

Use of Enzymes in the Pre-Treatment of Cotton

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Due to the ever-growing costs for water and energy worldwide investigations are carried out to substitute conventional chemical textile processes by environment-friendly and economically attractive bioprocesses using enzymes. Here, a successful strategy for the combined use of α -amylase, hemicellulase and pectinase in the pre-treatment of cotton is described. After hot bleaching enzymatically pre-treated cotton exhibits similar or even better properties compared to conventionally desized, alkaline scoured and bleached cotton. The combined use of the enzymes allows to omit the alkaline scouring without a loss of quality in the finishing result. The described enzymatic procedure is accompanied by a significant lower demand of energy, water, chemicals, time and therefore costs. So it has advantages as well in terms of ecology as in economy. Another field of investigations targets the subsequent bleaching process. The authors developed a new concept combining the use of two oxidoreductases in bleaching processes. Starting with glucose as substrate for glucose oxidase (GOD) hydrogen peroxide is generated in situ. The freshly built substrate H_2O_2 is used immediately by peroxidases (POD) to oxidize colored impurities on cotton yielding a higher degree of whiteness.

1. Introduction

The potential of the so-called "white biotechnology" as an ecological advantageous and moreover economical beneficial technology is beyond all question. Caused of the ever-growing costs for energy and polluted waste water, enzymatic technologies will stay in the focus of science and technique, and their relevance will increase significantly in the future. Enzymes, biological catalysts with high selectivities, have been used in the food industry for hundreds of years, and play an important role in many other industries (washing agents, textile manufacturing, pharmaceuticals, pulp and paper). Currently, enzymes are becoming increasingly important in sustainable technology and green chemistry. In the opinion of many experts and based on different studies, by 2010, 20 % of all chemical products in a dimension of 300 billion US dollar will be produced using biotechnology. This would represent a tenfold increase compared to 2001 [1-3].

Especially in textile manufacturing the use of enzymes has a long tradition. The enzymatic desizing of cotton with α -amylases is state-of-the-art since many decades [4]. Moreover, cellulases, pectinases, hemicellulases, lipases and catalases are used in different cotton pre-treatment and finishing processes [5]. Other natural fibers are also treated with enzymes. Examples are the enzymatic degumming of silk with sericinases [6], the felt-free-finishing of wool with proteases [7] or the softening of jute with cellulases and xylanases [8]. In future, also synthetic fibers such as polyester [9] or

polyacrylonitrile [10] will be modified by an enzymatic treatment. The application of enzymes has many advantages compared to conventional, non-enzymatic processes. Enzymes can be used in catalytic concentrations at low temperatures and at pH-values near to neutral. Their high substrate selectivity allows a very gentle treatment of the goods. Moreover, enzymes are biologically degradable and can be handled without risk [11,12].

Besides cellulose cotton contains in the so-called primary wall natural compounds such as pectins, hemicelluloses, proteins, waxes and lignin, which can impair the finishing results. In conventional pre-treatment these substances are removed by a strong alkaline treatment at high temperatures after the enzymatic desizing of raw cotton fabrics with α -amylases. This inspecific alkaline scouring process has a high energy, water and alkali consumption and can also cause a damage of the cellulosic material [13].

The aim of this work is the development of a comprehensive concept for the enzymatic pre-treatment of cotton to replace the alkaline scouring (**Figure 1**). The material should be desized and prepared for following bleaching and dyeing in a single step using α -amylases and further enzymes such as pectinases and hemicellulases. The renouncement of the alkaline scouring process leads to ecological and economic advantages for industrial users.

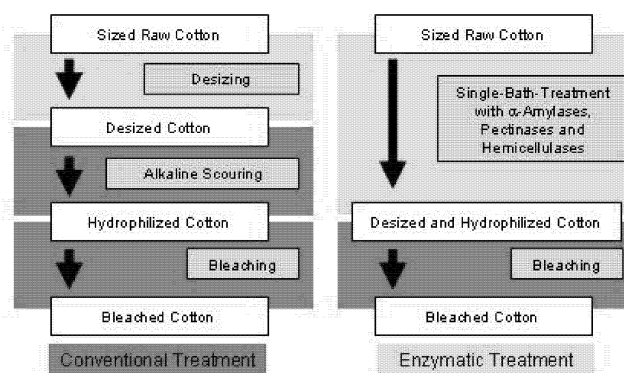


Figure 1: Processing steps for the conventional and enzymatic pre-treatment of cotton.

Moreover, investigations were carried out to substitute the subsequent hot and alkaline bleaching step by the use of oxidoreductases under moderate conditions. Former investigations were focussed on the enzymatic generation of hydrogen peroxide as bleaching agent with glucose oxidases (GOD) using sugar from desizing liquors [14-16]. This process failed in technical applications because of its general problems. At first, the used glucose oxidase has to be free of catalase, which is often found as a by-product in technical GOD preparations. This enzyme destroys any generated hydrogen peroxide immediately. Moreover, in bleaching processes the decomposition of hydrogen peroxide into radicals needs alkaline conditions at high pH-values and high temperatures - so the process has only a small benefit in terms of economy and ecology.

Another approach was to use peroxidases (POD), which are successfully applied in the decoloration of dyeing baths [17,18], for cotton bleaching [14]. While decoloration of dissolved dyestuffs works fast, in the two-phase system POD-solution/cotton the enzymatic oxidation of cotton impurities is significantly slower. During the process POD is attacked by hydrogen peroxide resulting in a high loss in activity.

Therefore, a new concept for a simultaneous application of glucose oxidase and peroxidase was developed as illustrated in **Figure 2**. Starting with glucose as substrate for the GOD hydrogen peroxide is generated in situ, which is immediately used by the POD to oxidize colored compounds in dyeing baths. In this way the stationary peroxide concentration is nearly zero during the whole process and the enzymes are not degraded by the oxidizing agent. Moreover, experiments are carried out to check if this two-compound-system is suitable for textile bleaching of natural cotton fibers.

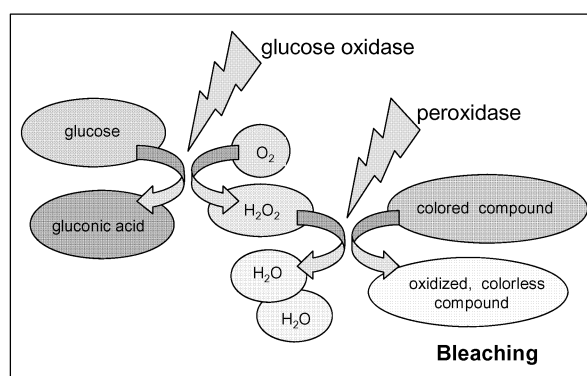


Figure 2: Use of oxidoreductases in bleaching processes.

2. Experimental

2.1 Combination of Desizing and BioScouring

A common industrial, sized cotton fabric with a starch layer of 8 wt.-% is used (187 g/m², width 160 cm). As Enzymes the α -amylase Beisol T 2090[®] (CHT, Tübingen, Germany) and the mixed hemicellulase/pectinase product Beisol DHP[®] (CHT) are used. All enzymes are industrial products. As auxiliaries the non-ionic surfactant Felosan Jet[®] (CHT), the biological degradable complexing agent Beixon NE[®] (CHT) and the buffer Neutracid NVM 200[®] (CHT) are used. After optimizing the process on lab scale industrial experiments are carried out at the "Textilveredlung an der Wiese" at Lörrach (Germany). Raw cotton is treated with enzymes via a cold pad-batch procedure. The conventional treatment (desizing with α -amylases) is followed by a common alkaline scouring (experiment C). The enzymatic treatment (experiment E) is done with a mixture of Beisol T 2090[®] and Beisol DHP[®] without following alkaline scouring. Finally the cotton is dried in a stenter frame. The differently treated materials are subsequently stitched together and bleached hot. All procedures correspond to conventional industrial practise.

2.2 Enzymatic Bleaching

The bleaching was done with a commercial desized and alkaline scoured cotton fabric. A nearly catalase-free GOD was delivered from Erbslöh (Geisenheim, Germany). Glucose was purchased from Fluka. POD is a commercial Baylase[®]RP system from Lanxess (Germany). The incubation investigations of the POD were done in combination with Baylase[®] Assist RP, which contains the hydrogen peroxide. Moreover, decoloration and bleaching experiments were done using a new chloro-peroxidase (CPO) from ASA-Spezialenzyme (Wolfenbüttel, Germany).

Decoloration of dyestuff with GOD, glucose and POD

20 mg Sirius Supra Blue[®]FGG 200 %, 1 mg GOD and 0.5 ml Baylase[®]RP respectively 0.5 ml CPO were added to 50 ml buffer pH 7 (T = 37 °C). The reaction was started by adding 125 mg glucose. After different times (range 0 min to 75 min) the absorption of the solutions was measured with an UV-Vis-spectrometer (Cary 5E).

Bleaching of cotton with GOD, glucose and POD

1 g cotton was stirred in 50 ml buffer with 50 mg GOD and 0.5 ml Baylase[®]RP respectively 0.5 ml CPO at 37 °C. The bleaching process was started by adding 600 mg glucose. After 90 min the cotton was removed and washed with distilled water.

3. Results

3.1 Combination of Desizing and BioScouring

It had to be examined, whether the alkaline scouring can be substituted by an alternative one-step enzymatically catalyzed process. After detailed preparatory work on lab scale industrial experiments are realized on sized raw cotton. The fabrics are wetted with an enzyme containing liquor (α -amylases, pectinases and hemicellulases, experiment E) with no subsequent alkaline scouring. PH-value is 5 due to the pH-optima of the enzymes. For comparison a classical desizing followed by a conventional alkaline scouring is carried out (experiment C). Both experiments are completed by a subsequent hot bleaching step, which is necessary to remove the coloured compounds of cotton (chromophores, pigments) and to fulfil a degree of whiteness of at least 90 (Stensby).

Table 1 shows the most important parameters of textile interest for the cotton fabrics after the bleaching process. All samples reach an excellent degree of whiteness of more than 90. The enzymatic treatment E gives the highest levels. Moreover the materials show no visible residues of seeds capsules. In both cases the degree of desizing is excellent. The level of 8 of the enzymatic treatment using α -amylase and hemicellulase/pectinase (E) is only insignificantly lower than the level of the conventional treatment C. Moreover hot bleaching shows a particularly dramatic effect in terms of the wettability. The enzymatic decomposition of pectins and hemicelluloses improves the accessibility of the fiber for the bleaching chemicals, which remove remaining hydrophobic residues completely. So the wettability of the enzymatically treated material is excellent and as good as the conventional treated fabric. Pre-treatment - especially inspecific alkaline scouring - has a direct influence on the weight and the mechanical properties of cotton. Weight loss is mainly caused by desizing and

removal of undesired cotton components. But moreover the alkaline treatment causes a partial hydrolysis of the cellulosic material, which might damage the fiber material. As expected the conventionally treated material shows the highest weight loss. The mass per unit area decreases from 187 g/m² to 166 g/m² (- 11.2 %). Compared to this the enzymatic procedure using the hemicellulase/pectinase formulation reduces the weight only by 8.0 %. The different damage of cotton fabric is shown best regarding the tensile strength, the elongation and the average degree of polymerisation. The enzymatic procedure E delivers the best mechanical properties.

Table 1: Parameters of different treated cotton after hot bleaching.

Parameter	C	E
Degree of desizing	8 - 9	8
Drop penetration time [s]	3	1
Mass per unit area [g/m ²]	166	172
rel. Weight loss to raw material [%]	- 11.2	- 8.0
Degree of whiteness (Stensby)	90.2	90.5
Seeds capsules	none	none
Tensile strength [daN]	61.6	63.0
Elongation at F _{max} [%]	24.4	24.7
Degree of polymerisation DP	1590	1694

Table 2: Saving potential of combined use of Beisol T 2090 and Beisol DHP compared to conventional cotton pre-treatment related to one metric ton cotton (*calculated for an average German middle-class textile finishing company)

Water demand	6,000 l
Energy	2.21 GJ or 615 kWh
CO ₂	115 - 250 kg
NaOH	75 kg
Costs*	226.-- €

With regard to all producing parameters such as e.g. water consumption, energy input, productivity, working staff **Table 2** summarizes the economic and ecological advantages of the combined enzymatic pre-treatment using α -amylase, hemicellulase and pectinase (E) compared to the conventional procedure including alkaline scouring (C). In both cases the quality of the treated goods after a bleaching step is equivalent. All items are related to one metric ton cotton and can be easily transferred to each manufacturing amount to find the specific potential saving of a cotton pre-treating company.

3.2 Enzymatic Bleaching

As described in the introduction peroxidases (POD) are used in textile decoloration processes, but their activity is limited by the hydrogen peroxide concentration, which

attack the POD during the reactions. To maintain the activity of the POD over a long time period the new combined system of GOD and glucose as the hydrogen peroxide source and additional the POD as an oxidation catalyst was used exemplarily for the slow decoloration of Sirius Supra Blue[®]FGG 200 %.

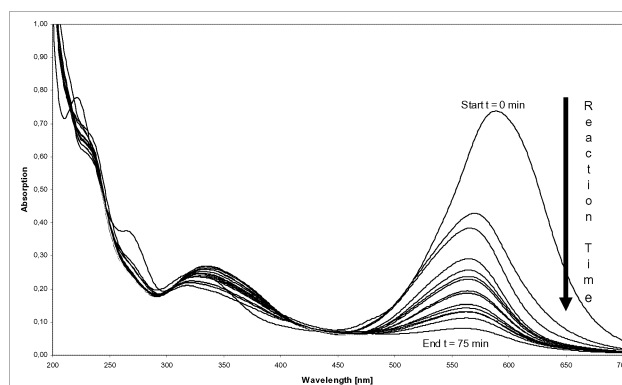


Figure 3: Decoloration of Sirius Supra Blue[®]FGG 200 % with glucose oxidase, glucose and peroxidase (Baylase[®]RP).

Figure 3 shows UV-Vis-spectra of the Sirius Supra Blue[®]FGG 200 % solutions after different reaction times. The dyestuff has an absorption maximum at approximately 600 nm and the intensity decreased during the reaction time. The GOD oxidizes glucose to gluconic acid and generates hydrogen peroxide, which can be used by the POD for the oxidation of the dyestuff over a long period without inactivating the enzymes. After 75 min the decoloration has finished. Similar results can be achieved using the new chloroperoxidase from ASA-Spezialenzyme. In this case the oxidation of the dyestuff is finished already after 60 minutes.

After showing that the suggested combined use of both oxidoreductases works in principle for decoloration processes investigations were carried out to use this enzyme cascade for textile bleaching processes. In **Figure 4** the results of different bleaching procedures of cotton are summarized. The starting material has a degree of whiteness (according to Berger) of 55. Bleaching of cotton with POD (Baylase[®]RP) in the presence of hydrogen peroxide fails, the degree of whiteness remains at 55, because the POD is inactivated by H₂O₂ after a short time. Using the combined system with GOD, glucose and POD (Baylase[®]RP) the degree of whiteness increases up to 64. Applying GOD and the new chlor-peroxidase from ASA-Spezialenzyme simultaneously in the presence of glucose a degree of whiteness of 66 can be reached. Compared to a conventional non-enzymatic bleaching process using only hydrogen peroxide at high pH-value and high temperature the bleaching result is not satisfactory but the investigations show that this environment-friendly enzymatic bleaching procedure at low temperature and a pH-value near to neutral basically works.

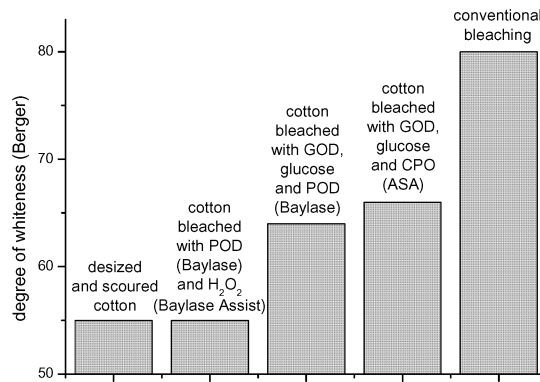


Figure 4: Bleaching of cotton with glucose oxidase, glucose and different peroxidases.

4. Conclusions

Due to the ever-growing costs for water and energy worldwide investigations were carried out to substitute conventional chemical textile processes by environment-friendly and even economic attractive bioprocesses using enzymes.

Here, a successful strategy for the combined use of α -amylase and hemicellulase/pectinase in the pre-treatment of cotton is developed. The pectin and hemicellulose degrading enzymes are given to the desizing liquor. Besides the desizing the removal of undesired substances can be fulfilled in one step. In the subsequent bleaching step residual hydrophobic components such as fats and waxes can be mobilised and removed. It can be shown that the enzymatic pre-treated cotton has similar or even better properties after a hot bleaching than the conventional desized and alkaline scoured material. So the combined use of the α -amylase Beisol T 2090 and the hemicellulase/pectinase Beisol DHP in the pre-treatment of cotton allows to renounce the alkaline scouring without a loss of quality in the finishing result. Neither the wettability nor the degree of whiteness or the dyeability of the bleached material are worse than for the conventional treated cotton; moreover the material is treated more gently and shows better mechanical properties. The new enzymatic procedure is corresponding with a significant lower demand of energy, water, chemicals, time and therefore costs. So it has advantages as well in terms of ecology as in economy.

An enzymatic cotton bleaching process is not realized up to now because of different problems such as the use of catalase contaminated GODs or the inactivation of PODs by hydrogen peroxide. Therefore a new concept was developed combining the use of two oxidoreductases, GOD and POD, in textile decoloration and bleaching processes. Under gentle conditions in terms of temperature, pH-value and even the peroxide concentration different peroxidases (commercial POD from Lanxess and a new chloro-peroxidase from ASA-Spezialenzyme) are stable over a long period of time, what offers the

possibility to use this enzyme cascade also in heterogeneous systems such as the bleaching of cotton fabrics. First results are of great promise. Up to now, indeed this concept is not able to compete with conventional bleaching, but the authors show that the combined use of oxidoreductases works in principle in decoloration and bleaching processes. In future further investigations have to be carried out to open this environment-friendly concept for an even economic attractive application in large-scale industrial manufacturing.

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