OPTIMIZING ENZYMATIC PRETREATMENT OF RECYCLED FIBER TO IMPROVE ITS DRAINING ABILITY USING RESPONSE SURFACE METHODOLOGY

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A three-factor, three-level Box-Behnken Design (BBD) was used with enzyme dosage (0.05-0.15‰ o.d. fiber), enzymatic contact time (20-40 min), and pulp consistency (3-7%, o.d.) as independent variables to understand and optimize the enzymatic pretreatment conditions of mixed office waste (MOW) for maximum improvement of its drainability. All the independent variables considered were found to have significant influence on the drainability of the pulp. The enzyme dosage had a predominate effect, the pulp consistency took second place, and the contact time seemed to have low priority. A quadratic polynomial model had high Adj-R^2 value and low p value for predicting the decrement of beating degree of the pulp. Applying desirability function method, the optimal pretreatment conditions were found to be an enzyme dosage of 0.11‰ o.d., enzymatic contact time of 31.0 min, and pulp consistency of 4.50% o.d. The optimal pretreatment resulted in a maximum decrement of 10 units of beating degree, a decrease by 20% when compared with the control sample. The observed and predicted values of beating degrees were in close agreement. Results of fiber morphology analysis and physical property tests showed that the optimal pretreatment partially recovered the fiber flexibility and retained the strength properties of the handsheet even under the lower beating degree. Isothermal (Thermogravimetric Analyzer) TGA experiments of the fibers confirmed that the enzymatic pretreatment decreased the fiber hornification.

Keywords: Response surface methodology (RSM); Box-Behnken Design (BBD); Recycled fiber; Drainage; Cellulase modification

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INTRODUCTION

Recycled fibers have become increasingly important as raw material in the pulp and paper industry (Dienes et al. 2004). Paper recycling offers several advantages. Substitution of virgin pulp with recycled fibers preserves and economizes scarce forest resources, reducing the exploitation of forests, and contributing to the sustainable development of the forests resources. Recycling also minimizes environmental pollution and contributes to water and energy conservation. Wastepaper is a low-cost source of fiber for paper and board manufacture, and the use of recycled fibers can greatly reduce the cost of the final paper (Verma et al. 2010; Singh and Bhardwaj 2011). Although utilization of secondary fibers has environmental and economic advantages, unfortunately the deteriorated drainability of secondary fibers undermines the advantages of the recycled fibers and causes decreased productivity (Dienes et al. 2004). The mechanical properties of fibers, as well as their ability to swell, are diminished after they are exposed to pulping and drying conditions imposed during the paper making cycle (Pala et al. 2001; Pommier, Fuentes, and Goma 1989; Nazhad and Paszner 1994; Marton et al. 1993). Several problems are associated with the recycling of fibers. The main problems include the deteriorated drainability of recycled fiber by the inferior fiber quality (shortened length, more fines, and less flexibility) which causes higher drainage resistance, hornification of the fibers (inability to regain the original water–swollen state due to stiffening of the polymer structure of fibers during drying), less effective inter-fiber bonding (which consequently decreases paper strength properties), and higher energy consumption in the dryers. Chemical analysis of the recycled fibers suggested that the low molecular weight xylans in the fibers become gradually depleted with the recycling of the pulp. The aging and heating stiffen the fibrils and xylan that attached to the fibers, thereby inactivating the surface of the fibers.

Mixed office waste (MOW) is an available and inexpensive source of high quality bleached chemical fiber. It can be hand-sorted to exclude lower value fiber and debris, leaving a relatively clean pulp furnish. Novel and highly effective methods to modify the deteriorated properties of MOW fibers will be bound to releave the shortage of fiber material and be benefit to the promising sustainable development of pulping and paper making industry (Kraschowetz 2009; Sykes 1995).

There are a variety of approaches, such as mechanical treatment, chemical treatment, or chemical additive and enzymatic treatments, that are available for improving the drainage or restoring strength of recycled fibers. Among all these methods, the use of enzymes for modification of the recycled fibers is the most promising approach. It is gaining popularity in the pulp and paper industry and providing benefits to mills. It has been suggested that these problems in the reuse of the recycled fibers can be overcome by treating the pulp slurry with enzymes (Garg and Singh 1997; Bajpai and Mishra 2003). Since the 1970s, enzymes have been introduced into biomechanical pulping, modification of fiber properties, deinking, and improvement of drainage (Dienes et al. 2004). Enzymes have great potential in solving many problems associated with the use of recycled fiber, especially related to deinking, drainability, hornification, refining, and strength. The potentials of cellulase have also been demonstrated for reducing the energy requirement in pulp refining, improving the machine runnability, and controlling stickies when recycled fibers are used. Enzymes can also be considered as “green” products (Bajpai 2010). The cellulase system hydrolyzes cellulose chains with a synergistic effect: Cellobiohydrolases hydrolyze cellulose chains from the ends and release cellobiose; endoglucanases randomly attack the amorphous regions of the cellulose substrate, yielding a high degree of polymerization oligomers; and β-D-glucosidases further break down dimers into glucose (Liu and Hu 2012). Researchers from La Cellulose du Pin were the first to show that the reduced dewatering properties of recovered paper can be improved by enzymatic treatment (Pin 1990). The improved drainability of the pulp resulted in higher productivity in the manufacture of corrugated board (Stork et al. 1995). Dienes et al. (2004) used cellulase to treat lab-recycled kraft sack paper, and the drainage of the pulp increased by 16%; similarly Shaikh and Luo (2009) used cellulase to treat the mixed pulp of old newsprint and old corrugated containers; such treatment increased the freeness by 13 to 40% (Dienes et al. 2004; Shaikh and Luo 2009).

Optimization of pretreatment conditions is one of the most important stages in the development of an efficient and economic pretreatment method. The response surface methodology (RSM) has been recognized as a useful technique to optimize the process variables, and is used only when a few significant factors are involved in optimization (Palamakula et al. 2004). Various types of RSM designs are available now, including 3-level factorial design, central composite design (CCD), Box-Behnken design, and D-optimal design (Boza et al. 2000; Singh et al. 1995; Sánchez-Lafuente et al. 2002). Among all these RSM designs, the Box-Behnken design requires fewer runs (17 runs) in a 3-factors 3-levels experimental design. In addition, the Box-Behnken design, a modified central composite experimental design, is an independent, rotatable or nearly rotatable quadratic design (contains no embedded factorial or fractional factorial design), in which the treatment combinations are at the midpoints of the edges of the process space and at the center (Ragonese et al. 2002). Hence, the Box-Behnken design was applied to optimize the combination levels of the process parameters that play important roles in modification of the recycled fiber. These parameters include the enzyme dosage, contact time, pulp consistency, temperature, pH value, etc., which have been considered by previous researchers. However, little research has been carried out to optimize the interaction of these main influential factors. According to our previous study, the enzyme dosage, contact time, and pulp consistency were three of the most important influential factors (Liu et al. 2012). Thus, the main objective of the present study was to maximize the drainability of the pulp by using the RSM to optimize the main influential factors during the enzymatic modification of recycled fibers. Meanwhile, the present study also tries to investigate the influence of enzymatic pretreatment on the physical properties of the final paper.

EXPERIMENTAL

Material

The cellulase used in the present study is a commercially available pure endoglucanase, with an EG activity of 1070 u/mL, obtained from *Trichoderma reesei* supplied by an enzyme corporation in the USA. The optimal temperature and pH of the cellulase were 40 to 50 ^{o}C and 4.0 to 6.0, respectively. It has been proved to be one of the most effective enzymes during the modification of recycled pulp in our previous screening experiments. The mixed office waste (MOW) used in this study was supplied by the Chen Ming paper company in Shandong Province, China. The pulps were obtained from the end of the deinking process.

Experimental Design and Analysis

The main objective of the present study was to use a Box-Behnken design with three variables to statistically determine the optimum combination levels of the enzyme dosage, contact time, and pulp consistency for maximizing the drainability of the deinked pulp (DIP) and to evaluate main effects, interaction effects, and quadratic effects of the formulation variables on the modification of the pulp by enzymatic pretreatment. A 3-factor, 3-level design was adopted to explore quadratic response surfaces and constructing second-order polynomial models. Thus, each variable was coded and run at three levels: -1, 0, and 1, as shown in Table 1. The interaction of the three parameters was also investigated. The nonlinear quadratic model is given as:

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} b_{ij} X_i X_j + \varepsilon \quad (1)$$

where Y is the predicted response associated with each factor level combination, b_0 is the interception coefficient, b_i is the linear coefficient, b_ii is the quadratic coefficient, and b_ij is the interaction coefficient. X_i is the independent variable under study, and ε is random error. Regression analysis and estimation of coefficients were performed using Design Expert ® (Statease Inc., Minneapolis, USA). The optimal pretreatment conditions from the RSM were validated by pretreating the pulp at the identified optimum enzyme dosage, contact time, and pulp consistency.

Table 1. Box-Behnken Design Showing Both Coded and Actual Values of Independent Variable (X_i) and Dependent Responses (Y)

Order	X_1: Enzyme dosage (‰，o.d.)	X_2: Contact Time (min)	X_3: Pulp consistency (%，o.d.)	Y: Beating degree (ºSR)
1	0 (0.10)	0 (30)	0 (5.0)	40.0
2	-1 (0.05)	1 (40)	0 (5.0)	44.0
3	0 (0.10)	-1 (20)	-1 (3.0)	43.5
4	0 (0.10)	1 (40)	-1 (3.0)	42.0
5	0 (0.10)	0 (30)	0 (5.0)	40.5
6	-1 (0.05)	-1 (20)	0 (5.0)	45.0
7	1 (0.15)	-1 (20)	0 (5.0)	42.0
8	0 (0.10)	0 (30)	0 (5.0)	40.0
9	-1 (0.05)	0 (30)	1 (7.0)	46.0
10	0 (0.10)	0 (30)	0 (5.0)	40.5
11	1 (0.15)	0 (30)	-1 (3.0)	42.0
12	-1 (0.05)	0 (30)	-1 (3.0)	45.0
13	1 (0.15)	0 (30)	1 (7.0)	44.0
14	0 (0.10)	0 (30)	0 (5.0)	41.0
15	0 (0.10)	-1 (20)	1 (7.0)	46.0
16	1 (0.15)	1 (40)	0 (5.0)	43.0
17	0 (0.10)	1 (40)	1 (7.0)	44.0

Characterization of the Hornification of Fibers

For the quantitative study of the hornification of the recycled fiber, Jayme's method (Luo et al. 2011; Jayme 1944), which characterizes the hornification degree by water retention value (WRV), is still prevalent and is given as,

$$Hornification = [(WRV_0 - WRV_1) / WRV_0]*100\% \quad (2)$$

where WRV_0 is the original water retention value of the fibers and WRV_1 is the water retention value of the fibers that had undergone a papermaking process in Jayme's research. In this study WRV_1 represent the water retention value of the fibers after the enzymatic pretreatment, and WRV_0 is the water retention value of the fibers without enzymatic pretreatment.

A thermogravimetric analyzer (TGA) was used to measure the "hard-to-remove (HR) water content," which was defined as the ratio of water mass to fiber mass at the transition between the constant rate zone and the falling rate zone from the isothermal TGA experiment, so as to evaluate fiber hornification (Park et al. 2005). A 10 mg fiber sample (10 g/g moisture ratio) was placed in the furnace, which was under the flow of dry nitrogen. The TGA experiment was conducted isothermally at 90 ^{o}C to dry the fiber sample until the weight change was less than 0.001%/min in the derivative of the weight loss.

Enzymatic Treatment

30 g oven-dried (o.d.) MOW were processed in each experiment. The pulp suspensions were prepared by disintegrating the samples in a disintegrator for 10000 revolutions following the ISO 5263 procedure, which can separate the fibers completely and without appreciably changing their structural properties. Then, a certain amount of water was added to adjust the pulp consistency to the desired values. The pulp suspensions were warmed up to the desired temperature (45 ^{o}C), and pH values were adjusted by addition of sulfuric acid (0.1% w/w).

After the disintegration and adjustment steps, the enzymes were added to the pulp suspensions according to the values listed in Table 1. The enzyme preparation was diluted 100 times with pure water before being added to the pulp suspensions so as to achieve better dispersion. The enzymatic reactions were carried out with intermittent manual mixing (10 minutes per time) under constant temperature and pH value. The enzymatic reaction was stopped by boiling the pulp suspension for 5 minutes to deactivate the enzymes. The control samples were prepared following the same procedures without the addition of enzymes.

Evaluation of Pulp and Handsheet Properties

Schopper-Riegler beating degree values were measured according to the ISO 5267-1 standard procedure. The water retention values (WRV) of the pulp were determined according to ISO 23714:2007(E) standard procedure. Laboratory handsheets were prepared on a standard sheet former, according to ISO 5269-1 method. Tensile index and tear index were determined following the standard procedures of ISO 1924-2 and ISO 1974 respectively.

The fiber morphology was measured by using the Fiber tester 912 (L&W). Scanning electron microscopy (SEM) images of fibers were obtained in a JSM-6380 scanning electron microscope after combined dehydration treatment of fibers with increasing concentration of ethanol and the freeze dehydration treatment.

RESULTS AND DISCUSSION

Model Fitting

To check whether the second-order polynomial response surface model (Eq. (1)) was well fitted to the response variables (*Y*) and for the corresponding fitting of the explanatory model and variation of the enzyme dosage, contact time, and pulp consistency, the sum of squares of the sequential model was analyzed.

Table 2. Sequential Model Sum of Squares

Source	DF	Beating degree	
		Sum of squares	Pr>F
Mean vs. Total	1	31218.37	
Linear vs. Mean	3	18.69	0.2165
Interaction vs. Linear	3	1.31	0.9614
Quadratic vs. Interaction	3	43.74	<0.0001
Cubic vs. Quadratic	3	1.94	0.1197
Residual	4	0.70	
Total	17	31284.75	

In Table 2, the mean, linear, interaction, quadratic, and cubic headings represent the sequential sum of squares for the linear terms, the two-factor interaction (AB, BC, AC) terms, the quadratic (A-squared, B-squared, C-squared) terms, and the cubic terms respectively. The F-value tests the significance of adding higher terms to the lower terms model. A small p-value (Pr>F) indicates that adding terms has improved the model. In Table 2, it is obvious that adding terms up to quadratic significantly improved the model (Table 2, p value<0.0001, sum of squares is maximum) and, therefore, could be the most appropriate model for the response.

To further examine the statistical significance of the terms, as well as fitting the model, the regression analysis and ANOVA were calculated by software based on the data in Table 1. Table 3 lists the estimated regression coefficients of the quadratic polynomial models for the response variables, along with the corresponding coefficients of determination (R^2). In addition, adj-R^2 and coefficient of variation (*CV*) were calculated to check the model adequacy.

Lack of fit measures how well the model fits the data, indicating the failure of a model as a means of explaining the experimental data. Specifically, the observed data cannot be attributed to random error that by chance happens to fit the model (Neter et al. 1983; Koocheki et al. 2009). If the model does not fit the data well, this will be significant. Lack of fit is an undesirable characteristic for a model, so we generally would want this to be insignificant (P>0.10 is better). Strong lack of fit (p<.05) is an undesirable property, because it indicates that the model is a poor choice for representing the data. The lack of fit illustrated in Table 3 (p value of 0.1193) did not result in a significant p-value for selected variables, meaning that these models were sufficiently accurate for predicting the relevant responses.

R^2 is a measure of the amount of variation around the mean explained by the model, it is the proportion of variation in the response attributed to the model rather than to random error. It has been suggested that for a good fitted model, R^2 should not be less than 80%. The higher of R^2 value (approaching to 1.0), the higher likelihood of fitting a model to the actual data is (Koocheki et al. 2009). By contrast, a lower value of R^2 shows the inappropriateness of the model to explain the relation between variables. The R^2 value shown in Table 3 was higher than 0.80, indicating the regression models were suitable to explain the behavior. However, adding of variables to the model will always increase R^2, regardless of whether the additional terms are statistically significant or not. Thus, a large value of R^2 cannot always imply the adequacy of the model. For this reason, it is more appropriate to use an adj-R^2 of over 90% as a criterion of model adequacy. Adj-R^2 is a measure of the amount of variation around the mean explained by the model, adjusted for the number of terms in the model. The adj-R^2 decreases as the number of terms in the model increases if those additional terms do not add substantial value to the model (Koocheki et al. 2009; Little and Hills 1978). The adj-R^2 value listed in Table 3 is higher than 0.90, indicating that non-significant terms have not been included in the model.

Moreover, the coefficient of variation (*CV*) describes the extent to which the data were dispersed. It is the standard deviation expressed as a percentage of the mean. As a general rule, the coefficient of variation (*CV*) should not be greater than 10% (Koocheki et al. 2009; Daniel 1991). The *CV* shown in Table 3 is 1.43% and less than 10%, suggesting that the model developed was feasible and adequate for response.

Table 3. ANOVA and Regression Coefficients of the Response Surface Quadratic Model for the Response Variables

Source	DF	Schopper-Riegler Beating Degree				
		Coefficient Estimate	Sum of squares	Standard Error	F value	P value Prob.>F
Model	9	40.40	63.74	0.27	18.80	0.0004
A-enzyme dosage	1	-1.12	10.12	0.22	26.87	0.0013
B-Contact time	1	-0.44	1.53	0.22	4.06	0.0836
C-pulp consistency	1	0.94	7.03	0.22	18.66	0.0035
AB	1	0.50	1.00	0.31	2.65	0.1473
AC	1	0.25	0.25	0.31	0.66	0.4422
BC	1	-0.12	0.063	0.31	0.17	0.6960
A2	1	1.74	12.71	0.30	33.74	0.0007
B2	1	1.36	7.82	0.30	20.75	0.0026
C2	1	2.11	18.79	0.30	49.87	0.0002
Residual	7		2.64			
Lack of fit	3		1.94		3.69	0.1197
Pure error	4		0.70			
Total	16		66.38			
R^2		0.96				
Adj-R^2		0.91				
C.V. %		1.43				

As shown in Fig. 1, the predicted values calculated from the polynomial regression model and the actual observed values of the beating degree of the pulp were found to be in good agreement. All the results discussed suggest that the model used in this research can be used to navigate the design space and to identify operating conditions for modifying the drainability of the pulp.

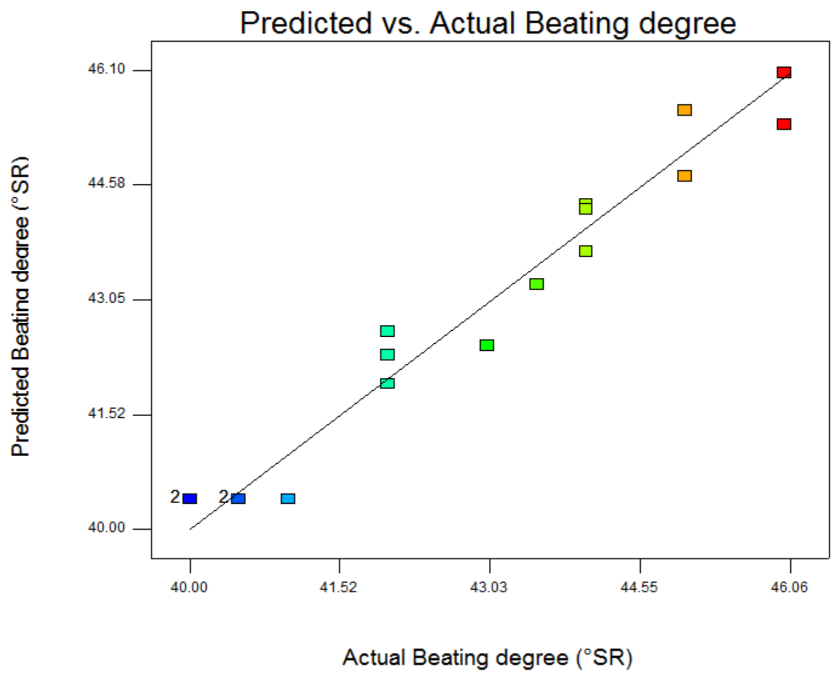


Fig. 1. Comparison between the predicted values calculated from the polynomial regression model and the actual observed values of the beating degree of the pulp

Table 3 also lists the *F* values and *P* values for the model and each term. The *p* value is the probability value that is associated with the *F* value for this term. It is the probability of getting an *F* value of this size if the term did not have an effect on the response. In general, a term that has a probability value less than 0.05 would be considered a significant effect, and a probability value greater than 0.10 is generally regarded as not significant (Kanakambaran et al. 2011). In Table 3, the Model F-value of 18.80 implies that the model is significant. There is only a 0.04% chance that a "model *F*-value" this large could occur due to noise. In this case, *A*, *C*, A^2, B^2, and C^2 are significant model terms. The "lack of fit *F*-value" of 3.69 implies the lack of fit is not significant relative to the pure error. There is an 11.97% chance that a "lack of fit *F*-value" this large could occur due to noise. Non-significant lack of fit is what is desired.

After generating the polynomial regression model relating the dependent and independent variables presented in Table 1, the pretreatment processes were optimized for the response of *Y*. Optimization was performed to obtain the levels of X_1, X_2, and X_3, which minimized *Y*. The responses obtained for each experimental run performed according to Box-Behnken design were analyzed by multiple regression analysis, and the second order polynomial equation was derived to represent the beating degree of the pulp as a function of the variables tested as below:

$$Y = 77.3\text{-}204*X_1 - 0.930*X_2 - 4.88*X_3 \\ +1.00*X_1X_2 + 2.50*X_1X_3 - 6.25*10^{-3}*X_2X_3 \\ +695*X_1^2 + 0.014*X_2^2 + 0.528*X_3^2 \quad (3)$$

where Y=predicted response (beating degree), and X_1, X_2 , and X_3 are actual factors of enzyme dosage (‰, o.d.), contact time (min), and pulp consistency (%, o.d.) respectively. The polynomial equation represents the quantitative effect of process variables (X_1, X_2, and X_3) and their interactions on the response Y. The coefficient values of X_1, X_2, and X_3 are related to the effect of these variables on the response Y. Coefficients with more than one factor term and those with higher order terms represent interaction terms and quadratic relationships, respectively. A negative value represents an effect that favors the optimization (decreasing the beating degree), while a positive value indicates an antagonistic effect. The values of X_1, X_2, and X_3 were substituted into the equation with actual values to obtain the theoretical values of Y. (Palamakula et al. 2004). It was confirmed from the p values of linear terms shown in Table 3 that the enzyme dosage, contact time, and pulp consistency had a strong influence on the beating degree of the pulp. Among the independent variables, enzyme dosage had a more prominent effect on decreasing the beating degree than that of other variables, as evident from their linear coefficients and sum of squares in Table 3. Enzyme dosage also had a favorable influence (decreasing the beating degree as we desire). The magnitude of the pulp consistency was lower than that of enzyme dosage for decreasing the beating degree, whereas it was higher than that of the contact time and had a positive influence (increase of pulp consistency might result in the increase of beating). The linear terms and cubic terms of enzyme dosage and pulp consistency contributed to the decreasing of beating degree, as evident from the coefficients and sum of squares in Table 3.

Interaction Effects of Variables on the Modification of the Drainage of the Pulp

In order to visualize and understand the interaction effects of the variables and for identifying optimal levels of each parameter for attaining minimal beating degree of the pulp, significant interaction response surfaces generated by the model are plotted in Figs. 2 to 4. The response surfaces were based on the coefficients presented in Table 3, and the data were generated through keeping one variable at its respective zero level (center value of the testing ranges) and varying the other two within the experimental range. In general, exploration of the response surfaces indicated a complex interaction between the variables (Koocheki et al. 2009).

Figures 2, 3, and 4 illustrate the interaction effects of X_1, X_2, and X_3 on the response Y. The variation of beating degree with enzyme dosage and contact time at a given pulp consistency (5%, o.d.) is presented in Fig. 2. At both medium levels of enzyme dosage and contact time, beating degree of the pulp was lower. At lower enzyme dosage, a longer contact time was needed for decreasing the beating degree, but even extending the time to 40 min, the decrease of the beating degree was still insignificant.

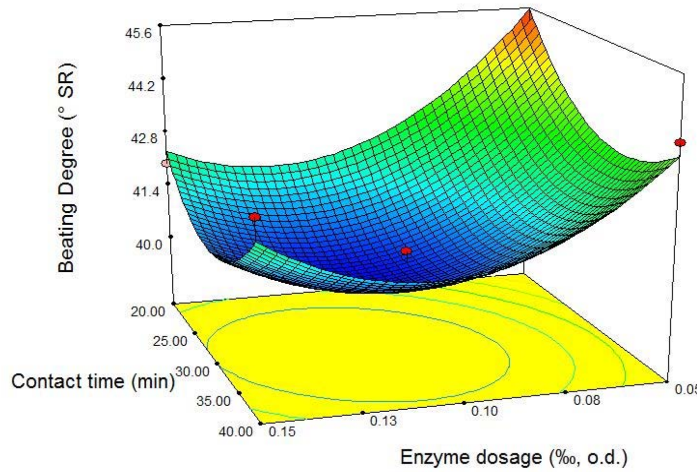


Fig. 2. Response surface plots showing the effect enzyme dosage (X_1) and contact time (X_2) on response *Y* (i.e., the beating degree of the pulp) (Pulp consistency=5%, o.d.)

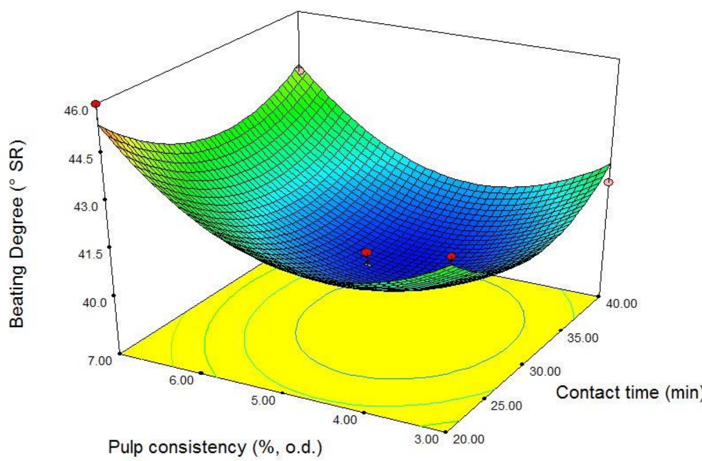


Fig. 3. Response surface plots showing the effect contact time (X_2) and pulp consistency (X_3) on response *Y* (i.e., the beating degree of the pulp) (Enzyme dosage= 0.1‰, o.d.)

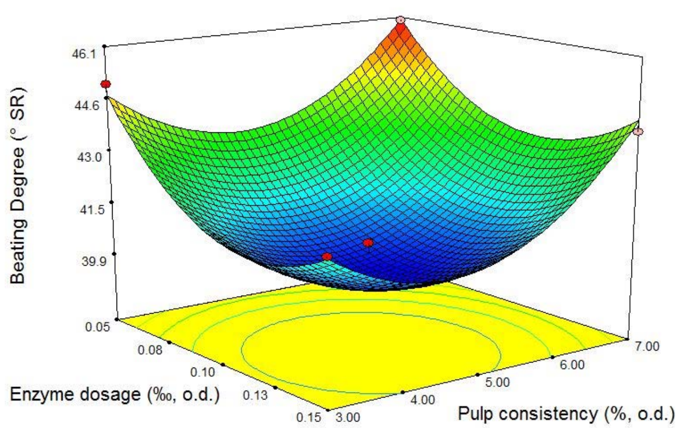


Fig. 4. Response surface plots showing the effect enzyme dosage (X_1) and pulp consistency (X_3) on response Y (i.e., the beating degree of the pulp) （Contact time= 30min）

However, at a higher enzyme dosage the situation was somewhat complex. Shorter and longer contact times in combination with a higher enzyme dosage were determined to be detrimental to the decrement of beating degree. Since the modification of fibers by enzyme requires several steps (transportation, adsorption, hydrolyzation, and desorption) which might take a certain amount of time, the shorter time may not reach the requirement. On the other hand, longer contact time and higher enzyme dosage might result in excessive hydrolysis of the fibers, and such an effect might release more fiber fragments and fines, which undermine the decrement of the beating degree of the pulp.

Figure 3 presents the variation of beating degree with contact time and pulp consistency when the enzyme dosage is constant (0.1‰，o.d.). From the plot it can be seen that when pulp consistency was decreased from 7.0% to 3.0% the beating degree changed in a parabolic manner, dramatically decreasing to a trough and then increasing slightly. At medium consistency, efficiency of enzymatic hydrolysis is favorable. Lower pulp consistency is instrumental in the dispersing of the enzymes. More free water content heightens the efficiency of enzyme transportation mass transfer of intermediates and enzymes (Felby et al. 2008). However, too much free water can also adversely affect the decrease of beating degree, with the decreased modification efficiency at very low pulp consistency being due to the lessened adsorption of cellulase on the cellulose fibers (Kristensen et al. 2009). In addition, the contact time also demonstrated a similar change as in Fig. 2.

Figure 4 shows the interaction between enzyme dosage and pulp consistency on the beating degree of the pulp. As the previous ANOVA revealed, enzyme dosage had a more prominent effect on the modification of the drainability than that of other variables, and it had a favorable influence (decreased the beating degree as we desired). The magnitude of the pulp consistency was lower than that of enzyme dosage for decreasing the beating degree, whereas it was higher than that of the contact time and had a negative influence (increase of pulp consistency might result in the increase of beating that we dislike). In the examined range of each parameter, with the increase of the enzyme dosage and decrease of pulp consistency, the beating degree decreased rapidly. Similarly, higher enzyme dosage and lower pulp consistency also caused damage to the improvement of the drainability of the pulp.

Therefore, choice of enzyme dosage, contact time, and pulp consistency has to be made very judiciously to decrease the beating degree of the pulp. An enzyme dosage of about 0.1‰ o.d., a contact time of about 30 min, and pulp consistency of about 5% o.d. were found to be optimal for minimizing the beating degree of the pulp.

Optimization of the Enzymatic Pretreatment to Increase the Drainability of the Pulp

The response surface pilots discussed above revealed the trends of variation of the responses with the independent variables, but there were no accurate conditions obtained; therefore further optimization is needed. Optimal conditions for minimizing the beating degree of the pulp were predicted using numerical optimization function in Design Expert software.

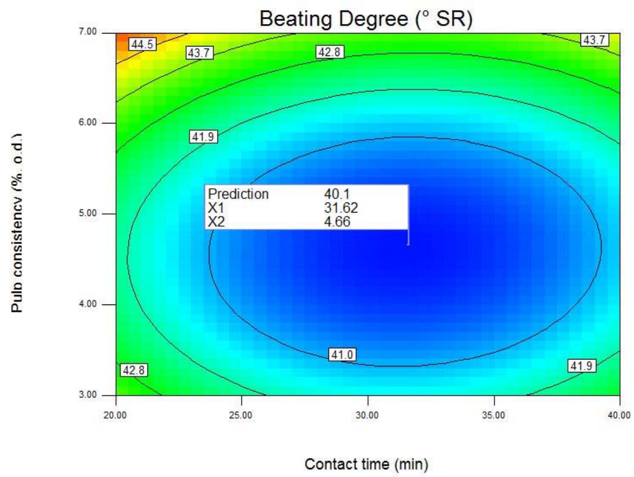


Fig. 5. Optimum conditions by contour plot of the responses evaluated as a function of pulp consistency vs. contact time (at a given enzyme dosage of 0.1‰ o.d.)

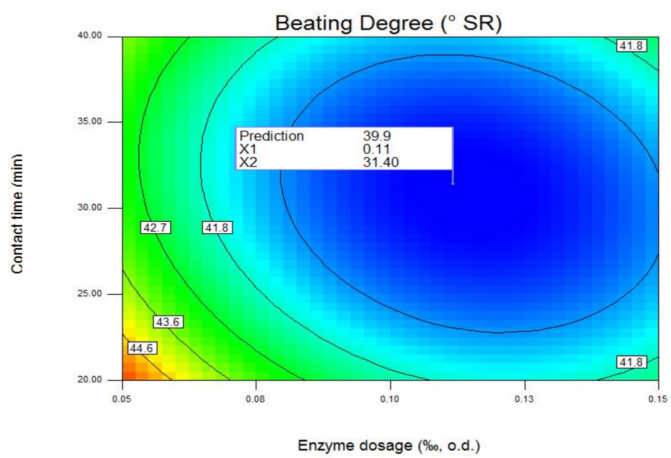


Fig. 6. Optimum conditions by contour plot of the responses evaluated as a function of enzyme dosage vs. contact time (at the optimal pulp consistency of 4.6%)

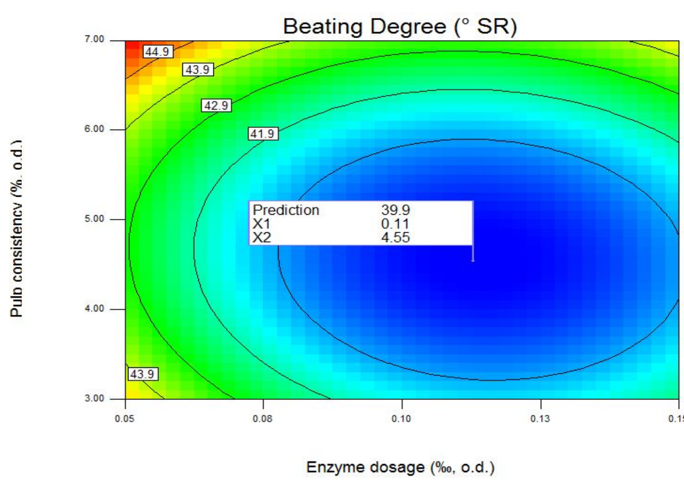


Fig. 7. The optimum conditions by contour plot of the responses evaluated as a function of pulp consistency vs. enzyme dosage (at the optimal contact time of 31 min)

The levels of independent variables are tabulated in Table 4, and the objective of the optimization is to minimize the beating degree of the pulp. The optimum pretreatment conditions were determined by the contour plots of the responses, and the contour plots were evaluated as functions of each pair of variables when other parameters were given. For instance, when the enzyme dosage was given as 0.1‰ o.d., the optimum conditions of contact time and pulp consistency were 31.62 min and 4.66% o.d. respectively, and it brought a beating degree of 40.1 ºSR. When the optimized pulp consistency was given as 4.6 % o.d., the optimum conditions of enzyme dosage was 0.11‰ o.d., contact time was 31.40 min, and the beating degree under these conditions was 39.9 ºSR. When the optimized contact time was given as 31 min, the optimum conditions of the enzyme dosage and pulp consistency were 0.11‰ o.d. and 4.55% o.d. respectively, and the beating degree was predicted as 39.9 ºSR. In summary, the final optimal enzyme dosage was 0.11 ‰ o.d., pulp consistency was 4.55% o.d., and contact time was 30.88 min; under these optimal conditions, the predicted beating degree was 39.9 (Table 4). The actual beating degree of the pulp that was treated under the same condition was found as 40.5 ºSR, which was close to the predicted value.

Table 4. Predicted Optimum Condition to Minimize Beating Degree of the Pulp

Factor	Goal	Lower Limit	Higher limit	Optimum	Actual value
Enzyme dosage (‰, o.d.)	In range	0.05	0.15	0.11	0.11
Contact time (min)	In range	20	40	30.88	31.00
Pulp consistency (%, o.d.)	In range	3	7	4.55	4.50
Beating Degree (ºSR)	Minimize	0	46	39.9	40.5

Effect of the Enzymatic Pretreatment under the Optimum Condition on the Properties of Pulp and Handsheet

According to data of optimization experiments, the effect of enzymatic treatment on the drainage of the pulp and physical properties of the handsheet at the optimal enzyme dosage, contact time, and pulp consistency was investigated, and the results are shown in Table 5.

Table 5. Effect of Enzymatic Treatment on the Pulp and Handsheet Properties

	Optimum results	Control
Beating degree (ºSR)	40.5	51.0
WRV (%)	155.3	138.7
Hornification degree (%)	-11.97	0
Fiber length (mm)	0.908	0.885
Fines content (%)	13.1	14.2
Tensile index (Nm/g)	37.16	36.83
Tear index ($mN{\cdot}m^2/g$)	5.74	6.66
Air permeation (ml/s)	3.97	3.28

Note: Handsheet grammage is 40 g/m^2.

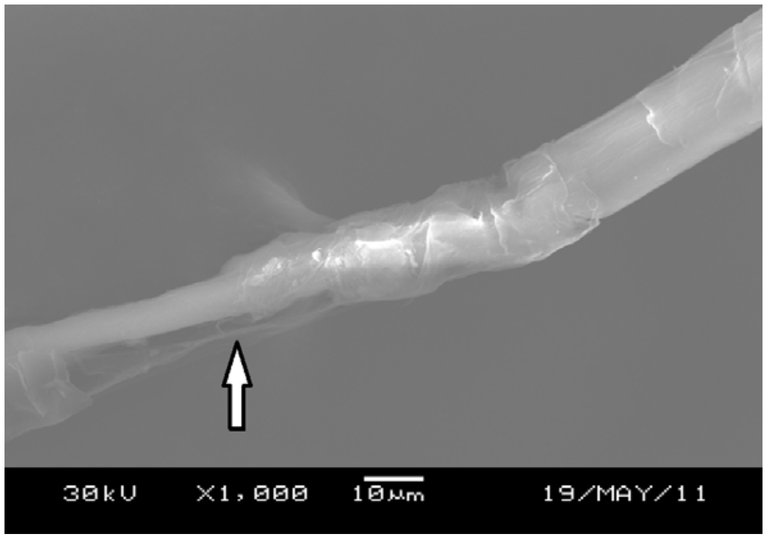
As shown in Table 5, the beating degree of the pulp, which was pretreated at the optimal conditions, decreased by ca. 20%, indicating that the drainability of the pulp was significantly improved. A similar trend has been reported (Bhardwaj1995; Bajpai 2003; Shaikh and Luo 2009), where the drainage of the recycled fiber decreased by 5% to 40%, depending on the kind of fibers. The cellulase preferentially hydrolyzes the fines with high specific surface area and the gel-like polysaccharide layer on the surface; such features ordinarily would contribute to the deteriorated drainability of recycled fibers. Some authors also have pointed out that cellulases may behave in a manner similar to retention aids, facilitating the flocculation of the small fiber particles, thereby enhance the drainability of the recycled fibers (Jackson et al. 1993; Mansfield and Wong 1999). However, most of these improvements of the drainability were at the expense of the strength properties of the paper. Our results in Table 5 suggested that the tear index of the handsheet decreased slightly, the tensile index remained unchanged or increased slightly, while the air permeability was improved due to the enzymatic treatment. The lower value of the tear index can be explained in terms of a weakening effect of the intrinsic fiber strength. Surprisingly, the tensile index of the optimal treated sample remained unchanged even with a lower beating degree. It is likely that the fiber bonding surface, which may contribute to the interfiber bonding, was recovered and increased by enzyme acting on the surface of the fibers. Besides, the decreased fines content alleviated its negative effect (non-effective bonding and filling among the inter-fibers) on the development of strength properties of the handsheets.

The effect of enzymatic treatment on the WRV was investigated as well, and according to equation (2) the hornification degree of the fibers was calculated. Results show that the enzymatic treatment at the optimal conditions could improve the WRV of fibers that were deteriorated due to drying in the paper making process causing hornification of fiber. We hypothesize that the hornification degree of the control sample is zero. Therefore, the negative values of the hornification degree means that hornification degrees of the fibers were decreased, and the fibers become more flexible. Such a finding was in agreement with Stork et al. (1995) who treated recycled pulps with cellulase and found that WRV value could be recovered.

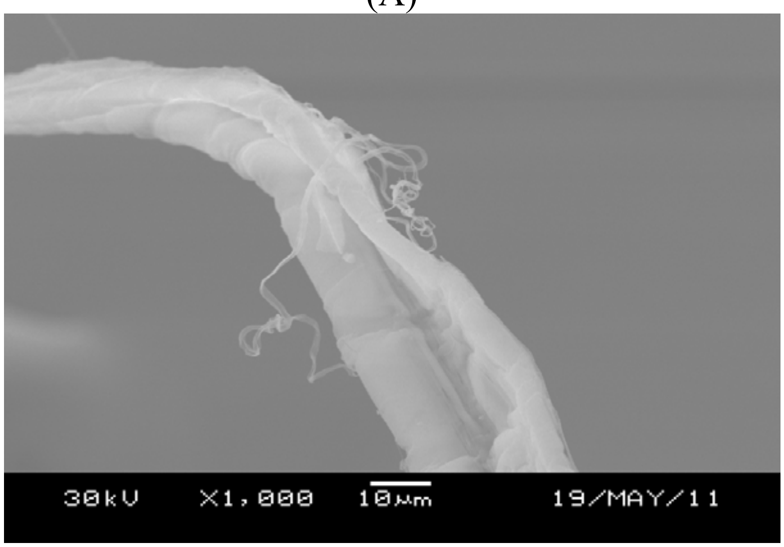
Results from the fiber quality analysis proved that the enzymatic treatment hydrolyzed partial fines and fiber fragments and increased the proportion of the long fibers, which plays an important role in the strength properties of the handsheet. With the decrease of the fines in the pulp, the increase of the air permeability of the handsheet is inevitable.

Analysis of the Fiber Morphology by SEM

As shown in Fig. 8(A), scanning electron micrography of the single control fiber revealed a smooth surface which seemed as if it were covered by a layer of hard crust, and a few fines can be seen attached with the fiber surface. This may be due to the hornification effect of the fiber caused by the previous drying step in the papermaking process. When a fiber is dried, physical continuities in the cell wall are collapsed by high surface tension forces that pull the surface together. Therefore, much of the fibrillation that was developed in the previous beating process had become flattened, lying on the fiber surface, where it had also become hydrogen-bonded, forming the crust, which reduces swelling in the next cycle (Fig. 8(A)) (Bajpai 2010; James 1994; Dienes et al. 2004). However, when the cellulases were applied at the optimal conditions, the hydrolysis of fibrils from the crust of the fiber proceeded readily. The electron microscopic analysis showed that the crust covering the fiber surface was digested or destroyed by enzyme, restoring the rough surface with large specific area, and releasing some fibrils from the surface, which might have a positive effect on the development of the strength properties of the handsheet.



(A)



(B)

Fig. 8. Electron micrographs of untreated (A) and optimal enzyme treated (B) recycled fibers

Analysis of the Fiber Hornification by TGA

The thermogravimetric analysis (TGA) method makes it possible to use the moisture ratio at the transition of constant rate zone or the increasing rate zone and falling rate zone during isothermal drying of fibers to characterize fiber-water interactions.

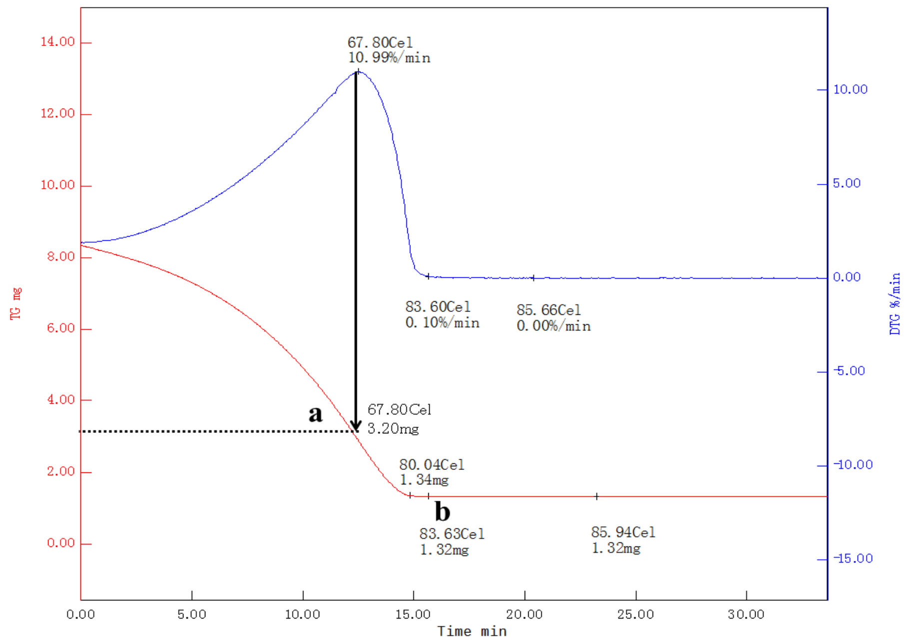


Fig. 9. Thermogravimetric analysis of the hornification of the control fibers

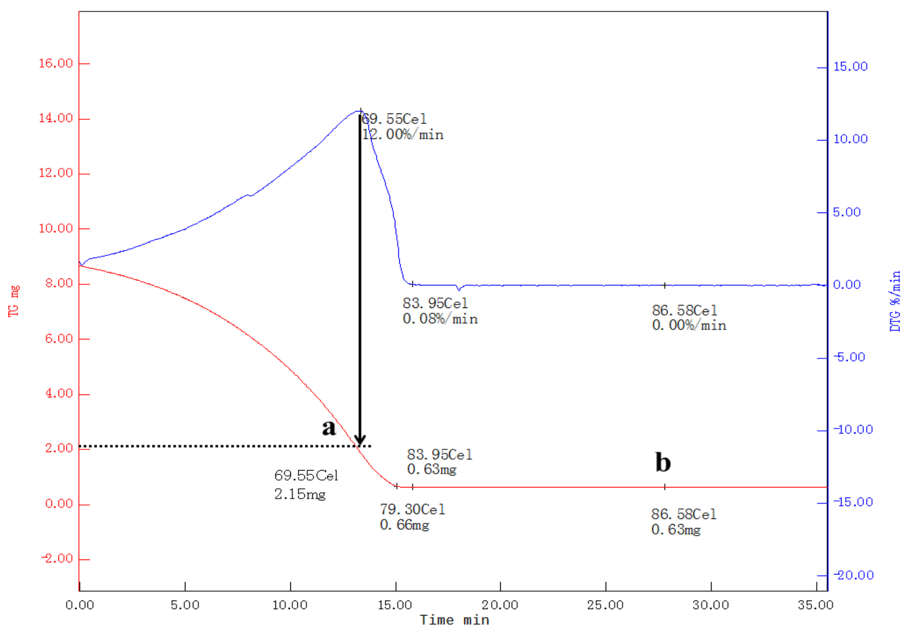


Fig. 10. Thermogravimetric analysis of the hornification of the fibers treated at the optimal conditions

The hard-to remove (HR) water content is defined as the moisture ratio of the fibers at the transition between the constant rate zone or the increasing rate zone and the falling zone (Park et al. 2006). In Figs. 9 and 10, there were no constant rate zones observed. This might be due to the difference of the temperature program. However, it does not hinder the observation of the falling zone. As shown in Fig. 9, the HR content is the weight of water at point “a” divided by the weight of the dried fiber at point “b”. As shown in Figs. 9 and 10, the drying response of the control sample and enzymatic treated sample reveal a similar trend. However, the HR water content was 1.42 (g of water/ g of dry sample) for the control sample and 2.41 (g of water/ g of dry sample) for the cellulase treated sample. The results were in agreement with the WRV values shown in Table 5 and with the report of Park et al. (2006). As the fibers were exposed to the drying and wetting process during papermaking, the cellulose chains were packed closed upon heating and water removal and were not fully reopened when subsequently exposed to water. After the cellulase pretreatment, the packed cellulose chains and pores were digested or reopened and thus, more HR water was detected.

CONCLUSIONS

1. Optimization of the pretreatment conditions for maximizing the drainability of the pulp was performed with the use of response surface methodology (RSM) and a Box-Behnken experimental design. Results showed that the effect of pretreatment variables including enzyme dosage, contact time, and pulp consistency were statistically significant for increasing the drainability. The enzyme dosage plays a predominate effect, the pulp consistency takes the second place, and the contact time seemed low priority on the modification of the MOW for improving its drainability.
2. The optimum levels of these three factors were predicted based on their quantitative effect and the polynomial equations generated by RSM. The optimal conditions were revealed as an enzyme dosage of 0.11‰ o.d., an enzymatic contact time of 31.0 min, and a pulp consistency of 4.50% o.d. The optimal pretreatment resulted in a maximum decrement of 10 units of beating degree, which represents a decrease by 20% when compared with the control sample.
3. The flexibility and swelling ability of the fibers were recovered after the optimal pretreatment. The tensile index of the handsheet increased slightly even at a lower beating degree.
4. Fiber morphology analysis suggested that the enzymatic pretreatment partially hydrolyzed fines and fiber fragments and increased the proportion of the long fiber. By comparison the SEM images of the control and pretreated fibers we found: the crust covering the fiber surface (control sample) was digested or destroyed by enzyme, restoring a rough surface with a large specific area and releasing some fibrils attached the surface (pretreated sample).
5. Analysis of the fiber hornification by the TGA and WRV confirmed that the hornification degree of the fibers was decreased by the cellulase pretreatment.

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