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Combined ultrasound-laccase assisted bleaching of cotton

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Abstract

This study evaluates the potential of using ultrasound to enhance the bleaching efficiency of laccase enzyme on cotton fabrics. Ultrasound of low intensity (7 W) and relatively short reaction time (30 min) seems to act in a synergistic way with the enzyme in the oxidation/removal of the natural colouring matter of cotton. The increased bleaching effect could be attributed to improved diffusion of the enzyme from the liquid phase to the fibres surface and throughout the textile structure. On the other hand inactivation of the laccase occurred increasing the intensity of the ultrasound. However, at the ultrasound power applied in the bleaching experiments the loss of enzyme activity was not significant enough to justify the use stabilizer such as polyvinyl alcohol. Furthermore, the polyvinyl alcohol appears to be a substrate for the laccase.

Keywords: Laccase; Ultrasound; Cotton; Bleaching

1. Introduction

Laccase (EC 1.10.3.2) is a multi-copper oxidase using a broad range of aromatic compounds as substrates and oxygen as a terminal electron acceptor [1]. Laccases have found various biotechnical and environmental applications, among which colour removal from both liquors and materials (bleaching) is of particular interest [2]. In the preparation technology of cellulose textile fibres, the bleaching process is not only concerned with brightening of the fibres, removing the natural colouring matter, e.g. fats, waxes, pectines, proteins and pigments, but it is directly related to the success of subsequent wet processing operations such as dyeing, printing and finishing. The whitening of the textiles is achieved traditionally at acidic to alkaline conditions, and in a wide temperature range, with different oxidizing agents. The whiteness level aimed in bleaching depends on the end use of the fabrics. When higher whiteness is needed it is necessary to perform a repeated oxidizing treatment. The bleaching chemicals normally are dosed in excess to the fibres, which necessitates repeated washing to remove the residual, harmful to the next processing operations, oxidants. This renders the bleaching process high chemicals, water, energy, and time consuming, discharging environmentally hazardous waste liquors. In a previous work we demonstrated that a short-time laccase pre-treatment enhanced the whiteness of cotton fabrics and reduced significantly the hydrogen peroxide dosage in subsequent chemical bleaching [2]. Enzymatic treatment of textiles involves mass transfer from the enzyme solution across the interior of the textile substrate, but in general, has low diffusion rates and the effect is concentrated on the outer fibres in the yarns [3]. Ultrasound could be a way to improve the diffusion of the enzyme to the interior of the yarns. Ultrasonic energy has been used successfully in conventional processes of desizing, scoring, bleaching, mercerization and dyeing of cotton [3,4]. Combined ultrasound/hydrolytic enzymes (e.g. cellulases, pectinases, amylases) applications provided reduction in the consumption of enzymes, shorter process time, less fibre damage and greater uniformity of the treatment [3,4]. However, data about the effect of ultrasound on the

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stability of oxidative enzymes such as laccases and their bleaching capacity have not been reported so far. The objective of the present research was to study the effect of the ultrasound on the bleaching ability of laccase as an alternative to the conventional chemical bleaching process of cotton.

2. Materials and methods

2.1. Enzyme and enzyme activity

Laccase (EC 1.10.3.2) from *Trametes villosa* (5.3 mg protein/mL) was provided by Novozymes (Bagsvaerd, Denmark). Laccase activity was assayed spectrophotometrically (Helyos γ, Unicam) by measuring the increase of absorbance at 420 nm ($\varepsilon_{420} = 3.6 \times 10^4\ M^{-1}\ cm^{-1}$) due to the oxidation of 0.5×10^{-3} M 2.2′-azinobis(3-ethylbenzthiazoline-6-sulfonate) (ABTS) (from Sigma) by a suitable amount of enzyme in 0.1 M sodium acetate buffer pH 5, at 50 °C. Enzyme activity (U) was defined as μmol of substrate oxidized per min. Lacccase activity at bleaching applications was followed by measuring the oxygen consumption (data acquired every 30 s) as a direct function of the oxidation of the colouring matter in cotton by means of CellOx 325 oxygen sensor, (from WTW GMH & Co. KG) in a thermostated, hermetically sealed vessel.

2.2. Ultrasound equipment

The equipment (Fig. 1) is composed of an electrical generator (Sonics & Materials, USA) with fixed frequency of 20 kHz and power intensity ranging from 7 to 100 W supplied by a piezoelectric transducer with probe diameter 13 mm. The diameter of the thermostated (50 ± 2 °C) glass cell, where all experiments were conducted, was 60 mm and the height was 200 mm. The ultrasound intensity used in this study and the distance between the transducer and the sample were selected according to preliminary experiments (ultrasound with intensity of 7, 30, and 50 W was applied to aluminium foil samples fixed perpendicularly at 0–2 cm from the transducer in acetate buffer pH 5.0 for 30 min) and the data for laccase stability in presence of ultrasound. The optimal distance between the transducer and material surface, providing maximum affected area (about 35%) was 0.5 cm for the intensity power of 7 W. The perpendicular positioning of the sample to the probe was chosen due to the specific application – to improve the diffusion of the biocatalysts towards and throughout the pores of the textile material, while a parallel positioning of the fabric would promote the removal of impurities from the surface [5] and not the biocatalyst/fibre contact.

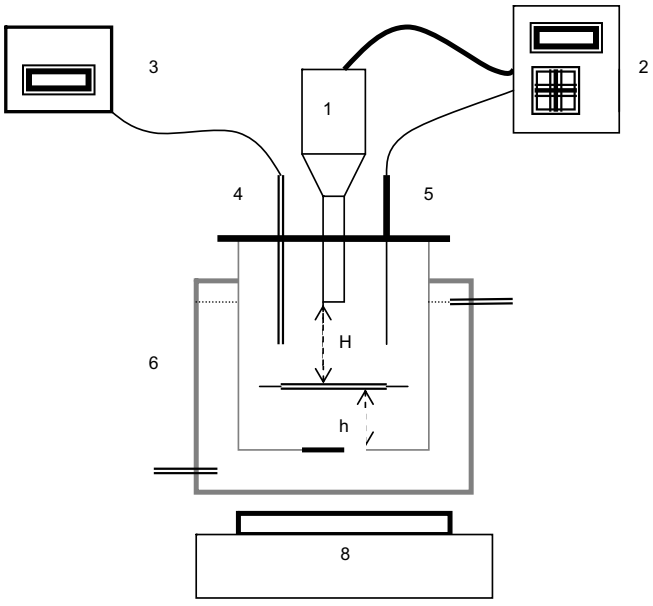

Fig. 1. Experimental set-up: (1) ultrasound probe, (2) ultrasonic generator, (3) oxygen measuring apparatus, (4) oxygen sensor, (5) temperature sensor, (6) thermostated and hermetically sealed vessel, (7) sample, (8) stirring equipment, (H) distance between the sample and the probe and (h) distance from the sample to the bottom of the reaction vessel.

2.3. Stability of laccase in the presence of ultrasound

Laccase solutions (125 mg prot./L) prepared in 0.1 M acetate buffer pH 5 were subjected for up to 40 min to ultrasound with intensity of 7, 30 and 50 W at 20 kHz, and 50 °C. Then the activity of the enzyme was measured against ABTS as described above. The enzyme was further stabilized using 1%, 5% and 10% w/v of high-hydrolyzed grade polyvinyl alcohol (DP. 682–1591 from Sigma).

2.4. Combined laccase/ultrasound assisted bleaching of cotton

The textile substrate used in the experiments was alkali scoured, twill weave, 120 g/cm^2, 100% cotton fabric. Samples of 1 g (60 mm × 60 mm) were fixed perpendicularly to the ultrasound transducer at a distance of 0.5 cm and treated simultaneously with 125 mg prot./L laccase and 7 W, 20 kHz ultrasound in 0.1 M acetate buffer pH 5, at 50 °C for 30 min. The oxygen consumption during the reaction was followed continuously.

The fabrics were bleached chemically afterwards following the recipe: 1.75 g/L Na-silicate, 0.5 g/L Na_2CO_3, 1 g/L NaOH, and 2 g/L 35% H_2O_2 (all reagents are from analytical grade). The bleaching was carried out at 90 °C for 1 h, in Ahiba Spectradye-Datacolor dyeing apparatus at liquor to fabric ratio 20:1. The whiteness index Berger (W^*) of the fabrics was determined using a reflectance measuring Datacolor apparatus at standard illuminant D_{65} (LAV/Spec. Excl., d/8, D_{65}/10°).

3. Results and discussion

Most of the physical and chemical effects of ultrasound are due to the cavitation phenomenon, e.g. collapsing of

the bubbles produced in aqueous medium at sonication, generating high local temperature and pressure. If the collapse occurs in the vicinity of a solid surface such as textile fabric the bubbles undergo deformation, which results in formation of a high velocity microjet directed towards the textile surface. This microjet would accelerate the transportation of the biocatalysts from the liquor to the textile substrate and subsequently throughout its structure. However, the microjet formation, together with the formation of hydroxy radicals and heat generation during collapsing of the bubbles would affect also the biocatalyst stability, what appears as a limiting factor in combined ultrasound/enzymatic applications. Enzymes are easily denaturated by slight change of the environmental conditions such as temperature, pressure, shear stress, pH and ionic strength. In our experiment the activity of the laccase decreased with the increase of ultrasound intensity from 7 to 50 W and this tendency was more pronounced at sonication longer than 10 min (Fig. 2). Slight decrease in activity was also observed at stirring (shear stress) of the enzyme solution.

The loss of catalytic activity at ultrasound application implies the use of stabilizing agents for the enzyme. Aqueous solutions containing 125 mg prot./L laccase and 1%, 5%, and 10% w/v polyvinylalcohol (PVA) have been tested for stability performance against sonication at pH 5, 50 °C for 30 min. The increase of PVA concentration did not affect significantly the laccase activity at the above conditions. Nevertheless, the highest laccase stability at all ultrasound intensities was induced by 10% w/v PVA. The denaturation of the enzymes in aqueous solution proceeds through hydratation of the protein. The role of the polyols in enzyme stabilization is as a water-structure maker, which depresses the hydration of the protein. The polyol molecules are preferentially excluded from the surface layer of the protein molecule and the water shell around the protein molecule is preserved [6]. The PVA would also prevent the protein from crosslinking and aggregation promoted by the radicals formed in the sonication process.

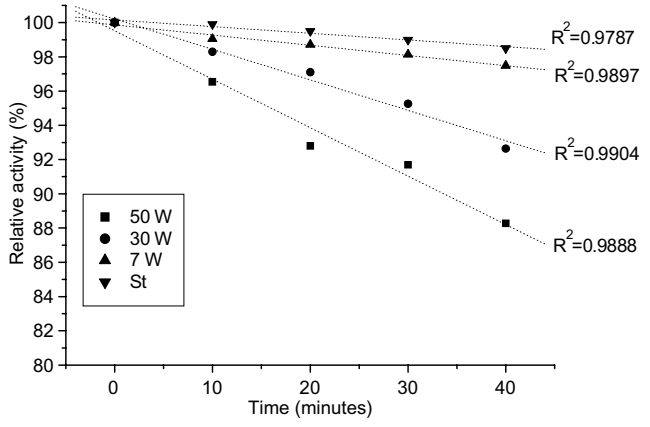

Fig. 2. Laccase (125 mg prot./L) activity after application of 20 kHz ultrasound with intensity 7, 30 and 50 W in buffered solution pH 5, 50 °C. Activity of 100% corresponds to 1000 U (μmol ABTS oxidized per min).

3.1. Bleaching experiments

The laccase treatment, previous to conventional peroxide bleaching of cotton samples, was carried out at the optimum for this enzyme temperature and pH [2] for 30 min. The results in Fig. 3 showed that the laccase pre-treatment alone did not improve fabric's whiteness, compared to the pre-treatment with either ultrasound or stirring at the same reaction conditions (ΔW* laccase was calculated as a difference between the whiteness index of the ultrasound and/or enzyme-treated samples and the blank treated with buffer). The enzyme application deteriorated the whiteness of the textile material and apparently generated colour. This might be due to laccase-catalyzed oxidative transformation of the cotton flavonoids into another coloured product, which was easier to remove during the oxidative bleaching [2,7].

Nevertheless, after conventional oxidative bleaching of the enzymatically pre-treated samples a whiteness improvement ($\Delta w^*_{\text{lac-US/perox.}} = w^*_{\text{lac-US./perox.}} - w^*_{\text{perox}}$) of 1.67 Berger units was obtained compared to the whiteness of the peroxide bleached non-treated fabrics (the base line). Application of combined ultrasound/enzyme pre-treatment further increased the whiteness level of the fabrics by 0.56. Thus the sonication had a positive effect in the enzyme-assisted bleaching of cotton.

Improvement of fabric whiteness was observed also after pre-treatment with PVA stabilized laccase and ultrasound. Normally the colouring matter of cotton cellulose, mainly composed by nitrogen-free flavone pigments, suffers oxidation and browning after application of laccase [7–9]. Thus, some darkening of the fabrics should be expected if the stabilized with polyvinyl alcohol enzyme would have oxidized these pigments. These contradictory at first glance results could be explained considering the data for oxygen consumption during the enzymatic reaction in the presence of PVA (Fig. 4).

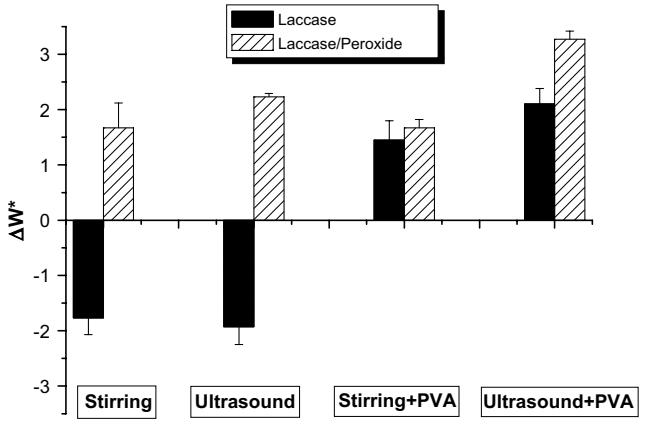

Fig. 3. Effect of laccase (125 mg prot./L) and/or ultrasound (7 W, 20 kHz) pre-treatment at pH 5, 50 °C, 30 min, on the whiteness of cotton fabrics before and after hydrogen peroxide bleaching. The baseline represents treatment with buffer solution without enzyme at either stirring or sonication.

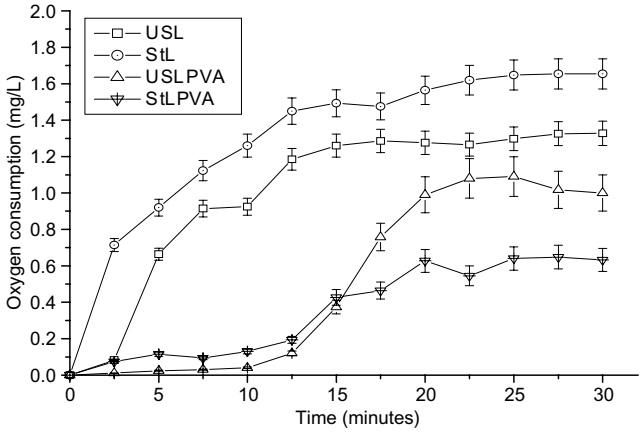

Fig. 4. Oxygen consumption in individual or combined treatment of cotton fabric with 125 mg prot./L laccase and 7 W, 20 kHz ultrasound at pH 5, 50 °C. The enzyme was stabilized with 10% w/v of PVA.

The lower lever of oxygen consumption in the ultrasound assisted laccase reaction was clear evidence for decreased enzyme activity during sonication supported also by the results in Fig. 2. Referring to Fig. 3, however, the bleaching effect in the combined ultrasound/enzyme treatment was higher than in individual laccase application. Thus for the short time of treatment (30 min) the decrease of laccase activity was compensated by the cleaning efficiency of the ultrasound [5] besides of improvement of mass transfer aspects in the enzymatic reaction.

In case of using stabilizer PVA both stabilizing (avoiding aggregation and crosslinking of the protein due to the generated during the sonication $HO_2^{\cdot}$ radicals) and deactivation (due to the high viscosity of the medium and PVA adhesion on the fibres preventing them from oxidation) effects on the biocatalysts should be considered. It should be noted that the stability measurement (Table 1) were against the soluble ABTS substrate (homogeneous catalysis) and not the insoluble cotton pigments, which oxidation occurs at the liquid–solid interface. Aqueous solutions of PVA of high degree of hydrolysis with concentration about 10% w/v (DP. 1200–2000) tend to form a network structure of hydrogen-bonded molecules [10]. However, the continuous application of shear stress, provided by stirring (200 rpm) or ultrasound waves (7 W, 20 kHz), was able to disrupt the entanglement of PVA molecules and the solution underwent an apparent decrease of viscosity after 10–15 min of treatment. Stirring and ultrasound with the above characteristics appear to have similar effect on the viscosity of the PVA solution. This would help the penetration of the solution, presumably affected at concentrations above 3–4% only by the viscosity [10], into the porous structure of the textile material. Accordingly, the oxygen consumption increased and thus oxidation of enzyme substrate occurred. Surprisingly, browning (oxidation of cotton pigments) of the fabrics has not been observed (Fig. 3). This suggests that except for the cotton pigments, the only other possible substrate for laccase could be the PVA. Oxidases have been identified to be able to degrade PVA [11,12]. The degradation of PVA consists of two types of reactions – oxidation of OH groups (C=O) and cleavage of C–C linkages, with production of hydrogen peroxide.

Table 1
Relative activity of laccase after ultrasound treatment

Ultrasound intensity	Relative activity of laccase (%)		
20 kHz, 30 min, pH 5, 50 °C	(1% w/v PVA)	(5% w/v PVA)	(10% w/v PVA)
7 W	98.56 ± 0.39	98.78 ± 0.20	99.67 ± 0.66
30 W	96.65 ± 0.68	98.14 ± 0.51	98.34 ± 0.33
50 W	92.36 ± 1.11	93.94 ± 0.69	97.90 ± 0.82

The oxidative degradation of PVA would also decrease the viscosity of the solution. Thus the bleaching improvement achieved at application of PVA-stabilized enzyme and ultrasound could be attributed to ultrasonic activation (radical formation) of the generated peroxide [13,14] and not to enzymatic oxidation of the cotton colouring matter.

4. Conclusions

The authors have previously reported on the bleaching capacity of laccases on cotton and the present work is a trial to intensify the enzymatic process by sonication. The supply of low ultrasound energy (7 W) to the laccase pretreatment of cotton fabrics increased the whiteness effect in conventional peroxide bleaching. Biocatalysts and ultrasound seem to have a synergistic effect in the bleaching enhancement, though inactivation of the laccase was observed during the process confirmed by the data for oxygen consumption. The enzyme was further stabilized using 10% w/v polyvinyl alcohol. However, besides of stabilizing effect the PVA also impeded the oxidation of the textile substrate either due to viscosity constraints to the diffusion of the enzyme to the fibre surface or due to high affinity of polyvinyl alcohol towards cellulose and formation of protective film on the fibres. The PVA seems also to be substrate for the laccase and the peroxide generated during its oxidation/degradation might contribute together with the ultrasound energy to non-enzymatic bleaching effects. Thus stabilization of the enzyme revealed to be unnecessary for relatively short application time e.g. up to 30 min. The bleaching improvement in the combined enzyme-ultrasound process could be explained by accelerated heterogeneous enzyme–cotton fibres reaction and the formation of reactive transient species due to the cavitation phenomenon at sonication.

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