Bleach boosting ability of endoxylanase from *Bacillus pumilus*

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Abstract— A thermo tolerant alkalophilic and cellulase-poor endoxylanase (E.C. 3.2.1.8) from *Bacillus pumilus* was evaluated for prebleaching of Kraft pulp. Enzyme was produced by solid-state cultivation using wheat bran as carbon source. Experimentation demonstrates the combination of enzyme pretreatment before chlorine and peroxide treatment resulted in complete utilization of bleaching chemical thereby reducing elemental chlorine in the effluent. The UV absorption spectrum of the compounds released by enzyme treatment of Kraft pulp exhibited characteristic peak at 280 and 465 nm indicating the presence of lignin in the released colouring matter. Enzymatic prebleaching of Kraft pulp showed reduction in kappa number of the pulp and complete utilization of chlorine dioxide (ClO_2) resulting in high final brightness. The cost of enzymes production is far below than that of elemental chlorine and ClO_2, because the crude enzyme can be directly utilized for treatment without purification, so this technology may be less expensive and ecofriendly for paper manufacturers than traditional industrial processes.

Index Terms—alkalophilic, *Bacillus pumilus*, biobleaching, chlorine compounds, cellulose,endoxylanase, Kraft pulp, total chlorine free

1 INTRODUCTION

In the pulp bleaching process the destruction, alteration or solubilization of the lignin, colored organic matter and other undesirable residues on the fibers occur. The hemicelluloses and lignin dissolved and partially degraded during heating process. In the subsequent phase of the process, the pH drops sharply because of the discharge of xylan side groups, and it precipitates with readsorption of lignin on top of the cellulosic micro fibrils. During Kraft pulping cellulosic fibers get darkly stained due to the dark colour of lignin. Usually, one or more bleach sequences are required to remove the dark colour caused by this deposition of lignin. For this usually large amounts of chlorine-based chemicals and sodium hydrosulfite where used, which cause several effluent-based problems in the pulp and paper industries. These chemicals produces stable chlorinated organic substances, some of which are toxic, mutagenic, persistent, and highly resistant to biodegradation, besides it also causes numerous harmful disturbances in biological systems and forms as one of the major sources of environmental pollution (Motta et al. 2013, Demuner et al. 2011).

Oxygen delignification and application of new agents like enzyme in biobleaching result in further reduction or elimination of chlorinated organic formation. Incorporation of xylanase into pulp mill-bleach sequence is simple and economically feasible which was one of the greatest success stories of enzyme in the pulp and paper industry (Viikari et. al. 1993, Kenealy and Jeffries 2003). Due to heterogeneous nature of xylan, complete degradation of it requires several hydrolytic enzymes, of which the best known are endo-β - 1, 4-xylanase (E.C. 3.2.1.8), which attack the main chain of xylan, and β – xylosidase (E.C.3.2.1.37), which hydrolyze xylooligosaccharides to D-xylose.

Xylanase cleave and solubilize reprecipitated xylan and lignin located on the surface of the micro-fibrils that facilitates pulp bleaching and lowers chlorine consumption thereby reducing the discharge of toxic organochlorine compounds in the environment (Zhao et al. 2006). Earlier industries have been using mildly acidic pH range by lowering the concentration of xylanase, now they go for new generation of enzymes that operate close to mill conditions, like alkaline pH, thermostable and short retention time (Kenealy and Jeffries, 2003, Bajpai 1999). The importance of xylanase in the pulp and paper industries is related to the hydrolysis of xylan, which facilitates the release of lignin from paper pulp and, consequently, reduces the usage of chlorine as the bleaching agent. The effectiveness of xylanase treatments was evaluated by determining the amount of sugars present after enzyme incubations and by observing increased bleachability with conventional methods after treatments (Khandeparkar and Bhosle, 2007). The break down of lignin carbohydrate bonds improves delignification. It also supposed to hydrolyze the xylan in the fiber thus enhancing the free penetration of bleaching chemicals. This resulted in an increase of final brightness of the pulp, which is very important in developing a chlorine free bleaching process. Use of xylanase improves the fiber swelling that result in good refining and upshot in better physical properties of paper (Poorna and Prema 2007). The enzyme treated pulp is more difficult to refine and requires more beating to achieve an equivalent tensile strength and freeness (Torres et al. 2000, Bajpai 1999). In this paper the application of endoxylanase from *B. pumilus* in Kraft pulp treatment was evaluated. The surface morphology of the pulp fiber examined at each bleaching stages by Scanning electron micrograph (SEM).

2. MATERIALS AND METHODS

2.1 Microorganism And Xylanase Production

B.pumilus isolated from soil was maintained of xylan agar slant at 4°C. Xylanase production was carried out in Erlenmeyer flasks (500ml) using dry wheat bran (500mm particle size) as solid substrates at 30°C pH-7 (Poorna and Prema, 2007). The crude enzyme extraction was done after 72h, of incubation in

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phosphate buffer (pH -7), and vortexed thoroughly to recover the maximum possible xylanase yield in the supernatant; it was centrifuged at 10,000 x g for 20 min at 4°C.

2.2. Kraft pulp preparation

The xylanase applications on Kraft or Chemical pulp (CP) - Bamboo and Reed, pulps collected from a commercial paper mill (Hindustan News Print Ltd, India). The investigation aimed at the effectiveness of xylanase treatments on conventional sequence used in the mill. The pulp with kappa number 18.2 with pH - 9 at 106°C was taken shred and packed in polythene bags separately; the moisture content of the pulp was estimated. Known weight of pulp was taken filtered through Buchner funnel, pressed to sheet, dried in hot air oven, and dry weight was taken, from this the weight of oven dried pulp (Od pulp) was calculated which help to fix on the consistency of the pulp for further experiments. Enzyme, ClO_2 and peroxide treatment where given sequentially to the pulp. The enzyme treatment given before chlorination as well as control run parallel without treatment and results compared. For this purpose, crude enzyme extracts from *B. pumilus*, prepared as described used.

2.3. Conventional treatment and Xylanase application on kraft pulp

Firstly the wood made to chips and cooked in active alkali mixture (Na_2S and NaOH) at 160 °C for 4 hour, which partially remove the hemicelluloses and lignin. This is followed by thickening process where the water content was lowered and unbleached pulp was stored in storage tower. This pulp is given chemical bleaching using ClO_2 followed by alkali extraction to obtain pulp with different properties. D_0 Eop (A), where D_0 is the ClO_2 treatment; E_{op} is the H_2O_2 reinforced extraction. The enzymatic treatment (X- xylanase) made as follows in the conventional process X D_0 E_{op} (B).

In all sequences studies 20g oven-dried pulp used as samples, the xylanase treatment given to the pulp separately. The pulp treated with different concentration of xylanase for optimization of enzyme concentration as explained below (2.4). The enzymes diluted in distilled water before application, and applied in the final pulp. The pulp consistency was 3 % w/v -1 ie. 20g of Od pulp, until and otherwise mentioned. After application of enzyme, the mixture was incubated in a thermostatic bath at 50 °C for 60 min. At every 15 min intervals, the pulp suspension mixed well for proper distribution of enzyme. The pulp filtered through Buchner funnel and washed with distilled water. The pulp samples where subsequently submitted to the other bleaching stages. At the D_0 stage in Kraft pulp was subjected to chlorine dosage (5 % consistency), where it was tested with different concentrations of Chlorine dioxide: without chlorine, 1, 2, 3, and 4 % (w/w). Followed by treatment with H_2O_2 (12 % consistency), concentration tried were-1.5, 2, 3, 4, 5 and 6 %. The treated pulp washed and hand sheets prepared by standard Test Method T 236 cm-85 (TAAPI methods). The ISO brightness of pulp measured on a brightness analyzer (GLRPHO- brightness color tester according to the TAPPI standards) and pulp properties analyzed by SEM. All experiments run in triplicate and results taken as the mean for tabulation. All experiments where done in triplicate.

2.4. Optimization of condition for xylanase treatment

The optimization of enzyme concentration for biobleaching carried out by treating pulp with different concentration – raw pulp, 2, 4, 6, 8, 12 and 14.0 U g –1OdP in (3 % consistency) for variable time intervals upto 2 hour and pulp properties studied at regular intervals. Optimization of temperature carried out at 40, 50 and 60 °C. The effect of pH of the pulp on enzyme action done at different pH - 8, 9 and 10 (pH of the pulp was adjusted using 1N -H_2SO_4 / NaOH) with xylanase in a total volume of 200 mL at 50 °C. The chemical pulp requires more bleaching to attain required brightness so it was subjected to chlorination before alkali extraction. Untreated and enzyme treated pulp where washed to neutral pH and subjected to chlorination with ClO_2 (4%) at 3 % consistency for 45 min at room temperature without shaking and proper action of chemical on the pulp was attained by giving regular kneading. The pulp washed to neutral pH, followed by alkali extraction (1.5 % peroxide) at 12 % consistency for 3 hour at 75 °C. The pulp washed to neutral pH, hand sheets made, dried and brightness attained estimated. The pulp samples subjected to scanning electron micrograph.

2.5. Optimization of bleach sequence

Kraft pulp after enzyme treatment subjected to peroxide treatment of different concentration devoid of chlorination (3, 4, 5 and 6 %). Enzyme concentration (4 and 7 U g –1 OdP) was optimized for 4 and 5 % peroxide treatment. In the next experiment, low concentration of Cl_2 treatment (1 and 2 %) given to enzyme (4 U g-1 OdP) treated pulp and it was subjected to peroxide treatment (4 and 5 %). Next experiment the enzyme treated (4 Ug –1 OdP) pulp subjected to Cl_2 treatment (2 %) and H_2O_2 4 % followed by varying NaOH charge (1N) 22, 24, 26, 28 and 30 (% OdP).

2.6. Release of chromophoric material, reducing sugar and Kappa number of the pulps

For estimation of chromophoric material released the pulp samples treated with different enzyme concentration as mentioned above were sealed in plastic bags (3 % consistency) in 100 mM phosphate buffer, pH -8 at 50 oC for 1hour. Intermittent kneading was given; control sample was treated under the same conditions with inactivated (boiled) enzyme. The lignin-derived compounds and chromophoric material released by enzyme-mediated treatment of pulp was estimated by monitoring the absorbance at 280 and 465 nm of the filtrate (Wong et al. 1997). Amount of reducing sugar released from pulp was determined spectrophotometrically at 540 nm according to the DNS method (Miller, 1959) and the degree of solubilisation of pulp determined from the reducing sugar released.

Degree of solubilisation = Reducing sugar released / weight of pulp x 100

The kappa number of unbleached and the bleached pulp estimated by potassium permagnate method. The sample of pulp (3- 4 g) was exposed to the action of 0.1 N KMnO4, (acidified) in 1000 ml at room temperature for 10 min. Addition of excess KI solution terminate the reaction and $KMnO_4$, consumed determined from the results by back-titrating the liberated iodine with standard sodium thiosulphate. The k number so obtained was the mL of 0.1 N KMnO4, consumed per gram of pulp (TAPPI method T -236) that indicates the presences of residual

lignin in the pulp. All experiments done in triplicate, individually and optimized condition of one carried over to another. All values represent the mean of the values from three independent experiments, with a standard deviation of < +/-5 %.

2.7. Scanning electron micrograph (SEM)

Change on the surface morphology of the enzyme bleached and non-enzyme bleached pulp examined by SEM to obtain a better understanding of the effect of xylanase treatment on enhancement of pulp bleaching. Samples of control and enzyme treated pulp were processed for normal scanning procedure. The fibers suspended in distilled water, placed on a cover slip, and air-dried. The preparation coated with gold particles (24 carat, 12 nm- layer 20 nm- thick) and examined at 15kV under SEM (JOEL JSM- 6400) at various magnifications and micrographs prepared.

3. RESULTS

The crude xylanase from *Bacillus pumilus* was cellulase poor. Specificity and Characteristics of endoxylanase extract was that it is free of cellulase activity (xylanase to cellulase ratio 1: 0.0117); the enzyme evaluated for prebleaching properties. The crude enzyme extract characterized to determine the optimum activity at different pH and temperature as well as the stability. The pH analyzed ranged from 5-11 and temperature 30 – 65 ^{o}C (Poorna and Prema, 2006). The reason for multiple xylanase during Solid state cultivation was due to the heterogeneous nature of the wheat bran used as substrate for enzyme production (Gunasekaran, 2001, Biely, 1985). The simultaneous action of these multiple enzymes improves the solubilisation of pulp, by action of each of these enzymes on different location of the xylan polymer, due to these specialties, the crude enzyme as such used for the application studies.

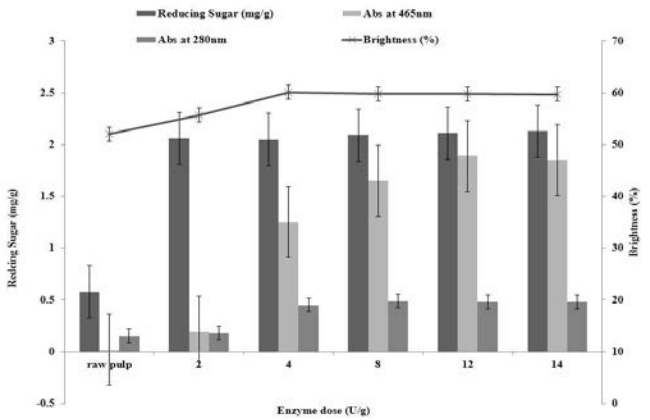


Figure 1. Optimization of enzyme dosage for Kraft pulp bleaching, reducing sugar, UV absorbance of coloured compounds released at 280 and 465nm during enzyme treatment at different enzyme level and Brightness observed after chlorination and alkali extraction.

3.1. Optimization of xylanase treatment

The samples were incubated for 2 h resulted in the release of chromophore with an OD ~1.45 at 280 nm and ISO brightness of 60.1 % with 4 Ug –1 of crude extract (Figure 1). After 1 h, there was no considerable change in the result so it was selected. Low concentration of 4 U/g Odp observed to be more effective than higher concentration, which has exhibited an increase in reducing sugar and chromophoric material, the ISO brightness of resulted sheet was only of marginal difference. Low enzyme concentration is preferred for further experiments to make the process more economical. The results of effect of pH and temperature of Kraft pulp illustrate that pH 9 and temperature 50 ^{o}C was optimum for the enzymatic pretreatment of pulp (Figure 2 and 3).

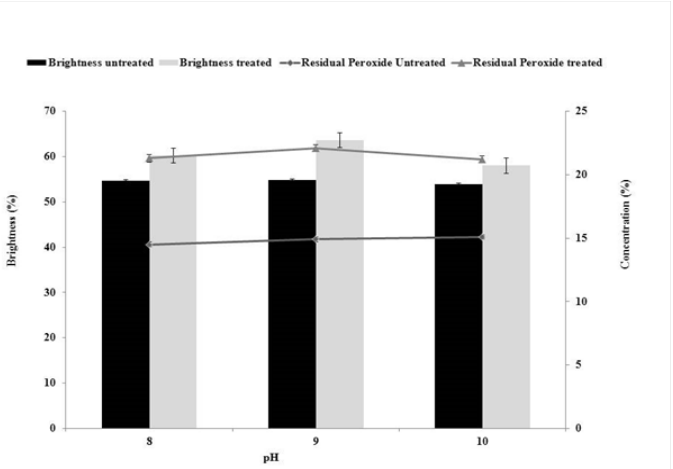
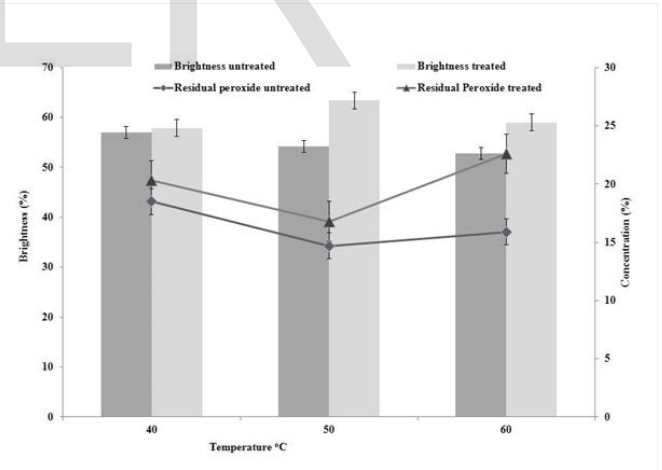


Figure. 2. Effect of pH of the pulp on enzyme treatment. Comparison of final brightness of untreated and treated pulp

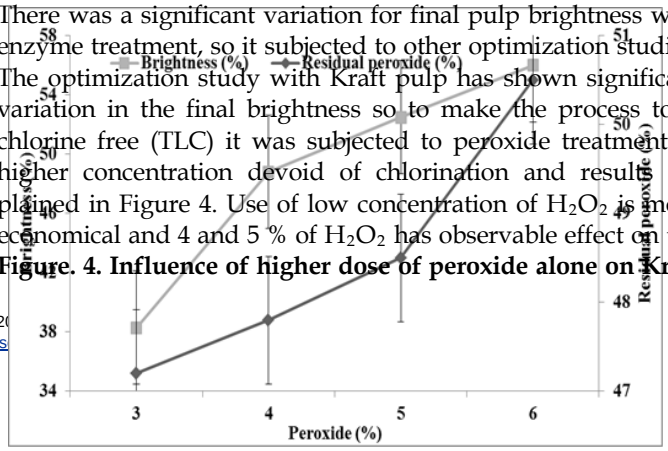
Figure.3.Effect of temperature of the pulp on enzyme treatment. Comparison of final brightness of untreated and treated pulp evaluated



3.2. Optimization of bleach sequence

There was a significant variation for final pulp brightness with enzyme treatment, so it subjected to other optimization studies. The optimization study with Kraft pulp has shown significant variation in the final brightness so to make the process total chlorine free (TLC) it was subjected to peroxide treatment of higher concentration devoid of chlorination and results explained in Figure 4. Use of low concentration of H_2O_2 is more economical and 4 and 5 % of H_2O_2 has observable effect on the

Figure. 4. Influence of higher dose of peroxide alone on Kraft



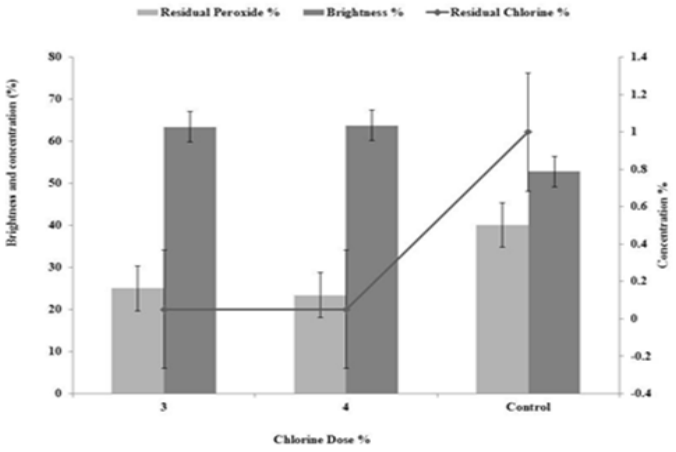
pulp bleaching. The brightness was checked without enzyme treatment and chlorination.

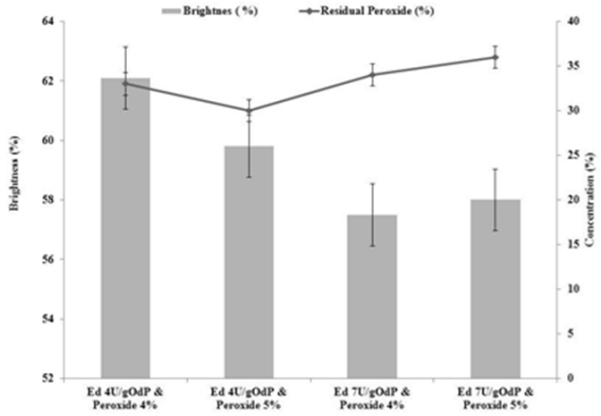
pulp brightness, so it was selected for further studies. Enzyme concentration (4 and 7 U g -1Odp) was then optimized for 4 and 5 % peroxide treatment and result given in Figure 5. The pulp treated with xylanase 4 U g-1 Odp has given a final brightness of 62.1 U with 4 % peroxide treatment. The pulp after enzyme treatment (4 U g –1 Odp) subjected to low dosage of chlorine (1 and 2 %) and peroxide treatment (4 and 5 %), the results given in Table. 1. The effluent was total chlorine free and traces of residual peroxide observed which shows that the enzyme treatment has helped in the complete utilization of chlorine on the pulp. The blank was given with 2 % chlorination and 4 % H_2O_2 treatment which was run parallel exhibited an ISO brightness of 54.2U. The final ISO brightness of the pulp was observed to be 62.1 and 62.5 for 1% chlorine treated followed by 4 and 5% H_2O_2 treatment where as the pulp, which was given 2%, chlorination exhibited 64.7 and 64.5 U ISO brightness.

Cl_2 Dose (%)	Peroxide dose 4%)			Peroxide dose (5%)		
	Res. Cl_2 (%)	Res. H_2O_2 (%)	Bht (%)	Res. Cl_2 (%)	Res. H_2O_2 (%)	Bht (%)
1	0	30.8	62.1	0	26.3	62.5
2	0	11.5	64.7	0	8.0	64.5
C (1&2)	1	42.0	54.2	1	42.0	53.2

Table. 1. Effect of higher dose of H_2O_2 (4 and 5 %) and low dose of ClO_2 (1 and 2 %) on kraft pulp samples treated with xylanase (4 U/g OdP). The left over chlorine and peroxide tabulated. C- control without enzyme treatment.

Figure.5. Effect of higher dose of peroxide (4 and 5 %) on Kraft pulp samples treated with different dose of xylanase (4 and 7 U/g OdP). Total chlorine free bleaching was performed, residual peroxide and final brightness of the pulp taken.





Higher concentration of chlorination (3 and 4 %) exhibited ginal increase in final brightness (Figure 6.) with marginal residual chlorine present. Higher concentration of ClO_2 with enzyme treatment in not economical in paper pulp processing.

Figure.6. Effect of higher dose of Chlorine dioxide (3 and 4 %) with 4 % peroxide treatment on Kraft pulp samples treated with xylanase (4 U/g OdP). The residual chlorine and peroxide tabulated.

To make the process more economical low concentration of enzyme, ClO_2 and peroxide selected and amount of alkali varied. The enzyme treated (4 U g -1 Odp) pulp subjected to Chlorine treatment (2%) followed by H_2O_2 (4%) and different NaOH charge (1N), result given in Figure. 7.

Suitable NaOH charge applied to avoid notable viscosity losses without significant lignin removal. The result show that the pulp treated with 30% OdP of 1 N NaOH exhibited higher final brightness of 65.9 U and followed by 28%, 65.1 and 64.4 and 63.2 U by 26 and 24% respectively.

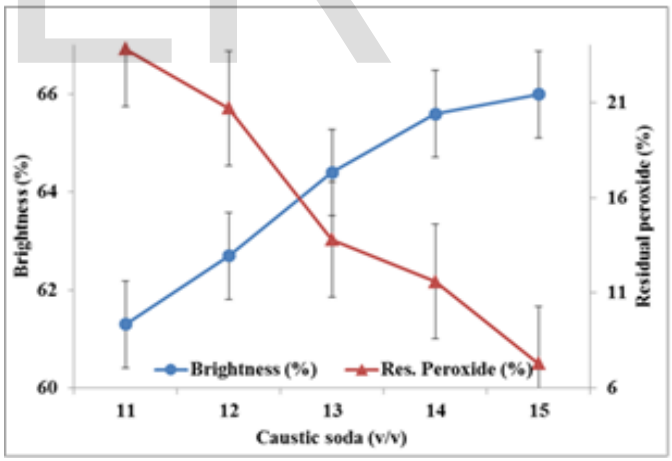


Figure.7. Effect of higher dose of Caustic soda with 4 % peroxide treatment on Kraft pulp samples treated with xylanase (4 U g -1 OdP) and chlorine dioxide (2 %).

The control exhibited a brightness of 57.4 U with low amount of chlorination and peroxide treatment. Difference related about 8.5U to control. In the effluent, there was low amount of residual peroxide where as residual chlorine absent. This shows that the treatment of enzyme prior to chlorination and peroxide treatment has made the fibers to swell and have effected in easy penetration of chemical to the interior.

3.3. Degree of solubilisation and Kappa No. reduction of pulp

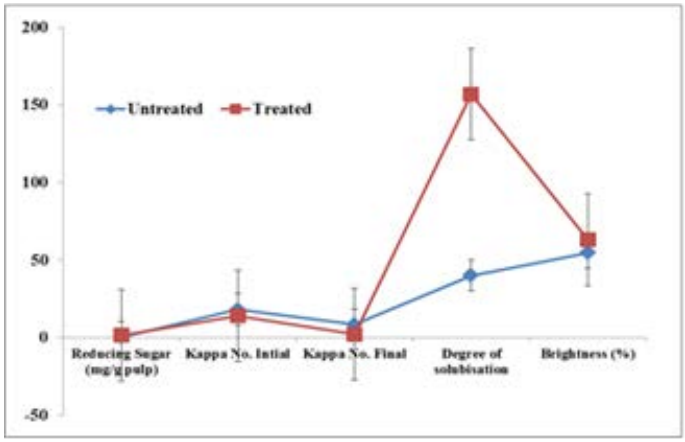


Figure. 8. Reducing sugar, Kappa No. (Initial and final-after treatment), Degree of solubilisation and brightness of untreated and xylanase treated (4U/g Od pulp) Kraft pulp.

The reducing sugar released measured using DNS method; the degree of solubilisation of pulp determined from the reducing sugar released and the kappa number as well as the brightness of optimized condition was explained in Figure. 8. The pulp treated with enzyme 4 U g -1 Odp, the kappa no. before and after treatment measured. The final Kappa number measured after the chlorination and alkali extraction. There is an observable change in the enzyme treated pulp than untreated pulp samples. The major factors affecting enzyme treatment efficiency includes pH, temperature, reaction time, enzyme concentration and dispersion consistency. The optimum pH for xylanase treatment varies among enzymes, generally enzyme from bacterial origin were effective at pH range 6 to 9 and fungal 4 to 6 and temperature ranges from 35 to 60 ^{o}C as well as optimum enzyme concentration ranges from 2 to 5 U g -1 of dry pulp.

3.4. Scanning electron micrograph (SEM)

In order to obtain a better under standing of the effect of xylanase treatment on enhanced bleachability, SEM used to evaluate the surface morphology of the pulps. The photomicrographs showed significant changes on the surface of xylanase treated pulps as result of xylan hydrolysis. SEM of chemical pulp (Figure 9). (a) untreated + ClO_2, (b) enzyme treated + ClO_2, (c) untreated + ClO_2 + H_2O_2, (d) enzyme treated + ClO_2 + H_2O_2). Fiber of treated pulp underwent a peeling processes giving rise to flakes and filaments of material detached from the fiber surface. There was no significant change on the surface of untreated it looks smooth than that of xylanase treated ones. This suggests that the enzyme not only assisted in surface modification but also penetrated pulp fibres allowing for a much improved xylan hydrolysis.

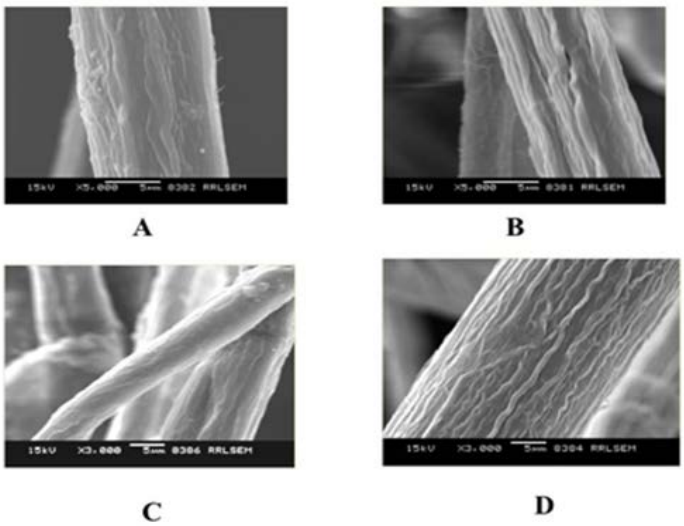


Figure. 9. SEM micrograph of fiber surface of Untreated and enzyme treated Kraft pulp (A) - untreated + ClO_2, (B)- enzyme treated + ClO_2, (C)-untreated + ClO_2 + H_2O_2, (D) - enzyme treated ClO_2 + H_2O_2.

4. Discussion

The effectiveness of xylanase treatments can be evaluated by two aspects: i). by determining the amount of sugars present after enzyme incubations, where 0.5 % to 1.0 % of the pulp carbohydrate content is liberated. ii). by observing increased bleachability with conventional methods after xylanase treatments (Viikari et al. 1993). The results shows that when there is an increase in enzyme concentration and time of treatment there is an enhancement in the amount of reducing sugar released from the brown pulp, similar observation made by the application of xylanase from A. fischeri Fxn 1 (Gunansekaran, 2001). Similarly, 5 g of eucalyptus pulp using 7 IU g -1 of xylanase from *B. circulans* AB 16 when incubated at 55 °C for 3 h resulted in the release of chromophore with an OD of 0.2414 at 465 nm (Dhillon et al. 2000). The UV absorption spectrum of the compounds released by enzyme treatment showed a characteristic peak at 280 nm, indicating the presence of lignin in the released colouring matter. Chromophore release by xylanase treatment is one of the significant factors of enzyme attack on the pulp. The enzyme when act upon the pulp help in the release of material that have absorbance at UV and visible region, which originates from the colour of the pulp. Reducing sugar, chromophore release and kappa number have been widely monitored to check the solubilisation of pulp following xylanase treatment (Shah et al. 1999, Jeffries, 1996).

The direct use of SSF enzymes in bleaching was a relatively new biobleaching approach and similar result of direct used of crude xylanase from *T. lanuginosus* strain ATCC 36350 and ATCC 46882 most efficiently used in biobleaching, resulted in improving brightness by 2.0 and 1.8 points respectively (Christopher et

al. 2005). There were reports related to the utilization of xylanase enzyme for the bleach boosting of baggase soda pulp (Bissoon et al. 2002) and wheat straw enriched soda pulp (pH 9.5 - 10) at 65°C (Li et al. 2005). Crude enzymes produced from *Penicillium A10 and Aspergillus L22* were used in the production of leached wheat straw with xylanase concentration of 4 IU/g prior to pulping, decreased pulp kappa number by 6.29% and 12.07% respectively as compared to the control (Zhao et al. 2006). Cellulase-free xylanase produced from *Arthrobacter* sp. TCC 5214 by SSF using wheat bran, which was thermo alkalophilic evaluated for prebleaching of kraft pulp that resulted 20 % reduction in kappa number of the pulp without much change in viscosity. It also reduced the amount of chlorine by 9 % without any decrease in brightness (Khandeparkar and Bhosle, 2007). The pulp consistency should be such as effective distribution of enzyme throughout the system. During alkaline digestion, modified carbohydrates deposited on the cellulose fibers imparting colour (Wong et al. 1997). When xylans break down xylooligomers, LCC formed which persist in the pulp, and later reprecipitate, and the brightness of the paper affected. All breakdown products are susceptible to potassium permagnate oxidation and easily removed by alkali extraction after enzyme treatment of pulp. Similar reports were there, Xylanase from Streptomyces thermoviolaceus active at 50°C, which reduced the kappa number and increased the brightness of kraft pulp in conventional CEDED bleach sequence at 4 % chlorine concentration (Garg et al. 1998). Earlier reports pertained to xylanase treatment at neutral or acidic pH, which made possible by adjusting the pH after alkaline extraction. Alkali stable xylanase have clear advantage in that there was no need of washing the pulp to reduce its pH. In this study, it was observed that pH- 8 as more effective in the final brightness. Therefore, adjustment pH of the pulp was not required. In case of *Bacillus* sp. Sam -3, a pH of 8.0 was suitable for solubilisation of pulp, it was also reported that increase in enzyme dosage 1.2 U/g pulp has effected in increased production of reducing sugar and lignin derivative compounds (LDC) (Shah et al. 1999). Xylanase from *A. japonicas* was reported as potential candidate for biobleaching and best result was obtained with 10U/g dry pulp for 3h resulted in 3.9 point decrease in kappa number compared to control 25.2% and brightness improved from 2.8, 2.2 and 3.1points (Guimarães et al. 2013). Xylanase (1027.65 U/g DBP) from *Cellulosimicrobium cellulans* CKMX1 was used for pretreatment of wheat straw pulp and 5U/g reported as effective which help in the decrease of kappa number by 1.4 points and reduced the chlorine charge by 12.5% and increase in final brightness by 1.42% (Walia et al. 2015). Beg et al. (2000) has reported xylanase from *Streptomyces* sp. QG- 11-3 (3.5 U g -1 of Odp) which support maximum bleach boosting effect at 50 °C and pH- 8.5 for a 2 h treatment. Endoxylanase from Thermotoga maritime cloned into *E. coli* was capable of releasing reducing sugar in the pH 5.0 - 10.0 with maximum at 5.0 (Shah et al. 2000). Pulp processing is generally carried out at high temperature so thermostable xylanase are more acceptable for bleaching process. Xylanase from *Bacillus* sp. Sam-3 solubilized pulp maximally at 60°C (Shah et al. 1999). Xylanase from *Thermomyces lanuginosus* produced by SSF was applied on bagasse pulp at pH 6.0; 60°C; 10 % pulp consistency for 3 h (Christopher et al. 2005). In biobleaching process, the xylanase act upon the left over xylan in the pulp and degrades it, which enhances the penetration of chemical bleaching agents with ease (Bajpai, 1999). Therefore, before the treatment of xylanase in the pulp bleaching sequence maximization of solubilisation achieved by optimization of enzyme concentration, pH and temperature. There are reports related to use of combination of enzyme for treatment of pulp which help to reduce the chemicals. When xylanase and laccase were used jointly, no improvement was detected; however, when the xylanase application preceded the laccase stage, the beneficial effects of laccase were boosted. Thus, the final XLEP bleached pulp showed a kappa number of 5.4 and a brightness of 60.5% ISO, although the H_2O_2 consumption increased (77.0% vs. 64.5% and 73.8% for EP and LEP respectively). Finally, after subjecting the bleached pulps to accelerated ageing, the best optical properties were observed in the XLEP pulp (Martín et al. 2012). Torres et al. (2000) has also reported similar results of the effect of xylanase on ECF bleaching of oxygen delignified Eucalytus Kraft pulps. Xylanase treatment observed to be effective in the opening the closed cell - wall - pores of Kraft pulps.

Changes in fiber morphology such as cracks, flakes filaments and peeling observed during the bleaching due to enzyme treatment. These crakes facilitate the diffusion out the fiber cell wall of the larger lignin macromolecules (Torres et al. 2000). Thus, the xylanase enzyme treatment improves the accessibility of pulps for the bleaching chemicals. It decreases the diffusion resistances to the outward movement of degraded lignin fragments and allows the removal of the less degraded lignin fragments from the fiber wall. This was the reason for the decreased kappa number and higher brightness of the enzyme treated pulps at the same bleaching reagent consumption (Torres et al. 2000). Unbleached bagasse soda pulp was pretreated with a purified xylanase (150 U g-1) from *Thermomyces lanuginosus* SSBP, exhibited direct brightening abilities on bagasse pulp improving brightness by 1.5 points, however, kappa number decreased by 0.5 points SEM revealed significant surface modification of bagasse pulp fibres without marked fibre disruptions (Bissoon et al. 2002).

5. Conclusions

Biobleaching process needs xylanases that are active at high temperature and pH. *Bacillus pumilus* produced a xylanase with high temperature and pH optima, thereby suggesting its potential in biobleaching processes. When crude enzyme extract used for the biobleaching process, reduction in kappa number of the Kraft pulp without much change in viscosity was observed. Complete utilization of chlorine as well as peroxide was the significant observation made during the bleaching process. Moreover, the enzyme treatment enhanced the brightness of the pulp by 8.5 units compared to the untreated pulp. This suggests that xylanase could be useful in the paper and pulp industry as it produced better quality Kraft pulp than that obtained with xylanase produced by other bacterial cultures.

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