British Biotechnology Journal
8(4): 1-13, 2015, Article no.BBJ.18845
ISSN: 2231–2927

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Bio-bleaching of *Anthocephalus cadamba* Kraft Pulp through Direct Fungal Treatment by FEQP Sequence

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Authors' contributions

This work was carried out in collaboration between all authors. Author ML designed the study, wrote the protocol, performed experiments and statistical analysis. Author AK wrote the first draft of the manuscript. Authors DD and AG managed the literature searches and analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BBJ/2015/18845
Editor(s):
(1) Ana Lúcia Leitão, Faculty of Sciences and Technology, New University of Lisbon, Portugal.
Reviewers:
(1) EMAD Sadeghi Nezhad, Mechanical Engineering, University of Malaya, Malaysia.
(2) Anonymous, University of Pardubice, Czech Republic.
Complete Peer review History: http://sciencedomain.org/review-history/10126

Original Research Article

Received 12th May 2015
Accepted 29th June 2015
Published 10th July 2015

ABSTRACT

Aims: The study aims at mitigating pulp kappa number before bleaching to minimize pollution load.
Study Design: An experimental study.
Methodology: The various parameters of direct fungal (*Coprinellus disseminatus* MLK01) treatment (F-stage) of unbleached kraft pulp of *Anthocephalus cadamba* were optimized and compared with the results of enzymatically pre-bleaching. Finally, the pulp was bleached by EQP three-stage and XECEHH six stages bleaching sequences.
Results: Direct fungal treatment (F-stage) delignified the *Anthocephalus cadamba* kraft pulp more selectively with *Coprinellus disseminatus* MLK01 compared to xylanase prebleached pulp from the same fungus and oxygen delignification. F-stage mitigated the unbleached pulp kappa number by 55.0% and improved brightness and viscosity by 17.3 and 7.63% respectively. Kappa number reduction and brightness improvement were 22.1 and 6.3% more in F-stage compared to XE-stage. The kappa number and pulp brightness of oxygen delignified were 0.9 and 5.2% less compared to F-stage. The viscosity of oxygen delignified pulp reduced drastically due to alkaline peeling reactions compared to XE-stage (–26.86%) and F-stage (–27.09%). The brightness and viscosity of XECEHH bleached pulp were 80.1% and 7.4 cps at a chlorine demand of 4.3% while FEQP bleached pulp produced brightness of 79.7% and viscosity 8.2 cps. COD and colour values in

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effluent generated during FEQP bleaching were 53.29% and 54.36% less compared to CEHH bleaching.

Keywords: Coprinellus disseminatus MLK01; Anthocephalus cadamba; kraft pulp; prebleaching; fungal treatment; oxygen delignification.

ABBREVIATION

F-stage	*Direct fungal treatment*
XE-stage	*Xylanase prebleaching followed by alkaline extraction*
C	*Chlorination*
E	*Alkaline extraction*
H_1	*Hypochlorite Ist stage*
H_2	*Hypochlorite IInd stage*
Q	*Chelating stage*
P	*Peroxide stage*
COD	*Chemical Oxygen Demand*
AOX	*Adsorable organic halides*

1. INTRODUCTION

Pulp bleaching has become an issue of great concern due to public awareness and more stringent laws against pollution generating industries about environmental hazards caused by the release of AOX. Chlorine-based pulp bleaching generates chlorinated organic compounds which are recalcitrant to degradation [1], acute or even chronic toxins and can induce genetic changes in exposed organisms [2]. Since, pulp kappa number decides bleach chemical requirements during pulp bleaching, various pulping technologies except conventional pulping methods are available to reduce kappa number during pulping such as: Modified continuous kraft pulping (MCKP) [3], modified continuous cooking (MCC) [4], polysulphide cooking [5], rapid displacement heating (RDH) process [6], Black Liquor Impregnation (BLI) [7], isothermal cooking (ITC) [8], extended modified continuous cooking (EMCC) [9], displacement batch cooking [10], use of cooking aids such as anthraquinone during delignification of conventional kraft and polysulphide pulping of hardwoods [11] and oxygen delignification [12].

Moreover, reduction in kappa number can also be achieved by the process of bio-pulping in which lignin is selectively removed from the wood chips by colonizing the white-rot fungi in suitable conditions. *Phanerochaete chrysosporium* Burds. (strain BKM F-1767) is highly selective and removed 73% of the lignin and only 15% of the glucan, in 12 weeks. *Heterobasidion annosum* (Fr.) Bref. removes nearly equal proportions of lignin and glucan (26% and 23%) from pine, whereas, *Ceriporiopsis subvermispora* (Pil.) Gilbn. et Ryv. removes 50% and 3% of lignin and glucan, respectively [13,14]. The white-rot fungus *Phanerochaete chrysosporium*, mitigates the kappa number of soda-AQ pulps of kenaf bast fibers by 67% for the pulp from Japan and by 33% for the pulp from China after a six-day treatment [15]. The pulp and paper industries are attempting to bring changes to the bleaching process to minimize the use of chlorine to satisfy regulatory and market demands. Xylanases offer the cost effective way to along with environmental benefits [16]. Enzymes are also successfully employed during pulp prebleaching but thermal stability and alkali tolerance are desirable attributes for pulp biobleaching with xylanases, as the enzyme is often incubated with the pulp in high-density (HD) brown-stock towers where pulp pH varies from 8 to 10, depending on the washing efficiency and temperature from 60 to 85ºC [17,18]. Battan et al. [19] observed 11% reduction in kappa number during prebleaching of hardwood pulp by cellulase-free xylanase from *Bacillus pumilus* at conditions: xylanase dose of 5 IU/ml, temperature 60^0C, pH 7.0 and pulp consistency 10%. Guimaraes et al. [20] reported 25.2% decrease in kappa number by xylanase pretreatment from *Aspergillus japonicus* var. *aculeatus* with an enzyme dose of 10 IU/g dry pulps after 3 h of treatment. Li et al. [21] showed that xylanase from *Streptomyces chartreusis* reduced the kappa number of wheat straw pulps by 9.7 and 10.1% at an enzyme dose at 20 and 40 IU/g pulp respectively. Bankeeree et al. [22] reported the treatment of the rice straw soda pulp with xylanase prior to treatment with 10% (v/v) H_2O_2 and handsheets showed an increase in brightness by 13.5% (ISO) with improvement in tensile and tear strengths by 1.16 and 1.71 folds, respectively, compared to pulps treated with H_2O_2 alone.

The present study aimed at delignifying the kraft pulp of *Anthocephalus cadamba* by direct fungal treatment with white-rot fungus *Coprinellus disseminatus* MLK01 and comparing the results with prebleaching with xylanase from the same fungus followed by alkaline extraction and oxygen delignification. Finally, the *A. cadamba* kraft pulp was bleached by FEQP and CEHH

bleaching sequence to study the effect on pulp brightness and effluent characteristics.

2. MATERIAL AND METHODS

2.1 Kraft Pulp Preparation

The screened chips of *Anthocephalus cadamba* (age 12 years) were digested in a WEVERK electrically heated rotary digester of capacity 0.02 m^3 by kraft pulping process at an active alkali dose of 16% (as Na_2O), sulphidity 20%, maximum cooking temperature 165 ^{0}C, time 90 min and liquor to wood ratio of 3.5:1. The screened pulp yield of *A. cadamba* is found to be 48.74% at kappa number of 24.0±0.4, pulp brightness 33.0±0.3% (ISO) and viscosity 28.80±0.25 [23].

2.2 Microorganism

Alkali-tolerant strain of *Coprinellus disseminatus* MLK01 was isolated from decaying wood of *Shoria robusta* by enrichment culture technique. The decaying wood sample was kept in glass Petri plate containing moist wheat bran, incubated at 40 ^{0}C and growth was observed every day. The successive degradation resulted into appearance of white threads like structures. Further, it was purified on wheat bran-agar medium (2% wheat bran). National Type Culture Collection (NTCC), Pathology Division, Forest Research Institute, Dehradun (India) confirmed the purified strain of white-rot fungus as *Coprinellus disseminatus* (Pers.:Fr.) Lange and allotted the NTCC Culture Nos. 1180. This was maintained on wheat bran-agar (20 g wheat bran powder [retained on +100 mesh] and 20 g agar in 1,000 ml of distilled water) slants at 4°C and also stored at -20°C in 20% glycerol.

2.3 Fungal Treatment of *A. cadamba* kraft Pulp

10 g oven dry unbleached kraft (UBK) pulp was taken in a 500 ml Erlenmeyer flask and mixed with 80 ml of nutrient salt solution (NSS) -a culture medium. In regard to its composition, this culture medium contained the following in one liter of distilled water: 2.0 g of dextrose, 1.5 g of KH_2PO_4, 4.0 g of NH_4Cl, 0.5 g of $MgSO_4$, 0.5 g of KCl, and 1.0 g of yeast extract with 0.4 ml of trace element solution ($FeSO_4.7H_2O$ 200 µg/l, $MnSO_4$ 180 µg/l and $ZnSO_4.7H_2O$ 20 µg/l). Before autoclaving, the pH of flasks was adjusted at 8.0 and autoclaved at 121°C for 15 min. Ten ml of mycelium culture suspension was prepared by homogenizing five disks of eight mm diameter from growing culture of white-rot fungi in deionized water and each flask was inoculated maintain a pulp consistency of 10%. These flasks were incubated at 40°C in static condition and harvested on 4th to 12th day of incubation with an interval of one day. In another set of experiments, the samples were harvested at different pH levels varying from 4.0 to 9.0 with a gap of one unit on 8th day of incubation and temperature 40°C. In 3rd set, the flasks were incubated at temperature varying from 25 to 50°C with an interval of 5°C at optimum pH 7.0 and harvested on 8th day of incubation. The 4th and 5th set of experiments aimed at supplementing the nutrient media with different doses of D-glucose, D-xylose, D-galactose and urea separately varying from 1 to 5 g/l while keeping other conditions constant as optimized above.

All these samples after harvesting were filtered on a laboratory flat stationary screen with a 300 mesh wire bottom and filtrates were kept for estimation of residual enzyme activities. Then, the pulps were washed thoroughly with deionized water on a laboratory flat stationary screen and analyzed for kappa number (TAPPI T 236 cm-85 “Kappa number of pulp”), brightness (TAPPI T452 om-02 “Brightness of pulp, paper and paperboard [Directional reflectance at 457 nm]”), viscosity (TAPPI T 230 om-04 “Viscosity of pulp [capillary viscometer method”) and yield losses [24].

2.4 Enzyme Assays

The xylanase activity was determined by measuring the release of reducing sugars using birch wood xylan (Sigma Chemicals Co.) as the substrate by 3,5 dinitrosalicylic acid reagent (DNS) method [25]. 1.6 ml of enzyme preparation was added to 0.4 ml of substrate suspension (10 mg/ml birch wood xylan in 0.1 M potassium phosphate buffer at pH 6.5). The assay mixture was incubated at 55°C for 15 min with constant shaking at 100 rpm. Then, assay mixtures was cooled and centrifuged at 10,000xg. 1ml of supernatant was poured in a fresh tube which contained 3 ml of 3, 5-dinitrosalicylic acid (DNS) reagent, kept for 5 min on boiling water bath and optical density was measured at 540 nm. The cellulase activity was measured in terms of CMCase by using 2% (w/v) carboxyl methyl cellulose (CMC) as the substrate in 0.05 M citrate buffer at pH 4.8. Two ml of crude enzyme preparation diluted to 10 times be added to a test tube containing 2 ml CMC and incubated at 50°C for 30 min. Lignin peroxidase activity was measured spectrophotometerically as described

by Mercer [26] using 2, 4-di-chlorophenol as the substrate. One ml of reaction mixture was taken in a test tube, which contained 200 µl of 50 mM 2, 4-4-di-chlorophenol, 200 µl of 1 mM 4-aminoantipyrine and 200 µl crude enzyme, maintained at pH 6.5 with 200 µL of 100 mM phosphate buffer and finally, 200 µl of 50 mM H_2O_2 was added in to the reaction mixture. The absorbance was determined after every 30 s until it becomes constant at 510 nm. The enzyme activity was expressed as the amount of enzyme produced with an increase of 1.0 absorbance unit per 30s.

2.5 Pulp Bleaching

The unbleached kraft pulp of *A. cadamba* was bleached by a FEQP four-stage sequence. An eight-day fungal treatment (F) was followed by alkaline extraction (E), diethylene triamine penta acetic acid (DTPA) treatment and hydrogen peroxide bleaching. During the alkaline-extraction (E) stage, the pulp at 10% consistency was extracted with 1.55% NaOH for 90 min at 70±2ºC and pH 11.4. The washed alkaline-extracted pulp was followed by a chelating pretreatment stage (Q), conducted at pH 5.0 for 45 min at 55±2°C. The hydrogen peroxide bleaching was carried out with charge of 2% H_2O_2, at 10% consistency for 120 min at 80±2ºC.

Coprinellus disseminatus MLK-01 produced xylanase, CMCase and lignin peroxidase activities 30.32±1.2, 0.32±0.02 and 0.25±0.03 IU/ml respectively at an incubation period of six day and pH 10.0 at 40°C under sub-merged fermentation (SmF) conditions using 2% wheat bran as the substrate. Enzymes from *Coprinellus disseminates* showed 100% activity at pH 7.5 and retained 95.04±0.8 and 85.43±0.7% activities at pH 6.5 and 8.5 respectively. Similarly, it showed 100% activity at 75°C and 90.32±0.5 and 92.12±0.4 % activities at 70 and 80°C respectively [27]. *A. cadamba* kraft pulp, disintegrated in a L&W laboratory disintegrator to loosen fiber bundles or lumps without cutting, was taken in a polyethylene bag and treated with an enzyme dose of 5IU/g at a consistency of 10% and pH 7.5 and reaction time 90 min. The enzymatically treated pulp was subjected to alkaline-extraction (E) stage at 10% consistency, 1.5% NaOH for 90 min at 70±2ºC and pH 11.5. The extraction stage after enzymatic prebleaching facilitated the dissolution of lignin-carbohydrate complexes (LCC) in pulp that were previously modified by enzymes but still remained in pulp because of their large molecular weight thus, alkaline treatment swells up the cellulose fiber and increases pore size [28]. The pulp was evaluated for kappa number (TAPPI T 236 cm-85 “Kappa number of pulp”), brightness (TAPPI T452 om-02 “Brightness of pulp, paper and paperboard [Directional reflectance at 457 nm]”) and viscosity (TAPPI T 230 om-04 “Viscosity of pulp [capillary viscometer method”). The enzymatic prebleached pulp was bleached by a CEHH bleaching sequence at 4.3% chlorine demand. 70% of the total chlorine charge was applied in a ‘C’ stage and the remaining 30% in equal amounts in two distinct hypochlorite H_1 and H_2 stages. Pulp obtained after each bleaching stage was filtered through four-layered cheese cloth and filtrate was analyzed for residual chlorine except alkali extraction stage. Rest of the filtrate was preserved at 4°C for further analysis and the pulp was washed with 2 l of tap after each bleaching stage, including enzyme treatment, for proper impregnation of subsequent bleaching chemicals and then squeezed and crumbled. Finally, the pulp was analyzed for brightness and viscosity.

The pulps obtained after fungal treatment and enzymatic prebleaching followed by extraction (XE) stage were compared in terms of pulp kappa number, viscosity and brightness. Both the bleaching sequences i.e. FEQP and XECEHH were also compared for pulp brightness and viscosity.

The kraft pulp of *A. cadamba* was delignified with O_2 in electrically heated Weverk rotary digester of capacity 0.02 m^3 having four bombs of capacity 2l each at conditions: Consistency 15%, pH 11.0 maintained with 2% NaOH (as such), oxygen pressure 4.902 bar, temperature 110°C and reaction time 90 min in presence of carbohydrate stabilizer Epsom salt (0.1% $MgSO_4$). After completion of cooking, the pulps were washed on a laboratory flat stationary screen having 300 mesh wire bottom for the removal of residual cooking chemicals, squeezed and crumbled. The oxygen delignified pulp was evaluated for kappa number, brightness and the viscosity [24].

2.6 Characterization of Pulp and Bleach Effluent

The bleach effluent generated from each stage of bleaching sequence was mixed in equal amounts and were analyzed for COD (closed reflux titrimetric method using Thermoreactor CR2010) (Test method No-508 B), colour (Test method No-204A) as per standard methods for the

examination of water and waste water, American Public Health Association, 1985 and AOX by column method [29] Greenberg et al. [30] with AOX Analyzer Dextar ECS 1200 [29].

3. RESULTS AND DISCUSSION

3.1 Prebleaching Studies with Direct Fungal Treatment

3.1.1 Effect of incubation period on pulp properties and extracellular enzymes production

Fungal treatment of pulp produced maximum xylanase production (28.4 IU/ml) on 8th day while maximum cellulase production was observed (0.44 IU/ml) on 7th day of incubation and decreased thereafter. Extracellular xylanase and cellulases are produced in chorus and catabolically repressed by the release of monomer sugars and enzyme action on pulp (Table 1). Steiner reported that fungal xylanase are generally associated with cellulases [31]. Both enzymes are primary metabolites, therefore, produced during exponential phase of growth begin to decrease on the onset of death phase with fungal growth. Beside this, the metabolic enzymes such as, proteases and transglycosidases secreted by xylanase producing microorganisms may hydrolyze xylanase and cellulase, which can affect the enzyme yield adversely [32].

Table 1 also reveals that the peroxidase activity almost was constant from 8th to 12th day of incubation. Lignin degrading enzymes are secondary metabolites and produced in the stationary phase of growth. Mono-sugars produced from xylanase and cellulase action on pulp may be enhanced the production of lignin peroxidase. Lignin removal started from 4th day and increased up to 12th day of incubation. On 8th day of incubation, kappa number reduced by 36.67% compared to control and further increase in incubation time increased the pulp yield losses rapidly. The fungal treatment improved the pulp brightness by 41.21% on 8th day of incubation. However, the pulp brightness improved after 8th day of incubation but pulp yield losses were more compared to decrease in kappa number. The increase in pulp brightness with decrease in pulp kappa number indicating that fungal treatment removed residual lignin in the pulp. It indicated that the maximum pulp yield losses up to 8th day of incubation were due to removal of lignin-carbohydrates complexes. Beyond that, the maximum pulp yield losses occurred due to depolymerization of carbohydrates on account of increased in cellulase activity and longer reaction time. Therefore, reduction in kappa number and improvement in brightness were due to combined action of xylanase and lignin peroxidase during fungal treatment. Xylanase primarily removes the lignin-carbohydrates complexes, which are generated during pulping and act as physical barrier for bleaching chemicals [33]. At the initial stage of incubation, xylanase removes precipitated xylan from fiber surface and subsequently, lignin peroxidase oxidizes the remaining lignin from secondary cell wall and may be from lumen of fiber. Lignin peroxidases catalyze the H_2O_2-dependent oxidative depolymerization of lignin. Lignin peroxidases are known to oxidize phenolic aromatic substrates and also a variety of non-phenolic lignin model compounds as well as a range of organic compounds with a redox potential up to 1.4 V [34,35]. Nezamoleslami, [15] reported maximum reduction in kappa number and maximum improvement in brightness after six day of fungal treatment of Chinese and Japanese pulps by using *Phanerochaete chrysosporium.* Fujita et al. [36] also studied the bleaching of unbleached kraft pulp with white rot-fungi IZU-154 and observed 49-52% and 63% improvement in brightness on 5th and 11 day of treatment respectively.

3.1.2 Effect of initial pH and temperature on pulp properties and extracellular enzymes production

Table 2 shows that xylanase activity increased up to an initial pH 8.0 and decreased thereafter while cellulase and lignin peroxidase activities decreased continuously on increasing the initial pH and 91.60% of xylanase activity was retained at pH 7.0. The higher fungal biomass achieved at higher pH may be due to the effect of pH on nutrients availability. At pH 5.0, maximum cellulase (0.49 IU/ml) and lignin peroxidase (0.54 IU/ml) productions was observed but pulp yield losses increased up to 10.40%. The reason is that, degrading wood, a natural habitat for the growth of white-rot fungi has an acidic environment. The high cellulase activity resulted into cellulase-induced disintegration of fibers [37]. The fiber surface was stripped through the enzymatic hydrolysis of subsequent layers or fibrils called as “peeling effect” [38]. Although, maximum lignin peroxidase activity was found at pH 5.0 and 81.48% of lignin peroxide was retained at pH 7.0 but lignin peroxidase with xylanase reduced the pulp kappa number to 15.5 and improved pulp brightness up to 47.4%. The

pulp yield losses reduced to 6.40% due to decrease in cellulase activity at pH 7.0. Therefore, pH 7.0 was selected for further studies due to balance between pulp yield losses and kappa numbers. Tran and Chambers, [39] reported the pH 3.5 as optimum pH for maximum delignification (33.5%) of unbleached hardwood kraft pulp by *Phanerochaete chrysosporium.*

Table 3 reveals that the maximum xylanase (27.7 IU/ml), cellulase (0.42 IU/ml) and lignin peroxidase (0.44 U/ml) activities at 40 ^{0}C and beyond that, the decrease or increase in temperature resulted loss in enzyme activities. The decreased enzyme production at lower temperatures was possibly due to lower transport of substrate across the cells. On proceeding towards optimum temperature for enzyme production, increased kinetic energy of reacting molecules increased the reaction rate. At higher temperatures, thermal denaturation of enzymes of the metabolic pathway occurred, which increased the maintenance energy requirement of cellular growth, thereby resulting in poorer production of the metabolites and even loss of enzyme activity signifying that the end-point of fermentation ought to be controlled carefully [40]. Reduction in pulp kappa number and improvement in brightness were observed with increase in temperature up to 40°C and beyond that increase in kappa number and decrease in pulp brightness might be due poor growth and denaturation of enzymes [41]. Tran and Chambers [39] reported 38°C as optimum temperature for delignification of unbleached hardwood kraft pulp by *Phanerochaete chrysosporium.*

3.1.3 Effect of sugars addition on pulp properties and extracellular enzyme production

Table 4 reveals that reducing sugars D-glucose, D-xylose and D-galactose repressed the xylanase as well as cellulase synthesis under semi-solid fermentation conditions. D-xylose was found to be major catabolic repressor and produced minimum xylanase activity (4.0 IU/ml) at 5.0 g/l of dose. At the same dose, D-glucose and D-galactose showed no cellulase activities and D-xylose showed a minimal cellulase activity of 0.10 IU/ml. While de Souza et al. [42] found resistance of *Aspergillus tamari* to catabolic repression with glucose when wheat bran used as the sole substrate under SSF condition.

Table 1. Effect of incubation period on pulp kappa number, brightness and yield loss during fungal treatment of unbleached kraft pulp of *A. cadamba*

Incubation period, day	Xylanase activity, IU/ml	CMCase activity, IU/ml	Lignin Peroxidase activity, U/ml	Kappa number	ISO brightness,%	Losses,%
0	0	0	0	24.0±0.4	33.0±0.3	0
4	13.4±0.8	0.25±0.02	0.15±0.02	22.4±0.3	34.5±0.3	1.50±0.02
5	15.2±0.2	0.32±0.02	0.22±0.03	20.5±0.2	36.4±0.3	3.00±0.04
6	16.3±0.6	0.36±0.01	0.24±0.02	17.1±0.2	39.1±0.3	5.45±0.08
7	21.2±1.0	0.44±0.02	0.34±0.04	16.3±0.1	44.7±0.2	6.50±0.10
8	28.4±2.8	0.40±0.02	0.41±0.03	15.2±0.3	46.6±0.2	6.80±0.12
10	20.5±0.7	0.40±0.02	0.40±0.03	14.4±0.4	48.4±0.3	8.00±0.09
12	17.3±0.9	0.52±0.02	0.40±0.03	12.5±0.5	49.8±0.3	14.22±0.11

Conditions for fermentation: Initial pH, 8.0, temperature, 40ºC

Table 2. Effect of pH on pulp kappa number, brightness and yield loss during fungal treatment of unbleached kraft pulp of *A. cadamba*

Initial pH	Xylanase activity, IU/ml	CMCase activity, IU/ml	Lignin peroxidase activity, U/ml	Kappa number	ISO brightness, %	Losses, %
4.0	15.7±0.5	0.47±0.03	0.51±0.04	13.6±0.2	48.0±0.3	10.30±0.09
5.0	16.6±1.0	0.49±0.04	0.54±0.04	13.0±0.1	48.5±0.2	10.40±0.14
6.0	20.5±0.7	0.46±0.02	0.47±0.02	14.0±0.3	48.8±0.3	9.00±0.15
7.0	26.2±0.5	0.41±0.02	0.44±0.03	15.5±0.4	47.4±0.3	6.40±0.08
8.0	28.6±0.7	0.34±0.02	0.32±0.05	15.1±0.3	46.5±0.2	5.60±0.09
9.0	20.2±0.6	0.25±0.02	0.15±0.04	20.7±0.2	37.3±0.3	3.50±0.07

Conditions for fermentation: Incubation time, 8 days, temperature, 40°C

The lignin peroxidase activity increased with increasing D-xylose dose up to 4 g/l and beyond that there was no significant change in lignin peroxidase activity. Compared to D-galactose, D-glucose and D-xylose were found to improve lignin peroxidase production. It means that the reducing sugars act as a catabolic repressor for xylanase as well as cellulase production. Lignin peroxidase is a secondary metabolite when the culture medium is deficient of carbon, sulphur or nitrogen; the lignin peroxidase activity increases due to increased mycelial growth [32]. Lignin peroxidase was efficient to reduce pulp kappa number and improve the pulp brightness to its maximum level at a dose of 4 g/l. Further, the increased dose of D-glucose and D-xylose did not improve the pulp properties significantly. These results indicate that without addition of sugars, carbohydrates in pulp are consumed by fungus for their growth and enzyme production. While sugars added to the pulp during treatment are consumed by fungus and resulting in to low pulp losses. Tran and Chambers [39] studied delignification of unbleached hardwood kraft pulp by *Phanerochaete chrysosporium* and found 12.4% increase in delignification by the addition of glucose (0.47%, w/v) compared to control (without glucose).

3.1.4 Effect of different concentration of urea on the growth and enzyme production

Table 5 shows that lignin peroxidase activity decreases with increasing the dose of urea. The minimum lignin peroxidase activity is found 0.09 IU/ml at 5 g/l of urea. It means that the high dose of urea represses the lignin peroxidase induction and it may be due to consumption of lower dose of urea during primary growth of fungi while lignin peroxidase is produced as secondary metabolite. Fem and Kirk [43] reported that urea repressed 57% lignolytic activity in *Phanerochaete chrysosporium.* Kachilishvili et al. [44] reported that the yield of hydrolytic enzymes and laccase produced by four white-rot fungi namely *Funalia trogii* IBB146, *Lentinus edodes* IBB363, *Pleurotus dryinus* IBB 903 and *P. tuberregium* IBB624 increased and manganese peroxidase activity repressed by supplementation of medium with an additional nitrogen source during solid-state fermentation state of wheat straw and beech tree leaves.

On the other hand, cellulase and xylanase activities increased with increasing dose of urea and maximum cellulase (1.77 IU/ml) and xylanase (40.1 IU/ml) productions were obtained at 5g/l the dose of urea. The wood is a natural site for the growth of white-rot fungi and has very low nitrogen content i.e. 0.303% to 0.073% dry weight [45]. Addition of nitrogen to the wood leads to cellulose degradation. Table 5 also reveals that additional dose of urea lead to fiber losses due to increased cellulase production. The addition of 5 g/l urea in pulp reduced the kappa number by 24% while improvement in brightness was 2.3% only compared to control. Urea caused 24.20% in pulp yield loss at a dose of 5 g/l; therefore, addition of urea is not advisable due to drastic pulp yield losses.

3.2 Chemical Bleaching of Pulp

Pre-bleaching studies reveal that crude xylanase from *Coprinellus disseminatus* MLK01 showed 32.91% reduction in kappa number and 11.0 and 7.29% improvement in pulp brightness and viscosity after xylanase treatment and followed by alkaline extraction (XE-stage) (Fig. 1). The extraction after pulp prebleaching facilitates the dissolution of lignin-carbohydrate fragments in pulp that were previously modified by these enzymes but still remain in pulp because of their large molecular weight [28]. Gupta et al. [46] reported that xylanase and laccase treatment followed by alkali extraction and peroxide (E_P) treatment resulted in marked increase in brightness (13%) and whiteness (106.15%).

Table 3. Effect of temperature pulp kappa number, brightness and yield loss during fungal treatment of unbleached kraft pulp of *A. cadamba*

Temper-ature, °C	Xylanase activity, IU/ml	CMCase activity, IU/ml	Lignin peroxidase activity, U/ml	Kappa number	ISO brightness, %	Losses, %
25	18.5±0.4	0.15±0.02	0.08±0.02	23.5±0.2	34.1±0.3	2.60±0.05
30	22.9±0.4	0.25±0.02	0.15±0.04	22.2±0.1	35.5±0.3	3.00±0.08
35	24.6±0.6	0.38±0.02	0.42±0.03	19.7±0.3	40.3±0.2	6.20±0.09
40	27.7±0.7	0.42±0.02	0.44±0.03	15.4±0.3	47.8±0.2	6.80±0.12
45	23.5±0.8	0.40±0.02	0.38±0.03	16.2±0.3	44.6±0.2	5.80±0.11
50	20.4±0.6	0.26±0.02	0.16±0.04	21.5±0.2	36.4±0.3	4.50±0.10

Conditions for fermentation: Incubation time, 8 days, pH 7.0

Table 4. Effect of reducing sugars on pulp kappa number, brightness and yield loss during fungal treatment of unbleached kraft pulp of *A. cadamba*

	Dose, g/L	Xylanase activity, IU/ml	CMCase activity, IU/ml	Lignin peroxidase activity, U/ml	Kappa number	ISO brightness, %	Losses, %
D-glucose	Control	27.1±1.2	0.41±0.03	0.42±0.05	15.0±0.1	47.0±0.3	7.00±0.16
	1.0	15.4±3.1	0.26±0.04	0.72±0.04	13.5±0.1	47.3±0.3	6.54±0.15
	2.0	12.9±2.2	0.16±0.02	1.11±0.03	12.0±0.2	48.6±0.3	6.05±0.08
	3.0	12.4±1.6	0.10±0.03	1.25±0.03	11.0±0.1	49.2±0.3	5.75±0.06
	4.0	10.1±2.6	0.09±0.02	1.35±0.02	10.8±0.2	50.3±0.2	5.50±0.10
	5.0	09.2±1.1	nd	1.37±0.03	10.2±0.2	50.4±0.2	5.40±0.12
D-xylose	1.0	11.9±1.3	0.28±0.05	0.84±0.05	13.2±0.3	47.4±0.3	6.15±0.13
	2.0	10.7±1.7	0.19±0.04	1.20±0.02	12.0±0.1	48.3±0.3	6.25±0.10
	3.0	08.1±0.8	0.17±0.03	1.25±0.03	10.8±0.1	50.0±0.3	6.00±0.08
	4.0	04.8±1.0	0.12±0.02	1.34±0.04	10.5±0.2	50.3±0.3	5.10±0.11
	5.0	04.0±0.3	0.10±0.02	1.37±0.02	10.1±0.1	50.6±0.2	4.70±0.06
D-galactose	1.0	15.1±0.6	0.37±0.05	0.60±0.03	14.9±0.1	46.0±0.2	6.68±0.12
	2.0	15.0±0.8	0.25±0.04	0.72±0.03	14.0±0.2	46.3±0.3	6.65±0.11
	3.0	10.0±1.1	0.22±0.03	0.81±0.04	13.0±0.2	47.0±0.3	5.67±0.09
	4.0	05.5±0.5	0.11±0.04	0.83±0.01	12.8±0.1	48.3±0.3	5.60±0.09
	5.0	01.9±0.3	Nd	0.80±0.03	15.2±0.3	45.0±0.3	5.55±0.07

Conditions for fermentation: Incubation period, 8 days, Temperature, 40°C, pH, 7.0

Table 5. Effect of urea on pulp kappa number, brightness and yield loss during fungal treatment of unbleached kraft pulp of *A. cadamba*

Dose, g/l	Xylanase activity, IU/ml	CMCase activity, IU/ml	Lignin peroxidase activity, U/ml	Kappa number	Brightness, % ISO	Losses, %
Control	28.2±1.0	0.42±0.06	0.42±0.03	15.0±0.3	47.0±0.2	6.04±0.08
1.0	29.3±1.2	0.78±0.04	0.17±0.02	13.5±0.2	48.5±0.3	13.32±0.14
2.0	31.5±1.1	0.92±0.05	0.15±0.05	12.5±0.3	48.8±0.4	15.24±0.09
3.0	34.1±1.7	1.28±0.03	0.13±0.03	12.2±0.4	48.5±0.2	19.54±0.12
4.0	38.7±1.8	1.52±0.04	0.12±0.04	11.5±0.3	49.5±0.3	22.33±0.11
5.0	40.1±1.0	1.77±0.02	0.09±0.01	11.4±0.3	49.3±0.4	24.20±0.08

Conditions for fermentation: Incubation period, 8 days, Temperature, 40°C, pH, 7.0

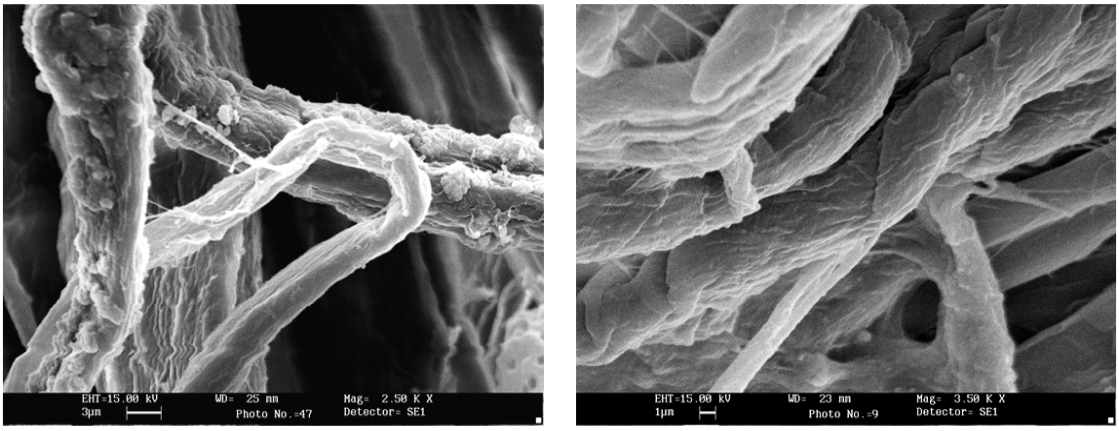

Fig. 1 (A) Scanning electron microphotograph showing the point of incomplete delignification and the flattened fibers after kraft pulping at a magnification of 2.50 K X (B) scanning electron microphotograph of enzyme treated kraft pulp of *A. cadamba* without beating at magnifications of 3.5 K x showing smooth surface

Xylanase-laccase treatment also showed an improvement in breaking length, burst factor, tear factor and viscosity by 49,6.9,23 and 11.68% respectively [46]. The direct fungal treatment (*Coprinellus disseminatus* MLK01) of pulp reduced the pulp kappa number by 55.0% and improved the pulp brightness (ISO) and viscosity by 17.3 and 7.63% respectively compared to control (Table 6). The pulp fibers when merged in water contain mobile and immobile layers enveloping the pulp fibers (Fig. 2A). As the consistency of pulp increases, the mobile layer is progressively eliminated leaving only the thin immobile layer surrounding the fiber, thus decreasing considerably the diffusion path length of reactant to the fiber.

Table 6. Comparison of results of prebleaching stage followed by alkaline extraction stage (XE-stage) and fungal treatment of unbleached kraft pulp of *A. cadamba* (F-stage) and results of XECEHH and FEQP bleaching sequences

Particulars	**Unbleached pulp properties**		
Pulp kappa number	24.0±0.4		
Pulp brightness, % (ISO)	33.0±0.3		
Pulp viscosity, cps	28.80±0.25		
Post pulp treatments	**XE-stage**	**Fungal treatment of unbleached kraft pulp (F-stage)**	**Oxygen delignification**
Kappa number	16.1±0.4(−32.91)	10.8±0.4(−55.0)	9.9±0.3
Brightness, % (ISO)	44.0±0.2(+11.0)	50.3±0.2(+17.3)	45.1±0.3
Viscosity, cps	30.90±0.26 (+7.29)	31.0±0.26 (+7.63)	22.60±0.22
Bleaching sequence	**CEHH**	**EQP**	—
Chlorination (C) stage			
Total chlorine charged, % (as available Cl_2)	4.30	—	—
Cl_2 applied in 'C' stage, % as available Cl_2	3.00	—	—
Cl_2 consumed in 'C' stage as available Cl_2	2.95	—	—
Brightness, % (ISO)	50.6	—	—
Extraction (E) stage			
NaOH applied in E stage	1.55	1.55	—
Chelating (Q) stage			
DTPA applied in 'Q' stage, %	—	0.2	—
Peroxide (P) stage			
Peroxide applied in 'P' stage	—	2.0	—
$MgSO_4$ applied, %	—	1.0	—
Sodium silicate applied, %	—	2.0	—
Hypochlorite (H_1) stage			
$Ca(OCl)_2$ applied, % as available Cl_2	0.90	—	—
$Ca(OCl)_2$ consumed, % as available Cl_2	0.88	—	—
Hypochlorite (H_2) stage			
$Ca(OCl)_2$ applied, % as available Cl_2	0.40	—	—
$Ca(OCl)_2$ applied, % as available Cl_2	0.38	—	
Final brightness, % (ISO)	80.1±0.2	79.7±0.3 (–0.4)	—
Final viscosity, cps	7.40±0.05	8.20±0.06 (−9.76)	—
Combined bleach effluent characteristics			
AOX, kg/T	2.394	—	
COD, mg/l	1199	560 (–53.29)	—
Colour, PTU	1490	680 (–54.36)	—

Note: ± refers to standard deviation and +/– % difference compared to control pulp; Bleaching conditions: XE–stage: Reaction time 90 min, pH 7.5, enzyme dose 5 IU/g of oven dried pulp, temperature 75 ^{0}C; alkali extraction with 1.5% NaOH at 70^{0}C temperature for 90 min; C– stage: Reaction time 45 min, pH 2.5, ambient temperature, consistency 3%. E –stage: Reaction time 90 min, pH 11.4, temperature 70±2 ^{0}C, consistency 10%; H_1 and H_2–stages: Reaction time 90 min, pH 11.3, temperature 70±2 ^{0}C, consistency 10%; Q –stage: Reaction time 45 min, pH 5.0, temperature 55±2 ^{0}C, consistency 3%; P –stage: Reaction time 120 min, pH 11.5, temperature 80±2 ^{0}C, consistency 12%; O_2 delignification (O) stage: O_2 pressure, 4.902 bar, NaOH applied, 2.0 % (o.d. pulp), $MgSO_4$ applied, 0.1 % (o.d. pulp), pH 11.0, temperature 110 ^{0}C, reaction time 90 min

Water layer thickness now becomes the rate-determining step [47]. In case of direct fungal treatment, mycelia protrude through the pulp fibers and secrete the enzymes directly at the reaction sites. After digestion, the released soluble products are absorbed by the hyphae (Fig. 2B). The removal of lignin-carbohydrates complexes (LCC) reduces the pulp kappa number and improves the pulp brightness. Tran and Chambers [39] showed 33.5 to 56.5% delignification of unbleached hardwood kraft pulp by the treatment of *Phanerochaete chrysosporium.* Fujita et al. [36], also reported 21% increase in brightness after five day of fungal treatment and kappa number reduced from 20.9 to 9.2 after eleven days incubation.

Oxygen delignification mitigated the kappa number from 24.0 to 9.9 and pulp viscosity 28.8 to 26.6 cps and improved the brightness from 33.0 to 45.1% (ISO). The pulp kappa number obtained after direct fungal treatment is comparable to kappa number of oxygen delignified pulp but pulp brightness improved by 5.2%. Meng et al., [48] found 14.20% reduction in kappa number and improvement in brightness by 2.3% (ISO) after pretreatment with xylanase (50 IU/g oven-dried pulp).

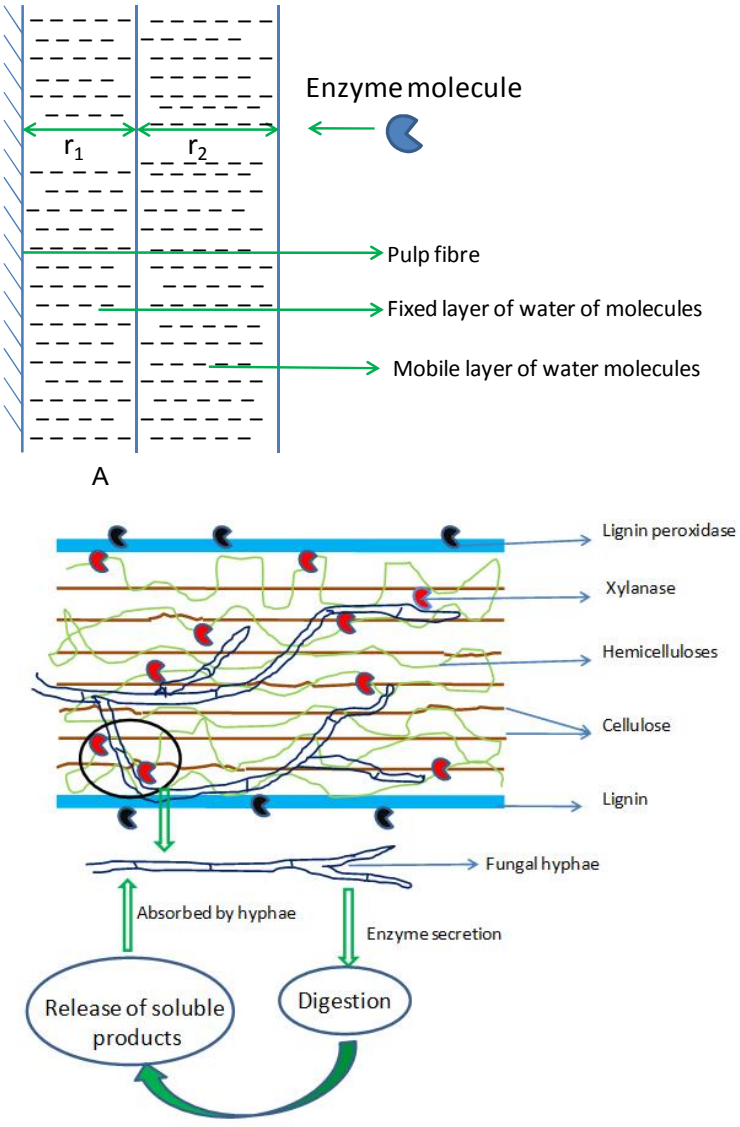

Fig. 2 (A) Showing fixed and mobile layers of water on pulp fibre (B) Mechanism of action of fungal treatment on pulp fibre

The reduction in pulp viscosity is due to alkaline hydrolysis (depolymerization) of the polysaccharide chains in addition to the peeling reactions and is subjected to further degradation reactions (secondary peeling) [49].

The enzymatically prebleached pulp produced pulp brightness of 80.1% (ISO) of viscosity 7.4 cps at a chlorine demand of 4.3% (as available chlorine) by CEHH bleaching sequence. The pulp obtained after direct fungal treatment produced a pulp brightness of 79.7% (ISO) and pulp viscosity 8.2 cps by total chlorine free bleaching sequence (EQP). The pulp viscosity of EQP bleached pulp was slightly higher than CEHH bleached pulp. It may be due to the detrimental effect of molecular chlorine on carbohydrates during bleaching. AOX in effluent generated during CEHH bleaching was 2.4 kg/tone of pulp. On the other hand, the COD and colour in effluent generated during FEQP bleaching were 53.29% and 54.36% less compared to CEHH bleaching (Table 6).

4. CONCLUSIONS

The crude xylanase from *Coprinellus disseminatus* MLK01 reduced kappa number by 32.91% and improved pulp brightness and viscosity after xylanase treatment followed by alkaline extraction (XE-stage) by 11.0 and 7.29% respectively compared to control**.** Comparatively, the direct fungal treatment reduced the pulp kappa number by 55.0% and improved the pulp brightness and viscosity by 17.3 and 7.63% respectively compared to control. The enzymatically prebleached pulp produced a pulp brightness of 80.1% (ISO) of viscosity 7.40 cps at a chlorine demand of 4.3% (as available chlorine) by CEHH bleaching sequence. Whereas, direct fungal treatment produced a pulp brightness of 79.7% (ISO) and pulp viscosity 8.20 cps by total chlorine free bleaching sequence (EQP).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nestmann ER. Detection of genetic activity in effluent from pulp and paper mills: Mutagenicity in *Saccharomyces cerevisiae*. In: Zimmerman FK, Taylor-Mayer RE, editors. Testing in Environmental Pollution Control, Horwood, London. 1985;105-117.
2. Taneja K, Gupta S, Kuhad RC. Properties and application of a partially purified alkaline xylanase from an alkalophilic fungus *Aspergillus nidulans* KK-99. Bioresour. Technol. 2002;85:39-42.
3. Johansson B, Mjoberg J, Sandstrom P, Teder A. Modified continuous kraft pulping-now a reality, Svensk Papperstid. 1984;87:30.
4. Lloyd JA, Allison RW, Wrathall SW. MCC (Modified Continuous Cooking) pulping with additives and modified sulfidity profiles. Appita. 1995;48:284.
5. Berthold F, Lindström ME. Polysulfide addition as a means to increase delignification in kraft pulping. Nordic Pulp Paper Res. 1997;12:230.
6. Pu Q, McKean W, Gustafson R. Pulping chemistry and kinetics of RDH (Rapid Displacement Heating) process. Appita. 1993;46:277-281.
7. Engström J, Lönnberg B, Pekkala O. Black liquor pretreatment in kraft cooking. Paperi Puu. 1994;76:59-66.
8. Hakanen A, Teder A. Modified kraft cooking with polysulfide: Yield, viscosity, and physical properties. Tappi J. 1997;87:189-196.
9. Jiang JE. Extended modified cooking-with polysulfide for simultaneous pulp yield and strength improvements. Tappi Pulping Conference Proceedings. 1992;683-690.
10. Tikka P, Kovasin K. Displacement vs. conventional batch kraft pulping: Delignification patterns and pulp strength delivery. Paperi ja Puu. 1990;72:773-779.
11. Parthasarthy VR, Smith GC, Rudie GF, Detty AE, Steffy JJ. Application of anthraquinone in extending the delignification of kraft and polysulphide pulps part-1: Pulping and bleaching of mixed hardwoods. Tappi J. 1995:78(2):113-125.
12. Olm L, Teder A. The kinetics of oxygen bleaching. Tappi. 1979;62:43–46.
13. Otjen L, Blanchette RA. A discussion of microstructural changes in wood during decomposition by white rot basidiomycetes. Can. J. Bot. 1986;64:905-911.
14. Blanchette RA, Otjen L, Effland M J, Eslyn WE. Changes in structural and chemical components of wood delignified by fungi. Wood Sci. Technol. 1985;19:35-46.
15. Nezamoleslami A, Suzuki K, Nishida T, Ueno T. Biobleaching of kenaf bast fiber,

soda–AQ pulp using white-rot fungus. Tappi J. 1998;81(6):179-183.
16. Kumar A, Gangwar N, Prakash T, Prakash R. Applicability of microbial xylanases in paper pulp bleaching: A review. Bioresources. 2014;9(2):3733-3754.
17. Lundgren KR, Bergkvist L, Hogman S, Joves H, Ericksson G, Bartfai T. TCF mill trial on softwood pulp with korsnas thermostable and alkaline stable xylanase T6, FEMS Microbiol. Rev. 1994;13:365–368.
18. Tolan JS, Olson DRE, Dines RE. Survey of xylanase enzyme usage in bleaching in Canada. Pulp Paper Can. 1995;96:107–110.
19. Battan B, Sharma J, Dhiman SS, Kuhad RC. Enhanced production of cellulase-free thermostable xylanase by *Bacillus pumilus* ASH and its potential application in paper industry. Enzyme Microb. Technol. 2007; 41:733–739.
20. Guimaraes NCA, Sorgatto M, Peixoto-Nogueira SC, Betini JHA, Zanoelo FF, Marques MR, et al. Xylanase production from *Aspergillus japonicus* var. *aculeatus*: Production using agroindustrial residues and biobleaching effect on pulp. J. Biocatal. Biotransformation. 2013;2:1-6.
21. Li X, Sun B, Zhao J, Lv Y, Song H, Li E, et al. Production and improved bleaching abilities of a thermostable xylanase from a newly isolated *Streptomyces chartreusis* strain. Afr. J. Biotechnol. 2011;10:14132-14142.
22. Bankeeree W, Lotrakul P, Prasongsuk S, Chaiareekij S, Eveleigh DE, Kim SW, et al. Effect of polyols on thermostability of xylanase from a tropical isolate of Aureobasidium pullulans and its application in prebleaching of rice straw pulp. SpringerPlus. 2014;3(37):1-11.
23. Lal M, Dutt D, Tyagi CH, Upadhyaya JS. Anatomy, morphology and chemical studies of *Anthocephalus cadamba*. TAPPI J. 2010;9:30-37.
24. TAPPI Test Methods. Technical association of the pulp and paper industry. TAPPI press, Atlanta, GA; 2007.
25. Miller GL. Use of dinitrosalicylic reagent for determination of reducing sugar. Anal. Chem. 1959;31:238-244.
26. Mercer DK, Iqbal M, Miller PGG, McCarthy AJ. Screening actinomycetes for extracellular peroxidase activity, Appl. Environ. Microbiol. 1996;62:2186–2190.
27. Lal M, Dutt D, Tyagi CH, Kaur H, Kumar A. Effect of residual enzyme on effluent load during biobleaching of kraft-AQ pulp of *A. cadamba* by crude xylanase from *Coprinellus disseminatus* MLK-03. BioResources. 2011;6:1019-1028.
28. Christov LP, Szakacs G, Balakrishnan H. Production partial characterization and use of fungal cellulose free xylanase in pulp bleaching. Process Biochem. 1999;34: 511–517.
29. User manual ECS 1200 Rev. 3.1.0, Themo electron corporation. 2006;40.
30. Greenberg AE, Clesceri LS, Eatonne AD. Standard methods for the of water examination and wastewater, American Public Health Association (USA), American Water Works Association (USA), Water Environment Federation (USA), Washington, DC (USA); 1992.
31. Steiner W, Laierty RM, Gomes I, Esterbauer H. Studies on a wild type strain of *Schizophyllum commune*: Cellulase and xylanase production and formation of the extracellular polysaccharide schizophyllan, Biotechnol. Bioeng. 1987;30:169-178.
32. Jeffries TW, Choi S, Kirk TK. Nutritional regulation of lignin degradation by Phanerocheate chrysosporium, Appl. Environ. Microbiol. 1981;42:290-306.
33. Nagar S, Jain R K, Thakur VV, Gupta VK. Biobleaching application of cellulase poor and alkali stable xylanase from *Bacillus pumilus* SV-85S, 3 Biotech, 2013;3:277–285.
34. Wong DWS. Structure and action mechanism of ligninolytic enzymes, Appl. Biochem. Biotechnol. 2009;157:174–209.
35. Valli K, Wariishi H, Gold MH. Oxidation of monomethoxylated aromatic compounds by lignin peroxidase: Role of veratryl alcohol in lignin biodegradation, Biochemistry. 1990;29:8535-8539.
36. Fujita K, Kondo R, Sakai K. Biobleaching of kraft pulp using white-rot fungus IZU-154. Tappi J. 1991;11:123-127.
37. Oltus E, Mato J, Bauer S, Farkas V. Enzymatic hydrolysis of waste paper, Cellulose Chem. Technol. 1987;21:663-72.
38. Jackson LS, Heitmann JA, Joyce TW. Enzymatic modifications of secondary fiber. Tappi. J. 1993;76(3):147-154.
39. Tran AV, Chambers RP. Delignification of an unbleached hardwood kraft pulp by *Phanerochaete chrysosporium,* Appl. Microbiol. Biotechnol. 1987;25:484-490.
40. Pal A, Khanum F. Production and extraction optimization of xylanase from

Aspergillus niger DFR-5 through solid-state-fermentation. Bioresour. Technol. 2010;101:7563–7569.
41. Yoon LW, Nam T, Ngoh GC, Chua ASM. Fungal solid-state fermentation and various methods of enhancement in cellulase production, Biomass Bioenerg. 2014;67:319-338.
42. De Souza DF, De Souza CG, Peralta MR M. Effect of easily metabolizable sugars in the production of xylanase by *Aspergillus tamarii* in solid-state fermentation. Process Biochem. 2001;36:835–838.
43. Fenn P, Kirk TK. Relationship of nitrogen to the onset and repression of ligninolytic activity and secondary metabolism in *Phanerochaete chrysosporium*. Arch. Microbiol. 1981;130:59-65.
44. Kachlishvili E, Penninckx M, Tsiklauri N, Elisashvili V. Effect of nitrogen source on lignocellulolytic enzyme production by white-rot. basidiomycetes under solid-state cultivation. World J. Microbiol. Biotechnol. 2006;22:391-397.
45. Dill I, Kraepelin G. Palo Podrido: Model for extensive delignification of wood by *Ganoderma applanatum*, Appl. Environ. Microbiol. 1986;52:1305-1312.
46. Gupta V, Garg S, Capalash N, Gupta N, Sharma P. Production of thermo-alkali-stable laccase and xylanase by co-culturing of *Bacillus* sp. and *B. halodurans* for biobleaching of kraft pulp and deinking of waste paper bioprocess Biosyst. Eng. 2015;38:947–956.
47. Reeve DW, Earl PF. Mixing gases, water and pulp in bleaching. TAPPI J. 1986;69(7):84-88.
48. Qijun Meng, Shiyu Fu, Bingyun Li, Lucian A. Lucia. The impact of xylanase and hot acid pretreatment on HexAs in eucalyptus kraft pulp bleaching, Journal of Wood Chemistry and Technology. 2015; 35(4):239-250.
49. McGinnis GD, Shafizadeh F. In: Pulp and paper chemistry and chemical technology, (Casey JP, Ed.), 3rd edn., Wiley Interscience, New York, Chap. 1. 1980;1.

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