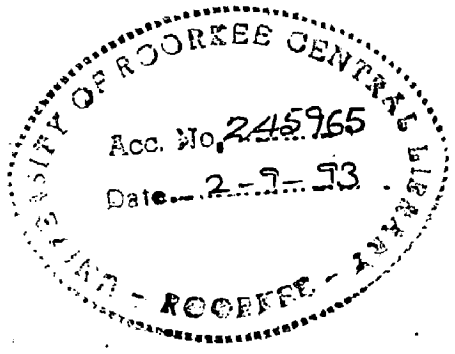
APPLICATION OF XYLANASE FOR BLEACH BOOSTING AND FIBER PROPERTIES MODIFICATION



A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of **MASTER'S OF ENGINEERING** in Pulp and Paper Technology from Institute of Paper Technology (UOR) Saharanpur.

by,

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B.Tech.(Chem.Engg.); HBTI-Kanpur, 1990

under the guidance of,

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January, 1993.

Candidate's Dec lqration

I hereby declare that the work which is being presented in the thesis entitled "Application of xylanase for bleach boosting and fiber properties modification" submitted to the University of Roorkee in partial fulfillment of the requirements for the award of the degree of `Master's of Engineering' in Pulp and Paper Technology from Institute of Paper Technology, (Saharanpur) is an authentic record of my own work carried out during the period from July, 1992 to January, 1993 under the guidance of Dr. Ashok Kumar and co-guideship of Dr. J.S. Upadhyaya.

The matter presented in this thesis has not been submitted by me for the award of any other degree.

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This is to certify that the above statement made by the candidate is correct to the best of our knowledge.

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Abstract

Enzymatic bleach boosting and fiber properties modification are among the few strategies developed to influence the efforts for conserving energy and safeguarding the environment. This aspect was studied with reference to Bagasse and Eucalyptus pulps.

A 0.05% xylanase pretreatment for 60 minute was given to bagasse and eucalyptus pulps, cooked by modified techniques, this resulted in an increase of 2.7 and 2.8 points in brightness for bagasse and eucalyptus pulps respectively. This amounts to a saving of about 50% of chlorine dioxide consumption to achieve the same level of brightness. Thus, it was possible to obtain an ISO brightness of about 90 + with only 1.45% and 2.4% of total ClO_2 application on bagasse and eucalyptus pulps respectively following XDED bleaching sequence. The bonding ability of pulps is only slightly affected due to the hydrolysis of xylan. The results obtained suggest that this technology is also pertinent for the pulps cooked by modified techniques.

The experiments were also carried out to assess the use of xylanase for the modification of fiber properties. When fully bleached bagasse and eucalyptus pulps were treated with 0.1% of enzyme solution for 180 minutes, a 5 point and 7 point rise in °SR were obtained with minor loss in the yield. The xylanase seems to give some external as well as internal fibrillation by hydrolysing native xylan. Thus, as a result of some internal modification in the fiber skeleton, the beatability of pulps as well as is strength properties were improved even after xylan hydrolysis. This may be due to the fact that such internal modification occurring in the fiber wall more than compensates the effect of the removal of xylans.

In order to make these processes more effective and economical, it is recommended to develop better strains which produce lower molecular size enzyme. A study to investigate the possibility of reutilisation of enzymes is also recommended.

Contents

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Chapter - 1

INTRODUCTION

1.1 Pulping and Environment

Chemical pulping process is the most widely used process for papermaking. The organic chlorine compounds formed during the bleaching of chemical pulp have perhaps attracted most attention in recent years. Earlier measures taken by the pulp industry to solve the chlorine problem have focused on improving effluent treatment methods, i.e. measures external to the process. Today, the emphasis of research in this area has shifted more towards improving the processes. The efficient and more selective pulping processes reduce the amount of lignin going with the pulp to the bleaching process. Also the amount of chlorine required for bleaching is being reduced by the use of bleaching agents such as chlorine dioxide, oxygen, hydrogen peroxide and ozone with the ultimate aim of completely chlorine free bleaching.

The environmental loading caused by final bleaching is very small compared with that from earlier bleaching stages. Thus, the often proposed reduction of final pulp brightness would do little to reduce the amount of chlorinated compounds discharged with the effluents. The aim of the researches working in this field is to overcome the chlorine problems associated with bleaching by modifying the cooking stage (extended cooking, various additives) or the subsequent chlorine free residual delignification stages (oxygen-ozone, intensified washing) in order to minimize the amount of lignin going for chlorine bleaching. Enzymatic bleaching serves this aim, and gives positive results with modified processes too.

1.2 Recent Developments in Biological Bleaching Technology

The idea of biological bleaching probably originated from observations of color changes in wood during its natural degradation by fungi. The so-called white-rot of wood is chemically characterized by a more or less specific removal of lignin. During the very beginning of 1980's, a lot of work was done on biobleaching especially in STFI - Sweden. After a considerable research, it could be possible to reduce the treatment times from 42 days to 72 hours and could be possible to check degradation of cellulose by developing cellulase-less mutants of fungi. But still treatment times and provision of controlled environment to avoid any other microbial attack, makes the process far from realisation. To retify this problem, assuming that biological bleaching is primarily due to attack on lignin and it has long been realized that phenoloxidases are necessary for lignin degradation to occur (1). With the discovery of ligninases (peroxidases), this fact has been emphatically confirmed (2,3). Attempts were made to bleach the pulps with isolated lignin modifying enzymes from white rot fungi (4). But in addition to known lignin degrading enzymes, other enzymes and co-enzymes are also necessary since the main lignin degrading enzyme, phenloxidases produce phenoloxy-radicals that spontaneously polymerizes. These polymerisation reactions must be prevented or reversed for actual depolymerisation of lignin polymer to occur. Till today, it has not been possible to construct a enzyme mixture that prevents the polymerisation reaction from taking place in a cell-free state. Now this matter is in active research and its future success for practicle exploitation may be anticipated.

A new dimension was added to the challenge of biological bleaching when it was realized that hemicellulases could play an important role considering lignin's close relationship with hemicelluloses in wood. This basic research idea has resulted from cooperation between the Biotechnical Laboratory of the Technical Research Centre of Finland (VTT) and the Finnish Pulp and Paper Research Institute (KCL). This began in 1985 as part of a technology programme financed by the Technology Development Centre (TEKES) Finland. Since then this research has resulted in its practical implementation, or exploitation.

1.3 Present State of Art of Enzyme Treatment Technology in Pulp and Paper Industry

The application of xylanase as pre-bleaching stage should be considered as modification over modifications as its applicability may be harnessed for both conventional and modified processes to reduce the consumption of bleach chemicals with same effectiveness. Its applicability for the production of high brightness bagasse and eucalyptus pulps using chlorine-dioxide has been investigated in the present study.

Moreover, since pulps and paper industry is energy intensive; the use of xylanase may also be helpful in energy saving if a enzymatic treatment stage is carried out before beating \ refining. Since fiber's accessibility towards enzymes is also limited by the median pure width (space between fibrils) and the size of enzyme molecule; the effective applicability is only for fully bleached pulps. The applicability of xylanases for this purpose has also been carried out in the present study for bagasse and eucalyptus fully bleached pulps.

Another use of enzyme in paper industry is the reduction of pitch. Pitch is conventionally reduced by seasoning during storage, but the yield loss and brightness reduction are undesirable side effects. Even the addition of a control agent such as talc does not necessarily eliminate pitch problems. Enzymatic pitch reduction has now reached industrial scale at Jujo Paper Mill in Japan (5,6), using Navo Nordisk's enzyme Resinase A.

The other significant application of enzyme in paper industry is the use of cellulase for deinking the secondary fibers with its quality upgradation (7,8). So far, many patents have been granted for the process which virtually doesn't require surfactants, sodium silicate, caustic soda, collector chemicals or H_2O_2 and further saves disintegration times or energy. The other promising applications of enzyme treatment technology in pulp and paper industry are enzymatic debarking, bast fiber pulping employing pectinases and increased stock freeness, enzymatic beating employing cellulases.

In pulp and paper industry, this technology is in primitive stage. However, it is anticipated that its future development will make it more friendly both economically and technically.

1.4 Enzymes

It is important to understand that enzymes are not microorganisms as many people may think because of the biological origin of enzymes. Enzymes are only kinds of chemicals used by a microorganism to digest the particular food that is usually fed (true for all kinds of life). Enzymes are bio-catalytic protein in nature and are not always high tech. specialty products. As papermakers, we some times use enzymes for starch liquefaction with a minimum of supervision. In fact, they behave just like other chemicals except that they work in very mild conditions. The enzymes are not always expensive. Otherwise, it is difficult to imagine their intensive use in such cheap products such as detergent. If we put it all together, no restriction remains against the use of enzymes in paper making.

1.5 Enzymatic Action

The best definition of enzymes is that they are super catalyst. Many chemical reactions require the presence of a catalyst in order to decrease the energy barrier existing between initial and final state. The only difference with enzymes is that since they are used in living organisms, the conditions in which they are most efficient are very mild not vary far from room temperature and without pressure. Enzymes must be used in those mild conditions, otherwise as they are complex chemicals, they would be denatured and destroyed.

Another strong peculiarity concerns their selectivity. They are designed to bind to complex molecules in order to decrease their bonding strength. Since, these molecules, in general are large and complex, enzymes are very selective, even in the tiny details of the structure of the receiving molecules (9). For instance, an enzyme able to catalyze the hydrolysis of starch can't have the same ability with cellulose even if the molecule

is constituted with the same basic unit-glucose, but arranged in a different steriochemical network. In short, selectivity and mild working conditions are the main advantages of enzymatic catalysis. However, several thousands of different enzymes are involved in directing and controlling the complex chemical reactions of life.

Chapter - 2

LITERATURE REVIEW

2A. Bleaching with Hemicellulolytic Enzyme

It has been discussed in an earlier section that the idea of using a hemicellulase treatment to promote bleaching arose at a time when no enzyme that would attack lignin direct was available. The credit for this, goes to the team of Finnish scientists under the leadership of Prof. Jorma Sundquist of KCL and Prof. Matti Linko of VTT. It was thought that hemicellulases might promote bleaching due to its close association with lignin which is mainly linked to hemicelluloses in wood (10). The side chain groups; arabinose, galactose and 4-O-methylglucoronic acid have been proposed to be the links connecting hemicellulose to lignin (11,12,13). Both ether and ester linkages have been proposed.

The kraft process is an efficient pulping method leading to pulp normally containing about 5% residual lignin in a batch cook. Further more, the formation of new linkages between lignin and carbohydrates have been demonstrated to occur during the batch pulping process (14,15).

Depending on the species, wood contains 20-30% hemicelluloses. Hard wood hemicellulose is mainly glucoronoxylan. Softwood hemicelluloses are mainly glactoglucomannan and arabinoglucuronoxylan (16) while in non woods, particularly in bagasse, hemicelluloses are constituted by arbinoglucoronoxylan, arabinoxylan, O-acetylated arabino glucorono xylan and galactoglucomannan (17).

2A.1 Barriers

The removal of residual lignin from kraft pulps may by physically and chemically restricted by hemicelluloses. Under alkaline batch pulping conditions xylan is first dissolved and then reprecipitated on to the fibers. During the heating period of the kraft cook, when the alkali concentration is comparatively high, the partial degradation of xylan by peeling and hydrolysis results in dissolution of degraded xylans, free of side chain units in the pulping liquors to a considerable extent. But as the cook proceeds, the alkali concentration decreases below the critical point and degraded short chain xylan precipitates in a more or less crystalline form on the surface of cellulose fibers. This behavior in batch digestion, which is absent in continuously or modified batch cooked pulps, has been verified by many investigations (18,19,20,21). The resistance of this type of xylan in kraft pulp towards both hot and cold alkaline extraction is remarkable. A considerable part of the xylan is readsorbed onto the cellulose although a part remains undissolved at its original location in the fiber (22,23). Also, the reprecipitation of xylan has been found to follow the redeposition of dissolved lignin during kraft pulping. These redeposited polymers have also been suggested to be chemically linked to each other (24,25). However, glucomannan molecules dissolved in alkali are completely degraded in a very short time at 130°C only (18,26) and the residual glucomannan is rather stable to further degradation and dissolution. So this behaviour of batch-wise cooked pulps, either soft wood, hardwood or non wood should be considered universal.

The reprecipitated and readsorbed alkali resistant xylan, possibly together with lignin, seems to form a physical barrier against extraction of residual lignin molecules from the fibers. In absence of this barrier, the pulps cooked continuously or by a modified batch method have different morphological properties compared with those produced by conventional digestion to same

level of kappa number. The permeability of such pulps should be more pronounced.

2A.2 Mechanism of Enzyme Pretreatment

After a thorough investigation (27,28), it has been suggested for kraft pulps cooked by conventional batch process that xylanase is able to efficiently hydrolyze the alkali resistant reprecipitated xylans on the fiber surface. However, the residual xylans located within the fiber were also found to be hydrolyzed. this enzyme pretreatment simultaneously increases the permeability of the pulp to delignifying bleaching chemicals and permits the passage of molecules of residual lignin from the fiber. This explains why xylanases alone bring about most of the bleaching effect even in softwood pulps. Mannan has not been found to be reprecipitated or readsorbed in the same manner as xylan during alkaline pulping, so it does not seem to form a physical barrier on the surface of fiber. The addetion of mannanase for soft-wood pulps has been shown to improve the bleaching results (29,30) which seems to add to the permeability of fiber by hydrolysing native mannans.

For kraft pulps cooked of continuous method or modified batch process, xylanase seems to hydrolyse native xylan only thus rendering pulps more permeable towards delegnifying bleaching chemicals. This behaviour seems to be the case as in the present study for both eucalyptus and bagasse pulps (see section 3A). However, for soft wood pulps cooked with modified processes, it is recommended to enrich the xylanase solution with mannase, otherwise xylanase alone can also give desired results.

2A.3 Type of Enzyme

Earlier bleaching studies were carried out using the hemicellulases produced by fungi; containing several different enzyme activities. It was noticed that this enzyme treatment caused an unacceptably high decrease in pulp yield and viscosity (31). The fall in viscosity was due to the presence of cellulase in the enzyme preparations. Even low cellulase activity causes hydrolysis of cellulose chains, which in turns weakens the fibers. The decrease in yield was due to the degradation and subsequent dissolution of hemicellulose and cellulose both.

This loss of viscosity was prevented and yield losses reduced to an acceptable level by optimizing the enzyme dosages and reaction times and by changing over to enzyme preparations with very low cellulase activities (27). Of the different microbes, in particular the fungi, often produces cellulases along side hemnicellulases. By contrast, certain bacteria have proved to be very efficient and specific producers of hemicellulases. On comparing different types of hemicellulases, it was found that the effect of the enzyme pretreatment on pulp bleachability is due largely to the action of endo 1-4 -β-D xylanases (E C 3.2.1.8). This has since been verified with purified xylanases isolated from a number of different microbes. However, no other hemicellulolytic enzyme, when used alone, have been shown to exert the same effect (32,33). This addition of other enzyme such as mannanase has been shown to improve bleaching results of softwoods slightly (29).

2A.4 Benefits from Enzyme Treatment

Treating unbleached pulp with xylanase does not, in itself, remove lignin from the pulp. Instead, it merely renders lignin easier to extract in following bleaching stage after this treatment. Enzyme pretreatment gives either a higher final brightness or a lower bleach chemical consumption (Cl_2 or any other bleach chemical if applied instead of chlorine).

(i) ICI Canada has reported on a xylanase that was effective for a treatment of hardwood pulp - a 40% reduction of the

chlorine charge was attained while continuing to reach brightness of 91% + in the final bleaching stage (34). Even greater chlorine savings (60 to 70%) were reported for bleaching when low substitution (10%) was employed,

(ii) Novo enzyme, Pulpzyme - HB (xylanase) which is active under alkaline pH range has been lab. tested on Eucalyptus pulp following (D50 C50) E D sequence and a DED - sequence (35). In the delignification stage, the active chlorine requirement is reduced by 22 and 28% respectively. The NaOH dosage was reduced correspondingly. The enzyme pretreatment makes it possible to reach full market brightness of 91% ISO with 20 Kg/Ton of ClO_2 in three stage Cl_2-free sequence with same properties as that of control pulps.

(iii) The Metsa-Sellu mill, Aanekoski(Finland) has conducted trials in May 1991 demonstrating that enzyme application can reduce active chlorine by 25% and total (including chlorinedioxide equivalent) by 15% for softwood pulp. (36). The mill points out that investment necessary for oxygen bleaching is about 20-50 times more than that for enzyme treatment. The brown stock storage tank was used as reactor vessel for xylanase treatment.

(iv) A xylanase preparation, Cartazyme -HT, a product being developed by Sandoz, UK and Swiss Federal Institute of Technology is able to work upto 80°C in the pH range 5-9. The laboratory tests on both hardwood and softwood pulps following sequence X(EOP)D(EOP)D produced pulp of 89% brightness. The bleached yield with the enzyme (92%) was about the same as in the control sequence.

2A.5 Amount of Enzyme

The hemicelluloses are important in determining the chemical interaction between fiber, water and pulping chemicals. In the enzymatic pretreatment for bleaching, the hydrolysis of hemicelluloses should be restricted to a minimum by using only very small amount of enzymes in order to maintain a high yield and the advantageous properties of hemicelluloses, at the same

time obtaining the desired results. Hemicellulases can promote bleaching by degrading less than 10% of the xylan present in the unbleached pulp with little effect on its final paper making properties.

In the reported literature (27-36), it may be concluded that an optimum activity of 15-30 IU per gram of OD pulp for 30-60 minutes gives the desired results with minimum effect on yield and virtually no adverse effect on pulp properties, which has been verified in the present study also.

2A.6 Access of the Enzymes to the Substrate

When considering the effeciency of enzymatic treatment of pulps, the critical parameter is the pore volume or pore size distribution, which determines the accessibility of the enzymes to the fibers. In general, the inter fibrillar space within the cell wall ranges between 1.0 and 5.0 nm in the wet pulp. However, wood itself has a median pore size of 1.0 - 1.2 nm, a value which increases as a function of the decreasing yield of pulp, reaching 5-6 nm at a common yield level of 50% in the sulfite and kraft processes (37). It has also been shown that an exact correlation exists between the mean pore size and the size of lignin molecules leaving the cell wall at various stages during the pulping (38).

According to the results of molecular modelling of the core protein of the cellulase (CBHII) from Trichoderma reesei of 56 kD molecular weight, the approximate diameter of the enzyme is 5 nm (39). Although the molecular weights of different enzymes very considerably depending on type of producing strain, the molecular weight of xylanase (Pulpzyme HB-Navo Nordisk A/S,Denmark) is about 26 kD, preparation derived from bacterial origin while the molecular weight of Pulpzyme - HA is about 30 - 40 kD, xylanase preparation derived from fungal strain Trichoderma reesei (40). It is evident that penetration of enzymes (cellulase, xylanase, ligninase) in native wood is limited and efficacy of enzyme treatment of pulp should depend on molecular size of enzyme also. Efforts should be made to produce the xylanase as small as possible. Moreover, scientists at NCL, Puna (India) have

discovered a fungal strain Chainia producing xylanase of 6 kD only, probably smallest enzyme known so far. No further information is available in this regard.

It has been shown using immunoelectron microscopy that the enzymes of white-rot fungi invade the wood cell-walls only on superficial areas in the near proximity of hyphae or in places where predegradation has already been occurred (41,42). By contrast, a large part of the fibers in pulps were fully accessible to enzymatic attack by lignin peroxidase (43), although great differences were observed with respect to the penetrability of individual fibers in the same pulp, correlating with the morphological appearance of the fibers.

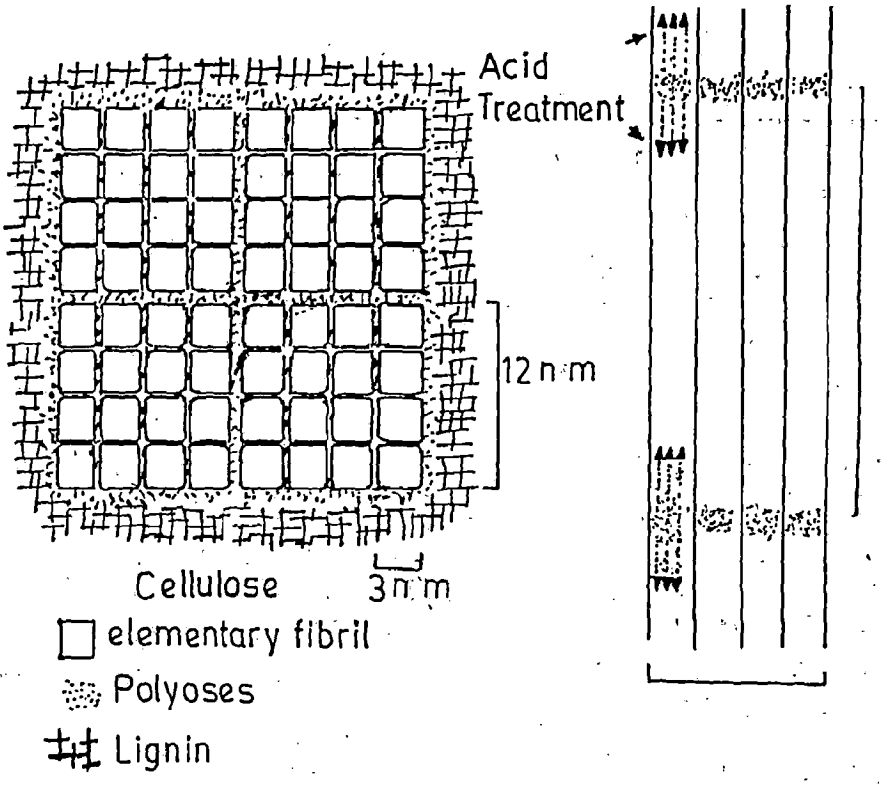

Fig. 2·1 Structure model of microfibril showing arrangement of cellulose fibrils, hemicellulose (polyoses), and lignin in cross section according to Fengel(44)

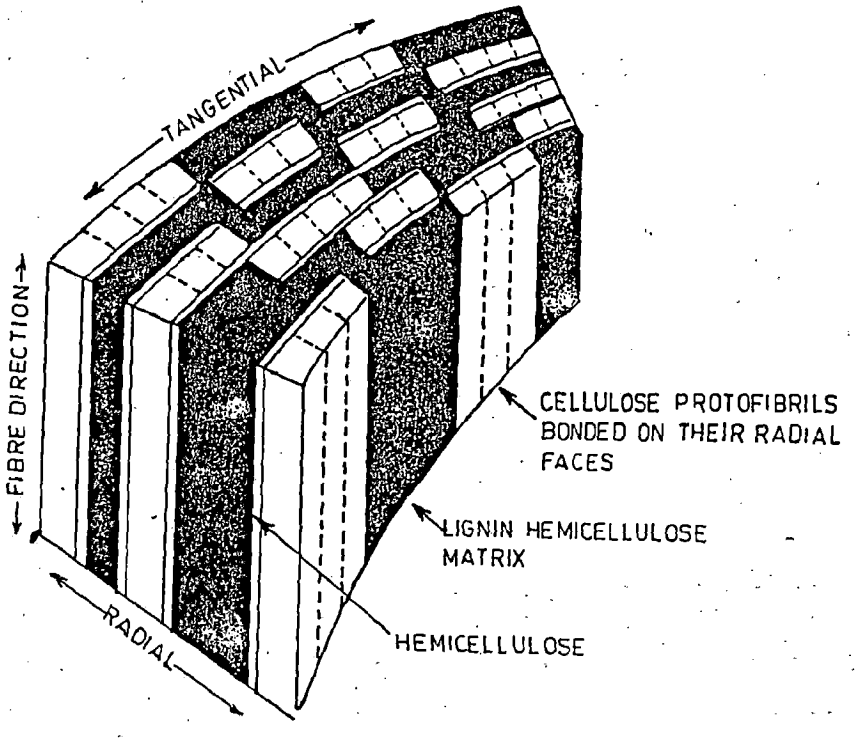

Fig. 2·2 Interrupted Lamella Model For The Ultrastructural Arrangement of Carbohydrates and Lignin in The Cell Wall According to Goring (45,46)

2B. Enzymatic Fiber Properties Modification

To manufacture paper from wood pulp fibers, the mechanical action is required to improve the formation and to produce the paper sheet with desired properties. This operation is known as beating or refining which consumes about 20% of total electrical energy required by a paper mill using kraft process. However, the paper industry itself is a major energy intensive unit. Any measure to cut this energy demands would obviously be advantageous. A measure other than design or operational modification is through enzymes. However, when a enzymatic bleach boosting approach has already been adopted through hemiceullulolytic enzyme; a further decrease in hemicellulose contents do not seem to be logical. But incontrast a further action of hemicellulolytic enzyme, seems to compensate the previous losses by modification of internal fiber structure. In addition, a decrease in energy demand is also realized.

In order to understand the applicability of enzyme for the purpose, it is very necessary to understand the ultrastructure of fiber and various changes brought about by beating and refining.

2B.1 Ultra Structure of Fiber

Models for ultra structure of wood fiber that take into account the hemicelluloses and lignin in the fiber cell-wall have been proposed by Fengel (44) and Goring (45). In Fengel's model (fig. 2.1), the elementary fibrils are surrounded by monolayers of hemicelluloses, with the larger units enclosed by hemicelluloses and lignin. Variable thickness for the hemicellulose layers would explain differences in microfibrillar thickness. Based on extensive studies on the distribution of lignin in the cell wall by ultraviolet microscopy; Goring and coworkers (45,46) proposed an interrupted lamellar structure for the wood cell wall (fig. 2.2). The model shows the cellulose microfibrills as ribbon like structures consisting of 2-4 proto (elementary) fibrills bonded on their radial phases with their tangential surface coplaner and parallel to middle lamella. The

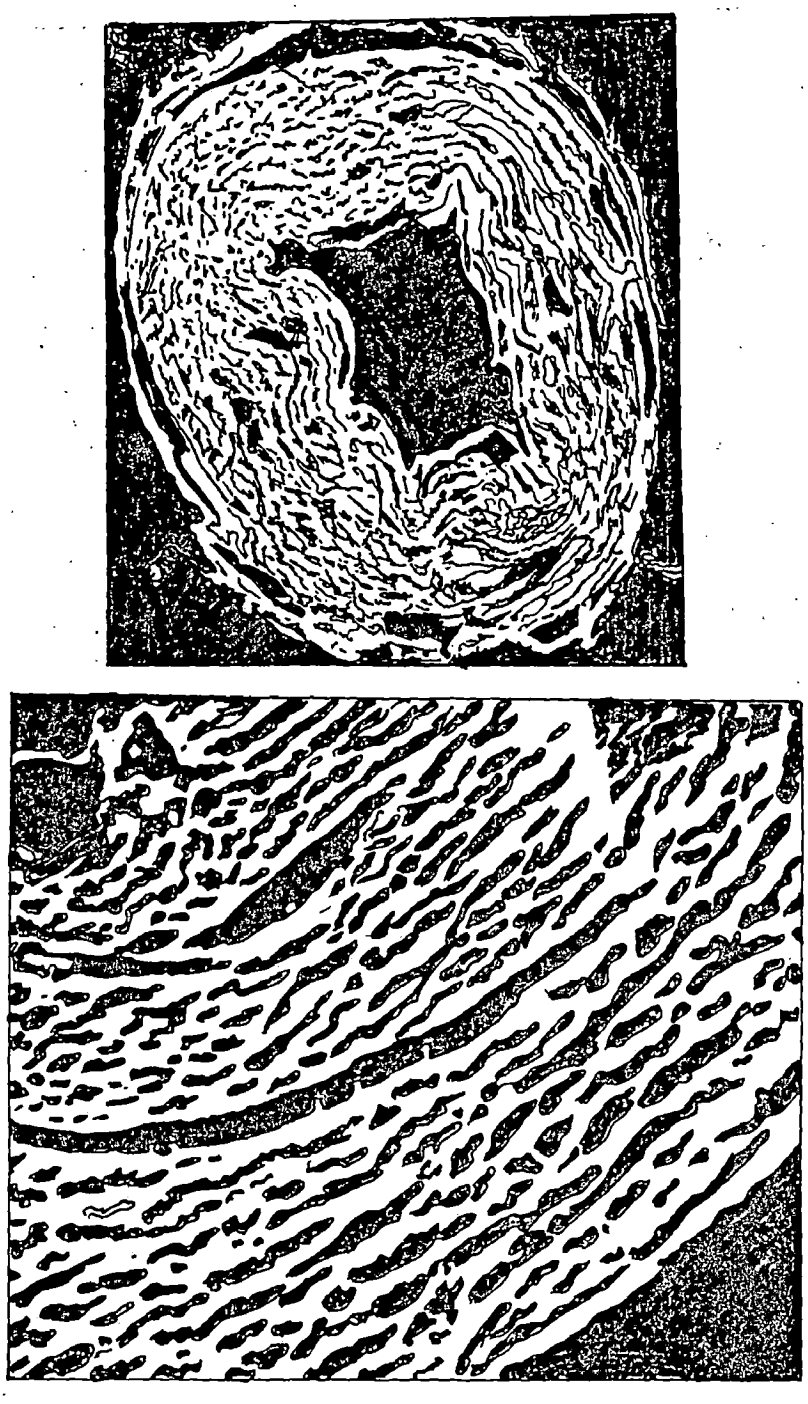

Fig. 2-3- Cross-section of solvent-exchange-dried bleached sulfite fiber embedded in methyl acrylate; Stone, Scallan (47)

lignin was visualized as layers between the cellulose ribbons. The realization of this model was based partially on the postulated multilamellar structure for the cell-wall of spruce pulp fiber based on data derived from nitrogen adsorption measurements and on electron microscopic examination of cell walls in corss-section (47). The extensive honey combed structure of an expended cell wall can be seen in fig. 2.3. McIntosh (48) obtained similar evidence of lamellar separation on beating of pine holocellulose.

2B.1.1 Cell wall Pore Structure and its Importance for Fiber Properties

As Caufield has pointed out (49), water does not interact with glucose moieties burried within the cellulose crystallite but only on the surfaces of cellulose crystallites that is in the spaces between the cellulose fibrills and micro fibrills. Thus, the microporous structure of cellulose fibers is of great importance to the properties and reactivity of cellulose fiber in the presence of water. It influences the extensive imbibition of water, which imparts desirable bonding properties to fibers in wet paper making. It becomes a critical parameter for efficacy and accessibility of enzymes to the substrate (pulp fiber).

Pores arise from imperfections in the lateral packing of microstructural elements. With paper making fibers (wood and non woods), the extensive microporous structure is also closely related to the removal of hemicelluloses and lignin in the pulping process. In 1968, Stone and Scallan (37, 50) convincingly demonstrated by solute exclusion techniques that the cumulative pore volume in wood pulp fibers increases with decreasing yield. The median pere size increases from 1.2 nm to almost 6.0 nm at the lowest yield. Pores smaller than 2.5 nm in diameter were attributed to intralamellar spaces and pores greater than 2.5 nm diameter to interlamellar spaces, the lamellar thickness was 6.2 nm, and the authors proposed that the pores are widest between the outer lamellae of the fiber and that they become progressively narrower towards the lumen.

The pore size distribution varies with the type of

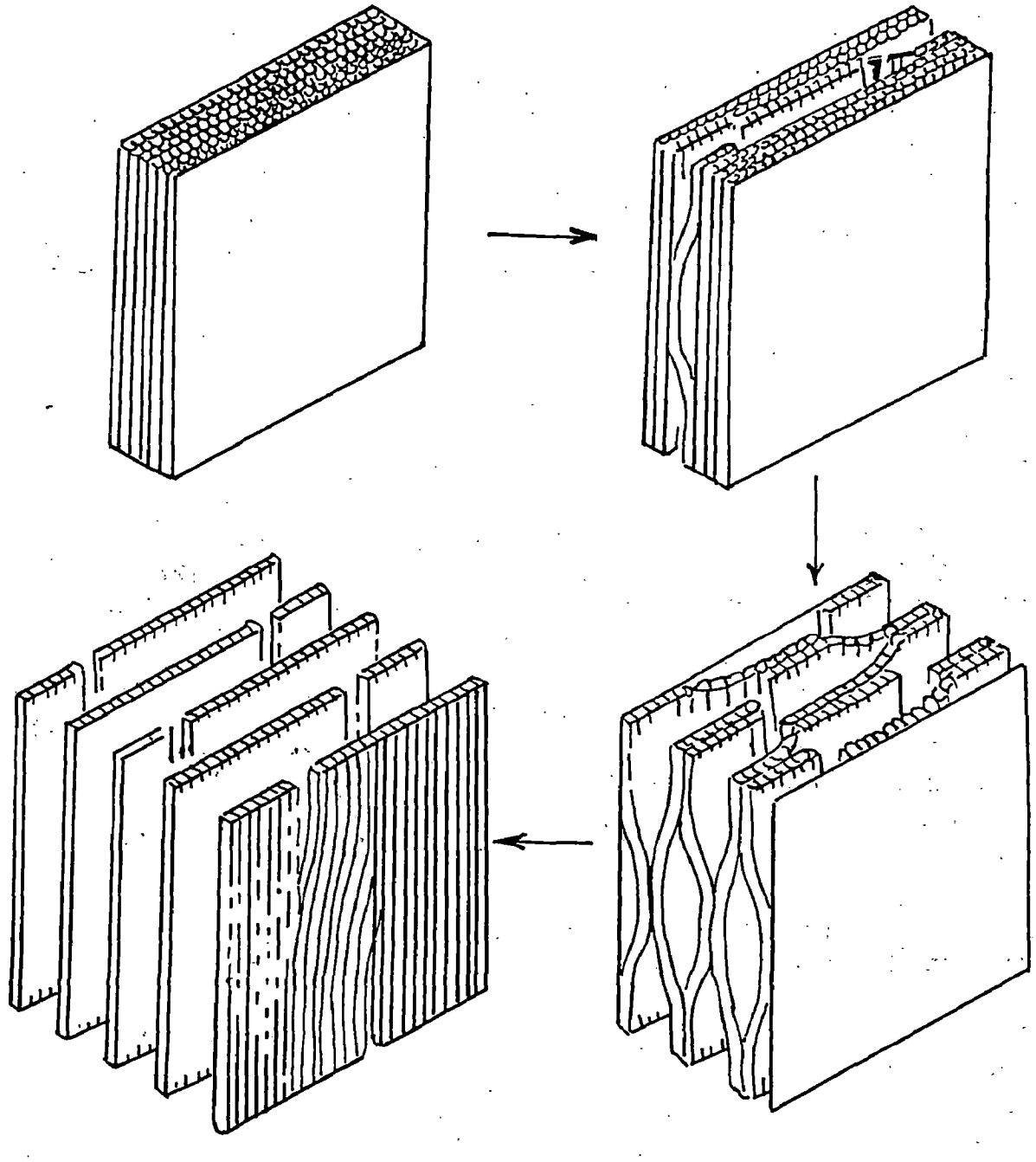

Fig. 2·4–Pattern of internal fibrillation of the cellwall according to Scallan (50)

cellulosic raw material. And its pulping, not only increases the pore volume, but it also creates a wider distribution of pores in the pulp fiber (51,52). The pore structure of fibers can also considerably influence the reactivity in modification reactions. Young and Fujita (53) have similarly found that the bonding properties of wood fiber are substantially improved by treatment with 3N-sodium hydroxide which further expends the micropore structure.

2B.2 Beating or Refining Vs. Ultra structure of Cellulosic Fiber

This mechanical treatment, referred to as beating or refining, causes a variety of changes in the fiber, but the main effects are fibre shortening, external fibrillation and internal fibrillation (54). As a result of beating, the fibers can absorb much greater quantities of water. The increased amount of water in the cell wall plasticizes the fiber with the result that fiber becomes more conformable when randomly laid into a fibrous web by wet forming processes. The greater conformability and collapse of the fiber lumens gives more bonded area at fiber crossover points.

The major changes that occur on beating are at the fibrillar or lamellar level in the cellulose structure (see fig. 2.4). Emerton (55) following others particularly Campbell (56), has proposed that the increase in strength is caused primarily by an increase in the flexibility and plasticity of the wet fibers, which when pressed and dried, form a denser sheet. It was suggested that this enhancement of flexibility arises from the break down of the wall into separate lamellas by the stress of flexure in the beating zone. The term "internal fibrillation" has been coined by Campbell to describe the structural change. External fibrillation is a more obvious change when viewed under the microscope owing to extensive disruption of outer layers of the fiber cell wall. Fine fibrillar fragments are left attached to the fiber surface, which results in further hydration (57). Considerable portions of the S-1 and even of S-2 layers are stripped from the fiber during beating and together with other

cell elements such as perenchyma and fragments creases the fine or crill fraction in the pulp (58).

The importance of external versus internal fibrillation has been discussed by several authors with differenting view points as to relative importance of these two factors (50,54,57,58). Recent evidence strongly indicates that external fibrillation and fine formation during beating or refining are the main factors affecting drainage and network porosity while the swelling and delamination (internal fibrillation) are critical to sheet consolidation and fiber-fiber bonding (54,59).

2B.3 Hemicellulolytic Enzyme Promoting Beatability and Fiber Properties

The desired structural changes in the fiber which are created during beating or refining have already been described in section 2B.2. The role of xylans in the fiber properties was studied using xylanases in the treatment of fully bleached sulfite and birch kraft pulps. Electron microscopic examination revealed (60) external fibrillation and good flexibility of fibers implying internal modification. The water retention value, which describes fiber swelling, increased by 20%. The conclusion was that the xylans were hydrolysed in the whole delignified cell walls of pulp. The enzymatically treated pulps were comparable with slightly beaten pulps. The beatability was enhanced and energy demand was reduced further (61). It was also revealed that (62) with a higher concentration of xylanase and with prolonged time of hydrolysis, the bundles of cellulose micro fibrills and single micro fibrill were isolated when seen in TEM, which confirmed the important role of xylans as cohesive agent between cellulose microfibrills inside the secondary wall.

The degree of polymerisation (DP) of pulps treated with a cellulase free xylanase obviously would increase due to selective removal of low DP xylan. However, even low cellulase activities in the enzyme preparations results in decreased viscosities.

Moreover, cellulase side activity would hydrolyse cellulose microfibrills where ever exposed to it which is the typical exploitation of cellulase enzyme when added to detergents (63).

It has also been observed (64) that even at very high enzyme dosages with limiting treatment times in bleached hardwood sulfite pulps, the xylan content was decreased from 4 to only 3.5%.

However, very little work have been done in this direction and the information regarding dosage and effects, brought about by treatment are not available.

2B.3.1 Access of Enzymes to the Pulps

This has also been discussed in section (2A.6) when considering the efficiency of enzymatic treatments of pulps, the critical parameters should be the pore volume or pore size distribution which will determine the accessibility of enzymes to the fibers. It was also shown by Stone etal. (65) that the digestibility by cellulase of a series of celluloses of different degrees of swelling was directly proportional to the accessibility to a enzyme molecule diameter of 3-4 nm. However, for a fully bleached pulp, on an average pore size distribution should be higher as compared to unbleached pulps. Moreover nature of raw material would also decide average pore size distribution depending on their morphological and anatomical features.

According to the results of molecular modeling of the core protein of cellulase CBHII from T. reesei, the approximate diameter of enzyme (MW 56 kD) is 5 nm (39). The molecular weight of Pulpzyme - HB, the enzyme used here is 26 kD and its size should be smaller accordingly. However, the size of enzyme molecule will vary with varying strains. In this context, however, leaving aside other aspects, a strain producing lower molecular enzyme would be considered better, as it should have higher efficiency. Efforts should be to develop such strains.

2B.3.2 Amount of Enzyme

Considering the importance of xylan in swelling behavior of fiber, it was decided to get desired results with least possible impact on its content while deciding on the doses of enzyme during experimental runs, almost nothing happens when the reducing power equivalent to bleaching within same time span was applied. After a through study, it was decided to apply 5400 μ mols/(gm OD pulp), of minimum reducing power to get the desired results.

Chapter - 3

OBJECTIVES AND THEIR EXPERIMENTAL EXECUTION

3A. Applicability of Xylanase for Bleach Boosting

The aim of this study was to carry out the bleach boosting by xylanase particularly when the pulps are cooked by modified methods ie. when no redeposition or adsorption of xylan takes place. This was done by using bagasse pulps as bagasse is a potential future raw material for Indian Pulp and Paper Industry. No such observation on bagasse pulp has so far been reported in the literature. However, eucalyptus pulp was also included in the study.

3A.1 Raw Materials

Sugar cane rind covers which can be considered as 100% depithed bagasse and eucalyptus chips were used as raw materials for pulping.

3A.2 Cooking Conditions

The cooking conditions are taken from another study. For this purpose, two cooks were performed for each pulp under identical conditions obtaining similar results. The cooking time for eucalyptus pulp was higher to have it at lower kappa number. The pulps were produced in the standard laboratory batch digester. As it is well known that during the pulping of

eucalyptus in its delignification three phases exists namely initial, bulk and final while only two phases during bagasse pulping. In order to simulate the effects of extended delignification the spent liquor from bulk phase and final phase was used in the initial phase with sodium sulfide fortification with an aim to set in sulpher inside the chips during initial phase which affects the delignification in bulk phase (66), while fresh liquor was used in other phase or phases. Thus spent liquor from initial phase which has been used twice may be sent to recovery section depending on mill practice.

3A.2.1 Bagasse Pulping

Table 3.1 Cooking Conditions for Bagasse Pulp

Phase	Effective alkali %, as Na_2O	Sulfidity %	Addetive % AQ.	Bath ratio	Time to Temperature °C / min.	Time at Temperature °C, in min.	Remarks
I^{st}	6	24	--	4:1	150 / 60	30	--
II^{nd}	11	24	0.1	3.5:1	160 / 30	30	stop

3A.2.2 Eucalyptus Pulping

Table 3.2 Cooking Conditions for Eucalyptus Pulp

Phase	Effective alkali %, as Na_2O	Sulfidity %	Additive %AQ.	Bath ratio	Time to Temperature °C / min.	Time at Temperature °C, in min.	Rem.
I^{st}	6.5	25	--	4:1	125 / 60	30	--
II^{nd}	13	25	--	3.5:1	155 / 60	30	--
III^{rd}	13	25	0.15	3.5:1	165 / 60	30	stop

3A.3 Pulp Properties

Table 3.3 Properties of Bagasse and Eucalyptus Pulps

Pulp	Kappa Number (T-236 cm 85)	Yield %	intrinsic viscosity (Scan C-15:62) (dm^3/Kg)	Brightness % ISO	aCl demand (Scan 29:72) Kg/(Ton of Od Pulp)
Bagasse	4.2	47	1225	51.5	10.93
Eucalyptus	9.8	38	897	43.1	21.2

A total of 100 g of each pulp was prepared and each pulp was divided into 50-50 g for bleach boosting analysis and for the fiber properties modification. The pulps were stored in a refrigerator under wet conditions.

3A.4 Enzyme Used

A pure xylanase preparation (Pulpzyme-HB) was received from Navo Nordisk-A/S (Denmark), as a gift. It is derived from bacterial origin containing endo-1,4-beta-D-xylanase activity (EC 3.2.1.8), and is free of cellulase side activity.

Its activity is 30,000 IU/g (at pH 7.5, 45°C)

Where, 1 IU = 1(μmol/min.) of reducing power.

3A.4.1 Scheme

Each Bagasse (B) and Eucalyptus (E) pulps was divided into two parts of 25 g each. One sample of 25 g of each pulp was subjected to enzyme treatment [B(X), E(X))] and other sample of 25 g of each pulp was subjected as control [B(C),E(C)]. The treatment was given on a shaker built in temperature bath and during treatment, pulps were contained in plastic containers. After prescribed (section 3A.4.2) treatment, the pulps were tested for their properties such as kappa number, brightness,

intrinsic viscosity and °SR values. However the control samples were also subjected to same treatment except addition of enzyme.

3A.4.2 Treatment Conditions

It has been reported that a total treatment of 900 μmols of enzyme reducing power per g of OD pulp in minimum 30 minutes time is sufficient for desired results (27,28,31,33,35). Moreover the amount of enzyme and treatment times are inter changeable so we can reduce or increase the enzyme doses depending on treatment time or vice versa.

(contd...on P.23)

Enzyme dose	:	15 $\frac{\text{IU}}{\text{g of OD pulp}}$ ($\approx$ 0.5 $\frac{\text{Kg}}{\text{Ton OD pulps}}$)
Time	:	60 minutes
Temperature	:	45 °C
pH	:	7.7 (Britton - Robinson Buffer)
Consistency	:	2.5%

Just after 60 minutes, the pulps were washed with cold distilled water on a 200 mesh screen. The pulp properties following this treatment are given under results in Table 4.1.

3A.5 Bleaching

Just after enzyme treatment, all pulp samples [B(X),B(C),E(X) and E(C)] were subjected to DED bleaching sequence under same conditions with exactly same amount of dosage. However, sodium chlorite was used as an alternative to chlorine dioxide (67). After each stage pulps were washed on a 200 mesh screen.

Table 3.4 Bleaching Conditions of DED Sequence

Bleaching Stage	Pulp/ Parameters	B(C)	B(X)	E(C)	E(X)
Chlorine Dioxide Delignifict-ion Stage (D)	$\equiv ClO_2$ Kg / Ton OD Pulp	4.5	4.5	8	8
	Temperature °C	70	70	70	70
	pH*	2.8	2.8	2.8	2.8
	Consistency %	2.5	2.5	2.5	2.5
	Time, minutes	60	60	60	60
Extraction Stage (E)	NaOH** Kg / Ton OD Pulp	5.25	5.25	7	7
	Temperature °C	60	60	60	60
	Consistency %	2.5	2.5	2.5	2.5
	Time, minutes	60	60	60	60
Chlorine Dioxide Brightning Stage (D)	$\equiv ClO_2$ Kg / Ton OD Pulp	4.5	4.5	8	8
	Temperature °C	70	70	70	70
	pH*	2.8	2.8	2.8	2.8
	Consistency %	2.5	2.5	2.5	2.5
	Time, minutes	180	180	180	180

* Citrate Buffer was used.

** Amount of NaOH = $\left[\frac{1}{2} \cdot \frac{KgClO_2}{\text{Ton OD Pulp}} + 3\right], \frac{Kg}{\text{Ton OD Pulp}}$

The bleached pulp samples were tested for their properties, the results are given in Table 4.2.

3A.6 Preparation of Hand Sheets

The bleached pulp samples were beaten to 45°SR in a PFI - mill. The hand sheets were prepared and tested as per standards. The results are given in Table 4.3.

3B. Applicability of Xylanase for Modification of Fiber Properties

The aim of this work was to study the role of xylanase in the beating of pulps and to see the effects of this treatment on sheet properties.

3B.1 Raw Materials

It has already been discussed earlier that only fully bleached pulps are accessible to enzymes due to limitation of pore size distribution vis a vis enzyme molecule size. Fully bleached bagasse and eucalyptus pulps were employed for the purpose. The pulps were bleached following XDED sequence. The bleaching conditions and bleached pulp properties of bagasse [B(XDED)] and eucalyptus (E(XDED)] pulps are given in Tables 3.5 and 3.6. A total amount of 50 g OD of each pulp was bleached with a yield loss of 5.6% and 5.7% for bagasse and eucalyptus pulps respectively. The samples were divided into two parts, each containing equal amounts of pulp. One sample of each pulp was treated as control (C) while the other was subjected to enzyme treatment (X).

Table 3.5 Bleaching Conditions of XDED Sequence

Stage	Pulp/ Parameters	Bagasse	Eucalyptus
Xylanase Pretreatment Stage (X)	IU / g OD Pulp	15	15
	Temperature °C	45	45
	pH*	7.7	7.7
	Time, minute	60	60
Chlorine Dioxide Delignification Stage (D)	≡ ClO_2 Kg / Ton OD Pulp	4.5	8
	Temperature °C	70	70
	pH**	2.8	2.8
	Time, minutes	60	60
Extraction Stage (E)	NaOH Kg / Ton OD Pulp	5.25	7
	Temperature °C	60	60
	Time, minutes	60	60
Chlorine Dioxide Brightning Stage (D)	≡ ClO_2 Kg / Ton OD Pulp	10	16
	Temperature °C	70	70
	pH**	2.8	2.8
	Time, minutes	180	180

* During treatment B-R Buffer was used.
** During Treatment Citrate Buffer was used.

Table 3.6 Bleached Pulp Properties

Pulp/Properties	B(XDED)	E(XDED)
Viscosity, dm^3/kg	1212	847
Brightness, % ISO	90.2	90.8
°SR	13	14

3B.2 Enzyme Used

The enzyme preparation used for the purpose was Pulpzyme-HB containing endo-1,4-β-D-xylanase activity (EC 3.2.1.8). This was completely free of cellulase side activity. It is a product of Navo-Nordisks-A/S(Denmark), having activity of 30,000 IU/(g of Enz. Soln.), at pH 7.5, 45°C.

3B.3 Enzyme Treatment

The treatment conditions are given in Table 3.7. The treatment was given on a shaker built in temperature bath. The samples to be treated as control were also subjected to the same treatment conditions except the addition of enzyme.

Table 3.7 Enzyme Treatment Conditions

Pulp/ Treatment Conditions	B(XDED)	E(XDED)
Amount of Enzyme, IU/(g OD Pulp)	30	30
Time, minutes	180	180
Temperature, °C	45	45
pH*	7.7	7.7

* Britton- Robinson Buffer was used during the treatment.

Just after this treatment, the pulps were washed on a 200 mesh screen with cold distilled water. The properties of enzyme treated and control pulps are given under results in Table 4.4.

3B.4 Beatability of Pulps

The enzyme treated and control pulps were beaten in a PFI-mill under analytical beating conditions. First, the enzyme treated pulps were beaten to reach a °SR level of 45. Then the control pulps were beaten to same number of PFI revolutions. The control pulps reached the lower °SR levels. The control pulps were further beaten to reach the same °SR levels. The results obtained are given in Tables 4.4 and 4.5 for bagasse and eucalyptus pulps respectively. The reproducibility of these experimental results was found to be very good. The beatability results are given in Table 4.5 and 4.6.

3B.5 Preparation of Handsheets

After achieving the same °SR for enzyme treated as well as control pulps. The handsheets were prepared and tested. The hand sheet's properties are given in Table 4.7.

RESULTS AND DISCUSSION

4A. Bleach Boosting Following Xylanase Pre-treatment Stage

4A.1 Results

As described in section - 3A, the pulps were obtained following XDED sequence and CDED (where C, showing control). The results are shown in Tables 4.1 and 4.2. The properties of hand sheets prepared from these pulps (3A.6) are given in Table 4.3.

Table 4.1 Pulp Properties After Enzyme Pretreatment Stage

Pulp/	Bagasse (B)		Eucalypts (E)	
Properties	B(C)	B(X)	E(C)	E(X)
Yield Loss (%)	0	0.40	0	0.35
Kappa Number	4.2	4.2	9.8	9.8
Brightness (% ISO)	52	53.0	43.5	44.5
Intrinsic viscosisy (dm^3/Kg)	1225	1268	897	919
°SR	11	11	12	12

Table 4.2 Properties of Bleached Pulps

Pulp/ Parameter	B(CDED)	B(XDED)	E(CDED)	E (XDED)
Yield Loss (%)	4.8	5.2	5.0	5.4
Brightness (% ISO)	85.7	88.4	86.3	89.1
Intrinsic visicoisy (dm^3/Kg)	1220	1208	872	843

Table 4.3 Properties of Hand Sheet's

Pulps Sheet Properties	B(CDED)	B(XDED)	E(CDED)	E(XDED)
Burst Index ($\frac{kPa\ m^2}{g}$)	4.08	3.56	2.76	2.38
Tear Index ($\frac{mN\ m^2}{g}$)	11.6	12.8	8.28	8.97
Breaking Length (m)	5232	5100	3708	3588
Folding Endurance, Log_{10}(No. of double fold)	2.61	2.68	1.85	1.89
Brightness (% ISO)	85.7	88.4	86.3	89.1

4A.2 Discussion

As is evident from Table 4.1 the results obtained from enzyme pre-teeatment show a minor loss in the yield but the viscosity increases. It seems that there is selective removal of low-DP xylan following the enzyme treatment. Further more, viscosity rise indicates that the enzyme preparation (Pulpzyme-HB) is free from cellulase side activity. Following this enzyme treatment, kappa number of pulps remains unchanged. However a marginal increase of 0.5 and 0.4 point brightness of bagasse and eucalyptus control pulps may be due to the buffer action but additional 1-point brightness rise of enzyme treated pulps is difficult to explain. There is no change in °SR level which probably indicates that the enzyme treatment is limited to outer surface of the fiber and in case some interfiber xylan is acessible to xylanase, no fibrillation occurs. When the xylanase treatment is given to fully bleached pulps (during fiber properties modification studies) an appreciable rise in °SR was obtained (Table 4.4). This may be due to the fact that xylan hydrolysis gives same fibrillaltion only after complete delignification.

When enzyme pretreated and control pulps were bleached (Table 4.2), there was a higher yield loss and drop in the viscosity for enzyme pretreated pulps. This probably indicates that enzyme pretreatment makes fiber more susceptible to bleach chemicals. this is also evident from the fact that 2.7 and 2.8 points brightness rise is also observed for enzyme pretreated bagasse and eucalyptus pulps. It is worth mentioning that for this much increase in brightness, we in general, have to apply more than double the amount of chlorine dioxide in the brightening stage with subsequent higher amounts of chlorine dioxide in delignification stage.

The properties of hand sheets made from these pulps showed lower strength properties (burst and breaking length) but a higher tear. This may be due to the hydrolysis of xylans decreasing the bonding ability of fibers. But when this bonding

loss is compared to the brightness rise, it is not significant. The higher folding endurance of enzyme pretreated pulps probably indicates that the fibers have become more flexible.

As a result of 0.05% Enzyme treatment for 60 minute, the 88.4% ISO and 89.1% ISO brightness levels are obtained with only 9 Kg/Ton and 16 Kg/Ton of chlorine dioxide for bagasse and eucalyptus. This resulted in a 2.7 and 2.8 brightness rise compared to control pulps. Moreover, the repeatability of results was found to be excellent as fully bleached pulps following XDED sequence were obtained later for the studies on fiber properties modification giving almost identical properties.

4B. Effect of Xylanase Treatment on Fiber Properties

4B.1. Results

As described earlier in section -3B, the bleached pulps were treated with xylanase (3B.3). The results are given in Table 4.4.. An increased beatability for enzyme treated pulps was observed for which the results are given in Tables 4.5 and 4.6. The hand sheet's properties are given in Table 4.7.

Table 4.4 Pulp Properties Following Treatment 3B.3.

Pulp/ Properties	B(XDEDEC)	B(XDEDX)	E(XDEDC)	E(XDEDX)
Yield Loss; (%)	0	0.42	0	0.45
Visicosity; (dm^3/Kg)	1212	1248	846	862
Brightness;				
(% ISO)	90.2	90.2	90.8	90.8
; °SR	13	18	14	21

Table 4.5 Beatability of Bagasse Pulps

Pulp/ Parameters	B(XDEDC)	B(XDEDX)	Remarks
initial °SR	13	18	
°SR at PFI revolution of 3950	33	45	20 pt °SR rise in control pulp 27 pt °SR rise in enzyme treated pulp
No. of extra RPM needed to reach 45°SR for control pulp	2450	--	
Total RPM needed to reach 45°SR	6400	3950	Total energy thus saved = 38.3%
Actual energy saved as a result of increased beatability, %	--	26.9	

Table 4.6 Beatability of Eucalyptus pulps

Pulp/ Parameters	E(XDEDC)	E(XDEDX)	Remarks
initial °SR	14	21	
°SR at PFI RPM of 3250	31	45	17 pt °SR rise in control pulp 24 pt °SR rise in enzyme treated pulp
No. of extra RPM needed to reach 45°SR for control pulp	2750	--	
Total RPM needed to reach 45°SR	6000	3250	Total energy thus saved = 45.8%
Actual energy saved as a result of increased beatability, %	--	30.0	

Tablle 4.7 Properties of Handsheets

Pulp/ Sheet Properties	B(XDEDC)	B(XDEDX)	E(XDEDC)	E(XDEDX)
Burst Index ($\frac{kPa\ m^2}{g}$)	3.62	4.15	2.48	2.86
Tear Index ($\frac{mN\ m^2}{g}$)	12.1	11.5	8.7	7.9
Breaking Length (m)	5139	5247	3603	3728
Folding Endurance Log_{10} (No. of double fold)	2.69	2.76	1.89	1.97

4B.2 Discussion

When fully bleached bagasse [B(XDED)] and eucalyptus [E(XDED)] pulps were treated with pure xylanase preparation (Pulpzyme-HB) a minor yield loss was observed with somewhat increased viscosities. The yield loss is due to removal (hydrolysis) of xylans while the rise in viscosity should be due to the selective removal of lowDP-xylans. However a lesser accessibility of xylanase is noticed to bagasse pulps when a lower °SR rise [5°SR rise for bagasse {B(XDEDX)} and 7°SR rise for eucalyptus {E(XDED)} pulp] is obtained of bagasse pulp. While the rise in °SR levels for both pulps following enzyme treatment may be due to external fibrillation, some internal fibrillation may also be there thus higher water retention ability. However, a lesser accessibility of bagasse pulp to enzyme may be due to lower average pore size distribution in bagasse pulp which could not be confirmed due to non availability of such information. There is no effect on brightness of pulps. But when unbleached pulps were given such treatment (for bleach boosting), the brightness was affected with both enzyme and buffer.

When enzyme treated pulps were beaten in PFI - mill (Table 4.5 and 4.6) to reach 45°SR levels. The PFI revolutions needed were 3950 and 3250 giving °SR rise of 27 and 24 points for bagasse and eucalyptus pulps respectively. When control pulps were beaten to same revolutions. The increase in °SR was 20 and 17 points for bagasse and eucalyptus pulps respectively. The increased beatability of enzyme treated pulps indicates that some internal modification may be occruing at fibrillar or lamellar level. However, when control pulps were further beaten to reach same °SR levels the PFI revolutions require were 2450 and 2750 for bagasse [B(XDEDC)] and eucalyptus [E(XDEDC)] pulps respectively. This results in overall energy saving of 38.3% and 45.8%. While considering higher initial °SR levels of enzyme treated pulps; as a result of increased beatability, a 26.9% and 30.0% of net energy saving are obtained for bagasse [B(XDEDX)] and eucalyptus [E(XDEDX)] pulps.

When handsheets made from these pulps were tested for their properties (Table 4.7), a little higher level of burst, and breaking length were found for enzyme treated pulps with reduced tear. This increase in strength properties was not expected because of lower xylan contents in enzyme treated pulps which should have decreased the bonding ability of fibers. It has already been observed that bonding ability of fibers reduces following xylanase treatment due to xylan removal (4A.2). But here increased bonding even after the removal of xylans may be an indication of some internal modification at fibrillar or lamellar level (internal fibrillation). It was earlier discussed in section 2B.2 on the issue of the importance of internal fibrillation vis a vis external fibrillation for strength properties. The control pulps are expected to have higher internal fibrillation due to higher beating times and higher xylan contents which should have imparted higher swelling. In the present case it may be possible that as a result of xylanase treatment, we may be getting such internal modifications which are over shadowing xylan losses at such a level of enzyme action.

Chapter -5

SUMMARY AND CONCLUSIONS

5.1 Summary

The experimental studies of the application of xylanase for bleach boosting indicate that a 0.05% enzyme pretreatment for 60 minutes to unbleached bagasse and eucalyptus pulps cooked by modified techniques resulted in an increase of 2.7 and 2.8 points brightness for bagasse and eucalyptus pulps respectively, with minor yield loss when control pulps are bleached with same amount of ClO_2. The bagasse pulp could be bleached to an ISO brightness of 88.4 with 0.9% of total ClO_2 applied and to ISO brightness of 90.2 with 1.45% of total ClO_2 applied. The eucalyptus pulp could be bleached to 89.1% ISO and 90.8% ISO brightness with total ClO_2 application of 1.6% and 2.4% respectively. The bonding ability of pulps is only slightly affected due to the removal of xylan. Thus it may therefore be concluded that enzymes are also pertinent with the pulps cooked by modified techniques giving either a higher brightness or the saving in the amount of bleach chemicals required to achieve the same brightness.

The experiments were also carried out to assess the use of xylanase for the modification of fiber properties. When fully bleached bagasse and eucalyptus pulps were treated with 0.1% of enzyme for 180 minutes, a 5 point rise in °SR for bagasse pulp and a 7 point rise in °SR for eucalyptus pulp were obtained with minor loss in the yield. The beatability of these pulps is

further improved as some external as well as internal fibrillation (modification) takes place due to xylan hydrolysis, giving a total energy saving of 38% and 46% for bagasse and eucalyptus pulps compared to control pulps. About 27% and 30% of net energy saving was obtained as a result of improved beatability. Furthermore, there was an improvement in the strength properties of these pulps even after the removal of xylans. This may be due to the fact that some internal modification is occurring in the fiber wall which more than compensates the effect of the removal of xylans.

5.2 Conclusions

From the present study the efficacy of hemicellulolytic enzyme for pulps cooked by modified techniques becomes quite evident. Based on this limited study, it can be said that enzyme pretreatment is also pertinent with such pulps. However, enzyme preparations should be free from any cellulase side activity and the treatment should be mild in order to minimize yield losses.

The need of such modifications has been emphasized in the literature with increasing awareness towards cleaner environment, the modified cooking processes are likely to become a usual practice and bleach chemical demands can also be reduced further by such pretreatments. In countries like India where bleach chemicals such as ozone will take time before they are utilized due to their higher installation costs, this type of technology may be exploited as an alternative where brown stock storage tank can be a suitable reactor. This will give pulps with brightness levels of 88% or more for pulps such as bagasse with only 9 Kg/Ton of chlorine dioxide consumed on O.D. pulp. This is a lesser detrimental chemical whose effluents are biotreatable (68).

Bagasse, an annually renewable potential raw material for countries like India should be successfully used with the least impact on the environment. With Chlorine dioxide, consumption of 14.5 Kg per tonne of pulp a brightness value of 90 + may be achieved as has been obtained for bagasse pulp during the studies of enzymatic fiber properties modification.

The cost of enzymes which at present is about Rs.350/- per Kg. may be reduced further if its industrial application increases thus the production and new competitors.

The analytical results obtained during the studies of enzymatic fiber properties modification are encouraging. A minimum xylanase dose of 0.1% and minimum treatment times of 180 minutes are required to obtain the desired results. A higher reducing power of enzyme action is required to get the desired results as compared to that in bleach boosting. This may be due to location of xylans. With tight energy situation, such technology may become important. The possibility already exists to further reduce the enzyme doses and treatment times by increasing their (enzymes) efficacy with proper selection of strains or developing such strains which produce lesser molecular size enzymes.

An overall cost saving may be obtained in case of papers such as tracing and condenser tissues by reducing the energy costs involved in the beating processes. There are obvious advantages with paper tissues and other absorbent papers if the degree of water retention can be increased by treatment of the fiber alone which here is implied by internal modifications otherwise this has also been observed elsewhere (60,71) as a result of xylanase treatment. Till date, there is very little work being done in this direction and the details are not available. The further elaborate study is needed to determine the changes brought about by xylanases in the internal structure of fiber, which obviously will require advanced facilities and resources. It is also required to critically evaluate the importance of the changes brought about in the internal structure of fiber by such treatments vis a vis hemicellulose contents for their bonding ability.

In order to make these processes economically competitive, the possibilities of enzyme reutilisation, should be investigated. That is to see whether enzymes are released into solution automatically once the substrate has been hydrolyzed to a desired extent or can they be desorbed cheaply and efficiently?

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