Enzymatic Refining Of Pulps: An Overview

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ABSTRACT

This review highlights the progress of application of enzymes in refining including various types of enzymes, mechanism of action and their effects. Refining is a papermaking process where the cellulosic fibres are mechanically treated, resulting in morphological and structural changes to produce desired fibre properties for a better quality paper. As different types of fibres react differently to refining, the refining process is carried out so as to take into account the type of fibres concerned. Refining requires a large amount of energy and is performed in beaters or refiners. Due to high energy requirement and problems related to fines generation by the conventional refining process, use of enzymes especially the cellulases and hemicellulases is gaining popularity. Enzymes are valuable in order to develop better fibrillation or net formation for better quality of paper, saving electrical energy, reduced steam consumption in drying operation and improved drainability of pulp. But, there is a limitation of using enzyme in that it can reduce the pulp viscosity. Although, the applications of the enzymes have been reported recently at mill scale but more research is needed to develop effective enzymes and to optimize process parameters to the interest of the papermakers.

The pulp produced in the pulp mill without mechanical treatment is not suitable for making most paper grades. A paper sheet made from unbeaten virgin pulp is characterized by low strength, bulkiness and rough surface. For producing good quality paper, fibres must be capable of being matted into a uniform sheet and they must also develop strong bonds at the points of contact, Bhardwaj *et al* (1). During refining, outer layers of fibres are removed, which allows swelling of fibres by hydration, decrease in length due to the cutting effect i.e. fibre shortening, internal fibrillation, external fibrillation and increase of flexibility etc. This in turn leads to an increase of the fibre surface and specific volume. In laboratory Valley beater and PFI mill are used for performing refining operation whereas in mills suitable equipment like disc or conical refiners is used. The old papermakers saying 'the paper is made in beater' illustrate the significance of this process. Any damage done to the fibres at this stage can not be corrected elsewhere.

In pulp and paper industry energy costs account for about 25% of the total manufacturing cost. Refining/ beating, which is one of the steps in papermaking process essential for the development of desired pulp properties, requires substantial energy to produce paper from pulp which accounts approximately 15-18% of the total electrical energy, Shamim *et al* (2). The consumption of electrical energy has increased many folds with the pace of development, and now energy is a scarce and costly commodity. With the depletion in available energy and the high energetic cost, energy conservation has become a necessity for the paper industry. Consequently, any pulp treatment that promotes a decrease in energy consumption, particularly with regard to the refining process, will have a beneficial effect on the global consumption of energy in the paper industry.

Spurred by the recent advances in technologies, one of the steps forward in making good quality paper with energy conservation process is the introduction of 'Enzymatic refining'. In the last few years, interest in the use of enzymes as a way of modifying fibre properties to improve the beatability/ refinability of pulps has increased (3-9). The use of cellulase and hemicellulase enzymes has been found helpful in energy saving if an enzymatic treatment stage is carried out before beating or refining (1-4, 9).

LITERATURE

CONCEPT OF ENZYMATIC REFINING

Enzymes are used in paper industry from a long time but gained popularity in last one decade. In 1942, a patent claimed that hemicellulase enzymes from *Bacillus* and *Aspergillus* species aid refining and hydration of pulp fibres, Diehm (10**)**. In 1952, Bolaski *et al* (11) patented the use of cellulase enzyme from *Aspergillus niger* to separate and fibrillate pulp. In 1968 Yerkes (12) patented for cellulase enzymes from a white rot fungus to reduce beating or refining time. After that Noe *et al* (3) reported external fibrillation of bleached chemical pulps and reduced energy demand in the papermaking process.

Enzymes can give a gentler, targeted refining. As in case of mechanical refining, fines generation may be a problem which results in a decrease of paper strength properties, drainability of pulp and therefore it needs an expense for strength and drainage additives. Cochaux A. and D'aveni A. (13) have described the fundamental differences between beating and enzymatic actions using cellulases on softwood Kraft fibres. They studied beaten pulp and cellulasic treated pulp in order to compare the effects of these treatments on the fibres. According to them, beating is a mechanical action and enzymatic hydrolysis a chemical action, their effects seems to be similar if one considers the shortening of softwood Kraft fibres. Indeed, the fibre length distribution (amount of long fibres and short fibres) evolves in the same way for both the beaten and the enzymatic treated pulps.

Enzymes are complex protein molecules that act as catalysts speeding up the chemical reactions under a narrow range of conditions. The activity of enzyme depends on the chemical and physical environment (i.e. temperature, pH etc.), enzyme dose

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TABLE- 1
EFFECT OF VARIOUS COMMERCIAL ENZYMES ON PULP BEATABILITY

Enzyme	Optimum pH	Optimum temperature (^oC)	Reduction in beating time (%)	Reducing sugars (kg/tonne pulp)
Pulpzyme HC	7- 8	60- 70	22.7	0.53
Hemicellulase 'Amano'90	4.5- 5	45- 50	25.0	4.59
Cartazyme HS 10	4.5- 5	45- 55	17.9	4.67
Irgazyme 40s	7- 8	50- 60	17.9	3.93
Bleachzyme F	6.5- 7.0	40- 50	20.4	3.61

and reaction time, the type and concentration of the substrate. An enzyme (E) molecule has a highly specific binding site or active site to which its substrate (S) binds to produce enzyme-substrate complex (ES). The reaction proceeds at the binding site to produce the products (P), which remains associated with the enzyme-product complex (EP). The product is then liberated, and the enzyme molecules are freed in an active state to initiate another round of catalysis as depicted in the following equation.

$$E+S \leftrightarrow ES \leftrightarrow EP \leftrightarrow E+P$$

Enzymes can be added at a pre-refining stage by optimizing different process variables like temperature, pH, pulp consistency, enzyme dose, reaction time.

ENZYMES AND THEIR MECHANISM OF ACTION DURING REFINING

The main enzymes used for refining are cellulases and hemicellulases which act on cellulose and hemicelluloses respectively. Cellulose chains existing at fibre surfaces contain hydroxyl groups that participate in fibre-to-fibre bonding in paper. Cellulose hydroxyl groups on the fibre surface and within the fibre wall also interact strongly with water and are important in the swelling phenomenon associated with refining. Hemicelluloses interact strongly with water and contribute greatly to the swelling of wood fibre due to beating and promote development of fibre-fibre bonding in paper. A number of studies have been performed using enzymes for refining. Some results have been summarized in table-2.

Cellulases

Cellulases hydrolyze β 1, 4-glycosidic linkage in cellulose chain. Two major types of cellulases are exocellulases and endocellulases. Exocellulase acts on the end of cellulose chains, cutting bonds on the end of fibre by splitting off cellobiose or glucose causing disruption in cellulose hydrogen bonding. Endocellulases act in the middle of cellulase chain causing hydrolysis of the accessible cellulose, in a synergistic sequence of events (14). Microorganisms have grown as powerful source of enzymes and proved to have effective applications including refining in paper industry. The main cellulase producers used are white rot fungus, *Phanaerochaete chrysosporium, Aspergillus niger*, *Trichoderma ressei*, *Penicillium funiculosum*, *Chrysosporium lucknowens* and species of *Acremonium, Talaromyces.*

Hemicellulases

Xylanases and mannanases are hemicellulases with different actions. Xylanases (endoxylanases) hydrolyze the 1, 4 β-d-xylopyranosyl linkage of xylans. Mannanases (1, 4 β-d-mannanase) hydrolyze the 1, 4 β-d-mannopyranosyl linkage of D-

TABLE- 2
MAJOR STUDIES PERFORMED ON ENZYMATIC REFINING OF PULPS

Year	Researchers	Enzymes used	Materials used	Major effects
1968	Yerkes	Cellulases (obtained from white-rot fungus *Trametes suaveolens*)	Chemical pulps or cotton linters	Improved strength properties and reduced beating time
1986	Noe *et al*.	Xylanase	Bleached chemical pulps	Reduced energy demand
1996	Bhardwaj *et al.*	Hemicellulase	Unbleached Kraft pulps (softwood, bamboo and mixed pulp)	Reduction in beating time by 17-25%
1997	Mansfield *et al*.	Cellulase and Xylanase	Douglas fir Kraft pulp	Changes in fibre at both micro and macro structural level
1999	Mansfield *et al*.	Cellulases	Douglas fir Kraft pulp	Enhanced in-plane strength of handsheets
1999	Kibblewhite and Wong	Endoglucanases and Xylanase	Radiata pine Kraft pulp	Increased tear index-apparent density and tear index- tensile index properties of handsheets
2000	Seo B. *et al*.	Cellulase	Chemical pulp	Shortened refining time by causing extensive fibre cutting and improved drainage without significantly lowering fibre length.

2001	Sigoillot *et al*	Manganese peroxidise	Alkaline peroxide pulp derived from poplar	Decrease in refining energy was found to develop pulp freeness by 25%
2006	Bajpai *et al*.	Mixtures of cellulase and hemicellulases	Different type of pulps including bleached hardwood Kraft, long fibre fraction bamboo, old corrugated containers etc.	Reduced the refining energy requirement by 18-45%
2006	Shamim *et al*.	Cellulase/hemicellul oses	Unbleached and bleached mixed (hardwood and bamboo) chemical pulp	Increased freeness value, reduced beating time and also reduced energy by 15-20%
2008	Sandeep *et al*.	Hemicellulases	Bleached mixed hardwood pulp containing acacia and MTH	6-30% energy savings, improved drainage and strength properties.
2009	Gill *et al.*	Cellulases and carbohydrases mixture	Bleached Eucalyptus globulus Kraft pulp	Improved pulp drainability (°SR) by up to 80% at the same level of refining energy (1500 PFI revolutions)

mannans and D-galacto-D-mannans (14). Some examples of hemicellulase producing microbes are *Coriolus versicolor, Glionatix trabeum, Trichocladium candense, Trichoderma ressei, Sporotrichum pulverulentum*, *S. Diorphosporum* and species of *Bacillus* and *Aspergillus*.

The exact mechanism by which enzymatic pulp fibrillation occurs is still not understood, so basic research is still underway in this area. The basic mechanism of enzymes in refining can be described as an increase in fibre swelling, hydration, fibrillation and inter- fibre bonding.

EFFECTS OF ENZYMES

On refining energy

Through the application of enzymes before refining, mills can reduce their energy requirement for refining of pulps and realize a saving in steam consumption. For a number of years it has been suggested that hydrolytic enzymes could be used to decrease the energy consumed during refining of chemical pulps, Diehm (10). Release papers, used as backings to hold adhesive labels, are high density papers made by extensive refining of chemical pulp. Mill trials have shown that treatment with a cellulase reduces the refining energy required by 7.5%, Freiermuth *et al* (15). Other product grades in this category include high density papers used in the food industry, condenser papers and glassine, the refining of which have all been shown to be enhanced by cellulase treatments. Other mill trials on recycled Kraft fibres and old corrugated container pulp successfully demonstrated savings in refining energy, Caram *et al* (16). The cellulase component cellobiohydrolase I, from *Trichoderma reesei*, has been reported to enhance mechanical pulp refining by reducing energy consumption, apparently as the result of selective action on crystalline cellulose, Pere *et al* (17).

Bajpai *et al* (18) performed laboratory and process scale studies with mixtures of cellulases and hemicellulases enzymes for reducing the refining energy requirement of different type of pulps including hardwood Kraft pulp, long fibre fraction of bamboo pulp, old corrugated containers etc. In the laboratory, the refining energies were reduced by 18-45% for different enzymes whereas strength properties of pulps were not affected by enzyme treatment. In the process scale trials, use of one of enzymes products in the production of high strength extensible sack Kraft paper reduced the refining energy consumption by 25 kWh/metric ton of pulp. It also yielded savings in steam consumption per ton of paper about 20% from various section of paper machine operation. By using enzymes, the mill eliminated a refining bottleneck in its softwood line and increased production by 12%. Tripathi *et al* (19) performed laboratory scale study on mill bleached mixed hardwood pulp with different commercial enzymes. He reported 6-30% energy savings.

Shamim *et al* (2) performed a laboratory study using different enzyme cellulase/ hemicellulase on unbleached and bleached mixed (hardwood and bamboo) chemical pulp and found reduction in energy by 15-20% as shown in figure 1 and concluded that enzymes perform better in the bleached pulp as compared to unbleached chemical pulp probably due to removal of lignin and exposure of hydroxyl groups of celluloses and hemicelluloses.

On beating time

Enzymes result in a decrease of time required for beating. Early in 1968 a process was patented for application of cellulases obtained from white-rot fungus *Trametes suaveolens* on Kraft pulp fibres to reduce refining or beating time, Yerkes (12). Similar effects were thought to be achievable using xylanase treatments, Noe *et al* (3). The advantage of using xylanases over cellulases is that there is little or no loss in intrinsic fibre strength and only minor changes to handsheet properties. Moreover the beneficial effects seemed to be more representative of mill scale refining.

In 1996, Bhardwaj *et al* (1) used hemicellulases for unbleached kraft pulps viz. softwood, bamboo and mixed pulp (60% waste corrugated kraft cuttings and 40% unbleached softwood pulp) under the conditions of optimum pH and temperature; enzyme dose 0.05% on o.d. pulp, residence time 180 min and found the results as shown in table- 1 below.

Yung *et al* (20) performed enzymatic and mechanical treatment of chemical pulp for two furnishes NBKP and LBKP with a cellulase rich enzyme. The enzyme treatment shortened refining time by causing extensive fibre cutting and improved drainage without significantly lowering fibre length.

On fibre, pulp and paper properties

The fibres themselves have different lengths from 1.5 to 5.5 mm and the cross sectional area is about 1/100th of their length. Inside the fibre, there is an empty space called the lumen and the fibre wall is composed of several layers. The fibre has an outer primary wall (P) and an inner secondary wall which is made up of three components (S1, S2 and S3). These inner components are rich in cellulose and are considered to be the main body of the fibre. As the fibres are treated by enzymatic refining, the primary wall P, and the first layer of the inner wall, S1, are removed, which allows fibrillation of the S2 wall (21).

Refining results in delamination of fibre which allows swelling by hydration, increase of flexibility and an increase of the fibre surface and specific volume, decrease in length due to the cutting effect i.e. fibre shortening, internal fibrillation, external fibrillation etc. Paper has traditionally been defined as "felted sheet of fibres formed on a fine screen from a water suspension". Enzymes produce a better fibrillation, so paper properties that depend on fibril content are generally affected. Employing the enzymatic approach significant improvement in sheet density, smoothness, burst and tensile indices and stiffness can be obtained with a decrease in opacity, bulk, tear strength, porosity and roughness.

Nomura (22) reported that cellulase plus cellobiase when added to pulps facilitated fibrillation without strength loss. Mansfield *et al* (23) treated Douglas- fir Kraft pulp fibres with cellulase and xylanase and investigated the nature of changes in fibre at both micro and macro structural level and reported the substantial reduction in pore volume of the fibres indicating that the enzymatic treatment had eroded the surfaces of fibres. In another study treatment of CTMP spruce fibres with endoxylanases resulted in improved surface area of the fibres by fibrillation, Lorenzo *et al* (24). A few studies have also been performed taking recycled fibres (25- 27) and found to have good results.

Oksanen *et al* (28) have studied the effect of *Trichoderma reesei* cellulases and hemicellulases on the paper technical properties of never-dried bleached Kraft pulp taking four purified cellulases, a xylanase and mannanase from *Trichoderma reesei*. They treated never-dried bleached pine Kraft pulp prior to refining, and evaluated the effects on pulp properties. Pretreatment with cellobiohydrolases I (CBH I) and II (CBH II) had virtually no effect on the development of pulp properties during refining, except for a slight decrease in strength properties. On the contrary, endoglucanase I (EG I) and endoglucanase II (EG II) improved the beatability of the pulp as measured by Schopper Riegler value, sheet density and Gurley air resistance. Of the endoglucanases, EG II was most effective in improving the beating response. The combinations of CBH I with EG I and EG II had similar effects on the pulp properties as the endoglucanases alone, although the amount of hydrolysed cellulose was increased. Pretreatments with xylanase or mannanase did not appear to modify the pulp properties. The same enzyme treatments which improved the beatability, however, slightly impaired the pulp strength, especially tear index at the enzyme dosages used. When compared at a given level of cellulose hydrolysis, the negative effect of EG II on strength properties was more pronounced compared with EG I. Thus, the exploitation of cellulases for fibre treatments requires careful optimization of both enzyme composition and dosage. Since the endoglucanases had no positive effect on the development of tensile strength, it was suggested that the explanation for the increased beating response is increased fibre breakage and formation of fines.

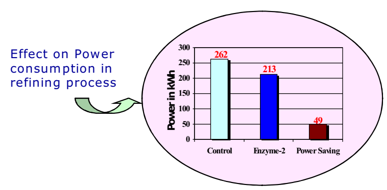

Figure 1. Effect on power consumption

Kibblewhite and Wong (29) used five endoglucanases and one xylanase from *Trichoderma ressei* and *Humicola insolens* on radiata pine Kraft pulp and found an increase in the tear index-apparent density and tear index- tensile index properties of handsheets. Pulp treatment with endoglucanases caused fibre strength degradation normally without significant dissolution of carbohydrate material when low doses were used. The change to fibre dimensions during refining was not consistent among all the endoglucanases. Dickson A. R. *et al.,* (30) studied the effects of treating an unbleached kraft pulp derived from Pinus radiata with a commercial xylanase enzyme using Escher-Wyss and PFI refining. The Escher-Wyss mill, a laboratory scale conical refiner, was considered to give a closer representation of mill scale refining than the PFI mill. The results of investigation were found to have a greater development of handsheet properties after PFI than Escher-Wyss refining. Escher-Wyss refining, however, revealed greater modification of fibre characteristics by xylanase treatment, when compared to the control, than was observed after PFI refining.

In a work performed by Janardhan and Sain (31) using fungal enzyme for refining there was observed a significant impact on the defibrillation characteristics of the bleached kraft pulp of northern black spruce fibres which is a major step in the isolation of cellulose microfibrils. Tripathi *et al* (19) performed laboratory scale study on mill bleached mixed hardwood pulp with different commercial enzyme while Shamim *et al* (2) used mixture of

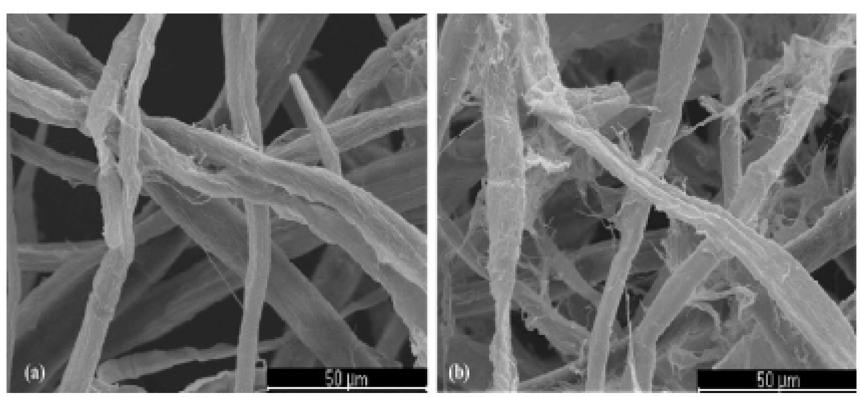

Figure 2. SEM micrographs of eucalyptus Kraft pulp refined for 1500 PFI revolutions without enzyme treatment (a), and treated with 4 IU of cellulases (b)

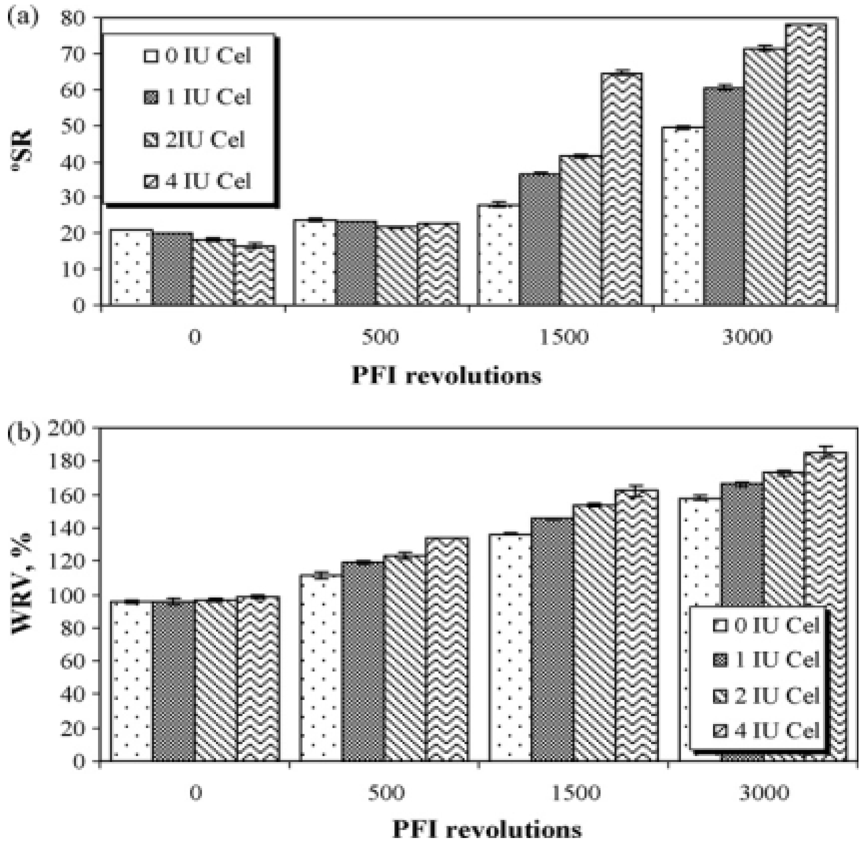

Figure 3. Effect on pulp treated with cellulases for refining process

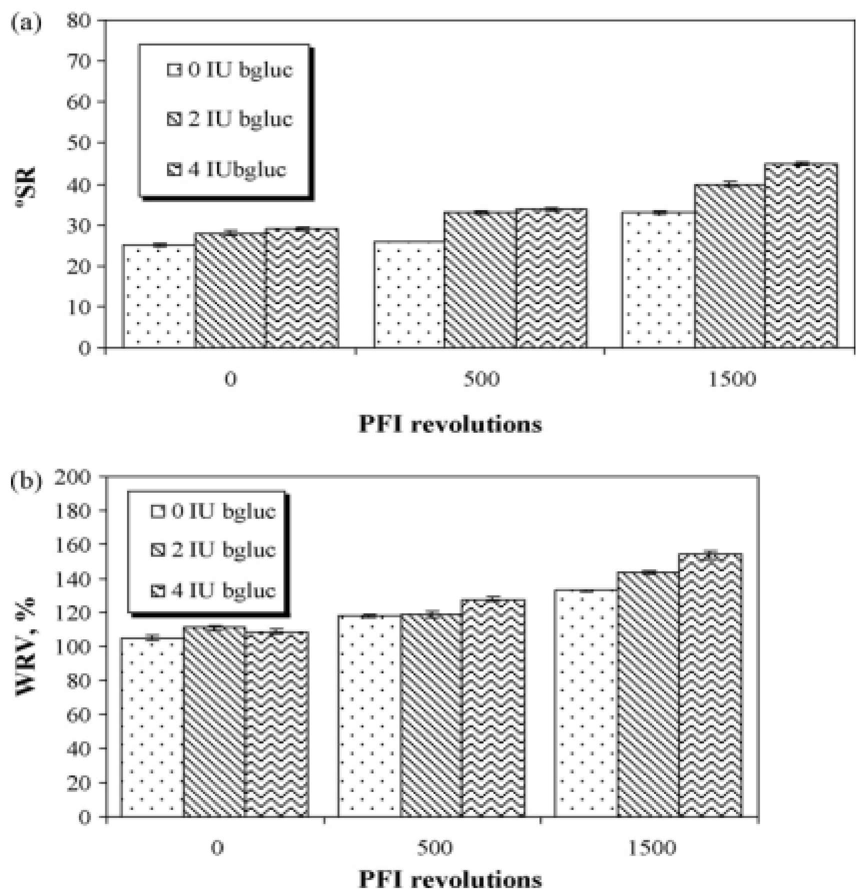

Figure 4. Effect on pulp treated with beta- glucanases for refining process

cellulase/hemicellulase for unbleached and bleached mixed (hardwood and bamboo) chemical pulp and reported an improvement in drainage and better strength properties. Gill *et al*., (32) evaluated the effect of Celluclast 1.5 L® (cellulases mixture) and Viscozyme L® (carbohydrases mixture) for bleached *Eucalyptus globulus* Kraft pulp and found an improvement in drainability by upto 80% (°SR) and found an increment in external fibrillation through scanning electron micrography as shown in figure 2. Effect on pulp taking different doses of cellulases for refining process is shown in figure 3, and with beta-glucanases the results are explained in figure 4.

Recently, Yong-sheng MU *et al* (33) have studied the effect of cellulase and xylanase treatment on fast-growing poplar wood APMP. The effects of the factors influencing the pulp energy consumption, pulp physical strength properties and optical properties such as enzyme dosage, pH, temperature and time were studied. The results showed that the fiber structure gets looser, the fiber swells fast, the interfibre bonding force gets stronger, the energy consumption decreases remarkably and the physical properties and optical properties of the MP were improved effectively. It was found that cellulase was better in physical properties improvement and energy consumption reduction and xylanase was better in raising the brightness properties.

ADVANTAGES OR BENEFITS

Since refining requires significant energy input as well as capital investment for equipment, facilitating the refining process could provide numerous benefits including stronger paper and more use of recycled paper.

Reduction in electrical energy

By applying enzymes, mills can reduce their pulp refining energy requirement. Enzymes are expected to give more benefits to those mills, which are not having captive power generation and/or limited by refining capacity.

Drainage improvement

Many researchers have explored the use of enzymatic treatment for increasing pulp freeness. The enzymatic attack involves peeling mechanisms which removes fibrils and leaves the fibrils less hydrophilic and easier to drain. The increase in drainage may also attribute to the cleaving of cellulose on the surface of fines.

Reduction in steam consumption

Enzymatic refining results in improved drainability i.e. better dewatering, so there is decreased steam load for paper drying and reduced steam consumption.

Improvement in paper properties

Enzymes produce a better fibrillation, so paper properties that depend on fibril content are improved. These properties are tensile strength, burst strength, tensile energy absorption.

Better machine runnability

Efficient drainage of pulp furnishes on the wire of paper machines is desired to maximize machine speed. Improved drainage results in shorter time for drying period so paper machine runnability increases and results in enhanced productivity.

Cleaner recycled water

Enzymes partially eliminate fine fibrils and colloidal materials, mainly those contained in the white water loop due to longer contact time with the residual enzyme activities in water. This produces cleaner recycled water, with optimum fine and fibrils content, Bajpai *et al* (18). Enzymatic treatments are useful in cleaning the process water within the mill. Enzymes partially hydrolyze cellulose debris to low molecular weight saccharides that are easily biodegraded in the wastewater treatment system.

CHALLENGES

The main challenge in using enzymes to enhance fibre bonding is to increase fibrillation without reducing pulp viscosity. Viscosity decreases when cellulases cleave cellulose chains lowering the degree of cellulose polymerisation and destroying the fibre integrity. Though promising results have been achieved, yet more intensive studies on enzymatic pulp refining are required to make the process cost effective for easy adoption by paper mills.

FUTURE PROSPECTS

The application of enzymes in refining seems to have great potential with rising power cost and possible reduction in enzymes cost in the near future. Thus, there is a need for carrying out detailed studies to develop cost effective enzymes. In order to get enzymes to act in an effective manner, papermaking technologists need to do some careful work. The enzyme also needs to be well chosen with respect to the temperature, pH etc. Reaction conditions, especially exposure time and dosage, need to be chosen with care. Creative thinking is also needed as technologists attempt to improve the effectiveness of various enzymatic treatments. There may be potential to save costs or increase throughput by increasing the catalytic activity of a given enzyme.

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