APPLICATION OF A LOW-LEVEL, UNIFORM ULTRASOUND FIELD FOR THE ACCELERATION OF ENZYMATIC BIO-PROCESSING OF COTTON

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Enzymatic bio-processing of cotton generates significantly less hazardous, readily biodegradable wastewater effluents, yet having several critical shortcomings, such as expensive processing costs and slow reaction rates, which impedes its acceptance at an industrial level. Our research showed that the introduction of a low-energy, uniform ultrasound field in enzyme-processing solutions greatly improved enzyme efficiency by significantly increasing their reaction rate. It has been established that the following specific features of combined enzyme/ultrasound bio-processing of cotton are critically important: a) the cavitation effects caused by the introduction of an ultrasound field in the enzyme processing solution greatly enhance the transport of enzyme macromolecules towards the substrate surface, b) the mechanical impact, produced by the collapse of the cavitation bubbles, provides an important benefit, that of "*opening up*" the surface of the solid substrates to the action of enzymes, c) the effect of cavitation is several hundred times higher in heterogeneous (solid substrate-liquid) than in homogeneous systems, and d) in water, the maximum effects of cavitation occur at ~50 °C, which is the optimum temperature for many industrial enzymes. At a laboratory scale, the introduction of ultrasonic energy in the reaction chamber during enzymatic bio-preparation of greige cotton fabrics and enzymatic bio-conversion of cotton gin and cotton lint waste biomass in sugars resulted in a significant improvement in enzyme efficiency.

Keywords: enzyme, ultrasound, cellulose, cotton, cotton gin/lint waste and bio-fuel

INTRODUCTION

Since the middle 1990s, the use of various enzymes in the textile industry has considerably increased, especially in the processing of natural, high value fibers such as cotton. A major advantage of enzymatic bio-processing is that the application of enzymes is much more environmentally benign and the reactions catalyzed are very specific, thus assuring a higher performance. In contrast, the traditional use of harsh organic/inorganic chemicals for cotton processing generates large quantities of toxic wastewater effluents, much less specific, often inducing undesirable side effects, such as reduction in the polymerization degree of cellulose. The enzymes used in cotton bio-processing, acting as catalysts, speed up complex bio-chemical reactions such as the hydrolysis of cellulose (by cellulases), pectins (by pectinases), starches (by amylases), and triglyceride-based compounds in fats and oils (by lipases).

Once they act as catalysts, relatively small concentrations of enzymes are required; if the applied conditions are favorable to the specific enzyme, the action will be repeated several times during the process.

Other potential benefits of enzymatic bio-processing include cost reduction through energy and water savings, and improved product quality. Even a larger acceptance of enzymatic bio-processing by the textile industry in the near future will probably result from increasing legislative pressures, from the part of the governments worldwide, to sharply decrease the quantity and toxicity of textile wastewater effluents.

In recent years, the high worldwide demand for energy and unstable and progressively more expensive petroleum sources imposed the development of new alternative transportation fuels,[1,2] such as bio-ethanol from various biomass feedstocks, including the underutilized sources of plant cellulose, such as cotton gin and lint waste. Currently, the cost-competitive production of cellulosic bio-ethanol is prohibited mostly by the high cost and low efficiency of enzymatic hydrolysis of plant celluloses. Despite the recent,[3] substantial reduction in the production cost of cellulolytic enzymes, the actual conversion of plant cellulose into sugars still remains an expensive and slow step. One of the most critical stages of this conversion of plant celluloses into biofuels employs hydrolysis reactions between a highly specific enzyme and the matching substrate (*e.g.* cotton gin/lint waste cellulose with cellulase), soluble sugars, to be easily converted into ethanol in a subsequent step, thus resulting. The typical applications of enzymes for bio-processing of cotton and cotton waste celluloses are summarized[4-7] in Table 1.

Table 1
Typical examples of enzymes used in cotton bio-processing

Application	Enzyme(s)	Benefit
Desizing of cotton	Amylase	Removal of starch from the fiber surface
Scouring of greige cotton	Pectinases, Cellulases, Lipases	Removal of waxes, proteins, pectins and natural fats from the cotton fiber surface
Peroxide breakdown	Catalase	Effluent treatment to remove residual H_2O_2
Bio-finishing of cotton	Cellulases	Improvement of the appearance of cotton fabrics and garments by removal of fiber fuzz and pills from the substrate surface
Bio-stoning of denim	Cellulases	"Stone-washing" of denim fabrics to produce fashionable aged appearance
Bio-bleaching of denim	Laccases	"Stone-washing" effects without loss of fabric strength
Laundry washing	Proprietary mixtures of enzymes	Removal of soils and stains
Hydrolytic conversion of cotton gin and lint waste celluloses	Cellulases	Produce soluble sugars, subsequently easily converted into bio-ethanol

In addition to the numerous advantages of enzymatic bio-processing of cotton and cotton waste celluloses, several critical shortcomings – such as added processing costs and most important, slow reaction rates – should be mentioned. Enzymatic bio-processing of cotton, like any other wet processing system, involves transfer of mass (enzyme macromolecules) from the processing liquid medium (enzyme solution) across the surface of the substrate. The detailed mechanism of enzymatic reactions, quite complicated, is still being investigated. In very general terms, the enzymatic reaction could be described according to the stages from Figure 1. At least two stages of the enzymatic reaction (1 and 4) involve transport of the enzyme macromolecules and of the enzymatic reaction products to and from the substrate surface. Since both stages are controlled by diffusion, the overall reaction rate of enzymatic hydrolysis is governed by the diffusion rate of the enzyme macromolecules. Generally, the large three-dimensional enzyme macromolecules have very low diffusion rates and also tend to react with the outlaying cellulose fibers from the cotton yarn, which could result in

excessive fiber damage. It was suggested[8] that sonication of the enzyme processing solution under certain specific conditions could provide a far more efficient transport mechanism for the "bulky" enzyme macromolecules throughout the immediate border layer of liquid at the substrate surface.

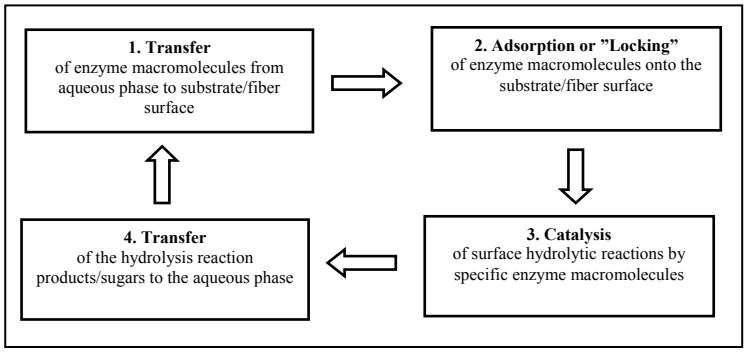

Figure 1: Schematic diagram of the general stages of an enzymatic reaction on a solid substrate

Technical aspects of using ultrasound to accelerate enzymatic bio-processing

Generally, the introduction of ultrasound energy into the liquid medium has two primary effects: cavitation and heating (Fig. 2). In enzymatic bio-processing, the more important of these two is cavitation formation, growth and implosive collapse of bubbles in a liquid. The dynamics of cavity growth and collapse are highly dependent on the type of liquid, on the presence of dissolved species and gases in the liquid and on the liquid temperature. The imploding cavitation bubble causes a nearly adiabatic compression of the excess vapors inside the cavity, thus raising its pressure (~500 atm) and temperature (~5,500 °C; plasma conditions). Quite important, liquid sonication at low frequencies dissipates most of the ultrasound energy through cavitation phenomena, while sonication at high frequencies dissipates a significant amount of energy through heating (at the expense of cavitational dissipation). As the excess vapors inside the cavitational bubble are compressed by its collapse, and the vapors reach several thousand degrees Celsius, these trapped vapors are largely dissociated.

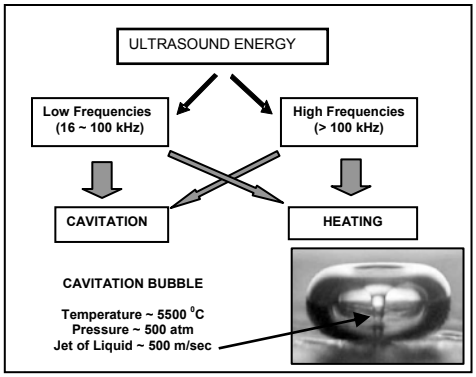

Figure 2: Schematic diagram of the basic properties of ultrasound

As a result, a powerful ultrasonic irradiation of liquids produces a plethora of high energy chemical reactions that have been studied for many years.[9] For water, the collapse of the cavitational bubble produces high-energy intermediates, such as H• (atomic hydrogen), OH• (hydroxyl), $e^-_{(aq)}$ (solvated electrons), H_2O_2 (hydrogen peroxide), HO_2 (superoxide) and, H_2 (molecular hydrogen). A special distinctive brand of chemistry – sonochemistry – specifically studies the reactive interactions of these high-energy intermediates with various dissolved species in a liquid:

$$O_2 \rightarrow 2\,O\bullet$$
$$H_2O \rightarrow H\bullet + OH\bullet$$
$$OH\bullet + OH\bullet \rightarrow H_2O_2 \quad (1)$$
$$2\,O\bullet + H\bullet \rightarrow HO_2$$
$$H\bullet + H\bullet \rightarrow H_2$$

It appears that the formation of such highly reactive intermediates by collapsing cavitational bubbles should significantly affect the long-term catalytic stability/ activity of the dissolved enzyme macromolecules. The common perception was that these highly reactive intermediates and powerful shock waves, resulting from the collapse of the cavitation bubbles, could severely damage or, at least, inactivate the very sensitive and intricate structures of enzyme proteins. However, when sonication was specifically tried to inactivate enzymes and terminate enzymatic activity, its inactivation efficiency was quite low.[10] In another example, it was reported[11] that the combined effects of heat, ultrasonic waves and pressure were applied, with limited success, for the inactivation of certain thermostable enzymes. The authors showed that the synergistic effects of manothermosonication could reduce enzyme resistance to thermal inactivation only to a small degree. In another comprehensive overview[12] on the combined effects of heat, pressure and ultrasound on microorganisms and enzymes, the authors concluded that the resistance of most microorganisms and enzymes to ultrasound is so high that the required intensity of an ultrasound treatment would be impractical. One possible explanation of the apparent inefficiency of ultrasound to inactivate enzyme macromolecules could be just their extremely low ratio to the huge quantity of solvent molecules (*e.g.* water) at typically used enzyme processing concentrations of 4-5 g/L. Therefore, the probability of enzyme macromolecules to be seized into a cavitation bubble and to encounter the highly reactive intermediates created by the collapsing bubbles should be very low.

If the ultrasound, as it appears from the literature, does not affect the *specific activity* of industrial enzymes in any significant way, it could be used for intensifying the enzymatic processing of cellulose-based substrates by improving the *transport* of enzyme macromolecules towards the substrate surface.

Unlike the collapse of cavitation bubbles in homogenous systems (liquid–liquid interface), in heterogeneous systems (*e.g.* enzyme solution–cellulose substrate) cavitation bubbles collapsing on or near a surface are non-symmetrical, since the surface provides resistance to liquid flow. The result is an in-rush of liquid predominantly from the opposite side of the bubble (remote from the substrate surface), a powerful liquid jet (roughly 500 m/sec) being formed and targeted at the surface. Also, because of the reduced liquid tensile strength at the liquid-solid interface, lower sonication intensities can be used in heterogeneous systems.

It is critically important that the rapid collapse of the cavitation bubbles generates significant shear forces in the bulk liquid immediately surrounding the bubble and, as a result, produces a strong stirring mechanical effect. This effect can significantly increase mass and heat transfer to the surface of the substrate, by disrupting the interfacial boundary layers, on also activating the catalytic performance of the enzyme macromolecules adsorbed onto the substrate surface.

Generally, the diffusion transport of enzyme macromolecules toward the surface of a solid substrate could be also enhanced to a certain degree by simple mechanical agitation[13] of the processing solution, although it is well-known that mechanical agitation is not a very effective stirring mechanism for the immediate border layer of liquid at a solid–liquid interface, where the enzymatic reaction actually occurs. Figure 3 presents the schematic distribution of the velocities in the layers of liquid concentrically surrounding the solid particle (substrate).

The first, immediate layer of liquid at the solid–liquid interface is motionless, while the velocities of the following layers quickly increase to the maximum constant value defined by the agitation power of the bulk solution. Since the immediate, adjusted layer of liquid at a solid–liquid interface is practically immobile, the only available transport mechanism for enzyme macromo-

lecules to reach the substrate surface is diffusion which, in the case of such large protein macromolecules (50 000-250 000 Da), is highly inefficient. When microscopic cavitation bubbles collapse in the immediate vicinity of a substrate surface, they generate powerful shock waves that cause effective stirring/mixing of this adjusted layer of liquid. These shock waves, generated by cavitation bubbles collapsing on and near the surface of the substrate (*e.g*. cellulosic fibers), are an ideal stirring mechanism for the immediate layer of liquid at the solid–liquid interface, where enzyme reactions take place. The forceful stirring/ mixing of this normally immobile layer of liquid greatly improves the supply of enzyme macromolecules to the surface of a substrate.

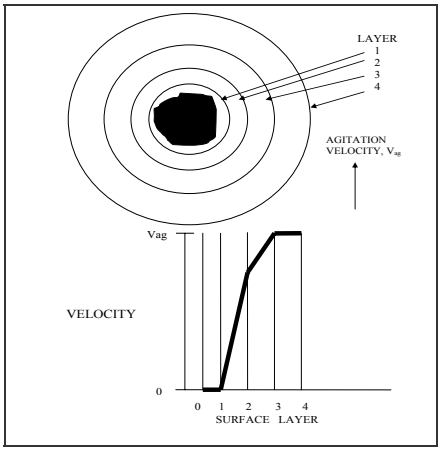

Figure 3: Schematic distribution of the velocities of the liquid layers concentrically surrounding the solid particle (substrate)

Therefore, the specific features of cavitation phenomena are very important for practical bio-processing applications: a) effect of cavitation is several hundred times higher in heterogeneous (*e.g*. textile wet processes, enzymatic hydrolysis of plant celluloses) than in homogeneous systems and b) in water, the maximum effects of cavitation occur at ~50 °C, which is the near optimum temperature for many enzymatic bio-processing applications.[14]

Essentially, the *uniform* introduction of ultrasound energy into heterogeneous systems will generate the majority of cavitation bubbles in the immediate vicinity of the solid–liquid interface, because of the asymmetry of surface tension while, in the case of homogeneous systems, cavitation bubbles are distributed evenly throughout the bulk of the processing solution.

It is also important that, in the case of heterogeneous systems, most of the cavitation bubbles are generated close to the substrate surface, thus providing an important additional benefit of the "*opening up*" of the surface of solid substrates to the action of the enzyme macromolecules, as a result of the mechanical impacts produced by the collapse of the cavitational bubbles.

Another imperative consideration is that, despite their close-packed and generally well-ordered structures, the enzyme macromolecules are usually not entirely rigid and have some conformational flexibility in solution, which helps them to properly position their active domain relative to the substrate. Therefore, vigorous stirring/agitation of the normally immobile border layer of the liquid at the liquid–solid interface, caused by sonication, should help the enzyme macromolecules to more easily position themselves "*fittingly*" onto the substrate.

Finally, another valuable benefit of the intensive stirring/agitation of this border layer by collapsing cavitation bubbles is an enhanced removal of the hydrolysis reaction products from the reaction zone, which should also contribute to an overall increase in the reaction rate.

In summary, the necessary requirements to maximize the benefits of ultrasound

energy for enzymatic bio-processing can be expressed as follows:

- **Ultrasound frequency**: it appears that optimum sonication frequency should be in the 20-100 kHz range. Such low sonication frequencies are more beneficial because:

  a) most of the introduced ultrasound energy is dissipated through the cavitation mechanism rather than through wasteful heating;

  b) lower sonication frequencies produce larger cavitation bubbles and therefore, more powerful "jets", thus providing more vigorous stirring/mixing of the border layer of the liquid at the solid–liquid interface;

- **Ultrasound energy**: it appears that the optimum sonication power should be in the 2-10 W/cm^3 range. The low energy sonication of the enzyme processing solution enhances the transport of enzyme macromolecules, without generating excessive amounts of highly reactive intermediates;

- **Uniform introduction of ultrasound energy**: it is critically important to introduce ultrasound energy into the processing bath in the most uniform way. This assures a uniform generation of the cavitation bubbles throughout the reaction chamber, resulting in uniform enhancement of the transport of enzyme macromolecules toward the substrate;

- **Application of ultrasound in heterogeneous *vs.* homogeneous systems**: since the effects of cavitation are several hundred times greater in heterogeneous than in homogenous systems, it appears that the introduction of ultrasound could be economically justified only for solid–liquid systems. In homogeneous systems, the much less expensive mechanical agitation will be probably sufficient.

On the whole, despite the apparent attractiveness of introducing ultrasound energy for intensifying the enzymatic bio-processing of natural fibers, it was unclear to what degree sonication would affect the complex structures of the enzyme macromolecules and how significant the benefits of ultrasound energy introduction could be.

The objectives of our experiments were to study the effects of a low-level, uniform ultrasound field on: a) enzymatic bio-scouring of cotton textiles with pectinase, and b) enzymatic bio-conversion of cotton gin and lint waste celluloses into sugars (for subsequent conversion into bio-ethanol).

EXPERIMENTAL

Ultrasound hexagon reactor

All experimental studies of the effects of a low-level uniform ultrasound field on enzymatic bio-processing were carried out with an Ultrasound Hexagon Reactor (UHR), manufactured by Advanced Sonics Company. This medium scale sonication reactor introduces ultrasound energy *via* six sets of identical transducers attached to the six sides of the hexagonal reaction chamber (volume ~4.0 L), thus assuring a very uniform and controlled sonication of the sample. Figures 4A and 4B show the experimental set-up for studying the effect of ultrasound on pectinase bio-scouring and hydrolytic conversion of cotton gin and lint waste celluloses into sugars.

Combined pectinase/ultrasound bio-scouring test

Two different types of cotton fabrics, both supplied by Testfabrics, Inc., were used for bio-scouring tests: in **test 1 –** light-weight original greige cotton printcloth (118 g/m^2) and in **test 2 –** heavy greige cotton duck cloth (501 g/m^2). To prepare samples for pectinase bio-scouring tests, all fabric samples (457 x 127 mm) were sewn around the edges (to prevent unraveling during processing) and desized with an Amylase enzyme solution at 50 °C, for 90 min. After completing the desizing procedure, all samples were tested for the remaining sizing agent (starch) with the Iodine/Potassium Iodide indicator. At the beginning of every experiment, the working enzyme solution was "degassed" for 1 h at a UHR generator setting of 20 amps. After degassing, a cylindrically shaped sample of cotton fabric was attached (hooked) to the supporting wire ring with alligator clips, then dipped into the enzyme processing solution in a UHR reactor chamber and sonicated for 30, 45, 60 and 90 min, at a UHR generator setting of 20 A and magnetic stirrer setting of 200 rpm. After the treatment, all samples were boiled in DI water for 6 min and washed thoroughly with DI water, to remove the remnants of enzyme and/or buffer solution. Finally, the samples were padded on a Mathis HVF padder and dried in a Mathis LTE

pop-out oven at 140 °C, for 1 min, prior to laboratory testing. In all tests, at least six samples (3 warps + 3 fills) of the same fabric were treated under identical conditions, so that each trial would assure good data reproducibility. All fabric measurements were performed under constant conditions: 21 °C and 65% humidity. The resulting wettability and whiteness values for all samples, treated under various conditions with pectinase enzyme and/or ultrasound, were determined and compared with those of the original, untreated cotton samples. The wettability of the treated samples was evaluated in accordance with the AATCC RA63 Water Resistance, Absorbency and Wetting Agent Evaluation Test (based on the measurement of the time during which water is wicked up at a distance of 3 cm on a strip of tested fabric in warp and fills direction). In the experiments with cotton printcloth, the test was stopped after 600 sec, whether it reached the marks or not, and it was recorded as >600 sec; in the experiments with cotton duck cloth, the test was run up to 1200 sec. The CIE whiteness index was determined by measuring the average of the front and back of each treated fabric sample with a Milton Roy Color Mate Color Analyzer. α-Amylase was acquired from Sigma Aldrich, Multifect Pectinase FE and Accelerase 1000 were provided by the courtesy of Danisco Division of GENENCOR. Enzyme assays and reaction conditions for all enzymes used in the experiments are listed in Table 2.

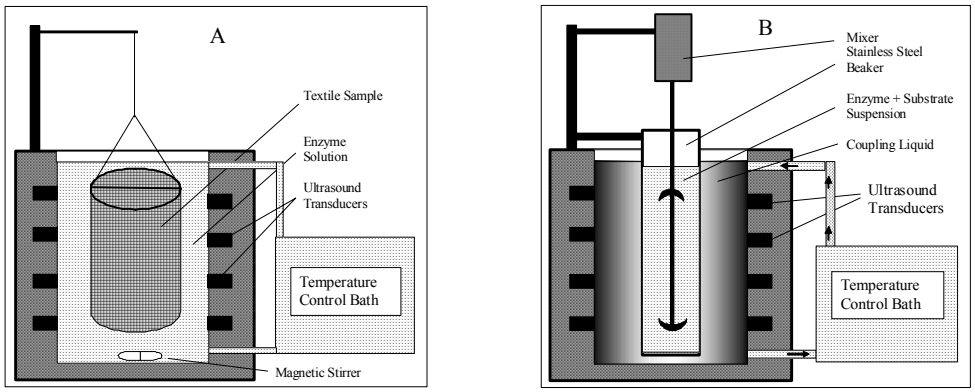

Figure 4: Schematic diagrams of the experimental set-up of an Ultrasound Hexagon Reactor for combined enzyme/ultrasound bio-scouring of cotton fabrics (A), and enzymatic hydrolysis of cotton gin and lint trash samples (B)

Table 2
Enzyme assays and reaction conditions

Enzyme	Activity, U/g	Buffer, M	pH	T, °C
α-Amylase from *Bacillus amyloliquefaciens*	250	Acetate: 0.05	5.0	50
Multifect Pectinase FE	145-180	Formate: 0.02	3.85	45
Accelerase 1000	Endoglucanase: 2500 against CMC Beta-Glucanase: 400 against pNG	Acetate: 0.05	5.0	50

Combined accelerase/ultrasound hydrolytic bio-conversion tests. A stainless steel beaker (64 mm in diameter; 305 mm height; ~500 mL), containing finely ground cotton gin (**test 3**) or lint (**test 4**) cellulose samples (Wiley Mill; 1-mm screen) was placed in the center of a UHR reactor. For all Accelerase hydrolytic conversion tests, the UHR ultrasound generator was set up at 13 amps and a special agitation mechanism was employed inside the SS beaker: a slowly rotating shaft (27 rpm) with two impeller blades stirring the sample suspension into counter flow mode (to assure a uniform distribution of the cellulose substrate particles throughout the volume of the SS beaker). The suspensions of Accelerase and cotton gin or lint trash sample were run with and without ultrasound, for 5 and 8 h, respectively. The first liquor sample (~5 mL) was taken immediately after stirring was stabilized (~5 min), and placed into a boiling water bath for 5 min (the same for buffer controls), to inactivate the Accelerase cellulase enzyme. After inactiva-

tion, the liquor sample was cooled in an ice bath, poured into a 15 mL centrifuge tube and centrifuged at 4000 rpm for 15 min. The following liquor samples (~5 mL) were taken every half hour for the first 3 h, and every hour for the remaining duration of the test. The degree of conversion of the cotton gin or lint trash cellulose into sugars was determined by measuring the actual concentration of glucose in the processing solution, in accordance with the DNS method[15] (Fisher DNS-reagent; Milton Roy Spectronic 21 spectrophotometer; 540 nm wavelength). The automatic temperature control of the coupling liquid (and of the samples in the SS beaker) in the reactor chamber was maintained by a NesLab RTE-211 temperature control bath.

EXPERIMENTAL RESULTS

Effects of ultrasound on pectinase bio-scouring of greige cotton fabrics

Raw unscoured (greige) cotton contains ~90% cellulose and various non-cellulosics, such as waxes, pectins, proteins, fats and coloring matter. To remove these hydrophobic non-cellulosics and to produce a highly absorbent fiber that can be uniformly dyed and finished, the greige cotton is traditionally processed by boiling a sodium hydroxide solution in the presence of wetting and sequestering agents.[16] This industrial process requires large quantities of water and energy, and generates a highly alkaline wastewater effluent. It was suggested that pectinase enzymes might be a valuable alternative to harsh alkaline solutions in the preparation of cotton. At present, enzymatic bio-preparation of greige cotton, representing a fairly new approach, is mostly in the developmental stage.[17]

Two bio-preparation tests were carried out at identical enzyme concentration, temperatures, pH, and sonication power: a) light-weight cotton fabric (printcloth; **test 1**) and, b) heavy-weight cotton fabric (duck cloth; **test 2**). In addition, control experiments were carried out in which both tests were replicated using only buffer solution, to determine if sonication by itself could affect the fabric samples. The average wettabilities – (warps + fill)/2 – of the printcloth samples treated with/without ultrasound are presented in Figure 5, and the average wettabilities of the duck cloth samples – in Figure 6. For comparative purposes, Figures 5 and 6 also present the wettabilities of untreated/desized samples and of those treated by conventional alkaline scouring. The data indicate that, in both tests, the wettabilities of the original, untreated samples were far in excess, of 1500 sec.

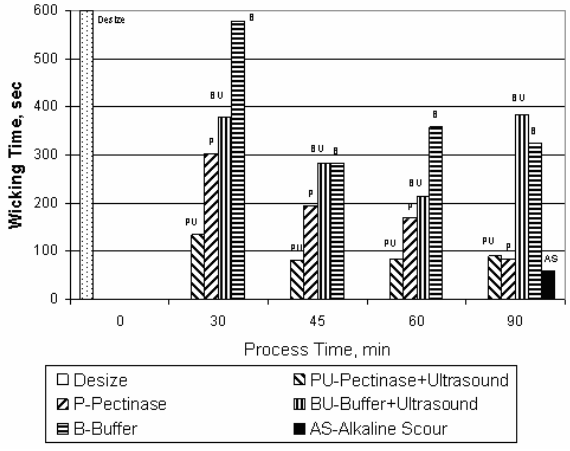

Figure 5: Evaluation of the influence of treatment time on average wettabilities (warp + fill)/2 of cotton printcloth samples after pectinase bio-scouring under sonication conditions (**test 1**)

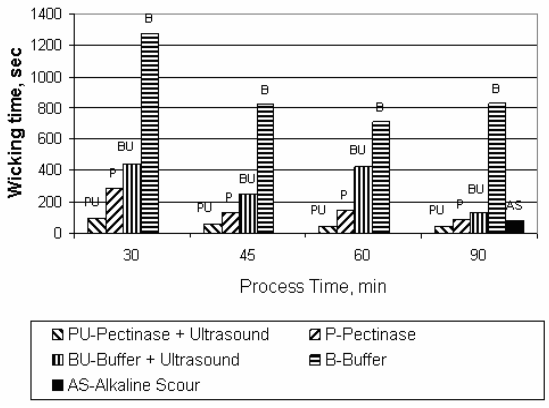

Figure 6: Evaluation of the influence of the treatment time on average wettabilities (warp + fill)/2 of cotton duck cloth samples after pectinase bio-scouring under sonication conditions (**test 2**)

Experimental data on the wettability of all treated samples for both types of cotton fabric clearly show that the introduction of a low-level uniform ultrasound field during pectinase bio-scouring greatly accelerated the process. In **test 1** (cotton printcloth), the

wettability values of the samples treated with a combination of enzyme and ultrasounds were comparable with those of the samples treated with a conventional alkaline scouring after a treatment of only 45 min. The effect of ultrasound by itself (as the difference between the wetting time of the sonicated sample *vs*. that of the un-sonicated one) was well-pronounced at treatment times of 30, 45 and 60 min, but not at treatment times of 90 min. Similarly, in **test 2** (cotton duck cloth), the wettability values of the samples after combined enzyme/ultrasound treatments were comparable with those of the alkaline scoured samples even after just a 30-min treatment. Also, the effect of the ultrasound by itself was well-pronounced at all treatment times. Interestingly, in both tests, the introduction of ultrasound energy also improved to some extent the performance of the buffer solution used for control runs.

Figure 7 presents the whiteness index of the bio-scoured samples of cotton printcloth and Figure 8 – the whiteness index of cotton duck cloth.

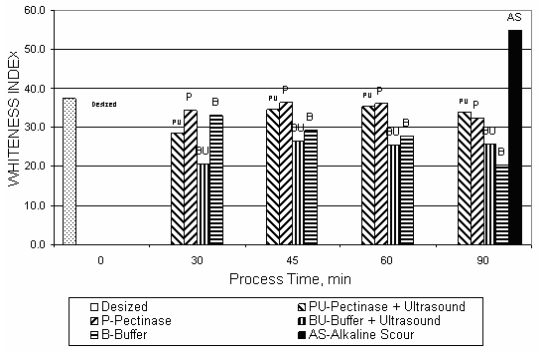

Figure 7: Evaluation of the influence of the treatment time on the CIE Whiteness index of cotton printcloth samples after pectinase bio-scouring under sonication conditions (**test 1**)

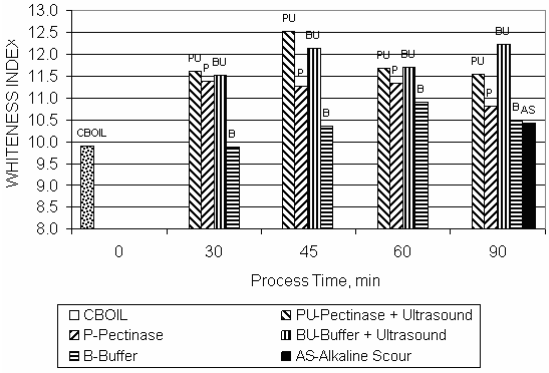

Figure 8: Evaluation of the influence of the treatment time on the CIE Whiteness index of cotton duck cloth samples after pectinase bio-scouring under sonication conditions (**test 2**)

Generally, the data indicate that neither single pectinase bio-scouring nor pectinase bio-scouring in combination with ultrasound significantly affected the whiteness index of the treated samples. In **test 1**, the bio-scored samples had slightly lower whiteness index values, while in **test 2** – slightly higher whiteness indexes than those recorded after alkaline scouring. It appears that, if bio-scoured with pectinase, the cotton fabrics intended to be dyed (especially for light color shades), have to undergo an additional bleaching treatment.

Effects of ultrasound on enzymatic bio-conversion of cotton gin and lint trash into sugars

In the US, cotton is usually harvested by spindle-picker harvesters or stripper harvesters. An average of 1500 lbs of spindle-picked seed cotton or 2000 lbs of stripper-harvested seed cotton is required to produce a standard 500 lb bale of cotton fiber. The annual production of 15 to 20 million bales of cotton in the US leaves over two million tons of gin and lint trash to be disposed of. Approximately half of the gin mills in the US must actually spend money to dispose of the cotton gin and lint trash.[18] The enzymatic conversion of such underutilized sources of plant celluloses as cotton gin and lint trash into valuable bio-fuels could be beneficial to the US cotton growers.

Two experimental studies on the influence of ultrasound on the hydrolytic conversion of cotton waste celluloses into sugars were carried out with/without ultrasound: a) **test 3** (cotton gin trash cellulose; Accelerase concentration – 8 ml/L; sample suspension – 40.0 g/L; duration 5 h), and b) **test 4** (cotton lint trash cellulose; Accelerase concentration – 4 ml/L; sample suspension – 40.0 g/L; duration 8 h). The results of the enzymatic conversion of cotton

gin trash cellulose into sugar are presented in Figure 9, and those of cotton lint trash cellulose conversion – in Figure 10. The experimental data of **test 3** unambiguously indicate that the introduction of a low-level uniform ultrasound field significantly improved the enzymatic conversion of the samples of cotton gin trash cellulose to glucose, when compared to the run without sonication, for the entire duration of the experiment. The overall improvement in the enzymatic conversion of cotton gin trash cellulose into sugars, caused by the introduction of ultrasound, was up to ~22% at the end of the experiment. The experimental data of **test 4** indicate that the introduction of ultrasound energy during hydrolytic conversion of cotton lint trash cellulose to glucose resulted in an even more pronounced improvement in Accelerase enzyme performance, as the reaction time progressed.

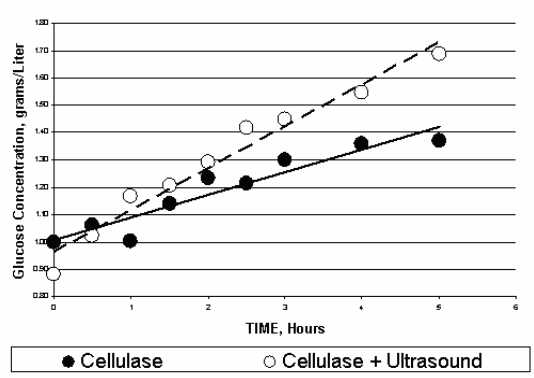

Figure 9: Enzymatic hydrolysis of cotton gin trash (**test 3**). Concentration of glucose *vs*. reaction time under sonication conditions

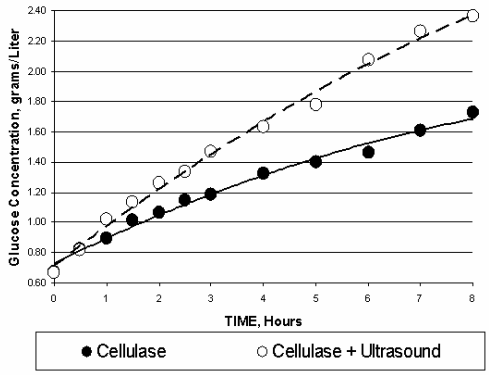

Figure 10: Enzymatic hydrolysis of cotton lint trash (**test 4**). Concentration of glucose *vs*. reaction time under sonication conditions

The overall improvement in the enzymatic conversion of cotton lint trash cellulose into sugars caused by sonication was up to ~41% at the end of the experiment. Interestingly, the enhancement in the hydrolytic conversion of cotton waste cellulose into glucose caused by sonication was more significant for cotton lint trash cellulose (~29%) than that for cotton gin trash cellulose (~22%), even with a lower concentration of Accelerase enzyme (4.0 ml/L *vs*. 8.0 mL/L). The most probable explanation of this phenomenon is the reduced accessibility of the pure plant cellulose (normally, more or less tightly bonded with the hemicelluloses/lignin matter) to the hydrolytic action of the Accelerase enzyme in cotton gin trash *versus* cotton lint trash.

DISCUSSION

The general trend observed during the experimental studies on enzymatic bio-scouring of cotton fabrics and hydrolytic conversion of cotton waste celluloses into sugars indicates that the introduction of a low-level uniform ultrasound field into the reaction chamber considerably enhanced the performance of enzymes by significantly increasing their overall reaction rates. The beneficial effects of the introduction of ultrasonic energy could be summarized as follows:

a) acceleration of the transport of the enzyme macromolecules toward the substrate/fiber surface through the border layer of the liquid at the liquid–solid interface. The concentration of enzyme macromolecules in this layer is a controlling factor, which defines the overall reaction rate;

b) vigorous agitation of the normally immobile border layer of the liquid at the liquid–solid interface, caused by sonication, helps the enzyme macromolecules to position themselves "*fittingly*" onto the substrate;

c) prevention of any possible agglomeration of enzyme macromolecules, which could decrease enzyme activity;

d) improved removal of the enzymatic hydrolysis products from the reaction zone,

which accelerates the overall enzymatic reaction rate;

e) "*opening up*" of the surface of the substrate/fibers as a result of the mechanical impacts produced by the collapsing cavitational bubbles.

CONCLUSIONS

- It appears that sonication of the enzyme processing solution does not reduce the specific activity of the enzyme macromolecules in any significant way.
- At a laboratory scale, the introduction of ultrasonic energy in the reaction chamber during the enzymatic bio-preparation of cotton fabrics or enzymatic bio-conversion of cotton waste celluloses into sugars resulted in a significant improvement in enzyme efficiency.
- The combination of enzymatic bio-preparation and enzymatic bio-conversion of cotton waste celluloses with a low-level, uniform ultrasound irradiation could significantly advance these new "green chemistry" processes and make them more suitable for widespread industrial implementation. This could considerably reduce the amount of wastewater effluents, energy consumption and overall processing costs.
- This study also provides a good potential for intensifying other technological processes that involve various types of enzymes and matching substrates. One can assume that, practically, any solid/liquid system that involves a reaction between the enzyme macromolecules and the solid substrate would greatly benefit from the introduction of ultrasonic energy into the system.

DISCLAIMER: Specific company, product and equipment names are given to provide exact description of experimental details. Their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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