Carbohydrate Polymers 96 (2013) 474–480

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Ultrasonic effect on the desizing efficiency of α-amylase on starch-sized cotton fabrics

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ARTICLE INFO

Article history:
Received 14 January 2013
Received in revised form 4 April 2013
Accepted 5 April 2013
Available online 12 April 2013

Keywords:
α-Amylase
Enzymatic desizing
Ultrasound
Starch
Cotton fabrics

ABSTRACT

Enzymatic desizing by α-amylase and ultrasound irradiation are the two important clean technologies in the textile industry. In the present work, with the aim of giving a further insight to the influence of ultrasound on α-amylase activity and its desizing efficiency, the ultrasound-based experiments were afforded in two ways: (i) step-wise treatment of α-amylase by ultrasound and then enzymatic desizing, as well as; (ii) simultaneous utilization of ultrasound and α-amylase for the desizing. By the step-wise strategy, it is found that the ultrasound has negative impact on the α-amylase activity using soluble starch as substrate. However, the sonicated α-amylase possesses higher desizing efficiency because there are higher hydrophobic interactions between sonicated α-amylase protein and starch-sized cotton and thus intensifies its catalytic activity. By the simultaneous procedure, the enhancement to desizing efficiency is more pronounced than that by the step-wise procedure. This can be attributed to comprehensive actions of several reasons such as more effective stirring/mixing mechanism, damages or changes to substrate, more effective catalysis to hydrolytic reactions and faster removal of loosened products from the fabric bulk.

1. Introduction

Ultrasound (US) is a sound beyond human audible range of 18–20 kHz, which can be used for various purposes in diverse industrial fields(Kwiatkowska, Bennett, Akunna, Walker, & Bremner, 2011; Vankar, Shanker, & Verma, 2007). Low frequency (20–100 kHz) US is considered a green technology due to its economically viable performance, high efficiency and low instrumental requirements (Rokhina, Lens, & Virkutyte, 2009). It can be used for cleaning, welding and sonochemistry (Basto, Tzanov, & Cavaco-Paulo, 2007; Hao, Wang, Liu, & Liu, 2012b; Rokhina et al., 2009). US can promote a wide variety of chemical, physical and biological processes mainly through the cavitation phenomenon in the liquid medium. Acoustic cavitation is the formation, growth and explosive collapse of microscopic bubbles in liquid, which causes drastic increase in the local temperature and pressure, releases large amounts of highly localized energy and generates highly reactive hydroxyl radicals (Merdan, Akalin, Kocak, & Usta, 2004; Yachmenev, Blanchard, & Lambert, 2004). In heterogeneous systems, when microscopic cavitation bubbles collapse at the surface of the solid substrate, they generate powerful shock waves that cause effective stirring/mixing of the border layer of liquid at a solid/liquid interface (Yachmenev et al., 2004).

US has the potential to improve the performance of various enzyme in textile wet processing such as the hydrolysis of cellulose by cellulase, pectins by pectinase and/or starches by amylase (Hao, Wang, Liu, & Liu, 2012a; Imai, Ikari, & Suzuki, 2004; Wang, Yu, & Zhong, 2012; Yachmenev, Bertoniere, & Blanchard, 2001). α-Amylase, an important industrial enzyme for the removal of starch size from woven textiles, can hydrolyze starch, glucogen and related polysaccharides by randomly cleaving the internal α-1,4-glucosidic linkages (Dong & Lu, 2008; Muralikrishna & Nirnala, 2005). However, very little is known about the actual effect of US on α-amylase because contradictory results of inactivation and activation of enzymes upon US treatment have been reported. Apar, Turhan, and Ozbek investigated the effect of US (20 kHz) on the activity of alpha-amylase enzymes produced by *Bacillus subtilis* on corn, rice and wheat starch at 50 °C under pH 6.5. They found that the activity of alpha-amylase decreased from 89% to 75% by increasing the duty cycle from 10% to 80% at acoustic power 100 W (Apar, Turhan, & Ozbek, 2006). Kadhodaee and Povey confirmed that the sonication at a constant operating frequency of 30 kHz and maximum nominal power output of 50 W would result in the apparent inactivation of bacterial α-amylase. It was found that the inactivation rate varied depending on the temperature and the radiating face of the sonotrode used (Kadkhodaee & Povey, 2008). Yaldagard, Mortazavi, and Tabatabaie studied the effect of 20 kHz US on the germinated barley's alpha-amylase activity at different

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http://dx.doi.org/10.1016/j.carbpol.2013.04.003

temperatures (30, 50 and 70 °C) and ultrasonic intensities (20, 60 and 100% setting from total power of 460 W) by using the Taguchi statistical method. They concluded that the US had a destructive effect on barley's alpha-amylase and caused apparent inactivation of this enzyme (Yaldagard, Mortazavi, & Tabatabaie, 2008). On the contrary, Souza et al. reported that the α-amylase activity determined in 40 kHz US bath was higher than that without sonication when temperature below 45 °C. In this work, the activation energies of amylase catalysis in the presence and absence of US irradiation were calculated to be 12.11 and 58.54 kJ/mol, respectively (Souza et al., 2013). Interestingly, positive and consistent results about sonication were obtained when α-amylase was used for the enzymatic desizing of woven textiles instead of the hydrolysis to soluble starch. Sahinbaskan and Kahraman treated the starch-sized 100% cotton plain woven fabric with α-amylase in conventional and ultrasonic bath procedures, respectively, and found that this US-based method brought about a significant increase in the starch-size removal. They attributed this effect to the acceleration of enzyme diffusion toward the fabric surface by the US (Sahinbaskan & Kahraman, 2011). Wang, Yu, and Zhong utilized 53 kHz US to improve the desizing efficiency of a commercial amylase. They suggested that the US assisted system could save half the processing time and improve about 5% point in desizing efficiency (Wang et al., 2012).

Clearly, the effect of US on enzyme performance is substrate dependent. What should be noted is that the starch size on the textile surfaces consists of non-soluble starch with higher molecular weight than the soluble starch, so the hydrolysis catalyzed by α-amylase for desizing is a heterogeneous procedure. As a heterogeneous system, much more factors are involved because it is a comprehensive impact of US on the amylase, fabric substrates and their mutual actions, and it is hard to tell which factor might be predominant and how to balance them. In this research, with the aim of giving a further insight to the influence of US on desizing efficiency of amylase, we performed the US-based experiments in two ways: (i) step-wise treatment of α-amylase by US and then enzymatic desizing, as well as; (ii) simultaneous utilization of US and α-amylase for the desizing. By this strategy, the effects of US on the α-amylase, fabric and their interactions will be thoroughly discussed. It will be clear as to how significant the sonication could be in reducing energy consumption and improving the starch size removal during the enzymatic desizing process by α-amylase.

2. Experimental

2.1. Materials

Untreated, starch-sized, 100% cotton plain woven fabrics used throughout this work were obtained from Binzhou Dyeing Factory, China. The fabric weight is 167 g/m^2 (47 ends/cm and 36 picks/cm) with 6.5% starch size add-on. A commercial α-amylase (HY-ktm, 40,000 U/mL) produced by selected strain of *B. subtilis* for desizing at medium and high temperature was supplied by Haiyi Chemical Company. The non-ionic wetting agent HWT (based on alkylaryl polyglycol ether) was also kindly provided by Haiyi Chemical Company The soluble starch (potato, AR) was bought from Tianjin Chemical Ltd. and used as substrate for estimating α-amylase activity.

3,5-Dinitrosalicylic acid solution was prepared for measuring the reducing sugar. 10 g of 3,5-dinitrosalicylic acid and 10 g of sodium hydroxide were dissolved in 500 mL distilled water. 200 g of sodium potassium tartrate was then added to the solution with constant mixing. Afterward by adding 2 g of phenol and 0.5 g of metabisulphite, the total volume of the solution was adjusted to 1 L with distilled water (Shukla & Jajpura, 2004).

2.2. Equipment

Experiments were performed in a Kudos (Japan) US cleaner with thermostatic water bath (temperature accuracy of ±1 °C) at a frequency of 49 kHz. The output power was set at 200 W and supplied by two transducers at the bottom horizontally along the length of the bath. The apparatuses have accurate digital time controller (0–90 min) and degas functions. All enzymatic reactions were carried out in a 250 mL cylindrical glass reactor (7 cm in diameter and 10 cm in height).

2.3. Methods

Desizing of woven fabrics was carried out in two ways:

(i) Two steps (step-wise): 50 mL of α-amylase (40 U/mL, pH 6.0) was filled in the glass tube and placed in the center of the 49 kHz ultrasonic field. Different temperatures (30 °C and 60 °C) were set during the sonication for various exposed time of 5, 10 and 15 min. After sonication, the residual activity of amylase toward soluble starch was immediately measured according to the method of reducing sugars. And then, the starch-sized cotton fabrics were desized according to following conditions: α-amylase dosage 2 mL/L, NaCl 5 g/L, nonionic wetting agent 2 g/L, pH 6.0, liquor ratio 30:1, temperature 50–90 °C and time 15 min. The binding ratio of amylase protein on fabrics was estimated by means of the loss of protein in solution during the process using the Bradford method (Bradford, 1976). Upon the completion of the reaction, the solution was drained, and then the treated samples were thoroughly washed with 95 °C water for 15 min. At last, the fabrics were rinsed using tap water and then dried at 105 °C to a constant weight.

(ii) One step (simultaneous): the cotton fabrics were desized in the presence of US by following conditions: α-amylase dosage 2 mL/L, NaCl 5 g/L, nonionic wetting agent 2 g/L, pH 6.0, liquor ratio 30:1, temperature 50–90 °C and time 15 min. Upon the completion of the reaction, the solution was drained, and then the treated samples were thoroughly washed with 95 °C water for 15 min. At last, the fabrics were rinsed using tap water and then dried at 105 °C to a constant weight.

2.4. Measurements

2.4.1. α-Amylase activity assay according to the method of reducing sugars

Amylase activity was determined by measuring the decrease/increase in absorbance at 540 nm at pH 6.0 and 25 °C. Two milliliters of starch solution (1%, w/v) was added to 2 mL of diluted α-amylase enzyme solution. The mixture was incubated at 50 °C for 10 min in a thermostated water bath and stirred during the incubation period. After the incubation, 2 mL of 3,5-dinitrosalicylic acid was added to the solution and then held in boiling water bath for 5 min. The intensity of the red-brown color developed during heating was measured spectrophotometrically against the blank solution at 540 nm after cooling to room temperature. The residual activity of α-amylase was calculated from the slope of the calibration curve prepared for standard solutions of glucose. All assays were performed in duplicate.

2.4.2. Determination of the desizing efficiency

For determining the desizing efficiency, samples were taken to test the residual starch content on them. The starch content on fabric was analyzed by referring to the perchloric acid method (Dong &