mean width (µm)	16.73	16.67	16.83	17.37	17.60	17.90	29.27
Macro-fibrillation index (%)	15.95	16.55	17.39	18.92	19.55	20.36	20.96
Mean fines (%)	20.28	21.20	24.88	27.11	28.54	29.10	30.31
Coarseness (mg/m)	0.05	0.06	0.06	0.06	0.06	0.06	0.06
Mean kink index (%)	41.83	35.94	35.39	33.80	33.43	32.48	32.17
SEL- CBH I							
Mean length (µm)	828	820	814	803	795	790	784
Mean width (µm)	16.70	16.57	16.77	17.00	17.33	17.70	17.97
Macro-fibrillation index (%)	16.00	15.25	16.47	16.95	17.74	18.63	19.44
Mean fines (%)	23.68	22.81	23.90	24.25	25.63	26.31	27.65
Coarseness (mg/m)	0.05	0.06	0.05	0.06	0.06	0.06	0.06
Mean kink index (%)	39.66	40.25	35.22	33.79	32.28	31.71	31.98
SEL+ CBH I							
Mean length (µm)	817	809	795	780	770	764	756
Mean width (µm)	16.53	16.43	17.03	17.50	17.73	18.20	18.33
Macro-fibrillation index (%)	15.35	15.67	17.69	18.86	19.56	20.23	21.56
Mean fines (%)	22.56	23.20	26.69	30.01	31.73	32.69	33.26
Coarseness (mg/m)	0.05	0.06	0.06	0.06	0.06	0.06	0.06
Mean kink index (%)	42.31	41.98	34.66	32.88	32.71	31.27	31.68
GA CBH I							
Mean length (µm)	819	800	792	776	756	740	727
Mean width (µm)	16.67	16.17	16.70	17.20	17.57	17.80	18.40
Macro-fibrillation index (%)	15.35	14.33	15.96	17.23	18.41	19.98	21.43
Mean fines (%)	19.51	20.77	26.36	30.07	31.83	32.94	33.29
Coarseness (mg/m)	0.06	0.06	0.06	0.07	0.07	0.07	0.07
Mean kink index (%)	41.29	42.53	36.40	35.05	34.57	33.95	32.06
GC CBH I							
Mean length (µm)	827	821	806	795	783	770	762
Mean width (µm)	16.97	16.63	16.60	16.50	17.43	17.60	17.53
Macro-fibrillation index (%)	16.17	16.15	17.50	18.35	19.26	19.94	21.01
Mean fines (%)	20.64	20.53	22.99	23.33	24.93	27.15	29.96
Coarseness (mg/m)	0.05	0.05	0.05	0.05	0.06	0.06	0.07
Mean kink index (%)	40.60	39.63	33.08	32.85	27.94	26.35	25.80

Table D-5: Fibre morphology results obtained for the six bijective refining runs conducted for the FBSW pulp

REF NO CBH I							
Fibre morphology	0	1	2	3	4	5	6
Mean length (µm)	1792	1835	1683	1442	1252	1090	945
Mean width (µm)	26.40	26.70	27.33	27.67	28.20	28.50	28.93
Macro-fibrillation index (%)	2.97	2.70	6.53	10.80	14.77	18.77	22.57
Mean fines (%)	19.97	18.93	20.57	23.73	26.90	29.60	32.40
Coarseness (mg/m)	0.12	0.15	0.14	0.14	0.12	0.11	0.09
Mean kink index (%)	2.02	1.92	1.40	1.27	1.25	1.38	1.48
REF CBH I							
Mean length (µm)	1823	1838	1630	1336	1088	900	769
Mean width (µm)	26.43	26.63	27.40	28.07	28.60	29.07	29.27
Macro-fibrillation index (%)	2.63	2.70	7.30	13.43	18.77	23.47	26.67
Mean fines (%)	17.50	17.23	19.17	22.83	26.73	30.20	33.73
Coarseness (mg/m)	0.13	0.13	0.14	0.12	0.09	0.08	0.08
Mean kink index (%)	1.31	1.34	1.45	1.26	1.08	1.12	0.74
SEL- CBH I							
Mean length (µm)	1830	1817	1606	1418	1271	1188	1001
Mean width (µm)	26.60	26.60	27.37	28.17	28.80	29.27	29.33
Macro-fibrillation index (%)	2.63	2.80	7.60	13.70	20.07	25.43	29.60
Mean fines (%)	17.57	17.70	19.53	23.10	27.33	31.63	35.97
Coarseness (mg/m)	0.14	0.11	0.12	0.13	0.10	0.08	0.06
Mean kink index (%)	2.11	2.07	1.38	1.31	1.44	1.70	1.95
SEL+ CBH I							
Mean length (µm)	1838	1853	1553	1296	1084	941	831
Mean width (µm)	26.60	26.80	27.43	28.17	28.60	29.00	29.27
Macro-fibrillation index (%)	2.60	2.60	7.97	13.13	17.07	20.47	23.33
Mean fines (%)	17.53	17.43	20.23	24.17	28.03	30.50	33.23
Coarseness (mg/m)	0.16	0.19	0.12	0.12	0.12	0.11	0.10
Mean kink index (%)	2.09	2.03	1.54	1.52	1.54	1.67	1.76
GA CBH I							
Mean length (µm)	1850	1850	1711	1588	1420	1365	1283
Mean width (µm)	26.77	26.77	27.43	27.97	28.50	28.93	29.20
Macro-fibrillation index (%)	2.67	2.53	7.60	13.37	19.00	24.10	28.93
Mean fines (%)	17.27	17.53	19.50	22.43	25.57	28.77	31.53
Coarseness (mg/m)	0.12	0.17	0.15	0.13	0.11	0.10	0.08
Mean kink index (%)	2.09	2.03	1.43	1.46	1.94	1.58	1.75
GC CBH I							
Mean length (µm)	1840	1835	1688	1483	1364	1241	1117
Mean width (µm)	26.67	26.70	27.10	27.47	27.47	27.70	27.60
Macro-fibrillation index (%)	2.63	2.60	7.53	11.97	16.73	21.70	25.67
Mean fines (%)	17.63	18.07	22.47	23.33	27.10	29.93	31.90
Coarseness (mg/m)	0.13	0.15	0.12	0.13	0.12	0.10	0.11
Mean kink index (%)	2.13	2.05	1.45	1.39	1.42	1.49	1.59

Table D-6: Fibre morphology results obtained for the six bijective refining runs conducted for the UBSW pulp

REF NO CBH I							
Fibre morphology	0	1	2	3	4	5	6
Mean length (µm)	2291	2297	1714	1314	1078	1079	876
Mean width (µm)	35.57	35.97	36.10	36.07	35.80	35.93	35.57
Macro-fibrillation index (%)	6.60	6.00	14.90	22.60	27.63	28.13	32.70
Mean fines (%)	45.60	32.70	38.10	42.23	46.13	46.80	48.30
Coarseness (mg/m)	0.29	0.28	0.31	0.21	0.21	0.19	0.16
Mean kink index (%)	0.75	0.75	0.60	0.78	1.06	1.12	1.43
REF CBH I							
Mean length (µm)	2264	2198	1755	1368	1091	928	820
Mean width (µm)	36.03	88.60	88.23	86.60	85.07	83.97	83.00
Macro-fibrillation index (%)	6.97	6.97	14.03	20.67	26.13	30.23	33.47
Mean fines (%)	35.73	37.83	46.30	46.03	48.87	50.77	51.37
Coarseness (mg/m)	0.19	0.23	0.18	0.16	0.14	0.12	0.11
Mean kink index (%)	0.69	0.61	0.53	0.71	0.99	1.07	1.59
SEL- CBH I							
Mean length (µm)	2273	2273	1752	1317	1090	897	779
Mean width (µm)	35.80	35.67	35.80	35.73	35.57	35.07	34.57
Macro-fibrillation index (%)	7.20	6.53	15.40	23.17	29.27	33.37	36.10
Mean fines (%)	44.70	32.93	38.20	44.60	46.43	48.70	49.20
Coarseness (mg/m)	0.23	0.26	0.19	0.19	0.17	0.14	0.13
Mean kink index (%)	0.75	0.65	0.56	0.80	1.13	1.47	1.82
SEL+ CBH I							
Mean length (µm)	2258	2273	1752	1317	1090	897	779
Mean width (µm)	35.53	35.40	35.87	36.17	35.93	35.87	35.60
Macro-fibrillation index (%)	6.87	6.47	14.73	22.53	26.70	29.53	31.93
Mean fines (%)	39.37	35.50	37.00	39.43	45.43	47.07	48.27
Coarseness (mg/m)	0.25	0.31	0.24	0.19	0.16	0.14	0.14
Mean kink index (%)	0.70	0.62	0.59	0.80	1.03	1.23	1.44
GA CBH I							
Mean length (µm)	2240	2254	2051	1830	1607	1429	1281
Mean width (µm)	35.47	35.50	35.73	36.03	35.97	35.87	36.07
Macro-fibrillation index (%)	7.83	8.70	11.60	16.90	22.07	26.60	29.10
Mean fines (%)	48.00	46.00	35.47	39.47	45.13	45.43	47.17
Coarseness (mg/m)	0.18	0.17	0.24	0.20	0.17	0.15	0.14
Mean kink index (%)	0.70	0.67	0.54	0.59	0.73	0.93	1.66
GC CBH I							
Mean length (µm)	2290	2263	2128	1921	1779	1628	1434
Mean width (µm)	35.77	35.57	35.30	35.10	35.17	34.73	34.20
Macro-fibrillation index (%)	6.53	6.87	11.27	15.93	20.33	24.57	29.17
Mean fines (%)	31.87	35.27	42.70	39.87	41.87	46.90	47.90
Coarseness (mg/m)	0.23	0.21	0.22	0.22	0.19	0.20	0.16
Mean kink index (%)	0.77	0.67	0.51	0.49	0.60	0.75	0.93

Table D-7: Handsheet properties results obtained for the six bijective refining runs conducted for the BHKP

REF NO CBH I								SEL- CBH I							
Handsheet properties	0	1	2	3	4	5	6	Handsheet properties	0	1	2	3	4	5	6
Grammage (g/m ²)	71.11	71.25	69.34	72.10	71.71	69.94	70.31	Grammage (g/m ²)	560.00	530.00	390.00	300.00	250.00	190.00	150.00
Bulk specific (cm ³ /g)	1.86	1.77	1.70	1.65	1.60	1.52	1.47	Bulk specific (cm ³ /g)	1.96	1.80	1.78	1.62	1.55	1.49	1.44
Moisture (%)	7.08	7.26	7.23	7.49	7.61	7.29	7.33	Moisture (%)	7.33	7.43	7.41	7.49	7.48	7.59	7.67
Tensile index (Nm/g)	28.96	31.52	37.38	44.99	48.80	59.69	73.28	Tensile index (Nm/g)	28.52	28.29	45.26	55.38	65.26	70.55	72.80
Stretch (%)	1.74	1.67	2.17	2.69	2.96	3.12	4.15	Stretch (%)	1.62	1.55	2.94	3.50	3.87	3.70	3.99
Breaking Length (m)	2954	3215	3813	4589	5590	6089	7669	Breaking Length (m)	2910	2886	4616	5649	6657	7196	7425
Opacity (%)	82.53	82.19	80.81	80.38	79.24	77.97	77.69	Opacity (%)	81.46	82.04	80.02	79.73	78.52	77.67	76.43
Burst index (kPa.m ² /g)	1.32	1.45	2.39	3.11	3.74	4.53	5.03	Burst index (kPa.m ² /g)	1.27	1.50	2.72	3.72	4.64	4.99	5.81
Tear index (mN.m ² /g)	5.58	5.82	7.99	8.62	8.84	8.66	7.76	Tear index (mN.m ² /g)	5.31	5.92	7.87	9.03	9.72	9.02	8.46
T.E.A (J/m ²)	25.75	26.26	40.73	63.73	85.62	95.14	159.62	T.E.A (J/m ²)	23.50	22.76	67.38	104.24	129.13	135.10	149.11
Porosity (mL/min)	5000.00	5000.00	2582.00	1193.50	454.25	221.00	102.00	Porosity (mL/min)	5000.00	5000.00	2623.25	955.00	345.50	125.00	53.75
REF CBH I								GA CBH I							
Grammage (g/m ²)	71.27	71.34	69.81	72.40	69.60	71.04	72.91	Grammage (g/m ²)	550.00	520.00	380.00	270.00	210.00	150.00	110.00
Bulk specific (cm ³ /g)	1.86	1.84	1.70	1.55	1.50	1.40	1.39	Bulk specific (cm ³ /g)	1.84	1.87	1.77	1.72	1.59	1.54	1.40
Moisture (%)	7.21	7.03	7.10	7.14	7.18	7.26	7.19	Moisture (%)	7.12	4.87	5.80	6.09	7.17	7.36	7.21
Tensile index (Nm/g)	23.79	30.10	43.10	52.06	63.02	67.86	75.19	Tensile index (Nm/g)	30.38	31.33	41.27	46.70	57.87	58.31	62.30
Stretch (%)	1.43	2.01	2.91	3.53	3.83	3.88	4.43	Stretch (%)	1.71	1.80	2.67	2.62	3.07	2.90	3.29
Breaking Length (m)	2427	3070	4396	5311	6428	6922	7474	Breaking Length (m)	3099	3196	4210	4763	5903	5948	6355
Opacity (%)	82.62	82.27	80.09	79.84	77.77	76.71	76.33	Opacity (%)	82.24	82.21	81.25	81.04	78.55	77.71	77.78
Burst index (kPa.m ² /g)	1.32	1.76	2.98	3.77	4.70	4.62	5.31	Burst index (kPa.m ² /g)	1.21	1.57	2.73	2.75	3.89	3.99	3.59
Tear index (mN.m ² /g)	4.90	5.66	8.40	8.69	8.01	7.64	7.47	Tear index (mN.m ² /g)	5.73	5.80	7.22	7.73	8.00	7.63	7.07
T.E.A (J/m ²)	17.13	31.88	64.17	97.49	121.93	135.54	171.15	T.E.A (J/m ²)	26.81	28.95	57.83	64.09	91.38	90.91	98.92
Porosity (mL/min)	5000.00	5000.00	2237.50	758.00	303.00	89.25	35.00	Porosity (mL/min)	5000.00	4858.25	2432.25	1290.50	298.25	120.75	123.00
SEL+ CBH I								GC CBH I							
Grammage (g/m ²)	520.00	510.00	310.00	140.00	100.00	90.00	70.00	Grammage (g/m ²)	560.00	560.00	410.00	350.00	290.00	230.00	150.00
Bulk specific (cm ³ /g)	1.93	1.94	1.63	1.55	1.44	1.37	1.34	Bulk specific (cm ³ /g)	1.94	1.86	1.70	1.58	1.50	1.50	1.49
Moisture (%)	7.36	7.37	7.43	7.38	7.41	7.46	7.55	Moisture (%)	5.70	7.17	7.24	7.20	7.33	7.32	7.36
Tensile index (Nm/g)	23.05	25.21	48.58	63.38	69.46	70.28	73.08	Tensile index (Nm/g)	24.62	28.83	42.39	53.02	59.76	68.94	68.54
Stretch (%)	1.36	1.63	3.20	3.92	4.18	3.97	3.67	Stretch (%)	1.39	1.58	2.52	2.87	3.46	3.70	3.64
Breaking Length (m)	2351	2571	4955	6465	7085	6658	7455	Breaking Length (m)	2511	2940	4324	5408	6096	7032	6991
Opacity (%)	82.47	81.81	79.67	79.22	78.16	76.66	74.59	Opacity (%)	83.42	81.87	82.40	81.83	80.84	79.63	79.57
Burst index (kPa.m ² /g)	1.11	1.34	3.60	4.63	5.10	5.06	5.87	Burst index (kPa.m ² /g)	1.10	1.22	2.46	3.43	4.19	4.70	5.06
Tear index (mN.m ² /g)	4.94	5.26	9.21	8.34	7.95	7.57	6.85	Tear index (mN.m ² /g)	5.39	5.57	8.72	8.55	8.32	7.59	7.44
T.E.A (J/m ²)	15.64	20.69	80.24	124.17	150.36	136.47	139.83	T.E.A (J/m ²)	17.82	22.83	57.90	82.49	116.11	131.42	130.22
Porosity (mL/min)	5000.00	5000.00	932.00	379.50	127.75	45.75	13.00	Porosity (mL/min)	5000.00	5000.00	1990.50	714.50	288.50	140.50	73.50

Table D-8: Handsheet properties results obtained for the six bijective refining runs conducted for the FBSW pulp

REF NO CBH I								SEL- CBH I							
Handsheet properties	0	1	2	3	4	5	6	Handsheet properties	0	1	2	3	4	5	6
Grammage (g/m ²)	75.76	77.56	70.31	69.81	71.44	68.63	68.54	Grammage (g/m ²)	73.68	72.63	76.26	74.10	77.71	78.30	75.55
Bulk specific (cm ³ /g)	0.34	0.36	0.34	0.31	0.30	0.29	0.28	Bulk specific (cm ³ /g)	0.38	0.38	0.32	0.30	0.28	0.25	0.25
Moisture (%)	6.46	6.45	6.33	6.30	6.27	4.91	6.39	Moisture (%)	5.15	6.68	4.58	6.77	6.86	7.01	5.04
Tensile index (Nm/g)	20.21	18.38	39.81	43.36	47.98	65.50	73.05	Tensile index (Nm/g)	19.67	20.19	44.89	55.54	65.39	71.49	81.65
Stretch (%)	2.42	1.96	3.72	3.19	4.67	3.56	4.30	Stretch (%)	1.89	2.28	3.09	3.74	3.90	4.20	4.91
Breaking Length (m)	2061	1875	4061	4423	4894	6681	7451	Breaking Length (m)	2006	2060	4579	5665	6670	7292	8328
Opacity (%)	78.84	78.43	78.46	77.44	76.34	74.43	72.46	Opacity (%)	78.09	76.99	74.55	71.26	71.01	69.67	68.52
Burst index (kPa.m ² /g)	1.31	1.60	3.07	3.85	4.33	4.93	4.82	Burst index (kPa.m ² /g)	1.40	1.67	3.08	4.04	4.44	4.52	4.87
Tear index (mN.m ² /g)	18.96	17.66	18.17	15.64	12.84	11.00	9.39	Tear index (mN.m ² /g)	18.72	18.75	13.85	10.64	8.45	6.81	6.45
T.E.A (J/m ²)	32.67	21.98	84.74	84.53	138.43	136.44	170.38	T.E.A (J/m ²)	20.62	25.35	76.76	101.07	142.63	174.23	180.52
Porosity (mL/min)	5000.00	5000.00	2304.00	636.75	211.75	61.33	19.00	Porosity (mL/min)	5000.00	5000	2143.5	567.75	91.75	45.75	0.00
REF CBH I								GC CBH I							
Grammage (g/m ²)	72.67	71.18	76.10	76.71	77.25	70.10	74.58	Grammage (g/m ²)	72.84	72.86	79.33	70.68	78.49	74.47	70.77
Bulk specific (cm ³ /g)	0.38	0.39	0.31	0.30	0.27	0.28	0.25	Bulk specific (cm ³ /g)	0.40	0.39	0.33	0.34	0.29	0.29	0.28
Moisture (%)	3.95	8.09	12.52	6.51	5.87	6.01	6.60	Moisture (%)	4.64	6.76	6.39	6.69	6.70	6.80	6.73
Tensile index (Nm/g)	20.79	21.81	37.55	47.80	58.07	70.18	69.87	Tensile index (Nm/g)	17.48	17.89	41.31	55.71	60.65	74.87	83.48
Stretch (%)	2.21	2.29	3.45	3.74	3.65	3.88	4.91	Stretch (%)	1.93	2.04	3.59	3.94	3.82	3.89	3.98
Breaking Length (m)	2121	2225	3830	4875	5923	7434	7126	Breaking Length (m)	1783	1825	4214	5682	6187	7636	8515
Opacity (%)	78.84	76.07	75.73	73.35	73.32	71.31	68.52	Opacity (%)	77.62	77.44	77.97	74.53	74.13	71.97	70.05
Burst index (kPa.m ² /g)	1.25	1.77	3.34	4.22	4.25	4.95	5.15	Burst index (kPa.m ² /g)	1.16	1.37	3.44	4.54	4.70	5.33	5.93
Tear index (mN.m ² /g)	14.74	13.25	9.21	8.14	6.14	4.93	4.56	Tear index (mN.m ² /g)	14.64	15.15	11.66	9.33	8.62	7.88	7.00
T.E.A (J/m ²)	24.30	25.19	74.74	101.07	120.50	143.68	143.85	T.E.A (J/m ²)	18.38	19.65	86.04	108.88	126.00	150.50	161.27
Porosity (mL/min)	5000.00	5000.00	2357.00	552.75	139.25	45.25	0.00	Porosity (mL/min)	5000.00	5000.00	2077.25	636.00	192.00	51.25	11.50
SEL+ CBH I								GA CBH I							
Grammage (g/m ²)	74.40	82.91	75.06	74.51	76.23	72.17	73.22	Grammage (g/m ²)	78.25	75.15	79.84	78.85	75.09	74.24	74.31
Bulk specific (cm ³ /g)	0.37	0.34	0.33	0.30	0.29	0.28	0.27	Bulk specific (cm ³ /g)	0.38	0.37	0.31	0.29	0.28	0.28	0.26
Moisture (%)	6.59	6.45	6.33	6.30	6.27	4.91	6.39	Moisture (%)	6.18	5.26	6.48	8.43	6.61	4.39	6.46
Tensile index (Nm/g)	20.20	17.52	37.95	49.93	59.92	61.98	69.52	Tensile index (Nm/g)	17.56	20.04	41.85	60.91	66.24	76.44	80.98
Stretch (%)	2.30	2.51	3.51	3.70	3.98	3.73	3.71	Stretch (%)	2.13	2.35	3.40	4.29	4.28	4.13	4.33
Breaking Length (m)	2059	1787	3871	5093	6112	6322	7091	Breaking Length (m)	1791	2044	4268	6213	6552	8103	8260
Opacity (%)	75.96	76.71	74.26	74.21	73.73	71.64	69.51	Opacity (%)	77.38	77.14	75.14	73.11	70.04	69.12	66.64
Burst index (kPa.m ² /g)	1.66	1.39	3.01	3.93	3.60	4.46	4.45	Burst index (kPa.m ² /g)	1.34	1.22	3.42	4.61	5.35	5.85	6.06
Tear index (mN.m ² /g)	13.73	15.95	11.47	9.30	8.46	8.28	6.58	Tear index (mN.m ² /g)	17.79	16.42	11.41	8.41	7.08	6.03	5.35
T.E.A (J/m ²)	24.31	28.18	71.58	105.06	130.80	119.62	133.10	T.E.A (J/m ²)	21.90	26.47	89.86	143.14	151.01	168.47	180.22
Porosity (mL/min)	5000.00	5000.00	2501.75	490.50	120.25	41.00	0.00	Porosity (mL/min)	5000.00	5000.00	2229.00	381.50	72.25	10.00	0.00

Table D-9: Handsheet properties results obtained for the six bijective refining runs conducted for the UBSW pulp

REF NO CBH I								SEL- CBH I							
Handsheet properties	0	1	2	3	4	5	6	Handsheet properties	0	1	2	3	4	5	6
Grammage (g/m ²)	76.12	77.17	81.72	78.14	77.27	77.73	77.84	Grammage (g/m ²)	75.50	75.96	74.26	72.97	77.71	78.00	67.35
Bulk specific (cm ³ /g)	0.38	0.38	0.34	0.32	0.29	0.27	0.26	Bulk specific (cm ³ /g)	0.42	0.43	0.34	0.31	0.29	0.25	0.26
Moisture (%)	7.88	7.94	8.96	7.68	7.73	7.82	7.89	Moisture (%)	7.57	7.28	7.92	7.79	10.51	7.79	7.79
Tensile index (Nm/g)	30.24	30.14	50.85	61.79	70.14	71.45	72.75	Tensile index (Nm/g)	30.67	34.45	51.07	65.87	72.30	74.03	94.26
Stretch (%)	2.17	2.19	2.79	3.15	3.16	2.90	2.84	Stretch (%)	2.17	2.11	2.38	2.88	3.42	2.80	3.08
Breaking Length (m)	3056	3075	5187	6302	7562	7288	7421	Breaking Length (m)	3126	3513	5209	6719	7449	7375	9615
Burst index (kPa.m ² /g)	2.20	2.46	3.56	4.02	4.08	3.94	4.38	Burst index (kPa.m ² /g)	2.31	2.39	3.65	4.17	4.42	4.41	4.99
Tear index (mN.m ² /g)	19.62	18.91	13.64	10.53	8.88	7.45	5.91	Tear index (mN.m ² /g)	19.59	15.37	11.11	8.46	7.15	6.06	4.31
T.E.A (J/m ²)	38.87	36.83	74.29	101.18	300.07	111.61	112.80	T.E.A (J/m ²)	34.22	36.99	60.47	93.24	137.21	108.40	129.63
Porosity (mL/min)	5000.00	5000.00	4760.00	520.50	83.50	15.75	0.00	Porosity (mL/min)	5000.00	5000.00	3910.50	717.00	20.25	10.00	0.00
SCT (kN/m)	3.00	3.11	3.86	4.04	4.19	4.47	5.39	SCT (kN/m)	2.64	2.85	3.18	4.03	4.45	5.34	5.52
CMT(N)	131.32	132.75	194.00	210.50	233.25	245.33	270.00	CMT(N)	140.32	140.25	181.00	188.50	235.25	236.50	241.67
REF CBH I								GA CBH I							
Grammage (g/m ²)	76.35	81.42	75.89	76.55	77.50	78.57	75.39	Grammage (g/m ²)	76.13	74.79	78.58	78.74	84.02	76.55	76.79
Bulk specific (cm ³ /g)	0.39	0.39	0.31	0.29	0.28	0.28	0.24	Bulk specific (cm ³ /g)	0.39	0.41	0.34	0.31	0.27	0.29	0.27
Moisture (%)	7.99	8.01	7.89	7.87	7.96	7.98	7.99	Moisture (%)	7.39	6.44	7.78	8.10	8.29	8.31	8.26
Tensile index (Nm/g)	30.34	33.15	59.08	66.18	76.04	79.84	76.00	Tensile index (Nm/g)	36.90	38.09	50.80	59.46	63.79	66.37	67.24
Stretch (%)	2.35	2.62	3.08	3.38	2.46	3.18	2.84	Stretch (%)	2.45	2.31	2.84	3.11	3.14	3.34	2.96
Breaking Length (m)	3156	3381	6026	6751	7756	8143	7752	Breaking Length (m)	3367	3886	5182	6065	5487	6858	6769
Burst index (kPa.m ² /g)	2.26	2.44	3.68	4.56	4.63	4.71	4.58	Burst index (kPa.m ² /g)	2.73	2.75	3.80	4.20	4.22	4.97	4.94
Tear index (mN.m ² /g)	20.16	19.00	14.09	10.70	8.34	8.05	6.14	Tear index (mN.m ² /g)	17.87	18.30	13.05	12.39	11.09	9.08	7.96
T.E.A (J/m ²)	43.90	42.22	102.66	117.81	92.64	136.69	109.67	T.E.A (J/m ²)	41.78	45.48	78.19	99.68	96.41	118.54	108.57
Porosity (mL/min)	5000.00	5000.00	2509.75	176.75	20.00	14.75	0.00	Porosity (mL/min)	5000.00	5000.00	5000.00	1307.00	287.00	50.00	12.00
SCT (kN/m)	3.11	3.49	4.11	4.34	4.63	5.18	5.64	SCT (kN/m)	2.17	2.18	3.30	4.10	4.46	4.84	4.59
CMT(N)	142.67	152.00	224.50	254.00	261.50	244.25	268.00	CMT(N)	156.87	168.50	180.75	212.12	227.75	233.75	241.25
SEL+ CBH I								GC CBH I							
Grammage (g/m ²)	75.60	76.49	78.71	78.32	80.11	75.68	77.08	Grammage (g/m ²)	78.13	81.88	76.36	75.23	79.15	79.70	75.64
Bulk specific (cm ³ /g)	0.40	0.39	0.33	0.31	0.27	0.28	0.26	Bulk specific (cm ³ /g)	0.37	0.38	0.35	0.33	0.31	0.28	0.29
Moisture (%)	7.58	7.48	7.49	8.14	8.36	6.27	9.74	Moisture (%)	7.43	7.53	7.38	7.68	7.70	8.02	8.08
Tensile index (Nm/g)	30.90	34.95	51.38	55.36	60.07	67.76	71.04	Tensile index (Nm/g)	31.77	34.00	49.46	57.69	67.02	72.07	74.93
Stretch (%)	2.45	2.22	2.85	2.65	3.04	2.89	3.06	Stretch (%)	2.28	2.37	2.97	2.94	3.30	3.04	3.19
Breaking Length (m)	3265	3565	5241	5647	6128	6912	7246	Breaking Length (m)	3301	3468	5044	5884	7351	6837	7643
Burst index (kPa.m ² /g)	2.39	2.49	3.39	3.79	3.71	4.18	4.35	Burst index (kPa.m ² /g)	2.34	2.33	3.55	3.95	3.76	3.97	4.43
Tear index (mN.m ² /g)	16.32	15.91	10.83	9.07	7.35	6.63	6.00	Tear index (mN.m ² /g)	18.45	18.21	14.68	13.47	12.12	11.82	10.65
T.E.A (J/m ²)	39.74	37.18	80.02	78.18	106.34	97.59	111.80	T.E.A (J/m ²)	43.99	45.72	74.94	86.34	128.47	97.59	123.34
Porosity (mL/min)	5000.00	5000.00	2149.25	143.00	27.75	0.00	0.00	Porosity (mL/min)	5000.00	5000.00	5000.00	3027.00	399.25	113.75	23.50
SCT (kN/m)	3.12	3.14	3.59	3.84	4.67	4.81	5.27	SCT (kN/m)	2.94	2.96	3.51	3.46	3.92	4.34	4.11
CMT(N)	148.23	154.75	193.50	204.25	229.50	236.75	235.67	CMT(N)	126.99	128.00	191.75	194.50	240.50	241.50	242.50

ANNEXURE E: OPTIMISATION OF REFINING PARAMETERS

The refining data obtained for the pilot refining bijective studies was modelled using a customised modelling software to determine the optimum refining parameters for each pulp. The raw data obtained for the three optimum runs conducted for each pulp is shown in Table E-1, Table E-2, and Table E-3. The green shaded column indicates the optimum refining point as indicated by the customised bijective modelling software.

E.1 Optimisation of refining parameters raw data

Table E-1: Refining energy and the corresponding pulp properties obtained for the BHKP optimum runs

BHKP Optimisation						
Run 1						
Refining Energy (kWh/t)	0	23.3	45.8	67.5	88.8	108.8
CSF (mL)	490	390	340	270	200	180
Tensile Index (Nm/g)	26.40	44.27	46.44	52.20	59.42	63.00
Tear Index (mN.m ² /g)	6.29	8.10	8.03	8.04	7.46	7.36
Run 2						
Refining Energy (kWh/t)	0	23.3	45.8	67.5	88.8	108.8
CSF (mL)	520	420	370	300	270	220
Tensile Index (Nm/g)	26.25	39.35	45.21	56.42	59.80	62.41
Tear Index (mN.m ² /g)	5.90	8.43	9.75	9.66	9.59	9.54
Run 3						
Refining Energy (kWh/t)	0	23.3	45.8	67.5	88.8	108.8
CSF (mL)	510	400	330	280	250	200
Tensile Index (Nm/g)	26.5	42.87	49.89	57.72	59.56	61.52
Tear Index (mN.m ² /g)	5.88	8.05	7.98	7.78	7.75	7.59

Table E-2: Refining energy and the corresponding pulp properties obtained for the FBSW optimum runs

FBSW Optimisation						
Run 1						
Refining Energy (kWh/t)	0	61.4	121	178.9	235.1	289.1
CSF (mL)	620	540	380	250	150	110
Tensile index (Nm/g)	18.69	46.90	62.82	68.63	75.81	83.17
Tear (mN.m2/g)	22.36	15.39	8.87	8.75	8.52	7.76
Run 2						
Refining Energy (kWh/t)	0	61.4	121	178.9	235.1	289.1
CSF (mL)	660	550	400	270	150	100
Tensile index (Nm/g)	18.47	39.95	61.13	69.93	77.58	81.39
Tear (mN.m2/g)	20.99	17.41	9.72	9.21	8.78	6.98
Run 3						
Refining Energy (kWh/t)	0	61.4	121	178.9	235.1	289.1
CSF (mL)	690	550	430	280	170	100
Tensile index (Nm/g)	20.17	44.32	61.13	69.93	76.67	79.68
Tear (mN.m2/g)	21.73	15.77	9.92	9.53	9.07	7.50

Table E-3: Refining energy and the corresponding pulp properties obtained for the UBSW optimum runs

UBSW Optimisation						
Run 1						
Refining Energy (kWh/t)	0	26.8	52.9	78.4	103.2	127.4
CSF (mL)	710	650	530	390	270	130
SCT (kN/m)	3.11	3.86	4.06	4.19	4.47	5.14
CMT(N)	132.75	194.00	227.25	238.25	245.33	270.00
Run 2						
Refining Energy (kWh/t)	0	26.8	52.9	78.4	103.2	127.4
CSF (mL)	720	640	550	400	300	180
SCT (kN/m)	3.38	3.65	3.92	4.14	4.33	4.93
CMT(N)	132.75	191.75	222.25	229.50	240.25	267.50
Run 3						
Refining Energy (kWh/t)	0	26.8	52.9	78.4	103.2	127.4
CSF (mL)	730	670	580	450	310	190
SCT (kN/m)	3.51	3.75	3.95	4.32	4.37	5.07
CMT(N)	136.25	192.25	225.00	237.75	257.25	266.00

E.2 One-way ANOVA statistical analysis raw data

Minitab was used to perform one-way ANOVA statistical analysis to determine statistically significant differences between the three optimum runs for each pulp. A confidence level of $\alpha = 0.05$ was used. The raw data for each pulp is shown in Figure E-1, Figure E-2, and Figure E-3.

Method					
Null hypothesis	All	means	are		
		equal			
Alternative hypothesis	Not all means are				
	equal				
Significance level	$\alpha = 0.05$				
Equal variances were assumed for the analysis.					
Factor Information					
Factor	Levels	Values			
Factor 3	Run 1, Run 2,				
	Run 3				
Analysis of Variance					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	15.51	7.757	0.05	0.954
Error	15	2489.20	165.947		
Total	17	2504.71			

Figure E-1: Minitab one-way ANOVA results for the three BHKP optimum runs

Method					
Null hypothesis	All	means	are		
		equal			
Alternative hypothesis	Not all means are				
	equal				
Significance level	$\alpha = 0.05$				
Equal variances were assumed for the analysis.					
Factor Information					
Factor	Levels	Values			
Factor 3	R1, R2,				
	R3				
Analysis of Variance					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	4.78	2.391	0.00	0.996
Error	15	8301.83	553.455		
Total	17	8306.61			

Figure E-2: Minitab one-way ANOVA results for the three FBSW optimum runs

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	$\alpha = 0.05$

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Factor 3		R1, R2, R3

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Factor	2	0.03565	0.01783	0.05	0.951
Error	15	5.28671	0.35245		
Total	17	5.32236			

Figure E-3: Minitab one-way ANOVA results for the three UBSW pulp optimum runs