Development of Xylanase Based Pulp Bleaching Process

A thesis submitted in fulfilment of the requirement for the award of the degree of

DOCTOR OF PHILOSOPHY

by

Avdhesh Kumar Gangwar

(Reg. No. 951000002)

Department of Biotechnology
Thapar University
Patiala-147004
India

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CERTIFICATE

Certified that the thesis "Development of Xylanase Based Pulp Bleaching Process" which is submitted by Mr. Avdhesh Kumar Gangwar, in fulfillment of the requirement for the award of the degree of Doctor of Philosophy in the Department of Biotechnology, Thapar University, Patiala, is a record of candidate's own independent and original research work carried out by himself under my supervision and guidance. The material embodied in the thesis has not been submitted in part or full to any other university or institute for the award of the any degree in India or Abroad.

(Supervisor)

Dr. Ranjana Prakash
Associate Professor
School of Chemistry and Biochemistry
Thapar University
Patiala- 147004
Punjab, India

(Supervisor)

Dr. N. Tejo Prakash
Professor& Head
School of Energy and Environment
Thapar University
Patiala- 147004
Punjab, India

CANDIDATE'S DECLARATION

I, hereby declare that the work presented in the thesis entitled "Development of Xylanase Based Pulp Bleaching Process" in fulfillment of the requirement for the award of the degree of Doctor of Philosophy in the Department of Biotechnology, Thapar University, Patiala, is an authentic record of my own work carried out under the supervision of Dr. Ranjana Prakash, Associate Professor, School of Chemistry and Biochemistry and Dr. N. Tejo Prakash, Professor & Head, School of Energy and Environment, Thapar University, Patiala, Punjab, India. The matter embodied in the thesis has not been submitted in part or full to any other university or institute for the award of the any degree in India or Abroad.

Place: Thapar University, Patiala
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Avdhesh Kumar Gangwar

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…….....…."live in present and accept the belongings at its own way"

Avdhesh Kumar Gangwar
June, 2016, TU, Patiala, India

Dedicated to my

Beloved parents, wife

&

Lovable Son

CONTENTS

LIST OF SYMBOLS/ABBREVIATIONS

g	Gram
kg	Kilogram
B	Beta
µ	Micron
%	Percentage
°C	Degree centigrade
kgt^{-1}	Kilogram per ton
M	Molarity
N	Normality
µmole	Micromoles
mL	Milliliter
L	Liter
µl	Microliter
nm	Nanometer
µmol/ml	Micromole per milliliter
min.	Minutes
hr.	Hour
g/l	Gram per liter
cp	Centipoise
psi	Pounds per square inch
$mN.m^2/g$	Milli newton meter square per gram
mN	Milli newton
g/m^2 / *GSM*	Gram per meter square
kN/g	Kilo-newton per gram
kPa	Kilo Pascal
mmol/kg	Millimole per kilogram
mg/l	Milligram per liter
g/cc	Gram per cubic centimeter
IU/ml	International units per milliliter
viz;	Namely
e.g.	For example

i.e.	That is
Mtpa	Metric Tonnes Per Annum
O / ODL	Oxygen delignification
X	Enzyme stage
C / C_D	Chlorine / Chlorine with chlorine dioxide stage
D / D_0 / D_1 / D_2	Chlorine dioxide stage
E / E_P	Extraction / Extraction with peroxide stage
P	Peroxide stage
H	Hypochlorite stage
Q	Pulp chelating stage
°SR	Schopper Riegler freeness
AOX	Adsorbable Organic Halide
BCTMP	Bleached Chemi-Thermo-Mechanical Pulping
BOD	Biochemical / Biological Oxygen Demand
CAGR	Compounded Annual Growth Rate
CED	Cupriethylenediamine
CIE	International Commission on Illumination
CMC	Carboxymethyl Cellulose
COD	Chemical Oxygen Demand
CSF	Canadian Standard Freeness
DNS	Di-nitrosalicylic acid
DP	Degree of polymerization
ECF	Elemental Chlorine-free
Hex-A	Hexenuronic acid
ISO	Indian Organization of Standardization
KF	Kappa factor
LCCs	Lignin Carbohydrate Complexes
MeGlcA	Methyl-glucuronic acid
MOE	Modulus of elasticity
NOx	Nitrogen Oxide
PC No.	Post color number
SD	Standard deviation
SEM	Scanning Electron Microscopy

SOx	Sulpher Oxide
TAPPI	Technical Association of the Pulp and Paper Industry
TCF	Total Chlorine-free
TEA	Tensile Energy Absorption
TOC	Total Organic Carbon
USD	United States Dollars
VOC	Volatile Organic Compounds

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ABSTRACT

As per the industries norms in regulating and maintaining the environmental conditions, current interest in the implementing of biological treatment in pulp bleaching processes has arisen very fast in the last few years globally. Due to use of huge quantities of hazardous chemicals in bleaching process, generation of pollutants is rapidly increased in environment. To overcome this problem researchers and industrialist are working continuously and finding alternative solutions of these hazardous chemicals. Use of biotechnology in pulp bleaching process shows its high impact towards green technology. Enzymes manufactured from microbial sources are very helpful and useful for reducing the use of chemicals; resulting in reducing huge percentage of pollutants generation to save our environment. There are many commercial enzymes are easily available in the market now, which is highly alkalistable and thermostable in nature which are the best desired conditions for enzymatic bleaching. Laccases and xylanases suits and helps a lot in enzymatic bleaching for achieving higher pulp qualities with cost effective process as compared to conventional process. Xylanase bleaching is very much popular rather than laccase and delivers high quality pulp at very low cost. Due to a mediator needed for the use of laccase in the process; it leads to increase production cost as compared to xylanase bleaching process.

In this study, we used four different commercial enzymes and studied their effect on bleaching of two different hardwood eucalyptus (*E. globulus*) and acacia (*A. mangium*) kraft pulps using different bleaching sequences. Out of these four enzymes; two enzymes were manufactured and gifted by Novozyme, India, one from DuPont Genencor Sciences, USA, and another from Dyadic International, USA and coded as EnzyA, EnzyB, EnzyC and EnzyD respectively for the study.

Screening of these four enzymes was done on the basis of hexenuronic acid (Hex-A) removal on eucalyptus pulps using elemental chlorine-free (ECF) bleaching sequence ($XD_0E_PD_1D_2$). Enzyme (X) stage resulted in removal of hexenuronic acid up to 20.0% with EnzyC in addition to improved brightness by 3.4 units and 19.7% reduction in kappa number as compared to control. Hexenuronic acid content was reduced by 21.3% in the final bleached pulp with 1.5 units of brightness gain over control. Due to reduced Hex-A components in bleached pulps, post color number (brightness reversion) was also reduced up to 45.0% with EnzyC. In comparison to control, EnzyC resulted in maximum

pollution load reduction of chemical oxygen demand (COD) and biological oxygen demand (BOD) up to 27.1 and 23.8% respectively in the bleach effluent. Out of these four enzymes; EnzyC was also helpful in maximum saving 22.0 and 31.8% of chlorine dioxide (ClO_2) and sodium hydroxide (NaOH) respectively with improvement in optical properties and reduction in pollution load. The observations, thus, demonstrated Hex-A reduction profile, brightness improvement and reduction in pollution during the enzymatic bleaching with xylanases.

Further, a comparative study was carried out with EnzyC in enzymatic stage followed by ECF bleaching sequence ($D_0E_PD_1D_2$) on two different hardwood kraft pulps (eucalyptus and acacia) for improvement in optical and physical strength properties. Reduction in bleach chemicals consumption was observed after the use of xylanase in enzymatic treatment. At equivalent charge of bleaching chemicals, a brightness improvement of 1.7 and 2.1 units and whiteness gain of 2.3 and 2.7 units were obtained with enzyme treatment in eucalyptus and acacia pulps, respectively. A final brightness of 89%ISO and 90%ISO was achieved easily for eucalyptus and acacia pulps respectively by following ECF bleaching sequence. During this study, brightness reversion was also studied and 45% reduction in post color number was observed for both hardwood pulps as compared to control. After xylanase pre-bleaching, a 17% and 23% reduction in chlorine dioxide (ClO_2) and caustic (NaOH) were observed for both hardwood pulps; indicating an environmentally friendly approach to the process. Out of these two hardwood pulps, acacia was observed highly beneficial for the effect of bleachability over the use of eucalyptus.

The effect of xylanase stage incorporation at different places of bleaching sequences was also studied to examine the outcome on the various parameters. For this study, nine different bleaching sequences were chosen and compared using eucalyptus hardwood kraft pulps and divided in three categories of bleaching sequences (pre-treatment, intermediate, and post-treatment). Efficacy of xylanase stage on final pulp brightness, whiteness, brightness reversion and bleach effluent characteristics were studied with EnzyC. Pre-treatment bleaching sequences resulted in increased final pulp brightness by 1.6 units with 32% reduction in adsorbable organic halogens (AOX) at 0.5 kgt^{-1} doses of xylanase, in addition to improved ratio of biological oxygen demand (BOD) to chemical oxygen demand (COD), which shows better bio-degradability of discharge effluents in a secondary treatment stage. Post-treatment bleaching sequences were found helpful in boosting of final pulp whiteness by 3.4 units and to improve

brightness stability by 48% at the same dose of xylanase as in pre-treatment bleaching. Post-treatment bleaching sequences also showed a significant improvement in BOD-to-COD ratio at 0.5 kgt^{-1} xylanase doses. Intermediate incorporations of enzyme stage have not showed any significant benefits over pre and post-treatment bleaching sequences.

This study, thus, presents findings regarding the application of xylanases in the bleaching of pulp, with emphasis on the mechanism and effects of xylanase treatment on pulp and paper and the factors affecting the bleaching process and its efficiency.

CHAPTER 1.0 INTRODUCTION

India is becoming one of the world's fastest growing economics, catalyzing a dynamic role in industrial growth including pulp and paper sector. As per the report on opportunities for green chemistry initiatives: Pulp and paper industry, the average consumption of paper in India in 2012, was 9.3 kg/capita vis-à-vis global average of 58 kg/capita. In India, the estimated production of paper is 10.9 Mtpa, placing it at 20th position among the top producers of paper globally. Indian pulp and paper mills produce about 2.6% of paper (~390 Mtpa) of the total global production.

In addition to the requirement of high quality and affordability in bleached kraft pulp, the market is also now demanding and expecting bleached pulp that could be produced by a process that is more eco-friendly and within the regulatory guidelines. Lignin in wood fibres is removed during the pulp bleaching process by diverse variety of chemical approaches. Lignin is chemically removed from the fibers during kraft cooking process. After the pulping process, rest of the lignin is removed in bleaching process. In conventional bleaching method like total chlorine-free (TCF) and elemental chlorine-free (ECF) bleaching process, use of chlorine and its components is highly common and popular. But the discovery of toxic chlorinated organic components in bleached effluents in kraft paper mills increased public attention and concerns of regulatory agencies towards its drawbacks for the environment. The paper industries have been focusing on finding the alternative bleaching agents that can replace or minimize the use of chlorine in the bleaching sequence. Both TCF and ECF bleaching sequences are being analyzed and presently being following by most of the paper mills. Paper industries need advancement and development in their process to produce pulp effectively and therefore need to evaluate advanced methods and develop the most appropriate bleaching sequences by using easily available bleach chemicals (Nelson 1992; Johnson 1994; Lachenal and Nguyen-Thi 1994; Gangwar *et al*. 2014; 2015 & 2016b).

Seerampur paper mill was listed as the first known paper mill in India which was established in the year 1812 and situated in West Bengal. In India, at the initial stage paper production was started at very small scale, while in early 1905, mechanized technology of papermaking was introduced at large scale across the sector. As per the requirement of industry and quality improvement, raw material was also modified and has gone through a number of changes in last few years. Earlier wood and bamboo were the main raw material sources for the papermaking, but other non-conventional raw

furnishes have also been identified and industrialized for their use in paper production. Pulp and paper industries are now being categorized based on their raw material sources *viz*; waste paper and secondary fibres, market pulp, bast fibres, forest and agro-residues. In India, the pulp and paper industries have also been categorized into small and large scale. Pulp and paper industries with more than 24000 tons per annum production capacity are categorized as large scale paper mills (Verma 2012).

At present, approximate 40% of total production of paper is based on wood and rest is on non-wood raw materials sources. In paper consumption ratio per annum, India ranks amongst the top 15 globally amounting more than 6 Mtpa. Growth in per capita paper consumption is recorded 10 per cent each year. In 2007-2008, India has become as the fastest growing paper market in the world at the rate of 7.5 kg per capita consumption; the consumption has now gone up to 8.3 kg. Pulp and paper industries are growing rapidly with an estimated compounded annual growth rate (CAGR) of 7-8% projected over the next decade. The production of paper and paperboard in India accounts to about 1.6% of the world's production. The estimated turnover of the pulp and paper industries is about USD 5.95 billion approximately. India has more than 660 paper mills across the country, out of which only 38 mills are responsible for 60% of total production. This clearly indicates the demand of investment and technology up-gradation of existing pulp and paper mills.

For paper industries in India, apart from capacity augmentation, energy efficiency of the individual units is the main concern to be improved in the present scenario. Keeping consideration of our environment and to follow the statutory norms, a number of Indian paper mills have become active towards better and cleaner production of paper. In future prospects, the Indian pulp and paper industries have to become globally competitive with energy efficient and environmentally green in operations for production of high quality pulp and paper.

First step in papermaking process is the selection of appropriate raw materials. Paper production is a multistep process where forest and/or agro based raw materials (wood, bamboo, waste paper, recycled secondary fibres and market pulps) is used. The second step involves chemical and mechanical processing of the fibres to convert it into the pulps by removing lignin from the wood though cooking and bleaching process followed by rolling of these pulps into large rolls of paper by passing through the paper machine. The final step in paper industries is the conversion process, where paper are

being used and converted into end products *viz*; corrugated boxes, office paper or paper towels etc.

Among all the industrial processes, papermaking is supposed to be a major source of hazardous pollutants worldwide. During pulping and bleaching many hazardous chemicals are being used in significant quantities. During papermaking, pulping and bleaching process generates large volumes of solid, liquid, and gaseous wastes. In cooking process, mechanical or chemical treatment is given to the wood log/chips for lignin removal in order to facilitate release of hemicellulose and celluloses. For the removing of residual lignin components in fibres, pulp is treated with bleach chemicals to whiten and brighten the pulp. For this, multi-stage bleaching process is followed, typically consuming ample amount of water and chemicals *viz*; elemental chlorine or chlorine dioxide, sodium carbonate, sodium hydroxide, bisulfites, calcium oxide, hydrochloric acid, sodium sulfide, and hydrogen peroxide etc. These chemicals and their byproducts results in generation of many hazardous pollutants in the bleach effluents. The bleach effluents generated by the pulp and paper industries; resulted in the following listed major environmental concerns (Verma 2012; Gangwar *et al*. 2014);

- Depletion in dissolved oxygen and increase in biochemical oxygen demand due to huge amount of organic matter in bleach mill effluents;
- High chemical oxygen demand (COD) in the water due to use of huge amount of elemental chorine and chlorine dioxide;
- Increased adsorbable organic halide (AOX) loads; and
- Presence of toxic pollutants those are bio-accumulative and persistent in nature.

In addition to bleach effluent, other waste forms like solid wastes are also generated in large quantities from paper mills. These solid wastes include sludge that is generated from wastewater treatment plant in addition to reject fibers, green liquor dregs, lime mud, barks, scrubber sludge, boiler and furnace ash. In paper industries, the major cause of odour issues originate from kraft cooking and chemical recovery process. During digestion of wood chips, and during evaporation of spent liquor and bleaching process, different organics compounds are generated as volatile organic compounds (VOC) that are very hazardous for the environment. Combustion processes are also lead to generation of gaseous wastes *viz*; nitrogen oxides (NOx) and sulphur oxide (SOx) etc.

The degree of toxicity and pollution totally depends upon the selection of raw materials, type of cooking methods and bleaching sequences adopted by the paper

industries. In general, most of the pollutants are generated when softwood is used as raw materials as compared to hardwood. On the other hand, with the use of non-wood fibres during pulping process, spent liquor generates has high load of silica content. Thus, the requirement of wastewater treatment technologies and that of pollution prevention differs with each mill due to variability of effluent characteristics and volume in each of the pulp and paper mills. Due to environmental concern and regulatory requirements for industrial discharge, many of the mills are seeking and adopting the alternative methods to reduce or prevent the generation of the hazardous pollutants in environment.

One of the best alternatives envisaged has been the incorporation of enzymatic process for papermaking. This approach is being attempted at many papermaking units for replacing the chemicals from conventional method to reduce the chemicals consumption (Nelson 1992).

Many research groups are working continuously and developing enzymes for use in many areas such as bio-pulping, bio-bleaching, bio-deinking, bio-refining, for stickies and pitch control and starch modification etc. Enzymes are also being used on routine basis for the improvement of pulp drainage at plant scale. Enzymatic deinking of recycled fibres is also well established at mill scale and begin used in many paper mills globally. However, other applications of enzyme in pulp and paper industries are still in experimental stage in phases such as enzymatic debarking, removal of shives and slime, retting of flax fibers and reduction of vessel picking, in addition to reduction in refining energy by modifying the fibres before the refining process in paper mills (Bajpai 1999).

Currently, the most important application of enzymes is in the bleaching of pulps. Pulp bleaching process generates large quantities of pollutants due to use of lots of chemicals loading in this particular step. Therefore, pulp and paper industries are always looking for bleach boosting agents or enhancers to improve the final pulp qualities without increasing the production cost when implemented in the bleaching sequence. The use of the enzymes is also becoming an alternative source for degrading or modifying the lignin and its removal to improve the bleachability of pulps. Among various enzyme tested, the effectiveness of xylanase as a pretreatment on softwood and hardwood kraft pulps in a ECF and TCF bleaching sequences has been addressed in both laboratory and mill scale. Xylanase pretreatment greatly enhances the bleaching capability of active chlorine and chlorine dioxide. Many alternative techniques of using enzyme in the paper manufacturing process are now followed by many paper mills.

However, there are many aspects for using of enzyme and its benefits in paper mills to be identified and understood at level of application.

Keeping in view the above aspects, we used four different commercial xylanases and studied them to elaborate their applications in hardwood kraft pulp bleaching. First we screened enzymes on the basis of their efficiency of hexenuronic acid removal from the hardwood kraft pulps in bleaching. Further, enzyme was used for its application in enzymatic bleaching for improving the optical properties at same and reduced chemical consumption. In later study, incorporation of enzyme stage at appropriate point of bleaching sequence was identified by experiment through different bleaching sequence. Further, we also studied on step-wise hexenuronic acid removal during enzymatic bleaching.

Thus, the observations obtained in this study can lead to defining an eco-friendly approach could be useful for improved pulp quality with cost effective process in pulp and paper industries.

CHAPTER 2.0 LITERATURE REVIEW

2.1 Composition of Hardwood

The main components of wood are cellulose, hemicellulose, lignin and extractives. These components differ quantitatively and qualitatively not only between different species of tree but within the age group of trees, sites where tree has grown and even between wood taken from different parts of the same tree. Cellulose content is also different between different softwood and hardwood species. Cellulose components are ultimately responsible for fiber strength and form the main platform on to which the hemicellulose and lignin are deposited. After the kraft pulping, the complex substrates are formed depending on the amount and composition of the lignin and hemicellulose fraction in wood. Xylanase acts upon these complex substrates directly or indirectly.

Composition of cellulose, hemicellulose and lignin are different in hardwood and softwood and between the species of wood types. The approximate ranges of these components are shown in Table 2.1.

Table 2.1 Composition of cellulose, hemicellulose and lignin in hardwood with softwood (Betts *et al*. 1991; Fang 2013)

Wood	Cellulose	Hemicellulose	Lignin
Hardwood	45-55	24-40	18-25
Softwood	45-50	25-35	25-35

2.1.1 Cellulose

Cellulose is a linear polysaccharide derived from D-glucose units, which are linked by β (l-4)-glycosidic bonds. A strong tendency is shown by cellulose molecules to form intra and intermolecular hydrogen bonds. Cellulose molecule bundles are aggregated together in the form of microfibrils and these microfibrils are organized in two different regions *viz*; crystalline and amorphous. These microfibrils are further bound together to form cellulose fibers. Hydrogen bonds with these fibrous structures and provides high tensile strength to cellulose fibers and makes it insoluble in most of the organic solvents (Sjostrom 1981; Sathisuksanoh *et al*. 2009). To maintain the fibre strength in paper and its products, cellulose is required to be protected from its

degradation by either mechanical processes or use of chemicals and enzymes throughout the papermaking process.

2.1.2 Hemicellulose

Other than cellulose, hemicellulose is a component that participates as a supporting material in the cell wall of plants. Unlike cellulose, hemicelluloses are branched to various extents and have relatively lower molecular masses than cellulose. Hemicellulose also differs from cellulose in degree of polymerization (DP). Cellulose can have a degree of polymerization (DP) of 10,000 or more, while hemicelluloses usually have a DP of around 200 sugar units. The content and types of hemicellulose in hardwood differ from those in softwoods. Hemicelluloses are closely linked with lignin and cellulose in the cell wall of plant species. There are several other polysaccharides that are considered to be hemicelluloses in plant, such as the xylans, mannans, arabinans, arabinogalactans and glucans (Hui *et al*. 2010).

Due to branched structure of hemicellulose, xylan traps lignin components after pulping process and has to be removed in pulp bleaching process. The type and nature of the xylan present in the lignocellulosic complex depends on the plant species. Xylans constitute the major hemicellulose in hardwoods, while galactoglucomannans are the major constitute of hemicellulose components in softwoods. The xylan backbone is derived of (H1-4) linked D-xylopyranose units (Bastawde 1992). Softwood xylans have fewer 4-O-methyl-a-D-glucuronic acid side chains than hardwood xylans. In hardwood xylan, there is usually one acetyl group present for every two xylose residues. Softwood xylan can also contain other residues such as L-arabinofuranose. The ability to modify both cellulose and hemicellulose has become an integral research area as industry search for new production processes and alternative methods to achieve the same.

2.1.3 Lignin

Lignin is an aromatic polymer which is formed by polymerization of a mixture of different 4-hydroxypropenyl alcohols in wood. The ratios of the three basic alcohols are different in wood species and even within the same species of a tree. These alcohols produce large, amorphous, three dimensional structures which are linked together in various ways. Lignin is a highly branched structure and bound by variety of chemical bonds. The molecular weight of the complex structure is still not known because of difficulties in degradation of wood. For softwood lignins, molecular weight is about

20,000 using milled wood, whereas lower values have been reported for hardwood lignins. Concentration is also different across the cell wall of both softwood and hardwood fibers. Hardwood lignin is primarily derived from sinapyl alcohol and coniferyl alcohol, whereas softwood lignin is mainly derived from coniferyl alcohol (Fiebach and Grimm 2000; Fang 2013). In cell wall layers, the middle lamella contains the highest concentration of lignin (Sjostrom 1981; Nelson 1992).

Lignin acts as bonding agents between the fibers and responsible for providing the rigidity to the fiber walls providing mechanical strength to the plant. Probably, lignin is linked to hemicelluloses chemically in wood (Yamasaki *et al*. 1981; Nie *et al*. 2015). It is presumed that lignin is bound to the hemicellulose which binds cellulose fibrils together. It is supposed that cellulose forms the main skeleton of fibre which is surrounded by hemicelluloses that form a supporting matrix, and everything is glued together with lignin.

2.1.4 Fiber morphology

There are many specific differences in the chemical and physical composition of the different species of wood before pulping. During and after the kraft pulping process these differences decrease and the chemical composition of the wood is drastically changed by reduction in the hemicellulose and lignin content in the pulp fiber while conserving the cellulose portion during the whole process. The other extractives are solubilized and removed from the fiber along with black liquor. The re-precipitation of the hemicellulose xylan onto the pulp fiber that occurs during the kraft cooking process would occur for all wood species. After the kraft cooking process, characteristics in fiber morphology is maintained in all wood species. Each wood species has a certain type of pulp fiber with specific characteristics differences these can be identified such as; thickness and length of fiber, porosity and surface area.

2.2 Pulp and paper process

The multi-stages process exists from wood chipping to final paper in pulp and paper process, which mainly contributes five major steps *viz*; wood preparation/chipping, pulping/cooking, bleaching, chemical recovery and papermaking process.

2.2.1 Wood preparation

First step for paper making is wood preparation where wood is processed through chipping to break wood logs into small pieces to make suitable for further cooking operations. Basically wood processing involves two major steps like chipping and debarking of wood logs (Schumacher *et al*. 1997).

2.2.2 Pulping/cooking process

Cooking/pulping of wood materials is a crucial step to prepare the unbleached pulp by removing lignin content using chemicals. In this process, black liquor is generated and is sent to chemical recovery. After pulping process, bleaching is carries out to remove the residual lignin and brighten the pulps. The wood cooking process is categorized in three major types; chemical, mechanical and semi-chemical pulping processes (Verma 2012).

2.2.2.1 Mechanical pulping process

To free the fibres, wood or non-wood chips are processed mechanically by grinding and shredding. In mechanical pulping process, pressure and temperature might be applied to assist the process to produce mechanical pulp. This type of pulping process is helpful for producing high yield pulp and produce minimal air pollution and relatively less water loads. Mechanical pulping is generally used for producing low grade pulp with high color and short fibres (McDonald *et al*. 2004).

2.2.2.2 Chemical pulping process

Chemical pulping is widely accepted and a commonly used procedure to generate free fibres of wood or non-wood raw materials with the help of chemicals. This is carried out through digestion of lignin binding materials from the wood chips or non-wood raw materials such as bamboo, straw, grass and cotton. This method is very helpful in removing more than 95% of the lignin from the wood chips and facilitate pulp yield in the range of 35 to 57% (McCubbin 1984). There are many types of chemical pulping methods and further divided into sulphite, sulphate (kraft), soda and semi-chemical pulping methods.

2.2.2.3 Kraft pulping process

Kraft pulping is also known as sulfate pulping and was first used in 1879 (Verma 2012). It is a process where wood is converted into wood pulp consisting of pure cellulose fibres. In this process to modify the caustic soda, sodium disulphide (Na_2S) is added in white cooking liquor. In kraft pulping, sodium hydroxide is suitable for cooking of all wood species and sodium sulphate acts as buffer in cooking liquor.

There are several reasons why sulfate pulping has become the most common chemical pulping process world-wide. Kraft cooking has high tolerance for large amounts of extractives as well as portions of decayed wood and barks residues and thus allows considerable variabilities in the wood substrate entering the kraft pulping cycle. Another reason for using the sulfate pulping process is the relatively short cooking times, usually less than two hours for each stage, and results in excellent pulp strength properties which makes the process economically advantageous. Well established processing of the spent liquid, including the recovery of the pulping chemicals, generation of process heat, and the production of valuable by-products such as tall oil and turpentine from pine species makes the kraft pulping process economically favourable (Fuller 1987; Santos *et al*. 2015).

To make high quality paper, cellulose microfibrils must be protected during the removal of lignin in the raw wood substrate. It is also desirable to maintain high quantity of hemicellulose to improve pulp yield within the fiber during the production of wood pulp. Consumption of most of the alkali in the pulping process is due to hemicellulose fraction degraded by alkali and the extent of delignification is partially controlled by the amount of hemicellulose removed (Browning 1967). In the kraft pulping process, highly strong alkali conditions are maintained by adding caustic soda and sodium disulfide in the digester to degrade the wood chips and dissolve the major portion of the lignin. Huge amount of lignin is extracted from the middle lamella layer of cell wall. Unreacted woody pieces are removed by screening process and individual fibers within the wood chips are loosened and separated after cooking process. Black liquor is formed after the completion of chemical reaction with the wood, containing huge amount of extracted lignin. This filtrate containing significant concentrations of organic material, can be removed and burned for producing energy. The kraft pulping process results in production of higher pulp yields and superior quality of fiber in comparison to other chemical pulping processes (Fengel and Wegener 1984; Nelson 1992, Gangwar *et al*. 2014).

Hemicelluloses are responsible for partially covering the lignin and blocking the bleaching chemicals from reacting with it thus affect the reactivity of residual lignin in kraft pulp (Clark *et al*. 1991). The hemicellulose fraction of the fiber is extensively modified during the kraft cooking process. Xylan parts are dissolved in pulping liquor during heating period of the cooking, when the alkali concentration is comparatively high (Viikari *et al*. 1994). During the kraft pulping process, lignin in the pulp become darkly colored and if they are not soluble, they are usually re-deposited on the pulp fibers during the last stage of cooking (Sjostrom 1981). Alkali concentration decreases at the end of cooking process which leads for re-precipitation of short xylans in a more or less crystalline form onto the cellulose fibres. Side groups from the most of the xylan are removed during the kraft pulping resulting in increasing tendency of the xylan to crystallize. Once the initial dissolution of xylan has occurred, polysaccharide peeling reactions uncover more lignin which can be subsequently released during the kraft cooking (Buchert *et al*. 1993).

During the kraft cooking process, the re-precipitation of xylan is followed by the re-precipitation of lignin. These re-deposited compounds have been reported to be chemically linked to each other (Iversen and Wannsrtom 1986; Yang and Eriksson 1992b; Daneault *et al*. 1994; Spence *et al*. 2009). These compounds increase the difficulty to remove the residual lignin from the fibres after formation of lignin-carbohydrate complexes (LCCs), even though the major part of the lignin network has been torn apart. The extent of re-precipitation of xylan has been suggested to decrease in continuous kraft cooking procedures (Pedersen e*t al*. 1992; Buchert *et al*. 1993; Spence *et al*. 2009).

After the kraft process, only 3-6% lignin is left in the pulp. Bleaching chemicals are used in order to facilitate the removal of this small percentage of residual lignin left in the pulp after the kraft pulping process. These residual lignins darken the pulp and are very difficult to remove without damaging the pulp fibers. Functional groups of partially degraded and modified residual lignin are responsible for the light absorbing chromophoric components in unbleached pulp. To remove these residual lignin form the unbleached pulp, multi stages bleaching process is required to remove color-causing components with minimum loss to pulp quality. Bleaching can be performed either by removing the lignin from the carbohydrate fraction of the pulp or by converting and stabilizing the chromophoric groups (lignin-preserving bleaching). Other compounds such as extractives, ash and some hemicelluloses are also removed along with the lignin.

Bark specks and shives (larger particles) may also be bleached partially or completely and removed from the pulp mixture during the various bleaching stages. Therefore, bleaching can also be known as a brightening process as well as a purification process.

2.2.2.4 Bleached chemi-thermo-mechanical pulping (BCTMP) process

BCTMP process is a type of chemi-mechanical pulping process that results in high yields of unbleached pulps. It is totally different form the others chemicals pulping methods such as sulphate and sulphite, as the lignin is re-collected in the process. Almost double pulp yield can be achieved in BCTMP as compared to chemical pulping where lignin dissolved away from the pulp by using chemicals in the process (Verma 2012).

BCTMP process is highly productive when woods like birch, aspen, pine, maple and spruce are used as raw materials. A variety of softwoods and hardwoods can be used to make BCTMP. Color component, lignin is removed from pulp in bleaching process that give brighter and whiter pulp and these brightness and whiteness index in paper are measured through as ISO (International Organization of Standardization) brightness. Pulp recovery is also measured on the basis of raw material used and pulp obtained after the process. A brightness (%ISO) range of 80-83 and 90% pulp yield could be obtained in softwood BCTMP whereas in hardwood BCTMP, brightness in the range of 85-87 and 88% of pulp yield could be achieved easily. In specific hardwood, aspen has an economic rotation length of 25 years which are longer rotation lengths of other hardwoods and has a typical pulp yield of 87% (Johnson 1991).

2.2.3 Pulp Bleaching

Bleaching is the process to brighten and whiten the wood pulps by removing color compounds and residual lignin. The process modifies or eliminates the attached lignin to the wood cellulose fiber. Many oxidizing agents, alkali solutions and other chemicals are being used in chemical bleaching process. Darker pulps are produced during kraft process which requires more bleaching. Hydrogen peroxide and/or sodium hydrosulfite are used in mechanical pulps to minimize the absorption of light by lignin components instead of removing it (Schumacher *et al*. 1997). This is basically completed in different stages; initially, the pulp is treated with NaOH in the presence of oxygen, where hydrogen ions are removed by the action of NaOH on lignin and then the oxygen helps in breaking down the polymers, the pulp is then treated with chlorine dioxide

(ClO_2), sodium hydroxide, oxygen and H_2O_2 and finally with chlorine dioxide again to remove the residual lignin from pulp fibres.

2.2.3.1 History of pulp bleaching

Initially, sunlight and/or chemicals such as potash and hypochlorite were the major sources for the brightened the non-wood fibers, such as cotton rags. The utilization of wood fibers came later but the bleaching was still slow and on a small scale as compared to modern bleaching processes. Towards the end of the nineteenth century, newer means of industrial bleaching of wood pulps were initiated. Firstly, the use of hypochlorite and then chlorine, in combination with an alkali extraction stage was started at small scale. Quality pulps in larger amounts could be produced when chlorine and chlorine dioxide were introduced into the bleaching process. The pulp and paper industry began to grow and the bleaching of pulps with various types of chlorine-based chemicals was considered the best and cheapest process available (Nelson 1992).

However, during the past decade, huge changes have happened in response to environmental concerns. Bleaching has become a complex and expensive step in the production of market kraft pulp due to use of huge quantity of bleach chemicals. Many different chemical agents and bleaching sequences are now being used. Currently, individual mills must analyze their options carefully and choose the best bleaching alternatives suiting their own requirements. The drive towards using fewer chlorine containing compounds tends to make that each mill follow more and more specialized bleaching process.

2.2.3.2 Environmental concerns in bleaching process

The effluents from pulp and paper industries are considered as the highly polluted discharge at industrial scale. The presence of chlorinated organics in bleach effluents and paper products and the discharge of colorants into the receiving waters are considered as major environmental concern (Brunner and Pulliam 1993; Gangwar *et al*. 2014). For reducing the pollutants in bleach effluents, many strategies are being developed to increase delignification of the pulp before it reaches the bleach plant. Other methods for reducing the environmental impacts include use of alternative bleaching sequences and chemicals, increased effluent treatment and adaptation of partially or completely closed the mill cycle.

Work at the Swedish Pulp and Paper Research Institute and the Pulp and Paper Research Institute of Canada (PAPRICAN) determined that various dioxins and organochlorines that were identified as the most toxic came from several defoamers and oils, as well as the wood and water used in the pulping process (Sharma *et al*. 2014). Chlorine and its components (elemental chlorine and other chlorine containing chemicals) are very corrosive. They tend to undergo electrochemical reactions such as the reduction of chlorine to chloride ions (Laliberte and Sharp 1979; Laliberte and Garner 1981). The chlorides ions react with metal pipes and containers used in the system and corrode it slowly. The effect is made worse by the recirculation of chlorine containing water in the bleach plant, thus increased concentration the chloride ions. Effluents containing chlorine and its components cannot easily be recycled or burned because of their associated impact on the environment. Currently, the filtrate from the final chlorination stage can been recirculated to various dilution points in the chlorination stage, resulting in substantial lowering of effluent volume discharged into the environment. The amount of organochlorines generated can be reduced by modifying the bleaching conditions and decreasing the use of elemental chlorine.

In traditional methods, chlorine was used in bleaching process for delignification of lignin from unbleached pulp, but the discovery of toxic chlorinated organics within bleach effluents in kraft mill encouraged the pulp and paper industries to search for alternative bleaching methods and bleaching agents. However, government legislation and public pressure is still motivating pulp and paper industry to decrease or even eliminate the use of chlorinated chemicals in the production of high quality bleached kraft pulp (Nelson 1992).

2.3 Chlorine-free bleaching of kraft pulps

It is desirable and required by current market that pulp and paper industries should be produce paper with little or no chlorine (Beaton 1994). Researchers are working and developing newer technologies and methods for delignification and bleach the pulp, thereby facilitating the elimination of elemental chlorine and decreasing the amounts of other chlorinated bleaching agents required (McDonough 1995; Gangwar *et al*. 2014). Bleaching alternatives are necessary and required in order to eliminate or reduce the chlorine consumption, which leads to decrease the organochlorine contents in bleach effluent thus increasing the possibility of closed loop system (Glowacki 1994;

Sharma *et al*. 2014). There are two new technologies (elemental and total chlorine free bleaching) already developed and being followed by many pulp and paper industries globally.

2.3.1 Elemental chlorine-free (ECF) bleaching

After using elemental chlorine for a long time in conventional bleaching process, many researchers have developed elemental chlorine-free bleaching, where chlorine dioxide is used instead of elemental chlorine for bleaching of pulp fibres. Use of ClO_2 also reduces or prevents the formation of carcinogenic pollutants like dioxins and other chlorinated components. Chlorine dioxide is emerging out as the main bleaching agent for kraft pulp bleaching and it is believed to be the most efficient bleaching agent available at this time. Chlorine dioxide can also degrade phenolic residues in the pulp and it is very effective at removing bacteria and fungi that may be present in the pulp (Croon 1993; Lin *et al*. 2013). There are no observed chronic effects and toxic bioaccumulations in the effluent generated from the pulps that was treated with 100% of chlorine dioxide (Haglind *et al*. 1991).

2.3.2 Total chlorine-free (TCF) bleaching

In the search for a compound that will help the bleaching industry eliminate the use of chlorinated bleaching compounds, Wearing (1993) and Khider *et al*. (2012) indicated that it is important to ensure that new bleaching technologies are environmentally sound and represent an improvement over current technology. Effluent generated from new bleaching sequences should be easily treated and disposed in a way that will meet any present or proposed government legislation, and they should improve the chances of a closed mill system being developed.

Totally chlorine-free (TCF) bleaching process are those where chlorine and its other components are not being used in the bleaching sequence. The production of TCF paper is currently one of the most intensely researched areas of pulp and paper technology and it is the subject of several debates. A considerable amount of effort is being spent on trying to find chlorine-free bleaching agents that are affordable and effective. In the search for the perfect bleaching alternative, the driving forces which often conflict with one another include: the effectiveness of the new bleaching technologies; the quality of the final paper; operation costs; product selling price;

consumer demands; and pressure from environmental groups. Totally chlorine-free pulps derived from hardwood, such as birch and eucalyptus, have been produced but those from softwoods pulps are still problematic. High brightness has been achieved in softwood pulps but often with a loss of yield and strength. Keeping in view the current trends, newer mills will be able to produce total chlorine-free pulp by increasing production cost slightly (due to the use of more expensive bleaching chemicals) but older mills will continue and face higher costs due to the limitation of retro-fitting equipment (Cockram 1993). However, the market for chlorine-free pulp is growing and producers have been attempting to fill these demands using chlorine-free chemicals (Koncel 1991; Cox 1992; Gangwar *et al*. 2014).

Although industry is actively trying to decrease chlorine consumption, it may be impossible to completely eliminate organochlorine generation. Berry (1993) opined that 0.1 kgt^{-1} of pulp is taken in with process water alone. Further, wood naturally contains enough chlorine to generate approximately 0.1 g of organochlorine compounds per ton of pulp. The two chlorine-free alternatives used in this study were oxygen delignification and hydrogen peroxide. Oxygen delignification uses relatively high temperatures and pressures, and requires a substantial capital cost to implement within the bleach plant operations. However, the production capacity of O_2-delignified pulp has proved the dramatic growth during the last several years globally, and oxygen delignification is considered a prerequisite for TCF pulp production (Johnson 1993).

The brightening effects of hydrogen peroxide have been recognized for years. However, the effectiveness of peroxide as a bleaching agent has been limited by its degradative effects on pulp strength (Van Lierop *et al*. 1993; Loureiro *et al*. 2010). Current research has focused on improving the action of peroxide on the pulp without damaging the pulp fiber. For this study, the enzyme xylanase was used in conjunction with oxygen delignification and multiple peroxide stages in order to determine if enzymatic pretreatment of the kraft pulp could facilitate subsequent bleaching stage.

2.4 Papermaking

Papermaking is a four step process starting from preparation of pulp fibres through beating to make them more flexible and addition of dyes, pigments, sizing and filler materials, followed by forming and pressing. Forming process involves distribution the pulp fibres on a screen. In pressing stage, pressing is done to remove the water from

the paper. Last step stands drying the paper by applying steam to remove remaining moisture from paper. Preparation and drying of the paper are the most energy consuming processes among these all stages during papermaking. There are two different types of methods in papermaking; the most common being the continuous type where, continuous process is being followed for the pressing, draining and for the drying of the paper while in another type, water is removed in between the deposits by forming layers of paper matt (Schumacher *et al*. 1997; Verma 2012).

2.5 Xylanases

2.5.1 Background

Recently, a lot of attention has already been given to the bleach boosting effect that the use of xylanase has in the chemical bleaching of both hardwood and softwood kraft pulps. Hemicellulases have previously been tested for the removal of hemicellulose from pulp in order to facilitate the production of dissolving pulp (Paice and Jurasek 1984; Bajpai and Bajpai 2001). The authors concluded that a xylanase mixture does not affectively remove hemicellulose completely. Viikari *et al*. (1986) and Kantelinen *et al*. (1988) introduced the idea of using xylanase to decrease the amounts of chlorinated chemicals required to bleach kraft pulp. It has been found that xylanases are the most effective hemicellulases for enhancing the extractability of lignins by conventional bleaching chemicals (Viikari *et al*. 1986; Gangwar *et al*. 2015). Several reviews have summarized the current status of xylanases being studied for use in the pulp and paper industry (Bajpai and Bajpai 1992; Nelson 1992; Lavielle 1993; Wong and Saddler 1993; Daneault *et al*. 1994; Viikari *et al*. 1994; Tolan *et al*. 1995; Gangwar *et al*. 2014; 2015; 2016a & 2016b).

Environmental concerns have further driven the research and development of xylanases with as focus on reducing the use of elemental chlorine in pulp bleaching. However, it was soon realized that, when combined with chlorine-free chemicals such as hydrogen peroxide, xylanase treatment was not able to boost pulp brightness to the same extent achieved by chemicals containing chlorine.

Many researchers believe that a greater understanding of the mechanism of xylanase bleaching is a pre-requisite to improve the applicability of these enzymes in a TCF bleaching sequence. It has also become apparent that conditions within the pulp matrix complicate xylanase action when compared to those with the isolated substrates

often used in the laboratory. Research is currently focusing on the optimization of xylanase action on the kraft pulp in terms of reducing the consumption of bleach chemicals for pulp bleaching. Because the pulp and paper industries have been forced to consider many new bleaching alternatives, the potential of using xylanases without requiring significant capital expenditure has made them worthy of study.

2.5.2 What are xylanases?

Xylanases are a type of hemicellulases required by many microorganisms to degrade plant biomass. Hemicellulases, responsible for the degradation of hemicelluloses, are generally classified according to the substrate that they degrade. Collectively, hemicellulases, pectinases, amylases and cellulases are referred to as glycan hydrolases (EC 3.2.1). Xylanase, the enzyme responsible for xylan breakdown, occurs in both prokaryotes and eukaryotes (Dekker and Richards 1976). Various fungal and bacterial sources have been extensively studied and identified for the production of intracellular and extracellular xylanases (Reilly 1981; Woodward 1984; Dekker 1985; Bastawde 1992; Gangwar *et al*. 2014). Endo-P-(l-4)-D-xylanase acts randomly on xylan branch to produce xylo-oligosaccharides of various chain lengths. Exo-P-(l-4)-D-xylanase removes single xylose units from the non-reducing end of the xylan chain. P-xylosidase (or xylobiase) hydrolyses disaccharides like xylobiose and xylo-oligosaccharides with decreasing specific affinity. Of the three types of enzymes, the endoxylanase appears to be the most important enzyme for pulp and paper industries (Bastawde 1992; Kantelinen *et al*. 1993; Chauhan *et al*. 2006; Gallardo *et al*. 2010; Batalha *et al*. 2011).

Two general methods are currently being used for the production of xylanases. Batches of a particular microorganism that produces large amounts of extracellular xylanases, such as the fungus *Trichoderma sp*., are grown, and the filtrates are collected and partially purified by gel filtration. Unfortunately, it is extremely difficult to remove all of the other contaminating hydrolytic enzymes, such as cellulase, that are also produced by most microorganisms using this method. The second method involves the cloning of the gene encoding a xylanase from microorganism. This method is particularly successful for the production of cellulase-free xylanases required by the pulp and paper industry. There are, however, researchers who believe that by removing the other enzymes from the native xylanase preparation, additive and synergistic activities

that may otherwise improve the overall bleach boosting results are decreased, if not completely eliminated (Nelson, 1992).

There are few microorganisms that are known to produce only a single type of xylanase. A multiplicity of xylanases, especially endoxylanases, has been reported from bacteria and yeasts. The stability and activity of the different xylanases often depends on factors such as pH, temperature, ion concentration, and variability within the substrate itself. Xylanases appear to have a wide range of pH and temperature optima, from 4-7 and 37-80°C, respectively. Most of the characterized xylanases presented by Bastawde (1992) remained active within several pH units above and below the reported pH optimum for each xylanase.

2.5.3 Factors affecting xylanase activity

Enzymes are derived from biological organisms and they tend to require relatively specific conditions for optimal activity. It is possible to formulate an optimal mixture of xylanases that could completely hydrolyze the xylan in an isolated substrate (Viikari *et al*. 1994). Xylanases have been used for the selective removal of xylan from pulps but several factors often limit the action of the enzyme on the substrate. When hemicelluloses are bound to the pulp fiber, other factors such as porosity, specific surface area, charge of the fibers and distribution within the substrate tend to affect the efficiency of the enzymatic hydrolysis. The inaccessibility of the substrate to the xylanase may be due to physical limitations and the influence of lignin-carbohydrate linkages on xylan hydrolysis. Other inhibiting factors include difficulty in hydrolyzing the xylan due to its branched nature; thermal instability of the enzyme preparations; non-selective adsorption of xylanases onto the cellulose and lignin components of the pulps; and the inactivation or inhibition of the xylanase by constituents of the pulp. The adsorption and/or inactivation of xylanase would reduce the concentration of the enzyme available for the hydrolysis of xylan. Senior *et al*. (1990) determined that the incubation of xylanase with unbleached pulps resulted in a significant loss of activity that was mainly due to the presence of leachable materials from the pulp. They also reported that non-selective adsorption of the xylanases onto components of the pulp other than xylan severely limited the effective concentration of the enzyme.

Most enzymes are sensitive to both temperature and pH extremes. A bleach plant may have difficulty adjusting to and maintaining optimal conditions for enzyme activity, although this has not been found to be a serious problem in modern mills. Current

research is being conducted to develop xylanases that are active at higher temperatures (Simpson *et al*. 1991; Gangwar *et al*. 2014) and broader pH ranges (Daneault *et al*. 1994). Recently, the activity of a commercially available xylanase was tested at various pH values and temperatures and there appeared to be an increase in pulp brightness for a wide range of conditions (Gangwar *et al*. 2015). In order to achieve the highest brightness possible from a TCF bleaching sequence, the action of the xylanase treatment must be optimized. One TCF bleaching sequence that has already been used in industry is oxygen delignification (O) in conjunction with multiple peroxide stages (P). This sequence has been able to produce pulp brightness of around 80 for softwoods and 85 for hardwoods, without compromising pulp strength (Roncero *et al*. 2003b). If a xylanase treatment could enhance this final brightness then it would be worthwhile incorporating a xylanase treatment step into the bleaching sequence. There are several conditions for kraft pulp bleaching that require close monitoring if it is being used in conjunction with xylanases. Important conditions, other than the pH and temperature of the pulp prior to and during the xylanase stage, include the duration of the xylanase stage, the presence of ions in the pulp, and the extent of pulp washing before and after the xylanase stage. If any of these conditions are not appropriately set then the action of the xylanase will not be as efficient as it could otherwise be.

2.5.4 Mechanism of xylanase action in pulp and paper

To eliminate lignin from the unbleached pulp breakage of its links with cellulose is necessary and desired to brighten the pulp. It is readily possible during the subsequent bleaching stages (Turner *et al.* 1992; Pham *et al.* 1995). During kraft pulping process, free native xylan re-deposited again onto the surface of fibres. These re-precipitated xylans prevent penetration of bleach chemicals onto the fibre surface and act as a physical barrier in bleaching process (Kantelinen *et al.* 1993) and increased the consumption of ClO_2 and other chemicals in bleaching process. Lignin entrapped in between these re-deposited xylans, influencing fiber swelling and very difficult to remove with chemicals (Shobhit *et al.* 2005). Xylanase enzymes promotes efficient pulp bleaching by hydrolyze re-precipitated xylan on the fiber surface.

Enzymatic treatment is responsible for the reduction of xylan concentration on to fibres surface due to hydrolyzing of re-precipitated xylans, particularly in hardwood kraft pulps. Permeability of fibres surfaces increased by reducing the xylan concentration; thus improved bleachability (Paice *et al.* 1992; Bajpai *et al.* 1994; Viikari *et al.* 1996; Gliese

et al. 1998; Roncero *et al.* 2000; Shah *et al*. 2000; Torres *et al.* 2000). Another mechanism of action of xylanases involves hemicellulases. As per the mechanism, hemicellulase acts on the particular points of attachment of hemicellulose to lignin and break the bonds of hemicelluloses, therefore this improves lignin solubility.

A possible hypothesis for the xylanase action in pulp and paper was suggested by Henriksson and Teeri (2009);

- Lignin components that are covalently bound to xylan or physically entrapped lignin by the xylan can be removed out after the xylanase treatment;
- Xylanase treatment is responsible for the partly removal of the re-precipitated xylan from the fiber surface and provide more surface area for better penetration of chemicals onto the fibre during bleaching process (Sharma *et al.* 2007); and
- Regions with high hexenuronic acid content can be removed by xylanase treatment; results in reduction of consumption of bleaching chemicals.

Hexenuronic acids are responsible for the significant brightness reduction in totally chlorine-free bleached pulp (Roncero *et al.*2003a). This problem can readily be removed by the use of xylanases in enzymatic bleaching (Cadena *et al.* 2010). In addition, enzyme treatment is also responsible for reduction of lignin content in the pulp which is measured through kappa number, thus improves the pulp brightness.

For directing these benefits of enzyme treatment, certain specific qualities are necessary and required in xylanase enzymes to assist their optimal use in the bleaching processes;

- Implementing cellulase-free xylanase with minimum or negligible cellulolytic activity to avoid hydrolysis of cellulose fibers (Archana and Satyanarayana 2003);
- To facilitate easy diffusion of enzyme into the pulp fibers, low molecular mass is required; and
- Cost effective process with high yield output (Niehaus *et al.* 1999).

2.5.5 Commercially available xylanases

Natural sources (bacterial and fungal strains) are being used for the production of enzymes. Researchers are working on producing such type of enzymes which can be implemented as on commercial basis to the industrialist. Commercially enzymes are easily available in the market now for the production of quality paper with cost effective

process by lowering kappa number, improved optical and strength properties in the final paper.

Valls *et al.* (2010) stated that the enzymatic stage can removed 14% of hexenuronic acid by hydrolyzing re-precipitated xylans from the fiber surfaces. The effects of some commercially available xylanase enzyme *viz*; Pulpzyme HC, Irgazyme-10 and 40S, and Bleachzyme-B and F, *etc.* were also examined by Bajpai and Bajpai (1996) with respect to subsequent bleaching stages. Authors obtained a decrease of 0.4 to 1.2 units in the kappa number after the extraction stage as compared to control when pulp was treated with xylanases. Brightness gain of 0.8 to 1.5 units was achieved at equivalent bleaching chemicals with different enzymes. Bleachzyme F and Irgazyme-40S were noted as the best enzymes among all enzymes for improving the brightening effect in paper. The above both enzymes were also responsible in kappa number reduction after extraction stage; resulted in 20% reduction in bleach chemicals.

Bajpai and Bajpai (2001a) also worked on six xylanase enzymes with a wide range of incubation temperature, incubation times, and pH values. They used bleaching sequence as CEHED (where C-chlorine, E-extraction, H-hypochlorite, and D-chlorine dioxide) after enzyme treatment. They observed that a higher pulp yield was achieved at lesser energy consumption due to high level of pentosans in the unbleached pulp after mild hydrolysis with xylanases. The enzyme treatment was also resulted in reduced pollutant release into the pre-hydrolyzate liquor, reduced consumption of bleaching chemicals and higher pulp brightness. Some of the prominent suppliers (*viz*; Advanced enzyme, Novozymes, Dyadic International, Genencor Dupont Sciences, AB Enzymes etc.) are fully involved in manufacturing of enzymes and selling these commercially to pulp and paper industries globally.

2.5.6 Applications of xylanase in pulp and paper

2.5.6.1 Effect of xylanase on carbohydrate composition

When enzyme treatment was done before the oxygen delignification (ODL) then hydrolysis of xylan dramatically affected. A reduction in xylan content by 13.4% was observed during enzymatic treatment itself and this reduction in xylan content increased up to 15.5% when xylanase treatment used in conjunction with oxygen delignification. Roncero *et al.* (2005) also studied the xylanase influence on the carbohydrate composition. Enzyme treatment and oxygen delignification both affected the ratio of

amorphous and crystalline structures. The degree of crystallinity was also increased after the enzymatic process.

Shatalov and Pereira (2008) studied on peroxide bleaching after enzyme treatment. They used *Eucalyptus globulus* and treated it with two commercial enzymes (Pulpzyme and Ecopulp). After enzyme treatment, subsequent peroxide stages were followed in bleaching sequence (XQPPP; where X-xylanase treatment, Q-pulp chelating, and P-hydrogen peroxide). Ecopulp was found more effective in removing xylose during the peroxide bleaching when the pulp was treated with xylanase treatment. A year later, Shatalov and Pereira (2009) studied on the same peroxide bleaching sequences for removing lignin compounds from *E. globules* pulp i.e. XQPPP. They used enzyme followed by chelating stage then followed by different stages of peroxide beaching. Significant results were achieved in removal of lignin components from the eucalyptus pulps as compared to control. Reduction in lignin was improved with addition of peroxide stage after the chelating stage. Authors found that Ecopulp showed better results in lignin removal then pulpzyme.

Shirkolaee *et al.* (2008) studied on the lignin removal by using three different raw materials (Bagasse, Rice straw and Wheat straw) and reported that the reduction of lignin content depends on raw material used in the process after xylanase pretreatment of the pulp. They used two types of xylanases and applied enzyme doses of 1.0, 5.0 and 10.0 IU/ml of pulp. According to them kappa number reduction was increased with increase in enzyme dose and higher reduction was observed in Bagasse followed by Rice straw and then in Wheat straw.

2.5.6.2 Effect of xylanase on fiber morphology

Roncero *et al.* (2000) stated that xylanase treatment removes re-precipitated xylan from the fibre surface therefore it opens the pores of fibre. During enzymatic treatment some morphological changes occur on fibre like holes, flakes, filaments, cracks, and peeling. As reported by some authors, these cracks and holes share some free spaces for the diffusion of larger lignin macromolecules, (Paice *et al*. 1995; Wang *et al*. 1997). According to Roncero *et al.* (2005), accessibility of bleaching chemicals improved towards pulps by increasing outward movement of degraded lignin fragments due to increased diffusion. Therefore, removal of less-degraded lignin fragments increased from fibre cell wall, results in reducing kappa number.

With attention to the effects of xylanase in pre-bleaching process, ECF and TCF bleaching sequences were also studied for the effect of enzyme treatment on fiber morphology using eucalyptus hardwood pulps. As per scanning electron microscopy (SEM) analysis, xylanase changes the surface of the fibers during the enzyme treatment. Xylanase treated pulp fibres have more splits (open pores) over rough surfaces, which leads to increase the contact between the substrate and bleaching chemicals (Viikari *et al.* 1986; Roncero *et al.* 1999 & 2000).

Pulp bleaching is a multi-stages process and it is already reported that enzyme treatment is much more effective on changing fibre morphology in initial stages of bleaching process (Roncero *et al.* 2000). Xylanase treatment is responsible for a remarkable peeling effect i.e. xylan is removed as flakes from the fibres surface; results in fibre modification. Xylanase treatment delivers rough surface to the fibre in contrast of smoother surface in untreated pulps due to many filaments of materials and flakes detached from their surface. The authors also studied untreated and xylanase-treated pulps after the oxygen delignification (O) stage, where fibre modification was observed in XO-pulp due to peeling effect as compared to O-pulp and rough surface of fibres was seen as compared to smooth surface of untreated fibres.

2.5.6.3 Effect of xylanase on Hexeneuronic acid content

Hexenuronic acid (Hex-A) is widely distributed among other natural polysaccharides such as chondroitin, heparin and lepidimoide (Adorjan *et al.* 2006). About 75-90% of 4-O-methyl-glucuronic acid side groups (MeGlcA) linked to heteroxylan are lost during the alkaline pulping process and the residual MeGlcA are almost completely (83-88%) converted to unsaturated hexenuronic acid (Hex-A or 4-deoxy-l-threo-hex-4-enopyranosyluronic acid) *via* the intermediate product, 4-O-methyliduronic acid (Farrell *et al.* 1996; Senior *et al.* 1999; Bim and Franco 2000; Beg *et al.* 2001; Chauhan *et al.* 2006).

The H-factor and the alkali charge are the main factors that influence the formation of hexenuronic acid during kraft pulping. Considering *Eucalyptus globulus* wood as a raw material for kraft pulp, the alkali charge is the main variable that contributes to the degradation and formation of hexenuronic acid during pulping. Hexenuronic acids attached with lignin by forming covalent bonds during the kraft pulping process. The methyl glucoronic acid side groups are found in xylans which are

totally responsible for the formation of hexenuronic acid. Due to hexenuronic acid components consumption of beaching chemicals increased because of its undesired neutralization of electrophilic bleaching chemicals such as chlorine dioxide, ozone, and peracids (Vuorinen *et al.* 1999). Bajpai *et al.* (2005) stated that the Hex-A percentage differ species to species of wood. According to them casuarina wood pulp having the highest and subabul wood pulp the lowest content of hexenuronic acid. Similarly, Assam bamboo pulp having the highest hexenuronic acid content while Maharashtra bamboo pulp having the lowest content.

Sometimes hexenuronic acid helps in preserving the pulp yield in the pulping process by protecting xylan against terminal depolymerization reactions. However, in extreme conditions of alkali dosage and temperature, these polysaccharides as well as other composites, suffer alkaline hydrolysis and are degraded to some extent that results in lower pulp yield in the pulping process. In spite of extreme alkaline conditions, acidic environment is also responsible for the hydrolysis by hexenuronic acid as it is vulnerable to the attack of electrophilic oxidants. According to Almeida (2004) study on removal of Hex-A from the pulp, an acid environment is responsible for hydrolysis of hexenuronic acid at a reaction pH of 3.0 and temperature of 80 to 100°C.

Shatalov and Pereira (2009) studied on xylanase aided biobleaching to see the impact of hexenuronic acid on chemicals pulps. They observed that Hex-A removal with solubilized xylooligosaccharide fractions during xylanase treatment results in improving brightness of the pulp. As per their findings, a strong positive correlation was also established between the bleaching profile of Hex-A and xylanase bleach boosting effect during the enzymatic bleaching process. They also studied on the effects of oxygen and alkali extractions on xylanase aided bleaching using kraft pulps as well. The authors noted a significant improvement in final brightness of up to 1.4 to 2.1 units with reductions in the kappa number and Hex-A of xylanase-pretreated pulps compared to the corresponding reference pulps (Wong *et al.* 2001). Based on the data from Shatalov and Pereira (2008), xylanase pretreatment followed by three-stage peroxide bleaching also reduced the hexenuronic acid content in the *E. globulus* pulp and Ecopulp showed better performance than Pulpzyme. Peroxide stage was also responsible for better reduction in hexenuronic acid from eucalyptus kraft pulp.

2.5.6.4 Effect of xylanases on bleaching chemical consumption

Enzymes are being used alone or in combination of other enzymes in the enzymatic bleaching of the pulps. A number of reports are available on the reduction of bleach chemicals consumption with the use of xylanase enzymes. According to Chakar *et al.* (2000), hexenuronic acid contribution to kappa number is always remaining different in hardwood (33 to 67%) and softwood (5 to 12%) kraft pulps. Bleaching chemicals specially consumed by that part of hexenuronic acid which are attached with xylan, leads to increase consumption of bleaching chemicals during bleaching. During xylanase treatment, hydrolysis of xylan occurs and therefore high contents of hexenuronic acid bounded with xylan eliminated from the pulps, resulting in decreasing the bleach chemicals consumption in pulp bleaching process.

Some of the laboratory studies and mill trials indicate savings in total elemental chlorine of about 20 to 25% (Senior and Hamilton 1992a; 1992b & 1992c; Tolan and Canovas 1992; Senior and Hamilton 1993; Senior *et al.* 1999; Bim and Franco 2000; Shobhit *et al.* 2005). Xylanase enzymes hydrolyze the re-precipitated xylan onto the fiber surface and therefore improve fiber permeability to bleaching reagents. Compared to chemical bleaching, enzymatic bleaching is beneficial to reduce chemical consumption with maintaining the brightness and kappa number in the final bleached pulp. Xylanase treatment reduces chlorine consumption by 10% when bleaching wheat straw pulps to the same brightness and kappa number as compared to reference pulps (Lin *et al.* 2013).

A mill-scale study conducted on hardwood pulps by Thakur *et al.* (2012) and concluded that 15% of bleach chemical requirements reduced upon implementing of xylanase enzyme before bleaching. Xylanase treatment was carried out at a temperature range of 50 to 60°C and pH range of 9 to 10. They observed kappa number reduction in the range of 8.7 to 12.0% during the study. After the chlorine stage, kappa number reduction was obtained 14.3%, while after the first extraction stage, a kappa number drop was noted up to 20% and it was increased up to 25% after the second extraction stage with 2.0 to 3.0 units of brightness gain in each stage. In enzymatic bleaching process, the average chlorine consumption was reduced by 20% as compared to the conventional process where no enzyme was added. Effect of xylanase treatment also leads to reduction in hypochlorite flow rate in the range of 13.33 to 20%; resulting in reduction of hypochlorite consumption by 11.11 to 15.55%. To maintain brightness (%ISO) level of 82 to 83% with enzyme treatment, 10 to 12% reduction in hypochlorite was achieved

with 15% saving in chlorine consumption at plant scale using hardwood pulp.

2.5.6.5 Effect of xylanase on pulp brightness

In a study, unbleached industrial eucalyptus kraft pulp was treated with two different commercial xylanase; Ecopulp and Pulpzyme to assess the effect of xylanases on the three-stage hydrogen peroxide (QPPP) bleachability of *Eucalyptus globulus* kraft pulps. Samples were examined and compared with reference pulp (treated identically except without the adding of enzyme) for change in pulp properties after each bleaching stage. Hydrogen peroxide bleaching was used, though it is generally used as a separate bleaching stage incorporated into multistage, industrial bleaching sequences (Shatalov and Pereira 2008).

Jimenez *et al.* (1996) studied on biobleaching of wheat straw pulp and reported a brightness gain of 2.4 points for enzyme peroxide bleaching and 3.0 points in the case of enzyme peroxide active chlorine bleaching. The use of *Bacillus coagulans* xylanase on three non-woody pulps (wheat straw, rice straw, and jute) was explored using a totally chlorine-free bleaching sequence (Chauhan *et al*. 2006). A maximum brightness gain of 5.1 points was achieved in rice straw pulps at an initial pH value of 8.5. Similarly, in the cases of wheat straw and jute straw pulps, maximum brightness gains were obtained at pH values of 8.5. Post-treatment with xylanase using bleached hardwood kraft pulp, resulted in reducing yellowness from the pulps. Pulp yields could suffer significantly after the post-treatment with enzyme (Simeonova *et al*. 2007). After the post-treatment bleaching they achieved a brightness gain of 1.5% with reduction in post color number of up to 15% and a decrease in hexenuronic acid by 10 to 15%.

Bajpai and Bajpai (1996) studied various cellulase-free commercial xylanases (Bleachzyme B, Bleachzyme F, VAI xylanase, Ecopulp-X200, Cartazyme HS-10, Pulpzyme HC, Pulpzyme HB, Irgazyme-10 and Irgazyme 40S) and used C_DEHD (C_D-Chlorination; E-Extraction; H-Hypochlorite; D-chlorine dioxide) bleaching sequence to compare their effect on the brightness gain with enzymatic process. Out of these nine enzymes Bleachzyme F, Cartazyme HS-10 and Irgazyme-10 showed higher gain of 1.5 units in brightness as compared to control while minimum gain of 0.8 units was observed in the case of Bleachzyme B. In another study done by Shatalov and Pereira (2009) for improvement in brightness of eucalyptus pulp, they reported that use of xylanase before three-stage hydrogen peroxide (QPPP) bleaching can also affected the brightness of *Eucalyptus globulus* pulp and Ecopulp again proved it over Pulpzymes in better

performance in terms of brightness gain. They also stated that number of hydrogen peroxide stages is also equally responsible in getting higher brightness after adding chelating agent.

2.5.6.6 Effect of xylanases on paper properties

Enzymatic modification of bleached pulp fiber structure is a very attractive means for improvement of pulp and paper properties. Enzymatic treatment with xylanases modifies the structure and characteristics of fibers, internal fibrillation, resulting in improved hydration, and delamination. These structural changes lead to alter or modify the final paper properties.

The enzyme-treated pulp showed unchanged or improved strength properties as compared to control pulps (Viikari *et al.* 1991 & 1993; Tolan *et al.* 1996; Tolan and Guenette 1997; Kim and Paik 2000) and was easier to beat in refining process than the untreated reference pulps (Wong *et al.* 1999). During the papermaking process, hemicelluloses play an important role to provide and maintain better strength properties in paper. Hemicelluloses provide better inter-fibril bonding to the fibres and have a favorable effect on improving the physical properties. Therefore during an enzymatic bleaching stage, xylan removal sometimes interferes with the strength of treated pulps as compared to untreated pulps. The decrease in tensile and burst strength (11 to 25% and 32 to 40% respectively) was more notable than that of tear strength (8.2 to 10.3%). Tear resistance is may be affected by the length of fibers and the bonding between them (Batalha *et al.* 2011). The tear resistance is the measurement of force required to tear the paper. Pulp refining can minimize the difference in strength properties; particularly tear strength, between enzyme-treated and untreated pulps (Wong *et al.* 1996; Roncero *et al.* 2005). Enzymatic elimination of the re-deposited xylan from the fibre surface may be the crucial step for superior external fibrillation of treated pulps (Roncero *et al.* 2005; Shatalov and Pereira 2008).

Bajpai and Bajpai (1996) used a commercial enzyme (Bleachzyme F) and studied its effect on physical strength properties of bamboo pulp. In first category they kept same chlorine dose in enzymatic bleaching process as compared to control and observed higher tensile and burst index during enzymatic bleaching as compared to control. They also obtained higher double fold of paper when used enzyme in the bleaching process but slight reduction in tear index was observed after using enzyme in pulp bleaching. In the

second category they reduced chlorine dioxide by 20% in enzymatic process as compared to control. An improvement in tensile and burst index was observed with slight loss in tear index in enzymatic process as compared to control. Double fold was also increased upon using enzyme in the process over control. It was also concluded by Shatalov and Pereira (2008), that physical strength properties could also be affected by the limited degradation of carbohydrates during the xylanase treated and in peroxide bleached pulps as compared to control using eucalyptus hardwood pulps.

A notable work on improving tensile strength of paper was done by Batalha *et al*. (2011). They worked on both ultrasonic processes and enzymatic treatments and reported their effect on the tensile strength of paper (Batalha *et al.* 2011). Tensile strength is basically related with utility and durability of the paper particularly in packaging papers that are subject to direct tension forces. The strength required for the sheet to withstand such forces is generated by inter-fibril linkages occurring during the formation of the paper. Such linkages cannot occur without the external fibrillation and collapse of the fibers. The xylanase is responsible for removing superficial hemicelluloses, which decrease the inter-fiber linkages. The tensile index, therefore, also decreases, as it is favored by increased inter-fibril linkages. During tensile tests, force-deformation relations were produced and, through these relations, the specific elastic modulus (MOE) and tensile energy absorption (TEA) properties were derived. In this study, it was also concluded that combined effect of ultrasonic and enzymatic treatments leads to increase in MOE and TEA by 48.0 and 12.1% respectively as compared to the initial pulp. It was also observed that opacity of paper was improved after the ultrasonic treatment when the ultrasonic treatment was applied before the xylanase treatment.

2.5.6.7 Effect of xylanases on the effluent characteristics

The kraft pulp bleaching is highly responsible for the generation of a huge volume of effluent in pulp and paper industries. Chlorine and its components are the main cause of pollutants generation during chlorine-based pulp bleaching process. Organochlorine compounds (AOX), contaminants are the major components within this effluent for pollutants generation (Vidal *et al.* 1997). Now industrialist form paper mills are looking for the alternative methods for bleaching process in order to minimize the impact of these hazardous effluents on the environment. In traditional methods, chlorine was the only solution to bleach the pulp but now the use of enzymes in pre-bleaching stages is more effective to improve effluent quality and to reduce the major pollutants by

reducing the amount of organochlorine compounds in the effluent (Faleiros 2008).Xylanase before the bleaching process is highly effective in reducing the use of chlorine or chlorine dioxide (Call and Mucke 1997). It can also lower the AOX percentage in the effluent by 25% while improving the optical properties of the pulp (Saleem and Akhtar 2002; Hart and Harry 2005; Atik *et al.* 2006; Manji 2006).

In 1991, it was studied and concluded that xylanase is also responsible for increasing the biochemical oxygen demand (BOD) of the filtrate by almost two times as compared to untreated pulp. Similarly, other parameters like total organic carbon (TOC) and chemical oxygen demand (COD) were also increased, and the ratio of BOD-to-COD was significantly higher for the xylanase pre-bleaching filtrates than non-treated pulps, indicating the increased biodegradability of the effluents (Senior and Hamilton 1991). Energy and aeration demand in treatment plant is increased by increasing organic matter content in the filtrates. Therefore, the final chemical oxygen demand of the treated effluents was higher in enzymatic bleaching sequences than in those generated in conventional bleaching sequences (Borges *et al.* 2010).

Xylanases affect the level of AOX by reducing the chlorine consumption during the bleaching process therefore it significantly lower AOX levels in its effluents as compared to the effluents generated from the conventional bleaching methods (Viikari *et al.* 1986). Shobhit *et al.* (2005) stated that xylanase pre-treatment helps in reducing the AOX level by 20-30%.

2.5.6.8 Effect of mill operations on xylanase performance

Industry infrastructures and mill operations affect the performance of chemicals and enzymes using in the process. Raw material type used in the process is the crucial step for the performance of xylanase enzyme. Methods of pulping and bleaching processes in the mills also affect the performance of xylanase (Tolan and Guenette 1997). Xylanase treatment is much more helpful in reducing the percentage of bleaching chemicals consumption and it is greater for hardwoods than for softwoods as the xylan content is greater in hardwood rather than softwood. Under favorable enzyme treatment conditions, 20% of chlorine reduction has been seen for hardwood while 15% for softwoods pulps only.

Digester performance during the cooking process decides the xylan content of the pulp. For example, sulfite pulping is not suitable for enhancing the bleaching efficiency

of the pulps with enzyme as it destroys most of the xylan during pulping process. In conventional kraft pulping, the xylan content depends strongly on the effective alkalinity. The type of bleaching stages and sequences that are being followed by the particular mill is also equally important for the maximum efficiency and better performance of the xylanase in enzymatic bleaching.

2.6 Lacunae

Use of huge amount of chlorine, chlorine components and alkali during the bleaching of kraft pulp in the pulp and paper industries generate bleach effluent with high levels of corrosive chloride and other organochlorine compounds. This type of effluents creates many problems in treatment plant and cannot be recycled back easily into the chemical recovery furnace. Effluent generated from these stages contains huge amounts of hazardous chemicals, which are known to have carcinogenic and mutagenic effects. Presently, regulatory agencies are very much concerned to protect the environment from these hazardous pollutants. The environmental effects of these chlorinated organic compounds have driven pulp and paper industries to seek out new alternative techniques and bleaching technologies that eliminate or minimize the consumption of these hazardous chemicals during the bleaching process.

Enzymes are very helpful and eco-friendly in nature and could be used as a better substitute for these hazardous chemicals in the pulp bleaching process. Xylanases bleaching process is a cost effective process as it helps in reducing the consumption of hazardous chemicals. Byproducts generated from biological reactions of microorganisms are generally non-hazardous in nature and easily bio-degradable. Therefore, enzymes produced *via* microbial sources have become alternatives to polluting chemical technologies.

Temperature and pH are the main and crucial factors in enzymatic bleaching process. Unbleached pulp coming from the brown stock washers carries higher pH and temperature. This high temperature and pH are favorable conditions for bleaching processes. But these requirements act as limiting factors for the use of enzyme in process. Now-a-days, such commercial enzymes are available in the market which can work at high pH and temperature. Still, an innovative approach needs be explored for the cost effective process with improvement of the pulp qualities at industrial level.

Keeping the lacunae in view, the study was focused at:

- Determining the comparative efficacy of commercially available xylanases in removing hexenuronic acid from hardwood pulps
- Optimization of xylanase treatment on different hardwood pulps and its impact on the properties of bleached pulp
- Optimization of bleaching sequence of the xylanase treated pulps

CHAPTER 3.0 MATERIALS AND METHODS

3.1 Enzyme

Four xylanases manufactured and gifted by Novozyme-India - two enzymes (EnzyA and EnzyB), Genencor (Dupont Genencor Sciences, USA) - EnzyC and Dyadic (USA) - EnzyD were taken for the study. Two different methods Bailey *et al.* (1992) and Ghose (1987) were used to measure xylanase and CMCase activities respectively in the above enzyme samples. Birch wood xylan and carboxymethyl cellulose (CMC) were used as substrates and release of reducing sugars (xylose and glucose) was measured for assaying the xylanase and CMCase activities respectively.

The proper dilutions and mixing of enzymes were made and used in the experiments by following the kneading mechanism. The untreated pulps; where enzyme was replaced with water treated in the similar way as xylanase-treated pulps to negligible the effect of process parameters. Different hardwood kraft pulps (acacia and eucalyptus) were used for the study and treated with the enzyme to see the efficacy of xylanase based on reduction in kappa number, pulp yield, and gain in brightness (%ISO) of the pulp. During the pre-treatment bleaching sequences, xylanase treatment (X stage) is given first to the pulps and then washed and subjected to different bleaching sequences. Efficacy of xylanases was also seen in intermediate and post-treatment bleaching sequences, where X-stage was given in between and after the bleaching sequences.

3.1.1 Xylanase assays

Xylanase activity was determined following range of dilutions using 0.05*M* Na-phosphate buffer. Different dilutions were used as shown in Annexure tables (Table 1.1-Table 1.8) as per their optimum activity at different pH and temperature and stored under refrigerated conditions. Each enzyme dilution was made using buffer and stored under refrigerated conditions. The di-nitrosalicylic acid (DNS) xylanase assay was used to determine xylanase activity (Bailey *et al.* 1992). This assay is used to detect reducing sugars, such as xylose, that are released during an enzyme reaction on a specific substrate. The activity measurements were carried out in replicates and expressed as IU/ml, which represents; one international unit of enzyme was defined as the amount of enzyme that released 1 µmole of xylose or glucose sugars per minute per mL of enzyme.

Representative standards were run concurrently in order to determine relative carbohydrate concentrations.

Substrate; 1.0% birch wood 4 O-methyl glucorono xylan (Sigma Aldrich) was prepared by adding 1.0 g of xylan in 80 ml 0.05*M* Na- phosphate buffer and kept at 60°C on a magnetic stirrer (with heating) for homogenous mixing. After mixing, the sample was allowed to cool with continued stirring for 30 min. On cooling, the sample was made to 100 ml with buffer 1.8 ml of substrate was added in reaction tube and kept at desired temperature (50 or 55°C) then added 200 µl enzyme and mixed it and kept this solution at desired temperature for 5 minutes. After exactly 5 min., the reaction was stopped by adding 3.0 ml of the DNS solution. Enzyme blank (200 µl of highest enzyme dilution) and xylose standards (200 µL of concentrations 2.0, 3.33, 5.0 and 10 µmol/ml) were added to incubated substrate along with addition of DNS. All of the test tubes were then placed in a boiling water bath for 5 minutes and then allowed to cool. The absorbance of each reaction mixture was measured using a UV spectrophotometer at a wavelength of 540 nm. Xylanase activity in IU/ml was calculated as follows;

Xylanase activity (IU/ml) = X* x DF (Dilution factor) / Incubation time

Where,

X = concentration at 540 nm

3.1.2 Determination of optimal pH for Xylanase activity

The DNS xylanase assay was carried out at pH 6.0, 7.0, 8.0 and 9.0 to determine optimal pH for xylanase activity. All pH adjustments were carried out using sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH). Enzyme blanks were run concurrently for each pH tested. In these experiments, the final pH of the reaction was used for evaluating optimal pH for the activity of each of the xylanases. EnzyA, EnzyB and EnzyC showed the maximum xylanase activity at pH 8.0 while EnzyD shows the maximum xylanase activity at pH 7.0 (Table 4.2). All four enzymes were used at pH 8.0 in enzyme (X) stage to simulate the pulp mill condition.

3.1.3 Optimum temperature for xylanase activity

All four enzymes were tested at 50 and 55°C. Except, EnzyB all other three enzymes (EnzyA, EnzyC and EnzyD) showed their maximum activity at 55°C while EnzyB showed its maximum activity at 50°C as shown in Table 4.2. All four enzymes were used at 55°C in X stage to simulate the pulp mill condition.

3.1.4 Activity of other enzymes

Cellulase activity was assayed using CMCase method by Ghose (1987). Substrate; 2.0% carboxymethyl-cellulose (Sigma Aldrich) was prepared in 0.05*M* sodium phosphate buffer. An incubation time of 30 min. was given before addition of DNS. Enzyme blank and glucose standard were added to incubated substrate following the addition of DNS. The absorbance of each reaction mixture was measured using a UV spectrophotometer at a wavelength of 540 nm. Despite use of much higher enzyme concentrations (100 times more concentrated than those used for the xylanase assay) negligible amount of cellulase activity was observed in these xylanase commercial enzymes. So, further optimization experiments were not done for optimal pH and temperature for the cellulase activity.

3.2 Raw materials and kraft pulps

Wood chips of eucalyptus (*E. globulus*) and acacia (*A. mangium*) were collected from different pulp and paper industries in India. Kraft unbleached pulps were made using lab-scale stationary digester for the study. To optimize the pulping conditions, cooking was done at different active alkali doses and for the different cooking time. Kraft pulps were produced of different kappa number in the range of 18 to 20 for the study.

Optimization of kraft pulping was done as shown in Table 4.3 (for eucalyptus) and Table 4.5 (for acacia). To achieve kappa no. 18-20, kraft cooking conditions were optimized for eucalyptus as in Table 4.4 with active alkali as Na_2O- 19.0%, Sulphidity- 24.21%, cooking temperature- 160°C and cooking time at 160°C- 180 minutes. Same approach was followed for acacia kraft pulps cooking conditions under optimized conditions as shown in Table 4.6, with active alkali as Na_2O is 19.5% and cooking time of 200 min. at 160°C, which were higher in acacia pulp production to reach at the same extent of kappa no.~18-20 than eucalyptus, other parameters were maintained same as of those applied for eucalyptus; Sulphidity- 24.21% and cooking temperature- 160°C. Bath ratio of 1:3 (solid to liquid) was maintained for producing both hardwood kraft pulps. Table 4.4 and 4.6 present the properties of the initial kraft pulp after cooking. This unbleached hardwood kraft pulp was used in further experiments.

3.3 Enzymatic bleaching sequences and conditions

In this work, enzyme (X) stage was used at pre, post and in intermediate stages of pulp bleaching. Three different bleaching sequences ($C_DE_PD_1D_2$, $D_0E_PD_1D_2$ and $D_0E_PD_1E_P$) were used in this study (where; C_D represents the chlorination stage with elemental chlorine and chlorine dioxide, D_0 represents chlorine dioxide stage, E_P indicates extraction stage with caustic and hydrogen peroxide and D_1 and D_2 represent first and second dioxide stages after extraction stage). All of the enzyme (X) and bleaching (C_D, D_0, E_P, D_1 and D_2) stages were done in heat sealable polyester bags in an appropriately heated water bath. All pH adjustments in pulp slurry were done using diluted acid (H_2SO_4) or alkali (NaOH).

3.3.1 Enzyme (X) stage

To simulate the paper mill conditions in brown stock washer pulps for enzyme stage, all experiments were performed at pH 8.0 by maintaining the reaction temperature of 55°C in pulp slurry and pulp consistency was maintained at 10% throughout the treatment process as discussed in Table 4.21. A 90 min treatment time was given to all the sets in enzyme stage. Enzyme doses were maintained at 0.1, 0.3 and 0.5 kgt^{-1} of pulp as demanded in the scope of approach planned and desired parameters of interest. The proper dilutions and mixing was done of enzymes by following kneading mechanism for their better use in further experiments. Untreated pulps were also prepared following similar process wherein enzyme was replaced with water. After the enzyme stage, pulp was washed with the known amount of the water and kept in oven at 105°C for overnight to know the amount of moisture in the pulp for further study. Pulp pads were also made in Buchner funnel for checking brightness of the X stage pulps. Kappa number was also checked as discussed the method in 3.4.1 section and viscosity was measured by viscosity capillary method as described in 3.4.3 section.

3.3.2 Chlorination (C_D) stage

Chlorination was carried out (Section 4.4), wherein nine different bleaching sequences were studied by incorporating enzyme stage at different position *viz*; pre ($XC_DE_PD_1D_2$) and post ($C_DE_PD_1D_2X$) where; X represents enzyme stage, C_D represents the chlorination stage with elemental chlorine and chlorine dioxide, D_0 represents chlorine dioxide stage, E_P indicates extraction stage with caustic and hydrogen peroxide

and D_1 and D_2 represent first and second dioxide stages after extraction stage. Chlorination of pulp sample was done in heat sealable polyester bags in pre-heated water bath set at 60°C, for 45 min., by maintaining the pulp consistency of 5%. Diluted acid ($4N$ H_2SO_4) was used for maintaining pH (1.8-2.2) of pulp slurry. Elemental chlorine was applied to the pulp on the basis of lignin amount present in the pulp sample. This lignin amount is measured by knowing kappa no. of the pulp. Elemental chlorine was calculated as per the equation given here (Hise 1996):

Elemental chlorine (%) = Kappa factor (KF)* x Kappa number

** Kappa factor is optimized on the basis of residual amount of chlorine after the reaction. In our studies we applied kappa factor 0.22 for the same chemicals charge and 0.187 after reducing the elemental chlorine by 15% (at reduced chemicals charge).*

3.3.2.1 Analysis of residual chlorine in C_D stage

Residual chlorine was checked after the completion of C_D stage to know the excess amount of chlorine given to pulp samples for treatment. 100 ml of chlorination stage effluent was collected in a 250 ml of Erlenmeyer flask; followed by addition of 10 ml each of 20% KI and 20% acetic acid solution and titrating the liberated iodine with 0.1N sodium thiosulphate solution to a starch end point (titer reading - V_2).

The concentration of chlorine in C-stage effluent in (g/l), was calculated as follows:

$$\textbf{Residual chlorine (g/l)} = \frac{V_2 \times N \times 35.5}{V_1}$$

Where;

V_2 = volume of sodium thiosulphate solution consumed, ml

N = normality of sodium thiosulphate solution

V_1 = volume of C-stage effluent taken, ml

35.5 = equivalent weight of chlorine

3.3.3 Chlorine dioxide (D_0) stage

D_0 is the first chlorine dioxide (D_0) stage which was applied before the extraction stage in bleaching process. D_0 stage was also simulated in heat sealable polyester bags in heated water bath with rest of the conditions as mentioned earlier in section 3.3.2.

Chlorine dioxide was calculated on the basis of elemental chlorine dose as the below equation;

Chlorine dioxide (%) = Elemental chlorine (%) / 2.63*

** 2.63 is the conversion figure as chlorine dioxide is 2.63 times as reactive as Cl_2, so it is needed to convert it on chlorine dioxide on chlorine basis.*

3.3.3.1 Analysis of residual chlorine dioxide in D_0 stage

Residual chlorine dioxide was checked after the completion of D_0 stage to know the excess amount of chlorine dioxide was applied to pulp samples for treatment. 25 ml of D-stage effluent was taken in Erlenmeyer flask (250 ml capacity); added 20% KI and 4.0*N* H_2SO_4 each 5 ml respectively and titrated the liberated iodine with 0.1*N* sodium thiosulphate solution to a starch end point and noted down the titer reading (V_2).
Calculated the concentration of chlorine dioxide in D-stage effluent in (g/l) as follows;

$$\textbf{Residual chlorine dioxide (g/l)} = \frac{V_2 \times N \times 13.5}{V_1}$$

Where;

V_2 = volume of sodium thiosulphate solution consumed, ml

N = normality of sodium thiosulphate solution

V_1 = volume of D-stage effluent taken, ml

13.5 = equivalent weight of chlorine dioxide

3.3.4 Extraction (E_P) stage

Extraction stage was carried out with alkali in the presence of hydrogen peroxide (H_2O_2). Extraction stage was also done in heat sealable polyester bags in heated water bath by maintaining the pulp consistency of 10% and kept in water bath for 120 min. Treatment temperature and pH were maintained 80°C and 10.5-11.0 respectively. 1*N* alkali (NaOH) was used to maintain pH of pulp slurry. Caustic addition was done on the basis of elemental/active chlorine demand in the bleaching process. For hardwood pulps (eucalyptus and acacia), it usually being divided by 0.4-0.5 of elemental/active chlorine as below equation;

Alkali (NaOH) amount (%) = Elemental/active chlorine (%) / 0.4-0.5

Hydrogen peroxide was also applied in the extraction stage for the bleaching of fibres. Hydrogen peroxide was kept in refrigerator and fresh dilutions were used in each experiment. It is thermodynamically unstable and its rate of decomposition increases with increase of temperature, pH and concentration. Hydrogen peroxide is used as strong oxidizing agent in the bleaching process. The dose of hydrogen peroxide was calculated in the optimization study by determining the residual hydrogen peroxide amount in the

pulps fixing the supplemental hydrogen peroxide to 0.5% as fixed in all the extraction stages during bleaching sequences.

3.3.4.1 Analysis of residual hydrogen peroxide in E_P stage

Residual hydrogen peroxide was checked after the completion of E_P stage to know the excess amount of H_2O_2 applied to pulp samples for treatment. 50 ml of H_2O_2 stage effluent was taken in Erlenmeyer flask (250 ml capacity) and added 20% KI solution and 4.0*N* H_2SO_4 as 5 and 10 ml respectively and 5 drops of ammonium molybdate. The liberated iodine was titrated with 0.1*N* sodium thiosulphate solution to a starch end point (titer reading-V_2).

The concentration of $H_2O_2$was calculated in bleach effluent (from P, E_P, E_{OP} -stages) in (g/l) as follows:

$$\textbf{Residual } H_2O_2 \text{ (g/l)} = \frac{V_2 \times N \times 17.008}{V_1}$$

Where;

V_2 = volume of sodium thiosulphate solution consumed

N = normality of sodium thiosulphate solution

V_1 = volume of H_2O_2 -stage effluent taken

17.008 = equivalent weight of H_2O_2

3.3.5 Chlorine dioxide (D_1 and D_2) stages

After the extraction stage in the bleaching process, D_1 and D_2 stages were given to the pulps. Chlorine dioxide stages were also done in heat sealable polyester bags in heated water bath by maintaining the pulp consistency of 10% and kept in water bath for 180 min. Treatment temperature and pH were maintained 75°C and 2.8-3.0 respectively. Diluted acid (4*N* H_2SO_4) was used to maintain pH of pulp slurry. In D_1 stage; chlorine dioxide was applied in the range of 0.7-0.8% and in the last chlorine dioxide (D_2) stage, it was applied as 0.3%. It was calculated in the optimization study by calculating the residual chlorine dioxide amount in the pulps.

3.3.5.1 Analysis of residual chlorine dioxide in D_1 and D_2 stage

Residual chlorine dioxide in D_1 and D_2 stage was calculated as discussed in section 3.3.3.1.

3.4 Pulp characterization

After each stages in enzymatic bleaching process, untreated and treated pulps were characterized for the different properties *viz*; kappa number, pulp yield/shrinkage, pulp viscosity, brightness (%ISO), CIE whiteness, tearing strength, bursting strength, post color number/brightness reversion and hexenuronic acid.

3.4.1 Kappa number

Kappa number is defined as the volume in milliliters of 0.1*N* potassium permanganate ($KMnO_4$) solution consumed by 1 g of moisture-free pulp under the specified conditions. Kappa number indicates hardness (lignin content) and bleachability (degree of delignification of pulp).Residual lignin was checked in the pulp samples collected after each stage of enzyme and bleaching process, using the micro-kappa or kappa number method.

For measuring kappa number, 4 g of dry weight pulp was taken and a pad was prepared by filtering the same using plastic Buchner funnel to avoid fiber losses from pulps. The pad was kept for air dry and torn to pieces for further use. The test samples were conditioned in normal temperature and humidity for at least 20 min and weighed. Weighed the required sample and dispersed in distilled water (700 ml) and agitation was applied to get undispersed fiber bundles and free of fiber clots for better mixing of chemicals. Reaction temperature was maintained as 25°C. Sample was continuously stirred at slow speed to avoid any interference of the air. A mixture of 100 ml of potassium permanganate solution was prepared in 100 ml of the sulfuric acid solution and quickly added to disintegrate the test specimen. The total volume was made up to 1 liter. After completing 10 min., added 20 ml of potassium iodide (KI) solution to stop the chemicals reactions. Sodium thiosulfate was used to titrate the residual iodine by adding a few drop of starch indicator. When sodium thiosulfate reading was obtained below or above the 50 ml then it was corrected as per the given table 3.1 to find the exact value of f factor. Therefore, results were corrected to 50% consumption of $KMnO_4$ added for kappa number testing. Control pulp was also tested for kappa number by following the same procedure. Kappa number was estimated as per TAPPI test method (T 236 om-99). Calculation for kappa number was done as follows;

$$K = \frac{p \times f}{W} \quad \text{and,} \quad p = \frac{(b - a)\, N}{0.1}$$

Where;

K = kappa number

p = amount of actual consumed 0.1N $KMnO_4$ by the pulp sample, ml

w = weight of moisture-free pulp sample, g

f = correction factor to 50% $KMnO_4$ consumption, depend on p value (see Table 3.1)

N = normality of the sodium thiosulfate

b = volume of $Na_2S_2O_3$ consumed in the blank, ml

a = volume of $Na_2S_2O_3$consumed by the test specimen, ml

Table 3.1 Value of correction factor

P +	0	1	2	3	4	5	6	7	8	9
30	0.958	0.960	0.962	0.964	0.966	0.968	0.970	0.973	0.957	0.977
40	0.979	0.981	0.983	0.985	0.987	0.989	0.991	0.994	0.996	0.998
50	1.000	1.002	1.004	1.006	1.009	1.011	1.013	1.015	1.017	1.019
60	1.022	1.024	1.026	1.028	1.030	1.033	1.035	1.037	1.039	1.042
70	1.044									

3.4.2 Pulp yield / Pulp shrinkage

Due to use of chemicals and enzymes in the pulp fibres, some loss in pulp yield or shrinkage was observed. To measure pulp yield loss, 10-15 g of uniformly mixed air-dried pulp samples were taken in triplicate and noted down the exact weight and kept in an oven at 105°C for overnight. Next day, all samples were taken out from oven in to the desicator and kept for cooling for some time. Took the weight of the oven dried samples and calculated the yield as follows;

$$\textit{Dryness (\%)} = (A \times 100) / B$$

$$\textit{Pulp yield (\%)} = (W \times \textit{Dryness of pulp}) / \textit{O.D. weight of pulps taken}$$

$$\textit{Shrinkage (\%)} = 100 - \textit{Pulp yield (\%)}$$

Where;

A = O.D. wt of pulp taken for dryness

B = A.D. wt of the pulp taken for dryness

W = Total A.D. wt. of the pulp

3.4.3 Pulp viscosity

Measurement of degree of polymerization of cellulose refers as viscosity. Viscosity gives an idea of cellulose degradation or decrease in molecular weight of cellulose during the pulping and/or bleaching process in papermaking. In this study, capillary viscometer method (TAPPI test method T 230 om-99) was used for the measurement of pulp viscosity (cp).

In capillary viscometer method, 0.5*M* cupriethylenediamine (CED) solvent was used for viscosity measurement in 0.5% cellulose solutions. To measure viscosity of pulp, 0.125 ± 0.005 g of oven-dry pulp in triplicate was weighed and added to beaker containing 25 ml of CED solution. The sample was stirred for about 15 minutes for complete dissolution. The lower bulb of the viscometer was filled with the solution and placed in the viscometer at 25°C temperature. On attaining the said temperature, the solution up drawn into the measuring leg of the viscometer with a suction bulb, and then allowed to drain down to wet the inner surfaces of the viscometer bulb. The efflux time was determined by drawing the liquid above the upper mark and the time required for the meniscus to pass between the two marks was recorded. The viscosity of the pulp solution was determined from the formula;

$$V = C \times t \times d$$

Where;

V = viscosity of CED solution, mPa.s (cp)

C = viscometer constant (known by calibration)

t = efflux time, sec.

d = density of the pulp slurry, g/cc

3.4.4 ISO brightness and CIE whiteness

Brightness is the measurement of percent reflectance of blue light while whiteness refers to diffusely reflect light on the paper. Brightness is measured at 457 nm wavelength whereas whiteness is measured at all wave lengths in visible spectrum. Diffuse reflectance is measured in the wavelength range of 400-520 nm with an effective wavelength of 457 nm by using a suitable filter set or an equivalent device and an instrument having diffuse illumination and perpendicular observation geometry. The measurements are made in terms of absolute reflectance factors.

Diffuse brightness of pulps was measured by utilizing an integrating sphere to provide diffuse illumination and perpendicular (0°) observation geometry designated in

optical terminology as *d*/0. With this geometry, specimen surface structure and azimuthal orientation have a negligible effect on brightness. This method was used to evaluate the diffuse blue reflectance factor (diffuse brightness) of pulp. The Buchner pads and handsheets were prepared for brightness and whiteness of treated and untreated pulp samples. The ISO brightness (Indian organization of standardization) and CIE whiteness (International Commission on Illumination) of pulp was measured using Konica Minolta (Model No.-CM 3600A) instrument. ISO Brightness and CIE whiteness were measured as per the TAPPI test methods T 452 om-02 (2002) and T 560 pm-96 (1996) respectively. Optical properties were measured at several points of the pulp matrix and the observations reported are mean of the measurements

3.4.5 Beating of pulp

Treated and untreated samples were beaten in PFI mills as per TAPPI test method T 248 sp-00 (2000). Pulp sample of 30.0 g oven dry weight was taken for each set. The pulps sample was soaked and softened to before the disintegration process. 15000 revolutions were given for disintegration of pulp slurry after adding distilled water at 20°C to make up the total volume of 2.0 liter. After disintegration, pulp sample was drained on a coarse filter paper to make 20% consistency of pulp slurry by using Buchner funnel and this process was repeated 2-3 times to avoid loss of fines. The pad was weighed and added distilled water to make a total weight of 300 g corresponding to a 10% pulp suspension. This pulp stock was transferred to the beater in this way that no pulp remained on the bottom of beater housing. After closing the cover of beater the beating pressure was gradually applied as required and the revolutions of the roll was started. On the basis of desired pulp freeness, number of revolutions was given. The beating was discontinued by removing pressure from the roll. The pulp was now ready for freeness determination and preparation of handsheets for further testing.

3.4.6 Freeness of pulp (Canadian standard method)

The freeness of pulp is a measurement of the rate at which a dilute suspension of pulp, drained through a fibre mat, which forms a mat of fibres during the test, on a perforated screen plate. The filtrate is discharged into a funnel, which has a bottom and side orifice. CSF number is the filtrate volume expressed in ml that received from the side orifice of the CSF tester.

Freeness of the test sample was checked by taking 3 g (od. weight) in 1 L total volume. Deionized water was used to maintain 0.3% consistency and temperature 20 ± 3°C during the test. The stock was thoroughly stirred in the bucket to ensure a homogeneous mix. One liter of sample was mixed thoroughly and poured into the chamber. The top of the chamber was closed air tight and the bottom of chamber opened After 5 sec. of complete addition of sample, the air-cock was opened quickly in a single motion. When the discharge from the side orifice had ceased, the volume discharged was recorded to the maximum accuracy possible. CSF was determined as per the TAPPI test method T 227 om-94 (1994).

3.4.7 Hand Sheets Preparation

Handsheets were prepared for the testing of reflectance and physical strength properties of bleached or unbleached pulp whose fibres were equally dispersed in water. Former handsheet was used for making handsheets by sheet machine procedure. This method is the best for producing handsheets with smooth and reproducible surface for measuring reflectance during measurement of optical properties.

For making handsheets, 4.0 g (o.d) pulp samples were weighed in a beaker and 1000 ml distilled water was added. The pulp slurry was disintegrated for about 5 min. (15000 revolutions) till the fibres get dispersed. The pulp suspension was mixed properly and poured on to the sheet machine. The pulp slurry was stirred across the deckle until the fibres were distributed uniformly. After 3-4 sec., the drain valve was opened and the water was drained off. The sheet thus formed was picked from the wire using blotters after pressing from the couch roll. The sheet was then pressed under high pressure in hydraulic sheet pressure machine. The press was covered and tightened and the pressure was raised to 50 psi for 5 and 3 minutes respectively by changing the blotters in between both pressing. At the end, the pressure was released and sheets with blotters were removed out from the pressing machine. The handsheets were then dried in drying rings. After the sheet dried, the sheets were removed from drying rings then stored in a conditioned room for optical and physical testing. Handsheets were prepared with handsheets former as per the TAPPI test method T 205 sp-02 (2002).

3.4.8 Tearing strength

Internal tearing resistance is the force perpendicular to the plane of the paper sheet required to tear multiple plies through a specified distance after the tear has been

started. An Elmendorf-type tearing tester was used and the result was quoted in mN/ply. The Elmendorf-type tester is a pendulum that applies the force and measures a loss in the potential energy of the pendulum equating to the work done in tearing the paper.

Tearing resistance depends on the degree of fiber refining; and is related to inter fiber bonding, the fiber strength, the fiber length, the quality and quantity of fillers used. Among of them length and bonding of fibers are the most important factors. Longer fibers increase the tear strength because it is able to allocate the pressure over the more fiber surface area and more bonds between the fibers; whereas the stress will be less on the smaller region in short fibers. The direction of the fiber is another important parameter for tear strength. It is larger in lateral direction of the fiber than in longitudinal direction. Appropriate refining increases the tearing strength, whereas insufficient and extra refining decreases.

Tearing strength was measured according to T 414 om-98 (1998), in which multiple sheets of the sample material were torn together through a fixed distance using the pendulum. Tearing strength was measured using tearing tester by raising the pendulum sector to its initial position and setting the pointer against its stop position. The handsheet was clamped with the bottom edge using approximately the same pressure on both clamps. The pendulum was quickly depressed as far as possible to release the pendulum back. The pendulum stop was held until after the tear completed and the pendulum was caught on the return swing without disturbing the position of the pointer. Number of plies was kept same for all the samples. The scale readings were recorded and the tear index was calculated as follows;

$$\textit{Tear index } (mN.m^2/g) = \textit{Tear strength* } (mN) / \textit{Grammage } (g/m^2)$$

Tearing strength (mN) = (16 x 9.8 x average scale reading) / number of plies

3.4.9 Bursting strength

Bursting strength was determined as per the TAPPI test method T 403 om-97 (1997), and is defined as the maximum hydrostatic pressure required to produce a rupture of the material when a controlled and constantly increasing pressure is applied through a rubber diaphragm. Bursting strength depends on basis weight of paper.

To determine the same, handsheet was placed in clamps, overlapping the specimen at all points. The pressure gauge was set at zero and the hydrostatic pressure was applied as specified until the specimen ruptured. The maximum pressure applied at

the time of rupturing of the handsheet was recorded. To standardize the bursting strength for various handsheets, bursting strength is measured as;

Burst Index (kN/g) = Bursting Strength (kPa) / Grammage (g/m^2)

3.4.10 Post color number (Brightness reversion)

Post color number is determined in the measurement of pulp and paper color reversion before and after heat aging. It is the way of expressing the brightness loss independent of the original brightness. The brightness reversion of the pulp was estimated in terms of post color (PC) number according to TAPPI test method T 260 om-85 (1985).

The brightness of pulp specimen was measured before and after 4 h exposure of the pulp at high temperature (105°C) in oven and results are reported either as loss in brightness or as a post color number. Calculation of post color number was carried out following the equations given below;

PC number = 100 (K/S* after aging - K/S* before aging)

Where; K/S = $(1-R_\infty)^2 / 2R_\infty$

Where; R approaches R_∞ as the pulp pad becomes thick, i.e.., 566 g/m^2

R = Reflectance (brightness)

K = Absorption coefficient

S = Scattering coefficient

3.4.11 Hexenuronic acid (Hex-A)

Pulp samples were collected for both treated and untreated pulps at each stage of enzymatic bleaching. Hex-A profile was seen during the enzymatic ECF bleaching of eucalyptus hardwood pulps at same and reduced chemicals charge. The hydrolyzed product of Hex-A absorbs UV light therefore, it can be determined by UV-spectroscopy. HUT method (Tenkanen *et al*. 1999) was used for the determination of Hexenuronic acids (mmol/kg) in the pulps sample. As per this method, Hex-A groups are selectively converted to furan derivatives (5-carboxy-2-furaldehyde and 2-furoic acid) and formic acid (Vuorinen 1999), which were than quantified using UV-visible spectrometer. In the UV spectra, two absorption maxima appeared at 245 and 285 nm, corresponding to 5-carboxy-2-furaldehyde and 2-furoic acid, respectively.

In this method, combination of two buffers was used as Solution A (~ 0.01 formic acid) and Solution B (~ 0.01*M* Sodium formate) mixed in a fixed ratio of 2:1 (Solution A: Solution B). After preparation of the buffer solutions, 3 g o.d weight of pulp samples was taken in 100 ml of the above buffer. The sample was disintegrated and loaded to the bombs by removing all the content of sample with 50 ml of buffer. Nitrogen (N_2) gas was passed to the sample in bombs for creating the inert atmosphere inside the bombs and the bombs were loaded to the digester at 110°C for 60 minutes. After completing the digestion, the sample was carefully removed with the help of deionized water and then diluted to 1 liter by filtering the sample with Buckner funnel and the absorbance was taken at 243 nm. Hex-A calculated as follows;

$$\textit{Hexenuronic acid (mmol/kg)} = \frac{\textit{Absorbance at 243 nm} \times 10^6}{10900}$$

3.5 Filtrate characterization

Bleaching effluents were characterized for BOD, COD and AOX for treated and untreated samples.

3.5.1 Preparation of filtrate samples

Required amount of filtrates from all bleaching stages *viz*; chlorine (C_D), chlorine dioxide (D_0, D_1 and D_2) and hydrogen peroxide (E_P) were collected and titrated to determine the level of residual chlorine, chlorine dioxide and hydrogen peroxide respectively as discussed in section 3.3. The control was analyzed simultaneously with enzyme treated samples. Bleach effluents samples were also taken after each bleaching stage for effluent characterization and pollution load. Due to consistency and amount of effluent released are different at each stages, mixed effluent of approximately 250 mL of all bleaching stages (C_D/D_0, E_P, D_1 and D_2) was collected in the ratio of 2:1:0.5:0.5 and checked for BOD and COD.

3.5.2 Biochemical/Biological oxygen demand (BOD)

The biochemical oxygen demand (BOD) is the measurement of degradable organic material present in a water sample and can be defined as the amount of oxygen required by the microorganisms in stabilizing the biologically degraded organic matter under aerobic conditions. The standard test condition includes incubating the sample in an air tight bottle, in dark at a specified temperature for a specific time.

Bleach effluent were collected during each stage of bleaching process of both treated and untreated samples for the BOD test. Samples were collected in clean plastic or glass bottles. Most reliable results were obtained when samples were analyzed as soon as possible after collection. Precautions were maintained to avoid excessive agitation or prolonged contact with air.

BOD was determined in bleach effluents by following process; first step was preparing of the dilution water by adding 1 ml of $MgSO_4$, $FeCl_3$, phosphate buffer and $CaCl_2$ solutions per liter of water and saturated with dissolved oxygen by aerating with organic-free filtered air for 8-12 hrs. Samples were added in the BOD bottle of unknown capacity. Filled the BOD bottle by siphoning to avoid air bubble and placed the stopper and mixed well. Prepared four bottles of same dilution as required in titrimetric iodometric methods for DO measurement then determined the initial DO of two bottles. Next step was of determination of initial dissolved oxygen just after filling of BOD bottle with diluted samples. Water blank was used to check cleanliness of incubation bottles and quality of unseeded dilution water. Blank bottle was incubated without sample i.e. dilution water only, together with each batch of sample. Also used 2% glucose-glutamic acid in BOD bottle and filled the BOD bottle with dilution water by siphoning. Mixed well and determined the initial DO of one set by azide modification of the iodometric method. Incubated the other set in the incubation at 27°C for 3 days. After 3 days determined the final DO was measured. BOD ($kgtp^{-1}$) were characterized using and APHA 5210 methods to measure of degradable organic material present in a sample and can be defined as the amount of oxygen required by the microorganisms in stabilizing the biologically degraded organic matter under aerobic conditions. BOD was calculated as follows;

When sample was undiluted

$$\textbf{\textit{BOD, mgl}}^{-1} = \textbf{\textit{DO before incubation - DO after incubation,}}$$

When unseeded dilution water is used,

$$\textbf{\textit{BOD, mgl}}^{-1} = \textbf{\textit{(D}}_1\textbf{\textit{-D}}_2\textbf{\textit{ / P) x 1000}}$$

Where;

D_1 = initial dissolved oxygen of diluted/ standard, mg/l

D_2 = final dissolved oxygen of diluted/ standard incubation, mg/l

P = percentage dilution of sample (sample volume in ml / 10)

3.5.3 Chemical oxygen demand (COD)

The chemical oxygen demand (COD) is defined as a determination of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant.

Bleach effluent were also collected during each stage of bleaching process of treated and untreated both samples for the COD test. Samples were collected in glass bottles and tested immediate using open reflux method. The open reflux method is suitable for a wide range of wastes where a large sample size is preferred. Samples were kept in COD digestor, after completing digestion process, ferrous ammonium sulfate was used to titrate the residual amount of potassium dichromate to quantify the consumed amount of potassium dichromate and therefore the oxidizable organic matter was measured in terms of equivalent oxygen.

COD ($kgtp^{-1}$) was determined using APHA 5220, to measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant. For estimation of COD amount in bleach effluent following procedure was followed;

100 ml of sample was taken and homogenized for 30 seconds in a blender and turned on the COD digestor, preheated to 150°C and placed 50.0 ml sample suitably diluted in a refluxing flask of 500 ml capacity. Several glass beads were added with 1 g of $HgSO_4$, and 5 ml of H_2SO_4 reagent. Further added 25.0 ml 0.0417*M* $K_2Cr_2O_7$ solution and mixed. After cooling, added remaining sulfuric acid reagent (70 ml) through upper end of the condenser. Digestion of samples was carried out for 2 hrs. in digestor. After digestion of 2 hrs., disconnected reflux condenser and cooled it to room temperature and titrated excess $K_2Cr_2O_7$ with FAS using 2-3 drops ferroin indicator till reddish brown end point achieved. In the same way blank was refluxed and titrated. COD was calculated as follows;

$$\textit{COD, mgl}^{-1} = \{(A-B) \times M \times 8000\} / \textit{ml of sample}$$

Where;

A = amount of ferrous ammonium sulfate consumed for blank, ml

B = amount of ferrous ammonium sulfate consumed for sample, ml

M = Molarity of ferrous ammonium sulfate

Chemical oxygen demand (COD) was calculated in kgt^{-1} as follows;

$$\textit{COD (kgtp}^{-1}) = (R \times F \times V) / 1000$$

Where;

R = COD reading, mg/l

F = dilution factor

V = effluent volume, m^3 /TP V = effluent volume, m^3 /TP

3.5.4 Adsorbed organic halides (AOX)

AOX is the amount of the halogens (chlorine and bromine) contained in organic compounds determined as chloride. The bleach effluents were collected from each stage of bleaching process in glass bottles and 0.5 ml of sodium sulfite solution (0.2*M*) was added to 100 ml of sample immediately. The pH of the samples was adjusted in between 1.5 and 2.0 with nitric acid and it was allowed to stand for 8 hrs. for further analysis.

Acidification of the water sample with nitric acid was required first then stripping of the water sample and combustion of volatile organic halogens in a separate step was also required. Adsorption on activated carbon of the organic substrate in the water sample was done. Sodium nitrate solution was used for the displacement of the inorganic halogen by rinsing. Combustion of the loaded carbon in an oxygen stream was preceded followed by the absorption of the halogen halides and determination of their mass concentration done by Micro Coulometry (Micro Coulometry is capable of determining 1 μg of chlorine with a standard deviation of less than 10% or an equivalent device to detect the chloride ions). AOX of effluents was measured using a standard procedure mentioned above using ThermoFisher Scientific instrument (Model No.-ECS 1200).

CHAPTER 4.0 RESULTS AND DISCUSSION

4.1 Characterization of four commercial xylanases

4.1.1 Background

Four xylanases manufactured and gifted by Novozyme-India, Genencor (Dupont Genencor Sciences, USA) and Dyadic (USA) were taken for the study. Enzyme codes are used for the study as shown in Table 4.1 with their physical appearance.

Table 4.1 Enzyme coding

Source	Product code	Code used	Physical appearance	
			State	Color
Novozyme	NS 51024	EnzyA	Liquid	Pale
	NS 51025	EnzyB		Yellowish
DuPont Genencor Sciences	Optimase CX72 L	EnzyC		Amber
Dyadic	Fiberzyme LBL conc.	EnzyD		Amber

Before testing their effectiveness on kraft pulp, the four commercial xylanases were characterized for their xylanase activities. All enzymes were tested over a dilution range to determine xylanase activity in a buffered system using 1% birch wood xylan. An appropriate dilution was chosen for each enzyme to evaluate the optimal pH and optimum temperature for xylanase activity. All commercial enzymes were marketed as having negligible contaminating cellulase. This determination is important as the presence of cellulase can cause significant damage to the pulp fibers, decreasing the strength and final pulp quality by destroying the long cellulose chains (Bajpai *et al*. 1993).

4.1.2 Xylanase assays

A method outlined by Bailey *et al*. (1992) was used to determine the activity of xylanases. Xylanase assays were done at two different reaction temperature; 50 and 55 ^{o}C and four different reaction pH; 6.0, 7.0, 8.0 and 9.0 for the optimization of optimum pH and temperature. *Further information about their characteristics has not been provided by sources due to commercial confidentiality.*

The absorbance measured at 540 nm for making the xylose standard curve for calculating the xylanase activity in the enzymes samples. Xylanase activities were measured at two dilutions and 5 minutes of incubation time with DNS. Detailed data are presented in the tables shown in Annexure.

4.1.3 Summary of xylanase assays

Using the activity assays with birch wood xylan as the substrate, EnzyC was found to have the most concentrated xylanase activity (46876±226 IU/ml), followed by EnzyA (19192±553 IU/ml), EnzyB (14355±399 IU/ml) and EnzyD (7564±245 IU/ml). The four commercial enzymes have different optimal pH's for xylanase activity (Table 4.2). EnzyA and EnzyC were found to have an optimal pH of about 8 for xylanase activity on buffered isolated birch wood xylan at 55°C. The optimal pHs for xylanase activity in EnzyB and EnzyD activities in a buffered solution were 8.0 and 7.0 respectively at 50°C and 55°C respectively. After determining the optimal pH and temperature ranges, it was decided that pH 8 would be the most representative for studying the four commercial enzymes at 55°C, which are the most favourable conditions for the kraft pulps coming from brown stock washer for bleaching process without adding extra amount of acid or alkali to change the pH of pulp slurry in enzyme treatment stage.

Table 4.2 Summary of xylanase assays optimization for EnzyA, EnzyB, EnzyC and EnzyD

Temperature (°C)	Enzyme	pH 6.0	pH 7.0	pH 8.0	pH 9.0
50°C	EnzyA	9021±68	11331±202	17044±178	10556±361
	EnzyB	4891±149	10441±238	**14355**±399	13232±550
	EnzyC	10850±759	18979±353	35312±441	33622±229
	EnzyD	4431±207	5177±75	4891±240	926±69
55°C	EnzyA	12156±235	15925±270	**19192**±553	12370±256
	EnzyB	4266±105	8211±470	14123±350	13133±163
	EnzyC	18556±752	21719±625	**46876**±226	39476±997
	EnzyD	5920±221	**7564**±245	5315±59	1414±79

It is probably not correct to directly compare the activity on isolated xylan to that of the xylan within the pulp fiber which is closely associated with lignin, cellulose and other hemicelluloses in the fiber matrix. For this reason, it is difficult to assume that the activity determined for the xylanases on the isolated substrate is the same as that achieved on the pulp. Furthermore, there is often a shift in the pH of the pulp mixture during the 90 minutes of xylanase treatment that is not seen during the 5 minutes DNS assay using a buffered system to determine the enzyme activities. It is probable that a shift of 1 pH unit will affect the activity of the xylanase over the 90 minutes of reaction time with pulps. Although it is recognized that there are drawbacks to using an isolated xylan substrate as a means of determining enzyme activity, it is generally known that this is one of the important and well accepted ways to standardize the xylanase loadings used for laboratory studies.

4.1.4 Enzyme stability

Enzymes were stored in refrigerator (4^0C) to minimize their activity loss with time. Stability of enzyme was checked before each enzyme stage and stability drop were observed not more than 5% during 6 months of time period. After 6 months fresh enzyme products were used for each time. Enzymes were charged on the basis of percentage or gram per ton of pulp to simulate the industrial process conditions for enzyme dosing.

4.2 Optimization of cooking conditions

4.2.1 Optimization of cooking conditions for eucalyptus pulp

Optimization studies were carried out to get kappa number in the range of 18 to 20 using eucalyptus hardwood kraft pulps. Pulping results at different conditions of active alkali are presented in Table 4.3. The requirement of active alkali to reach kappa numbers of 23.1, 20.9 and 19.7 is 17.0%, 18.0% and 19.0% respectively. This indicates that high active alkali amount leads to higher reduction of lignin components from the pulp fibres, therefore reduced more kappa number of kraft pulps. The unbleached yields are 47.8, 46.9 and 46.3% for 23.1, 20.9 and 19.7 kappa pulps respectively. High kappa pulps also generate more rejects, rejects generation were 1.21, 0.87 and 0.36% in 23.1, 20.9 and 19.7 kappa pulps respectively.

Table 4.3 Pulping for eucalyptus to Kappa No. ~ 18-20

Parameter	Set 1	Set 2	Set 3
Active alkali (AA) as Na_2O, %	17.0	18.0	19.0
Kappa no.	23.1	20.9	19.7
Unbleached yield, %	47.8	46.9	46.3
Rejects, %	1.21	0.87	0.36
Unbleached brightness, % ISO	24.4	26.9	28.6
Free alkali as Na_2O (g/l) at 20% solids	9.5	8.9	8.0
Unbleached viscosity, cp	14.3	13.6	12.8
Sulphidity- 24.21%; cooking temperature- 160°C; cooking time- 180 min.; bath ratio- 1:3			

For further study, experiments were performed at optimized conditions of cooking (AA~19.0%; cooking temperature~160°C; cooking time~180 min.). The bulk production of eucalyptus kraft pulps was done for enzyme treatment and ECF bleaching. Table 4.4 shows the requirement of active alkali is 19.0% to reach kappa numbers in the range of 18-20. The unbleached yields are 46.6, 46.1 and 46.0% for 19.8, 19.7 and 19.3 kappa pulps respectively. Here the rejects generations were 0.42, 0.35 and 0.33% in 19.8, 19.7 and 19.3 kappa pulps respectively.

Table 4.4 Pulping for eucalyptus to Kappa No. ~ 18-20 at optimized conditions

Parameter	Mean	S.D
Kappa no.	19.6	$\pm$0.26
Unbleached yield, %	46.2	$\pm$0.32
Rejects, %	0.37	$\pm$0.05
Unbleached brightness, % ISO	28.3	$\pm$0.15
Free alkali as Na_2O (g/l) at 20% solids	7.9	$\pm$0.85
Unbleached viscosity, cp	12.8	$\pm$0.10
AA as Na_2O- 19.0%; sulphidity- 24.21%; cooking temperature- 160°C; cooking time- 180 min.; bath ratio- 1:3		

4.2.2 Optimization of cooking conditions for Acacia pulp

Optimization studies were carried out to get kappa no. in the range of 18-20 for acacia hardwood kraft pulps. Pulping results at different conditions of active alkali are

presented in Tables 4.5. The requirement of active alkali to reach kappa numbers of 23.2, 20.9 and 19.7 is 17.5%, 18.5% and 19.5% respectively. The unbleached yields are 50.6, 49.8 and 49.2% for 23.2, 20.9 and 19.7 kappa pulps respectively. Rejects generation are obtained lesser then eucalyptus kraft pulps, rejects generation are 0.42, 0.23 and 0.11% in 23.2, 20.9 and 19.7 kappa pulps respectively.

Table 4.5 Pulping for acacia to Kappa No. ~ 18-20

Parameter	Set 1	Set 2	Set 3
Active alkali as Na_2O, %	17.5	18.5	19.5
Kappa no.	23.2	20.9	19.7
Unbleached yield, %	50.6	49.8	49.2
Rejects, %	0.42	0.23	0.11
Unbleached brightness, % ISO	24.0	26.2	28.4
Free alkali as Na_2O (g/l) at 20% solids	6.9	6.7	6.4
Unbleached viscosity, cp	15.6	14.7	13.6
Sulphidity-24.21%; cooking temperature- 160°C; cooking time- 200 min.; bath ratio- 1:3			

For further study, experiments were performed at optimized conditions of cooking (AA~19.5%; cooking temperature~160°C; cooking time~200 min.). The bulk production of acacia kraft pulps was done for enzyme treatment and ECF bleaching.

Table 4.6 Pulping for acacia to Kappa No. ~ 18-20 at optimized conditions

Parameter	Mean	S.D
Kappa no.	20.1	$\pm$0.20
Unbleached yield, %	49.23	$\pm$0.15
Rejects, %	0.16	$\pm$0.07
Unbleached brightness, % ISO	28.1	$\pm$0.20
Free alkali as Na_2O (g/l) at 20% solids	6.9	$\pm$0.15
Unbleached viscosity, cp	13.4	$\pm$0.21
AA as Na_2O- 19.5%; sulphidity- 24.21%; cooking temperature- 160°C; cooking time- 200 min.; bath ratio- 1:3		

Table 4.6 shows the requirement of active alkali is 19.5% to reach kappa numbers~19. The unbleached yields are 49.4, 49.1 and 49.2% for 20.3, 19.9 and 20.1 kappa pulps respectively. Rejects were comparable in all sets. In support of our results, Afrida *et al*. (2009) stated in their article that *Acacia spp.* are very suitable for the production of hardwood kraft pulp due to generation of woodchips with high quality and gives high unbleached pulp yield of 50-52% in kraft cooking process.

Solid vs. liquid bath ratio, sulphidity and cooking temperature were maintained same for both eucalyptus and acacia hardwood for the optimization of pulping conditions to get the same kappa number. Table 4.3 and 4.5 indicate that AA demand for acacia hardwood is more than the eucalyptus hardwood to achieve kappa number around 20. Demand of cooking time is also more in acacia than eucalyptus hardwood for making of same kappa number pulp. But the fibre recovery was better in acacia than eucalyptus due to less rejects remained after cooking of acacia hardwood. Cellulose protection was also found better in cooking of acacia than eucalyptus for the same kappa number pulps.

4.3 Optimization of xylanase treatment on different hardwood pulps and its impact on the properties of bleached pulp

Eucalyptus and acacia hardwood kraft pulps were used for the optimization of xylanase treatment. Screening of these four enzymes (EnzyA, EnzyB, EnzyC and EnzyD) was done on the basis of kappa number reduction, the pulp yield, and gain in brightness in enzyme stage. After screening of commercial xylanases, enzyme treatment was given with the enzyme giving optimal results (EnzyC) for further ECF bleaching ($D_0E_PD_1D_2$) process to see the impact of the enzyme stage on brightness and whiteness along with the physical strength properties of the bleached pulps at equivalent as well as at reduced kappa factor.

4.3.1 Screening of commercial xylanases

Screening of commercial xylanases (EnzyA, EnzyB, EnzyC and EnzyD) was done on the basis of their performance towards the hardwood kraft pulps at enzyme (X) stage. Same process conditions *viz*; incubation pH (8.0) and temperature (55ºC) were given to all the enzymes for the comparative study. Effect of all four enzymes at 0.1, 0.3 and 0.5 kgt^{-1} doses were observed for both hardwood pulps (Tables 4.7 and 4.8). Enzyme solutions were used in the experiments after making proper dilutions and mixing it by

following the kneading mechanism. Untreated pulp (where no enzyme was added and replaced with water) was also treated in the similar way as the xylanase-treated pulp in each experiment. After X-stage, the treated and untreated both pulps were washed with the same amount of water and subjected to elemental chlorine free bleaching process.

Table 4.7 and 4.8 indicate that out of these four commercial enzymes, EnzyC is giving better results in enzyme stage towards pulp yield, kappa no. reduction and brightness gain. Therefore, enzyme treatment with EnzyC was given (Table 4.9) to produce pulp in bulk amount for carried out to ECF bleaching to see its impact on optical and physical properties at equivalent and reduced kappa factor for eucalyptus and acacia kraft pulps.

Table 4.7 Enzymatic treatment of eucalyptus hardwood pulp

Enzyme	Set	Enzyme Dose, kgt^{-1}	End pH	End Kappa No.	End Brightness, %ISO	Pulp yield, %
EnzyA	Control	---	8.05	19.3±0.30	28.6±0.38	99.7±0.15
	1	0.1	7.79	19.0±0.31	29.5±0.35	99.3±0.26
	2	0.3	7.72	18.6±0.15	29.8±0.57	99.1±0.15
	3	0.5	7.70	18.0±0.06	30.4±0.20	98.8±0.21
EnzyB	Control	---	8.05	19.3±0.30	28.6±0.38	99.7±0.15
	1	0.1	7.81	18.9±0.38	29.6±0.45	99.5±0.06
	2	0.3	7.78	18.4±0.26	30.0±0.44	99.4±0.32
	3	0.5	7.75	17.8±0.35	30.7±0.31	99.1±0.21
EnzyC	Control	---	8.05	19.3±0.30	28.6±0.38	99.7±0.15
	1	0.1	7.96	18.6±0.15	30.7±0.46	99.6±0.12
	2	0.3	7.94	17.9±0.32	31.1±0.40	99.5±0.23
	3	0.5	7.82	17.5±0.26	31.4±0.32	99.3±0.06
EnzyD	Control	---	8.05	19.3±0.30	28.6±0.38	99.7±0.15
	1	0.1	7.93	19.0±0.55	30.2±0.40	99.5±0.25
	2	0.3	7.87	18.4±0.40	30.5±0.44	99.3±0.23
	3	0.5	7.76	17.9±0.10	30.9±0.49	99.2±0.10

Reaction time- 90 min.; consistency- 10.0 %; Reaction temp.- 55.0°C; Initial pH- 8.0; Initial Kappa No.- 19.6; Initial Brightness- 28.3 %ISO

Table 4.8 Enzymatic treatment of acacia hardwood pulp

Enzyme	Set	Enzyme Dose, kgt^{-1}	End pH	End Kappa No.	End Brightness, %ISO	Pulp yield, %
EnzyA	Control	---	8.25	19.5±0.25	28.6±0.31	99.8±0.17
	1	0.1	7.92	19.1±0.10	29.8±0.21	99.1±0.32
	2	0.3	7.87	19.0±0.21	30.3±0.25	99.0±0.20
	3	0.5	7.81	18.8±0.15	30.5±0.42	98.7±0.17
EnzyB	Control	---	8.25	19.5±0.25	28.6±0.31	99.8±0.17
	1	0.1	7.90	18.8±0.06	30.2±0.15	99.3±0.06
	2	0.3	7.82	18.4±0.26	30.6±0.21	99.1±0.26
	3	0.5	7.86	18.1±0.12	30.7±0.23	99.0±0.16
EnzyC	Control	---	8.25	19.5±0.25	28.6±0.31	99.8±0.17
	1	0.1	7.93	18.6±0.35	30.9±0.17	99.5±0.21
	2	0.3	7.83	18.0±0.55	31.2±0.40	99.4±0.06
	3	0.5	7.80	17.6±0.12	31.4±0.40	99.2±0.21
EnzyD	Control	---	8.25	19.5±0.25	28.6±0.31	99.8±0.17
	1	0.1	7.92	18.7±0.50	30.5±0.20	99.3±0.15
	2	0.3	7.81	18.2±0.29	30.8±0.45	99.2±0.20
	3	0.5	7.85	17.8±0.36	31.0±0.25	99.0±0.06

Reaction time- 90 min.; consistency- 10.0%; Reaction temp.- 55°C; Initial pH- 8.0; Initial Kappa No.- 20.1; Initial Brightness- 28.1%ISO

4.3.2 Effectiveness of Optimase CX 72 L (EnzyC) for improving optical properties in eucalyptus and acacia hardwood kraft pulps

4.3.2.1 Enzyme treatment of eucalyptus hardwood pulps at optimized conditions prior to ECF bleaching

Enzyme-treated pulps showed a reduction in kappa number of 4.1, 7.8, and 9.8% for eucalyptus pulp at xylanase doses of 0.1, 0.3, and 0.5 kgt^{-1} compared with the control (Table 4.9).

These observations on the reduction in kappa number by xylanase were also supported by Thakur *et al*. (2012), wherein Pulpzyme HC was used and a 4.2% reduction in kappa number of eucalyptus pulp was achieved. Brightness gains of 2.3, 2.4, and 2.8 units were observed in eucalyptus pulp at enzyme doses of 0.1, 0.3, and 0.5 kgt^{-1} compared with the control. Shatalov and Pereira (2008) obtained 1.2 to 1.6 units of

brightness gain after xylanase treatment on eucalyptus hardwood pulp, which is relatively lower than the observations obtained in the present study. A minor loss in yield was also observed in eucalyptus wood pulps in the range of 0.2% to 0.6% with xylanase doses of 0.1, 0.3, and 0.5 kgt^{-1} compared with the control (Table 4.9).

Table 4.9 Enzymatic treatment of Eucalyptus pulp with EnzyC

Parameter	Control	Set 1	Set 2	Set 3
Enzyme Dose, kgt^{-1}	---	0.1	0.3	0.5
End pH	8.05	7.96	7.94	7.82
End Kappa No.	19.3±0.30	18.5±0.17	17.8±0.45	17.4±0.32
End Brightness, %ISO	28.7±0.50	31.0±0.40	31.1±0.31	31.5±0.42
Pulp yield, %	99.7±0.15	99.5±0.20	99.3±0.21	99.1±0.10
Reaction time- 90 min.; consistency- 10.0%; Reaction temp.- 55°C; Initial pH- 8.0; Initial Kappa No.- 19.6; Initial Brightness- 28.4%ISO				

4.3.2.2 ECF bleaching of eucalyptus hardwood pulps at equivalent and reduced chemicals charge

Enzyme treated and untreated pulps from Table 4.9 were carried out for ECF bleaching using $D_0E_PD_1D_2$ sequence at equivalent (Table 4.10) and reduced (Table 4.11) chemical charge. Equivalent kappa factor was maintained as 0.22 while it was decreased by 15% (i.e. 0.187) in reduced chemical charge. Handsheets of the final pulp were prepared and evaluated for the desired optical properties. The final pulp brightness and whiteness of the enzyme-treated pulps was superior to untreated pulp at both equivalent and reduced amounts of bleach chemicals. Brightness gains of approximately 1.7 and 0.8 units were observed at equivalent and reduced bleach chemical consumption in the bleaching of eucalyptus pulp. The observations of Gallardo *et al*. (2010) on hardwood pulp showed a brightness gain in the range of 0.7 to 1.0 units more than the control pulp with the xylanase enzyme.

In another study done by Qy *et al*. (1996), the brightness gain of 6.8% was observed in enzyme treated kraft pulp as compared to untreated kraft pulps. A similar trend was observed in whiteness improvement (up to 2.3 and 1.5 units) at equivalent and reduced bleach chemical consumption in the enzymatic treatment of eucalyptus pulp. The viscosity of the final eucalyptus pulp was also determined and found comparable to

that of untreated pulp. The reduction in post-color (PC) number increased with increasing doses of xylanase (Table 4.10 & 4.11) and higher reduction of 45% was seen when the pulp was treated at the equivalent chemical dosages. According to Kim and Paik (2000), xylanase treatment was responsible for the removal of carboxylic acids and their counter ions, which resulted in color reversion. By the partial removal of xylan in the pulps, xylanase treatment increased brightness stability.

Table 4.10 Enzymatic bleaching behavior using $D_0E_PD_1D_2$ sequence after enzymatic treatment at equivalent chemicals charge- EUCALYPTUS

Parameter	Control	Set 1	Set 2	Set 3
Kappa No.	19.3	18.5	17.8	17.4
Kappa factor	0.22	0.22	0.22	0.22
D_0 stage				
ClO_2 added, %	1.63	1.56	1.50	1.47
End pH	2.36	2.41	2.41	2.42
ClO_2 consumed, %	100	99.1	98.6	98.0
E_P stage				
NaOH added, %	2.12	2.04	1.96	1.91
H_2O_2 added, %	0.5	0.5	0.5	0.5
Initial pH	11.40	11.41	11.39	11.42
End pH	10.51	10.65	10.81	10.64
Kappa No.	2.1±0.15	1.9±0.26	1.8±0.21	1.7±0.10
Brightness, %ISO	74.9±0.66	76.7±0.50	77.2±0.38	78.1±0.49
H_2O_2 consumed, %	100	100	100	100
D_1 stage				
ClO_2 added, %	0.8	0.8	0.8	0.8
Initial pH	2.78	2.71	2.72	2.68
End pH	3.28	3.24	3.26	3.11
Brightness, %ISO	84.3±0.50	85.6±0.60	86.1±0.42	86.5±0.51
ClO_2 consumed, %	99.6	99.0	98.4	98.0

contd..

D_2 stage				
ClO_2 added, %	0.3	0.3	0.3	0.3
Initial pH	2.71	2.66	2.68	2.74
End pH	3.20	3.11	3.21	3.31
ClO_2 consumed,%	88.5	81.4	81.0	80.6
Brightness, %ISO	87.1±0.50	88.0±0.29	88.4±0.17	88.8±0.45
CIE Whiteness	74.48±0.34	75.59±0.47	76.18±0.23	76.77±0.38
L*	97.12	97.39	97.39	97.42
a*	-0.15	-0.18	-0.16	-0.14
b*	4.90	4.00	3.87	3.75
PC No.	0.65±0.04	0.40±0.03	0.39±0.05	0.36±0.04
PC No. reduction, %	---	38.46	40.00	44.62
Shrinkage, %	5.5±0.32	5.8±0.45	6.2±0.29	6.4±0.32
Viscosity, cp	12.4±0.26	12.2±0.35	12.1±0.15	12.0±0.26

Table 4.11 Enzymatic bleaching behavior using $D_0E_PD_1D_2$ sequence after enzymatic treatment at reduced chemicals charge- EUCALYPTUS

Parameter	Control	Set 1	Set 2	Set 3
Kappa No.	19.3	18.5	17.8	17.4
Active chlorine reduction, %	---	15.0	15.0	15.0
Kappa factor	0.22	0.187	0.187	0.187
D_0 stage				
ClO_2 added, %	1.63	1.33	1.28	1.25
End pH	2.36	2.31	2.28	2.30
ClO_2 consumed, %	100	100	99.8	99.7
E_P stage				
Caustic reduction, %	---	18.40	21.70	23.11
NaOH added, %	2.12	1.73	1.66	1.63
H_2O_2 added, %	0.5	0.5	0.5	0.5
Initial pH	11.40	11.25	11.20	11.22
End pH	10.51	10.55	10.61	10.54

contd..

Kappa No.	2.1±0.15	2.1±0.10	2.0±0.25	1.9±0.21
Brightness, %ISO	74.9±0.66	75.1±0.35	76.1±0.32	76.9±0.40
H_2O_2 consumed, %	100	100	100	100
D_1 stage				
ClO_2 reduction, %	---	12.5	12.5	12.5
ClO_2 added, %	0.8	0.7	0.7	0.7
Initial pH	2.78	2.76	2.74	2.71
End pH	3.28	3.25	3.24	3.22
Brightness, %ISO	84.3±0.50	84.3±0.40	85.0±0.55	85.4±0.55
ClO_2 consumed, %	99.6	99.2	98.8	98.4
D_2 stage				
ClO_2 added, %	0.3	0.3	0.3	0.3
Initial pH	2.71	2.75	2.72	2.76
End pH	3.20	3.28	3.27	3.24
ClO_2 consumed, %	88.5	83.4	82.2	81.5
Brightness, %ISO	87.1±0.50	86.9±0.55	87.5±0.56	87.9±0.36
CIE Whiteness	74.48±0.34	74.25±0.12	75.28±0.52	75.98±0.30
L*	97.12	97.09	97.19	97.28
a*	-0.15	-0.16	-0.15	-0.15
b*	4.90	4.94	4.20	4.00
PC No.	0.65±0.06	0.41±0.04	0.40±0.03	0.38±0.05
PC No. reduction, %	---	36.92	38.46	41.54
Shrinkage, %	5.5±0.32	5.7±0.23	5.1±0.21	6.2±0.25
Viscosity, cp	12.4±0.26	12.3±0.25	12.2±0.26	12.1±0.15

Table 4.12 shows the strength properties of eucalyptus hardwood pulp. Enzyme-treated and untreated eucalyptus hardwood pulps were refined at 2000 PFI revolutions. Handsheets were also prepared of approximate 70 GSM to check the physical strength properties. Comparable results were observed in terms of the bursting strength of enzyme-treated and untreated eucalyptus wood pulps. The enzyme treatment showed a slightly negative impact on the tearing strength of eucalyptus pulps as the action of the xylanases reduced the intrinsic fibrillar strength due to the removal of superficial

hemicelluloses. This is presumably the reason underlying the reduced tear index after the enzymatic pretreatment of wood pulps (Bajpai 1999).

Table 4.12 Physical strength properties of eucalyptus pulp

Particular	Control	EnzyC					
		At equivalent chemical			At reduced chemical dose		
Enzyme dose, kgt^{-1}	---	0.1	0.3	0.5	0.1	0.3	0.5
PFI Revolutions	2000	2000	2000	2000	2000	2000	2000
Initial CSF	584	587	591	593	592	594	597
End CSF	422	438	447	455	441	456	465
°SR	30.0	29.0	28.5	28.0	29.0	28.0	27.5
Grammage, g/m^2	70.22	70.59	70.79	70.46	71.01	70.76	70.43
Bulk, cc/g	1.30	1.30	1.31	1.29	1.29	1.31	1.30
Burst index, kN/g	4.2±0.23	4.2±0.10	4.2±0.15	4.3±0.17	4.2±0.06	4.1±0.15	4.1±0.25
Tear index, mNm^2/g	7.3 ±0.44	7.2 ±0.21	7.1 ±0.31	7.1 ±0.25	7.2 ±0.38	7.2 ±0.42	7.0 ±0.30

4.3.2.3 Enzyme treatment of acacia hardwood pulps at optimized conditions prior to ECF bleaching

Enzyme-treated pulps showed a reduction in kappa number of 4.6, 8.1, and 10.2% for acacia pulp at xylanase doses of 0.1, 0.3, and 0.5 kgt^{-1} compared with the control (Table 4.13).

Brightness gains of 2.4, 2.7, and 2.9 units were observed in acacia pulp at enzyme doses of 0.1, 0.3, and 0.5 kgt^{-1} compared with the control. A minor loss in yield was also observed in acacia wood pulps in the range of 0.3% to 0.6% with xylanase doses of 0.1, 0.3, and 0.5 kgt^{-1} compared with the control (Table 4.13).

Table 4.13 Enzymatic treatment of Acacia pulp with EnzyC

Parameter	Control	Set 1	Set 2	Set 3
Enzyme Dose, kgt^{-1}	---	0.1	0.3	0.5
End pH	8.25	7.92	7.87	7.81
End Kappa No.	19.7±0.40	18.8±0.47	18.1±0.31	17.7±0.40
End Brightness, %ISO	28.5±0.40	30.9±0.44	31.2±0.32	31.4±0.32
Pulp yield, %	99.8±0.15	99.5±0.20	99.4±0.31	99.2±0.21

Reaction time- 90 min.; Consistency- 10.0%; Reaction temp.- 55°C; Initial pH- 8.0; Initial Kappa No.- 20.1; Initial Brightness- 28.1%ISO

4.3.2.4 ECF bleaching of acacia hardwood pulps at equivalent and reduced chemicals charge

Enzyme treated and untreated pulps from Table 4.13 were carried out for ECF bleaching using $D_0E_PD_1D_2$ sequence at equivalent (Table 4.14) and reduced (Table 4.15) chemicals charge. Equivalent kappa factor was maintained as mentioned earlier. Handsheets of the final pulp were prepared and evaluated for the desired optical properties. The final pulp brightness and whiteness of the enzyme-treated pulps was superior to untreated pulp at both equivalent and reduced amounts of bleach chemicals. Brightness gains of approximately 2.1 and 0.9 units were observed at equivalent and reduced bleach chemical consumption in the bleaching of acacia pulp.

Table 4.14 Enzymatic bleaching behavior using $D_0E_PD_1D_2$ sequence after enzymatic treatment at equivalent chemicals charge- ACACIA

Parameter	Control	Set 1	Set 2	Set 3
Kappa No.	19.7	18.8	18.1	17.7
Kappa factor	0.22	0.22	0.22	0.22
D_0 stage				
ClO_2 added, %	1.66	1.58	1.53	1.49
End pH	2.41	2.36	2.39	2.40
ClO_2 consumed, %	100	98.9	98.4	98.1
E_P stage				
NaOH added, %	2.17	2.07	1.99	1.95

contd..

H_2O_2 added, %	0.5	0.5	0.5	0.5
Initial pH	11.51	11.43	11.46	11.48
End pH	10.49	10.56	10.63	10.61
Kappa No.	2.3±0.15	2.1±0.15	2.0±0.30	1.9±0.17
Brightness, %ISO	74.2±0.40	76.4±0.50	77.0±0.25	77.6±0.35
H_2O_2 consumed, %	100	100	100	100
D_1 stage				
ClO_2 added, %	0.8	0.8	0.8	0.8
Initial pH	2.83	2.84	2.78	2.80
End pH	3.32	3.34	3.36	3.30
Brightness, %ISO	83.8±0.30	85.3±0.36	84.8±0.38	86.3±0.23
ClO_2 consumed, %	99.4	98.7	98.2	98.0
D_2 stage				
ClO_2 added, %	0.3	0.3	0.3	0.3
Initial pH	2.86	2.82	2.84	2.82
End pH	3.35	3.35	3.40	3.41
ClO_2 consumed, %	87.8	82.4	81.8	80.3
Brightness, %ISO	87.7±0.67	88.9±0.35	89.3±0.36	89.8±0.36
CIE Whiteness	76.56±0.31	77.82±0.20	78.77±0.52	79.27±0.23
L*	95.87	95.93	96.09	96.22
a*	0.08	0.10	0.11	0.11
b*	3.43	3.38	3.34	3.30
PC No.	0.58±0.06	0.35±0.05	0.33±0.03	0.32±0.04
PC No. reduction, %	---	39.66	43.10	44.82
Shrinkage, %	5.1±0.38	5.3±0.40	5.6±0.50	5.9±0.35
Viscosity, cp	13.6±0.45	13.5±0.26	13.4±0.46	13.4±0.38

A similar trend was observed in whiteness improvement (up to 2.7 and 1.6 units) at equivalent and reduced bleach chemical consumption in the enzymatic treatment of acacia pulp. The viscosity of the final acacia pulp was also determined and found to be similar to that of untreated pulp. The reduction in post-color (PC) number increased with increasing doses of xylanase and was higher (45%) when the pulp was treated at the same chemical dosages (Table 4.14 and 4.15).

Table 4.15 Enzymatic bleaching behavior using $D_0E_PD_1D_2$ sequence after enzymatic treatment at reduced chemicals charge- ACACIA

Parameter	Control	Set 1	Set 2	Set 3
Kappa No.	19.7	18.8	18.1	17.7
Active chlorine reduction, %	---	15.0	15.0	15.0
Kappa factor	0.22	0.187	0.187	0.187
D_0 stage				
ClO_2 added, %	1.66	1.35	1.30	1.27
End pH	2.41	2.35	2.31	2.33
ClO_2 consumed, %	100	99.9	99.6	99.6
E_P stage				
Caustic reduction, %	---	18.40	21.70	23.11
NaOH added, %	2.17	1.76	1.69	1.66
H_2O_2 added, %	0.5	0.5	0.5	0.5
Initial pH	11.51	11.23	11.22	11.18
End pH	10.49	10.52	10.60	10.64
Kappa No.	2.3±0.15	2.2±0.17	2.0±0.31	1.9±0.21
Brightness, %ISO	74.2±0.40	74.8±0.51	75.4±0.29	76.4±0.65
H_2O_2 consumed, %	100	100	100	100
D_1 stage				
ClO_2 reduction, %	---	12.5	12.5	12.5
ClO_2 added, %	0.8	0.7	0.7	0.7
Initial pH	2.83	2.81	2.78	2.73
End pH	3.32	3.22	3.29	3.32
Brightness, %ISO	83.8±0.30	84.3±0.36	84.9±0.47	85.1±0.36
ClO_2 consumed, %	99.4	99.0	99.2	99.3
D_2 stage				
ClO_2 added, %	0.3	0.3	0.3	0.3
Initial pH	2.86	2.79	2.73	2.81
End pH	3.35	3.38	3.31	3.34
ClO_2 consumed, %	87.8	83.1	82.7	82.0

contd..

Brightness, %ISO	87.7±0.67	87.8±0.35	88.4±0.49	88.6±0.67
CIE Whiteness	76.56±0.31	77.12±0.44	77.86±0.32	78.17±0.26
L*	95.87	95.91	95.98	96.10
a*	0.08	0.07	0.09	0.11
b*	3.43	3.42	3.38	3.36
PC No.	0.58±0.06	0.36±0.03	0.34±0.04	0.33±0.04
PC No. reduction, %	---	37.93	41.38	43.10
Shrinkage, %	5.1±0.38	5.2±0.35	5.5±0.30	5.7±0.30
Viscosity, cp	13.6±0.45	13.6±0.15	13.5±0.35	13.4±0.29

Table 4.16 shows the strength properties of acacia hardwood pulp. Enzyme-treated and untreated acacia hardwood pulps were refined at 3600 PFI revolutions. Results show that the requirement of refining energy is more in acacia than eucalyptus to get the same °SR/CSF levels. Handsheets were also prepared of approximate 70 GSM to check the physical strength properties. Like eucalyptus hardwood pulps, comparable results were observed in terms of the bursting strength of enzyme-treated and untreated acacia wood pulps. The enzyme treatment showed a slightly negative impact on the tearing strength of acacia pulps too.

Table 4.16 Physical strength properties of acacia pulp

Particular	Control	EnzyC					
		At equivalent chemical			At reduced chemical dose		
Enzyme dose, kgt^{-1}	---	0.1	0.3	0.5	0.1	0.3	0.5
PFI Revolutions	3600	3600	3600	3600	3600	3600	3600
Initial CSF	619	625	628	630	628	631	634
End CSF	431	436	445	452	443	454	461
°SR	29.5	29.5	29.0	28.5	29.0	28.5	28.0
Grammage, g/m^2	70.81	70.25	70.19	70.67	70.89	71.17	71.03
Bulk, cc/g	1.32	1.29	1.32	1.31	1.28	1.30	1.30
Burst index, kN/g	2.6±0.31	2.6±0.29	2.5±0.15	2.6±0.45	2.5±0.38	2.6±0.25	2.6±0.17
Tear index, mNm^2/g	6.2 ±0.45	6.2 ±0.35	6.2 ±0.29	6.0 ±0.25	6.1 ±0.26	6.0 ±0.35	5.9 ±0.31

4.3.3 Comparative study between eucalyptus and acacia ECF bleaching

A comparative study of eucalyptus and acacia kraft pulps was carried out in an ECF bleaching sequence after xylanase treatment with commercial xylanase (EnzyC), in which the optical and physical properties of the final bleached pulp and paper were tested. In this study, it was observed that optical properties of hardwood kraft pulps were getting improved with xylanase treatment, while physical strength properties were observed comparable. Similar observations were also reported by Atik *et al*. (2006), wherein, application of xylanase did not affect the strength properties of hardwood pulps significantly, while optical properties were improved. The results showed enzymatic bleaching exhibited higher selectivity in the case of acacia kraft pulp (Figure 4.1 and 4.2). The reduction of bleach chemicals consumption was also observed to be higher in acacia than eucalyptus wood pulps with xylanase enzyme treatment before the ECF bleaching sequence.

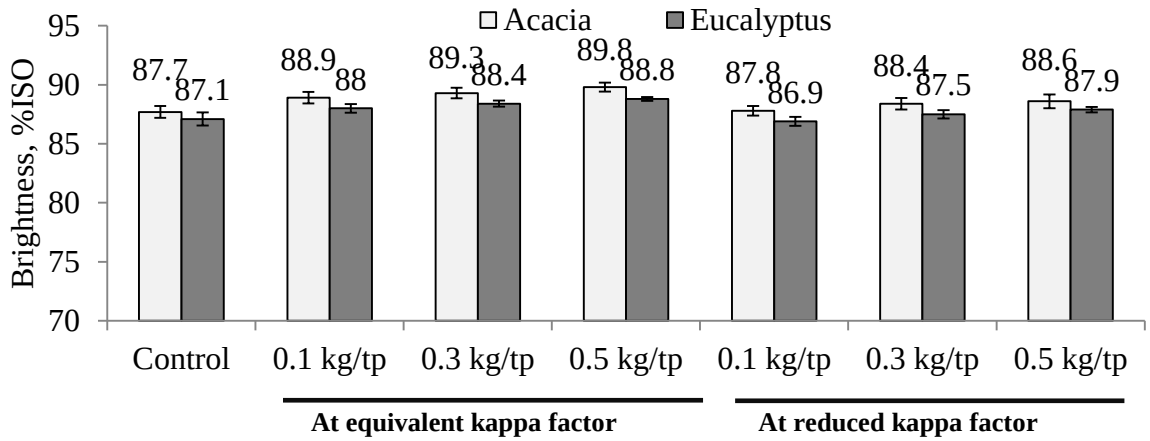

Fig. 4.1 Brightness (% ISO) of the final bleached pulp samples at equivalent and reduced kappa factor for eucalyptus and acacia

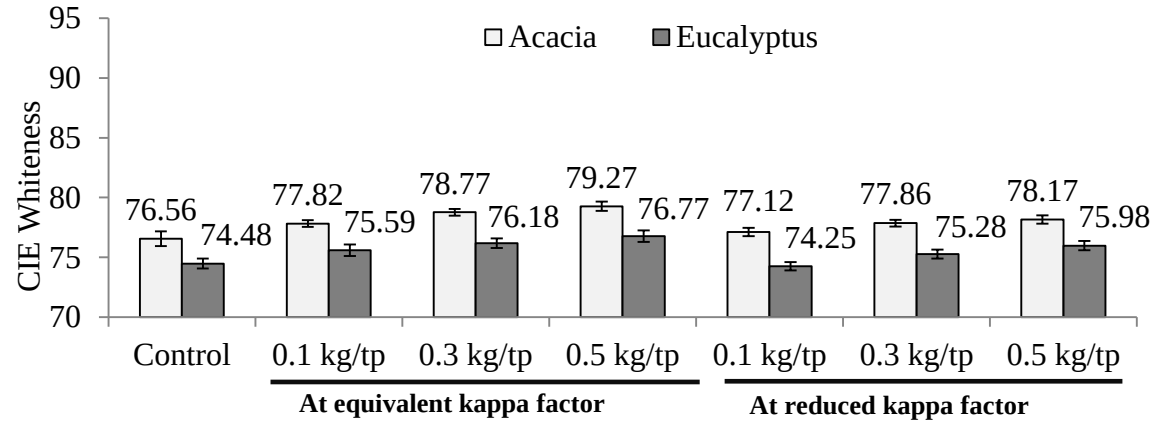

Fig. 4.2 CIE whiteness of the final bleached pulp samples at equivalent and reduced kappa factor for eucalyptus and acacia

After enzymatic bleaching with EnzyC, burst index for eucalyptus and acacia was around 4.5 and 2.5 kN/g respectively at equivalent and reduced KFs. Tear index was achieved 7.0 and 6.0 mNm^2/g for eucalyptus and acacia respectively at equivalent and reduced KFs. The requirement of refining energy is also less in eucalyptus than acacia to achieve the same °SR/CSF level. Therefore, the strength properties of eucalyptus pulp were observably better than acacia pulp (Table 4.12 and 4.16). Marginal increment in pulp shrinkage was observed in both hardwood pulps as compared to their respective control with lesser shrinkage in acacia (5.2-5.9) than eucalyptus (5.7-6.4) pulps at equivalent and reduced KFs (Table 4.10, 4.11, 4.14 and 4.15). According to Tolan and Popovici (2002), the enzyme treated pulp requires 10-20% less bleaching chemicals to reach up to same brightness level as compared to untreated pulp. In another study carried out by Nair *et al*. (2010), 14.3% of elemental chlorine could be reduced with xylanase treatment on kraft pulps.

Table 4.17 also indicate that bleaching experiments, carried out with acacia, performed at both an equivalent kappa factor (KF~0.22) and reduced kappa factor (KF~0.187), resulted in decreasing consumption of 14.9 to 17.8% chlorine dioxide, along with a reduction of sodium hydroxide in the range of 18.9 to 23.5% compared with the control at a reduced kappa number. A similar reduction was also observed for eucalyptus wood pulp, wherein a reduced use of chlorine dioxide and sodium hydroxide to 14.7 to 17.6% and 18.4 to 23.1%, respectively, was required at the reduced kappa factor compared with the control with an improvement in the optical properties of the pulps. During xylanase treatment, due to disruptive action of xylanase on xylan chain, the bond between lignin-carbohydrate interrupt and therefore lignin releases from the pulp fibres during subsequent chemical bleaching stages. It results in reduction of bleach chemical consumption like chlorine dioxide in bleaching process (Kapoor *et al*. 2007; Sharma *et al*. 2014).

Comparable results were obtained for viscosity of pulp at equivalent and reduced kappa factor for both acacia and eucalyptus pulps (Figure 4.3). Reduction in post color number by more than 44% was observed in both hardwood pulps (Figure 4.4). According to Sharma *et al*. (2014), enzyme treatment is responsible for reduction of 50% post color number in hardwood kraft pulps.

Table 4.17 Bleach chemical consumption in ECF bleaching of eucalyptus and acacia pulps

	Particulars	Control	At equivalent KF			At reduced KF		
			0.1 kgt^{-1}	0.3 kgt^{-1}	0.5 kgt^{-1}	0.1 kgt^{-1}	0.3 kgt^{-1}	0.5 kgt^{-1}
Eucalyptus pulp	Applied ClO_2, kgt^{-1}	27.3	26.6	26.0	25.7	23.3	22.8	22.5
	ClO_2 reduction, %	---	2.56	4.76	5.86	14.65	16.48	17.58
	NaOH, kgt^{-1}	21.2	20.4	19.6	19.1	17.3	16.6	16.3
	NaOH reduction, %	---	3.77	7.55	9.91	18.40	21.70	23.11
Acacia pulp	Applied ClO_2, kgt^{-1}	27.6	26.8	26.3	25.9	23.5	23.0	22.7
	ClO_2 reduction, %	---	2.90	4.71	6.16	14.86	16.67	17.75
	NaOH, kgt^{-1}	21.7	20.7	19.9	19.5	17.6	16.9	16.6
	NaOH reduction, %	---	4.61	8.29	10.14	18.89	22.12	23.50

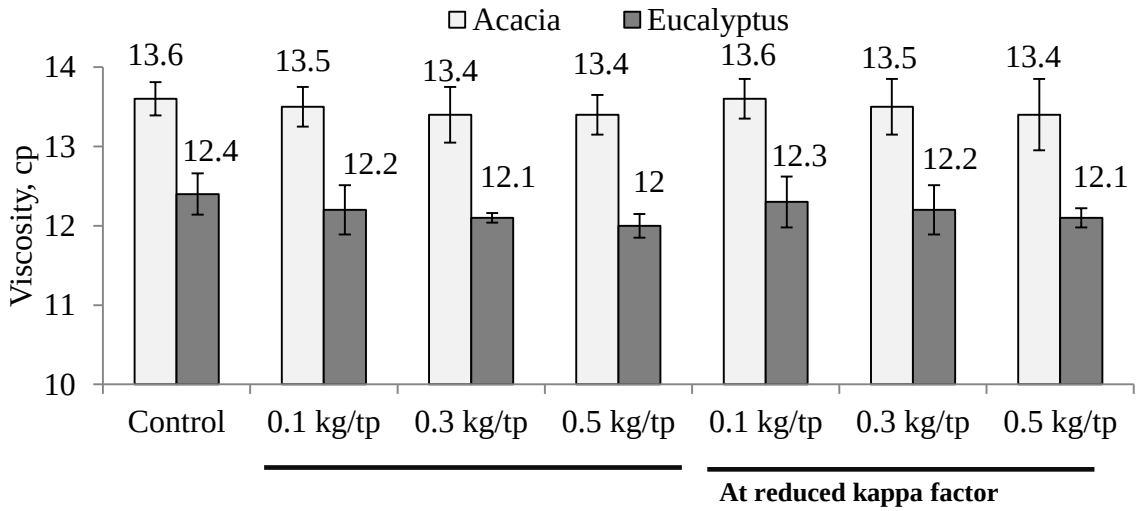

Fig. 4.3 Viscosity of the final bleached pulp samples at equivalent and reduced chemical charges

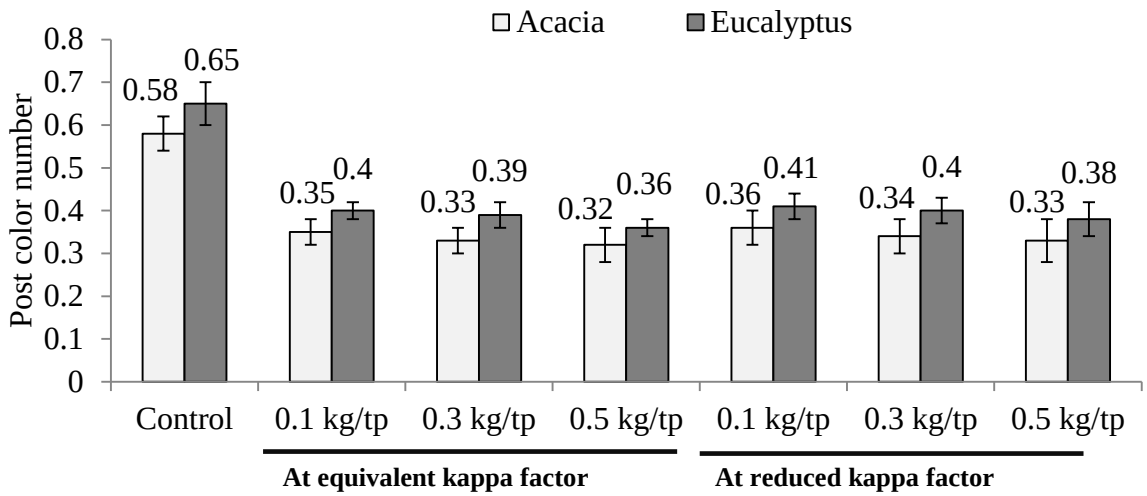

Fig. 4.4 Post-color number of the final bleached pulp samples at equivalent and reduced chemical charges

The present results provide a clear indication for the better selectively of acacia over eucalyptus for use in the paper-making process, as these approaches resulted in the decreasing consumption of bleach chemicals, leading to a reduced cost in the treatment of hazardous chemicals and improved qualities of the final paper.

4.4 Suitability of the best bleaching sequence of xylanase treated eucalyptus hardwood pulps

In general, enzymes are being used before the bleaching sequences to improve the pulp brightness while enzymes are also being used after the bleaching sequences to improve the whiteness of the pulp. In this approach, observations are presented on different bleaching sequences in which enzyme treatment (X) stage has been incorporated at different stages across the bleaching sequence and its effect on the AOX, BOD and COD in bleach effluents has been studied. This approach is very beneficial to find out the best enzymatic bleaching sequences for hardwood kraft pulps in the industrial processing. Application of EnzyC, that had exhibited better properties over other commercial xylanases considered in the study.

Three different bleaching sequences ($C_DE_PD_1D_2$, $D_0E_PD_1D_2$ and $D_0E_PD_1E_P$) were used for this approach (where; C_D represents the chlorination stage with elemental chlorine and chlorine dioxide, D_0 represents chlorine dioxide stage, E_P indicates extraction stage with caustic and hydrogen peroxide and D_1 and D_2 represent first and

second dioxide stages after extraction stage). For each category of pre, post and intermediate bleaching process, three bleaching sequences were designed as shown in Table 4.18.

Table 4.18 Incorporation of the enzymatic stage before, after and within the bleaching sequences

Sequence type	Enzymatic bleaching sequence		
Pre-treatment	$XC_DE_PD_1D_2$	$XD_0E_PD_1D_2$	$XD_0E_PD_1E_P$
Post-treatment	$C_DE_PD_1D_2X$	$D_0E_PD_1D_2X$	$D_0E_PD_1E_PX$
Intermediate	$D_0XE_PD_1D_2$	$D_0E_PD_1XD_2$	$D_0E_PXD_1E_P$

4.4.1 Enzyme treatment of the pulp

Enzyme treatment with EnzyC was performed in the same manner as done before in first approach as per the Table 4.2 which indicates the optimized process conditions *viz*; incubation pH and temperature for EnzyC and the optimum activity was observed at pH and temperature 8.0 and 55°C respectively. Kneading mechanism was followed for proper dilutions and mixing of enzyme to be used in experiments. Control pulp was also treated in the same way as the xylanase treated pulp in each and every experiment, where enzyme was replaced with water. In pre-treatment enzymatic bleaching category, pulps were taken for bleaching sequences after the enzymatic stage, while in case of post-treatment enzymatic bleaching, pulps were first treated with different bleaching stages, washed and subjected to enzymatic stage. Enzyme stage was also incorporated in between the bleaching sequences for steps involving intermediate enzymatic bleaching.

4.4.2 Pre-treatment bleaching sequences

In this category, enzyme (X) stage was introduced before bleaching process ($XC_DE_PD_1D_2$, $XD_0E_PD_1D_2$ and $XD_0E_PD_1E_P$), where enzyme was applied at different doses (0.1, 0.3 and 0.5 kgt^{-1}) to see the efficacy of xylanase treatment on optical pulp properties (%ISO brightness and CIE whiteness). Untreated pulp was also treated at the similar conditions as treated pulp except the addition of xylanase to neglect the effect of pH, temperature and incubation time (Annexure -Table 1.9). After enzymatic treatment process, pulp was subjected to different bleaching sequences and bleached pulps were

analyzed for brightness, whiteness and PC number to determine the best bleaching sequence in these three bleaching sequences with X stage at pre-treatment.

With the bleaching sequence $XC_DE_PD_1D_2$, final brightness gain of 0.9, 1.3 and 1.6 units and whiteness of 1.1, 2.2 and 2.8 units were obtained in eucalyptus wood pulps at enzyme doses of 0.1, 0.3 and 0.5 kgt^{-1} respectively (Annexure -Table 1.10). Similar results were also obtained by Nagar *et al*. (2013) with the same bleaching sequence for eucalyptus hardwood pulp. As reported by them, the final gain in brightness of enzyme pretreated pulp was higher by 2.7 points as compared with the control demonstrating efficacy of the enzymatic pre-bleaching. While Li *et al*. (2005) reported that pretreatment with cellulase-free xylanase isolated from the fungus *Thermomyces lanuginosus* brightened the pulp by 1.8 %ISO over the control. When chlorination stage (C_D) was replaced with chlorine dioxide stage (D_0) then final brightness of 0.7, 1.1 and 1.5 units and whiteness gain of 1.4, 2.0 and 2.5 units were obtained when compared to control (Annexure -Table 1.11). Similar to our results, Brijilall *et al*. (2011) observed that treatment of pulp with xylanase increased pulp brightness by 2.1 units. Several researchers (Jiang *et al*. 2006; Ahlawat *et al*. 2007; Sanghi *et al*. 2008; Garg *et al*. 2011; Nagar *et al*. 2013) have also reported an increase in brightness by 2.0 points after pre-treatment of pulp with xylanase indicating effectiveness of the enzyme in pulp bleaching. When the second chlorine dioxide (D_2) stage was replaced with extraction (E_P) stage, the final brightness gain of 0.6, 1.0 and 1.3 units and whiteness gain of 0.9, 1.6 and 2.0 units were observed (Annexure -Table 1.12). A minor loss in pulp yield was seen across all pre-treatment bleaching sequences with xylanase treatment (Annexure -Table 1.9, 1.10, 1.11 and 1.12).

4.4.3 Post-treatment bleaching sequences

For the post-treatment bleaching studies, three sequences, similar to those mentioned earlier, were used ($C_DE_PD_1D_2$, $D_0E_PD_1D_2$ and $D_0E_PD_1E_P$). Here, X stage was introduced after the bleaching process *viz*; $C_DE_PD_1D_2X$, $D_0E_PD_1D_2X$ and $D_0E_PD_1E_PX$ to determine the effect of xylanase on brightness and whiteness of the bleached pulp. Firstly, the unbleached pulp sample was characterized for kappa number and brightness (%ISO) and then subjected to the bleaching sequences followed by the X stage. In enzymatic stage of post-bleaching sequence, enzyme doses of 0.1, 0.3 and 0.5 kgt^{-1} were also maintained as in pre-treatment bleaching sequences. After the enzymatic stage, pulps were analyzed for the brightness, whiteness and PC number of the bleached pulp.

Table 1.13 (Annexure) presents the observations on introduction of X stage post treatment. In case of $C_DE_PD_1D_2X$, results of gain in final brightness of 0.5, 1.0 and 1.2 units and whiteness of 1.7, 2.8 and 3.4 units was obtained on hardwood pulps. When ECF bleaching sequence ($D_0E_PD_1D_2X$) was used then the final brightness and whiteness gain of 0.5, 0.8 and 1.1 units and 1.5, 2.5 and 3.2 units were obtained respectively as compared to control (Annexure -Table 1.14). While in the third bleaching sequence ($D_0E_PD_1E_PX$), the final brightness of 0.4, 0.7 and 1.0 units and whiteness gain of 1.3, 2.3 and 2.8 units were recorded (Annexure -Table 1.15). A minor pulp yield loss was observed for all post-treatment bleaching sequences with xylanase as compared to control. Post-treatment results show that the enzymatic stage (X stage) after the bleaching sequence is more beneficial to improve the final whiteness of the bleached pulp than the pre-treatment bleaching sequences.

4.4.4 Intermediate bleaching sequences

We also applied the X stage at intermediate stage of bleaching sequences to see the xylanase efficacy if it is incorporated within the bleaching sequences. These intermediate sequences *viz*; $D_0XE_PD_1D_2$, $D_0E_PD_1XD_2$ and $D_0E_PXD_1E_P$ were tried. The kappa number and brightness (%ISO) were determined prior to bleaching. In X stage, enzyme doses of 0.1, 0.3 and 0.5 kgt^{-1} were applied as reported earlier. After completion of the bleaching process, pulps were analyzed for the brightness, whiteness and PC number of the bleached pulp.

In Table 1.16 (Annexure), the final brightness and whiteness gain of 0.4, 0.7 and 1.0 units and 0.7, 1.1 and 1.2 units were obtained in the case of $D_0XE_PD_1D_2$ bleaching sequence. When X stage was incorporated in between of last two dioxide stages ($D_0E_PD_1XD_2$) then the final brightness and whiteness gain of 0.4, 0.7 and 1.1 units and 0.8, 1.1 and 1.4 units were obtained (Annexure -Table 1.17). While integration of X stage between the extraction and first chlorine dioxide stage ($D_0E_PXD_1E_P$), resulted in the final brightness gain of 0.9, 1.2 and 1.4 units and whiteness gain of 1.3, 1.8 and 2.3 units as compared to control (Annexure -Table 1.18). When X stage was incorporated just after the extraction stage, improved brightness and whiteness was obtained as compared to other intermediate bleaching process. A minor pulp yield loss was observed for all intermediate bleaching sequences with xylanase as compared to control.

4.4.5 Effect of enzymatic bleaching sequence on brightness reversion (Post color number)

Oxidized groups (carboxyl groups and their counter ions) in pulp, such as the native uronic acids and the unsaturated Hex-A (which generated during the kraft cooking process) effect the brightness reversion of the bleached pulp (Buchert *et al*. 1997; Loureiro *et al*. 2010). The specific role of Hex-A on the brightness reversion of bleached pulps has already been the subject of many studies and a positive correlation between the amount of Hex-A and the extent of reversion has been reported (Vuorinen *et al*. 1999; Sevastyanova *et al*. 2006; Loureiro 2012). Removal of carboxylic acid components and Hex-A by xylanase treatment significantly influences in decreasing the brightness reversion as they directly contribute in color reversion by decreasing brightness of the final bleached pulp.

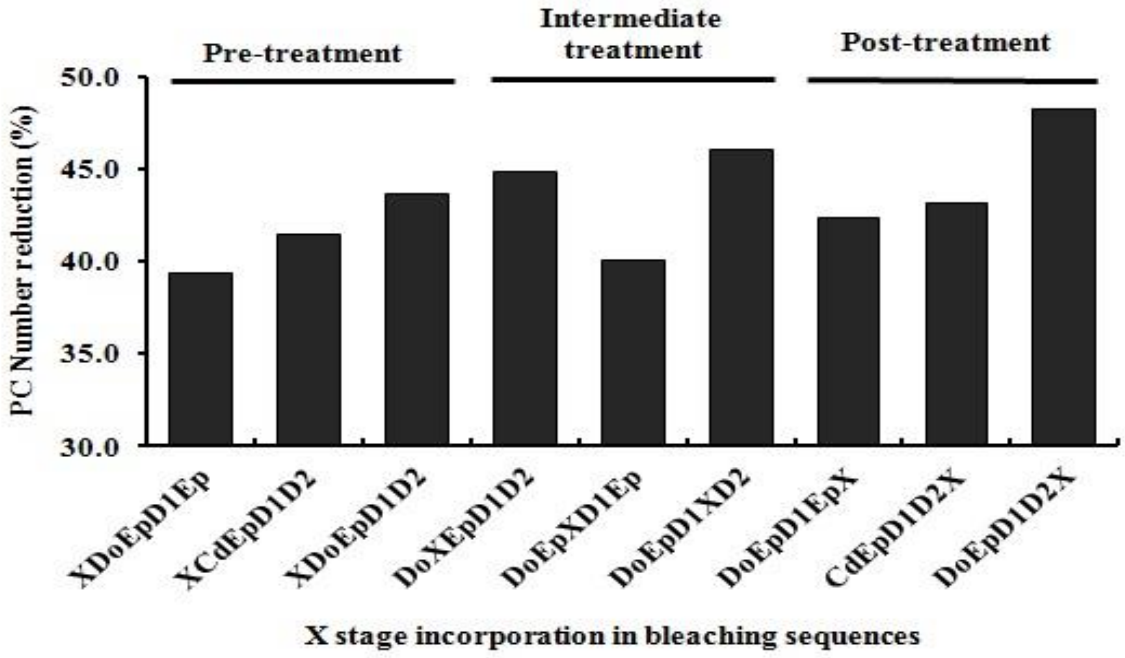

Fig. 4.5 Effect of enzymatic stage incorporation on post color number reduction (%) in bleaching sequences

In this study, we obtained maximum PC number reduction of 48% with ECF bleaching sequence, in which X stage was introduced after the bleaching stages (post-treatment bleaching-$D_0E_PD_1D_2X$) at xylanase dose of 0.5 kgt^{-1} (Annexure -Table 1.14). While minimum PC number reduction of 39% has been observed in pre-treatment bleaching sequence ($XD_0E_PD_1E_P$), in which last chlorine dioxide stage was replaced with

extraction stage at the same xylanase dose of 0.5 kgt^{-1} (Annexure -Table 1.12). Increasing order of reduction in PC number (%) for all nine bleaching sequences is shown in Figure 4.5.

4.4.6 Effectiveness of bleaching sequences on the effluent characteristic

The pollution load of enzyme treated and untreated pulp bleach effluents were analyzed for COD, BOD and AOX to determine the environmental benefits of enzyme bleaching process. To check the pollutant load in bleach effluent, composite effluent solutions were collected from all respective stages of bleaching process which were treated at xylanase dose of 0.5 kgt^{-1}. Untreated pulp effluents were also collected for each subsequent bleaching sequence of pre, post and intermediate bleaching sequences.

As shown in Table 4.19, In pre-treatment bleaching sequences ($XC_DE_PD_1D_2$, $XD_0E_PD_1D_2$ and $XD_0E_PD_1E_P$), reduction in AOX was observed in the range of 29-32%, as pulps on treatment with xylanase, results in reduced demand of bleach chemicals mainly elemental chlorine, chlorine dioxide and caustic. In contrast, with X stage at post-treatment ($C_DE_PD_1D_2X$, $D_0E_PD_1D_2X$ and $D_0E_PD_1E_PX$), the reduction in AOX was recorded less when compared to pre-treatment bleaching sequences and it was observed in the range of 16-22%. When X stage was incorporated in between the bleaching sequences, variable results were noted in AOX reduction in the range of 17-22%. X stage, when incorporated between chlorine dioxide and extraction stage ($D_0XE_PD_1D_2$), the AOX reduction was relatively higher when compared to other stages where intermediate incorporation of X stage was carried out.

COD and BOD of the bleach effluents were examined across all the X stage incorporated bleaching sequences when compared to control. Overall, xylanase treatment showed promising results towards reduction in release of pollutants in the bleach effluents of chemical pulps. Other research groups have also reported that the xylanase bleaching led to a reduction in effluent AOX, and the ratio of BOD to COD was significantly higher for the xylanase pre-bleaching filtrates (Senior and Hamilton 1991; Bajpai 2010; Thakur *et al*. 2012; Gangwar *et al*. 2014).The authors (Fillat *et al*. 2012; Thakur *et al*. 2012; Sharma *et al*. 2014) reported 34% AOX reduction in bleach effluent of eucalyptus hardwood kraft pulps after enzymatic pre-bleaching. According to Nie *et al*. (2015), the AOX content in bleach effluent could be reduced by 37.6% during chlorine dioxide bleaching after xylanase treatment.

Table 4.19 Comparison of bleach effluent properties in pre, post and intermediate bleaching sequences at 0.5 kgt^{-1} enzyme dose

Enzyme treatment	Bleaching sequence	BOD, $kgtp^{-1}$	COD, $kgtp^{-1}$	AOX, $kgtp^{-1}$	AOX, % reduction
Pre-treatment bleaching sequences	Control	45.2±0.40	75.1±0.66	0.87±0.05	--
	$XC_DE_PD_1D_2$	36.1±0.47	74.1±0.46	0.59±0.05	32.2
	Control	41.3±0.51	69.9±0.55	0.78±0.04	---
	$XD_0E_PD_1D_2$	34.7±0.51	66.7±0.47	0.54±0.03	30.8
	Control	39.3±0.49	67.3±0.42	0.72±0.03	---
	$XD_0E_PD_1E_P$	33.3±0.50	64.4±0.45	0.51±0.04	29.2
Post-treatment bleaching sequences	Control	51.5±0.51	77.9±0.55	0.96±0.05	---
	$C_DE_PD_1D_2X$	40.1±0.69	72.1±0.25	0.75±0.05	21.9
	Control	46.3±0.31	73.4±0.45	0.91±0.04	---
	$D_0E_PD_1D_2X$	38.7±0.40	64.7±0.15	0.76±0.02	16.5
	Control	43.5±0.51	70.3±0.40	0.82±0.05	---
	$D_0E_PD_1E_PX$	36.3±0.40	61.4±0.35	0.69±0.05	15.9
Intermediate bleaching sequences	Control	33.5±0.46	55.6±0.30	0.72±0.05	---
	$D_0XE_PD_1D_2$	32.6±0.50	51.2±0.46	0.56±0.03	22.2
	Control	38.1±0.53	57.7±0.38	0.75±0.07	---
	$D_0E_PD_1XD_2$	37.4±0.35	56.7±0.59	0.62±0.04	17.3
	Control	32.1±0.61	53.2±0.36	0.69±0.04	---
	$D_0E_PXD_1E_P$	31.5±0.29	52.1±0.42	0.56±0.07	18.8

4.5 Determining the comparative efficacy of commercially available xylanases in removing Hexenuronic acid from eucalyptus hardwoods pulps

The fate of xylanase bleach boosting effect of eucalyptus (*E. globulus*) was studied to know the exact profile of the hexenuronic acid reduction at each stage of ECF bleaching and in terms of change in optical properties of pulp. Effluent from each bleaching stages were collected and characterized to investigate their remedial efficiency in terms of pollution load (BOD and COD). Reduction in bleach chemicals were also studied with four different xylanases. We chose all four enzymes (EnzyA, EnzyB, EnzyC and EnzyD) to examine their efficacy in removing Hex-A from eucalyptus hardwood pulps. For this study, bulk production of hardwood kraft pulps was required and done by

following kraft cooking process at optimized conditions (AA~19.5%; cooking temperature~160°C; cooking time~180 min.).

Table 4.20 Pulping for Eucalyptus to Kappa No. ~ 18-19- At optimized conditions

Parameter	Mean	S.D
Kappa no.	18.5	±0.21
Unbleached yield, %	45.4	±0.40
Rejects, %	0.22	±0.10
Unbleached brightness, % ISO	31.7	±0.28
Free alkali as Na_2O (g/l) at 20% solids	7.5	±0.76
Hexenuronic acid content, mmol/kg	21.3	±0.31
Unbleached viscosity, cp	12.5	±0.10

AA as Na_2O- 19.5%; sulphidity- 24.21%; cooking temperature- 160°C; cooking time- 180 min.; bath ratio- 1:3

Table 4.20 shows the requirement of active alkali is 19.5% to reach kappa number in the range of 18-19 with unbleached brightness in the range of 31-32 %ISO. Hexenuronic acid content was obtained in kraft pulp in the range of 21-22 mmol/kg with the unbleached pulp viscosity in the range of 12-13 cp. This kraft pulp was carried out for further enzyme treatment stage to see its impact on kappa number and hexenuronic acid reduction with improvement in brightness of the pulp due to lignin removal (Table 4.22). After the cooking process (Table 4.20), pulp samples were subjected to enzyme treatment, as per the conditions shown in Table 4.21.

Table 4.21 Application conditions used during enzymatic bleaching ($XD_0E_PD_1D_2$) of eucalyptus pulp

Parameter	X stage	D_0 stage	E_P stage	D_1 stage	D_2 stage
Consistency, %	10	5	10	10	10
Treatment time, min.	90	45	120	180	180
Treatment Temp., °C	55	60	80	75	75
pH	8.0	1.8-2.0	10.5-11.0	2.8-3.0	2.8-3.0

Table 4.22, presents the variations in kappa number, brightness and Hex-A content obtained with the enzymes (at X stage) compared with control sample. All the

enzymes tested *viz*; EnzyA, EnzyB, EnzyC and EnzyD reduced the Kappa number by 12.6% (EnzyB) to 19.7% (EnzyC) after the X stage. A similar reduction was accomplished in studies with different xylanases. Brightness gain was in the range of 2.0-3.4 units, with maximum for EnzyC (3.4 units) and minimum with EnzyB (2.0 units). A decrease in Kappa number and increase in brightness would indicate a better efficiency of the enzyme treatment.

Table 4.22 Enzyme treatment of eucalyptus hardwood pulp with EnzyA, EnzyB, EnzyC and EnzyD for Hexenuronic acid removal

Parameter	Control	EnzyA	EnzyB	EnzyC	EnzyD
End Kappa No.	18.3±0.10	15.7±0.17	16.0±0.20	14.7±0.10	15.3±0.20
Kappa No. reduction, %	---	14.2	12.6	19.7	16.4
End brightness, %ISO	31.9±0.10	34.3±0.17	33.9±0.35	35.3±0.20	34.7±0.10
Brightness gain, Unit	---	2.4	2.0	3.4	2.8
Hex-A, mmol/kg	20.9±0.69	18.15±0.46	18.53±0.59	16.74±0.37	17.66±0.44
Hex-A reduction, %	**---**	**13.2**	**11.3**	**19.9**	**15.5**
Viscosity, cp	12.1±0.10	12.4±0.10	12.1±0.0	12.9±0.20	12.6±0.10

Effects of different xylanases are clearly seen in the enzyme stage in this study, while some other authors (Shatalov & Pereira 2007b) reported that the effects of different xylanases on kappa number and brightness are not always correlated. Although, the xylanase pretreatment facilitates the penetration of bleaching chemicals in subsequent bleaching stages, it is expected that its effect may not appear at the enzymatic stage but in later bleaching stages. However in some studies, results showed that in some cases the enzymatic stage caused reduction of kappa number with improved brightness. This effect was probably produced by the release of chromophores compounds derived from xylan, producing direct delignification or by the attack of some xylan-lignin complexes during bleaching as suggested by different authors (Patel *et al*. 1993; Jong *et al*. 1997; Shatalov & Pereira 2007b).

The extent of Hex-A removal in the treated pulps was in the range of 11.3-19.9% as compared to control pulps. Previous studies also reported reduction in Hex-A content by 14.5% and 11.1% after the enzyme treatment with Ecopulp® and Pulpzyme®

respectively (Shatalov and Pereira 2008) due to Hex-A losses in the enzyme stage resulting from enzymatic solubilization of HexA-carrying xylooligosaccharide fractions. In this study, EnzyC showed relatively higher reduction of Hex-A when compared to other enzymes. Xylanases hydrolyze xylans on fibre surfaces, resulting in removing Hex-A compounds that are bound as side groups to xylan. By hydrolyzing the xylan, xylanase acts as a bleaching aid rather than as a true delignification agent, since the enzyme does not directly degrade lignin. Another proposed mechanism of xylanase action involves removal of Hex-A residues that consume bleach chemicals and contribute to Kappa number. Xylanase reduces the hexenuronic acid content and facilitates increase in the pulp brightness as well.

4.5.1 Effect of xylanase treatment on removal of hexenuronic acid with optical properties improvement of eucalyptus hardwood at equivalent and reduced chemicals charge

Pulp samples, both treated and untreated, were subjected to $D_0E_PD_1D_2$ bleaching sequence and characterized for Hex-A contents and brightness improvement at each stage *viz*; XD_0, XD_0E_P, $XD_0E_PD_1$ and $XD_0E_PD_1D_2$ after enzyme treatment.

Table 4.23 & 4.24 show the profile of Hex-A contents at same and reduced KF across the bleaching stage with treatment of EnzyA, EnzyB, EnzyC and EnzyD as compared to control. Although all enzymes notably reduced the Hex-A contents, the extent of Hex-A removal in the presence of EnzyC was better throughout the bleaching sequence.

At KF- 0.22; after the XD_0 stage, Hex-A contents of the pulp reduced by 29.7, 28.5, 32.3 and 30.2% with EnzyA, EnzyB, EnzyC and EnzyD respectively with respect to control (Table 4.23). Costa & Colodette (2007) studied the effect of xylanase on eucalyptus hardwood for the reduction of Hex-A. They obtained significant reduction in Hex-A levels after the dioxide stage to the extent of up to 50% and hypothesized that chlorine dioxide might act as an electrophylic oxidant that may attack and destroy the Hex-A double bond during the bleaching process. Similarly, Hex-A after XD_0E_P sequence reduced by 31.0, 26.6, 40.8 and 35.1%; after $XD_0E_PD_1$ by 18.5, 16.3, 25.9 and 21.1% and at the end of $XD_0E_PD_1D_2$ bleaching sequence by 16.7, 12.5, 21.3 and 17.9% with enzymes EnzyA, EnzyB, EnzyC and EnzyD respectively as compared to control (Table 4.23). Shatalov and Pereira (2008), also observed 13.0-15.0% reduction in Hex-A after the final bleaching process with xylanase treatment. Whereas; at KF- 0.187, Hex-A content of the pulp reduced by 25.1, 22.1, 29.3 and 26.9% after XD_0; 21.2, 16.1, 31.5 and

27.6% after XD_0E_P; 10.7, 6.3, 17.0 and 12.6 after $XD_0E_PD_1$ and 5.8, 2.1, 15.0 and 9.2% after $XD_0E_PD_1D_2$ with EnzyA, EnzyB, EnzyC and EnzyD respectively with respect to control (Table 4.24). Across the various stages of bleaching, the efficacy of enzyme EnzyC on removal of Hex-A was notably better over other enzymes (Table 4.23 & 4.24).

Table 4.23 & 4.24 also present the bleach boosting effect of xylanases during pulp bleaching and it is normally assessed through examination of brightness (%ISO) and CIE whiteness of the bleached pulp at same and reduced kappa factor (KF). On the basis of residual chlorine dioxide amount in same KF (Table 4.23) experiments at stages (D_0, D_1 and D_2); we also examined optical properties and Hex-A reduction at reduced kappa factor (Table 4.24). The target for same and reducing KF was to get increased and same brightness levels respectively as compared to control. Although all enzymes have shown significant improvement in the brightness and whiteness of the bleached pulp, EnzyC resulted in relatively higher gain of final brightness and whiteness, when compared to other enzymes.

Table 4.23 Effect of xylanases for hexenuronic acid removal and improvement in optical properties of eucalyptus hardwood pulp at equivalent kappa factor

Parameter	Control	EnzyA	EnzyB	EnzyC	EnzyD
Kappa No.	18.3	15.7	16.0	14.7	15.3
Kappa factor	0.22	0.22	0.22	0.22	0.22
D_0 stage					
ClO_2 added, %	1.53	1.31	1.34	1.23	1.28
ClO_2 consumed, %	98.9	98.5	98.6	98.1	98.3
Brightness, %ISO	54.7±0.55	56.8±0.67	56.5±0.65	57.8±0.50	57.1±0.40
Hex-A, mmol/kg	13.1±0.40	9.21±0.71	9.37±0.64	8.87±0.33	9.14±0.49
E_P stage					
NaOH added, %	2.01	1.73	1.76	1.62	1.68
H_2O_2 added, %	0.5	0.5	0.5	0.5	0.5
Brightness, %ISO	75.6±0.61	77.4±0.57	77.1±0.40	77.9±0.47	77.5±0.60
Hex-A, mmol/kg	4.10±0.19	2.83±0.17	3.01±0.34	2.43±0.30	2.66±0.35

contd..

D_1 stage					
ClO_2 added, %	0.8	0.8	0.8	0.8	0.8
ClO_2 consumed, %	99.6	99.4	99.5	98.9	99.3
Brightness, %ISO	85.3±0.42	86.3±0.47	86.1±0.35	86.9±0.36	86.4±0.29
Hex-A, mmol/kg	2.70±0.24	2.20±0.35	2.26±0.33	2.00±0.26	2.13±0.12
D_2 stage					
ClO_2 added, %	0.3	0.3	0.3	0.3	0.3
ClO_2 consumed, %	88.4	88.3	88.2	87.9	88.0
Brightness, %ISO	87.6±0.62	88.5±0.67	88.4±0.82	89.1±0.68	88.8±0.57
CIE Whiteness	76.11±0.23	78.32±0.28	77.79±0.22	78.98±0.52	78.34±0.23
Shrinkage, %	4.6±0.20	5.9±0.31	6.1±0.45	5.4±0.20	5.8±0.25
Hex-A, mmol/kg	2.40±0.28	2.00±0.09	2.10±0.11	1.89±0.31	1.97±0.26

Table 4.24 Effect of xylanases for hexenuronic acid removal and improvement in optical properties of eucalyptus hardwood pulp at reduced kappa factor

Parameter	Control	EnzyA	EnzyB	EnzyC	EnzyD
Kappa No.	18.3	15.7	16.0	14.7	15.3
Kappa factor	0.22	0.187	0.187	0.187	0.187
D_0 stage					
ClO_2 added, %	1.53	1.12	1.14	1.05	1.09
ClO_2 consumed, %	98.9	99.3	99.1	99.6	99.4
Brightness, %ISO	54.7±0.55	56.1±0.47	56.0±0.45	57.2±0.25	56.4±0.36
Hex-A, mmol/kg	13.1±0.40	9.81±0.76	10.2±0.45	9.26±0.23	9.57±0.56
E_P stage					
NaOH added, %	2.01	1.47	1.50	1.37	1.43
H_2O_2 added, %	0.5	0.5	0.5	0.5	0.5
Brightness, %ISO	75.6±0.61	76.6±0.57	76.5±0.61	77.2±0.46	76.9±0.40
Hex-A, mmol/kg	4.10±0.19	3.23±0.39	3.44±0.46	2.81±0.52	2.97±0.42

contd..

D_1 stage					
ClO_2 added, %	0.8	0.7	0.7	0.7	0.7
ClO_2 consumed, %	99.6	99.9	99.9	99.8	99.9
Brightness, %ISO	85.3±0.42	85.7±0.78	85.5±0.61	86.1±0.50	85.8±0.60
Hex-A, mmol/kg	2.70±0.24	2.41±0.46	2.53±0.55	2.24±0.37	2.36±0.16
D_2 stage					
ClO_2 added, %	0.3	0.3	0.3	0.3	0.3
ClO_2 consumed, %	88.4	89.3	89.6	89.8	89.5
Brightness, %ISO	87.6±0.62	87.5±0.62	87.3±0.35	88.2±0.36	87.9±0.72
CIE Whiteness	76.11±0.23	77.05±0.38	76.74±0.34	77.76±0.32	77.25±0.26
Shrinkage, %	4.6±0.20	5.4±0.26	5.6±0.30	5.1±0.15	5.2±0.23
Hex-A, mmol/kg	2.40±0.28	2.26±0.18	2.35±0.39	2.04±0.20	2.18±0.33

As shown in Table 4.23, after the XD_0 stage, brightness gain of 2.1, 1.8, 3.1 and 2.4 units were obtained for enzyme EnzyA, EnzyB, EnzyC and EnzyD respectively as compared to control at same KF. The trend of brightness gain was observed decreasing as we carried out for further stages and it was 1.8, 1.5, 2.3 and 1.9 units after XD_0E_P; 1.0, 0.8, 1.6 and 1.1 units after $XD_0E_PD_1$ and at the end of the bleaching sequence ($XD_0E_PD_1D_2$) the final brightness gain of 0.9, 0.8, 1.5 and 1.2 units were observed. It is also reported earlier that improvement in final brightness of up to 1.4 to 2.1 units was observed with reductions in Hex-A and the kappa number of xylanase pretreated pulps compared to the corresponding control pulps.

During $XD_0E_PD_1D_2$ bleaching sequence, highest brightness gain was observed after the dioxide (D_0) stage before the extraction stage and it was also supported by observations of Fillat *et al*. (2012) where effect of different xylanases on eucalyptus hardwood pulps during enzymatic bleaching was examined. At reduced KF by 15% (Table 4.24), brightness gain was observed only for EnzyC and Enzy D and maximum of 0.6 units was obtained with EnzyC with the reduction of 22.0 and 31.8% of chlorine dioxide (ClO_2) and sodium hydroxide (NaOH). It clearly shows better candidature of EnzyC out of these four enzymes.

After the bleaching process, whiteness gain at same KF was recorded 2.21, 1.68, 2.87 and 2.23 units, while at reduced KF it was 0.94, 0.63, 1.65 and 1.14 units in

bleached pulp with enzymes EnzyA, EnzyB, EnzyC and EnzyD respectively as compared to control (Table 4.23 and 4.24). The present observations show that the effect of bleach boosting differs for each bleaching stage and varies substantially within the bleaching sequence with continued decrease in Hex-A content resulting in reduced bleach boosting effects towards the end of the sequence.

4.5.2 Effect of xylanases treatment on brightness reversion (PC no.) at equivalent and reduced chemicals charge

Due to lesser amount of Hex-A contents in bleached pulp, brightness reversion is also reduced (as chromophores are removed by xylanases treatment) as shown in Table 4.25. Here also EnzyC showed better results in reduction of post color number of the final bleached pulps in comparison to other enzymes. Table 4.25 shows the post color number as 0.60, 0.38, 0.39, 0.33 and 0.35 unit with control and enzymes (EnzyA, EnzyB, EnzyC and EnzyD) respectively at same kappa factor while at reduced kappa factor PC no. was observed as 0.60, 0.44, 0.47, 0.38 and 0.40 unit with control and enzymes (EnzyA, EnzyB, EnzyC and EnzyD) respectively.

Table 4.25 Effect of EnzyA, EnzyB, EnzyC and EnzyD on final pulp and bleach effluent properties*

Parameter	KF	Control	EnzyA	EnzyB	EnzyC	EnzyD
Post Color no.	0.22	0.60±0.03	0.38±0.03	0.39±0.04	0.33±0.01	0.35±0.04
	0.187	---	0.44±0.04	0.47± 0.04	0.38±0.03	0.40±0.02
Viscosity, cp	0.22	10.6±0.58	10.4±0.31	10.5±0.20	10.2±0.10	10.3±0.35
	0.187	---	10.5±0.30	10.6±0.15	10.4±0.12	10.4±0.15
BOD, $kgtp^{-1}$	0.22	45.4±0.87	38.1±0.60	40.5±0.70	34.6±0.42	36.2±0.35
	0.187	---	41.8±0.45	43.5±0.50	38.7±0.45	39.9±0.50
COD, $kgtp^{-1}$	0.22	75.6±0.26	66.2±0.59	68.2±0.46	55.1±0.40	65.4±0.60
	0.187	---	70.1±0.70	71.8±0.64	63.2±0.40	68.8±0.38

*Mixed effluent of all stages (D_0, E_P, D_1, D_2) in the ratio- 2:1:0.5:0.5

The content of Hex-A in the pulp after bleaching plays a major role for the discoloration reactions causing the brightness reversion. Brightness reversion in eucalyptus hardwood pulp is a complex phenomenon influenced by various factors and

directly related to its chemical composition with acid groups, such as hexenuronic acids, playing a major role. The effect of removing Hex-A (by selective hydrolysis) clearly indicates that Hex-A are the precursors of some chromophores, which results in modifying the optical properties of bleached pulps by decreasing brightness and increasing brightness reversion. The xylanase assisted removal of xylan derived chromophores (e.g. hexenuronic acids) with dissolved xylooligosaccharide fractions can also contribute to brightness as well as brightness stability improvement. Further, xylanase treatment that is responsible in removal of Hex-A indirectly facilitates improvement in brightness of pulp due to removal of residual lignin by bleach chemicals. The carbohydrate derived chromophores have a pronounced effect on brightness development of chemical pulps during xylanase aided bio-bleaching.

4.5.3 Effect of xylanases treatment on pulp viscosity

Table 4.25 depicts the change in the intrinsic viscosity of enzyme treated and untreated pulps, measured after enzyme treatment and bleaching process. Intrinsic viscosity of xylanase treated pulps, was found to be better (EnzyA, EnzyC and EnzyD) over or similar (EnzyB) to control after the X stage (Table 4.22). It is probably due to the enzyme induced dissolution of low molecular weight xylan components (oligosaccharides) causing increase in pulp viscosity of treated pulps. While, at the end of bleaching process, drop in viscosity in enzyme treated pulps was noted over the control (Table 4.25) due to the enhanced delignification of enzyme treated pulps during bleaching process at same and reduced kappa factor.

4.5.4 Effect of xylanases treatment on the effluent characteristics

However, in present investigation, effluent of different bleaching stages was also characterized for BOD and COD to study the effects of xylanase pretreatment on pollution load reduction (Table 4.25). Low values of BOD and COD in bleach effluent clearly indicated the remedial efficiency of treated pulp samples over to control.

Table 4.25 shows reduction of BOD in the range 10.8-23.8% and 4.2-14.8% for KF 0.22 and 0.187 respectively. Same trend was also followed for COD reduction and it was in range 9.8-27.1% and 5.0-16.4% for KF 0.22 and 0.187 respectively. Table 4.25 also indicates maximum reduction in BOD and COD with EnzyC and the ratio of BOD to COD was also increased for EnzyC, which leads to easier treatment of bleach effluent in secondary treatment at effluent treatment plant. Table 4.23 and 4.24 specifies more

yield loss (pulp shrinkage) in enzymatic process over to control. It is clearly shows the enzymatic process lead to reduction of organic loads in the pulps as it reduces Hex-A components from the pulps in to the effluents, sometimes it also destroy part of xylan or hemicellulose in the pulps, which may cause of yield loss or increases pulp shrinkage as compared to control.

4.5.5 Effect of xylanases treatment in reduction of bleaching chemicals consumption

Reduction in Kappa number is directly proportional to reduction in Hex-A vis-à-vis increase in the brightness of the pulp. During alkaline cooking process of hardwood pulps, hexenuronic acid bound with the xylan entraps lignin and this bounded hexenuronic acid cause hindrance to penetration of chemicals onto the fibre surface, giving higher Kappa number and thus indicating higher lignin content than actual. This results in higher addition vis-à-vis consumption of bleaching chemicals. Xylanase effectively hydrolyzes the re-precipitated xylan and removes the bounded Hex-A from the pulp fibre thus releasing the entrapped lignin content. Thus, xylanase treatment facilitates measurement of the true Kappa number vis-à-vis the lignin content leading to lesser use and better penetration of bleaching chemicals.

Various authors (Yang *et al*. 1992a; Tolan *et al*. 1996; Shatalov and Pereira 2009) also stated that xylanases treatment allows to reduce consumption of active bleaching chemicals (particularly the chlorine-based) and to increase the final brightness ceiling of bleached pulps.

Table 4.26 Reduction in bleaching chemicals consumption in ECF bleaching of eucalyptus hardwood pulp at reduced kappa factor

Particulars	ClO_2, $kgtp^{-1}$	Savings, $kgtp^{-1}$	NaOH, $kgtp^{-1}$	Savings, $kgtp^{-1}$
Control	26.3	---	20.1	---
EnzyA	21.2	5.1	14.7	5.4
EnzyB	21.4	4.9	15.0	5.1
EnzyC	20.5	5.8	13.7	6.4
EnzyD	20.9	5.4	14.3	5.8

Out of these four enzymes; EnzyC shows better reduction in bleach chemicals consumption (Table 4.26) during bleaching of eucalyptus hardwood pulps and obtained

the possible reduction of 22.0 and 31.8% respectively for ClO_2 and NaOH at reduced KF with improvement in optical properties of the final pulp. It leads to its promising behavior and better suitability at industrial level. Results reported by Battan *et al*. (2007) supported our findings about reduction in chlorine consumption indicating 20.0% reduction in chlorine consumption due to enzymatic bio-bleaching with xylanase without any change in brightness. Dhiman *et al*. (2008) also reported that xylanase treatment was responsible for the reduction of 15.0% in chlorine consumption.

Removal of hexenuronic acid, reduction in kappa number and improvement in brightness were tested across all the stages (X, XD_0, XD_0E_P, $XD_0E_PD_1$ and $XD_0E_PD_1D_2$) as shown in Figure 4.6 and 4.8a & 4.8b. Figure 4.8a and 4.8b show the trend of hexenuronic acid reduction throughout the bleaching process at equivalent and reduced chemicals charge. Effect of enzyme stage was also seen on pulp viscosity before and after the bleaching process with all four enzymes; EnzyA, EnzyB EnzyC and EnzyD (Figure 4.9). Figure 4.10 shows the pattern of post color number after the enzyme treatment with all four enzymes. Final pulp whiteness was also observed after the enzyme treatment as shown in Figure 4.11.

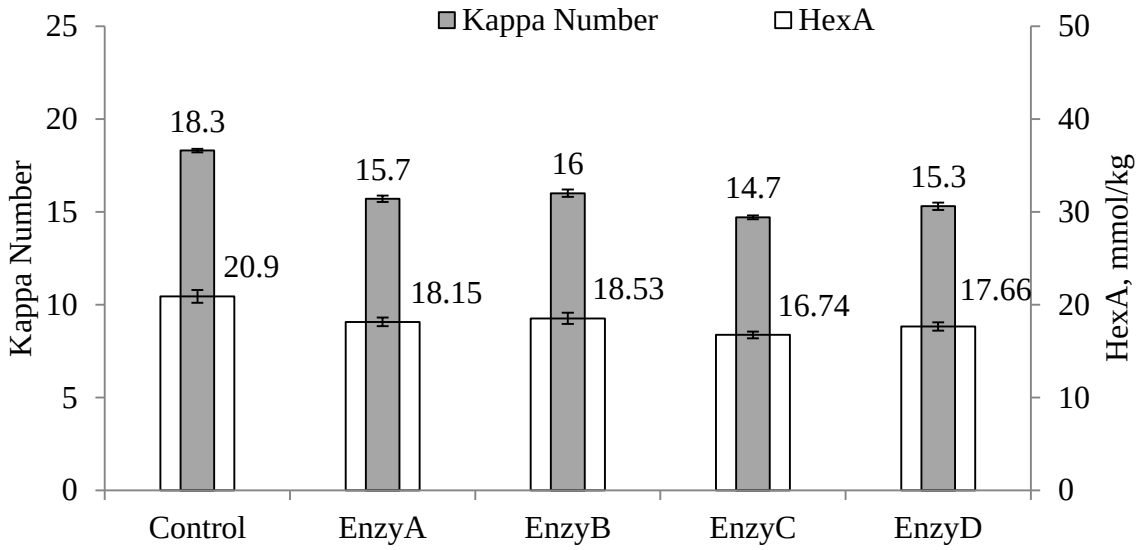

Fig. 4.6 Effect of enzyme treatment on Hex-A and Kappa number reduction in X stage

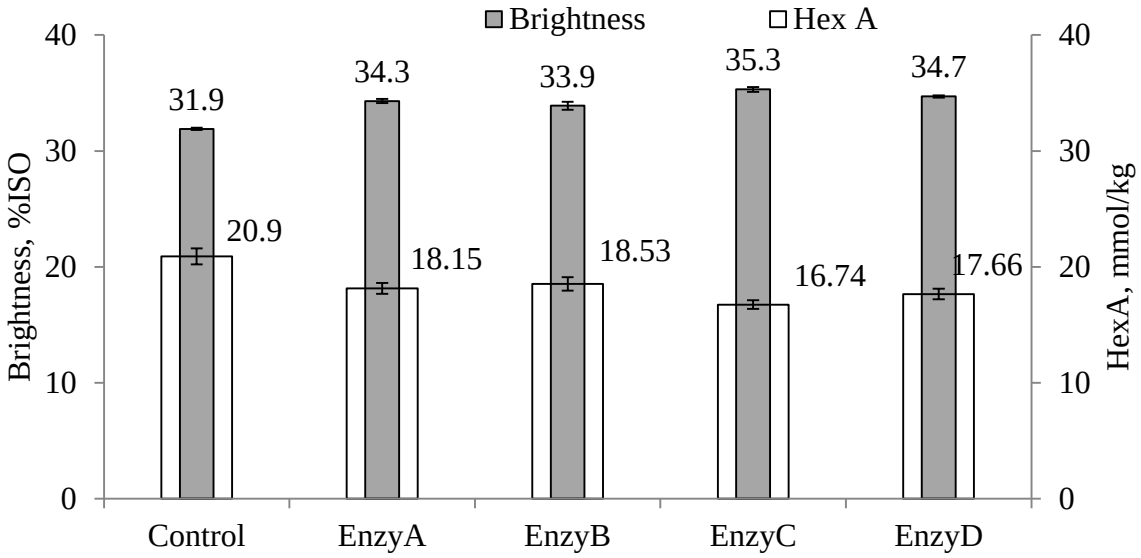

Fig. 4.7 Effect of enzyme treatment on Hex-A and Brightness improvement in X stage

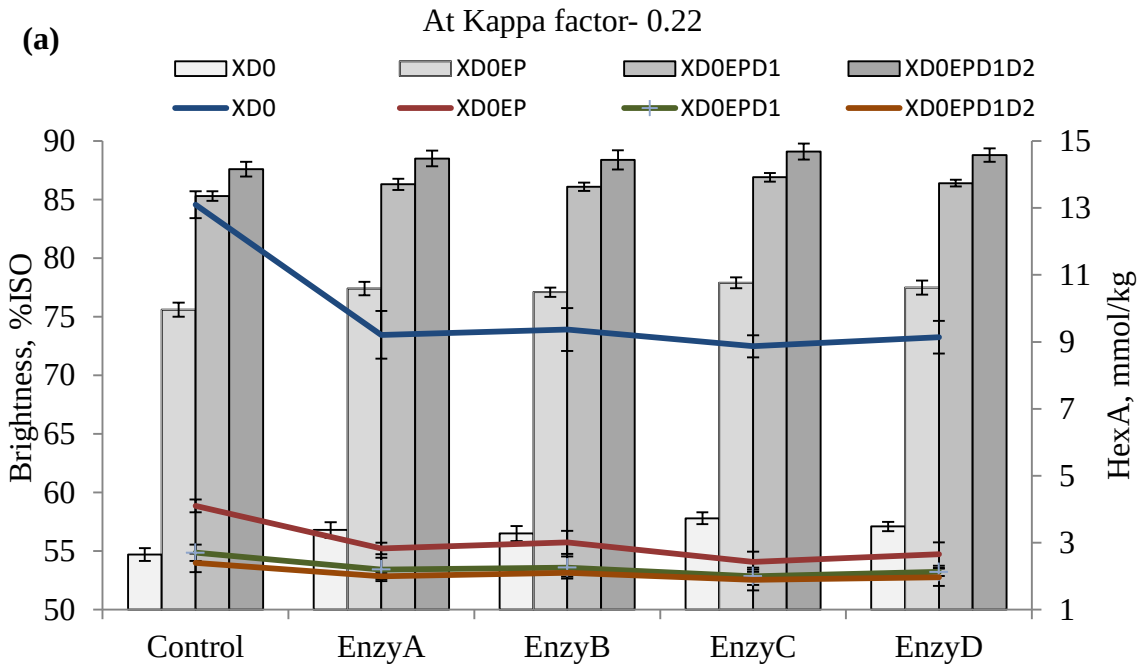

Fig. 4.8a Trend of Hex-A and Brightness (%ISO) at each stage during ECF bleaching process at equivalent kappa factor

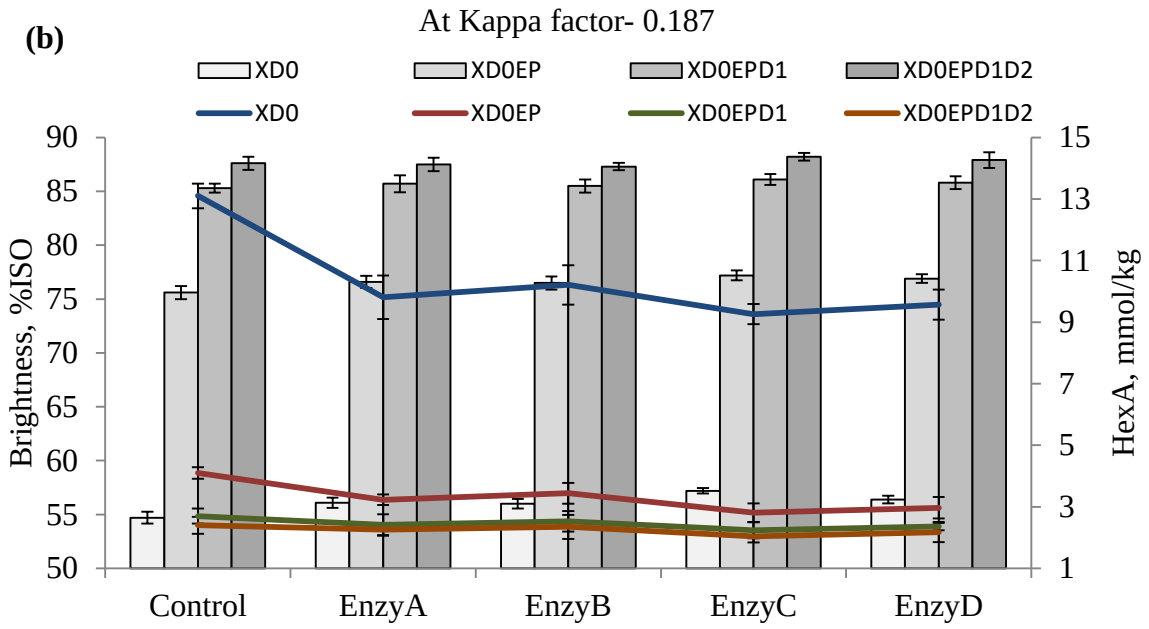

Fig. 4.8b Trend of Hex-A and Brightness (%ISO) at each stage during ECF bleaching process at reduced kappa factor

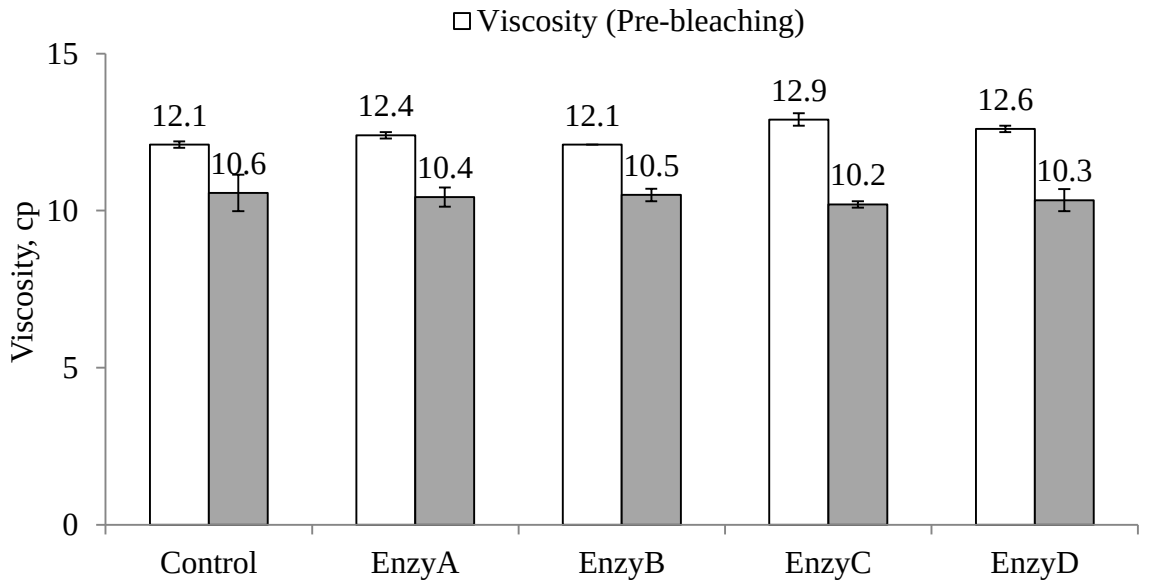

Fig. 4.9 Effect of enzyme stage on viscosity of pulp (Pre and post bleaching)

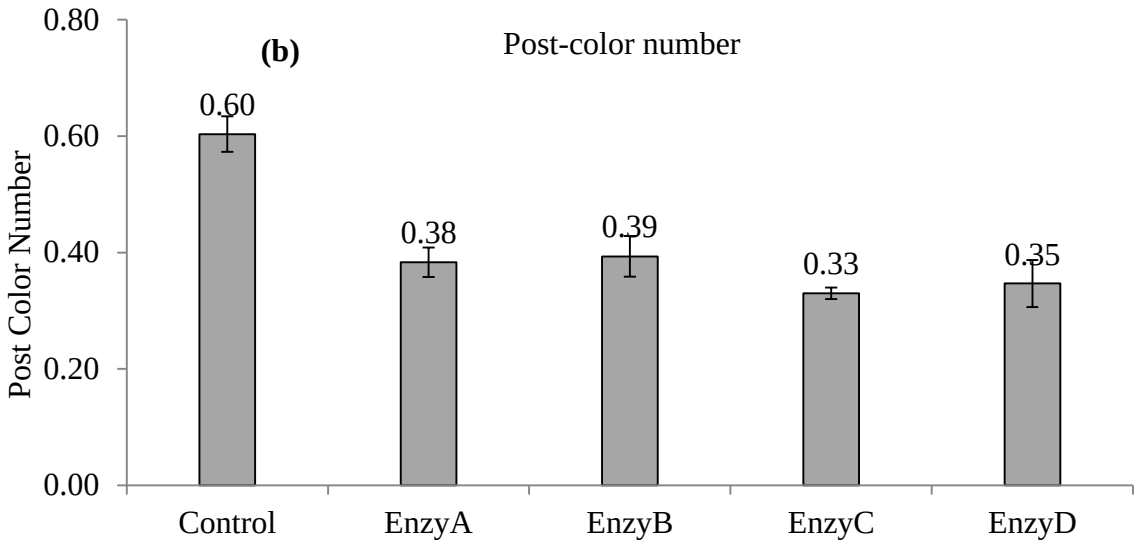

Fig. 4.10 Effect of enzyme stage on post color number reduction

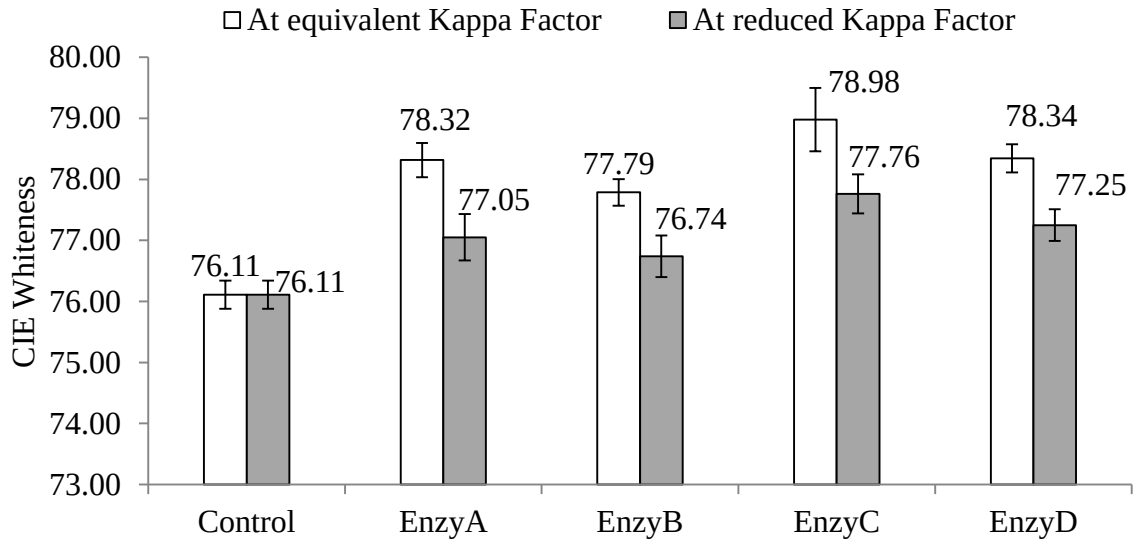

Fig. 4.11 Effect of enzymatic stage on CIE whiteness of bleached pulp

The incorporation of enzyme treatment prior to conventional bleaching sequence and its efficacy in removal of Hex-A vis-à-vis improvement in optical properties of pulp was studied in this approach.

This approach is helpful in knowing the hexenuronic acid reduction at each bleaching stages during the bleaching process. Out of these four enzymes, EnzyC reported as the best suitable enzyme in reduction of hexenuronic acid along with the improvement of optical properties in hardwood kraft pulps. This is the best beneficial approach for the industrialists, who are working on the enzymatic reduction of hexenuronic acid from the hardwood kraft pulps.

CONCLUSIONS

This study is concluded the effect of four different cellulase-free commercial xylanases on different hardwood kraft pulps using different bleaching sequences. Comparative study of acacia and eucalyptus hardwood pulps was done with all four enzymes (EnzyA, EnzyB, EnzyC and EnzyD) to find out the best suitable enzyme and hardwood for industrial purpose.

The present results provide a clear indication of the advantage of xylanase treatment, and a better selectively of acacia over eucalyptus for use in the paper-making process, as these approaches resulted in the decreasing consumption of bleach chemicals, leading to a reduced cost in the treatment of hazardous chemicals and improved qualities of the final paper. In this approach, reduction in bleach chemicals consumption was observed to be higher in acacia than eucalyptus wood pulps with xylanase enzyme treatment before the ECF bleaching sequence. A reduction in chlorine dioxide (ClO_2) and sodium hydroxide (NaOH) by more than 17.0% and 23.0%, respectively, was noticed for both hardwood pulps, with an improvement in the optical properties of the pulps. Marginal increment in pulp shrinkage was observed in both hardwood pulps as compared to their respective control with shrinkage lesser in acacia than eucalyptus pulp while the strength properties of eucalyptus pulp were observably better than acacia pulp. This approach results in better selectivity of acacia over eucalyptus use at industrial level.

Enzyme (EnzyC) stage was also tested and incorporated at different places of eucalyptus hardwood bleaching. In conclusion, pre-treatment bleaching sequences is observed more effective in increasing the final brightness (1.6 units for $XC_DE_PD_1D_2$ and 1.5 units for ECF bleaching sequence, $XD_0E_PD_1D_2$) in addition to reduction in bleach chemicals and their subsequent release into the effluent. In addition, a reduction of 32% in AOX was observed for pre-treatment bleaching sequence over post-treatment and intermediate bleaching sequences. On contrast, if the desired target is to increase the final whiteness in paper, post-treatment was observably a better option over others as gain in whiteness of 3.4 units was achieved for $C_DE_PD_1D_2X$ and 3.2 units for $D_0E_PD_1D_2X$. It is also concluded that out of nine different sequences examined, maximum reduction in PC number (48%) was possible with post-treatment bleaching sequences and minimum (39%) for pre-treatment bleaching sequence. Incorporation of enzyme stage at any place in bleaching sequence improved BOD to COD ratio indicating

better degradability of the bleach effluents in secondary treatment when compared to control.

Among the enzymes tested, EnzyC was also found to exhibit better performance with reference in removal of Hex-A contents across all the stages (X, XD_0, XD_0E_P, $XD_0E_PD_1$ and $XD_0E_PD_1D_2$) tested respectively over to control. These results also demonstrate the clear trend of Hex-A profile at all bleaching stages for eucalyptus hardwood pulp. This approach can be used as bleach boosting process and can be potentially used to develop the green technology for pulp and paper industry. Maximum reduction in kappa number and bleach chemicals consumption with improvement in optical properties was observed with EnzyC over other enzymes (EnzyA, EnzyB and EnzyD) throughout the enzymatic bleaching process. Pulp viscosity (pre and post bleaching) was comparable with all enzymes. EnzyC showed higher reduction in the post color number as compared to all other enzymes. Improvement in CIE whiteness was also observed higher in case of EnzyC treated pulps. Performance of EnzyC was found better for reducing the pollution load in bleach effluent as compared to other enzymes. Highest chemicals savings for chlorine dioxide and caustic were shown with EnzyC among the all four enzymes used in the study.

Thus, with reference to all the observations obtained during the study, EnzyC is able to deliver the promising results and can be concluded as the best bleach boosting enzyme for hardwood kraft pulps bleaching.

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Table 1.1 Xylanase activities for EnzyA, EnzyB, EnzyC and EnzyD at 50°C and pH 6.0

Enzyme	Absorbance at 540nm	Concentration	Dilution factor	Incubation time	Individual activity	Xylanase activity (IU/ml)
EnzyA	0.419	4.48	10000	5	8,966	9,021 ±68
	0.424	4.54	10000	5	9,080	
	0.2888	2.99	15000	5	8,959	
	0.2923	3.03	15000	5	9,079	
EnzyB	0.247	2.51	10000	5	5,011	4,891 ±149
	0.2477	2.52	10000	5	5,028	
	0.1668	1.58	15000	5	4,752	
	0.1674	1.59	15000	5	4,772	
EnzyC	0.364	3.85	15000	5	11,552	10,850 ±759
	0.3611	3.82	15000	5	11,452	
	0.2531	2.58	20000	5	10,303	
	0.2485	2.52	20000	5	10,092	
EnzyD	0.401	4.28	5000	5	4,276	4,431 ±207
	0.397	4.23	5000	5	4,230	
	0.229	2.29	10000	5	4,598	
	0.23	2.31	10000	5	4,621	

Table 1.2 Xylanase activities for EnzyA, EnzyB, EnzyC and EnzyD at 50°C and pH 7.0

Enzyme	Absorbance at 540nm	Concentration	Dilution factor	Incubation time	Individual activity	Xylanase activity (IU/ml)
EnzyA	0.627	3.75	15000	5	11,245	11,331 ±202
	0.6406	3.84	15000	5	11,530	
	0.489	2.78	20000	5	11,133	
	0.5032	2.88	20000	5	11,530	
EnzyB	0.8498	5.31	10000	5	10,613	10,441 ±238
	0.821	5.10	10000	5	10,210	
	0.5787	3.41	15000	5	10,231	
	0.5987	3.55	15000	5	10,651	
EnzyC	0.624	3.73	25000	5	18,636	18,979 ±353
	0.642	3.85	25000	5	19,266	
	0.537	3.12	30000	5	18,713	
	0.551	3.22	30000	5	19,301	
EnzyD	0.84	5.24	5000	5	5,238	5,177 ±75
	0.821	5.10	5000	5	5,105	
	0.457	2.56	10000	5	5,119	
	0.466	2.62	10000	5	5,245	

Table 1.3 Xylanase activities for EnzyA, EnzyB, EnzyC and EnzyD at 50°C and pH 8.0

Enzyme	Absorbance at 540nm	Concentration	Dilution factor	Incubation time	Individual activity	Xylanase activity (IU/ml)
EnzyA	0.891	5.64	15000	5	16,909	17,044 ±178
	0.906	5.73	15000	5	17,182	
	0.657	4.22	20000	5	16,873	
	0.671	4.30	20000	5	17,212	
EnzyB	0.767	4.88	15000	5	14,655	14,355 ±399
	0.729	4.65	15000	5	13,964	
	0.569	3.68	20000	5	14,739	
	0.541	3.52	20000	5	14,061	
EnzyC	0.943	5.95	30000	5	35,709	35,312 ±441
	0.922	5.82	30000	5	34,945	
	0.784	4.99	35000	5	34,915	
	0.802	5.10	35000	5	35,679	
EnzyD	0.739	4.72	5000	5	4,715	4,891 ±240
	0.729	4.65	5000	5	4,655	
	0.379	2.53	10000	5	5,067	
	0.384	2.56	10000	5	5,127	

Table 1.4 Xylanase activities for EnzyA, EnzyB, EnzyC and EnzyD at 50°C and pH 9.0

Enzyme	Absorbance at 540nm	Concentration	Dilution factor	Incubation time	Individual activity	Xylanase activity (IU/ml)
EnzyA	0.635	3.42	15000	5	10,252	10,556 ±361
	0.662	3.61	15000	5	10,835	
	0.5388	2.73	20000	5	10,901	
	0.5157	2.56	20000	5	10,236	
EnzyB	0.753	4.27	15000	5	12,799	13,232 ±550
	0.798	4.59	15000	5	13,770	
	0.634	3.41	20000	5	13,640	
	0.602	3.18	20000	5	12,719	
EnzyC	0.934	5.57	30000	5	33,410	33,622 ±229
	0.943	5.63	30000	5	33,799	
	0.824	4.78	35000	5	33,439	
	0.832	4.83	35000	5	33,842	
EnzyD	0.281	0.87	5000	5	871	926 ±69
	0.296	0.98	5000	5	978	
	0.229	0.50	10000	5	993	
	0.22	0.43	10000	5	863	

Table 1.5 Xylanase activities for EnzyA, EnzyB, EnzyC and EnzyD at 55°C and pH 6.0

Enzyme	Absorbance at 540nm	Concentration	Dilution factor	Incubation time	Individual activity	Xylanase activity (IU/ml)
EnzyA	0.308	6.19	10000	5	12,377	12,156 ±235
	0.297	5.98	10000	5	11,962	
	0.191	3.98	15000	5	11,943	
	0.198	4.11	15000	5	12,340	
EnzyB	0.0903	2.08	10000	5	4,162	4,266 ±105
	0.091	2.09	10000	5	4,189	
	0.0568	1.45	15000	5	4,347	
	0.0571	1.45	15000	5	4,364	
EnzyC	0.319	6.40	15000	5	19,189	18,566 ±752
	0.297	5.98	15000	5	17,943	
	0.217	4.47	20000	5	17,887	
	0.235	4.81	20000	5	19,245	
EnzyD	0.2851	5.76	5000	5	5,757	5,920 ±221
	0.3035	6.10	5000	5	6,104	
	0.1311	2.85	10000	5	5,702	
	0.1421	3.06	10000	5	6,117	

Table 1.6 Xylanase activities for EnzyA, EnzyB, EnzyC and EnzyD at 55°C and pH 7.0

Enzyme	Absorbance at 540nm	Concentration	Dilution factor	Incubation time	Individual activity	Xylanase activity (IU/ml)
EnzyA	0.9088	7.85	10000	5	15,698	15,925 ±270
	0.9081	7.84	10000	5	15,686	
	0.629	5.39	15000	5	16,184	
	0.627	5.38	15000	5	16,132	
EnzyB	0.91	7.86	5000	5	7,860	8,211 ±470
	0.898	7.75	5000	5	7,754	
	0.508	4.33	10000	5	8,667	
	0.502	4.28	10000	5	8,561	
EnzyC	0.821	7.08	15000	5	21,237	21,719 ±625
	0.859	7.41	15000	5	22,237	
	0.649	5.57	20000	5	22,281	
	0.616	5.28	20000	5	21,123	
EnzyD	0.8533	7.36	5000	5	7,362	7,564 ±245
	0.899	7.76	5000	5	7,763	
	0.4325	3.67	10000	5	7,342	
	0.458	3.89	10000	5	7,789	

Table 1.7 Xylanase activities for EnzyA, EnzyB, EnzyC and EnzyD at 55°C and pH 8.0

Enzyme	Absorbance at 540nm	Concentration	Dilution factor	Incubation time	Individual activity	Xylanase activity (IU/ml)
EnzyA	0.8775	6.57	15000	5	19,713	19,192 ±553
	0.8383	6.26	15000	5	18,787	
	0.635	4.66	20000	5	18,646	
	0.666	4.91	20000	5	19,622	
EnzyB	0.9197	6.90	10000	5	13,806	14,123 ±350
	0.9484	7.13	10000	5	14,258	
	0.6592	4.85	15000	5	14,556	
	0.6303	4.62	15000	5	13,873	
EnzyC	0.8972	6.73	35000	5	47,082	46,876 ±226
	0.89	6.67	35000	5	46,685	
	0.784	5.83	40000	5	46,677	
	0.7901	5.88	40000	5	47,061	
EnzyD	0.712	5.27	5000	5	5,268	5,315 ±59
	0.724	5.36	5000	5	5,362	
	0.377	2.63	10000	5	5,260	
	0.384	2.69	10000	5	5,370	

Table 1.8 Xylanase activities for EnzyA, EnzyB, EnzyC and EnzyD at 55°C and pH 9.0

Enzyme	Absorbance at 540nm	Concentration	Dilution factor	Incubation time	Individual activity	Xylanase activity (IU/ml)
EnzyA	0.649	4.20	15000	5	12,609	12,370 ±256
	0.63	4.06	15000	5	12,180	
	0.508	3.14	20000	5	12,571	
	0.493	3.03	20000	5	12,120	
EnzyB	0.679	4.43	15000	5	13,286	13,133 ±163
	0.666	4.33	15000	5	12,992	
	0.531	3.32	20000	5	13,263	
	0.522	3.23	20000	5	12,992	
EnzyC	0.826	5.53	35000	5	38,737	39,476 ±997
	0.857	5.77	35000	5	40,368	
	0.73	4.81	40000	5	38,496	
	0.76	5.04	40000	5	40,301	
EnzyD	0.27	1.35	5000	5	1,353	1,414 ±79
	0.286	1.47	5000	5	1,474	
	0.179	0.67	10000	5	1,338	
	0.189	0.74	10000	5	1,489	

Table 1.9 Enzymatic treatment of Eucalyptus pulp with EnzyC for $C_DE_PD_1D_2$, $D_0E_PD_1D_2$ and $D_0E_PD_1E_P$ bleaching sequence

Parameter	Control	Set 1	Set 2	Set 3
Enzyme Dose, kgt^{-1}	---	0.1	0.3	0.5
Reaction time, min.		90		
Consistency, %		10		
Reaction temp., °C		55		
Initial pH		8.0		
End pH	7.64	7.71	7.75	7.77
Initial Kappa No.	20.4	20.4	20.4	20.4
End Kappa No.	20.0±0.53	19.3±0.46	18.6±0.46	18.0±0.45
Kappa No. reduction, %	---	3.5	7.0	10.0
Initial Brightness, %ISO	27.7	27.7	27.7	27.7
End Brightness, %ISO	28.0±0.44	29.2±0.49	30.1±0.46	30.8±0.52
Brightness gain, unit	---	1.2	2.1	2.7
Pulp yield, %	99.8±0.23	99.7±0.06	99.5±0.23	99.2±0.36

Table 1.10 Enzymatic bleaching behavior using $XC_DE_PD_1D_2$ sequence

Parameter	Control	Set 1	Set 2	Set 3
Kappa No.	20.0	19.3	18.6	18.0
Kappa factor	0.22	0.22	0.22	0.22
C_D stage				
Elemental chlorine, %	4.40	4.25	4.09	3.96
ClO_2 added, %	1.67	1.61	1.56	1.51
End pH	2.35	2.34	2.38	2.30
Cl_2 consumed, %	99.8	99.5	99.4	99.2
E_P stage				
NaOH added, %	2.20	2.13	2.05	1.98
H_2O_2 added, %	0.5	0.5	0.5	0.5
Initial pH	11.46	11.42	11.40	11.43
End pH	10.58	10.52	10.53	10.54
Kappa No.	2.2±0.12	2.1±0.06	2.0±0.25	1.8±0.06
Brightness, %ISO	76.4±0.61	77.5±0.36	78.1±0.42	79.0±0.47
H_2O_2 consumed, %	100	100	100	100
D_1 stage				
ClO_2 added, %	0.8	0.8	0.8	0.8
Initial pH	2.85	2.80	2.79	2.84
End pH	3.37	3.36	3.31	3.36
Brightness, %ISO	85.5±0.40	86.5±0.51	86.9±0.45	87.3±0.35
ClO_2 consumed,%	99.7	99.2	99.0	98.8
D_2 stage				
ClO_2 added, %	0.3	0.3	0.3	0.3
Initial pH	2.88	2.87	2.89	2.90
End pH	3.40	3.38	3.40	3.44
ClO_2 consumed, %	85.1	82.0	81.7	81.5
Brightness, %ISO	87.5±0.67	88.4±0.42	88.8±0.49	89.1±0.36
CIE Whiteness	77.14±0.38	78.26±0.45	79.29±0.31	79.97±0.36
L*	97.56	97.81	97.99	98.10
a*	-0.15	-0.13	-0.12	-0.10
b*	4.86	3.90	3.78	3.70
PC No.	0.58±0.07	0.48±0.06	0.40±0.05	0.34±0.04
PC No. reduction, %	---	17.2	31.0	41.4
Shrinkage, %	5.7±0.49	5.9±0.46	6.1±0.30	6.3±0.45

Table 1.11 Enzymatic bleaching behavior using $XD_0E_PD_1D_2$ sequence

Parameter	Control	Set 1	Set 2	Set 3
Kappa No.	20.0	19.3	18.6	18.0
Kappa factor	0.22	0.22	0.22	0.22
D_0 stage				
ClO_2 added, %	1.69	1.61	1.56	1.51
End pH	2.35	2.34	2.38	2.30
ClO_2 consumed, %	99.9	99.6	99.4	99.3
E_P stage				
NaOH added, %	2.20	2.13	2.05	1.98
H_2O_2 added, %	0.5	0.5	0.5	0.5
Initial pH	11.51	11.48	11.46	11.48
End pH	10.60	10.55	10.52	10.51
Kappa No.	2.3±0.29	2.2±0.17	2.1±0.17	1.9±0.15
Brightness, %ISO	76.0±0.57	77.2±0.21	77.6±0.17	78.2±0.26
H_2O_2 consumed, %	100	100	100	100
D_1 stage				
ClO_2 added, %	0.8	0.8	0.8	0.8
Initial pH	2.78	2.80	2.81	2.84
End pH	3.39	3.38	3.41	3.43
Brightness, %ISO	85.1±0.40	85.9±0.29	86.4±0.45	86.8±0.26
ClO_2 consumed,%	99.6	99.1	98.5	98.1
D_2 stage				
ClO_2 added, %	0.3	0.3	0.3	0.3
Initial pH	2.78	2.74	2.79	2.74
End pH	3.30	3.33	3.31	3.37
ClO_2 consumed, %	84.6	81.6	81.2	80.8
Brightness, %ISO	87.3±0.46	88.0±0.38	88.4±0.17	88.8±0.36
CIE Whiteness	76.86±0.57	78.26±0.49	78.81±0.34	79.31±0.34
L*	97.13	97.44	97.48	97.51
a*	-0.17	-0.15	-0.14	-0.13
b*	4.91	4.00	3.78	3.71
PC No.	0.62±0.07	0.50±0.04	0.42±0.04	0.35±0.03
PC No. reduction, %	---	19.4	32.3	43.5
Shrinkage, %	5.5±0.46	5.7±0.32	5.9±0.32	6.1±0.26

Table 1.12 Enzymatic bleaching behavior using $XD_0E_PD_1E_P$ sequence

Parameter	Control	Set 1	Set 2	Set 3
Kappa No.	20.0	19.3	18.6	18.0
Kappa factor	0.22	0.22	0.22	0.22
D_0 stage				
ClO_2 added, %	1.67	1.61	1.56	1.51
End pH	2.35	2.34	2.38	2.30
ClO_2 consumed, %	99.9	99.6	99.4	99.3
E_P stage				
NaOH added, %	2.20	2.13	2.05	1.98
H_2O_2 added, %	0.5	0.5	0.5	0.5
Initial pH	11.51	11.48	11.46	11.48
End pH	10.60	10.55	10.52	10.51
Kappa No.	2.3±0.26	2.2±0.12	2.1±0.15	1.9±0.17
Brightness, %ISO	75.2±0.44	76.3±0.35	76.8±0.42	77.2±0.26
H_2O_2 consumed, %	100	100	100	100
D_1 stage				
ClO_2 added, %	0.8	0.8	0.8	0.8
Initial pH	2.78	2.80	2.81	2.84
End pH	3.39	3.38	3.41	3.43
Brightness, %ISO	84.8±0.42	85.6±0.45	86.0±0.42	86.3±0.42
ClO_2 consumed, %	99.6	99.1	98.5	98.1
E_P stage				
NaOH added, %	1.76	1.70	1.63	1.58
H_2O_2 added, %	0.5	0.5	0.5	0.5
Initial pH	11.26	11.25	11.21	11.20
End pH	10.36	10.35	10.30	10.31
H_2O_2 consumed, %	99.9	99.8	99.8	99.7
Brightness, %ISO	87.1±0.50	87.7±0.38	88.1±0.17	88.4±0.36
CIE Whiteness	76.01±0.32	76.91±0.31	77.61±0.23	78.01±0.38
L*	97.10	97.21	97.42	97.49
a*	-0.17	0.16	0.15	0.15
b*	4.96	4.11	4.00	3.91
PC No.	0.61±0.06	0.51±0.02	0.44±0.04	0.37±0.05
PC No. reduction, %	---	16.4	27.9	39.3
Shrinkage, %	5.4±0.40	5.7±0.26	5.8±0.25	6.0±0.20

Table 1.13 Enzymatic bleaching behavior using $C_DE_PD_1D_2X$ sequence

Parameter	SET 1			
Kappa No.	20.4			
Kappa factor	0.22			
C_D stage				
Elemental chlorine,%	4.49			
ClO_2 added, %	1.71			
End pH	2.26			
Cl_2 consumed, %	99.8			
E_P stage				
NaOH added, %	2.24			
H_2O_2 added, %	0.5			
Initial pH	11.50			
End pH	10.64			
Kappa No.	2.2±0.31			
Brightness, %ISO	76.0±0.38			
H_2O_2 consumed, %	100			
D_1 stage				
ClO_2 added, %	0.8			
Initial pH	2.86			
End pH	3.41			
Brightness, %ISO	85.3±0.46			
ClO_2 consumed, %	99.7			
D_2 stage				
ClO_2 added, %	0.3			
Initial pH	2.90			
End pH	3.42			
ClO_2 consumed, %	85.2			
Brightness, %ISO	87.4±0.53			
	SET 1	SET 2	SET 3	SET 4
X stage				
Enzyme Dose,kgt^{-1}	---	0.1	0.3	0.5

contd..

Reaction time, min.	90	90	90	90
Consistency, %	10	10	10	10
Reaction temp. ,°C	55	55	55	55
Initial pH	8.0	8.0	8.0	8.0
Final pH	7.61	7.69	7.70	7.72
Final Brightness, %ISO	87.3±0.55	87.8±0.50	88.3±0.25	88.5±0.36
CIE Whiteness	76.54±0.49	78.24±0.42	79.33±0.39	79.91±0.41
L*	97.58	97.98	98.08	98.21
a*	-0.16	-0.14	-0.14	-0.13
b*	4.84	3.92	3.81	3.76
PC No.	0.51±0.05	0.40±0.05	0.34±0.06	0.29±0.02
PC No. reduction, %	---	21.6	33.3	43.1
Shrinkage, %	6.0±0.36	6.0±0.35	6.3±0.46	6.6±0.46

Table 1.14 Enzymatic bleaching behavior using $D_0E_PD_1D_2X$ sequence

Parameter	SET 1
Kappa No.	20.4
Kappa factor	0.22
D_0 stage	
ClO_2 added, %	1.71
End pH	2.28
ClO_2 consumed, %	99.9
E_P stage	
NaOH added, %	2.24
H_2O_2 added, %	0.5
Initial pH	11.48
End pH	10.58
Kappa No.	2.3±0.26
Brightness, %ISO	75.8±0.40
H_2O_2 consumed, %	100
D_1 stage	
ClO_2 added, %	0.8
Initial pH	2.90
End pH	3.42
Brightness, %ISO	84.8±0.51
ClO_2 consumed, %	99.6
D_2 stage	
ClO_2 added, %	0.3
Initial pH	2.88
End pH	3.33
ClO_2 consumed, %	85.0
Brightness, %ISO	86.9±0.61

	SET 1	SET 2	SET 3	SET 4
X stage				
Enzyme Dose,kgt^{-1}	---	0.1	0.3	0.5

contd..

Reaction time, min.	90	90	90	90
Consistency, %	10	10	10	10
Reaction temp. ,°C	55	55	55	55
Initial pH	8.0	8.0	8.0	8.0
Final pH	7.58	7.66	7.70	7.71
Final Brightness, %ISO	87.1±0.51	87.6±0.59	87.9±0.36	88.2±0.21
CIE Whiteness	76.00±0.58	77.50±0.31	78.52±0.33	79.21±0.27
L*	97.01	97.18	97.28	97.31
a*	-0.17	-0.15	-0.14	-0.14
b*	4.84	4.21	4.11	4.01
PC No.	0.54±0.04	0.40±0.04	0.32±0.06	0.28±0.06
PC No. reduction, %	---	25.9	40.7	48.1
Shrinkage, %	5.7±0.31	5.8±0.21	6.0±0.21	6.2±0.35

Table 1.15 Enzymatic bleaching behavior using $D_0E_PD_1E_PX$ sequence

Parameter	SET 1			
Kappa No.	20.4			
Kappa factor	0.22			
D_0 stage				
ClO_2 added, %	1.71			
End pH	2.30			
ClO_2 consumed, %	99.8			
E_P stage				
NaOH added, %	2.24			
H_2O_2 added, %	0.5			
Initial pH	11.51			
End pH	10.60			
Kappa No.	2.3±0.40			
Brightness, %ISO	74.8±0.56			
H_2O_2 consumed, %	100			
D_1 stage				
ClO_2 added, %	0.8			
Initial pH	2.88			
End pH	3.44			
Brightness, %ISO	84.1±0.32			
ClO_2 consumed, %	99.5			
E_P stage				
NaOH added, %	1.80			
H_2O_2 added, %	0.5			
Initial pH	11.48			
End pH	10.65			
H_2O_2 consumed, %	100			
Brightness, %ISO	86.7±0.26			
	SET 1	SET 2	SET 3	SET 4
X stage				
Enzyme Dose,kgt^{-1}	---	0.1	0.3	0.5

contd..

Reaction time, min.	90	90	90	90
Consistency, %	10	10	10	10
Reaction temp. ,°C	55	55	55	55
Initial pH	8.0	8.0	8.0	8.0
Final pH	7.64	7.69	7.74	7.76
Final Brightness, %ISO	86.9±0.55	87.3±0.32	87.6±0.35	87.9±0.40
CIE Whiteness	75.60±0.38	76.93±0.23	77.91±0.40	78.42±0.22
L*	97.15	97.08	97.18	97.32
a*	-0.17	-0.14	-0.13	-0.12
b*	4.81	4.18	4.05	3.99
PC No.	0.52±0.05	0.42±0.03	0.36±0.03	0.30±0.02
PC No. reduction, %	---	19.2	30.8	42.3
Shrinkage, %	5.5±0.23	5.6±0.35	5.7±0.35	5.9±0.21

Table 1.16 Enzymatic bleaching behavior using $D_0XE_PD_1D_2$ sequence

Parameter	Set 1			
Kappa No.	20.4			
Kappa factor	0.22			
D_0 stage				
ClO_2 added, %	1.72			
End pH	2.28			
ClO_2 consumed, %	99.8			
X stage				
	Set 1	Set 2	Set 3	Set 4
Enzyme Dose,kgt^{-1}	---	0.1	0.3	0.5
Reaction time, min.	90	90	90	90
Consistency, %	10	10	10	10
Reaction temp. ,°C	55	55	55	55
Initial pH	8.0	8.0	8.0	8.0
Final pH	7.47	7.53	7.58	7.63
Final Kappa No.	10.3±0.40	9.1±0.50	7.8±0.31	6.5±0.40
Final Brightness, %ISO	57.1±0.60	57.9±0.47	58.5±0.45	59.4±0.49
E_P stage				
NaOH added,%	2.24	2.24	2.24	2.24
H_2O_2 added, %	0.5	0.5	0.5	0.5
Initial pH	11.54	11.46	11.45	11.54
End pH	10.56	10.49	10.41	10.50
Kappa No.	2.5±0.40	2.3±0.44	2.2±0.10	2.0±0.15
Brightness, %ISO	74.6±0.60	75.5±0.40	75.9±0.31	76.3±0.50
H_2O_2 consumed, %	100	100	99.5	99.2
D_1 stage				
ClO_2 added, %	0.8	0.8	0.8	0.8
Initial pH	2.61	2.64	2.61	2.60
End pH	3.23	3.24	3.28	3.26
Brightness, %ISO	83.8±0.42	84.3±0.49	84.6±0.44	85.3±0.58
ClO_2 consumed, %	99.4	98.5	98.2	97.9

contd..

D_2 stage				
ClO_2 added, %	0.3	0.3	0.3	0.3
Initial pH	2.74	2.81	2.80	2.82
End pH	3.34	3.31	3.30	3.36
ClO_2 consumed, %	87.4	87.0	86.5	86.0
Brightness, %ISO	85.8±0.38	86.2±0.20	86.5±0.44	86.8±0.40
CIE Whiteness	73.95±0.27	74.62±0.34	75.00±0.53	75.11±0.41
L*	96.39	96.77	96.85	96.98
a*	-0.03	-0.03	-0.04	-0.09
b*	5.75	5.02	4.82	4.42
PC No.	0.58±0.07	0.46±0.01	0.38±0.03	0.32±0.06
PC No. reduction, %	---	20.7	34.5	44.8
Shrinkage, %	5.4±0.42	5.5±0.26	5.7±0.46	5.8±0.15

Table 1.17 Enzymatic bleaching behavior using $D_0E_PD_1XD_2$ sequence

Parameter	Set 1
Kappa No.	20.4
Kappa factor	0.22
D_0 stage	
ClO_2 added, %	1.71
End pH	2.30
ClO_2 consumed, %	99.8
E_P stage	
NaOH added, %	2.24
H_2O_2 added, %	0.5
Initial pH	11.51
End pH	10.60
Kappa No.	2.3±0.31
Brightness, %ISO	74.8±0.49
H_2O_2 consumed, %	100
D_1 stage	
ClO_2 added, %	0.8
Initial pH	2.90
End pH	3.44
Brightness, %ISO	84.1±0.48
ClO_2 consumed, %	99.5
X stage	

	Set 1	Set 2	Set 3	Set 4
Enzyme Dose,kgt^{-1}	---	0.1	0.3	0.5
Reaction time, min.	90	90	90	90
Consistency, %	10	10	10	10
Reaction temp. ,°C	55	55	55	55
Initial pH	8.0	8.0	8.0	8.0
Final pH	7.56	7.61	7.70	7.72
Final Brightness, %ISO	84.4±0.48	85.1	85.5	85.9

contd..

D_2 stage				
ClO_2 added, %	0.3	0.3	0.3	0.3
Initial pH	2.81	2.80	2.82	2.83
End pH	3.30	3.31	3.35	3.30
ClO_2 consumed, %	86.9	82.5	82.0	81.8
Brightness, %ISO	87.2±0.60	87.6±0.35	87.9±0.44	88.3±0.50
CIE Whiteness	76.21±0.41	76.96±0.49	77.32±0.50	77.58±0.39
L*	96.91	97.18	97.21	97.34
a*	-0.18	-0.18	-0.17	-0.16
b*	4.92	4.02	3.86	3.71
PC No.	0.63±0.05	0.49±0.05	0.39±0.07	0.34±0.05
PC No. reduction, %	---	22.2	38.1	46.0
Shrinkage, %	5.4±0.49	5.6±0.10	5.7±0.36	5.9±0.44

Table 1.18 Enzymatic bleaching behavior using $D_0E_PXD_1E_P$ sequence

Parameter	Set 1			
Kappa No.	20.4			
Kappa factor	0.22			
D_0 stage				
ClO_2 added, %	1.71			
End pH	2.30			
ClO_2 consumed, %	99.8			
E_P stage				
NaOH added, %	2.24			
H_2O_2 added, %	0.5			
Initial pH	11.51			
End pH	10.60			
Kappa No.	2.3±0.31			
Brightness, %ISO	74.8±0.49			
H_2O_2 consumed, %	100			
X stage				
	Set 1	Set 2	Set 3	Set 4
Enzyme Dose,kgt^{-1}	---	0.1	0.3	0.5
Reaction time, min.	90	90	90	90
Consistency, %	10	10	10	10
Reaction temp. ,°C	55	55	55	55
Initial pH	8.0	8.0	8.0	8.0
Final pH	7.62	7.74	7.79	7.82
Final Kappa No.	2.2±0.40	2.0±0.12	1.8±0.32	1.7±0.21
Final Brightness,	75.9±0.50	76.8±0.56	77.3±0.53	77.7±0.55
D_1 stage				
ClO_2 added, %	0.8	0.8	0.8	0.8
Initial pH	2.89	2.88	2.86	2.90
End pH	3.36	3.32	3.30	3.40
Brightness, %ISO	85.0±0.52	86.0±0.44	86.4±0.23	86.8±0.57
ClO_2 consumed,%	99.7	99.2	99.1	99.0

contd..

E_P stage				
NaOH added, %	1.80	1.80	1.80	1.80
H_2O_2 added,%	0.5	0.5	0.5	0.5
Initial pH	11.30	11.34	11.35	11.31
End pH	10.34	10.31	10.30	10.31
H_2O_2 consumed, %	99.9	99.7	99.7	99.6
Final Brightness, %ISO	87.0±0.30	87.9±0.40	88.2±0.31	88.4±0.45
CIE Whiteness	75.94±0.33	77.26±0.34	77.71±0.35	78.22±0.50
L*	96.99	97.34	97.61	97.74
a*	-0.19	-0.18	-0.17	-0.17
b*	4.94	3.89	3.81	3.76
PC No.	0.60±0.04	0.49±0.04	0.43±0.05	0.36±0.04
PC No. reduction, %	---	18.3	28.3	40.0
Shrinkage, %	5.5±0.42	5.6±0.12	5.8±0.29	6.0±0.38

LIST OF PUBLICATIONS

1. **Gangwar, A. K.,** Tejo Prakash, N., and Prakash, R. (2016). "An eco-friendly approach: Incorporating a xylanase stage at various places in ECF and chlorine-based bleaching of Eucalyptus pulp," *BioResources* 11(2), 5381-5388.
2. **Gangwar, A. K.,** Prakash, N. T., and Prakash, R., (2016). "Questioning Conventional Wisdom Regarding the Most Suitable Sequence of Enzyme Usage in Pulp Bleaching: Editorial," Bioresources 11(1), 6-7.
3. **Gangwar, A. K.,** Tejo Prakash, N., and Prakash, R. (2015). "Amenability of Acacia and Eucalyptus Hardwood Pulps to Elemental Chlorine-Free Bleaching: Application and Efficacy of Microbial Xylanase," *Bioresources* 10(4), 8405-8413.
4. **Gangwar, A. K.,** Prakash, N. T., and Prakash, R., (2014). "Applicability of microbial xylanases in paper pulp bleaching: A Review," Bioresources 9(2), 3733-3754.

An Eco-Friendly Approach: Incorporating a Xylanase Stage at Various Places in ECF and Chlorine-based Bleaching of Eucalyptus Pulp

Avdhesh K. Gangwar,[a] N. Tejo Prakash,[b] and Ranjana Prakash [c]*

A potentially more environmentally compatible approach was evaluated, involving the use of an enzyme (X) stage optimally inserted into various bleaching sequences for *Eucalyptus* kraft pulps. The efficacy of the X stage was evaluated in terms of final brightness, CIE whiteness, post-color number (brightness reversion), effluent characteristics, *etc*. The results showed considerable benefits with an enzymatic pre-treatment bleaching sequence for improved final pulp brightness (1.6 units higher) and reduced adsorbable organic halogens (AOX) (32% lower), in addition to improved biological oxygen demand (BOD) to chemical oxygen demand (COD) ratio, when using 0.5 kg/t pulp dosage of xylanase; enzymatic post-treatment bleaching sequences were observed to boost final CIE whiteness up to 3.4 units and to reduce post color number by 48% at 0.5 kg/t pulp dosage of xylanase. In addition, approximately 32% reductions in AOX released, as well as appreciable improvement in BOD-to-COD ratio, were observed in the bleach effluents. An improved ratio of BOD-to-COD facilitates possible enhancement in the bio-degradability of discharge effluents in a secondary treatment stage. Nine different bleaching sequences were compared. Three sequences for each category (pre-treatment, intermediate, and post-treatment bleaching sequences) were performed to provide an overview of the influence of xylanase treatment on various pulp properties and environmental sum parameters of the ensuing effluents.

Keywords: Adsorbable organic halides (AOX); Bleaching sequences; Biological oxygen demand (BOD); Chemical oxygen demand (COD); Optical properties; Xylanase

Contact information: a: Department of Biotechnology, Thapar University, Patiala, India; b: School of Energy and Environment, Thapar University, Patiala, India; c: School of Chemistry and Biochemistry, Thapar University, P. O. Box 32, Pin- 147004, Patiala, India;
** Corresponding author: rprakash@thapar.edu*

INTRODUCTION

Environmental concerns have influenced the pulp and paper industry to alter the traditional approaches to more environmentally compatible and sustainable processes. Pulp bleaching is one of those processes, in which elemental chlorine is effective at a very low capital cost but is a major environmental threat because of the release of toxic chlorinated compounds and other dioxins that are generated in mill effluents. Elemental chlorine-free (ECF) and total chlorine-free (TCF) bleaching process have been introduced to overcome these environmental issues to some extent. However, the optimum use of an enzyme stage incorporated into a bleach sequence is still a point of research interest for reducing the cost of bleaching (Gangwar *et al*. 2016).

Environmental issues in the pulp bleaching process comprise generation of absorbable organic halogens (AOX), including chlorinated dioxins in mill effluents.

Chlorine-based bleaching sequences in the pulp and paper industry have negative environmental impacts, such as the production of toxic and mutagenic chloroorganic compounds (Comlekcioglu *et al*. 2014). To improve the delignification of pulp with lower bleach chemical usage and costs, alternative methods to traditional bleaching process are being developed. One such method is enzymatic bleaching. Enzymes have been shown to bleach pulp effectively with reduced costs and minimized environmental impacts (Moore 1999; Hart and Rudie 2012). In recent years, there has been increasing interest in the use of xylanases, particularly in the bleaching process of the pulp and paper industry (Sandrim *et al*. 2005). Xylanase enhances the effects of bleaching agents rather than removing lignin directly (Gangwar *et al*. 2015).

In general, enzymes are being used before the bleaching sequences to improve the pulp brightness, while enzymes are also being used after the bleaching sequences to improve the whiteness of the pulp. In this study, we present observations on various bleaching sequences in which the enzyme treatment (X) stage has been incorporated into the bleaching sequence. We examine the impact of the X stage on AOX, BOD, and COD in the ensuing bleaching effluents, which affects the environmental impact of the bleaching process. This comparative approach is very beneficial to find out the best enzymatic bleaching sequences for hardwood kraft pulps in the industrial processing.

EXPERIMENTAL

Eucalyptus (*Eucalyptus globulus*) wood chips, obtained from Ballarpur Industries Ltd, India, were cooked (AA as Na_2O- 19.0%; sulphidity- 24.21%; cooking temperature- 160 ^oC; cooking time- 180 min.; bath ratio- 1:3) in stationary laboratory digester to produce an unbleached kraft pulp with a kappa number of approximately 20 to 21. Cellulase-free commercial xylanase (Optimase CX 72L) was procured from DuPont Genencor Sciences, USA, for this study. The activities of xylanase and cellulase were determined according to the procedure of Bailey *et al*. (1992) and Ghose (1987), respectively.

The optimum temperature, pH, and activity of Optimase CX 72L were 55 °C, 8.0, and 46,876 IU/mL, respectively. A control pulp was also treated in the same way as the xylanase-treated pulp in each and every experiment, where the enzyme was replaced with water. Three different types of bleaching sequences were examined with and without xylanase: (CD)(EP)D_1D_2, D_0(EP)D_1D_2, and $D_0(EP)_1D_1(EP)_2$ (where (CD) represents a chlorination stage with partial chlorine dioxide substitution, D_0 represents a chlorine dioxide delignification stage, (EP) indicates an extraction stage with caustic and hydrogen peroxide reinforcement, and D_1 and D_2 represent the first and second chlorine dioxide brightening stages after the (EP) stage). Experimental conditions for each bleaching sequences are shown in Table 1.

Different bleaching sequences were designed for this study, which included the direct action of chlorine at the first stage and substitution of a chorine stage with chlorine dioxide (ECF) in another sequence. In another design for a sequence, the last chlorine dioxide (D_2) stage was replaced with extraction stage to study the effect of alkali and peroxide on pulp properties. In total, nine different bleaching sequences were studied for each category (pre, post, and intermediate) to determine the best enzymatic beaching sequence in terms of brightness, CIE whiteness, and PC number of bleached pulps. These nine sequences are given in Table 2.

Table 1. Experimental Conditions and Dosages Used for Bleaching

Parameter	X stage	CD stage	D_0 stage	EP stage	D_1 stage	D_2 stage
Temperature, ^oC	55	Ambient	60	80	75	75
pH	8.0	1.8-2.0	1.8-2.0	10.5	2.8-3.0	2.8-3.0
Time, min.	90	45	45	120	180	180
Consistency, %	10	5	5	10	10	10
Dosage: *Kappa factor- 0.22; Active chlorine- based on kappa number; Chlorine dioxide- based on active chlorine in D_0 stage, 0.8% in D_1 and 0.3% in D_2 stage; Caustic- half of active chlorine dose; Hydrogen peroxide- 0.5%*						

Table 2. Incorporation of the Enzymatic Stage Before, Within, and After the Bleach Sequence

Sequence Type	Enzymatic Bleaching Sequence		
Pre-Treatment	$X(CD)(EP)D_1D_2$	$XD_0(EP)D_1D_2$	$XD_0(EP)_1D_1(EP)_2$
Post-Treatment	$(CD)(EP)D_1D_2X$	$D_0(EP)D_1D_2X$	$D_0(EP)_1D_1(EP)_2X$
Intermediate	$D_0X(EP)D_1D_2$	$D_0(EP)D_1XD_2$	$D_0(EP)_1XD_1(EP)_2$

After bleaching, the effluents from enzyme-treated and untreated bleaching sequences (*i.e.*, controls) were characterized to determine the contents of adsorbable organic halogens (AOX), chemical oxygen demand (COD), and biological oxygen demand (BOD). Inter-stage washing was followed for the collection of mixed bleach effluent for all stages (CD/D_0, EP, D_1, D_2) in the ratio 2:1:0.5:0.5 as per the consistency maintained in the particular bleaching stage.

Kappa number, a measure of residual lignin in the pulp, was determined using TAPPI Test Method T236 om-99 (1999). Handsheets were prepared with a smooth and reproducible surface for reflectance measurements in accordance with TAPPI Test Method T205 sp-02 (2002). The brightness (% ISO) and CIE whiteness of pulp were measured using a Konica Minolta (Model No.-CM 3600A) in accordance to TAPPI Test Methods T452 om-02 (2002) and T560 om-96 (1996), respectively. The brightness reversion of the pulp was estimated in terms of the post color (PC) number in accordance with TAPPI Test Method T260 om-85 (1985). COD (kg/t pulp) and BOD (kg/t pulp) were characterized using test methods APHA 5220:B (1999) and APHA:B 5210 (1999), respectively. AOX of the bleaching effluents was measured using a ThermoFisher Scientific instrument (Model No. ECS 1200) in accordance with ISO-9562 test procedure; the values obtained by the AOX determination included most chlorinated organics and are reported as organically bound chlorine (ppm).

RESULTS AND DISCUSSION

Nine bleaching sequences were studied, in which a xylanase (X) stage was included at various sequence positions, as shown in Table 2. The optical properties and pulp shrinkages of these various bleaching sequences of Table 2 are shown in Table 3. In xylanase pre-treatment bleaching sequences, the final brightness increased by 0.9, 1.3, and 1.6 units and the CIE whiteness increased by 1.1, 2.2, and 2.8 units when xylanase was

applied at dosages of 0.01%, 0.03%, and 0.05% on pulp, respectively, for X(CD)(EP)D_1D_2 sequence. When the chlorination stage (*i.e.*, (CD)) was replaced with a chlorine dioxide delignification stage (D_0), the final brightness increased by 0.7, 1.1, and 1.5 units and the CIE whiteness increased by 1.4, 2.0, and 2.5 units when xylanase was applied at dosages of 0.01%, 0.03%, and 0.05% on pulp, respectively, *versus* the control. When the ECF sequence was changed by replacing the second chlorine dioxide (D_2) stage with a second extraction stage (*i.e.*, $(EP)_2$), the final brightness increased by 0.6, 1.0, and 1.3 units and the CIE whiteness increased by 0.9, 1.6, and 2.0 units when xylanase was applied at dosages of 0.01%, 0.03%, and 0.05% on pulp, respectively.

During the xylanase post-treatment bleaching sequences, in the case of (CD)(EP)D_1D_2X, the final brightness increased by 0.5, 1.0, and 1.2 units, whereas the CIE whiteness increased by 1.6, 2.7, and 3.4 units *versus* the control. When the ECF bleaching sequence, D_0(EP)D_1D_2X, was used, the final brightness and CIE whiteness values were augmented by 0.5, 0.8, and 1.1 units and 1.5, 2.5, and 3.2 units, respectively, as compared with the control (Table 3). When the other ECF sequence, $D_0(EP)_1D_1(EP)_2$X, was used, the final brightness went up by 0.4, 0.7, and 1.0 units, whereas the CIE whiteness went up by 1.3, 2.4, and 2.8 units when compared with the control. Post-treatment results (Table 3) showed that the enzymatic (X) stage after the bleaching sequence was more beneficial for improved final CIE whiteness of the bleached pulp than X stage pre-treatment in the bleach sequences.

Intermediate application of the X stage in the various bleaching sequences was examined to see if xylanase could further improve the optical properties of the eucalypt pulps *versus* the other X stage bleach sequence placements (Table 3). Kappa number and brightness (% ISO) of the pulp were determined prior to bleaching. In the X stage, enzyme dosages of 0.01%, 0.03%, and 0.05% on pulp were applied, as has been reported earlier. After the completion of the bleach sequence, the bleached pulps were analyzed for the brightness, CIE whiteness, and PC number. In Table 3, the final brightness and CIE whiteness gains versus the control were 0.4, 0.7, and 1.0 units and 0.7, 1.0, and 1.2 units, respectively, for the $D_0X(EP)D_1D_2$ bleaching sequence. When the X stage was incorporated within the ECF bleaching sequence (*i.e.*, $D_0(EP)D_1XD_2$), the final brightness and CIE whiteness gains over the control were 0.4, 0.7, and 1.1 units and 0.75, 1.1, and 1.4 units, respectively. While integrating the X stage between $(EP)_1$ and D_1 stages (*i.e.*, $D_0(EP)_1XD_1(EP)_2$), the final brightness increased by 0.9, 1.2, and 1.4 units, whereas the CIE whiteness increased by 1.3, 1.8, and 2.3 units as compared with the control. The increase in the brightness and whiteness level with xylanase treatment was presumably due to the influence of xylanases through variety of mechanisms such as increased accessibility of bleach chemicals on the pulp fibers, removal of hexenuronic acid components after the chlorine dioxide stages, removal of xylan derived chromophores, and hydrolysis of re-precipitated xylan on the cellulose fibre during the kraft pulping.

Table 3 also indicates that the maximum PC number reduction was 48% for the ECF bleaching sequence, where the X stage was used at the end (*i.e.*, post-treatment sequence D_0(EP)D_1D_2X) and had a xylanase dose of 0.05%. The minimum PC number reduction of 39% was obtained when a pre-treatment X stage sequence was employed (*i.e.*, $XD_0(EP)_1D_1(EP)_2$), where the D_2 stage was replaced with $(EP)_2$ stage while using a xylanase dose of 0.05%. A minimal pulp yield loss (*i.e.*, shrinkage) was observed across all the enzymatic bleaching sequences (pre, post, and intermediate) as compared with the respective controls.

Table 3. Effect of Bleaching Sequences with an Incorporated Xylanase Stage on Eucalyptus Pulp

Bleaching Sequences		Set	Final Brightness (% ISO)	CIE Whiteness	Post Color Number	Pulp Shrinkage (%)
Pre-treatment Bleaching	$X(CD)(EP)D_1D_2$	Control	87.5±0.67	77.2±0.38	0.58±0.07	5.7±0.49
		0.01%	88.4±0.42	78.3±0.45	0.48±0.06	5.9±0.46
		0.03%	88.8±0.49	79.3±0.31	0.40±0.05	6.1±0.30
		0.05%	89.1±0.36	79.9±0.36	0.34±0.04	6.3±0.45
	$XD_0(EP)D_1D_2$	Control	87.3±0.46	76.9±0.57	0.62±0.07	5.5±0.46
		0.01%	88.0±0.38	78.3±0.49	0.50±0.04	5.7±0.32
		0.03%	88.4±0.17	78.8±0.34	0.42±0.04	5.9±0.32
		0.05%	88.8±0.36	79.3±0.34	0.35±0.03	6.1±0.26
	$XD_0(EP)_1D_1(EP)_2$	Control	87.1±0.50	76.01±0.32	0.61±0.06	5.4±0.40
		0.01%	87.7±0.38	76.91±0.31	0.51±0.02	5.7±0.26
		0.03%	88.1±0.17	77.61±0.23	0.44±0.04	5.8±0.25
		0.05%	88.4±0.36	78.01±0.38	0.37±0.05	6.0±0.20
Post-treatment Bleaching	$(CD)(EP)D_1D_2X$	Control	87.3±0.55	76.5±0.49	0.51±0.05	6.0±0.36
		0.01%	87.8±0.50	78.2±0.42	0.40±0.05	6.0±0.35
		0.03%	88.3±0.25	79.3±0.39	0.34±0.06	6.3±0.46
		0.05%	88.5±0.36	79.9±0.41	0.29±0.02	6.6±0.46
	$D_0(EP)D_1D_2X$	Control	87.1±0.51	76.0±0.58	0.54±0.04	5.7±0.31
		0.01%	87.6±0.59	77.5±0.31	0.40±0.04	5.8±0.21
		0.03%	87.9±0.36	78.5±0.33	0.32±0.06	6.0±0.21
		0.05%	88.2±0.21	79.2±0.27	0.28±0.06	6.2±0.35
	$D_0(EP)_1D_1(EP)_2X$	Control	86.9±0.55	75.6±0.38	0.52±0.05	5.5±0.23
		0.01%	87.3±0.32	76.9±0.23	0.42±0.03	5.6±0.35
		0.03%	87.6±0.35	77.9±0.40	0.36±0.03	5.7±0.35
		0.05%	87.9±0.40	78.4±0.22	0.30±0.02	5.9±0.21
Intermediate Bleaching	$D_0X(EP)D_1D_2$	Control	85.8±0.38	74.0±0.27	0.58±0.07	5.4±0.42
		0.01%	86.2±0.20	74.6±0.34	0.46±0.01	5.5±0.26
		0.03%	86.5±0.44	75.0±0.53	0.38±0.03	5.7±0.46
		0.05%	86.8±0.40	75.1±0.41	0.32±0.06	5.8±0.15
	$D_0(EP)D_1XD_2$	Control	87.2±0.60	76.2±0.41	0.63±0.05	5.4±0.49
		0.01%	87.6±0.35	77.0±0.49	0.49±0.05	5.6±0.10
		0.03%	87.9±0.44	77.3±0.50	0.39±0.07	5.7±0.36
		0.05%	88.3±0.50	77.6±0.39	0.34±0.05	5.9±0.44
	$D_0(EP)_1XD_1(EP)_2$	Control	87.0±0.30	76.0±0.33	0.60±0.04	5.5±0.42
		0.01%	87.9±0.40	77.3±0.34	0.49±0.04	5.6±0.12
		0.03%	88.2±0.31	77.7±0.35	0.43±0.05	5.8±0.29
		0.05%	88.4±0.45	78.2±0.50	0.36±0.04	6.0±0.38

The pollution loads of enzyme-treated and control bleach effluents for various bleach sequences were determined in terms of COD, BOD, and AOX to evaluate the environmental impacts of enzymatic treatment. Composite effluent solutions from each bleach sequence were collected from all respective stages where the xylanase dosage was 0.05%. Similar composite effluent solutions from each bleach sequence were collected for the corresponding control sequences.

As shown in Table 4, bleaching sequences with enzyme pre-treatment (*i.e.*, $X(CD)(EP)D_1D_2$, $XD_0(EP)D_1D_2$, and $XD_0(EP)_1D_1(EP)_2$) had AOX reductions in the range of 29 to 32% *versus* the controls. In contrast, bleach sequences with X stage post-treatment (*i.e.*, $CD)(EP)D_1D_2X$, $D_0(EP)D_1D_2X$ and $D_0(EP)_1D_1(EP)_2X$) had reductions in AOX (16% to 22% less) when compared with X stage pre-treatment bleaching sequences. When the X

stage was incorporated within the bleaching sequences, the amount of AOX reduction when compared with the controls was on the order of 17% to 22%.

Incorporating an X stage between chlorine dioxide delignification and extraction stages (*i.e*., $D_0X(EP)D_1D_2$) reduced the AOX by a relatively higher amount than the other intermediate ECF bleaching sequences that incorporated an X stage.

Table 4. Comparison of Bleach Effluent Properties Where Enzyme Treatment is Pre, Post, or Intermediate Sequence Placement at a 0.5 kg/t Pulp Enzyme Dosage

Enzyme treatment	Bleaching sequence	BOD (kg/t pulp)	COD (kg/t pulp)	AOX (kg/t pulp)	AOX (% reduction)
Pre-treatment bleaching sequences	Control	45.2±0.40	75.1±0.66	0.87±0.05	--
	$X(CD)(EP)D_1D_2$	36.1±0.47	74.1±0.46	0.59±0.05	32.2
	Control	41.3±0.51	69.9±0.55	0.78±0.04	---
	$XD_0(EP)D_1D_2$	34.7±0.51	66.7±0.47	0.54±0.03	30.8
	Control	39.3±0.49	67.3±0.42	0.72±0.03	---
	$XD_0(EP)_1D_1(EP)_2$	33.3±0.50	64.4±0.45	0.51±0.04	29.2
Post-treatment bleaching sequences	Control	51.5±0.51	77.9±0.55	0.96±0.05	---
	$(CD)(EP)D_1D_2X$	40.1±0.69	72.1±0.25	0.75±0.05	21.9
	Control	46.3±0.31	73.4±0.45	0.91±0.04	---
	$D_0(EP)D_1D_2X$	38.7±0.40	64.7±0.15	0.76±0.02	16.5
	Control	43.5±0.51	70.3±0.40	0.82±0.05	---
	$D_0(EP)_1D_1(EP)_2X$	36.3±0.40	61.4±0.35	0.69±0.05	15.9
Intermediate bleaching sequences	Control	33.5±0.46	55.6±0.30	0.72±0.05	---
	$D_0X(EP)D_1D_2$	32.6±0.50	51.2±0.46	0.56±0.03	22.2
	Control	38.1±0.53	57.7±0.38	0.75±0.07	---
	$D_0(EP)D_1XD_2$	37.4±0.35	56.7±0.59	0.62±0.04	17.3
	Control	32.1±0.61	53.2±0.36	0.69±0.04	---
	$D_0(EP)_1XD_1(EP)_2$	31.5±0.29	52.1±0.42	0.56±0.07	18.8

COD and BOD levels of the bleach effluents were examined for the various sequences with an X stage at various positions and compared with the controls. Overall, xylanase treatments were promising and showed reduction in COD and BOD levels in combined bleaching effluents for each sequence *versus* the respective controls. Other investigators have also reported that xylanase bleaching led to a reduction in AOX found in bleaching filtrates and to an appreciably higher BOD-to-COD ratio for the xylanase pre-bleaching filtrates (Senior and Hamilton 1991; Bajpai 2010; Thakur *et al*. 2012; Gangwar *et al*. 2014).

CONCLUSIONS

1. The study facilitates a better understanding of the influence of xylanase incorporation at various stages of bleaching sequence.
2. Enzyme pre-treatment bleaching sequences are more effective at boosting final pulp brightness (1.6 units for $X(CD)(EP)D_1D_2$ and 1.5 units for ECF bleaching sequence, $XD_0(EP)D_1D_2$).
3. In addition, a 32% reduction of AOX was observed for enzyme pre-treatment bleaching sequence *versus* enzyme post-treatment and intermediate bleaching sequences.

4. In contrast, enzyme post-treatment was observably a better option *versus* enzyme pre-treatment or intermediate bleaching when the desired target is to increase the CIE final whiteness of the pulp (*e.g.*, 3.4 units higher for (CD)(EP)D_1D_2X and 3.2 units higher for D_0(EP)D_1D_2X).
5. It is also concluded that out of the nine different sequences examined, the maximum reduction in PC number (48%) was obtained with enzyme post-treatment bleaching sequences and the minimum reduction (39%) was obtained with enzyme pre-treatment bleaching sequence.
6. Incorporation of an enzyme stage at any position in a bleaching sequence improved the BOD-to-COD ratio of the resulting effluents, which indicated better degradability of the bleach effluents in secondary treatment when compared to the control.
7. A marginal increase in pulp shrinkage was also observed across all the enzymatic bleaching sequences versus their respective controls.

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Questioning Conventional Wisdom Regarding the Most Suitable Sequence of Enzyme Usage in Pulp Bleaching

Avdhesh K. Gangwar,[a] N. Tejo Prakash,[b] and Ranjana Prakash [c,*]

Increased public scrutiny and governmental legislation towards the pulp and paper industries have motivated industrialists and researchers to seek improved bleaching sequences having the potential to minimize pollutants in bleach effluent generated during manufacturing of paper. Discovery of toxic chlorinated organics and their components in bleach effluents has focused people's attention towards finding alternative ways of bleaching pulp. Use of enzymes at industrial scale has become well known, but still it is not clear whether the sequence of enzymatic treatment most often employed in industrial applications represents the best overall practice. The point of enzyme addition is critically important to maximize benefits. Many publications describe the use of an enzyme treatment stage before the use of chemicals in a bleaching process. Insufficient attention has been paid to the alternatives of adding an enzyme in between chemical bleaching agents (intermediate) or at the end of the bleaching process.

Keywords: Bleach effluent; Bleaching sequence; Elemental chlorine; Laccase; Pollution load; Xylanase

*Contact information: a: Department of Biotechnology, Thapar University, India; b: School of Energy and Environment, Thapar University, India; c: School of Chemistry and Biochemistry, Thapar University, P. O. Box 32, Pin- 147004, Patiala, India; *Corresponding author: rprakash@thapar.edu*

Are we ready to accept nature's challenge?

According to conventional wisdom, the environmental impact of pulp bleaching often can be reduced by pretreating the fibers with enzyme. The goal is to maximize benefits in terms of pulp brightness with reduced pollution load in bleach effluent in a system that can be implemented at an industrial level. We all are living in, surrounded by, or in direct contact with air, water, and everything that is required for survival on this planet; thus anything implemented on an industrial scale is likely to affect our health, either immediately or after some delay. The pulp and paper industry can be regarded as a major generator of industrial pollution, though substantial progress has been achieved in either reducing the generation of toxic substances or in treating effluent that contains them. The most serious contribution to pollution consists of the bleaching chemicals. Elemental chlorine and its derivatives, as well as hydrogen peroxide and caustic soda (NaOH) are the major chemicals used in pulp bleaching that are very hazardous towards nature. Elemental chlorine is being used in huge amounts in the traditional bleaching process in various parts of the world. Replacement of elemental chlorine by chlorine dioxide in the first stage of bleaching has been helpful to some extent in reducing the formation of pollutants; this bleaching sequence is known as the "elemental chlorine free" (ECF) bleaching sequence. But chlorine dioxide can be regarded as a form of chlorine, so in many paper mills it is also being replaced by ozone and enzymes. Such approaches make it possible for these paper mills to meet and exceed environmental regulations.

Though the industry has converted much of its production to either elemental chlorine-free (ECF) or total chlorine-free (TCF) bleaching sequences, and enzymatic treatments also have come into common use, it is still not clear what is the best bleaching sequence with respect to the use of enzymes to get maximum benefits for improving the optical properties of the paper while reducing the pollutant loads in the bleach effluent. Enzymes, which are produced by microbes, may be in blended or in single form for its use at an industrial level. Mostly xylanases are being used to improve bleaching outcomes and in order to reduce the consumption of bleaching chemicals. Laccase with mediators are also beneficial for this purpose. They can be more efficient contributors towards bleaching, but due to cost-effectiveness, xylanase has been regarded as the most favorable option for industrial use.

Do we really know the best bleaching sequence with enzymes?

An enzyme can degrade or modify the lignin component. If one uses an enzyme before the bleaching chemicals, it can help in reducing the lignin amount either by degrading (laccase) or by modifying (xylanase) it. Xylanase is capable of hydrolyzing xylan, which is found bound with hexenuronic acid (Hex-A). Lignin is entrapped in between the Hex-A components; therefore there is a higher consumption of bleaching chemicals during bleaching process and a corresponding greater generation of pollution loads. When xylanase is used in bleaching, it breaks the bond between xylan and Hex-A and helps in extracting the lignin. Consequently, less bleach chemicals are required, reducing the generation of hazardous chemicals in bleach effluent.

Though the mechanism just described might appear to justify initial treatment with enzymes, it is far more important to find out what system works best in practice. Do industrialists really know that adding the enzymes first, before the chemical bleaching agents, will give the best results? Do they know which sequence of enzymes usage will be able to reduce the pollutants or to save our environment or to produce paper at very low cost? The answer would appear to be "NO". We simply do not know, and research is needed to find out the best stage for the addition of the enzymes for bleaching of pulp and paper. Much literature is available for enzyme use before the bleaching process, while very few publications have considered its use in between or after the chemical additives of the bleaching system.

It has already been discovered that the chlorine dioxide stages in ECF bleaching can generate new unsaturated structures, and xylan is a major source of these colored chromophores, which affect brightness development and stability of bleached pulps. Implementation of xylanase treatment as an intermediate or after bleaching process may be expected to reduce these chromophores as associated with xylan and result in improving the optical properties of the pulps. To target the same brightness level as in conventional bleaching processes, there would be chances of reducing bleaching chemicals in previous bleaching stages, which will further reduce the generation of pollution load in the process. Therefore, it might be economically beneficial to add enzyme after the bleaching process or in between the bleaching stages.

The use of enzymes in between and after the bleaching process has not been implemented yet at an industrial level. In the future, individual paper companies will have to effectively evaluate their options and to develop appropriate bleaching sequences using enzymes to reduced impacts on our environment and for the progress of pulp and paper industries globally.

Amenability of Acacia and Eucalyptus Hardwood Pulps to Elemental Chlorine-Free Bleaching: Application and Efficacy of Microbial Xylanase

Avdhesh K. Gangwar,[a] N. Tejo Prakash,[b] and Ranjana Prakash [c,*]

This study outlines the results of a biobleaching study of acacia (*A. mangium*) and eucalyptus (*E. globulus*) hardwood kraft pulps with commercial xylanase (Optimase CX 72 L). The comparative study was carried out using an elemental chlorine-free (ECF) bleaching sequence ($D_0E_PD_1D_2$) after the enzyme (X) stage. The enzyme treatment resulted in improved optical properties with a reduction in bleach chemical consumption. At an equivalent bleach chemical consumption, a brightness gain of 2.1 and 1.7 units and a whiteness gain of 2.7 and 2.3 units were observed with xylanase treatment in acacia and eucalyptus pulps, respectively. In ECF bleaching using the $D_0E_PD_1D_2$ sequence, a final brightness was achieved to the extent of 90% ISO and 89% ISO for acacia and eucalyptus, respectively, at an equivalent charge of bleach chemicals. The post-color (PC) number was also reduced by up to 45% for both hardwood pulps compared with the control. The bleachability of acacia was observed to be significantly higher than that of eucalyptus. In addition, a 17.0% and 23.0% reduction in chlorine dioxide and sodium hydroxide, respectively, were obtained for both hardwood pulps after xylanase pre-bleaching, thus indicating an environmentally friendly approach to the process.

Keywords: Chlorine dioxide; ECF bleaching; Post-color number; Pulp properties; Xylanase

*Contact information: a: Department of Biotechnology, Thapar University, India; b: School of Energy and Environment, Thapar University, India; c: School of Chemistry and Biochemistry, Thapar University, P. O. Box 32, Pin- 147004, Patiala, India; *Corresponding author: rprakash@thapar.edu*

INTRODUCTION

Several pulp and paper mills are currently adopting enzymatic bleaching as an alternative to chlorine-based chemical bleaching, as such an approach can reduce or eliminate the use of chlorine and its compounds and therefore address environmental concerns and governmental regulations (Selvam and Arungandhi 2013; Sharma *et al.* 2014). In recent years, there has been increasing interest in applying green technology to the bleaching process to reduce pollution as well as to improve the quality of the pulp produced (Torres *et al.* 2000; Zhao *et al.* 2006). Chlorine dioxide-based elemental chlorine-free (ECF) bleaching for the pulp and paper industry offers a number of obvious benefits over the traditional methods. ECF bleaching results in significantly superior pulp quality in addition to providing an environmentally beneficial process. The enzymatic pretreatment of pulps before ECF bleaching can be of further advantage in terms of reducing the consumption of bleach chemicals, and therefore reducing the amount of pollutants generated (Bajpai 1999).

The concept of the addition of enzymes for improving the delignification of pulp is well known, and xylanase is the most common type of enzyme used for this purpose.

Biobleaching, *i.e.*, the enzymatic pre-treatment of pulp with xylanases (endo-1,4-xylanase activity, EC 3.2.1.8.) before chemical bleaching is an alternative and cost-effective process for reducing the consumption of bleach chemicals and for minimizing the generation of toxic chlorinated organic substances in the effluents of bleach mills (Viikari *et al.* 1994; Call and Mycke 1997; Suurnakki *et al.* 1997; Bajpai *et al.* 2006; Shatalov and Pereira 2008). The use of xylanases results in better optical properties and reduces the use of bleaching chemicals (Valls and Roncero 2009). Xylanase treatment reduces the xylan concentration in the secondary wall of the fiber surface, particularly in hardwood pulps, thus enhancing pulp bleachability and increasing the permeability of the fiber surface (Viikari *et al.* 1996; Kim and Paik 2000; Roncero *et al.* 2000). Xylan concentration is present in the range of 11 to 12.5% and 16 to 20% in *Acacia mangium* and *Eucalyptus globulus*, respectively (Pinto *et al.* 2005; Evtuguin and Neto 2007).

Xylanase enhances the effects of bleaching chemicals rather than removing lignin directly. The enzyme does not attack lignin-based chromophores, but rather the xylan network by which residual lignin particles are trapped in the pulp. Limited hydrolysis of the xylan network is often sufficient to facilitate the subsequent chemical removal of lignin without sacrificing yield. The viscosity of the pulp is also improved as a result of xylanase treatment. However, the viscosity of the pulp is adversely affected when cellulase activity is present. Therefore, cellulase activity by the enzyme preparation is undesirable in enzymatic bleaching (Bajpai and Bajpai 1997).

Studies have demonstrated that factors such as type of raw material, sequence of bleaching, and type of xylanase could significantly affect enzymatic treatment (Gallardo *et al.* 2010). Presently, xylanases are being modified to work at alkaline pH values and higher temperatures (Gangwar *et al.* 2014). Keeping in view the limited reports available on the bleach-boosting properties of xylanase on acacia (*A. mangium*) kraft pulp and its comparative study with eucalyptus (*E. globulus*) kraft pulps, the present study aimed at examining the influence of xylanase on the optical and physical properties of both pulps and bleach chemical consumption over the conventional bleaching process.

EXPERIMENTAL

The plantation of acacia and eucalyptus is usually done in several parts of India viz; West Bengal, Tamil Nadu, Uttar Pradesh, Madhya Pradesh, Maharashtra and Karnataka. Acacia (*A. mangium*) and eucalyptus (*E. globulus*) wood chips were procured from different pulp and paper mills in India. The cooking was done in a stationary digester using the kraft process. The cooking experiments were performed at different temperatures, durations, and active alkali doses to optimize the pulping conditions and obtain pulps with a kappa number in the range of 18 to 20 for both types of wood pulp.

Optimase CX 72L sourced from Dupont Genencor Sciences was used in the present study. It has low or negligible cellulase activity and is designed for a bleach-boosting effect. This enzyme is alkali and thermostable in nature, which are favorable conditions for the enzymatic bleaching process. The optimum temperature, pH, and activity of Optimase CX 72L were 55 °C, 8.0, and 46,876 IU, respectively.

The activity of xylanase and CMCase were determined according to the methods outlined by Bailey *et al.* (1992) and Ghose (1987), respectively. The activities were assayed by measuring the release of reducing sugars (xylose and glucose) from birch wood xylan and carboxymethyl cellulose (CMC). One international unit of enzyme was

defined as the amount of enzyme that released 1 µmole of xylose or glucose sugars per minute per mL of enzyme.

The kneading mechanism was followed for the proper dilutions and mix of enzymes to be used in the experiments. The control pulp was also treated in the same way as the xylanase-treated pulp in each experiment, where the enzyme was replaced with water. The efficacy of the xylanase enzyme was determined for acacia and eucalyptus hardwood pulps based on pulp yield, kappa number reduction, and brightness gain of the pulp. After the enzyme treatment (X stage), the pulps were washed and subjected to ECF bleaching.

Enzyme-treated and untreated wood pulps were bleached using the ECF bleaching sequence ($D_0E_PD_1D_2$) to explore the potential savings on chlorine dioxide and sodium hydroxide and to determine the gain in final brightness and whiteness of the bleached pulps. The processing conditions (pH, temperature, time, and consistency) used at various bleaching stages were maintained as indicated in Table 1.

Table 1. Processing Conditions Used during ECF Bleaching ($D_0E_PD_1D_2$)

Parameter	Chlorine Dioxide (D_0) Stage	Alkali Extraction (E_P) Stage	Chlorine Dioxide (D_1) Stage	Chlorine Dioxide (D_2) Stage
Consistency (%)	5	10	10	10
Time (min)	45	120	180	180
Temperature (°C)	60	80	75	75
pH	1.8 to 2.0	10.5 to 11.0	3.0 to 4.0	3.0 to 4.0

Analytical Methods

Kappa number was determined according to TAPPI test method T236 om-99 (1999). Kappa number is the volume (in milliliters) of 0.1 N potassium permanganate solution consumed by one gram of moisture-free pulp under the specified conditions. The results were corrected to 50% consumption of the permanganate added. The viscosity of the pulp was determined by the capillary viscometer method according to TAPPI test method T230 om-99 (1999). The viscosity of the 0.5% cellulose solution was determined, using 0.5 M cupriethylenediamine as a solvent and a capillary viscometer. Viscosity gives an indication of the average degree of polymerization of the cellulose. The brightness (% ISO) and CIE whiteness of the pulps were measured using a Konica Minolta instrument according to TAPPI test methods T452 om-02 (2002) and T560 om-96 (1996), respectively. The post-color (PC) number was measured according to TAPPI test method T260 om-85 (1985). It is a measurement of reversion in brightness of the paper at a specified time and temperature.

Canadian Standard Freeness (CSF) was measured according to TAPPI test method T227 om-94 (1994), in which the freeness of the pulp was designed to give a measure of the rate at which a dilute suspension of pulp (3 g of pulp in 1 L of water) may be drained. Handsheets were prepared with a smooth and reproducible surface for reflectance measurements according to T205 sp-02 (2002). Bursting strength was checked using TAPPI test method T403 om-97 (1997), and is defined as the maximum hydrostatic pressure required to produce a rupture of the material when a controlled and constantly increasing pressure is applied through a rubber diaphragm. Tearing strength was also measured according to T414 om-98 (1998), in which multiple sheets of the sample material were torn together through a fixed distance using the pendulum.

RESULTS AND DISCUSSION

Enzyme-treated pulps showed a reduction in kappa number of 4.6%, 8.0%, and 10.1% for acacia pulp and 4.1%, 7.8%, and 9.8% for eucalyptus pulp at xylanase doses of 0.1, 0.3, and 0.5 kg/tp, respectively, compared with the control (Table 2). These observations on the reduction in kappa number by xylanase were also supported by Thakur *et al.* (2012), wherein Pulpzyme HC was used and a 4.2% reduction in kappa number of eucalyptus pulp was achieved. Brightness gains of 2.4, 2.7, and 2.9 units were obtained in acacia wood pulp, while brightness gains of 2.3, 2.4, and 2.8 units were observed in eucalyptus pulp at enzyme doses of 0.1, 0.3, and 0.5 kg/tp, respectively, compared with the control (Table 2). Shatalov and Pereira (2008) obtained 1.2 to 1.6 units of brightness gain after xylanase treatment on eucalyptus hardwood pulp, which is relatively lower than the observations obtained in the present study. A minor loss in yield was also observed in both acacia and eucalyptus wood pulps in the range of 0.2% to 0.6% with xylanase doses of 0.1, 0.3, and 0.5 kg/tp compared with the control (Table 2).

Table 2. Characterization of Enzyme-treated and Untreated Pulp after Enzymatic Treatment

Particulars	Set	Xylanase Dose (kg/tp)	Yield (%)	Kappa Number		Brightness	
				Value	Reduction (%)	Value (%ISO)	Gain (unit)
Acacia Kraft pulp	Control	---	99.8 ± 0.23	19.7 ± 0.50	---	28.5 ± 0.58	---
	Xylanase -treated	0.1	99.5 ± 0.31	18.8 ± 0.42	4.6	30.9 ± 0.35	2.4
		0.3	99.4 ± 0.35	18.1 ± 0.42	8.1	31.2 ± 0.38	2.7
		0.5	99.2 ± 0.45	17.7 ± 0.25	10.2	31.4 ± 0.36	2.9
Eucalyptus Kraft pulp	Control	---	99.7 ± 0.40	19.3 ± 0.42	---	28.7 ± 0.40	---
	Xylanase -treated	0.1	99.5 ± 0.32	18.5 ± 0.35	4.1	31.0 ± 0.31	2.3
		0.3	99.3 ± 0.25	17.8 ± 0.40	7.8	31.1 ± 0.44	2.4
		0.5	99.1 ± 0.38	17.4 ± 0.35	9.8	31.5 ± 0.15	2.8

Xylanase indirectly affects the lignin degradation in the pulps as it breaks the linkage between xylan and the hexenuronic acid component, facilitating better penetration of bleach chemicals into the fibre. Increased penetration of bleach chemicals invariably reduced their consumption in the process. In addition, a key reduction was seen in the consumption of chlorine dioxide and sodium hydroxide in ECF bleaching, as shown in Table 3. A reduction in chlorine dioxide of 14.3% was also reported in the eucalyptus pulp during enzymatic bleaching (Thakur *et al.* 2012).

Bleaching experiments, performed at both an equivalent kappa factor (KF=0.22) and reduced kappa factor (KF=0.187), resulted in decreasing consumption of 14.9% to 17.8% chlorine dioxide, along with a reduction of sodium hydroxide in the range of 18.9% to 23.5% for acacia pulp compared with the control (Table 3: AcK pulp) at a reduced kappa number. A similar reduction was also observed for eucalyptus wood pulp (Table 3: EuK pulp), wherein a reduced use of chlorine dioxide and sodium hydroxide to 14.6% to 17.6% and 18.4% to 23.1%, respectively, was required at the reduced kappa factor compared with the control.

Table 3. Bleach Chemical Consumption in ECF Bleaching of Acacia (AcK) and Eucalyptus (EuK) Pulps

Particulars		Control	At Equivalent KF (0.22)			At Reduced KF (0.187)		
			0.1 (kg/t)	0.3 (kg/t)	0.5 (kg/t)	0.1 (kg/t)	0.3 (kg/t)	0.5 (kg/t)
AcK Pulp	Applied ClO_2 (kg/tp)	27.6	26.8	26.3	25.9	23.5	23.0	22.7
	ClO_2 reduction (%)	---	2.90	4.71	6.16	14.86	16.67	17.75
	NaOH (kg/tp)	21.7	20.7	19.9	19.5	17.6	16.9	16.6
	NaOH reduction (%)	---	4.61	8.29	10.14	18.89	22.12	23.50
EuK Pulp	Applied ClO_2 (kg/tp)	27.3	26.6	26.0	25.7	23.3	22.8	22.5
	ClO_2 reduction (%)	---	2.56	4.76	5.86	14.65	16.48	17.58
	NaOH (kg/tp)	21.2	20.4	19.6	19.1	17.3	16.6	16.3
	NaOH reduction (%)	---	3.77	7.55	9.91	18.40	21.70	23.11

Handsheets of the final pulp were prepared and evaluated for the desired optical properties. The results indicated that the bleachability of acacia was significantly higher than that of eucalyptus pulp. The final brightness and efficacy of the enzyme were higher in acacia than in eucalyptus pulp. The final pulp brightness and whiteness of the enzyme-treated pulps was superior to untreated pulp at both equivalent and reduced amounts of bleach chemicals. Brightness gains of approximately 2.1 and 0.9 units were observed at equivalent and reduced bleach chemical consumption in the bleaching of acacia pulp, respectively (Fig. 1(a)). A similar trend was observed in whiteness improvement (up to 2.7 and 1.6 units) at equivalent and reduced bleach chemical consumption in the enzymatic treatment of acacia pulp (Fig. 1(b)). Correspondingly, 1.7 and 0.8 units of brightness gain and whiteness gains of 2.3 and 1.5 units were obtained in the bleaching process of eucalyptus pulp at equivalent and reduced bleach chemical consumption, respectively (Figs. 1a and 1b). The observations of Gallardo *et al.* (2010) on hardwood pulp showed a brightness gain in the range of 0.7 to 1.0 units more than the control pulp with the xylanase enzyme.

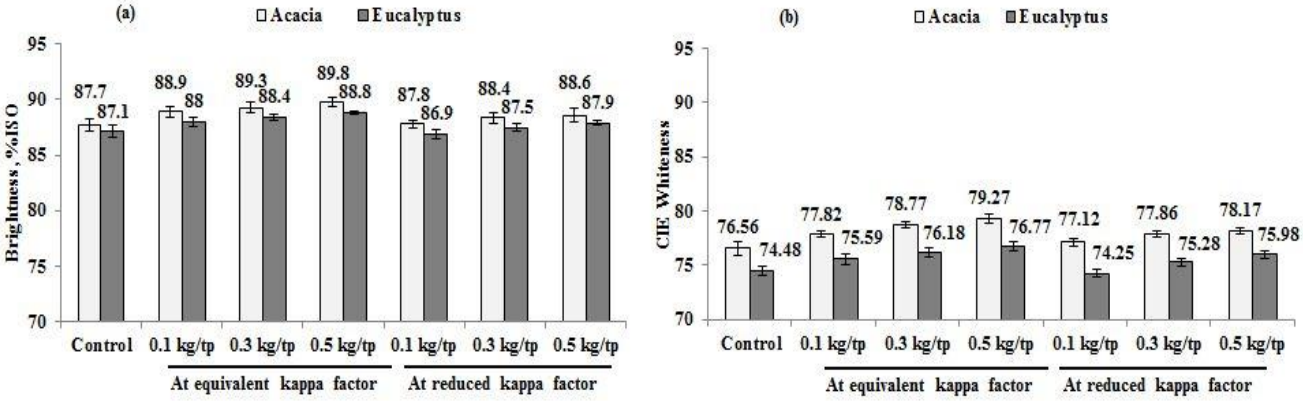

Fig. 1. (a) Brightness (% ISO) and (b) CIE whiteness of the final bleached pulp samples at equivalent and reduced kappa factors

The viscosity of the final acacia pulp was also determined and found to be similar to that of untreated pulp (Fig. 2a). The reduction in post-color (PC) number increased with increasing doses of xylanase and was higher when the pulp was treated at the same chemical dosages (Fig. 2b).

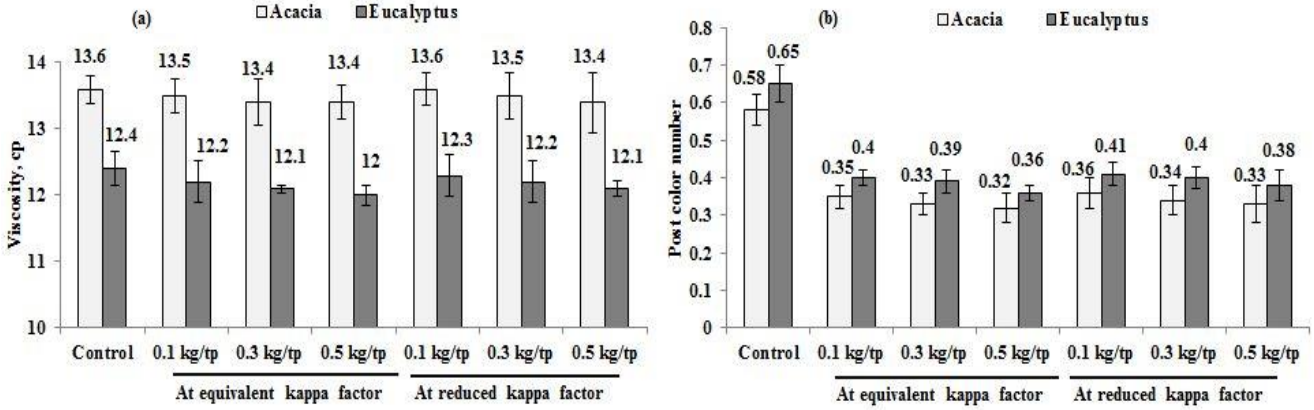

Fig. 2. (a) Viscosity and (b) Post-color number of the final bleached pulp samples at equivalent and reduced chemical charges

Here as well, the same trend of final bleached pulp viscosity was obtained for eucalyptus wood pulp (Fig. 2a), and it was nearly similar to that of untreated pulp. As in acacia pulp, a reduction in PC number was observed with eucalyptus pulp.

Table 4. Final Pulp Properties of Enzyme-treated and Untreated Acacia (AcK) and Eucalyptus (EuK) Pulps

Particulars		Control	At Equivalent KF (0.22)			At Reduced KF (0.187)		
			0.1 (kg/t)	0.3 (kg/t)	0.5 (kg/t)	0.1 (kg/t)	0.3 (kg/t)	0.5 (kg/t)
AcK Pulp	Shrinkage (%)	5.1 ± 0.32	5.3 ± 0.15	5.6 ± 0.35	5.9 ± 0.40	5.2 ± 0.15	5.5 ± 0.20	5.7 ± 0.26
	Strength properties							
	PFI Revolutions (PFI)	3600	3600	3600	3600	3600	3600	3600
	Initial CSF	619	625	628	630	628	631	634
	End CSF	431	436	445	452	443	454	461
	Grammage (g/m^2)	70.81	70.25	70.19	70.67	70.89	71.17	71.03
	Burst index (kN/g)	2.6 ± 0.31	2.6 ± 0.29	2.5 ± 0.15	2.6 ± 0.45	2.5 ± 0.38	2.6 ± 0.25	2.6 ± 0.17
	Tear index (mN.m^2/g)	6.2 ± 0.45	6.2 ± 0.35	6.2 ± 0.29	6.0 ± 0.25	6.1 ± 0.26	6.0 ± 0.35	5.9 ± 0.31
EuK Pulp	Shrinkage (%)	5.5 ± 0.30	5.8 ± 0.32	6.2 ± 0.10	6.4 ± 0.15	5.7 ± 0.45	6.1 ± 0.21	6.2 ± 0.21
	Strength properties							
	PFI Revolutions (PFI)	2000	2000	2000	2000	2000	2000	2000
	Initial CSF	584	587	591	593	592	594	597
	End CSF	422	438	447	455	441	456	465
	Grammage (g/m^2)	70.22	70.59	70.79	70.46	71.01	70.76	70.43
	Burst index (kN/g)	4.2 ± 0.23	4.2 ± 0.10	4.2 ± 0.15	4.3 ± 0.17	4.2 ± 0.06	4.1 ± 0.15	4.1 ± 0.25
	Tear index (mN.m^2/g)	7.3 ± 0.44	7.2 ± 0.21	7.1 ± 0.31	7.1 ± 0.25	7.2 ± 0.38	7.2 ± 0.42	7.0 ± 0.30

However, it was higher when the pulp was treated at reduced chemical doses, as shown in Figs. 2a and b. According to Kim and Paik (2000), xylanase treatment was responsible for the removal of carboxylic acids and their counter ions, which resulted in color reversion. By the partial removal of xylan in the pulps, xylanase treatment increased brightness stability.

The strength properties of eucalyptus were found to be higher than in acacia pulp. Enzyme-treated and untreated acacia and eucalyptus hardwood pulps were refined at 3600 and 2000 PFI revolutions, respectively. To achieve the same CSF level, more PFI revolutions were required for acacia wood fibers. Comparable results were observed in terms of the bursting strength of enzyme-treated and untreated acacia (Table 4: AcK) and eucalyptus (Table 4: EuK) wood pulps. The enzyme treatment showed a slightly negative impact on the tearing strength of acacia and eucalyptus pulps as the action of the xylanases reduced the intrinsic fibrillar strength due to the removal of superficial hemicelluloses (Bajpai 1999). This is presumably the reason underlying the reduced tear index after the enzymatic pretreatment of wood pulps.

CONCLUSIONS

1. A comparative study of acacia and eucalyptus kraft pulps was carried out in an ECF bleaching sequence after xylanase treatment with commercial xylanase (Optimase CX 72L), in which the optical and physical properties of the final bleached pulp and paper were tested.
2. The results showed enzymatic bleaching exhibited higher selectivity in the case of acacia kraft pulp.
3. The reduction of bleach chemicals consumption was also observed to be higher in acacia than eucalyptus wood pulps with xylanase enzyme treatment before the ECF bleaching sequence. A reduction in chlorine dioxide (ClO_2) and sodium hydroxide (NaOH) by more than 17.0% and 23.0%, respectively, was noticed for both hardwood pulps, with an improvement in the optical properties of the pulps.
4. The strength properties of eucalyptus pulp were observably better than acacia pulp.
5. Marginal increment in pulp shrinkage was observed in both hardwood pulps as compared to their respective control with shrinkage lesser in acacia than eucalyptus pulp.
6. Reduction in post color number by more than 44% was observed in both hardwood pulps.
7. The present results provide a clear indication of the advantage of xylanase treatment, and a better selectively of acacia over eucalyptus for use in the paper-making process, as these approaches resulted in the decreasing consumption of bleach chemicals, leading to a reduced cost in the treatment of hazardous chemicals and improved qualities of the final paper.
8. Many commercial xylanase products are available in market now and there may be significant variations in the bleaching response with different xylanase products towards final bleached pulp qualities.

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Applicability of Microbial Xylanases in Paper Pulp Bleaching: A Review

Avdhesh K. Gangwar,[a] N. Tejo Prakash,[b] and Ranjana Prakash [c,*]

The pulp and paper industries are attempting to bring changes to the bleaching process to minimize the use of chlorine to satisfy regulatory and market demands. Xylanases offer a cost-effective way for mills to realize a variety of benefits in bleaching. One main benefit is reducing Adsorbable Organic Halides (AOX) discharge. This is achieved primarily by decreasing chlorine gas usage. Other benefits include eliminating chlorine gas usage in mills with high chlorine dioxide substitution levels and increasing the brightness ceiling (particularly for mills contemplating Elemental Chlorine Free (ECF) and Totally Chlorine Free (TCF) bleaching sequences and in mills using large amounts of peroxide or chlorine dioxide). These benefits are achieved in the long term when the enzymes are properly selected and integrated into the process. This review summarizes the application of xylanases in the bleaching of pulp, with emphasis on the mechanism and effects of xylanase treatment on pulp and paper and the factors affecting the bleaching process and its efficiency. Brightness gains of up to 1.4 to 2.1 units have been achieved with xylanase treatment with the reduction of chlorine consumption by 15.0%. Xylanase treatment can lower the AOX amount in filtrate by 25.0% as compared to references. The Chemical Oxygen Demand (COD) can be reduced by 85%.

Keywords: Adsorbable organic halides; Bleaching effluents; Chlorine compounds; Enzymatic bleaching; Kraft pulps; Pulp properties; Xylanases

*Contact information: a: Department of Biotechnology, Thapar University, India; b: School of Energy and Environment, Thapar University, India; c: School of Chemistry and Biochemistry, Thapar University, P. O. Box 32, Pin- 147004, Patiala, India; *Corresponding author: rprakash@thapar.edu*

INTRODUCTION

Biotechnology is an area with a high potential to improve various aspects of pulp and papermaking processes through cost reduction, quality improvement, and reduction of the environmental impact of an industry that has historically been considered a polluting industry (Valls *et al.* 2010a; Senior *et al.* 1999). Increased environmental awareness has forced paper manufacturers to consider new bleaching strategies, as chlorine-based bleaching leads to the formation of dangerous adsorbable organic halides (AOX). In this regard, the use of biotechnology in the paper industry has provided some fascinating and eco-friendly results. Spence *et al.* (2009) observed that enzymatic pretreatment of hardwood kraft pulp reduced the overall cost of bleaching to 89% ISO brightness by about US $ 2.32 per oven-dry ton of pulp.

According to mill-scale experiments, xylanase treatment can substantially improve the final brightness of bleached pulps while simultaneously decreasing bleaching costs, when it is used with hydrogen peroxide and ozone (non-chlorine bleaching chemicals) within totally chlorine-free (TCF) bleaching sequences (Allison *et al.* 1994).

It has also been demonstrated that xylanase pretreatment usually results in up to a 20 to 25% reduction in total elemental chlorine for hardwoods and 10 to 15% for softwoods, all while decreasing AOX by 12 to 25% (Shatalov and Pereira 2008; Tolan *et al.* 1996).

Organochlorine compounds are very important to the pulp and paper industry. These compounds are generated by the reactions between the residual lignin present in wood fibers and the chlorine used in some mills for bleaching. According to Bajpai *et al.* (2006), some of these compounds are toxic, mutagenic, persistent, and bioaccumulating and are therefore very harmful to biological systems.

Due to concerns about the short- and long-term environmental effects of chlorinated organic compounds, regulatory agencies in many countries have imposed limits on their discharge. In order to prevent production of organochlorine compounds, the most commonly used enzymes in bleaching are hydrolytic enzymes such as xylanases. Prebleaching of kraft pulps using xylanase has provided many advantages to pulp mills such as improving environmental performance, reducing bleaching cost, increasing productivity, and enhancing pulp properties. This technology has been well-received worldwide (Nguyen *et al.* 2008).

The use of enzymes in pulp bleaching is known as biobleaching. The use of xylanase for biobleaching yields pulps with high brightness and saves bleaching chemicals (Bajpai and Bajpai 1999). Xylanase is also widely used in the bleaching of non-woody pulps (Bajpai and Bajpai 1996; Chauhan *et al.* 2006; Roncero *et al.* 2003b; Shirkolaee *et al.* 2008). The use of *Bacillus coagulans* xylanase on three non-woody pulps (wheat straw, rice straw, and jute) was explored using a TCF bleaching sequence (Chauhan *et al.* 2006). A maximum brightness gain of 5.1 points was achieved in rice straw pulp at an initial pH value of 8.5. In the case of wheat straw pulp, maximum brightness gains of 4.4 points has been obtained only after bleaching stage with the enzyme-treated pulp at a pH of 8.5. Similarly, in the cases of jute straw pulps, maximum brightness gains of 4.0 points were obtained at pH values of 7.0. Biobleaching (*i.e.*, enzymatic pre-treatment/prebleaching of pulp with xylanases such as endo-1,4-β-xylanase, EC 3.2.1.8.) before chemical bleaching is an alternative and cost-effective way to reduce the consumption of bleaching chemicals (especially chlorine) in pulp mills. It further works to minimize the formation of toxic chlorinated organic substances in bleach plant effluent (Shatalov and Pereira 2008; Suurnakki *et al.* 1997).

The application of xylanase is often referred to as "prebleaching" or "bleach boosting" because it enhances the effects of bleaching chemicals by breaking the xylan network, which helps in removing the trapped lignin from the pulp rather than removing lignin directly or attacking lignin-based chromophores (Bajpai and Bajpai 1997). Roncero *et al.* (2003c) also reported effects of enzyme pretreatment before the ozone bleaching. They worked with two different pulps, eucalyptus and straw pulp and treated both pulp with enzyme treatment (X stage) before oxygen delignification (O) followed by ozone (Z stage) bleaching and compared individually with reference pulp which was not treated with enzyme before O stage and followed by Z stage. They observed that there was no significant effect of enzyme pretreatment on the elimination of lignin during the Z stage with straw pulp, while with eucalyptus O pulp and XO pulp, decrease in lignin was parallel, but the difference in the kappa number was maintained at 1.5 in XO pulp and 2.0 in O pulp. On the basis of these results they concluded that the X treatment eliminates a certain amount of lignin that cannot be eliminated in the Z stage.

The aim of enzymatic bleaching depends on mill conditions and may be related to environmental demands, reduction of chemical costs, and improvement and maintenance

of product quality. The concept of using xylanase enzymes to increase the efficiency of bleaching pulp was introduced in 1986 (Viikari *et al.* 1986) and commercialized in 1991.

Bleaching with xylanase requires strict control of parameters including pH, temperature, and retention time (Farrell *et al.* 1996; Suurnakki *et al.* 1997). The performance of enzymes depends heavily on the types of the raw material, the pulping process, brown stock washing, and the bleaching sequence. The xylan content in the pulp depends on the performance of the digester. In conventional kraft pulping, the xylan content depends on the effective alkalinity. Bleaching using xylanase, as compared to conventional bleaching, increases bleaching efficiency and decreases the need for bleaching chemicals.

There are, however, some disadvantages of using xylanase in the bleaching process. It has been observed in mills that it is responsible for accelerated corrosion of equipment and increased time for maintenance of the brown stock (Tolan *et al.* 1996**)**, a very common problem with enzymatic bleaching. In mills, sulfuric acid is used in huge amounts to lower the pulp's pH for more effective application of enzymes. This results in corrosion of flexible steel facilities. Thus, the effects of xylanase prebleaching are limited due to its indirect operation and problems related to strength properties. It was observed in a study by Bajpai *et al.* (2006) that enzymatic bleaching results in reduced tear strength of the final paper.

The production and use of paper has a number of adverse effects on the environment by generating pollutants. In 2011, Keefe and Teschke reported that wood derivatives dissolved in the pulping liquors, including oligosaccharides, simple sugars, low molecular weight lignin derivatives, acetic acid, and solubilized cellulose fibres, are the main contributors to both biological oxygen demand (BOD) and chemical oxygen demand (COD). Compounds that are toxic to aquatic organisms include chlorinated organic (AOX) generated from bleaching, especially kraft pulp. Contaminated wastewater from pulp and paper mills may cause of death for aquatic organisms, allow bioaccumulation of toxic compounds in fish, and impair the taste of downstream drinking water. Sulphur compounds generated in paper industries are the main cause of mucous membrane irritation and headache in human being. Particulate matters are the main cause of respiratory problems in children. Those paper mills, which are using chemical methods for producing pulp particularly in kraft pulping, generate more pollutants in air.

MECHANISM OF XYLANASE ACTION IN PULP AND PAPER

Xylanase enzymes remove xylan by breaking the link between cellulose and lignin. During subsequent stages of the bleaching process, lignin is readily eliminated by the breakage of its links with cellulose (Woolridge, 2014; Roncero *et al.* 2005; Torres *et al.* 2000; Pham *et al.* 1995; Turner *et al.* 1992). It is known that kraft pulping causes precipitation of short xylan chains onto the surface of fibers. These xylans re-deposit on the fiber and act as a physical barrier against penetration by bleaching agents (Kantelinen *et al.* 1993). Re-deposited xylan becomes an obstacle for bleaching chemicals and results in increased consumption of chlorine dioxide (ClO_2) in the first bleaching stage. Xylan physically entraps the lignin, influencing fiber swelling (Shobhit *et al.* 2005).

Xylanases catalyze the hydrolysis of xylans and therefore can hydrolyze precipitated xylans. This results in a reduction of the xylan concentration on the secondary wall of the fiber surface during enzymatic treatment with xylanase, particularly

in hardwood pulps. Reducing the xylan concentration increases the permeability of fiber surfaces, improving bleachability (Paice *et al.* 1992; Bajpai *et al.* 1994; Viikari *et al.* 1996; Gliese *et al.* 1998; Shah *et al*. 2000; Torres *et al.* 2000; Roncero *et al.* 2000a). Another mechanism of the action of xylanases involves hemicellulases. According to this mechanism, hemicellulases cleave hemicellulose bonds near their points of attachment to lignin. It is possible that this improves the extraction of trapped lignin from the pulp. Xylanases are thought to promote efficient pulp bleaching *via* the hydrolysis of the re-precipitated xylan on the fiber.

Henriksson and Teeri (2009) suggested a possible mechanism for xylanase action in pulp and paper, wherein:

- Lignin that is covalently bound to xylan (LCC) or lignin entrapped physically by xylan can be extracted from the fiber after xylanase treatment;
- Xylanase treatment partly removes the xylan layer that is re-precipitated onto the fiber surface and opens more spaces for bleaching chemicals to enter the fiber (Sharma *et al.* 2007); and
- Xylanase treatment removes the region with high hexenuronic acid content and thereby reduces the consumption of bleaching chemicals.

According to Roncero *et al.* (2003a), hexenuronic acids can cause significant reversion of brightness in TCF bleached pulp, a problem which can be combatted by the application of xylanases (Cadena *et al.* 2010). In addition, it has been observed that the kappa number, which reflects the lignin content in pulp, also decreases with xylanase treatment as xylanases are responsible for better penetration of bleaching chemicals after removing hexenuronic acid form the pulp.

However, certain characteristic features are desired in xylanases to facilitate their use in the pulping and bleaching processes. These include:

- Minimum cellulolytic activity to avoid hydrolysis of cellulose fibers (Archana and Satyanarayana 2003);
- Low molecular mass to facilitate their diffusion into the pulp fibers; and
- High yield of enzymes through cost-effective processes (Niehaus *et al.* 1999).

XYLANASE WITH A LACCASE MEDIATOR SYSTEM

There has been a wealth of research regarding the use of xylanase with a laccase mediator system. It has been reported that xylanase modifies surfaces of the pulp fiber and results in increased penetration by bleaching agents (Roncero *et al.* 2000b; 2005; Salles *et al.* 2005). An enzyme pretreatment stage (X) with milder application conditions for laccase mediator systems (L) was introduced. At high levels of the variables in this system (the XLE sequence, where E is an alkaline extraction stage) the kappa number dropped by 55%, 11% more than with the LE sequence, and an ISO brightness gain of 6% was found in all XLE-treated pulp samples compared to the corresponding LE sequence pulps (Valls *et al.* 2010a).

Another study was done on oxygen-delignified eucalyptus kraft pulp under optimal conditions for a laccase mediator treatment. Experiments were run with and without a xylanase pretreatment using a statistical plan for the dose of laccase, the dose of mediator (1-hydroxybenzotriazole, HBT), and the reaction time. Kappa number and brightness results showed that some lignin in the pulp remained inaccessible and the

bleaching system started to remove or alter other chromophoric compounds present in the pulp. The optimum points for the LE and XLE sequences were achieved at the lowest HBT dose, highest laccase dose, and at a reaction time of 3.4 or 4.6 h. The xylanase pretreatment increases enzyme access to cellulose fibers, thereby boosting the effect of the laccase mediator system in reducing the residual lignin content and releasing more hexenuronic acids (Valls and Roncero 2009). Further, it has been reported that increasing HBT dose affects the kappa number and brightness. It was also seen that low HBT doses provide the better quality pulp. Another advantage of using a lower dose of HBT is that it reduces laccase inactivation and effluent toxicity (Valls *et al.* 2010a).

COMMERCIALLY AVAILABLE XYLANASES

Enzymes are produced by natural sources (typically fungal or bacterial strains), which are available on the market for the production of bleached pulp with higher brightness and lower kappa number. These enzymes are also applicable for reducing brightness reversion and improving physical strength. Mainly, bacterial and fungal strains are used to produce xylanase for pulp and paper industries. In 2002, Subramaniyan and Prema reported that bacterial xylanase have more advantages over fungal xylanase due to their alkaline-thermostable xylanase producing trait. In general, the optimum pH and temperature of bacterial xylanases are slightly higher than the optimal pH and temperature of fungal xylanases, which is a suitable characteristic in most industrial applications, especially in the pulp and paper industries (Ratanachomsri *et al.* 2006; Khasin *et al.* 1993). *Bacillus* strains are attractive producers of high levels of extracellular cellulase free xylanases stable at both high temperature and alkaline pH (Nagar *et al.* 2013). The usual drawbacks of fungal xylanases are that their optimal activity occurs in a pH range that is too low and too narrow for direct treatment of brownstock pulp. The ideal xylanase should maintain all or most of its activity through as broad pH range as possible. Ideally, the optimal pH range should allow brownstock to be treated with no acidification. Bacterial xylanases fulfill the criteria of having lower residual cellulase activity (which would reduce the fibre strength and pulp yield) in comparison of fungal xylanases (Ledoux *et al.* 1993).

Valls *et al.* (2010b) reported that the enzymatic stage removed 14% of Hex-A as a result of xylanase hydrolyzing xylans on fiber surfaces. The effects of commercially available xylanases (Pulpzyme HC, Irgazyme-10 and 40S, and Bleachzyme-B and F, *etc.*) were also examined by Bajpai and Bajpai in 1996 with respect to subsequent bleaching and the improvement of pulp quality. They observed that the enzyme treatments led to a decrease in extraction stage kappa number by 0.4 to 1.2 units relative to untreated pulp. The brightness gain in final pulp under the same total bleaching chemical charge was 0.8 to 1.5 units with Bleachzyme-B, Cartazyme HS-10, and Irgazyme-40S. The maximum brightening effect was noted in the cases of Bleachzyme F, Cartazyme HS-10, and Irgazyme-40S. In this study, about 20% reduction in chlorine was observed on the basis of the kappa number at the extraction stage with both enzymes.

In 2001, Bajpai and Bajpai tested six different enzymes under a wide range of pH values, incubation temperatures, incubation times, and enzyme doses at 5 to 10% stock concentration. After enzyme treatment the pulp was bleached using a CEHED (where C-chlorine, E-extraction, H-hypochlorite, and D-chlorine dioxide) bleaching sequence.

Observations indicated that the levels of pentosans were high in the unbleached pulp after mild hydrolysis, resulting in lesser energy consumption and a higher pulp yield. The treatment also resulted in reduced pollutant release into the prehydrolyzate liquor, higher pulp brightness, and reduced consumption of bleaching chemicals.

APPLICATIONS OF XYLANASE IN PULP AND PAPER

Effect of Xylanase Treatment on Carbohydrate Composition

Hydrolysis of xylan occurs in the presence of the xylanases used in enzymatic treatment (Roncero *et al.* 2005). Hydrolysis was found to be more dramatically affected when the enzymatic treatment was done prior to oxygen delignification (ODL). Enzymatic treatment by itself yielded a 13.4% reduction in xylan content and a 15.5% reduction when used in conjunction with oxygen delignification. The authors also studied the influence of xylanase on the carbohydrate composition. Results obtained from XRD indicated that the ratio of crystalline and amorphous regions was affected in both cases by enzymatic treatment and oxygen delignification. The degree of crystallinity was increased.

Limited hydrolysis of the xylan network is often sufficient to facilitate the subsequent chemical removal of lignin without sacrificing yield. The viscosity of the pulp is also improved as a result of xylanase treatment. However, the viscosity of the pulp is adversely affected when cellulase activity is present as it increases the degradation of cellulose (Jeffries 1992). Therefore, cellulase activity by the enzyme preparation is undesirable in enzymatic bleaching.

Shatalov and Pereira (2008) studied two commercial enzymes (Ecopulp and Pulpzyme) using *Eucalyptus globulus* pulp bleached with an XQPPP bleaching sequence (where X-xylanase treatment, Q-pulp chelating, and P-hydrogen peroxide). The main polysaccharide constituent was xylose and the change in its content generally reflected the bleaching behavior of the heteroxylan. Xylanase pre-treatment with Ecopulp was more effective in removing xylose during the three stages of peroxide bleaching after enzymatic treatment, dissolving about 9% of xylan while pulpzyme dissolved only 5.8%. Both xylanase preparations caused additional xylan removal, in comparison to the reference pulp during each subsequent peroxide bleaching stage. They also noted lignin removal by 65.4% and 63.7% with enzyme treated with Ecopulp and Pulpzyme, respectively, and 58.0% and 57.9% was noted for and corresponding control pulps, within the specified range of peroxide charge of 3-9%. Peroxide also affects the viscosity of the bleached pulp. In the sequence XQPPP, viscosity drop was observed due to enhanced degradation of lignin associated carbohydrates and cellulose under deep delignification of enzyme treated pulps.

A study by Shatalov and Pereira (2009) on the removal of lignin compounds in *E. globulus* pulp with three-stage peroxide bleaching after xylanase pretreatment with Ecopulp and Pulpzyme was completed. Lignin content was determined as a Klason and acid soluble lignin according to T 222 om-88 and UM 250 TAPPI standards. Results are shown in Table 1.

Table 1. Effect of Xylanase-aided Three-stage Hydrogen Peroxide Bleaching on the Reduction of Lignin in *E. globulus* Kraft Pulp*

Bleaching stage		Lignin (%, Oven dry pulp)	
		Ecopulp	Pulpzyme
X	Control	3.33	3.06
	Enzyme	2.98	2.85
XQP	Control	1.95	1.87
	Enzyme	1.57	1.60
XQPP	Control	1.63	1.61
	Enzyme	1.24	1.30
XQPPP	Control	1.40	1.29
	Enzyme	1.01	1.03
* X-Enzymatic pretreatment; Q-Chelating; P-Peroxide bleaching			
* Based on data from Shatalov and Pereira (2009)			

The reduction of lignin content after xylanase pretreatment of the pulp also depends on the types of raw material used, as shown in Table 2.

Table 2. Effect of Xylanase Pretreatment on Kappa No. Reduction in Bagasse, Rice Straw, and Wheat Straw*

Sample		Enzyme dose (U/g Pulp)	Decrease in Kappa No.
Bagasse	Xylanase (*T. lanuginosus*)	1	0.4
		5	0.7
		10	0.8
	Xylanase HS	1	1.1
		5	1.3
		10	1.9
Rice straw	Xylanase (*T. lanuginosus*)	1	0.3
		5	0.6
		10	0.6
	Xylanase HS	1	0.8
		5	0.8
		10	0.9
Wheat straw	Xylanase (*T. lanuginosus*)	1	0.2
		5	0.6
		10	0.7
	Xylanase HS	1	0.5
		5	0.9
		10	1.7
* Xylanase pretreatment was carried out at 60 °C for 3 h in sodium citrate buffer (pH 6.5)			
* Xylanase HS pretreatment was carried out at 60 °C for 3 h in citrate/phosphate buffer (pH 5.5)			
* Based on data from Shirkolaee *et al.* (2008).			

Effect of Xylanase Treatment on Fiber Morphology

Roncero *et al.* (2000a) concluded that xylanase treatment of kraft pulps is responsible for opening pores in the cell walls of fibers. Some morphological changes such as cracks, flakes, holes, filaments, and peeling are caused by enzyme treatment. These cracks and holes allow for the diffusion of larger lignin macromolecules, as reported by some authors (Paice *et al.* 1995; Wang *et al.* 1997). According to Roncero *et*

al. (2005) xylanase treatment improves the accessibility of bleaching chemicals to the pulps by increasing diffusion to outward movement of degraded lignin fragments. This results in the removal of less-degraded lignin fragments from the cell wall, yielding a reduction in kappa number and enhanced brightness. The viscosity of pulp also increases in xylanase-treated pulps as compared to untreated pulps.

With attention to the effects of enzymatic treatment, eucalyptus pulp has been studied to determine effects of the treatment on fiber morphology after TCF and ECF bleaching sequences. Xylanase changes the surface of the fiber as observed in an analysis done by scanning electron microscopy (SEM). Treated fibers have rough surfaces with splits (*i.e.*, are more open), which in turn increases contact between the bleaching agent and the substrate (Roncero *et al.* 1999; 2000a; Viikari *et al.* 1986).

In another study, it was reported that the effects of enzymatic treatment on fiber surfaces were more evident in the earlier bleaching stages. Unbleached eucalyptus kraft pulps were treated with xylanase (Roncero *et al.* 2000a). There was a remarkable flaking found in the fibers of enzyme treated pulps. Many flakes and filaments of material detached from their surface. In contrast, smoother fiber surfaces were seen in untreated pulps. The group also studied untreated (O-Pulp) and xylanase-treated (XO-Pulp) pulps after the ODL stage. Fibers with very smooth surfaces were observed in untreated (O-Pulp) pulp. In contrast, fibers with a remarkable peeling effect were observed in treated pulp (XO-Pulp). The XO-Pulp appeared to continue undergoing a peeling process in which xylans were removed as flakes. These flakes of material removed from the surface caused the surface modification.

Effect of Xylanase on Hexeneuronic Acid Content

Hexenuronic acid (Hex-A) is widely distributed among natural polysaccharides such as heparin, chondroitin, and lepidimoide (Adorjan *et al.* 2006). During the alkaline pulping process, about 75 to 90% of 4-O-methyl-glucuronic acid side groups (MeGlcA) linked to heteroxylan are lost and the residual MeGlcA are almost completely (83 to 88%) converted to unsaturated hexenuronic acid (Hex-A or 4-deoxy-l-threo-hex-4-enopyranosyluronic acid) *via* the intermediate product, 4-O-methyliduronic acid (Shatalov and Pereira 2008; Chauhan *et al.* 2006; Beg *et al.* 2001; Bim and Franco 2000; Senior *et al.* 1999; Farrell *et al.* 1996).

The alkali charge and the H-factor are the main variables that influence the formation of hexenuronic acid during kraft pulping. Considering *Eucalyptus globulus* wood as a raw material for kraft pulp, the alkali charge is the main factor that contributes to the formation and degradation of hexenuronic acid during pulping. During the cooking process, hexenuronic acids form covalent bonds with lignin (Vuorinen *et al.* 1999). Hex-A is formed in kraft cooking from the methyl glucoronic acid side group found in xylans. It plays an important role in bleaching because of its undesired neutralization of electrophilic bleaching chemicals such as chlorine dioxide, ozone, and peracids, increasing the consumption of these chemicals. Bajpai *et al.* (2005) reported that the Hex-A percentage in particular raw material depends on the growing region and its species. As in hardwood unbleached pulp, it is found in the range of 7.1 to 30.5 mmol/kg, *Casuarina* pulp having the highest and Subabul pulp has the lowest Hex-A content. In bamboos, it is found in the range of 3.1 to 6.6 mmol/kg; Assam bamboo having the highest and Maharashtra bamboo having the lowest Hex-A content.

Hexenuronic acid protects xylan against terminal depolymerization reactions, thus preserving the yield of the pulping process. However, in extreme temperature and alkali

dosage conditions, these composites, as well as other polysaccharides, suffer alkaline hydrolysis and are degraded. Hexenuronic acid also suffers hydrolysis under acidic conditions as it is vulnerable to the attack of electrophilic oxidants (Marechal *et al.* 1993). Since the discovery of hexenuronic acid structures in kraft pulps (Vuorinen *et al.* 1999), several strategies have been proposed for removing the composites from the pulp during the bleaching phase, which are based on an acid hydrolysis stage conducted at a temperature of about 80 to 100 °C and a pH of about 3.0 (Almeida 2004).

Shatalov and Pereira (2009) used chemical pulps to determine that the impact of hexenuronic acids on xylanase aided biobleaching. They found that xylanase assisted in direct pulp brightening. This was presumed to be due to Hex-A removal with solubilized xylooligosaccharide fractions. A strong positive correlation was established between the xylanase bleach boosting effect and the bleaching profile of Hex-A. The effects of alkali and oxygen extractions of kraft pulp on xylanase aided bleaching were studied as well. The group noted an improvement in final brightness of up to 1.4 to 2.1 units with reductions in Hex-A and the kappa number of xylanase-pretreated pulps compared to the corresponding control pulps (Wong *et al.* 2001).

According to Shatalov and Pereira (2009), the carbohydrate derived chromophores have a pronounced effect on brightness development of chemical pulps during xylanase aided bio-bleaching. The xylanase assisted direct pulp brightening was caused by HexA removal with solubilized xylooligosaccharide fractions. Strong positive correlation was established between xylanase bleach boosting effect and bleaching profile of HexA. The results are summarized in Table 3.

Table 3. Effect of Xylanase-aided Three-stage Hydrogen Peroxide Bleaching on Hexenuronic Acid Content in *E. globulus* Kraft Pulp*

Bleaching stage		Hexenuronic acid content (µmol/g dry pulp)	
		Ecopulp	Pulpzyme
X	Control	50.45	49.16
	Enzyme	43.13	43.69
XQP	Control	44.13	44.04
	Enzyme	34.69	35.92
XQPP	Control	39.81	40.19
	Enzyme	32.19	33.77
XQPPP	Control	36.60	37.14
	Enzyme	30.63	31.88
* X-Enzymatic pretreatment; Q-Chelating; P-Peroxide bleaching			
* Based on data from Shatalov and Pereira 2009.			

Effect of Xylanases on Bleaching Chemical Consumption

A number of reports on the reduction of bleaching chemical usage with xylanase enzymes (alone or in combination of other enzymes) in the enzymatic bleaching of pulp are available. According to Chakar *et al.* (2000), hexenuronic acids contributed 33 to 67% of the kappa number of hardwood kraft pulps, whereas for softwood kraft pulps, hexenuronic acids contributed only 5 to 12% of the pulp's kappa number. Xylan contains hexenuronic acid, which consumes bleaching chemicals, resulting in more consumption of chemicals during bleaching. Xylanase treatment removes regions with high contents of hexenuronic acid, thereby decreasing the consumption of bleaching chemicals.

Results obtained from laboratory studies and mill trials indicate savings in total active chlorine of about 20 to 25% (Bim and Franco 2000; Senior and Hamilton 1992; 1992a,b; 1993; Senior *et al.* 1999; Shobhit *et al.* 2005; Tolan and Canovas 1992). Xylanase enzymes hydrolyze the xylan re-precipitated onto the fiber surface and therefore improve fiber permeability to bleaching reagents (Kantelinen *et al.* 1993). Compared to chemical bleaching, enzymatic bleaching is beneficial in that it reduces chlorine consumption by 10% when bleaching wheat straw pulp to the same brightness and kappa number (Lin *et al.* 2013).

In a different study, Bajpai and Bajpai (2001) examined six commercial enzymes (Pulpzyme HB, Bleachzyme F, Irgazyme 40 S, VAI Xylanase, and Cartazyme HS-10) to determine their effects on bleaching chemical consumption. They found that xylanase pretreatment of pulp is responsible for reducing chemical consumption *via* removal of hexenuronic acid from the pulp, which results in removing of trapped lignin in the pulp.

A mill-scale study on xylanase prebleaching of hardwood pulp conducted by Thakur *et al.* (2012) concluded that it reduced bleaching chemical requirements by 15%. In their work, enzymatic treatment was carried out at a pH of 9 to 10 and a temperature of 50 to 60 °C. Reductions in kappa number from 23.0-25.0 to 21.0-22.0 were observed. After the chlorine (C) stage, a kappa number drop from 7.0-8.0 to 6.0-7.0 was achieved. After the first extraction (E_P) stage, a drop from 5.0-5.9 to 4.0-5.0 was noted, and in the second extraction stage, a drop from 3.6-3.8 to 3.0-3.5 was observed, with a brightness gain of 2.0 to 3.0 units in each stage. The average chlorine charge in the mill was 5 kg/t pulp before treatment. It was reduced to 4 kg/t pulp and the hypochlorite flow rate was reduced from 75% to 60-65% (15 m^3/h to 13 m^3/h). Therefore, hypochlorite consumption was reduced from 45 kg/t pulp to 38-40 kg/t pulp, a hypochlorite savings of 10 to 12% while maintaining the target ISO brightness of 82 to 83%. In that way it was achieved to reduce 15% of chlorine in the C and H stages on the plant scale using hardwood pulp.

Shirkolaee *et al.* (2008) reported that xylanase pretreatment reduces the consumption of chlorine dioxide as shown in Table 4.

Table 4. Effect of Xylanase Pretreatment on Chlorine Dioxide Consumption in a DED Bleaching Sequence*

Brightness (%)						Reduction of chlorine dioxide (kg/t)
Bagasse pulp		Rice straw pulp		Wheat straw pulp		
Xylanase (*T. lanuginosus*)	Xylanase HS	Xylanase (*T. lanuginosus*)	Xylanase HS	Xylanase (*T. lanuginosus*)	Xylanase HS	
79.5	79.7	79.1	79.9	79.2	79.6	Control
80.6	81.7	81.1	82.0	80.1	80.8	0.0
79.9	81.1	80.8	81.7	80.0	80.4	0.5
78.9	80.5	80.6	81.5	79.7	80.2	1.0
79.7	80.2	80.6	81.6	79.8	79.9	1.5
79.5	80.0	79.8	81.0	79.6	79.7	2.0
79.1	79.8	79.3	80.5	79.2	79.6	2.5
78.7	79.8	79.1	80.1	79.2	79.1	3.0
78.0	79.7	N.D*	79.9	79.1	N.D*	3.5
N.D*	79.1	N.D*	79.9	79.0	N.D*	4.0
* D_1 (Chlorine dioxide) conditions: 10% consistency, 67 °C, 113 min						
* E (alkali extraction) conditions: 10% consistency, 67 °C, 67 min						
* D_2 (Chlorine dioxide) conditions: 10% consistency, 67 °C, 180 min						
* Based on data from Shirkolaee *et al.* (2008).						

Effect of Xylanase on Pulp Brightness

To assess the effect of xylanases on the peroxide bleachability of *Eucalyptus globulus* kraft pulp, unbleached industrial eucalyptus kraft pulp was treated with two commercial xylanase preparations: Ecopulp® TX-200A and Pulpzyme® HC (endo-1, 4-β-xylanase activity; EC 3.2.1.8). The pulp was bleached using a totally chlorine-free (TCF), three-stage hydrogen peroxide bleaching sequence (QPPP, where Q is a pulp chelating stage and P is a hydrogen peroxide bleaching stage) without oxygen pre-delignification. The change in pulp properties after each bleaching stage was examined and compared with those of control samples treated identically except without the addition of enzyme. Hydrogen peroxide bleaching was used, though it is generally used as a separate bleaching stage incorporated into multistage, industrial bleaching sequences (Shatalov and Pereira 2008).

Jimenez *et al.* (1996) reported that biobleaching of wheat straw pulp yielded a brightness gain of 2.4 points for enzyme peroxide bleaching and 3.0 points in the case of enzyme peroxide active chlorine bleaching. Xylanase post-treatment of bleached hardwood kraft pulp resulted in significantly reduced yellowing. In spite of the reduction of yellowing, yield could suffer significantly from enzymatic treatment (Simeonova *et al.* 2007). The effects of Pulpzyme HC, a commercial enzyme, were also studied as potential post-treatment enzymes for use after bleaching processes. They yielded a 1.5% brightness improvement, reductions in PC number of up to 15%, and a 10 to 15% decrease in Hex-A. These effects were possibly due to hydrolysis of xylan located on the fiber surface but were probably due to the extraction of stabilized quinine chromophoric structures otherwise retained by the pulp.

Bajpai and Bajpai (1996) reported the effects of various cellulase-free commercial xylanases on the brightness of pulps. Their results are shown in Table 5.

Table 5. Increase in the Brightness of Pulps using Various Cellulase-free Commercial Xylanases in a C_DEHD Bleaching Sequence*

Sample Tested	Reaction pH	Reaction temp. (°C)	Reaction time (h)	Enzyme dose (IU/g)	Brightness (%ISO)	Increase in brightness (%ISO)
Control	---	---	3.0	---	87.1	---
Bleachzyme B	7 to 7.5	40 to 50		10.0	87.9	0.8
Bleachzyme F	6 to 6.5	45 to 50		6.0	88.6	1.5
VAI xylanase	5 to 7	50 to 60		3.5	88.4	1.3
Ecopulp-X200	5 to 6	50 to 55		10.0	88.5	1.4
Cartazyme HS-10	4 to 5	40 to 55		13.0	88.6	1.5
Pulpzyme HC	8 to 9	60 to 70		14.0	88.0	0.9
Pulpzyme HB	7 to 8	45 to 55		14.0	88.2	1.1
Irgazyme-10	5.5 to 6	40 to 50		12.0	88.5	1.4
Irgazyme 40S	7 to 8	50 to 60		7.5	88.6	1.5
* C_D-Chlorination; E-Extraction; H-Hypochlorite; D-chlorine dioxide						
* Based on data from Bajpai and Bajpai (1996).						

Xylanase-aided three-stage hydrogen peroxide bleaching also affected the brightness of *E. globulus* pulp, as shown in Table 6.

Table 6. Effect of Xylanase-aided Three-stage Hydrogen Peroxide Bleaching on the Brightness of *E. globulus* Kraft Pulp*

Bleaching stage		Brightness (%ISO)	
		Ecopulp	Pulpzyme
X	Control	42.4	42.0
	Enzyme	43.9	43.2
XQP	Control	75.8	74.8
	Enzyme	77.9	76.9
XQPP	Control	81.2	80.1
	Enzyme	82.9	81.5
XQPPP	Control	85.0	84.5
	Enzyme	86.4	85.7
* X-Enzymatic pretreatment; Q-Chelating; P-Peroxide bleaching			
* Based on data from Shatalov and Pereira (2009).			

Effect of Xylanases on Pulp Yield

Cellulase-free xylanases are more favorable for enzymatic bleaching, as hydrolysis of cellulose components results in reducing yield and viscosity of pulps. Cheng *et al.* 2013 worked on isolation of cellulase-free crude xylanase (*S. griseorubens* LH-3) and used it in enzymatic bleaching. They observed that xylanase treatments of eucalyptus kraft pulps did not cause significant reduction in pulp yield due to non-degradation of cellulose, as there was no cellulase activity present in their isolated xylanase enzyme. According to Gubitz *et al.* 1997, 16% loss of yield was observed due to the presence of cellulase in the fungal xylanase extract, whereas Manimaran *et al.* 2009 reported that treatment of bagasse pulp with cellulase-free xylanase extract yielded a loss of only 2.5%.

Thakur *et al.* 2012 studied the effect of enzymatic pre-treatment on hardwood and nonwood kraft pulps of eucalyptus and bagasse. They charged 500g/t dose of Pulpzyme HC (from Novozymes) in X stage. Pulp after enzymatic treatment was carried out for ECF bleaching. They observed pulp yield loss of 0.5% in eucalyptus while 0.6% in bagasse pulp in comparison to control. Pulp kappa number was reduced by 4.2% and 14.0% in eucalyptus and Bagasse pulp respectively as compare to control. Brightness gain was observed of 1.20 and 2.17 units in eucalyptus and bagasse pulp respectively.

Lian *et al.* 2011 reported the combined effect of a xylanase-laccase system on the same dosage levels on pulp yield loss with or without refining of the pulp. A significant reduction in pulp yield, about 1.8%, from 96.6% to 94.8% was obtained with the Laccase/Xylanase System (LXS) without refining. Correspondingly, pulp yield loss, about 2.5% from 95.9% to 93.4% was observed in addition of refining. This is probably due to the fact that fines are lost which generated during refining.

Blomstedt *et al.* (2010) studied the selective hydrolysis of xylan using xylanase, Ecopulp TX 200 A from the AB Enzyme, Finland. A dose of 200 nkat/g xylanase in X stage resulted in pulp yield loss of 0.58% of pulp dry weight. They concluded that the filtrate from the xylanase treatment mainly contained sugars originating from xylan, indicating that the used commercial xylanase was applicable for the selective hydrolysis of xylan.

Cheng *et al.* (2013) found the influence of enzymatic treatment on peroxide bleaching in respect of yield and viscosity drop in pulp. In set 1, they treated the pulp with xylanase (X stage) at 20 IU/g of dry pulp followed by bleaching with hydrogen peroxide (P stage) at 3.0%, while in set 2, they bleached the pulp with hydrogen peroxide only at 3.6%. They concluded that yield and viscosity of eucalyptus pulp were higher in set 1 by 2.13% and 1.8%, respectively as compared to set 2. Chemical reduction of 17% was also observed with the use of xylanase enzyme in biobleaching of eucalyptus pulp.

Effect of Xylanases on Paper Properties

The modification of the fiber structure of bleached hardwood pulp is a very attractive means for improvement of paper properties. Enzymatic treatment with xylanases modifies the structure and characteristics of fibers, resulting in improved hydration, internal fibrillation, and delamination.

The enzyme-treated pulp showed unchanged or improved strength properties (Kim and Paik 2000; Tolan and Guenette 1997; Tolan *et al.* 1996; Viikari *et al.* 1991; 1993) and was easier to refine than the untreated reference pulps (Wong *et al.* 1999). During the papermaking process, hemicelluloses strengthen inter-fibril bonding and have a favorable effect on the physical properties of fibers themselves. The removal of xylan during an enzymatic bleaching stage interferes with the strength of treated pulps. The loss in burst and tensile strength (32 to 40% and 11 to 25%, respectively) was more notable than that of tear strength (8.2 to 10.3%). The tear resistance measures the work required to tear the paper. The length of fibers and the linkages between them are factors that may affect the tear resistance (Batalha *et al.* 2011). The difference in strength properties, particularly tear strength, between enzyme-treated and untreated pulps is normally minimized by pulp refining (Roncero *et al.* 2005; Wong *et al.* 1996). This may be due to superior external fibrillation of treated pulps after enzymatic elimination of re-deposited xylan from the surface of the fibers (Roncero *et al.* 2005; Shatalov and Pereira 2008). Similar results were also found in a study by Roncero *et al.* (2003b) wherein enzymatically treated hardwood pulps obtained a higher tear resistance value and kept the same tensile index compared to the untreated reference pulp. Znidarsic *et al.* (2009) worked on hardwood pulp and reported that better external fibrillation is observed in the case of the enzyme-treated pulp. Such increased fibrillation favors increased tear resistance. With refining, these fibrils were then probably more or less removed from the fiber surface, causing a weaker tear resistance.

A study was done on a commercial enzyme (Bleachzyme F) by Bajpai and Bajpai (1996) to determine its effects on the physical strength properties of bamboo pulp. Their results are shown in Table 7.

It was also reported (Shatalov and Pereira 2008) that limited degradation of carbohydrates during the bleaching process could be the cause of similar changes in the physical properties of xylanase-treated, peroxide-bleached pulps compared to untreated, unbleached eucalyptus pulps.

A notable work regarding both enzymatic treatments and ultrasonic processes was carried out to determine their effects on the tensile strength of paper (Batalha *et al.* 2011). Tensile strength is related to the durability and utility of the paper. For example, packaging papers are subject to direct tension forces.

Table 7. Effects of Bleachzyme F on the Physical Strength Properties of Bamboo Pulp*

Parameter	Bleachzyme F							
	A*				B*			
	C_DEHD		XC_DEHD		C_DEHD		XC_DEHD	
°SR	30	40	30	40	30	40	30	40
PFI revolution (No.)	1800	2800	1895	2900	1800	2800	1895	2900
Bulk (cm^3/g)	1.65	1.45	1.60	1.40	1.61	1.40	1.58	1.40
Tensile index (N.m/g)	56.72	60.60	57.40	61.54	55.45	59.60	57.78	61.99
Burst index (kN/g)	3.706	3.795	3.667	4.070	3.65	3.82	3.75	3.92
Tear index ($mN.m^2/g$)	7.74	7.61	7.74	7.04	7.60	7.50	7.80	7.11
Double fold (No.)	53	110	78	115	50	105	80	120
A*-Same chemical dose in XC_DEHD as in control								
B*-20% less chlorine dose in XC_DEHD compared to the control								
* Based on data from Bajpai and Bajpai (1996).								

In this work it was also observed that combined enzymatic and ultrasonic treatments resulted in 48.0 and 12.1% increases in MOE and TEA compared to the initial pulp, respectively. It was also shown that the ultrasonic treatment improved opacity when the ultrasound was applied before xylanase treatment.

Effect of Xylanases on the Effluent Characteristics

The bleaching of kraft pulp is responsible for the generation of a large effluent volume in paper mills. Organochlorine compounds, contaminants generated during chlorine-based bleaching, are major components within this effluent (Vidal *et al.* 1997). Presently, the paper industry is looking for new bleaching processes in order to minimize the impact of these effluents on the environment. In the 1990s, the use of enzymes in pre-bleaching stages was intended to improve effluent quality, particularly by reducing the amount of organochlorine compounds (AOX) in the effluent (Faleiros 2008).

Xylanase is very efficient in reducing the consumption of bleaching chemicals (Call and Mucke 1997) such as chlorine or chlorine dioxide. It can lower the AOX in the filtrates by as much as 25% while increasing the brightness of the pulp (Atik *et al.* 2006; Hart and Harry 2005; Manji 2006; Saleem and Akhtar 2002).

In 1991, it was determined that after xylanase pre-bleaching of softwood, the biochemical oxygen demand (BOD) of the filtrate increased by almost two times as compared to non-treated pulp. Similarly, the chemical oxygen demand (COD) and total organic carbon (TOC) were increased, and the ratio of BOD to COD was significantly higher for the xylanase pre-bleaching filtrates, indicating that the effluents were more biodegradable (Senior and Hamilton 1991). In spite of the increase in the COD of the enzymatic prebleaching stage filtrates, treatment of the generated effluents was efficient in aerobic bioreactors. The COD removed was found to be above 85%, similar to the reference. Increases in the organic matter contents of the filtrates led to higher aeration and energy demands in the treatment plant. Moreover, the final COD of the treated effluents from enzymatic pre-bleaching stages was higher than in those generated in conventional bleaching sequences (Borges *et al.* 2010).

Xylanase-treated pulp has significantly lower levels of AOX in its effluents as compared to the effluents of conventionally bleached control pulps (Viikari *et al.* 1986).

In a study by Shobhit *et al.* (2005), it was observed that enzymatic pretreatment of pulp reduced AOX levels by 20 to 30%. During the enzyme treatment period, the amount of AOX being discharged into the receiving waters decreased from 2.4 kg/air-dry metric ton to 2.2 kg/air-dry metric ton.

Effect of Mill Operations on Xylanase Performance

Mill operations also affect the performance of the xylanase enzyme. The performance of xylanase depends on the types of raw materials, the pulping process, and the bleaching sequence (Tolan and Guenette 1997). Among raw materials, the important distinction is between hardwoods and softwoods. The percentage of bleaching chemicals saved by xylanase treatment is greater for hardwoods than for softwoods as the xylan content is greater in hardwood. Under favorable treatment conditions, the decrease in chlorine chemicals is about 20% for hardwoods and 15% for softwoods.

The xylan content of the pulp significantly depends on digester performance. For example, sulfite pulping destroys most of the xylan, so sulfite pulp is not suitable for enhanced bleaching with enzymes. In conventional kraft pulping, the xylan content depends strongly on the effective alkalinity. The bleaching sequence that is being used by the mill is also equally important to the performance of the xylanase in enzymatic bleaching.

CONCLUSIONS AND FUTURE PROSPECTS

Chlorine and alkaline extraction stages have historically been used as the main stages for the bleaching of kraft pulp in the paper industry. These stages generate effluent with high levels of corrosive chloride that cannot be recycled back into the chemical recovery furnace. Generated effluent from these stages contains large amounts of hazardous chemicals in the form of chlorinated organic compounds, which are known to have mutagenic and carcinogenic effects. Presently, regulatory agencies are very concerned with protecting the environment from these pollutants (Vidal *et al.* 1997). The environmental effects of these chlorinated organic compounds have driven pulp mills to seek out new bleaching technologies that reduce or eliminate the consumption of these hazardous chemicals during the bleaching process.

Enzymes are eco-friendly in nature and could be used as a substitute for these hazardous chemicals in the pulp bleaching process. Xylanases, which are capable of reducing the consumption of hazardous chemicals, could prove cost-effective. Byproducts generated from biochemical reactions of microorganisms are generally non-hazardous in nature. Therefore, enzymes produced *via* microbial sources have become alternatives to polluting chemical technologies. However, implementation of these enzymes on the industrial level is still a challenge for the pulp and paper industry.

Bleaching process parameters such as temperature and pH act as limiting factors preventing the best possible use of bleaching chemicals. High pH and temperature are favorable for bleaching processes. Many xylanase-producing commercial strains which are highly active and stable at high pH and temperatures are available. Still, an innovative approach should be explored for the screening of such novel xylanolytic microbial strains. Such an approach would need to be able to work at high pH and temperature within cost-effective processes on an industrial scale. Microbial and recombinant DNA methods for obtaining xylanase enzymes with new properties must be explored so that

enzymes can be commercialized easily. When enzymes become cost-effective to produce and use, the paper industry will enjoy benefits like the prevention of environmental degradation and reduction of health hazards.

In order to bring about such a revolution in paper production and industrial applications, microbiologists, biotechnologists, and biochemists should work cooperatively with the paper industry towards a pollutant-free future.

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