Enzymatic Upgradation of Secondary Fibres

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ABSTRACT

Enzymatic technologies provide a natural solution for various problems encountered in the papermaking process. The key to a successful enzyme application is the careful selection of the right enzymes for a mill's specific furnish, process conditions, and water chemistry. This paper presents a possible action of endoglucanase (EG) and cellobiohydrolase (CBH) components of cellulases on cellulosic pulp. It reviews the knowledge of complex "fibre enzyme" interactions and identification of the most effective enzyme component (EG or CBH) for pulp drainage improvement. It covers the understanding whether the effectiveness or strength loss depends on a specific type (EG or CBH) of enzyme activity. Better understanding of the enzyme−fines interaction will improve the selection of enzymes for drainage improvement in recycled fibres.

Key words: Recycled fibre, pulp properties, drainage, dewater ability, enzymes, endo/exo cellulase

Introduction

Paper recycling offers several advantages; substitution of virgin pulp with recycled fibres saves wood for making pulp, which reduces the exploitation of forests important for the biodiversity. Every ton of recycled fibre saves an average of 17 trees plus reqired pulping energy. Producing paper with recycled fibres involves 28 to 70% less energy consumption than that made with virgin fibre and the process uses less water. This is because a large part of the energy used in papermaking is used during pulping. Wastepaper pulp requires less refining than virgin pulp. Recycled paper is not usually re-bleached and when it is, oxygen is normally used instead of chlorine; hence recycled paper produces fewer polluting emissions to air and water. High-grade papers can be recycled quite a few times, providing environmental savings every time. Deinked pulp suitable for making printing grade papers usually imparts special properties to the finished papers compared with papers made from wood pulp, such as increased opacity, less curling tendency, less fuzziness, and better formation.

Several problems are also associated with the recycling of fibre. These are deinking of different types of post consumer paper, drainability of recycled fibre, hornification of the fibres and stickies contamination.

The wet end operation of papermaking involves the removal of water from a dilute fibre slurry stock by uniformly distributing the stock onto a moving forming wire. Aided by applied vacuum water drains through the narrow pores of the wire while the furnish solids, comprised of fibres and other materials, are trapped atop the wire as a wet mat. The mat in turn traps the fines and filler materials in the drainage zone, then by vacuum applied by foils and suction boxes in the vacuum zone. This description suggests that an operative mechanism in the initial phase of papermaking is filtration. As filtration proceeds, and the mass of fibres and fines forming the paper mat builds up, the retention and flow resistance increase. Consequently, at a constant pressure drop, the drainage rate decreases.

By definition, fines have been identified as the fraction of the furnish solids that passes through a 200 mesh screen (nominally a 76 mm hole size) (Mansfield and Wong, 1999). The main problem with fines is that due to their high relative surface area dewatering rate is lower compared to primary pulp. Thus, the productivity of the paper making process is considerably decreased compared to operation using virgin fibres.

Fines have properties very distinct from their associated fibre fractions. They are often regarded as a special paper furnish component that must be controlled to maintain the optimum properties and wet end characteristics. Fines have a significant impact at the wet end, affecting features such as drainage and retention. They have a marked impact on formation and properties of paper produced. Hydrocolloidal material/fines within the furnishes has high specific surface area and a high level of amorphous cellulose. These characteristics restrict the free drainage of water and retain bound water within the pressed sheet.

Enzymes can be used to overcome these problems to a great extent in addition to assist in refining the fibres recovered from old kraft paper and paperboard products such as old corrugated cartons (OCC), which require very high energy.

Enzyme Treatment To Restore Fibre Quality

Application of cellulases and hemicellulases could improve the drainability in an environmentally friendly way. Several explanations for the possible effects of enzymes have been suggested and various experimental observations exist. It has been also shown that although enzymatic treatment of fibres decreased the amount of amorphous and gel-like polysaccharide layer on the surface, it did not affect the amount of fines (Kantelinen and Jokinen, 1997). On the other hand, enzymes may behave in a manner similar to retention aids and polymers facilitating the flocculation of the small fibre particles (Mansfield and Wong, 1999).

The fines are held responsible for the deteriorated drainability, decreasing the amount of fines could be the key solution for improving drainage. When cellulolytic enzymes are used for

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partial hydrolysis of cellulose chains, and thus to form a better recycled fibre structure, it is important to find the balance between two opposite directions. On one hand, by the hydrolysis of fines improved dewatering rate is obtained. On the other hand, enough fines have to be left in the pulp in order to obtain optimal interfibre bonding, which is required for good strength properties of the end product. Moreover, enzyme action should not result in excessive hydrolysis, as this means loss of weight and thus production.

When papermakers treat recycled fibres with cellulase, their main goal usually is to allow water to drain more quickly during paper forming. Such an effect can increase the rate of production on drainage- limited paper machines. More importantly, faster drainage often makes it possible to apply higher levels of refining, while running the paper machine at top speed. Studies have shown that a judicious choice of the enzyme component, its dosage and the duration of treatment can make it possible to achieve significant freeness increase, while still not causing unacceptable levels of degradation to the fibres (Bhat et al.1991; Eriksson et al.1998).

Cellulases

Cellulases and hemicellulases have been used for saccharification of lignocelluloses for a long time. Filamentous fungi and cellulolytic bacteria are capable of producing extracellular cellulolytic enzymes. Historically, development of enzyme application began with studies on cellulases to enhance fibrillation, i.e. to improve the strength of produced paper due to increased inter-fibre bonding. Furthermore, cellulases have been investigated to enhance improve beatability, increase runability and water retention value, enhance fibre flexibility, and thereby improve pulp properties, drainage and deinking of recycled paper.

In submerged culture most cellulolytic fungi secrete a complex array of degradative enzymes. Three classes of hydrolytic enzymes are recognized on the basis of substrate specifity or mode of action:

- Endo-1, 4- β glucanases (EG) cleave randomly at 1,4- β linkages within the cellulose chain. They randomly attack the amorphous regions of cellulose substrate, yielding high degree of polymerization oligomers. The endoglucanases are commonly assayed by viscosity reductions in carboxymethyl cellulose (CMC) solutions. Crystalline cellulose is commonly not degraded by endoglucanases.
- Exo-1, 4- β glucanases (exo-1, 4- β-D-glucan cellobiohydrolases, CBH) Cellobiohydrolases are exoenzymes and hydrolyze crystalline cellulose, releasing cellobiose. *Cellobiohydrolase have virtually no activity on CMC and slowly degrade crystalline cellulose.* Both types of cellulase enzymes whether exo or endo hydrolyze β-1, 4-glycosidic bonds.
- 1, 4- β- glucosidases hydrolyze cellobiose to glucose, and cellobionic acid to glucose and gluconolactone. The β-D-glucosidase or cellobiase converts cellooligosaccharides and cellobiose to glucose.

Mechanism of enzyme action

The two cellobiohydrolases attack the crystalline areas at the opposite chain ends and endoglucanases (EG) in the middle of the more disordered regions of cellulose. The filled circles, denoted R, represent the reducing ends and the open circles, denoted NR, represent the non-reducing ends. C indicates the highly ordered crystalline regions.

Since the presence of fines and highly fibrillated fibres are associated with low pulp freeness, several theories have been proposed to explain the freeness increase occurring after enzymatic treatment. The enzymatic attack may involve a peeling mechanism, which removes fibrils and fibre bundles that naturally have a high affinity for water, and leaves the fibres less hydrophilic and easier to drain. Alternatively it has been suggested that enzymes act preferentially on fines which have a propensity to block up interstices in the fibre network. The increase in drainage has also been attributed to the cleaving of amorphous cellulose on the surface of fines.

One of the explanations suggested that due to the high specific surface area of the fines, the attack of cellulases was specific towards this fraction (i.e. fines) of the pulp. It has been pointed out in literature that the fibre surface is stripped through the enzymatic

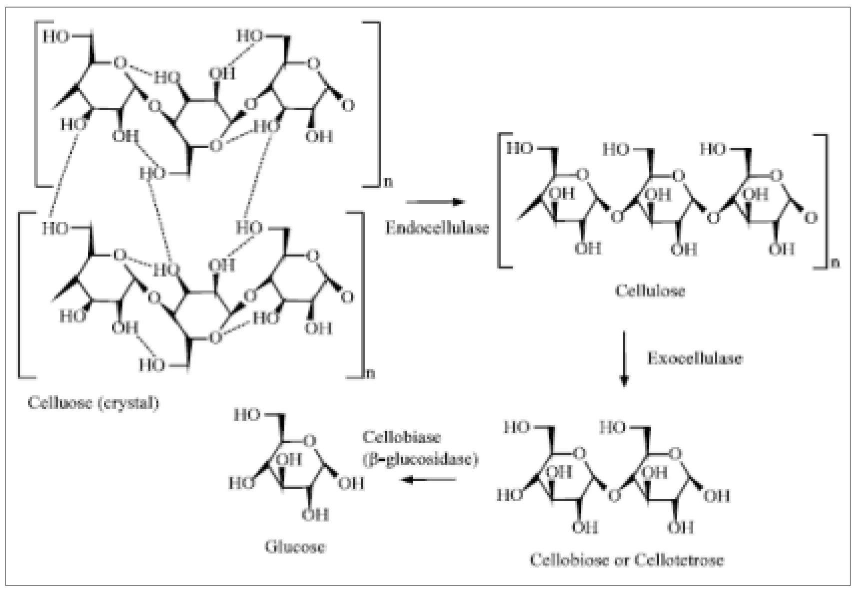

Fig. 1 : Mode of action of various components of cellulase

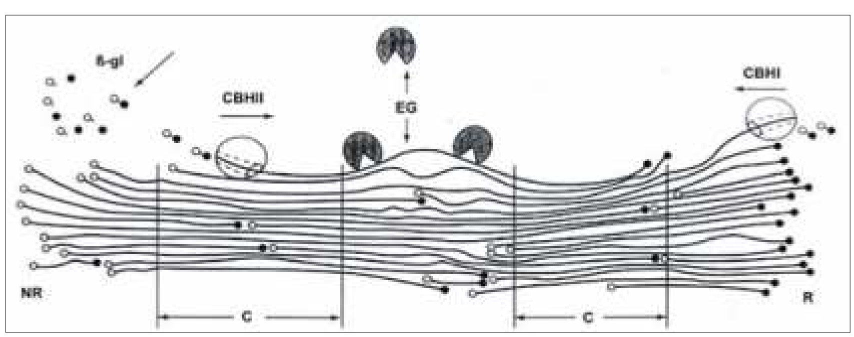

Fig. 2: Mechanism of enzymatic hydrolysis of cellulose (Teeri, 1997).

hydrolysis of subsequent layers or fibrils.

Jackson et al. (1993) suggested that enzymes can either flocculate or hydrolyze fines and remove fibrils from the surface of large fines. The enzyme aided flocculation occurs when a low enzyme dosage is used. In this case, fines and small fibre particles aggregate with each other or with the larger fibres, decreasing the amount of small particles in pulp and consequently improving pulp drainage. On the other hand, at higher enzyme concentration, flocculation becomes less significant, and hydrolysis of fines begins to predominate.

Enzyme specifity also plays very important role. As shown by Pere et al. (1995), endo and exoglucanases affect paper properties differently. Endoglucanase can lower pulp viscosity and thus dramatically reduce the pulp strength. The differences in enzyme activity are attributed to different modes of action. Endoglucanase are more active on amorphous cellulose and randomly attack the inner part of the cellulosic chain, whereas exoglucanases can hydrolyze both crystalline and amorphous cellulose by removing cellobiose from the terminal part of cellulose chains (Henrissat et al. 1995). Although considered more detrimental to fibres, endoglucanase action is probably the main determinant of drainage improvement (Jackson et al., 1993; Stork et al. 1995).

Cellulase complex vs Monocomponent cellulases

Several commercial cellulose enzymes are available which claim to improve the drainage of secondary fibres. These types of enzymes are to be applied after refining/ beating of the pulp, mainly to improve the dewatering. But using mixtures of cellulases can be disadvantageous for certain pulp properties. By applying purified enzymes on specific regions of the cellulose fibres, the desired part of the pulp could be modified for a particular enzyme application. When applying cellulase enzyme mixtures, identification of the key component, responsible for the required effect on pulp and paper properties, is very difficult. In secondary fibres, the fines and fibrils, which cause low rate of drainage, decisively consist of amorphous cellulose. Since, the amorphous cellulose is more accessible than crystalline cellulose, it is not necessary to use the whole cellulase complex for the hydrolysis. That is applying specific cellulose component may be effective enough.

Monocomponent cellulase have also been recently evaluated for their potential to upgrade recycled furnishes. It is well known that the drying operation decreases the accessibility of cellulose and hemicelluloses due to losses in fibre wall porosity. Interestingly, the increased solubilisation of amorphous cellulose mediated by endoglucanase treatments (EGI& EGII) improved water retention values, and consequently improved drainability. Furthermore, combinations of endoglucanases and hemicellulases acted synergistically to improve drainage beyond that accomplished by single enzymes alone. In contrast, cellobiohydrolase treatments (CBH I) failed to improve the fibre drainage, and only in combination with hemicellulases, there were slight positive effects observed. Tensile and tear indices were decreased by endoglucanases alone and endoglucanases supplemented with hemicellulases, while very little strength loss was recorded after CBH I and CBH I/hemicellulase treatment. It was concluded that drying and refining have important but unique effects on recycled fibre properties and enzymatic treatment can substantially improve drainage.

The endoglucanases (EG I and EG II) of *Trichoderma reesei* have been reported to significantly improve pulp drainage at low dosage levels, and EG II was found to be more effective at a given level of carbohydrate solubilization endoglucanase treatments (high dosage) a slight loss in strength was observed. EG alone has been found to be more detrimental to strength properties as compared to EG+Xyl at a given level of cellulose hydrolysis. Although the drainage properties of the pulps could be improved by selected enzymes, the water retention capacity of the dried hornified fibres could not be recovered by any of the enzymes tested. It was reported that endoglucanases enhance dewatering by hydrolyzing the amorphous hydrophilic cellulose which is the main constituent of the fines formed during refining.CBH I did not influence pulp drainage at any level of refining (Oksanen et al. 2000). All enzyme treatments except CBH1 alone reduced the SR Value to some extent (Table 1).CBH as well as xylanase did not markedly affect the strength properties.

Stork et al. (1995) used isolated cellobiohydrolases and endoglucanases of *Penicillium pinophilum* to treat recycled pulps and measured the effects on the water retention value. They found that the action of endoglucanases was necessary for an improvement in the drainage of recovered paper. The effect did not appear to be due to a selective hydrolysis of the fines fraction but was a consequence of the hydrolysis of amorphous cellulose on the surface of the fibres. Depending on the origin and history of primary and secondary fibres, the endoglucanase treatment decreased the strength properties to differing degrees

Table1. Effect of enzymatic treatment on recycled soft wood pulp after refining

Enzyme	Dosage (mg per g of pulp d.w.)	Solubilized carbohydrates (% of d.w.)		°SR
		Cellulose	Hemicellulose	
REF	–	0	0	39
EG I	0.02	0.1	0.1	35
EG I+XYL	0.02+200[a]	0.13	0.85	32
EG II	0.02	0.08	0	33
EG II+XYL	0.02+200[a]	0.11	0.85	31
CBH I	0.2	0.05	0	38
CBH I+XYL	0.2+200[a]	0.08	0.93	n.d.

[a] *values are in nkat.*

(Oksanen et al. 2000). Combining hemicellulases with the endoglucanase treatments increased the positive effects of the endoglucanases on pulp drainage. However, as a result of the

.It has been reported by Pere et al. 1995 that EGs dramatically decreased pulp viscosity. EG appears to attack cellulose at sites where even a low level of hydrolysis reduces pulp viscosity, resulting in a marked deterioration of strength properties. In secondary fibres the fines and fibrils that cause lower rate of drainage consist decisively amorphous cellulose. Since amorphous cellulose is more accessible it is not necessary to hydrolyze with the whole cellulase system, endoglucanases are effective. They concluded that endoglucanases play the main role in drainage improvement.

While comparing two commercial products, Pergalase (mixture of cellulases and hemicellulases) and Indiage Super L (pure endoglucanase) at similar dosage, Dienes et al.2004 have reported Indiage Super L proved to be more efficient in improvement of drainage.

The effectiveness of a cellulase enzyme having predominantly endoglucanase (EG) activity was evaluated in the laboratory and paper mills for improving the freeness and drainability of different types of recycled pulps (Shaikh and Luo 2009). On treating the refined pulps with monocellulase component, the improvement in Canadian standard freeness (CSF) was observed by 13.1% in ONP, 19.3% in old corrugated container (OCC) and 40.5% in mixed waste (MW), as shown in Table 2.

Using OCC and addition of enzyme at different levels, the CSF value increased with the increase in enzyme dose. However, there was no appreciable change in the tensile and compression strengths of the hand sheets prepared from the enzyme-treated pulps (Table 3).

Table 2. Impact of monocomponent cellulase enzyme treatment on CSF value of different grades of pulp

Pulp Grade	CSF,ml	
	Control (before enzyme treatment)	After enzyme treatment*
OCC	419	500(19.3%)
MW	304	427 (40.5%)
ONP	168	190(13.1%)

values in paranthesis indicate improvement

Table 3. Effect of enzyme dose on CSF and strength properties of pulp (OCC)

Enzyme dose,g/TP	CSF,ml	Compression strength, Nm/g	Tensile strength, Nm/g
Nil	300	22.0	45.0
50	353	22.5	45.5
100	368	23.0	45.5
200	388	21.5	43.0
500	422	22.0	43.0

Conclusions

The presence of endoglucanase activity is a prerequisite for improvements of drainage of recovered paper by enzymatic means. When cellobiohydrolase and xylanase activity were present, they acted synergistically with the endoglucanase to improve its effects. Endoglucanases enhance dewatering by hydrolyzing the amorphous hydrophilic cellulose which is the main constituent of the fines formed during refining. Fines fraction are more susceptible to enzymatic degradation due to their higher surface areas. Considering all these findings, it can be concluded that the improvement of drainage of recycled fibres having high fines content is caused by the degradation of amorphous cellulosic material on the surface of fines. By applying purified enzymes on specific regions of the cellulose fibres, the desired part of the pulp could be modified for a particular enzyme application.

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