# Biobleaching of bagasse pulp with xylanase enzymes and hydrogen peroxide

**Saeed Soltanali, Yaser Ziaie-Shirkolaee***

*Department of Chemical Engineering, Faculty of Engineering, Tehran University, P.O Box 11365-4563, Tehran, I.R. Iran*

***Abstract***
The effect of the operating conditions (temperature, time, pH, enzyme level and pulp consistency) used in the enzymatic step of a XP (Cartazyme-hydrogen peroxide) sequence for bleaching Kraft pulp from bagasse on various properties of the resulting pulp and paper sheet products was studied. The quality of bagasse used, was examined by its yield, brightness,viscosity and the Kappa number. Finally, the quality of paper sheets produced were examined by their brightness, breaking length, burst index and tear index. The total number of experiments required for five independent variables was calculated from $N=2^k+2k+1$ equation, in which k is the number of independent variables. The results of 43 experiments performed, were processed using the MINITAB software suite which provided equations that reproduced the values of the dependent variables with less error. The application of the steepest ascent method has been carefully inserted in the experimental design section the identification of the most suitable conditions for optimizing the values of the dependent variables. Based on the results, using enzyme level 6 (IU/g) , temperature 35°C, pH 5 and pulp consistency 12% for 2 hrs. in the enzymatic step provided paper sheets of acceptable quality.
***Keywords***: Xylanase; bagasse; hydrogen peroxide; biobleaching; Central Composite Design (CCD).

## INTRODUCTION

Bagasse is an important source of raw material for pulp and paper manufacture in the countries that produce large amounts of this type of waste and have scantly wood resources. Iran is one of these countries which have produced about 4.1 million tones of sugar cane annually, during the resent past years (FAO, 2004). An estimation based on a planted area of 41000 ha, and a production rate of dry sugar cane biomass of 89 ton/ha.year (15 ton/ha dry bagasse), shows that Iran sugar cane industry produced approximately 615000 ton of air dry lignocellulosic biomass in 2003 (Rezayati-Charani *et al*., 2006).

Plants as raw materials of pulp-making are usually cooked at high temperature and pressure in the conventional pulping process. There are major problems associated with this process such as energy consumed and large amount of chemicals leading to environmental pollution (Hongzhang *et al.,* 2002). Therefore, the need to decrease pollution from cellulose pulp mills has promoted the search for alternative bleaching and especially ozone, hydrogen peroxide and biobleaching processes (Jiménez *et al*., 1999; Roncero *et al.,* 2003; Khristova *et al.,* 2006).

The biobleaching process is based on the action of the microorganisms and/or enzymes. Microbial xylanases that are thermostable and cellulose-free are generally preferred for biobleaching of paper pulp. The interest for xylan degrading enzyme and its applications in the pulp and paper industries has advanced significantly during past few years (Adachi and Chen, 2007; Han *et al.,* 2007; Rajasekar, 2007; Shen Liu, 2007; Bajpai *et al.,* 1994; Garg *et al.,* 1998; Christov *et al.,* 1999; Srinivasan and Rele, 1999). In this process, the bond between lignin and hemicelluloses is primarily between lignin and xylan which can be removed by xylanase. Once this layer of hemicellulose is removed, the lignin layer is easily available for degradative action of the ligninolytic enzymes (Eriksson, 1993). However, the cellulases produced with xylanases in the microbial based processes simultaneously, alter cellulose fibers and diminish pulp

*Correspondence to: **Yaser Ziaie-Shirkolaee,** Ph.D.
*Tel: +98 9173376809; Fax: +98 21 66957781*
*E-mail: ya_ziaee@yahoo.com*

quality. Therefore, xylanases with high degree of purity are required (Jiménez *et al.,* 1997). The studies on xylanase bleaching of hardwood and softwood pulps have received much attention, but studies on bagasse are few (Neeta and Mala, 2002; Charin *et al.,* 2003; Sandrim *et al.,* 2005). Prior to this research, the bagasse biodegradation with commercial xylanase enzyme Cartazyme HS (Sandoz) was investigated by Denise and his colleague (Denise and Adilson, 2002); but the effect of operation variables in enzymatic pulp bleaching was not published. The main operational variables in enzymatic pulp bleaching processes are temperature, time, pH, enzyme level and pulp consistency. Danault examined the operating conditions used by several authors and found enzyme concentration ranging from 1 to 15 IU/g dry pulp, temperature from 40 to 70°C, time from 0.5 to 24 hrs., pH from 3.5 to 8, and consistency from 2.5 to 12% (Daneault *et al.,* 1994). However, each enzyme processes specific catalytic properties and requires special operating conditions, so no generalization can be made in this respect.

In few studies on operational variables of the pulp biobleaching proess, a factorial design has been used to develop empirical models. These models are involved in several independent variables regarding performance examining and predicting pulp properties and paper sheets in terms of the conditions used in the enzymic step (Allison and Clark, 1994; Abdul-Karimand and Rab, 1995; Ziaie-Shirkolaee *et al.,* 2007a,b). In this work, a main composition design was used to study the influence of bleaching variables in bagasse with the xylanase and peroxide hydrogen on different obtained properties such as yield, viscosity, brightness, breaking length, bear index and tear index.

## MATERIALS AND METHODS

**Raw material** : The bagasse used in this study, obtained from the local sugar field in central area of Iran. Before pulping, the raw material was cleaned, cut to pieces, approximately 3cm length and sun-dried. The chemical composition of bagasse was determined as follows: 50.33% cellulose, 20.29% lignin, 74.27% holocellulose, 46.05% α-cellulose, 7.07% ash and 1.57% ethanol/ dichloromethane extractable, on an oven-dry weight basis (moisture content 9.8%). Deviations of these contents from their respective means were less than 10%.

**Analysis of raw material, pulp and paper sheets:** Analysis of raw material and pulp made according to Tappi Standard Methods (TAPPI, 2002) with the exception of hemicellulose that determined by decreasing of cellulose content from holocellulose (holocellulose determined by Wise's sodium chlorite method) (Wise and Murphy, 1946), cellulose according to Kurscher and Hoffner's nitric acid method (Rowell, 1984) and viscosity of pulp was measured in cupri-ethylenediamin (CED) solution according to SCAN-CM 15:88 standard (SCAN, 1998).

Pulp yield was determined gravimetrically following drying at 105°C ± 2 for 24 h.

**Pulping:** Pulps were made in a 21-l batch cylindrical mini digester (stainless steel 321), that described by Ziaie-Shirkolaee *et al.* (Ziaie-Shirkolaee *et al.,* 2007a,b). Wheat straw was cooked in the reactor, using the following conditions: temperature 160°C, time 80min, sulfidity 20%, Active alkali $NaO_2$ 14% and solid/liquor ratio, 1/10 (d. w.).

**Bleaching process:** The pulp was bleached using an XP sequence. The enzymatic treatment, based on the enzyme Cartazyme HS (Sandoz), was carried out in the transparent plastic bags containing the pulp, under different conditions.

For hydrogen peroxide treatment, enzyme-treated pulp was placed with $H_2O_2$ at 70°C for 3 hrs. In addition, in order to reduce the role of transition metals on alkaline decomposition of peroxide and also, to limit the degradation and deterioration of carbohydrates, the materials such as EDTA (diethylene-diamine-tetra-acetic acid), Epson salt (magnesium sulfate) and DTPA (diethylene-triamine-penta-acetic acid) are used in bleaching process (Table 1). Finally, after each bleaching stage, the pulp was washed by deionized water.

**Experimental design:** The tested model uses a series of points (experiments) around a central one (central experiment), and several additional points (additional experiments), to estimate the first- and second-order interaction terms of a polynomial. This design needs the general requirement that each parameter in the mathematical model can be estimated from a fairly small number of experiments (Montgomery, 1991).

The total number of observations (experiments) required for the five independent variables (viz. temperature -T-, cooking time -t-, pH – pH-, enzyme level -D- and pulp consistency –C-) was calculated from the following equation (Akhnazarovaand and Kafarov, 1982):

$$N=2^k + 2k + 1 \quad (1)$$

and found to be 43. In this equation, k is the number of independent variables.

In this work, all independent variables (temperature, time, pH, enzyme level and pulp consistency) normalized from −1 to +1 according to following formula (Rodríguez *et al.,* 1998):

$$X_n = 2\frac{X - X_{mean}}{X_{\max} - X_{\min}} \quad (2)$$

This normalization also results in more accurate estimates of the regression coefficients as it reduces interrelationships between linear and quadratic terms (Montgomery, 1991). Normalized independent variables and experimental data of pulp properties were used for the development of empirical models, in which the dependent variables were evaluated by the following general equation:

$$Z = a_0 + \sum_{i=1}^{5} b_i X_{ni} + \sum_{i=1}^{5} c_i X_{ni}^2 + \sum_{i=1; j=1}^{5} d_{ij} X_{ni} X_{nj} \quad (3)$$

where Z is the response or dependent variable [viz. Pulp yield following step X ($Yield_X$), Pulp brightness following step X ($Brightness_X$), Final yield (Yield), Viscosity, Kappa number (K.N), Final brightness, Breaking length (BL), Burst index (BI) and Tear index (TI)]; $X_n$ is the normalized value of the independent variable concerned; and $a_0$, $b_i$, $c_i$ and $d_{ij}$ are unknown characteristic constants estimated from the experimental data.

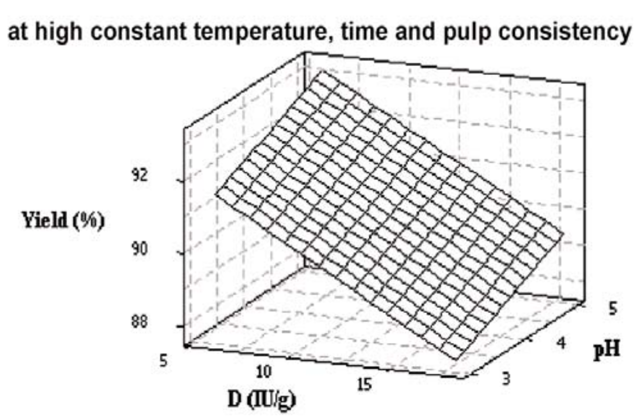


**Figure 1.** Variation of the yield of bagasse pulp after the enzymatic step in the XP bleaching sequence with pH and pX at low temperature ,low consistency and long time.

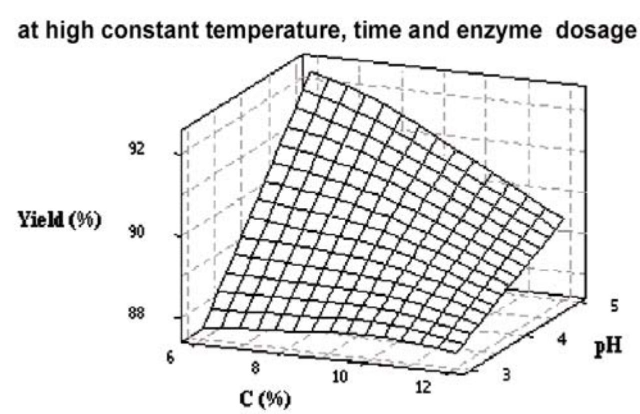


**Figure 2.** Variation of the yield of bagasse pulp after the enzymatic step in the XP bleaching sequence with pH and time at high pX value, low temperature and low consistency.

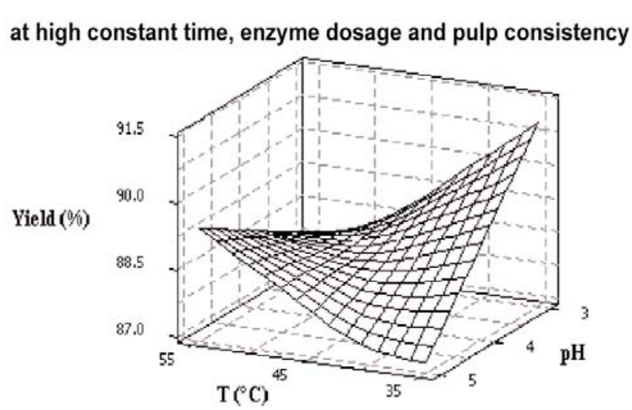


**Figure 3.** Variation of the yield of bagasse pulp after the enzymatic step in the XP bleaching sequence with pH and temperature at low consistency, high pX value and long time.

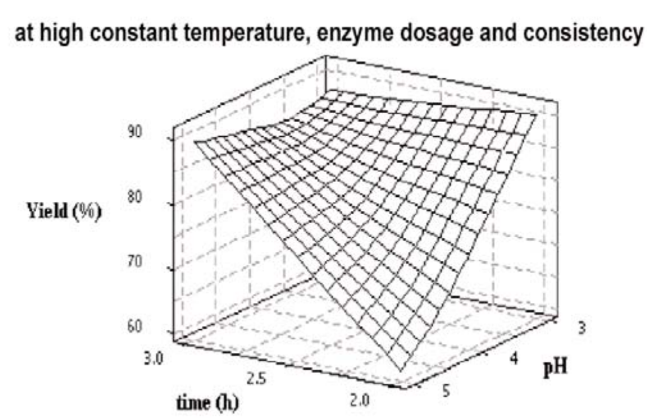


**Figure 4.** Variation of the yield of bagasse pulp after the enzymatic step in the XP bleaching sequence with pH and temperature at low consistency, high pX value and long time.

The values of responses obtained allow the calculation of mathematical estimation models for each response, which were subsequently used to characterize the nature of the response surface.

## RESULTS

Table 2 and 3 show the average experimental results of the pulp and paper properties studied in the 43 bleaching experiments. By correlating the experimental pulp yield values obtained from the enzyme treatment step (Table 2) with those of independent values (Table 1) via Eq. (4), the following equation was obtained once all the terms with R-sq at a level of more than 82% were eliminated using the step wise method:

$$\text{Yield (X)} = 89.56 + 1.96\, T^2 - 0.89\, D + 0.52\, pH - 0.46\, t*D - 0.48\, T - 0.44\, C + 0.44\, T*pH - 0.41\, pH*C - 0.38\, D*C - 0.30\, t \quad (4)$$

This equation reproduces the experimental results with error less than 3% (Table 4,5, 6). The S, R-sq, R-sq (adj) and P-Value are statistical items of obtained Eq. (3) which all of them has been added and explained in the margin of Table 4 and experimental design section. Appling steepest ascent method (Press *et al.,* 1992) to Eq. (4) provided the values of independent variables that maximized the yield. Within the range of normalized values for the independent variables from −1 to +1, the maximum yield (93.28%) was achieved at high pH (normalized value of +1 for it), in addition to low temperature, enzyme dosage and short time (normalized values of −1 for three variables).

Eq. (4) allows the estimation of changes in the yield with one of the independent variables over the range considered on constancy of all other variables. With the most suitable values for all the independent variables that are to be kept constant (viz. high pH, low temperature and enzyme dosage, short time and each pulp consistency), if a high yield is to be achieved, then the greatest variation of the yield is obtained by altering the time (1.52 units, 1.6%) and the smallest by the changing the enzyme level (0.08 units, 0.1%); an intermediate variation is obtained by altering temperature (1.18 units, 1.2%), pH (0.98 units, 1.1%) or pulp consistency (0.82 units, 0.9%). Figures 1-4 support these conclusions. From the foregoing analysis, it follows that, by high pulp consisten-

**Table 1.** Operating conditions used in step X of the XP sequence for bleaching bagasse pulp.

| No. | $X_T$ | $X_t$ | $X_D$ | $X_{pH}$ | $X_C$ | T(°C) | t(h) | D(IU/g) | pH | C(%) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 45 | 2.5 | 12 | 4 | 9 |
| 2 | 1 | 1 | 1 | 1 | -1 | 55 | 3 | 18 | 5 | 6 |
| 3 | 1 | 1 | 1 | 1 | 1 | 55 | 3 | 18 | 5 | 12 |
| 4 | 1 | -1 | 1 | 1 | -1 | 55 | 2 | 18 | 5 | 6 |
| 5 | 1 | -1 | 1 | 1 | 1 | 55 | 2 | 18 | 5 | 12 |
| 6 | -1 | 1 | 1 | 1 | -1 | 35 | 3 | 18 | 5 | 6 |
| 7 | -1 | 1 | 1 | 1 | 1 | 35 | 3 | 18 | 5 | 12 |
| 8 | -1 | -1 | 1 | 1 | -1 | 35 | 2 | 18 | 5 | 6 |
| 9 | -1 | -1 | 1 | 1 | 1 | 35 | 2 | 18 | 5 | 12 |
| 10 | 1 | 1 | 1 | -1 | -1 | 55 | 3 | 6 | 3 | 6 |
| 11 | 1 | 1 | 1 | -1 | 1 | 55 | 3 | 6 | 3 | 12 |
| 12 | 1 | -1 | 1 | -1 | -1 | 55 | 2 | 6 | 3 | 6 |
| 13 | 1 | -1 | 1 | -1 | 1 | 55 | 2 | 6 | 3 | 12 |
| 14 | -1 | 1 | 1 | -1 | -1 | 35 | 3 | 6 | 3 | 6 |
| 15 | -1 | 1 | 1 | -1 | 1 | 35 | 3 | 6 | 3 | 12 |
| 16 | -1 | -1 | 1 | -1 | -1 | 35 | 2 | 6 | 3 | 6 |
| 17 | -1 | -1 | 1 | -1 | 1 | 35 | 2 | 6 | 3 | 12 |
| 18 | 1 | 1 | -1 | 1 | -1 | 55 | 3 | 18 | 5 | 6 |
| 19 | 1 | 1 | -1 | 1 | 1 | 55 | 3 | 18 | 5 | 12 |
| 20 | 1 | -1 | -1 | 1 | -1 | 55 | 2 | 18 | 5 | 6 |
| 21 | 1 | -1 | -1 | 1 | 1 | 55 | 2 | 18 | 5 | 12 |
| 22 | -1 | 1 | -1 | 1 | -1 | 35 | 3 | 18 | 5 | 6 |
| 23 | -1 | 1 | -1 | 1 | 1 | 35 | 3 | 18 | 5 | 12 |
| 24 | -1 | -1 | -1 | 1 | -1 | 35 | 2 | 18 | 5 | 6 |
| 25 | -1 | -1 | -1 | 1 | 1 | 35 | 2 | 18 | 5 | 12 |
| 26 | 1 | 1 | -1 | -1 | -1 | 55 | 3 | 6 | 3 | 6 |
| 27 | 1 | 1 | -1 | -1 | 1 | 55 | 3 | 6 | 3 | 12 |
| 28 | 1 | -1 | -1 | -1 | -1 | 55 | 2 | 6 | 3 | 6 |
| 29 | 1 | -1 | -1 | -1 | 1 | 55 | 2 | 6 | 3 | 12 |
| 30 | -1 | 1 | -1 | -1 | -1 | 35 | 3 | 6 | 3 | 6 |
| 31 | -1 | 1 | -1 | -1 | 1 | 35 | 3 | 6 | 3 | 12 |
| 32 | -1 | -1 | -1 | -1 | -1 | 35 | 2 | 6 | 3 | 6 |
| 33 | -1 | -1 | -1 | -1 | 1 | 35 | 2 | 6 | 3 | 12 |
| 34 | 0 | 0 | 0 | 0 | -1 | 45 | 2.5 | 12 | 4 | 6 |
| 35 | 0 | 0 | 0 | 0 | 1 | 45 | 2.5 | 12 | 4 | 12 |
| 36 | 0 | 1 | 0 | 0 | 0 | 45 | 3 | 12 | 4 | 9 |
| 37 | 0 | -1 | 0 | 0 | 0 | 45 | 2 | 12 | 4 | 9 |
| 38 | 1 | 0 | 0 | 0 | 0 | 55 | 2.5 | 12 | 4 | 9 |
| 39 | -1 | 0 | 0 | 0 | 0 | 35 | 2.5 | 12 | 4 | 9 |
| 40 | 0 | 0 | 0 | 1 | 0 | 45 | 2.5 | 18 | 5 | 9 |
| 41 | 0 | 0 | 0 | -1 | 0 | 45 | 2.5 | 6 | 3 | 9 |
| 42 | 0 | 0 | 1 | 0 | 0 | 45 | 2.5 | 12 | 4 | 9 |
| 43 | 0 | 0 | -1 | 0 | 0 | 45 | 2.5 | 12 | 4 | 9 |

**Table 2.** Experimental results for bagasse pulp subjected to XP bleaching sequence.

| No. | $Yield_X$ [a] (%) | $Brightness_X$ [b] (%) | Final Yield (%) | Viscosity ($cm^3/g$) | Kappa |
|---|---|---|---|---|---|
| 1 | 89.8 | 43.9 | 73.1 | 709 | 15.2 |
| 2 | 92.3 | 43.5 | 70.8 | 610 | 14.7 |
| 3 | 89.4 | 43.8 | 69.6 | 623 | 14.4 |
| 4 | 92.4 | 43.2 | 71.4 | 626 | 14.6 |
| 5 | 90.5 | 44.4 | 69.9 | 631 | 14.2 |
| 6 | 92.7 | 43.2 | 72.9 | 645 | 15.5 |
| 7 | 87.1 | 42.8 | 71.4 | 647 | 15.7 |
| 8 | 92.9 | 41.7 | 73.7 | 650 | 15.5 |
| 9 | 91.1 | 40.1 | 72.1 | 658 | 15.8 |
| 10 | 87.7 | 43.2 | 71.6 | 619 | 14.3 |
| 11 | 87.8 | 44.8 | 72.1 | 609 | 13.9 |
| 12 | 90.5 | 41.9 | 72.6 | 629 | 14.1 |
| 13 | 90.3 | 43.1 | 72.7 | 627 | 14.4 |
| 14 | 91.4 | 44.7 | 71.3 | 704 | 15.4 |
| 15 | 91.0 | 42.4 | 72.2 | 712 | 14.9 |
| 16 | 92.0 | 43.4 | 72.9 | 809 | 14.5 |
| 17 | 91.8 | 42.3 | 73.9 | 763 | 14.3 |
| 18 | 93.0 | 43.1 | 75.1 | 615 | 14.9 |
| 19 | 93.1 | 43.4 | 73.3 | 625 | 14.3 |
| 20 | 92.8 | 42.7 | 73.0 | 633 | 14.8 |
| 21 | 92.7 | 43.0 | 71.8 | 640 | 15.1 |
| 22 | 93.2 | 42.6 | 72.4 | 639 | 16.1 |
| 23 | 93.3 | 42.7 | 71.2 | 637 | 15.9 |
| 24 | 93.3 | 42.3 | 72.4 | 673 | 15.8 |
| 25 | 92.1 | 39.8 | 71.0 | 687 | 17.1 |
| 26 | 91.8 | 42.9 | 72.5 | 628 | 14.4 |
| 27 | 91.6 | 44.7 | 74.5 | 611 | 13.9 |
| 28 | 90.9 | 38.8 | 71.7 | 636 | 14.2 |
| 29 | 91.1 | 41.4 | 72.7 | 643 | 14.7 |
| 30 | 92.1 | 44.1 | 71.5 | 644 | 16.0 |
| 31 | 92.2 | 42.2 | 71.9 | 640 | 14.8 |
| 32 | 92.2 | 43.3 | 69.2 | 652 | 14.6 |
| 33 | 92.5 | 42.1 | 70.6 | 649 | 14.0 |
| 34 | 90.6 | 43.2 | * | 709 | 15.3 |
| 35 | 89.3 | 44.4 | * | 708 | 15.0 |
| 36 | 89.4 | 44.1 | 73.0 | 733 | 15.1 |
| 37 | 90.1 | 43.7 | 73.5 | 697 | 15.2 |
| 38 | 87.2 | 43.9 | 71.5 | 742 | 14.7 |
| 39 | 90.6 | 42.5 | 73.2 | 664 | 15.3 |
| 40 | 91.1 | 43.0 | 73.9 | 700 | 15.5 |
| 41 | 88.5 | 44.0 | 72.5 | 692 | 15.1 |
| 42 | 87.7 | 44.5 | 72.2 | 700 | 14.9 |
| 43 | 90.9 | 43.3 | 74.1 | 710 | 15.3 |

[a] Pulp yield following step X
[b] Pulp brightness following step X

**Table 3.** Experimental results for paper sheets obtained from pulp bleached by the XP sequence.

| No. | Final brightness (%) | Breaking length (m) | Burst index (KN/g) | Tear index ($mNm^3/g$) |
|---|---|---|---|---|
| 1 | 73.8 | 5690 | 3.59 | 5.94 |
| 2 | 73.6 | 5741 | 3.62 | 5.86 |
| 3 | 73.2 | 5897 | 3.61 | 5.64 |
| 4 | 73.0 | 6001 | 3.61 | 5.85 |
| 5 | 70.4 | 5910 | 3.63 | 5.91 |
| 6 | 73.2 | 5800 | 3.53 | 4.20 |
| 7 | 72.0 | 5710 | 3.55 | 4.45 |
| 8 | 69.9 | 6120 | 3.51 | 4.06 |
| 9 | 71.0 | 5810 | 3.52 | 4.37 |
| 10 | 72.2 | 5760 | 3.66 | 4.83 |
| 11 | 74.5 | 5991 | 3.64 | 4.53 |
| 12 | 70.9 | 5611 | 3.64 | 5.60 |
| 13 | 71.9 | 5543 | 3.63 | 5.53 |
| 14 | 72.6 | 5791 | 3.55 | 4.48 |
| 15 | 71.4 | 5873 | 3.55 | 5.33 |
| 16 | 73.6 | 5650 | 3.54 | 3.84 |
| 17 | 74.4 | 5462 | 3.55 | 4.52 |
| 18 | 70.2 | 5694 | 3.64 | 5.87 |
| 19 | 71.4 | 5742 | 3.65 | 5.60 |
| 20 | 72.3 | 5893 | 3.63 | 5.28 |
| 21 | 73.3 | 5990 | 3.62 | 5.45 |
| 22 | 72.6 | 5911 | 3.52 | 3.89 |
| 23 | 71.8 | 5793 | 3.53 | 5.54 |
| 24 | 73.0 | 5708 | 3.54 | 3.61 |
| 25 | 70.9 | 6119 | 3.55 | 4.44 |
| 26 | 73.0 | 5810 | 3.68 | 4.64 |
| 27 | 72.6 | 5760 | 3.67 | 4.72 |
| 28 | 69.9 | 5993 | 3.64 | 5.58 |
| 29 | 71.7 | 5871 | 3.65 | 5.91 |
| 30 | 72.4 | 5651 | 3.57 | 4.45 |
| 31 | 71.9 | 5461 | 3.59 | 4.41 |
| 32 | 72.1 | 5698 | 3.56 | 3.84 |
| 33 | 71.6 | 5740 | 3.54 | 4.10 |
| 34 | 70.9 | 5765 | 3.59 | 6.14 |
| 35 | 73.3 | 5791 | 3.59 | 6.09 |
| 36 | 71.9 | 5697 | 3.60 | 6.88 |
| 37 | 72.7 | 5688 | 3.58 | 5.87 |
| 38 | 73.9 | 5807 | 3.61 | 5.79 |
| 39 | 72.0 | 5776 | 3.58 | 4.48 |
| 40 | 73.1 | 5660 | 3.62 | 6.11 |
| 41 | 73.5 | 5621 | 3.59 | 6.49 |
| 42 | 73.0 | 5690 | 3.59 | 6.29 |
| 43 | 71.6 | 5683 | 3.59 | 5.90 |

**Table 4.** Parameters for the fits of Eqs. (4) - (12)

| Equation | S[a] | R-sq[b] | R-sq(adj)[c] | P-Value[d] |
|---|---|---|---|---|
| Pulp yield following step X: Eq. (4) | 0.833 | 82.91 | 77.58 | 0.046 |
| Pulp brightness following step X: Eq. (5) | 0.747 | 71.05 | 65.26 | 0.139 |
| Final Yield: Eq. (6) | 0.590 | 82.32 | 78.56 | 0.112 |
| Viscosity Eq. (7) | 25.8 | 74.18 | 68.10 | 0.055 |
| Kappa number: Eq. (8) | 0.313 | 83.84 | 78.79 | 0.124 |
| Final brightness: Eq. (9) | 0.911 | 66.11 | 60.23 | 0.100 |
| Breaking length: Eq. (10) | 117 | 45.69 | 38.35 | 0.099 |
| Burst index: Eq. (11) | 0.014 | 91.37 | 90.20 | 0.142 |
| Tear index: Eq. (12) | 0.411 | 80.49 | 76.58 | 0.130 |

[a] **S**, estimate of the standard deviation of the error term in model. In general, the smaller the S, the better the model fits the data.
[b] **R-sq** the proportion of the variation in the response data explained by the model.
[c] **R-sq (adj)**, modified version of R that has been adjusted for the number of predictors in the model.
[d] **P_Value,** For the data of project, Alpha-to enter is 0.15, thus at each step of procedure, a predictor is added to the model if it has the smallest P_value among those predictors with P_value less than 0.15.

cy, the yield decreases to 73.43%, i.e., by only 0.9% with respect to its highest level (74.33%). Therefore, low temperature, enzyme level, short time , high pulp consistency and pH may be used in order to decrease operating costs and immobilized capital depreciation.

Using the same products, the following equations were obtained for the other response variables:

$$\text{Brightness (X)} = 43.68 + 0.62\ t + 0.63\ T*C + 0.49\ T*pH - 0.94\ T^2 + 0.31\ D + 0.28\ T + 0.20T*D \quad (5)$$

$$\text{Total Yield} = 73.00 - 0.75\ T*D - 0.58\ pH*C - 0.54\ t*D - 0.94\ T^2 - 0.41\ D*pH - 0.28\ T*pH - 0.17D \quad (6)$$

$$\text{Viscosity} = 705 - 55.4\ T^2 - 21.4\ T - 13.4\ D*pH - 13.3\ T*D - 10.6\ t + 10.6\ T*pH - 9.6\ pH + 8.8\ D \quad (7)$$

$$K.N = 15.15 - 0.459\ T + 0.365\ pH - 0.181\ T*pH - 0.156\ t*C - 0.141\ D - 0.131\ t*pH - 0.125\ T*t - 0.25\ T^2 + 0.100\ pH*C - 0.088\ D*pH \quad (8)$$

$$\text{Total brightness} = 71.98 + 0.31\ pH - 0.3\ D - 0.7\ pH^2 + 0.3\ t*pH - 0.4\ D*pH + 0.4\ C*pH \quad (9)$$

$$BL = 5715 + 65\ pH - 56\ t*pH + 51t*D + 82\ T^2 - 35\ T*pH \quad (10)$$

$$BI = 3.592 + 0.0456\ T - 0.0109\ pH - 0.0071\ D + 0.0065\ t - 0.0037\ t*pH \quad (11)$$

$$TI = 5.998 + 0.546\ T - 1.11\ T^2 - 0.231\ T*t - 0.157\ T*C + 0.142\ T*pH + 0.133\ C + 0.113\ t*pH \quad (12)$$

Eqs. (5)-(12) reproduce the experimental pulp brightness following step X, and the final yield, viscosity, kappa number, final brightness, breaking length, burst index and tear index which errors less than 3, 5, 8, 2, 6, 4, 10 and 9% respectively.

**Table 5.** Values of independent variables providing the optimum values for the response variables.

| Response variables | Optimum response values | variation [a] | Independent values | | | | |
|---|---|---|---|---|---|---|---|
| | | | T | t | D | pH | C |
| Yield following step X (%) | 93.28 | 0.02 | -1 | -1 | -1 | 1 | -1, 1 |
| Brightness following step X (%) | 44.29 | 1.15 | 1 | 1 | 1 | -1 | 1 |
| Final Yield (%) | 74.23 | 1.17 | 1 | 1 | -1 | 1 | -1 |
| Viscosity ($cm^3/g$) | 738.1 | 9.61 | -1 | -1 | 1 | -1 | -1 |
| Kappa number | 15.22 | 5.78 | -1 | -1 | -1 | 1 | 1 |
| Final brightness (%) | 72.99 | 1.37 | -1 | -1 | 1 | -1 | 1 |
| Breaking length (m) | 5902 | 3.69 | -1 | -1 | 1 | 1 | -1 |
| Burst index (kN/g) | 3.66 | 0.55 | 1 | 1 | -1 | -1 | 1 |
| Tear index ($mNm^2/g$) | 6.00 | 14.66 | 0 | 1 | 0 | 0 | 0 |

[a] Percentages variation relative to the optimum value and estimated value of the response variable

**Table 6.** Maximum variation in the response variables with changes in each individual independent variable with constancy of the others at their optimum values [a] .

| Response Variable | Maximum variation by the independent variable changing | | | | |
|---|---|---|---|---|---|
| | T (°C) | t (min) | D (IU/g) | pH | C (%) |
| Yield following step X (%) | 1.18(1.2) | 1.52(1.6) | 0.08(0.1) | 0.98(1.1) | 0.82(0.9) |
| Brightness following step X (%) | 1.04(2.3) | 1.03(2.3) | 1.01(2.2) | 0.98(2.2) | 1.06(2.4) |
| Final Yield (%) | 0.94(1.3) | 1.08(1.5) | 3.70(5.0) | 1.40(1.9) | 1.10(1.5) |
| Viscosity ($cm^3$/g) | 99.6(13) | 21.2(2.9) | 71.0(9.6) | 67.2(9.1) | — |
| Kappa number | 1.03(6.8) | 0.32(2.1) | 0.46(3.0) | 1.73(11) | 0.51(3.4) |
| Final brightness (%) | — | 0.6(0.82) | 0.2(0.27) | 0.1(0.15) | 0.8(1.09) |
| Breaking length (m) | 75(1.27) | 10(0.17) | 102(1.7) | 310(5.3) | — |
| Burst index (kN/g) | 0.1(2.74) | 0.02(0.5) | 0.01(0.4) | 0.03(0.8) | — |
| Tear index ($mNm^2$/g) | 0.32(5.3) | 0.46(7.7) | — | — | 0.27(4.5) |

[a] Variations are given as percentages in brackets.

The S, R-sq, R-sq (adj) and P-Values for the fits of Eqs. (5)-(12) are given in Table 4.

The results of Tables 5 and 6 were obtained by using a procedure, similar to that applied to Eq. (4) above.

Varying one of the independent variables between the normalized values from −1 to +1 while keeping the others constant at the values shown in Table 5, resulted in the greatest changes in the dependent variables relative to the optimum values (also shown in Table 5) given in Table 6. As it can be seen, temperature changes was specially influential on the viscosity and burst index; time changes on yield in step X and tear index; D changes on the final yield; pH changes on the Kappa number and breaking length of the paper sheets and consistency changes on brightness in step X and Final brightness. On the other hand, temperature changes had little effect on the brightness in step X, Final brightness and final yield; time changes on the Kappa number and breaking length; D changes on the Yield following step X, Brightness following X, burst and tear index; pH changes on brightness in step *X*, Final brightness and tear index; and consistency changes on the viscosity, breaking length and burst index.

## DISCUSSION

The data in Table 6 and previous conclusions show that the dependent variables vary little from their optimum values if low temperature and D values, high pH and consistency values in addition to short time are used. The estimates for the dependent variables are given in Table 7, which also shows the percentage variations of such values with respect to their optimum levels (Table 5). These new operating conditions are more suitable than those in Table 6 in which they are milder. This reduces energy and immobilized capital expenses, with only slight losses in some proper-

**Table 7.** Values of dependent variables obtained at low temperature and ,low enzyme level, short time, high pH and high pulp consistency.

| Response variable | Estimated value of the response variables | Percentage variation relative to the optimum value |
|---|---|---|
| Pulp yield following step X | 92.34 | -1.00% |
| Brightness following step X | 40.61 | -8.31% |
| Final Yield | 71.05 | -4.28% |
| Viscosity | 679.3 | -7.96% |
| Kappa number | 16.4 | +7.75% |
| Final brightness | 72.39 | -0.83% |
| Breaking length | 6001 | +1.67% |
| Burst index | 3.54 | -3.27% |
| Tear index | 5.446 | -9.25% |

ties of the pulps and paper sheets obtained from them. In summary, using a temperature of 35 (°C), an enzyme concentration of 6 (IU/g) by dry pulp weight, pH 5 and a consistency of 12% for 2 (h) provides the best results.

## References


Abdul-Karim LA, Rab A (1995). An optimization approach for the oxygen bleaching of kraft wheat straw cellulose. *Tappi J*. 78: 83-90.

Adachi S, Chen K (2007). Environmentally pulping of rice straw by ethylene glycol solvent. *Asian J Biochem Chem Eng*. 1: 18-22.

Akhnazarova S, Kafarov V (1982). *Experiment Optimization in Chemistry and Chemical Engineering*. Mir Publisher, Moscow, PP. 120-121.

Allison RW, Clark TA (1994). Effect of enzyme pretreatment on ozone bleaching. *Tappi J*. 77: 127-34.

Bajpai B, Bhardwaj NK, Bajpai PK, Jauhari MB (1994). The impact of xylanases on bleaching of eucalyptus kraft pulp. *J Biotechnol*. 38: 1-6.

Charin T, Naiyatat P, Masanori W, Ken S (2003). Thermostable and alkaline-tolerant microbial cellulase-free xylanases produced from agricultural wastes and the properties required for use in pulp bleaching bioprocesses: a review. *Proc Biochem*. 38: 431-433.

Christov LP, Szakacs G, Balakrishnan H (1999). Production partial characterization and use of fungal cellulase free xylanase in pulp bleaching. *Pro Biochem*. 34: 511-517.

Daneault C, Leduc C, Valade JL (1994). The Use of Xylanasea in Kraft Pulp Bleaching. a Review. *Tappi J*. 77: 125-31.

Denise SR, Adilson RG (2002). *Ethanol/Water pulp of sugarcane bagasse bleached with xylanase and classified by filter-PCA*. IBER-AMERICA congress on pulp and paper research. Washington, U.S.A.

Eriksson KE (1993). Concluding remarks: Where do we stand and where are we going? Lignin biodegradation and practical utilization. *J Biotechnol*. 30: 149-158.

FAO, http://faostal.fao.org/faostal/, (2004).

Garg AP, Roberts JC, McCarthy AJ (1998). Bleach boosting effect of cellulase-free xylanase of *Streptomyces thermoiolaceus* and its comparison with two commercial enzyme preparations on birchwood kraft pulp. *Enz Microbiol Technol*. 22: 594-598.

Han J, Shen Liu Y, Han C (2007). Organosolv pulping of nonwoods by high molecular organic solvent. *Asian JBiochem Chem Eng*. 1: 7-12.

Hongzhang Ch, Fujian Xu, Zuohu L (2002). Solid-state production of biopulp by phanerochaete chrysosporium using steam-exploded wheat straw as substrate. *Bioreso Technol*. 81: 261-263.

Jiménez L, Navarro E, Ferrer JL (1997). Biobleaching procedures for pulp from agricultural residues using Phanerochaete chrysosporium and enzymes. *Pro Biochem*. 32: 297-304.

Jiménez L, Navarro E, Ferrer L, Lopéz F, Ariza J (1999). Biobleaching of cellulose pulp from wheat straw with enzymes and hydrogen peroxide. *Pro Biochem*. 35: 149-157.

Khristova P, Kordsachia O, Patt R, Karar I, Khider T (2006). Environmentally friendly pulping and bleaching of bagasse. *Ind Crop Prod*. 23: 131-139.

Montgomery DC (1991). *Análisis De Experimentos*. Grupo Editorial Iberoamericana, Mexico, PP. 52-56.

Neeta K, Mala R (2002). Thermostable and alkaline-tolerant cellulase-free xylanase produced by thermotolerant Streptomyces sp. Ab106. *J Biosci Bioeng*. 93: 431-433.

Press WH, Teukolsky SA, Vetterling WT, Flannery BB (1992). *Numerical Recipes in C: The Art of Scientific Computing*. Cambridge: Cambridge University Press, PP. 221-223.

Rajasekar T (2007). Study on Biobleaching of nonwoods with fungal enzymes. *Asian J Biochemical Chem Eng*. 1: 1-6.

Rezayati-Charani P, Mohammadi-Rovshandeh J, Hashemi SJ, Kazemi-Najafi S (2006). Influence of dimethyl formamide pulping of bagasse on pulp properties. *Bioreso Technol*. 97: 2435-2442.

Rodríguez F, Gilarranz MA, Oliet M, Tijero J (1998). Pulping of lignocellulosics by Organosolv processes. *Recent Res Dev Chem Eng*. 2: 9-17.

Roncero MB, Torres AL, Colom JF, Vidal T (2003). TCF bleaching of wheat straw pulp using ozone and xylanase. Part B: kinetic studies. *Bioreso Technol*. 87: 315-323.

Rowell R (1984). *The chemistry of Solid wood: Based on Short course and symposium sponsored by the division of cellulose, paper and textile chemistry*, the 185th meeting of the American Chemical Society, Seattle, Washington, PP. 70-72.

Sandrim VC, Rizzatti ACS, Terenzi HF, Jorge JA, Milagres AMF, Polizeli, ML (2005). Purification and biochemical characterization of two xylanases produced by Aspergillus caespitosus and their potential for kraft pulp bleaching. *Proc Biochem*. 40: 1823-1828.

SCAN (1998). SCAN-test standards. *Viscosity in cupri-ethylenediamin (CED) solution, SCAN-CM 15:88*. Scandinavian Pulp, Paper and Board testing committee, Stockholm, PP. 121-125.

Shen Liu Y (2007). TCF bleaching on xylanase treated pulps. *Asian J Biochem Chem Eng*. 1: 13-17.

Srinivasan MC, Rele MV (1999). Microbial xylanases for paper industry. *Curr Sci*. 77: 137-142.

TAPPI Committee (2000-2002). *TAPPI Test Methods*. TAPPI Press, Atlanta, GA, U.S.A. PP. 243-249.

Wise LE, Murphy M (1946). A Chlorite holocellulose, its fractionation and bearing on summative wood analysis and studies on the hemicelluloses. *Paper Trade J*. 122: 35-43.

Ziaie-Shirkolaee Y, Mohammadi-Rovshandeh J, Rezayati-Charani P, Khajeheian MB (2007 a). Study on cellulose degradation during organosolv delignification of wheat straw and evaluation of pulp properties. *Iran Polym J*. 16: 86-94.

Ziaie-Shirkolaee Y, Mohammadi-Rovshandeh J, Rezayati-Charani P, Khajeheian MB (2007 b). Influence of dimethyl formamide pulping of wheat straw on cellulose degradation and comparison with Kraft process. *Bioreso Technol*. (In press).