PAPERmaking!
FROM THE PUBLISHERS OF PAPER TECHNOLOGY
Volume 5, Number 1, 2019

Paper Industry Technical Association
5 Frecheville Court, Bury, Lancashire BL9 0UF, United Kingdom
Tel: +44 (0)300 3020 150 Fax: +44 (0)300 3020 160
Email: info@pita.org.uk Website: www.pita.org.uk

Biobleaching for pulp and paper industry in India: Emerging enzyme technology

Gursharan Singh, Satinderpal Kaur, Madhu Khatri, Shailendra Kumar Arya.

Indian pulp and paper industry is one of the fastest emerging business sector of the country which has shown tremendous growth in last few years. Governments policies are creating sustain pressure on paper industries to preserve the clean and pollution free environment at any price. As a result industries are pondering to replace the chemical bleaching processes with facile bio-based cost effective technologies. Eco-friendly bleaching enzymes like xylanases and laccases have the potential for biobleaching of wood and agro-based pulps at industrial scale. In India, enzymatic prebleaching of pulp is widely being investigated and has achieved favourable outcomes but at laboratory scales only and commercial application of enzymes for the delignification of pulp is still at budding stage. This article tends to draw the attention on significant efforts which have been continually attributed by indigenous research laboratories and industries to replace the chemical bleaching with enzymes.

Contact information: Department of Biotechnology, University Institute of Engineering Technology, Panjab University, Chandigarh, India E-mail address: skarya@pu.ac.in

Biocatalysis and Agricultural Biotechnology 17 (2019) 558–565.

https://doi.org/10.1016/j.bcab.2019.01.019

Biocatalysis and Agricultural Biotechnology 17 (2019) 558–565

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

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Gursharan Singh, Satinderpal Kaur, Madhu Khatri, Shailendra Kumar Arya*

Department of Biotechnology, University Institute of Engineering Technology, Panjab University, Chandigarh, India

ARTICLE INFO

Keywords:
Biobleaching
Eco-friendly
Laccase
Pulp and paper
Xylanase

ABSTRACT

Indian pulp and paper industry is one of the fastest emerging business sector of the country which has shown tremendous growth in last few years. Governments policies are creating sustain pressure on paper industries to preserve the clean and pollution free environment at any price. As a result industries are pondering to replace the chemical bleaching processes with facile bio-based cost effective technologies. Eco-friendly bleaching enzymes like xylanases and laccases have the potential for biobleaching of wood and agro-based pulps at industrial scale. In India, enzymatic prebleaching of pulp is widely being investigated and has achieved favorable outcomes but at laboratory scales only and commercial application of enzymes for the delignification of pulp is still at budding stage. This article tends to draw the attention on significant efforts which have been continually attributed by indigenous research laboratories and industries to replace the chemical bleaching with enzymes.

1. Introduction

Currently Indian pulp and paper industrial units account for ~ 3.0% of the world's production of paper. The estimated turnover of the industry is US$ ~ 8.0 billion. The industry provides employment to more than 0.5 million people directly and 1.5 million indirectly. During 2015–16, domestic production of paper was estimated to be 12.2 million tons (http://ipma.co.in). Paper industry in country is becoming more promising as the domestic demand of paper is increasing due to the growing population and literacy rate, growth in gross domestic product (GDP) and lifestyle of the individuals (Sharma et al., 2015a; Sharma et al., 2015b; Sharma et al., 2015c). The focus of paper industry is now shifting towards eco-friendly production of paper. The paper is produced from pulps generated from wood, agricultural residues like wheat straw or from waste paper. The use of wood based technology is constantly on the decline because of capital and raw material availability constraints. The production of pulp and paper involves three important steps viz. pulping, bleaching, and final paper finishing. The removal of recalcitrant lignin from pulp is called bleaching which is necessary for making the bright and white paper. Till the end of 20th century, bleaching of pulps, irrespective of their origin from soft or hard wood, employed large amounts of chlorine and chlorine based chemicals. But now most of the pulp and paper mills worldwide use chlorine dioxide (ClO_2) as the elemental chlorine free (ECF) bleaching agent for the production of high quality white paper (Dwivedi et al., 2010; Bajpai, 2012). The high organic content (especially in the wood based pulp), coupled with chlorine dioxide used in the bleaching process, results in the production of organo-chlorine compounds, which are finally discharged as bleach effluents in water bodies. These organo-chlorine compounds (measured as Adsorbable Organic Halogens, AOX) have been reported to cause genetic and reproductive damages in aquatic as well as terrestrial animals including humans (Sharma et al., 2014). Although more eco-friendly options for bleaching are open to pulp mills in the form of alternatives to ClO_2 like extended cooking or oxygen, hydrogen peroxide or ozone based delignification, but implementation of these alternates needs process modifications and is considered as cost intensive proposition at large scale. Enzymes provide a simpler and cost effective way to reduce the use of ClO_2, chlorine compounds and other bleaching chemicals. Enzymes also offer the simple approach that allows for a higher brightness ceiling to be reached (Abhay et al., 2018). This can all be achieved without major capital investment. The applications of xylanase enzyme as prebleaching agent has been established in several laboratories and has also been commercially exploited in Europe, North America and in few Asian countries (Bajpai, 2012).

2. Structure of the Indian paper industry

The Indian paper industry recognized as the aggregation of small, medium and large sized paper mills with different paper making

* Corresponding author.
E-mail address: skarya@pu.ac.in (S.K. Arya).

https://doi.org/10.1016/j.bcab.2019.01.019
Received 8 November 2018; Received in revised form 9 January 2019; Accepted 10 January 2019
Available online 11 January 2019
1878-8181/

capacities, 10–1150 t per day. Paper production in the country is widely based on wood and agricultural waste as the major raw materials. The Indian paper industry prominently produces writing, newsprint and commercial grade paper. Newsprint grade paper is produced by mills utilizing mainly of recycled waste paper as the raw material. In 2012, India recorded the paper consumption of 9.3 kg/capita besides global average was 58 kg/capita. Presently there are 759 paper mills in the country and producing ~10.9 Mt of paper annually (http://psa.gov.in;initiatives-pulp-and-paper-industry-2014). Indian paper manufacturers association (IPMA) representing the platform to project paper industry's views and articulate its strategies. IPMA promoted the interests of paper industry in the country and help it achieve global competitiveness while striving to be an active participant in the policy making process. The important activities of IPMA are following, work as the interface with government, non-governmental organizations (NGOs) and industrial associations so as to present the perspective and interests of Indian paper mills. Promote the excellence in paper manufacturing through presentation of awards, networking with international bodies with a view to gain better visibility for Indian paper industry. IPMA also synchronize the R&D projects in collaboration with academic institutions of India.

3. Manufacturing process of paper in Indian paper mills

The manufacturing process of paper industry can be divided in to three steps, pulping, bleaching and papermaking. Among all of the three steps, bleaching is tedious and combination of chemical and physical treatment of lignin contained pulp (Fig. 1).

3.1. Pulping

Pulping is the first step of paper making procedure in which separation of cellulose fibers from the lignin components. Commonly two different methods of pulping are applying in the Indian pulp and paper industries, chemical pulping and chemi-mechanical pulping.

3.2. Chemical pulping - Kraft sulphate process

In this procedure the wood chips usually cooked at higher temperature, 165–170 °C in the presence of sodium hydroxide (caustic soda) and sodium sulphide to separate the lignin and wood resins from the cellulose. About 92–95% of the chemicals (sodium hydroxide, sodium sulphide and lime) can be recovered and reuse further.

3.3. Chemical pulping – soda process

The soda pulping is used for the conversion of agro residues (like wheat and rice straw and bagasse) to pulp. In this case raw materials usually cooked in the presence of caustic soda at a temperature of 150–160 °C to separate lignin from the cellulosic material.

3.4. Chemi-mechanical pulping (CMP)

In the chemi-mechanical pulping the wood chips initially treated with the mild caustic soda based chemicals to extract resin and lignin from the cellulose prior to mechanical refining.

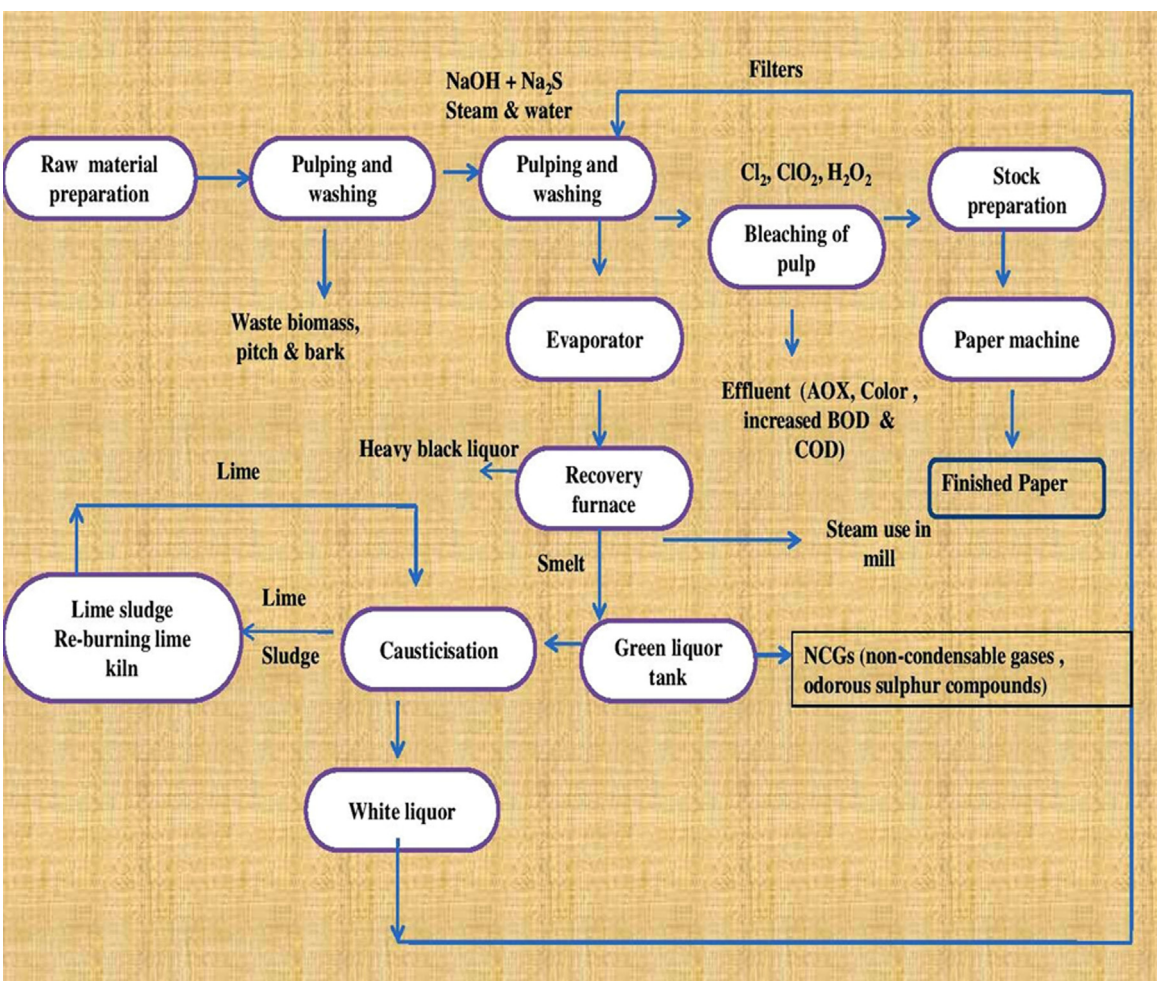

Fig. 1. Common manufacturing process of paper in Indian paper mills.

3.5. De-inking of RCF

Recycled fibers (RCF) dispersion or floatation pulping process is applied for the de-inking of the news papers/print papers. For de-inking, chemicals such as detergents, dispersants and foaming agents added and ink is separated from the pulp.

3.6. Pre-bleaching of pulp with enzymes

The term bleaching is generally referred to the removal of lignin from any kind of the pulp by use of chemicals/gases/steam etc. but prebleaching terminology is used for the enzymatic treatment of the pulp for removal of lignin. Prebleaching is an eco-friendly and cleaner process of lignin removal that can save the chlorine based and other chemicals 10–15% (Bajpai, 2004; Camarero et al., 2007; Garg et al., 2011). Prebleaching of pulp with enzymes is still under trial or at pilot scale in paper mills of India.

3.7. Chlorine bleaching of pulp

The process is used to remove the residual lignin in the range 5–10%. This process is followed by several stages of treatment of pulp with chlorine dioxide or hypochlorite to whiten the pulp. Bleaching process employed in most of the medium and small mills is based on elemental chlorine. However, few of the large sized wood based/agro based mills have introduced elemental chlorine free (ECF) bleaching process making use of chlorine dioxide ClO_2.

3.8. Elemental chlorine free (ECF) bleaching

ECF bleaching technology is being practiced in few large mills of the country where it uses oxygen delignification (ODL), followed by ClO_2 to enhance the brightness of the pulp.

4. Eco-friendly bleaching enzymes (xylanases and laccases) studied by the Indian research laboratories

There are numerous commercially available enzyme cocktails are available, but due to the differences in paper making process in the developed countries and in India, it has been felt to characterize enzymatic pre-bleaching process indigenously with enzymes produced from locally isolated cultures or with commercially available enzymes that match with the interests of Indian pulp industries. One of the major differences is the use of different sort of raw materials for pulp making in India (Sharma et al., 2015a; Sharma et al., 2015b; Sharma et al., 2015c; Dutt et al., 2009; Bajpai et al., 1994; Singh et al., 2008; Singh et al., 2010). Up to the 1980, there was no university or institute was associated in research and development (R&D) that can directly involved for giving the technical guidelines to Indian paper industry. R&D progress on enzymes for paper industry is still in its beginning and only single institute works in a direction to undertake industry related issues and emphasized on applied research, is Central Pulp and Paper Research Institute (CPPRI). There were only a few reports on xylanases for the biobleaching of pulp in country before 2000, e.g. treatment of eucalyptus pulp with commercial xylanases such as Novozyme 473, and Cartazyme HS-10 reduced the chlorine consumption by 31% and increased the final brightness by 2.1–4.9 points (Bajpai et al., 1994). Thermostable cellulase-free xylanase from *Streptomyces* sp. QG-11-3 was produced and applied for delignification of eucalyptus kraft pulp at pH 8.5 and 50 °C for 2 h. There was reduction in kappa number and increase in brightness of pulp by 25% and 20% respectively (Beg et al., 2000). Bajpai, reported, properties of many commercial xylanases make them unsuitable for the real process of pulp bleaching (Bajpai, 2004). So industries need xylanases which can function efficiently in their existing papermaking processes. Xylanase from *Bacillus megaterium* showed 8.1% decrease in kappa number and 13% increase in brightness of eucalyptus kraft pulp with 31% reduction in chlorine consumption (Sindhu et al., 2006). Extracellular cellulase free xylanase produced from *Bacillus subtilis* C01 increased the brightness by 19% of banana pulp Ayyachamy and Vatsala (Ayyachamy and Vatsala, 2007). Purified alkali stable xylanase from *Aspergillus fischeri* was immobilized on polystyrene that reduced the kappa number of paper pulp by 87% (Senthilkumar et al., 2008). A synergistic action of xylano-pectinolytic enzymes from *Bacillus pumilus* was evaluated for the prebleaching of kraft pulp; as a result 8.5% and 25% reduction was noticed in kappa number and chlorine consumption respectively (Kaur et al., 2010). Alkali stable and thermo tolerant xylanase from *B. pumilus* SV-85S showed (at pH 9.0, 55 °C for 2.0 h) the reduction in kappa number by 1.6 points and increased brightness by 1.9 points. The pretreatment of pulp with xylanase resulted in 29% reduction in chlorine consumption (Nagar et al., 2013). First report on a bacterial system involving direct growth of xylanase -producing *B. halodurans* FNP 135 on kraft (eucalyptus) pulp under submerged fermentation conditions, showed 35% reduction in kappa number and 5.8% enhancement in brightness with 20% reduction in chlorine consumption (Gupta et al., 2015). Kumar et al. (2016) emphasized that significant application of thermostable xylanases is biobleaching in pulp and paper industry, where these enzymes acted as delignifying agents, showing clear economic and environmental advantages over chemical alternatives. After xylanases, laccases are the next extensively explored enzymes for biobleaching of pulp; these are oxidative biocatalysts that have influenced the researchers by their numerous merits over any other bleaching enzyme (Singh et al., 2008; Singh et al., 2010; Singh et al., 2009; Singh et al., 2015). Laccases, together with mediators are able to delignify the pulp by the oxidation chain reaction leading to lignin oxidation without the degradation of cellulose. In India pioneering work on alkalophilic laccases was started by Bains et al. (Bains et al., 2003), through isolation of a novel strain named as γ-proteobacterium JB. An alkalophilic cellulase-free laccase from γ-proteobacterium JB was applied to wheat straw-rich soda pulp to evaluate its bleaching potential by optimizing the conditions statistically using response surface methodology based on central composite design in the presence of ABTS at pH 8.0 which enhanced the brightness by 5.8 and reduced the kappa number by 21% within 4 h of incubation at 55 °C. It was noticed that pre-bleaching of eucalyptus kraft pulp with xylanase or laccase individually avoided the ClO_2 by 15% and 25% respectively. When both enzymes were applied together at pilot scale (50 kg pulp), there was reduced organo-chlorine compounds consumption by 34% in bleach effluent (Sharma et al., 2014). Tables 1, 2 shows the year wise isolation of new laccase and xylanase producing organisms and enzyme characterization, but there were very few enzymes either xylanase or laccase evaluated for bioleaching of pulps. Recently, also many reports published on xylanases and laccases from Indian laboratories but none of them studied on delignification of biomass (Sharma et al., 2015a; Sharma et al., 2015b; Sharma et al., 2015c; Desai and Iyer, 2016; Nikam et al., 2017; Afreen et al., 2017; Dharmesh et al., 2017; Raj et al., 2018; Kumar et al., 2018; Ranimol et al., 2018).

5. Commercial use and availability of Indian patents on bleaching enzymes

R&D work on isolation and screening of microbial cultures, capable of producing low molecular weight xylanases was started initially at National Chemical Laboratory Pune in early 1990s. Later, IIT Delhi, Birla Institute of Scientific and Industrial Research Jaipur and few other research and academic institutions began working on culture development for the production of alkaline thermo-tolerant xylanase enzymes. A national research laboratory CPPRI and a premier educational institution in the country, Institute of Paper Technology (IPT) also initiated R&D on xylanase enzyme based pre-bleaching of the pulp. The first ever mill trial of xylanase pre-bleaching in India was conducted in a pulp and paper mill of Ballarpur Industries Ltd. (BILT) in 1992 using

Table 1
Laccase enzymes from different sources studied by the Indian laboratories.

Laccase producing organisms	Optimum conditions for growth		Optimum conditions for enzyme catalysis		Outcome of the study	References
	Temp. °C	pH	Temp. °C	pH		
γ-proteobacterium JB*	37	7.2	55	6.0[a],6.0[b], 6.5[c],6.5[d],7.0[e], 7.2[f]	Molecular weight of the purified laccase was determined (120 kDa), successfully degradation of indigo carmine to anthranilic acid via isatin.	Singh et al. (2007)
γ-proteobacterium JB**	37	7.2	55	8.0	Successfully biobleaching of agro-based wheat straw rich soda pulp in presence of ABTS as a mediator.	Singh et al. (2008)
Cladosporium cladosporioides	37	NA	40–70	3.5[g]	Purified the laccase (71 kDa) up to homogeneity and successfully degradation of 11 structurally different polyaromatic and sulfonated azo dyes.	Halaburgi et al. (2011)
Pleurotus sp.	NA	NA	65	4.5[g]	New organism for laccase production was found and purified (40 kDa)	More et al. (2011)
Escherichia coli AKL2	37	8.5	50	8.5[a]	Study concluded that Cu_2O nanoparticles enhanced the thermostability and activity by 36 and 4.0 fold respectively.	Mukhopadhyay et al. (2013)
Trametes hirsuta (MTCC 11397)	25	5.5–7.5	20–25	2.6[g]	$CuSO_4$ and acetone stimulated the production of laccase up to 2 fold	Dhakar and Pandey (2013)
Streptomycetes sp.	30	7.5	35	6.0[g]	Extracellular laccase producing novel bacteria was isolated from soil	Demissie and Kumar (2014)
Aspergillus nidulans	30	NA	40	6.0[d] and 5.0[g]	Molecular weight of the purified laccase was 66 kDa	Vivekanandan et al. (2014)
Serrtia marcescens	25	5.0	25	5.0[g]	Psychrotolerant laccase producing bacterium was isolated and characterized	Kaira et al. (2015)
Cotylidia pannosa	30	5.0	50	5.0[g]	Laccase was purified (43 kDa), fungal biomass as well as the crude laccase were able to decolorized the congo red, bromophenol blue and coomassie brilliant blue R-250 to different extent.	Sharma et al. (2015a), Sharma et al. (2015b), Sharma et al. (2015c)
Ganoderma lucidum MDU-7	30	5.2	50	4.0[d]	Two laccase isozymes (Glac H1 and Glac L1) were purified from native-PAGE protein purification method and both of the laccase isozymes have same optimum temperature and pH for catalytic activity	Kumar et al. (2015)
Bacillus subtilis MTCC 2414	30	7.0	70	9.0[d]	First report on the maximum production (270 Uml^{-1}) of bacterial laccase.	Muthukumarasamy et al. (2015)
Aspergillus flavus	35	7.0	27	5.0[g]	Optimization of nutritional and cultural paramaters for the laccase production by using statistical method, design of experiment (DOE).	Kumar et al. (2016)
Lysinibacillus and Bacillus Bhargavaea	37	7.0	55	7.0[a,d,g]	Observed the impact of phosphate and other medium components like tryptone and glucose on physiological regulation of laccase production.	Kaur et al. (2016)

*First report from India, alkalophilic bacterial laccase was purified.
**First report on application of alkalophilic bacterial laccase for biobleaching of agro-based pulp.
NA: Not available.
[a] Syringaldazine.
[b] Catechol.
[c] Pyrogallol.
[d] Guaiacol.
[e] L-Methyl DOPA.
[f] *p*-Phenylenediamine.
[g] ABTS.

Table 2
Xylanases enzymes from different sources studied by the Indian laboratories.

Xylanase producing organisms	Optimum conditions for growth		Optimum conditions for enzyme catalysis		Outcome of the study	References
	Temp. °C	pH	Temp. °C	pH		
Streptomyces sp. QG-11-3	37	8.0	60	8.6	Optimization of production media, contained amino acids like L-leucine, DL-isoleucine, L-lysine increased the xylanase production.	Beg et al. (2000)
Emericella nidulans NK-62	45	6.5	60	6.0	Lignocellulosic material, corn cob was used for increasing the cellulase free xylanase production by 318Uml^{-1}	Kango et al. (2003)
Aspergillus fischeri	30	NA	60	9.0	A cellulase free, alkali tolerant xylanase was produced (1024Ug^{-1})	Senthilkumar et al. (2005)
Bacillus megaterium	40	8.0	40	8.0	The use of cellulase free xylanase for biobleaching of kraft pulp, 8.1% increased in brightness and 13% decreased in kappa number of pulp	Sindhu et al. (2006)
Bacillus subtilis	37	7.0	37	7.0	Biobleaching of kraft pulp by using xylanase, increased the brightness by 4.9% and reduced the chlorine consumption by 28%	Sanghi et al. (2009)
Bacillus pumilus	50	9.0	37	8.5	Biobleaching of kraft pulp with xylanase as a result, reduced the 8.5% in kappa number and 25% reduction in consumption of chlorine without any increase in brightness.	Kaur et al. (2010)
Bacillus stearothermophilus	60	9.0	60	9.0	Pretreatment of wheat straw pulp with xylanase, as a result reduction came in kappa number by 7.1% and chlorine consumption by 20% and increased in brightness by 1.7%.	Garg et al. (2011)
Bacillus pumilus SV-85S	37	8.0	50	6.0	Alkali stable xylanase was produced from cost effective and easily available agro-residues.	Nagar et al. (2011)
Bacillus pumilus SV-205	37	7.0	50	7.0	Xylanase was 100% stable at pH 6.0–11 for 24 h.	Nagar et al. (2012)
Cellulosi microbium sp.	40	7.0	40	7.0	Production (4962 ± 45Ug^{-1}) of xylanase was observed in large enamel trays	Goluguri et al. (2016)
Aspergillus lentus	30	NA	70	9.0	Cellulase free xylanase production was more on wheat bran (158 Ug^{-1}) followed by corn cob (153 Ug^{-1}), sugarcane bagasse (129 Ug^{-1}) and wheat straw (49 Ug^{-1})	Kamble and Jadhav (2012)
Sporotrichum thermophile	35	7.0	35	9.5	Useful in food industries, xylan hydrolysis to produce xylo-oligosaccharides i.e. 73 xylotetraose, 15 xylotriose and 10% xylobiose.	Kaushik et al. (2014)
Bacillus sp. *and B. halodurans* (co-culture)	37	10	70	9.0	Xylanase, increased the brightness by 13%, breaking length 49%, viscosity by 11% and decreased in kappa number by 15% of kraft pulp.	Sharma et al. (2015a), Sharma et al. (2015b), Sharma et al. (2015c)
Bacillus halodurans	37	7–12	37	7–12	This study revealed the potential application of *B. halodurans* for biobleaching of hard wood kraft pulp that reduced the cost-intensive steps of enzyme production and extraction before their use in biobleaching.	Chutani and Sharma (2015)
Aspergillus oryzae	28	8.0	60	6.0	Xylanase increased the brightness by 57% during the deinking of newspaper pulp	Boruah et al. (2016)
Thielaviopsis basicola	60	5.0	30	7.2	Alkali and thermostale xylanase was produced (1360Umg^{-1})	Garg et al. (2012)
Penicillium meleagrinum	30	5.5	30	NA	The kappa number was reduced from 13 to 8.5, with increased in brightness by 69% and viscosity 8.9 cP of kraft pulp.	Goswami et al. (2014)

Table 3
Patents reported from the India on bleaching enzymes.

Name of the enzyme	Proposed application	Patent/Application number	Patentee/Institute/Industry	Publication year of patent
Xylanase	Process for the production of xylanase from *Termitomyces clypeatus*	US6569646 B2	Subhabrata Sengupta/Indian Institute of Chemical Biology (CSIR), Calcutta	May, 2003
Xylanase	Process for the production of thermophilic and alkalophilic extracellular xylanase by *Pseudomonas stutzeri*	US6833259 B2	Narayan Baburao Bhosle and Asha Giriyan/ (CSIR), Goa	Dec., 2004
Consortium of xylanolytic bacterium, *Providencia rettgeri*, MTCC 5096 and three ligninolytic bacteria, *Serratia marcescens*, MTCC5094, *Pseudomonas aeruginosa*, MTCC5095 and *Pseudomonas aeruginosa*, MTCC 5098.	Bacterial consortium was capable for biobleaching of kraft pulp up to the 8.0%	US 11/236,819	Rita Kumar and Anil Kumar/CSIR-Institute of Genomics and Integrative Biology at Delhi.	Sep., 2005
Xylanase	A process for the production of cellulase free xylanase	225/DEL/2001	A. Lachke and Chinnathambi Sathivel/ NationalChemical Laboratory, Pune, India.	May,2008
Laccase	Antimicrobial properties of enzyme	IN/PCT/2001/615/CHE	Novozymes A/S, India	March 2009
Xylanase	Process for the xylan-degradation was optimized	389/DEL/2000	Chandralata R and Usha Devi/National Institute of Oceanography Dona Paula, Goa, India.	March 2009
Laccase	Production of polylactide polymers	PCT/IN2010/000010	Venkata R Sonti and group/Praj industries Ltd. India	Aug., 2010
Xylanase	A process for the production of alkalo philic and thermophilic xylanase	491/DEL/1999	Narayan B Bhosle/National institute of Oceanography Dona Paula, Goa, India.	Feb., 2012
Laccase	Invention of process for production of enzyme from *Arthrographis* sp. MTCC5479	WO2012023021 A1	Vijay Sonawane/IMTECH, Chandigarh, India	Feb., 2012

low pH-xylanase, provided by M/s Biocon India Pvt. Ltd. Subsequently, several other trials were organized in different mills by using different types of raw materials and practicing different pulping and bleaching processes, using xylanase enzymes of different qualities (including alkaline and thermo-tolerant) (Singh et al., 2016). According to the news bulletin on pulp and paper R&D, CPPRI Saharanpur (2011), evaluated the potential of bleaching enzymes at pilot and mill scale that was sponsored by the Department of Biotechnology, Govt. of India, New Delhi. Aim of the project was to evaluate prebleaching efficiency of xylanase/laccase biocatalysts produced by Department of Microbiology, South Campus, Delhi University and Kurukshetra University on hardwood pulps at both pilot and mill scale. The outcome of this trial was quite favorable to commercialize the biobleaching process (http://www.cppri.org.in). Through the extensive literature search, there were several patents found on xylanases and laccases on the name of Indian laboratories and industries up to the extent for the production of bleaching enzymes but not yet evaluated for their biobleaching potential. Moreover Biocon India, Bangalore alone is, selling its commercial xyalnase under the name of Bleachzyme F for the delignification of pulps (Table 3).

6. Heterologous expression of xylanase, laccase and protein engineering

Garg et al. (2012) reported the cloning and expression of *Cyathus bulleri* laccase in *Pichia pastoris.* In this study, complete cDNA encoding laccase (Lac) from white rot fungus *C. bulleri* was amplified by RACE-PCR, cloned and expressed in *P. pastoris* under the control of alcohol oxidase (*AOX)1* promoter. Later it was also observed, $CuSO_4$ increased the synthesis of laccase up to 12-fold when added in production media. Goswami et al. (2014) reported cloning and heterologous expression of cellulase free thermostable xylanase from *Bacillus brevis.* Xylanase gene was isolated and expressed in *E. coli* BL21. The recombinant xylanase was predominantly secreted to culture medium and showed mesophilic nature (optimum working was at 55 °C, pH 7.0). Both rational design and directed evolution has been widely applied for designing of proteins in technically advanced molecular biology laboratories worldwide. Generally most of the enzymes are produced by mesophilic organisms, like fungi, molds, yeasts and several bacteria. Commonly enzymes produced by these types of organisms have less thermo-stability/pH stability and least consistency in the presence of salts and also less specificity of enzymes towards their substrates. Therefore it is necessary to bring an improvement in the catalytic performance of enzymes by applying the protein engineering that is the vital tool of molecular biology. Verma et al. (2013) reported increased thermostability of xylanase (Mxyl) retrieved from a compost-soil-based metagenomic library. After scrutinizing the structure of xylanase by molecular dynamics simulation exposed more structural fluctuations in β-sheets. The surface of *β*-sheets was enriched with arginine residues by substituting serine/threonine by site-directed mutagenesis; the enzyme with four arginine substitutions (MxylM4) exhibited enhanced thermostability at 80 °C. The Half life ($t_{1/2}$) of MxylM4 at 80 °C, in the presence of birchwood xylan, increased from 130 to 150 min, without any alteration in optimum pH and temperature. The K_m of MxylM4 was also, increased from 8.01 ± 0.56 of Mxyl to 12.5 ± 0.32 mg ml^{-1} but reduced the affinity as well as specific enzyme activity. Both Mxyl and MxylM4 xylanases remained effective for delignification of pulp. Laccase enzyme is metallic biocatalyst unlikely xylanases, it needs mediators (small molecules) for the delignification of pulps. Biobleaching of pulps by laccases in absence of mediator component is not feasible due to the less redox potential (E0) of laccases (470 to ~ 800 mV) than non phenolic structures of lignin (1400 mV) like veratryl alcohol (Camarero et al., 2007; Singh et al., 2015). In the case of laccases, protein engineering may not consider as worthy if there is only increase in enzyme activity not E0. Kenzom et al. (2014) have performed the random mutagenesis to *Cyathus bulleri lcc* gene (WtLcc) by using an error prone

PCR. The 816-bp fragment (toward the C terminus) of the WtLcc was manipulated and enzyme variants (Lcc35, Lcc61, and Lcc62) were chosen best on the criteria of enhanced enzyme activity against ABTS. In this study the mutant laccase variants have the same E0 like the parent WtLcc.

7. Environmental regulations for the paper industry and policy measures

Pulp and paper industry presently consuming the large quantities of fresh water, 80–150 m^3 t^{-1} of paper, depends on the type of raw material being used. Commonly agro based paper mills are expending more water than recycled fibre (RCF) mills for removing the chemicals from processed pulp. Disposal of waste water contained severely environmental toxic compounds (AOX), bleaching (hydrogen peroxide, chlorine dioxide and caustic soda) and whitening agents (kaolin, calcium carbonate and titanium dioxide). Consequences of growing awareness about healthy and clean environment, paper industries facing stringent criticisms from Government as well as from general public due to release of untreated or partially treated effluent (Bajpai, 2012). In response to environmental concerns the paper industry has reacted by making process modifications based on existing and new proven technologies. The Central Pollution Control Board (CPCB) has taken several initiatives for reducing the pollution caused by paper mills, up to 2020. CPCB will make sure that none of the paper industry can discharge untreated industrial effluent to the water bodies like rivers and canals. Some of large paper industries recently upgraded their effluent treatment plant (ETP) with installation of tertiary treatment system for better effluent quality, particularly colour and suspended solids. Some of the medium sized agro-based paper mills have installed the non-conventional chemical recovery system, to incinerate the black liquor, which is one of the major causes of pollution. Effluent from all the operational units is mixed together and collected in effluent treatment plant for a common treatment, this is current practice adopted by most of the small and medium paper industries. This mixing up of all types of wastes poses a problem of handling large volumes of effluents with a variety of effluent parameters. It is suggested that coloured and non-coloured effluents should be segregated and treated separately thereby reducing the overall chemical load and possibly improving the treated wastewater quality. Therefore, mills may initiate actions to reengineer and modernize the existing ETP to phase out unlined lagoons by providing efficient coagulation and flocculation processes and converting the existing anaerobic lagoons into a lined lagoon for active aerobic process, thereby avoiding any groundwater pollution problem, improving the quality of treated effluent as well as reducing the holding time (http://psa.gov.in).

8. Conclusion and future prospects

Irrespective of continuous progress of Indian paper industries, only few of the large wood based paper mills have made progress by adoption of new green technologies but fully fledged, total chlorine free (TCF) bleaching of pulp with cocktail of enzymes is still under observations. ECF and TCF paper production offers opportunities for emerging enzyme technology which provide a simple and cost-effective way to satisfy the consumers and environmental protection agencies' concerns. The day may not be far when paper products manufactured with chlorine compound-based technology will be prohibited for wrapping of food products and other consumer items. If industry will not implement the international standards, as a result paper export market may face the undesirable consequences in future. It is also imperative to generate new technologies for economical xylanase and laccase production. Realistic cost estimate and improvement in process economics shall be the key factors for commercial success of any technology and therefore it must be clearly understood that enzyme-based process for bleaching must be as inexpensive as using chlorine or even organic chlorine compounds.

Acknowledgments

Authors are thankful to the SERB/DST, Delhi, India, for providing the research funding under Fast Track Young Scientist Program (SB/FT/LS-315/2012).

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