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Enzymatic Pre-Treatment of Cotton. Part 2: Peroxide Generation in Desizing Liquor and Bleaching

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Abstract
The objective of this study was to utilise desizing liquors of starch-sized fabrics using glucoseoxidase enzymes for bleach to produce hydrogen peroxide from glucose units of the starch removed; glucoseoxidase enzymes are efficient only at high glucose doses. In the first part of this paper, glucose generation in a desizing bath was discussed and an optimum recipe was obtained with an amyloglucodidase/pullanase mixture enzyme. In this study, process optimisation for the glucose oxidase enzyme was undertaken in order to generate hydrogen peroxide in the desizing liquor and then bleaching with the peroxide generated. Results indicated that sufficient hydrogen peroxide, about 800 mg l^{-1}, could be generated to perform successful enzymatic bleaching; however, the bleaching was compatible with the conventional peroxide type only in the alkali pH range. The maximum whiteness obtained by enzymatic treatment was 73.8 Stensby degree, whereas the whiteness of the conventionally treated fabric was 79.4 Stensby degree.

Key words: *enzymatic bleaching, cotton, glucose, glucose oxidase, peroxide, whiteness.*

Introduction

This paper reports the second stage of a series of experiments conducted to investigate the performance of the pre-treatment of cotton undertaken entirely with enzymes. An amyloglucodidase/pullanase mixture enzyme was used for desizing and producing glucose in a desizing bath, and a glucose oxidase enzyme was used to produce hydrogen peroxide from this glucose. Enzymes, desizing and enzymatic bleaching was reported in the first part but not here.

Experimental

Material

The fabric used was a plain weave 100% cotton raw fabric with surface density of 175 g m^{-2} with equal weft and warp counts of Nm16 and densities of 14 ends cm^{-1}. The fabric was sized with a 100% starch sizing agent, and 4% (owf) starch was present on the sized fabric.

The fabrics were desized with the amyloglucodidase/pullanase mixture enzyme in a desizing bath to produce glucose, as described in part 1 of this study. Glucose containing desizing liquors and fabrics were used in this study.

Glucose oxidase (EC 1.1.3.4) from Aspergillus niger (Biozymes) was used for peroxide generation.

The acetic acid, sodium hydroxide and sodium acetate used were products of Merck. The *D-(+)* Glucose was a product of Sigma.

A peroxide activator was used in the acidic and neutral bleaching baths. The activator was an anionic, slightly yellow liquid consisting of a combination of organic and inorganic buffering compounds, a product of BASF-Germany. A peroxide stabiliser, a sequestering/dispersing agent, was used in the alkali bleaching baths, a product of Eksoy Chemicals-Turkey.

Method

Process optimisation was made to generate hydrogen peroxide in the desizing liquor using the glucose oxidase (GOx) enzyme. Bleaching trials with the generated hydrogen peroxide were then performed at three different pH values:

1. Acidic pH range: the pH 4 ± 01, adjusted during desizing without any further adjustment after peroxide generation. Bleaching was performed at 85 °C for 60 min, in which a peroxide activator (10% owf) was used.
2. Neutral pH range: the pH was adjusted to a neutral range using NaOH after peroxide generation with the glucose oxidase enzyme. Bleaching was performed at 85 °C for 60 min, in which the peroxide activator (10% owf) was used.
3. Alkali pH range: the pH was adjusted to the alkali range (pH 9 - 12) using NaOH after peroxide generation with the glucose oxidase enzyme. Bleaching was performed at 98 °C for 60 min, in which a peroxide stabiliser (1.5 ml l^{-1}) was used.

The breaking load and water absorbency of the fabrics were determined according to the standard methods AATCC 173 and DIN 53924, respectively. Whiteness measurements were made via a Macbeth MS2020 spectrophotometer.

Results and discussion

Peroxide generation by GOx

pH optimisation for GOx

The glucose oxidase enzyme was added to the desizing liquor after desizing. The pH of the desizing liquors were adjusted to pH 4.1 using 2.25 ml l^{-1} of acetic acid, which remained almost unchanged during the desizing. Consequently, the initial pH of the liquor before the addition of GOx was about pH 4.1.

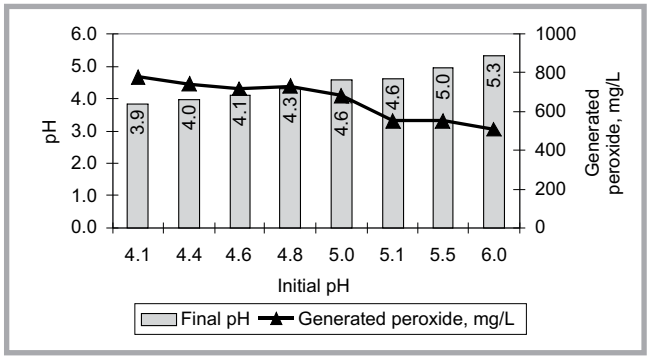

Figure 1. *pH dependence of peroxide generation using a glucoseoxidase enzyme. (Process parameters: enzyme 0.01%, sodium acetate 2 g l-1, 55 °C, 45 minutes).*

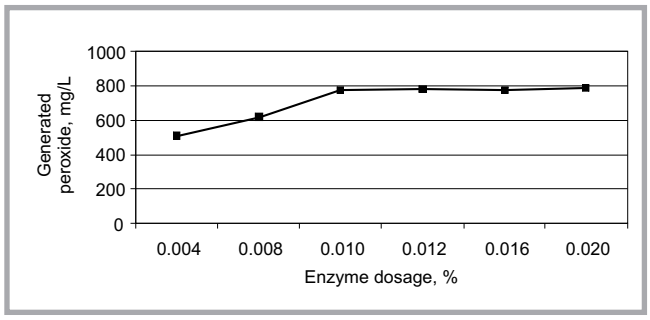

Figure 2. *Enzyme dosage dependence of peroxide generation using a glucoseoxidase enzyme. (Process parameters: sodium acetate 2 g l-1, 55 °C, 45 minutes).*

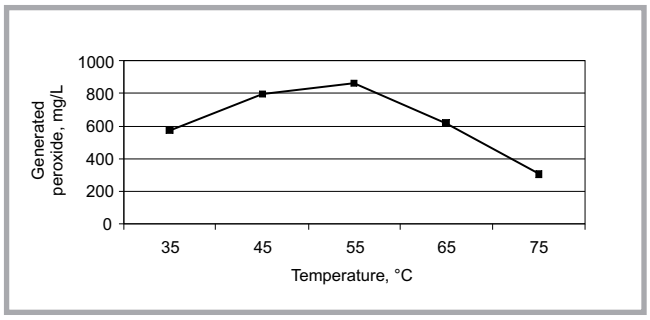

Figure 4. *Temperature dependence of peroxide generation using a glucoseoxidase enzyme. (Process parameters: enzyme 0.01%, sodium acetate 2 g l-1, 45 minutes).*

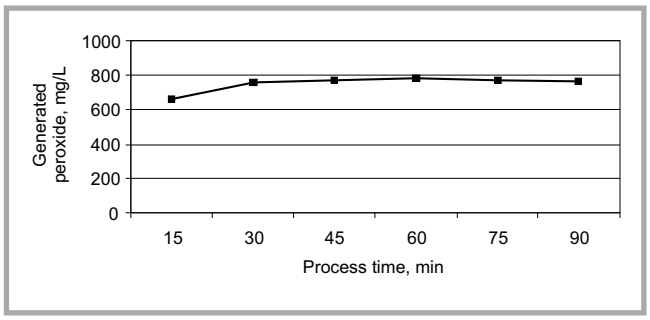

Figure 3. *Process time dependence of peroxide generation using a glucoseoxidase enzyme. (Process parameters: enzyme 0.01%, sodium acetate 2 g l-1, 55 °C).*

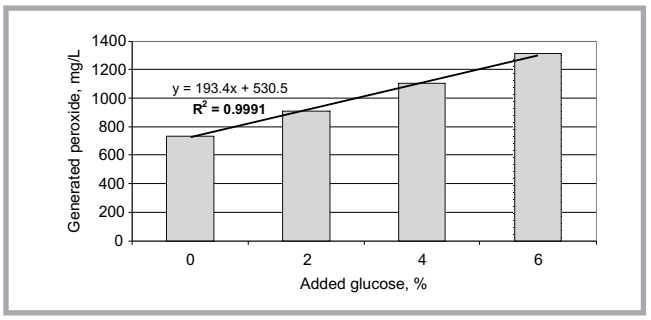

Figure 5. *Peroxide generation using a glucoseoxidase enzyme after the addition of glucose . (Process parameters: enzyme 0.01%, sodium acetate 2 g l-1, 55 °C, 45 minutes).*

The pH effect was tested between pH values of 4 - 6 to see the effect of the pH on peroxide generation. Different amounts of soda was added to the desizing liquors before the addition of GOx to maintain the pH of the glucose generation bath. Results in ***Figure 1*** show that the maximum amount of peroxide was generated at pH 4.1; the pH adjusted during desizing. Therefore the pH of the desizing liquor did not change during the peroxide generation experiments; only the use of 2 g l^{-1} sodium acetate was involved in order to prevent a pH decrease.

Enzyme dosage optimisation for GOx

The pure GOx enzyme had a very high activity (1 million U g^{-1}), thus very low enzyme amounts were tested. ***Figure 2*** illustrates the minimum enzyme dosage necessary - 0.01% (owf). Increasing the enzyme dosage did not change the amount of peroxide generated. Therefore, the appropriate enzyme dosage for the GOx enzyme was chosen as 0.01% (owf).

Process time optimisation for GOx

A set of trials were performed for 90 minutes with 15 minute intervals to investigate the effect of the process time on peroxide generation. The results shown in ***Figure 3*** indicate an optimum process time of 45 minutes, where a prolonged process time did not contribute to peroxide generation.

Temperature optimisation for GOx

The optimum temperature for peroxide generation in a desizing bath was found to be 55 ºC, shown in ***Figure 4***. The optimum recipe was obtained for the GOx enzyme (glucose oxidase) as a result of the optimisation trials made, which was the following:

- 0.01% (owf) enzyme
- 2 g l^{-1} acetate (pH of the desizing liquor 4.1)
- 55 °C, 45 minutes.

The optimum recipe was tested by directly adding glucose to the liquor. The results are illustrated in ***Figure 5***. Glucose was added to the desizing bath, which already contained glucose generated from the starch during desizing. Therefore, all the liquors initially contained approximately 4000 mg l^{-1} of glucose produced in the desizing bath, and glucose additions (2 - 4 - 6%) were made to those liquors. The increase in peroxide by adding glucose is shown in ***Figure 5***, which shows that the glucose in the liquor is effectively converted into hydrogen peroxide. The amount of peroxide generated drastically increased after further additions of glucose. The linearised line in ***Figure 5***, with $R^2 = 0.9991$, shows a direct proportion between the amount of glucose and peroxide generated.

Bleaching with the glucose generated

Bleaching at the acidic pH range

The aim of these trials was to check the effectiveness of the process without any pH changes at every stage of the pre-treatment. The pH of the liquor was adjusted before desizing, and no further adjustment was made during the whole pre-treatment, only a 2 g l^{-1} buffer was added before the GOx treatment, during the set of trials conducted at the acidic pH range. An unchanged pH has the advantage of a lower chemical (acid/alkali) consumption and easier process. However, the whiteteness degrees were not high enough at the acidic pH range. The results are given in ***Table 1***.

The whiteness degrees of the untreated fabric and conventionally prepared fabric were given as control. Bleaching trials were also conducted with glucose added to the desizing liquors; the corresponding peroxide amounts are given in ***Figure 5***. Results indicate a poor bleaching effect when compared to conventional preparation. An increase in the whiteness degree of the fabric was observed after the addition of glucose, the reason for which was the increase in peroxide generated in the desizing bath (***Figure 5***). However,

whiteness degrees were still poor even after the addition of glucose.

The reason for the low whiteness was thought to be the activation problem of peroxide, not the amount of peroxide generated. Hydrogen peroxide is not an active bleaching agent for cellulosic fibers in acid or neutral solutions because the liberation of perhydroxl ions is too slow; but excessive alkali will cause instability accompanied by decomposition with the evolution of oxygen. A suitable activator is used for bleaching with hydrogen peroxide in a neutral or weakly acidic medium [1 - 3]. Consequently, new sets of trials were conducted at neutral and alkali pH ranges

Bleaching at the neutral pH range

The initial pH 4.1 ± 0.1 of the liquor after peroxide generation using the GOx enzyme was adjusted to neutral by NaOH (38 Bé) titration, the amounts of peroxide present were measured and bleaching trials were conducted. The results given in ***Table 2*** show a minor increase in the Stensby whiteness degrees; however, the effect was still too far from that achieved by conventional preparation. The reason was assumed to be the activation problem of peroxide, as described above.

Bleaching at the alkali pH range

The best whiteness results with the enzymatically generated peroxide were achieved at the alkali pH range, the results of which are given in ***Table 3***. Whiteness degrees increased to over 70 in the alkali baths. The maximum whiteness index of 73.8 was reached at pH 11.95. A progress of early 10 Stensby degrees was achieved when compared to the trials in acid and neutral baths.

The whiteness index of the raw fabric increased to 73.8 from 52.0 after the enzymatic pre-treatment. This value was still lower than the value of 79.4 achieved by conventional pre-treatment, which is, however, very promising. The lower values of the enzymatic bleaching, when compared to conventional bleaching, can be attributed to the peroxide consumption of the impurities in the treatment bath. The enzymatic treatment bath was more contaminated than the conventional bleaching bath because the desizing bath, containing impurities, was utilised during the enzymatic bleaching, although fresh water was used during the conventional bleaching. A similar conclusion was made in an earlier study to explain the lower whiteness degree, concluding that the single step procedure was not

Table 1. *Stensby whiteness index values of the fabrics after bleaching at the acidic pH range. (Process parameters: pH 4.1±0.1 remained unchanged after peroxide generation using glucoseoxidase, peroxide activator 10% , 85 °C, 60 minutes; conventional preparation: desizing with thermo-stable α-amylase 0.2%, 90°C, 20 min, pH 7; bleaching hydrogen peroxide 3 ml l^{-1} , peroxide stabilisers 0.5% , 98 °C, 60 min, pH 11);* The amounts of peroxide generated in the bleaching bath is given in Figure 5.*

Stensby whiteness					
Controls		Bleached fabrics with and without glucose addition*			
Raw fabric	Conventionally prepared	---	+2%	+4%	+6%
52.0	79.4	59.7	60.9	62.2	63.1

Table 2. *Stensby whiteness index values of the fabrics after bleaching at a neutral pH range. (Process parameters: the initial pH of 4.1±0.1 of the liquor was adjusted to neutral by NaOH (38 Bé) titration, peroxide activator 10%, 85 °C, 60 minutes; conventional preparation: desizing with thermo-stable α-amylase 0.2% 90°C, 20 min, pH 7; bleaching with hydrogen peroxide 3 ml l^{-1} , peroxide stabiliser 0.5% , 98 °C, 60 min, pH 11).*

Quantity	Controls		Bleaching baths with generated peroxide					
	Raw fabric	Conventionally prepared						
Peroxide amount, mg l^{-1}	-	-	725	700	660	700	690	685
pH	-	-	7.13	6.85	6.95	6.90	6.85	6.80
Stensby whiteness	52.0	79.4	65.0	64.9	63.9	64.7	64.7	64.6

Table 3. Stensby whiteness index values of the fabrics after bleaching at the alkali pH range. (Process parameters: the initial pH of 4.1±0.1 of the liquor was adjusted to neutral by the addition of NaOH (38 Bé) , peroxide stabiliser 0.5%, 98 °C, 60 minutes; conventional preparation: desizing thermo-stable α-amylase 0.2% 90°C, 20 min, pH 7; bleaching hydrogen peroxide 3 ml l^{-1} , peroxide stabiliser 0.5% , 98 °C, 60 min, pH 11).

Fabric	NaOH (38 Bé) amount, ml l^{-1}	pH	Peroxide amount, mgl^{-1}	Stensby whiteness
Raw fabric	**-**	**-**	**-**	**52.0**
Conventionally prepared	**-**	**-**	**-**	**79.4**
Trial 1	5	9.65	710	64.1
Trial 2	6	10.62	700	71.0
Trial 3	7	11.95	720	73.8
Trial 4	8	12.10	690	71.3

able to achieve the whiteness of the fabric treated with the conventional bleaching process, which was probably due to the contamination of the desizing/scouring/bleaching bath reused, as well as to the decrease in the bleaching power of the peroxide [4].

The decrease in trial 4, despite the increase in pH (***Table 3***), was attributed to two factors: the first factor is the lower amount of peroxide in the bleaching bath, and the second is the rapid decomposition of peroxide over pH 12 [1].

Comparison of the strength and water absorbency properties

Comparisons were made only for the alkali range since the whiteness values of the aci and neutral range were inadequate. Results are given in ***Table 4***. The breaking load did not show considerable variation during the various treatments.
The water absorbency of the conventionally treated fabric was higher than that of the enzymatically treated ones. The conventional process included two baths (desizing-bleaching was separate), although the enzymatic treatment was completed in one bath; this probably caused a higher extraction of hydrophobic impurities.

Conclusions

Approximately 800 mg l^{-1} of hydrogen peroxide was generated in a desizing bath of starch sized cotton fabric using a glucoseoxidase enzyme from approximately 4000 mg l^{-1} of glucose, generated in the desizing bath using an amyloglucodidase/pullanase mixture enzyme before peroxide generation.

The hydrogen peroxide generated was utilised in bleaching with three different recipes at acidic, neutral and alkali pH values. The best Stensby whiteness index values were obtained at alkali pH. The Stensby whiteness index values of the raw fabric increased to over 70 from 52 after the enzymatic pre-treatment. The Stensby whiteness index values of

Table 4. Breaking load and water absorbency values of the raw, conventioanlly pre-treated and enzymatically pre-treated fabrics. (Conventional preparation: desizing, thermo-stable α-amylase 0.2%, 90 °C, 20 min, pH 7; bleaching with 3 ml l^{-1} of hydrogen peroxide, peroxide stabiliser 0.5%, 98 °C, 60 min, pH 11; trials 1 - 4: details were given in Table 3).

Fabric	Breaking load, N mm^{-2}	Water column, mm
Raw fabric	22.2	0
Conventionally prepared	15.6	66.5
Trial 1	15.6	61.3
Trial 2	16.9	63.3
Trial 3	16.0	64.0
Trial 4	16.4	63.7

the conventionally pre-treated fabric was 79.4 and could not be reached by enzymatic treatment. This was explained by the peroxide consumption of impurities during enzymatic bleaching.

The ttrength properties of the fabric did not vary in the conventional and enzymatic treatments. The water absorbency of conventionally treated fabric was a little higher due to the use of two baths during the conventional treatment, although enzymatic treatment was completed in one bath.

These results, together with the results of the first part of the paper, showed the promising success of entirely enzymatic pre-treatment. More research has to be done to increase the whiteness degrees of enzymatically treated fabrics to the levels of conventional treatment. Commercially available glucose oxidase enzymes with higher activities and easier to handle is also a problem to be solved.

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