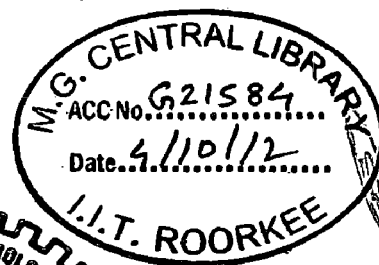# REDUCTION IN ENVIRONMENTAL LOADS BY USING ENZYME DURING BLEACHING OF CHEMICAL PULPS

A THESIS

Submitted in partial fulfilment of the
requirements for the award of the degree
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DOCTOR OF PHILOSOPHY

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ENVIRONMENTAL ENGINEERING

by

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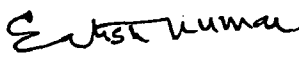
CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in this thesis entitled **Reduction in Environmental Loads by Using Enzyme During Bleaching of Chemical Pulps** in partial fulfilment of the requirements for the award of the Degree of Doctor of Philosophy and submitted in the Department of Paper Technology, Indian Institute of Technology Roorkee, Roorkee is an authentic record of my own work carried out at Department of Paper Technology during the period from **July 2006 to June 2012** under the supervision of Dr. Chhaya Sharma, Assistant Professor & Dr. Satish Kumar, Professor & Head, Department of Paper Technology, Indian Institute of Technology Roorkee, Roorkee, India.
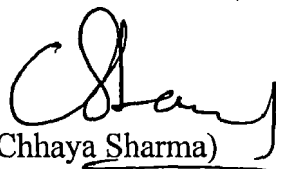
The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other Institute.

Date:14/06/2012   **(SHIV KUMAR)**

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

(Satish Kumar)   (Chhaya Sharma)
Supervisor   Supervisor

Date:14/06/2012

The Ph.D. Viva-Voce Examination of **Mr. Shiv Kumar**, Research Scholar, has been held on _____.

Supervisors   Chairman SRC   External Examiner

Head of the Department/Chairman ODC

The Indian paper industries are using bleaching sequences where chlorine is a predominant bleaching chemical. As a consequence the bleach plants have become a major source of environmental pollution contributing to high chloroganics release with high BOD, COD, AOX, color loads, various organic & inorganic compounds. There have been continuous efforts to minimize their generation, during pulp bleaching.

The enzyme pre-bleaching is one of such option, which is expected to decrease the bleach chemical demand and the chloroorganics load in the pulp and paper mill effluent. The xylanase is also considered as bleach boosting enzyme. It can be successfully used as a pre-bleaching step with existing conventional, ECF and TCF sequences.

The aim of the present work is to look at enzyme pre-bleaching of pulps followed by bleaching. Though several studies using various xylanases have been carried out for pre-bleaching hardwood and softwood pulps, the data for non wood pulps is quite scanty.

It is not apparent how xylanase enhances the bleaching of pulp, as xylan-degrading enzyme is not expected to act directly on residual lignin. It is not clear how the enzymatic process works due to variable response of different pulping and bleaching parameters. Xylanase may act by hydrolyzing xylan present on the pulp in different forms. Moreover, the mechanical strength properties of enzyme pretreated hardwood & softwood pulps also give contradicting results for different enzymes. Very little data is available on agricultural residue pulps, which are important in Indian context due to decreasing wood resources. Very few researchers worked on wild species of sugar cane which can be very useful to conserve wood resource. Specific analysis for the above parameters is necessary and the results have to be carefully quantified to give more flexibility in xylanase pretreatment technology of the pulp.

Both the pulps, non-oxygen mixed hardwood and bagasse, were bleached to a target brightness of 85% ISO using different bleaching sequences i.e. DED, $D_{50}/C_{50}ED$ and ODED. Results of mixed hardwood pulp indicate that total bleach chemical consumption for different sequences followed the order DED>$D_{50}/C_{50}ED$ >ODED. BOD and COD also follow the same order but the AOX is lowest for ODED sequence and the highest for $D_{50}/C_{50}ED$ sequence. The substitution of chlorine by chlorine dioxide reduces the AOX values of the effluent, the BOD and the COD values increase and the strength properties are comparable for all the three sequence, though the $D_{50}/C_{50}ED$ sequence shows the lowest strength properties. The minimum drop in viscosity

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was found in DED while maximum was observed in $D_{50}/C_{50}ED$ mixed hardwood pulp. Bagasse pulp also shows similar behavior with regard to bleach chemical consumption, BOD, COD, AOX and strength properties as observed with mixed hardwood pulp. Bleached mixed hardwood pulp is stronger than bleached bagasse pulp of same brightness when bleached with same bleach chemical sequence. The pollution load of bagasse pulp bleached with different sequences was more compared to pollution load of mixed hardwood pulp from similar sequence.

The mixed hardwood pulp is more responsive to oxygen delignification than bagasse pulp. The oxygen delignified DED bleached mixed hardwood pulp of 85% brightness (medium brightness) required 48.45% less bleach chemicals compared to the non-oxygen pulp. The BOD, COD and the AOX values of the combined effluents were also substantially lower. The mechanical strength properties showed no change at the same level of beating. The oxygen delignified bagasse pulp showed similar behavior as oxygen delignified mixed hardwood pulp at medium brightness levels. Bleaching of oxygen delignified bleached pulps using $OD_{50}/C_{50}EpD$ sequence yields pulp, which is weaker in comparison to oxygen delignified DED bleached pulp for both the raw materials. The BOD, COD and the AOX of the combined effluents were higher for $D_{50}/C_{50}EpD$ sequence. The higher values of environmental parameters and higher pulp degradation observed with $OD_{50}/C_{50}EpD$ bleached pulps is due to longer bleaching sequences required to reach 90% brightness than 85% observed with DED sequence.

Oxygen delignified bleached mixed hard pulp is a stronger pulp than bleached bagasse pulp of same brightness when bleached with similar bleaching sequence for both medium and high brightness levels. The range of drop in viscosity of bagasse pulp 7.7 - 17.30% was observed. The minimum drop was observed in enzyme pre-treated DED sequence and maximum was in $OD_{50}/C_{50}EpD$ sequence. The pollution load of bagasse pulps bleached with different sequences was more compared to pollution load of the mixed hardwood pulp from similar sequences.

The enzyme pre-bleaching is possible for both mixed hardwood and bagasse pulps with and without oxygen delignification. The optimized conditions of enzymes pre-bleaching are different for different pulps. Oxygen delignified pulps required lower enzyme dose compared to their respective non-oxygen pulps.

There is a substantial decrease in bleach chemical consumption to obtain 85% brightness pulp (medium) brightness, the order of effectiveness of enzyme pretreatment on mixed hardwood pulp is DED>ODED>$D_{50}/C_{50}ED$. The bleaching behavior of enzymatic prebleached bagasse pulp is quite different from enzymatic pre-bleached mixed hardwood

pulp.

AOX value of combined effluent for both the pulps, show a decrease of 23 – 42% for different bleaching sequences. The BOD, COD and BOD/COD of combined effluents are higher for enzyme pretreated pulps compared to non treated pulps. The enzyme pretreated bleached pulps show marginal increase in tensile, tear and burst index at same target brightness. The impact of enzymatic pretreatment on bleach chemical consumption of oxygen delignified pulps of medium brightness is more compared to the respective non oxygen pulps but the impact is lower for high brightness pulp. The BOD, COD and AOX values follow the same trend as non oxygen pulps i.e. decrease in AOX, increase in BOD, COD and BOD/COD ratio with minor change in mechanical strength at the same level of beating .The results are comparable with the results reported in the literature. The AOX load in $XD_{50}/C_{50}EpD$ bleached bagasse pulp is 30% reduced in comparison to control pulp.

The above studies clearly indicate the effectiveness of xylanase pre-bleaching of mixed hardwood pulp and bagasse pulps not only as a means for improving environmental performance of bleach plants but also have significant reduction in bleach chemical consumption with marginal increase in strength properties.

Many researchers have worked on xylanase and reported the potential and benefits of xylanase aided bleaching. Experiments were planned to systematically evaluate how xylanase acts in pre-bleaching to elucidate the mechanism of xylanase aided bleaching of non oxygen and oxygen delignified mixed hardwood and baggase pulps.

It is already reported in literature that xylanase tends to hydrolyze surface xylan and remove hexeneuronic acid more due to its higher accessibility. The effectiveness of xylanase aided bleaching is relatively higher in oxygen-delignified pulp where part of xylan and lignin could have been redistributed to the outer fiber surface. This is also confirmed by reduced pentosan content that showed that xylanase hydrolyze the xylan. As reported in literature, it has been observed that after enzyme pretreatment, color of effluent shows the xylanase attack on chromophoric groups. It is further confirmed by a minor decrease in kappa number, presence of lignin in enzyme pretreated effluents (infrared spectroscopy) and increase in UV absorbing materials detected (measuring absorbance at 280°A). The increase of color of the effluent with decrease in kappa number correlates well with stronger lignin peaks detected in infrared spectrum, more UV absorbing material and higher reducing sugars in enzyme pretreated effluents. No change or marginal change in tensile index in enzyme pretreated pulps suggests that enzyme does not cause a change in fiber bonding. This is

further confirmed by retaining of peaks associated with cellulose in enzyme pretreated pulps by infrared spectroscopy. The water retention value, which is a measure of fiber swelling, shows a sharp increase in enzyme pretreated bleached (beaten) pulp suggesting that the enzyme causes fiber swelling. The infrared and UV spectroscopy studies also indicate xylanase attack on LCC or lignin-hemi-cellulose bond.

The substitution $D_{50}/C_{50}ED$ bleaching of chemical mixed hardwood and bagasse pulps show that the environmental loads particularly with regard to AOX are high. The modified bleaching sequences ($D_{50}/C_{50}EpD$) in some cases meet the AOX stipulations but they are found to be economically at a disadvantage.

The enzyme pre-bleaching using xylanase is effective in reducing bleach chemical demand in all cases with reduced AOX loads and increased BOD and COD loads. The environmental and economical evaluation shows that enzyme pre-bleaching with sequences like $D_{50}/C_{50}ED$ can be adopted.

The response of enzymatic pre-bleaching to oxygen delignified mixed hardwood and bagasse pulps were found to be positive with reduction in bleach chemical demand and associated reduction in AOX loads. However adoption of these options will require addition of costly oxygen delignification step. The cost advantages in such cases may be obtained if pulp is bleached to higher brightness levels (90% ISO). Bleaching of oxygen delignified enzyme pre-bleached pulps show that bleaching sequence like $D_{50}/C_{50}ED$ was effective for both mixed hardwood and bagasse pulps though the magnitude of brightness gain becomes smaller with high pulp brightness, there remain a potential for chemical savings (10%).

The experiments on mechanism of xylanase pre-bleaching clearly show that surface xylan is not the sole target substrate for xylanase. Enzyme seems to be attacking the lignin carbohydrate complex (LCC) or is hydrolyzing the bond between lignin and hemicellulose and does not cause a major change in fiber bonding.

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1.1 STATUS OF PAPER INDUSTRY IN INDIA

Paper industry in India is the 15th largest paper industry in the world. It provides employment to nearly 1.5 million people and contributes Rs 25 billion to the government's kitty. The government regards the paper industry as one of the 35 high priority industries of the country.

Paper industry is primarily dependent upon forest-based raw materials. The first paper mill in India was set up at Sreerampur, West Bengal, in the year 1812. It was based on grasses and jute as raw material. Large scale mechanized technology of papermaking was introduced in India in early 1905. Since then the raw material for the paper industry underwent a number of changes and over a period of time, besides wood and bamboo, other non-conventional raw materials have been developed for use in the papermaking. The Indian pulp and paper industry at present is very well developed and established. Now, the paper industry is categorized as forest-based, agro-based [70] and others (waste paper, secondary fibre, bast fibre and market pulp) [230].

In 1951, there were 17 paper mills, and today there are more than 525 units engaged in the manufacturing of paper and paperboards and newsprint in India. The pulp & paper industries in India have been categorized into large-scale and small-scale. Those paper industries, which have capacity above 24,000 tonnes per annum, are designated as large-scale paper industries. India is self-sufficient in manufacture of most varieties of paper and paperboards. Import is confined only to certain specialty papers. To meet part of its raw material needs the industry has to rely on imported wood pulp and waste paper.

Indian paper industry has been de-licensed under the Industries (Development & Regulation) Act, 1951 with effect from 17th July, 1997. The interested entrepreneurs are now required to file an Industrial Entrepreneurs' Memorandum (IEM) with the Secretariat for Industrial Assistance (SIA) for setting up a new paper unit or substantial expansion of the existing unit in permissible locations. Foreign Direct Investment (FDI) up to 100% is allowed on automatic route on all activities except those requiring industrial licenses where prior governmental approval is required.

Growth of paper industry in India has been constrained due to high cost of production caused by inadequate availability and high cost of raw materials, power cost and concentration of mills in one particular area. Government has taken several policy measures to remove the bottlenecks of availability of raw materials and infrastructure development. For example, to overcome short supply of raw materials, duty on pulp and waste paper and wood logs/chips has been reduced. Today the paper industry accounts for almost 2.5% of world's production and 2% of world trade.

More than 90% of the total annual world production of pulp is obtained by kraft pulping process and it removes most of the constituent lignin, the residual lignin (4-5%) covalently bonded to carbohydrate moieties. Dark brown color of the kraft pulp is attributed to 3-4% of the residual lignin and its reaction products, degraded polysaccharides and some extractives [167, 226]. These are subsequently bleached in a multistage chlorine based chemical sequence to increase the brightness of the pulp to marketable grades but produce highly colored toxic chlorinated phenols and dioxins in effluent that are resistant to degradation, thus, causing the serious environmental problems [87, 242].

The major bleaching parameters such as, incoming kappa number, chlorine dosage, pH and temperature of chlorination and extraction stages have considerable effect on the effluent BOD, COD and color. The formation of chlorinated materials, produced in the chlorination and extraction stages are a function of the amount of chlorine applied to the pulp, which is determined by kappa number of pulp [11, 229]. An increase in the chlorine dosage results an increase in BOD, COD and chlorolignins in the bleach effluent [229].

In a softwood kraft mill using a conventional bleaching sequence, approximately 5 kg of total organically bound chlorine (TOCl) is discharged per ton of bleached pulp [59] and more than 300 different organic compounds along with a small quantity of highly toxic dioxins have been detected in waste bleach water. Conventionally bleached pulps effluent contains 10-15 μg/g absorbable organic halides (AOX) [69, 87]. The kappa number of Indian wood based mills vary from 14-25 and kappa number of agro-based mills vary from 10-30. The chlorine consumption of agro-based and wood based mills is 130-200 and 60-100 kg/T of pulp respectively. The AOX range in final discharge of agro based mills [71, 72] with and without chemical recovery process is 7-11 and 14.2-21.5 mg/L. Where, AOX in final discharge of wood based mills is 0.60-9 mg/L. It was found out that the chlorination stage was generally the

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first point in which 2,3,7,8-TCDD, 2,3,7,8-TCDF and 1,2,7,8-TCDF congeners were always present [103, 169, 191, 205].

Ministry of environment and forest has a constant watch on Indian paper industry because it is an environment sensitive sector and falls under Red Category of industries [158]. The pulp and paper industry is categorized under 17 highly polluting industries in India [105] and the quality and the quantity of effluents generated depends on the various unit processes, size of unit, waste recycling capability etc. [215].

1.2 BLEACH PLANT EFFLUENT CHARACTERISTICS

The pulp and paper industry are using many technological processes to transform the wood and non wood raw materials into use-able fibers [104]. The fibrous raw materials essentially consist of cellulose, lignin and other extraneous substances. 80% of the lignin and other extraneous substances are dissolved in cooking liquor [as black liquor] during pulping process. The aim of pulping process is to remove the maximum amount of lignin without affecting cellulose. It is not possible to remove 100% lignin by the chemical pulping process and degradation reactions of hemicelluloses and celluloses occur simultaneously along with lignin removal at these stages [67]. A small amount of modified lignin is left after the pulping process. The residual lignin imparts brownish color to the pulp, due to the presence of chromophoric compounds which are the functional groups of degraded and altered lignin bound to the fibers. This must be removed or converted in the bleaching process to be followed to obtain a pulp high brightness which can be further used to make a high grade white paper [52].

The amount of bleach chemical required for the effective bleaching depends on the type of lignocellulosic material used to obtain fiber [pulp] and residual lignin content of the pulp, measured by kappa or K number. All the coloring material cannot be removed or eliminated by any single chemical process, so multi step bleaching is the process which is capable to remove maximum amount of lignin content and give a desired brightness. The two types of bleaching chemicals are used to remove the coloring substances which are as follows-

1. Oxidants are used to degrade and decolorize lignin.
2. Alkali is used to degrade lignin by hydrolysis and to aid in its dissolution.

The bleaching of pulp with chlorine and chlorine based chemicals generates various chloroorganics which include chlorinated resins, fatty acids, chlorophenolic compounds, dioxins & furans, are found to have high toxic effects on the environment [55, 56, 141, 181,

189, 229, 238]. Hence bleach plants are major source of environmental pollution and the effluent released from bleach plant have high BOD, COD, AOX, color loads.

More than last two decades the pulping and bleaching technologies have undergone extensive changes in order to reduce the discharge of chlorinated compounds to the environment [201]. Major changes have been made for the replacement of elemental chlorine to chlorine dioxide in bleaching process to give elemental chlorine free [ECF] bleaching sequence, [35, 73] without elemental chlorine or chlorine containing chemicals, termed as total chlorine free [TCF] bleaching process. Modern bleaching sequence of both ECF and TCF types can meet the tough environmental demands of Best Available Technology [BAT] set up by European commission [159]. In India, the introduction of Charter on Corporate Responsibility for Environmental Protection [CREP] in the year 2003, by Central Pollution Control Board has brought out a time bound implementation schedule to address major environment issue particularly in bleaching [182].

1.3   TOXICITY OF BLEACH PLANT EFFLUENT

The toxicity of bleach plant effluent have been study extensively [12, 18, 91, 92, 101, 109, 113] that whole effluent of a given mill is directly depend on the total organically bound chlorine the extractive content of raw material being used and to what extent these extractives are removed during pulping and other processes [160]. Most of the researchers separate the high molecular weight fraction (HMW>1000 Dalton) from the low molecular weight (LMW < 1000 Dalton) where molecular weight is operationally defined by ultra filtration (UF) process [53]. The compounds from HMW fraction (~ 80% of total) are probably biologically inert, because they cannot penetrate the cell membranes of living organisms, while the compounds from LMW fraction (~ 20% of total) are more problematic [130, 104].

Out of 20%, of LMW AOX, nearly 19% are hydrophilic and can be metabolized while 1% is extractable only by non polar solvents, is referred as Extractable Organic Halide (EOX). Compounds of this fraction are potentially toxic and bio-accumulable [130]. Toxicity is a vague and variable parameter. The toxicity of the whole mill effluent of a given mill directly depends on the total organically bound chlorine, the extractive content of raw material is being used and to what extent these extractives are removing during pulping and other processes [76].

A small increase in the concentration of chlorinated phenolic compounds, change the toxic effect from zero to 100%. The chlorinated phenolic compounds are the most toxic compounds in the bleach plant effluent. This toxicity fully depends on residual lignin content of

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the pulp which invariably increases in the kappa number of unbleached pulp [51]. Quantification of toxicity in effluent is difficult as the results are strongly influenced by the intricacies in the test methods. Lethal concentration (LC) is usually expressed as $^{96}LC_{50}$ which indicate the level of the effluent toxicity [107, 108]. The sub-lethal effects of bleach plant effluent are probably of more importance for the environment than the lethal effect, because they show the long term effects from the accumulation of toxic substances in the organisms [58, 111]. Dioxins are a family of 210 poly-chlorinated dibenzo-p-dioxin and dibenzo-p-furan (PCDD & PCDF). The most toxic compound of this group is 2378 TCDD about 100,000 times more toxic than the least potent in them [106]. Pulp chlorination results in the production of PCDD's and PCDF's [77]. 2378 TCDD has been shown to be highly toxic, bioaccumulation, mutagenic and carcinogenic [148].

Research has indicated that the major part of dioxin and furan formation occurs during the first chlorination stage (C) and then carried through successive bleaching stages. The effluents partially contributing effluent color, acute/chronic toxicity, mutagenicity and carcinogenicity [48, 140].

The E-stage effluent contributes to 90% of acute toxicity, with majir species being 3,4,5-trichlorophenol and tetrachloroguaiacol [140]. They also persist in the environment and pose the threat living organisms [18, 55, 113]. 2,4 di chlorophenol, 2,4,5 trichlorophenol, pentachlorophenol, chlorinated dioxins dibenzofurans and chloroforms are carcinogenic, whereas chlorocatechols are strongly mutagenic [170]. A large number of organic compounds present in pulp and paper mill effluent are acute toxic to aquatic organisms [9, 36, 61, 79, 1].

Recent studies have been shown that reproduction of fish is affected at concentration of the pulp mill effluents. The analysis of samples of fish species showed that they contained small amounts of chlorinated phenols and guaiacols in their extractable fats. Similarly mill effluents were found to have an inhibitory effect on growth of plants and animals. Inhibition of photosynthesis may be caused due to toxic properties of the effluent. It has been found that physical parameters such as pH, temperature etc. in the aquatic environment may strongly influence the toxicity of pollutants [58].

In the present study attempts to minimize the generation of organochlorine compounds during pulp bleaching by substituting chlorine gas by chlorine dioxide or by eliminating the use of chlorine contain compounds [156, 90].

Bleaching without chlorine gas has been termed as 'Elemental Chlorine Free' (ECF) bleaching while that without chlorine containing chemicals has been termed as 'Totally Chlorine Free' (TCF) bleaching process. The demand for ECF and TCF pulps has really forced pulp and paper producers to stretch technology to meet these tough demands.

1.4 ORIGIN AND IDENTIFICATION OF CHLOROORGANICS

Chlorinated phenolic compounds such as chlorophenols, guaiacols, and catechols are produced as degradation products of lignin during pulp bleaching using chlorination procedures [114, 239]. The lignin molecule in softwood and hardwood is composed of p-coumaryl, coniferyl and sinapyl alcohols. Softwood lignin is guaiacyl lignin derived lignin derived mainly from coniferyl alcohol while hardwood lignin is guaiacyl – syringyl lignin formed by copolymerization of coferyl and sinapyl alcohols. 1% to 5% of p-coumaryl alcohol also participates in the formation of both softwood and hardwood lignin. Grass lignin appears to be like hardwood lignin but some grass lignin is thought to contain p-coumryl units aswell [118].

New development in the field of analytical chemistry, applying computer-assisted GCMS and Capillary column of Gas Chromatography have led to successful estimation of th e presence of chloroorganics in the bleach plant effluent. Lindstorm and Nordin were the first to categorize chloroorganics present in spent bleach liquor [118]. Most of the studies on the identification of chlorophenolic compounds have been performed on softwoods [26, 102].

Nature and quantities of various chlorophenolic compounds present in bleach plant effluent formed [133, 207] from Indian varieties of hardwoods and agro-residues have been reported by Sharma et.al. 1996 [179, 180].

1.5 ABATEMENT OF BLEACH PLANT POLLUTION

Increasing awareness towards pollution abatement with the implementation of strict environmental regulations, the pulp and paper industry is forced to look alternatives [97]. All pollution reduction strategies of the bleach plant effluents are based on controlling, reducing or eliminating the main agents responsible for the formation of chlorinated organic compounds. In connection with innovation in the paper industry, it is at pulp bleaching plants that the greatest changes have taken place, since this is the section of pulp and paper manufacturing process generation the greatest degree of pollution [145]. Some of the major approaches for

detoxification of bleach plant effluent have been in combination of the following one or more approaches:

- Process modifications
- Control of outside contaminations
- External control measures [end of pipe treatment]

1.5.1 Process Modifications: Several process changes have been made or being considered for meeting the environmental restrictions. Pulp and paper industry is committed to implement changes in the bleach plant to reduce the discharge of undesirable byproducts into the receiving water environment [28].

Delignification of pulp should be done as much as possible, to decrease residual lignin in pulp before bleaching. Improve brown stock washing and screening to reduce carryover of dissolved organics and particles to bleach plants. Improvement in bleaching sequence to reduce or eliminate use of elemental chlorine compounds.

1.5.2 Control of Outside Contamination

Prohibiting use of Pentachlorophenol (PCP) as wood preservative [27, 49, 190, 228] improved deknotting and screening [119] result in reduced environmental loads in bleach plant effluents.

1.5.3 Maximum Lignin Removal Before Bleaching

The quantity of chlorine necessary to bleach pulp is a function of kappa number (or K number) of pulp, a lower kappa number before the bleaching stage means lower bleach chemical usage [119].

Extended delignification based on the system design modification in batch digester are – Superbatch, Rapid displacement heating (RDH), Enterbatch [163]. Extended delignification in continuous digester based on system design modification are Extended modified cooking, Isothermal cooking [155]. MCC^{TM}, $EMCC^{TM}$ & ITC^{TM} methods are based on the principle that the alkali profile should be leveled out throughout the cook, so that the hydrogen sulfide concentration should be high in initial phase and at the beginning of bulk phase and temperature should be low as possible, especially at beginning of cook [83]. Compact cooking and kobudo cooking method, improves selectivity by maintaining the concentration of hydroxide and hydrogen sulfide ions, in both initial and bulk phases leading to increased pulp yield [10]. The

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use of pulping additives mainly polysulfide (PS) or anthraquinone (AQ) or both also gives low kappa pulps [120]. Improved pulp washing decreases the black liquor carryover and generates lower pollution loads [77].

1.5.4 Modification of Lignin Before Bleaching

Modification of lignin structure before bleaching results in reduced chemical consumption in the bleach plant consequently generates fewer amounts of chloroganics without effecting pulp properties. Enzyme prebleaching is one such option, which is a specific biocatalytic treatment to hydrolyze the xylosidic bonds in lignin carbohydrate complex (LCC) structures thus increasing the removal of lignin in subsequent bleaching sequences [161, 188, 219].

Enzymatic pretreatment of pulps is no longer a laboratory curiosity, but rather chemical ability. Enzyme suppliers include Genencor International, Iogen, Novo Nordisk, Repligen, Sandoz, Voest-Alpine, Biocon and ICI [22].

1.5.5 Improvement in Bleaching Sequence to Reduce or Eliminate Use of Elemental Chlorine:

1.5.5.1 Modifying Chlorine Stage:

Slow and multipoint addition of chlorine – Adding chlorine water continuously throughout various lengths of time at either 3.5 or 10% pulp consistency, increase pulp viscosity and decreases the formation of AOX compounds in the effluent [119]. In split addition of chlorine, dividing the total required chlorine charge of molecular chlorine in the C-stage into two or three separate applications without any washing in between helps reduce dioxin formation. The reduction in the formation of chloroorganics is apparently caused by the lower rate of chlorination reactions at lower chlorine concentration [78].

1.5.5.2 High or Total Substitution of first Chlorination Stage with Chlorine Dioxide –

Chlorine dioxide reacts exclusively with free phenolic group in lignin and residual lignin could be totally removed by ClO_2 [115]. At 90-100% ClO_2 substitution BOD decreased by 20-30% and color by 50-80% [84]. Pryke and Singh also reported that effluent toxicity decreases with increase ClO_2 substitution [154, 184] ClO_2 substitution in C stage results in optimum pulp quality also [16]. 100% ClO_2 substitution in C stage decreases AOX in the

effluent by 70-80% and chlorinated phenolic compounds by 95% as confirmed in the mill trial [154].

1.5.5.3 Use of oxygen and H_2O_2 in extraction stage:

Another approach for reduction of elemental chlorine application involves distribution of delignification load over both the C and E stages instead of just C stage. This can be achieved by converting E stage into an oxidative stage by addition of hydrogen peroxide (E_p), oxygen (E_o) alone or both (E_{op}) [152]. Oxidative extraction reduces chlorine consumption, enhances delignification and reduces bleach chemical demands in the next bleaching stage and generates wastewater with reduced pollution load particularly AOX and color. A mill of an agro based mill confirms that oxidative extraction reduces BOD, COD and color by 10-20% in bleach plant effluent [183].

1.5.5.4 Chlorine Free Bleaching:

Ozone-bleaching system is considered as one of the popular chlorine free bleaching system. Ozone is a powerful oxidizer, and bleaching conditions must be rigorously specified and adhered to avoid unnecessary pulp degradation. Loss of pulp strength in ozone treatments has been a major demerit to its serious consideration as a commercial bleaching agent [124, 172].

1.5.5.5 End of Pipeline Treatment Methods:

Although toxicity of bleach plant effluent can be reduced appreciably by various methods internal control measures and process modification, however, unless the fully closed mill is achieved, external methods for treatment is always essential in order to meet the regulatory standards for chlorinated organic matter including AOX [121]. The Indian pulp and paper mills essentially treat effluents by primary treatment followed by secondary treatment. Primary treatment comprises of removal of suspended solids (SS). The two principal methods employed in the pulp and paper industry for primary treatments are gravity sedimentation and dissolved air flotation. Secondary or biological treatment is nothing more than a duplication of nature's own purification process, except that it is carried out under controlled conditions, usually at accelerated rates. The most common biological treatments used in the pulp and paper industry include.

- Oxidation lagoon

- Aerated stabilization basin [ASB]
- Activated sludge process [ASP]
- Anaerobic process
- Biological filter processes such as trickling filter, rotatory disc etc.

In most cases, it is necessary to add nutrient chemicals (nitrogen and phosphorous) to stimulate metabolic activity. The secondary treatment reduces the BOD by biological treatment (aerobic or anaerobic or a combination). Any treatment beyond primary and secondary treatment is usually termed as tertiary treatment for removal of effluent color, COD and toxicity. The tertiary treatment techniques (Physico-chemical treatments) have been developed to control the discharge of deleterious substances from chemical pulp bleach plants.

The major tertiary treatment techniques include [231].

- Adsorption and ion exchange methods
- Flocculation and chemical precipitation methods
- Membrane methods

1.6 APPLICATION OF FUNGI AND ENZYMES

Many studies have been carried out on the use of fungi and enzymes derived from them for the treatment of effluents from pulp mills. Some of the most commonly used fungi have been white rot fungi *Trametes versicolor* and *Phanerochaete chrysosporium*. These have been reported to degrade a variety of chlorinated toxics including chlorolignin and pentachlorophenols. It is believed that peroxidase enzyme secreted from this fungus is responsible for this action. Laccase enzyme secreted by *Trametes versicolor* has also been shown to act similarly [202].

The current practice in the paper mills is to treat the combined effluent at the end of pipe i.e. primary and secondary treatment plants which reduce the suspended solids and biochemical oxygen demand (BOD) significantly, with small reduction in chemical oxygen demand (COD) AOX and color loads. The tertiary treatment techniques are quite expensive to be implemented successfully in operating mill conditions.

Therefore, it is apparent that the direction of the pulp and paper industry should be heading towards upgrading and modernizing the process itself rather than trying to perform end of pipeline treatment or in some case attempt at combination of both.

Using enzymes as a pre-bleaching step is an example of process modification and can be combined effectively as a pre-bleaching step with conventional [85, 86, 175, 177] and TCF [232, 240] bleaching options for reduced bleach chemical usage in subsequent bleaching stages.

1.7 THE USE OF HEMICELLULASE ENZYME IN BLEACHING

1.7.1 The Origin of Hemicellulase Enzyme

During the decade of 1970-80 biotechnology grew rapidly worldwide. Much of the attention was focused on the conversion of waste biomass to value added chemicals and fuels. Such conversion requires that the biomass be hydrolyzed into the component sugars prior to fermentation, e.g. to alcohol. To accomplish the hydrolysis, workers applied living cultures of wood inhibiting fungi and bacteria. In nature theses microbes secrete enzymes that decay biomass, thus providing soluble sugars for their growth. Wood degrading microbes produce a variety of enzymes that degrade all components of wood.

In the early 1980's saw attempts to separate and isolate the enzyme produced by these microbes. These enzymes include ligninases, hemicellulases degrade cellulose and hemicellulose respectively [22]. One type of hemicellulase that has been isolated is called xylanase. Xylanases are generally xylan degrading enzymes that are secreted in large quantities by many of these fungi and microbes.

1.8 PRODUCTION AND PROPERTIES OF HEMICELLULASE

Numerous articles on the production and properties of hemicellulases especially xylanases have been published [47, 63, 194, 195]. The productivity has been increased both by developing more efficient production strains and by optimizing the production methods [138, 151, 196].

Fungal xylanases of *Aspergillus* and *Trichoderma* species, as good as bacteria xylanases of *Bacillus, Streptomycin* and *Clostridium* have been intensively studied by many researchers [197, 198, 234].

The optimum pH for most of the fungal xylanases is around five and they are generally stable between two and nine pH values. The optimum pH for bacterial xylanases is generally slightly higher than that of fungal xylanases. Most of the fungi and bacteria produce xylanases which can efficiently work on temperature of 70°C [216]. Endo-xylanases show the highes

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activity against polymeric xylan, and the rate of hydrolysis reaction normally decreases with the decreasing chain length.

Xylan is the second most abundant polysaccharide and a major component in plant cell walls, consists of β-1-4-linked xylopyranosyl residues. The plant cell wall is a composite material in which lignin, cellulose and hemicellulose are closely associated. Xylan is a hemicellulose and has binding properties mediated by covalent and non-covalent interactions with lignin, cellulose and other polymers. Lignin is bound to 4-O-methyl-glucoronic-residue of xylan by an ester linkage [147].

Xylanase enzymes partially hydrolyze the hemicelluloses portion of pulp. Unlike the conventional bleaching chemicals, these enzymes do not brighten or delignify the pulp. Rather, these enzymes act on the pulp to make the subsequent bleaching by the oxidative chemicals more efficient. The precise mechanism of xylanase action on the pulp is not known. There is evidence that xylanase allows larger molecular weight lignin to be removed from the pulp in the alkali extraction stage, so it is postulated that removal of a portion of the xylan either releases xylan-bound lignin or increases the ability of higher molecular weight lignin to diffuse through the surface of the fiber. In either case, removing a portion of the xylan from the pulp increases the efficiency of the oxidative chemicals.

Xylanses are fast becoming a major group of industrial enzymes, finding significant application in pulp and paper industry because the hydrolysis of xylan facilitates the release of lignin from the pulp and reduces the level of bleaching chemicals in subsequent bleaching.

1.9 APPLICATION OF XYLANASE IN BLEACHING

The pretreatment of pulp with crude hemicellulase, prepared from a fungus and an actinomycete, was found to improve peroxide delignification of birch and pine kraft pulps. The amounts of lignin remaining in the pulp as measured by (DC) E kappa number reduced, permitting a reduction in the amount of chlorine used in the prebleaching stages [99]. The basic idea of prebleaching with hemicellulase enzyme was published in 1986 and originally developed at the VTT biotechnical laboratory in cooperation with the Finnish pulp and paper research institute [224].

Viikari et al. (1986) [224] first reported that endoxylanases decrease chemicals needed for bleaching kraft pulp. Many researchers have confirmed and extended this observation, and the technology is now being commercialized [45, 62, 162, 192]. Enzymatic biobleaching of

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kraft pulp with xylanases enhances lignin extraction and is an effective means of attaining the target brightness with lower chlorine dose or increasing the final brightness of pulp produced by totally chlorine-free blenching procedures [21, 164, 192].

The action of xylanase on pulp and its bleachability have been studied by different researchers by different methods [20]. The enzyme is usually characterized by using xylan substrate, which is used to determine its activity. Combination of the enzymatic treatments with different bleaching sequences gives the economic and practically acceptable results. The improvement in bleachability was evaluated by using by different ECF bleaching sequences. It was clear that the brightness was substantially increased by the enzymatic treatment. This improved brightness could be exploited to reduce use of chlorine compounds in subsequent bleaching. The great concern on environmental protection, explore the possibility to minimize the consumption of chlorine in bleaching of pulp. When these results were introduced, it was generally considered that the reduction of chlorine consumption was not cost-effective. However, since the end of 1980s, the pulp and paper industry has been forced to explore any new technique available for reducing chlorine consumption or increasing the brightness of pulp with decrease in environmental load. The enzymatic treatment was combined with more efficient pulping and non-chlorine bleaching methods [34].

Many other enzyme applications are possible based on properties confirmed in the laboratory. These include eliminating caustic chemicals for cleaning paper machines, enhancing kraft pulping, reducing refining time, decreasing vessels picking, facilitating retting, selectively removing fiber components, modifying fiber properties, increasing fiber flexibility, and covalently linking side chains or functional groups [89]. Commercial development of these applications need enhanced knowledge of enzyme mechanisms and actions on fibers, development of improved processes for their use, and changing enzymes to function better under present working conditions.

The present investigation aims at use of crude enzyme preparation and its effect on brightness, viscosity and mechanical strength properties and pollution load was studied during biobleaching of mixed hardwood and bagasse pulp.

It is revealed that effectiveness of enzyme pretreatment could be achieved using low amount of enzymes and a relatively short incubation time in preliminary optimization of the amount of enzyme and the hydrolysis time. In order to maintain a high pulp yield and the advantageous properties of hemicelluloses in the pulp it is necessary to minimization overall

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hydrolysis of hemicelluloses [34, 224]. Only small differences in the bleaching efficiency have been observed between xylanase from different organisms, although the degree of hydrolysis has varied [212].

Numerous research papers and reports have been published on the application of xylanases in the pre-bleaching of pulps. Xylanase aided bleaching appears to be most effective on the pulp produced by conventional batch process [95].

Laboratory results show about 10-15% reduction in active chlorine at the chlorination stage for softwoods and 35-41% for hardwoods, whereas the savings in the total active chlorine is found to be 10-15% for softwoods and 25-25% for hardwoods, after xylanase pretreatment. The AOX load of the xylanase pre-bleaching effluent has been reduced by 20-30%. The xylanase pre-bleaching enhance the capacity of the bleaching plant in elemental chlorine free (ECF) bleaching sequences, when the capacity of chlorine dioxide (ClO_2) is a limiting factor. Enzymes increases the final brightness value, in totally chlorine free (TCF) bleaching sequences, which is a key parameter in marketing of chlorine free pulps. The strength properties of enzymatically treated pulps have been improved to the reference pulps, both in laboratory and mill trials [22, 85].

Numerous patent applications have filed, concerning the hemicellulases facilitated bleaching of kraft pulps. Several patents specify compatibility of xylanase in combination with conventional chlorine bleaching, and some claim the use of xylanase pre-treatments with oxygen bleaching. One of the patents specifies xylanase from *S. livindans* while another specifies the use of thermostable alkaline xylanase from *T.fusca*. Some other patent indicates that xylanase from *Aureobasidium pulluloans* may be used to bleaching of different kinds of pulps. Several other patents claim that low molecular weight xylanases from various *Chaini* species that facilitate bleaching [22].

1.10 XYLANASE AIDED BLEACHING: A MECHANISM

In softwoods, the main hemicellulose is, arabino-4-O-methylglucoronoxylan comprises about one third of the total hemicelluloses whereas, in hardwoods, the main hemicellulose is O-acetyl-O-methylglucoronoxylan. The softwood xylan contains 4-O-methylgluronic acid and L-arabinofuranoside groups linked to the backbone by α (1-2) and α (1-3)-glycosidic bonds, respectively. Hardwood xylan contains 4-O-methylgluronic acid and acetyl side-groups. Methylgrlucoronic acid is linked to the backbone by α(1-2) glycosidic bonds. Acetic acid is

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esterified at the hydroxyl group of carbon 2 and/or 3. The average molar ratio of xylose: 4-O-methyl-glucoronic acid: arabinose sugar units in softwood xylans is 8:1:6:1 and that of xylose: 4-O-methylgluronic acid: acetic acid in hardwood xylan is 10:1:7 [34]. A number of changes occurred in hemicelluloses during the processes of pulp production. On account of higher alkali concentration a part of the xylan is dissolved in the pulping liquor, during the heating period of the conventional kraft cooking. As the cooking proceeds the alkali concentration decreases, and degraded short chain xylan is reprecipitated in a more or less crystalline from on the surface of cellulose microfibrils.

It has been a decade since the first report that xylanase enhances the chemical bleaching of kraft pulp [223]. This hydrolytic enzyme depolymerizes xylan, a polysaccharide found in plant cell walls and wood pulps [39, 40]. Xylanase is not expected to directly modify residual lignin, the polyphenolic component of wood pulp commonly thought to be responsible for its color [74, 173]. Therefore, it is not clear how xylanase can reduce the amounts of chemicals required to bleach kraft pulp to desired brightness levels. A number of hypotheses have been forwarded concerning the nature of the target substrates for xylanase [233] and they include xylan-derived chromophores, xylan in lignin–carbohydrate complexes, xylan that physically entraps lignin, and xylan that influence fiber swelling. An apparent consensus has been that xylanase makes kraft pulp more amenable to chemical bleaching, without brightening or delignifying the pulp during the xylanase pre-bleaching stage [223]. This phenomenon, where the brightness gain achieved after subsequent chemical bleaching is substantial while that before chemical bleaching is negligible, has been coined "bleach boosting." It differs from direct brightening in which an increase in pulp brightness occurs immediately following the enzyme stage. Bleach boosting has been advocated as the main mechanism of xylanase action on kraft pulp, primarily because any report of direct brightening [146, 157, 175, 243] or direct decrease in the kappa number of the pulp [81, 139] has been considered insignificant. However, past work in our laboratories has consistently shown that direct brightening occurs in oxygen delignified kraft pulps (henceforth referred to as kraft-oxygen pulps) derived from softwoods, and peroxide bleached kraft pulps [135, 232].

Furthermore, very recent work in another laboratory has led to the hypothesis that xylan contributes to the kappa number of pulp because its methylglucuronic acid substituents are converted to hexenuronic acid substituents under alkaline pulping and bleaching conditions [33]. It is therefore important to compare systematically the contribution that bleach boosting

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and direct brightening have on pulp brightness because one process might be more dependent on subsequent lignin removal and the other on direct xylan removal. Not only would this evaluation provide new insights on the mechanisms by which xylanase enhances pulp bleaching, it may also have important implications for the application of xylanase in kraft mills. Three basic factors are involved in the enzyme pre-treatment of pulps-

i.   Role of re-precipitated xylan,

ii. Role of lignin carbohydrate bond,

iii. Physical loosening of cell wall.

It has been reported that the removal of reprecipitated xylan enhance the bleaching by several means one involves solubilization of chromophores formed in xylan by kraft cooking, other talks swelling of fiber matrix by disrupting adsorptive interactions between xylan and cellulose, solubilization of xylan deposits which entrap residual lignin and cleavage of the carbohydrate portion of the lignin-carbohydrate bond to produce smaller residual lignin molecules, which are easier to remove.

1.11   PERFORMANCE OF XYLANASE IN MILLS

It has been reported that the first mill trial has performed in 1988; thereafter so many trials have been performed in Canada and Europe. Most of the trials were chlorine based, although many TCF trials were also performed [88, 100, 174, 187, 211, 217]. When a mill wants to make its TCF bleaching process economically viable or wants to reduce the cost of conventional bleaching process, then it is necessary either modify its process or introduce newer technology like enzyme pretreatment.

To obtain the better results with enzyme pretreatment, enzyme pretreatment conditions like dose, pH, consistency, retention time and temperature must be optimized. The best enzyme pre-treatment result can be obtained only after one hour of treatment. Usually the reaction time is set to two hours. Long retention times should be avoided if cellulase activity is present.

The aqueous solution of enzyme is in the pulp in the final brown stock washer and best results can be observed with small amount of enzyme.

1.12   BENEFITS OF XYLANASE PRETREATMENT

Depending upon the needs of various mills, xylanase have been used to obtain the following benefits [88, 100, 174, 187, 211, 217]

(i)   Minimum modification in bleaching process.

(ii)   Decrease in pollution load especially AOX.

(iii)   Decrease in bleach chemical consumption.

(iv)   Reduce bleaching costs.

(v)   Increase in final brightness at same chemical dose

1.13   BACKGROUND OF THE PROBLEM

The pulp and paper mill effluent has shown to be one of the highly polluted. It consists of both biodegradable and non-biodegradable compounds like pulp fibers and some chemical species like organic halides etc [134]. The BOD: COD ratio that denotes the biodegradability of pulp mill is also very low so it is very difficult to treat the effluent by end of pipeline treatment.

Most of the large paper industries are based on the forest based raw materials. Some of Indian pulp and paper mills are using varying amounts of agro based non woody pulps like bamboos, wheat straw, bagasse, sarkanda etc. Most of the Indian pulp and paper mills are based on conventional pulping and bleaching technologies because of low operations cost.

The bleaching process is essentially being chlorination followed by alkali extraction and calcium hypochlorite. Most common bleaching sequences are CEH, CEHH or CE_pHH. In the past very few mills were attempting the oxygen as pre-bleaching but now a days most of the paper industries are following oxygen pre-treatment. In spite of so many limitations, Indian pulp and paper mills are managing paper brightness above 80% ISO which is at the cost of increased chlorine consumption resulting in high level of AOX generation.

In small scale mills the scenario of AOX generation is more alarming while in large scale integrated mills it varies in the range of 2.0-4.5 kg/t paper. On account of economic reasons paper mills are using very high dose of chlorine to bleach the pulp. The AOX generation in these paper mills varies between 6-10 kg/t of paper in 2002 [143]. The limit of AOX is fixed to 1 kg/t in India [65].

The analysis of the present situations in pulping and bleaching in Indian mills indicate that

(i)   More and more mills either switch to non-wood raw materials or utilize available forest based raw materials sustainably.

(ii)   As the first bleaching step, chlorination may continue for next few years for better bleaching ability and to reduce the cost.

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(iii)   Now, most of the mills attempt for partial substitution of chlorine by chlorine dioxide.

(iv)   Alkali extraction and oxidative extraction can be continued.

In view of the above, following conditions there is a need to develop some new eco-friendly technologies for Indian mills that could be incorporated in the existing mill conditions easily.

Enzyme pretreatment is one of the technologies that is supposed to reduce the bleach chemical demand in order to achieve the same target brightness and also help to reduce the chloroorganic release. The enzymatic pre-bleaching step is successfully used with existing conventional, elemental chlorine free (ECF) and total chlorine free (TCF) bleaching sequences.

However, before enzyme pretreatment, mills have to plan the application of enzymes under optimized conditions like dose, pH, consistency, retention time and temperature and assess the benefits in terms of environmental improvement and economic advantage. It is not clear how xylanase improve the brightness of pulp because this xylan degrading enzyme is not expected to act directly on residual lignin. It is not apparent how the enzyme works due to variable response of different pulping and bleaching parameters. It may be possible that xylanase hydrolyzes xylan, present on the pulp in different forms. The mechanical strength properties and optical properties also give contradicting results for different enzymes on enzyme pretreatment of hardwood and softwood pulps.

Sufficient data are not available on agro-residue raw materials, which can be important for the whole world due to decreasing wood resources. A qualitative study and analysis for the above parameters is necessary and the results have to be carefully analyzed and quantified to give more flexibility in xylanase pretreatment technology of the pulp.

1.14   STATEMENT OF THE PROBLEM

In view of the above, it is proposed to conduct laboratory studies on enzymatic pre-bleaching with commercially available enzyme on mixed hardwood and bagasse pulps to establish pulp bleaching conditions, bleach chemicals demand, bleach pulp characteristics and pollution loads using conventional and ECF sequences.

The main objective is to aim for environmental friendly bleaching to reduce generation of chlorooganics from bleach plants. For this various objectives formulated for the project are:

(i)   To optimize conditions of enzymatic pre-bleaching for mixed hardwood and oxygen delignified mixed hardwood pulps and bagasse.

(ii)   To study the impact of enzymatic pre-bleaching on different bleaching sequences with regard to

   •   Bleach chemical consumption

   •   Pulp characteristics

   •   Pollution load generation.

(iii) Analysis of the enzyme pre-bleaching data to understand the manner in which the enzyme works on the substrate.

(iv)   To study economic viability of enzyme pre-bleaching.

(v)   To study the impact of enzyme pretreatment on the strength properties of the pulp.

The experimental results of this project are expected to provide a basis to establish suitable process sequences, optimized operational parameters that can help to understand the proper enzyme pre-bleaching process and integrating them with existing mill configurations. This project also helps to establish a new eco-friendly approach, saving of energy, bleach chemicals and improve bleaching in real mill situations.

All these are expected to provide significant benefits in terms of improved produc quality, improved environmental management associated with improved economics.

2.1 MATERIALS AND REAGENTS

2.1.1 MATERIALS

2.1.1.1 Pulps

Unbleached mixed hardwood (Eucalyptus:Poplar, 70:30) and bagasse pulps were procured from a paper mill in India. The pulps were washed with plenty of water to remove chemicals, screened & dried in air, finally stored in polythene bags for studies.

2.1.2 Reagents

• Chlorine water, chlorine dioxide, sodium hydroxide.

• Potassium permanganate, sodium thio sulphate, sodium oxalate, potassium dichromate, potassium iodide, starch, and sulphuric acid, etc.

• Various standards of chlorophenols (Aldrich, USA) were used as reference compounds.

• Solvent n-hexane and acetone (HPLC grade) and diethyl ether (LR grade).

• Analytical grade acetic anhydride.

• Oat splet xylan (Aldrich), DNS (Thomas Baker), DMSO (Qualigens).

• Other reagents were of Laboratory grade.

2.1.3 Enzyme

Commercial xylanase enzyme has been used for all enzyme pretreatment experiments. Details of enzyme are as follows:

Name of Supplier	Star enterprises
Name of product	Aquabrite
Manufacturer	S.M.B. Biotech Industries, Amritsar.
State of enzyme formulation	Liquid
Source of enzyme	Fungi
Activity level	500 IU/ml
Any other activity	Nil

2.2 TEST METHODS

The xylanase activity was measured by estimating reducing sugars formed from oat splet xylan (20). Enzyme units (International units) are defined as the amount of enzyme necessary for the production of 1 micro mole product/min. TAPPI and SCAN standard test methods were used to determine the kappa number, viscosity (CED), tensile index, tear index, burst index.

Pentosan content was estimated using tappi test method no. T-223 om-01 [208]. The details and different standard procedures used for the analysis of pulp and hand-sheet are given in Table 2.1.

- TECHNIBRITE ERIC 950 from Technibrite Corporation, USA was used for brightness measurement [209].

- BOD and COD were estimated by standard test method [193].

2.3 TEST PROCEDURES

2.3.1 Analysis of Bleach Liquor

2.3.1.1 Sodium Chlorite and Chlorine Water

Solution of sodium chlorite 40 g/l was prepared in distilled water. To 2-5 ml of sodium chlorite solution added 10 ml of 10% acetic acid, 10 ml of 10% potassium iodide and this solution was titrated with 0.1N of sodium thio sulphate ($Na_2S_2O_3$) solution with 5% starch solution as an indicator. The end point was noted when blue color changed to colorless.

The active chlorine was calculated in gram per liter = Normality of bleach liquor x 35.5

2.3.1.2 Analysis of Residual Chlorine

Same procedure as described above was used except volume of effluent (bleach liquor) was increased to 100 ml and titrated with 0.1N sodium thiosulphate solution [110].

2.3.1.3 Analysis of Hydrogen Peroxide Solution (H_2O_2)

10 ml of concentrated H_2O_2 solution was diluted and was made upto 250 ml in a volumetric flask. To 5 ml of this diluted solution, 10 ml of 10% KI, 10 ml of 4N H_2SO_4 and 1 ml of ammonium molybdate was added. This solution was titrated with 0.1N $Na_2S_2O_3$ solution with 0.5% starch as indicator [227]. The end point was blue to colorless.

2.3.2 Disintegration of Pulps

The air dried pulp was disintegrated to remove lumps of fibers. For this, the pulp was soaked in water for few hours and then disintegrated in disintegrator (20-25 gm of pulp can be disintegrated in a single run). The pulp was squeezed and kept in refrigerators for 6-7 days. This pulp was used to perform various bleaching experiments.

2.3.3 Preparation of Sheets for Brightness and Kappa Number Determination

The consistency of the disintegrated pulp was adjusted to 1%. The pulp suspension was then transferred to Buchner funnel. Water was drained through a screen of 22 mesh size, so as to give sheet of about 5 gm OD weight. The pulp sheet was picked with the help of blotting papers and then pressed using hydraulic pressure to remove excess of water. The sheets were air dried and protected from dirt and dust. The brightness was determined after 4-5 hours of drying. For kappa number determination the sheets were stored in polythene bags.

Table 2.1: Standard test methods followed for analysis

TEST NO.	DESCRIPTION
SCAN-C 18:65	Disintigration of chemicals pulp for testing
SCAN-C 24:67	Beating of pulp in PFI mill
SCAN-C 26:76	Forming handmade sheets for physical testing of pulp and paper
SCAN-C 15:62	Viscosity of cellulose in cupra di-eyhylene di-amine
ISO standards 2469	Brightness of pulp
BOD (APHA)	Biochemical oxygen demands (5210 B) 5-day BOD test
T-236	Determination of kappa number of pulp
T-403	Burst strength of paper
T-404	Tensile strength of paper
T-414 om-98	Internal tearing resistance of paper
T-227 om-99	Determination of CSF, ml
T-610 om-87	Preparation of indicators and standard solutions

2.3.4 Oxygen Delignification Procedure

Oxygen delignification was performed in autoclaves (which have the capacity of 100 g of pulp) revolving in a hot glycol bath NaOH (40gpl) at various doses (1-3% on oven dried basis) and $MgSO_4.7H_2O$ (0.2g/100g of OD pulp) were mixed with the disintegrated wet pulp and pulp consistency after mixing was adjusted to 10%. These pulps were then transferred to autoclaves from the top. After removing air from the autoclave oxygen was fed into the autoclave at pressure of $6kg/cm^2$. The autoclaves were checked for leakage and then placed in glycol bath preheated to a temperature of 100^0C for 75 minutes [110].

The autoclaves were taken out and cooled to room temperature. Pulps were then washed properly and squeezed and pulps were stored in poly bags for further bleaching experiments.

The oxygen delignification optimization was targeted to a kappa number drop of 40-50% without compromising much on pulp viscosity. The oxygen and non-oxygen pulps were characterized for kappa number, viscosity and brightness. The range of variables studied, given in **Table-2.2.**

Table 2.2: Variables studied for oxygen delignification and xylanase optimization studies

Experiment	Range of variables studied	Pulp type			
		Mixed hardwood	Oxygen delignified mixed hardwood	Bagasse	Oxygen delignified Bagasse
Oxygen delignification Optimization	Alkali charge (kg/t)	10-20	-	15-20	-
	Temperature (^0C)	100	-	100	-
	Retention time (min.)	75	-	75	-
Xylanase Optimization	Enzyme dose (IU/g)	10-20	6-14	16-26	10-20
	Temperature (^0C)	40-70	40-70	40-70	40-70
	pH	4.5-9.5	4.5-9.5	4.5-9.5	4.5-9.5

2.3.5 Enzyme Pretreatment Optimization Method

The enzyme activity was measured at different operating conditions like pH, temperature, retention time and dose. The impact of one parameter was studied by maintaining other parameters constant. The reducing sugar level gave better results on enzyme pretreatment optimization. Enzyme pretreatment studies were carried out at optimized conditions discussed in chapter - 4.

2.3.6 Bleaching of Pulps

All the bleaching experiments were performed in plastic bags, in a temperature controlled water bath. The chlorine dioxide substitution stage was performed in plastic bottles at ambient temperature. The chemicals were mixed properly by kneading the pulp with hands at a desired pulp consistency or by shaking the bottle time to time during bleaching experiments.

The non oxygen and oxygen mixed hardwood and bagasse pulps were bleached to 85% ISO brightness using different bleaching sequences like DED, $D_{50}/C_{50}ED$, ODED. The oxygen delignified mixed hardwood and bagasse pulps were also bleached to 90% ISO brightness using $OD_{50}/C_{50}E_pD$ bleaching sequence.

All the bleaching experiments were performed in triplicate and brightness measured as % ISO. Different doses of bleach chemicals were applied to oxygen and non oxygen mixed hardwood and bagasse pulps to achieve the desired target brightness.

2.3.6.1 Substituted (D_{50}/C_{50}) Stage Bleaching

The disintegrated desired quantity of pulp at 3% consistency was taken in a plastic bottle and pH was adjusted to below or equal to 2 by adding sulfuric acid (H_2SO_4) and sodium hydroxide (NaOH) drop by drop. Calculated amount of chlorine dioxide ($NaClO_2$) was added to pulp, shake well to mix the chlorine dioxide with the pulp and after 15 – 30 seconds the chlorine water was added to the suspension (50:50 as active chlorine) if necessary adjusted to pH 2 and shaken well to mix the chlorine water with the pulp and checked the pH again (it should be < 2). The suspension was then left for 45 minutes at an ambient temperature with intermittent shaking (5 – 6 times within the duration of 45

minutes). After 45 minutes the effluent was collected and analyzed for final pH, residual chlorine and environmental parameters.

2.3.6.2 Chlorine Dioxide (D) Stage

Calculated amount of chlorine dioxide liquor and water were mixed to the disintegrated pulp in a plastic bag by maintaining its consistency (10%) and pH (3-4). Sealed the bag and kept in hot water bath at 70^0C temperature for 180 minutes with intermittent kneading. The bag was removed, the effluent was collected for further analysis and pulp was washed.

2.3.6.3 Alkali Extraction (E) Stage

After first stage of bleaching, the washed pulp was taken into a plastic bag and calculated amount of sodium hydroxide (NaOH) solution and water were mixed to the pulp to make a pulp desired consistency of 10% with distilled water and pH of 11-12. The plastic bag was tightly bound with rubber bands and placed in a water bath at 70^0C temperature for 90 minutes with intermittent mixing. After completion of extraction stage, the effluent was collected for further analysis and pulp was washed.

2.3.6.4 Peroxide Reinforced Extraction (Ep) Stage

For H_2O_2 reinforced alkaline extraction stage 0.4% on OD pulp basis was added to pulp suspension along with alkali, all other parameters were same as E stage.

2.4 CHARACTERIZATION OF BLEACHING EFFLUENTS

The target brightness was achieved with the chemical doses for both the pulps **(Table 2.3)** and the effluents after each stage were mixed and analyzed for BOD, COD, AOX, color and chlorophenolic compounds in laboratory 100 ml of combined effluent was taken and adjusted to pH below 2 by HNO_3 and then stored in bottles in refrigerator for AOX analysis.

Table 2.3: Bleach chemical charge (as active chlorine) to achieve target brightness level (kg/t)

Pulp type	Bleaching sequence	Total bleach chemical charge (kg/t)	
		Mixed hardwood	Bagasse
Non oxygen untreated	DED	45	31
	$D_{50}/C_{50}ED$	43.5	30
Non oxygen xylanase Pretreated	DED	40.5	28
	$D_{50}/C_{50}ED$	39	27
Oxygen delignified Untreated	ODED	23.2	17.7
	$OD_{50}/C_{50}E_pD$	24.8	18.3
Oxygen delignified xylanase pretreated	ODED	21.6	16.1
	$OD_{50}/C_{50}E_pD$	22.4	17.7

2.4.1 Color Measurement

2.4.1.1 Preparation of Standard Color Solution of 2500 Pt-Co Units

250 mg of platinum (purity 99.99%) was dissolved in hot aqua-regia. This solution was heated to dryness to remove nitric acid (as oxides of nitrogen) by repeated evaporation and addition of fresh quantity of concentrated HCl. The residual was dissolved in distilled water and thereafter, 500 mg of $CoCl_2.6H_2O$ (equivalent to 125 mg Co) was added along with 5 ml of HCl. The total volume of the solution was made upto 100 ml with distilled water to yield a color standard solution of 2500 Pt-Co units [46].

2.4.1.2 Determination of Color

Standard solutions of 50, 250, 350, 500, 1000, 1500, 2000, Pt-Co units were prepared by diluting 0.5, 2.5, 3.5, 5, 10, 15 and 20 ml of standard stock solution to 25 ml in a volumetric flask. Absorbance of different standard solutions (Table 2.4) was determined at 465 nm on a spectrophotometer [206] and calibration curve was plotted Figure 2.1. This curve was used to determine the color of the effluent. The pH of the effluent was adjusted to 7.6 and the suspended particles were removed by centrifuging the effluent for 5 minutes at 1500 rpm. The absorbance of the solution was determined at 465 nm and color was calculated from calibration curve.

Table 2.4: Absorbance of standard color solutions

Pt-Co units	Absorbance
50	0.012
100	0.027
250	0.066
350	0.09
450	0.122
500	0.125
750	0.198
1000	0.264
1200	0.319
1500	0.382
2000	0.51
2500	0.66
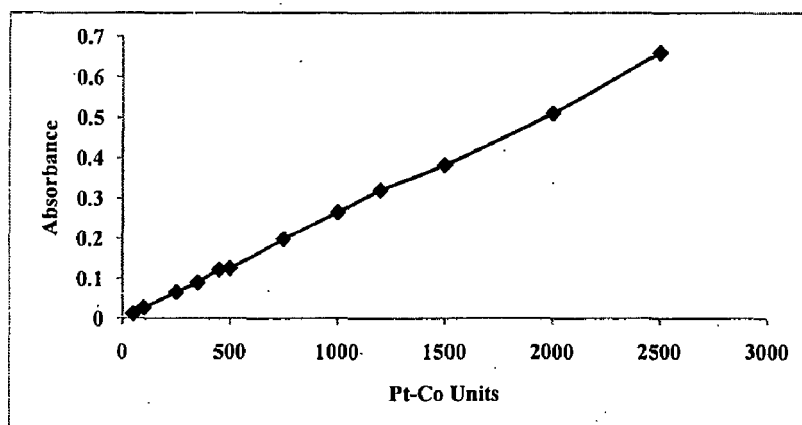
Figure 2.1: Calibration curve used for color determination

2.4.2 Analysis of Various Chorophenolics

Modern analytical methods such as, High Performance Liquid Chromatography (HPLC) and Gas chromatography (GC) alone and in combination with Mass Spectrometry have been used for determining chlorophenolic compounds in the pulp and paper effluent. Among all these methods, GC or GC-MS are the most commonly used techniques.

In principle, solvent extraction should provide a simpler and faster method for isolation of chlorophenols from waste water sample. Mainly, two extraction procedures are used today for chlorophenols analysis in pulp and paper mill effluent. One proposed by BCRC (British Columbia Research Canada) and another by Lindstrom et al. [118]. For present study,

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extraction of various chlorophenolic compounds has been done, as per procedure using diethyl ether [118].

The chlorophenols are converted to readily volatilized acetyl derivatives prior to GC analysis. Different procedures for derivatising phenols directly in the water sample have been suggested, including extractive alkylation with acetic anhydride. For the present study, acetylation was done with acetic anhydride based on procedure [1, 179].

2.4.2.1 GCMS Conditions

Trace GC Ultra (Thermo Electron Corporation, Italy) was used to analyze various chlorophenolics. The separation of the acetyl derivative was achieved on DB-5 fused silica capillary column (30m X 0.25 mm Id, 0.25 µm). The injection was splitless for two minutes. The GCMS conditions used are given in the **Table 2.5.**

2.4.2.2 Derivatization Procedure

4.5 ml of the sample of standard chlorophenolics was taken in a PTFE lined screw capped glass tube and 0.5 ml of buffer solution (0.5M Na2HPO4) was added, and then 0.5 ml of acetic anhydride added to the sample tube, followed by extraction with 1ml of n-hexane. The mixture was shaken for 3 minutes. Hexane layer was taken in sample vials and 1µl of hexane layer (acetyl derivative) was injected into the GC column by auto sampler [179].

2.4.2.3 Retention Time Determination

Standard solutions of various chlorophenolic compounds (20—30 mg/l) were prepared in 10% acetone water. 1ml of the sample was derivatized and 1µl of the hexane extract was injected into the column. The retention time was recorded.

2.4.2.4 Separation of Chlorophenols from the Effluent

The procedure as given in figure 2.2 was followed to achieve the separation of chlorophenolics from the effluents (118). For each bleaching sequence, two liters of each stage effluents were mixed and then 1.8 liters of combined effluent was taken. The pH of the effluent was adjusted to 2 with dilute H_2SO_4. The effluent was transferred to separating funnel and extracted for 48 h with 400ml diethyl ether and acetone solution (90% diethyl ether and 10% acetone). The emulsion formed in ether layer was broken by using a heat gun. The whole

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etheral extract of the effluent was transferred into another separating funnel and shaken with 5ml of 0.5M $NaHCO_3$ solution to remove acidic impurities. Thereafter, the ether layer was shaken with 5ml of 0.5M NaOH to extract the chlorophenolics.

Aqueous NaOH layer, which contained chlorophenolics from bleaching effluent, was separated and washed with 10ml of fresh diethyl ether to remove the neutrals. The extracted chlorophenolics were derivatized as under. 4ml of the extracted liquor was diluted to 4.5ml with water and was acetylated using 0.1ml of the acetic anhydride. The remaining process was same as per **2.4.2.2 procedure.** 0.1μl of the derivatized sample was injected into column for identification and quantitative analysis of chlorophenolics.

2.4.2.5 Quantitative Analysis of Various Chlorophenols

The chlorophenols were determined quantitatively by GCMS.

- **Extraction Efficiency**

For the extraction of various standard chlorophenolics, 1ml of standard solution of various chlorophenols was diluted to 1liter with distilled water. The pH of the solution was adjusted to 2. The solution was then extracted with 400ml of 90% ethyl ether and 10% acetone mixture for 48 hours and derivatized as acetyl derivative as described under **2.4.2.2** for chlorophenolics. 1μl of the derivatized sample was injected into the colum and the peak area was recorded. From the area of peak, the quantity of chlorophenolics present in the extracted sample was determined. The percentage extraction efficiency was calculated as under:

$$\text{Extraction efficiency (\%)} = \frac{\text{Quantity in the extracted sample}}{\text{Quantity in the sample before extraction}} \times 100$$

The values of retention time, response factors and extraction efficiency values of various chlorophenolics determined are given in **Table 2.6.** These values are the average of minimum three values.

2.5 MECHANICAL STRENGTH PROPERTIES

Finally bleached pulps were beaten in PFI mill to a fixed CSF of 300±10. Hand sheets of (60±2 gsm) were made in Swedish hand sheet maker. Air dried sheets were used to analyze various strength properties like tear index, tensile index and burst index.

2.6 DETERMINATION OF WATER RETENTION VALUE (WRV)

Untreated and enzyme pretreated bleached pulps were taken for WRV [119]. A thick pad of wet pulp (OD 0.7-1.0 g) was made which was squeezed in a centrifuge tube at 3000 g force for 15 min. The pulp was weighed and then put into an oven and dried for 4 hours and then again weighed. Then WRV was calculated using the formula given below:

WRV (g/100g pulp) = [(wet wt. of pulp-Dry wt. of pulp)/ Dry wt. of the pulp] x 100

2.7 DI METHYL SULPHOXIDE (DMSO) EXTRACTION PROCEDURE

After enzyme pretreatment, re-precipitated xylan is redeposited on the outer cell wall of the pulp fiber so that DMSO extraction is required to remove re-precipitated xylan from the pulp fiber in order to enhance the bleachability of pulp. Air dried pulp was soaked in water over night and disintegrated. The disintegrated pulp samples were suspended in DMSO at 2.5% consistency for 3 hours with an intermittent shaking. The pulp was squeezed and washed to remove DMSO. All enzyme pretreatment and bleaching studies were performed on DMSO extracted pulps [3].

2.8 INFRARED SPECTROSCOPY OF XYLANASE PRETREATED PULP AND EFFLUENTS

Thin sheets of 10 gsm were prepared for infrared (IR) studies [127]. 20g OD pulp sample was taken and soaked in water overnight, disintegrated for 5 minutes and dispersed in water and made the final volume of 2000 ml (1% consistency). After thorough mixing, a 20 ml aliquot was diluted to 400 ml in a measuring cylinder and poured in conventional hand-sheet maker so that thin sheet of uniform thickness can be picked on the nylon cloth. The sheet was air dried for 1 hour on the nylon cloth. The dried sheets was removed and used for IR studies. Effluents were directly used for IR studies. PERKLIN-ELMER 1600 series FT-IR spectrophotometer was used for all IR studies (PERKIN-ELMER 1600 series FT-IR spectrophotometer, Instruction manual).

Table 2.5: GCMS conditions for the analysis of chlorophenols

Parameters	Details
Detector	Mass spectrometer
Column	DB-5 fused silica capillary column (30mx0.25 mm Id, 0.25 μm)
Injection port temperature	210^0C
Temperature Programming	45 ^{0}C for 1 min, 45 – 280 ^{0}C at 6 ^{0}C/min, 280 ^{0}C for 25 min.
Mass scan range (m/z)	42 – 336
Scan speed	216.7 amu/sec
Carrier gas	Helium
Carrier gas flow rate	1 ml/min
Mode	Split-less
Amount of sample injected	1μl

Table 2.6: Retention time (RT), base peak (m/z) and extraction efficiency (EE) of various chlorophenolic compounds

S.No.	Chlorophenolics	RT	m/z	EE
1	3-chlorophenol	14.20	127.9	49
2	4-chlorophenol	14.36	127.9	98
3	2,3-dichlorophenol	17.69	161.8	108
4	2,4-dichlorophenol	16.98	161.8	87
5	2,5-dichlorophenol	16.96	161.9	106
6	2,6-dichlorophenol	16.52	161.9	64
7	3,4-dichlorophenol	18.27	161.9	81
8	2,3,4-trichlorophenol	21.23	195.8	63
9	2,3,5-trichlorophenol	20.17	195.9	88
10	2,3,6-trichlorophenol	20.01	195.8	93
11	2,4,5-trichlorophenol	19.07	195.8	77
12	2,4,6-trichlorophenol	20.31	195.8	83
13	4-chloroguaiacol	18.70	157.9	104
14	4,5-dichloroguaiacol	21.19	191.9	79
15	4,6-dichloroguaiacol	22.27	191.9	102
16	3,4,5-trichloroguaiacol	24.40	225.8	99
17	3,4,6-trichloroguaiacol	23.16	225.9	70
18	4,5,6-trichloroguaiacol	25.07	225.9	100
19	2,3,5,6-tetrachloroguaiacol	26.66	261.8	51
20	3,5-dichlorocatechol	22.77	177.9	5
21	3,6-dichlorocatechol	22.50	177.9	3
22	Tetrachlorocatechol	28.31	247.8	33

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                    ┌─────────────────────────┐
                    │   EFFLUENT (pH - 2)     │
                    └─────────────────────────┘
                              │   Extracted with 90% diethyl ether + 10% acetone
          ┌───────────────────┴──────────────────────┐
┌─────────────────────┐                    ┌─────────────────────┐
│   ETHER LAYER       │ 0.5M NaHCO₃        │  AQUEOUS LAYER      │
└─────────────────────┘                    └─────────────────────┘
          │
┌─────────────────────┐                    ┌─────────────────────┐
│   ETHER LAYER       │                    │  AQUEOUS LAYER      │
└─────────────────────┘                    │     (Acids)         │
          │  Washed with 0.5M NaOH          └─────────────────────┘
          │
┌─────────────────────┐                    ┌─────────────────────┐
│   ETHER LAYER       │                    │  AQUEOUS LAYER      │
└─────────────────────┘                    │   (Chlorophenol)    │
                                            └─────────────────────┘
                                                 │  Acetylation
                                            ┌─────────────────────────┐
                                            │ ACETYLATED DERIVATIVE   │
                                            │  OF CHLOROPHENOLS       │
                                            └─────────────────────────┘
                                                 │
                                            ┌─────────────────────────┐
                                            │  QUALITATIVE AND        │
                                            │ QUANTITATIVE ANALYSIS BY│
                                            │       GC-MS             │
                                            └─────────────────────────┘
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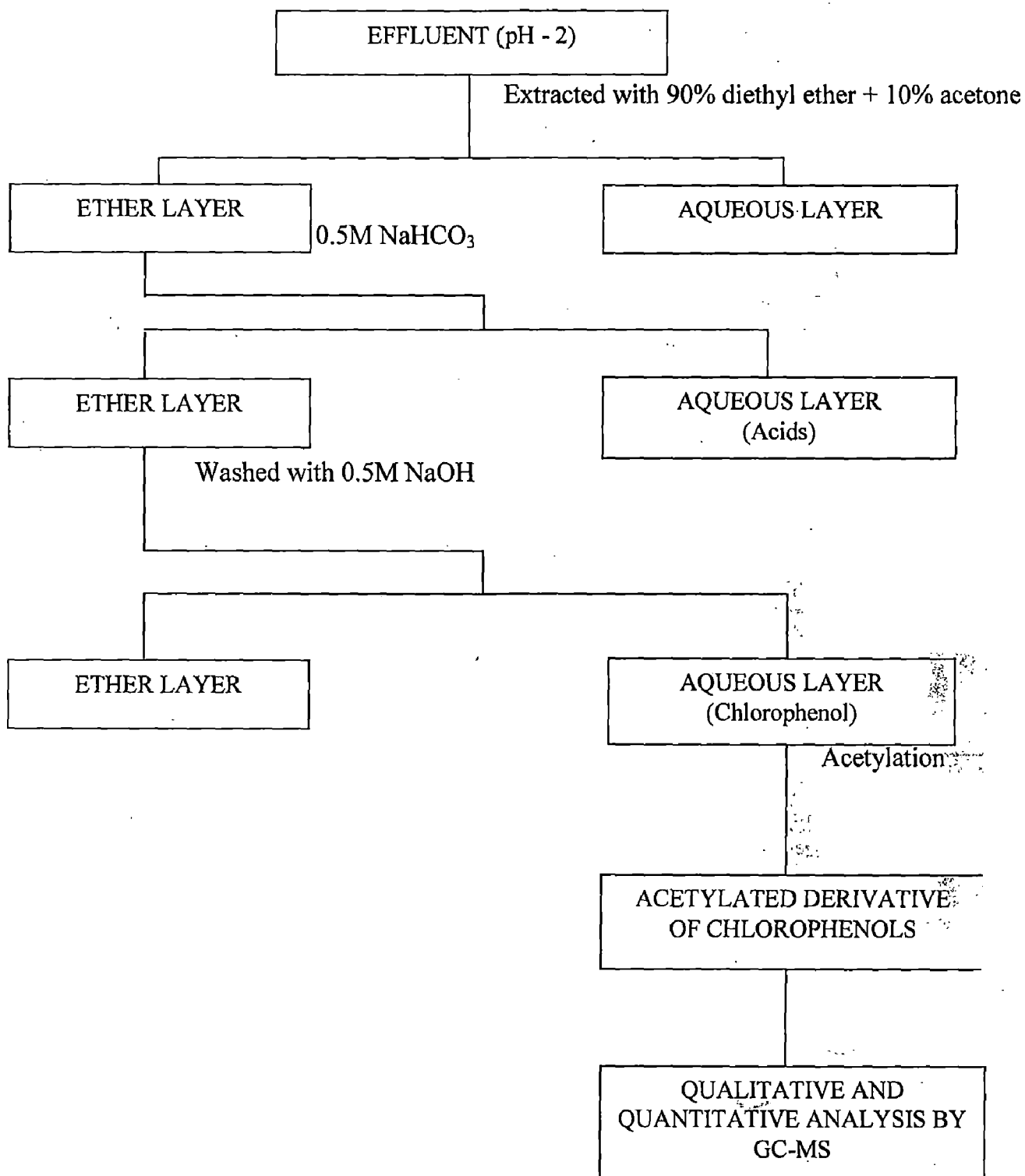
Figure 2.2: Separation of phenolic compounds from effluents

3.1 INTRODUCTION

Bleaching is a chemical process applied to cellulosic material to achieve the desired pulp brightness. Bleaching process increases the pulp brightness either by lignin removal or decolorization. The conventional pulp bleaching process uses chlorine compounds to selectively degrade lignin but causing serious environmental impact. The chlorine in these chemicals binds with residual lignin and generates chloro-lignin compounds i.e. chlorophenolics and dioxins, which are highly toxic even at low concentrations. The use of oxygen and hydrogen peroxide in bleaching, instead of chlorinated chemicals, can reduce toxic contaminants in the effluents. However, due to their poorer selectivity towards lignin, these agents are not as efficient as chlorine derivatives and they produce bleached pulps of lower quality.

Recently, applications of biotechnology in this field have attracted considerable interest. Its first application in bio-bleaching was proposed by Viikari et al. (1986), who used xylanases as bleaching booster. Since then, many researchers have used enzymes, such as xylanases and laccases, to treat pulps before applying the standard bleaching sequences [23, 60, 96, 185, 218]. These experiences showed that the pre-treated pulps required smaller number of chemical agents in further bleaching than the non pre-treated pulps, maintaining the same pulp quality and reducing the pollutant load in the process effluents [224]. Paper industry has undergone marked changes over the past decades, particularly as regards to the pulp bleaching section. Environmental pressure has fostered the reduction or total avoidance of chlorine and chlorine-containing products in bleaching processes.

After recognizing the chemical agents which are responsible for chloroorganic compounds/AOX generation, two main approaches have been considered to control the samples.

1. By changing or modifying the conventional pulping and bleaching process to control the formation of chloro-organics/AOX.

2. By maintaining the regulatory standards of discharge by applying the advanced treatment methods that is not easy.

Approach 1 is easy, better and economically viable to control the generation of chloroorganics.

3.2 PROCESS MODIFICATIONS

3.2.1 Oxygen Delignification

Oxygen delignification performed by using oxygen and alkali to remove a fraction of lignin from the unbleached pulp. This process is usually conducted under pressure and oxygen delignification is normally in the range of 35-50% [52, 68, 128, 149, 150, 178]. It is generally a pre-bleaching stage that can help to reduce the kappa number of the pulp prior to bleaching and also reduce the environmental loads (COD, BOD, AOX, color, chloro-organics and other toxic compounds) produced in the bleach plant [116]. Oxygen delignifications were studied and successfully performed by many researchers in early days and highlighted the advantages and disadvantages of oxygen delignification.

Now a days, oxygen delignification is being done by implementing two stages delignification by optimizing standard conditions for both the stages (first stage and second stage oxygen delignification). By implementing the two stage oxygen delignification it is possible to reduce cellulose degradation and up to 60% delignification seems to represent a practical limit even after two steps [115]. In addition, catalysis and activation also enhance efficiency of oxygen delignification [13].

Oxygen delignification helps to reduce BOD, color and chloro-organics discharge up to 50, 60, 35-50% respectively. Capital cost is the main disadvantage of oxygen delignification [14]. A further drawback is the possibility of overloading the recovery system of the mill [52].

3.2.2 Increased Chlorine Dioxide Substitution

Chlorine dioxide reacts with the free phenolic hydroxyl aromatic compounds which are abundantly found in residual lignin [37]. Chlorine dioxide substitution leads to substantial decrease in the AOX concentration and varies linearly with the elemental chlorine used in first stage of bleaching [19]. Liebergot also found a linear decrease in effluent AOX accompanied by increasing ClO_2 substitution [116, 117]. An increase in percentage of chlorine dioxide substitution up to 75% gives up to 45% of AOX reduction in effluent [66]. At very high level of chlorine dioxide substitution, AOX was lower than the anticipated based on linear relationship observed at lower substitution levels [16, 17].

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At a very high level of chlorine dioxide substitution i.e. 90-100%, BOD and color decreased by 20-30% and 50-80% respectively [84]. There are many other studies which highlight the impact of chlorine dioxide substitution on COD and color. These studies have shown that COD and color can be reduced by increasing chlorine dioxide substitution [153, 184, 214]. Other studies have also shown the impact of chlorine dioxide substitution on AOX formation [17, 52].

The greatest consideration in the use of chlorine dioxide in delignification is the chemical cost. In many studies lowest cost is obtained in the range of 30-50% substitution with the cost being relatively insensitive over this range [16, 241]. As 100% substitution is approached, chemical cost increase sharply [52]. The change of 100% substitution of chlorine dioxide is a response to environment and market pressure [125].

3.2.3 Peroxide Reinforced Oxidative Extraction

Peroxide has been recognized as an environmentally friendly and strong oxidizing agent for bleaching. The loss of delignifying power in the C-stage due to decrease in chlorine charge can be made up by decreasing the chemical charge in the first stage of bleaching and using oxygen as pre-bleaching and hydrogen peroxide in the subsequent alkaline extraction. This process can help to decrease the generation of AOX in the bleach plant effluent.

In view of the above, following experiments were formulated:

i. To study the partial and 100% substitution of chlorine dioxide on mixed hardwood and bagasse pulps.

ii. To reduce the incoming kappa number of mixed hardwood and bagasse pulps using optimized oxygen delignification.

iii. To campare the brightness of untreated and oxygen pretreated mixed hardwood pulp and bagasse pulp by different bleaching sequences.

iv. To compare the environmental loads of enzyme and non enzyme treated bleached pulp effluents.

3.3   RESULT AND DISCUSSION

The results given in this chapter will be used for comparison with the results on enzyme pretreatment given in chapter – 4.

3.3.1 Effect of Bleaching on Mixed Hardwood and Bagasse Pulps

Both the mixed hardwood and bagasse pulps were bleached to a target brightness of 85% ISO using different bleaching sequences i.e. DED, $D_{50}/C_{50}ED$. The bleaching conditions, pulp characteristics and effluent load generation are given in the **Tables 3.1 – 3.6**. Results indicate that for mixed hardwood pulp, total active chlorine (aCl) consumption for different sequences followed the order DED > $D_{50}/C_{50}ED$ i.e. 45 kg/t and 43.5 kg/t (**Table 3.1**).

Table 3.1: Bleaching conditions for mixed hardwood pulp (kappa number 15)

Parameter	DED			$D_{50}/C_{50}ED$		
	D	**E**	**D**	**D_{50}/C_{50}**	**E**	**D**
Temperature (^{0}C)	70	70	70	Ambient	70	70
Consistency (%)	10	10	10	3	10	10
Retention time (min)	180	90	180	45	90	180
End pH	3.2	11.1	3.22	2.3	10.9	3.27
Charge as aCl (kg/t)	31.5	-	13.5	30.45	-	13.05
Alkali charge as NaOH (kg/t)	-	10	-	-	10	-
Brightness (% ISO)	84.9			85.1		
Total active Cl (kg/t)	45			43.5		

BOD follows the same order as above but COD value of DED is higher than $D_{50}/C_{50}ED$ while AOX value in $D_{50}/C_{50}ED$ is higher than DED sequence i.e. 1.4 kg/t and 0.95 kg/t. BOD/COD (Biodegradability of effluents) of $D_{50}/C_{50}ED$ is slightly higher than DED sequence. The 100% replacement of chlorine by chlorine dioxide reduces the AOX value of the effluent, the BOD and COD values increase and the mechanical strength properties are comparable for both the sequences (**Table 3.2 and 3.3**).

Table 3.2: Combined bleached effluent characteristics from mixed hardwood pulp

Parameter	Bleaching sequence	(σ)	Bleaching sequence	(σ)
	DED		**$D_{50}/C_{50}ED$**	
BOD (kg/t)	9.1	0.16	8.6	0.17
COD (kg/t)	39	0.4	37.6	0.36
COLOR (kg/t)	26	2.2	32.9	0.2
AOX (kg/t)	0.9	1.5	1.4	0.1
BOD/COD	0.230	-	0.228	-

(σ): Standard deviation

Table 3.3: Mechanical properties of bleached mixed hardwood pulp

Parameter	Bleaching sequence DED	(σ)	Bleaching sequence $D_{50}/C_{50}ED$	(σ)
Tensile index (Nm/g)	70.4	0.28	65.3	0.5
Burst index (kPam²/g)	6.1	0.08	5.5	0.12
Tear index (mNm²/g)	6.5	0.16	7.5	0.35

(σ): Standard deviation

The bagasse pulp shows the similar behavior for the bleach chemical consumptior **(Table 3.4)** BOD, COD, AOX, color **(Table 3.5)** and mechanical strength properties **(Tablε 3.6)** as observed with bagasse pulp.

Table 3.4: Bleaching conditions of bagasse pulp (kappa number 10)

Parameter	DED			$D_{50}/C_{50}ED$		
	D	E	D	D_{50}/C_{50}	E	D
Temperature (°C)	70	70	70	Ambient	70	70
Consistency (%)	10	10	10	3	10	10
Retention time (min)	180	90	180	45	90	180
End pH	3.1	11.2	3.1	2.9	11.3	3.4
Charge as aCl (kg/t)	21.7	-	9.3	21	-	9
Alkali charge as NaOH (kg/t)	-	10	-	-	10	-
Brightness (% ISO)	85.1			85.3		
Total active Cl (kg/t)	31			30		

Table 3.5: Combined bleached effluent characteristics of bagasse pulp

Parameter	Bleaching sequence DED	(σ)	Bleaching sequence $D_{50}/C_{50}ED$	(σ)
BOD (kg/t)	8.7	0.28	9.8	0.11
COD (kg/t)	43.1	0.27	42.2	0.08
COLOR (kg/t)	23.2	0.08	38.6	0.10
AOX (kg/t)	0.60	0.04	1.8	0.08
BOD/COD	0.203	-	0.233	-

(σ): Standard deviation

Table 3.6: Mechanical properties of bleached bagasse pulp

Parameter	Bleaching sequence DED	(σ)	Bleaching sequence $D_{50}/C_{50}ED$	(σ)
Tensile index (Nm/g)	68.5	0.25	64.8	0.4
Burst index (kPam^2/g)	4.4	0.14	3.7	0.2
Tear index (mNm^2/g)	4.9	0.16	3.2	0.14

(σ): Standard deviation

Bleached mixed hardwood pulp is stronger than bleached bagasse pulp of same target brightness using same bleaching sequence. The pollution load for bagasse was more compared to mixed hardwood pulp.

The bleach chemical consumption of mixed hardwood pulp is higher than the bagasse pulp. This is due to the higher kappa number. The bleach chemical demand of $D_{50}/C_{50}ED$ and DED bleaching sequence suggests that mixed hardwood pulp is less responsive to chlorine dioxide bleaching compared to bagasse pulp.

The response of chlorine dioxide substitution, towards various bleaching sequences i.e. $D_{50}/C_{50}ED$ and DED for mixed hardwood and bagasse pulps agreed with the reported literature on softwood and hardwood pulps. Maximum delignification efficiency was achieved when the chlorine dioxide substitution was between 40 - 60% [75, 82]. BOD decreases very slightly with chlorine dioxide substitution [123, 125].

It has also been reported in the literature that BOD changes very little upto 90% substitution and then decline very slightly thereafter [116]. COD decreases about 10% with increasing in chlorine dioxide substitution from 0-10%, but some literature also reports considerably scattered data in this regard [116, 123, 125].

Literature survey reported AOX values for bleaching sequence involving chlorine dioxide substitution and varies linearly with the elemental chlorine used in the first stage. At very high levels of substitution, AOX was lower than the anticipated based on the linear relationship observed at lower substitution levels [52] There are many other studies of the impact of chlorine dioxide substitution on AOX formation showing the same results [17, 52].

3.3.2 Oxygen Delignification

The objective of oxygen delignification was to target the residual lignin and remove it up to 50% by oxygen delignification. The optimization of alkali charge was carried out by

performing oxygen stage under optimum conditions of oxygen pressure at 6 kg/cm^2, pulp consistency of 10%, retention time 75 minutes, temperature of 100 °C and magnesium sulfate charge 2 kg/t **(Tables 3.14 and 3.15).**

The results of these studies on mixed hardwood pulp are shown in **Table 3.7**. At 15 kg/t of alkali charge, 46.66 % reduction in kappa number was obtained.

Table 3.7: Optimized conditions for oxygen pre-bleaching and pulp characteristics of oxygen delignified pulps

Parameter		Mixed hardwood	Bagasse
O$_2$ charge (kg/cm^2)		6	6
Consistency (%)		10	10
Time (min)		75	75
Temperature (°C)		100	100
Alkali charge (kg/t)		15	8
Magnesium sulphate (kg/t)		2	2
Kappa number	Non oxygen	15	10
	Oxygen delignified	8	5.4
Viscosity (cm^3/g)	Non oxygen	450	520
	Oxygen delignified	430	490
Brightness (% ISO)	Non oxygen	27.7	45.3
	Oxygen delignified	42.1	57.8

Similar studies were performed with bagasse pulp. As the kappa number of bagasse pulp is lower than the mixed hardwood pulp, oxygen delignification alkali charge was reduced to 8 kg/t to obtain 46.2 % reduction in kappa number. The optimum conditions and the properties of oxygen delignified mixed hardwood and bagasse pulp are given in **Table 3.7**. The oxygen delignified mixed hardwood and bagasse pulps were used for enzyme pre-bleaching studies.

The reduction in pulp kappa number and increase in brightness for both the pulps mixed hardwood and bagasse **(Figure 3.1 & 3.2)** are principally due to dissolution of lignin fraction **[50, 186].**
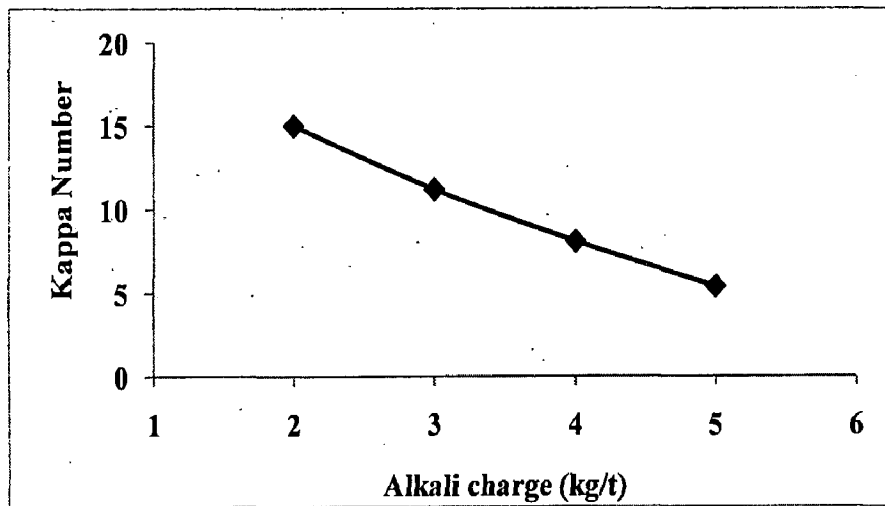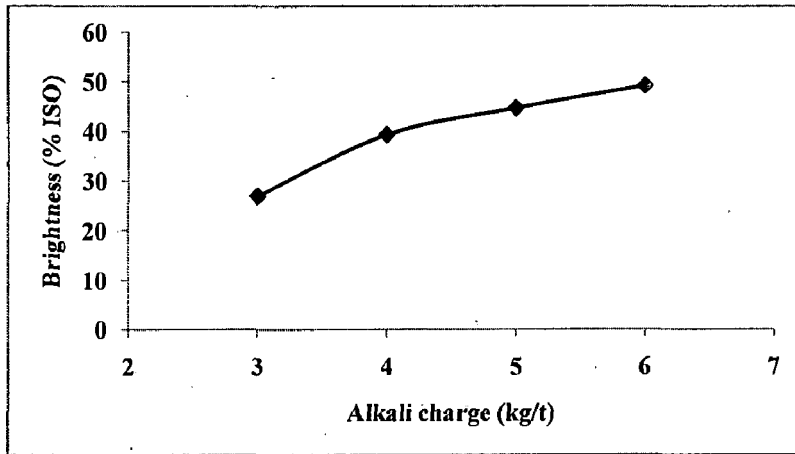
Figure 3.1: Effect of alkali charge on kappa number and brightness of mixed hardwood pulp
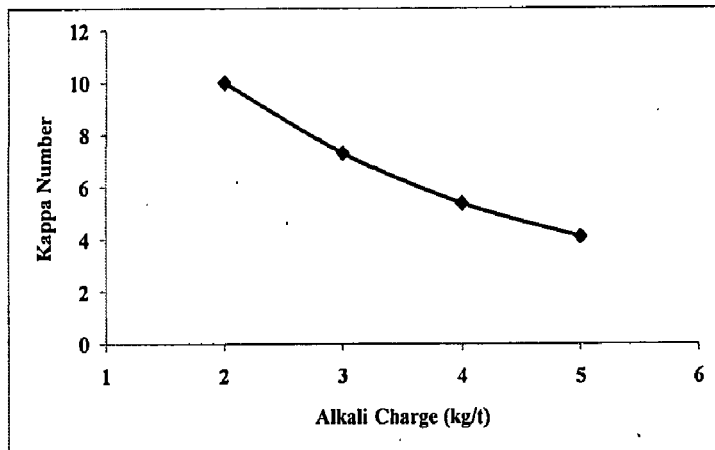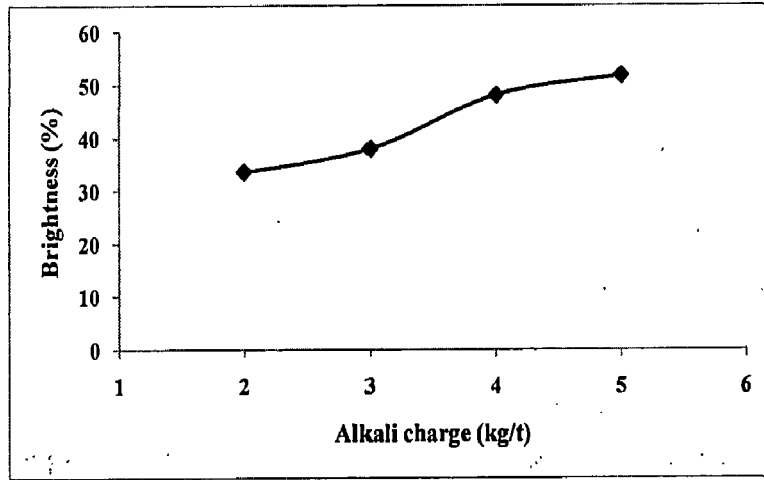
Figure 3.2: Effect of alkali charge on kappa number and brightness of bagasse pulp

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3.3.3 Bleaching of Oxygen Delignified Pulps

3.3.3.1 ODED Bleaching (85% ISO)

Mixed Hardwood Pulp

Oxygen delignified mixed hardwood pulp was bleached using DED sequence to get 85% ISO brightness. The total active chlorine required was 23.20 kg/t. This is about 48.45% lower than total requirement of total active chlorine to bleach mixed hardwood pulp to 85% ISO brightness using DED sequence. The reduction in bleach chemical demand is due to lower kappa number of oxygen delignified pulp (**Tables 3.1 and 3.8**).

The BOD, COD, AOX and color values of combined effluent from DED bleached oxygen delignified pulp are lower by 41, 42.2, 68.4 and 50.7% respectively in comparison to combined effluent from DED bleached mixed hardwood pulp (**Table 3.2 and 3.9**).

Bagasse Pulp

Oxygen delignified bagasse pulp also give the similar results during DED bleaching. The reduction in total active chlorine demand 42.7% (**Tables 3.4 and 3.11**). BOD, COD, AOX and color values of combined effluent were lower by 50.2, 38.1, 25 and 50.5% respectively in comparison to DED bleached bagasse pulp (**Tables 3.5 and 3.12**). However the strength properties (tear, tensile and burst strength) of DED bleached oxygen delignified pulp improved about 2-4% at the same level of beating to DED bleached non oxygen pulps (**Tables 3.6 and 3.13**).

3.3.3.2 $OD_{50}/C_{50}E_pD$ Bleaching (90% ISO)

Mixed Hardwood Pulp

The response of chlorine dioxide to oxygen delignified mixed hardwood pulp is comparatively better. Increase in chlorine dioxide dose improved the brightness significantly, 50% substitution of chlorine with chlorine dioxide (D_{50}/C_{50}) reinforced with 3 kg/t peroxide in extraction stage improved brightness to 89.9 that too at total active chlorine charge of 24.8 kg/t (**Table 3.8**).

pulp effluent the maximum fall in the quantity of chlorophenolics is observed in $D_{50}/C_{50}ED$ i.e. 33.2 %. The quantity of chlorophenolics in enzyme and non enzyme mixed hardwood pulp effluent follows the same trend as bagasse. The highest concentration of chlorophenolics is observed in $D_{50}/C_{50}ED$ bleaching sequence effluent i.e. 151.7 µg/l in hardwood. The maximum fall in the concentration of chlorophenolics is observed in enzyme pretreated ODED bleaching sequence effluent 58%. The reduction in the concentration is due to xylanase pretreatment. The generation of chlorophenolic compounds depends on lignin content i.e. kappa number and bleach chemical dose. The total chemical demand of a bleaching sequence is reduced after xylanase pretreatment due to which the phenolic compounds present in the pulp less exposed to chlorinated bleaching agents, resulting reduction of chlorophenolic compounds in the bleaching effluent.

Table 3.16: Quantity of various chlorophenolic compounds identified in the bleach effluents of various enzyme and non enzyme sequences of mixed hardwood pulp

S.No	CHLOROPHENOL	SEQUENCES							
		DED (µg/l)	XDED (µg/l)	$D_{50}/C_{50}ED$ (µg/l)	$XD_{50}/C_{50}ED$ (µg/l)	ODED (µg/l)	OXDED (µg/l)	$OD_{50}/C_{50}EpD$ (µg/l)	$OXD_{50}/C_{50}EpD$ (µg/l)
1	4-Chlorophenol	2.75	1.77	1.22	0.24	0.08	ND*	6.82	4.05
2	2,6-Diichlorophenol	0.18	ND*	0.38	0.06	0.13	0.01	1.03	ND*
3	2,4-Dichlorophenol	0.20	0.07	15.40	10.03	0.06	0.13	6.24	5.55
4	2,4,5-Trichlorophenol	0.56	0.32	35.62	29.12	1.31	0.01	2.13	2.03
5	2,4,6-Trichlorophenol	0.05	ND*	0.08	ND*	0.05	ND*	0.35	ND*
6	3-Chlorophenol	5.34	3.01	3.50	1.32	0.34	ND*	18.23	12.22
7	2,3-Dichlorophenol	0.19	0.12	0.08	ND*	0.05	ND*	ND*	ND*
8	2,3,6-Trichlorophenol	0.17	0.45	0.18	0.22	0.06	0.07	1.3	0.05
9	2,3,4-Trichlorophenol	0.23	0.10	0.91	1.06	0.26	0.11	3.17	1.33
10	3,4,5-Trichloroguaiacol	0.05	ND*	24.51	15.80	0.06	ND*	4.52	3.44
11	2,5-Dichlorophenol	0.46	1.33	40.67	33.54	0.50	0.01	21.11	18.76
12	3,4-Dichlorophenol	0.11	ND*	1.25	3.66	0.14	0.01	2.12	1.37.
13	4-Chloroguaiacol	14.65	9.54	2.47	0.44	2.32	0.92	2.43	1.95
14	2,3,5-Trichlorophenol	0.30	0.02	0.41	ND*	0.13	0.10	1.21	0.60
15	4,5-Dichloroguaiacol	23.63	16.94	17.94	15.55	10.34	7.00	3.34	1.83
16	4,6-Dichloroguaiacol	0.53	0.10	23.88	21.01	0.36	0.23	15.36	8.50
17	3,6-Dichlorocatechol	7.30	3.07	24.93	21.08	2.66	1.01	14.54	11.00
18	3,5-Dichlorocatechol	4.65	1.35	3.85	2.77	1.40	0.04	2.59	3.36
19	3,4,6-Trichloroguaiacol	0.34	0.11	9.05	5.55	0.23	0.01	ND*	ND*
20	4,5,6-Trichloroguaiacol	0.31	0.56	3.50	2.00	ND*	ND*	1.45	0.85
21	2,3,5,6-Tetrachloroguaiacol	0.30	0.09	13.53	11.45	ND*	ND*	13.40	9.04
22	Tetrachlorocatechol	2.01	0.22	2.27	0.01	0.24	0.53	ND*	ND*
	TOTAL	64.30	39.16	225	175.45	20.74	10.17	121.34	84.56

ND*- Not Detected

Table 3.14: Optimization of alkali charge during oxygen delignification on mixed hardwood pulp

Parameter	Untreated	Set 1	Set 2	Set 3
Alkali charge (as NaOH) (kg/t)	-	10	15	20
Kappa No.	15	11.2	8	5.4
Brightness (% ISO)	27	39.3	44.7	49.2
Oxygen pressure (kg/cm^2)	-	6	6	6
Consistency (%)	-	10	10	10
Retention time (min)	-	75	75	75
Temperature (^{0}C)	-	100	100	100
MgSO$_4$ (kg/t)	-	2	2	2

Table 3.15: Optimization of alkali charge during oxygen delignification on bagasse pulp

Parameter	Untreated	Set 1	Set 2	Set3
Alkali charge (as NaOH) (kg/t)	-	6	8	12
Kappa No.	10	7.31	5.38	4.1
Brightness (% ISO)	33.6	38.00	48.1	51.8
Oxygen pressure (kg/cm^2)	-	6	6	6
Consistency (%)	-	10	10	10
Retention time (min)	-	75	75	75
Temperature (^{0}C)	-	100	100	100
MgSO$_4$ (kg/t)	-	2	2	2

3.4 QUALITATIVE AND QUANTITATIVE ANALYSIS OF CHLOROPHENOLIC COMPOUNDS

The qualitative and quantitative analysis have been carried out for various chlorophenolic compounds present in laboratory generated wastewater from various enzyme and non enzyme bleaching sequences of mixed hardwood and bagasse pulps. The analysis of chlorophenolics is performed for both the pulps and results of qualitative and quantitative analysis are given in **Table 3.16 & 3.17**. The formation of chlorophenolics in the bleaching effluents is dependent on chlorine charge. The highest concentration is obtained in $D_{50}/C_{50}ED$ (957.4 µg/l) bleaching sequence in bagasse. Although after enzyme pretreatment the quantity is considerably decreased in all the bleaching sequence but in case of enzyme pretreated bagasse

Table 3.12: Combined bleached effluent characteristics of bleached oxygen delignified bagasse pulp

Parameter	Bleaching sequence DED	(σ)	Bleaching sequence $D_{50}/C_{50}E_pD$	(σ)
BOD (kg/t)	4.40	0.28	5.7	0.34
COD (kg/t)	26.6	0.36	29.1	0.37
COLOR (kg/t)	11.5	0.40	7.3	0.29
AOX (kg/t)	0.6	0.04	1.2	0.14
BOD/COD	0.165	-	0.197	-

(σ): Standard deviation

Table 3.13: Mechanical properties of bleached oxygen delignified bagasse pulp

Parameter	Bleaching sequence DED	(σ)	Bleaching sequence $D_{50}/C_{50}E_pD$	(σ)
Tensile index (Nm/g)	71.3	0.53	68.5	0.47
Burst index (kPam^2/g)	4.5	0.35	3.8	0.52
Tear index (mNm^2/g)	5	0.40	3.4	0.24

(σ): Standard deviation

It has been concluded on the basis of above studies that both mixed hardwood and bagasse pulps can be bleached to a brightness of 85% using DED, $D_{50}/C_{50}ED$ and ODED bleaching sequences. The total active chlorine consumption follows the order DED>$D_{50}/C_{50}ED$>ODED. BOD and COD also follow the same order but AOX is lowest in ODED sequence. The $D_{50}/C_{50}ED$ bleached pulp showed the lowest strength properties. The mixed hardwood pulp required higher dose of alkali charge to target 40-50% reduction in kappa number by oxygen delignification. A higher brightness of 90 % ISO can be achieved for both the pulps using $OD_{50}/C_{50}E_pD$ sequences.

The study further shows that environmental loads particularly AOX is higher in the combined effluents of $D_{50}/C_{50}ED$ bleached mixed hardwood and bagasse pulps. The 100 % replacement of chlorine by chlorine dioxide meets the AOX discharge norms, but economically at a disadvantage. Adoption of $OD_{50}/C_{50}E_pD$ bleaching sequence for higher brightness levels will require additional oxygen delignification process which requires investments but operation cost are lower than $D_{50}/C_{50}ED$. The optimum conditions for mixed hardwood and bagasse pulps at different alkali doses are given in **Tables 3.14-3.15**.

Table 3.10: Mechanical properties of bleached oxygen delignified mixed hardwood pulp

Parameter	Bleaching sequence	(σ)	Bleaching sequence	(σ)
	DED		$D_{50}/C_{50}E_pD$	
Tensile index (Nm/g)	69	0.16	53.2	0.27
Burst index (kPam^2/g)	6.5	0.24	6.0	0.22
Tear index (mNm^2/g)	7.7	0.32	6.3	0.29

(σ): Standard deviation

Bagasse Pulp

The optimum conditions and bleach chemical dose for baggase pulp are given in **Table 3.11**. The BOD, COD, AOX and strength properties follow the similar behavior as observed with oxygen delignified mixed hardwood pulp (**Tables 3.12 and 3.13**). The oxygen delignified pulps of both the raw materials showed poorer response to chlorine dioxide than their respective non-oxygen pulps. The above results are in agreement with the literature, which suggests that in going from unbleached to oxygen delignified pulp, the free phenolic content of the pulp decreases thereby increasing the difficulty of delignification with chlorine dioxide alone [52].

Table 3.11: Bleaching conditions for oxygen delignified bagasse pulp

Parameter	Bleaching sequence					
	D	E	D	D_{50}/C_{50}	E_p	D
Temperature (^{0}C)	70	70	70	ambient	70	70
Consistency (%)	10	10	10	3	10	10
Retention time (min)	180	90	180	180	90	180
End Ph	3.2	11.3	3.1	2.4	10.8	3.2
Charge as aCl (kg/t)	12.43	-	5.32	12.81	-	5.49
Alkali charge as NaOH (kg/t)	-	10	-	-	10	-
Hydrogen peroxide (kg/t)	-	-	-	-	3	-
Brightness (% ISO)	85.3			90.2		
Total active Cl (kg/t)	17.75			18.3		

Kappa number of oxygen delignified bagasse pulp 5.4

Table 3.8: Bleaching conditions for oxygen delignified mixed hardwood pulp

Parameter	Bleaching Sequence					
	D	E	D	D_{50}/C_{50}	E_p	D
Temperature (^{0}C)	70	70	70	Ambient	70	70
Consistency (%)	10	10	10	3	10	10
Retention time (min)	180	90	180	45	90	180
End pH	3.4	11.3	3.2	2.6	11.9	3.3
Charge as aCl (kg/t)	16.24	-	6.96	17.36	-	7.44
Alkali charge as NaOH (kg/t)	-	10	-	-	10	-
Hydrogen peroxide (kg/t)		-		-	3	-
Brightness (% ISO)	85.2			89.9		
Total active Cl (kg/t)	23.2			24.8		

Kappa number of oxygen delignified mixed hardwood pulp 8

The effluent parameters i.e. BOD, COD and effluent color from bleached oxygen delignified pulp with reinforced alkali extraction by the addition hydrogen peroxide in extraction stages are given in **Table 3.9**. The higher BOD, COD and color values observed with $OD_{50}/C_{50}E_pD$ bleached pulp effluent compared to oxygen delignified DED bleached pulp effluent is due to higher chemical charge and longer bleaching sequence required to obtain 90% brightness than 85% observed with ODED bleaching sequence. The AOX value of combined effluent of $OD_{50}/C_{50}E_pD$ sequence is less compared to ODED sequence. The oxygen delignified bleached pulp using $D_{50}/C_{50}E_pD$ sequence is weaker in comparison to ODED bleached pulp as shown by the strength properties (**Table 3.10**). The increased pulp degradation due to higher chemical charge and longer bleaching sequence is responsible for lowering of strength properties.

Table 3.9: Combined effluent characteristics of bleached oxygen delignified mixed hardwood pulp

Parameter	Bleaching sequence	(σ)	Bleaching sequence	(σ)
	DED		$D_{50}/C_{50}E_pD$	
BOD (kg/t)	5.3	0.2	6	0.16
COD (kg/t)	22.7	0.25	27.4	0.09
COLOR (kg/t)	12.8	0.11	14.0	0.19
AOX (kg/t)	0.4	0.4	0.6	0.04
BOD/COD	0.233	-	0.218	-

(σ): Standard deviation
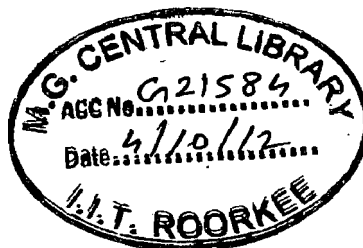
XYLANASE PREBLEACHING OF MIXED HARDWOOD AND BAGASSE PULPS

4.1 INTRODUCTION

The main target in the xylanase aided pre-bleaching of pulps have been directed towards reducing environmental loads mainly chloro-organic compounds (AOX) content of the effluent thereby reducing chemical doses in bleaching process and making the effluent more amenable to biological treatment. It has been reported that the main enzyme used in the xylanase aided bleaching is endo-β-xylanase. Vikari et al. (1986) were the first to successfully demonstrate that xylanase pre-bleaching facilitates the subsequent chemical bleaching of pulps [224]. The enzyme pre-bleaching, substantially improves the pulp brightness that could be exploited for reduction of chlorine consumption or for value addition [41].

The impact of xylanase pretreatment has also been reported for non-woods [25, 199, 245] and agricultural residue pulps [85, 86, 129, 171, 244]. The effectiveness of xylanase pretreatment on bleaching of chemical pulps is briefly summarized under the following paragraphs.

4.2 FACTORS AFFECTING TREATMENT EFFICIENCY

The pre-treatment conditions for xylanase enzyme with respect to dose, pH, retention time and temperature should be optimized for given raw materials. It has been suggested that different commercial xylanases have different optimum pretreatment conditions for effective action [25, 29, 85, 219]. Many studies have indicated that fungal xylanases have optimum pH in the range of 4-6 while bacterial xylanases have optimum pH in the range of 6-9 [25].

4.3 EFFECT ON BLEACHING CHEMICAL CONSUMPTION AND FINAL BRIGHTNESS

The effect of xylanase aided bleaching has been studied on various ECF [5, 54, 124, 175, 177, 204] TCF [4, 15, 124, 220, 232] and conventional bleaching sequences [5, 24, 25, 85, 86, 161, 188]. A considerable reduction in bleach chemical consumption in the range of 15-35% has been reported. A higher final brightness can be achieved at the same level of bleach chemical dose after xylanase pretreatment [64]. The xylanase treatment can also be applied to

the pulp before, after and between two successive oxygen delignification stages [15]. The enzyme offers an alternative approach to biological bleaching. Lignin is known to be covalently bound to hemicelluloses in wood and pulp. The cleavage of this linkage will allow lignin to be released. Hydrolysis of the lignin hemicelluloses linkage would have to be specific in order to prevent deterioration of pulp qualities, e.g. viscosity, due to cellulose hydrolysis. In hardwood, the linkage is mainly between lignin and xylan, possibly through arabinose chains. It was found that the enzymatic treatment resulted in an increased rate of lignin removal from the pulp as shown by the increase in brightness. The magnitude of brightness gain may be less, but potential of chemical savings seems to be large [175, 177].

4.4 EFFECT OF XYLANASE ON BLEACH EFFLUENTS

In the production of paper, residual lignin from wood pulp is chemically liberated by using chlorine and chlorine based bleaching chemicals. Elemental chlorine reacts with lignin and other organic matter in the pulp, forming chlorinated compounds. The effluents have high BOD, COD, color and chlorinated compounds measured as adsorbable organic halides. The Biobleacching is an important alternative to reduce the use of chlorine and chlorine compounds in the bleaching process. Xylan is the predominant hemicelluloses in both the pulps mixed hardwood and bagasse that are used for paper making. The cellulose fibers are protected by lignin and hemicellolose. The bond between lignin and hemicelluloses is primarily between xylan and lignin, which can be removed by xylanase. For this reason, xylanase is most important hemicellulase. Once this layer of hemicellulose is removed, the lignin layer is easily removed by bleaching chemicals. In addition, during chlorine bleaching, the exposed layer of lignin requires less bleaching chemicals to achieve the target brightness. As a result, reduced amounts of AOX concentration/chlorinated compounds of lignin are discharged as effluent. About 20-40% reductions in AOX have been reported in the literature [85, 112, 129, 175]. There have been some inconsistent results with regard to BOD and COD values of the xylanase treated effluents. Some studies indicated an increase in BOD and COD of xylanase treated effluents [85, 109, 176] while others have indicated a decrease in BOD and COD values of xylanase pretreated effluents [161, 175].

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In all the cases the biodegradability of the xylanase pretreated effluent is increased. These effluents are more amenable to biological degradation, due to higher proportion of degraded xylan has been confirmed in the literature [137, 176].

4.5   EFFECT ON PULP AND PAPER PROPERTIES

The various reports show that there is a small but measurable drop in kappa number in xylanase pretreated pulps [2, 85, 136, 146, 168, 232]. Evidence for direct brightening of hardwood and nonwood pulps just after xylanase pretreatment has been found in the literature [85, 112, 146, 168, 236]. The xylanase-pretreated pulps show no change or improved strength properties [85, 100, 131, 222, 224]. Some studies indicate lower beating of xylanase pretreated unbleached pulp seems to be required to reach a given freeness [30]. In contrast some other studies suggested that xylanase pretreated bleached pulps are more difficult to refine, requiring more beating to achieve an equivalent freeness and tensile strength [25, 219].

4.6   ECONOMICS OF XYLANASE PRETREATMENT

Xylanases have been used not only to enhance brightness in ECF bleaching sequences but also to reduce the cost of bleaching by reducing the bleach chemical demands to achieve the target brightness [41, 210, 213]. The approximate cost of xylanase was around US $ 2 per metric ton of pulp in 1995 and thereafter the price of xylanases has decreased. A estimated saving of US $ 10,000 to US $ 100,000 in US is reported [22]. An eco-friendly & economically attractive technology for the reduction of peroxide consumption in mechanical grade pulps of bagasse and wood has been reported [188]. In view of the above facts, the experiments were planned to study the impact of xylanase pretreatment on non-oxygen & oxygen delignified pulps of mixed hardwood and bagasse with respect to bleach chemical consumption, pulp and paper properties, pollution load generation and economic viability.

4.7   RESULTS & DISCUSSION

4.7.1   Xylanase Pretreatment Optimization

The xylanase pretreatment parameters optimization of untreated pulps is performed by measuring activity of xylanase or reducing sugar equivalents released.

4.7.1.1 Xylanase Activity Measurement

The relative activity of xylanase was approximately 35% and 70% respectively. Xylanase activity was present over the temperature range 30-75°C, with the maximum activity at 55°C. At 65°C the relative xylanase activity was approximately 70% of the maximum (**Figure 4.1**). The xylanase shows the maximum relative activity at optimum pH 6.5 and at a temperature of 55°C (**Figures 4.2 a & b**). This is in agreement with xylanases of bacterial origin, which show highest activity in pH range of 6-9 [25]. The results of xylanase pretreated mixed hardwood pulp also show a similar behavior, the value being higher at pH 6.5 and at a temperature of 55°C. The final brightness values follow similar trend with respect to pH but not with respect to temperature.
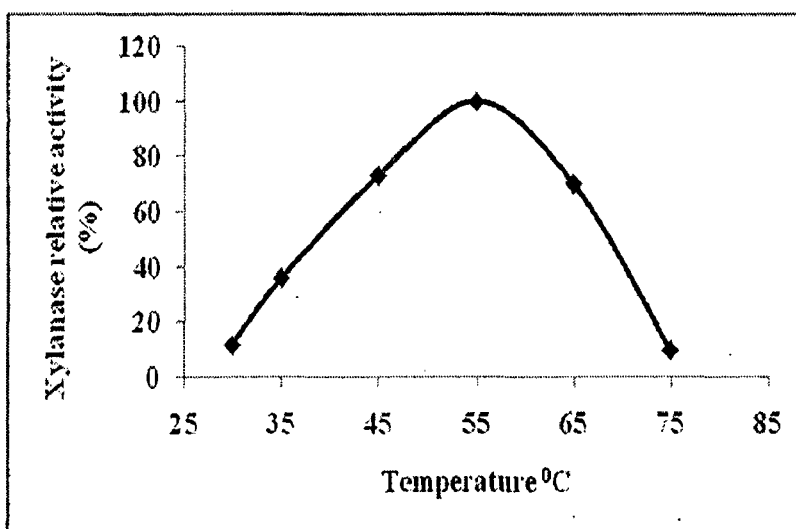
Figure 4.1: Effect of temperature on xylanase activity. Enzyme activities are compared to the highest value, considered as 100%

Table 3.17: Quantity of various chlorophenolic compounds identified in the bleach effluents of various enzyme and non enzyme sequences of bagasse pulp

S.No.	CHLOROPHENOLS	DED (µg/l)	XDED (µg/l)	$D_{50}/C_{50}ED$ (µg/l)	$XD_{50}/C_{50}ED$ (µg/l)	ODED (µg/l)	OXDED (µg/l)	$OD_{50}/C_{50}E_pD$ (µg/l)	$OXD_{50}/C_{50}E_pD$ (µg/l)
1	4-Chlorophenol	2.20	1.22	23.84	17.87	0.09	0.08	5.82	3.05
2	2,6 Dichlorophenol	0.1	0.12	0.22	0.13	0.04	0.03	0.20	ND*
3	2,4 Dichlorophenol	0.9	0.59	14.98	38.24	0.71	0.08	8.24	6.55
4	2,4,5 Trichlorophenol	0.63	1.18	102.03	61.53	0.44	0.42	1.07	1.06
5	2,4,6 Trichlorophenol	0.41	ND*	0.08	0.09	0.02	0.21	0.04	0.04
6	3 Thlorophenol	5.27	2.97	57.50	43.11	0.12	0.24	14.05	14.58
7	2,3 Dichlorophenol	0.1	0.03	0.04	ND*	0.2	0.02	0.02	0.02
8	2,3,6 Trichlorophenol	0.07	0.12	0.16	0.67	ND*	ND*	0.05	0.05
9	2,3,4 Trichlorophenol	0.11	0.11	1.22	0.43	0.05	0.45	0.13	0.12
10	3,4,5 Trichloroguaiacol	0.36	0.41	23.87	5.23	0.02	0.02	1.12	1.26
11	2,5 Dichlorophenol	2.11	1.48	197.47	90.01	0.25	0.25	19.46	17.14
12	3,4 Dichlorophenol	0.2	0.13	0.12	0.10	0.10	0.09	0.13	0.10
13	4 Chloroguaiacol	12.85	5.25	8.81	7.65	1.55	1.53	0.30	0.15
14	2,3,5 Trichlorophenol	0.18	0.24	0.37	0.13	0.09	ND*	0.12	0.10
15	4,5 Tichloro guaiacol	3.97	0.65	32.93	16.02	0.91	1.09	2.34	1.03
16	4,6,Dichloroguaiacol	0.50	0.39	223.00	159.54	0.36	0.36	12.96	6.50
17	3,6, Dichlorocatechol	4.00	4.62	245.44	184.71	3.18	2.67	2.82	1.00
18	3,5 Dichlorocatechol	2.08	2.51	ND*	ND*	1.59	1.4	1.43	1.36
19	3,4,6 Trichloroguaiacol	ND*	0.14	3.19	1.05	ND*	ND*	ND*	ND*
20	4,5,6 Trichloroguaiacol	0.06	0.19	14.44	6.09	ND*	ND*	0.22	0.25
21	2,3,5,6 Tetrachloroguaiacol	0.39	0.30	4.05	1.10	0.16	ND*	0.17	ND*
22	Tetrachlorocatecols	0.52	0.57	3.67	6.19	ND*	ND*	ND*	ND*
	TOTAL QUANTITY	37.01	23.22	957.43	639.89	9.88	8.94	70.69	54.36

ND* - Not Detected

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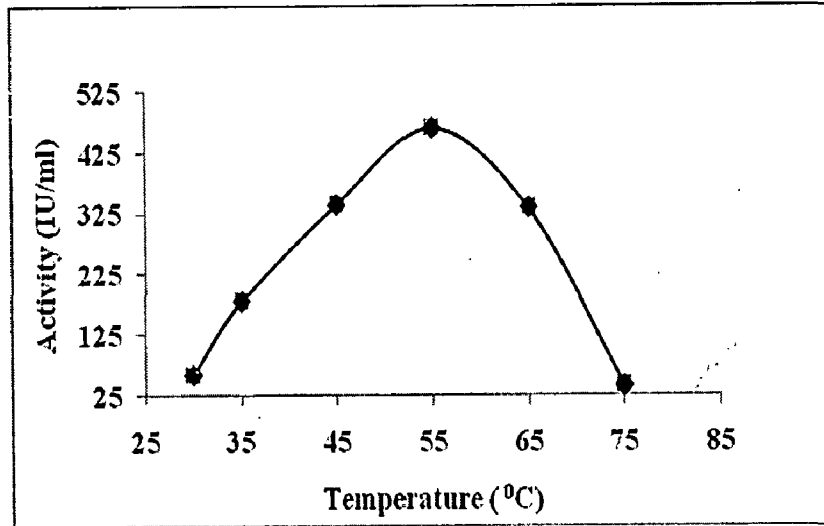
(a)
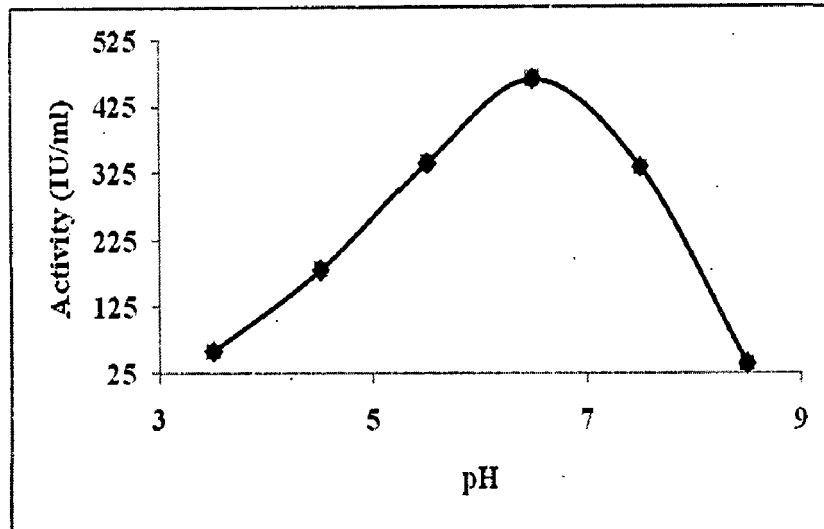
(b)

Figure 4.2: Activity of Xylanase (a) at various temperatures (b) at various pH

4.7.2 Reducing Sugar Equivalents Released

4.7.2.1 Xylanase Dose

Different doses of xylanase were given to the pulps, pH was adjusted to 6.5, consistency to 10% and temperature was kept at 55°C (**Figure 4.3**). The reducing sugars released from the pulp were found to depend on the xylanase dose. Reducing sugars showed a sharp increase with

xylanase dose from 12 to 20 IU/g, the increase was sharp upto 16 IU/g. A similar trend was seen in bagasse pulp though the release of reducing sugars was sharp upto 20 IU/g.
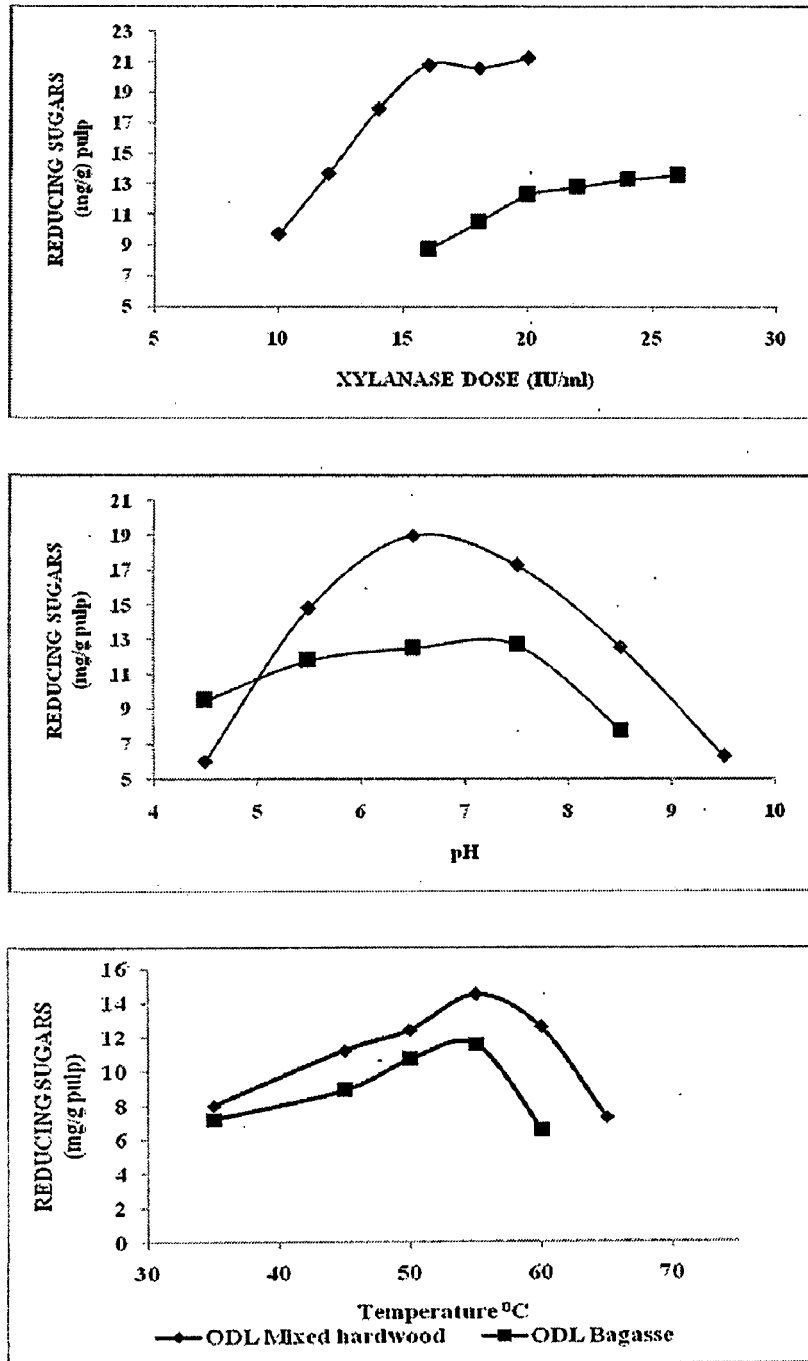
Figure 4.3: Reducing sugars at different xylanase pretreatment conditions for non oxygen bagasse pulp

The concentration of reducing sugars released in bagasse was less than in mixed hardwood pulps. It has been shown that reducing sugars release was maximum at a certain level of xylanase dose and further increase of xylanase dose did not increase solubilization. The above phenomenon may be due to the restricted accessibility of substrate during xylanase action or saturation of xylanase substrate complex. In general the effects that xylanase has on pulp bleaching increase with xylanase dose until an optima is reached [2, 3, 194] this depends on the type of the pulp used [3].

4.7.2.2 pH

The xylanase worked on acidic as well as alkaline pH but the xylanase used in this study is working on slightly acidic/near neutral pH [166]. The amount of reducing sugars released was maximum at pH 6.5 (near neutral) compared to both acidic and alkaline range.

4.7.2.3 Temperature

The effect of temperature was studied at optimized dose and at pH 6.5. Maximum reducing sugar released was at 55^0C. Optimized parameters for xylanase pretreatment are shown in **Table 4.1.**

4.8   EFFECT OF XYLANASE PRETREATMENT ON PULP PROPERTIES

All bleaching experiments were performed at optimized xylanase dose, constant process conditions and the effectiveness of xylanase pretreatment. The activity of xylanase with respect to temperature and pH has been studied (Figure 4.2 a & b). The results show maximum relative activity at a temperature of 55^0C and pH of 6.5, which were taken as optimum parameters for further studies. Xylanase improved delignification and bleaching during the sequence and the effect of the enzyme was observed on several pulp and paper properties [6, 42] (Tables 4.1 & 4.3). The process conditions used for oxygen and non oxygen mixed hardwood and bagasse pulps are given in **Table 4.2.** The improvement in brightness was more for mixed hardwood pulp compared to bagasse pulp. There was also a minor improvement in pulp viscosity for xylanase pretreated pulps in both cases. The increases in viscosity may be attributed to the fact that xylanase degrades / depolymerizes low DP xylan. Improvement in pulp viscosity and the minor reduction in kappa number are of the similar order as reported in the literature [85, 86].

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Table 4.1: Optimum parameters of xylanase pretreatment for non oxygen pulps

Parameter	Mixed hardwood	Bagasse
Enzyme dose (IU/g)	16	20
Pulp consistency (%)	10	10
pH	6.5	6.5
Retention (min.)	120	120
Temperature (^{0}C)	60	60

Table 4.2: Process conditions used during mixed hardwood and bagasse pulps bleaching of non oxygen and oxygen delignified pulps

Bleaching stage	Parameter			
	Temperature (^{0}C)	Consistency (%)	Time (min.)	End pH
First dioxide	70	10	180	3-4
Final dioxide	70	10	180	3-4
D_{50}/C_{50}	Ambient	10	45	<2
Alkali extraction	70	10	90	10-12

Table 4.3: Result of xylanase pretreatment for non oxygen pulps

Parameter	Mixed Hardwood		Bagasse	
	Untreated	Xylanase pretreated	Untreated	Xylanase pretreated
Inlet kappa No.	15	14.4	10	9.1
Brightness (%)	27.7	28.3	45.3	45.7
Viscosity (cm3/g)	450	470	520	530

4.9 IMPACT OF XYLANASE PREBLEACHING ON BLEACHING OF MIXED HARDWOOD PULP

4.9.1 Elemental Chlorine Free Bleaching (DED Sequence for 85% ISO Brightness)

4.9.1.1 Bleach Chemicals Consumption

Xylanase treatment is shown to improve the accessibility of the bleaching chemicals to the fibres [165]. Xylanase pretreated mixed hardwood pulp was bleached using different chlorine dioxide charges. This is done to decide the bleach chemical charge to get 85% ISO target brightness. The results indicate that brightness increases with the xylanase pretreatment with same chlorine dioxide dose. A target brightness of 85% was achieved at a total active chlorine dose of 40.5 kg/t. The xylanase pretreatment of mixed hardwood pulp and subsequent DED bleaching to 85% brightness requires lower bleach chemicals compared to untreated pulp (Table 4.4). The saving of chlorine dioxide in chlorine dioxide stage was 10%. At the constant bleach chemical dose, the brightness of xylanase pretreated bleached pulps increased by 1-2 points compared to the untreated pulps (Table 4.4).

4.9.1.2 Effluent Characteristics

Pre-treatment of pulp with xylanases facilitate the subsequent steps in bio-bleaching, thus lowering the amount of polluting reagents used [31]. The effluent characteristics are shown in Figure 4.4. At a target brightness of 85%, xylanase pretreatment shows a reduction in AOX and color in combined effluent by 22.2 and 11.5% while BOD and COD values increased by 17 and 8.1% respectively.

4.9.1.3 Mechanical Properties

As depicted in the Figure 4.5, the xylanase pretreated bleached pulp shows, at a constant pulp refining (3500 PFI revolutions), some improvement in tensile, tear and burst index values at same pulp brightness.
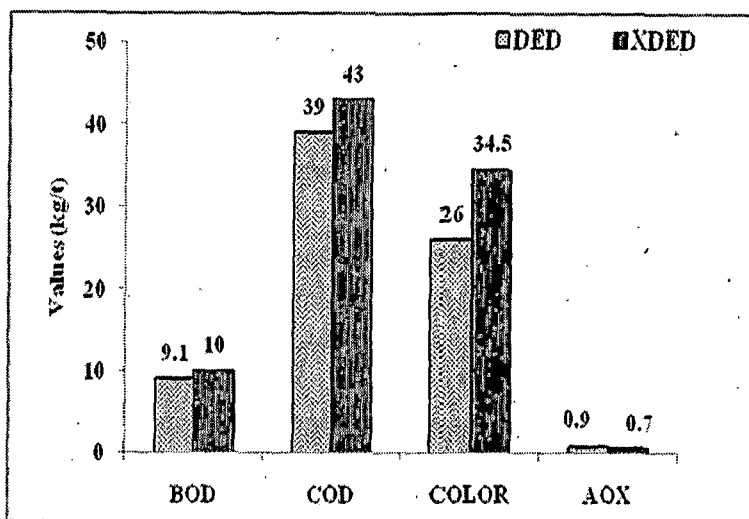
Figure 4.4: Characteristics of untreated and xylanase pretreated mixed hardwood combined DED effluent
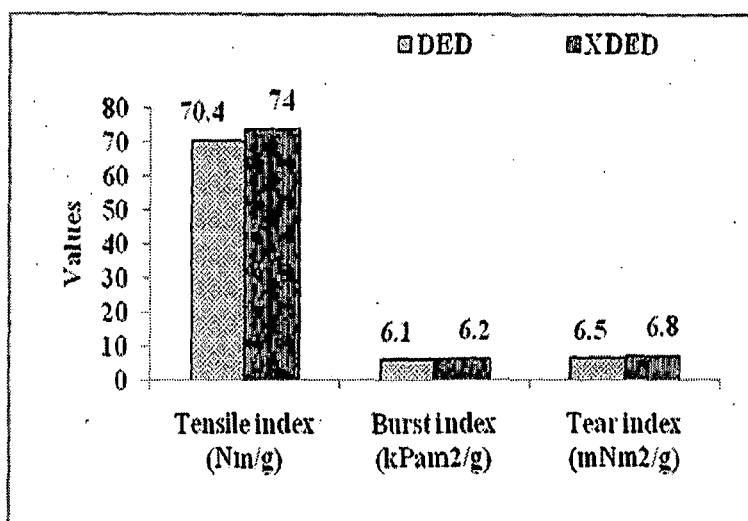
Figure 4.5: Mechanical strength properties of untreated and xylanase pretreated bleached DED mixed hardwood pulp at 3500 PFI revolutions

4.9.2 Substituted Bleaching Sequences ($D_{50}/C_{50}ED$)

A similar behavior is observed with $D_{50}/C_{50}ED$ bleaching sequence as observed with chlorine dioxide bleaching sequence. The results given in the **Table 4.4** also show that bleach chemical requirement of xylanase pretreated mixed hardwood pulp is lower as compared to

untreated mixed hardwood pulp. However, the bleach chemical savings are higher in $D_{50}/C_{50}ED$ sequence compared to DED sequence. At reduced bleach chemical charge, similar brightness (85% ISO) was observed as with xylanase pretreated $D_{50}/C_{50}ED$ sequence. The saving of active chlorine in D_{50}/C_{50} stage was 10.34%. At the same bleach chemical dose, the brightness of xylanase pretreated bleached pulps increased by 1-2 points compared to the untreated pulps (Table 4.4).

4.9.2.1 Effluent Characteristics

The BOD, COD and color values of combined effluents $D_{50}/C_{50}ED$ from bleaching of xylanase pretreated pulp from bleaching of $D_{50}/C_{50}ED$ sequence are higher than non-treated pulps (Figure 4.6). The AOX values are however lower (Figure 4.6). The lower AOX values are due to lesser bleach chemical used in xylanase pretreated pulp. A similar behavior was observed with DED sequence also.
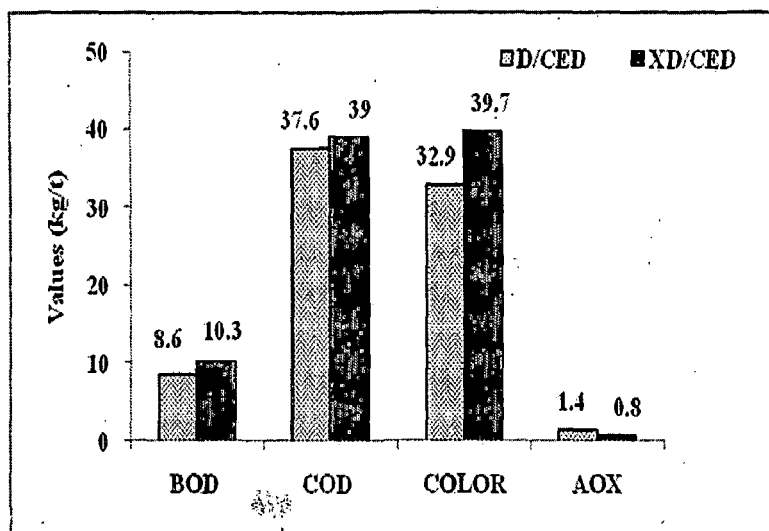
Figure 4.6: Characteristics of untreated and xylanase pretreated mixed hardwood combined $D_{50}/C_{50}ED$ effluent

4.9.2.2 Mechanical Properties

The tensile, tear and burst strength properties of xylanase pretreated $D_{50}/C_{50}ED$ bleached pulp refined at constant PFI revolutions (3500), show improvement in a similar way as observed with DED bleached pulp of same target brightness level (Figures 4.5 & 4.7).
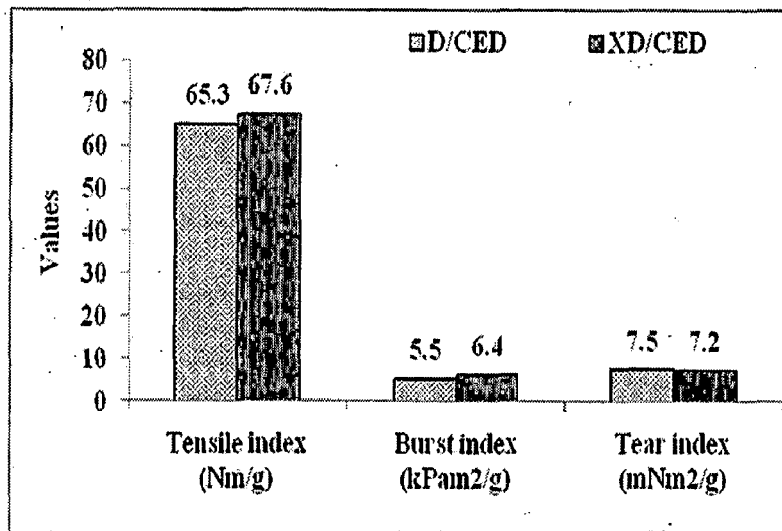
Figure 4.7: Mechanical strength properties of untreated and xylanase pretreated bleached $D_{50}/C_{50}ED$ mixed hardwood pulp at 3500 PFI revolutions

4.10 IMPACT OF XYLANASE PREBLEACHING ON BLEACHING OF BAGASSE. PULP

4.10.1 Elemental Chlorine Free Bleaching (DED Sequence for 85% ISO Brightness)

4.10.1.1 Bleach Chemicals Consumption

Xylanase pretreated bagasse pulp was bleached using different chlorine dioxide charges. This was done to decide the bleach chemical charge in different bleaching stages to get 85% ISO target brightness, which was achieved at a total active chlorine dose of 28 kg/t.

The xylanase pretreatment of bagasse pulp followed by DED bleaching to 85% brightness requires lower bleach chemicals compared to untreated pulp (Table 4.5). The saving of chlorine dioxide in chlorine dioxide stage was 9.67%. [At the reduced bleach chemical dose the brightness of xylanase pretreated bleached pulps increased by 0.1% compared to the untreated pulps (Table 4.5)].

4.10.1.2Effluent Characteristics

The effluent characteristics are shown in **Figure 4.8.** At a target brightness of 85% xylanase pretreatment shows a reduction of AOX and color in combined effluent by 8.3 and 25.8% while BOD and COD values increased by 11.80 and 4.4% respectively.
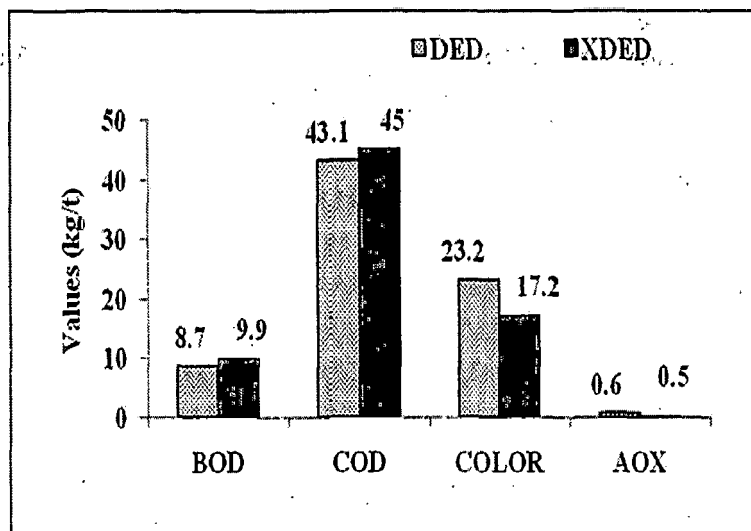
Figure 4.8: Characteristics of untreated and xylanase pretreated bagas; combined DED effluent

4.10.1.3Mechanical Properties

Tensile and burst values showed some improvement (5.3% and 4.75% respectively) while tear index decreased by 32.7% in enzyme pretreated bleached bagasse pulp of the same pulp brightness beaten at 2500 PFI revolutions **(Figure 4.9).**
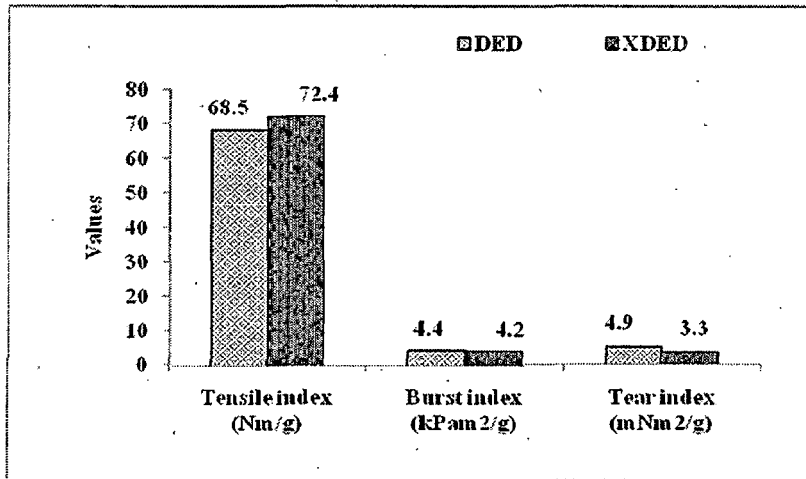
Figure 4.9: Mechanical strength properties of untreated and xylanase pretreated bleached DED bagasse pulp at 2500 PFI revolutions

Table 4.4: Bleach chemical charge (kg/t) required to obtain 85% (ISO) brightness of untreated and xylanase pretreated mixed hardwood pulps

Stage	Chemical (kg/t)	Bleach chemical charge (kg/t) as such					
		DED			$D_{50}/C_{50}ED$		
		Untreated Pulp	Xylanase pretreated pulp		Untreated pulp	Xylanase pretreated pulp	
			Constant charge	Constant brightness 85%		Constant charge	Constant brightness 85%
1st	Cl_2	-	-	-	15.225	15.225	13.65
	ClO_2	31.5	31.5	28.35	15.225	15.225	13.65
2nd	NaOH	10	10	10	10	10	10
3rd	ClO_2	13.5	13.5	12.15	13.05	13.05	11.7
Total chemical charge (kg/t)		45	45	40.5	43.5	43.5	39
Final brightness (% ISO)		84.9	87	85.2	85.1	86.8	85.1
Standard deviation (σ)		(0.34)	(1.15)	(0.23)	(0.4)	(0.47)	(0.15)

Table 4.5: Bleach chemical charge (kg/t) required to obtain 85% (ISO) brightness of untreated and xylanase pretreated bagasse pulps

Stage	Chemical (kg/t)	Bleach chemical charge (kg/t) as such					
		DED			$D_{50}/C_{50}ED$		
		Untreated	Xylanase pretreated pulp		Untreated	Xylanase pretreated pulp	
			Constant charge	Constant brightness 85%		Constant charge	Constant brightness 85%
1st	Cl_2	-	-	-	10.5	10.5	9.45
	ClO_2	21.7	21.7	19.6	10.5	10.5	9.45
2nd	NaOH	10	10	10	10	10	10
3rd	ClO_2	9.3	9.3	8.4	9	9	8.1
Total chemical charge (kg/t)		31	31	28	30	30	27
Final brightness (% ISO)		85.1	86.7	85.2	85.3	87	84.8
Standard deviation (σ)		(0.40)	(0.34)	(0.4)	(0.43)	(1.15)	(0.34)

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4.10.2 Substituted Bleaching ($D_{50}/C_{50}ED$ Sequence)

4.10.2.1 Bleach Chemical Consumption

At a target brightness of 85% for $D_{50}/C_{50}ED$ sequence, the saving of chlorine and chlorine dioxide in D_{50}/C_{50} stage was 10% but at the constant bleach chemical dose, the improvement in brightness was 2 to 3 points for xylanase pretreated pulps compared to the control pulps **(Table 4.5)**.

4.10.2.2 Effluent Characteristics and Mechanical Properties

Xylanase pretreated bagasse pulp behaved in a similar manner as compared to xylanase pretreated mixed hardwood pulp in terms of effluent characteristics i.e. BOD, COD and AOX while it is reverse in case of color. Mechanical properties using $D_{50}/C_{50}ED$ sequence shows minor change in its value **(Figures. 4.10 & 4.11)**.
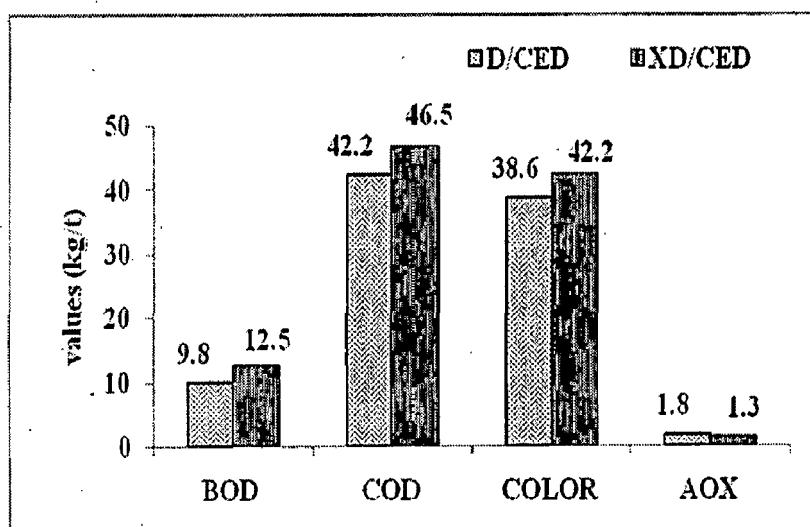
Figure 4.10: Characteristics of untreated and xylanase pretreated bagasse combined $D_{50}/C_{50}ED$ effluent

ⒹD/CED ⒶXD/CED

70
64.8 62.5
60
50
Values
40
30
20
10
3.7 4   3.2 3.5
0

Tensile index   Burst index   Tear index
(Nm/g)   (kPam2/g)   (mNm2/g)
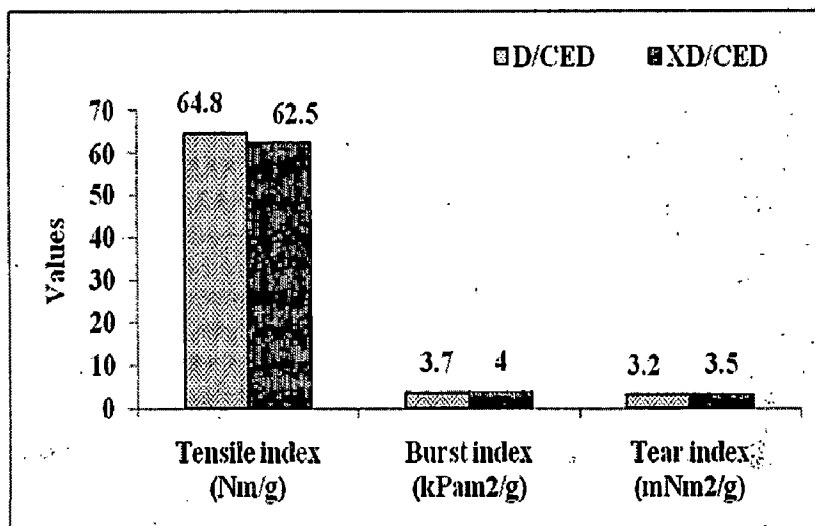
Figure 4.11: Mechanical strength properties of untreated and xylanase pretreated bleached $D_{50}/C_{50}ED$ bagasse pulp at 2500 PFI revolutions

Results shown in **Figures 4.6 & 4.7** for mixed hardwood and in **Figures 4.10 & 4.11** for bagasse pulp clearly show that both the pulps respond to xylanase pretreatment. The optimized dose for both pulps is different. It has been reported by many researchers that different commercial xylanases show different effectiveness for pulp bleaching [86, 135, 129, 157, 168, 177, 221, 240] and this variation also depends on the type of pulp used [243].

The response of xylanase pre-bleaching is different for different bleaching sequences for both the pulps. At 85% target brightness, the order of effectiveness (depend on the decrease in bleach chemical consumption) of two bleaching sequences is $D_{50}/C_{50}ED>DED$ for both the pulps i.e. mixed hardwood and bagasse pulps. The response of xylanase pretreatment appears to depend on the degree of substitution of elemental chlorine with chlorine dioxide in the chlorination stage.

A similar trend has also been reported for hardwood and softwood pulps. The hardwood pulps respond better at lower substitution [54] and softwood pulp at higher (100%) substitution [4]. The increase in brightness at the same bleach chemical dose as observed in the present case is of the similar order as reported for non wood pulps [220] but lower than hardwood pulps bleached with conventional sequence [86]. The xylanase pretreatment has similar effect on BOD, COD and AOX of the combined effluent as observed with mixed hardwood pulps. The behavior is just the reverse for color.

Xylanase pretreatment yields effluent of lower absorbable organic halides in the combined effluent of both the pulps for both the bleaching sequences. A reduction of 20-45% in

AOX value of xylanase pretreated effluents has been reported in the literature for different bleaching sequences [2, 176, 220, 221].

Bleaching of enzyme treated pulp yields effluent of higher BOD and COD. The increase in BOD and COD is lower at same target brightness level than at same bleach chemical dose. Inconsistent results on BOD and COD have been reported in the literature. Some studies indicate increase in values of BOD and COD of enzymatic treated effluents [168, 219] and others report decrease in BOD and COD values [161, 176] but common feature is that BOD/COD ratio increased making them more amenable to biological treatments [137]. The results obtained suggest that xylanase pretreatment increases the BOD/COD ratio. Minor or no change in mechanical strength properties due to enzymatic pretreatment is reported [25, 85]. The results obtained are in agreement with the above fact.

4.12 OPTIMIZATION OF XYLANASE PRETREATMENT FOR OXYGEN DELIGNIFIED PULPS

The optimization of xylanase pretreatment on oxygen delignified mixed hardwood and bagasse pulps was carried out on the same lines as done for non oxygen pulps based on reducing sugar equivalents released.

4.12.1 Reducing Sugar Equivalents Released

4.1.2.1.1 Xylanase Dose

The behavior of xylanase pretreatment was similar to as observed for non-oxygen pulps. Reducing sugars showed a sharp increase with increase in xylanase dose upto 18 IU/g and on further increase in xylanase dose upto 23 IU/g there was no appreciable change. The concentration of reducing sugars released from bagasse pulp was less than from mixed hardwood pulp (Figure 4.12).

4.1.2.1.1 pH

The amount of reducing sugars released was maximum at pH 6.5 (near to neutral) compared to either acidic or alkaline range. All xylanase pretreatment experiments were performed at optimized xylanase dose (Table 4.6).

Table 4.6: Optimum parameters of xylanase pretreatment for oxygen delignified pulps

Parameters	Mixed Hardwood	Bagasse
Enzyme dose (IU/g)	12	16
Pulp Consistency (%)	10	10
pH	6.5	6.5
Retention Time (h)	2	2
Tempetature (^{0}C)	55	55
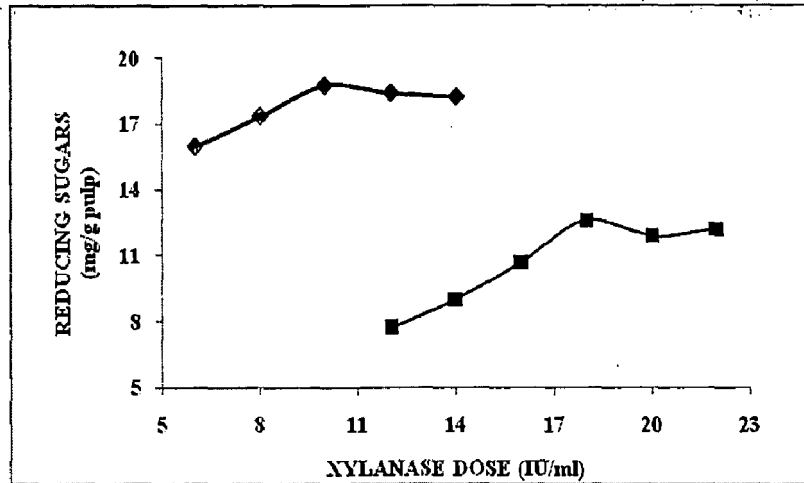
(a)
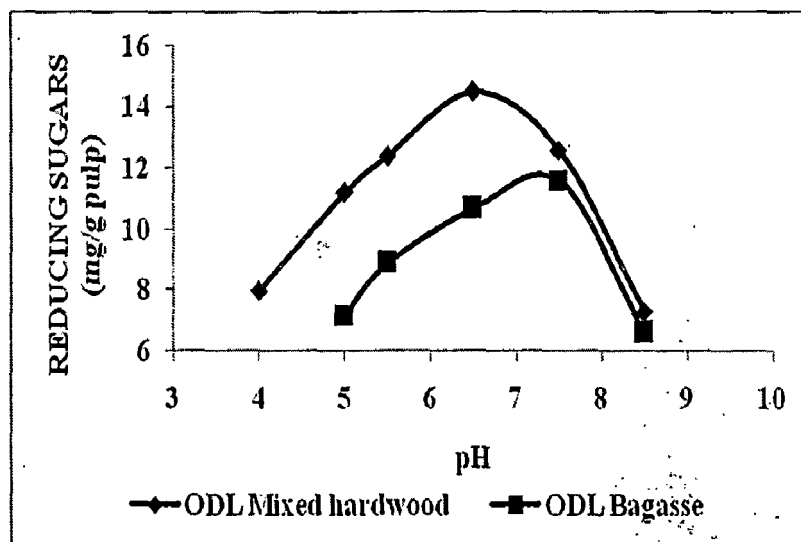
(b)

Figure 4.12: Reducing sugars from xylanase pretreatment (a) non oxygen and (b) oxygen delignified pulps

4.12.2 Pulp Properties

The results given in **Table 4.7**, confirm the effectiveness of xylanase pretreatment of both oxygen-delignified pulps. A reduction of (0.8–0.9 points) kappa no. was observed for both pulps. Higher brightness gain was observed with oxygen delignified bagasse pulp as compared to oxygen delignified mixed hardwood pulp. There was also an increase in viscosity for xylanase pretreated pulps in both cases **(Table 4.7)**. The increase in viscosity may be attributed to the fact that xylanase degrades/depolymerizes on treatment with xylanase. The increase in brightness and decrease in kappa number was more for oxygen delignified pulp than for non-oxygen pulps.

Table 4.7: Result of xylanase pretreatment on oxygen delignified pulps

Parameter	Mixed Hardwood		Bagasse	
	Untreated	Xylanase pretreated	Untreated	Xylanase pretreated
Inlet kappa No.	8	7.8	5.4	5.2
Brightness (%)	42.1	43.3	57.8	58.4
Viscosity (cm³/g)	430	440	490	510

4.13 IMPACT OF XYLANASE PRETREATMENT ON BLEACHING OF OXYGEN DELIGNIFIED MIXED HARDWOOD PULP

4.13.1 DED Bleaching (85% ISO brightness)

4.13.1.1 Bleach Chemical Consumption

The oxygen delignified xylanase pretreated DED bleached pulps follow the same trend towards bleach chemical consumption as observed for non-oxygen pulps. The xylanase pretreatment of oxygen delignified mixed hardwood pulp followed by DED bleaching required lower bleach chemical dose to achieve a target brightness of 85% as compared to untreated pulp. The total saving of $NaClO_2$ in DED stage was 6.9%. At the same bleach chemical dose for xylanase pretreated pulps, brightness was 1 to 2 points higher as compared to the control pulps. **(Table 4.8)**.

4.13.1.2 Effluent Characteristics

The BOD, COD and AOX values of combined effluents from xylanase pretreated oxygen delignified pulps were lower compared to xylanase pretreated non-oxygen pulps. This is due to higher amounts of bleach chemicals used in non-oxygen pulps (**Figures. 4.4 & 4.13**). Xylanase pre-treatment gives lower concentration of adsorbable organic halides in the combined effluent. The AOX decreased by 22.2-25% upon xylanase pretreatment. The BOD and COD results are similar to one observed with non-oxygen pulps.

Figure 4.13: Characteristics of untreated and xylanase pretreated oxygen delignified mixed hardwood bleached combined DED effluent

4.13.1.3 Mechanical Properties

The mechanical properties of xylanase pretreated oxygen delignified mixed hardwood pulp vary in a similar manner as xylanase pretreated non-oxygen mixed hardwood pulp i.e. a minor change in tear, tensile and burst indexes values at constant (3500) PFI revolutions (**Figures. 4.5 & 4.14**).

Figure 4.14: Mechanical strength properties of untreated and xylanase pretreated oxygen delignified bleached DED mixed hardwood pulp at 3500 PFI revolutions

4.13.2 $D_{50}/C_{50}E_pD$ Bleaching 90% ISO Brightness)

4.13.2.1 Bleach Chemical Consumption

Xylanase pretreated oxygen delignified mixed hardwood pulp was bleached with chlorine dioxide substitution sequence (D_{50}/C_{50}), alkali charge reinforced with peroxide (E_p) in the E stage and the chlorine dioxide dose in D_2 stage was kept the same as in the control pulp. The xylanase bleach boosting substantially improved subsequent chemical bleaching of pulps by hydrogen peroxide. The savings in bleaching chemicals with simultaneous increase in brightness and degree of delignification were observed [7]. This was done to decide the bleach chemical charge in different bleaching stages to get 90% ISO pulp brightness.

4.13.2.2 Effect of Xylanase Pretreatment in $D_{50}/C_{50}E_pD$ Sequence (90% Brightness)

The impact of xylanase pretreatment for a 90% ISO brightness reinforced with peroxide was similar to other sequences. At a brightness of 90%, the saving in total active chlorine was 9.7% compared to the control pulp. At the same bleach chemical dose, increase in brightness for xylanase pretreated pulps compared to the control pulps was approximately 1 point (Table 4.8).

4.13.2.3 Effluent Characteristics

The BOD, COD, color and AOX values of combined effluents from oxygen delignified xylanase pretreated $D_{50}/C_{50}E_pD$ pulp were higher than corresponding values for effluents from

oxygen delignified xylanase pretreated DED bleached pulp (Figures 4.15). Higher chemical charge in non enzyme oxygen delignified pulp results in higher dissolution of pulp components resulting in higher effluent loads.

Figure 4.15: Characteristics of untreated and xylanase pretreated oxygen delignified mixed hardwood bleached (90% ISO brightness) combined $D_{50}/C_{50}E_pD$ effluent

4.13.2.4 Mechanical Properties

The mechanical strength properties of oxygen delignified xylanase pretreated DED were higher than the $D_{50}/C_{50}ED$ bleached pulp (Figure. 4.16). The selectivity of chlorine dioxide is very high with lignin but selectivity of chlorine is much less which degrade cellulose and weakens the pulp fibers resulting in the decrease in pulp strength properties.

Figure 4.16: Mechanical strength properties of untreated and xylanase pretreated oxygen delignified bleached $D_{50}/C_{50}E_pD$ mixed hardwood pulp at 3500 PFI revolutions (90% ISO brightness)

4.14 IMPACT OF XYLANASE PRETREATMENT ON BLEACHING OF OXYGEN DELIGNIFIED BAGASSE PULP

4.14.1 DED Bleaching (85% ISO Brightness)

4.14.1.1Bleach Chemical Consumption

Xylanase prebleached oxygen delignified bagasse pulp responds to DED bleaching in a similar way as xylanase prebleached oxygen delignified mixed hardwood pulp. The chemical consumption is higher in mixed hardwood pulp as the kappa number of mixed hardwood pulp is higher compared to bagasse pulp. A target brightness of 85% was achieved at a total active chlorine dose of 16.14 kg/t (Table 4.9).

The saving of $NaClO_2$ in chlorine dioxide stage was 9%, with increase 1-2 point in brightness and is comparable to xylanase pretreated oxygen delignified mixed hardwood pulp (Table 4.9).

Table 4.8: Bleach chemical charge (kg/t) required to obtain 85% and 90% (ISO) brightness of oxygen delignified untreated and xylanase pretreated mixed hardwood pulps

Stage	Chemical (kg/t)	Bleach chemical charge (kg/t) as such					
		ODED			$OD_{50}/C_{50}E_pD$		
		Untreated Pulp	Xylanase pretreated pulp		Untreated pulp	Xylanase pretreated pulp	
			Constant charge	Constant brightness 85%		Constant charge	Constant brightness 90%
1st	Cl_2	-	-	-	8.68	8.68	7.84
	ClO_2	16.24	16.24	15.12	8.68	8.68	7.84
2nd	NaOH	10	10	10	10	10	10
	H_2O_2	-	-	-	0.4	0.2	0.2
3rd	ClO_2	6.96	6.96	6.48	7.44	7.44	6.72
Total chemical charge (kg/t)		23.2	23.2	21.6	25.2	25.2	22.8
Final brightness (% ISO)		85.2	86.6	85.1	89.9	91.9	90.1
Standard deviation (σ)		(0.4)	(0.41)	(0.40)	(0.46)	(0.16)	(0.18)

Table 4.9: Bleach chemical charge (kg/t) required to obtain 85% and 90% (ISO) brightness of oxygen delignified untreated and xylanase pretreated bagasse pulps

Stage	Chemical (kg/t)	Bleach chemical charge (kg/t) as such					
		DED			$D_{50}/C_{50}E_pD$		
		Untreated pulp	Xylanase pretreated pulp		Untreated pulp	Xylanase pretreated pulp	
			Constant charge	Constant brightness 85%		Constant charge	Constant brightness 90%
1st	Cl_2	-	-	-	6.405	6.405	5.85
	ClO_2	12.43	12.43	11.298	6.405	6.405	5.85
	NaOH	10	10	10	10	10	10
2nd	H_2O_2	-	-	-	0.4	0.2	0.2
3rd	ClO_2	5.32	5.32	4.842	5.49	5.49	5.0
Total chemical charge (kg/t)		17.75	17.75	16.14	18.7	18.5	16.9
Final brightness (% ISO)		85.3	86.7	85.3	90.2	91.4	89.4
Standard deviation (σ)		(0.12)	(0.3)	(0.11)	(0.18)	(0.27)	(0.22)

4.14.1.2 Effluent Characteristics and Mechanical Properties

Xylanase pre-bleached oxygen delignified bagasse pulp behaves in a similar manner towards DED bleaching as xylanase pre-bleached oxygen delignified mixed hardwood pulp in terms of effluent characteristics and mechanical properties. The pulp effluent characteristics and mechanical properties are better shown in **Figures. 4.17 and 4.18.**
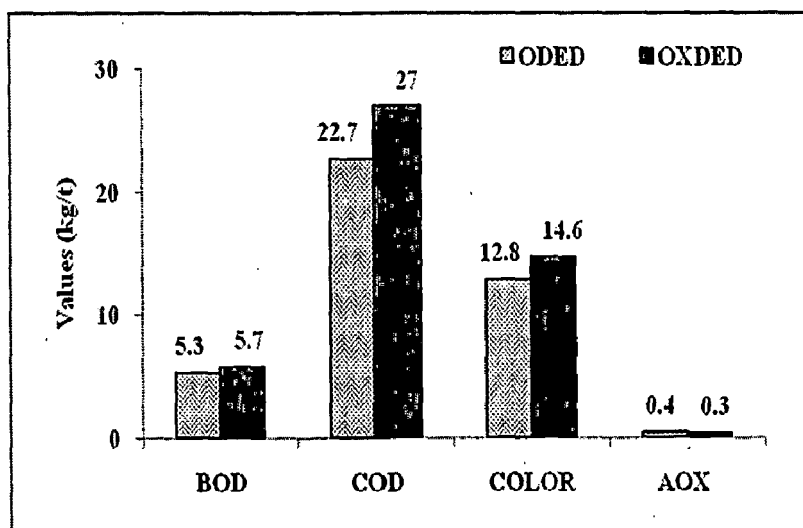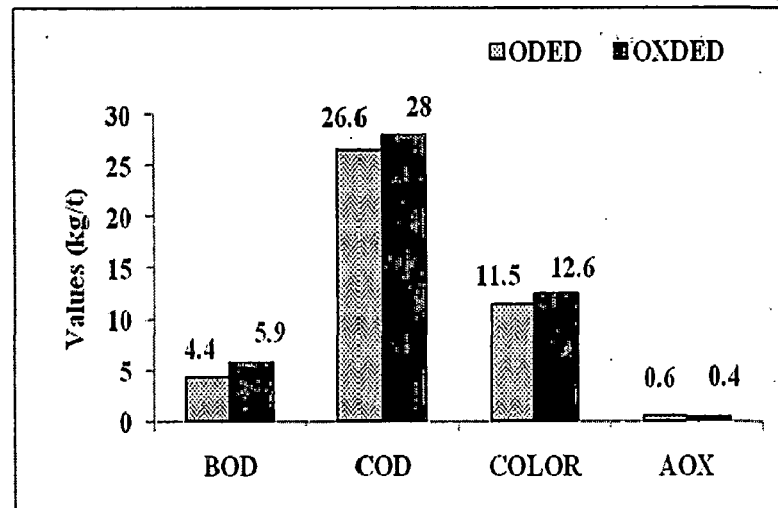

Figure 4.17: Characteristics of untreated and xylanase pretreated oxygen delignified bagasse bleached combined DED effluent
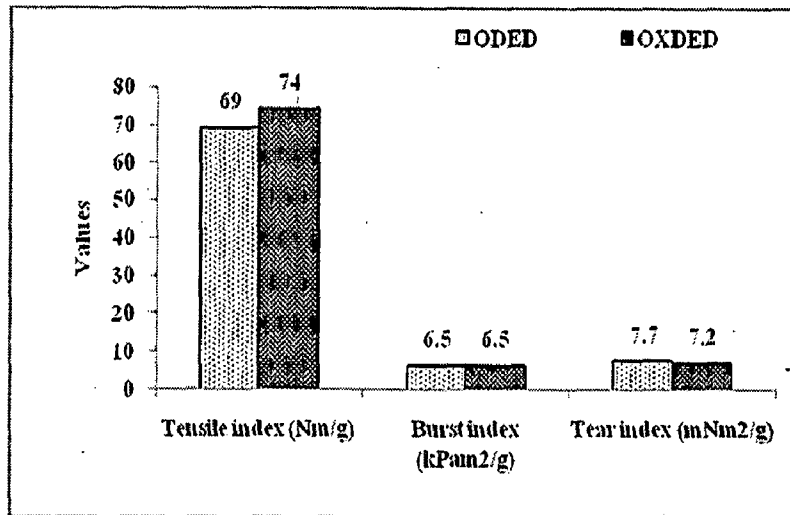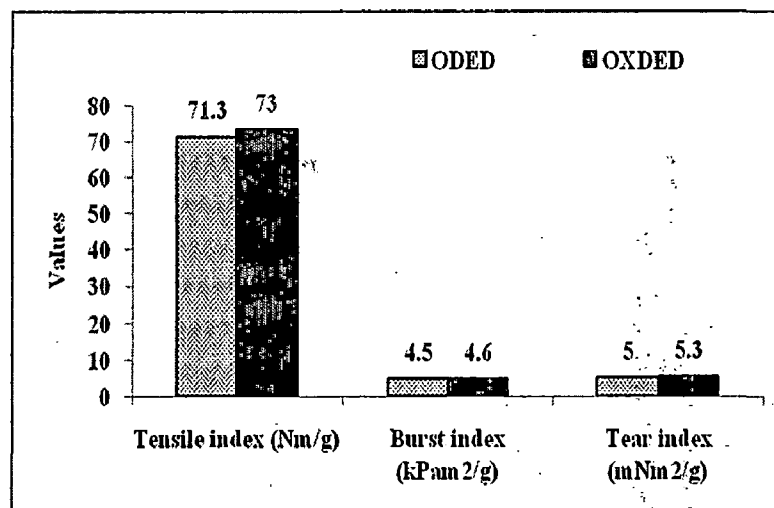

Figure 4.18: Mechanical strength properties of untreated and xylanase pretreated oxygen delignified bleached DED bagasse pulp at 2500 PFI revolutions

4.14.2 $D_{50}/C_{50}E_PD$ Bleaching (90% ISO)

4.14.2.1 Bleach Chemical Consumption

Xylanase pretreated oxygen delignified bagasse pulp was bleached with chlorine dioxide substitution sequence (D_{50}/C_{50}), alkali charge reinforced with peroxide (E_p) in the E stage and the chlorine dioxide D_2 stage was kept the same as the control pulp.

4.14.2.2 Effect of Xylanase Pretreatment on $D_{50}/C_{50}E_PD$ Sequence (90% Brightness)

The xylanase pretreatment for $D_{50}/C_{50}E_PD$ reduced the total bleach chemical demand. At a targeted brightness of 90%, the savings in total active chlorine was 8.75% compared to the control pulps. At the same bleach chemical dose increase in brightness for xylanase pretreated pulps compared to the control pulps was 1 to 2 point **(Table 4.9)**.

4.14.2.3 Effluent Characteristics and Mechanical Properties

Xylanase pretreated oxygen delignified bagasse pulp behaves in a similar manner to oxygen delignified mixed hardwood pulp in terms of effluent characteristics and mechanical properties **(Figures. 4.19 and 4.20)**.
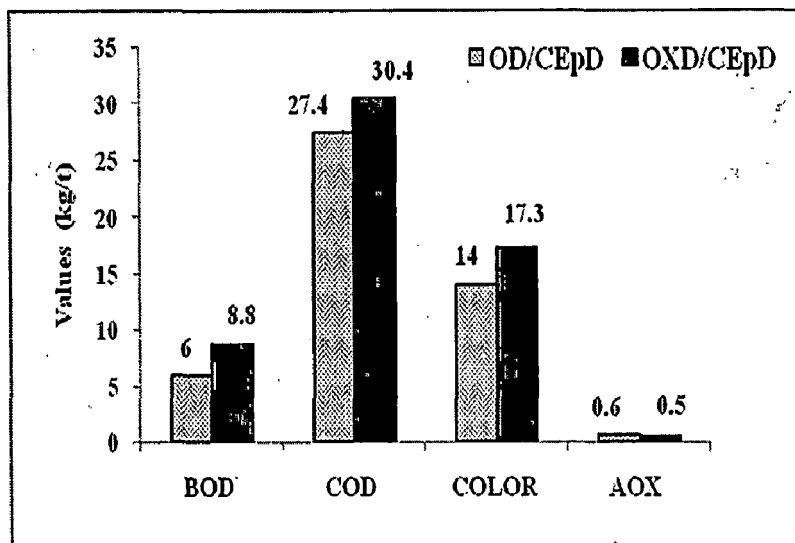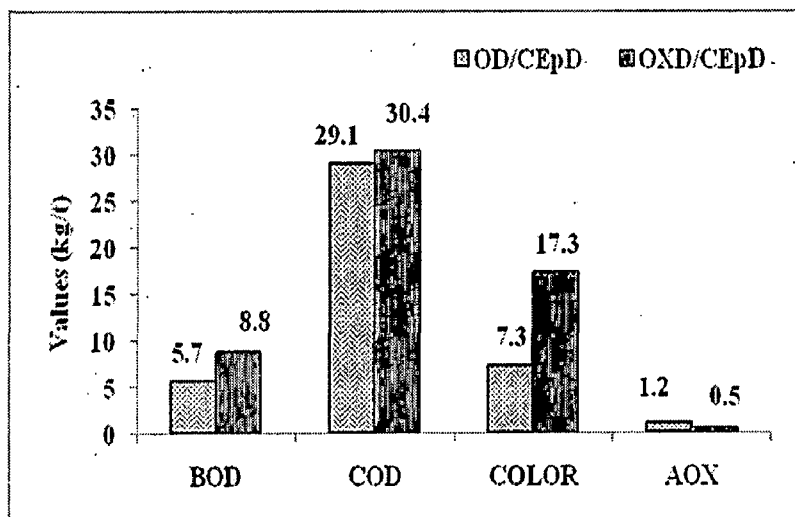

Figure **4.19: Characteristics of untreated and xylanase pretreated oxygen delignified bagasse bleached (90% ISO brightness) combined $D_{50}/C_{50}E_pD$ effluent**

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OD/CEpD OXD/CEpD

Values

80
70
60
50
40
30
20
10
0

68.5   69.6

3.8   3.6   3.4   3.7

Tensile index   Burst index   Tear index
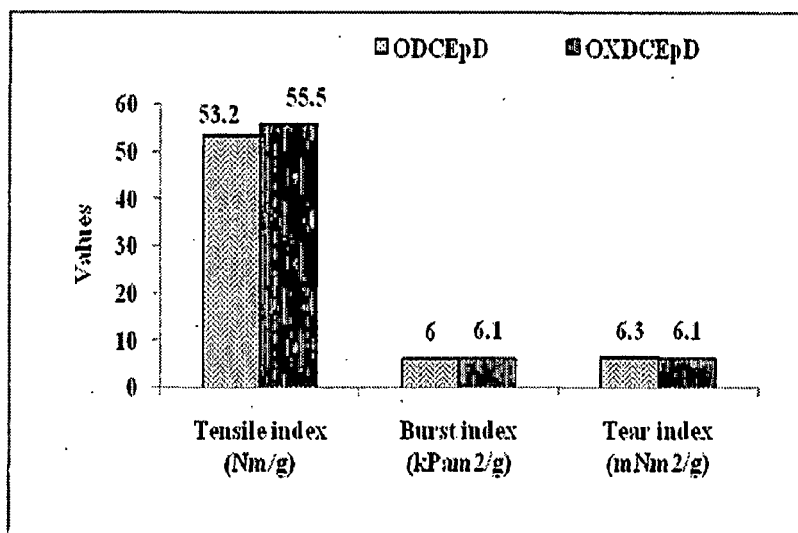(Nm/g)   (kPam2/g)   (mNm2/g)
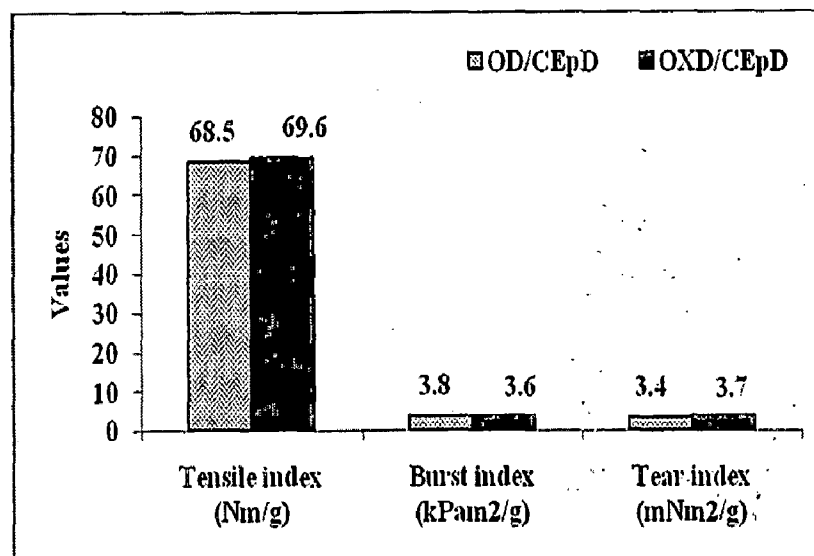
.Figure 4.20: Mechanical strength properties of untreated and xylanase pretreated oxygen delignified bleached $D_{50}/C_{50}E_pD$ bagasse pulp at 2500 PFI revolutions (90% ISO brightness)

The results of bleaching of xylanase pretreated oxygen delignified mixed hardwood and bagasse pulps are similar to xylanase pretreated non oxygen mixed hardwood and bagasse pulps i.e. decrease in bleach chemical consumption, decrease in AOX, minor increase in BOD, COD and color, there are no change in biodegradability and minor change in mechanical strength properties of the pulp bleached to same target brightness.

Bleaching of xylanase pre-bleached oxygen delignified pulp bleached with $D_{50}/C_{50}E_pD$ sequence yields pulp of high brightness (90%). The saving in bleach chemical consumption at same target brightness is higher at 85% brightness than at 90% brightness in bagasse pulp while in mixed hardwood pulp the saving in bleach chemical consumption is lower at 85% brightness than at 90% brightness. The value being 6.9 and 9.7% respectively for mixed hardwood and 9 and 8.75% for bagasse. The results are in agreement with the literature, which suggests that magnitude of brightness gain becomes smaller at higher pulp brightness, but there remains a potential for chemical savings [175, 177].

4.15   ECONOMICS OF XYLANASE PRETREATMENT

The impact of economic evaluation of any process modification is essentially made by evaluating the measurable economics in terms of gain in output cost (value addition) or either additional input costs (operating costs).

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However, it becomes difficult to assign an economic value or such a benefit, when the variables offer intangible benefits. On account of improved environmental performance of the process, one can see the viability of a process from its meeting the set regulatory standards. Non-compliance usually results in closure of an operation. In non-compliance situations one can consider the increased input costs for making a system operational as investment cost and the cost of closure for non-compliance as a measurable economic benefit of such an investment. In the economic analysis of xylanase pretreatment, the following costs have been considered towards input:

(i)   Quantity and cost of xylanase consumed.

(ii)   Reduction in the chemical costs for a given pulp brightness level during bleaching compared to such costs for xylanase untreated pulp bleaching.

(iii) The cost of reduction of environmental parameter like AOX through end of pipeline treatment technologies such as adsorption and ion exchange has been taken as an additional cost of operation to meet the set regulatory standards.

(iv)   CPCB, Ministry of Environment and Forests (GOI) has finalized and notified the following standards of AOX in case of large scale pulp and paper industries vide notification G.S.R.546(E) dated 30/08/2005. The maximum discharge limits for AOX specified in this notification are: [65]

       (a)   1.5 kg/t of paper produced with effect from the date of notification i.e. 30/08/2005.

       (b)   1 kg/t of paper produced with effect from the 1st day of March, 2008.

(v)   The intangible benefits of keeping operations sustainable due to environmental compliance with modified bleaching sequence involving xylanase pretreatment, which would otherwise have been closed for non compliance is not included in the current cost estimates.

The cost (In India in 2011) of different bleaching chemicals used in different bleaching stages is given in **Table 4.10.** The consumption of bleaching chemicals of untreated and xylanase pretreated (**Tables 4.8 & 4.9**) and the estimated costs of bleaching are shown in the **Tables 4.11 & 4.12.** The results indicate that total bleaching costs is higher with xylanase pretreatment (if the cost of AOX reduction is not considered). The increase is from Rs. 30/t ($OD_{50}/C_{50}E_pD$ sequence) to 87/t ($D_{50}/C_{50}ED$ sequence) for mixed hardwood pulp. The values given in the **Tables 4.11 &**

4.12 further indicate additional bleaching cost requirement for bagasse pulp and mixed hardwood pulp. **Table 4.11** also indicate that if the cost of AOX reduction is considered (Rs. 200 for reduction in AOX of 1 kg/t by adsorption and ion exchange method then at enzyme cost of Rs. 250/kg the xylanase pre-bleaching is a cost effective technology for mixed hardwood pulp. The total bleaching costs is higher for xylanase pretreatment for bagasse at an enzyme cost of Rs. 250/kg **(Table 4.12).**

Table 4.10: Cost of various bleaching chemicals (In India in 2011)

Chemical	Cost Rs./kg
Chlorine	12
NaOH	22
Hydrogen peroxide	46
Chlorine dioxide	80

The $D_{50}/C_{50}ED$ bleaching of chemical bagasse and mixed hardwood pulp show that the environmental loads with and without xylanase pretreatment with regard to AOX are high. The response of enzymatic pre-bleaching to oxygen delignified (ODED) bagasse and mixed hardwood pulps were found to be positive with reduction in bleach chemical demand and associated reduction in AOX loads. However, adoption of these options will require addition o: costly oxygen delignification step. The environmental and economical evaluation shows tha enzyme pre-bleaching with sequence like ODED can be adopted **(Tables 4.11-4.12).**

Over the years the international prices of enzymes have reduced considerably. In India enzyme prices are high and are expected to fall. The projected bleaching costs at enzyme price o Rs. 250/kg have also been calculated as shown in the **Tables 4.11 and 4.12**. Results indicate tha bleaching cost via xylanase route will be less compared to non xylanase route.

Today the environmental issues particularly control of AOX are the major challenges before the pulp and paper industry. With the pressure rising to limit the discharge of chlorinated organic compounds due to increased environmental awareness and imposition of stringent discharge norms the Indian pulp and paper industry is likely to face severe problems due to its high level of AOX generation. There is a need of time before alarming situations are reached that

Indian pulp and paper mill switch to new bleaching technologies. Xylanase pre-bleaching offers one such option. With likely reduction in enzyme prices xylanase pre-bleaching offers a cost effective and viable bleaching technology for Indian pulp and paper industry.

Table 4.11: Bleaching cost analysis of mixed hardwood pulp at xylanase cost of Rs 250/kg

Bleaching Sequence	Bleaching cost (Rs/t) of pulp					AOX (kg/t)				Difference in xylanase treatment cost (Rs/t)
	Xylanase pretreated			Untreated	Difference	Untreated (kg/t)	Xylanase pretreated (Rs/t) (@ 250/kg)	Reduction		
	Xylanase	Bleach chemical	Total					(kg/t)	Rs/t (@ 200/kg)	
DED	230	3430	3660	3820	-160	0.95	0.7	0.25	50	-110
ODED	200	1948	2148	2316	-168	0.35	0.25	0.10	20	-148
D$_{50}$/C$_{50}$ED	220	2411	2631	2664	-33	1.4	0.8	0.60	120	+87
OD$_{50}$/C$_{50}$EpD	200	1638	1838	1774	+64	0.65	0.48	0.17	34	+30

Table 4.12: Bleaching cost analysis of bagasse pulp at xylanase cost of Rs 250/kg

Bleaching Sequence	Bleaching cost (Rs/t) of pulp					AOX (kg/t)				Difference in xylanase treatment cost (Rs/t)
	Xylanase pretreated			Untreated	Difference	Untreated (kg/t)	Xylanase pretreated (@ 250/kg)	Reduction		
	Xylanase	Bleach chemical	Total					(kg/t)	Rs/t (@ 200/kg)	
DED	250	2460	2710	2700	+10	0.6	0.5	0.1	20	+10
ODED	220	1511	1731	1640	-91	0.6	0.4	0.2	40	-51
D$_{50}$/C$_{50}$ED	230	1737	1967	1906	+61	0.9	0.7	0.2	40	+21
OD$_{50}$/C$_{50}$EpD	220	1250	1470	1408	+62	1.0	0.7	0.3	60	+2

5.1   HEMICELLULOSE IN PULPS

Many other enzyme applications are possible based on properties confirmed in the laboratory. These include eliminating caustic chemicals used for cleaning paper machines, enhancing pulping, reducing refining time, decreasing vessels picking, facilitating retting, selectively removing fiber components, modifying fiber properties, increasing fiber flexibility, and covalently linking side chains or functional groups [89]. Commercial development of these applications need enhanced knowledge of enzyme mechanisms and actions on fibers, development of improved processes for their use, and changing enzymes to function better under present working conditions.

During pulping process, the native hemicellulose structure is heavily modified. The alkali concentration is comparatively high, during the heating period of the cook, the partial degradation of xylan by peeling and hydrolysis results in shorter chains free of acetyl groups and with less arabinose and uronic acid units. In the beginning of the cook, the majority of the 4-O–methylglucuronic acid side chains are converted to hexenuronic acid [236]. Un-degraded and degraded xylans are dissolved in the pulping liquor to a considerable extent, but as the cook proceeds the alkali concentration decreases below a critical point, the degraded short chain xylan re-precipitates in a more or less crystalline form on or within the surface of cellulose microfibrils [126].

The lignin present in raw material is dissolved during the pulping process by the action of alkali at very high temperature. This dissolved lignin has also been suggested to re-precipitate and re-deposit on the fibre surfaces [203]. Glucomannan molecules dissolved completely are degraded in a very short time at 130°C by the action of alkali. By contrast, the residual glucomannan is rather stable to further degradation and dissolution. As a result of solubilization and the distribution of hemicellulose during cooking, content of xylan and glucomannan in pulps differ from the native lignocellulosic material [94]. In softwood fibres, the xylan concentration is generally higher in outer layers and glucomannan is more

concentrated in middle layers of the fibre. However, due to the different methods of analysis, variations in the distribution of the polysaccharides in softwood pulps have been reported, although there is a general agreement that the outer surface layer of hard wood fibres is rich in xylan [122].

The residual lignin and xylan play a crucial role in xylanase aided bleaching. The xylanase pretreatment removes hexenuronic acid from the pulp fibers and facilitates the better conditions to increase brightness at the same chemical charge [8]. The mechanism of the action of xylanase is not known. However, the literature survey reveals some theories, which explain the possible mechanism of the enzyme. These are described below.

1.   The simplest concept behind the enzyme pretreatment is that it causes a physical loosening of the fibre wall due to partial depolymerisation of the hemicellulose chains, which facilitates the extraction of residual lignin during bleaching [44, 200]. Literature reported a significant decrease in xylan DP and removal of only small quantity of xylan during xylanase pre-bleaching [139]. The decrease in chain length of xylan or its removal results in increased freedom for lignin to diffuse from hemicellulose-lignin structure.

2.   Another hypothesis involves the role of re-precipitated xylans. Extraction of residual lignin is not possible due to re-precipitated xylan which acts as a barrier to extraction of residual lignin. In such conditions xylanase treatments partially or fully remove this xylan barrier and facilitates better access of bleach chemicals to the residual lignin and easier extraction of lignin from the pulp [93]. The result of the pulps delignified under high alkaline conditions does not support this hypothesis [38]. It has also been found that removal of re-deposited xylan with DMSO (dimethyl sulphoxide) does not improve the bleachability of pulp, in this situation it appears that xylanase specifically attack a small fraction of xylan in the pulp that is different from DMSO extractable xylan [144].

3.   The third hypothesis relates to the implied role of covalent bonds between residual lignin and hemicellulose, enzyme pretreatment partially attacks on the hemicellulose chain, release bond lignin fragments, which can then be more readily extracted during bleaching [142].

5.2 COMPOSITION OF XYLANASE TREATED PULPS

It is seen and proven that after xylanase treatment the xylan content is reduced in the pulps [164]. The xylanase pretreatment increased the accessibility of residual lignin and facilitated removal of hexenuronic acids [43]. In conventionally cooked pulps the decrease in xylan content was higher than mechanically cooked pulps [146].

A small drop in kappa number just after enzyme treatment is observed in brown stock hardwood, bagasse, softwood pulps and oxygen pretreated softwood pulps [2, 85, 86, 135, 236]. Extensive xylanase treatment of pine pulp increased the lignin coverage of fibre surface area from 17% to 28% thus exposing more lignin to extraction and bleaching chemicals [32].
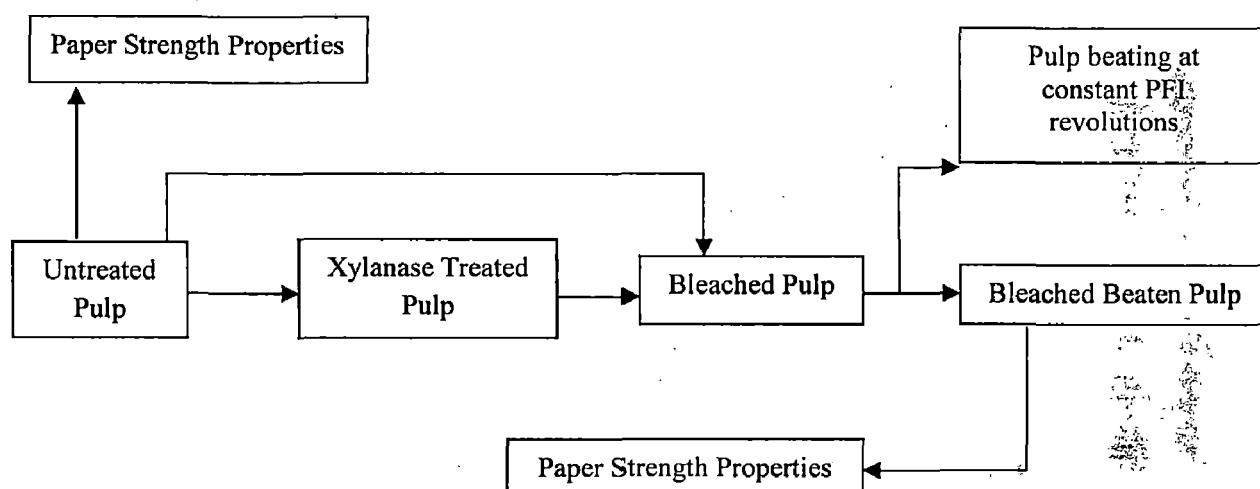
Figure A: Scheme showing sequence of various operations performed on unbleached pulp and evaluation of pulp/paper properties after each operation performed

The studies undertaken to the relative study roles of surface xylan and xylan present in the fibre wall on xylanase aided bleaching are shown in the Figure B. The DMSO extraction is discussed in a chapter 2 (sections 2.7).
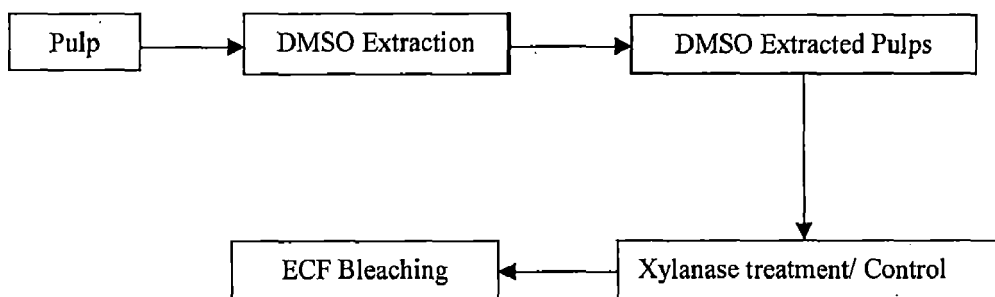
Figure B: Effect of pulp surfaces on xylanase aided bleaching of pulps

5.3   RESULTS AND DISCUSSION

5.3.1   Impact of Xylanase on Mechanical Properties of Paper

The impact of xylanase enzyme on mechanical strength properties of pulp and paper were studied. The flow chart showing the approach followed is shown in Figure A.

5.3.1.1 Bleaching and Beating of Non-Oxygen Pulp

The impact of bleaching and beating on the strength properties can be judged from the data given in Tables 5.1 & 5.2. The beating of bleached pulps open the fiber structure which results in the formation of stronger fibre bonding that improves mechanical properties of the pulp. This is reflected by substantial increase in the values of tensile and burst indices.

Table- 5.1: Mechanical properties of non xylanase and xylanase pretreated bleached mixed hardwood pulp at optimized xylanase dose.

Parameter	DED bleached pulp (85% ISO brightness)		
	Non xylanase	Xylanase treated	Change (%)
Tensile index (Nm/g)	70.4	74	+4.8
Burst index (kPam^2/g)	6.1	6.2	+1.6
Tear index (mNm^2/g)	6.5	6.8	+4.4

Table- 5.2: Mechanical properties of non xylanase and xylanase pretreated bleached bagasse pulp at optimized xylanase dose

Parameter	DED bleached pulp (85% ISO brightness)		
	Non xylanase	Xylanase treated	Change (%)
Tensile index (Nm/g)	68.5	72.2	+5.3
Burst index (kPam2/g)	4.4	4.2	-4.5
Tear index (mNm2/g)	4.9	3.3	-32.6

5.3.1.2 Xylanase Pretreatment of Non Oxygen Pulp

Very little impact of xylanase pretreatment on the strength properties of unbleached mixed hardwood & bagasse pulps was observed (Tables 5.1 & 5.2). A small reduction in the kappa number of the pulp was observed after xylanase pre-treatement that shows effectiveness of xylanase enzyme on the pulp. However, it is possible that xylanase release chromophoric groups associated with carbohydrates as suggested in the literature [142]. Chromophores are not necessarily derived or even associated with lignin. Certain studies have shown that carbohydrate degradation rather than lignin as a source of color in pulp and effluents [57, 63]. During pulping it has been reported that methyl glucuoronic acid, subsituents of xylan of the raw material is converted to hexenuronic acid [237]. Reported results further indicate that hexenuronic acid is a chromophore associated with xylan. The higher amount of chromophores formed at increasing xylanase dose indicate that at increased xylanase dose more of LCC attacked. Xylan derived chromophores therefore occur in pulp and their removal may be more effectively achieved using xylanase than bleaching chemicals. The increase in brightness just after xylanase pretreatment give an evidence of xylanase attack on chromophoric groups. This is further confirmed by our studies by decrease in kappa number, strong lignin peaks of infrared spectrum of effluents of xylanase pretreated pulp effluents.

Xylanase enzyme partially hydrolyzes the hemicelluloses portion of pulp. Unlike the conventional bleaching chemicals, the enzyme does not brighten or delignify the pulp. Rather, this enzyme act on the pulp to make the subsequent bleaching by the oxidative chemicals more efficient. The precise mechanism of xylanase action on the pulp is not known. There is reported evidence that xylanase allows larger molecular weight lignin to be removed from the pulp in the

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alkali extraction stage, so it is postulated that removal of a portion of the xylan either releases xylan-bound lignin or increases the ability of higher molecular weight lignin to diffuse through the surface of the fibre. In either case, removing a portion of the xylan from the pulp increases the efficiency of the oxidative chemicals.

Hemicelluloses in pulp play an important role in fibre morphology and contribute to the mechanical strength properties of paper. Hemicelluloses content increases the pulp yield, improves pulp strength and affects pulp quality. In the enzymatic pretreatment, the hydrolysis of hemicellulose is restricted to a minimum in order to maintain a high yield and the advantageous properties of hemicellulose. Furthermore, the absence of cellulase activity is important. The result do not show much change in strength properties of xylanase pretreated pulp. The above results are depicted in **Tables 5.1-5.6.** The results obtained are in agreement with the literature that reports that at 10% consistency the enzymatic pretreated pulps do not show a major change in mechanical properties [63].

5.3.1.3 Bleaching and Beating of Oxygen Delignified Pulps

During oxygen pretreatment of unbleached pulp removal of hemicellulose and cellulose takes place, and reduces mechanical properties. But after the beating of pulp, the mechanical properties of the pulp shows marked improvement due to opening of fibre structure. Bleached mixed hardwood & bagasse pulps of 85% ISO brightness has been obtained from both non-oxygen and oxygen pretreated pulps. Results show small drop in tensile index. The results depicted in the **Table 5.3 & 5.4** are in agreement with the above concept.

Table- 5.3: Mechanical properties of non oxygen and oxygen delignified bleached mixed hardwood pulp

Parameter	Unbleached			DED bleached beaten pulp (85% ISO brightness)		
	Non oxygen	Oxygen	Change (%)	Non oxygen	Oxygen	Change (%)
Tensile index (Nm/g)	41.2	38.7	- 6.3	70.4	69	- 2
Burst index (kPam^2/g)	2.5	2.3	- 7.9	6.1	6.5	+ 6.2
Tear index (mNm^2/g)	6.7	6.2	- 7.8	6.5	7.7	+ 15.6

Table- 5.4: Mechanical properties of non oxygen and oxygen delignified bleached bagasse pulp

Parameter	Unbleached			DED bleached beaten pulp (85% ISO brightness)		
	Non oxygen	Oxygen	Change (%)	Non oxygen	Oxygen	Change (%)
Tensile index (Nm/g)	35.1	33.8	-3.7	68.5	71.3	+3.9
Burst index (kPam²/g)	3.6	3.3	-8.3	4.4	4.5	+2.2
Tear index (mNm²/g)	5.2	4.7	-9.6	4.9	5	+2

5.3.1.4 Xylanase Pretreatment of Oxygen Delignified Pulp

The results on xylanase pretreatment of oxygen delignified unbleached pulp and oxygen delignified DED bleached pulp are similar to xylanase pretreated non oxygen DED bleached pulps i.e. minor lowering of strength properties due to hemicelluloses and cellulose degradation of the pulps **(Tables 5.5 & 5.6).**

Table- 5.5: Mechanical properties of non xylanase and xylanase treated oxygen delignified bleached mixed hardwood pulp

Parameter	DED bleached beaten pulp (85% ISO brightness)		
	Non Xylanase	Xylanase treated	Change (%)
Tensile index (Nm/g)	69	74	+ 6.8
Burst index (kPam²/g)	6.5	6.5	Nil
Tear index (mNm²/g)	7.7	8.2	+ 6.1

Table- 5.6: Mechanical properties of non xylanase and xylanase pretreated oxygen delignified bleached bagasse pulp

Parameter	DED bleached beaten pulp (85% ISO brightness)		
	Untreated	Xylanase treated	Change (%)
Tensile index (Nm/g)	71.3	73	+2.3
Burst index (kPam²/g)	4.5	4.6	+2.2
Tear index (mNm²/g)	5	5.3	+5.7

The tensile index is improved just after xylanase pretreatment in both mixed hardwood and bagasse pulps. A smaller change is found in burst & tears indices. Therefore, it appears that xylanase pretreatment does not cause a change in fiber bonding because it is known from the literature that beating of the pulp increases bonding of pulp fibers.

A minor decrease in tensile, tear and burst indices after enzyme pretreatment have also been reported in bamboo and hardwood bleached pulps at the same level of beating [25, 86].

5.4   ACTION OF XYLANASE ON FIBRE SWELLING

The fibre swelling is represented by water retention value (WRV) of the fibre. Water retention values of control and xylanase pretreated pulps at varying enzyme doses for oxygen & non oxygen mixed hardwood (10 & 16 IU/g) and bagasse (18 & 20 IU/g) respectively were studied. A minor reduction in water retention value has been observed. This may be due to the action of xylanase which break the bonding between lignin and cellulose by hydrolyzing the hemicelluloses in the pulps resulting reduction in bleach chemical demand of the pulp for the desired target brightness. Due to less exposure to bleach chemicals resulting less cellulose degradation pulps, show little improvement in the mechanical properties of the pulps. The hemicelluloses peaks found in the IR studies of xylanase treated effluents. The scheme followed is given in the **Figures 5.1 a & b & 5.2 a & b.** Results show that non oxygen pulp of mixed hardwood & bagasse pulps have higher water retention value (WRV) as compared to oxygen delignified pulps.

5.4.1   Effects of Beating and Bleaching on Water Retention Value of Non Oxygen Pulps

The effect of beating and bleaching on the water retention value of unbleached pulps are shown in **Figure 5.1.** Bleaching results in the loss of hemicelluloses, lignin and extractives. The removal of lignin, extractives and hemicelluloses result in the removal of certain amount of COOH group, which in turn decreases the water retention value. The results depicted in the **Figure 5.1,** are in agreement with the above concept.

The beating of bleached pulps results in fibre swelling and hence increases the WRV. This is reflected by a substantial increase in WRV and tensile index of beaten pulps.
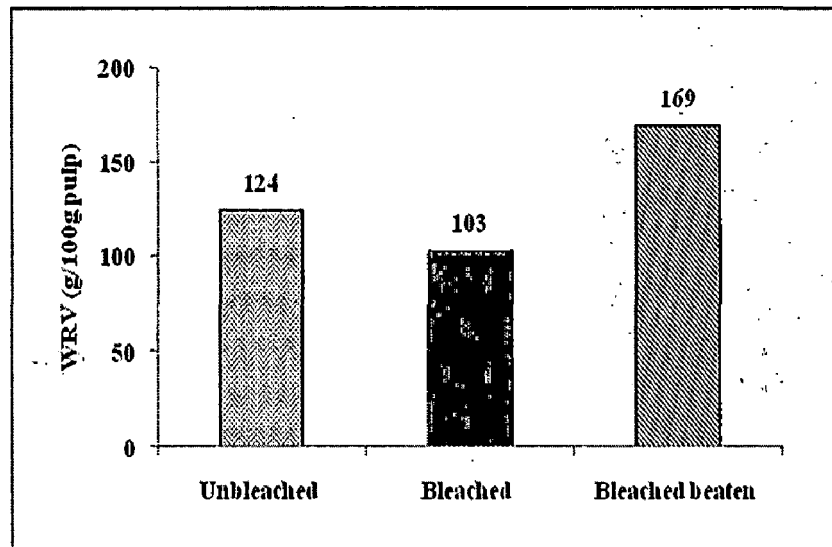
(a)
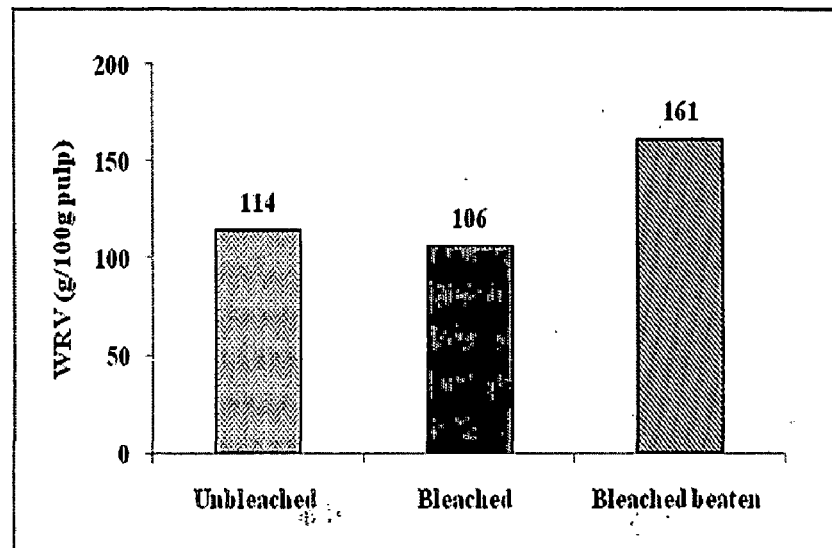
(b)

Figure 5.1: Water Retention Values of oxygen (12 IU/g) and non oxygen (16 IU/g) mixed hardwood pulp at an optimized xylanase dose

5.4.2 Effect of Beating and Bleaching on Water Retention Value of Oxygen Delignified Pulps

The WRV results (**Figures 5.2**) show that oxygen delignified pulps show similar behavior as non oxygen pulps. The oxygen pretreated unbleached pulps show lower water retention values than non oxygen pulps. This may be due to lower pentosan content in oxygen delignified pulp (28.8%) than non oxygen pulps (31.6%).
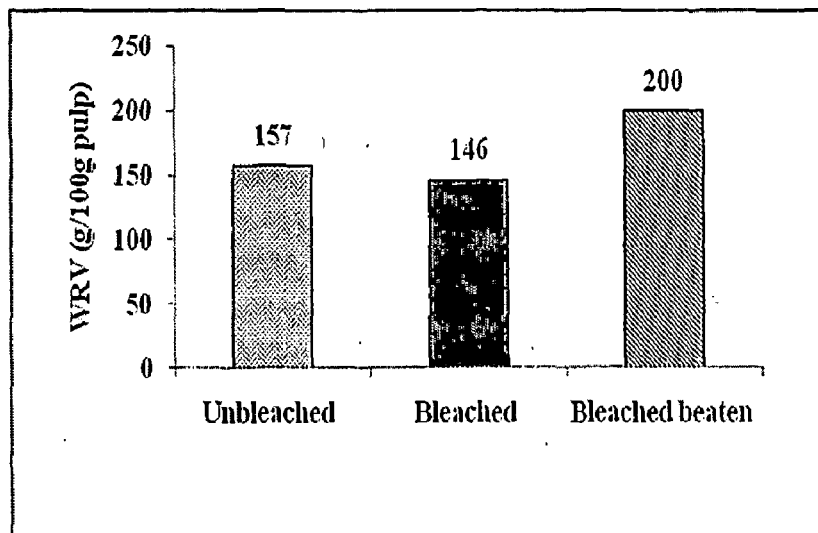
(a)
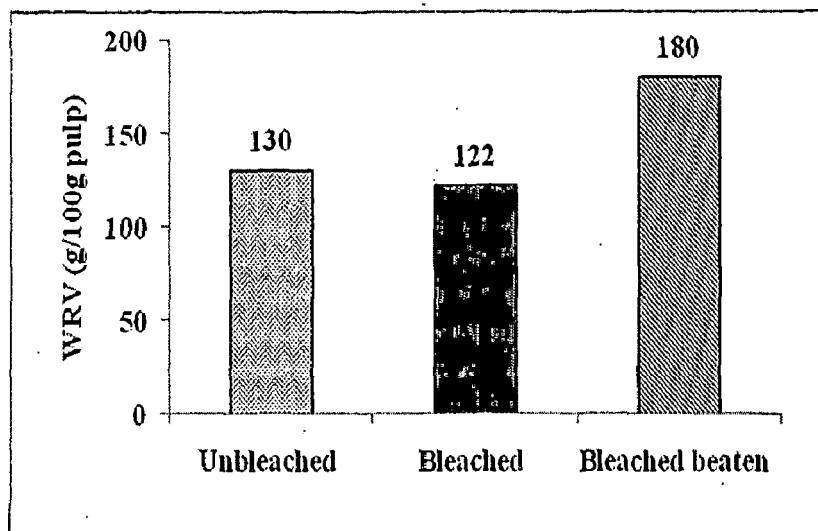
(b)

Figure 5.2: Water Retention Values of oxygen (16 IU/g) (a) and non oxygen (20 IU/g) (b) bagasse pulp at an optimized xylanase dose

5.4.3 Bleachability of DMSO Extracted Oxygen and Non Oxygen Pulps

The earlier research shows that the re-precipitated xylan can be removed by extraction with DMSO [132]. The results shown in **Figures 5.3 & 5.4** indicate that extraction with DMSO does not lead to any improvement in the final brightness of non oxygen and oxygen delignified pulps. Thus DMSO pretreatment does not affect the bleachability of non oxygen and oxygen delignified both types of pulp.
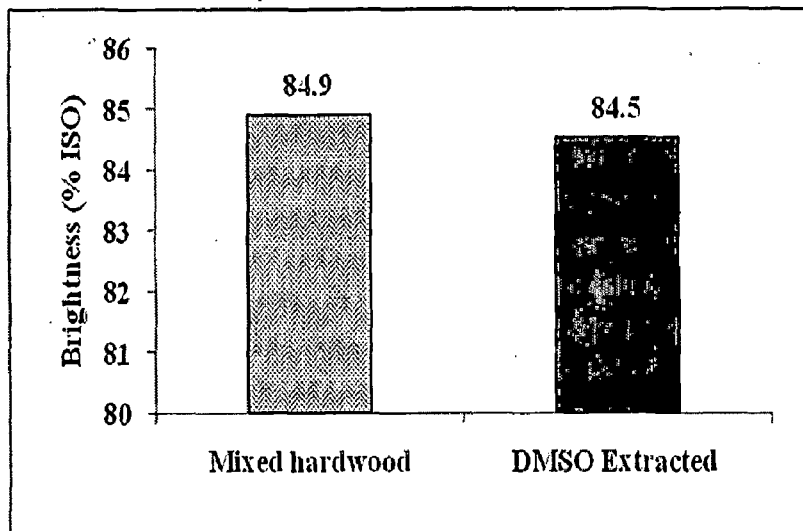
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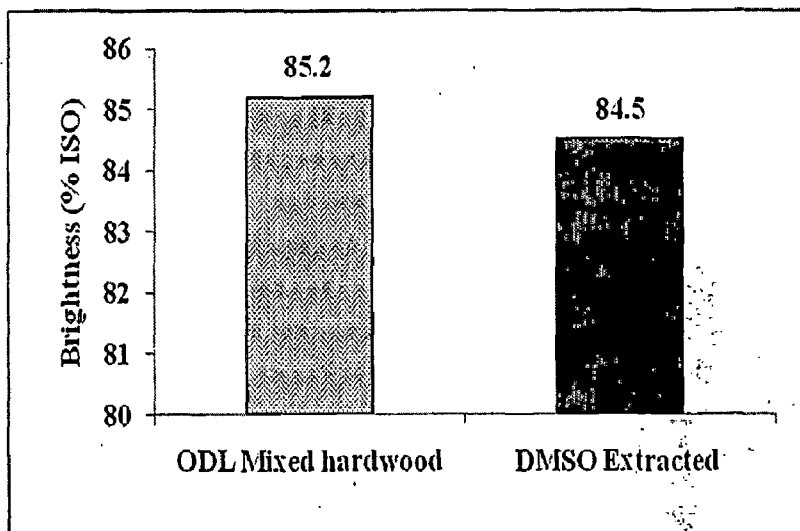
(b)

Figure- 5.3: Effect of DMSO extraction on bleachability of non oxygen mixed hardwood (a) and oxygen delignified mixed hardwood (b) pulps
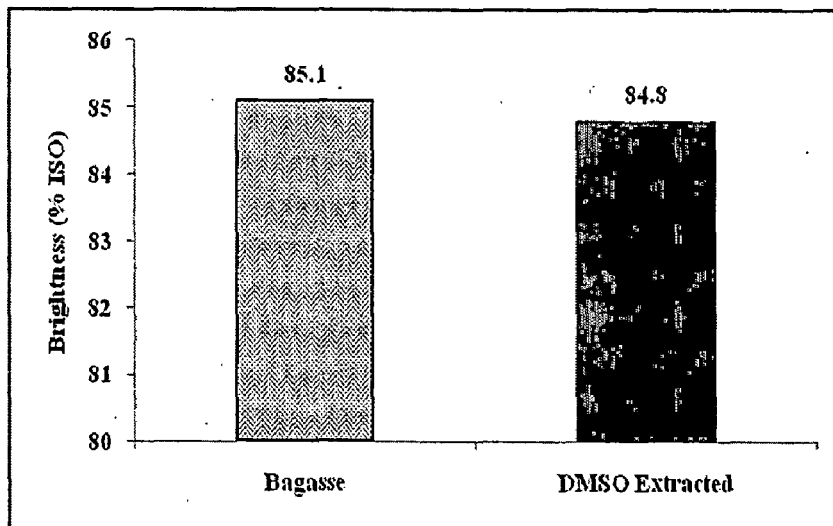
(a)
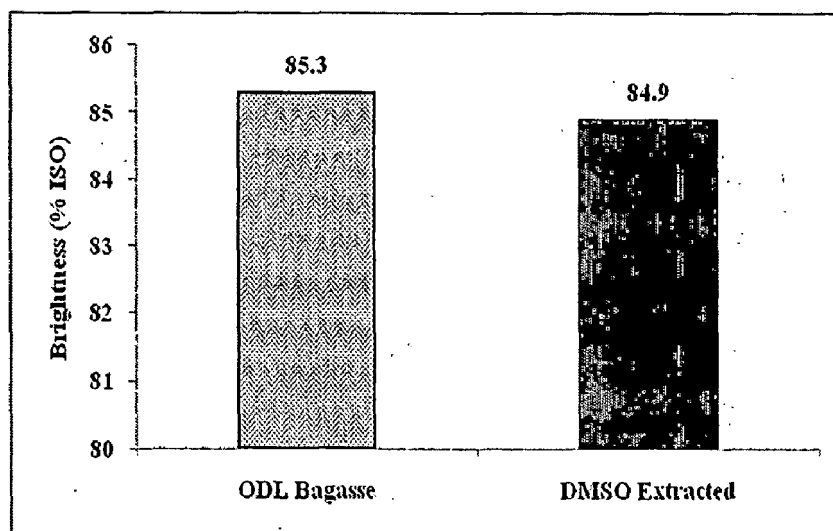
(b)

Figure- 5.4: Effect of DMSO extraction on bleachability of non oxygen bagasse (a) and oxygen delignified bagasse (b) pulps

5.4.4 Bleachability of DMSO Extracted on Xylanase Pretreated Pulps

The action of xylanase on both oxygen and non oxygen DMSO extracted pulp show improvement in final brightness (Figure 5.5 & 5.6). DMSO extraction is expected to remove re-precipitated surface xylan. It shows that in both the pulps, the fibre wall xylan is accessible for attack by xylanase.
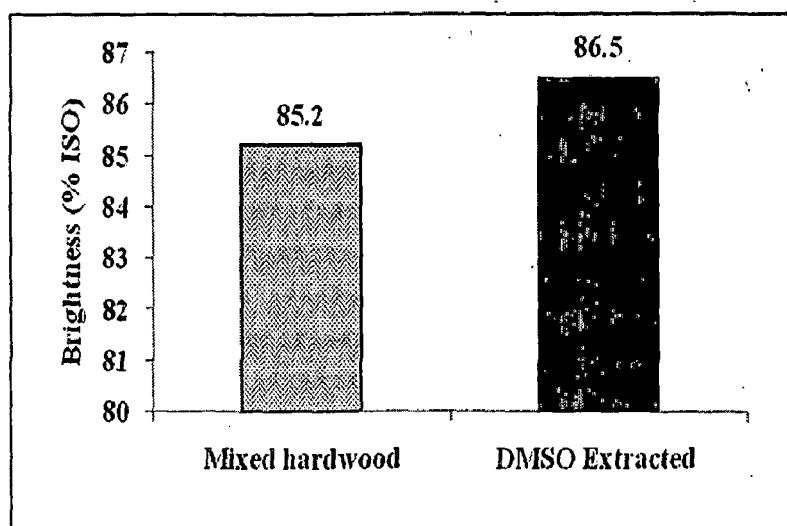
(a)
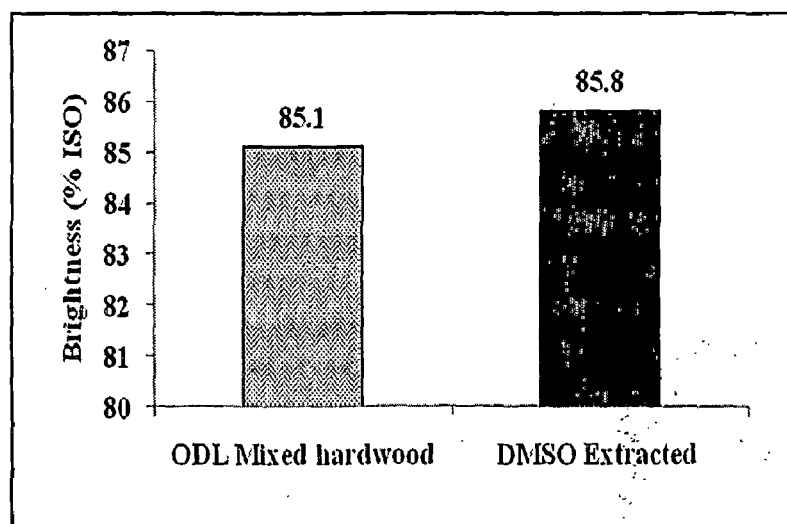
(b)

Figure- 5.5: Effect of DMSO extraction on bleachability of xylanase pretreated non oxygen mixed hardwood (a) and oxygen delignified mixed hardwood (b) pulps
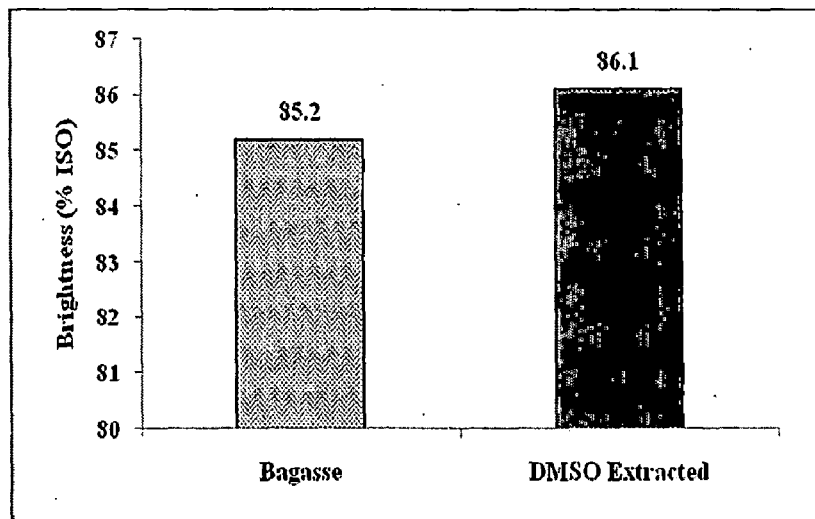
(a)
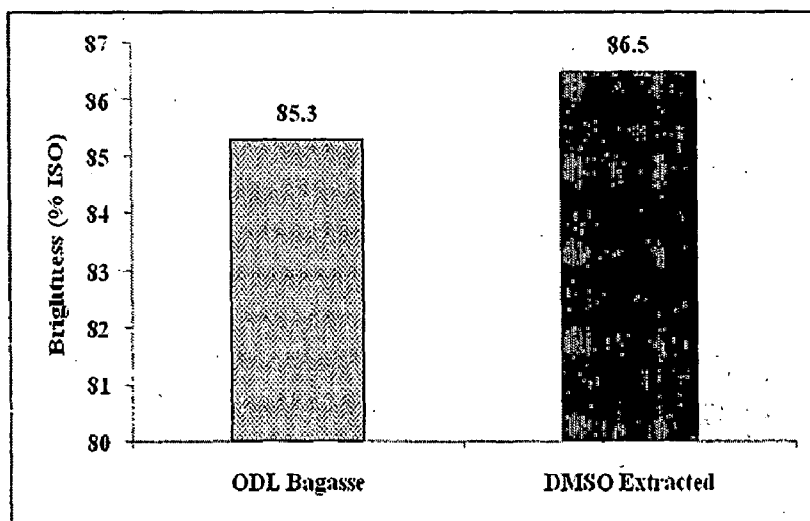
(b)

Figure- 5.6: Effect of DMSO extraction on bleachability of xylanase pretreated non oxygen bagasse (a) and oxygen delignified bagasse (b) pulps

The above results are in agreement with the literature. The pulp extracted with DMSO still remained amenable to xylanase aided bleaching. The importance of re-deposited xylan in xylanase aided bleaching has since been disputed with results from certain studies which indicate that low xylan pulps responded to xylanase aided bleaching [235] and the removal of surface xylan with DMSO did not eliminate pulp response to enzymatic pre-bleaching. The
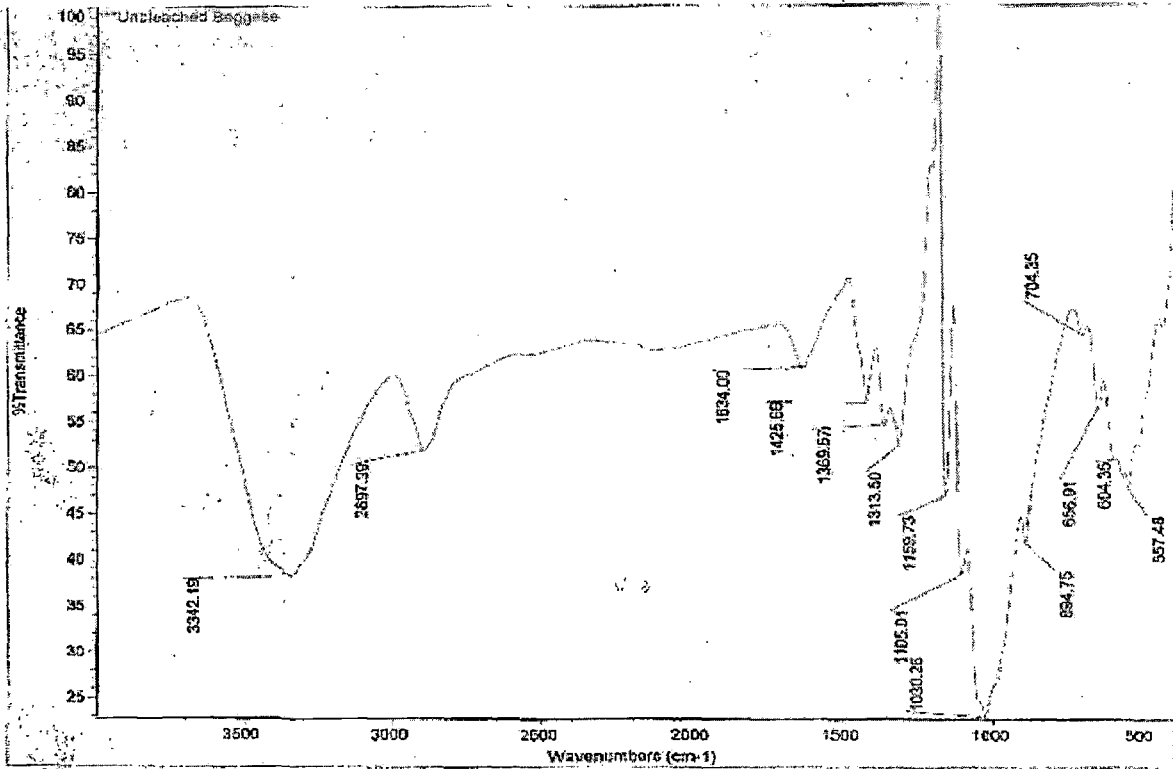
(a)
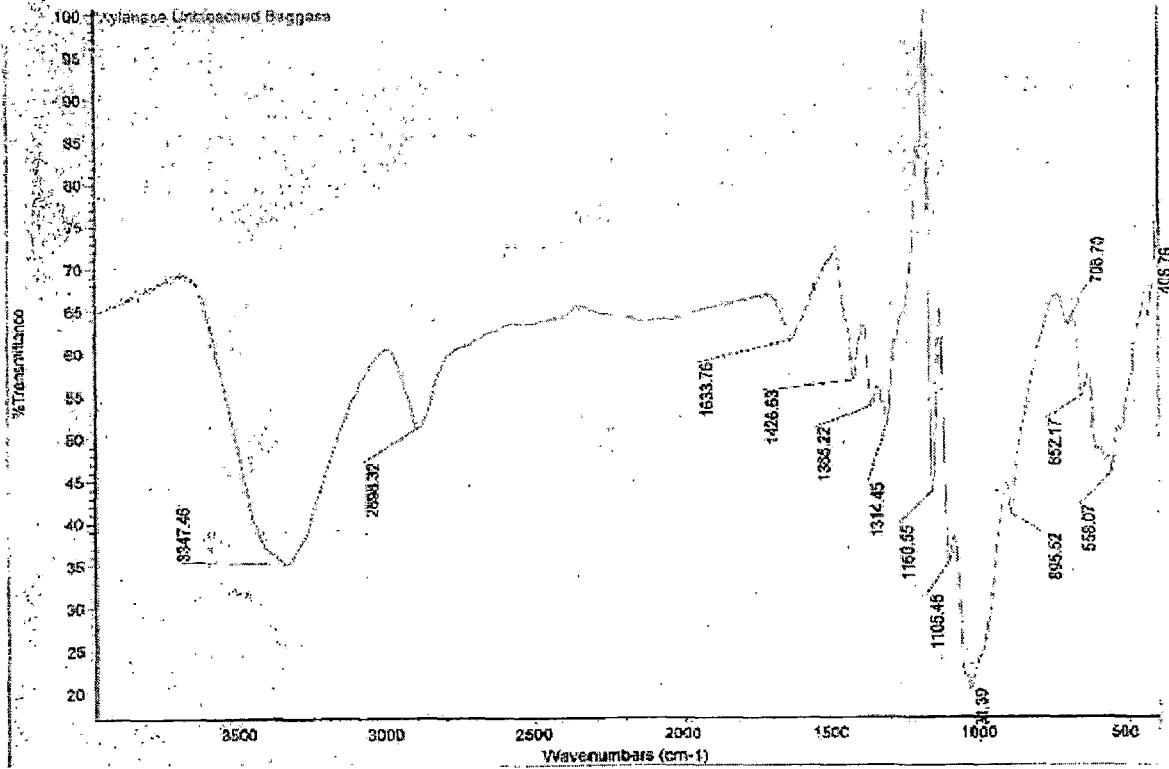
(b)

Figure 5.13: Infrared spectrum of untreated (a) and xylanase treated bagasse pulps (b)
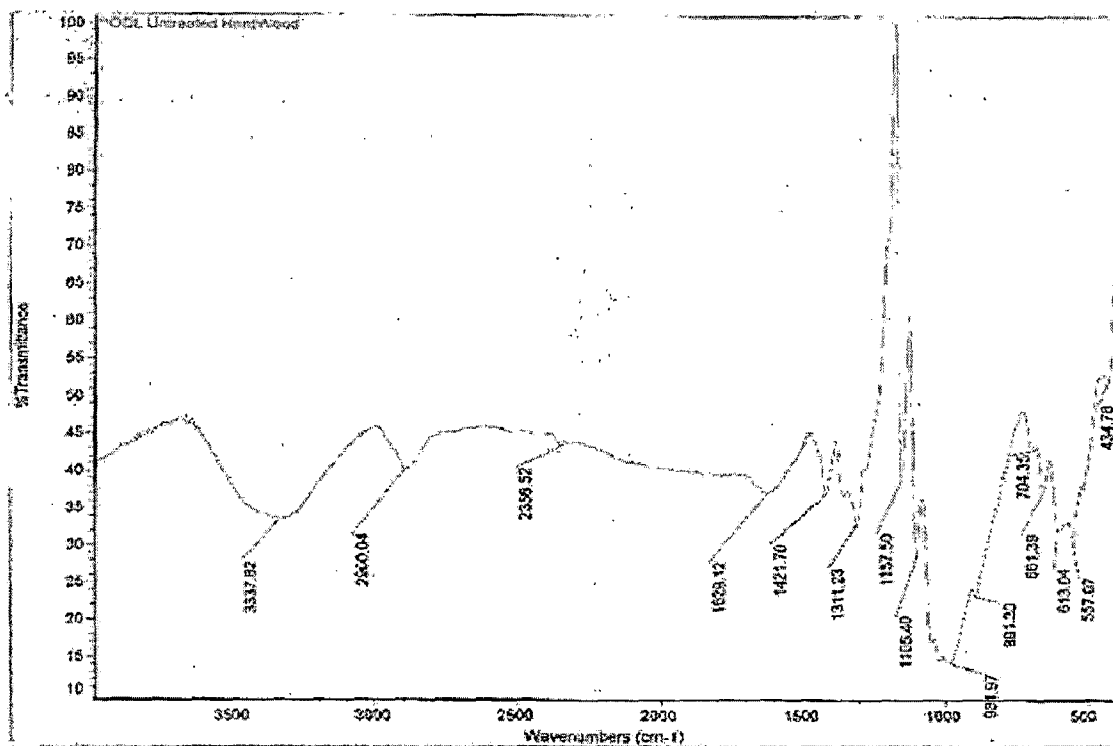
(a)
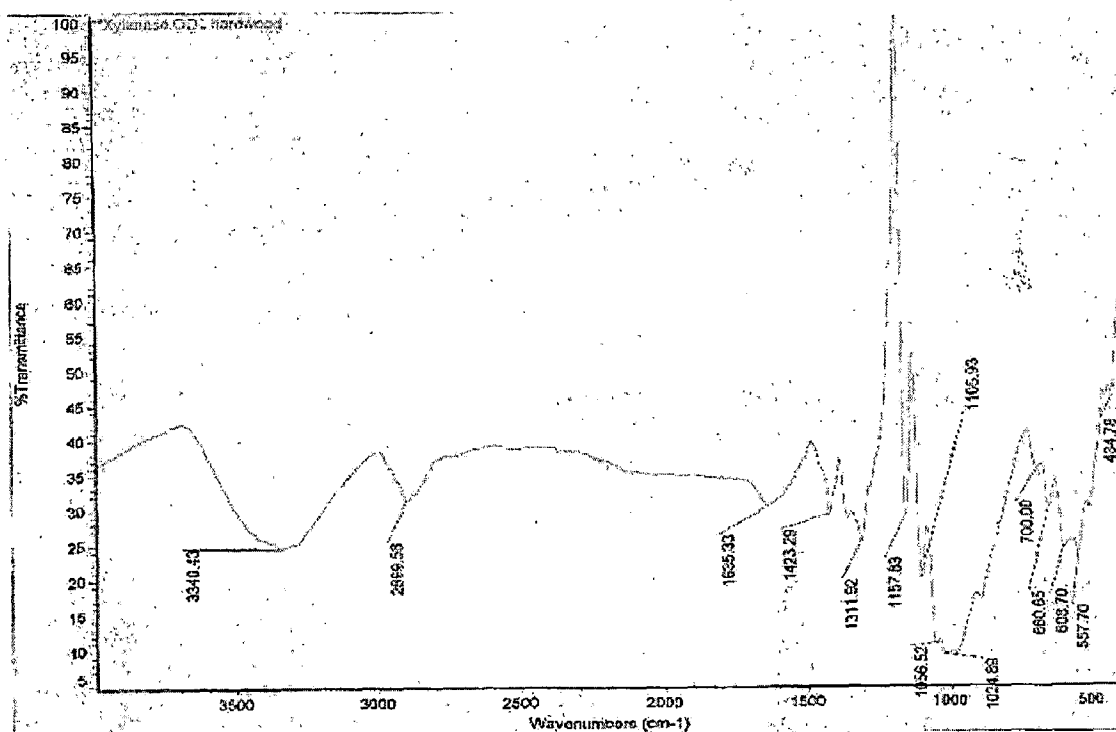
(b)

Figure 5.12: Infrared spectrum of untreated treated oxygen delignified (ODL) (a) and xylanase treated mixed hardwood pulps (b)
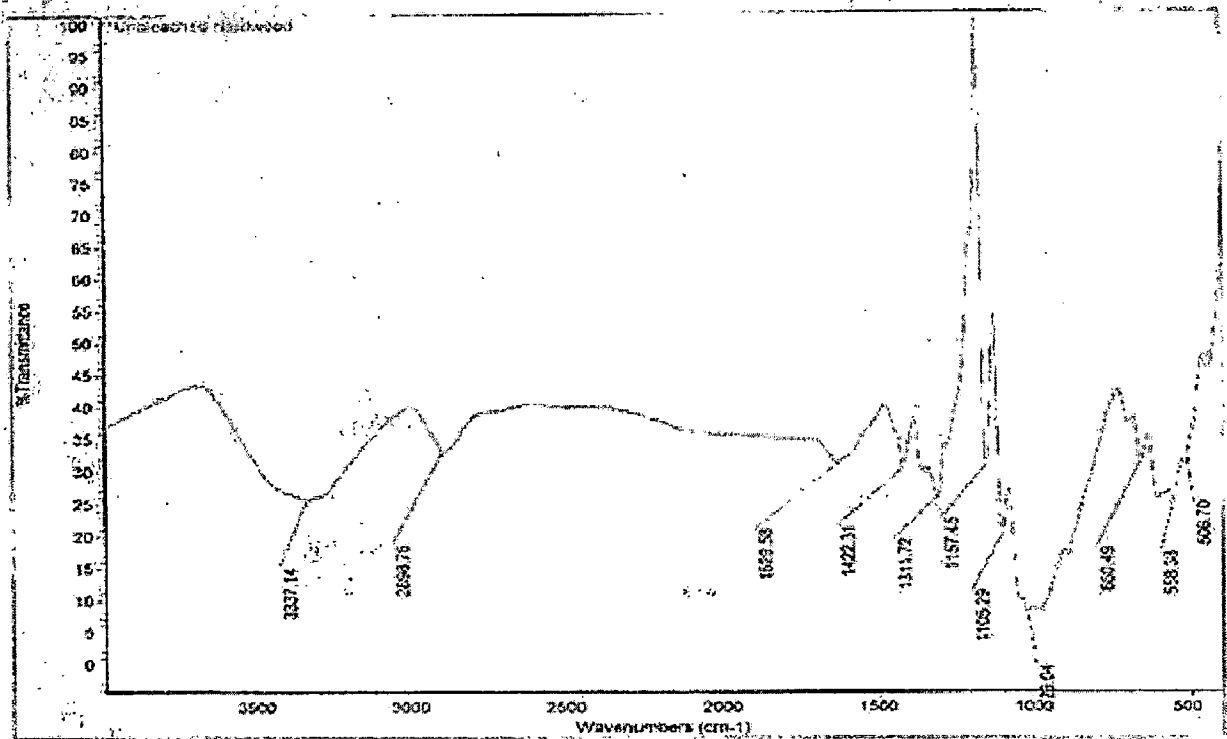
(a)
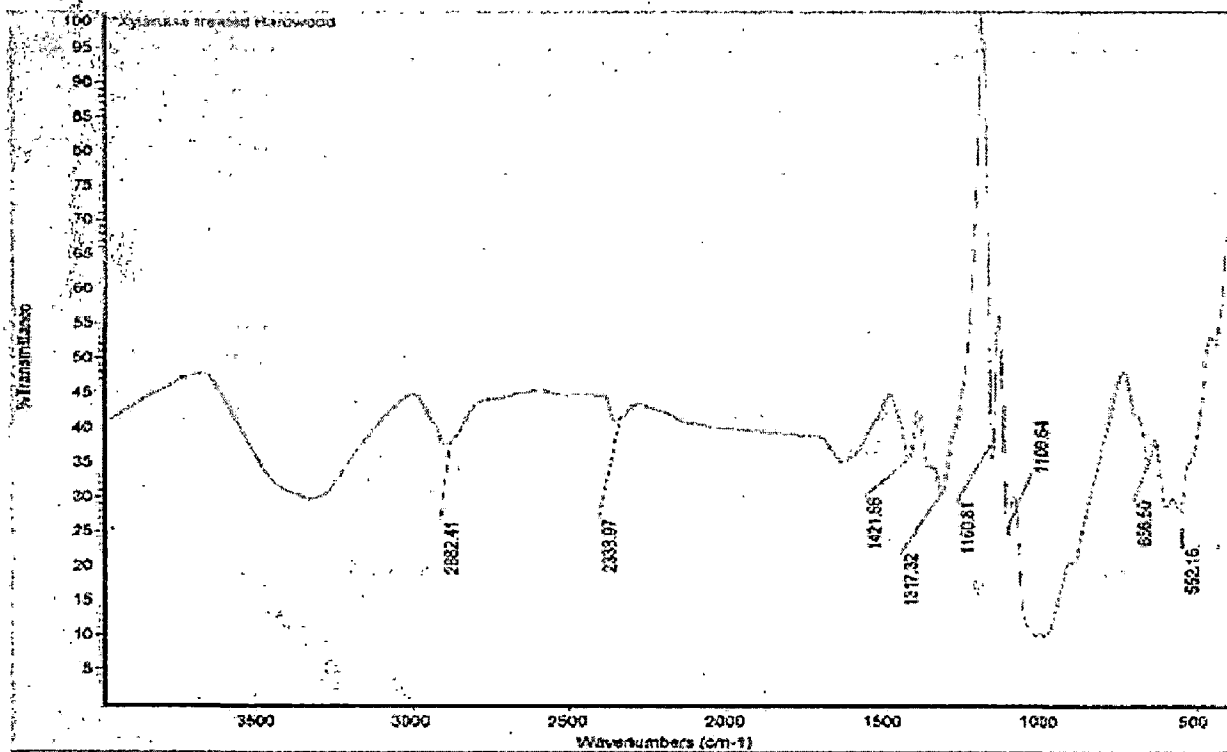
(b)

Figure 5.11: Infrared spectrum of untreated (a) and xylanase treated mixed hardwood pulps (b)

1313.19	1314.09	CH deformation
1365.22	1360.87	OH plane bending vibration of lignin
1425.39	1426.31	CH_2 scissors vibration in cellulose, C-H bending vibration in methoxyl groups
1635.27	1637.79	O-H stretching vibration of absorbed water
2898.21	2897.92	CH stretching vibration
3340.24	3339.26	OH stretching (intramolecular hydrogen bond in 101 plane)

Table 5.12: Comparison of IR Spectrum of xylanase treated non oxygen and oxygen delignified mixed hardwood pulp effluents

Untreated (cm^{-1})	Xylanase treated (cm^{-1})	Assignment
-	548.76	OH out of plane bend
690.88	-	OH out of plane bend
1382.53	1397.52	CH bending
1641.95	1636.5	O-H stretching vibration of absorbed water
2060.74	2075.52	Absorbed water, unassigned peak for cellulose
2357.84	2357.5	Unassigned cellulose peak
3256.29	-	CH stretching, OH stretching
-	3452.07	OH stretching
3615.72	-	OH stretching
3738.86	-	OH stretching

Table 5.13: Comparison of IR Spectrum of xylanase treated non oxygen and oxygen delignified bagasse pulp effluents

Untreated (cm^{-1})	Xylanase treated (cm^{-1})	Assignment
570.48	-	OH out of plane bend
669.54	664.23	OH out of plane bend
1460.78	-	CH deformation (asymmetric), lignin & CH_2 bending vibration in xylan
1547.73	1565.12	Aromatic skeleton vibrations of lignin, CH deformation (asymmetric), lignin & CH_2 bending vibration in xylan
1642.38	1642.10	O-H stretching vibration of absorbed water
2358	2358.05	CH stretching
3453.12	3451.46	OH stretching
3734.51	-	OH stretching

2900.04	2899.58	CH stretching vibration
3337.82	3340.43	OH stretching intramolecular hydrogen bond in cellulose & hemicellulose

Table 5.10: Comparison of IR Spectrum of untreated and xylanase treated bagasse pulps

Untreated (cm^{-1})	Xylanase treated (cm^{-1})	Assignment
-	408.76	C-O-C bending vibration
557.48	558.07	C-O-C bending vibration
604.35	-	OH out of plane deformation
656.91	652.17	OH out of plane bend
704.35	-	OH out of plane bend
-	895.52	Vibration involving the C_1 group in D(1-4) linked polymers of D-glucose & D-xylose
1030.26	1031.39	C-O & C-C stretching & CH_2 rocking vibration, C-O-C asymmetric stretching vibration in dialkyl ether linkages
1105.01	1105.46	C-O & C-C stretching and CH_2 rocking vibration
1159.73	1160.55	C-O & C-C stretching and CH_2 rocking vibration
1313.5	1314.45	CH deformation
1369.57	1365.22	CH deformation (symmetric)
1425.66	1426.63	CH_2 scissors vibration in cellulose, C-H bending vibration in methoxyl groups
1634	1633.76	O-H stretching vibration of absorbed water
2897.39	2898.32	CH stretching vibration
3342.19	3347.46	OH stretching (intramolecular hydrogen bond in 101 plane)

Table 5.11: Comparison of IR Spectrum of untreated and xylanase treated oxygen delignified bagasse pulps

Untreated (cm^{-1})	Xylanase treated (cm^{-1})	Assignment
443.48	-	C-O-C bending vibration
557.13	556.27	C-O-C bending vibration
658.37	656.52	OH out of plane bend
708.7	704.35	OH out of plane bend
891.3	895.13	Vibration involving the C_1 group in D(1-4) linked polymers of D-glucose & D-xylose
1029.79	1030.92	C-O & C-C stretching & CH_2 rocking vibration, C-O-C asymmetric stretching vibration in dialkyl ether linkages
1105.39	1106.13	C-O & C-C stretching and CH_2 rocking vibration
1159.77	1160.28	C-O & C-C stretching and CH_2 rocking vibration

Table 5.8: Comparison of IR Spectrum of untreated and xylanase treated mixed hardwood pulps

Untreated (cm⁻¹)	Xylanase treated (cm⁻¹)	Assignment
508.70	-	C-O-C bending vibration
558.38	552.16	C-O-C bending vibration
660.49	656.5	OH out of plane bend
1026.04	-	C-O & C-C stretching & CH_2 rocking vibration, C-O-C asymmetric stretching vibration in dialkyl ether linkages
1105.29	1108.64	C-O & C-C stretching and CH_2 rocking vibration
1157.45	1160.81	C-O & C-C stretching and CH_2 rocking vibration
1311.72	1317.32	CH deformation
1422.31	1421.66	CH_2 scissors vibration in cellulose, C-H bending vibration in methoxyl groups Aromatic skeleton vibrations of lignin
1629.58	-	O-H stretching vibration of absorbed water
-	2338.97	Unassigned cellulose peak
2898.76	2882.41	CH stretching
3337.14	-	OH stretching (intramolecular hydrogen bond in 101 plane)

Table 5.9: Comparison of IR Spectrum of untreated and xylanase treated oxygen delignified mixed hardwood pulps

Untreated (cm⁻¹)	Xylanase treated (cm⁻¹)	Assignment
434.78	434.78	C-O-C bending vibration
557.07	557.70	C-O-C bending vibration
613.04	608.7	OH out of plane bend
661.39	660.65	OH out of plane bend
704.35	700	OH out of plane bend
891.3	-	Vibration involving the C_1 group in D(1-4) linked polymers of D-glucose & D-xylose
981.97	-	C-O stretching
-	1024.89	C-O & C-C stretching & CH_2 rocking vibration, C-O-C asymmetric stretching vibration in dialkyl ether linkages
-	1056.52	C-O stretching
1105.4	1105.93	C-O & C-C stretching and CH_2 rocking vibration
1157.5	1157.83	C-O & C-C stretching and CH_2 rocking vibration
1311.23	1311.92	CH deformation
1421.7	1423.29	CH_2 scissors vibration in cellulose, C-H bending vibration in methoxyl groups
1629.12	1635.33	O-H stretching vibration of absorbed water
2356.52	-	Unassigned cellulose peak

1720	Absorbed water
2340	Unassigned cellulose peak
2500	Unassigned cellulose peak
2700	Unassigned cellulose peak
2820-2845	OH stretching in methyl & methylene groups, C-H stretching vibration in methoxyl and CH_2 symmetric stretching vibration
2850-2880	OH stretching in methyl & methylene groups, C-H stretching vibration in tertiary CH groups
2851	CH_2 symmetric stretching
2853	CH_2 symmetric stretching
2873	CH stretching
2897	CH stretching
2900	CH stretching vibration
2907	CH_2 stretching
2910	CH stretching
2914	CH streching
2920-2940	OH stretching in methyl & methylene groups, CH_2 antisymmetric stretching vibration
2945	CH_2 antisymmetric stretching
2967	CH_2 antisymmetric stretching
2970	CH stretching
3000	C-H stretching vibration
3245	Unassigned Cellulose peak
3275	Unassigned Cellulose peak
3300	OH stretching
3305	OH stretching (intramolecular hydrogen bond in 101 plane)
3350	OH stretching (intramolecular hydrogen bond in 101 plane)
3400	OH stretching
3405	OH stretching (intramolecular hydrogen bond in 101 plane)
3400-3450	OH stretching vibration

1130	Aromatic CH in plane deformation sryingyl type
1140-1145	Aromatic CH in plane deformation sryingyl type
1150	Vibration of less common type carbon oxygen linkages
1161	C-O & C-C stretching and CH_2 rocking vibration
1162	Antistretching bridge oxygen stretching
1180	Vibration of less common type carbon oxygen linkages
1202	CH_2 wagging & CH & OH deformation
1205	OH in plane bending of cellulose
1232	CH_2 wagging & CH & OH deformation
1235	Syringyl ring breathing with CO stretching
1249	CH_2 wagging & CH & OH deformation
1250	Unassigned cellulose peak
1257	CH_2 wagging & CH & OH deformation
1265	C-O-C asymmetric stretching vibrations of aryl ether linkages
1270-1275	Guaiacyl ring breathing with CO stretching
1282	CH bending
1310	CH deformation
1317	CH_2 wagging
1320	CH_2 wagging vibration in cellulose
1330-1325	Syringyl ring breathing with CO stretching
1333	OH in plane bending vibration of cellulose & hemicellulose
1335	CH in plane deformation
1336	CH n plane bending
1358	Unassigned cellulose peak
1360	OH plane bending vibration of lignin
1365	CH deformation
1365-1370	CH deformation (symmetric)
1374	CH bending
1425	CH_2 scissors vibration in cellulose, C-H bending vibration in methoxyl groups*
1426	CH_2 symmetric band
1430	CH_2 bending
1425-1440	Aromatic skeleton vibrations of lignin
1446	OH in plane deformation
1455	CH in plane bending
1460-1470	CH deformation (asymmetric), lignin & CH_2 bending vibration in xylan
1505-1515	Aromatic skeleton vibrations of lignin
1595-1600	Aromatic skeleton vibrations of lignin, COO^- ion in gloconoxylan after salt formation, C-C stretching vibration of benzene ring
1630	O-H stretching vibration of absorbed water
1635	O-H stretching vibration of absorbed water
1650	O-H stretching vibration of absorbed water
1660-1675	Carbonyl stretching, parasubstituted aryl ketone, C=O stretching vibration of 1 keto group
1710-1715	Carbonyl stretching, uncongugated ketone and carboxyl groups
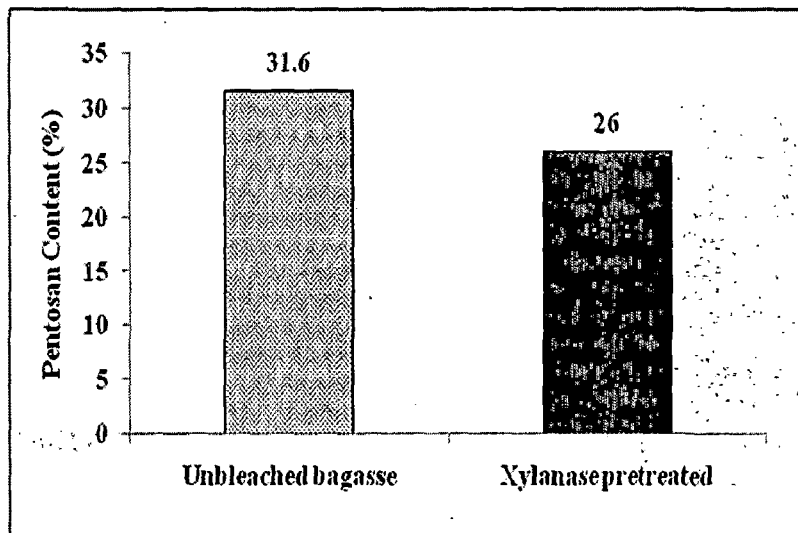
Figure 5.9: Pentosan contents of unbleached and xylanase pretreated bagasse pulps
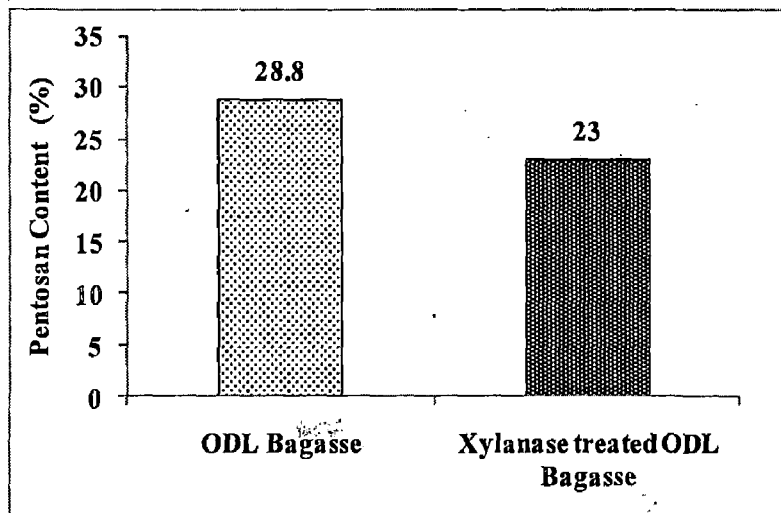
Figure 5.10: Pentosan contents of oxygen delignified (ODL) and xylanase pretreated oxygen delignified bagasse pulps

5.6 INFRARED SPECTROSCOPY STUDIES

The infrared spectroscopy studies have been performed on xylanase pretreated pulps and effluents generated. The scheme of performing the study is shown in the **Figure C**.
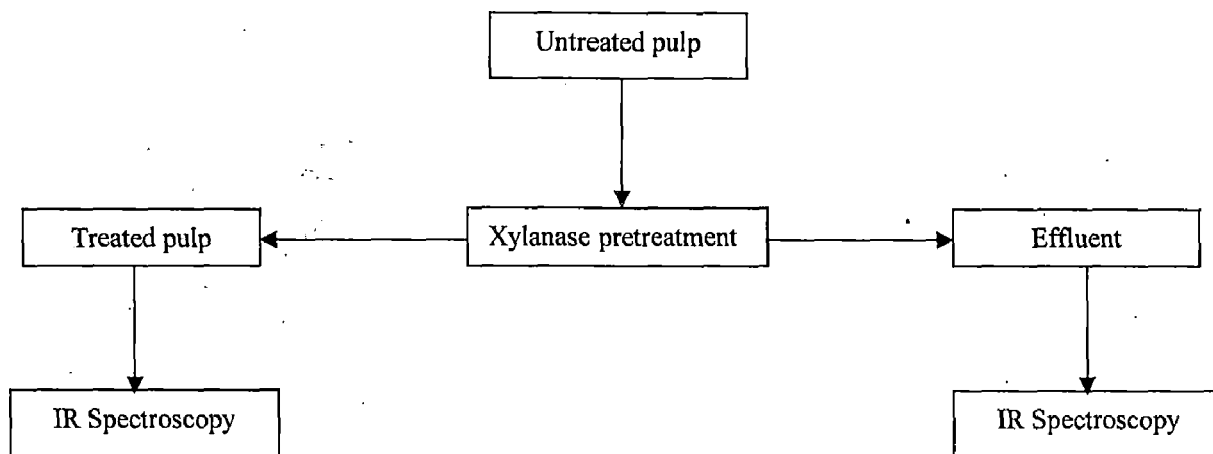
Figure C: IR Spectroscopy of Xylanase Pretreated Pulps and Effluents

5.6.1 Xylanase Pretreated Pulps

The IR peaks observed in the literature for lignin, hemicellulose and cellulose are given in **Table 5.7** (80, 127).

The various IR peaks observed with untreated and xylanase pretreated mixed hardwood pulp and their assignments are given in **Table 5.8**. Similar information for untreated and xylanase pretreated mixed hardwood pulp is given in **Figure 5.11 a & b**. The peak at 1422 cm^{-1} is assigned to CH_2 scissors vibration in cellulose, aromatic skeleton vibrations of lignin and peak at 3337 cm^{-1} is assigned to OH stretching intramolecular hydrogen bond in cellulose & hemicellulose. These peaks are observed with mixed hardwood untreated pulp. The peak around 1421 cm^{-1} is weakened during xylanase pretreatment of mixed hardwood untreated pulp. The weakening of 3337 peak indicates the dissolution of lignin whereas 1421 cm^{-1} is weakened during xylanase pretreatment of mixed hardwood untreated pulp. The weakening of 3337 cm^{-1} peak indicates action of xylanase on hemicelluloses content.

Information given in **Table 5.9** for untreated and xylanase pretreated oxygen delignified mixed hardwood pulp and **Figure 5.12 a & b** for untreated and xylanase pretreated oxygen delignified mixed hardwood pulp reveals the similar information. The weakening of 891 cm^{-1}

peak indicates partial hydrolysis of β-(1-4) linkages of xylan. The IR studies reveals similar information with bagasse pulp **Tables 5.10–5.11 & Figures 5.13 a, b & 5.14 a, b** as obtained with mixed hardwood pulp.

5.6.2 Effluents from Xylanase Pretreatment

The results obtained from IR studies of untreated and xylanase pretreated non oxygen and oxygen delignified mixed hardwood pulp effluents shows no significant changes in pulp content (**Table 5.12** & Figure 5·15). The presence of a very weak peak at 1547 cm^{-1} observed with xylanase treated bagasse pulp effluent is assigned to aromatic skeleton vibration of lignin indicating that lignin is present in enzyme effluent of bagasse which showed that xylanase working not only on hemicelluloses but also working on lignin content of the pulp (**Tables 5.13** and **Figures 5.16 a & b**)

The peaks observed at 1547 and 1565 cm^{-1} in the non oxygen & oxygen delignified, untreated & xylanase treated bagasse effluents are assigned to lignin (**Table 5.13 and Figures 5.16 a & b**). These peaks indicate that lignin is present in xylanase pretreated effluents. The presence of lignin in enzyme treated effluents has also been reported in the literature [146].

β-D-xylanase that are capable of hydrolyzing the (1-4) β-D-xylopyranosyl linkages of 4-0–methyl gluconoxylan have been isolated and characterized from bacteria, yeast and fungi. When the 4-0-methyl gluconoxylan isolated from birch was treated with the endo-xylanases purified from a fungus *Tyromyces palustris*, the neutral sugars in the hydrolyzates were xylose and xylobiose in the molar ratio 1:4. These aldouronic acids carry the 4-0 methyl-D-glucopyranosyluronic acid residue at the non-reducing xylose end groups. This fact indicates that the xylanase has a high affinity to the β(1-4) xylosidic linkage immediately to the left of the acid side chain. On the contrary the two β(1-4)-xylosidic linkage in the right vicinity of the acid side chain are very resistant to the enzyme action due to the steric hindrance offered by the subsitutent. This characteristic mode of xylanase action on 4-0 methyl glucoronoxylan has been reported by many workers [80].

Moreover, the peaks around 1500 cm^{-1} assigned to aromatic skeleton vibration of lignin are visible in enzyme pretreated effluent indicating that enzyme is attacking the lignin carbohydrate complex (LCC) or is hydrolyzing the bond between lignin and hemicellulose. Size exclusion chromatography has confirmed the enzyme attack on lignin hemicellulose bond [98].

The intensity of the lignin peaks in xylanase pretreated oxygen delignified effluents is stronger than the corresponding non oxygen pulps indicating that more lignin is present in effluents of oxygen pulps than non oxygen pulps.

Table 5.7: IR Peaks associated with lignin, hemicellulose and cellulose [80, 127]

| cm^{-1}	Assignment
530	C-O-C bending vibration
650	OH out of plane deformation
663	OH out of plane bend
700	OH out of plane bend
740	CH$_2$ rocking
750-770	Aromatic CH out of plane bend
800	Ring breading
815-835	Aromatic CH out of plane bend
855-860	Aromatic CH out of plane bend
893	Vibration involving the C$_1$ group in D(1-4) linked polymers of D-glucose & D-xylose
915	Aromatic CH out of plane defornation
965	C-O & C-C stretching & CH$_2$ rocking vibration
970	CH out of plane defornation
985	C-O stretching
988-990	C-O & C-C stretching & CH$_2$ rocking vibration
1000	C-O stretching
1005	C-O & C-C stretching & CH$_2$ rocking vibration
1015	C-O stretching
1030	C-O & C-C stretching & CH$_2$ rocking vibration, C-O-C asymmetric stretching vibration in dialkyl ether linkages
1035	C-O stretching, Aromatic CH in plane defornation guaiacyl type, C-O deformation, primary alcohol
1050	C-O stretching vibration in cellulose & hemicellulose
1058	C-O stretching
1069	C-O & C-C stretching and CH$_2$ rocking vibration
1078	C-O & C-C stretching and CH$_2$ rocking vibration
1085	CO deformation, secondary alcohol aliphatic ether
1090	Symmetric stretching analog either of the bend at 1265 or 1230
1106	C-O & C-C stretching and CH$_2$ rocking vibration
1110	Antisymmetric in plane ring stretching in cellulose and hemicellulose, OH associated bends in cellulose and hemicellulose
1117	C-O & C-C stretching and CH$_2$ rocking vibration
1120	C-O-C asymmetric stretching vibrations in dialkyl ether linkages
1125	Unassigned cellulose peak
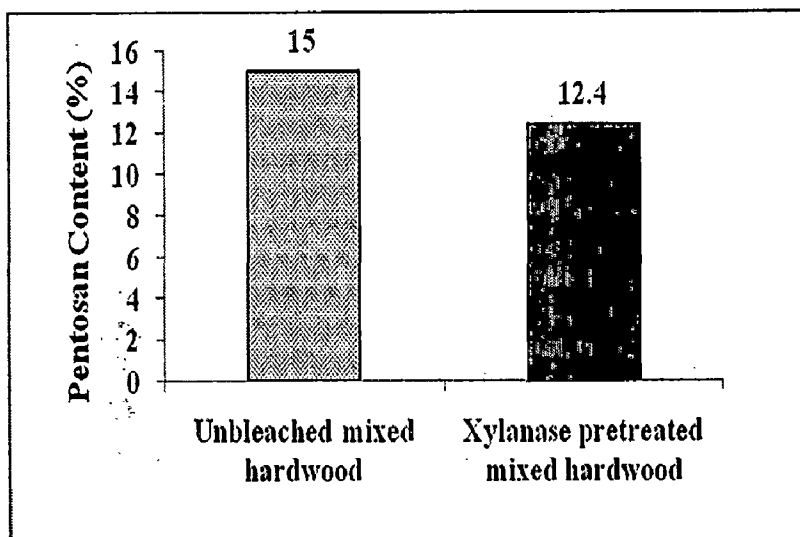
Figure 5.7: Pentosan contents of unbleached and xylanase pretreated mixed hardwood pulps
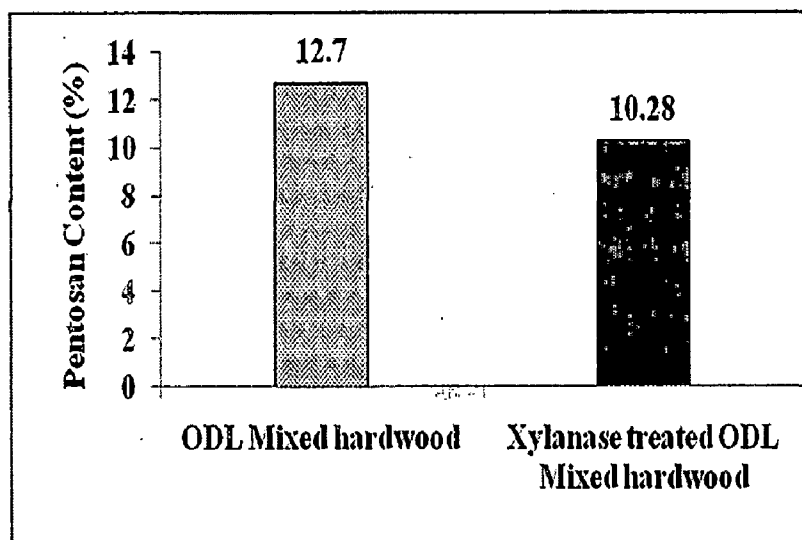
Figure 5.8: Pentosan contents of oxygen delignified (ODL) and xylanase pretreated oxygen delignified mixed hardwood pulps

DMSO does not help to improve brightness. However, the importance of removal of DMSO extractable xylan in the mechanism of xylanase aided may be questioned when radiata pine and oxygen pulps were treated with two commercial enzymes designated as enzyme A and enzyme B, the amount of DMSO extractable xylan increased in all pulps [144].

The results to date suggest that surface xylan may not be the sole target substrate for xylanase aided bleaching. It appears that xylanase tend to hydrolyze surface xylan more due to its higher accessibility. Preliminary studies have also suggested that xylanase can also penetrate the secondary wall of the pulp but it is not clear that the attack of xylan in the wall matrix is necessary to enhance pulp bleaching. However, when hemicelluloses are bound to the fibre matrix other factors such as localization and the structural organization of the substrate within the fibre also affect the efficiency of enzymatic treatment.

5.5 PENTOSAN CONTENT

The pentosan content represents the hemicelluloses content in the pulp. The hemicellulose is being hydrolyzed by the action of xylanase pretreatment of the pulp. The removal of hemicellulose content in the xylanase treated pulp improved the mechanical properties of the pulp due to reduced chemical charge and less exposure to bleach chemicals for the desired target brightness. The pentosan content in untreated pulps is higher than xylanase pretreated pulps. The xylanase plays an important role in the removal of hemicelluloses that is why pentosan content is lower in xylanase treated pulps. The oxygen delignified pulps also show similar behavior (Figures. 5.7 - 5.10).
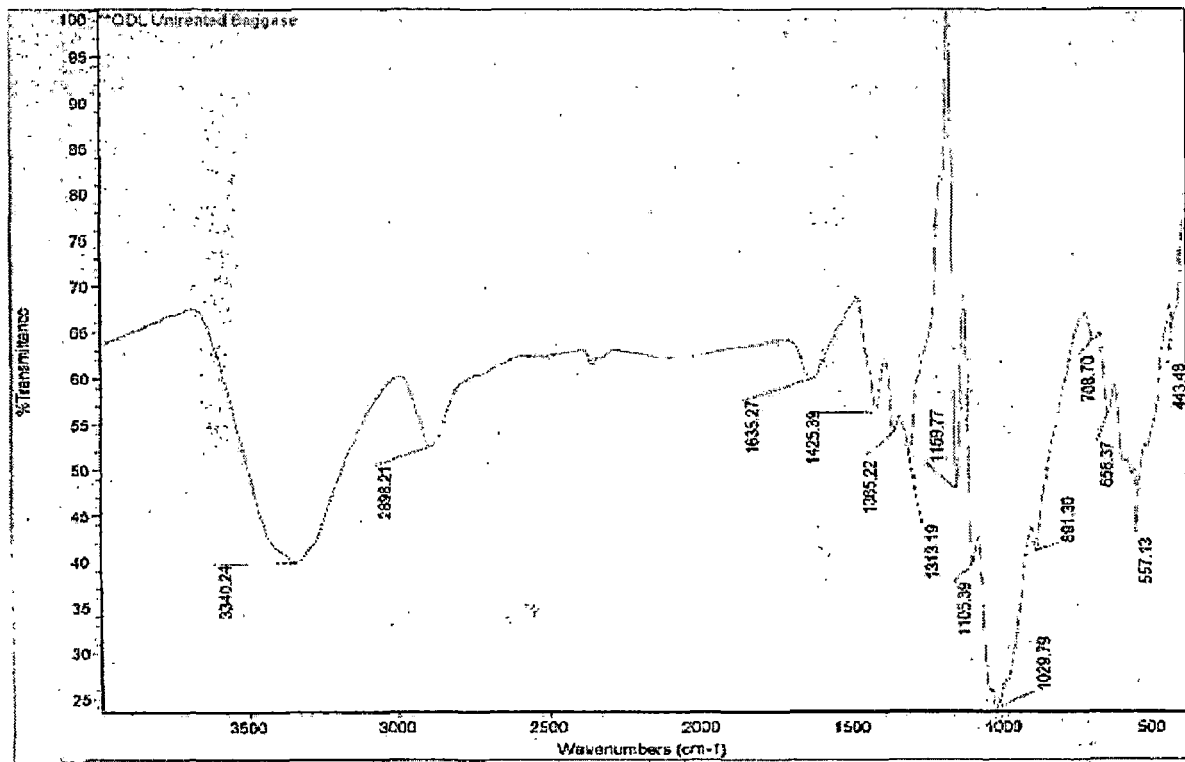
(a)
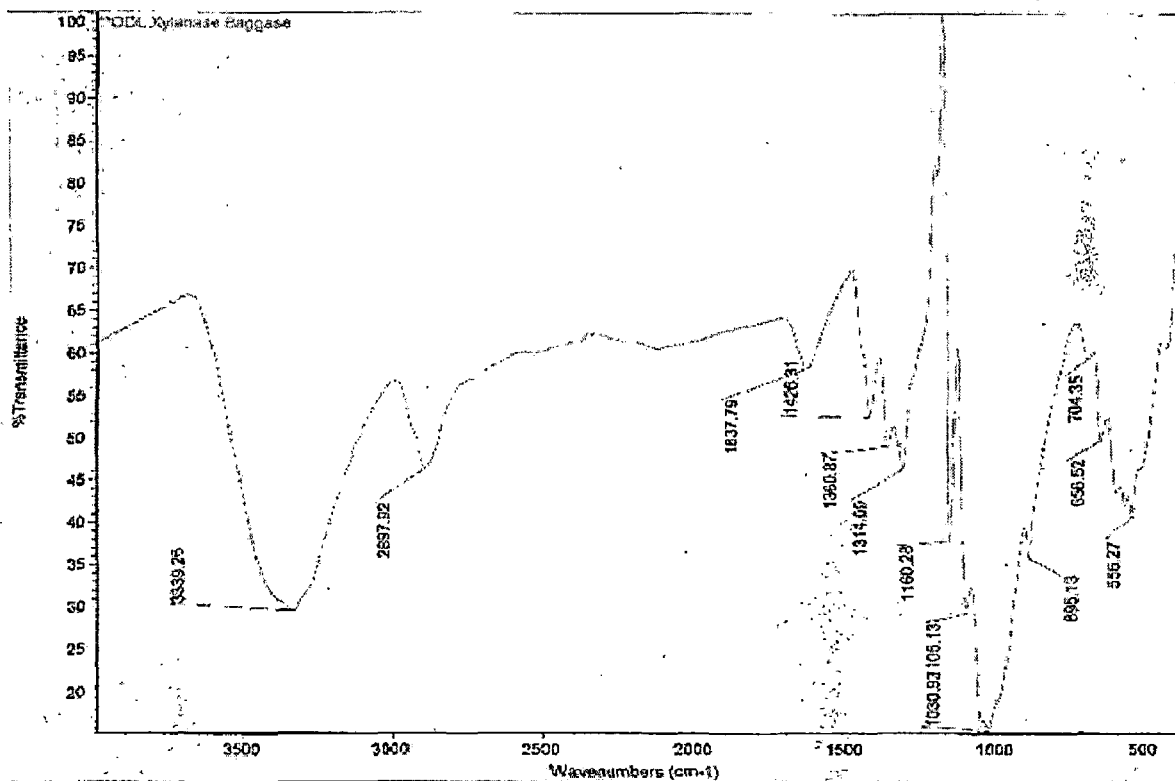
(b)

Figure 5.14: **Infrared spectrum of untreated (a) and xylanase treated oxygen delignified (ODL) bagasse pulps (b)**

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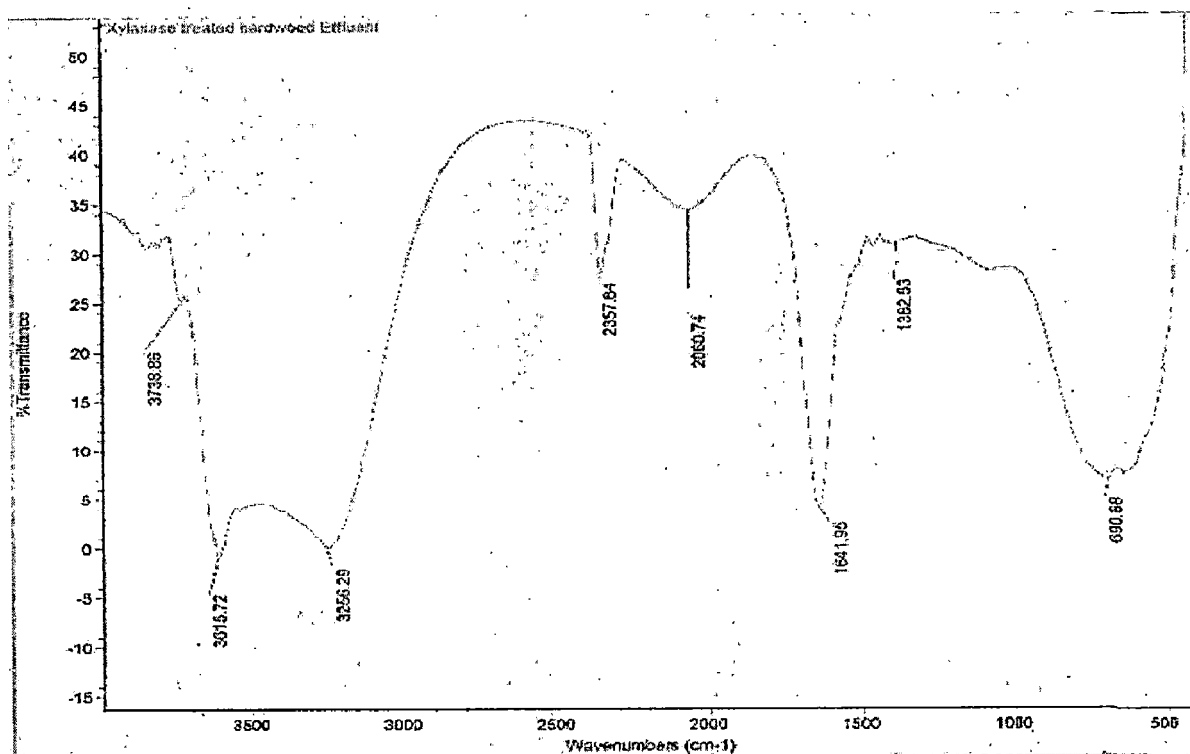
(a)
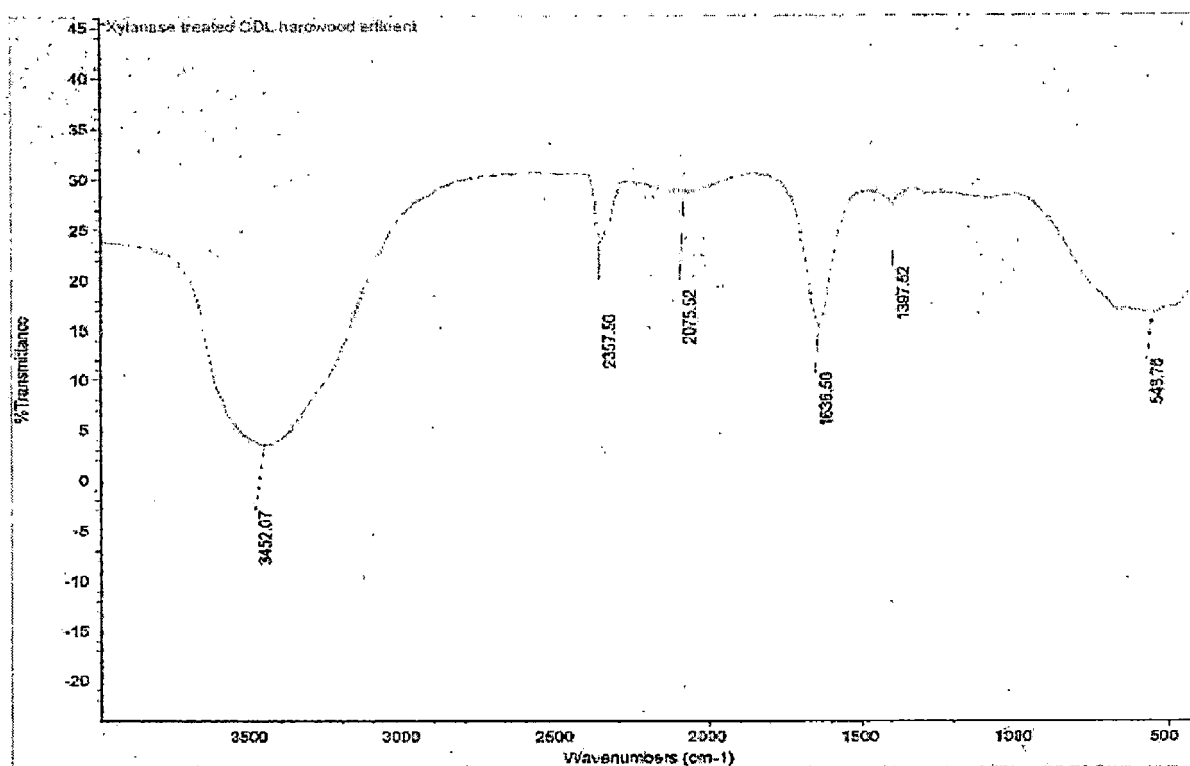
(b)

Figure 5.15: Infrared spectrum of xylanase treated non-oxygen (a) and xylanase treated oxygen delignified (ODL) (b) mixed hardwood pulp effluents.
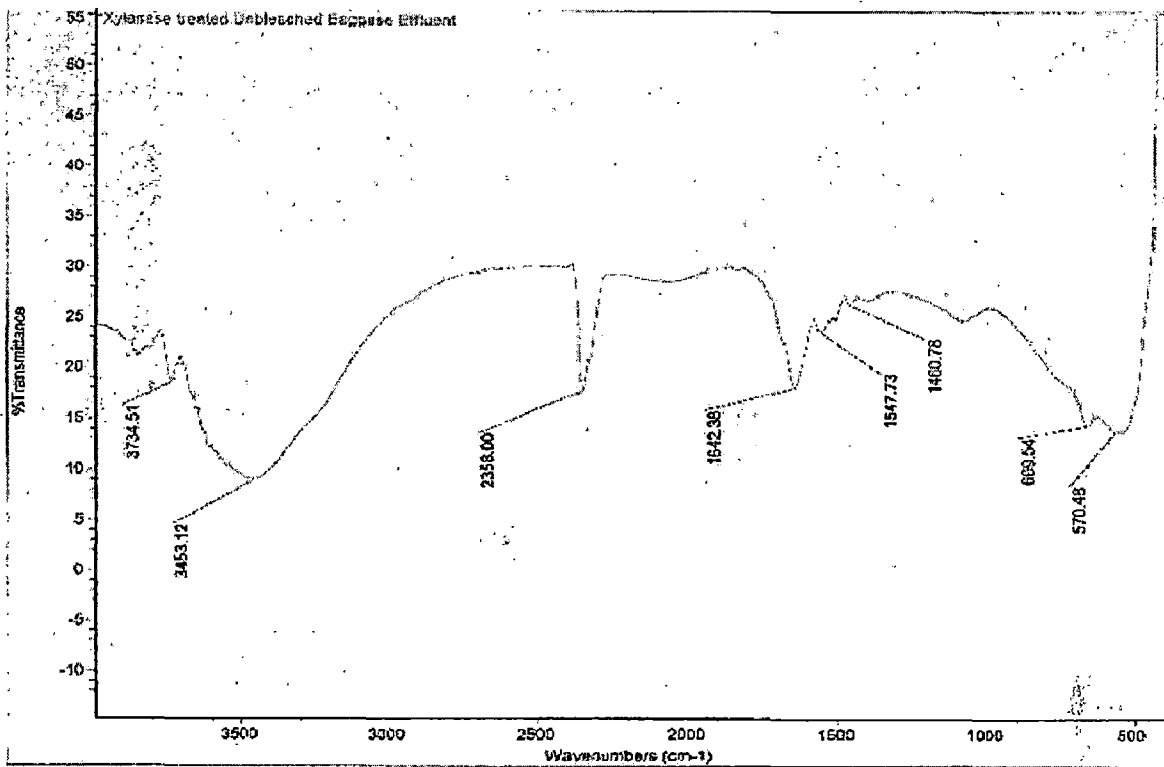
(a)
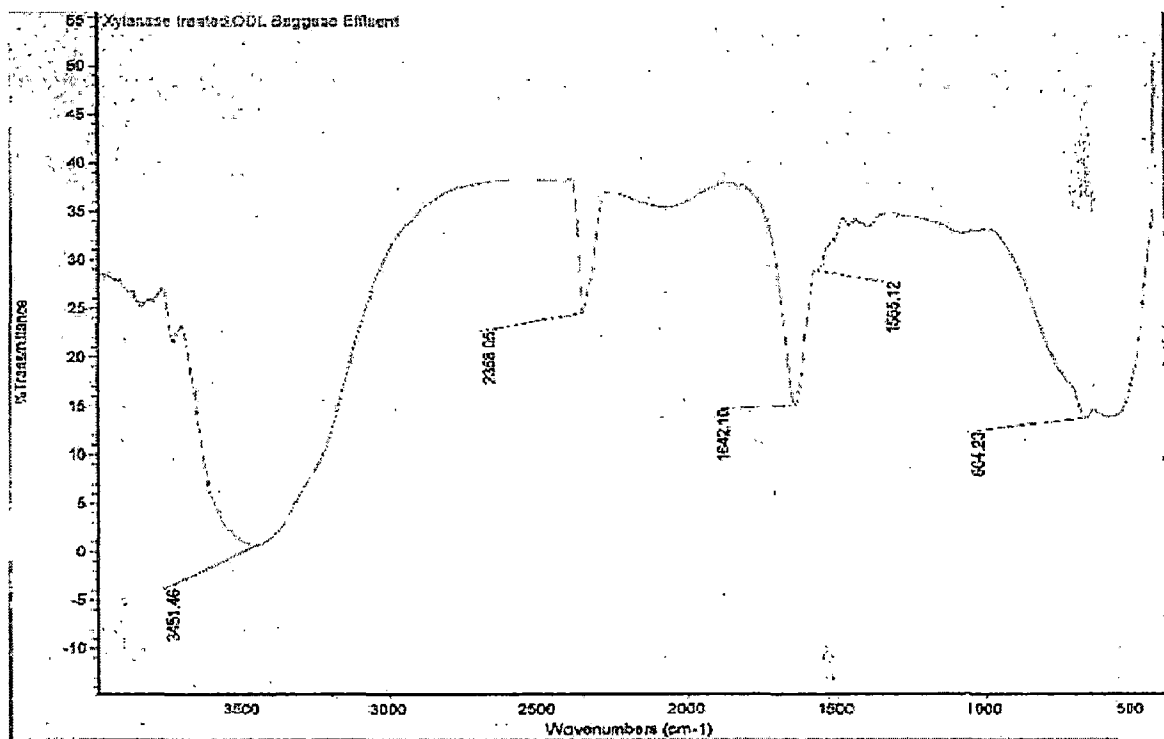
(b)

Figure 5.16: Infrared spectrum of xylanase treated non-oxygen (a) and oxygen delignified bagasse pulp effluents

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The present study concludes that:

1. A target brightness of 85% ISO is achievable for oxygen and non oxygen both mixed hardwood and bagasse pulps using DED, $D_{50}/C_{50}ED$ and ODED sequences. Higher brightness (90% ISO) can be achieved in both the pulps using the sequence $OD_{50}/C_{50}EpD$.

2. The xylanase pretreatment is possible on ECF sequences of mixed hardwood and bagasse pulps.

3. The enzyme prebleaching is possible for oxygen and non oxygen mixed hardwood and bagasse pulps. The oxygen delignified pulps required lower enzyme dose compared to their respective non oxygen pulps.

4. Xylanase pretreatment reduces substantially the bleach chemical consumption to get 85% ISO and 90% ISO target brightness pulp. The enzyme pretreatment is more effective for 85% ISO brightness than for 90% ISO brightness.

5. Oxygen delignified pulps responds comparatively better to enzyme pretreatment where part of xylan and lignin could have been redistributed to the outer surface of the fibre due to alkaline treatment.

6. Pentosan content is lower in xylanase pretreated pulps that shows the removal of hemicelluloses during the xylanase pretreatment.

7. At the same bleach chemical consumption, xylanase pretreatment improves the brightness as compared to untreated pulps.

8. Xylanase pretreatment lowers the AOX generation compared to their respective untreated pulps.

9. It is observed from the characterization of generated effluents that BOD, COD and color generally increased after xylanase pretreatment.

10. The BOD/COD ratio also increases, indicating that effluent is more amenable to biological treatment in the effluent treatment plant or increase in bio-degradability of the effluents.

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11. It has been observed that after xylanase pretreatment the amount of chloro-organic compounds is reduced in the combined effluent of mixed hardwood and bagasse pulps.

The above study and all above statements clearly indicate the effectiveness of enzyme prebleaching not only in reducing the bleach chemical consumption but also in improving the performance of bleach plant. The xylanase facilitates the extraction or removal of lignin from the pulps. The application of xylanase pre-bleaching is easy to attempt for any conventional and modern bleaching sequences without modifying the infrastructure. The xylanase pretreatment also plays a key role to reduce the generation of chloro-organic compounds which are very toxic to living and non living environment.

RECOMMENDATIONS FOR FUTURE STUDIES

1. Action of xylanase on the pulp and paper properties and composition of effluent under high xylanase dose needs investigation.

2. Size Exclusion Chromatography (SEC) Technique may be used to study the change in molecular weight profiles of lignin and carbohydrates in the pulp to confirm the action of xylanase enzyme on lignin carbohydrate compounds (LCC) in pulps.

3. Scanning Electronic Microscope (SEM) to be considered as a tool to visualize morphological changes in pulp structure.

4. Atomic Force Microscopy as a powerful technique to study the effect of xylanase on the penetration of secondary wall and pulp structure.

5. The role of other hemicelluloses-degrading enzymes in pulp and paper bleaching.

The adoption of xylanase pretreatment or pre-bleaching as a necessary step in treatment of pulps for woody and agro-residue pulps for bleaching must be undertaken to reduce the AOX generation in conventional and ECF bleaching sequences.

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