Research on cellulosic substrates for establishing environmentally-friendly and energy-saving technologies

OTKA 82044 - Final report

1. Introduction

In the last couple of years our research group has been working in the field of textile biotechnology. Enzymes can be used in different stages of the textile wet technologies. By now it was proved that the enzyme technologies are environmentally-friendly alternatives for the traditional chemical processes, they save chemicals, water and energy over the conventional treatments. Since all of the enzyme-aided textile processes are heterogeneous, the mass transfer is often the rate-limiting. As a result, the enzyme processes require relatively long processing time. In the present project we concentrated on the acceleration of the enzyme-aided heterogeneous processes of the cellulosic fibres by using low frequency ultrasound and non-thermal atmospheric air-plasma treatments.

Power ultrasound refers to sound waves with low frequencies (20–100 kHz) and high sound intensities (10–1000 W/cm^2). Its effect can be based on the cavitation phenomenon, which means the formation, growth and collapse of vapour or gas bubbles that occur with ultrasound. In a heterogeneous system, collapse of the bubbles near the solid surface results in high velocity micro jets, which accelerate the transport processes and significantly improve the mass transfer [1].

For non-thermal atmospheric plasma, the atomic temperatures are close to ambient, but electron temperatures reaching values up to orders of magnitude higher. Plasma with such characteristics readily interacts with solid surfaces, causing reactions that would otherwise occur only at elevated temperature [2]. Thus, ultrasound can accelerate the mass transfer in the treating bath by the local turbulences created by acoustic cavitation, and plasma can etch and activate the surface layers of the textile materials. Both can contribute significantly to the acceleration of the bioprocess.

At the beginning of the research, cellulosic substrates in fibrous and ground forms were characterized and then, they were used either in the ultrasound-aided or the cold-plasma-aided enzyme reactions. In the experiments with ultrasound a horn type ultrasound reactor with a driving frequency of 20 kHz, at 40, 60 and 80 % amplitude and with a stainless steel disc acted as a rigid reflector (placed at the bottom of the vessel, and used to intensify the reflectance of the ultrasound waves and to create a standing wave field) was used. Non-thermal plasma treatment was performed in ambient air, by a diffuse coplanar surface barrier discharge (DCSBD) type equipment. Both sides of the fabrics were treated applying a power of 300 W and treatment times of 30, 60, 120, 180 s.

Most of the results of the research were published predominantly in internationally recognised journals. This short overview is presented in the following sections: (1) characterization of the surface and bulk properties of cellulosic fibres in fibrous and ground form, (2) characterization of the ultrasound field in a homogeneous bath; effect of sonication on the activity of a commercial hydrolytic enzyme in homogeneous system, (3) studying of a heterogeneous enzyme reaction with sonication by a selected model enzyme reaction, (4) application of ultrasound for the enzyme extraction in solid state fermentation (SSF); effect of sonication on the activities of the crude hydrolytic and oxidative enzymes produced by SSF, (5) intensification of the biopreparation, biobleaching and biofinishing of cellulosic substrates by ultrasound, (6) effect of plasma treatment on the surface properties of cellulosic fibres, (7) increasing the accessibility of polymers to the respective enzymes by plasma surface etching; intensification of the enzymatic processes by plasma pretreatment.

2. Results

2.1. Characterization of the surface and bulk properties of cellulosic fibres in fibrous and ground forms

Cotton and flax fibres were ground in a ball-mill, and the effect of grinding on the microstructure and surface properties of the fibres was determined by combining a couple of simple tests with powerful techniques of surface and structure analysis. For both fibres, the degree of polymerization reduced. Furthermore, an increase in water sorption capacity of the ground substrates was in good agreement with the X-ray results, which proved a less perfect crystalline structure in the ground samples [3].

The multicellular and multicomponent flax is more susceptible to grinding than cotton. In general, grinding of flax fibres leads to a serious decrease in the degree of polymerization and an increase in water sorption capacity. Furthermore, grinding slightly destroys the crystalline structure of flax and the ground fractions contain smaller cellulose crystals with more imperfections. Grinding creates new surfaces where the O/C ratio is significantly higher than that of the original fibre. This can be explained by the destruction of the waxy surface layer [4]. Since the properties of the cellulosic materials can significantly be modified by grinding, in the ultrasound-aided enzyme reactions not only the fabrics but the ground fibres were also investigated.

2.2. Characterization of the ultrasound field in a homogeneous bath. Effect of sonication on the enzyme activity

For characterizing the intensity of the ultrasonic energy inside the reaction chamber in a homogeneous bath, the temperature change of the distilled water caused by sonication was observed for a period of 0–10 min. The power delivered to the system (P_{deliv}) and the actual power dissipated (P_{diss}) for each experimental configuration was determined by calorimetrically and the intensities of the ultrasound system (I_{diss}) in the reaction mixture were calculated [5]. Results revealed that at higher amplitudes the power delivered to the system and the rate of the energy dissipation was also higher. There are considerable differences between the P_{deliv} and P_{diss} values, indicating that 32, 35 and 40 % of the energy delivered to the horn at 40, 60 and 80 % amplitudes, respectively, were lost in the energy transfer. The intensities of the applied configurations (without the reflector) calculated from the dissipated energy are 16.2, 32.2 and 43.4 W/cm^2. The reflector has only a negligible effect on the power characteristics of the apparatus [6,7].

Ultrasound can have a direct effect on the enzyme molecules and enhances the mass transfer in the heterogeneous processes by acoustic cavitation. The enzyme-modifying effect of the ultrasound can contribute to alteration of the enzyme activity. Changes in activity can be influenced by the parameters of the sonication and the characteristics of the enzyme. Thus, the ‘enzyme macromolecule–ultrasound’ interaction has a significant effect on the efficiency of a bioprocess. Despite the fact that ultrasound is able to alter the enzyme activity, surprisingly, only a very few publications have focused on the behaviour of enzymes exposed to power ultrasound. Interestingly, most of the papers published in this field evaluate the efficiency of the ultrasound-aided processes, but draw the conclusions on the enzyme activity modifying effect of the ultrasound, without measuring the enzyme activity itself.

Therefore, in this research a commercial acidic cellulase enzyme complex was used in order to characterize the effect of sonication on the enzyme activity [6,7]. The diluted solutions of the enzyme were irradiated with ultrasound and subsequently the enzyme activity was measured. The filter paper activity (FPA) of the enzyme was determined as a function of the parameters (such as treatment time, amplitude, with and without a reflector) of sonication.

Sonicating the enzyme solution at different amplitudes, the cellulase enzyme underwent changes, and the reduction of the enzyme activity which occurred was highly dependent on the amplitude. At 40% amplitude, a 12% loss in enzyme activity was measured. By increasing the amplitude to 60 and 80%, the decrease in enzyme activity became more significant, and after 65 min the enzyme possessed only 80 and 75% of the original activity, respectively. Application of a reflector to intensify the sonication had a pronounced effect and the loss in enzyme activity was always higher in the presence of the reflector.

Results clearly prove that even a short sonication has a significant effect on the enzyme activity. Sensitivity of the enzyme macromolecules to sonication is different. The alteration in enzyme activity that occurred during sonication depended largely on both the characteristics of the enzyme and the sonication system [8]. It has not been established whether either the dissociation of the enzymes into subunits or the thermal denaturation of the enzymes by the shear forces or high localised temperatures, respectively, arising from the collapse of cavitation bubbles, are responsible for the damage of the enzyme occurring in the sonicated environment [9]. But we can conclude that sonication has a “sonochemical” effect on the enzyme molecule, which is manifested unambiguously in the diminished enzyme activity. Consequently, optimisation of the parameters of the sonicated system is required specifically for each of the enzymes used.

2.3. Effect of sonication on the efficiency of the cellulose-cellulase model reaction

Results presented above revealed that the selected cellulase enzyme is susceptible to ultrasound and its activity can significantly decrease, depending on the parameters of the sonication. In the scientific literature, however, many papers have shown that the overall effect of the ultrasound on the cellulase-catalysed reactions is very positive, and sonication results in a significant improvement in the enzyme efficiency.

For evaluating the hydrolytic potential of the enzyme toward a pure cellulosic substrate (i.e. bleached cotton fibre in ground form) in sonicated solution, a simple cellulose–cellulase model reaction was investigated. The parameters of the sonicated environment (i.e. amplitude, time and reflector) were varied to determine their effect on the yield of the enzyme catalysed reaction expressed in liberated reducing sugar concentration, which was monitored continuously [6].

Results clearly proved that despite the reduction of the enzyme activity, which occurred by sonication, the outcome of the enzyme catalysed hydrolysis is always positive. It means that the advantageous effects of sonication impressed on the heterogeneous enzyme reaction always overcome the undesirable, enzyme modifying effect of ultrasound. Interestingly, the highest yield was measured at 80 % amplitude and in the presence of the reflector, where the enzyme sustained a 27 % loss in enzyme activity.

In the next experiment the efficiency of the most often used agitating methods (i.e. magnetic stirring, horizontal shaking, vertical mixing) was compared to that of the ultrasonicated system in the cellulase–cotton cellulose model reaction [10]. The effect of position of the cotton fabric that measured from the tip of the horn on the cellulose hydrolysis was also evaluated. Results revealed that among the three traditional agitation methods magnetic stirrer proved to be the most effective in the model reaction, but it was less efficient than the sonicated system. Distance measured between the fabric and the tip of the horn has a significant effect on the efficiency of the enzyme reaction: the closest position resulted in the highest reducing sugars liberation. Thus, the ultrasonicated field is not homogenous concerning the efficiency of the ultrasound-aided enzyme reactions and optimisation of the position of the substrate is also required specifically for each of the experimental set-up.

2.4. Effect of ultrasound on the enzyme recovery from the SSF solids. Effect of sonication on the hydrolytic and oxidative enzyme activities of the crude enzymes produced by SSF

Solid state fermentation is an attractive alternative method to the widely used submerged fermentation for producing enzymes. Extraction of enzymes from the crude fermented products is a key-factor in SSF. In this research ligninolytic and hydrolytic enzymes were produced with many selected fungi on fibrous flax substrate by solid state fermentation. The SSF materials of the two best enzyme producers were further investigated. For improving the efficiency of the enzyme extraction, low frequency ultrasound at different amplitudes was applied and recovery of the enzymes was characterized. Single stage and multiple (three-stage) extractions with or without ultrasound were evaluated. The effect of sonication on the crude enzyme stability was also evaluated.

For the SSF material of *Trichoderma virens TUB F-498* [11], sonication (at 40 %, 60 % and 80 % amplitudes, for 60 min) did not result in significant reduction in the filter paper, lignin peroxidase (LiP) and laccase (Lac) activities of the crude enzyme solution, but has a enormous positive effect on the efficiency of enzyme extraction from the SSF material. Depending on the parameters of sonication, the enzyme activities in the extracts obtained can be increased up to 129–413 % of the original activities measured in the control extracts recovered by a common magnetic stirrer. For the SSF material of *Aspergillus oryzae NRRL 3485* [12], a three-stage extraction with sonication provided more than 2 times higher enzyme yield (2.3, 2.7 and 2.1 for FPA, PGal and LiP, respectively) when compared to the conventional single stage extraction.

The exceptionally positive effect of ultrasound on the extraction of enzymes can be attributed to the high-speed jets of buffer created by the collapse of cavitation bubbles, which disintegrate the cultivated solid particles into fibers, decreasing their size as well as increasing the accessible outer surface and the availability of the internal surfaces. And also to the intensive mechanical action, which results in the enhancement of the mass transfer [12]. To the best of our knowledge, the use of ultrasound in the enzyme recovery process from the SSF materials for improving the efficiency of extraction of the crude enzymes has not been studied by others so far.

2.5. Intensification of the biopreparation, biobleaching and biofinishing processes of cellulosic substrates by ultrasound

Biopreparation and biobleaching of cellulosic fabrics (i.e. greige linen) were investigated by using the crude SSF material of *Trichoderma virens TUB F-498* [11] and commercial pectinase enzymes [13]. In the former research, first, the extraction and the biobleaching were carried out simultaneously with or without the aid of sonication (SEB-US and SEB-S, respectively). Second, before the biotreatments, a 30-min long pre-extraction with or without the aid of sonication was applied to recover most of the enzymes from the SSF material. Then, this buffer extract with the solid material was used for the fabric treatment in a shaker (PE-US-B or PE-S-B, respectively).

ATR FT-IR method proved the removal of the waxy outer layer of the fibres by all the applied enzyme pretreatments. This method also revealed the decrease of the pectin peak in the ester region. Since the removal of the waxy outer layer of the fibre occurs indirectly by the enzymatic degradation of pectic substances, the latter process accelerates the removal of the non-cellulosic materials from the primary wall and of the waxy materials from the outer layer of the fibre. Thus, these processes produced water-wettable fibres and fabrics.

The efficiency of the applied enzyme treatments in degradation and removal of the colouring materials of the raw linen fabric was also characterized by colour measurement. Results revealed that sonication has a remarkable effect on the removal of colouring materials. The highest increase in lightness and decrease in colour content was achieved with the PE-US-B process. The shorter SEB-US process is also promising in biobleaching of linen.

An attempt was also made to characterize the effect of sonication on the chemical finishing of cotton fabrics by using an amino- aldehyde resin as a crosslinker. Furthermore, sonication was also tried to increase the hydrogen peroxide production in the reaction of glucose and a glucose oxidase enzyme. Unfortunately these experiments were unsuccessful.

2.6. Effect of plasma treatment on the surface properties of cellulosic fibres

In this research, non-thermal atmospheric air-plasma was used for the surface modification of raw linen fabrics. Changes in physical properties and chemical composition of the fibre surface as well as in colour of the fabric were measured as a function of time of the atmospheric air-plasma treatment. Wettability and wickability were measured and the surface free energy of the fibres was calculated. Changes in the chemical composition of the fibre surface were detected by X-ray photoelectron spectroscopy (XPS) and infrared spectroscopy (ATR FT-IR). The ageing of the surface modifications created by plasma treatment was also tested by characterizing the permanency of surface hydrophilicity and chemical composition as well as of fabric colour in a period of 1-14 days [14].

All of the applied analytical methods revealed differences in properties of the raw and the plasma treated fabrics and they all can measure also the differences as a function of time of the plasma treatment. In general, air-plasma treatment leads to a significant increase in hydrophilicity characterized by wicking and wetting properties and water contact angle, and to a slight but perceptible change in colour of the fabrics. Furthermore, plasma treatment creates modified surfaces where the O/C ratio measured by XPS is significantly higher than that of the original raw fibre. The O/C ratio increased steadily with increasing the duration of the plasma treatment. This can be explained mainly by the destruction of the waxy surface layer and creation of polar groups as well as exposure of pectin and other polymer components. Results obtained by ATR FT-IR technique indicated the exposure of pectin, which became more detectable on the surface with increasing the duration of the plasma treatment. Most of the results from wettability, wickability and colour measurements demonstrated the stability of the changes induced by plasma.

2.7. Increasing the accessibility of polymers to the respective enzymes by plasma surface etching

In this research we focused on how the air-plasma treatment changed the raw cotton fabric surface and how these changes improved the accessibility of polymers located on the surface of warp yarns (i.e. starch) or under the surface layers of cotton fibres (i.e. cellulose and pectin) in weft yarns to an amylase, cellulase and pectinase enzyme, respectively, and how the plasma pretreatment accelerated the subsequently applied enzyme processes [15].

Results proved that plasma slightly destroyed and oxidized the starch size on the surface of warp yarns and partially removed the thin and perfectly hydrophobic waxy coverage of the cotton fibres in the weft yarns, resulting in a more hydrophilic fabric with a significantly shorter wetting time and lower water contact angle. Plasma etching of the surface was accompanied by a creation of deep “pits” with a depth of 250 nm, which contributed to both the partial oxidation and removal of starch size from the warp yarns and the exposure of polymers such as cellulose and pectin located under the waxy outer layer of the fibres in weft yarns. The fabrics' breaking load and elongation, however, seemed to be unaffected by the applied cold plasma treatment.

An amylase and cellulase digestion of the plasma treated cotton fabrics confirmed that air-plasma significantly increased the accessibility of polymers (i.e. respective substrates) in the fibres surface to the enzymes, resulting in an enhanced solubilization of both starch and cellulose, respectively. Reducing sugars liberated from the starch-based size by amylase treatment proved that about 10-20 % of starch size in the raw cotton was removed by the air-plasma treatment. The increase in hydrophilicity and accessibility of the plasma etched surface and the more efficient hydrolysis of residual starch resulted in an increased desizing effect, which can lead to a significant shortening of the desizing process (from 60 to 10 minutes).

Raw cotton fibres in the weft yarns of the fabric had a low accessibility to cellulase, which is due to the hindering effect of the waxy outer layer. Almost 30 minutes were needed for the enzyme molecules to penetrate through the discontinuities of the wax to inner fibre structures and attack cellulose. By plasma-aided degradation and removal of the waxy layer, however, the accessibility of cellulose in the primary and secondary cell walls to cellulase enzyme improved significantly and a dramatic increase in the rate of cellulose hydrolysis was observed. Since the plasma treated substrates displayed significantly faster enzyme reactions, the enzymatic treatment time can shortened sharply.

3. Summary

In the last couple of years our research group has been working in the field of textile biotechnology. In the present project we concentrated on the acceleration of the enzyme-aided heterogeneous processes of cellulosic fibres by using low frequency ultrasound and non-thermal atmospheric air-plasma treatments. The project was successfully finished and several new aspects were investigated. Furthermore, new results and some prosperous outcomes were explored.

Results proved that despite the reduction of the enzyme activity in sonicated environment, the outcome of the enzyme reactions in sonicated environment was always positive. Very promising results were obtained in the field of the extraction of hydrolytic and oxidative enzymes from the solid substrate in solid state fermentation. The enzyme recovery was increased up to 400 %.

Plasma surface treatment partially destroyed and removed the thin and perfectly hydrophobic waxy coverage of the cotton fibres, resulting in a more hydrophilic fabric surface. Etching of the surface contributed to the exposure of cellulose and pectin located under the waxy outer layer and increased their accessibility to the respective enzymes. Both processes increased the efficiency of the subsequently applied enzyme treatments.

Plasma treatment slightly degraded the starch sizing agent on the surface of warp yarns of cotton fabric, too. Highly efficient hydrolysis of the lower amount of starch on the plasma treated fabrics resulted in an increased desizing effect, which can lead to a significant shortening of the enzymatic desizing process.

4. Publications/References

1. Szabó, O.E., Csiszár, E.: Enzimes textiltechnológiai folyamatok hatékonyságának növelése kisfrekvenciás ultrahang alkalmazásával, *Magyar Textiltechnika*, LXIV(3), 99-103 (2011)

2. Tóth, A., Csiszár, E.: Az atmoszférikus plazma és alkalmazási lehetőségei a textilkikészítésben, *Magyar Textiltechnika*, LXIV(4), 158-161 (2011)
3. Csiszár, E., Fekete, E.: Microstructure and surface properties of fibrous and ground cellulosic substrates, *Langmuir*, 27, 8444-8450 (2011) ***Impact factor: 4.186***
4. Csiszár, E., Fekete, E., Tóth, A., Bandi, É., Koczka, B., Sajó, I.: Effect of particle size on the surface properties and morphology of ground flax, *Carbohydrate Polymers*, 94, 927-933 (2013) ***Impact factor: 3.916***
5. Hagenson, L. C., Doraiswamy, L. K.: Comparison of the effects of ultrasound and mechanical agitation on the reacting solid–liquid system, *Chemical Engineering Science*, 53, 131–148 (1998)
6. Szabó, O.E., Csiszár, E.: The effect of low-frequency ultrasound on the activity and efficiency of a commercial cellulase enzyme, *Carbohydrate Polymers*, 98, 1483-1489 (2013) ***Impact factor: 3.916***
7. Szabó, O.E., Csiszár, E.: Kisfrekvenciás ultrahang alkalmazása az enzimes textiltechnológiai folyamatokban, *Magyar Textiltechnika*, LXVII(3), 2-5 (2014)
8. Macleod, R. M., & Dunn, F.: Ultrasonic irradiation of enzyme solutions, *Journal of the Acoustical Society of America*, 42, 527–529 (1967)
9. O'Donnell, C. P., Tiwari, B. K., Bourke, P., & Cullen, P. J.: Effect of ultrasound on food enzymes of industrial importance, *Trends in Food Science & Technology*, 21, 358–367 (2011)
10. Szabó, O.E., Csiszár, E.: Comparing the efficiency of different conventional agitating systems and low frequency ultrasound in the enzymatic hydrolysis of cotton cellulose, **manuscript**, 2015
11. Szabó, O.E., Csiszár, E., Tóth K., Szakács, G., Koczka, B.: Ultrasound-assisted extraction and characterization of hydrolytic and oxidative enzymes produced by solid state fermentation, *Ultrasound and Sonochemistry*, 22, 249-256 (2015)
12. Szabó, O.E., Csiszár, E., Koczka, B., Kiss K.: Ultrasonically assisted single stage and multiple extraction of enzymes produced by *Aspergillus oryzae* on a lignocellulosic substrate with solid-state fermentation, *Biomass and Bioenergy* (**submitted**), (2015) ***Impact factor: 3.411*** (in 2013)
13. Csiszar E: Surface properties and residual chromophore content of differently pretreated linen fabrics, *Textile Research Journal*, 82(19), 2030-2040 (2012) ***Impact factor: 1.135***
14. Szabó, O.E., Csiszár, E., Tóth, A.: Enhancing the surface properties of linen by non-thermal atmospheric air-plasma treatment, *Open Chemistry*, 13(1), 570-576 (2015) ***Impact factor: 1.329***
15. Szabó, O.E., Csiszár, E., Koczka, B., Tóth, A., Klébert, Sz.: Enhancing the accessibility of starch size and cellulose to enzymes in raw cotton fabric by plasma pretreatment, *Textile Research Journal* (**submitted**), (2015) ***Impact factor: 1.332*** (in 2013)

5. Further outcomes

In addition to the traditional methods of dissemination such as publishing in peer-reviewed journals (13), conference proceedings (3) and international/national conferences (14), the present project provided an excellent frame for creation a PhD thesis (by Szabo, O.E.; received absolutorium in 2013; passed the comprehensive exam in 2014; proposed date of the submission of the thesis: May, 2015) and 8 MSc/BSc diploma works, and contributed to the research work of several other students working on shorter topics of this project.