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Enzymatic Pre-Treatment of Cotton. Part 1. Desizing and Glucose Generation in Desizing Liquor

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Abstract
The objective of this study was to substitute cotton pretreatment chemicals with enzymes to create an environmentally friendly process for water and energy savings. In this study, enzyme selection and process optimisation was made in order to increase the glucose content of the desizing liquor of a starch-sized cotton fabric. Results indicated that commercial desizing enzyme formulations of α-amylase enzymes were not appropriate to produce a large quantity of glucose in the desizing bath; the glucose amounts obtained were about 200 mg/l. However, the food market enzyme used, an amyloglucodidase/pullanase mixture (amyloglucosidase 186 Units/g, pullanase 395 Units/g), produced approximately 4000 mg/l glucose in the desizing bath after process optimisation. The desizing effect of the amyloglucodidase/pullanase enzyme mixture was compatible with those of commercial desizing enzymes. The results of peroxide generation from glucose and bleaching trials are reported in the second part of the study.

Key words: *desizing, starch, α-amylase, amyloglucosidase, glucose, enzymatic bleach.*

Introduction

Starch is widely used as a sizing agent, being readily available, relatively cheap and based on natural, sustainable raw materials [1]. 75% of the sizing agents used worldwide are starch and its derivatives.[2]

Using amylase enzymes for the removal of starch sizes is one of the oldest enzyme applications.[1, 3-4] Amylases are enzymes which hydrolyse starch molecules to give diverse products, including dextrins and progressively smaller polymers composed of glucose units [5]. These partly degraded oligosaccarides can not be reused [2] and are usually discharged, contributing large amounts of Chemical Oxygen Demand (COD) and Biological Chemical Oxygen Demand (BOD) to effluent streams [6, 7]. 50-80% of the COD in the effluents of textile finishing industries is caused by sizing agents [2].

There are basically four groups of starch-converting enzymes: (i) endoamylases; (ii) exoamylases; (iii) debranching enzymes; and (iv) transferases. Endoamylases are able to cleave α,1-4 glycosidic bonds present in the inner part of the amylose or amylopectin chain. α-Amylase is a well-known endoamylase. The end products of α-amylase action are oligosaccharides. Enzymes belonging to the second group, the exoamylases, either exclusively cleave α,1-4 glycosidic bonds such as β-amylase, or cleave both α,1-4 and α,1-6 glycosidic bonds like amyloglucosidase or glucoamylase. Exoamylases act on the external glucose residues of amylose or amylopectin and thus produce only glucose (glucoamylase and α-glucosidase), maltose or dextrin (β-amylase). The third group of starch-converting enzymes are the debranching ones that exclusively hydrolyse α,1-6 glycosidic bonds: isoamylase and pullanase. The fourth group of starch-converting enzymes are transferases, which cleave the α,1-4 glycosidic bond of the donor molecule and transfer part of the donor to a glycosidic acceptor with the formation of a new glycosidic bond [8].

An enzymatic process is proposed to utilise desizing baths for bleaching in which glucose oxidase (GOx) enzymes generate hydrogen peroxide and gluconic acid using glucose as a substrate [9 - 11]. Advantages of the process are reducing the COD of the effluents by degrading glucose units, and reducing the use of peroxide stabilising agents with the help of gluconic acid, which is capable of complexing catalysts as well as saving water and energy by using desizing liquor for bleach [2, 9 - 12]. However, starch has to be degraded with glucose units in order to achieve process efficiency because of the high substrate selectivity of GOx enzyme. Conventional commercial desizing enzymes do not seem appropriate for this purpose since most include α-amylase in formulations [1, 4, 13 - 14], whereas amyloglucosidases are suitable amylase enzymes to degrade starch until it becomes glucose [2, 9 - 11].

The performance of an amyloglucosidase/pullanase mixture commercial enzyme used in the food industry to produce glucose syrup from corn starch was examined in this study, analysing the success of pure amyloglucosidase enzymes reported in former studies [2, 11].

Experimental

Material

The fabric used was a plain weave 100% raw cotton fabric with a mas per square meter of 175 g/m^2 with equal weft and warp counts of 62.5 tex and densities of 14 ends/cm. The fabric was sized with a 100% starch sizing agent and 4% (owf: over the weight of the fabric) starch was present in the sized fabric. 9 conventional commercial desizing enzymes of classical and thermo-stable types from 5 suppliers and 1 commercial enzyme used in the food industry to produce glucose syrup from corn starch was used in the experiments (***Table 1***).

Method

Desizing trials were performed according to the recipes listed in Table 1 with fabric specimens of 20 grams (approximately 30 × 30 cm^2) at a liquor ratio of 1:10 using distilled water. The process time for the commercial desizing enzymes was prolonged to 90 minutes (30-60-90 minutes) in order to investigate any further increase in the glucose content. Optimisation trials were performed for the amyloglucosidase/pullanase mixture.

Iodine tests were performed to determine the residual starch on the fabrics after desizing.

Glucose amounts of desizing liquor were measured by use of a Thermo-Trace GOx solution. The composition of this solution

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is as follows: glucoseoxidase > 15,000 U/l, peroxidase > 100 U/l, 4-aminoantiprin – 0.5 mmol/l, 4-hydroxybenzoic acid - 10 mmol/l, phosphate - 119 mmol/l and stabiliser. The parameters were:

Temperature:
37 °C

Primary wavelength:
500 nm (460-560 nm)

Secondary wavelength:
600-660 nm

Desizing liquor : GOx solution ratio
1:150

Incubation time:
5 minutes

Sensibility:
9-35 mmol/l (0-6300 mg/l)

Reactions can be written as:

1- Glucose + O_2 + H_2O →
→ Gluconoic acid + H_2O_2

2- H_2O_2 + *HBA* + 4-*AAP* →
→ Quinoneimine dye + H_2O

HBA = 4-Hydrobenzonoic acid

4-*AAP = 4-aminoantiprin*

The absorbance of the solution was measured using a spectrophotometer with a 460 - 560 nm interval; the darker the color, the greater the glucose amount. The absorbance of the desizing liquor was compared to the absorbance of standard glucose solution (5.55 mmol/l). The Glucose content of the desizing liquor was calculated by with formula $G_d = (A_d/A_s)\cdot G_s$, where G_d and A_d are the glucose amount (mg) and absorbance of the desizing liquor, and G_s and A_s are those of standard glucose solution. The calibration curve obtained is shown in ***Figure 1***.

Results and discussion

Table 2 compares the glucose generation and desizing effect of the enzymes used. Results indicate an acceptable desizing effect but very low glucose generation for α-amylases. The amount of hydrogen peroxide required to obtain a satisfactory whiteness is reported to be 400 - 600 mg/l, requiring a glucose amount of approximately 4,000 mg/l in the desizing bath for hydrogen peroxide generation of GOx [10]. The amounts of glucose reported in Table 2 for α-amylases were not enough even for prolonged processing times. The low glucose generation can be attributed to the reaction mechanism of α-amylases, which are endoamylase enzymes and do not involve the degradation of starch until single glucose units exists, despite their well-known and satisfactory desizing effect [1 - 7].

Table 1. *Enzyme types and desizing recipes recommended by manufacturers; CD: Commercial Desizing enzyme, [a] - Amyloglucosidase 186 Units/g, pullanase 395 Units/g. [b] - Commercial enzyme for food industry, no data available for desizing process.*

Enzyme Type	pH	Dosage	Temp. °C	Time, min.	Supplier
CD1- α-amylase	6 - 7	0.25-1.3 g/l	70	30	Novozymes, Bagsvaerd, Denmark.
CD2- α-amylase	6 - 7	0.06-0.3 g/l	70 - 110	30	
CD3- α-amylase	6 - 7	0.5-2 g/l	90 - 98	30	R-Duraner, Bursa, Turkey.
CD4- α-amylase	6 - 7	0.2-0.4 g/l	90 - 98	30	
CD5- α-amylase	6.5	0.02-0.05 g/l	80 - 90	30	AB Chem., Bursa, Turkey.
CD6- α-amylase	7 - 7.5	0.05-0.2 %	90 - 95	10 - 20	Gemsan, Istanbul, Turkey.
CD7- α-amylase	6.5 - 7	0.05-0.2 %	50 - 70	20 - 30	
CD8- α-amylase	5.4 - 8	1.2 g/l	30 - 60	30	CHT, Istanbul, Turkey.
CD8- α-amylase	5.4 - 8	0.5-2 g/l	60 - 100	30	
AMG-Amylo-glucosidase/ pullanase mixture[a]	4.1 - 4.3[b]	- [b]	60 - 63[b]	- [b]	Novozymes, Bagsvaerd, Denmark.

Table 2. *Glucose generated in desizing liquor during the desizing process; CD: Commercial Desizing enzyme, AMG: Amyloglucosidase/pullanase enzyme mixture.*

Enzyme	pH	Dosage	Temp., °C	Generated glucose, mg/l			Iodine test
				30 min.	60 min.	90 min.	
CD1	6.5	1.3 g/l	70	200	210	205	7 - 8
CD2	6.5	0.3 g/l	90	210	205	210	7 - 8
CD3	6.5	2.0 g/l	90	180	200	190	7 - 8
CD4	6.5	0.4 g/l	100	213	200	208	7 - 8
CD5	6.5	0.05 g/l	85	180	200	190	7 - 8
CD6	7.0	0.2%	90	180	190	185	7 - 8
CD7	6.5	0.2%	60	190	185	200	6 - 7
CD8	6.5	2.0 g/l	50	205	220	208	6 - 7
AMG	4.1	0.25%	62	3116	3920	3606	6 - 7

Amyloglucosidases, exoamylases and pullanases are debranching enzymes that produce starch degradation until single glucose units, as described before [8, 10]. The results reported in ***Table 2*** for amyloglucosidase (AMG) enzyme conformed this hypothesis, indicating a great increase in the amount of glucose: an average glucose generation of 199.8 mg/l for α-amylases and 3,544 mg/l for theamyloglucosidase/pullanase mixture.

Iodine test results indicate a sufficient desizing performance for AMG enzyme comparable to that of α-amylases.

Despite the increase in glucose amount while using the AMG enzyme, the glucose amounts were still under the required level of approximately 4,000 mg/l [10]. Recommended process parameters for the AMG enzyme were not available for desizing, but pH 4.1 - 4.3 and temperature of 60 - 63 °C have been applied to produce glucose syrup from corn starch in food industry. A set of trials were performed to find optimum circumstances to generate maximum glucose during the desizing process.

Above all it has to be stated that the acid consumption of untreated cotton fabrics

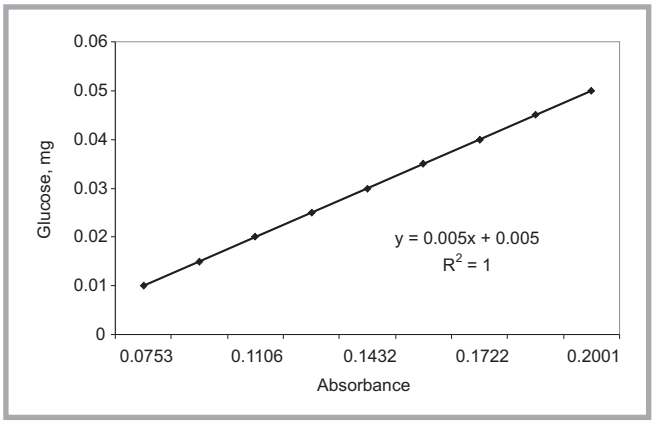

Figure 1. *Calibration curve obtained for a standard glucose solution to determine the amount of glucose in desizing liquor using a Thermo-Trace GOx kit.*

was found to be a very important factor and was considered during the trials (***Table 3***).

Table 3 shows the increase in liquor pH after 10 minutes of circulation with the fabric in the liquid. According to these results, a process of ten minute's circulation and re-adjustment of the pH before the addition of enzyme was applied in our experiments.

Enzyme dosage optimisation for AMG

Figure 2 illustrates the minimum necessary enzyme dosage - 0.5% (o.w.f.). However, increasing the enzyme dosage to 0.75% (o.w.f.) resulted in higher glucose amounts than 0.5% (o.w.f.). An appropriate enzyme dosage of AMG enzyme 0.75% (o.w.f.) was chosen from these results.

Process time optimisation for AMG

Since there were no data available concerning the use of AMG enzyme in the desizing process, the effect of the process time was also examined to a large scale in order to find optimum circumstances. Trials were performed for 90 minutes with 15 minute intervals.

The results illustrated in ***Figure 3*** indicated an optimum process time of 45 minutes, where a prolonged process time did not contribute to glucose generation.

pH optimisation for AMG

Although a pH of 4.1 - 4.3 was recommended as appropriate by the manufacturer of the enzyme, this pH was recommended for glucose syrup production from corn starch in food industry; however it lacked optimisation for desizing purposes. A set of trials were performed to find the optimum pH to generate maximum glucose during the desizing process.

Figure 4 illustrates that the optimum pH for glucose generation in desizing bath is pH 4.1, requiring an acetic amount of 2.25 ml/l.

Optimum circumstances obtained for the AMG enzyme (amyloglucosidase/ pullanase mixture) as a result of the trials made are:

- 0.75% (o.w.f.) enzyme,
- 2.25 ml/l of acetic acid (pH 4.1),
- at 62 °C, for 45 minutes.

The optimum recipe attained was tested by directly adding starch to the liquor, the results of which are illustrated in ***Figure 5*** where a linear relation between the amount of starch and generated glucose is observed. The linearized line with $R^2 = 0.9779$ indicated a direct proportion between the amount of starch and generated glucose.

Table 3. *Acid consumption of the untreated test fabrics. (Treatment: Blank dyeing at 45 °C for 10 minutes after acid addition).*

Acetic acid, ml/l	Initial pH	Final pH
1.00	3.50	4.55
1.25	3.45	4.45
1.50	3.38	4.40
1.75	3.28	4.25
2.00	3.21	4.19
2.25	3.18	4.17
2.50	3.16	4.14
2.75	3.16	4.10
3.00	3.15	4.03
3.25	3.14	4.00
3.50	3.12	3.96
3.75	3.10	3.98
4.00	3.08	3.94

Conclusions

The performance of a commercial enzyme (an amyloglucosidase/ pullanase mixture) was tested and process optimisation trials were performed. Optimum circumstances obtained were: 0.75% (o.w.f.) enzyme, pH 4.1, 62 °C and a process time of 45 minutes. Acid consump-

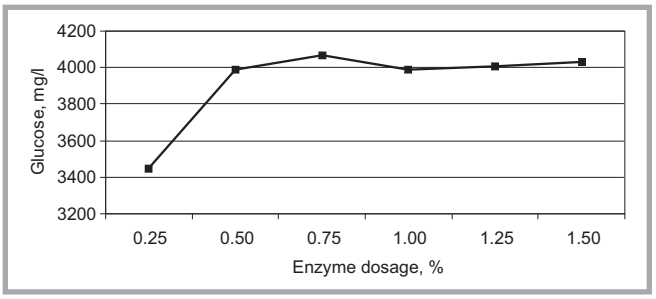

Figure 2. *Enzyme dosage dependency of glucose generation by the amyloglucosidase/pullanase enzyme mixture; Process parameters: pH 4.1 (acetic acid), 62 °C, 30 minutes.*

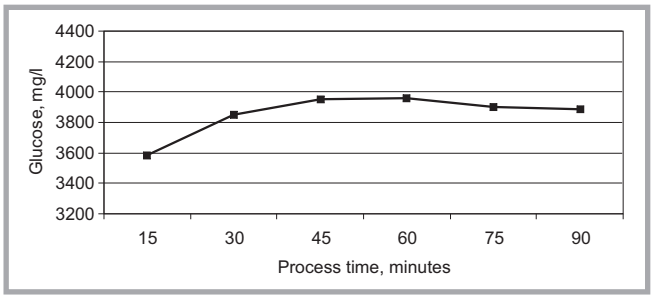

Figure 3. *Process time dependency of glucose generation by the amyloglucosidase/pullanase enzyme mixture; Process parameters: 0.75% enzyme, pH 4.1 (acetic acid), 62 °C.*

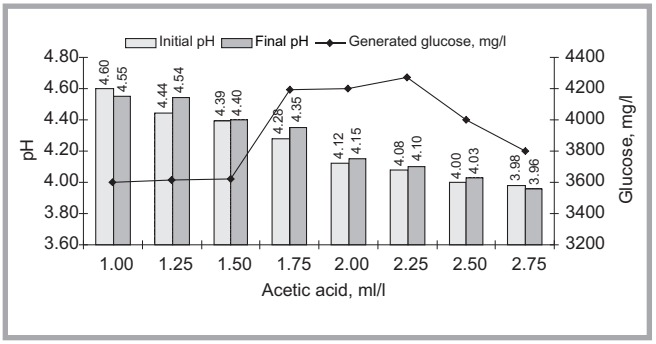

Figure 4. *pH dependency of glucose generation by the amyloglucosidase/pullanase enzyme mixture; Process parameters: 1% enzyme, 62 °C, 30 minutes.*

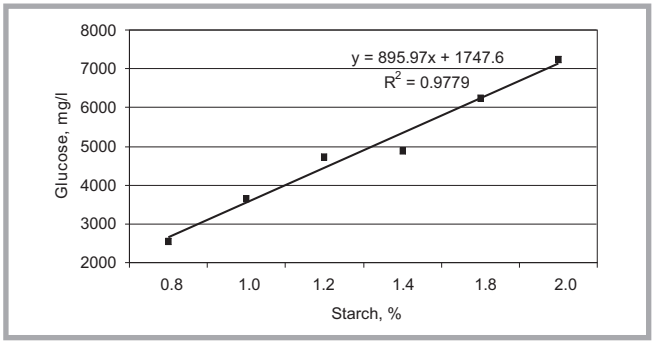

Figure 5. *Glucose generation by the amyloglucosidase/pullanase enzyme mixture after starch addition. (Process parameters: 0.75% enzyme, pH 4.1(acetic acid), 62 °C, 45 minutes).*

tion of the raw cotton fabric played an important role in pH adjustments.

Similar results were reported using pure amyloglucosidase enzymes [10]; however, storage and usage properties as well as the availability of pure enzymes may cause problems when compared to commercial ones. A commercial enzyme readily used in the food industry was utilized in this study.

The sufficiency of the generated glucose to utilise the desizing liquor for bleaching was tested by using glucose oxidase enzyme to produce hydrogen peroxide from this glucose. Bleaching trials were performed with the glucose generated. Compatible whiteness degrees were attained. Results are reported in the second part of the study.

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