INFLUENCE OF ULTRASOUND FIELD ON LACCASE DEGUMMING PROCESS

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The enzymatic degumming of flax becomes an important alternative to conventional procedures. This paper investigates the benefits of ultrasounds in the diffusion of the enzymes, as well as their effects on the substrate. The combined enzyme-ultrasound process improves the degumming by accelerated heterogeneous enzyme–flax fiber reaction and the cavitation phenomenon upon sonication of the substrate.

Keywords: flax-cotton, laccase, degumming, ultrasounds

INTRODUCTION

The conventional method for flax degumming is done using hot alkali solutions, with or without applying pressure.[1] The chemical treatment reduces the quality of the fiber and due to the consumption of a large amount of chemicals and energy, it becomes economically unfeasible. Even more, the waste waters from the textile industry are polluting the effluents. In recent years, the interest in using enzymes instead of the conventional non-biological methods for flax degumming has increased.[2,3] Different enzymes are used for degumming of raw materials such as pectinase, cellulase and laccases. Among them, laccases (benzenediol:oxygen oxidoreductases; EC 1.10.3.2) have been recently extensively studied due to a number of advantages they offer: laccases do not need to have a high level of stability in the extracellular environment, they present low substrate specificity, and the addition of a co-substrate is not required.[4] Usually, in the degumming process of flax with laccase, due to the nonspecificity of the enzymes, some low molecular mass mediators, such as *N*-heterocylic bearing N–OH groups (e.g. 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid, violuric acid, hydroxyl benzotryazole), are necessary.[5-8]

Enzymatic treatment of textiles involves mass transfer from the enzyme solution across the interior of the textile substrate, which in the case of flax degumming is the medium lamella.[9] The role of the medium lamella and pectin-lignin cement is to keep the elementary flax fibers bound. The enzymatic degumming process denotes the degradation of the pectin-lignin cement by enzymes (e.g. laccase, pectinase, cellulase and pectinase).[10,11] Different enzymes can be used for lignin degradation, such as lacasse,[12] pectinase[13,14] or their combination.[10]

The lignin degradation mechanism is very complex and includes the cleavage of the aromatic rings in reaction with phenoxy radicals. In general, enzymes have low diffusion rates and the effect is concentrated on the outer fibers. Ultrasound (US) could be a way to improve the diffusion of the enzymes to the interior of the technical fibers in order to release elementary fibers. Ultrasonic energy has been used in different processes of mercerization, desizing, bleaching, scoring, and dyeing of cotton.[15-17] It is already known that the use of ultrasounds in textile wet processes offers benefits in terms of time, energy and chemicals consumption.[18-20] Combined ultrasound/hydrolytic enzyme applications provide less fibre damage and higher uniformity of the treatment,[16,21] which leads to obtaining cottonised flax fiber with physical-

mechanical properties similar to those of cotton. Enzymatic ultrasound degumming of flax offers a novel route of fiber modification, which allows increasing the flax percentage (more than 50%) in the cotton blends used for fine yarns production. It was shown that even minor changes of the environmental conditions (e.g. temperature, pressure, pH, ionic strength) determined the denaturation of the enzymes.[22,23] Also, Basto *et al*.[24] demonstrated that the activity of laccase decreased slightly with the increase of ultrasound intensity upon sonication longer than 10 min.

Akin *et al*.[25] were among the first to report the results of pilot scale studies on flax fibers produced by the enzyme-retting method. The material is ready to use for some applications. The benefic role of the US on the substrate has already been mentioned by Moholkar *et al*.,[18,19] but the kinetics of the process was not demonstrated. In the present work, we attempted to determine the kinetics of the ultrasounds action in the laccase degumming process. Our hypothesis is that ultrasounds act not only on the substrate, but also on the enzymes.

EXPERIMENTAL

All chemicals were supplied by Sigma-Aldrich unless otherwise stated.

Enzyme concentrations used in the present experiment were 0.4, 1, 2, 3, and 4% related to fiber mass. For every experiment, 100 g of cleaned flax tows with impurities of max. 2.2% and Tex 0.23 have been used. The ratio between fiber and treatment solution was 1:25. A 0.1 M buffer solution of sodium acetate–acetic acid with a pH of 4.8 was used. Treatment time and temperature were 60 minutes and 55 °C, respectively. The flax fibers were treated by using a specific softening agent (Emolient® obtained as described by C. Sirghie *et al*.[26]) 0.25% over fiber (owf) and hydroxyl benzotryazole (HOBT) as mediator for laccase at 0.01% related to enzyme concentration. The ultrasound treatment was done at a fixed frequency of 32 kHz, using an ultrasonic thermostated bath with a degas feature (Cole-Parmer, Vernon Hills, Illinois, USA). The progress of the reaction was followed by measuring the reaction product absorbance at 290 nm using a spectrophotometer Specor 200 (AnalyticJena, Wembley, UK).

RESULTS AND DISCUSSION

The mechanism of lignin degradation by laccase has already been elucidated and it has been indicated that side-chains and aromatic rings of lignin substrates are cleaved via the aryl radical cation and phenoxy radical intermediates, in reactions mediated only by laccase/O_2.[27]

In order to prove the influence of US on the fibers in the absence of enzymes, we observed the evolution of absorbance in time. Figure 1 shows the kinetic curves obtained with and without US. As can be seen, the absorbance increases upon the ultrasound treatment (which is correlated with the yield of degumming). The increase in absorbance indicates a higher amount of lignin and pectin in the presence of ultrasounds. This is caused by the cavitation phenomenon, through the process of collapse of the bubbles produced in aqueous medium upon sonication, followed by generating high local temperature and pressure, which led to breaking of the medium lamella and release of lignin fragments.[28] In the absence of ultrasounds, the slight increase in absorbance can be explained by pectin solubility as a function of temperature.

In order to show the benefits of US on the enzymatic process, we measured the kinetic curves at five enzyme concentrations in the absence (Figure 2A) and presence (Figure 2B) of US for 60 minutes. It can be observed that in the presence of US, the absorbance increased by almost 50%, which is economically beneficial. The reaction rates of the degumming process increased with the enzyme concentration until reaching a maximum.

As the lignin content in the flax fiber is between 2.5 and 5%,[29] usually all the substrate (lignin) is transformed into reaction products in 1000 seconds. Although it is obvious from Figure 2 that US and enzymes work synergistically as regards degumming, we attempted to demonstrate the double effect of US on the enzyme and on the substrate by calculating the kinetic parameters of the enzymatic reactions.

The initial velocities, r_0, were determined by numerical derivation, at t = 0.01 s, after converting the absorbance vs. time curve to a concentration vs. time curve (for more details, see[30]). Mean values of initial velocities were calculated from 3-4 measurements at the same initial concentration of the substrate.

According to the well-known Michaelis-Menten equation:

$$r_0 = \frac{k[E]_0[S]}{K_M + [S]} = \frac{r_{\max}[S]}{K_M + [S]} \quad (1)$$

where $[E]_0$ and $[S]$ stand for the initial concentration of the enzyme and for the substrate concentration respectively, K_M and r_{max} are the Michaelis–Menten parameters, and k is the rate constant of breakdown of the enzyme–substrate complex to the product.

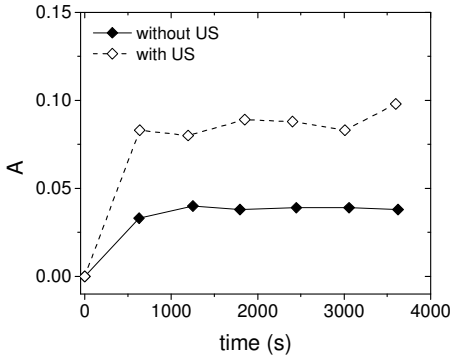

Figure 1: Time evolution of absorbance in the absence of enzymes, with (dash line) and without (bold line) ultrasound treatment of the fibers

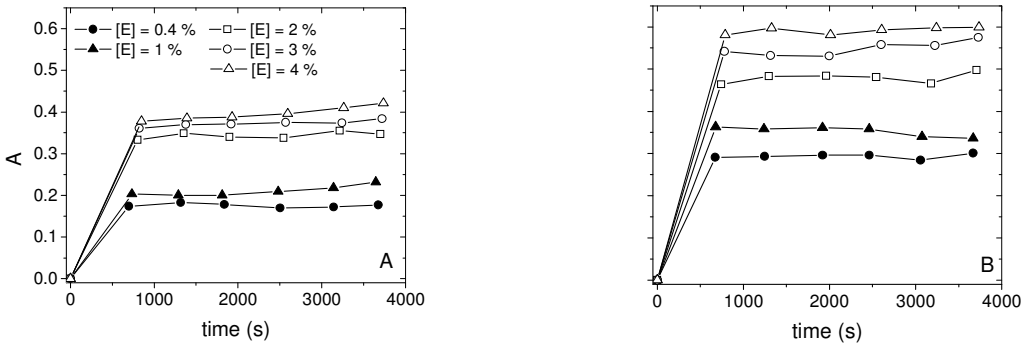

Figure 2: Time evolution of absorbance for 5 different concentrations of enzymes (owf) in the absence (A) and presence (B) of ultrasound treatment

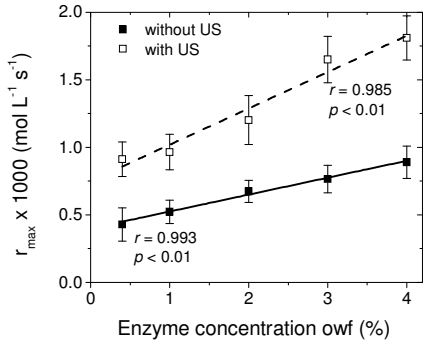

Figure 3: Dependence of maximum reaction rates (r_{max}) on enzyme concentration (owf)

Several methods were employed for determining the Michaelis–Menten parameters, as indicated below.

The double-reciprocal form of equation 1 (Lineweaver–Burk linearization):[31]

$$\frac{1}{r_0} = \frac{1}{r_{\max}} + \frac{K_M}{r_{\max}} \cdot \frac{1}{[S]} \quad (2)$$

where r_{max} resulted from the intercept and K_M from the slope of the graph.

The Eadie–Hofstee plot:[32]

$$r_0 = r_{\max} - K_M \cdot \frac{r_0}{[S]} \quad (3)$$

where again r_{max} resulted from the intercept and K_M from the slope of the graph.

The Hanes–Wolff plot:[33]

$$\frac{[S]}{r_0} = \frac{1}{r_{\max}} \cdot [S] + \frac{K_M}{r_{\max}} \quad (4)$$

where r_{max} was determined from the slope and K_M from the intercept of the plot. Maximum reaction

rates (r_{max}) provided the same results for all procedures with a deviation of less than 10%.

Figure 3 depicts the dependence of maximum reaction rates (r_{max}) on enzyme concentration (owf). Both in the presence and in the absence of the US treatment, the dependences are linear with very good correlation coefficients. The fact that the lines are not parallel suggests a synergetic effect of US on both: enzymes and substrate.[30,34]

The degumming improvement in the combined enzyme-ultrasound process could be explained by first the accelerated heterogeneous enzyme–flax fibers reaction, and second by the cavitation phenomenon upon sonication of the substrate. On the other hand, after 10 minutes, the enzymatic reaction is over regardless of whether or not the ultrasound treatment is applied, and the absorbance remains almost constant. The slight increase of absorbance after 30 minutes may appear due to other random side reactions.

CONCLUSION

Our experiment demonstrated that the acceleration of the degumming process can be explained by a synergetic effect of both parameters: cavitation and the heterogeneous reaction between fibers and enzymes. On the other hand, the synergetic effect of ultrasounds on the fibers and enzymes has been demonstrated using different linearization equations.

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