CHAPTER

1

LITERATURE REVIEW: FIBRE MOVEMENT IN MECHANICAL PULP AND PROCESSES FOR IMPROVEMENT OF STABILITY

ABSTRACT

Wood is the main source of fibres used industrially to produce paper. These fibres are subjected to different chemical or mechanical processes to produce pulp with specific characteristics. Mechanical fibres are generally stiff and difficult to collapse. When incorporated into paper these fibres often encounter water that may lead to movement within or out of the sheet. These movements include swelling, puffing and lifting, and are responsible for the breaking of inter-fibre bonds that could lead to high surface roughness and reduced paper strength. These potentially unstable fibres can be removed by fractionation or refining processes. Additional treatment of these unstable fibres such as chemical and enzymatic treatments can be combined with these conventional processes. Chemical treatment is often used to remove some of the lignin or hemicellulose, making fibres more flexible and collapsible leading to better paper formation. Enzymes on the other hand, hydrolyse glycosidic bonds in the cell-walls of fibre, to improve fibre collapse.

1.1. INTRODUCTION

Cellulosic fibres find wide application in many pulp and paper operations. These fibres are produced through several wood pulping methods that include chemical treatment or mechanical actions such as grinding.

Pulp fibres encounter water at many different stages during the manufacturing process and conversion to paper; its subsequent behaviour in the presence of moisture will determine its suitability for a given application. Distortion of the fibre network in the presence of water can often be observed. These moving fibres are described as unstable, while stable fibres are characterised by good fibrillation and conformability that allows them to retain their collapsed state even in the presence of moist conditions (Forseth and Lepoutre, 1994). Thick-walled fibres present in mechanical pulp are typically poorly fibrillated. These fibres cause problems during manufacturing of paper, due to their stiffness and low conformability. Different processes used to separate and develop the surface properties of fibres are already applied in the pulp and papermaking industry. However, these methods have limitations and new opportunities are being investigated at present.

Different processes for the treatment of the fibres with either chemicals or enzymes before refining have been investigated. Chemicals such as sulphate are already used in bleaching processes to improve the pulp quality by softening or removing lignin, which acts as a barrier to water during the pulping processes (Salmén, 1984; Lindström *et al*., 1988). Pre-treating fibres with chemicals can also make the fibre wall more responsive towards refining conditions (Strunk *et al*., 1990; Meyer-Pinson *et al*., 2004; Bian *et al*., 2008). The application of enzymes is also useful to modify components in the cell-wall structure for this purpose (Kibblewhite and Clark, 1996; Mansfield *et al*., 1996; Clark *et al*., 1997; Lumme *et al*., 1999) and can possibility lead to a reduction in chemical use (Hill, 1996). Enzymes can specifically attack compounds in the cell-wall, modifying these compounds to produce higher quality paper with greater strength and surface smoothness (Lumme *et al*., 1999). The aim of the present chapter is to review previous work on the behaviour of different fibres during papermaking, how environmental conditions influence their stability, and how different methods can be used to improve fibre stability.

1.2. MECHANICAL PULP

Pulp consists of wood fibres or other lignocellulosic materials that have been broken down into discrete fibres during pulping. These liberated fibres obtained after pulping can be dispersed in water and reformed into a web to produce a paper sheet. Pulping processes are generally divided into two broad classes, chemical and mechanical, which produce substantially different fibre characteristics. In many papermaking operations, a combination of chemical and mechanical pulps is used to obtain the desired paper characteristics.

Chemical pulping is based on the chemical actions of sodium hydroxide, sulphite or sulphate (Sjöström, 1993; Gullichsen and Fogelholm, 2000). These chemicals degrade and solubilise components of the wood, especially lignin and hemicelluloses, leading to easier separation of fibres (Lindholm and Kurdin, 1999). Very little to no mechanical action is necessary to separate fibres, therefore, making chemical pulping an energy efficient method. The separated fibres show little structural damage, and thereby strong and flexible fibres can be produced from both softwood and hardwood species. Strong papers are produced in this way, since the lignin, which interferes with hydrogen bonding of fibres, is largely removed. However, the high cost of chemicals and low pulp yield obtained (approximately 45% of the dry mass of the wood) are drawbacks of these chemical processes (Biermann, 1996).

Mechanical pulping offers different advantages in the production of pulp and paper. Wood fibres are disintegrated using mechanical actions such as grinding and refining, where heat is generated to soften the lignin for easier fibre separation (Sundholm, 1999). Since the lignin is only softened, it is retained in the pulp and, therefore, high yields of 90 to 95% can be obtained (Biermann, 1996). However, the presence of lignin can restrict fibre swelling during pulping (Ehrnrooth, 1982) and less collapsible fibres are, therefore, produced. It is likely that these lignin-rich fibres form weak paper, because of less inter-fibre bonds that are formed due to the small contact areas and limited hydrogen-bonding. Mechanical actions such as grinding and refining can also damage fibres and contribute further to reduction in strength (Page and El-Hosseiny, 1976; Page and Seth, 1980). The strength properties of mechanical pulp can be improved by either a chemical pre-treatment, mixing chemical and mechanical pulped fibres, or by additional refining steps.

Despite its lower quality, mechanical pulp constitutes 20 to 25% of the world paper production and is increasing due to the high yield of the process and growing competition for fibre resources (Biermann, 1996). Different fibre sources are available for mechanical pulping, and these play an important role in the quality of the pulp produced (Varhimo and Tuovinen, 1999). Softwood species such as spruce, fir, pine and hemlock, are most commonly used (Härkönen *et al.*, 1989; Harris, 1993). It is generally recognised that species from the spruce family are the most suitable raw materials for mechanical pulping, since this species provides pulp with properties ideal for various paper products (Varhimo and Tuovinen, 1999). The superiority of spruce is attributed to the favourable fibre characteristics (fibre length), as well as a lower extractives content and high initial brightness of the pulp.

Poplar is the best suited hardwood resource for mechanical pulping (Varhimo and Tuovinen, 1999), but the morphology of hardwood fibres is more complex than softwood due to the greater variation in fibre types (vessel elements, parenchyma cells etc). Significantly less lignin and correspondingly higher amounts of cellulose and hemicelluloses are present in hardwoods compared to softwoods (Sjöström, 1993). Mechanical pulps from hardwoods also exhibit good light-scattering and sheet properties (such as high surface smoothness), whereas the strength properties are usually poorer due to the short fibre length of 1.2 mm or less (Horn, 1974; 1978; Sjöström, 1993).

Softwood and hardwood species are usually mixed during mechanical pulping to produce higher quality pulp and improve the strength as well as surface properties of paper. However, these pulp blends are usually highly heterogeneous due to the wide variation in fibre morphology, mechanical properties and chemical composition (Biermann, 1996; Koljonen *et al.*, 1997). Pulp blends contain fibres that have good paper-making potential that can be exploited for the production of high quality papers. Mixed pulp also contains a fraction that contributes to poor paper quality unless these fibres are extensively processed. Fibres of low quality are usually present in mechanical pulps and are very stiff and difficult to form into smooth and strong paper. Processing of these stiff fibres fibrillates the cell-wall structure and leads to more flexible fibres. It has been proposed that different mechanical pulping processes can be applied to loosen up the cell-wall layers in different ways (Franzén, 1986; Salmén *et al.*, 1999) and some of these are illustrated in Fig. 1-1.

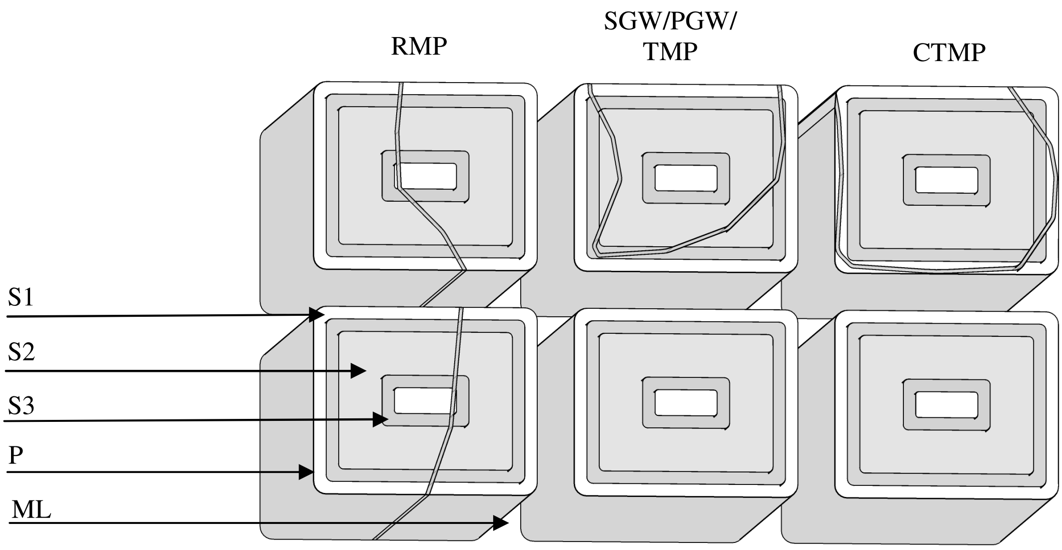

Figure 1 - 1: *Location of ruptures in fibres as a result of mechanical pulping. (P: primary wall, S1, S2 and S3: layers of the secondary wall, ML: middle lamella, RMP: refined mechanical pulp, SGW: stone groundwood, PGW: pressure groundwood, TMP: thermo-mechanical pulp and CTMP: chemi-thermo-mechanical pulp) (adapted from Franzén, 1986).*

Refiner mechanical pulping (RMP) is used to separate fibres only by mechanical actions, causing cracks in the S2-layer of the secondary cell-wall (Lindholm and Kurdin, 1999). These cracks in the cell-walls create fibre bundles that are not ideal for papermaking. However, the amount and size of these bundles can be reduced through extensive refining (Huusari, 1999). Stone groundwood (SGW) and pressure groundwood (PGW) are more common mechanical pulping methods, and separate fibres by pressurising the grinder with steam that heats and softens wood prior to the grinding action (Biermann, 1996). These two methods (SGW and PGW) split fibres between the S1- and S2-layers of the secondary cell-wall, leading to better separation, but some fibre damage may still occur (Lindholm and Kurdin, 1999; Fig. 1-1).

The thermo-mechanical pulping (TMP) method was developed 10 years after RMP, and has become the most important mechanical pulping method due to the higher strength of fibres that are produced. In TMP processes, wood chips are subjected to high temperatures (140°C to 160°C) in combination with mechanical refining (Lindholm and Kurdin, 1999). The effect on the fibre cell-wall structure is the same as observed with SGW and PGW.

A pulp yield of 91 to 95% can be attained with all these mechanical methods (Biermann, 1996), however, fibre damage that can degrade the pulp quality can occur. Less fibre damage is observed when chemi-thermo-mechanical pulp (CTMP) is produced. During this process heat is combined with small amounts of chemicals that partly dissolve or soften lignin. The chemical treatment before refining causes fibres to separate preferentially along the primary cell-wall (P) and the middle lamella (ML) (Lindholm and Kurdin, 1999; Fig. 1-1). These different mechanical pulping processes were investigated by Lindholm (1980), who demonstrated the effect of the typical action of each process on fibre properties.

1.3. FIBRE BEHAVIOUR

Mechanical pulp fibres can be significantly modified through refining processes, thus developing pulp properties to suit specific end-products, such as newsprint, uncoated papers and light weight coated (LWC) papers (Biermann, 1996). Two important requirements that need to be met for most end-products are high paper strength and smooth surfaces. Paper strength is necessary for good runability, or the ability of the web to withstand failure or breakages on the paper machine. In many cases when paper strength requirements are not met, the speed and productivity of the system may be limited by these web breaks. Surface smoothness, on the other hand, plays an important role in the quality of the printed images (Bristow and Ekman, 1981). These requirements for strength and smoothness are influenced by the ability of the fibres to collapse and conform during paper making.

1.3.1. Fibre collapse

The ability of fibres to collapse can be improved by removing some of the lignin in the cell-wall, modification of the cell-wall, or applying pressure on the fibre after drying. Lignin present in the cell-wall seems to act as a non-binding spacer between fibres and also limit fibre swelling during pulping (Lindström *et al.*, 1988; Ehrnrooth, 1982) and it is likely that these fibres will have poor collapsibility and less bonding. Some of the lignin can be modified or removed with chemical treatments (sodium sulphate, for example) followed by refining. Refining processes are used to modify the cell-walls of fibres (fibrillation), by removing the middle lamella, primary wall and S1-layer and parts of the S2-layer. Material that is only partly removed (fibrils) will give the fibre a larger surface area favourable for fibre bonding. Refining induces fibrillation, weakens the cell-walls and makes fibres more flexible and easier to collapse (Mohlin, 1975; Paavilainen, 1993). After chemical treatment and fibre development, these fibres are distributed unto a forming fabric, drained and some pressure is applied to induce fibre collapse. Wet pressing collapses fibres and brings them closer together (He *et al*., 2003). Additional methods such as calendering also apply pressure to achieve a smoother surface with higher gloss (Rounsley, 1991; Holmstad *et al*., 2004; Nesbakk and Helle (2002). While Skowronski (1990) showed that smoothness of a sheet made from TMP is achieved principally by fibre lumen collapse during calendering, little is known about the behaviour of fibres during calendering.

The degree of fibre collapsibility can vary, because of differences in fibre morphology among wood species and the effect of different pulping methods on the structure of the cell-walls. The lumen area is a good indication of the degree of collapsibility of fibres and is reduced or even eliminated when a fibre collapses (Fig. 1-2).

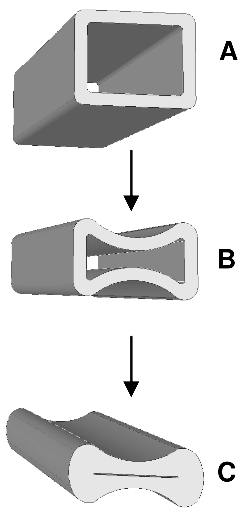

Figure 1 - 2: *Illustration of a fibre in cross-section showing changes in the lumen area for an A: uncollapsed fibre; B: a partially collapsed fibre and C: a totally collapsed fibre (adapted from Jang and Seth, 1998).*

Fibre collapsing is important in paper formation, because it can result in denser sheets with higher strength. The density of sheets is increased due to fibres that easily collapse and conform, creating fewer voids in the paper network. These highly collapsed fibres create large contact areas with more inter-fibre bonds, which are reflected in the stronger paper produced. Having recognised the importance of fibre collapse for papermaking, different methods for its measurement have been investigated (Robertson, 1964; Page, 1967; Kibblewhite and Bailey, 1988; Kibblewhite and Bawden, 1991; Jang *et al*., 1996, Jang and Seth, 1998 and Williams and Drummond, 2000).

Water retention measurements were used by Robertson (1964) to determine an index for collapse fibres and the reduction in the water content was associated with the loss in lumen volume. However, that method was only applicable to never-dried fibres, because the difference in the water content was associated not only with loss of lumen volume, but also with the fibre shrinkage and the collapse of fibrils. Page (1967) developed a sample preparation technique in which fibres were embedded in a medium that had the same refractive index as the fibre cell-wall. When this sample was viewed using a light microscope the totally collapsed fibres became invisible, while partially or uncollapsed fibres remained visible. While it was possible to discern the amount of collapsed fibres contained in the pulp, this method could not quantify the degree of collapse. Kibblewhite and Bailey (1988) investigated the shape of the fibres using microscopy and image analysis to measure fibre cross-sectional dimensions.

Examination of the cross-sectional shapes of fibres using microscopy appears to be the most accurate reflection of fibre collapse. The preparation procedure for this technique is tedious and requires aligning, resin embedding and microtome sectioning of the fibres (Kibblewhite and Bailey, 1988). The sample preparation technique is, however, very important for accurate results, and should not influence the state of the fibre present in the network. When representative cross-sections were obtained, image analysis could be applied to determine the dimensional parameters of fibers in micrographs (Jang *et al*., 1991; 1992; 1995; Fjerdingen *et al*., 1997; Reme *et al*., 1998; 1999). The most relevant of these dimensions are illustrated in Fig. 1-3.

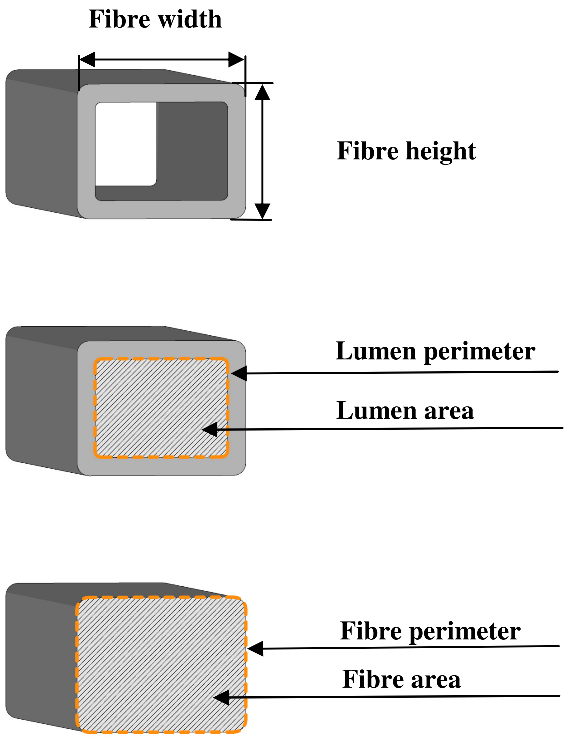

Figure 1 - 3: *Schematic representation of a fibre in cross- section to illustrate various cross-sectional parameters (adapted from Jang et al., 1995).*

The definition derived for fibre collapse is historically based upon the measuring techniques and image analysis software available. A collapse index (CI) was defined by Jang *et al*. (1995; 1996) using different area parameters such as fibre area (FA), lumen area (LA) and cell-wall area (CWA). The CI represents the change in the lumen compared to an estimated uncollapsed lumen. Kibblewhite and Bawden (1991) measured the fibre shape by using the aspect ratio (AR) as an index of fibre collapse. Fibre height is divided by fibre width, where a small AR implies a high degree of fibre collapse. Page (1967) expressed the collapse index as a function of the fibre-wall thickness only, since, wood fibres are tubular in shape and it is expected that thin-walled fibres collapse with greater ease than thick-walled fibres (Hallamaa *et al*., 1999). Xu *et al*. (1997) discussed a method that can determine the effect of the CWT at any stage of collapse, by calculating the ratio between fibre height and fibre width of a fibre cross-section. Image analysis software was also used by Reme *et al*. (2002) to measure the effect of CWT using the ratio of the CWA and the centreline perimeter, but this method has only limited application to fibres that are almost uncollapsed.

Those studies concluded that thin-walled fibres collapse to a higher degree than thick-walled fibres during paper production. Fibre morphology, therefore, can influence the paper structure, as well as its physical properties, but the impact of fibre collapse on paper properties is mainly due to its influence on conformation of fibres in the paper web.

1.3.2. Fibre conformation

Fibres that have the ability to bend and match the shape of adjacent fibres form large contact areas for fibre-to-fibre bonding, resulting in good sheet formation and stronger and smoother paper (Kure *et al*., 1999; Retulainen *et al*., 1998). Such fibres are described as conformable and the degree of conformability depends on the degree of fibre collapse. However, the conformability and collapsibility of a fibre is influenced by pulping conditions as well as the composition of the furnish.

Mechanical fibres are generally stiffer and less conformable when compared to chemical pulp fibres (Skowronski, 1990). Since mechanically-pulped fibres seldom collapse totally in the paper network, smaller contact areas are formed between fibres that lead to less bonding. It is, therefore, generally difficult to obtain well-formed sheets with the required strength and smoothness with mechanical pulps. However, different mechanical pulping methods are employed to produce specific end products, since CTMP fibres are, for example, more conformable with a higher degree of lumen collapsibility than TMP (Biermann, 1996). Fibres are tubular, and thus bend and conform more easily when collapsed or flattened. The degree of fibre collapse is, therefore, considered to be a good indicator of the fibre conformability (Jang and Seth, 1998).

It is clear that a high degree of fibre collapsibility and conformability are required to produce paper with good strength and printing surface. However, paper is subjected to processes such as coating and printing, where high moisture can reverse the collapsing of fibres (Retulainen *et al*., 1995; Forseth and Helle, 1996). Fibres with the ability to remain collapsed and resist movement can be described as stable fibres. High quality papers with good strength and surface properties are not only composed of collapsed fibres, but are also characterised by the stability of these fibres.

1.4. FIBRE STABILITY

The instability of fibres usually becomes evident when the relative humidity increases or when the fibres in the sheet come into contact with moisture. Paper has hygroscopic characteristics that attract moisture (Stone and Scallan, 1968). When moisture is present, water molecules penetrates between the hydrogen-bonded fibres in paper and into the cell-walls of the fibres, leading to changes in the paper and cell-wall structure (Skowronski and Lepoutre, 1985). The mechanism for individual fibre response under these conditions is still unclear.

The amount of water absorbed by the paper network depends on the fibre morphology and the state of collapse in the lumen (Aslund *et al*., 2005). Since pulps are characteristically heterogeneous, it is likely that paper sheets could contain stable fibres as well as unstable fibres that react differently with water molecules, and can cause undesirable surface roughness and a loss in paper strength. Unstable fibres can, therefore, move and cause negative changes in the paper structure. This is due to three types of fibre movement. Firstly, fibres that lift from the sheet surface (fibre rising); secondly, swelling of the fibre cell-wall and; thirdly, fibres that lose their collapsed shape (puffing). These movements become evident during operations such as coating and printing and are especially predominant in grades that contain mechanical pulp (Skowronski and Lepoutre, 1985; Aspler and Béland, 1994).

1.4.1. Fibre rising

Fibre rising can be observed in the fibre network after sizing, coating, printing or flexography (Hoc, 1989; Aspler and Béland, 1994). During these operations fibres are subjected to high moisture conditions, where one or both ends of the unstable fibres rise out of the paper surface (Forseth and Helle, 1997). In some cases when both ends are anchored, segments in the middle may rise. These movements are triggered by diffusion of water molecules into the fibre cell-wall (Hoc, 1989). Béland *et al*. (1993) showed that this movement can cause distortion in the fibre network and changes in the fibre dimensions, but the causal mechanism is still unclear. It was also observed by Skowronski and Lepoutre (1985) and Forseth and Helle (1997), that the distortion is irreversible and causes the surface to roughen and reduce the gloss in printed areas.

The extent to which paper roughness develops is influenced by the type of pulp used and when only mechanical pulp fibres were used, fibre rising was more evident, resulting in rougher sheets (Skowronski and Lepoutre, 1985; Hoc, 1989; Aspler and Béland, 1994). Fibre rising was also more noticeable in poorly-bonded sheets and, according to Hoc (1989), an increase in fibre length leads to a greater extent of fibre rising. Fibre rising is, therefore, expected to be more evident in softwood than in hardwood species, due to the relatively longer fibres. However, Mohlin (1989) emphasised that this problem was also due to the presence of thick-walled fibres typical in mechanical pulp.

1.4.2. Cell-wall swelling

Cell-wall swelling occurs when water molecules diffuse into the fibre wall, causing an increase in some the fibre dimensions (Page *et al.*, 1985). Cell-wall swelling in the present study refers to the reaction of fibres in a dried paper sheet when exposed to water during coating or printing. These swelling forces inside the paper can be extremely powerful, and it is likely to cause bond breakage, leading to unbalanced stress that may cause fibre segments to lift out of the sheet surface. According to Hoc (1989), these segments that lift out of the surface because of cell-wall swelling can contribute to fibre rising. The cell-wall swelling effect was primarily observed in chemical pulp by Forseth and Lepoutre (1994).

Cell-wall swelling can also contribute to increased bulk and surface roughness during coating (Skowronski *et al.*, 1988). Stone and Scallan (1968) used microscopy to observe the elementary fibrils present in the fibre wall (Fig. 1-4). In the model subsequently proposed, a dry fibre wall was described as being nonporous. The fibre wall in its dry state consists of fibrils bonded together in a close-packed array (Fig. 1-4 A). When these fibres start to adsorb water, hydrogen bonds are broken and the structure swells (Figs. 1-4 B to C). The fibrils are then separated by lamellar spaces, and water molecules can penetrate between the hydrogen bonded fibrils into the fibre cell-wall. The more water penetrates, the more internal bonds between the cell-walls are lost, to cause distortion or deformation of the fibres in the paper network (Figs. 1-4 C to D).

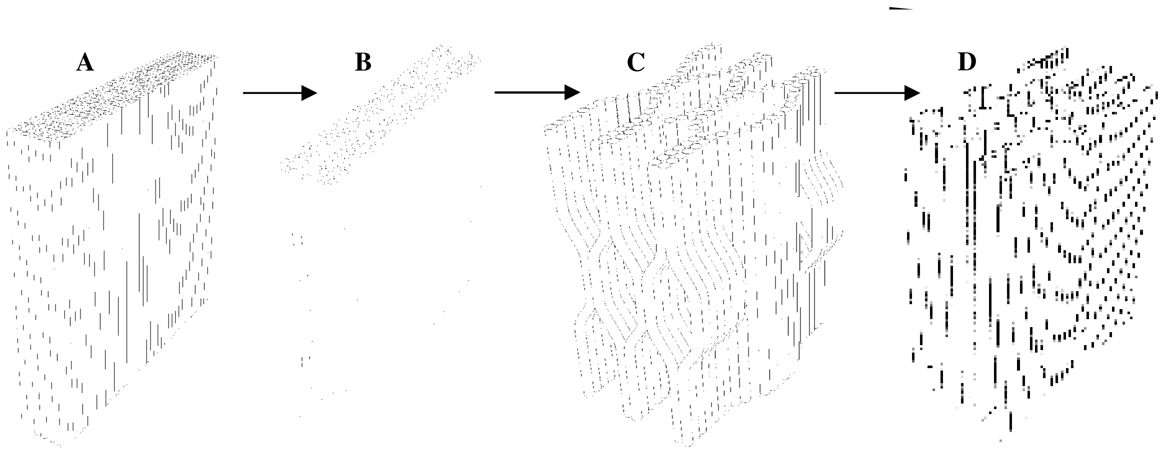

Figure 1 - 4: *Proposed model showing a successive decrease in bonding between fibrils in a sheet as result of water absorption (Stone and Scallan, 1968).*

1.4.3. Puffing

The recovery of the fibre shape to a tubular form has been referred to as decollapse (Reme *et al.,* 1998; 1999), but is better described as fibre puffing. Puffing can be defined as the expansion of the lumen of a fibre, under increasing moisture conditions. A study by Forseth and Helle (1997) showed that the puffing of fibres was not related to changes in the thickness of the cell-wall or the cell-wall area. Fibre puffing has not been widely investigated but a number of studies have been done to examine changes in the dimensions of the fibre lumen. In these studies, puffing of fibres was usually brought about by wetting of fibres (Skowronski, 1990; Forseth and Lepoutre, 1994; Forseth and Helle, 1996; 1997). Skowronski (1990) also investigated the wetting of fibres under different stress conditions that included uncalendered and calendered paper. It was evident that puffing of unstable fibres was more pronounced in calendered handsheets that were subjected to high moisture (Forseth *et al*., 1997).

1.5. IMPROVEMENT OF FIBRE STABILITY

Current technology such as refining and fractionation makes it possible to isolate and treat unstable fibres and thus control the fibre movements described above. Fibres are modified during refining, to increase cell-wall fibrillation for better bonding and conformability (Corson and Ekstam, 1994; Karnis, 1994; Mohlin, 1997). Since fibres react differently towards refining conditions, and since all fibres are not subjected to the same amount of refining action, a fraction of unfibrillated fibres often remains after refining. These unfibrillated fibres could add to instability, they are separated from the main stream using fractionation processes (Hautala *et al*., 1999; Kappel, 1999). Further improvement of such fibres can be achieved using these mechanical processes combined with either chemical treatments or enzymatic treatments aimed at making the cell-wall more susceptible to fibrillation and collapse.

1.5.1. Refining

The post-refining of mechanical pulp is one of the critical operations in the production of a desired end-product. One of the objectives of fibre modification during refining is to increase the bonding ability of fibres (Mohlin, 1975; 1997; Hiltunen, 2003). The increased bonding leads to stronger paper (Karnis, 1994; Kure, 1997) and reduced fibre movement. Fibre stability can, therefore, be improved by increasing the flexibility and fibrillation of the cell-walls. The flexibility of the cell-wall can be improved by internal fibrillation of fibres, while external fibrillation increases inter-fibre bonding. As mentioned earlier, internal fibrillation refers to loosening of the internal fibre-wall structure by rupture of bonds in the cell-wall (Mohlin, 1991), making it possible for the fibre to swell more during the refining process. As a result, the collapsibility and flexibility of fibres are increased and these fibres then conform effectively during sheet formation.

The high number of impacts associated with efficient refining causes cell-walls to also fibrillate externally. Scanning electron microscopy (SEM) showed that fine cellulosic bundles are loosened and in some cases parts of the cell-wall are peeled off, but not completely removed from the fibre (Kibblewhite, 1972; Kerekes, 2005; Molin and Lennholm, 2000). Since these external fibrils remain attached to the fibres, more fibre-to-fibre bonds are formed (Page *et al.*, 1985, Page, 1989). Strength properties such as tensile and Scott bond of Kraft pulp have been shown to increase by 20 and 46%, respectively, through efficient refining (Kang and Paulapuro, 2006).

The response of fibres to refining depends to a large extent on fibre morphology, such as cell-wall thickness (Huang *et al.*, 2007). It was observed that fibres with thin walls absorbed more energy and were easier to rupture under standard refining conditions when compared to thicker-walled fibres. After refining, most of the thin-walled fibres were split and collapsed and short elements and fines were also generated (Reme *et al*., 1999). When thick-walled fibres were refined, changes were not obvious, but higher refining levels caused reduction in the cross-sectional dimensions to produce more collapsed fibres (Corson and Ekstam, 1994; Karnis, 1994; Mohlin, 1997). Therefore, even thick-walled fibres can be modified through refining so that they can collapse to increase fibre and pulp stability (Norgren *et al*., 2004), and it is likely that these stable fibres can result in stronger paper with better surface properties. However, refining is an energy intensive process and mills include other processes to reduce these thick-walled poorly fibrillated fibre portions.

1.5.2. Fractionation

Mechanical pulp typically contains a wide range of fibres with different morphologies, including highly and poorly fibrillated fibres (Paavilainen, 1988). Fractionation refers to the selection processes that can be used to separate fibres which are suitable from those unsuitable for paper making (Vomhoff and Grundström, 2003). The result of using only desirable fibre is that pulp with a higher quality and more uniformity can be produced (Abubakr *et al*., 1994).

Fractionation processes are generally applied after refining, and consists of hydrocyclone cleaners and screens. Hydrocyclone cleaners use the principle of centrifugal separation, and remove fibres on the basis of specific gravity (Paavilainen, 1992). The result of cleaning is, therefore, to reject thick-walled fibres that are often responsible for printing defects (Hoc, 1989; Paavilainen, 1992; Kure *et al*., 1999). Handsheets made from the cleaned (thin-walled) fibres showed significantly higher surface smoothness and higher strength properties when compared to the rejected fibre fraction (Paavilainen, 1992; Vomhoff and Grundström, 2003).

Fractionation also includes screens that are usually constructed with foils to create pressure across the slots and are generally referred to as pressure screens (Biermann, 1996). Pressure screens primarily aim to separate long fibres as rejects (Kure *et al*., 1999). However, it was also observed that it was possible to separate out some of the coarse, thick-walled fibres using hydrocyclones (Kappel, 1999). By removing these long undeveloped fibres, paper properties such as smoothness can be improved (Mohlin, 1989).

Removal of unsuitable fibres (rejects) results in a reduction of pulp yield. In fractionation processes with low reject rates, fibres can be re-circulated by returning rejected fibres to the pulp stream before refining. Alternatively, with higher reject rates, rejected fibres can be improved through additional reject-refining stages. Reject refining specifically concentrates on promoting flexibility and collapsibility of these rejected fibres (Kure *et al*., 1999). Fibre development through only refining and fractionation are limited to the degree of fibre modification that can be achieved. Integrating these processes with pre-treatments such as chemicals or enzymes may offer new opportunities to improve pulp properties even further.

1.5.3. Chemical treatment

The effects of chemical pre-treatments before refining have been described in terms of pulp and paper properties (Franzén, 1983; Gummerus, 1986; Sferrazza *et al*., 1988; Strunk *et al*., 1988, 1990; Meyer-Pinson *et al*., 2004; Bian *et al*., 2008). The effect of different chemicals such as sodium sulphate, alkaline peroxide and oxalate were investigated on reject fibres. Sulphate treatments improved paper formation as well as surface properties (Franzén, 1983; Gummerus, 1986), while alkaline peroxide modified fibre characteristics and caused an increase in the paper strength and reduced refining energy requirements (Strunk *et al*., 1988, 1990; Bian *et al*., 2008). Paper strength can also be increased by using a biomimetic treatment, consisting of sodium oxalate (Meyer-Pinson *et al*., 2004). The benefit of chemical pre-treatments is that it can promote the quality of pulp, by softening or removing some of the lignin in the cell-walls before refining, (Heitner and Atack, 1983; Bian *et al*., 2008). Therefore, in most of the chemical treatments better fibrillation, increased fibre flexibility and swelling, followed by a high degree of fibre collapse and conformability are obtained. Oxalate treatment specifically modifies the hemicellulose-pectin complex, making the cell-wall more susceptible towards refining (Meyer-Pinson *et al*., 2004). Although chemical treatments could improve fibre characteristics, these have generally not been applied to improve the quality of mechanical pulp rejects. Therefore, other ways to pre-treatment of mechanical fibres before refining were investigated.

1.5.4. Enzymatic treatment

The natural polymeric components of paper (cellulose, hemicelluloses and lignin), has led to investigations into the potential use of enzymes to improve certain fibre characteristics by modifying these polymers (Pere *et al*., 1995; Bhardwaj *et al*., 1996; Clark *et al*., 1997; Mansfield and Saddler, 1999). Commercial enzymes that include cellulases and hemi-cellulases act on these polymers to promote specific fibre properties such as strength (Mansfield *et al*., 1996; Mansfield and Saddler, 1999; Kibblewhite and Wong, 1999). These enzyme applications were studied on a laboratory scale (Kibblewhite and Clark, 1996; Mansfield *et al*., 1996; Kibblewhite and Wong, 1999; Lumme *et al*., 1999; Mansfield *et al*., 1999a; 1999b; Mansfield and Saddler, 1999; Wong and Mansfield, 1999; Wong *et al*., 1999; 2000; Mansfield and Dickson, 2001), but until recently only a few processes have reached mill-scale application (Viikari *et al*., 1994).

Cellulases

Cellulases are used to modify the main component (cellulose) of the cell-wall and constitute the most widely studied of the fibre-treating enzymes. Cellulose forms approximately 80% of the dry material in all fibres and serves as an essential stress bearing constituent of all plant fibres (Sjöström, 1993). Cellulases have the ability to adsorb onto the cellulose molecule through a cellulosic binding region before attacking the glycosidic bonds (Béguin and Aubert, 1994; Lynd *et al*., 2002). Since cellulases occur in multiple forms with different specific activities, total degradation of the cellulose is possible when multi-component formulations are applied. When mono-component enzymes are used, the cellulose will be modified rather than totally degraded (Bledzki and Gassan, 1999; Lynd *et al*., 2002).

A multi-component cellulase system consists of three classes of enzymes with specific actions including endoglucanase (EG), cellobiohydrolases (CBH) and glucosidase (Béguin and Aubert, 1994). Endoglucanases attack the amorphous region of native cellulose, at multiple internal sites causing a rapid decrease in the length of the polymer chain (Pere *et al*., 1995). By opening the substrate, the surface area is increased for subsequent attack by CBH (Howard *et al*., 2003). Cellobiohydrolases form the major component of cellulase systems accounting for 40 to 71% of the total cellulase protein that hydrolyses highly crystalline cellulose structures (Béguin and Aubert, 1994). The CBH cleave mono and dimeric groups from different sites of the glucose chain. A-type CBH cleave from the reducing ends of the cellulose chain, while those enzymes that cleave from the non-reducing ends are known as the CBH II (B-type). Glucosidase is a considerably smaller enzyme that cannot bind or degrade crystalline cellulose. This enzyme can only hydrolyse cellobiose to D-glucose.

The potential applications of cellulases to the pulp and paper industry are numerous. As early as 1959, cellulases were applied to improve fibre fibrillation (Bolaski and Gallatin, 1959). Cellulases are also known to promote the drainage ability of pulp by decreasing finer fibre elements (Mansfield and Saddler, 1999). The ability to deink recycled fibres (Tausche, 2005) can also be improved by using these enzymes. Cellulases also enhance the potential for improvement of fibre stability through increased fibrillation. Treatments with different cellulases were evaluated on chemical pulp (Kibblewhite and Clark, 1996; Mansfield *et al*., 1996; Mansfield and Saddler, 1999) and strength properties such as tensile, tear and burst were increased. However, it was observed that these enzyme treatments reacted differently to unbleached and bleached chemical pulps. Unbleached fibres treated with a multi-component cellulase did not show any significant changes in length and coarseness and fibres remained essentially uncollapsed when compared with bleached fibres (Kibblewhite and Clark, 1996). They found that treatments with EG were more effective, resulting in more collapsible fibres. Although it was possible to increase fibre flexibility through EG treatment, fibre strength was negatively influenced and reflected in lower tear and tensile properties (Kibblewhite and Clark, 1996; Kibblewhite and Wong, 1999; Lumme *et al.*, 1999). The effect of the multi-component cellulase and EG were also evaluated on fully bleached fibres, and the effect of the enzymes was more evident. The fibres were more collapsed in both treatments, but strength loss was still a problem (Mansfield *et al*., 1996).

Strength properties of both bleached and unbleached chemical pulp were reduced when treated with enzymes (Clark *et al*., 1997). The highest strength loss was observed with EG treatment that mainly caused weakening of the individual fibres and not of inter-fibre bonds or overall fibre length (Clark *et al*., 1997). Strength reduction of the fibres due to EG treatment correlated strongly with loss of the degree of polymerisation in cellulose, suggesting that enzymatic degradation was focused on accessible areas of the fibre and created weak points within these areas.

To minimise the loss in fibre strength, only selected fibres fractions were treated with enzymes (Mansfield *et al*., 1996; Mansfield and Saddler, 1999). These selected fractions contained fibres that were more resistant to chemical treatments and refining processes and mostly consisted of thick-walled long fibres. Fractions that contained long and coarse fibres were collected on a laboratory scale, with the use of a Bauer-McNett classifier, and on a mill scale rejected fibres were collected after screening and cleaning (Mansfield *et al*., 1996; Mansfield and Saddler, 1999). Enzymatic treatments were applied to these pulp fractions and reduced fibre strength was observed. However, 10% less refining energy was required to reach the target freeness when enzyme treatments were applied compared with untreated samples. Furthermore, the integration of an enzymatic treatment stage with refining could provide a means of incorporating coarse fibres into the manufacture of fine paper (Mansfield *et al*., 1999b).

The influence of cellulases on long thick-walled fibres was also investigated on mechanical pulp (Pere *et al*., 1996). When these fibres were pre-treated with EG no significant changes were observed. However the application of CBH showed improvement in tensile strength of handsheets. The difference in the effect of the enzyme may be due to the selectivity of the enzyme action as well as the different fibre properties (Pere *et al*., 1996).

Fibre properties of thick-walled fibres are difficult to improve during refining, and can influence paper formation negatively (Hallamaa *et al*., 1999; Kure *et al*., 1999). Compared to thin-walled fibres, the thick-walled fibres are not as susceptible to refining, unless they were previously treated with cellulase. Strength properties of handsheets made with coarse fibres were also improved with cellulase treatment, although the effect of the enzyme was more evident on less-coarse fibres (Lumme *et al*., 1999). When coarse and less-coarse fractions were treated with endoglucanase (mono-component of cellulase), the tensile properties improved irrespective of the fibre coarseness.

Increased strength properties of paper can be related to weakening of the cell-wall to improve fibre collapse (Mansfield *et al*., 1996, 1998). In that study two commercial enzymes (a multi-component cellulase and EG) caused the densities of the handsheets to increase, suggesting that collapsibility had been improved. These results implied that the limited hydrolysis caused by the mono-component enzyme treatment was enough to enhance fibre fibrillation.

Hemicellulases

The hemicellulose content of dry wood is approximately 20%. The structure of hemicelluloses is very complex and consists of monomeric compounds that include D-glucose, D-mannose, D-galactose, D-xylose and L-arabinose (Sjöström, 1993). The composition of the heteropolysaccharides differs in hardwood and softwood species and xylan is the main hemicellulose in hardwood fibres comprising 25 to 30% of dry weight (Sjöström, 1993). Xylan has been shown to contribute to fibre-wall swelling and flexibility (Eriksson *et al*., 1991).

The heterogeneous nature of hemicelluloses makes hydrolysis very complex and the hemicellulytic system contains the following enzymes: (i) endo-1,4-β-D-xylanases (EC 3.2.1.8), (ii) exo-1,2-β-D-xylosidases (EC 3.2.1.37), (iii) endo-1,4-D-mannanases (EC 3.2.1.78), (iv) β-mannosidases, (v) α-D-glucuronidases, (vi) acetyl xylan esterases, (vii) α-L arabino-furanosidase and (viii) α-galactosidase (Viikari *et al*., 1994). Hemicellulases in the form of xylanases are commercially applied to enhance delignification during bleaching (Kantelinen *et al*., 1993; Viikari *et al*., 1994; Wong *et al*., 1999). The effect of xylanase treatment on improving individual fibre properties has also been investigated (Noé *et al*., 1986; Pham *et al*., 1995; Kibblewhite and Wong, 1999; Moss and Pere, 2006). Partial removal of xylan by enzymes improved the potential of fibres to collapse (Kibblewhite and Wong, 1999; Moss and Pere, 2006). Highly collapsible fibres resulted in increased tear strength of handsheets (Kibblewhite and Wong, 1999). Most of these studies with xylanase included chemical pulp and showed that strength properties were retained, contrasting with some of the results obtained with endoglucanase treatments.

The effect of xylanases was more evident on hardwood species because of the high xylan content. Xylanase-treatment reportedly did not cause any significant differences in any of the fibre or handsheet properties (Lumme *et al*., 1999). The main hemicellulosic component of softwood is mannanase and application of mannanase could potentially have the same benefits on softwood that xylanase had on hardwood.

1.6. CONCLUSIONS

Chemical or mechanically pulped fibres are used to produce paper with specific strength and surface properties, which depend on the collapsibility and conformation of fibres. Paper produced from mechanical pulp contains stiffer and less collapsible fibres, especially due to the presence of latewood (thick-walled) fractions (Skowronski, 1990). These less-collapsible fibres cause lower strength and rougher paper surfaces, because of the smaller bonding areas. These thick-walled fibres are unstable when subjected to moisture, and was reflected in fibre movement (Forseth and Helle, 1996). These movements (fibre rising; cell-wall swelling and puffing) caused higher surface roughness and lower paper strength (Page *et al*., 1985; Hoc, 1989; Reme *et al*., 1999).

There are two ways of dealing with unstable fibres, the fibres can either be removed through fractionation (Vomhoff and Grundström, 2003), or by improving their stability through cell-wall modification. Refining is the conventional way to modify the cell-wall to achieve improved collapsibility (Paavilainen, 1993). These highly collapsible fibres lead to stronger bonding (Kure *et al*., 1999) which is believed to improve fibre stability. However, in moist conditions, fibre movement still remains a problem. Alternative treatments that could lead to more stable fibres include chemical (Strunk *et al*., 1990; Meyer-Pinson *et al*., 2004) and/or enzymatic treatments (Mansfield and Saddler, 1999; Kibblewhite and Clark, 1999); but these treatments should be combined with refining for the best results.

Chemical treatments are often used to remove some of the lignin or hemicelluloses, making fibres more flexible and collapsible for better paper formation. Enzymatic treatments weaken the cell-wall through breaking glycosidic bonds that result in better collapsibility or fibrillation. When combined with refining, these treatments can have the potential to extend the efficiency of refiners to obtain more stable fibres that will produce stronger and smoother paper.

1.7. PROBLEM STATEMENT AND OBJECTIVES

The stability of fibres in a paper network is clearly important to obtain high quality printed images. When environmental conditions change (e.g. moisture), unstable fibres react and move to cause changes in sheet properties such as surface roughness as well as reduced strength. Improvement of fibre stability under moist conditions was, therefore, investigated in the present study. It was noticed that refining could improve the quality of the pulp by fibrillating the cell-wall and by fractionation to remove some of the long- and thick-walled fibres. The effect of these processes is to increase surface smoothness and sheet strength. Additional treatments with enzymes have the potential to improve the stability of fibres and were, therefore, also a focus of the research presented in this thesis.

It is important to quantify the changes in the fibre shape after treatments and a method to observe and quantify these changes was developed (Chapter 2). It was also important to understand the influence of different treatments on a variety of wood species and fibre types (Chapters 3 to 5). The relevance of these methods was evaluated on commercial pulps and sheets (Chapters 6 to 8). The potential of the enzyme treatment for improvement of stability in different fibre types and the proposed mechanism for such development are presented in conclusion (Chapter 9).

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CHAPTER

2

SAMPLE PREPARATION AND QUANTIFYING OF FIBRE MOVEMENT

ABSTRACT

When mechanical pulp fibres come in contact with water, they can move and cause poor surface properties such as high roughness. Microscopy can be applied to observe these movements and understand the basis for roughness. However, obtaining reliable images of the fibres in the paper formation is essential. In the present study, details of the surface and internal structure of the fibre network was examined. Different methods that included cutting (blade, freezing and laser) and embedding (wax and resin) of handsheet cross-sections were evaluated. Resin embedding was selected as the method that gave the best detailed and reliable fibre images, and was subjected to image analysis that allowed measuring of fibre dimensions. Fibre morphology, the cutting angle and degree of collapse can influence these fibre parameters. Fibres displaying different features were modelled to illustrate the impact of each of these features. Different parameters were evaluated and it was determined that puffing of fibres in cross-sections can be quantified with a ratio of the lumen area to fibre area. Resin-embedded samples imaged with a conventional scanning electron microscope allowed puffing to be quantified by using image analysis.

2.1. INTRODUCTION

When mechanical paper comes into contact with water; for example during coating and printing, the structure of the fibres in the paper network can change. These changes are caused by fibre movements leading to a rough paper surface (Mao, 2001), which lowers the quality of printed images (Ginman and Visti, 1973; Oittinen and Saarelma, 1998). Movement such as fibre lifting was observed by Hoc (1989) when fibres lifted from the surface of paper. Lifting was more evident in paper that contained large proportions of long fibres and especially after supercalendering. Skowronski and Lepoutre (1985) observed cell-wall swelling when fibres were exposed to coating suspensions, suggesting that the swelling movement was irreversible because of debonding and stress relaxation occurring in the surface structure. A fibre movement called fibre puffing and defined here as the expansion or "decollapsing" of the lumen area, was also observed. While all the fibre movements (fibre rising, cell-wall swelling and puffing) have been observed and described in mechanical pulp, quantifying these movements still remains a challenge.

Different methods were published to observe changes caused by fibre rising and cell-wall swelling under high-moisture conditions (Hoc, 1989; Jang *et al*., 1991; 1992; 1995; 1996; Forseth and Helle, 1997; Jenkins and Donald, 1997; Jang and Seth, 1998; Williams and Drummond, 2000; Reme *et al*., 2002). However, a standard method to quantify puffing is not available yet. Most of these published methods include microscopy that makes it possible to visualise changes that occur in the structure of the fibre in a paper network in the presence of water. The fibre structure in paper can be observed using non-invasive as well as invasive microscopy methods. Confocal laser scanning microscopy (CLSM) provides a non-invasive method of visualising a paper network in two or three dimensions (Jang and Seth, 1998; Singh *et al*., 2008). With this method, fibre morphology can be examined by means of optical sectioning of the paper network. Alternatively, fibres in the paper network can be exposed mechanically by sectioning.

Irrespectively of the microscopic techniques used, it is important to follow sample preparation protocols that will not distort fibres and allow free response to environmental changes. Therefore, in order to obtain sections of fibres by means other than CLSM, the fibre network needs to be supported to withstand the mechanical cutting process. Supporting media such as wax or resin can be used, and previous research has shown that fibre and paper structure can be preserved well and artefacts minimised after embedding (Page, 1965; Nanko and Ohsawa, 1990; Szikla and Paulapuro, 1989; Forseth and Helle, 1997). In contrast, other studies reported that areas within these thin sections fail to fully represent significant structural features, and that reliable structure assessment is made difficult by the frequent artefacts that are created during sectioning (e.g. Reid and Beesley, 1991). These artefacts include scratches, compressed fibres and distorted paper structure which can lead to inaccurate measurements. Gibbon *et al.* (1989) explored an alternative method to thin sectioning by grinding and polishing methods to evaluate the resin-embedded samples. However, this sample preparation method was less suitable for detailed examination in the scanning electron microscopes (SEM) due to the lack of contrast between the fibres and the surrounding embedding material after polishing. This lack of detail was overcome by Williams and Drummond (2000) who, in addition to grinding and polishing to produce the section plane, also removed a thin layer of the supporting resin by chemical etching to reveal the internal structure of paper. The surface of an embedded sample could, therefore, be examined for puffing by etching the surface.

Conventional scanning electron microscopy (cSEM) can be used to view dry samples (Reimer, 1985) and puffing could, therefore, only be imaged before and after rewetting, because samples have to be dried before viewing. In contrast, environmental scanning electron microscopy (ESEM) makes it possible to control the environmental conditions in the specimen chamber, and thus to observe fibre structural changes in real time as the paper is gradually wetted (Danilatos, 1993; Jenkins and Donald, 1997).

These changes observed by cSEM and ESEM can then be quantified by measuring fibre dimensions, therefore, contributing towards increased understanding of the nature of puffing. Changes in fibre structure, due to movement (such as puffing) can be converted into reliable measurements using image-analysis programmes. These programmes can be used to record fibre dimensions such as cell-wall thickness (CWT), fibre area (FA), lumen area (LA) and the outer circumference of the lumen and fibre (Reme *et al.*, 1998; 1999). These parameters can be statistically analysed to determine the effects of treatments on fibre stability after rewetting. Forseth and Helle (1997) successfully applied image analysis to cSEM images to quantify the changes in the LA during rewetting of mechanical pulped fibres. An image analysis programme was also used by Reme *et al.* (2002), to determine the variation in CWT in pulp fibres, using cSEM micrographs. Jang *et al.* (1996) used CLSM combined with image analysis to examine fibre collapse and changes in CWT in mechanical pulp at different refining levels. In further research the state of the lumen was defined by a collapse index (Jang and Seth, 1998).

The present study was motivated by the difficulties previously experienced in obtaining compression-free sections of handsheets, as well as in observing and quantifying puffing. Therefore, the objectives of the present study were to a) establish a reliable method to obtain artefact-free and detailed cross-sections of handsheets of chemi-thermo-mechanical pulp (CTMP); and b) to find a reliable method to visualise - and wherever possible quantify - fibre movement, following rewetting. Fibre movement was observed with cSEM and ESEM on both sheet surfaces and in cross-sections, and in keeping with previous work (Hoc, 1989; Jang *et al*., 1991; 1992; 1995; 1996; Forseth and Helle, 1997; Jenkins and Donald, 1997; Jang and Seth, 1998; Williams and Drummond, 2000; Reme *et al*., 2002), the present study also used image analysis as a tool to quantify puffing.

2.2. Materials and Methods

2.2.1. Pulp

Pulp containing 75% spruce (softwood) and 25% poplar (hardwood) was received from a CTMP mill. The samples were made up to a 3% consistency and disintegrated at 3000 revolutions using a MK IIIC disintegrator (Messmer Instruments Limited, UK) to separate fibres. Handsheets with a basis weight of 60 g m^{-2} were subsequently made with a Rapid Köthen handsheet-former according to the 5269/2 standard ISO method. Samples from the handsheets were then processed as detailed below.

2.2.2. Sample preparation and examination

Surface areas of handsheets

Four random samples were cut from the handsheets and mounted with double-sided tape onto a stub, with the sheet surface area uppermost. The surface of handsheets was imaged with ESEM (Philips XL 30) to examine the movement of the fibres when exposed to water (15.0 kV, GSE 10.0 and a 350x magnification). The fibres on the surface were initially viewed in the dry state after which the humidity was raised to the point where water was visible on the sample. Images were recorded at various stages during the wetting and subsequent drying process.

Cross-sections of handsheets

The following preparation methods were tested to identify the most reliable method to obtain clean cross-sections that did not distort fibre or handsheets structure. In each instance, three to five samples were taken randomly from handsheets and processed as described below.

Cutting:

The following cutting methods were investigated and include: (a) *razor-blade*: cut handsheet-samples by using a new razor blade, (b) *freeze drying*: razor-blade cutting after samples of dry handsheets were frozen in an attempt to immobilise fibres in the network and thus prevent distortion during cutting. Samples were put into liquid nitrogen for 30 s and quickly cut with a sharp blade before thawing and (c) *laser cutting* where handsheets were cut with a Coherent G-100 laser (LaserCAMM^{TM}) with a laser power of 100 W.

Microtome Cutting:

Before paper samples can be cut with a microtome a supporting medium is needed. Two mediums were investigated during the present study: (a) *wax embedding: s*amples of handsheets were embedded in histology-grade paraffin wax, as this method is relatively quicker than resin embedding. Samples were placed in plastic moulds, intrafiltrated with molten wax at 50°C and left for 30 min to cool. The embedded wax samples were then cut by hand using a microtome to expose the paper web and (b) r*esin embedding: s*amples were embedded in Quetol 651 resin, according to the method described by Van der Merwe and Coetzee (1992). Microtome sectioning took place from the middle of each sample to avoid the mechanical distortion at the edges of the sample after trimming with a razor blade.

The sectioned face was etched with a solution of saturated sodium methoxide for approximately 40 min to expose the fibre network (Iwadare *et al*., 1990).

After etching, the blocks were rinsed thoroughly in ethanol (3 x 30 s) with the aid of an ultrasonic water bath, and allowed to dry. Chemical etching was used to expose fibre detail and increase the contrast of the sample. However, as chemicals used for etching could cause changes in the fibre structure, an experiment was set-up initially to investigate this possibility. In this experiment, fibres from resin-embedded samples were measured before and after etching to determine if any differences in handsheets structure could be detected.

The sample cross-sections obtained using razor-blades, laser ablation or microtomes were mounted with double-sided carbon tape onto stubs, with the cut area uppermost. Samples were viewed with an ESEM and images were recorded under the same conditions as for the surface samples. All the handsheet cross-sections were also photographed using a cSEM (LEO 1450, Carl Zeiss, Germany; and JSM 840, JEOL, Tokyo, Japan). The samples were rendered conductive in the vapour of a 0.5% RuO_4 solution (Van der Merwe and Peacock, 1999) before viewing to avoid charging artefacts. The influence of the etching chemicals was also examined with cSEM. Samples embedded in resin and cut with a microtome were photographed in backscatter-electron imaging mode (BEI) before etching. After etching, samples were viewed in normal secondary-electron imaging mode (SEI). Before and after images were compared to establish if movement due to etching had occurred.

2.2.3. Quantifying fibre dimensions

The "ImageTool" software (www.http://ddsdx.uthscs.edu/, University of Texas, San Antonio) was used to measure the area and circumference of fibres and lumina (Fig. 2-1). Only fibres with an intact cell-wall and with a visible lumen (i.e. not fully collapsed) were measured. The diameter of the fibres was also measured, and the CWT was calculated as follows:

CWT = (fibre diameter – lumen diameter)/2.. *(1)*

Many fibres are not cut perpendicularly during sectioning, because of the random orientation of fibres in a handsheet network. These fibres were thus excluded from the measurements as they can lead to over- or underestimated fibre values. The influence of cutting angle and fibre dimensions is explored in the next section.

2.2.4. Modelling of fibre morphology and orientation

A graphics package (AutoCAD, 2004, Autodesk Inc., U.S) was used to investigate the extent to which fibre orientation (cutting angle) or fibre morphology influenced parameters that could indicate fibre collapsing or puffing. Arbitrary values (units) were assigned to the fibre outer circumference and fibre area (defined as the whole area within the outer circumference) for this modelling (Fig. 2-1). Parameters were defined to create thin-walled and thick-walled fibres with the same fibre circumference, but with different circumferences for their lumina.

The profiles of the thin-walled and thick-walled fibres were then manipulated to produce collapsed and uncollapsed fibres sectioned at 90º or 45° angles. During this manipulation, the circumference of the fibre and lumen were kept constant. The following parameters from the models were selected: fibre area (FA); lumen area (LA) and cell-wall thickness (CWT) in both the x- and y-directions (Fig. 2-1).

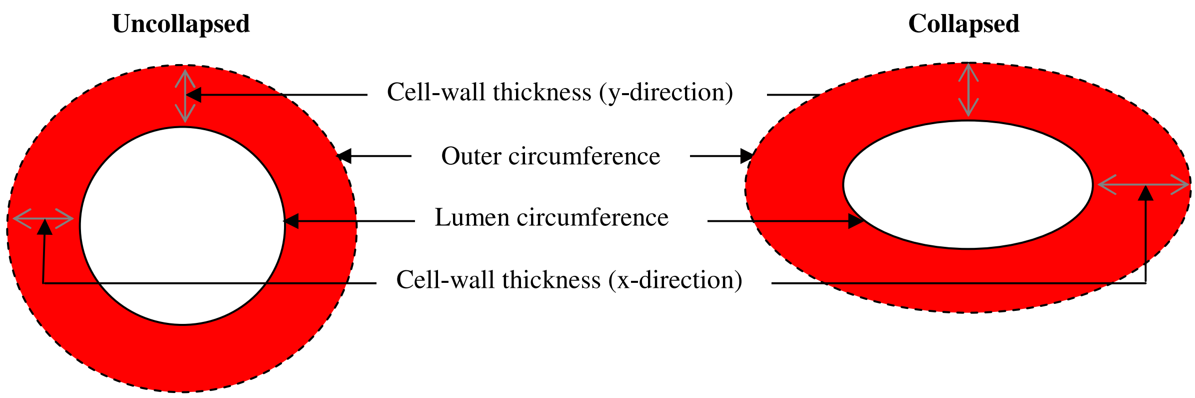


Figure 2 - 1: *Schematic representation of an uncollapsed and collapsed fibre in cross-section to illustrate parameters defined for modelling.*

2.3. RESULTS AND DISCUSSION

2.3.1. Surface examination using ESEM

Examination with ESEM proved to be an ideal technique to observe hydration of fibres on a paper surface in dynamic experiments. It was possible to examine the movement of fibres on the paper surface without any sample preparation method (Figs. 2-2 and 2-3).

After rewetting, some of the fibres appear to be twisted, possibly due to irregular cell-wall swelling as the cell-wall was gradually impregnated with water (Fig. 2-2).

In this study, other fibres appeared to remain rigid and lift out of the plane of the web when rewetted (Fig. 2-3). It was also observed by Skowronski and Lepoutre (1985) that fibre ends rise out of the paper surface, due to weak inter-fibre bonds that brake between fibres in the presence of water (Skowronski and Lepoutre, 1985). Fibre lifting was also observed during rewetting in previous studies done by Hoc (1989; 2005) and could cause irreversible changes such as increased paper roughness.

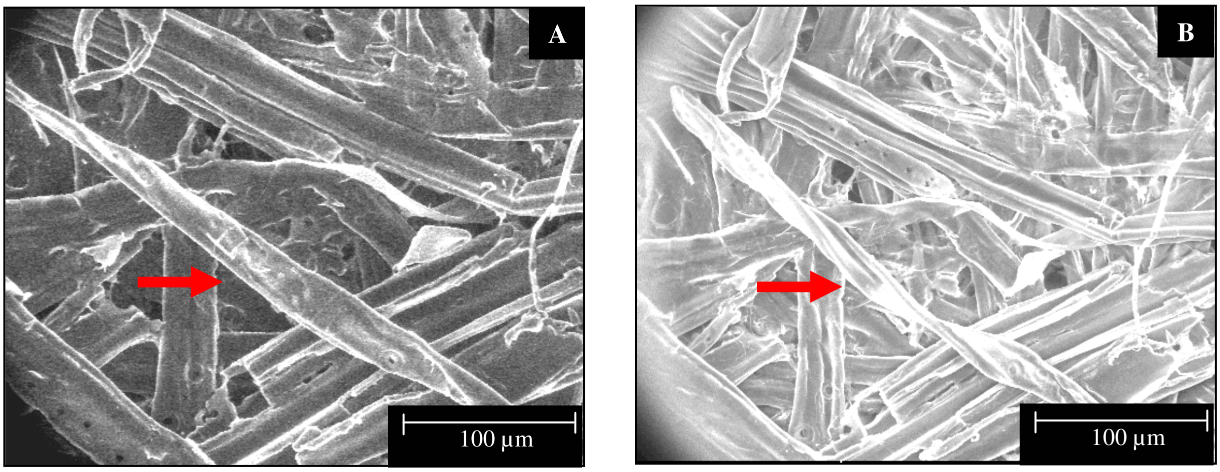


Figure 2 - 2: *Micrographs obtained with ESEM showed, A: a collapsed fibre in a dry handsheet and B: twisting behaviour of the same fibre in a rewetted handsheet.*

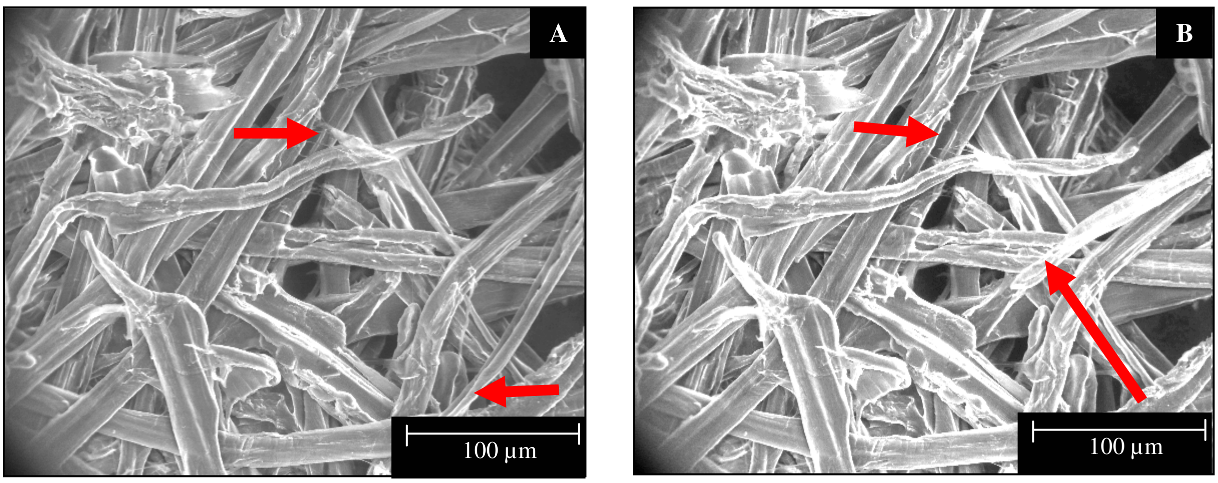


Figure 2 - 3: *Micrographs obtained with ESEM showed, A: a collapsed fibre in a dry handsheet and B: moving behaviour of the same fibre in a rewetted handsheet.*

2.3.2. Examination of cross-sections

The cutting of cross-sections in paper with a blade led to mechanical distortion and most of the fibres were compressed, obscuring lumina and making measurement of individual fibre dimensions impossible (Fig. 2-4A). When samples were frozen to make handsheets more rigid and amenable to sectioning, fibres were brittle and appeared to fracture rather than cut evenly (Fig. 2-4B). The image of the freeze cross-section also showed some debris present, which made the cross-section detail of fibres unclear. Laser cutting appeared to "melt" the cell-walls, making these samples unsuitable for measurements (Fig. 2-4C).

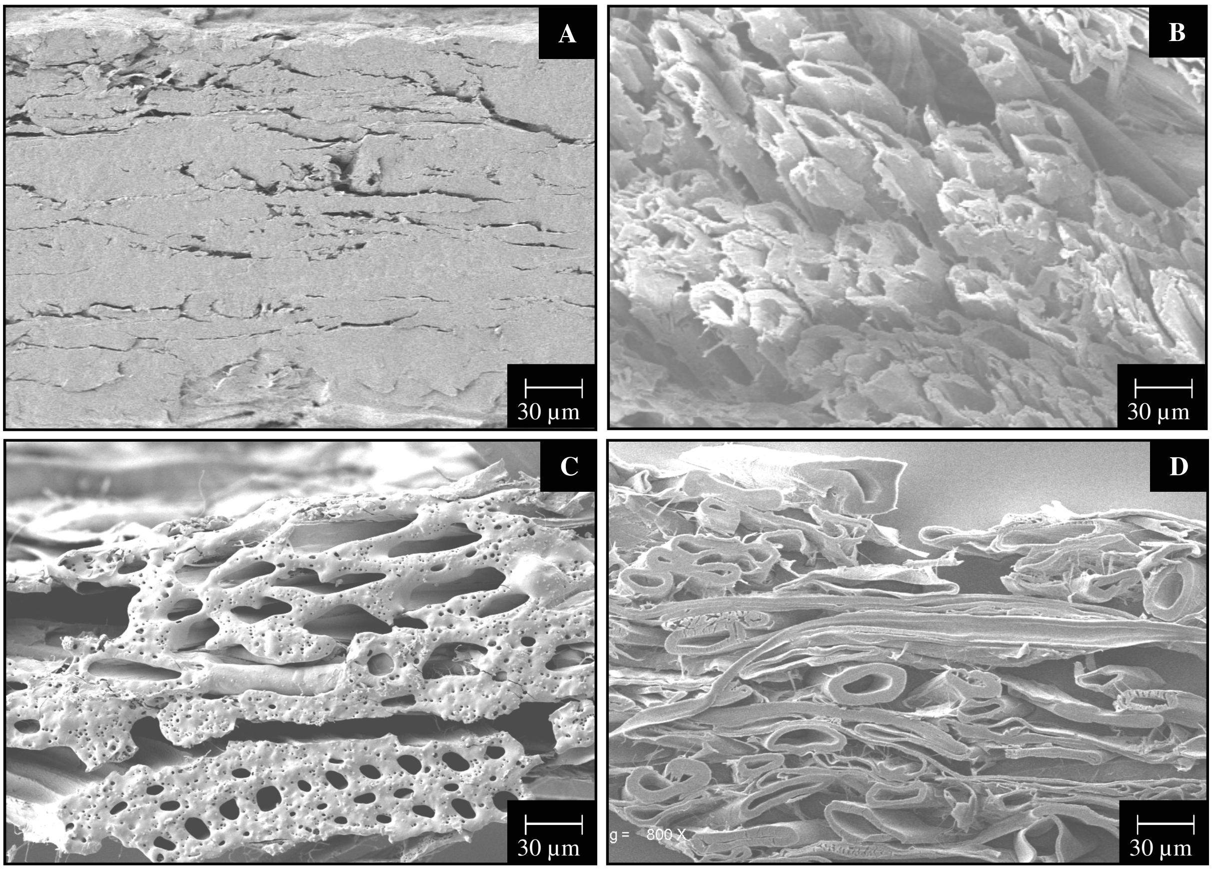


Figure 2 - 4: *Scanning electron micrographs of different sample preparation techniques including, A: blade hand-cutting, B: frozen and hand-cut, C: laser cut, and D: resin embedding and etch method. (working distance = 12 and 5 KV)*

While wax embedding was faster than resin embedding, samples lacked contrast during SEM viewing, which meant that fibre details were difficult to discern. Attempts to etch blocks with xylene to expose fibres before viewing with cSEM were also unsuccessful (not shown). Sectioning of resin embedded samples followed by etching appeared to be the best method, producing clear cross-sections (Fig. 2-4D). This method was, therefore, adopted for the rest of the study. One disadvantage of this method was that the method was lengthier than the others. It was also important to ensure that the etching solution was rinsed thoroughly before drying to prevent details being obscured by partially dissolved resin (Fig. 2-5).

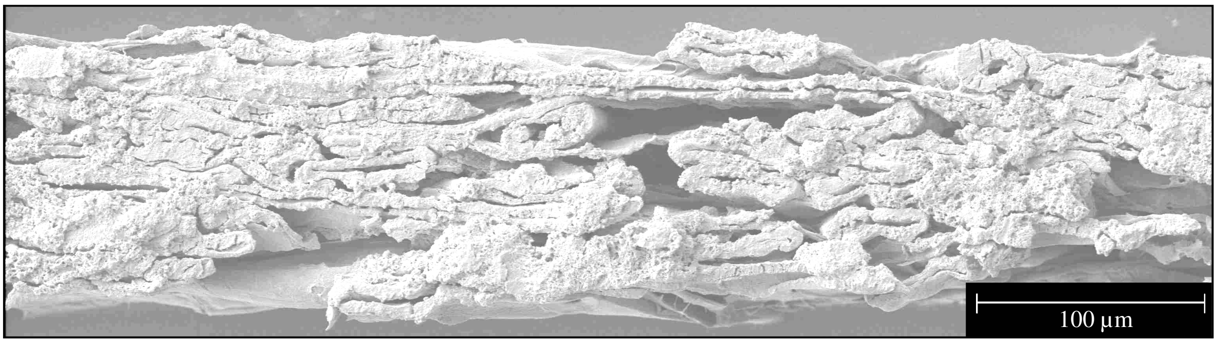


Figure 2 - 5: *Scanning electron micrograph of a paper cross-section that was not rinsed properly before drying. (working distance = 11 and 5 KV).*

Effect of etching solution on fibre structure

A comparison of the dimensions of fibres and handsheets before and after etching was done to establish that the etching solution did not distort the fibre or handsheet structure (Fig. 2-6). Backscattered-imaging showed sufficient differences in contrast between the fibres and the surrounding resin in the unetched sample to make it possible to measure fibre dimensions (Fig. 2-6A). The etched sample could be viewed better in normal secondary-electron imaging mode (SEI) (Fig. 2-6B). Measurements of the fibre dimensions using image analysis before and after etching showed no significant differences ($p \leq 0.05$) between any of the fibre dimensions measured (Table 2-1).

Table 2 - 1: *Mean fibre dimensions before and after etching. A Student's t-test showed no significant differences at 95% confidence interval.*

Measurements	Before etching	After etching
Fibre area (μm^2)	454.4	457.0
Fibre circumference (µm)	98.1	96.4
Lumen area (μm^2)	105.4	98.3
Lumen circumference (µm)	55.0	52.8
Cell-wall thickness (µm)	5.3	5.4

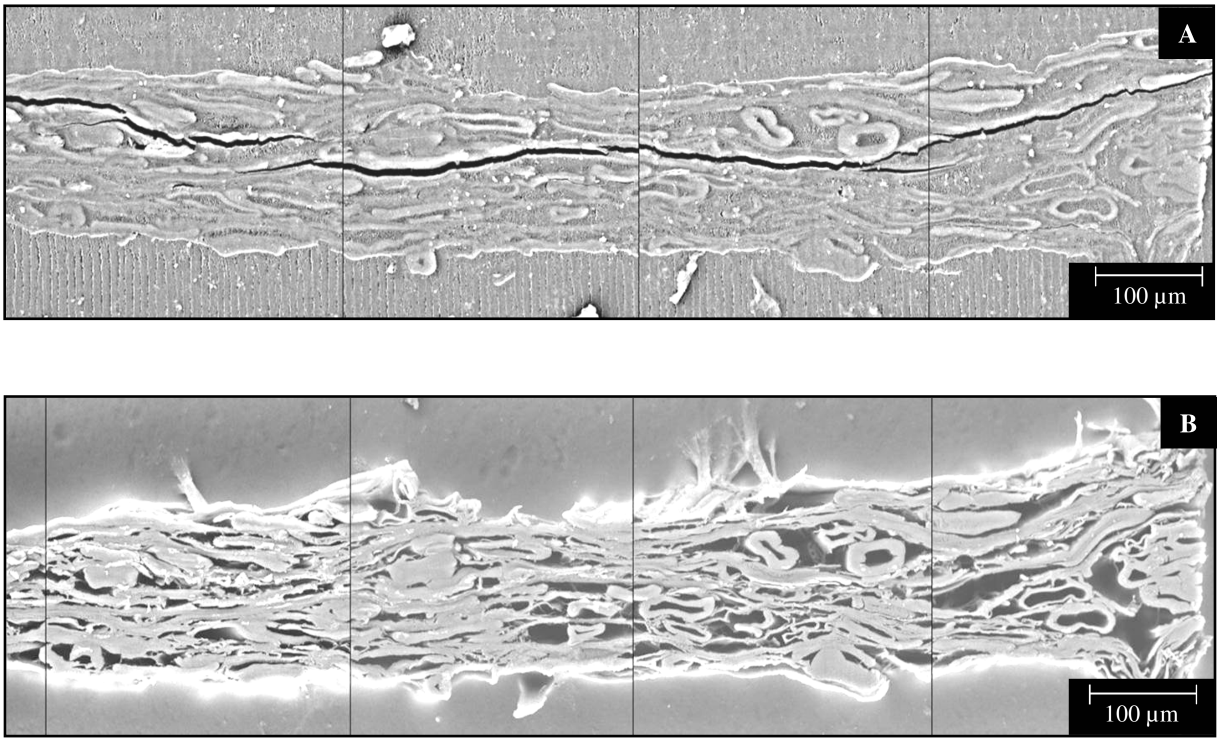
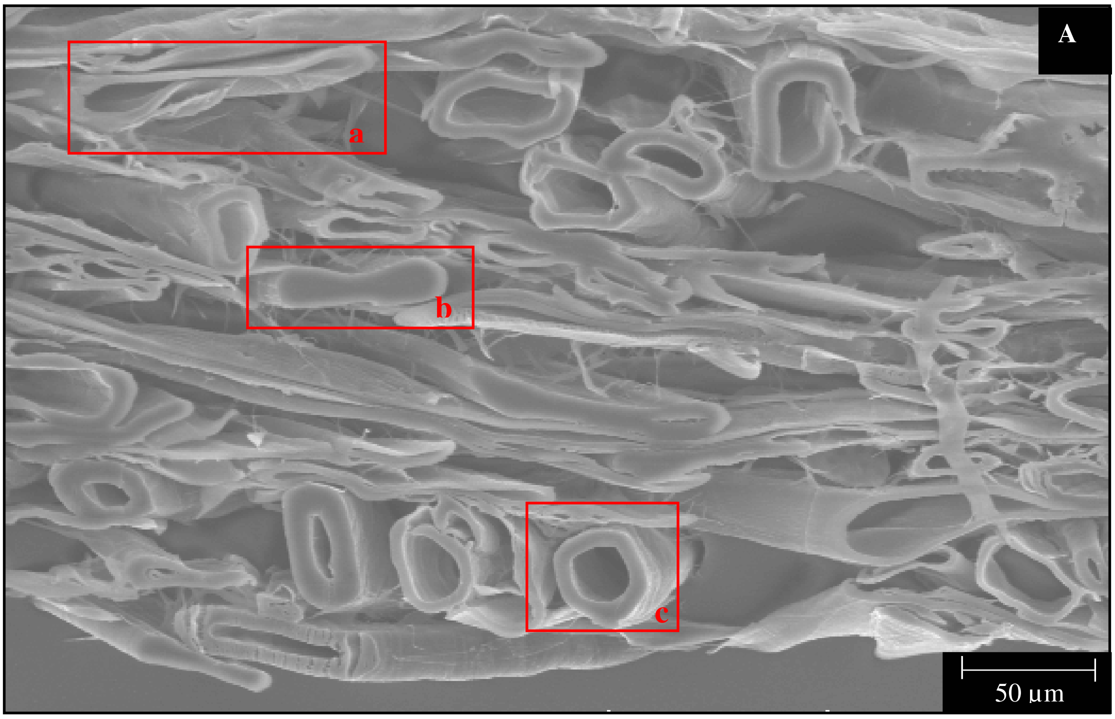


Figure 2 - 6: *Composite cSEM micrographs of cross-sections of handsheets embedded in resin after cutting with a microtome. A: backscatter-image of a sample before etching and the same sample B: after etching viewed in SEI mode. (working distance = 11 and 5 KV)*

The cross-section of the fibre structure in resin-embedded samples during rewetting was observed using ESEM. It was possible to examine dynamic changes in the LA and CWT (Fig. 2-7). In this study it was observed that fibre cell-walls can absorb water leading to an increase in thickness by up to 23% (Fig. 2-7B) and fibres also puffed during rewetting. However, puffing in these cross-sections during rewetting was not as marked as had been anticipated and occurred in relatively few fibres. A possible explanation for this is that swelling and puffing of fibres may have been restricted by resin remaining within the cell-walls of the fibres and thus limiting the degree of movement observed. Further etching of samples must be tempered against the possibility of interfering with the fibre network as more resin is removed deeper into the handsheet web.



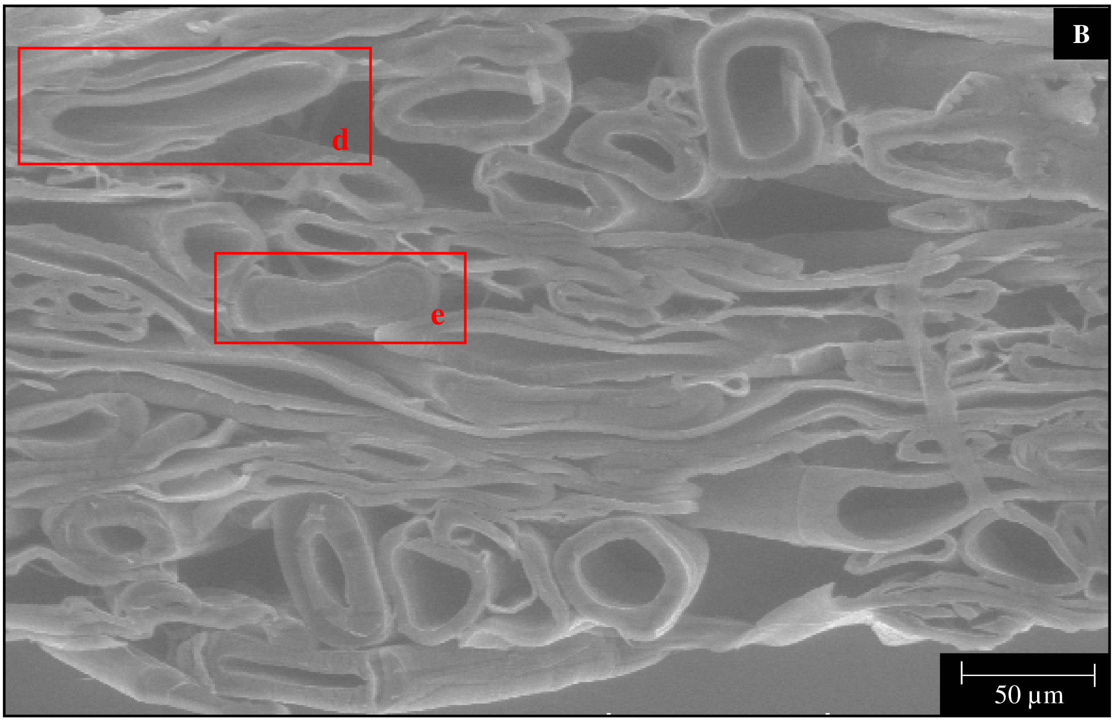
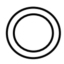
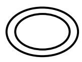
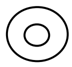
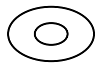
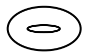
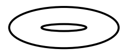


Figure 2 - 7: *ESEM micrographs of fibres, A: under dry conditions and B: during the rewetting stage. The fibre shapes that were observed can be described as, a: partially collapsed fibre, b: totally collapsed fibre, c: uncollapsed fibre, d: fibre puffing observed after rewetting and e: fibre swelling after rewetting.*

2.3.3. Image-analysis parameters and cutting angle

Image analysis was used to quantify fibre dimensions, but it was not clear initially which parameter was the most suitable to quantify puffing (Table 2-2). The models drawn with AutoCAD clearly showed that the cutting angle and the CWT influenced some of the parameters (Table 2-2). Ideally, fibres should be cut at a 90º angle, but fibres in sheets (especially handsheets) are randomly orientated and, therefore, it is not always possible to achieve this or to determine accurately at what angle fibres were cut. The models produced in this study showed that the CWT of fibres cut at 45° is overestimated in the x-direction, but not in the y-direction. These results were confirmed in models for both thin-walled and thick-walled fibres (Table 2-2).

***Table 2 - 2**: Relative dimensions using arbitrary units of computer-generated model fibres for collapsed and uncollapsed thin-walled and thick-walled fibres cut at a 90º or a 45º.*

	Thin-walled fibres				**Thick-walled fibres**			
	Uncollapsed		**Collapsed**		**Uncollapsed**		**Collapsed**	
	90º	**45º**	**90º**	**45º**	**90º**	**45º**	**90º**	**45º**
Appearance of fibre in cross-section								
x-CWT (units)	12.50	12.68	21.82	15.25	31.26	33.15	44.19	51.66
y-CWT (units)	12.50	12.50	12.50	12.50	31.26	31.26	31.26	31.26
FA (units^2)	7854	11271	4779	6387	7854	11272	7186	10748
LA (units^2)	4418	6370	1346	1649	1104	1624	543	784
LA:FA ratio	0.560	0.565	0.280	0.285	0.141	0.144	0.076	0.073

* x-CWT: cell-wall thickness measured in x-direction, y-CWT: cell-wall thickness measured in y-direction, FA: fibre area, LA: lumen area, LA:FA: lumen area to fibre area ratio, units: arbitrary units defined in the AutoCAD software.

When modelling was applied to fibres cut at different angles, it was possible to obtain the same FA (7854 units^2) for both thin- and thick-walled uncollapsed fibres cut at 90º (Table 2-2). At a 45° cutting angle, the FA of both these uncollapsed fibre types increased to 11271 units^2 and when the fibres cut at 90º were collapsed, the FA decreased for the thin-walled (4779 units^2) as well as for the thick-walled fibres (7186 units^2). When the collapsed thick-walled fibre was cut at a 45° angle, the FA increased to 10748 units^2 which is similar to the 11272 units^2 of the uncollapsed fibres. The FA increased when the same fibre was cut at an angle. The impression was, therefore, created that a collapsed fibre was uncollapsed.

A similar increase in the FA and LA was also observed for the thin-walled fibres. To overcome these discrepancies caused by the cutting angle, calculation of the LA:FA ratio proved useful. These ratios showed only small variation due to cutting angle. When a fibre collapses, the area of both the fibre and lumen becomes smaller and this is reflected in a smaller LA:FA ratio. This effect was illustrated with modelling when an uncollapsed thick-walled fibre cut 90º had an LA:FA ratio of 0.141 units but after collapsing a LA:FA of 0.076 units was calculated (Table 2-2).

A drawback of using LA:FA-ratios is that the CWT could potentially obscure differences between collapsed and uncollapsed fibres when pulp mixtures are examined. The models showed a small LA:FA ratio (0.144 units) for an uncollapsed thick-walled fibre compared to 0.280 units for a collapsed thin-walled fibre. According to these results, the thick-walled fibres appeared to be more collapsed that the thin-walled fibre. Separating the data according to CWT should, therefore, be considered to correctly interpret the state of the fibre collapse in heterogeneous pulp mixtures.

2.4. CONCLUSIONS

Environmental scanning electron microscopy was invaluable to capture fibre movement during rewetting in real time. Quantifying the movements (lifting and twisting) of fibres and other structural changes occurring in the surface of paper during rewetting was not possible, but quantification of cell-wall swelling as well as puffing in cross-sectional images was not a problem. However, using ESEM to evaluate puffing in cross-sectional images was time consuming, and embedded fibres were restricted in the amount of movement allowed by the resin even after etching. In contrast, even if cSEM allows no real-time observation, detailed images of samples before and after rewetting were deemed equally informative, and were used for the rest of the study. Image analysis was invaluable in quantifying the effect of rewetting on the fibre dimensions in the cross-sectional area.

Different sample preparation techniques were investigated and required representative cross-sectional images of the paper structure. Resin embedded samples that were sectioned and etched, optimally preserved the structure of the network and fibres being examined. The absence of changes in handsheet structure before and after etching (Fig. 2-6) added confidence that this step did not introduce any spurious puffing, swelling or any other form of fibre movement.

The influence of the cutting angle and degree of fibre collapsibility on the various parameters measured was investigated. Computer modelling of fibres indicated that the LA:FA ratio was the most suitable parameter to quantify puffing and the modelling data was supported by analysis of cSEM and ESEM micrographs. Based on this approach, the ratio of LA:FA was found to be a reliable measure to quantify puffing. Despite the fact that cutting angles can influence fibre dimensions, it was shown that the effect of the cutting angle on the LA:FA ratio was very small. Cell-wall thickness of fibres can influence the LA:FA ratio in such a way that this may obscure findings regarding puffed and/or collapsed fibres. This confusion can be overcome by dividing the fibre data into thin-walled and thick-walled fibre classes, an approach which is described in the following chapters.

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CHAPTER

3

THE INFLUENCE OF ENZYMATIC TREATMENT ON THE FIBRE STABILITY OF SPRUCE CTMP

This chapter was published as:

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ABSTRACT

An increase in moisture during printing can lead to movement of fibres such as puffing (return of a fibre to its uncollapsed state), twisting and lifting of fibres out of the paper web. Spruce fibres are especially subject to these movements contributing to weak bonding and increased surface roughness. The aim of the present study was to treat spruce pulp with enzymes to improve fibre bonding and stability. Spruce chemi-thermo-mechanical pulp was prepared in the laboratory and treated with mannanase, endoglucanase and with a combination of these enzymes. Strength properties were evaluated before and after wetting of handsheets and their transverse sections were examined by scanning electron microscopy. Handsheet properties before and after rewetting as well as measurement of lumen area of fibres proved to be effective in evaluating fibre movement and puffing. The treatment of pulp with mannanase or endoglucanase improved fibre stability; however, endoglucanase treatment resulted in loss of strength. Mannanase appeared to modify the cell-walls, making them more susceptible to beating and resulting in improved fibre development and inter-fibre bonding. Endoglucanase possibly damaged fibrils, and fibre degradation instead of fibre development occurred during beating. Stability of thin-walled fibres improved more than thick-walled fibres after enzyme treatment.

3.1. INTRODUCTION

The strength of paper is derived from both the strength of individual fibres in the network and the strength of the bonding between fibres (Page, 1969). Strength properties, such as tensile and burst indices, are especially dependant upon fibre-to-fibre bonding. Wetting of paper, such as during coating and printing, weakens hydrogen bonds and allows fibres to relax (Skowronski and Lepoutre, 1985; Hoc, 1989). During this process, fibres can return to their round, less collapsed state, undergo twisting movements and lift out of the paper web. The end result is that surface roughness is increased and strength properties are reduced.

Fibres with thick-walls, large diameters, and low degree of collapsibility are reportedly more subject to such movement under high moisture conditions (Forseth, 1997; Reme *et al.,* 1998) and are designated as unstable. Thick fibre cell-walls are also considered to be a dominant morphological factor that reduce the flexibility of wet fibres and weaken the bonding properties of the fibre network (Hattula and Niemi, 1988). Softwood fibres are included in paper for their positive contribution to strength, though they are coarse (16 to 67 mg 100 m^{-1}) and thick-walled with large diameters (Varhimo and Tuovinen, 1999). Softwood pulp can, therefore, be expected to contain a large number of unstable fibres that could contribute to weak bonding and increased sheet roughness after exposure to moisture.

Many attempts have been made to improve the paper-making quality of spruce pulp and these efforts have included methods, such as high-temperature refining, chemical treatments, and even fractionation (Kappel, 1999; Meyer-Pinson *et al*., 2004; Norgren *et al*., 2004). The aim of those studies has essentially been to modify cell-walls or to select fibres with suitable cell-wall properties. The modification of the cell-wall can potentially be achieved by removal of specific components from the cell-wall and thereby improving characteristics such as flexibility, swelling and collapsibility.

Improved fibre-wall properties have been achieved by increased fibrillation during refining (Mohlin and Pettersson, 2002) and also through selective enzymatic modifications with multi-component cellulase and endoglucanase (Mansfield and Saddler, 1999; Mansfield *et al*., 1999). These enzyme treatments resulted in reduced coarseness as well as denser and smoother handsheets (Mansfield *et al*., 1996). However, these fibre modifications were usually achieved at the expense of fibre and sheet strength (Edgar *et al*., 1998; Mohlin and Pettersson, 2002). Similarly, mannanase has been applied to reduce refining energy and improve tensile properties of mechanical pulp (Taylor *et al*., 2005). Although enzymes have not been widely applied for fibre improvement on an industrial scale, the enzymatic modification of fibre cell-walls holds promise, especially when combined with mechanical action.

The aim of the present study was, therefore, to treat spruce pulp with enzymes to improve fibre stability in order to retain surface smoothness and sheet strength by maintaining strong inter-fibre bonding in handsheets. Spruce, chemi-thermo-mechanical pulp (CTMP) was prepared in the laboratory and treated with mannanase (MAN), endoglucanase (EG), or a combination of these two enzymes (MAN+EG). The influence of the enzymes was determined by characterising fibre morphology, evaluating strength properties of handsheets and by examining the ultrastructure of fibres in transverse sections before and after rewetting. The return of fibres to an uncollapsed state was regarded as the critical response of unstable fibres to wetting and is referred to as puffing in this paper.

3.2. Materials and Methods

3.2.1. Pulp preparation

Simulated CTMP was done by cooking spruce chips (10.8 kg bone dry) for 60 min to 165°C and 60 min at 165°C with a sodium-bisulphite charge of 5.6% and liquor to wood ratio 2.5:1. The resulting pulp, at a chlorine number of 28, was subjected to two-stage refining for separation and fibrillation of the fibres. The first stage refining was performed with a Sprout Bauer refiner by the CSIR (Forestry and Forest Products Research Centre, Durban) and the second stage with a low-consistency pilot refiner by the Sappi Technology Centre (Pretoria).

3.2.2. Enzyme treatment

The pulp (35 g per sample) was made up to a consistency of 3.5% in 1ℓ of tap water and the pH adjusted to 7.0 with H_2SO_4 before treatment with mannanase NZ 510230, endoglucanase Novozyme 476 (both from Novozymes, Denmark) or a combination of the two. The enzymes are considered to be mono-component formulations of endo 1,4-βD-mannan mannanohydrolase (EC 3.2.1.78) and 1,4-(1,3;1,4)-beta-D-glucan 4-glucanohydrolase (EC 3.2.1.4), respectively. The activity of MAN was 10.7 KU mℓ$^{-1}$, assayed with locust-bean gum (Stålbrand *et al.*, 1993) and that of EG was 38.1 IU mℓ$^{-1}$, assayed with carboxymethyl cellulose (CMC) (Ghose, 1987). Each enzyme was applied at a relatively high dosage of 28.6 μℓ g^{-1} pulp to compensate for sub-optional physical conditions. The combined treatment consisted of 28.6 μℓ g^{-1} of each enzyme and no enzyme was added to the control. All treatments were incubated at 60°C for 30 min with constant stirring and the enzyme activity was not terminated after treatment. Following incubation, the pulp was disintegrated (1500 revolutions) and beaten for 3000 revolutions using a PFI mill. The samples were disintegrated again and diluted with tap water to approximately 6 ℓ and left over-night to reduce latency of fibres.

3.2.3. Fibre characterisation and pulp properties

Fibre characterisation of each pulp sample was done with a MorFi LB-01 Fibre Quality Analyser (TECHPAP, France) and fibre length (weighted in length), fibre width, coarseness, curl, kinked fibres, fines (% length and % area) were measured according to the user's manual. Handsheets with a base weight of approximately 60 g m^{-2} were prepared from each sample according to the ISO 5269/2 Rapid-Köthen method. The handsheets from each treatment were divided into two groups; the first was kept dry in a conditioning room (23°C, 50% relative humidity), while the second group was rewetted by dipping into water and then placed out to condition. Roughness, burst and tensile indices were determined for all the treatments (in dry and rewetted state) by the International Standard Methods (ISO 8791-2 1990; ISO 2758 2001; ISO 1924-3 2005). The tear strength of the pulps could not be determined due to the small amounts of treated pulp samples available. The response of the pulp to rewetting was calculated by comparing the properties of rewetted handsheets to the dry controls.

3.2.4. SEM examination

Samples (3 x 10 mm^2) were cut randomly out of each of three handsheets with a razor blade and embedded in Quetol 651 (Van der Merwe and Coetzee, 1992). Transverse sections were cut with a microtome from the middle of each sample, and the sectioned face was immersed in a saturated solution of sodium methoxide to etch the resin away from the fibres (Iwadare *et al*., 1990). The etching was done for approximately 40 min to expose a clean transverse section of the handsheet. Samples were then air dried, mounted and rendered conductive in the vapour of a 0.5% RuO_4 solution (Van der Merwe and Peacock, 1999). A JSM 840 instrument (JEOL, Japan) at 5 kV and a working distance of 10 mm was used to record ten SEM micrographs of each sample. Image Tools software (University of Texas, San Antonio) was used to measure the surface area of fibre walls in transverse section and the area of the lumina (lumen area or LA) in each image. The fibre area (FA) was calculated by adding the area of the fibre cell-wall and LA and these dimensions were then used to calculate the ratio of lumen area to fibre area (LA:FA) as the measure to quantify fibre collapse as a result of different treatments. The median of the measured cell-wall thicknesses was calculated, with the help of which the data for thin- and thick-walled fibres were separated for numerical analysis. The influence of different enzyme treatments on the movement of fibres in handsheets was also determined by measuring the mean height of each cross-sectional image of the handsheets. At least six measurements were done for each sample.

3.2.5. Experimental design and statistical analysis

A completely randomised experimental design was used to test the influence of the enzyme treatments on fibre characteristics as well as the response of the treated pulp in handsheets to rewetting. Each treatment was replicated three times and the data were subjected to one-way analysis of variance and means were tested for significant differences (Q-value) with Tukey's multiple-range test at a 95% confidence level.

Due to the small number of measurable fibres in some of the electron micrographs, data were grouped across replications. For comparison of LA:FA ratio and sheet thickness, all rewetted treatments (control, MAN, EG, and the combination of enzymes) were compared with the dry control sample by Student's t-test at a 95% confidence level.

3.3. Result and Discussion

3.3.1. Pulp characteristics

Neither of the fibre treatments with MAN, EG or the combination of these enzymes caused any significant changes in the morphological characteristics of fibres (Table 3-1). A previous report suggested that improvement of fibre characteristics should be reflected by a decrease in coarseness (Mansfield *et al*. 1996) and, therefore, a reduction in the cell-wall thickness (CWT). However, in the present study, the enzymes did not significantly change any of the fibre characteristics related to coarseness (Table 3-1).

Table 3 - 1: *Fibre characteristics after beating of untreated (control) pulp and pulp treated with mannanase (MAN) and endoglucanase (EG) or a combination of these enzymes (MAN+EG).*

Fibre characteristics	Control	MAN	EG	MAN+EG
Fibre length: weighted in length (µm)	739	760	745	741
Fibre width (µm)	32.83	32.97	32.80	32.70
Coarseness (mg g^{-1})	0.29	0.26	0.30	0.30
Curl (%)	9.67	9.53	9.60	9.53
Kinked fibres (%)	21.53	21.60	21.70	21.53
Fines (% length)	76.57	75.87	76.57	77.00
Fines (% area)	16.30 ^{ab}	15.73 ^{b}	17.71 ^{a}	16.46 ^{ab}

a b: Values in the row followed by the same letter do not differ significantly where applicable ($p \leq 0.05$, Tukey's multiple-range test).

3.3.2. Influence of enzymes on handsheet properties before rewetting

In comparison to the control, treatment with MAN, EG and the combination of the two enzymes significantly decreased roughness ($p \leq 0.05$) (Table 3-2). The in-plane strength properties (tensile and burst indices) were influenced differently by each enzyme treatment. Tensile strength increased significantly after MAN treatment, but the EG and the combination of the two enzymes resulted in a significant decrease in the tensile values. Burst index was not significantly increased by MAN treatment, but treatment with EG and the combined enzymes reduced the burst index significantly in comparison to the control (Table 3-2).

Table 3 - 2: *The influence of different enzyme treatments on properties of dry handsheets.*

Handsheet properties (dry)	Control	MAN	EG	MAN+EG
Roughness (ml min^{-1})	3334^{a}	2592^{b}	2504^{b}	2342^{b}
Tensile index (mN m^2 g^{-1})	50.30^{b}	53.70^{a}	44.19^{c}	37.70^{d}
Burst index (kPa m^2 g^{-1})	2.40^{a}	2.49^{a}	1.31^{c}	1.58^{b}

a b c d: Values in each row followed by the same letter do not differ significantly ($p \leq 0.05$, Tukey's multiple-range test).

Fibre modification with enzymes is often achieved at the expense of strength (Edgar *et al*., 1998), but in the present study, treatment with MAN not only maintained burst strength, it also significantly increased tensile strength in the sheets. The increased strength properties introduced by the MAN treatment, suggest that the enzyme degraded the mannans bound to the cellulose fibrils to some extent. This modification may have made the cell-wall structure more amenable to beating for better fibrillation or conformation. After treatment with EG, collapsibility of fibres increased (as evidenced by reduced roughness). However, the enzyme degraded the cellulose in cell-walls to the extent where fibrillation decreased and inter-fibre bonding weakened as a result. The strength properties were consequently reduced as described by Edgar *et al*. (1998). Furthermore, the combined action of the two enzymes (MAN+EG) caused even greater strength loss than the action of the EG alone. It is likely that the combined enzymes degraded the cell-walls to the extent where external fibrils were detached from the fibre during beating and could not contribute to bonding characteristics.

3.3.3. Response of handsheet properties to rewetting

When untreated handsheets were exposed to water, roughness increased while strength properties decreased, possibly as a result fibre movement. When MAN- or EG-treated handsheets were rewetted, roughness was significantly less when compared to the rewetted control sample (Fig. 3-1). These results can be interpreted that fibres were more stable or better conformed as a result of better fibre collapse or increased fibrillation. The increased stability caused less movement on rewetting. However, a combination of MAN and EG resulted in increased roughness, similar to that of the rewetted control, possibly indicating that the degradation caused a loss of fibrillation. With the loss of fibrillation, inter-fibre bonding decreased and it, therefore, reflects the important role that the fibrils play in anchoring fibres within the network to control movement.

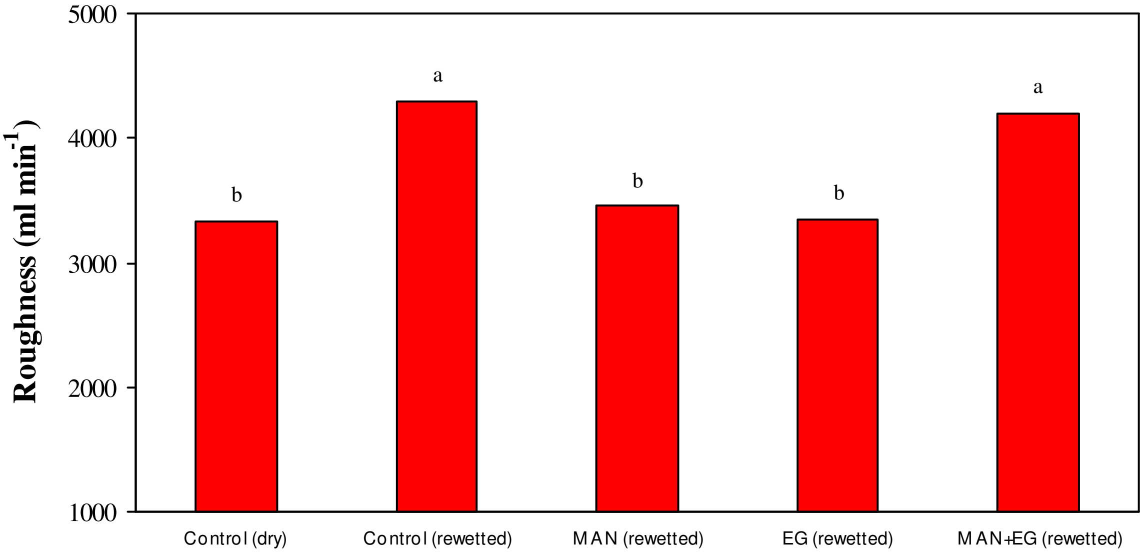

Figure 3 - 1: *The influence of rewetting on the roughness of untreated pulp and pulp treated with mannanase (MAN), endoglucanase (EG) or a combination of these enzymes (MAN+EG). Bars with the same letter (a, b) indicate that treatments did not differ significantly ($p \leq 0.05$, Tukey's multiple-range test).*

The tensile strength of the control decreased significantly ($p \leq 0.05$) after rewetting (Fig. 3-2), possibly due to breaking of hydrogen bonds between fibres and decreased contact between fibres. However, the tensile index from MAN-treated pulp after rewetting was significantly higher than the rewetted control. The high tensile strength observed despite rewetting supports the earlier hypothesis that the MAN treatment led to greater external fibrillation and stronger inter-fibre bonding. Rewetting of EG-treated pulp resulted in a significant ($p \leq 0.05$) decrease in the tensile index of handsheets and this effect was even more marked in the treatment with MAN+EG (Fig. 3-2).

The trends observed for burst after rewetting (Fig. 3-3) mirrored those observed for tensile strength (Fig. 3-3) and rewetting decreased the burst index of the control significantly ($p \leq 0.05$). However, when MAN treated handsheets were rewetted, the burst index remained significantly above that of both dry and rewetted controls. An even greater strength loss was observed in pulp treated with EG and the MAN+EG than in the rewetted control. These observations again reflected improved fibre development as result of MAN treatment and reduced inter-fibre bonding consequence of EG and MAN+EG treatment.

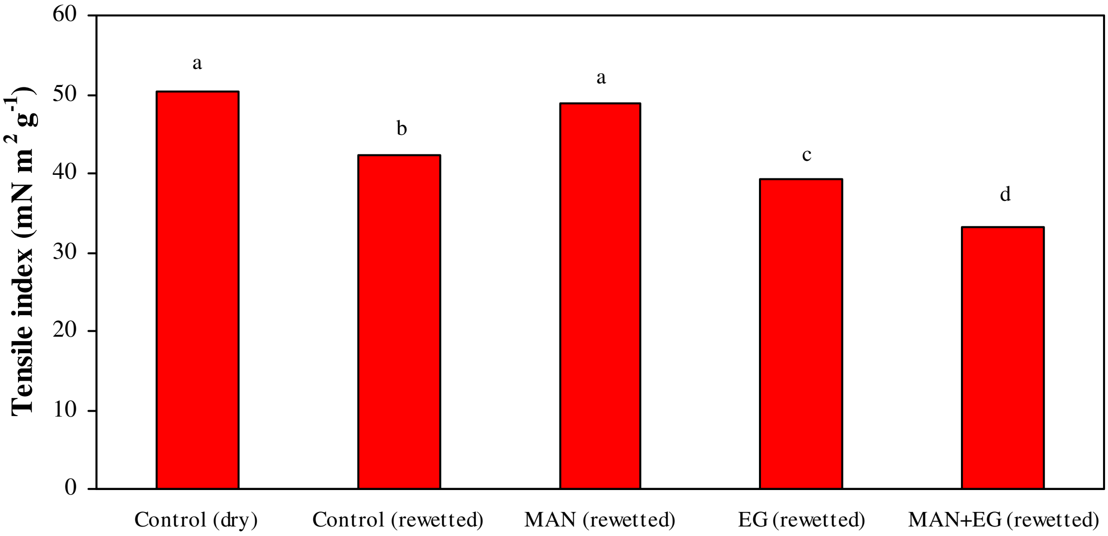

Figure 3 - 2: *The influence of rewetting on the tensile index of untreated pulp and pulp treated with mannanase (MAN), endoglucanase (EG) or a combination of these enzymes (MAN+EG). Bars with the same letter (a, b, c, d) indicate that treatments did not differ significantly ($p \leq 0.05$, Tukey's multiple-range test).*

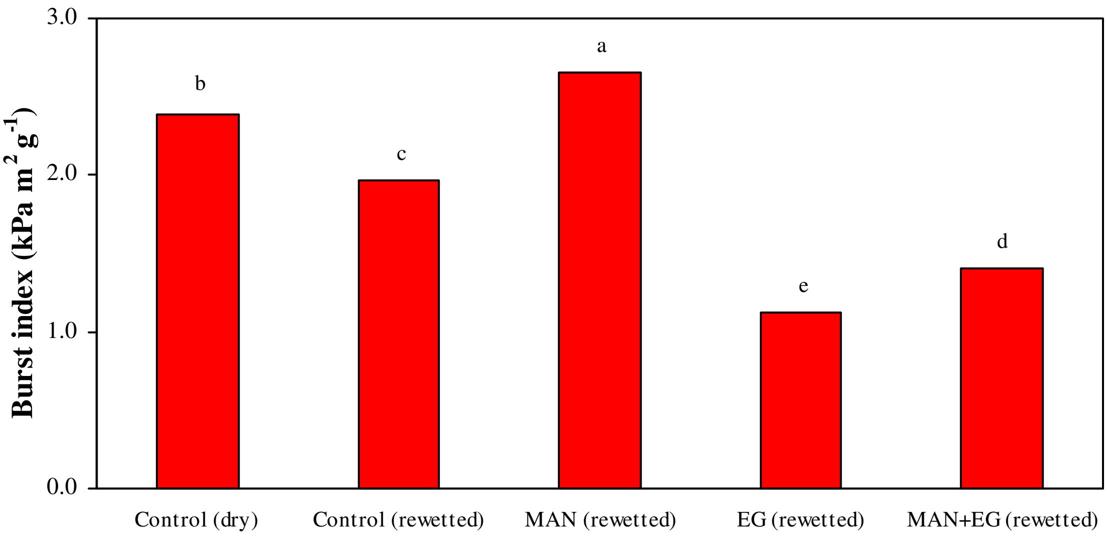

Figure 3 - 3: *The influence of rewetting on the burst index of untreated pulp and pulp treated with mannanase (MAN), endoglucanase (EG) or a combination of these enzymes (MAN+EG). Bars with the same letter (a, b, c, d, e) indicate that treatments did not differ significantly ($p \leq 0.05$, Tukey's multiple-range test).*

3.3.4. SEM examination

No differences in the collective response of pulp fibres between enzyme treatments could be measured by SEM and this was possibly due to large variation in the fibre morphology. However, trends became apparent when measured data were separated into thin- and thick-walled groups by means of the calculated median of the CWT (2.65 µm) (Fig. 3-4). Separate analysis of these groups highlighted morphological changes that were not previously evident and the ratio of lumen area to fibre area (LA:FA) appeared to be the best parameter to quantify puffing, especially after rewetting.

The LA:FA ratio of thin-walled fibres was significantly larger in handsheets that were rewetted than in those that were kept dry, indicating that lumen volume had increased when fibres came in contact with water (Fig. 3-4). The high LA:FA ratio, therefore, reflects the return of a fibre to its uncollapsed state (puffing). Pulp treated with MAN, EG or MAN+EG showed no significant change in the LA:FA ratio after rewetting when compared to the dry control. These enzyme treatments, therefore, appeared to modify the wall-structure of fibres sufficiently to ensure that fibres remained in the collapsed state after wetting.

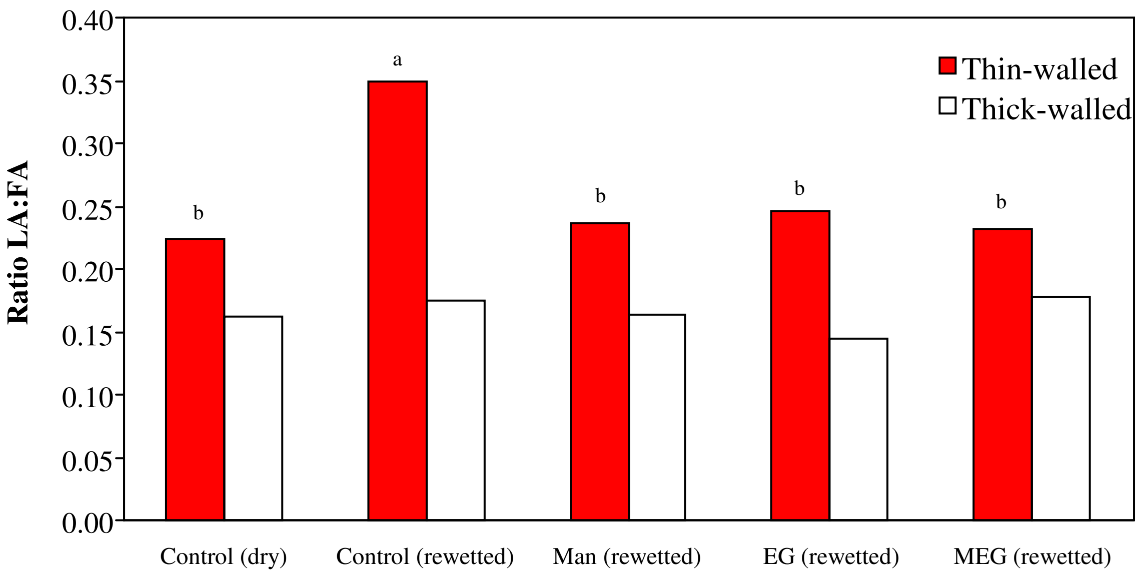

Figure 3 - 4: *The ratio of lumen area (LA) to fibre area (FA) reflects the degree of puffing for thin-walled and thick-walled fibres. Bars with the same letter (a, b) indicate that treatments of thin-walled fibres did not differ significantly. No significant differences were found between treatments of thick-walled fibres ($p \leq 0.05$, Tukey's multiple-range test).*

The changes in LA:FA ratio (or the puffing effect) were observed in cross-sections of handsheets (Fig. 3-5) and measurements of sheet thickness (Fig. 3-6). The untreated handsheets showed a dense cross-section with only a few lumina and inter-fibre spaces visible (Fig. 3-5A). Rewetting increased the thickness of the handsheet, with more lumina visible and larger inter-fibre spaces (Fig. 3-5B), likely reflecting fibre movement as result of disrupted inter-fibre bonding. Mannanase-treated fibres were more stable after rewetting and only a few puffed lumina were visible (Fig. 3-5C), appearing similar to the control. These measurements of individual fibres were confirmed by measurement of the thickness of whole handsheets (Fig. 3-6) and wetting of the control handsheets resulted in an increase in sheet thickness while no change was recorded after treatment with either MAN, EG or MAN+EG.

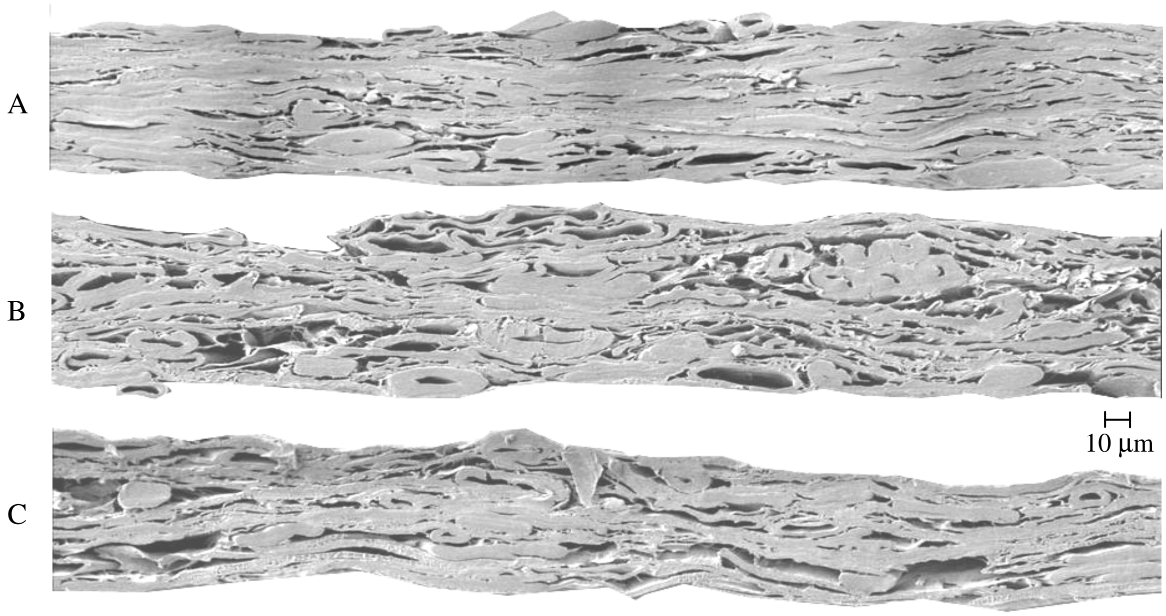

Figure 3 - 5: *Cross-sections of spruce CTMP handsheets. (A: Dry control, B: Rewetted control, C: Mannanase treated and rewetted).*

Results from thick-walled fibres were higly variable, and although small differences between treatments were observed, no significant effects of the enzyme treatments could be detected (Fig. 3-7). It is well-established that thick-walled fibres do not collapse readily, even after beating. However, the present study also demonstrated that these fibres are less responsive to rewetting than previously reported (Forseth, 1997; Reme *et al.*, 1998), especially when compared to thin-walled fibres.

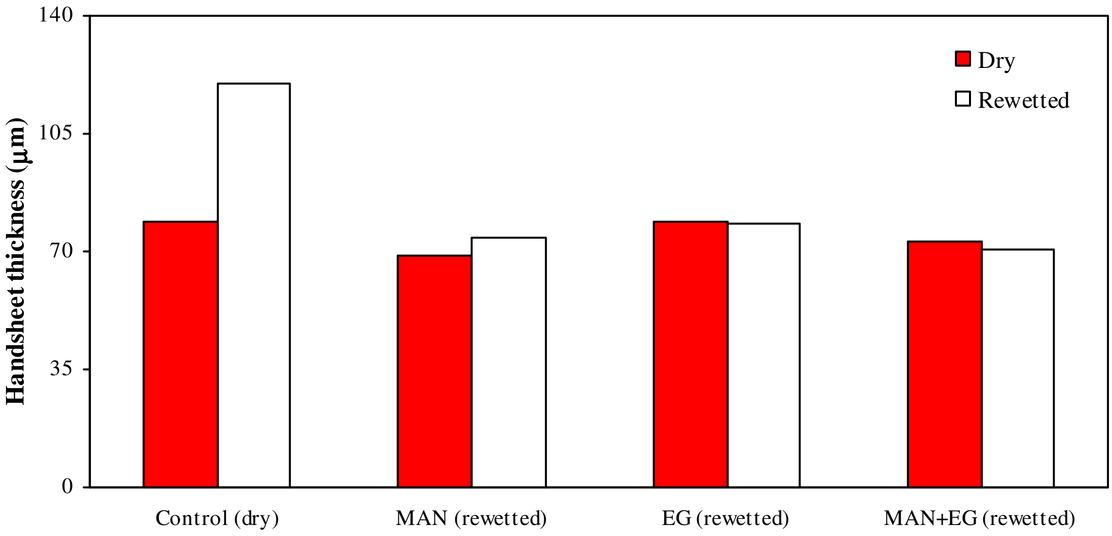

Figure 3 - 6: *The thickness of control and enzyme-treated handhseets before and after rewetting. Bars with the same letter (a, b, c) indicate that handsheets did not differ significantly in thickness (Student's t-test at 95% confidence level).*

3.3.5. Proposed mechanisms

The enzymes tested here apparently modified the cell-walls and predisposed fibres in different ways to the beating that followed. These differences became apparent in surface and strength properties of handsheets before and after wetting as well as in fibre puffing. However, interpretation is limited by the fact that surface morphology of fibres and sheets was not imaged and samples were too small to allow the testing of fibre or tear strength. Within the constraints of the present dataset, the following scenarios for enzymatic action, beating and rewetting on fibres are proposed (Fig. 3-7):

Modification of fibres possibly manifests itself as a weakening of the fibre wall through degradation of the cell-wall structure (Fig. 3-7B-D). The reduction of strength properties in dry handsheets (Table 3-2) appeared to indicate that this weakening was detrimental in the EG-treated fibres (Fig. 3-7C-D). Beating caused fibrillation of fibres in all treatments, but the slightly increased tensile strength of the MAN-treated pulp (Table 3-2) could reflect a possible increase in fibril length or number (Fig. 3-7F). However, the reduction in strength properties of the EG-treated pulps probably indicates a manifestation of fibrillation loss (Fig. 3-7G-H) when compared to the control. The amount of fines in beaten pulps did not differ significantly between treatments (Table 3-1), possibly indicating that only very small fibrils detached from the EG-treated pulp or that the fines were fully degraded.

The improved conformation of the three enzyme-treated pulps was reflected in reduced surface roughness of handsheets. Improved conformation is possibly due to increased collapsibility of the fibres (Table 3-2), where the enzyme-treated fibres (Fig. 3-7J-L) collapsed to a greater extent than the control fibres (Fig. 3-7I). However, when these handsheets were rewetted, puffing of the untreated fibres (Figs. 3-4 and 3-7M) took place and, in combination with relatively little fibrillation, led to increased roughness and reduced tensile and burst indices (Figs. 3-1 to 3-3). Puffing did not occur in the enzyme-treated pulps (Figs. 3-4 and 3-7N-P), but due to less fibrillation of the EG-treated pulps, inter-fibre bonds were disrupted. The breaking of these bonds is evidenced by reduced burst and tensile strength, but no change in surface roughness (Figs. 3-1 to 3-3) is observed. A higher level of fibrillation in the MAN-treated fibres and the resulting fibre stability retained the handsheet structure in terms of strength (Figs. 3-2 and 3-3) and surface smoothness (Fig. 3-1).

This proposed mechanism does not seem to describe the effect of enzymes on thick-walled fibres, since thicker fibre walls were less responsive to enzyme treatment. Differences in fibrillation levels, collapsibility and stability were, therefore, more difficult to observe. It is possible that the response of fibres to the enzymes could increase with longer incubation, but penetration of the enzyme into the fibre wall will play an important role in improving fibre collapsibility.

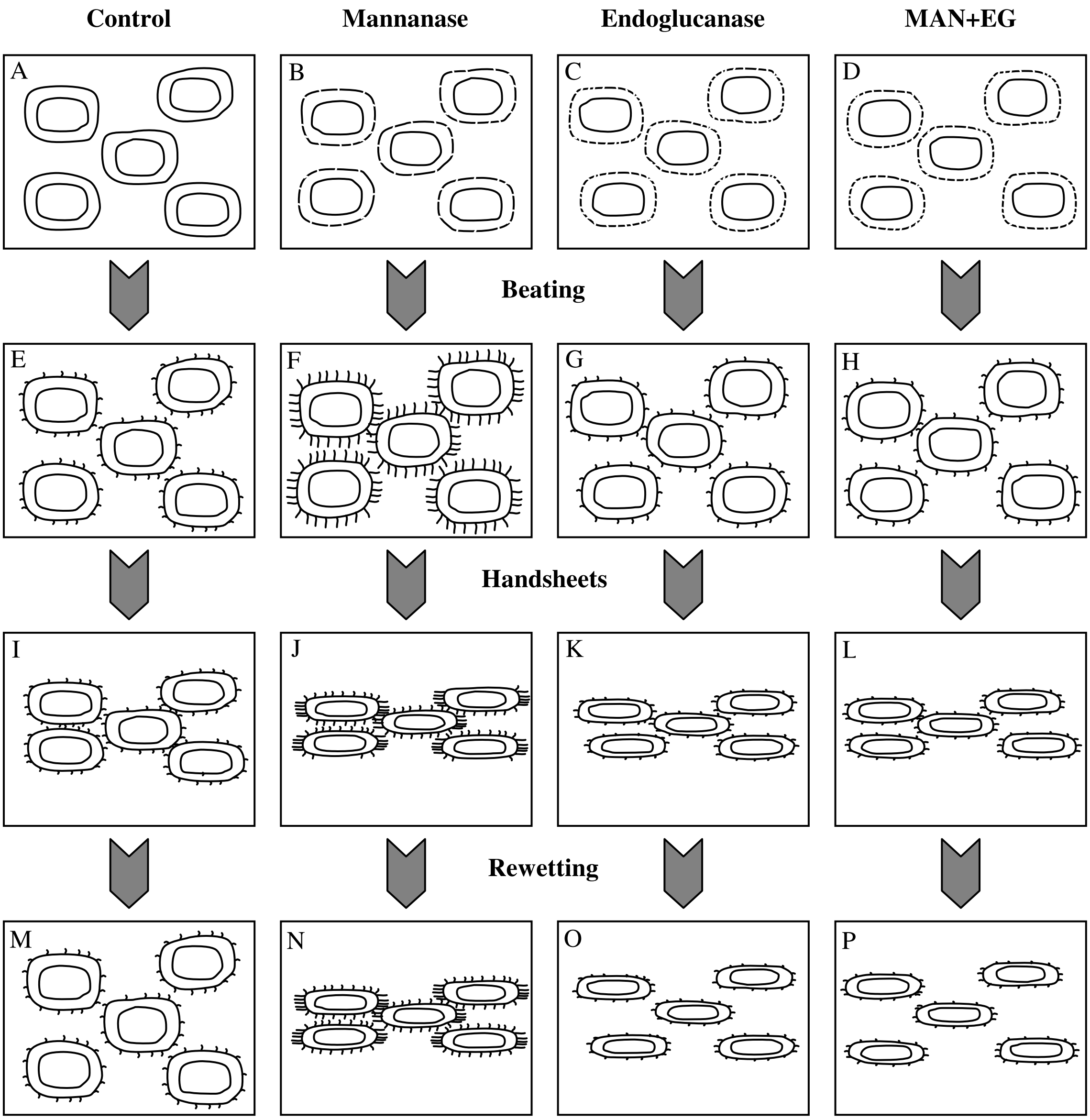

Figure 3 - 7: *Schematic representation of the proposed mechanism for enzymatic modification of thin-walled fibres when combined with beating and rewetting. (A to D: pulp subjected to enzyme treatments, E to H: pulp after beating, I to L: pulp formed into handsheets and, M to P: pulp after handsheets were rewetted).*

3.4. CONCLUSIONS

Moisture causes disruption of inter-fibre bonding and fibre movement, in the course of which the surface roughness deteriorates (Skowronski and Lepoutre 1985). Enzymes can be applied to improve fibrillation (Mohlin and Petterson, 2002) that can result in denser and smoother handsheets (Mansfield *et al*., 1996). Enzymatic modification can also improve fibre stability as shown in the present study. Handsheet properties and SEM studies, both demonstrated that enzymes such as EG or MAN improved fibre stability. The maintenance of strength and smoothness after MAN treatments were the result of better fibrillation and collapsibility for higher fibre stability in handsheets. However, the EG treatment was associated with strength loss. Mannanase increased the strength of dry en rewetted handsheets despite inclusion of thin-walled and thick-walled fibres in these sheets. The reduction in puffing was only observed in thin-walled fibres.

Fibre stability depends on two factors: firstly, collapsibility of fibres and their ability to remain collapsed results in a larger area for inter-fibre bonding that persists after rewetting of handsheets. This property was reflected in surface smoothness. Secondly, it is proposed that the degree of fibrillation determined the strength of fibre bonding as it was reflected in strength properties of handsheets. A high degree of fibrillation resulted in increased tensile and burst strengths that were retained after rewetting. The model for enzymatic modification to effect fibrillation, collapse, and stability of fibres supported the empirical data, but a similar model could not be developed for thick-walled fibres. The experiments and model also placed an emphasis on fibrillation and fibre bonding and future work should investigate the impact of the enzymes on fibre strength and tear strength of pulps.

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