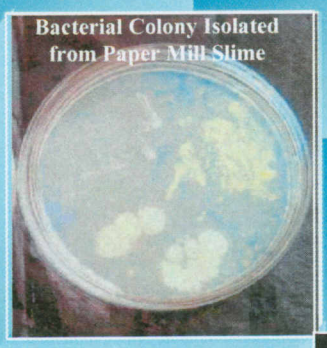
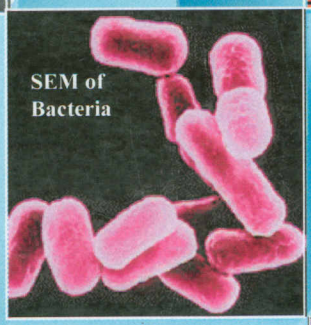
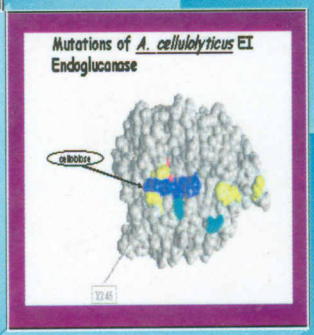
INTERACTION MEET ON BIOTECHNOLOGY IN PAPER INDUSTRY - A FRESH LOOK

7th December 2001







ORGANISED BY

CENTRAL PULP AND PAPER RESEARCH INSTITUTE

SAHARANPUR, U.P. (INDIA)

INTERACTION MEET

ON

BIOTECHNOLOGY IN PAPER INDUSTRY - A FRESH LOOK

7TH DECEMBER, 2001

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CENTRAL PULP & PAPER RESEARCH INSTITUTE
SAHARANPUR - 247 001(U.P.) INDIA

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वाणिज्य एवं उद्योग मंत्रालय
(औद्योगिक नीति और संवर्धन विभाग)
उद्योग भवन, नई दिल्ली—११००११
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FOREWORD

Pulp & Paper Industry which has the rare distinction of employing all the areas of Science & Technology, has been of great interest to Biotechnologists. Biotechnology being a greener and cleaner technology received a considerable attention in the last one decade. The major target areas of biotechnological applications in Pulp & Paper Industry are enzymatic pre-bleaching of pulps, use of enzymes in deinking & upgradation of waste paper quality, microbial delignification of cellulosic raw material i.e. biopulping as well as slime control in paper making. These areas are explored with prime objective of improving the status of energy & environment, product quality with cost effective production. Enzymatic prebleaching of pulp has been an area of major interest for paper industries not only in developed countries but also in developing countries. Pre enzymatic bleaching would help in phasing out the chlorine and chlorine based bleaching chemicals, ultimately resulting in the effluents with lower AOX levels.

Having realised the importance of biotechnological applications in Pulp & Paper, Central Pulp & Paper Research Institute took initiative to create required infrastructure in the laboratory in association with various industry associations, Research Institutions & laboratories as well as the enzyme manufacturers Institute has taken a lead in promotion of biotechnological applications in Indian Paper Industry. The Institute has been able to successfully conduct mill scale trials on enzymatic prebleaching of pulps at some of the large integrated pulp & paper mills. Institute has also made good progress in biopulping.

This Interaction meet on "**Biotechnology in Paper Industry - A Fresh Look**", is organised to discuss the status & recent developments that have taken place in the area of biotechnological applications in pulp & paper industry. This will further facilitate in exploring the possibilities of promoting biotechnology in Indian Paper Industry.

This souvenir, containing the articles contributed by eminent persons should become highly useful for Industry and research scientists.

I hope that the recommendations of the Interaction meet will facilitate in evolving the strategies for promotion of biotechnology in Indian Paper Industry.

I wish the Interaction meet a great success.

(S. Jagadeesan)

CONTENTS

Perspectives in Developing Enzyme Technology for Indian Paper Industry

Rele. M V, Srinivasan M. C and Balakrishnan H

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ABSTRACT:

In the search for environmentally compatible technologies for paper industry, global efforts have focussed on the use of xylanase enzymes for bio-pulping and bio-bleaching. Microbial xylanases free of cellulase activity active at high alkaline pH conditions have received particular attention in the western countries where technological feasibility of xylanase enzymes in paper biotechnology has been firmly established. In view of the stringent regulation abroad limiting the use of toxic chlorine compounds in paper industry, it will soon become obligatory that paper manufactured in India will also necessitate minimizing the use of chlorine compounds.

Raw materials for paper manufacture in India such as hard woods, grasses etc. are different in composition compared to the soft woods of the western world. The xylan content is higher and also the compatibility of xylanases would be significantly different from that of the xylans of soft woods. It is thus evident that xylanases compatible with the soft wood may not find direct application to our indigenous substrates and there is an urgent need for screening for xylanases compatible with indigenous substrates, in particular the agro-based lignocellulosic residues. Several laboratories in India including NCL have undertaken investigations to identify microbial strains with promise and potential for producing xylanases useful to the paper industry. There is a need for evolving a mechanism by which these xylanases are studied and standardized for pulp biotechnology with R&D inputs from the Indian paper industry. In view of the diversity of substrates employed in India for paper manufacture, it is quite possible that more than one xylanase suited to individual substrates may be required to be identified. The higher cost of enzyme application compared to chlorine bleaching will have to be accepted by the paper industry as a contribution towards making the paper technology environmentally compatible. Some of the researches generated at NCL on a cellulase-free alkaline xylanase from a *Bacillus* along with some of our limited studies on paper pulp bleaching are presented.

INTRODUCTION

The large scale pollution generated by the excessive use of molecular chlorine in the bleaching process by the paper industries have forced researchers to seek alternative eco-friendly technologies. This is more so in the developed western countries where public awareness coupled with stringent regulations have stimulated the paper mills to adopt newer and greener technologies such as pre- bleaching of the paper pulp with xylanases. The long term use of enzymes on regular production scale has been reported by a number of paper mills. This has been possible due to the availability of reasonably prized commercial enzyme preparations coupled with low investment and ease of process operations, compatible with the existing processes.

Chlorination is generally one of the stages of pulp bleaching. It involves the addition of chlorine or chlorine di-oxide to remove residual lignin from the pulp. Chlorine reacts with the lignin in the pulp to form chlorinated organic compounds which are discharged as effluents. These effluents containing chlorinated lignins are highly coloured, toxic and carcinogenic causing serious environmental problems. In response to these concerns, modification of the production process at the pulping and bleaching stages are being developed abroad. These include extending the cooking time for additional lignin removal, introduction of oxygen delignification as a pretreatment step, elemental chlorine free (ECF) or total chlorine free (TCF) bleaching using hydrogen peroxide instead of chlorine dioxide coupled with xylanases as pre-bleach agents (Viikari, *et al.* 1994).

ENZYMES IN PULP BIOTECHNOLOGY

A number of enzymes beside xylanases are being envisaged to have an impact on pulp processing. These are at various stages of development and are yet to become commercialized. eg. Cellulases and xylanases in deinking, laccases and peroxidases in lignin removal, proteases and lipases in removal of protein deposits and pith on paper machines. Pilot plant studies have been carried out for de-inking of recycled paper (Bajpai.,1997). Research over several years focussed on the use of ligninase enzymes to oxidize and remove the lignin from the pulp. However, this has not been suitable for mill applications because reaction times of several days are required. Also there is a lack of understanding of the degradation mechanism of residual lignin. But the single most significant application of enzymes in pulp biotechnology is the use of cellulase-free xylanases in bio-bleaching. Viikari *et al.* (1986) for the first time showed that xylanases could be used as pre-bleach agents. The first industrial application of xylanase was carried out in Finland in 1991 (Koponen, 1991). By 1994, 18 mills in Canada had run xylanase trials and 6 are presently regular users of the enzyme to treat 750,000tonnes of pulp, which represents 8% of Canada's bleached kraft pulp production (Tolan *et al.*1996).

Detailed laboratory work has been carried out to adapt the enzymatic treatment to existing mill conditions. No major modifications need to be implemented for full scale runs. The only requirement is the addition of pH adjustment facilities which will not be required in the case of alkaline xylanases. The application of xylanases in pre-bleaching of wood pulp in India is yet to reach a commercial stage

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due to several factors. Moreover, in-depth studies need to be carried out with indigenous substrates as the xylanases being used in the western world are more suited to softwood pulps which have a different composition to that of tropical hard woods.

ORIGIN OF CELLULASE- FREE XYLANASES

Xylanases are produced by a large number of bacteria, actinomycetes and fungi. Several reviews have appeared in the literature regarding microbial xylanase production (Wong *et al.* 1988, Bastawde, 1992). Although filamentous fungi such as *Trichoderma, Penicillium,* and *Aspergillus* are known to produce high levels of xylanases, they normally also co-secrete cellulases. For application in pulp biotechnology, it is preferable that the xylanase preparations are free of accompanying cellulase activity in order to retain the desired viscosity of the paper pulp. Earlier attempts to obtain xylanase preparations free of cellulase activity involved heat inactivation of the cellulase, treatment with mercurial compounds, selective expression of xylanase genes in homologous and heterologous systems etc. (Srinivasan and Rele, 1995). But perhaps the most successful approach has been screening for naturally occurring microorganisms capable of producing exclusively cellulase-free xylanases. Pioneering research work in the area of cellulase-free xylanases has been carried out at National Chemical Laboratory for paper and pulp industry. One of the earliest reports of the isolation of an organism capable of producing cellulase-free xylanase has been from our laboratory in the early eighties involving the isolation of a sclerotial actinomycete *Chainia* sp. (NCL 82-5-1) from the desert sands of Rajasthan (Srinivasan *et al.* 1984, Srinivasan *et al.* 1990). Since then, several cellulase-free xylanases have been reported in literature and these have been comprehensively reviewed by Srinivasan and Rele (1992).

Since pulping operations during paper production are carried out at high pH and temperature, it is advantageous if the xylanases are active and stable to alkaline pH and high temperature. Recent years have witnessed growing interest in the study of alkalophilic microrganisms and their enzymes and the first comprehensive treatise on the subject was published in 1982 (Horikoshi and Akiba, 1982). In our studies, an alkalophilic *Bacillus* strain (NCL 87-6-10) was isolated which secreted high levels of xylanase totally free of cellulase in a commercially viable medium containing wheat bran and organic

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nitrogen (Balakrishnan, *et al.* 1992). This xylanase has an optimum pH and temperature of 8.0 and 60°C respectively, both these features being ideal for paper and pulp applications. The work on cellulase-free xylanases from an alkalotolerant *Cephalosporium* NCL (87-11-9) active at high pH was reported for the first time from our laboratory for which a US patent was granted (Bansod, *et al.* 1993, Rele *et al.*1996). Subsequently, Kang *et al.* (1996) reported another *Cephalosporium* sp. co-secreting both alkaline cellulases and xylanases. Chandra and Chandra (1995) have reported a cellulase-free xylanase from an alkalotolerant *Aspergillus fischeri* strain.

ENZYME AIDED BLEACHING:

The non-availability of cellulase-free xylanases was a serious drawback in the commercial application of this concept as the associated cellulase in xylanase preparations caused weakening of the cellulose pulp and lowered its quality. During the heating period of kraft cooking when the alkali concentration is comparatively high part of the xylan is dissolved in the pulping liquor. As the cooking proceeds, the alkali concentration decreases and degraded short chain xylan reprecipitates in a more or less crystalline form on the surface of the cellulose microfibrils whereby hemicelluloses seem to physically restrict the passage of high molecular mass lignin out of the pulp fiber.

According to the simplest theory, the enzyme treatment causes a physical loosening of the fibre wall due to partial depolymerisation of the hemicellulose chains which facilitate extraction of residual lignin during bleaching. The other possible explanation for xylanase action in bleaching is that the disruption of xylan chain by xylanases disrupts the carbohydrate-lignin bonds thereby improving the accessibility of the bleaching chemicals to the fibre and facilitates the removal of solubilised lignin. The reprecipitated xylan may physically shield the residual lignin from bleach chemicals and xylanases hydrolyse part of the redeposited xylans allowing better access of bleach chemicals to the residual lignin.

The benefits obtained by using enzymes are dependent on the chemical bleaching sequences used as well as the residual lignin content of the pulp (Viikari, *et al.*1994). Enzymatic pretreatment has been reported to result in a higher final brightness and a reduction in the consumption of bleach chemicals

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with a concomitant reduction of AOX (Adsorbable organic halogens) in effluents (Koponen, 1991, Tolan *et al.*1996). An average reduction of 25% in active chlorine consumption in pre-bleaching or a reduction of 15% in total chlorine consumption has been reported with softwood kraft pulps (Koponen, 1991). In the TCF bleaching process the brightness gained obtained by enzymatic pretreatment is of significance.

The dosages of enzymes reported to be used in enzyme aided bleaching have varied between 2 units/gram pulp to as high as about 500 units/gram of pulp, the economically realistic dosage being 6 to 10 units/gram. The price of enzyme treatment today is 2-5 US dollars per tonne of pulp (Viikari, *et al.* 1991). Usually the price of the enzyme is only compared with that of the competing bleaching chemicals and it may be difficult to specify a price. The price of enzymes is expected to come down as more efficient production strains are used. The strength properties of enzymatically prebleached pulp have been reported to be rather similar to those of the reference pulps both in lab scale and mill scale (Christov and Prior, 1998).

RAW MATERIALS / SUBSTRATES

The efficacy of enzymatic pre-bleaching is largely dependent on the type of biomass, the pulping operations and the nature of the xylans. A large variety of ligno-cellulosic substrates are used by the paper industries. The composition and distribution of xylan in these substrates play an important role in the bio-bleaching process. The xylan content of hard wood, straw and bagasse is very different to that of soft woods. The xylan content in hard wood and non-woody tissue ranges between 20 - 35 % while the xylan content is 7-12% in soft wood and is characterized by a higher propotion of 4-O-methyl-D-glucuronic acid. In contrast, soft wood contains a higher content of mannans. The xylans from hard wood are devoid of arabinose residues, while xylans from softwood as well as grasses contain substantial quantities of arabinose. Moreover, soft wood is often preferred over hard wood as the cellulosic fibers obtained from soft woods are longer and devoid of vessels which often interfere in pulping operations. In India bagasse is one of the raw materials used in pulping operations. There are no detailed studies on the exact mechanism of action of xylanases on non-wood species such as bagasse, straw etc. These studies are essential to optimize xylanase treatment and develop tailor -made

enzymes suited to indigenous substrates for the soft woods may not be compatible with our raw materials for paper making (Timell, 1964, Joseleau *et al.* 1992).

ACTION OF XYLANASES:

The action of different xylanases on pulp have been evaluated on the basis of release of sugars, liberation of lignin derived compounds as measured by absorbance at 260 and 237 nanometers. These studies need to be correlated to the brightness increase in peroxide delignification of pulps. Combination of the enzyme treatment to different bleaching sequences gives the most reliable and practical result. Kappa number reduction as well as colour removal are the two other consequences of treating pulps with xylanases. These parameters are largely determined by several factors such as the origin and processing of pulps, access of enzymes to xylan substrate and the nature of the enzymes themselves. Reduction in kappa number has been attributed to lignin removal. However, recent work has shown that removal of hexeneuronic acid-xylo-oligosaccharides also brings about significant kappa number reduction (Gellerstedt, 1996). These are formed from the 4-O-methyl-α-D-glucoronic acid component of xylan during the kraft cooking of the pulp It accounts for the significant fraction of the oxidizable compounds removed by xylanases. Different xylanases release hexeneuronic acid - xylo-oligosaccharides to different extents from the kraft pulp.

Several researchers have shown that xylanases release materials that absorb in the visible region (Chromophores). It was earlier thought that these were derived from lignin but more recently, NMR and proton studies have revealed that the extracts have no aromatic moieties. These were shown to be due to sugar moieties having undergone degradation. Besides chromophore release, it has also been demonstrated that liberation of UV absorbing material correlates well with increased brightness following bleaching than sugar release. The extent of release of all these substances depends on the nature of xylanases used and the synergetic action of different iso-enzymes and accessory enzymes present in the crude culture filtrates (Elegir, *et al.*1995, Patel, *et al.* 1993, Davis *et al.* 1997, Jeffries, 1996).

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DEVELOPMENT OF NOVEL XYLANASES

Upgradation of any technology by screening for novel enzymes with enhanced properties, or developing tailor-made enzymes by site-directed mutagenesis is a continuous effort and is essential to be competitive. Some broad characteristics of xylanases suitable for bleaching have been arrived at from the studies carried out so far. These are

1) Cellulase-free nature of the xylanase
2) The enzyme should be of low molecular weight to have easy access to the pulp matrix.
3) Activity at high alkaline pH 10-11 so that they can function effectively on kraft or soda pulp.
4) Thermal stability.
5) The enzyme should be active on a wide variety of pulps.
6) Affinity for acidic side chains in order to maximize hexaneuronic acid -xylo-oligosaccharides and UV absorbing material.
7) The enzyme should not be inhibited by the impurities from the pulp.

Most microbial xylanases including the ones from alkalophiles have a molecular mass in the range of 20,000 to 50,000 daltons (Honda *et al.* 1986, Okazaki, *et al.* 1985, Nakamura, *et al.*1993, Rattoo *et al.* 1992, Tsujibo *et al.* 1990). A number of xylanases with apparent low molecular weights have been reported from many laboratories Gel-filtration data gives values for these xylanases in the range of 7000 to 12,000, while SDS-PAGE gives comparatively higher values (Grabaski and Jeffries, 1991, Holden and Walton, 1991). Since most of these proteins are not glyco-proteins the shape of the molecule appears to be far different from a globular structure. Xylanases having such a property would be advantageous in pulping operations, as the penetration of the cellulose pulps would be facilitated. Two low molecular weight xylanases (20,000 daltons) differing in pI (5.5 and 9.0) have been compared for their ability to hydrolyse wood xylan, pulp xylan and reprecipitated xylan from birch wood kraft pulp (Viikari, *et al.* 1992). Both xylanases could hydrolyse the wood xylan equally well, but after cooking, the xylans were more susceptible to the pI 9.0 enzyme. Similarly, xylanases having a high pI were found to have better binding properties with the pulp and therefore more efficient in the removal of xylan.

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Alkaline activity is a desirable property as kraft pulping accounts for 70% of the pulping operations. The incoming pulp is at high temperature and pH. In order to simplify pulping operations without drastic adjustment in pH, it would be advantageous to have an enzyme having optimal activity at pH 9.0 - 11.0. In the case of thermal stability, the enzyme should be active and stable preferably over a wide compatible range (40 - 80 ^{0}C) as pulping operations differ considerably with various wood substrates.

More recently, xylanases have been used in treating a wide variety of substrates eg. mechanical pulps, sulphite pulps beside kraft pulps in order to get enhanced brightness of pulps when H_2O_2 is used as a bleaching agent. It would be advantageous to have xylanases which act on a wide variety of substrates as well as suit different methods of pulping eg. kraft , mechanical and sulphite pulping. This is because pulping methods are likely to affect the composition and localization of hemicelluloses in the original pulp. The re-precipitation of xylan has been reported to diminish in the extended cooking of pulp and consequently the effect of enzymes in bleaching has been found to decrease. However, preliminary studies with kraft pulps having low kappa numbers have given increased brightness after peroxide delignification with enzyme treatment (Christov and Prior, 1998).

The use of xylanases in sulphite pulping has also been demonstrated recently. Dissolving pulp is used in the production of cellophane and rayon, cellulose esters (acetates, nitrates, propionates and butyrates), cellulose ethers such as carboxy methyl cellulose etc. The presence of hemicellulose in dissolving pulp is undesirable and the removal of this requires a large amount of chemicals. Besides, a part of the hemicellulose remains in the pulp after bleaching causing problems in the production of viscose rayon. The benefits of xylanase treatment of sulphite pulp are the potential increase in pulp yields, higher brightness, savings in pulping and bleaching chemicals besides reduction in the quality of the effluents in terms of chlorinated compounds. It is therefore obvious that various pulps would require specific types of xylanases or a combination of xylanases.

Our own studies have substantiated several of these factors. The use of xylanases in sulphite pulping are few compared to kraft pulping. Christov *et al.* (1999) in evaluating the xylanase from the

alkalotolerant *Cephalosporium* sp. on unbleached sulphite pulps from *Eucalyptus grandis* showed a brightness gain of three points in XP pulps and final brightness of one point in a five stage bleaching sequence involving $OD_1E_0D_2P$ where O is oxygen, D is chlorine dioxide E is alkali extraction and P is hydrogen peroxide. Bansod (1997) showed that a crude xylanase preparation from an alkalotolerant *Cephalosporium* species reduced the kappa number of bagasse pulp effectively over a broad range of pH 7 to 10 at 40 ^{0} C. The optimum enzyme concentration required was as low as 0.4 IU/g of oven dried pulp. The enzyme was neither adsorbed on the pulp nor inhibited by impurities from the pulp. Shah *et. al* (1999) in their studies too showed a considerable drop in kappa number over a pH range of 7.0 to 9.0 at 60 ^{0} C when bagasse pulp was treated with 1.2 IU/g of oven dried pulp. Moreover they showed that the effluent from enzymatic pretreatment of pulp can be utilized for enzyme production and that the effluent was not inhibitory to the organism.

Preliminary trials with the alkalophilic *Bacillus* strain (NCL 87-6-10) when evaluated by several commercial firms both within the country and abroad showed that the enzyme was an effective prebleach agent. (unpublished data, personal communication). This alkalophilic *Bacillus* enzyme has features such as low molecular weight, activity and stability at high pH besides the crude extract being virtually free of cellulase activity.

Multidimensional application of a product is an attractive feature for any enzyme manufacturer. The above *Bacillus* xylanase was also found to be compatible with several commercial detergents such as Surf Excel, Ariel, etc and hence has a potential application in detergents to remove soils of vegetable and the plant origin (Kamal Kumar, *et al.* (2001). An additional feature of this enzyme is that it is active and compatible with alkaline proteases such as Alcalase and *Conidioboluos* protease.This offers opportunities for marketing formulations containing several enzymes for use in both paper and pulp biotechnology and detergent industries.

CURRENT STATUS IN INDIA

Totally chlorine free technology in the pulp and paper industries has become a reality in many of the European countries and in Canada. However, the impact of their development is yet to percolate to the

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developing countries. In the absence of stringent environmental regulations, use of conventional hazardous chemicals is still being widely used in the paper mills and tanneries in India. Switch over to chlorine-free paper manufacture may soon become obligatory when environmental regulations abroad enforce restrictions even on food products exported to the developed world using paper products based on chlorine bleaching for wrapping. In view of the diversity of substrates employed for paper manufacture in our country, more than one xylanase suited to individual substrates may need to be developed. Besides, there is also a need to carry out studies on the application of other enzymes in pulp biotechnology. A closer interaction and linkage must be established immediately between the paper industry and biotechnologists and perhaps a national consortium established to make an in-depth analysis of the problem in perspective and find meaningful solution to establish a viable biotechnological approach to paper industry in our country.

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Biobleaching of kraft pulp using a cellulase free alkaline xylanase from *Streptomyces violaceoruber*

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ABSTRACT

A cellulase free extra-cellular xylanase from an alkalophilic *Streptomyces violaceoruber* was active over a broad pH range (4.0-9.5). The alkalostable xylanase was found to be highly stable at temperature 50-55°C for 2h, after which the stability decreased. The xylanase dose of 5 IU/g moisture free pulp, exhibited bleach boosting of kraft pulp optimally at pH 9.5-10.0 and 50°C after 2 h of reaction time. Pretreatment of pulp with xylanase and its subsequent treatment with 6% hypochlorite reduced the kappa number of the pulp by 30%, enhanced the brightness of handsheets by 4.5% and improved the tear factor by 4%. A reduction in hypochlorite consumption by 20% was achieved in biobleached pulp in order to obtain the same level of brightness as in the case of chemically bleached pulp (control).

INTRODUCTION

Xylan, a heterogeneous polysaccharide containing different substitute groups in the backbone and in the side chain, is the most renewable hemicellulose. It constitutes 35% of the total dry weight of higher plants (Puls and Poutanen, 1989). Xylanases, a repertoire of hydrolytic enzymes have many applications in industry. Although xylanases alone cannot hydrolyse plant heteroxylans completely, but they can be applied effectively in processes in which xylans are to be depolymerised. Therefore, in recent years, xylanase have received a great deal of attention due to their potential application in food, feed and most importantly in the pulp and paper industry (Viikari *et al.*, 1994; Sunna and Antranikian, 1997). A pre-requisite in the pulp and paper industry, is the use of cellulase free xylanases that ensure minimal damage to the pulp fibers and also generate rayon grade or superior quality dissolving pulp (Jurasek and Paice, 1987). The most important enzyme used in the enzyme aided bleaching of pulp is endoxylanase (Kantelinen *et al.*, 1988; Paice *et al.*, 1992). Endoxylanases are also the most widely studied and characterized hemicellulolytic enzyme (Kuhad and Singh, 1993). Endoxylanases have been reported from yeast (Biely, 1981; Basaran *et al.*, 2001), fungi (Tenkanen *et al.*, 1992; Khanongnuch *et al.*, 1998; Salles *et al.*, 2000) and bacteria (Elegir *et al.*, 1994; Chaudhary and Deobagkar, 1997; Gupta *et al.*, 2000). In this study we have reported the use of an abundantly

available and cost effective agro-residue to achieve high xylanase yield from *Streptomyces violaceoruber*. The xylanase, after partial purification was further applied as a biobleaching agent in the pulp and paper industry to develop an environment friendly technology.

MATERIALS AND METHODS

Micro-organism and culture conditions.

Streptomyces violaceoruber isolated from the industrial effluent of Shreyans pulp and paper mill, Ahmedgarh, Punjab has been used in the present study. The organism was maintained on Horikoshi agar slants (Ikura and Horikoshi, 1987). For xylanase production, the organism was cultivated in Horikoshi medium (glucose replaced by 2% w/v wheat bran, pH 8.5) under shaking (200 rpm) conditions at 37°C. After 60h, the fermented broth was centriguged at 10,000 g for 20 min. and the supernatant was subjected to purification.

Purification of xylanase.

The purification of xylanase (cell free supernatant) involved ammonium sulphate precipitation (between 0% to 50% saturation) of 600 ml cell free supernatant. The saturated solution was left overnight at 4°C, centrifuged, precipitates dissolved in 25 ml of Glycine-NaOH buffer (0.2 M; pH 9.0) and dialysed against the same buffer for 24 h and finally dissolved in 43.5 ml of buffer.

Enzyme Assay.

The xylanase (E.C. 3.2.1.8) activity was determined by measuring the release of reducing sugars from birch wood xylan (1% w/v) by dinitrosalicylic acid method (Miller, 1959). One unit of xylanase was defined as amount of enzyme required to release 1 µmol of xylose from birch wood xylan in 1min. under standard assay conditions (60°C, 0.2 M Glycine-NaOH, pH 9.0).

Biobleaching of kraft pulp.

Kraft pulp was used for the present study. Kraft pulp was kindly provided by Shreyans Pulp and Paper Industries Ltd. (Ahmedgarh, India). All the studies were performed at 12% pulp consistency, pH 9.5-10.0 at 50°C, unless otherwise mentioned.

Optimization of enzyme dose and reaction time for biobleaching.

The optimization of enzyme dose and reaction time was carried out by treating the pulp with different doses of xylanase from *Streptomyces violaceoruber*, ranging between 0 and 10.0 IU/g moisture free pulp for different intervals up to 3 h and the pulp properties were studied following standard procedures.

Chemical bleaching.

The kraft pulp samples were bleached in a multistage bleaching process using CEHH sequence (C, chlorination; E, alkali extraction; H, hypochlorite treatment). The different types of pulp obtained at each step of chemical bleaching were given a biotreatment with optimal xylanase dose and reaction time at 50°C. The kraft pulp was also enzymatically prebleached with xylanase and then subjected to 6.0% hypochlorite treatment at 50°C for 2h.

Physical and chemical characterization of kraft pulps.

The control and treated pulp samples were washed and the hand sheets were prepared. The physical and chemical characterization of kraft pulp were determined according to the standard methods of Technical Association of the Pulp and Paper Industry (TAPPI) (Anon, 1991); Kappa number (T 236 cm-85), brightness (T 452 om-92), burst factor (T 403 cm-50), tensile strength (T 231 cm-96), release of reducing sugars (Miller, 1959), release of phenolic compounds (A_{237nm}) and hydrophobic compounds (A_{465nm}) (Patel *et al.*, 1993; Gupta *et al.*, 2000).

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RESULTS AND DISCUSSION

Xylanase production by *Streptomyces violaceoruber.*

Streptomyces violaceoruber when grown in 2% (w/v) wheat bran supplemented in Horikoshi medium (devoid of glucose) produced 86.4 IU/ml of xylanase

Biobleaching of kraft pulp.

The xylanase from *Streptomyces violaceoruber* was precipitated using ammonium sulphate (0-50% saturation) and subsequently used for biobleaching studies. The xylanase dose for the biobleaching of eucalyptus kraft pulp, at 50°C, was optimized as 5.0 IU/g moisture free pulp (Table 1). The biobleaching efficiency of xylanase treatment on eucalyptus pulp was maximum after 2 h with a reduction in kappa number by 32% and release of reducing sugars increased by 7-fold. The release of chromophores (A_{237nm}) and hydrophobic compounds (A_{465nm}) were also maximum after 2 h reaction time (Table 2). The correlation between the release of chromophores (A_{237nm}), hydrophobic compounds (A_{465nm}) and the reduction in kappa number coupled to the release of reducing sugars suggested the dissociation of lignin-carbohydrate complex from the pulp fibers. Higher enzyme dose or longer period of incubation did not enhance the extent of biobleaching benefits significantly. Many workers have reported an improvement in pulp and paper properties by using the optimized dose xylanase in the biobleaching process. Xylanase from *Streptomyces thermoviolaceus* and *Staphylococcus* sp. SG-13 at a dose of 5 IU/g and 1.8 IU/g moisture free pulp at pH 5.0 and 30°C for 24h and pH 9.5-10.0 at 50°C for 4 h, respectively, reduced the kappa number by 18% and 30% and resulted in an increase in brightness of the paper (Garg *et al.*, 1998; Gupta *et al.*, 2000), whereas, xylanases from *Streptomyces roseiscleroticus* (Patel *et al.*, 1993) and *Bacillus* sp. NCIM59 (Kulkarni and Rao, 1996) when applied on pulp at a dose of 3 IU/g and 10 IU/g, respectively, reduced the kappa number and improved the strength properties of the pulp. Successful biotreatments of pulps with commercial xylanases have also been reported, e.g. pulpzyme HA (Novo Nordisk, Denmark)

(Pedderson, 1989) and Cartazyme (Sandoz, USA) (Fleming, 1991) at a dose of 0.75 IU/g at 50°C and 0.5-2.0 IU/g at 50°C (pH 4.8), respectively.

It was observed that in the CEHH based conventional chemical bleaching process, the biobleaching effects of xylanase from *Streptomyces violaceoruber* on kraft pulp was observed at first (chlorintion *i.e* CX) and second (chlorination and alkali extraction *i.e.* CEX) stages of the biobleaching process. The xylanase based post bleaching benefits were more pronounced after CE treatment as compared to C treatment only since it resulted in greater reduction in kappa number which was significantly more than former stage. The maximum biobleaching was, however, achieved at the first stage where the xylanase treated kraft pulp exhibited a 12% reduction in the kappa number (Table 3), which was significantly more compared to the other stages of bleaching process. Therefore, enzymatic prebleaching of kraft pulp was established as the most suitable step to facilitate bleach boosting of pulp. The application of xylanases as prebleaching agent for pulp improvement has previously been reported by some workers (Ragauskas *et al.*, 1994;Khanongnuch *et al.*, 1998; Gupta *et al.*, 2000).

The enzymatic experiments on kraft pulp were carried out with the xylanase from *Streptomyces violaceoruber* where in the pulps were prebleached with xylanase and then subjected to 6% hypochlorite treatment. The physical and chemical properties of the pulp revealed an increase in brightness index by 4.5% and reduction in kappa number by 30% which was also evident from the release of hydrophobic compounds (A_{465nm}) and phenolic compounds (A_{237nm}). The release of reducing sugars and the release of lignin and phenolic compounds (absorbance at A_{237nm}) are interrelated phenomena. When the kraft pulp was pretreated with xylanase, the sugar xylose was released from the xylan which ultimately resulted in the high free sugar content in the pulp sample. Since xylan is a part of hemicellulose which is sandwiched between the lignin and cellulose layers, when xylan was degraded by the xylanase, in addition to xylose, it also caused the release of lignin and phenolic compounds from the pulp fibers which ultimately cause the enhancement in the absorbance (A_{237nm})of xylanase treated pulp sample compared to the control (untreated pulp sample) and the chemical treated pulp (Table 4). A reduction in hypochlorite consumption by 20% could be achieved in biobleached pulp in order to obtain the same level of brightness as in the case of chemically bleached pulp (control). An increase in tear factor by 4% was also observed (Table 4) which indicated that enzymatic

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prebleaching process could have facilitated an increase in pulp fibrillation, water retention and restoration of bonding in fibers. Biobleaching benefits, such as reduction in kappa number by 25% and increase in brightness by 10% with the use xylanase from *Humicola* sp. (Silva *et al.*, 1994) has been reported. Commercial xylanase (Novozyme 473, VAI Xylanase and Cartazyme HS-10) resulted in a 31% reduction in chlorine consumption and a 30% reduction in total organic chlorine content in the extraction stage effluent, with an increase in the brightness, tensile strength and burst factor by 3%, 26% and 32%, respectively (Bajpai *et al.*, 1994). The biotreatment of pulp with xylanase from *Bacillus* sp. NCIM 59 resulted in the reduction of kappa number by 21% and increase in brightness by 2.5% (Kulkarni and Rao, 1996). A chlorine saving of 30-35% by the biotreatment with cellulase free xylanase from *Streptomyces thermoviolaceus* (Garg *et al.*, 1998) and an increase in the brightness by 8% using xylanase from *Bacillus subtilis* (Khanongnuch *et al.*, 1992) has been reported. The present study showed that the thermostable xylanase from *Streptomyces violaceoruber* active under alkaline conditions was qualified for use in the biobleaching of kraft pulps where it significantly reduced the kappa number by 30% and enhanced the brightness of paper by 4.5%, even at very low dose of 5.0 IU/g pulp.

Table 1. Optimization of xylanase dose for the biobleching of kraft pulp at 50°C, pH 9.5-10.0 after 3 h

Xylanase dose (U/g pulp)	Kappa number	Sugar released (mg/g pulp)	Absorbance (237 nm)	Absorbance (465 nm)
0.0	12.3	0.26	0.017	0.012
1.0	11.5	0.36	0.187	0.014
2.0	8.8	1.60	0.189	0.011
5.0	8.4	1.70	0.565	0.034
7.0	8.4	1.68	0.534	0.034
10.0	8.3	1.58	0.533	0.033

Table 2. Optimization of reaction time for the biobleching of kraft pulp at 50°C, pH 9.5-10.0 using a xylanase dose of 5 IU/g moisture free pulp

Reaction time (h)	Kappa number	Sugar released (mg/g pulp)	Absorbance (237 nm)	Absorbance (465 nm)
0	12.3	0.23	0.011	0.008
1	8.9	1.17	0.483	0.024
2	8.4	1.61	0.486	0.035
3	8.4	1.62	0.486	0.035

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Table 3. Effect of *Streptomyces violaceoruber* xylanase on physicochemical properties of pulp obtained at different stages during CEHH based bleaching of kraft pulp

Bleaching stage	Kappa number	Sugar released (mg/g pulp)	Absorbance (237 nm)	Absorbance (465 nm)
Untreated	18.4	0.01	0.026	0.011
X	16.2	0.41	0.232	0.134
C	9.3	0.04	0.131	0.462
CX	8.8	0.28	0.442	0.504
CE	5.9	0.10	0.112	0.500
CEX	5.2	0.28	0.433	0.582
CEH*	2.8	0.00	0.133	0.131
CEH*X	2.8	0.00	0.133	0.132
CEH*H**	2.2	0.00	0.198	0.092
CEH*H**X	2.2	0.00	0.199	0.090

X, xylanase treatment with 5 U/g pulp (55°C for 2h; pH 11.0); C, Chlorine treatment with 4.5% Cl2 (65-70°C for 0.5 h, pH 2.0); E, Caustic extraction with 0.8% NaOH (60-65°C for 1-1.15 h, pH 10.0-10.5); H*, Hypochlorite I treatment with 6.0% hypochlorite (45-50°C for 1.45-2h, pH 8.0); H**, Hypochlorite II treatment with 4.5% hypochlorite (40-45°C for 2-2.15 h, pH 7.0).

Table 4. Physicochemical properties of chemical treated and (xylanase + chemical) treated pulp

Pulp properties	Control (untreated)	Chemical treated pulp*	Xylanase (5 U/g pulp) treated	Xylanase (5 U/g pulp) + chemical treated pulp*	Xylanase (5 U/g pulp) + chemical treated pulp**	Xylanase (5 U/g pulp) + chemical treated pulp***
Kappa number	12.8	3.3	11.4	2.3	2.6	3.3
Brightness (% ISO)	14.4	73.1	45.2	77.5	75.6	73.5
Tear index (Nm^3/g)	8.2	7.8	8.1	8.1	8.1	8.1
Burst factor ($KPam^2/g$)	1.47	1.27	1.23	1.22	1.23	1.23
Absorbance at 237 nm	0.04	0.21	0.23	0.26	0.25	0.24
Absorbance at 465 nm	0.01	0.12	0.13	0.26	0.25	0.25
Reducing sugars (mg/g pulp)	0.05	0.24	0.11	0.43	0.44	0.44

*6.0% hypochlorite treatment
**5.4% hypochlorite treatment
***4.8% hypochlorite treatment

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Bio-pulping of whole jute plants with white-rot fungus in soda process

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ABSTRACT

The effects of pretreatment of whole jute plant with five different white rot fungi were studies. Control experiments were carried out without fungus under the same condition. Control and treated jute samples were digested using 17% alkali (as NaO_2) and o.05% anthraquinon for 1.5 hrs a 170°C.

After the successful trial of pulping with the five fungal strains, experiments were repeated with different alkali charge (17.0-14.0%) using three strains (C. *subvermispora – 2, P chrysosporium and F. lignosus)* which showed the maximum reduction of kappa number. The resulting pulps were washed and hand sheets were prepared. Chemical and physical properties were analyzed as mentioned above.

All the five strains reduced the kappa number significantly. But the sample treated with **F.lignosus** showed the highest reduction of kappa number by around 22% than the control without any significant loss of pulp yield. Using various alkali charge (14-17%) it appears that at the same AQ does alkali demand can be reduced by 10-12% to attain yield of 42-44%. Pretreatment of all the three strains resulted the total yield of 42% against 45% with control. But in control experiment at 14% and 15.5% alkali charge no digestion take place for which kappa number were significantly higher. All treated chips showed reduction of Kappa Number even at 14% and 15.5% alkali charge.

INTRODUCTION:

Annual world production of jute and allied fibres is around 3 million tons. In addition to fibre, it also produces 1.17 million tons of leaves and 8 million tons of stick. The taditional products of jute are facing severe competition due to the emergence of synthetics. There is an urgent need to develop new product of jute and allied fibre and their respective markets. The demand for pulp and paper has increase sharply in all the jute/kenaf producing countries namely Bangladesh, India China and Thailand. Moreover forest and land resources of these countries are limited. All these countries have started using jute/kenaf as raw material for pulp and paper.

In this study the whole jute plant was used as a raw material for pulp and paper. We were studying five fungal strains prior to soda process cooking. In this study chemical demand can be reduced due to the prior degradation of lignin by the fungus. With this idea in view the affect of best bio-pulping fungus was studied. The Kappa Number, pulp yield and physical properties of hand sheet after using soda-AQ pulping process were studied.

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EXPERIMENT:

Fresh Culture of three strains of *Ceriporiopsis subvermispora* (1, 2 & 3 which were received from FPL of USDA, Forest Service), *Phanerochaete chrysosporium* and *Fomes lignosus* (locally isolated strain) were used.

For bio-pulping initially dried jute plants (*Corchorus olitorius*) were cut into small pieces (2-3 cm). 350 gm of these dried samples were taken in polythene bags and 700 ml of water containing 35 mg of KH_2PO_4, 150 mg of $KgSO_4$ 35µg $MnSO_4$ was added. These polythene bags were autoclaved at 121°C for 15 minutes. Each polythene bag was inoculated separately with 3 strains of *Ceriporiopsis subvermispora* (1, 2 & 3), *Phanerochaete chrysosporium* and *Fomes lignosus* with 10 plugs of fresh culture of these strains. In each polythene bag 14gms of glucose was also added. These polythene bags were fitted with inlet and outlet tubes for aeration and incubated at 30°C for 14 days. Control experiments were carried out without fungus under the same condition.

Laboratory scale pulping trials were conducted after harvesting, control and chips treated with five fungal strains. The following conditions were used. The temperature was ramped from initial temperature 35°C over a period given in table 1, which also details yield and kappa number after washing. The chemical and physical properties of the pulp were analyzed according to TAPPI test methods: T-236 cm 85 (Kappa number), T-205 sp 95 (hand sheet preparation), T – 403 om 97 (burst index), T-414 om 98 (tear index), T-404 cm 92 (tensile index), and T-220 sp 96 (density).

RESULTS:

24 experiments were conducted. The main objective of trials was to reduce the lignin significantly. At first 17% alkali was used and it demonstrated clearly that all the treated chips reduced the Kappa Number significantly (20-22%) after cooking.

It appears from the result that pretreated sample can reduce the alkali charge and cooking time to attain the yield and kappa number compared to untreated chips.

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Strength Properties:

Fungal pretreatment with *C. subvermispora-2, P. chrysosporium* and *F. lignosus* improved the burst index, tear index and tensile strength. Densities of the hand sheets were lower by the fungal treatment except when treated with *C. subvermispora – 2.* Lower density of hand sheet indicates that fibre might be damaged with fungal pretreatment and less fine materials produced. Previous workers [91-4] reported that hand sheet of fungal pretreated samples decreased the brightness. But in this present study no significant change of brightness was observed with different fungal treatments.

C. subvermispora –2 pretreated samples also improve the burst and tensile strength around 60% and 9.66% respectively. However, sample treated with *P. chrysosporium* showed the highest improvement of tear strength (21.63%). It was also observed that reduction of alkali (by 16%) also resulted in the reduction of kappa number and improvement of physical properties of the resultant pulp. All the pretreated samples produced pulp with higher freeness than the untreated samples prior to beating. All these results clearly demonstrated the reduction of kappa number and improvement in the physical properties fo the resultant pulp in soda-AQ process.

Table I: Laboratory pulping results of whole jute plant (*C. capsularies and C. olitorius* CVLI) from control and chips treated with different fungal strain in soda process.

Sample treated with	Na_2O (%)	AQ (%)	M:L	Temp. °C	Cooking time(min)	Kappa no.	Yield (%)
Water	17	0.05	1;5	170	70 + 90	22.20	45.30
C.Suvermispora – 1	17	0.05	1;5	170	70 + 90	21.08	43.30
C.Suvermispora – 2	17	0.05	1;5	170	70 + 90	17.60	42.25
C.Suvermispora – 3	17	0.05	1;5	170	70 + 90	23.50	43.50
P. chrysosporium	17	0.05	1;5	170	70 + 90	17.52	42.00
F. lignosus	17	0.05	1;5	170	70 + 90	17.28	42.70

Table II: Properties of 60 g/m^2 hand sheet from unbleached pulp obtained from control and chips (*C. capsularies and C. olitorius)* treated with different fungal strain in soda process.

Sample treated with	Burst index Kpa. M^2/g	Tear index mN-m^2/g	Tensile index Nm/g	Density kg/m^3	Freeness °SR
Water	3.050	13.000	53.512	507.700	15
C.Suvermispora – 1	3.647	10.882	41.100	518.702	17
C.Suvermispora – 2	4.896	10,588	58,682	497.600	19
C.Suvermispora – 3	3.370	17.058	50.380	347.506	15
P. chrysosporium	5.025	15.813	59.860	468.024	18
F. lignosus	5.290	15.260	56.389	472.580	19

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Enzymatic deinking of Recycled fibres

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ABSTRACT:

Mixed office waste present technical and economic challenge to the recycler and of the wide variety of fibre and contaminants present in the paper stock, toner and other non contact polymer ink from laser printing process, is one of the most difficult to deal with. Toner and laser printing ink are synthetic polymers with embedded carbon blocks, they don't disperse readily during conventional repulping processes. Moreover, these are not readily recovered during floatation or washing. Because of these problems recycled papers contaminated with toner have a relatively low value. Most of the deinking chemicals and high-energy dispersion steps employed in current deinking technology are tedious, cost prohibitive and some times leads to loss in pulp yield. The enzymatic deinking process employing suitable enzymes cocktail effective in deinking of laser and xerographic waste paper show promise in Indian paper industry. The present article discuss an overview of enzymatic deinking technology for nonimpact printed toners covering the mechanism & factors influencing the enzyme deinking of waste fibres.

INTRODUCTION

Forest based pulps has continuously lost its share of the total pulp and paper furnish in the global paper industry. This is likely to continue but at significantly reduced pace. As per estimate the share of wood pulp out of the world furnish mix will be 44% by 2014 as against 52% in 1998 and 70% in year 1980. Recycling of fibre is a rapidly growing segment of the paper industry and will continue to gain share of the fibre furnish world wide. The major driving force for increased use of the recycled fibre has been the environmental concern form the pollution control authorities as well as the customers preference for the environmentally benign products which demand highest quality of DIP because of the high brightness level of the final product.

The main quality issues of paper products using recycled fibres have been runnability and printability that have been limiting the use of these furnish. These are normally related to the instability and

cleanliness of the raw materials and strength properties, filler contents and average fibre length of the furnishes. The recovered furnish is actually a mixture in which the different material contents can vary uncontrolled based on the type and quality of collected and mixed paper and boards. These running and printing problems have been solved by developing better deinking processes, machinery, screening and sorting technology, than can help in achieving better quality of DIP and will also allow to use more low quality of recycled paper and even unsorted materials in near future.

Though the recycled source of fiber, non-coated printed papers which includes xerographic and laser printed papers are the fast growing sources due to the increased usage of office photocopier and computer print outs. However due to the difficulties in deinking of these waste paper by conventional deinking methods, the volume of recycling for this high quality fibre is in less use. In order to effectively utilise larger volume of recovered non-contact printed laser papers there is a need to introduce an effective and efficient technology that will deink non contact ink to an acceptable residual ink count in an economically and environmentally acceptable manner. New deinking mills establish in response to these projected needs are already competing for the cleanest and most homogeneous post consumer paper sources e.g. sorted white ledger and soon will have to dip deeper in to the post consumer stream of unsorted mixed office waste (MOW) to remain competitive.

Current deinking technology is being stretched to accommodate both the hard to remove toner ink. Stickies and the coloured dyes and unbleached fibre present in unsorted MOW. Additional chemicals, multiple floatation steps and dispersion alleviate some of the limitation of the heterogeneous paper stocks. While pulp cleanliness resulting from this sequence is good enough, however the process is capital and energy intensive and sometime loss in pulp yield also occur. Moreover they are not readily removed during floatation or washing. Because of these problems, recycled paper contaminated toners have a relatively low value.

CPPRI having created its vast infrastructural facilities in the area of biotechnology is actively engaged in promotion of biotechnological application in Pulp & Paper Industry. Among various biotechnological applications, enzymatic prebleaching of pulp, biopulping & Slime control & enzymatic deinking of waste paper are the major areas of interest where thrust has been laid upon

evaluation of various enzymes available & being developed indigenously on pulps so that suitable enzymes are made available to the Industry which could be effectively utilised under existing mill conditions.

Recently the use of enzymes to deink recycle fibres has been proposed & being studied intensely in developed countries where in thrust has been laid upon evaluation of the various deinking enzymes available on different papers & inks. But there continues to be a lack of understanding of how these enzymes functions during deinking of papers. The present article highlights an overview of enzymatic deinking process how to remove toner inks from MOW covering the details of the process conditions underwhat conditions enzymes work efficiently while performing its specific tasks.

DISCUSSIONS:

Enzyme deinking :

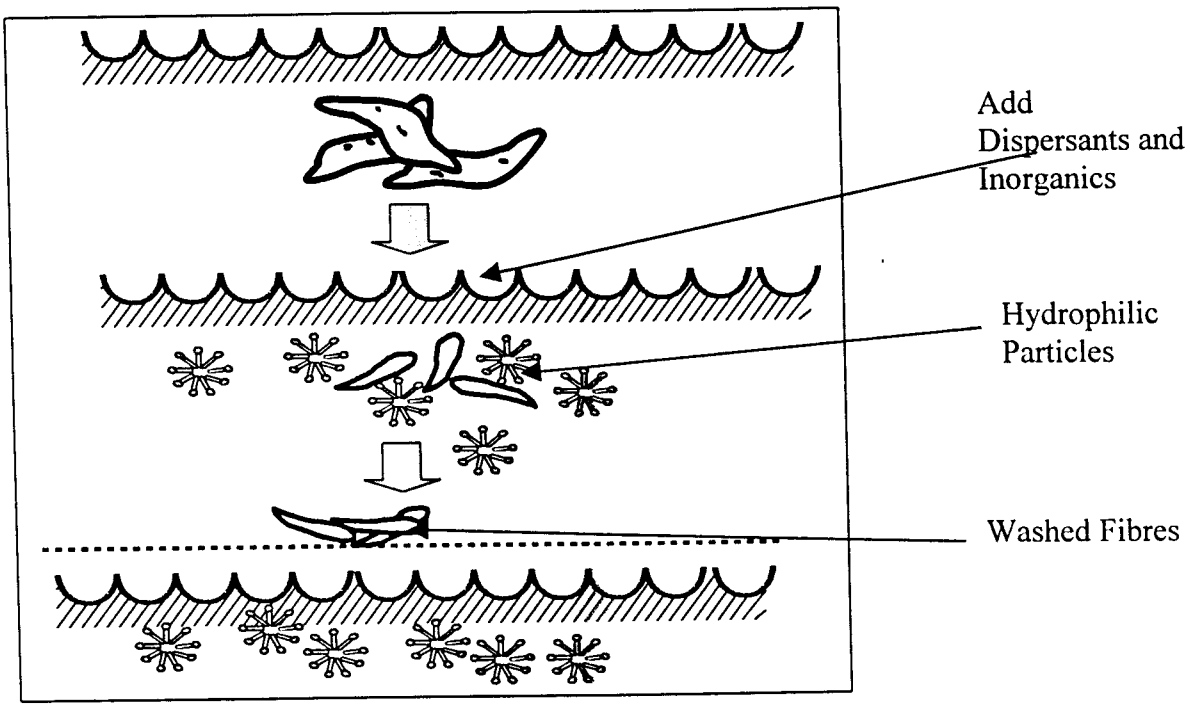
Laser & Xerographic printed paper are the major components of office paper & their use has increase day to day. Laser & Xerographic printing use thermo plastic toners that fuse on to fibre surfaces during high temperature non contact printing. These oven dispensable inks refuse special chemicals ,thermal and mechanical action to detach the ink from fibres so that the inks can be removed by flotation - a deinking process step that separates hydrophobic ink particles from hydrophilic pulp fibres. However costly dewatering & dispersion steps in addition to additional flotation & washing are necessary to improve the deinking of these types of waste paper. Microbial enzymes have also been shown to enhance the release of toners from office waste. Cellulases & Xylanases when applied to Xerographic printed papers in a medium consistency mixer are reported release toner particles and facilitated subsequent flotation & washing steps.

Deinking principles and Current deinking practices.

The common technique used for ink removal from laser and Xerox printing waste are washing and floatation. These kind of paper use thermoplastic toner that fuse on to the fibre surfaces during high

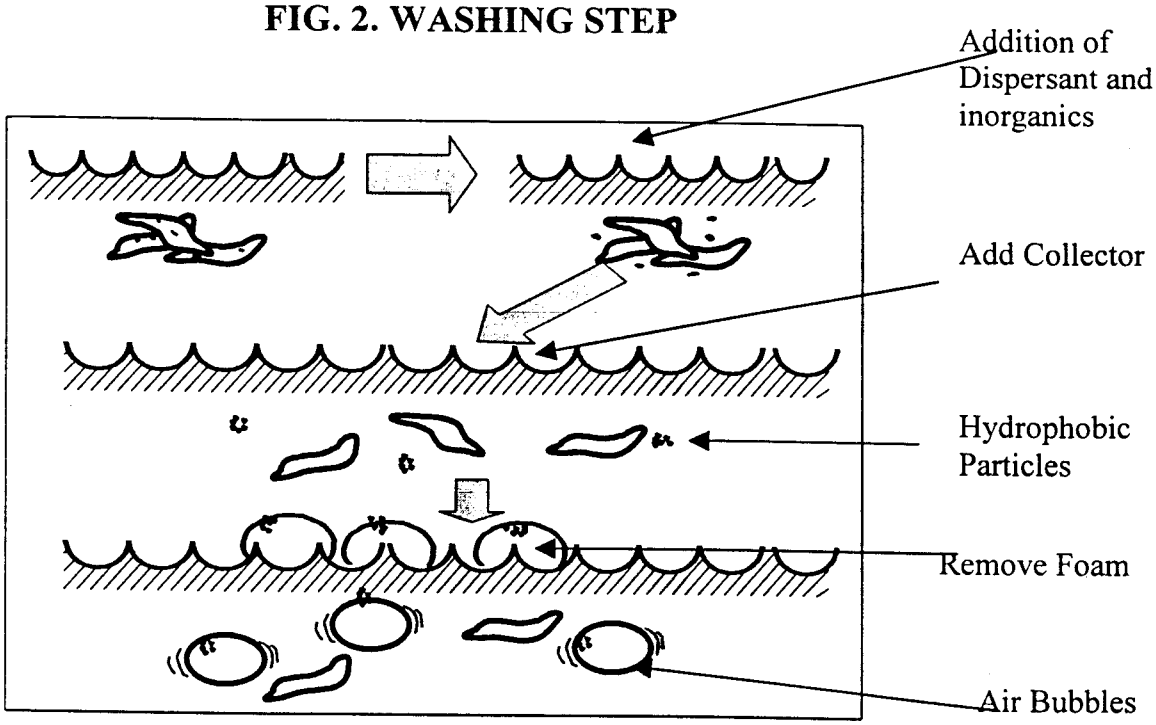
temperature non-contact printing. These non-dispersing inks require special chemicals, thermal and mechanical actions to detach the inks from the fibre so that the ink can be removed by floatation and washings. Floatation is a step in deinking process that separates hydrophobic particles from the hydrophilic fibres as shown in fig.-1. Floatation removes particles that are too small to be removed by screens and cleaners and yet are too big to remove by washing.

FIG. 1. FLOATATION STEP



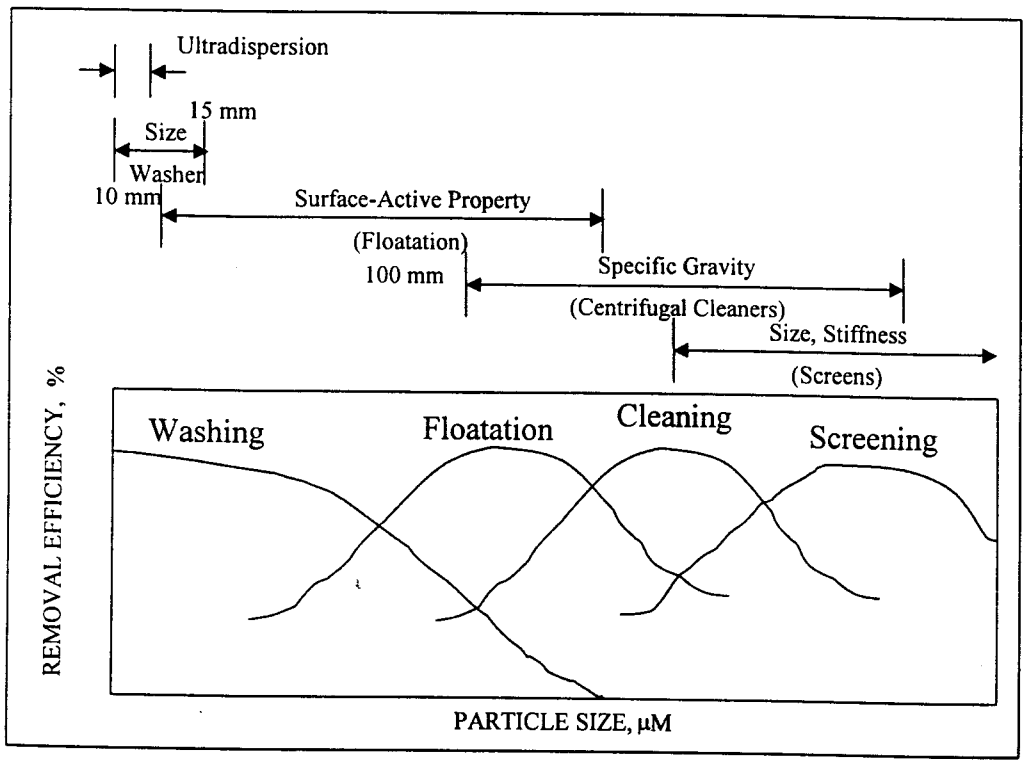
Washing is most efficient at removing the smallest particles of the ink. The objectives in washings are to keep the ink particles finally disperse and agglomerations. Washing requires the ink particles to be rendered hydrophilic so that they remain in the aqueous phase as shown fig.-2. Both of the process normally operates at high pH(10-11) with the use of conventional alkaline deinking agents such as sodium hydroxide, sodium carbonate, sodium silicate and hydrogen peroxide. In this environment the paper structure collapses rapidly and release the ink particles in to the suspension. A dispersant is added to stabilize the colloidal suspensions of ink particles in washing processes.

FIG. 2. WASHING STEP



The dispersion is replaced with a collector soap in floatation deinking. The optimum size range for the different unit operation are shown in fig.-3.

FIG.-3. OPTIMUM PARTICLE SIZE RANGES FROM VARIOUS UNIT OPERATIONS IN DEINKING



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LIMITATIONS AND PROBLEMS IN CURRENT DEINKING TECHNOLOGY

As discussed laser and xerographic inks are thermoplastic which are copolymer of styrene and acrylate designed to be non tacky at room temperature but it melt at temperature of 70-120^{0}C. During the fusion stage of the copolymer process at high temperature (100^{0}C). These thermoplastic resin binder set to form the printed film by cross linking.

These hard cross linked strongly bond system will only be fragmented to minimum size by strong mechanical forces, which in term leads to fibre degradation. Conventional chemical treatment is not effective in reducing particle size further and particles are visible as dirt in the finished product.

Due to this special treatment of laser and xerographic inks some modified deinking processes have been tried, among which washing/ floatation, two stage floatation and agglomeration and disintegration with subsequent removal of screening and cleaning are prominent are to mention. These requires some chemicals and the equipments but the process are capital and energy intensive too.

ENZYMATIC DEINKING

Predominant enzymes used for deinking of waste paper is mainly the cellulases and hemicellulases. Cellulases could work in several ways to enhance deinking. These reduce the hydrodynamic drag to increase the filtration and floatation rate. As cellulases are known to enhance drainage rates they can enhance any separation process such as filtration or floatation. Deinking with enzymes involves dislodging ink particles from fibre surfaces and then separating the disposed ink from the fibre suspension by washing and/ or floatation.

Mechanisms of enzymatic deinking

Deinking can be divided in to five different operations occur in partly consecutively and partly overlapping stages. These stages are:

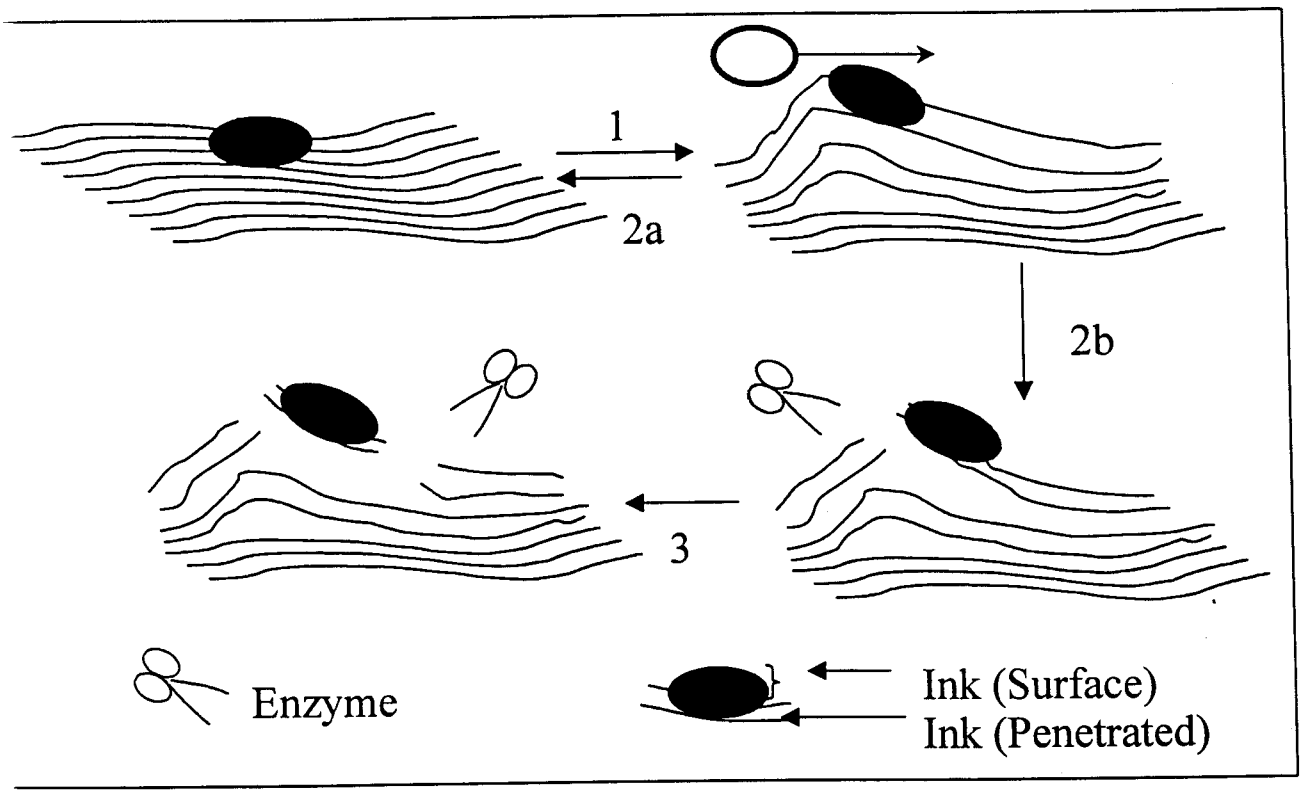
Disintegration, precleaning, chemical or enzyme treatment, floatation and/ or washing and finally bleaching.

During enzymatic treatment, the process is preceded by disintegration and followed by a cleaning operation, which makes it very difficult to determine the exact role of the enzyme. Cellulases action may also increase the specific surface area of the fibres and they reduce interaction with contaminants. That is to say that there might be microfibrils on the surface of these very frazzled, recycled fibres which could be trapping the ink particles, and by giving the fibre a haircut then reduce their adhesion. A model of enzymatic deinking is shown in Fig. -4.

FIG. 4. A MODEL OF ENZYMATIC DEINKING:

1- Friction open the fibre, 2a- Relaxation,

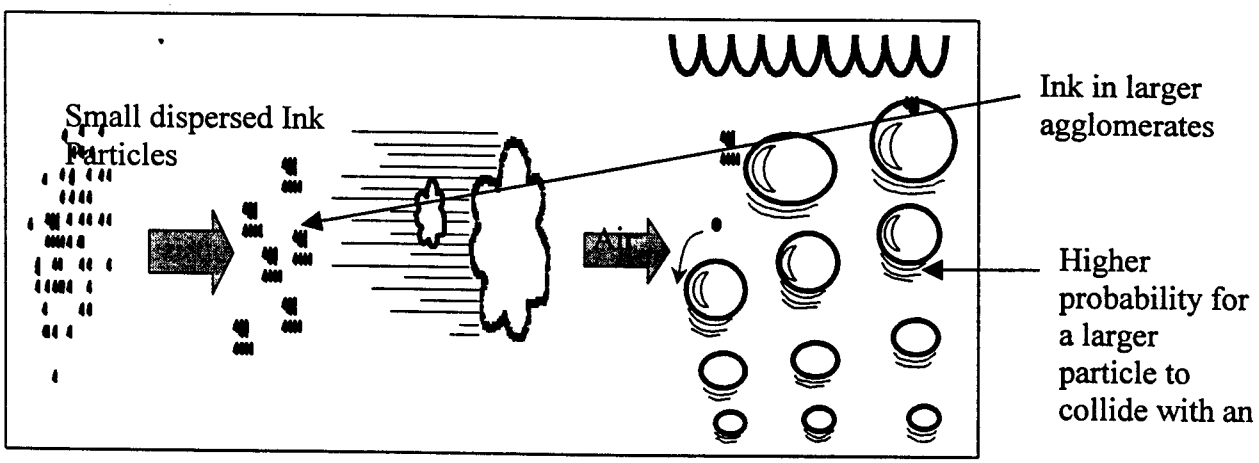
2b - Enzyme cutting, 3-Ink removed



However the studies have shown that the most important fraction with respect to toner removal is the increased floatation efficiency imparted by cellulase enzyme activities and the increased removal of toner has been observed during floatation stage. During floatation air bubbles rise to the surface of the

floatation tank through a relatively dilute pulp stock, approximately a 1% consistency. The surface of air bubbles being relatively hydrophobic, they carry the toner particle to the loop where they are removed by a skimming action. The operating principle of floatation deinking is shown in fig.5.

FIG. 5- OPERATING PRINCIPLE OF FLOATATION DEINKING



PROCESS CONDITIONS DURING ENZYMATIC DEINKING

Deinking Enzymes and the order of its addition

The preferable enzyme used for deinking wastepaper are commonly a mixture of cellulase and hemicellulase. The order for addition of material is to first added the proper amount of waste and waster in order to achieve desired consistency followed by the diluted solution of enzyme.

Access of cellulase enzyme to cellulase fibre is essential for achieving maximum activity of enzyme. Proper mixing of the enzyme at consistency 11-16% helps in dislodging toner particles from fibre and presence of surfactants help the enzyme penetrate through the paper additives. The increased cellulase available for cellulose attachment. Smoothing of fibre increase pulp freeness, which prevents toner particles from becoming trapped in the repulped fibre network.

Enzyme dosing point has been of great significant to achieve maximum enzyme efficiency. Enzyme activity being site specific, distribution of enzyme throughout the pulp is essential to achieve maximum enzyme efficiency. Addition of the enzyme after proper dilution at pulper near the beginning of the repulping process has been desirable. This allows the enzyme to react with waste furnish at higher consistency while under getting optimum mechanical agitation.

Pulp Consistency:

Results shown in the figure-5 clearly indicate that pulping at medium consistency, 12% is advantageous for recovering toner through the combined effect of enzyme and mechanical action. However from the results shown in table-1 clearly indicate that increasing the consistency to as high as 16% increase deinking efficiency. However at higher deinking pulping consistencies and extended pulping time the effect of mechanical action predominant with only little additional benefit from the enzyme.

Table -1 Effect of pulping consistency in ink removal[a]

Particulars	Residual ink at various levels of consistencies (PPM)	
	12%	16%
Control pulp	681	343
Enzyme treated pulp	329	220

a : Residual ink on hand sheets counted in 10-2000 μ range

Surfactants:

Addition of surfactants during enzymatic deinking process play an important role for separation of ink from pulp fibre during floatation. Surfactant make the cellulose more
accessible to cellulase enzymes and facilitate enzyme dispersion, thus making the enzyme available to attach to cellulose sites. Under appropriate conditions, surfactants increase cellulose effectiveness. The use of non-ionic surfactants to the paper prior to addition of enzymes has been preferred to achieve better deinking efficiency as indicated from the results shown in Table-II.

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Table-II . Effect of adding surfactant via floatation cell or pulper.

Specimen	Residual ink (PPM)	
	Floatation cell	Pulper
Control	326	231
Enzyme treated	278	168

Certain anionic surfactants containing sulphates or sulphonated functionally can reduce the efficiency of the enzyme to hydrolyze cellulase and in tern reduce the deinking efficiency of the enzyme.

Moreover there should always be synergy of enzyme with a particular surfactant which leads to be evaluated for an ideal enzyme preparation. Results of effectiveness of two of the identified enzymes and their synergy with the available surfactants is shown in table-III.

Table-III. Enzyme/ surfactant synergy

Variables	Residual ink (PPM)
Control	368
Enzyme A, Surfactant A	242
Enzyme A, Surfactant B	303
Enzyme B Surfactant A	278
Enzyme B, Surfactant B	214

pH :

The commercial cellulase enzyme preparation selected for deinking most active nearly to neutral pH range and around 6.5 to 7.5. increasing the waste paper stream containing alkali additional place some office waste paper repulped waste at the upper end of pH range of approximately 8.5 - 8.9, however better deinking efficiency have been obtained by adjusting the pH to 7.0 -. Results of the effect of pH on ink removal are shown in table- IV.

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Table -IV Effect of pH on Ink Removal

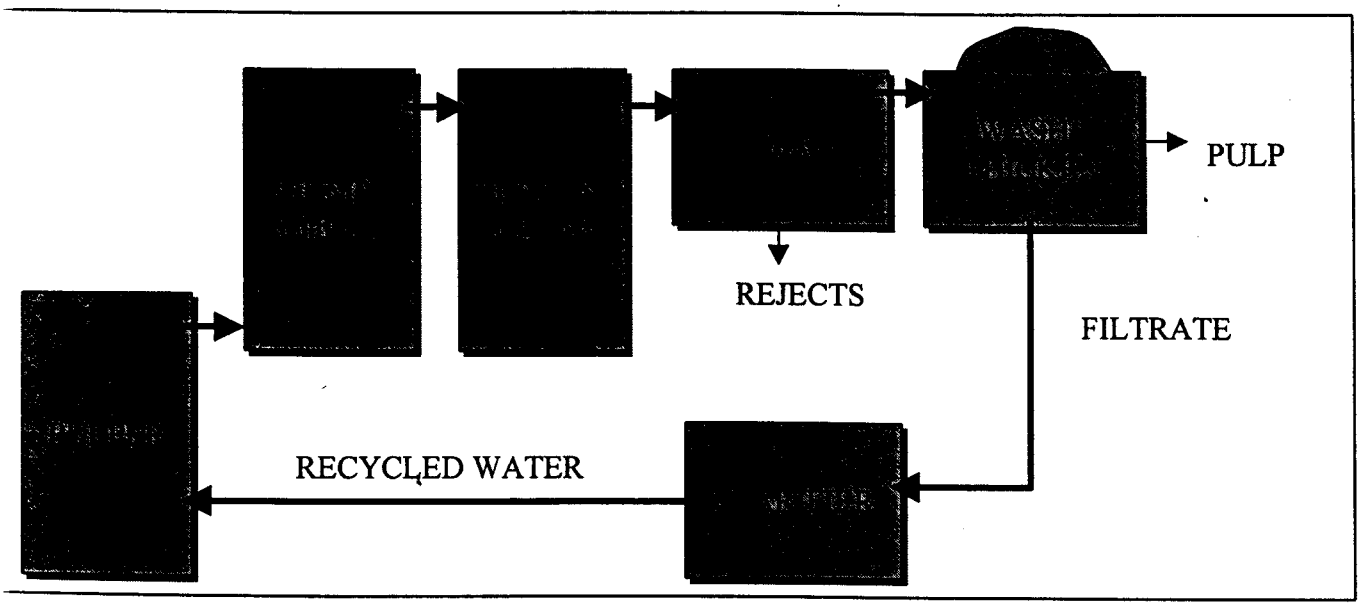
Particulars	PH	Residual Ink (PPM)
Control pulp	---	139
Enzyme treated pulp	8.6	64
Enzyme treated pulp	7.0	47

Depending on the mill performance sulphuric or phosphoric acids can be used to maintained proper pH. Relative enzyme activity at various pH levels is shown in fig.

Temperature:

The optimum temperature range for an conventional cellulase based enzyme has been 45- 55^{0}C. Having realized that the toner-based inks might began to fuse and reattach at higher temperature, the temperature below 65^{0}C is always preferable. The schematic of the proposed process flow sheet of the enzymatic deinking is shown in fig.- 6

FIG. - 6. PROPOSED PROCESS FLOW SHEET FOR ENZYMATIC



CONCLUSION

1. Enzymatic deinking of laser and xerographic waste paper appears to be promising technology for deinking of hard to remove toner inks.
2. Medium consistency pulping conditions of 11-13% appeared to be more effective than low consistency pulping conditions for toner removal.
3. Higher consistency and washing remove laser and xerographic toner from mixed office waste more effectively with a suitable blend of cellulase and xylanase.
4. Selection of an enzyme with optimum activity in the range of the pulped paper stock would minimize pH adjustment in the pulper and therefore simplifies the process and lowers the process cost.
5. Efforts are required to develop and evaluate enzymes preparation suitable for the kind of waste paper utilized by the Indian paper industry to make the technology techno-economically viable.

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Application of Sebrite-BB- a bleach boosting xylanase enzyme for pulp bleaching

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INTRODUCTION

Bleaching of kraft pulp using a combination of chlorination and alkaline extraction has been practiced for many years. Unfortunately, the effluent from chlorination and alkaline extraction stages can not be recycled back to chemical recovery furnace due to its high level of corrosive chlorides. In addition, the effluent contains large amounts of chlorinated organic compounds, which are known to have toxic, mutagenic and carcinogenic effects. The growing public concerns regarding the environmental effects of these chlorinated organic compounds have driven pulp mills to seek new bleaching technologies that reduce or eliminate the use of elemental chlorine in pulp bleaching. Chlorine dioxide, ozone and oxygen delignification have been adopted as alternatives or partial substitutes for chlorine, however, these alternatives due to their high capital cost, seem to have more viable at capacities more than 300 tpd.

The use of xylanase enzyme for bleaching Kraft pulp has generated a great deal of interest because it seems to offer a more cost effective solution in this direction. Due to the reduced application of chemicals, pollution load arising out of these chemicals is considerable reduced. Moreover, the pretreatment does not affect the strength properties of the pulp and is expected to be cost effective

Esvin Blosys international Ltd (EBIL) in association with M/S Advanced Biochemicals Ltd, (ABIL), has carried out successful mill trials with the xylanase enzyme **"SeBrite BB".** SeBrite BB xylanase enzyme is supplied as powder the activity of which, unless otherwise stated, is typically 5000 U/g of powder, The enzyme dosage varies from 0.3 kg/ton of pulp to 0.5 kg/ton of pulp. This enzyme has been found to be equally effective on desperate grades of pulp from low kappa dissolving grade, all agro based pulp to medium kappa chemical pulps and high kappa mechanical pulps.

THEORY AND MECHANISM OF XYLANASE TREATMENT

The distribution and composition of xylan in lignocellulosic raw materials play an important role in biobleaching process. It is observed that in bagasse, hardwood and straw, the xylan proportion is considerable more and hence pretreatment of such pulps with wylanases could be gainfully employed. A number of theories have been proposed to explain the improved delignification with hemicelluloses enzymes.

1. The simplest theory is that the enzyme treatment causes a physical loosening of the fiber wall due to partial depolymerisation of hemicellulose chains, which facilitates extraction of residual lignin during bleaching.

2. The other possible explanation for xylanase action in bleaching is that the disruption of xylan chain by xylanases interrupts lignin-carbohydrate bonds, improves the accessibility of bleaching chemicals to the fiber and facilitates the removal of solubilised lignin in bleaching.

3. Another explanation involves the role of redeposited xylans. It is well known that parts of xylan were initially dissolved in kraft pulping can be reabsorbed or redeposited on and within the pulp fibers. The redeposited xylan may physically shield the residual lignin from bleaching chemicals. Xylanase hydrolyse part of redeposited lignin, allowing better access of bleach chemicals to the residual lignin and easier extraction of lignin from pulp fibers.

The success of biological bleaching will depend upon the type of raw materials, such as, hardwood, softwood etc and also the nature and characteristics of xylanase enzyme.

SEBRITE BB ENZYME CHARACTERISTICS

Several features determine which xylanases are effective for bleaching. The following are the unique characteristics of SeBrite BB xylanase enzyme.

- This is a low molecular weight xylanase that is able to penetrate the fiber.

- It has an appropriate pH optimum.
- It has an alkaline pl in order to bind to negatively-charged fiber surfaces.
- Thermally stable at the temperature prevailing in the pulp stock.
- It possesses appropriate substrate specificity. The objective is to release chromophores and extract residual lignin not remove the bulk xylan.

Enzyme penetration throughout the cellulose fiber is essential in order to fully access and extract residual lignin. Complete penetration might not be achieved in a single enzyme application. Enzyme penetration is determined in part by molecular weight, but other factors such as substrate bindings may be more critical. High substrate affinity may be a positive factor when enzymes are applied to pulps in a dilute suspension, (because they would keep the enzyme in the pulp rather than free solutio9n) but at the relatively sigh consistencies used in bleaching plants (>10% total solids) they could work against enzyme diffusion to the interior of the fiber.

The second important characteristic, at least with respect to kraft pulp, is that the enzymes should have an alkaline pH optimum. If one uses a xylanase with an acidic pH optimum, it is possible to wash most of the alkali out of the pulp then neutralize the residual with sulfuric acid. Not much acid is required, but alkali keeps leaching out of these pulps even after extensive washing and pH adjustment. In fact, enzyme activity actually enhances the leaching of the alkali, and so one can observe a constantly increasing pH in the bulk solution. Moreover, neutralization may be uneven, and local pH may be critical. In order to maximize effectiveness, it is essential for the enzyme to act in the interior of the fiber where the residual alkali concentration is highest, so an alkaline pH optimum is very important.

A third feature that's useful in a xylanase is an alkaline isoelectric point. With an alkaline pl, the enzyme binds more readily to the fibers under the pH prevailing in the pulp, and this is important for determining its ability to attack the substrate. Pulp fibers are negatively charged due to the presence of sugar acids, and if an enzyme has an alkaline pl and hence a positive charge at the operational pH, it will bind more effectively.

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The fourth factor, thermal stability is not finally a problem for xylanases. Most microbial xylanases are stable at 30-60 deg.C, and this is within the range of prevailing temperatures in pulp mills.

THE PRE-REQUISITE FOR A GOOD BLEACH PLANTS ARE AS UNDER:

1. The entering pulp from mill should contain least amount of residual lignin (lowest Kappa number pulp).
2. the organics carry over with pulp entering the bleach plant should be minimum (COD should be least).
3. the knots, shives and particles in the pulp entering the bleach plant should be minimum.

The kraft process results in excellent pulp from a wide variety of wood species. Kraft pulp fibers are flexible, readily hydrated, and the pulp matrix is accessible to enzymes. Unfortunately, degradation products generated during pulping become trapped in this matrix and impart a brown color to kraft pulp. Cooking consumes pulping chemicals, and residual xylan (along with covalently-linked degradation productions) precipitates on the surfaces of the cellulosic fiber. The chromophores are believed to be composed of residual lignin and carbohydrate degradation products. They are hard to extract because they are covalently bound to the carbohydrate in the pulp matrix.

Manufacturers use elemental chlorine (CL2) and chlorine dioxide (Clo2) to bleach the chromphores, and then they extract the pulp to make white paper. Chlorine bleaching creates environmental problems. CL2 makes toxic and forms chlorinated aromatic hydrocarbons (including small amounts of dioxin), and pulp manufacturers must use special processes to remove them, from effluent streams. Consumers are becoming increasingly wary of chlorine bleached papers plates. More over, environmental regulations have largely eliminated chlorine bleaching as an acceptable process in several European countries.

SERBRITE BB APPLICATON BENEFITS

1. Increase in pulp brightness

Due to globalisation, paper mills in India are facing continuous pressure to produce paper with higher brightness levels. Chlorine Dioxide and Oxygen/ Ozone bleaching processes are highly capital intensive. Enzyme bleaching technique offers a cost effective route for these mills to achieve higher brightness levels.

CPPRI has carried out an extensive lab trials on Eucalyptus kraft pulp using "SeBrite BB" (earlier known as "Starzyme Alba") and made observations that brightness gain of 2 units could be achieved, while using Sebrite BB as prebleaching enzyme. Determination of the strength properties of enzyme treated pulps, showed similar or slight improvement in burst, tear & tensile Index. The results achieved in these trial are furnished in **Table 1.**

EBIL has also carried out an elaborate study on enzyme bleaching using "SeBrite BB" on hard wood kraft pulps in order to achieve higher brightness level. In these trials there is 3.0 unit brightness improvement, when the unbleached pulp is treated with SeBrite BB at a dosage level of 0.5 kgs/T of pulp at an unbleached pulp pH of 8.5 and at 35 C. The results achieved in these trials are furnished in Table 2.

Based on the above lab trials, five days mill scale trials were carried out in two big mills. In one mill about 1000 T of unbleached hard wood pulp was treated with SeBrite BB and in another paper mill, about 1500 T of unbleached hard wood pulp was treated with SeBrite BB. The procedure followed for enzyme solution preparation was as follows.

10 kg of the SeBrite was dumped into a sintex tank and 10% solution was prepared using 100 L of process water. Then the 10% solution was transferred into another sintex tank, which is connected, to a metering pump. The flow of enzyme stock solution was maintained by adjusting the stoke of the metering pump. The stock solution was pumped to a flow box where the enzyme got diluted

with process water (approximately 2 cu.m). The working solution thus obtained was then showered on the Decker pulp, through a shower pipe placed above, pulp pH was found to around 10.0 Therefore, a mild acid dosage was used to bring up the pulp pH to around 9.0. Also the enzyme was dosed @ 0.1 kg/T at the extraction stage along with peroxide addition.

The results achieved in various above trials has shown that it is feasible for the mills to enhance the final brightness of the bleached pulps by 1.5 to 2.0 point, thus enabling them to cope with growing market demand for higher sheet brightness.

2. Reduction in AOX Level

There is a new 'environmental' thrust in India to reduce the level of AOX (Absorbable Organic Halide) discharged by the paper mills. The classical approach of substituting gaseous chlorine with Chlorine Dioxide is found to be highly capital intensive. By using SeBrite BB Xylanase Enzyme, prior to chlorination stage, mills can now easily achieve reduction in gaseous chlorine consumption to the extent of 15%-20%, thereby realizing consequent reduction in AOX levels.

CPPRI has carried out a lab trial on enzymatic prebleaching using "SeBrite BB" and found that in case of enzyme treated pulp, even with 12% less chlorine, nearly 1.2 units brightness gain could be achieved. This has also resulted in 17% reduction in AOX toxicity of bleach plant effluent. The results achieved are furnished in **Table 3.**

3. Reduction in Bleach Chemical consumption/BOD/COD & Color Levels

Mills which have no necessity or incentive to increase brightness, can use the enzyme prior to or together with extraction stage to cut down caustic and hypo consumption, thus resulting in reduced pollution level but increased pulp strength.

EBIL has carried out a detailed study on use of SeBrite BB for agro based pulps, in order to achieve substantial reduction in chlorine, caustic and hypo. Wheat straw pulp was used for the

trials. Enzyme was applied in two stages, total dosage@ 0.3 kg/ton of pulp. Keeping the total enzyme dosage @ 0.3 kg/t and applying 0.1 kg/t at pre chlorination and 0.2 kg/t at post chlorination, resulted in:

- Brightness improvement
- Chlorine reduction (gas) @ 5 kg/T
- Caustic reduction @ 2 kg/T
- Hypo (as av. Cl2) @ 19 kg/T

The results are furnished in Table 4.

Mill scale trials were carried out in one big mill in south India for 10 days with a view to reduce the consumption of bleach chemicals viz., chlorine and hypo by using enzyme before and after chlorination. In total about 920 MT of unbleached hard wood pulp was treated with enzyme. The regular bleach sequence followed by the mill was C-Ep-H-H, by which they could able to achieve 80+ brightness. During the enzyme trials, the bleach sequence was changed to X-C-X-Ep-H and the target brightness was maintained at 80+.

Two barrels each of 200 L capacity was installed near the unbleached decker. Enzyme stock solution was prepare, which is sprayed over unbleached decker conveyor repulper. The flow was through gravity and no dosing pumps were used. Similar arrangement was made for adding enzyme to the chlorinated pulp (after adjusting chlorine washer pulp pH to 8.5). the hydrogen peroxide addition as switched over to the next stage(i.e H I stage), making way for single stage hypo addition only. The caustic was added at both enzyme stage (Buffering) and alkaline peroxide stage (extraction). The results indicated that-

- There is a reduction in elemental chlorine to the extent of 11% and hypo to the extent of 9%.
- The COD and BOD levels of bleach filtrates were reduced to the extent of 25%.

- The pulp brightness was maintained at 80+ and the strength properties, PC values and pulp viscosity were found comparable to the conventionally bleached pulp.

CONCLUSION

Enzymes can be used in many stages of pulp and paper processing. These include enhancing digestion, selectively removing xylan, removing pitch, and facilitating bleaching and removing contaminants. The use of enzymes in pulping, bleaching and deinking processes results in significant reduction in pollution load. The value addition by enzyme treatments can readily justify the cost of enzymes in many instances and therefore, has lot more and wider applications in future.

Table 1. Bleaching of Eucalyptus kraft pulp using conventional C-Ep-H sequence before and after enzyme treatment.

Aim: To determine the brightness gain due to SeBrite BB xylanase application.

Particulars	Control pulp	Enzyme treated pulp
% chlorine applied	3.4	3.4
%NaOH applied	2.0	2.0
% Peroxide applied	0.8	0.8
Brightness % ISO	59.3	61.5
% Hypo applied	1.5	1.5
Brightness % ISO	83.1	85.7

Strength and optical properties before and after enzyme treatment

Particulars	Control Pulp	Enzyme treated pulp
Freeness CSF	220	225
Apparent density,g/.m3	0.85	0.85
Burst Index , Kpa m2/g	5.65	5.10
Tensile Index, Nm/g	89.0	88.5
Tear Index, Mnm2/g	5.4	5.75
Optical Properties		
Opacity %	80.36	80.24

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Table 2. Bleaching of Eucalyptus kraft pulp using conventional C-Ep-H-H sequence before and after enzyme treatment.

Aim: To determine the brightness gain due to SeBrite BB xylanase application.

No	Particulars	Unit	Blank	Trial I	Trial II
1	Enzyme Stage:				
	- Pulp pH			8.5	9.5
	- Pulp Temp	Deg C		35	35
	- Dosage	Kgs/Ton		0.5	0.5
	- Retention time	Min		120	120
	- Consistency	%		8	8
2	Chlorination				
	- Chlorine dosage	%	3.5	3.5	3.5
	- Consistency	%	3.0	3.0	3.0
	- Temperature	Deg C	Amb.	Amb.	Amb.
	- Retention time	Min	60	60	60
3	Alkali extraction				
	- NaOH dosage	%	2.0	2.0	2.0
	- H2O2 dosage	%	0.8	0.8	0.8
	- Consistency	%	10	10	10
	- Temperature	Deg C	70	70	70
	- Retention time	Min	90	90	90
4	Hypo I Stage				
	- Hypo dosage	%	1.5	1.5	1.5
	- Consistency	%	10	10	10
	- Temperature	Deg C	40	40	40
	- Retention time	Min	120	120	120
5	Hypo II Stage				
	- Hypo dosage	%	0.5	0.5	0.5
	- Consistency	%	10	10	10
	- Temperature	Deg C	40	40	40
	- Retention time	Min	90	90	90

RESULTS

Particulars	Unit	Blank	Trial I	Trial II
Brightness				
- After extraction	%	58.0	65.5	65.0
- After Hypo II	%	84.5	87.5	86.5

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Table 3. Bleaching of Eucalyptus kraft pulp using conventional C-Ep-H sequence before and after enzyme treatment.

Aim: To determine the reduction chlorine & reduction in AOX in bleach plant filtrate due to SeBrite BB xylanase application.

Particulars	Control pulp	Enzyme treated pulp
% Chlorine applied	3.4	3.0
% NaOH applied	2.0	2.0
% Peroxide applied	0.8	0.8
Brightness % ISO	59.3	61.2
% Hypo applied	1.5	1.5
Brightness % ISO	83.1	84.3

Characteristics of bleach plant effluent before and after enzyme treatment

Parameters	Control Pulp	Enzyme treated pulp
AOX, Kg/T	1.47	1.22
AOX reduction %	-	17

Table 4. Bleaching of wheat straw pul using conventional C-Ep-H sequence before and after enzyme treatment.

Aim: To determine the maximum reduction in bleach chemical consumption, keeping the target brightness same, by SeBrite BB xylanase application.

No	Particulars	Unit	Blank	Trail 1	Trail 2
1	**Enzyme Stage**				
	- Pulp pH			7.8	7.8
	- Pulp Temp	Deg C		33.0	33.0
	- Dosage	Kgs/T		0.15	0.10
	- Retention time	Min		120	120
	- Consistency	%		8	8
2	**Chlorination**				
	- Chlorine	%	7.5	7.5	7.5
	- Consistency	%	3.0	3.0	3.0
	- Temp	Deg C	Amb	Amb	Amb
	- Retention time	Min	60	60	60

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3	**Alkali Extraction**				
	- NaOH	%	3.5	3.5	3.5
	- Enzyme	Kgs/T	0.0	0.15	0.20
	- Consistency	%	10	10	10
	- Temp	Deg C	60	60	60
	- Retention time	Min	90	90	90
4	**Hypo Stage**				
	- Hypo	%	4.5	2.6	2.6
	- Consistency	%	10	10	10
	- Temp	Deg C	40	40	40
	- Retention	Min	180	180	180
	RESULTS				
	Final Brightness	**%**	**79.0**	**9.0**	**80.0**

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Biotechnological applications in pulp & paper and activities of CPPRI

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INTRODUCTION

Biotechnology implies the technical exploitation of the biological processes. The technology has received considerable attention during the last one and half decade because of its commercial potential in number of areas. Pulp and paper is no longer an exception. Increased environmental pressures, high cost component due to increased usage and cost of energy and customer preference to environmentally benign products has forced the paper industry to look for new or greatly modified technologies, which will be necessary to carry the industry into the twenty- first century.

A brief look at the process technology trends world-wide show a clear leaning towards use of enzymes for various applications in pulp and paper industry and most important among various applications are the use of enzymes as an aid to pulping and paper making for reduction in use of cooking and bleaching chemicals, energy during pulping and refining, management ofwood and industrial waste minimisation. Enzyme technology is driven by the need for economic, efficient and ecologically friendly processing. Cost effective applications are made possible by the large-scale capacity for producing novel enzymes through application of modem technology. Enzymatic applications are now emerging in the area of pulp and paper where the potential areas which are being explored for their applications in commercial scale are bleach boosting, contaminant removal, lignin degradation, pitch removal, fibre modification and pollution control.

Recent interest for biological processes, in manufacture of pulp and paper particularly in the area of fungal de lignification of fibrous raw materials and enzymatic prebleaching of pulps, (biopulping & biobleaching) is a consequence of the possibiiities offered by biotechnology which imply the use of enzyme or the organism as a whole. In order to improve the energy and chemical efficiency together with environmental constraints derived from more strict legislation in pollution matters, these processes shows promising future where the selected enzyme or the selected strains of

microorganism are able to pretreat the fibrous raw material under controlled conditions. Although some work has been carried out in the developed countries using certain specific enzymes and/or microorganisms mainly on soft woods and certain specific hard woods, but not much of work has been carried out on screening of indigenous enzymes/ microorganisms in context to the fibrous raw materials used by Indian paper industry. Therefore studies are required to be undertaken and process conditions needs to be developed suiting the raw material, microorganisms and process conditions employed in Indian paper industry.

Central Pulp & Paper Research Institute (CPPRI), as part of its programme on promotion of clean & green technologies could identify biotechnological application as one of the promising up-coming cleaner production technology option in Indian Paper Industry .Extensive studies have been carried out at the institute and various enzyme preparations, both indigenous and the imported commercially available enzymes have been evaluated on mill pulps both from wood and non wood fibrous raw materials. Since the effectiveness of a particular enzyme may vary in respect of its activity, purity, enzyme pre-treatment conditions and the type of pulps, therefore evaluation studies are required to be carried out in order to assess its response on the pulps being produced in Indian paper industry and to develop tailor made enzyme for enzymatic prebleaching of pulp (biobleaching) and/ or biodegradation of lignin in the fibrous raw material(biopulping).

Thrust areas identified by CPPRI, for biotechnological application in pulp and paper industry.

1.	Microbial delignification of fibrous raw material employing white rot fungi	Biopulping
2.	Enzymatic prebleaching of pulp	Biobleaching
3.	Improving the drainage of wire part in paper making	Slime control
4.	Environmental pollution control	Biomethanation of pulp mill effluents in agrobased mills
5.	Removal of colour from mill effluents by use of microbes	Colour removal
6.	Enzymatic removal of ink particles from xerographic papers	biodeinking

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DISCUSSIONS:

ENZYMATIC PREBLEACHING OF PULP (BIOBLEACHING) :

The enzymes used commercially in pulp bleaching are hemicellulases, which selectively affect the accessible hemicellulose fraction of the pulps. Among various available hemicellulases, xylanases have been found to be more effective as prebleaching agents.

Xylanase producers are found both among bacteria and fungi. Several criteria are essential for choosing microorganisms to produce xylanase. In additions to possessing the desired biobleaching effect, the enzyme must be produced in sufficiently hige quantity and should be completely free of cellulase activity. Any cellulase activity will have serious economic implication in terms of cellulose loss, degraded pulp quality and increased effluent treatment cost. Now xylanolytic preparations could be produced by recombinant DNA technology, selective inactivation or bulk scale precipitation. High productivity could be achieved by exhaustive screening, genetic engineering and growth optimisation programmes.

ENZYMATIC PRE-TREATMENT OF WOOD KRAFT PULPS:

Effect of xylanase treatment on bleaching chemical requirement:

xylanase treated pulps differ in response to bleach chemical than untreated pulps. Bleaching of the control and enzyme treated pulps using conventional CEH bleach sequence showed remarkable reduction in the requirement of chemical chlorine which was reduced from 4.2% (42.00 kg/tp) to 3.6% (36.00 kg/tp) in case of enzyme treated pulp. Following the alkali extraction & hypo stage additional brightness gain in bleached pulp could be achieved to a level of3.0 % ISO points i.e. the final brightness was improved from 80.6 % ISO to 83.7 % ISO respectively with both xylanase enzymes (Table-l). Xylanase treatment has remarkable effect on the yield of the pulp as possible explanation for the effect of xylanase treatment as reduction in the requirement of the chlorine might be that xylanase effects the removal of specific lignin structures, leaving a residual lignin in the pulp which may be more responsive to bleach chemical oxidation than the residual lignin in conventional

pulps. Thus the quantities of the lignin represented by kappa factor of around 0.2 in conventional and enzyme treated pulp might not be identical but actually, lower in the enzyme treated pulp. Moreover the lignin in the enzyme treated pulp may respond better to oxidative bleach chemicals. The enzyme treated pulp samples which contains comparatively lower lignin are bleached to a higher brightness than conventional bleached pulp.

Effect of xylanase treatment on strength & optical properties of the pulp:

Extensive studies on evaluation of the xylanases treated pulps with both enzyme preparation indicate that such pulp required equivalent or sometimes more amount of refining energy to refine the pulps to same freeness level than control pulps (8). Determination of the strength properties of both enzyme treated pulps, showed that burst and tensile index could be maintained at par which were found to be 3.80 & 4.10 Kpa .m2/g against 4.10 and 60.5 and 64.0 NM./g against 64.0 of control pulp respectively. Little drop in burst and tensile index in case of enzyme-1 treated pulp could be resulted by reducing the treatment time and/or reduced enzyme dose. The tear factor was found to almost at par i.e. 4.9 mN.m2/g in both enzyme treated pulps as against 5.05 in case of control (Table-1b).

Environmental effect of enzyme treatment:

Reduction in chlorine demand of around 15% during CEH bleach sequence resulted in lowering the toxicity of the bleach plant effluent in enzyme treated pulp which could be reduced to the tune of around 20% as AOX level was reduced from 2.12 kg/tp to 1.8 kg/tp. Results are shown in the Table-2.

FUNGAL TREATMENT OF FIBROUS RAW MATERIAL(BIOPULPING):

Biopulping is the term applied to de lignify and involve either lignin depolymerising enzymes or growth of whole microorganism on fibrous raw materials. Either strategy would be a pre-treatment that lowers the use of subsequent chemical employed for biopulping and energy required for refining.

Biopulping processes shows promising future and certain microorganisms are able to delignify the fibrous raw material under controlled conditions. The process needs to be developed in Indian pulp and paper industry which might further improve the environmental situation.

White rot fungi produces a variety of extracellular enzymes that are involved in lignin degradation, the best characterised of which are laccase, MnP and LiP .These enzymes are capable of forming radicals inside the lignin polymer which results in destabilisation of bonds and finally the lignin macromolecule breakdown. Although still far &om completely understood the above enzyme systems are being characterised mechanistically and on a molecular level.

Studies carried out at the institute mainly stressed upon the screening of various identified white rot fungal strains like *Phanerochaete chrysosporium, Pleurotus ostreatus, Ceriporiopsis subvermispora* collected form reputed culture banks in India and abroad and two white rot fungal strains isolated from Indian paper mills renamed as CPPRI-ST-2 and ST-4, for their ability to delignify eucalyptus and bagasse, the major fibrous raw material for Indian paper industry .The preliminary studies indicated that these strains have different de lignifying efficiency. The fungal strain CPPRI-ST -2 could be screened as better delignifying fungus having efficiency at par with any imported strain. The result of pre-treated raw material of eucalyptus chips and depithed bagasse with various fungal strains are shown in Tables-3 and Table-4 respectively. These results show that the pre-treatment of the fibrous raw material with lignin specific strain of white rot fungi could raise the possibility for biopulping which could prove to be energy efficient and cleaner production technology.

However, scale up to the industrial process requirements presents challenges that are difficult to simulate in laboratory or pilot scale tests. Inoculation, aeration and heat dissipation are key parameters for maintaining fungal activity. It may be possible to monitor and maintain consistent treatments through a programme of wood chip pile management. Overcoming these challenges will determine, if biopulping becomes a reality.

To sum up biopulping research is currently following two tracks.

The first is to use the white rot organisms as pretreating agents for subsequent mechanical pulping.

he second is to use the isolated ligninase enzymes.

In the first case the challenge still is to optimise the organism for the wood being treated and thus bring down the treatment time. In the second case the thrust seems to be the produce the enzymes via recombinant DNA techniques and then to reconstitute an enzymes system that will duplicate in-vivo lignin degradation.

SLIME PROBLEM AND ITS CONTROL IN PAPER MAKING

Uncontrolled growth of microorganism in a paper mill can lead to a variety of process and quality problems resulting in down time and loss of nutrients to sustain growth and reproduction of microorganism. Microbiological contaminants can enter a mill from a number of sources such as fresh air, fresh water, pulps and waste papers etc. microorganism commonly found in paper mill system includes aerobic and anaerobic bacteria as well as molds & yeasts and when unchecked, biological contamination can result in the formation of slime deposit and biofouling. These deposits (shown in Fig.- 1), also known as biofilms can clog filters, screw and pipelines and results in spots and breaks in the paper sheet. Insufficient growth control may lead to problems that are identified as below

Area	**Problem**
Production	Slime deposits Breaks Corrosion Felt plugging Odour Reduced flows
Quality	Holes & spots Machine aesthetics Discolouration

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	Brightness losses Increased dirt count
Raw material	Fibre degradation Additive contamination Odour Reduction in strength properties Coating mass deterioration

In order to minimise the above said problem, microorganism level must kept under control. The use of slimicides in addition to good house keeping is the only practised way to control bacterial growth. Biological control involves more than just a haphazard addition of antimicrobial agent referred to as biocide or slimicide. Slimicides have been used in the pulp and paper industry since 1800s. many of the early active ingredients were used indiscriminently with little regards for workers health and safety on environmental concern. In the recent years there have been continuous efforts from biocide suppliers to provide safer products, offer an effective treatment programme can be implemented, the source, extent of contamination and nature of slime is necessary to be known.

CONTROL OF SLIME FORMATION IN PAPER MACHINE

In conventional slime control schemes, chemical slimicides are added to the system. However, microbial acclimation to the chemical and concern over the chemicals environmental impact are demanding safe use of environmental friendly and highly effective products.

SLIMICIDE DISPERSANT APPROACH

Biodispersants are typical chemical dispersant which have been screened and selected to be highly effective against biological deposits. Some have a fllming effect when added to a system. This film disrupt the contact between the biofilm and surface in addition the biocides/ dispersant penetrate the deposit, weakening the structure and making it easier to erode and remove the biofilm with the

normal flow of water through out the system. Biodispersant have very low toxicity, so they are readily acceptable in environmentally sensitive environment. They are easy to apply and can

provide both prevention and cure. They can be used alone in place of biocides or to supplement biocides. In either case use of biocides is minimised and treatment are more efficient. A dispersant aids the biocide performance in two ways: by wetting the various surfaces, thus interfering with the attachment of bacteria, and by allowing the biocide to penetrate the deposit and the cell at a faster rate, thereby minimising slime accretions.

ENZYME APPROACH

Enzyme based slimicides acts as biodispersants, which consists of stabilised enzymes. The enzymatic slimicide treatment in deposit control attack the pili with which bacteria attach themselves to surface and to each other. Degradation of these structures weaken the structural strength of the biofilm and allow it to be flushed away by the shearing force of the water. Traditionally, slime has been controlled by the use of slimicide or the combination of slimicide and the biodispersants. Recently, however efforts were being made to by a different approach to attack directly on the bacterial capsule improving the use of enzymes, enzymes that may have the ability to catalyse the breakdown of a microcapsular component, are being used in mill system. Theoretically if a reduction can be made in the formation ofbacterial capsule, then the ability of some bacteria to adhere to the machine surface and to other part can be reduced.

Having advantage of being environmentally friendly and non toxic in nature, the application of enzyme suffer with a set back of its specific nature, which limits its effect to a particular component (capsule varies greatly within species as well as between species), as well as the inability of the enzyme to act on macrocapsulated slime causing microbes, such as filamentous bacteria. Because of these limitations recently enzymes trend have usually included the use of traditional biocide as well. However, recently developed enzyme based slimicides by few of the enzyme/ slimicides manufacturing companies, have not proved to be effective in control of slime.

Fig. 1 Microphotograph of slime forming filamentous bacteria isolated from an agrobased paper mill slime

W ASTE MANAGEMENT

The pulp and paper industry already depends on microbial technology to treat its manufacturing wastes. Oxidation ponds, lagoons, Unox reactors, activated sludge beds, rotating biological contractors, and others are all ways of using mixed population of microbes to destroy or detoxify wastes. Although some of these systems are sophisticated and all can be highly effective, they can be improved through research, particularly as concern for the environment growths. Microbes screened from natural populations, generated via mutation and selection, or through genetic engineering are being developed to degrade specific industrial waste or recalcitrant products.

An example of the potential of biotechnology is the decolourising the efiluent of Kraft bleachers. Colour is due to polymeric lignin degradation products. These chromophoric material are resistant to the micro flora in current waste treatment facilities. Recent investigation has shown the use of white rot fungi to decolourise the highly coloured first extraction stage efiluent. Similar research has also shown the promising results for scale up of the process and further development.

CONCLUSION

Biotechnology has the potential to benefit the pulp and paper industry in many areas, including the growth of trees, the processing of wood and pulp, the utilisation of by- products, and the management of wastes.

In wood processing, biotechnology offers the tantalising prospects of biopulping, biobleaching and biological improving mechanical pulps. Alternate use of wood through bioprocessing can also be investigated, particularly for residues and wood not suited for pulping. These use include production of food and feed and conversion to fermentable sugars. Fermentation of carbohydrate rich byproduct streams to chemicals or protein is already being practised and can be expanded. Bioconversion of lignins to useful products may be possible. New improved microbial waste treatment processes are anticipated. The speed with which the many potentials of biotechnology are realised in the pulp and paper industry will depend in large part on the commitment of management to research and development. The most pressing research needs are probably in the basic biology, physiology and biochemistry of trees and key microorganisms.

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Table-1 a. Bleaching of pulp using conventional CEH sequence before & after enzyme treatment.

Particulars	Control Pulp	Enzyme- 1 Treated Pulp	Enzyme - 2 Treated Pulp
Chlorination Stage			
%, Chlorine Applied	4.2	3.6	3.6
% Chlorine Consumed	98.8	88,4	88.4
% Chlorine Savings	--	14	14
Alkali Extration Stage			
%, NaOH	1.5	1.5	1.5
% NaOH Consumed	57.7	46.6	58.3
Kappa No.	4.3	3.2	3.3
Hypo Stage			
% Hypo Applied	2.0	2.0	2.0
% Consumed	94.0	88.0	83.0
Final Brightness of the Pulp, % ISO	80.6	83.8	83.7
Brightness Gain, %	--	3.2	3.1

Table-1 b. Strength & Optical properties of wood pulp before and after enzyme treatment.			
Particulars	Control Pulp	Enzyme- 1 Treated Pulp	Enzyme - 2 Treated Pulp
Strength Properties			
Revolution, PFI	4000	4000	4000
Freeness, CSF	160	185	215
Apparent density, g/m3	0.82	0.84	0.79
Burst Index, Kpa.m2/g	4.1	3.8	4.1
Tensile Index, N.m/g	64.0	60.5	64.0
Hypo Stage			
Tear index M.nm2/g	5.05	4.9	4.9
Optical properties			
Brightness , % ISO	65.7	68.0	68.7
Opacity, %	92.2	91,2	91.2

Table-2. Characteristics of bleach effluent (CEH stage) of enzyme pre-treated & untreated Kraft pulp			
Particulars	Untreated pulp efluent	Enzyme-1 treated effluent	Enzyme-2 treated effluent
COD, kg/tp	19.57	25.87	31.92
BOD, kg/tp	3.42	7.11	9.06
AOX, kg/tp	2.12	1.80	1.80

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Table-3. Chemical composition of fungal treated & untreated depithed bagasse (21 days)			
Particulars	Untreated	S2 treated	S4 treated
%, Weight loss	0	6.1	5.85
Klasson Lignin* (after wt. loss)	0	7.01	2.3
α-Holocellulose	72.54	70.94	70.46
Cellulose	56.78	59.07	57.7
H/L ratio	3.02	3.23	3.05
Pentosans	22.38	21.83	19.97

* On total Lignin content

Table-4. Chemical composition of fungal treated (30 days) & untreated chips				
Parameters	Untreated	S2 treated	S4 treated	Ceriporiopsis subvermispora treated
Weight loss	0	03.84	3.81	1.86
Klasson Lignin	32.64	30.7	31.21	32.27
Holocellulose	66.9	63.85	64.06	65.93
K-Lignin loss	0	5.94	4.38	1.13
Cellulose	67.38	70.67	70.66	71.49
Pentosans	16.2	12.6	12.31	16.07
H/L Ratio	2.05	2.07	2.05	2.04

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Novel Microbial Xylanase for Paper Industry

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INTRODUCTION

In the last few years there has been growing pressure for change in the way that chemical pulps are bleached. The traditional and effective approach of using chlorine as a bleaching agent is in less use for environmental concern since the process produces dioxin and other chlorinated compounds which contribute to the discharge of AOX (adsorbable organic halides) in the recipient streams. Gaseous chlorine (Cl_2) in particular, but also other chlorine containing bleaching chemicals, such as chlorine dioxide(ClO_2) and hypo chlorites are blamed for the formation of chlorinated compounds.

Conventional bleaching of Kraft pulp has evolved over the years but there has been particular pressure to change to bleaching sequence like ECF and TCF in Europe and Scandinavian countries. A similar situation also exists in Asian countries, particularly in India, wherein pulp and paper mills started switching over to partial ECF bleaching. Regardless of the pace of change, a market has been created for ECF and TCF pulps and alternatives have to be found to chlorine containing bleaching chemicals or new technologies have to be developed. A number of options are open to paper mills and these include substitution of chlorine dioxide, oxygen delignification, extended delignification to reduce Kappa number before bleaching, replacement of chlorine based chemicals by hydrogen peroxide and ozone etc. While a number of advantages are associated with these alternatives, there are disadvantages such as, high capital cost, risk of loss in pulp viscosity and strength, high cost of bleaching chemicals.

This climate of change has provided an opportunity for enzymes as prebleaching agents with the basic aim of:

- Reduction in use of chlorine and chlorinated bleach.
- Reduction in discharge of AOX in bleach effluents.
- Gain in final brightness and improved pulp properties.
-

The enzymes used commercially in pulp bleaching are hemicellulases, which selectively affect the accessible hemicellulose fraction of the pulps. Among various available hemicellulases, xylanases have been found to be most effective as pre-bleaching agents.

The use of hemicellulolytic enzymes, particularly the xylan attacking enzymes xylanases, is widely reported in use in commercial bleaching sequence for production of bleached pulp from soft wood and certain species of hard woods in the developed countries .In developed countries it has been possible to reduce chlorine demand to a level of 15-20% during bleaching with corresponding reduction in AOX levels by 20% and improved brightness to a level of 2-3% ISO. Since the effectiveness of a particular xylanase enzyme may vary in respect of this activity, purity (particularly in terms of cellulase freeness), enzyme pre-treatment conditions and the type of pulps, evaluation studies for each type of enzyme is required to be carried out separately. This should be done to assess the response of the pulps being produced in paper industry and to develop tailor made enzymes for enzymatic pre-bleaching of pulp.

PRODUCTION OF XYLANASES AS PRE-BLEACHING AGENTS

The two main enzymes which depolymerise the hemicellulose backbone are endo-1,4-ø-D – xylanase and endo-1,4-ø-D–mannanases, referred to generally as xylanases and mannanases respectively. Xylanases and mannases are produced by many species of bacteria and fungi. Xylanases are the enzymes applied in commercial bleaching and are available from several different microbial sources.

Xylanase producers are found both among bacteria and fungi. Several criteria are essential for choosing microorganisms to produce xylanases. In addition to giving the desired biobleaching effect, the enzyme must be produced extracellularly in sufficiently large quantities. It should be free of cellulase activity. Any cellulase activity will have serious economic implication in terms of cellulose

loss, degraded pulp quality and increased effluent treatment cost. Some of the initial approaches in overcoming cellulase activities from microbial sources were treatment with mercurial compounds to selectively inhibit cellulase. The application of recombinant DNA technology was aimed at cloning and selective expression of xylanase genes in heterologous systems. The most practical approach has been in the selection of naturally occurring strains that produce cellulase free xylanases and a number of such strains have been reported possessing these features. Other important features, the enzyme should display, are thermal stability at high temperatures, particularly around 60-70^{0}C, (which is the temperature of the incoming pulp) and alkali stability. The processing of the incoming pulp makes it highly alkaline and enzyme with optimum temperature in the alkaline region would be a useful feature.

STRUCTURE OF HEMICELLULOSES IN KRAFT PULPS.

The native hemicellulose structure is heavily modified during pulping process. In the beginning of conventional sulphate i.e. Kraft cooking , Xylan in wood is partly solubilized in the alkaline cooking liquid and many of the side group and acetic acid residues are cleaved off. It has recently been observed that majority of the 4-O-methylglucuronic acid side groups in xylan are converted to hexanuronic acid already in the early phases of the Kraft cook. As the alkali concentration decreases towards the end of the Kraft cook, dissolved xylan tends to reabsorb on the surface of cellulose microfibrills. It has recently been suggested that pine Kraft xylan reprecipitates evenly over the accessible surface of the fibre wall. In addition to xylan chains, dissolved lignin and covalently bound lignin and xylan have been suggested to reprecipitate on fibre surfaces during cooking, resulting in relatively high amounts of lignin deposits on the fibre surfaces. The amount of xylan re-adsorbed during cooking depends on the wood species used in pulping. High amounts of xylan have been found to be present on the surface of birch Kraft fibres, probably partly due to reabsorption, where as in pine Kraft fibres the concentration of xylan on the fibre surfaces has not been observed to be higher than in the whole fibres. A large part of wood glucomannan is also dissolved in the beginning of the Kraft cook, but due to their instability in alkali, the solubilized polymers are completely degraded in the pulping liquor.

As a result of solubilization of hemicelluloses during cooking, the distribution and content of xylan and glucomannan in Kraft pulp fibres differ from that in the native wood fibres. In softwood Kraft fibres, the xylan concentration is generally higher in outer layers and glucomannan is more concentrated in the middle layers of the fibre. However, due to different methods of analysis, variations in the distribution of polysaccharides in soft wood Kraft fibres have been reported, although there is general agreement that the outersurface layer of hard work Kraft fibres is rich in xylan.

Recently, several modified Kraft pulping methods as well as totally new sulphate pulping methods have been introduced. In the pulps produced by these methods no or little reprecipitation of xylan and lignin is expected to occur due to the relatively constant alkali concentration throughout the cooking process. Consequently, the composition of outer surface pulp fibres is probably different from that of the conventional Kraft pulp fibres. In sulphite cooking, hemicellulose is extensively solubilized to mono- and oligomeric compounds and no reprecipitation occurs. Thus, The distribution of hemicellulose is relatively constant across the pulp fibres.

PROPOSED MECHANISM OF ENZYME ACTION

Hemicelluloses are polysaccharides associated with cellulose and lignin in plants. The two most common hemicellulose are xylans and mannans. Not only do the relative amounts of these two polysaccharides vary in hardwoods and softwoods, but their chemical composition varies as well. Extensive modification of hemicelluloses takes place during pulping processes. During the initial heating period in Kraft pulping, when the alkali concentration is comparatively high, the xylan is partially depolymerized and stripped of substituents such as acetyl- and arabinosyl- groups. As pulping proceeds, the alkali concentration decreases and degraded, short chain xylan precipitates in a more or less crystalline form on the surface of cellulose microfibrils. Overall, xylan constitutes over 90% of the hemicelluloses in hardwood Kraft pulp and 50% in softwood pulp. The re-precipitation results in concentration of hemicelluloses on the fibre surfaces of microfibrils, although a part remains at its original location in the fibres.

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One of the predominating hypothesis for the mechanism of xylanase activity in bleaching is that these enzymes catalyse the hydrolysis of reprecipitated xylan on the surface of the pulp fibres making the lignin fragments in and on the fibre easier to remove in the following bleaching and alkaline extraction stages. This hypothesis is not supported by the results of experiments in which pulp was delignified under high alkalinity conditions, which precluded xylan deposition. In that situation, xylanase treament led to a reduction in the amount of chemical required to reach a final brightness equivalent to that obtained for a conventional pulp in which the xylan was reprecipitated on the pulp surface. Another hypothesis is that xylanases , by catalysing the depolymerisation of xylan in the cell walls allow entrapped lignin to diffuse more easily out of the fibre.

APPLICATION OF XYLANASES PRODUCED FROM MELANOCARPUS ALBOMYCES

In an effort to identify a suitable enzyme which displays many of the favourable features discussed above, work has been done at IIT-Delhi on the enzymes produced by the fungus *Melanocarpus albomyces*. A detailed study has been made on the different isoenzymes produced by the fungus on different carbon sources and the results have been published. Detailed study has been undertaken on the bleaching potential of xylanases produced by this fungus and these results are summarized in the following paragraphs.

(1) Production of xylanases on different carbon and nitrogen source was studied at shake-flask level using standard medium described for *M. albomyces*. Various agricultural residues, differing sufficiently in their lignin, cellulose and hemicellulose content, were evaluated for their ability to induce xylanases in this fungus. Wheat straw and wheat bran were among the best raw materials. Enzyme activities of about 150 IU/ml were obtained at the end of 96 h. The optimisation of nitrogen in microbial enzyme production has been widely emphasized. We have also used both organic and inorganic nitrogen sources and find inorganic nitrogen favouring enzyme production. The maximum productivity of 3000 IU/l/h was obtained when wheat straw was used in combination with appropriate dosage of nitrogen content. The enzyme production has been successfully carried out at 14 L fermenter level and it is expected that media optimisation will lead to considerable increase in enzyme production.

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(2) Characterization of xylanases has been carried out in terms of its pH and temperature optima and stability. While the pH optimum has been observed to be at 6.0, enzyme performed equally well at higher pH (see below) in pre-bleaching experiments. The temperature optimum of the crude enzyme preparation was 70^0C and a loss in activity was observed only when the temperature increased beyond 70^0C. Thus, the enzyme is compatible with the operating conditions of 50-55^0C used at the mill site for enzymatic treatment of the pulp. The kinetic analysis of thermal deactivation indicated enzyme to be stable at 50^0C retaining about 90% of the original activity for 90 min.

(3) Evaluation of xylanase treatment was conducted on eucalyptus and bamboo pulp mixture and bagasse pulp procured from various mills. The pulp was subjected to enzyme treatment followed by conventional CEH bleaching sequence. Different parameters such as Kappa number, viscosity, savings in bleach chemicals, strength and optical properties and by-product (effluent) evaluation was carried out in detail. Following are the findings with this enzyme preparation

i) Reduction in Kappa number
ii) Gain in pulp brightness
iii) Chromophores released were higher indicating extraction of higher amounts of lignin.
iv) No appreciable reduction in viscosity of the pulp.
v) Reduction in chlorine requirement from 41 kg/tp to 35kg/tp.A significant reduction in alkali requirement during extraction stage was also observed.
vi) Strength and optical properties of the untreated and enzyme treated pulp were found to be almost at par in terms of tensile and burst. Little drop in tear was noticed which was observed to be arrested by reducing the treatment time and or enzyme dosage.
vii) Final brightness of the pulp was increased.

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viii) Effluents after enzyme treatment showed an improved environmental status in terms of AOX, colour and COD.

Enzyme preparation was compared with one of the currently available commercial enzymes and results indicated similar performance. Slight improvement in final brightness of the pulp was observed with enzymes from *M. albomyces* which was higher by 3%. Detailed analysis was also carried out with bagasse pulp and our results indicated that our fungal enzyme preparation was as effective on this pulp as on eucalyptus pulp. A highly significant finding has been that our enzyme preparation was effective at higher pH also.

TECHNOLOGICAL APPLICATION OF XYLANASES IN MILLS

Xylanases have been in use in various paper industries, particularly in the west , for a long time and extensive data is available on this subject. Enzymes of various activities, different pH and temperature optima and stabilities have been used .In general, various benefits are credited to the use of enzyme and these include improvement in pulp fibrillation and water retention, reduction of beating time in virgin pulps, restoration of bonding and increased freeness in recycled fibres, and selective removal of xylan. In the total chlorine free process, xylanases have been used in combination with hydrogen peroxide and oxygen in bleaching process. Several national and international companies are marketing xylanases. Any reduction in chlorine by about 12-15% is considered good . In addition to this, the effluent quality is significantly improved and may respond better to biological waste treatment schemes. Current efforts are directed towards cost reduction of enzyme production .Process optimisation may lead to very high enzyme titres bringing the cost of production low. Many of these enzymes may operate at higher pH also, but at high dosage. In the Indian scenario, commercial scale manufacture will be a major bottle neck and needs to be looked at carefully.

CONCLUSION

In the Indian context, the replacement of chlorine with suitable alternatives and complete chlorine free technologies become important, particularly, when paper products manufactured with chlorine based technology, are banned. Keeping in mind the nature of raw materials used in our paper industry, which

is largely agro-based, indigenous technologies have to be developed. Novel xylanases and mixture of enzymes (xylanases and some other hydrolytic enzymes) may have to be developed which would be effective on our raw materials. Input from several investigators with expertise in microbiology, biochemistry, biochemical engineering is required. Work should be coordinated with the paper industry to do effective and result-oriented research. While a lot of work is reported in literature on isolation of novel, alkalophilic strains , it is also important to improve existing good strains in terms of high enzyme titre, optimisation of process parameters, chemical modification of enzymes to improve enzyme characteristics. The cost of enzyme based technology will be slightly higher, but it will be off-set by the benefit to the environment and reduced health hazards to mankind.

Enzymatic prebleaching of kraft pulps : An option for cleaner production technology in Indian paper industry

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ABSTRACT:

Strict legislation and the environmental concerns regarding imposition of the effluent discharge norms in respect of Absorbable Organic Halides (AOX) in Indian paper industry is forcing the mills to look for alternate techniques to reduce or eliminate the use of elemental chlorine in bleach sequence. Among various technological options available, xylanase enzyme prebleaching could prove to be one of the promising options before the mills to reduce the generation of chlorinated organic compounds while improving the final brightness of bleached pulps. Although the technology has commercially been adopted in number of paper mills world-wide, but as far as Indian paper industry is concerned, it is still in the developing stage.

Central Pulp & Paper Research Institute carried out extensive studies in the area of xylanase prebleaching of pulps procured from wood and non-wood based mills. The present paper discusses the response of five identified xylanase enzyme preparations on the pulps procured from wood based (eucalyptus + bamboo) and bagasse based mills. Out of five enzyme preparations, four are available globally and one produced from an indigenous microbial strain. The response of three xylanase enzymes was found to be encouraging. The savings in terms of elemental chlorine is found to be to the tune of 15-20% during conventional CEH bleach sequence with simultaneous reduction of AOX level to 20-30% in both Kraft wood and Kraft bagasse pulps. Brightness gain to a level of 2.5-3.5 % ISO could be attained, while maintaining similar or slightly improved strength properties. Based on these findings, efforts are continued to commercialise the process in Indian paper mills employing identified xylanase enzymes.

INTRODUCTION

In the rapidly changing field of pulp bleaching, efforts have been made to adjust the process to meet the current challenges, which are driven by the environmental and economic forces. The environmental concern regarding imposition of the effluent discharges norms for AOX is forcing the industry to reduce or eliminate the use of chlorine or chlorine based chemicals in bleach sequence. Many of the alternate technologies have not yet proved feasible especially because of higher investment and operating cost and also the negative effect on the pulp quality. Under the prevalent conditions enzymatic prebleaching of pulps employing xylanases enzymes could prove to be a promising option as an environmental friendly technology & is early adaptable by the pulp and paper industry.

The primary goal of chemical pulp bleaching is to reduce the residual lignin of pulp without effecting the carbohydrate and/ or the physical properties of the pulps. Two different enzyme approaches have been generally in use for achieving the goal, which include.

1. Use of hemicellulases (specifically xylanases) enzymes as prebleaching agents (bleach booster) for enhancing the chemical removal of lignin in multistage bleaching sequence.

2. Another alternative approach is direct delignification of the unbleached pulps using enzymes called ligninases or laccases, which acts directly on the residual lignin in the pulp (2).

The first approach, which is the use of xylanase enzyme as prebleaching agents for chemical pulps has been successfully, applied in pulp and paper mills worldwide using various xylanases preparations of different origins. But the technology still is in nascent stage as far as Indian Paper Industry is concerned.

Xylanase enzymes used in bleaching of chemical pulps are being developed by Companies which claim their products suitable as prebleaching agents for the pulps produced in the paper industries. However, these enzymes, which are marketed by several suppliers are found to be highly sensitive to the conditions like temperature, pH & doses of the enzymes (3,4). Therefore it becomes difficult for the pulp and paper mills to decide which particular enzyme should suit to the specific requirement of their industry in order to achieve desired effect.

In view of the above, Central Pulp & Paper Research Institute (CPPRI) has been engaged for the last many years in conducting studies on evaluation of various xylanase enzyme preparations which are available globally and also being developed indigenously by several reputed biotechnological laboratories to study their response on the type of pulps produced in Indian paper mills(5).

The present paper highlights the studies carried out at the Institute in which several xylanase enzymes have been evaluated for their bleach response on Kraft wood and Kraft bagasse pulps, in order to

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explore potential for commercial application of the enzyme prebleaching technology in Indian paper industry.

MATERIALS & METHODS

2.1 Sources of Xylanase enzymes :

Several xylanase enzyme preparations used in the present investigations were procured from National & International enzyme manufacturing companies/ laboratories which are denoted as A,B,C,D & E The details about the xylanase preparation is given in Table-1.

2.2 Pulp Samples :

Wood Kraft Pulp samples were procured from a nearby large integrated Pulp & Paper Mill employing Eucalyptus as major raw material where as Kraft bagasse Pulp was obtained from an agrobased mill in Maharasthra equipped with chemical recovery system.

2.3 Enzyme assay Techniques :

All the enzymes were tested for filter paper activity for cellulase contamination by the method of Mandals & Weber and xylanase activity was measured by the method of Bailey et. al (1).

2.4 Xylanase Pretreatment of Pulps :

Enzymatic Pretreatment of the pulps was carried out under the optimised conditions as mentioned in Table-2.

2.5 Process conditions used during bleaching of Pulps :

Process conditions employed for bleaching of Pulps both with & with out enzyme preparation is shown in table-3.

RESULTS AND DISCUSSION:

While optimising the dosages of enzymes during enzyme treatment of woof Kraft and bagasse Kraft pulps, it was observed that excess dosages of xylanase enzymes for a longer time has resulted in decreased pulp yield and strength properties of the pulps. This could probably be due to loss of hemicelluloses by the action of enzyme. However under optimised dosages of enzymes i.e. 5-7 IU/gm of pulps and optimised treatment conditions (Table-2), no significant loss in pulp yield could be observed except with enzyme preparation E wherein even the treatment of the pulp at optimised

conditions of time and temperature could result in 1.5% pulp yield loss along with drop in viscosity which was reduced from 560 to 510 cm^3/g of the pulp leading to loss in strength properties (Table-4). Reasons for the same could be attributed to contamination of xylanase with cellulase activity, which could be ascertained by determination of the cellulase activity in the xylanase enzyme by filter paper activity. Therefore this enzyme named as E was found to be unsuitable for its application in bleaching of pulps.

Effect of xylanase treatment on bleach chemical requirement

Bleaching of the xylanase enzyme treated Kraft pulps, both wood and bagasse have been found to respond in a different manner than untreated pulps while bleaching with conventional CEH bleach sequence. Based on optimisation studies of the chlorine dosages during chlorination stage, it was observed that significantly less chlorine is required to bleach Kraft wood and Kraft bagasse pulps while bleaching to similar targeted brightness level.

Table - 5 shows the effect of various xylanase preparations on the bleach chemical requirement during conventional CEH bleach sequence of wood Kraft pulps with a targeted brightness level of 80% ISO. From the results shown it is clearly seen that there is a reduction of nearly 14 - 15% of chlorine demand with all the enzymes preparations in case of wood Kraft pulps except enzyme preparation D, where chlorine saving of only 9.3% could be noticed. Further the enzyme treated pulps could be bleached to higher brightness with a gain in brightness level of 2-3% ISO in all the cases while using similar chlorine dosages as in case of control pulp sample.

Reduction in chlorine requirement or improved pulp brightness in case of enzyme treated pulps was reflected from the reduction in Kappa number of the unbleached pulps after enzyme treatment and further after the alkali extraction stage. The reduction in kappa number by 7-8% after enzyme treatment and further reduction of kappa number by more than 25% after alkali extraction was achieved in enzyme treated pulps.

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Similar observation were made in case of Kraft bagasse pulps where nearly 18% reduction in chlorine demand could be observed in enzyme treated pulps with targeted brightness level of 83.0% wherein brightness gain of 2.0% ISO could be achieved when the enzyme treated pulp was bleached with similar chlorine dose of 5.1% as in case of control pulp. Results are shown in Table-8. This reduction in chlorine demand and gain in pulp brightness is also reflected from reduction in kappa number of the enzyme treated pulp where 20% reduction in kappa number after alkali extraction stage could be noticed.

Effect of enzyme treatment on unbleached pulp yield and physical properties of wood Kraft and bagasse Kraft pulps.

Table -6 shows the bleach chemical demand strength & optical properties of Kraft wood pulp treated with identified xylanase enzyme preparations B & D against control. From the results shown in Table-6, it is clearly evident that with a savings of nearly 15% chlorine demand employing enzyme preparation B, no loss in strength properties like burst, tensile & tear could be noticed, however the tear index was improved significantly from 5.3 Mn m^2/g to 6.0 Mn m^2/g whereas in case of enzyme D treated pulps, the strength properties, though were at par with control pulp but the chlorine savings were lower i.e 9.3% only. With regards to the optical properties of the enzyme treated pulps, there is an improvement in the yellowness of the pulp both with enzyme preparations B & D, since the yellowness of the enzyme treated pulps were decreased from 14.94 % to 9.79% & 10.65% respectively.

Similar observations could be made in case of Kraft bagasse pulps. Treatment of the Kraft bagasse pulp with xylanase enzyme B preparation showed no loss in pulp yield or pulp viscosity (Table-7).Bleach chemical demand of both enzyme treated and untreated pulps & the results of strength properties were shown in table-8. From the results it is clearly evident that strength properties of the enzyme treated pulps like Burst, Tear and Tensile could be maintained at par with control pulps with improvement in optical properties particularly in respect of yellowness which was dropped from 13.5 to 9.2 - 9.6% (Table-8).

Impact of enzyme treatment on environment

Characterisation of the resultant bleach effluent from wood Kraft pulps and Kraft bagasse pulps before and after enzyme treatment indicated that effluent properties namely AOX (Adosorbable Organic Halides) & COD are improved indirectly through the use of xylanase treatment which helps in release of lignin and other chromophore bearing compounds and allowing the use of lesser quantities of elemental chlorine or other chlorine based compounds in subsequent bleach sequence. Results of characterisation of enzyme treated wood Kraft pulp and Kraft bagasse pulps against control pulps are shown in Table-9. From the table it is clearly indicated that AOX level was reduced to 0.69 kg/tp in case of hard wood Kraft & 1.0 kg/tp in Kraft bagasse pulps compared with control pulps where AOX was reduced from 2.29 kg/tp to 1.6 kg/tp and from 4.13 kg/tp to 3.13 kg/tp respectively. Further decreased ratio of COD to BOD from 7.7 to 3.9 and from 4.12 to 3.33 in bleach effluent from Kraft wood and Kraft bagasse after enzyme treatment is an indication of improved biological treatability of the bleach effluent.

CONCLUSION

1. Enzymatic prebleaching of chemical pulps using xylanase enzyme could prove to be an effective technology in reducing the chlorine demand to the tune of 15% & 18% with gain in bleached pulp brightness of 2-3 % ISO while reducing the AOX level in bleach effluent to more than 25% in case of wood Kraft and Kraft bagasse pulps.

2. Xylanase enzyme respond better on wood Kraft pulps than non-woody raw materials due to the nature of the hemicelluloses wherein it has been observed that hard wood hemicelluloses are more responsive to xylanase enzyme action than those in Pulps from Kraft bagasse mills, due to the nature of the hemicelluloses. It has been observed that hard wood hemicelluloses are more responsive to xylanase enzyme action than those in pulps from agro residue raw materials.

3. Studies indicated that before selection of a particular xylanase enzyme preparation it is important to evaluate the enzymes for their activity and cellulase contamination, since excess dose of xylanase enzyme and cellulase contamination results in loss of pulp yield and strength properties of the pulp as in case of enzyme preparation E.

4. Before introducing enzyme-prebleaching technology in any mill it is important to evaluate particular enzyme preparation for its response towards pulp being produced in the mill for various parameters as discussed in the paper.

5. Enzyme being sensitive and specific in nature, it is very important to optimise the temperature, pH, dosages and proper dispersion or mixing of the enzyme with the pulp in order to achieve the targeted response.

6. The technology is relatively new as far as Indian paper industry is concerned. Efforts are required by researchers, technologists and enzyme manufacturers to isolate new xylanase enzymes suitable for pulps being produced in Indian paper industry from wood and non wood based raw materials and also to investigate the effect of other hemicellulases and lignolytic enzymes like laccase and lignin peroxidases on pulp bleaching to further reduce requirement of chlorine based chemicals.

TABLE-1 SOURCES OF XYLANASE ENZYMES	
Xylanase enzyme	**Source**
A	Produced indigenously from an indigenous microbial strain
B	Indigenously manufactured
C	Imported
D	Imported
E	Indigenously manufactured

TABLE- 2 ENZYME PRETREATMENT CONDITIONS					
Parameters	**Pulp treated with Enzymes**				
	A	**B**	**C**	**D**	**E**
Dose of enzyme, IU/g	7.0	10	5.0	5.0	10
Consistency of pulp ,%	10	10	10	10	10
Retention time, min`	120	120	120	120	120
PH at 25° C	6.2	8.2	8.5	8.5	8.5
Temperature, ° C	45-50	45-50	45-50	45-50	45-50

TABLE-3. PROCESS CONDITIONS USED DURING BLEACHING OF PULPS			
Particulars	**Chlorination stage**	**Alkali Extraction Stage**	**Hypo stage**
Temperature, ^{0}C	Ambient	60	40
Pulp Consistency, %	3.0	8.0	8.0
Retention Time, min	30	60	120
Final pH	1.8-2,0	>10.5	>9.0

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TABLE-4 CHARACTERIZATION OF UNBLEACHED PULP FOR YIELD & OTHER PARAMETERS OF WOOD KRAFT PULP BEFORE & AFTER ENZYME TREATMENT

Parameters	Control	Pulp treated with enzymes				
		A	B	C	D	E
Pulp yield , %	99.89	99.82	99.62	99.80	99.74	98.10
Kappa number of Pulp,	18.0	16.5	16.7	16.6	17.0	16.0
Brightness, % ISO	27.5	28.5	29.0	28.4	28.0	29.0
CED Viscosity, cm^3/g	560	620	590	557	604	510

TABLE- 5 EFFECT OF ENZYME PRETREATMENT ON BLEACH CHEMICAL REQUIREMENT USING VARIOUS XYLANASE PREPARATIONS ON KRAFT WOOD PULP

Parameters	Control	Pulp treated with Enzymes									
		A		B		C		D		E	
		ET1	ET2	ET1	ET2	ET1	ET2	ET1	ET2	ET1	ET2
Savings in elemental chlorine	--	--	15	--	15	--	14	--	9.3	--	
% Reduction in Kappa No. after X stage	---	8.0		7.2		7.3		5.5		11.1	
% Reduction in Kappa No. XCE Stage	---	28.0	--	25.9	14.5	26.0	16.0	27.0	23.0	--	--
Final brightness of the pulp, % ISO	80.0	82.5	80.0	82.5	81.3	83.0	80.2	82.0	80.0	83.0	80.0

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X - Enzyme treatment

XCE - Enzyme, Chlorination & Extraction

ETI - Enzyme treated Pulp treated with similar chlorine dose

ET2 - Enzyme treated Pulp treated with optimised less chlorine dose

TABLE-6 ENZYMATIC PREBLEACHING OF KRAFT WOOD PULP USING XYLANASE

Bleaching of Pulp using Conventional CEH Sequence Before & After Enzyme Treatment

Particulars	Control	Enzyme B	Enzyme D
Chlorination			
% Chlorine Applied	3.9	3.3	3.54
%, Chlorine Consumed	94	99	96
% , chlorine savings	--	15	9.23
Alkali Extraction			
% NaOH Applied	1.30	1.30	1.5
% NaOH Consumed	61.1	63.2	58.3
Final pH	10.88	10.76	11.21
Kappa no.	3.51	2.99	3.2
Hypo Stage			
% Hypo Applied	1.8	1.8	1.8
% Hypo Consumed	71.0	75.0	68
Brightness, % ISO	80.0	82.5	82.0
Brightness Gain, %	--	2.5	2.0

Strength and optical properties of the wood kraft pulps before & after enzyme treatment using two identified xylanase enzymes B & D

Parameters	Control	Enzyme B	Enzyme D
Revolution PFI	4000	4000	4000
Freeness, CSF	220	250	250
Apparent density, g/m^3	0.79	0.76	0.75
Burst index. $Kpa.m^2/g$	4.43	4.49	4.51
Tensile Index , Kpa.m2/g	64.4	64.3	64.4
Tear Index, Mn m2/g	5.3	6.0	5.3
Optical properties			
Opacity , %	92.2	91.2	91.0
Yellowness , %	14.94	9.79	10.65

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TABLE -7 YIELD & OTHER CHARACTERISTICS OF UNBLEACHED KRAFT BAGASSE PULP BEFORE & AFTER ENZYME TREATMENT USING IDENTIFIED XYLANASE (B) ENZYME'

Parameters	Pulp treated with Enzyme	
	control	Enzyme treated
Pulp yield , %	99.20	99.02
Kappa number of unbleached Pulp	26.2	25.5
Kappa No. of CE Stage	5.07	4.04
Brightness ,% ISO	30.3	30.8
CED Viscosity	535	520

TABLE-8 XYLANASE PRETREATMENT OF KRAFT BAGASSE PULPS & STRENGTH & OPTICAL PROPERTIES

Parameters	Control	ET1	ET2
Chlorination			
% Applied Chlorine	5.1	5.1	4.2
% Chlorine Consumed			
% Chlorine savings	--	--	17.6
Alkali Extraction			
% NaOH Applied	2.0	2.0	2.0
% NaOH Consumed	74.2	61.2	73.8
Final pH	10.24	11.55	10.25
Kappa no. of Pulp	5.07	4.04	4.30
Hypo stage			
% Hypo Applied	2.0	2.0	2.0
% Hypo Consumed	80.0	80.0	74.0
Final brightness of the pulp ,% ISO	83.0	85.0	84.0
Strength and Optical properties			
Parameters	**Control**	**ET1**	**ET2**
Revolution PFI	500	500	500
Freeness, CSF	335	350	355
Apparent density, g/m^3	0.66	0.74	0.72
Burst index. $Kpa.m^2/g$	2.60	3.05	2.55
Tensile Index , Kpa.m2/g	45.5	56.0	45.0
Tear Index, Mn m2/g	4.05	4.40	4.20
Optical Properties			
Brightness of pulps, % ISO	83.0	85.0	84.0
Yellowness	13.5	9.2	9.6

- ET1 -- Pulp treated with similar chlorine dose
- ET2 -- Pulp treated with less optimised Chlorine dose

TABLE-9 CHARACTERISTICS OF BLEACH EFFLUENTS OF KRAFT WOOD & KRAFT BAGASSE PULPS BEFORE & AFTER ENZYME TREATMENT USING XYLANASE ENZYME B

	Wood Kraft Pulp		Bagasse Kraft Pulp	
Parameters	**Control**	**Enzyme treated**	**Control**	**Enzyme treated**
AOX, Kg/tp	2.29	1.60	4.13	3.13
COD , Kg/tp	23.3	28.5	34.6	50.7
BOD, Kg/tp	3.02	7.31	8.5	15.0
COD: BOD ratio	7.7:1	3.9:1	4.1:1	3.3:1

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Pretreatment of paper pulps with RRL-T bacterial xylanases in bleaching process and its comparison with commercial enzymes

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ABSTRACT

The growing public concern regarding the environmental impact of pollutants from paper and pulp industry is increased and it is the strong driving force behind the endeavors leading to novel bleaching techniques. The conventional bleaching of paper pulp for the removal of adhering lignin and hemicelluloses has been effectively done by chlorine and its compounds. The resultant nonbiodegradable chlorinated biphenyls create enormous problems to the ecosystems. The different biotechnological approaches for the reduction of or even elimination of the usage of chlorine compounds in the bleaching experiments involved in the use of xylanases before bleaching to hydrolyse the hemicellulosic bond between the cellulose and lignin. This will effectively release the adhering lignin and release the free cellulose. Removal of even a small portion of the hemicellulose could be sufficient to open up the polymer and facilitate the removal of residual lignin by solvents. The ideal enzyme for the above process is thermostable alkaline xylanases. Xylanases are found commonly in microorganisms and they catalyse the hydrolysis of xylan. . Biobleaching trial runs of the Kraft pulp supplied from Hindustan News Print factory at Kottayam were done with the enzyme developed by RRLT and the results were compared with the commercial enzymes obtained from Eswin Biotech, Chennai & also from BIOCON Industries, Banglore. The encouraging results showed that RRLT enzyme performance was excellent and it was comparable with the commercial enzyme performance.

INTRODUCTION

The growing population of the world and its progressive adoption of an industrially-based lifestyle has inevitably led to an increased anthropogenic impact on the biosphere. The types and sources of pollutants are as diverse as their potential effects and fates within the environment. The aquatic system is much polluted by the indiscriminate discharge of effluents by the industries. Discharge of toxic heavy metals from different processing industries and mining; xenobiotic compounds discharge from chemical, pharmaceutical, detergent, textiles, pulp bleaching as well as other bleaching industries and also from other processing industries through their industrial effluents etc create enormous environmental problems. Because of their intrinsically persistent nature, these compounds are major contributors of environmental pollution. Hence the present global scenario of environmental destruction demands ameliorative measures by every branch of science. A vast paper and pulp industry exists around us to supply the great demand of paper products. Most of the paper pulp

industries adopt Kraft pulp process which is a cost effective one and during the bleaching of the pulp chlorine and its compounds are used. Chlorophenols, chlorobiphenyls and other chlorolignin derivatives formed during the above process and they are mainly non biodegradable. Most of the chloroaromatic compounds are toxic and accumulate in the biotic and abiotic components of the ecosystem (Subramaniyan and Prema, 2000; Subramaniyan et. al 2001). Several studies have been conducted to assess the deleterious effects of effluents from paper and pulp industries. Larsson *et al.* (1988) found a negative impact of Kraft mill effluents on fish populations, even 10 km away from the plant. The use of chemical pulp paper for the manufacture of baby diapers and food packaging is also of concern since it is sometimes associated with chlorinated compounds including the animal carcinogen dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) (Shoham *et al.*, 1992).

The growing public concern regarding environmental impact of pollutants from paper and pulp industry was the strong driving force behind the endeavours leading to novel bleaching techniques. Xylan, the second most abundant polysaccharide (Ali *et al.*, 1999) and a major component in plant cell wall is linked to lignin and cellulose (Puls, 1997) and its hydrolysis by xylanases eases the removal of lignin, the chromogenic precursor. Xylanases (1,4-β-D-xylan xylanohydrolase, EC 3.2.1.8.) are endo- enzymes which release xylooligosaccharides and xylose residues from xylan, while the exoacting β-xylosidases (1,4-β-D- xylan xylohydrolase, EC. 3.2.1.37) hydrolyse xylooligosaccharides resulting in the xylose residues. They are from different microbial origin and multiple forms of xylanases exist as isoenzymes. Fungal enzymes are acidic and thermophilic; actinomycetes enzymes are active in neutral range pH ; bacterial xylanases are neutral and alkaline pH range and highly thermophiles etc. Xylanases are of great importance to pulp and paper industries as the hydrolysis of xylan facilitates release of lignin from paper pulp and reduces the level of usage of chlorine as the bleaching agent (Shoham *et al.*, 1992). Viikari *et al.* (1986) were the first to demonstrate that xylanases could be useful in paper and pulp industry effecting delignification in bleaching process. Studies with fungal xylanases have resulted in the reduction of chlorine consumption; however, use of cellulase-free xylanases selectively remove hemicellulose components with minimal damage to cellulose (Srinivasan and Rele, 1995) and hydrolysis of reprecipitated and reabsorbed xylan or xylan-lignin complex. As a result the pulp becomes more accessible to bleaching chemicals.

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Xylanases require some basic associated characteristics for their application in biotechnology aided paper production. The nature of the paper pulp- high alkaline pH and temperature necessitates the use of alkaline thermostable xylanases for the pre-treatment process before the bleaching step. Such biological mediated process needs a cost effective enzyme for use.

In Regional Research Laboratory (RRL), Trivandrum, isolation and characterization of several xylanase producing cultures were done earlier. One of which (an alkalophilic Bacillus SSP-34) produced more than 600 IU/ ml of xylanase activity with a specific activity of 200. (Subramaniyan 2000) This xylanase has optimum activity at 50°C in a pH range 6-9 with negligible amounts of cellulolytic activities and application of the enzyme for the biobleaching experiments was tried. In this paper, there is a description of the experiments carried out on the pretreatment of paper pulps – Kraft pulps obtained from Hindustan News Print Ltd, Kottayam with the RRLT enzyme along with two commercial enzymes obtained from BIOCON, Banglore and ESWIN BIOTECH, Chennai. The pretreated pulps were subjected to bleaching sequences experiments and the results were evaluated by measuring the ISO brightness of the pulp.

MATERIALS AND METHODS

Enzyme sources taken for the studies.

Commercial enzyme samples were obtained from BIOCON, Banglore (Bio-xyl) and ESWIN BIOTECH, CHENNAI (Esw- xyl) respectively and they were analysed for their enzyme activities and concentration. Cell free crude extract of RRLT- xylanase was taken as the Rrlt-xyl enzyme.

Biobleaching experiments

The sequence for the entire process of pretreatment and bleaching are given below.

ε / E/ P / H

ε - Enzyme treatment.

E - Alkali extraction.

P - Hydrogen peroxide treatment.

H – Hypochlorite treatment.

Enzyme pre-treatment methods

Kraft Pulp samples were kindly provided by Hindustan Newsprint Ltd. Velloor. Pulp samples were washed thoroughly and then subjected to chelation treatment with 0.01 % EDTA for 30 minutes. Pulp samples from the above treatment were washed twice and the pH adjusted to 9. Xylanases – Bio-xyl, Esw-xyl and Rrlt-xyl were diluted and mixed individually to pulp samples at different unit levels keeping the mixing in a uniform way. The final consistency of the pulp samples were maintained at the level of 5%. The samples were incubated at 50^0C water bath for 2 hours. Appropriate controls were also kept along with the samples incubated for 2 hours for the completion of pretreatment. Enzyme pre-treated samples were washed thoroughly after this - (5 times water volume)

Alkali extraction

0.5 ml of 25% NaOH (high alkalinity) was then added for alkali extraction of the chromophores released during the pretreatment process.(5% consistency) After of 30 minutes the solution was washed well (10 times water volume)

Bleaching pulp with oxidising agents

The alkali extracted sample was then subjected first to H_2O_2 treatment while maintaining the conditions and components for super bleach (Rydholm, 1965b). The other components added during bleaching process were:

H_2O_2	-	1.5 % (w/w of pulp)	
NaOH	-	0.2%	„
$MgSO_4$	-	0.02%	„
Sod. Silicate	-	2.0%	„

The bleaching process was continued for 2 hours at 50^0 C. The peroxide bleached samples were washed thoroughly (5 times volume with water) and subjected to NaClO (sodium hypochlorite) treatment at 70^0 C. The active chlorine available for oxidation was 5%. Thus 1.5 ml NaClO (5 % sol.) ≡ 0.075 gm Cl_2 added, which is equal to 0.125 g / 5 gm of pulp (2.5 %).

Preparation of pulps for Brightness evaluation

The samples after 3 hours of incubation were washed thoroughly and filtered through Buchner funnel by the application of water pressure to uniform settling of round sheets. These sheets were pressed between filter paper sheets and then analysed for brightness studies.

ANALYTICAL PROCEDURES

Brightness measurements

The paper pulp sheets were measured for its ISO brightness against MgO (Color touch Spectrophotometer, Technidyne Corporation, USA.).

Assay of endoxylanases

Endoxylanase was estimated in the enzyme extract or in diluted enzyme preparation by the method of Bailey et al (1992). To 1.8 ml of pre incubated xylan solution (0.5% xylan in carbonate buffer pH 10), 0.2 ml of diluted enzyme was added. The mixture was incubated for 10 minutes at 50°C. The reaction was arrested by the addition of 3 ml of Dinitrosalicylic Acid (DNS reagent) and kept in a boiling water bath for 5 min and cooled. Suitable reagent blank and enzyme blanks were prepared along with the test. The color developed was read against a reagent blank at 540 nm. The reducing sugar liberated was compared with a standard graph of xylose treated in a similar way. One international unit (IU) of endoxylanase was defined as the μMol of xylanase liberated per min

RESULTS AND DISCUSSION

The Kraft cooked pulps were treated with different xylanases before their bleaching experiments. The enzyme units taken for each treatment was 5,10,15&20 U/ g of the pulps. Initial pretreatment of the pulps with EDTA would have removed any metal contamination in the pulp that will adversely affect the enzyme treatment. Fig1 gives the results of Brigtness achieved by the pulps after enzyme& bleach treatment and also the control value is given. The brightness of Paper pulp treated with water alone was 30 and that was taken as the baseline for the experiments. The control without enzyme pretreatment has given 36.8 and all the enzyme treated ones have given a hike of 1.5 to 2 Units more than the control. There was not much variation with 5,10,15&20 Units of enzyme used indicating that 5IU was sufficient to effect the required brightness. In the case of BIOCON enzyme, the results are given in fig.no 2. Here also the pulp pretreated with 5 and 10 Units have given more brightness than with higher enzyme units indicating lower enzyme units were sufficient to enhance the brightness. Fig-3 gives the results pertaining to treated pulps with ESWIN enzyme. This enzyme has given more than 2 units increase in comparison with the control. Here also the results indicated lower enzyme levels were sufficient to effect the opening of linkages. Experiments done with lesser than 5IU and also with higher H2O2 concentration did not effect much difference (data not shown) in all except with ESWIN which worked well with 3 IU/gm.

Kraft pulping involved the cooking with wood chips along with Na_2S and NaOH and it partially removed lignin and hemicelluloses. The residual lignin, covalently bound to carbohydrate moieties in the pulp (Yamasaki *et al.*, 1981) would be removed later by bleaching agents. During the final stages of cooking, xylan removed earlier would be reprecipitated on the surfaces of cellulosic fibres (Gierer and Wannstrom, 1984). The reprecipitated and relocated xylans trap the degradation products in the matrix, resulting in the characteristic brown colour of pulp. Even though lignin was the main contributor of pulp colour, there were other compounds, which according to Ziobro (1990) could add to the colour of the Kraft pulp. Sugars cooked under Kraft conditions gave rise to compounds with absorption spectra similar to those present in Kraft liquors. According to Kantelinen *et al.* (1993), during the pretreatment with xylanases, they act primarily by hydrolysing the reprecipitated xylan located on the surface of the pulp fibres. The average pore size in kraft pulp would be about 5-10 nm

allowing the penetration of enzymes with a molecular mass of 40 kDa or less, assuming a spherical conformation (Stone and Scallan, 1968). This necessitates search for xylanases with lower molecular weight. The RRLT enzyme has a molecular mass of 22 kDa . The above results (Fig1) showed it could be utilized as a pretreating agent before bleaching. The Esw-enzyme behaved in a better way and it could be due to its optimum pH at 10. Rrl-enzy optimum pH varies from 6 t0 9 having excellent activities expressed at pH 8.0. If the ideal condition for each enzyme could be given, the results could been also varied accordingly. In all these cases, the alkali treatment was applied as per previous reports (Rydholm, 1965a, b) because during treatment of pulp with high concentration alkaline solution (Alkalisation), the low molecular components are removed (Gamerith and Strutzenberger, 1992a). This high alkaline treatment was thus useful in xylanase pre-bleaching as the hydrolysed products of the reprecipitated and relocated xylans could be easily removed during the alkalisation process.

The application of xylanases was pioneered by Viikari *et al.* (1986) and Jurasek and Paice (1986), however the earlier studies were conducted with xylanases obtained from fungi. Those enzymes with considerable cellulase activity have effected viscosity loss of pulp. The alkaline nature of the pulp (traped alkali in the fibres even after extensive washing) and high temperature of the pulps require a thermophilic and thermostable as well as alkaline xylanases. The increase in brightness of the *Rrlt-enz* pretreated pulp was comparable with most of the earlier reports. Ratto *et al.* (1994) tried the applicability of xylanases from *Dictyoglomus* sp. in the biobleaching of kraft pulps and found minor increase, from 46.9 ISO units to 48.9 ISO units in brightness. Garg *et al.* (1998) reported the comparison of pretreatment effects of *S. thermoviolaceus* xylanases with two commercially available enzymes, Pulpazyme and Cartazyme and found that all the cases were having an 6-7 ISO unit increase in brightness, while Tenkanen *et al.* (1992) in a study using chlorine as the bleach sequence agent found that when xylanase was used, chemical consumption of active chlorine was reduced by 7% for achieving the same brightness obtained in the reference. The xylanase from *Aureobasidium pullulans* resulted in brightness increase of 2 ISO units (Yang *et al.*, 1992). Thus from the present study it is clear that the Rrlt xylanase was having characters suited for the application in pulp and paper industries and it is comparable with the commercial enzymes. In many reports bleach sequences are repeated to effect better brightness.

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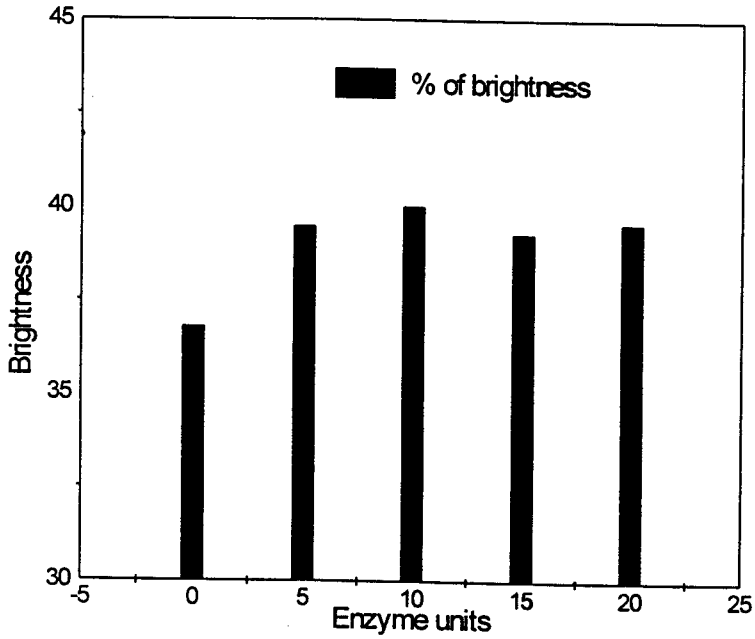
Fig.1. ISO Brightness of RRLT-enz treated pulps



Contorl without any treatment - 30
1.5% peroxide treated

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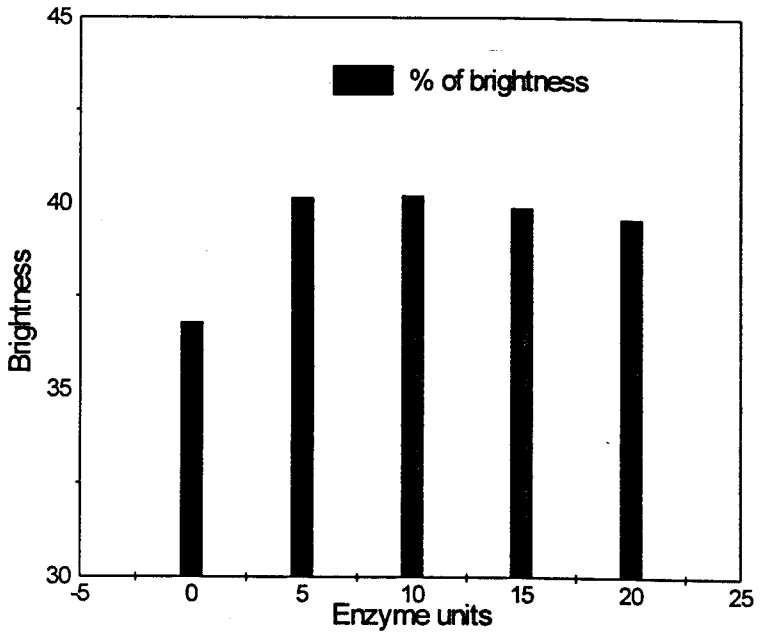
Fig.2. ISO Brightness of Biocon-enz treated pulps



Contorl without any treatment - 30
1.5% peroxide treated

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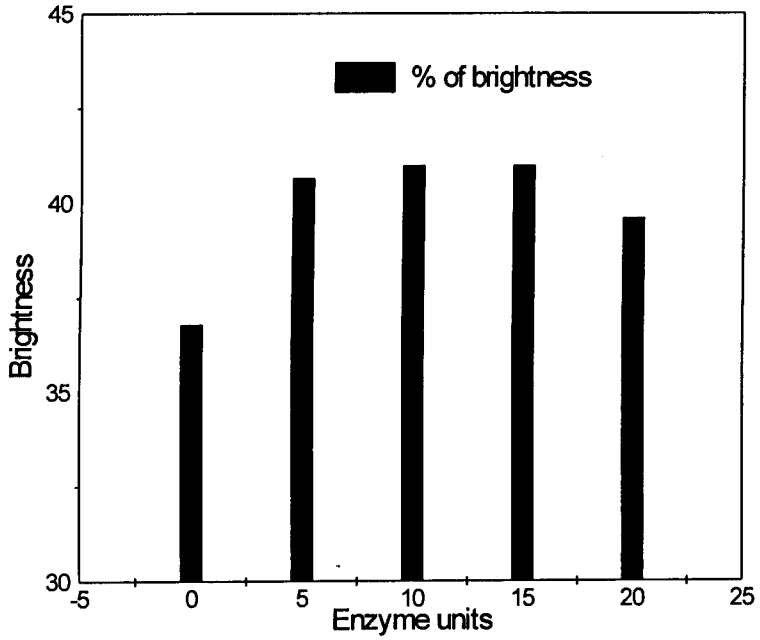
Fig.3. ISO Brightness of Eswin-enz treated pulps



Contorl without any treatment - 30
1.5% peroxide treated

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Alkaline xylanase in Pulp Biobleaching

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ABSTRACT

Xylanases hydrolyse the polysaccharide xylan that forms the major component of hemicellulose in the lignocellulose. The main application of xylanase is to remove the residual xylan in paper processing industries. Other applications include the food and feed processing industries. The conventional bleaching of pulp using chlorine emits carcinogenic chlorinated phenolics into the environment. The xylanase pretreatment of pulp prior chemical bleaching reduced the quantity of chlorine during the bleaching that also minimized the pollutant emission. For effective application of xylanases in biobleaching the xylanases should be capable of tolerating high temperature and alkaline pH. The alkaline stable xylanase produced from *Aspergillus fischeri* has been evaluated for solubilization of brown pulp. The crude enzyme preparation was obtained by extracting the extracellular xylanase from the solid state fermentation. Zymogram analysis showed two xylanases in the crude enzyme. The enzyme dose (upto 1500 U) and treatment time enhanced the amount of reducing sugars released from the pulp. The level of resuding sugars released from the brown pulp was higher than that of white pulp. The maximum pulp solubilization was achieved at 60°C.

INTRODUCTION

Recently, xylanases attracted attention for application in food, feed and pulp industries. Of these, the pulp processing industries are operated in large-scale, which are also considered as the major polluting industries. This is because in pulp industries the raw source is wood (lignocellulose) which undergoes various processes to isolate cellulose. Conventionally, after digesting the wood with alkali or acid, bleaching is done with chlorine based chemicals, which emits chlorinated phenolics into the environment. These pollutants are carcinogenic and hence harmful to human health and aquatic beings. To avoid the pollution, alternative strategies are being developed the use of hemicellulases has been suggested. Among them, xylanases play a major role in minimizing the consumption of chlorine. The required criteria for effective application of xylanases are free from cellulase activity; stable and active at alkaline pH; and stable and active at high temperature. Several thermophilic and alkalophilic bacteria were known to produce xylanases active and stable at high temperature (>80°C). Though many thermophilic fungi were known to produce xylanases tolerant to high temperature, only two fungi were reported to produce xlylanases tolerant to alkaline pH. The two fungi were not well studied

for their potential application. Therefore in the present study, the potential of xylanase of *Aspergillus fischeri* in bio-bleaching of pulp has been carried out.

MATERIAL & METHODS

ORGANISM AND FERMENTATION CONDITIONS

An alkali tolerant fungus *Aspergillus fischeri* Fxn1 (Chandra Raj and Chandra, 1995) was maintained on PDA medium. Spores obtained from fungal cultures grown in PDA at pH 9.5 were suspended in 0.05% Tween 80. The production medium consists of (in g/l) $NaNO_2$-3.0; KH_2PO_4-1.0; $MgSO_4$-0.5; $FeSO_4$ and Yeast extract – 5.0. pH 9.5. Wheat bran medium was inoculated with 10^7 spores / g for solid-state fermentation. The culture was grown on wheat bran (10g / 15 ml production medium) in solid in 500 ml Erlenmeyer flasks at pH 9.0 and room temperature (30±1°C). After 72h of incubation, the enzyme was extracted by squeezing the components of the culture broth with 0.05 M potassium phosphate buffer of pH 6 using a nylon cloth. The insoluble materials were removed by centrifugation at 15,000 rpm for 10 min. The supernatant was used as the crude enzyme source.

XYLANASE ASSAY

Xylanase activity was assayed by incubating 0.1 ml of appropriately diluted enzyme source with 0.5 ml of 1%(w/v) birch wood xylan dissolved in 0.05M potassium phosphate buffer (pH 6.0) and 0.4 ml of the same buffer at 50°C for 10 min. The amount of reducing sugars released in the assay mixture was quantified by DNS method using xylose as a standard. One unit of xylanase activity is defined as the amount of enzyme required to liberate one micro-mole of xylose equivalent per min. under the specified conditions.

ELECTROPHORESIS AND ZYMOGRAM ANALYSIS

SDS PAGE was carried out with separating and stacking gel of 12 and 4% polyacrylamide at pH 8.8 using 1.5M Tris-HCl buffer containing SDS (Laemmli, 1970). Protein sample was boiled with SDS (2%) and β-mercapto ethanol for 5 min. and loaded (100µl) into the wells. Electrophoreisis was run

using 0.5 M Tris-Glycine buffer (pH 8.8) containing SDS (0.1%) by supplying a current of 100V. The gel was washed with distilled water and stained with Coomassie Blue R250 dye in a solution of methanol (60%v/v) and acetic acid (7%v/v). Protein bands were visualized by destaining with the methanol-acetic acid solution.

Activity staining was carried out according to the method of Kubata *et al.*, (1994). A substrate gel was prepared on petriplate (13cm dia) using 10ml of 2% (w/v) agar containing 0.5% (w/v) birch wood xylan (Sigma Co. USA) in 0.05M Potassium phosphate buffer of pH 6.0. After the SDS polyacrylamide gel electrophoresis run as above, the polyacrylamide gel containing the protein was washed with distilled water and overlaid onto the substrate gel and incubated for 30 min. at 40°C. The substrate gel was stained with 1% (w/v) aqueous Congo Red. The zone of clearance was visualized by destaining with 1 M NaCl solution.

ENZYMATIC TREATMENT OF PULP

Unbleached pulp were obtained from Sun paper Mills, Cheranmahadevi, Tirunelveli, India. Pulp was washed with distilled water thoroughly until the pH was neutral, dried and stored at 5°C. Ten to 20 ml crudes purified xylanase of 100 U/ml was added to pulp suspension (1 % wlv) in a total volume of 100 ml of 0.05 M Potassium phosphate buffer (pH 8.0) and incubate at 40°C for 2 h. The reducing sugars released was measured using DNS reagent (Miller, 1959). The pulp was washed with distilled water and dried and stored at 5°C for further experiments.

EFFECT OF PH ON THE ENZYMATIC TREATMENT OF PULP

The effect of pH was studied by treating pulp suspension (1% w/v) with 15 ml xylanase of 100 U/ml, in a total volume of 100 ml from pH 5 to 10 (0.05 M sodium acetate buffer (pH 3.5-5.0); 0.05 M Potassium phosphate buffer (pH 5.5 -8.0); 0.05 M sodium borate buffer (pH 8.0-9.0) and carbonate - bicarbonate buffer (pH 10 -11) at 40°C for 2h.

EFFECT OF TEMPERATURE ON THE ENZYMATIC TREATMENT OF PULP

The effect of temperature from 40 to 70°C was studied by treating pulp suspension with 15 ml xylanase of 100 U/ml at pH 8.0 tor 2h. The reducing sugar released was measured, using DNS and xylose standard. The reducing sugars released from the pulp was also expressed as degree of solubilization as given below.

Degree of solublization = Reducing sugars released / weight of Pulp x 100

DETERMINATION OF KAPPA NUMBER

Kappa number of pulp was estimated by titration with pottassium permanganate solution according to TAPPI standard kappa number method-T236 cm-85. Moisture-free pulp sample (2g) was disintegrated in 800 ml of distilled water until free of fibre clots and placed in temperature bath maintained at 25°C. One hundred ml of 0.1 N potassium permanganate and 100 ml of 4 N sulfuric acid solution were taken in a beaker. This mixture was added to pulp suspension with stirring. At the end of 10 min the reaction was stopped by adding 20 ml solution (1.0N). Immediately after mixing, the free iodine was titrated with 0.2 N sodium thiosulfate solution, adding a few drops of starch indicator towards the end of the titration. A blank determination was carried out omitting the pulp. One unit of kappa number was defined as the amount of 0.1 N potassium permanganate solution consumed by 1.0 g of dry pulp.

RESULTS

ENZYME SOURCE

The extracellular xylanase was produced by cultivating A. fischeri in solid state fermentation using wheat bran. The crude enzyme showed a xylanase activity of 165 U/ml. Among several protein bands observed by polyacrylamide gel electrophoresis of the crude xylanase source, two were identified as xylanase proteins by zymogram using birch wood xylan agar gels. The high molecular weight

xylanase designated as xyl A had a stronger xylanase activity than the low molecular weight xylanase designated as xyl B (**Fig. 1**).

ENZYMATIC TREATMENT OF PULP

The concentration of pulp suspension was varied and the amount of reducing sugars released was measured at pH 5.0 and 40°C. The amount of reducing sugars released increased with the increase of pulp concentration (**Fig. 2**). Maximum amount of reducing sugars released was 18 mg at 5% (w/v) pulp suspension. But the degree of solubilization was maximum (6.5%) at 1% (w/v) pulp suspension. The rate of release of reducing sugars was high when the pulp suspension was increased. Even after 4 h of reaction, the release of reducing sugars was linear when the pulp suspension was at 1 % (w/v), whereas the reaction was completed at 3 h. when the pulp suspension was 5% (w/v). Hence, further work was proceeded using 5% (w/v) pulp suspension.

EFFECT OF ENZYME CONCENTRATION

The action of xylanase on pulp was analyzed by measuring the amount of reducing equivalents released from the pulp after the enzymatic treatment. The reaction mixture containing pulp with buffer but without enzyme was used as a control (**Fig. 3**) shows the release of reducing sugars from pulp treated with various doses of enzyme. The results indicated that the amount of reducing sugars released from the pulp was dependent on the xylanase dose. The buffer alone did not release reducing sugars under similar conditions. When the xylanase dose was increased to 330 U, the level of reducing sugar released improved to 20 mg/g pulp and further increase did not substantially improve sugar release. The time required to release the maximum reducing sugar was reduced at high xylanase concentration. The dependence of release of reducing sugar from the pulp indicates the action of xylanase on the pulp.

EFFECT OF PH

The effect of pH on the soubilization of pulp and the amount of reducing sugars released from the pulp was determined at various intervals of time. The effect of pH on the enzymatic solubilization of pulp is shown in **Fig. 4**. It is obvious that at various temperature the amount of reducing sugars released was more at acidic reaction pH compared to alkaline reaction pH. Maximum reducing sugars of 19 mg/g

pulp was released at pH 5.0. When the pH was reduced to 4.0 the reducing sugars released was 17 mg/g pulp which was relatively high. However, at higher reaction pH 6.0, the amount of reducing sugars released was 12.0 mg/g pulp which was considerably less. Further, increase of pH showed a decrease in the release of reducing sugars. It is obvious from the figure that the rate of release of reducing sugars decreased with increase of pH. These results indicated that the favorable condition for pulp solubilization was acidic pH.

EFFECT OF TEMPERATURE

Since the enzymatic solubilization of pulp was better at acidic pH, the effect of temperature on the solubilization of pulp was determined at acidic reaction conditions. The rate of enzymatic solubilization increased with increase in temperature. Maximum rate of solubilization of pulp was achieved at 60°C, but maximum amount of reducing sugars of 19mg/g pulp was released at 40°C. At 60°C, the degree of solubilization obtained was considerably high compared to low temperature hence, to reduce the time of the reaction. This enzyme can be used at high temperature.

EFFECT OF KAPPA NUMBER

The brown pulp and bleached pulp were treated with xylanase at optimum conditions (pH 5.0 and 40°C of solubilization. The kappa numbers of brown and bleached pulp were 30 and 10 respectively. The amount of reducing sugars released from the unbleached pulp was 19mg/g pulp which was more compared to the reducing sugars released (15mg/g pulp) from bleached pulp (Table 1).

Table 1.

Pulp	Kappa Number	Reducing sugars (mg/g pulp)	Degree of solubilization
Unbleached	30	19	1.9
Bleached	10	15	1.5

DISCUSSION

The crude xylanase of *A. fischeri* Fxn1 was free of cellulase and hence useful for bio- bleaching (Chandra Raj and Chandra, 1995). *A. fischeri* xylanase has been reported to produce multiple xylanases (Chandra Raj and Chandra, 1996). The zymogram technique revealed that the two xylanases Xyl A and Xyl B were produced with varying activities. The rationale for the production of multiple xylanases was the heterogeneous nature of xylan, its substrate (Biely, 1985). The simultaneous action of these multiple xylanases improves the solubilization of pulp, by action of each of these enzymes at different sites of xylan polymer. Due to the advantage of synergistic action of xylanases, the crude enzyme source comprising these multi-xylanases was used with out partial purification. In the process of bio-bleaching, when the xylanase is added to pulp, the xylan left over in the pulp after alkaline or acid digestion is degraded, which enables the penetration of chemical bleaching agents with ease (Coughlan and Hazlewood, 1993). Hence, prior to application of xylanase in pulp bleaching sequence, the solubilization of the residual xylan in the pulp must be at a maximum. Therefore, the conditions required for the maximum solubilization of pulp were optimized with respect to enzyme dose, pH, temperature and pulp concentration.

The increase of enzyme dose and treatment time enhanced the amount of reducing sugars released from the brown pulp and similar observation have been made in *Aureobasidium pullulans* xylanase (Christov *et al.*, 1993, 1994). In accordance with the present result, Chirstov *et al* (1993) have also showed that the maximum amount of reducing sugar released was maximum at a certain level of enzyme dose (1500 U) and further increase of enzyme dose did not improve solubilization. This must be due to the restricted accessibility of substrate for xylanase action or saturation of enzyme substrate complex.

The level of reducing sugar (19 mg/g pulp) released from the brown pulp, had a high kappa number, by the treatment of *A. fischeri* xylanase was due to more hemicellulose in the unbleached pulp. The observed low level of reducing sugars released (15 mg/g pulp) from bleached pulp with low kappa number was attributed to the removal of residual xylan in the subsequent bleaching step. Similarly the low level of solubilization of bleached pulp has been reported for *Saccharomonas viridis* xylanase

(Robert *et al.*, 1990). According to Paice *et al.*, (1984), the pentosans remaining in the bleached pulp are well shielded by other components, and therefore not susceptible to enzymatic attack, In contrast *Trichoderma harziamum* xylanase has been shown to hydrolyze the bleached pulp to a high level (54%) (Senior *et al.*, 1988). The bleachability of pulp is expressed in kappa number, which indicates the level of lignin in the pulp, Since the kappa number is measured from the amount of potassium permanganate consumed by the pulp, it includes lignin, the other coloring and oxidisable components in the pulp (Patel *et al* 1993). The bleached pulp with low kappa number led to the removal of lignin components by bleaching which renders accessibility for the enzyme to reach xylan. Hence, it appears that the intrinsic structure and the inter linkage of wood components decide the extent of solubilization of pulp. The solubilization of pulp depends on the pore size of pulp microfibrils (Roberts *et al.*, 1990 and Sinner *et al.*, 1976) and the molecular weight of xylanase. Due to the penetration of xylanase through the pulp, the low molecular weight xylanases are more ii efficient. Since the *A. fischeri* xylanases are of low molecular weight with 31 kDa (Chandra Raj and Chandra, 1996) and 20 kDa (Senthilprabu, 1999), this possibly favors permeability of xylanase through the pulp pores. The high specific activity (695 U /mg) (Chanda Raj and Chandra, 1996) also improves xylan hydrolysis.

The pH for enzymatic treatment of pulp depends on the bleaching sequence and also the step at which enzyme is applied. The alkaline and acidic pH were recommended for enzymatic treatment of pulp obtained from alkaline digested and sulfite digested step (Christov *et al.*, 1994, Senior *et al.*, 1988 and Roberts *et al.*, 1990 Patel *et al.*, 1993, Ragauskas et at., 1994 and Silva et at., 1994). In most of the cases, a neutral or acidic pH was chosen, because, after alkaline digestion the pulp was washed to remove degraded components, which leads to a decrease in the pH of the pulp. However if the xylanase is active and stable at alkaline pH it is advantageous. Pretreatment of pulp at alkaline pH (8.0) has been demonstrated in *Dictyoglomus* xylanase (Ratto *et al.*, 1994) although, maximum solubilization occurred at pH 6-7. Similarly, the present *A. fischeri* xylanase solubilized maximum level at pH 6-7, but reasonable level of solubilization could be achieved at pH 8-9. This suggests the possible application of xylanase of *A. fischeri* in pulp pre-treatment at alkaline conditions. The pulping processes involve a high temperature of more than 100°C and hence the thermotolerant xylanases are required for enzymatic treatment of pulp. Thermostable fungal xylanase have been reported in the application of xylanase in pulp solubilization at pH 5 and 60°C (Gilbert *et al.*, 1993, Silva *et al.*,

1994). The present results suggested the possible application of crude xylanase of *A.fischeri* in pulp biobleaching at neutral pH and 60°C.

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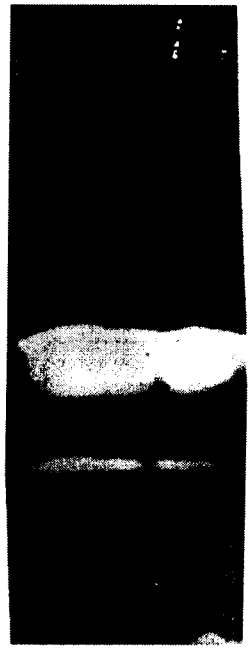

Fig. 1 Zymogram analaysis of crude xylanase from *A. fischeri* Fxn1

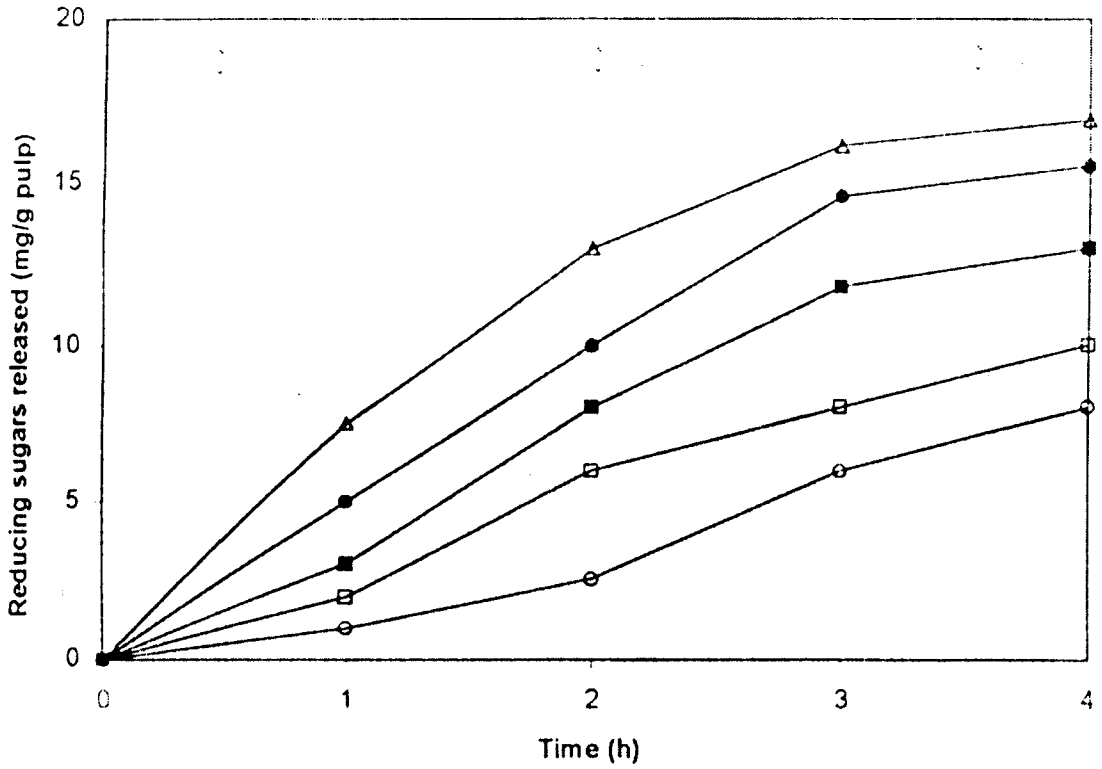

Fig. 2. Effect of concentration of pulp on the enzymatic solubilization of pulp

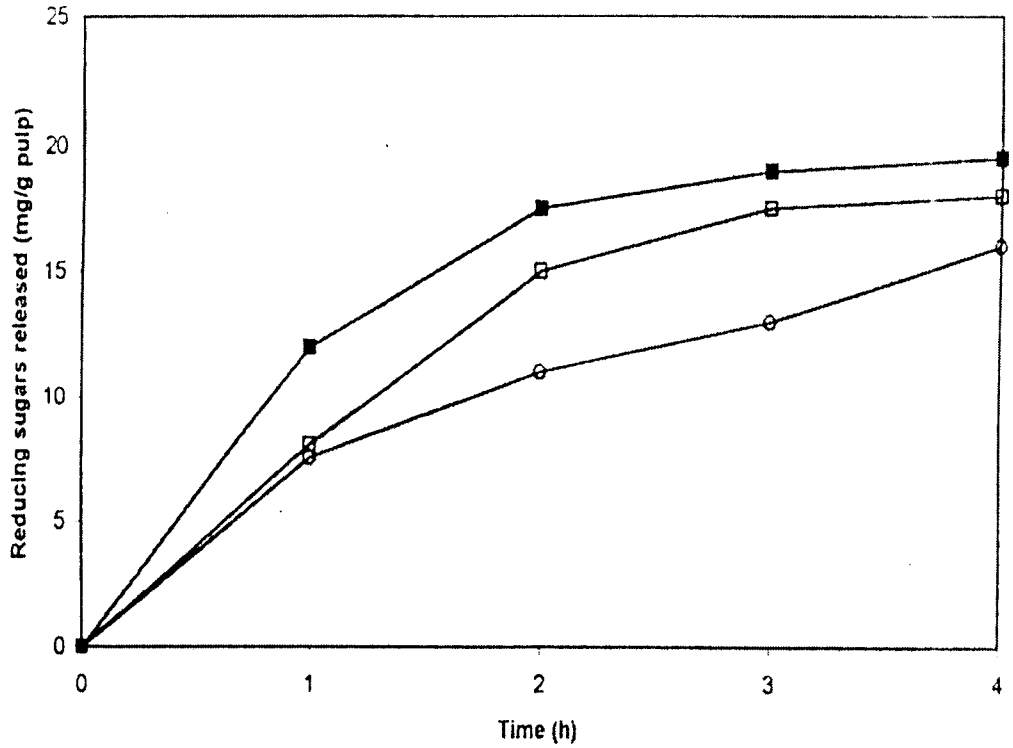

Fig. 3 Effect of xylanase dose on the solubilization of pulp

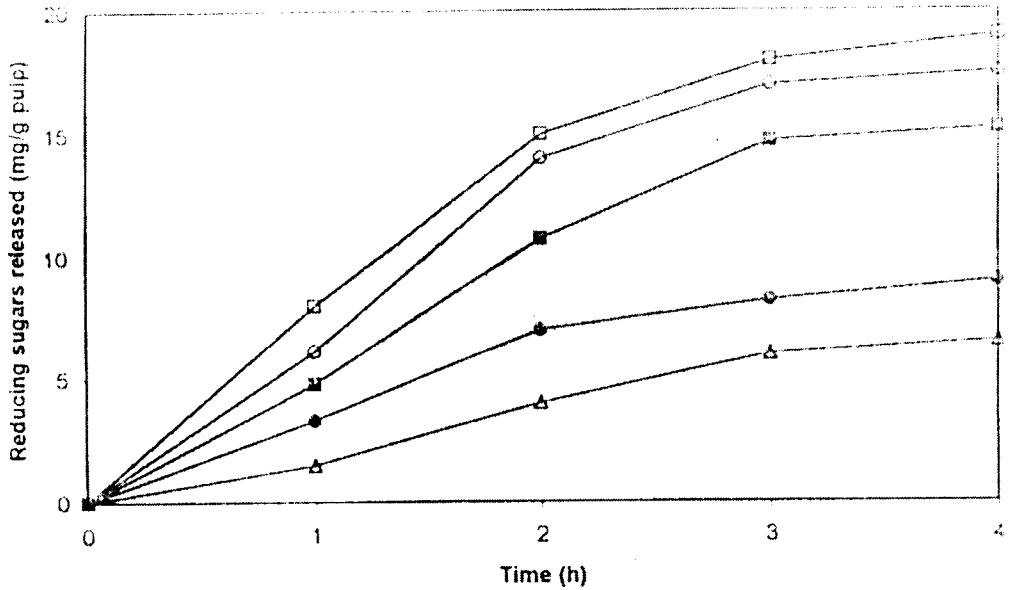

Fig. 4 Effect of pH on the enzymatic solubilization of pulp at 40C