# Enzymes improve the bleachability of bagasse mechanical pulp

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## ABSTRACT

*Two crude enzyme preparations were tested for their potential to enhance the bleachability of bagasse mechanical pulp. Enzyme preparations were obtained from fungi growing on wet bagasse in bulk storage. Pulp brightness after bleaching with hydrogen peroxide was 2–3 points higher for the enzyme-treated pulp than for an untreated control pulp. Strength properties of the enzyme-treated pulps were comparable with the control. Various chemical pretreatments were tested in an effort to enhance the enzyme treatment. Conventional chemicals had little effect, but a two-stage enzyme treatment—xylanase followed by treatment with one of the tested enzymes—boosted the bleached brightness by an additional 1.3 points.*

***Application:***
*Mills can use enzyme-bleaching technology to enhance the brightness ceiling of mechanical pulp.*


THE APPLICATION OF BIOTECHnology to improve pulp and paper manufacturing processes and products is gaining global attention. The use of enzymes—particularly in the area of debarking, pulping, bleaching, pitch control, pulp drainage, and deinking—offers far-reaching benefits *(1)*. Among the various enzymes relevant to the paper industry, the hemicellulolytic xylanases have been found to be commercially feasible for pulp bleaching *(2)*. Xylanase pretreatment facilitates chemical extraction of lignin from pulp, significantly reducing consumption of bleaching chemicals and the discharge of toxic compounds into the environment. Interest in xylanases continues to grow as potential pulp and papermaking applications are identified *(3)*.

Enzymes are widely used in a pretreatment stage to improve pulp bleaching *(4)*; but, so far, such pretreatments have been applied only to kraft pulps *(5)*. Enzymatic pretreatment of mechanical pulps has received little attention. The major enzymes implicated in lignin degradation of mechanical pulps are lignin peroxidase, manganese peroxide, and laccase *(6)*. The precise role of these enzymes, however, remains controversial.

Mechanical pulps are high-yield pulps used for newsprint furnish because of their high bulk, high light-scattering coefficient, and high ink receptivity. The final brightness of the newsprint sheet is influenced by the brightness of the mechanical component in the furnish, which in turn depends on the original brightness of the raw material. In this context, wood mechanical pulps possess high brightness and are very well suited for newsprint production. This is not the case for bagasse mechanical pulps.

Tamil Nadu Newsprint and Papers Limited (TNPL) is a large integrated bagasse-based plant producing 50,000 metric tons of newsprint and 40,000 metric tons of uncoated printing and writing paper annually. TNPL's newsprint contains mechanical pulp produced from bagasse, and the mill is the first in the world to produce newsprint with bagasse mechanical pulp. Unlike conventional wood species, bagasse has several inherent drawbacks, the most prominent of them being that it suffers rapid discoloration during storage, resulting in a mechanical pulp with a low initial brightness. To improve the bleachability of the bagasse mechanical pulps and to meet the newsprint target brightness, it would be desirable to have a low-cost pretreatment prior to bleaching with hydrogen peroxide.

This study investigated the application of two crude enzyme preparations obtained from fungal strains isolated from eight-month-old, wet, bulk-stored bagasse. Twelve different fungal strains were screened initially to determine the best isolate for use in biomechanical bleaching of bagasse. Subsequently, systematic studies were carried out on two strains that were found to be particularly effective in enhancing pulp brightness.

## RESULTS AND DISCUSSION

### *Enzyme pretreatment with lignase enzymes*

Previous research has mostly focused on enzyme treatment as a means of improving the bleachability of chemical pulps. Very little work has been done on mechanical pulps. Other studies have examined the use of enzymes (obtained from selected strains of white-rot fungi) to pretreat wood chips as a means of improving the quality of mechanical pulps and reducing the energy required to fiberize the pulp during refining. Such pretreatments have ultimately reduced pulp brightness and light-scattering coefficient *(7)*.

The present study focuses on enzyme pretreatment of bagasse mechanical pulp prior to bleaching. Bagasse mechanical pulps were treated with enzyme preparations

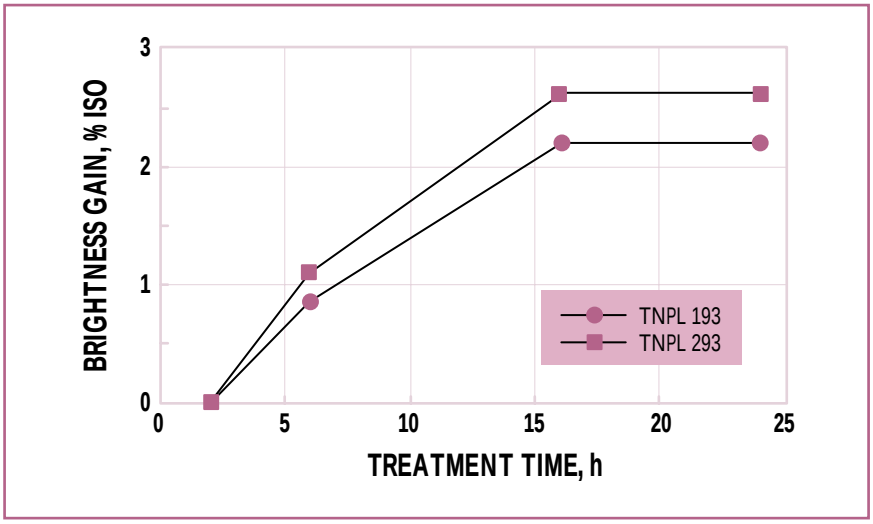
| Enzyme [b] | Brightness, % ISO mean [c] ± std. dev. |
|---|---|
| Control | 47.2 ± 0.01 |
| 1 | 47.8 ± 0.10 |
| 2 | 47.9 ± 0.12 |
| 3 | 45.5 ± 0.08 |
| 4 | 46.5 ± 0.09 |
| 5 | 43.5 ± 0.12 |
| 6 | 48.0 ± 0.12 |
| 7 | 48.0 ± 0.11 |
| 8 | 45.5 ± 0.09 |
| 9 | 48.0 ± 0.13 |
| 10 | 43.5 ± 0.08 |
| 11 (TNPL 193) | 49.5 ± 0.12 |
| 12 (TNPL 293) | 50.0 ± 0.11 |

[a] Treatment conditions: enzyme charge 2 mL/g o.d. pulp, 10% pulp consistency, 16-h treatment time at 30°C, pH 4.0. Bleaching conditions: $H_2O_2$ charge 1.5% on o.d. pulp, 10% pulp consistency, 2-h reaction time at 60°C.
[b] Enzymes derived from various white-rot fungal cultures isolated from wet bagasse after eight months storage
[c] Based on five replicates

***I. Brightness obtained with different crude ligninase enzymes after bleaching with hydrogen peroxide*** [a]

| | Ligninase | Cellulase | Hemi-cellulase |
|---|---|---|---|
| TNPL 193 | 5.0 | 0.5 | 1.2 |
| TNPL 293 | 8.0 | 0.2 | 3.0 |

***II. Activities in enzyme preparations, units/mL***




***1. Brightness improvement as a function of treatment time for two enzyme preparations***

obtained from various white-rot fungal cultures isolated from a bagasse storage pile. Pulp pH was maintained at 4.0 during treatment, which involved incubation at 30°C for 16 h. After the enzyme treatment, pulps were bleached with hydrogen peroxide.

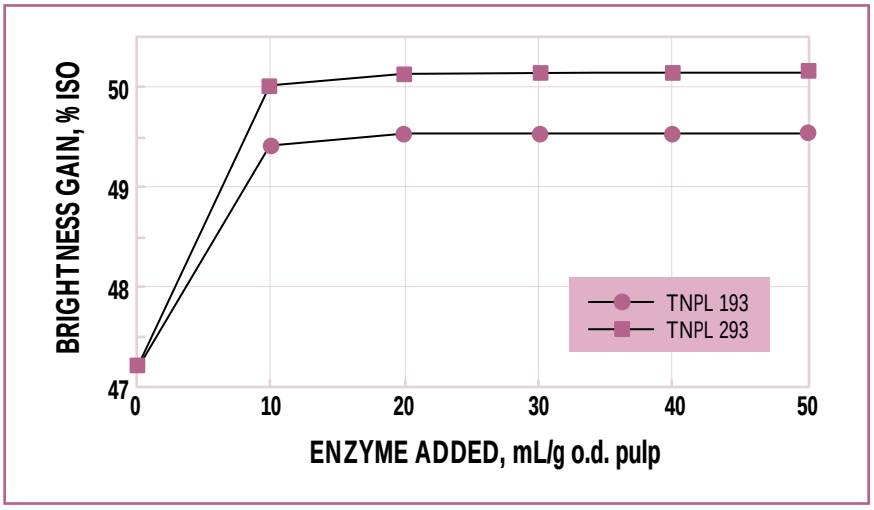
Brightnesses obtained after treatment with 12 different crude enzyme preparations are given in Table I. Brightness for several of the enzyme-treated pulps was significantly higher than that of the control pulp, indicating that bagasse mechanical pulp can be activated by enzymes. Two of the crude enzyme preparations—fungi 11 and 12 in Table I—showed the greatest potential as brightness enhancers. Consequently, further studies were confined to these two enzyme preparations, which are designated as fungal strains TNPL 193 and TNPL 293, respectively. The specific enzyme activities for these two preparations are listed in Table II.

*Pretreatment time.* The effectiveness of biological pretreatments on pulp greatly depends on the prior pulping processes. In the case of mechanical pulps, considerable portions of the fiber are completely inaccessible to enzymes, thus reducing the efficiency of enzymatic treatment. Consequently, an experiment was conducted to determine the pretreatment time required to achieve maximum brightness.

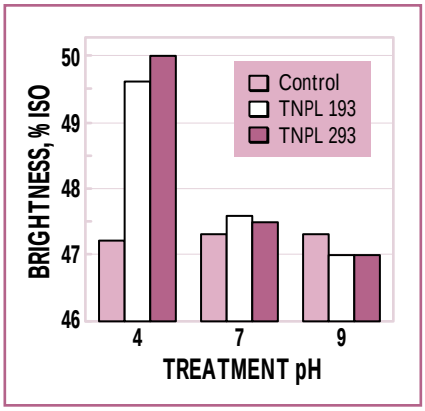
Bagasse mechanical pulp was treated with TNPL 193 and TNPL 293 crude enzyme preparations at a charge of 2 mL/g ovendry (o.d.) pulp, 30°C, and pH 4.0 for 2 h, 6 h, 16 h, and 24 h. After the treatment period, enzyme-treated pulps were bleached with hydrogen peroxide. The results in Fig. 1 show that a 16-h pretreatment with enzyme was required to obtain sufficient gains in pulp brightness. A further increase in time did not improve pulp brightness. Thus the pretreatment time for the mechanical pulps was fixed at 16 h for the laboratory studies. Chemical pulps respond to enzymatic pretreatment in less time *(8)*.

*Enzyme charge.* Experiments also were conducted to determine the optimum dosage of crude enzyme preparations required to attain maximum brightness. Unbleached ba-gasse mechanical pulp was treated with different dosages (2–10 mL/g o.d. pulp) of the TNPL 193 and TNPL 293 enzyme preparations. The pulps were treated (16 h at 30°C and pH 4.0) and subsequently bleached with peroxide. The results in Fig. 2 show that the brightness enhancement for both enzymes levels off at a charge of 2 mL/g, beyond which there is no appreciable brightness gain.

*Treatment pH* Xylanase enzymes typically operate most efficiently in an acidic environment *(9, 10)*. The recommended pH for enzymatic pretreatments of chemical pulps with xylanase enzymes is 4.0–6.0. However, the precise pH required for pretreatment of mechanical pulps is largely unknown. Therefore, the opti-




*2. Brightness as a function of enzyme charge for two enzyme preparations*




*3. Brightness improvement as a function of treatment pH for enzyme-treated pulps and untreated control pulp*

mum pH required to achieve maximum efficiency in these experiments was determined for each of the crude enzyme preparations. The unbleached bagasse mechanical pulps were treated with TNPL 193 and TNPL 293 enzyme preparations at different pH levels (4.0, 7.0, and 9.0) for 16 h and then bleached with hydrogen peroxide. The effect of treatment pH with respect to the final bleached brightness is presented in Fig. 3. The results show that both enzymes are efficient at the acidic pH of 4.0, but at higher pH values, the effect of the enzymes decreases sharply.

***Enzyme pretreatment followed by peroxide bleaching***

Based on the previous experiments, bagasse mechanical pulps were subjected to enzymatic pretreatment with TNPL 193 and TNPL 293 under optimized conditions for pH (4.0), enzyme charge (2 mL/g o.d. pulp), and pretreatment time (16 h). The results, after peroxide bleaching, are given in Table III, which shows that the enzyme-treated pulps were brighter than the control pulp. The brightness increase was 2.3 points for TNPL 193 and 2.7 points for TNPL 293.

Bagasse mechanical pulp has a lower initial brightness than hardwood and softwood pulps, so its bleaching response to hydrogen peroxide is also lower. The low brightness of unbleached bagasse pulp can be attributed to the low brightness of raw bagasse, which discolors during storage *(11)*. Thus the brightness gains of 2.3 points (47.2% to 49.5% ISO) and 2.7 points (47.3% to 50.0% ISO) over the untreated control are quite significant for a mechanical bagasse pulp.

The brightness of the bagasse newsprint furnish is dictated by the brightness of its mechanical bagasse component *(12)*, so any increase in the brightness of the mechanical pulp contributes directly to the quality of the newsprint. Thus further efforts were made to increase the brightness gain by applying additional chemical pretreatments prior to enzymatic pretreatment.

***Pretreatments to enhance pulp susceptibility to enzyme activity***

*Xylanase* Bagasse mechanical pulp was pretreated with crude TNPL enzyme preparations for 2 h at 50°C and pH 4.0. This xylanase-pretreated pulp was then treated with the TNPL 193 enzyme preparation under the stated optimum conditions and finally bleached with hydrogen peroxide. The results in Table IV show that the two-stage enzyme pretreatment provided an additional improvement of 1.3 points (49.5% to 50.8% ISO).

Arbeloa *et al. (13)* attributed an increase in pulp brightness after treatment with lignin peroxidase or laccase to lignin modification. Laccase enzymes, which are similar to lignin peroxidase, oxidize water-soluble lignin model compounds. However, the bulk of insoluble substrate lignin is not accessible to the active sites of an enzyme in the heterogeneous phase *(14)*. Ruel, Odier, and Joseleau *(15)* have shown that the hemicelluloses prevent the penetration of lignin peroxidase into the wood and pulp fibers. Given the increased brightness after the two-stage enzyme treatment, it appears that the first-stage xylanase pretreatment modified the bagasse mechanical pulp, making it more susceptible to the subsequent treatment with TNPL 193 enzyme.

*Conventional chemicals* The lignin in lignocellulosic materials can be activated by various chemical treatments. However, enzymatic action is hampered by lignin-covering polysaccharides in wood *(16)*, so biological, physical, and/or chemical pretreatments are required to enhance the enzymatic degradation of lignin. In the case of bagasse pulp, the fiber matrix is particularly inaccessible to enzymes. Consequently, several chemical pretreatments were evaluated to see if they enhanced the performance of the subsequent enzyme pretreatment.

Unbleached bagasse pulp was treated with a variety of chemicals, such as peroxide in acidic medium, calcium hypochlorite, sodium sulfite, sodium hydroxide, and sulfuric acid. The chemically treated pulps were

| | TNPL 193 | | TNPL 293 | |
|---|---|---|---|---|
| | Control | Treated | Control | Treated |
| Peroxide added, % on o.d. pulp | 1.5 | 1.5 | 1.5 | 1.5 |
| Peroxide consumed, % on o.d. pulp | 0.98 | 1.06 | 0.93 | 0.79 |
| Brightness, % ISO | 47.2 | 49.5 | 47.3 | 50 |
| Brightness gain, % ISO | … | 2.3 | … | 2.7 |

*Treatment conditions: enzyme charge 2 mL/g o.d. pulp, 10% pulp consistency, 16-h treatment time at 30°C, pH 4.0. Bleaching conditions: 10% pulp consistency, 2-h reaction time at 60°C.

***III. Effect of TNPL 193 and TNPL 293 enzyme treatment on brightness of bleached bagasse mechanical pulp****

then treated with enzyme and bleached in hydrogen peroxide. Table V shows the conditions maintained during the chemical treatments along with the brightnesses achieved after bleaching. The results show that none of the chemical pretreatments substantially improved the performance of the enzyme treatment. Indeed, the acid peroxide and the alkali pretreatments had a negative effect on pulp brightness. Earlier work has shown the same trend with other mechanical pulps *(17)*.

### *Comparison of white-rot fungus and TNPL cultures*

An enzyme preparation isolated from the white-rot fungus *Coriolus versicolor*, which is known to produce ligninases, was applied to bagasse mechanical pulp prior to peroxide bleaching. The efficiency of this preparation was then evaluated by comparing the results with those obtained with the TNPL crude enzyme preparations. While *C. versicolor* is reported to improve the bleachability of kraft pulps *(18)*, it produced only a marginal gain in brightness (0.9 point) for the bagasse mechanical pulp. The TNPL enzyme preparations were much more effective, increasing brightness by 2–3 points over the control.

### *Effect of enzyme pretreatment on bleached pulp quality*

The quality of the mechanical pulp before and after enzyme pretreatment was assessed. The fiber-classification results in Table VI indicate that there is no significant change in the fiber fractions across the treatments. Similarly, the strength properties given in Table VII show a slight decrease in strength without any change in light-scattering power. To date, studies of fungal–enzymatic treatments in mechanical pulps have resulted in either improvement of strength properties with a decrease in the light-scattering coefficient or vice versa. However, both ligninase enzymes of TNPL 193 and TNPL 293 improved pulp brightness without altering other properties.

| | | ENZYME TREATMENT | |
|---|---|---|---|
| | CONTROL | Xylanase (stage 1)[a] | Enzyme TNPL 193 (stage 2)[b] |
| Peroxide added, % | 1.5 | 1.5 | 1.5 |
| Peroxide consumed, % | 0.98 | 1.06 | 0.94 |
| Brightness, % ISO | 47.2 | 49.5 | 50.8 |
| Brightness gain, % ISO | … | 2.3 | 3.6 |

[a] Stage 1 pretreatment conditions: xylanase charge 1 U/g (2 mL/g) o.d. pulp, 10% pulp consistency, 2-h treatment time at 50°C, pH 4.0
[b] Stage 2 treatment conditions: enzyme TNPL 193 and 293 charge 2 mL/g o.d. pulp, 10% pulp consistency, 16-h treatment time at 30°C, pH 4.0. Bleaching conditions: 10% pulp consistency, 2-h reaction time at 60°C.

***IV. Effect of two-stage enzyme pretreatment—xylanase followed by ligninase—on brightness of bleached bagasse mechanical pulp***

## CONCLUSIONS

The crude enzyme preparations obtained from two fungal strains, TNPL 193 and TNPL 293, improved the bleachability of bagasse mechanical pulp. Enzymatic pretreatment followed by peroxide bleaching improved the brightness gain by 2–3 points over the untreated control.

Xylanase pretreatment, followed by treatment with the TNPL crude enzyme preparation, enhanced the brightness gain from 2.5 to 4.0 points. Other chemical pretreatments did not improve the bleachability.

Pulp quality and yield were not affected by enzymatic pretreatment with either TNPL 193 or TNPL 293.

## METHODS AND MATERIALS

### *Fungal strains: maintenance and culture conditions*

Twelve cultures were initially isolated from eight-month-old, wet, bulk-stored bagasse. The individual cultures were all identified as white-

| | Acid peroxide | Calcium hypo-chlorite | Sodium sulfite | Sodium hydroxide | Sulfuric acid |
|---|---|---|---|---|---|
| Chemical application, % on o.d. pulp | 1 | 10 | 5 | 2 | 1 |
| pH | 5 | 12 | 6.5 | 11.5 | 1.5 |
| Temperature, °C | 85 | Ambient | 80 | 60 | 60 |
| Time, min | 120 | 60 | 60 | 120 | 120 |
| Final brightness after peroxide bleaching, % ISO | | | | | |
| Control | 46.3 | 55.1 | 47.3 | 47.1 | 48.5 |
| Enzyme treated* | 44.2 | 56.1 | 45.6 | 46.6 | 48.7 |

*TNPL 293

***V. Effect of chemical pretreatment prior to enzyme treatment on brightness of bleached bagasse mechanical pulp***

| | ENZYME TREATMENT | | |
|---|---|---|---|
| | CONTROL | TNPL 193 | TNPL 293 |
| Freeness, mL CSF | 200 | 225 | 225 |
| Fiber fraction,* wt.% | | | |
| R30 (long fiber) | 13.7 | 14.7 | 14.3 |
| R50 | 25.8 | 25.1 | 25.0 |
| R100 | 24.9 | 24.6 | 24.5 |
| R200 | 7.9 | 8.6 | 11.3 |
| P200 (fines) | 27.7 | 27.0 | 24.9 |

*Fiber fractions obtained by retention on (R) or passage through (P) screens of various mesh in a fiber-length classifier

***VI. Fiber classification after enzyme treatment***

rot fungi and cultured by standard aseptic methods, i.e., by plating on agar media using serial dilution techniques *(19)*. Thereafter, pure cultures were maintained on potato dextrose agar slants at 30°C with subculturing every 3–4 weeks *(20)*. The isolates were grown on sterilized liquid culture medium comprising 1% glucose, 0.3% yeast extract, 0.3% malt extract, and 0.5% peptone. The medium was adjusted to pH 7.0, and 100 ppm of streptomycin sulfate was added to inhibit bacterial contamination. The culture medium was sterilized in an autoclave at 1.1 kg/cm$^2$ for 20 min.

A 100-mL volume of culture was put in a 500-mL baffled Erlenmeyer flask, and the flask was inoculated with an actively growing 4–5-day-old colony of the fungus. The flasks were incubated at 30°C for seven days under stationary conditions. The crude culture filtrates were extracted by filtering the mycelium through sterilized cotton, and the enzyme preparations were preserved in stoppered amber bottles at 4°C.

***Mechanical pulp***

Unbleached bagasse mechanical pulp was collected prior to entering the bleach plant. A uniform pulp was made by blending all the pulp and dewatering to 25% consistency (w/w). Pulp freeness was 180 mL CSF, and pulp brightness was 47.3% ISO.

***Enzyme pretreatment of pulp***

Enzyme treatments were carried out on 100 g ovendry (o.d.) unbleached bagasse mechanical pulp as obtained from the TNPL mill. Pulp pH was adjusted to 4.0 by addition of 4*N* $H_2SO_4$. The enzymatic treatment was carried out in polyethylene bags at a consistency of 10% (w/w). The enzyme reaction was performed at 30°C for 16 h. The pulp and enzyme were thoroughly kneaded manually. Enzyme dosage was defined as milliliters of crude enzyme preparation per gram of pulp (mL/g). The specific enzyme activities are given in Table II. After the treatment, the pulp was washed with demineralized water. "Control" refers to the pulps processed in a manner similar to the enzymatic treatments, except that no enzyme addition was made.

***Bleaching experiments***

Hydrogen peroxide was used to bleach the bagasse mechanical pulps. Exploratory bleaching experiments were performed with 10 g o.d. pulp, while control runs were performed with 100 g o.d. pulp. The alkaline hydrogen peroxide bleaching studies were carried out by thoroughly mixing the pulp in a Hobart mixer at 10% consistency (w/w) with the following: 1.5% $H_2O_2$, 5.0% sodium silicate, 0.05% $MgSO_4$, and 2.0% NaOH (all based on o.d. pulp). The pulp was then placed in a polyethylene bag and kept at 60°C for 2 h. Finally, the pulp was dispersed in water at 1% consistency (w/w), and the pH was adjusted to 5.0 with 4*N* $H_2SO_4$ prior to brightness measurements.

***Peroxide residual***

Bleach filtrate (25 mL) was combined with 20 mL of 4*N* $H_2SO_4$. Two drops of saturated ammonium molybdate solution were then added, followed by 10 mL of 10% potassium iodide. The liberated iodine was titrated against 0.025*N*

KEYWORDS
*Mechanical pulps, hydrogen peroxide, enzymes, xylanase, bleachability, bagasse, hemicellulase, pretreatment, newsprint, brightness, white rot fungi, enzymatic activity, peroxide bleaching, chemical treatment.*

| | ENZYME TREATMENT | | |
|---|---|---|---|
| | CONTROL | TNPL 193 | TNPL 293 |
| Freeness, mL CSF | 200 | 225 | 225 |
| Bulk, $cm^3/g$ | 2.61 | 2.63 | 2.69 |
| Tensile index, N·m/g | 31.08 | 28.92 | 26.56 |
| Tear index, $mN·m^2/g$ | 3.9 | 3.65 | 3.65 |
| Burst index, $kPa·m^2/g$ | 1.26 | 1.08 | 1.09 |
| Light-scattering coefficient, $m^2/kg$ | 45.2 | 45.8 | 45.0 |

***VII. Strength properties of control and enzyme-treated bagasse mechanical pulps***

sodium thiosulfate using a starch indicator.

***Brightness***

After acidification to pH 5.0 with 4*N* $H_2SO_4$, 3 g pulp was diluted to 2% consistency (w/w). Brightness pads were made after adjusting the pH to exactly 5.0. The pads were pressed at 4 $kg/cm^2$ in a laboratory sheet press and dried. Brightness was measured using an Elrepho brightness tester at 457 nm (TAPPI T 452 om-92).

***Light-scattering coefficient***

The pulp's light-scattering coefficient was measured on the same brightness pad using an Elrepho brightness tester at 575 nm (TAPPI T 220 om-88).

***Pulp evaluation***

Pulp freeness was determined according to TAPPI T 227 om-1992. Strength properties such as burst (TAPPI T 403 om-91), tensile (TAPPI T 404 cm-92), tear (TAPPI T 496 cm-85), and fiber classification (TAPPI T 233 cm-82) of the pulps were determined in accordance with TAPPI test methods. TJ

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