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Ultrasonic pilot-scale reactor for enzymatic bleaching of cotton fabrics

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ABSTRACT

The potential of ultrasound-assisted technology has been demonstrated by several laboratory scale studies. However, their successful industrial scaling-up is still a challenge due to the limited pilot and commercial sonochemical reactors. In this work, a pilot reactor for laccase-hydrogen peroxide cotton bleaching assisted by ultrasound was scaled-up. For this purpose, an existing dyeing machine was transformed and adapted by including piezoelectric ultrasonic devices. Laboratory experiments demonstrated that both low frequency, high power (22 kHz, 2100 W) and high frequency, low power ultrasounds (850 kHz, 400 W) were required to achieve satisfactory results. Standard half (4 g/L H_2O_2 at 90 °C for 60 min) and optical (8 g/L H_2O_2 at 103 °C for 40 min) cotton bleaching processes were used as references. Two sequential stages were established for cotton bleaching: (1) laccase pretreatment assisted by high frequency ultrasound (850 kHz, 400 W) and (2) bleaching using high power ultrasound (22 kHz, 2100 W). When compared with conventional methods, combined laccase-hydrogen peroxide cotton bleaching with ultrasound energy improved the whitening effectiveness. Subsequently, less energy (temperature) and chemicals (hydrogen peroxide) were needed for cotton bleaching thus resulting in costs reduction. This technology allowed the combination of enzyme and hydrogen peroxide treatment in a continuous process. The developed pilot-scale reactor offers an enhancement of the cotton bleaching process with lower environmental impact as well as a better performance of further finishing operations.

1. Introduction

The purpose of cotton bleaching is to decolorize natural pigments, mainly flavonoids conferring a pure white appearance to the fibres [1,2]. This process is directly related to the success of the subsequent wet processing operations such as dyeing, printing and finishing [3]. Nowadays hydrogen peroxide, due to its biodegradability, almost entirely replaced the conventional chlorine oxidizing chemicals [1]. It is applied at alkaline pH and temperatures closed to boiling, requiring therefore high energy consumption. Radical reactions of bleaching agents with the fibres can lead to a decrease in the polymerization degree and to fiber damage. Moreover, huge amounts of water are needed to remove hydrogen peroxide from fabrics which would cause dyeing difficulties [4]. Thus, more specific processes targeting only colored substances would be advantageous. Enzyme-based systems integrating bleaching of cotton have been developed in order to overcome these concerns and reduce processing costs. Based on the assumption that fungal laccases can oxidize phenolic moieties of lignin in pulp, it has been assumed that these enzymes could also decolorize or eliminate colored flavonoids of cotton attacking phenolic hydroxyl groups. Laccases (EC 1.10.3.2) are multi-copper-containing enzymes capable of oxidizing phenols and aromatic amines, reducing molecular oxygen to water. The reaction involves three types of copper centers with different functions: type 1 (blue copper) catalyzes the electron transfer from the substrate while type 2 and type 3 form a three-member cluster that collectively activate molecular oxygen [5].

Several authors have successfully described the use of laccases on cotton bleaching as a new environmentally friendly technology at laboratory scale [2,3,6–9]. Tzanov et al. reported for the first time the enhancement of the bleaching effect achieved on cotton using laccase. This enzyme applied in short-time batch wise or pad-dry processes prior to conventional bleaching, improved the

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end fabric whiteness. They postulate that the enzyme transforms the cellulose coloring matter in another colored compounds which are more easily susceptible to oxidation with peroxide and thus more easily degraded [9]. The advantages of this system rely on the hydrogen peroxide dosage reduction as well as on the reduced bleaching temperature and time [8].

Considering that enzymatic processes of cotton textiles require transfer of mass from the bulk solution to the fabrics surface, diffusion rates can be improved by mechanical agitation. Since this type of agitation is not very efficient, ultrasounds have been undertaken for the enhancement of mass transfer and speed-up of bleaching reactions [3,6,10–14]. Indeed, the use of ultrasound promotes an improvement on chemical reactivity (higher reaction speed and output, more efficient energy usage, performance improvement of phase transfer and increase in the reactivity of reagents or catalysts) mainly caused by cavitation. This is an acoustic phenomenon which lies in the formation, growth and implosive collapse of bubbles in a liquid. Once formed, small gas bubbles irradiated with ultrasound in a bulk of liquid are grown until it can no longer absorb energy efficiently and cannot sustain itself. When the cavity implodes, an increase of the local temperature and pressure of the surrounding liquid is created. Thus, the cohesion and adhesion forces within the liquid can be overcome. Some studies have identified the formation of high-energy intermediates during this process in aqueous solutions, including $HO_2^{\cdot}$ (superoxide), $H^{\cdot}$ (atomic hydrogen), $OH^{\cdot}$ (hydroxyl), and e- (aq.) (solvated electrons). Therefore, the sonolysis of water produces strong oxidants, such as hydroxyl radicals, capable of causing secondary oxidation reactions [12].

Although the reported effectiveness of the combined laccase-hydrogen peroxide/ultrasounds system on cotton bleaching, the scale-up of this process has not been successfully achieved. The existing conventional designs still do not give substantial efficiency at larger operation scales [15,16], since an intense cavitational activity is obtained very close to the transducers. Moreover, a lack of expertise is required in diverse fields, namely material science, acoustics and chemical engineering for scaling-up successful reactor design and scale-up strategies.

The main proposal of this work was to join the knowledge of experts in several areas, namely ultrasound field, enzymology and industrial engineering to scale-up a laccase-hydrogen peroxide system assisted by ultrasound for cotton bleaching. Standard operational conditions were optimized, namely temperature, processing time and hydrogen peroxide amount. All the process was studied at laboratory scale and further transferred to a pilot sonochemical reactor by adjusting existing dyeing machinery. Ultrasonic devices with different geometry and operational mode of action were tested. The final reactor was designed considering the optimal conditions attained at laboratory scale.

2. Materials, equipment and methods

2.1. Materials

100% of desized woven cotton fabrics and auxiliary products used on cotton bleaching experiments were supported by an industrial company, Acatel (Portugal). Laccase (EC 1.10.3.2) from ascomycete *Myceliophthora thermophila*, Novozym® 51003 (17 g protein/L, 4500 U/mL, at 50 °C), was obtained from Novozymes (Denmark). All the others chemical products were purchased from Sigma Aldrich and Panreac without further purification.

2.2. Equipment

The high frequency experiments were carried out using an ultrasonic power generator type K8 (850 kHz, 120 W) coupled with an ultrasonic high-power bath Type 5/1575 equipped with high-performed ultrasound plan-transmitter, double glass cylinder cooling system, ceramic, stainless construction and Titan-membrane (Fig. 1A). The maximum temperature reached by this equipment is 60 °C. Both equipments were purchased from Meinhardt Ultraschalltechnik (Germany). Low frequency assays were performed using piezoelectric transducers of mono-frequency (22 and 38 kHz) and multi-frequency (40–90 kHz), both with 400 W (Fig. 1B). Also magnetostrictive transducers of 40 kHz and 1400 W were used (Fig. 1C). All the equipment were made with stainless steel and equipped with temperature controlled vessels. One robotic arm was introduced on the ultrasonic systems to promote the agitation of bath solutions.

A prototype jet dyeing machine was adapted for the pilot scale experiments at Centre de Recerca i Innovació de Catalunya, SA – CRIC, Spain. Ultrasounds interact at two different levels, with concurrent effects. During pretreatment, ultrasounds are supposed to

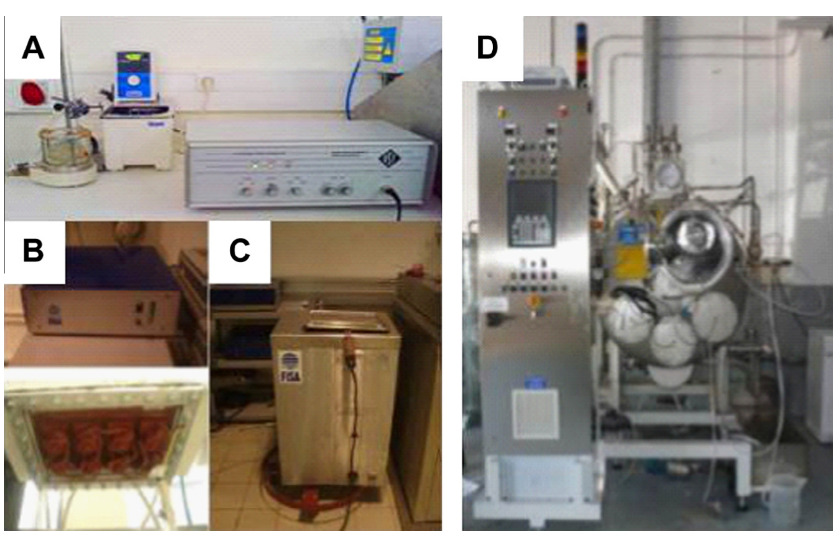

Fig. 1. (A) High frequency ultrasonic device (850 kHz, 120 W); (B) low frequency/high power ultrasonic devices: piezoelectric transducers with ultrasounds power supply (mono-frequency 22 kHz and 38 kHz, multi-frequency 40–90 kHz, 400 W); (C) vessel equipped with magnetostrictive transducers (40 kHz, 1400 W); (D) cotton bleach prototype containing low frequency/high power intensity (22 kHz, 140–2100 W) and high frequency (850 kHz, 400 W) devices.

boost diffusion rates, while at the bleaching step, are supposed to boost diffusion rates, as well as generate radicals able of whitening cotton fibres. For this reason, two different technologies were installed within the equipment, high power and high frequency ultrasonic devices. Low frequency, high power piezoelectric transducers (22 kHz, 140–2100 W) were strategically located at the main tank bottom aiming strong contribution to physical effects (mass transfer rates) from cavitation phenomena and reduction of mixing time plus better net power dissipation resulting from high intensity irradiation [17]. High frequency, low power (850 kHz, 400 W) piezoelectric transducers were positioned at the recirculation pipe, just before the jet in order to intensify the chemical effects such as free radicals production (Figs. 1D and 2) [18]. With this configuration it is expected that the oxidant agents reach the entire cotton fiber surface.

Before starting the process, the system was loaded with 5 kg of cotton (approximately 40 m * 0.5 width). Operation scheme is, firstly, pass the beginning of the cotton rope through the machine (reel + jet + body) manually, and connect it with the ending part (e.g. by magnets). This creates a continuous rope. Secondly, the remaining cotton is introduced using the reel. Once all the cotton length was inside the machine, the system is ready to start working (Fig. 2).

2.3. Methods

2.3.1. Protein concentration and enzymatic activity measurements of laccase

The total protein concentration of laccase was determined following the Lowry method using different concentrations of bovine serum albumin (BSA) as standard solutions [19]. The corresponding enzymatic activity was achieved by oxidation of 2,2′-azinobis-(3-ethylbenzothiazoline)-6-sulphonate (ABTS) in 0.1 M sodium acetate buffer at pH 5, at 50 °C [20]. One unit of laccase (U) was defined as 1 μmol of ABTS oxidized per minute. The protein quantification and enzymatic activity of laccase was done by monitoring the absorbance solutions at the wavelengths 750 nm and 420 nm, respectively, using a Helios Gamma UV–Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA). All measurements were performed using at least triplicate samples.

2.3.2. Standard half and optical cotton bleaching processes

Considering an industrial source, the conventional half-bleaching of cotton uses 4 g/L hydrogen peroxide, 4 g/L NaOH and the following auxiliary products: 1 g/L anti-wrinkle, 0.5 g/L wetting agent, 1.5 g/L sequestrant, 3 g/L equalizer using a bath ratio 1:50. The process is carried out at 90 °C for 60 min. For the optical cotton bleaching, the hydrogen peroxide concentration is increased (8 g/L) as well as the temperature (103 °C) for 40 min. Auxiliary products are 1 g/L anti-wrinkle, 0.5 g/L and 0.3 g/L wetting agents, 3 g/L equalizer and 3 g/L optical brightener. Washing procedures were realized with distilled water during 10 min (×3).

2.3.3. Whiteness measurement of cotton fabrics (Berger Index)

The whiteness of cotton fabrics was measured using a StellarNet colorimetric system connected to BLACK-Comet-SR spectrometer. The parameters monitored by spectrometer were luminosity (L^*), red/green (a^*) and yellow/blue (b^*) which were further converted into XYZ-CIE tristimulus values, allowing the calculation of Berger whiteness [21]. Data values were logged in real time by measuring the visible reflectance at the range 380–750 nm. For each test was considered the average of 8 measures.

2.3.4. Laboratory-scale process optimization

2.3.4.1. High frequency ultrasound. Previous researches demonstrated the enzymes deactivation after low frequency ultrasound application. The main reason for that was the aggregation phenomenon resulted from exposure of cysteine residues and reaction with highly reactive radicals [6,22]. Moreover, it is already published that low frequency, high intensity ultrasound usually denatures enzymes while high frequency, low intensity irradiation promotes a more stable cavitation, micro-streaming and fewer implosion occurrences which often stimulate enzymatic activity [23]. Therefore, in this work, the enzymatic pretreatment of cotton using laccase as catalyst was assisted by high frequency, low power ultrasound (850 kHz, 120 W). 2 U/mL of laccase with a half-life time of 37.5 min when exposed to high frequency ultrasound were used on enzymatic pretreatment of cotton. The bleaching effectiveness was tested at 50 and 60 °C (optimum temperatures already described by other authors [24,25]). All the experiments were carried out in 0.1 M sodium acetate buffer (pH 5) during 30 min (this

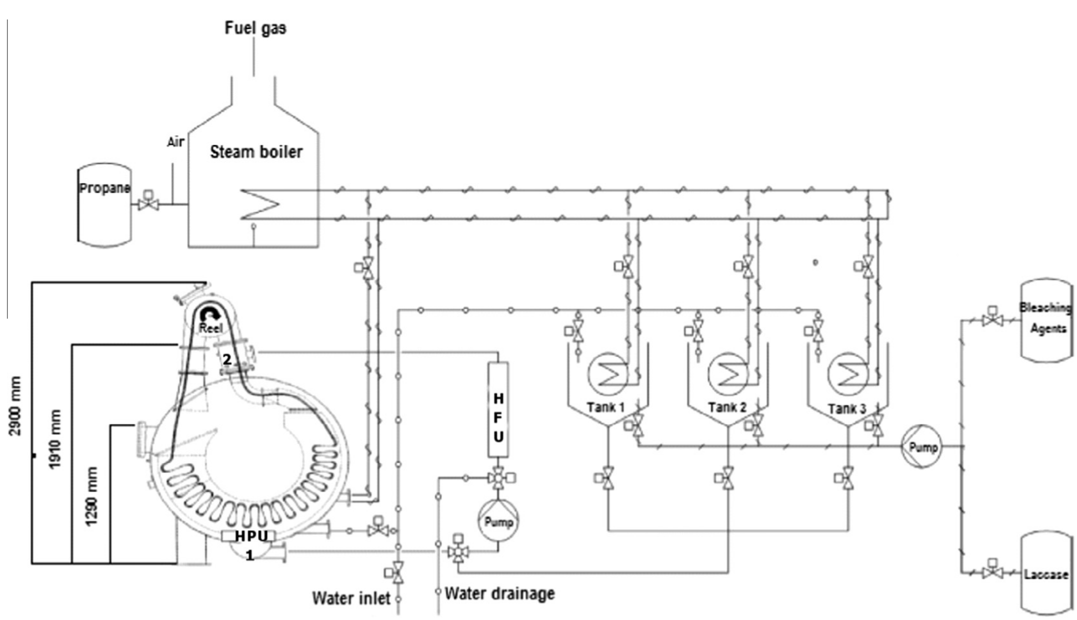

Fig. 2. Schematic representation of the experimental chamber for the combined laccase-hydrogen peroxide bleaching of cotton assisted by ultrasound; 1-high power piezoelectric transducers (22 kHz, 140–2100 W); 2-high frequency (850 kHz, 400 W) piezoelectric transducers. Tank 1, tank 2 and tank 3 will be used as water containers for dilute solutions as well as washing procedures.

incubation time was already optimized by us on a previous work) [26]. Washing procedures were performed with 2 g/L Lutensol AT25, at 80 °C for 10 min. Further, the effect of high ultrasound frequency on enzymatic cotton bleaching followed the half-bleaching recipe (Section 2.3.2) in a bath ratio 1:50, at 55 °C for 30 and 60 min aiming reducing the standard energy consumption.

2.3.4.2. Low frequency ultrasound. The ideal ultrasonic device for laboratory scale cotton bleaching was chosen according to the results attained after bleaching with 4 and 8 g/L H_2O_2, at 70 and 90 °C for 30 and 60 min following the half-bleaching recipe (Section 2.3.2) in a bath ratio of 1:50. The ultrasonic transducers tested were the piezoelectric and magnetostrictive devices (Fig. 1B and C).

Considering that whiteness reference values are overlapped using piezoelectric device, this equipment was chosen for the subsequent experiments. Once introduced laccase pretreatment using 2 U/mL of enzyme, at 50 °C for 30 min, the bleaching was carried out using lower amounts of hydrogen peroxide (2 and 4 g/L) aiming reducing the standard chemical consumption. Preliminary experiments performed with four hydrogen peroxide concentrations (2, 3, 3.5 and 4 g/L) revealed no significant whiteness differences between the lowest. For this reason, only two levels of H_2O_2 were applied. The experiments were tested at 70 and 90 °C for 30 and 60 min since energy consumption decrease is also a goal in this study. Three washes were done with distilled water for 10 min.

2.3.5. Process upgrading to pilot-scale

Considering the best conditions accomplished at laboratory scale, the cotton bleaching proceeded at pilot-scale using an adapted jet containing both high power low frequency (22 kHz, 140–2100 W piezoelectric transducers) and low power high frequency ultrasound (850 kHz, 400 W-power intensity available for scale-up high frequency ultrasound system) aiming to achieve the optimal performance for the enzymatic cotton bleaching. The pilot reactor was able to handle 5 kg cotton load per 240 L (1:50 bath ratio) batch and the characterization of the process was done for the half (4 g/L H_2O_2, 70 °C, 30 min) and the optical bleaching. The enzymatic pretreatment of cotton was assessed by using 2 U/mL of laccase in 0.1 M sodium acetate buffer (pH 5) at 60 °C for 30 min. Higher temperature than used on laboratory scale value was needed to promote reproducible whiteness values at pilot-scale. All of the treatments were evaluated in the presence of ultrasounds. The ultrasonic enhancement was studied for different power intensities and low frequency (22 kHz; 140 W, 700 W and 2100 W) as well as for high frequency of ultrasounds (850 kHz, 400 W). All the whiteness measurements were performed accordingly 2.3.3 method.

2.3.6. Statistical analysis

The effectiveness of each process studied was achieved statistically by analysis of variance results through two-way ANOVA test (GraphPad Prism 5.0 for Windows). Significant differences between variables were employed when it was observed $P < 0.05$.

3. Results

3.1. Laboratory-scale process optimization

3.1.1. High frequency ultrasound

Firstly, the ultrasound effects on laccase pretreatment of cotton fabrics were studied. Usually, high redox potential laccases such as laccase from *Trametes villosa* (E = +0.78 V) are widely used for biotechnological purposes due to their high oxidative abilities. However, laccases for industrial applications require robust expression systems aiming produce huge amounts of enzyme. Laccase from *Myceliophthora thermophila* is a commercially available enzyme with low redox potential (E = +0.48 V) that can be heterologously expressed in industrial hosts, while the difficult expression of high redox potential laccases limits their large-scale commercialization [27]. Once this work exploit the scale-up of an ultrasonic reactor for enzymatic cotton bleaching, laccase from *M. thermophila* was selected for further experiments. High frequency ultrasound (850 kHz, 120 W) was selected for this stage since more stable cavitation, micro-streaming and fewer implosion events usually results in stimulating enzyme activity [23]. For the optimization of bleaching process at high frequency ultrasound were selected two reaction times, 30 and 60 min, aiming to reduce the total operational time (Fig. 3). It is notable that the temperature selected was 55 °C due to the equipment limitation. As result, the introduction of ultrasound on conventional bleaching process was not enough to attain the pretended whitening levels. Only when laccase pretreatment was realized it was possible to overtake in 10 Berger the whitening values obtained by ultrasound bleaching. Statistical analysis showed that the introduction of laccase pretreatment allow whitening values significantly higher than the bleaching process carried out only with ultrasound ($P < 0.001$). These experiments confirmed the ability of laccase to oxidize and polymerize the phenolic compounds present on cotton surface. The end products were removed by ultrasound action improving the bleaching efficiency. Concerning the temperature of laccase pretreatment, no significant whitening changes were

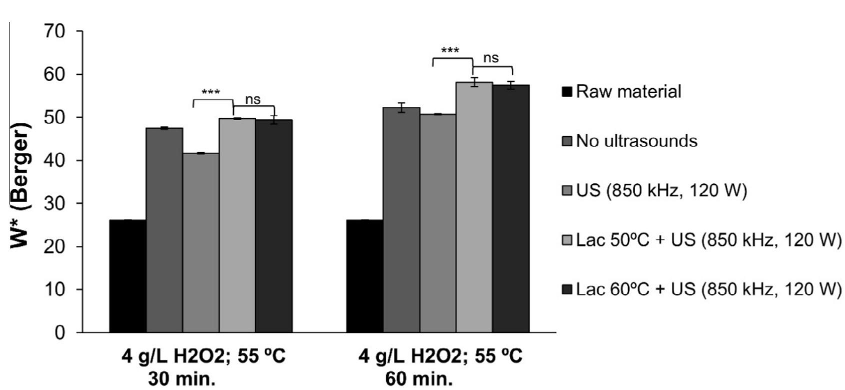

Fig. 3. Whiteness of cotton samples bleached using the combined laccase-hydrogen peroxide system assisted by high frequency ultrasound (850 kHz, 120 W). Pretreatment: 2 U/mL laccase at 50 and 60 °C for 30 min.; bleaching with 4 g/L H_2O_2 and auxiliary products at 55 °C for 30 and 60 min.). Statistically significant differences are indicated. *** = Ultrasound bleaching significantly different from enzymatic ultrasound bleaching ($P < 0.001$); ns = Pretreatment at 50 °C no significantly different from pretreatment at 60 °C ($P > 0.05$).

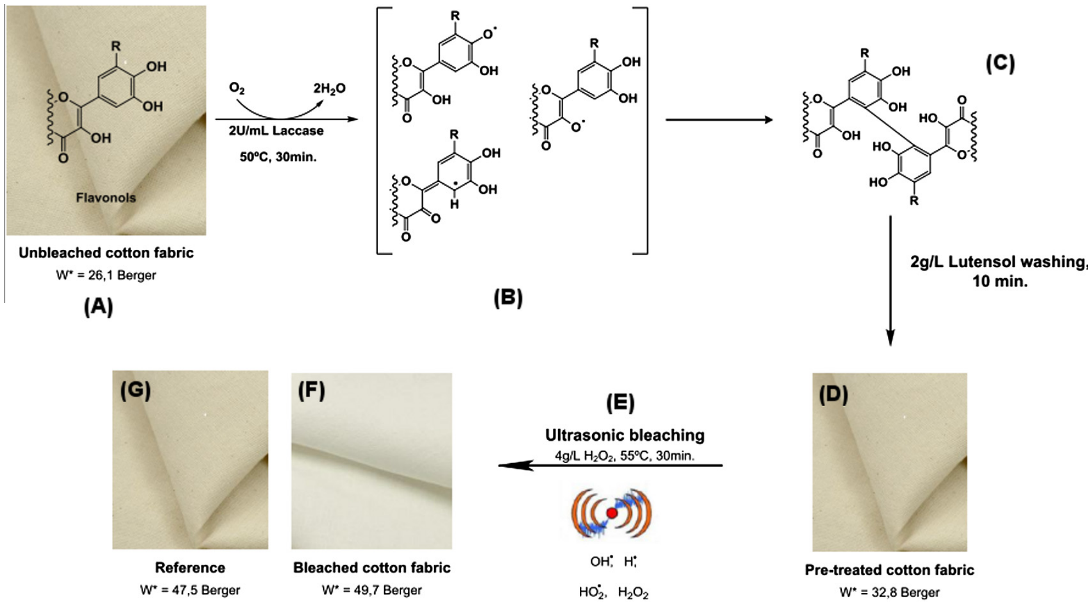

Fig. 4. Schematic representation of combined laccase-hydrogen peroxide cotton bleaching. (A) Unbleached cotton fabrics with naturally occurring flavonoids, (B) radicals and quinone intermediates resulting from enzymatic oxidation, (C) oxidized colored products, (D) aspect of cotton after laccase pretreatment, (E) bleaching of pre-treated fabrics assisted by ultrasounds, (F) aspect of cotton fabric after bleaching, (G) industrial reference processed without pretreatment and ultrasound [34,35].

observed when 50 or 60 °C were applied ($P > 0.05$) which means that the process can also be carried out at 60 °C. However, the energy consumption of cotton pretreatment will increase. Laccase and ultrasounds were the key requirements for the whiteness improvement. The mechanism of laccase bleaching action is not fully understood and described in literature. However, several authors already assume that this enzyme transforms the cellulose coloring matter in another colored compounds which are more susceptible to oxidation by peroxide and thus more easily removed from cotton's surface (Fig. 4) [9]. In a first stage, laccase oxidizes the surface naturally occurring flavonoids (Fig. 4A). Two mechanisms have been proposed for the formation of flavonoid oligomers: (a) nucleophilic addition of the A-ring of flavonoid to the B-ring of its oxidation product (the quinone) and (b) coupling of radicals produced from flavonoid oxidation. Values that have already been published suggest that laccase-catalyzed polymerization reaction proceeds mainly through the nucleophilic rather than radical mechanism [28]. The quinones formed are highly reactive and can undergo nucleophilic attack by other phenolic groups (Fig. 4B). Further, new polymerized colored species are produced at the cotton's surface (Fig. 4C), which are partially removed by surfactant washing (Fig. 4D). These new species are susceptible to oxidant attack and more easily removed at the bleaching stage (Fig. 4F). Laccase pretreatment alone did not improve fabrics whiteness since the natural coloring matter of cotton cellulose, mainly composed by nitrogen-free flavone pigments, suffers oxidation and browning after enzyme action [29]. Thus, it can be expected a lower whiteness increase after enzyme application. The final whiteness levels are only detectable after ultrasound assisted hydrogen peroxide bleaching (Fig. 4E). In this stage, the cavitation phenomena (expansion and collapse of micro bubbles) intensify the mass transfer from the bulk solution into the fabric, increasing enzyme and oxidant agent action [30]. Collapse near the surface produces an asymmetrical inrush of the fluid to fill the void forming a liquid jet targeted at the surface. These jets activate the solid catalyst and increase the mass transfer to the surface by the disruption of the interfacial boundary layers as well as dislodging the material occupying the inactive sites. The intensification of enzymatic reactions is due to the generation of cavitating conditions with the passage of ultrasound in the liquid medium. At the same time, when the strong collapse of bubbles occurs, the water vapor inside the bubbles is dissociated and chemical products such as OH·, O· and H·, as well as H_2O_2 are created. Those act as oxidant agents being responsible for the bleaching improvements. The results achieved confirm that ultrasonic cavitation was responsible for the swelling of fibres in water, for the increase in the diffusion coefficient of enzyme molecules and for the disintegration of aggregates with high molecular weight resulting from catalytic hydrolysis [12–14,31]. The introduction of ultrasonic energy on this system imparted the intensification of reactions due to the generation of cavitating conditions with the passage of ultrasound in the liquid medium. Comparing with industrial reference (Fig. 4G), a higher whiteness level was achieved (Fig. 4F) under the described operational conditions.

As confirmed by others, ultrasounds were responsible for the good enzyme performance and for the production of oxidant agent used at bleaching stage.

The combination of both enzyme pretreatment and ultrasound make possible the reduction of enzyme consumption allowing reducing the process final costs. It is also important to point out that the fabrics maintain their structural integrity in terms of tensile strength and elongation when exposed to this process (data not shown).

3.1.2. Low frequency ultrasound

According to literature, low frequency ultrasound should be employed where intense physical effects are required [17]. In this case, maximum energy gets dissipated near to the irradiating surface in a cone like structure. Due to this, there is a maximum cavitational activity very near to the irradiating surface and wide variation in energy dissipation rates in the remaining bulk of liquid [15]. In spite of that, the effect of low frequency ultrasonic transducers, piezoelectric (22 kHz) or magnetostrictive with (22–40 kHz), were studied. Standard bleaching temperature (90 °C) and a lower one (70 °C) were applied aiming to observe in which way it was possible to decrease the energy consumption. Fig. 5 expresses that both piezoelectric and magnetostrictive equipment allowed highest whitening values than the no

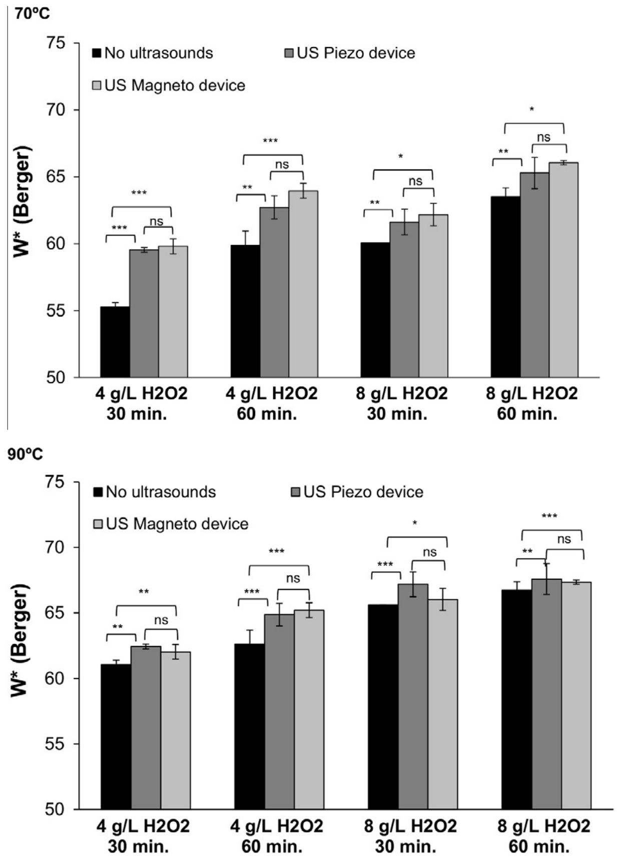

Fig. 5. Whiteness values (W^*) of cotton samples bleached at 70 °C and 90 °C using 4 and 8 g/L H_2O_2 for 30 and 60 min., assisted by low frequency ultrasounds (piezoelectric and magnetostrictive devices). The process without ultrasound was used as control. Statistically significant differences are indicated. *** = Ultrasound bleaching significantly different from the no sonicated bleaching ($P < 0.001$); ** = Ultrasound bleaching significantly different from the no sonicated bleaching ($P < 0.01$); * = Ultrasound bleaching significantly different from the no sonicated bleaching ($P < 0.05$); ^{ns} = Piezoelectric bleaching no significantly different from magnetostrictive bleaching ($P > 0.05$).

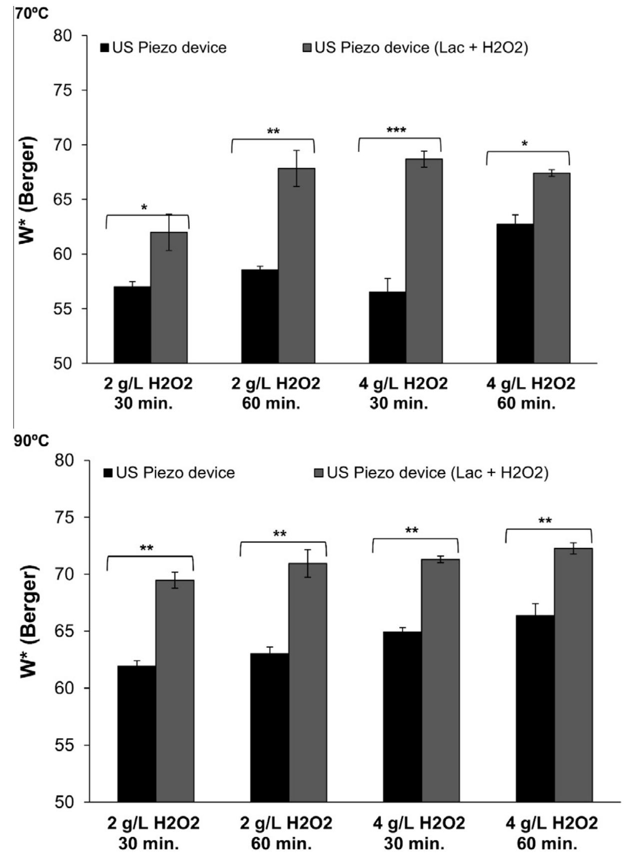

Fig. 6. Whiteness values (W^*) of cotton samples bleached with the combined laccase-hydrogen peroxide system assisted by low frequency ultrasound (piezoelectric device). Pretreatment: 2 U/mL laccase at 50 °C for 30 min.; bleaching with 2 and 4 g/L H_2O_2 at 70 and 90 °C for 30 and 60 min.). Statistically significant differences are indicated. *** = Ultrasound bleaching significantly different from enzymatic ultrasound bleaching ($P < 0.001$). ** = Ultrasound bleaching significantly different from enzymatic ultrasound bleaching ($P < 0.01$); * = Ultrasound bleaching significantly different from enzymatic ultrasound bleaching ($P < 0.05$).

ultrasound process. Even when a shorter processing time (30 min), lower temperature (70 °C) and hydrogen peroxide concentration (4 g/L) were applied it was observed a significant increase on the whitening value for both equipments used. However, the bleaching performance between these equipments did not show significant differences ($P > 0.05$). Indeed, the only difference between those transducers is related with the electric (piezoelectric) and magnetic fields (magnetostrictive) that generate acoustic energy. The piezoelectrics rely on the ability of certain materials to deform when exposed to an electric field mainly ceramics of Lead Zirconate Titanate (PZT), the magnetostrictives rely on the ability of certain materials to deform when exposed to a magnetic field. The magnetostrictive transducers are stronger and do not lose effectiveness over time as the piezoelectric, but in contrast, cannot operate at frequencies above 20 kHz and are very expensive due to costs of alloys and type of generator (for high power/frequency and low impedance). In this work, piezoelectric device were chosen for further experiments [32].

As confirmed by other authors [15], the use of multiple frequency operation can be considered has an efficient alternative to some drawbacks associated with high frequency ultrasounds, mainly related with the erosion of transducers in a continuous operational mode.

On Fig. 6 is presented the effect of laccase pretreatment on low frequency ultrasound cotton bleaching using a piezoelectric device. Once again, the conventional temperature (90 °C) and less than that (70 °C) were tested. The results confirmed the expected tendency that whiteness of cotton is improved when the concentrations of chemicals, incubation time or temperature increased. The results also demonstrate that the introduction of laccase as a pretreatment and the following hydrogen peroxide bleaching assisted by ultrasound significantly increased the whiteness values for all the set of conditions tested. At 90 °C, there was no significant difference on laccase-ultrasound bleaching for the different parameters studied which can be related with the possible maximum bleaching achievement. This high temperature by itself the key factor for the whiteness improvement. For 70 °C, the highest whiteness increment was only observed when 2 g/L H_2O_2 for 60 min ($P < 0.01$) and 4 g/L H_2O_2 for 30 min ($P < 0.001$) were applied. For that reason it could be possible to reduce the hydrogen peroxide amount from 4 g/L until 2 g/L extending the processing time. Nevertheless, the final laboratory scale recipe included 4 g/L of hydrogen peroxide at 70 °C for 30 min. The amount of oxidant agent was chosen considering the final processing costs. It is cheaper to use higher concentration of H_2O_2 during less time extent (30 min) instead of using low amount of this agent for longer periods. The time costs are considerably higher compared with the chemical agent price. Thus, the results achieved confirm that it is possible to reach the reference values using lower temperature (70 °C) and incubation time (30 min) than the standard bleaching process

(90 °C for 60 min). This gap can be attributed to the high energy induced by the ultrasonic system which replaces external temperature provided and can consequently reduce the processing time.

3.2. Process upgrading to pilot-scale

Based on the best results accomplished at laboratory scale, a new pilot scale reactor was designed. The design of the reactor had taken into account several parameters namely reactor diameter, liquid height and position of the transducers, for the best cavitational distribution and sonochemical efficacy. This new device is equipped with strategically located piezoelectric transducers that allow choose the appropriate ultrasound power intensity (140–2100 W) and the respective frequency (22 and 850 kHz). Using the best laboratory scale operational conditions for the half and optical bleaching processes, the combined laccase-hydrogen peroxide system assisted by ultrasound was studied.

3.2.1. Half-bleaching

The technology transfer from laboratory scale to pilot scale considered temperature (70 °C), high power and multi-frequency ultrasonic conditions and laccase pretreatment with 2 U/mL laccase at 60 °C (higher temperature than laboratory scale optimization was required to obtain desirable whiteness values). The study was carried out with low frequency ultrasound (22 kHz), at 70 °C for 30 min using different power intensities (140 W, 700 W and 2100 W). The results revealed a better bleaching performance when the process was carried out with high power ultrasound intensity (Fig. 7). On the other hand, under high frequency ultrasound (850 kHz, 400 W) conditions it was also increased the whiteness levels, however with lower impact than that achieved with low frequency device. The inclusion of laccase pretreatment on the system allowed increasing the cotton whiteness for both, low and high frequency ultrasounds ($P < 0.01$). Once again, on laccase-hydrogen peroxide system, the low frequency high power ultrasound was more efficient on cotton bleaching than high frequency low power ultrasound. Indeed, the frequency of ultrasonic systems can affect the temperature, collapse time, pressure and mass transfer properties on the cavitation site. Wayment et al. studied an ultrasonic system able to operate from 20 to 500 kHz and mentioned that the competition between the interfacial reaction volume of the bubbles resulted from cavitation, the heat loss at the bubble interface and the temperature achieved can affect the optimum sonication frequency of each system. The volume of bubble surface increased proportionally with frequency and also the volume of reactant at the interface of the bubble was proportional to the ultrasound frequency. Nevertheless, the heat loss of the medium was proportional to the sonication frequency increasing [33]. This can be an explanation for the lower bleaching efficiency achieved with 850 kHz since the heat induced by ultrasound influence the ability of hydrogen peroxide oxidize the colored phenolic compounds at the cotton surface, disturbing the whitening improvement.

Several authors already reported that combination of frequencies give synergistic results with yields greater that the algebraic sum of the single frequency yields [15]. Thus, further studies should consider this approach.

3.2.2. Optical-bleaching

So far, the optimum conditions achieved allowed us to improve the bleaching of cotton fabrics for the half-bleaching process. However, and due to the industrial and market needs, it was also our goal to improve and accomplish high levels of whiteness for the optical bleaching using the developed combined cotton bleaching. For this, the conditions optimized previously were applied on the optical bleaching and the results are presented in Table 1.

The results attained reveal that with the introduction of laccase and ultrasound parameters on cotton bleaching system, it was possible to increase the whiteness values from 107.92 to 118.23 Berger. This increase was achieved by introducing elements and energy on the system without any decrease in chemicals consumption. Thus, the potentialities of the combined laccase-hydrogen peroxide bleaching assisted by ultrasound should be exploited in order to reduce the amount of hydrogen peroxide and the bleaching temperature. The total costs would be reduced as well as the environmental impact.

3.3. Cost-benefit analysis

In order to carry out the final economic feasibility of the new developed technology, all the costs allied to chemical, water and energy consumptions involved in half-bleaching of cotton were detailed and broken down for each sub-process: laccase pretreatment, ultrasonic bleaching and washing procedures (Table 2).

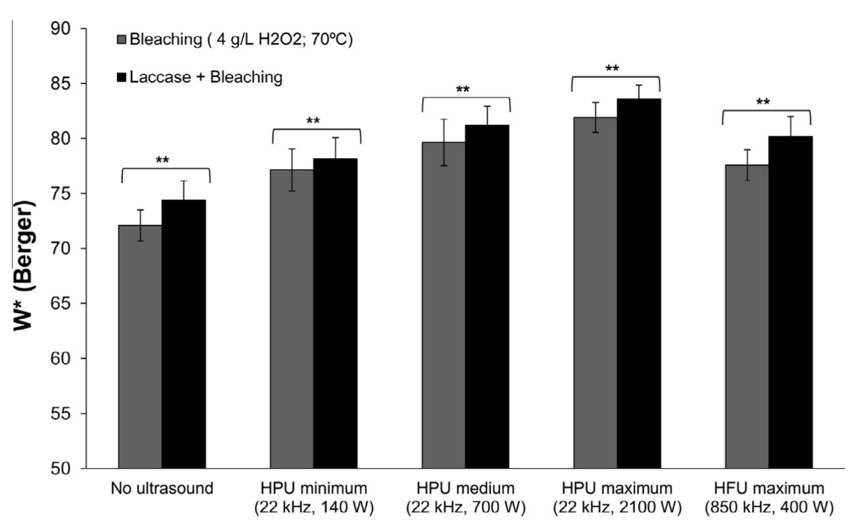

Fig. 7. Whiteness values (W^*) attained for half-bleaching using high power (22 kHz applying 140 W; 700 W and 2100 W) and high frequency (850 kHz applying 400 W) ultrasounds at pilot scale. The pretreatment was made using 2 U/mL laccase, 60 °C for 30 min. with 850 kHz piezoelectric device at 400 W. The ultrasonic bleaching was carried out using 4 g/L H_2O_2 at 70 °C for 30 min. Statistically significant differences are indicated. ** = Ultrasound bleaching significantly different from enzymatic ultrasound bleaching ($P < 0.01$).

Table 1
Optical bleaching conditions (8 g/L H_2O_2, at 103 °C for 40 min) and whiteness values using combined laccase-hydrogen peroxide system assisted by low-frequency high power ultrasounds (22 kHz, 2100 W) at pilot-scale reactor.

Process	Laccase pretreatment			Bleaching			
	Laccase (U/mL)	Temperature (°C)	US	H_2O_2 (g/L)	Temperature (°C)	US	W^* (Berger)
Standard optical bleaching	–	–	–	8	103	–	107.92
Combined Cotton bleach system	2	60	850 kHz 400 W	8	103	22 kHz 2100 W	118.23

Table 2
Costs estimation: Combined ultrasonic cotton bleaching/conventional bleaching; the costs calculation were based on the prices of water, energy and chemicals per kg of fabric.

Process	Pretreatment (€/kg cotton)	Bleaching (€/kg cotton)	Washings (€/kg cotton)	Total costs (€/kg cotton)
Conventional cotton bleaching	–	0.2239	0.1123	0.3362
Combined ultrasonic cotton bleaching	0.2788	0.1742	0.2376	0.6906

The cost analysis revealed that the new developed mechanism is still more expensive than the conventional process which is performed at highest temperature (90 °C). The main reasons for the higher cost lay on the chemical prices of Lutensol AT25 and laccase (23 €/kg and 11 €/L, respectively) that are too expensive and also on the energy consumption that was quite influenced by the heating energy provided by propane (high fee value). An alternative to this form of energy must be considered. Other solutions that could result on costs reducing are the study of minimum concentration of reagents needed to reach the whiteness values of cotton defined by the industrial source; the exploration of some ultrasound power intensity between 700 W and 2100 W that could obtain the aimed whiteness index; and also the analysis of all the additional washing processes that consume high quantities of water. Despite of this, it is important to mention that the huge evolution on enzymatic productions at industrial scale and ultrasound machinery will quickly allow decreasing the chemical and energy consumption of this new eco-friendly technology for cotton bleaching.

The goal of the work was to develop applicators that did not constitute a technological breaking for textile industries, mainly SMEs. The additional cost increment can still be lowered and further accepted if the quality improvements or savings are really achieved.

4. Conclusions

The combination of a laccase pretreatment with the conventional bleaching, both assisted by ultrasounds, resulted in a new cotton bleach technology that allowed increase whiteness levels of cotton samples. Despite the higher final costs compared with conventional process, some advantages should be considered with this new technology. At laboratory scale, the introduction of ultrasonic energy in the reaction chamber during enzymatic treatment of cotton fabric resulted in a significant improvement in enzyme efficiency, but it did not contribute to a decrease in tensile strength of the cotton fabric. Furthermore, the amount of oxidant agent required for half-bleaching was reduced. At pilot scale, it was not possible to decrease the amount of H_2O_2 since high quantity of sample is processed.

The transfer of the new technology from laboratory scale to pilot-scale stage was successfully achieved. The adaptation of a current jet dyeing machine by introducing multi-frequency ultrasonic devices promoted higher cavitational activity leading to higher processing yields in terms of catalyst activity and oxidant production. The operating and geometric parameters for maximizing the benefits of the sonochemical effects were reached for this specific application. Contrarily to current technologies which involve long processing times and high amounts of water, chemicals and high energy consumption, the efficiency of this wet finishing process was improved by increasing the mass transfer towards the inner parts of the textile material. The quality of the products is remarkably improved achieving whiteness levels above the values obtained by current methods. This innovative CottonBleach technology provide significant reduction bleaching temperature, from 90 to 70 °C, reducing therefore the energy involved on this process stage and the overall processing costs.

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