# Influence of Enzymatic Treatment on the Properties of Linen

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**ABSTRACT:** In this article, the effectiveness of cellulase treatment on linen was evaluated by means of phenol-sulfuric acid method. This method was performed by determining sugar liberation in the treatment bath with the amount expressed in glucose equivalence. As compared with conventional method, the measurement of amount of sugar liberated gave a more reliable and accurate result than the weight loss method. It was found that although weight loss of cellulose became negligible when the treatment was done under agitation-free condition, the amount of sugar liberated was still readily measurable. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 286–289, 2007

**Key words:** enzyme; fabric; modification

## INTRODUCTION

Cellulase enzymes are widely used for the treatment of cellulosic material, recently, most commonly on cotton materials. For example, cellulase is used on denim goods to achieve washed effect similar to stone washing with much better color uniformity and handle properties.[1–3] Cellulase enzymes are also used in biofinishing that produces deeper shades, improves appearance, and handle properties.[3–6] One of the common methods to evaluate the effectiveness of the cellulase treatment is by the measurement of fabric strength reduction (strength loss) and weight loss of the treated cellulose. However, during cellulase treatment, the cellulase will hydrolyze cellulose by reacting with the β-1-4-glycoside bonding of the cellulose molecule.[7,8] Unlike acid hydrolysis, the action of cellulase occurs mainly at the terminals of the polysaccharide chains. Reducing groups formed on the treated cellulose are therefore not abundant and it will have a negative response to the Fehling's solution test. On the other hand, sugar of reducing nature is liberated into the solution during such cellulase treatment and its amount can be measured readily.[8] Cellulases are secreted by various fungi and bacteria as complex mixtures of three major kinds namely endoglucanase (EG, EC 3.2.1.4), exocellobiohydrolase (CBH, EC 3.2.1.91), and β glucosidases (EC 3.2.1.21). The proposed mechanism of cellulase action onto cellulose is illustrated in Figure 1. EGs hydrolyze cellulase randomly along the chains, preferentially the amorphous region. CBHs attack the chain ends and produce primarily cellobiose coupled with the binding domains associated with the enzyme. The cellobiose and any small chain oligomers produced by CBHs are then hydrolyzed by the third enzyme beta glucosidase into glucose.

Therefore, it is possible to evaluate the extent of such hydrolytic effect by measuring the amount of the liberated sugars. Thus, the aim of this article was to evaluate the effectiveness of cellulase treatment and establish the correlation between the changing physical properties of cellulosic fiber, with an example of linen, by evaluating the degree of reducing sugars liberation.

## EXPERIMENTAL

### Materials

A 100% scoured and semibleached linen woven fabric was used. The fabric weight was 154 g/m$^2$ with a sett 21/20 per cm and yarn count 118 tex/118 tex. The linen fabric was first desized at 90°C with a heat stable α-amlyase, Thermozyl HTL (Novo Nordisk), to remove residual starch and polyvinyl alcohol sizes. The residual size if any was then detected by iodine/boric acid solution.

### Enzyme treatment

The enzyme used was Denimax BT (Novo Nordisk) which is a neutral cellulase of endo-glucanase. This cellulase is suitable for application at pH 6–8 so that the effect caused by acid hydrolysis could be eliminated. The following experimental conditions, in which five fabric samples were treated under the different experimental conditions, had been adopted for the cellu-

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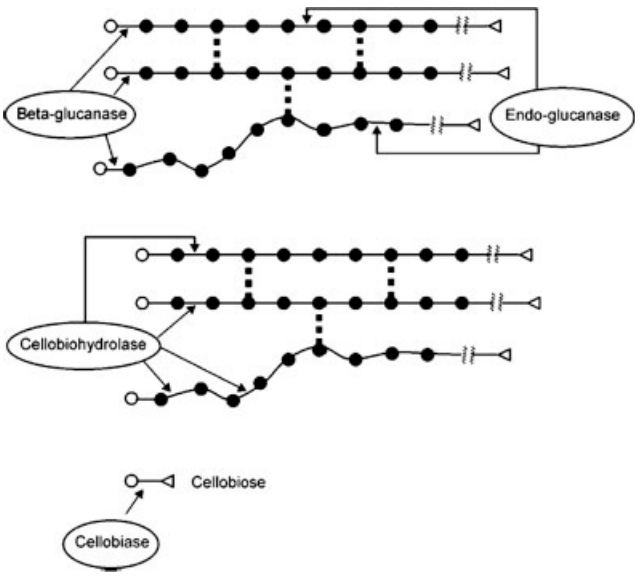


**Figure 1** Enzymatic reaction of cellulose by cellulase.[9]

lase treatment; Cellulase (Denimax BT, 140 EGU/g): 2.5, 5, 7.5, 10, 12.5, and 15 g/L; Treatment temperature: 60°C; pH: 7; Liquor-to-goods ratio: 40 : 1; Weight of material: 10 g; Treatment time: 120 min; Equipment: Atlas Launder-O-meter (washwheel speed 42 rpm).

Pots of 1000 mL volume were used and to each of the pots, 0, 10, 20 30, 40, 50, and 100 steel balls (1/4 inch) were added during the cellulase treatment. The addition of 0, 50, and 100 steel balls were to simulate treatment conditions with no agitation, normal agitation, and vigorous agitation respectively. The steel balls, which have uniform surface texture, could give a better and even effect than that of stone during the enzymatic treatment.

### Weight loss determination

The fabric samples were first conditioned in a standard atmosphere in accordance with ASTM D1776 and then weighed with an electronic balance. The weight change was calculated by eq. (1).

$$\text{Weight change}(\%) = \frac{A - B}{A} \times 100\% \qquad (1)$$

where $A$ is the weight of fabric sample before cellulase treatment and $B$ is the weight of fabric sample after cellulase treatment.

### Tearing strength determination

The fabric samples were first conditioned in a standard atmosphere according to ASTM D 1776 and the tearing strength of the samples were evaluated by ASTM D 1424. The change in tearing strength was calculated by eq. (2).

$$\text{Change in tearing strength}(\%) = \frac{A - B}{A} \times 100\% \qquad (2)$$

where $A$ is the tearing strength of fabric sample before cellulase treatment and $B$ is the tearing strength of fabric sample after cellulase treatment.

### Total sugar liberation

Phenol-sulfuric acid method[6] was used for determining the trace amount of sugar liberated during after the cellulase treatment. A Philips PU 8620 UV/VIS/IR spectrophotometer was used for measuring the absorbance at a wavelength of 490 nm. A calibration curve graph was established with D-glucose concentrations plotting against the absorbance values at 490 nm.[6] Mixture of residue enzymatic liquors, phenol, and sulfuric acid were measured for absorbance and the total sugar liberated was expressed as concentration of D-glucose equivalence by comparing with the calibration curve.

## RESULTS AND DISCUSSION

### Sugar liberated after enzymatic treatment

Although a number of works had been conducted to study the effect of agitation on the different properties of enzyme treated cellulosic fiber,[10,11] there is little discussion on the liberation of reducing sugars upon the effect of different levels of agitation during enzymatic treatment. Figure 2 showed the relationship between the amount of sugar liberated from the linen fabric after the cellulase treatment under different cellulase concentrations and agitation conditions. When no cellulase was added to the treatment bath, even different numbers of steel balls were added to the treatment bath for simulating agitation, no sugar was liberated and this confirmed that the cellulase was, other than agitation, responsible for reacting with the linen fabric for sugar liberation. When the cellulase concentration was increased, the amount of sugar liberated was increased accordingly. When taking the

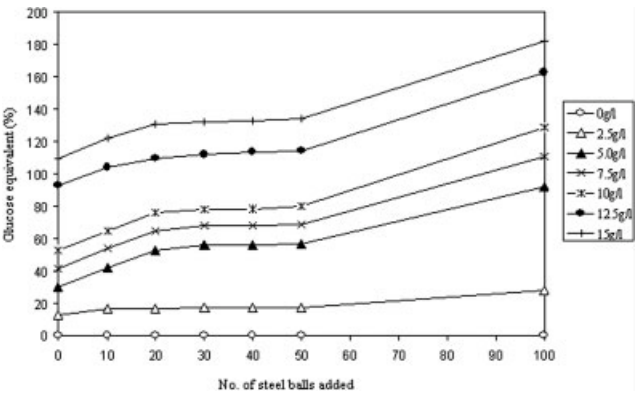


**Figure 2** Effect of agitation on sugar liberated after cellulase treatment (with analytical tolerance limit to 5%).

agitation into consideration, it was found that agitation did cause an initial increase in the amount of sugar liberated but further increase in the degree of agitation did not cause further increase in the sugar liberation which became fairly constant in the region between 20 and 50 steel balls applied, i.e., normal agitation. With the simulation of vigorous agitation, i.e., 100 steel balls, the sugar liberation was increased significantly. The vigorous agitation might impart certain degree of mechanical damage causing cellulosic molecular chains breakage of the linen fabric and hence, more accessible site could be provided for the enzymatic reaction. As a result, more sugar would be liberated but this did not reflect the extent of enzymatic hydrolysis. Therefore, this further confirmed that agitation was not the prime factor in governing the degree of enzymatic hydrolysis of linen.

### Strength loss after enzymatic treatment

Cellulase treatment will cause deterioration in the strength of the material possibly because of fiber loss and fiber breakage under the treatment condition. Figure 3 showed the strength loss of linen fabric during cellulase treatment under various degrees of simulated agitations. It could be observed that even no agitation was applied, there was still have certain degree of strength loss occurred in the cellulase treated linen fabric. This strength loss might be solely due to the enzymatic hydrolysis of the linen fabric. Under vigorous agitation condition with an addition of 100 steel balls into the treatment bath, there was a much higher degree of strength loss than when no steel ball was added during treatment. This effect is less pronounced when linen was treated under simulated normal agitation (50 steel balls). This reflected that the strength loss is not necessarily because of fiber loss or fiber breakage by enzymatic hydrolysis during the cellulase treatment but also the weakening of the fiber by mechanical agitation would probably be one of the contributing factors.

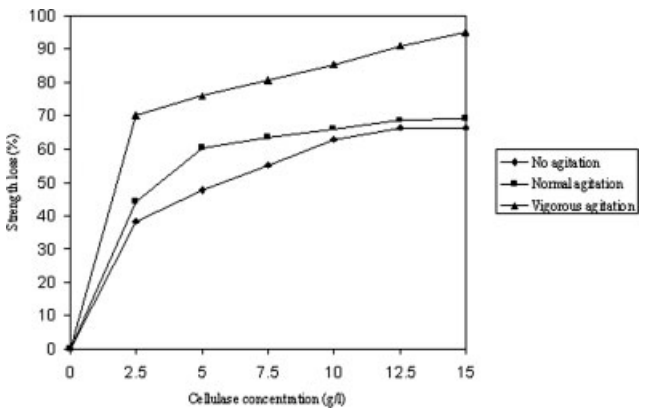


**Figure 3** Strength loss of linen under different agitation conditions (with analytical tolerance limit to 5%).

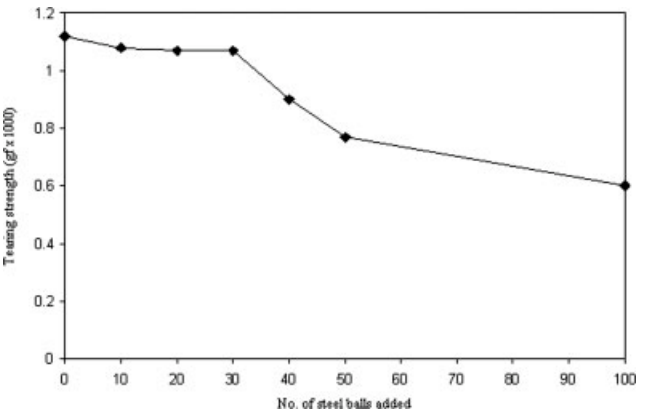


**Figure 4** Effect of agitation on tearing strength of linen fabric with 10 g/L cellulase treatment (with analytical tolerance limit to 5%).

Figure 4 illustrated the relationship between agitation and the tearing strength of cellulase treated linen fabrics (with concentration of 10 g/L as an example). The relationship showed that the agitation did not give a significant effect initially (within 30 steel balls) on the lowering of tearing strength unless agitation is becoming vigorous. In other words, optimization of the fabric strength during enzymatic treatment has to be achieved through controlling the operation parameters such as cellulase concentration and treatment time. However, if the agitation is too vigorous, the loss in fabric strength is also accountable to fiber loss and fiber breakage during the cellulase treatment.

Further evidence about the relationship between sugar liberated and the strength loss was shown in Figure 5. There is a fairly close relationship between the amount of sugar liberated during cellulase treatment and the strength loss of linen fabric under not excessive agitation conditions, i.e., normal agitation and agitation-free conditions. However, if the agitation became severe the relationship became less dis-

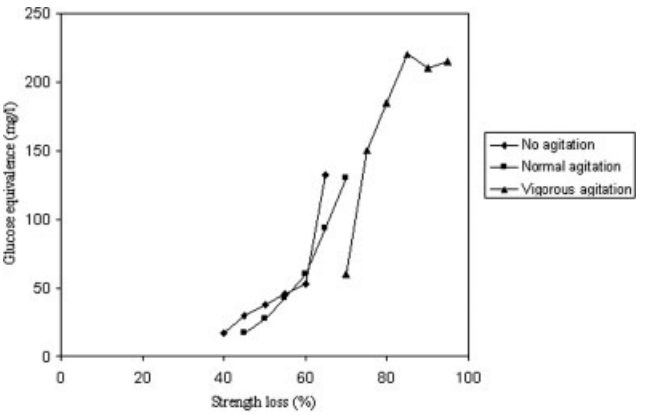


**Figure 5** Relationship between sugar liberated and strength loss after cellulase treatment under different agitation conditions (with analytical tolerance limit to 5%).

tinct. Therefore, providing that agitation during the enzymatic treatment is not too excessive, which may lead to extensive fiber loss and fiber breakage, the amount of sugar liberated during enzymatic treatment can be used to determine the effectiveness of cellulase treatment and can be used as an indicator on the extent of enzymatic hydrolysis.

### Weight loss after enzymatic treatment

Cellulase treatment under agitation, especially under excessive condition, often resulted in fiber loss due to the breaking-off of the weakened fibers formed by the effect of enzymatic hydrolysis. The weight loss during enzymatic treatment could therefore be used as a measure of the extent of the enzymatic hydrolysis. Figure 6 showed the effect of cellulase concentration on weight loss of linen under different agitation condition. Under normal agitation and agitation-free condition, the weight loss was comparatively small and was fairly constant even with an increased cellulase concentration. However, with reference to Figure 2, it could be observed that the amount of sugar liberated increased consistently with increasing cellulase concentration. It was also noted that there was an excessive weight loss when linen was treated under vigorous agitation. Thus, the amount of weight loss was a function of mechanical agitation during the enzymatic treatment and was of little relation to the extent of enzymatic hydrolysis.

This was further illustrated that in Figure 7 the relationship between the weight loss and strength loss. It could be seen that the strength loss was not closely related to the weight loss. Under normal agitation and agitation-free condition, the weight loss percentages only ranged from 0.5 to 2.0% and yet the strength loss had already over 50%. With vigorous agitation, a much higher strength loss was evident with a corresponding increase in weight loss. Apparently, the effect of enzymatic hydrolysis could not accurately be reflected by the weight loss arising from cellulase treatment.

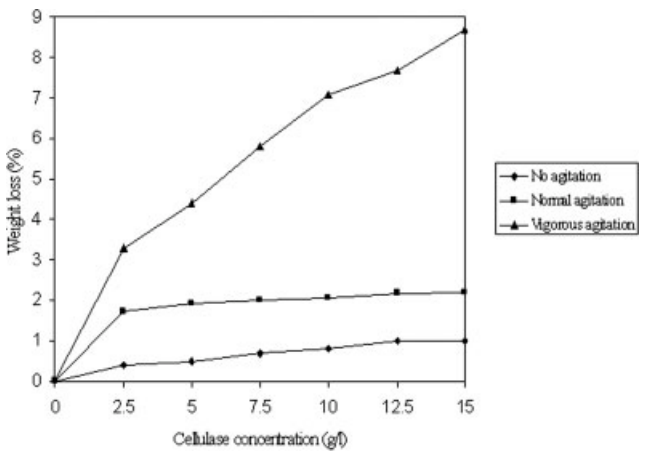


**Figure 6** Weight loss of linen under different agitation conditions (with analytical tolerance limit to 5%).

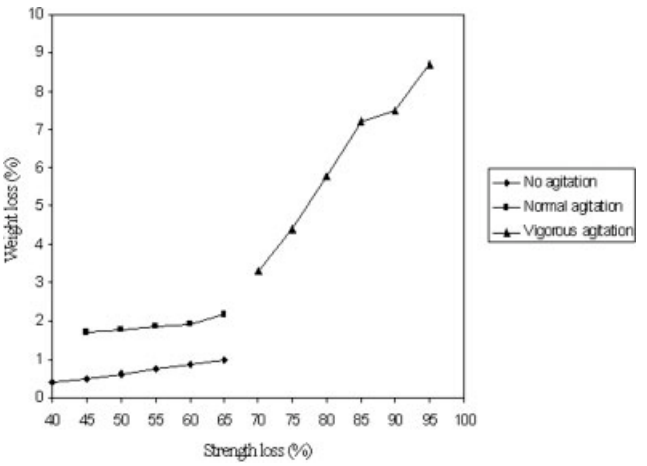


**Figure 7** Relationship between weight loss and strength loss of linen after cellulase treatment under different agitation conditions (with analytical tolerance limit to 5%).

## CONCLUSIONS

The phenol-sulfuric acid method was selected for the determination of sugar liberated during enzymatic hydrolysis of cellulose with cellulase and was found to be sensitive and consistent in representing the degree of hydrolysis of the linen fiber under test.

The weight loss resulted from cellulase treatment could not truly reflect the extent of enzymatic hydrolysis because it is highly dependent on the degree of agitation or mechanical action. When cellulase treatment was carried out under agitation-free condition, weight loss recorded is very small (less than 1%) and yet strength deterioration was still relatively high. Determination of the amount of sugar liberated during the cellulase treatment is more reliable in expressing the effectiveness of hydrolysis because it is less dependent on the degree of agitation. The possible deterioration in tearing strength of the material can also be predicted.

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