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Carbohydrate Polymers 67 (2007) 572–575
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Degradation kinetics of pectins by an alkaline pectinase in bioscouring of cotton fabrics

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Received 12 March 2006; received in revised form 21 June 2006; accepted 23 June 2006
Available online 4 August 2006

Abstract

Alkaline pectinases have been proven to be effective as bioscouring agents of cotton fabrics. In order to monitor the scouring degree of cotton fabrics quantificationally, a kinetic study of the degradation of pectins in cotton by an alkaline pectinase 'Bioprep 3000L' was performed and the influences of initial pectinase concentration and treatment time on bioscouring were evaluated quantitatively. The results showed that although the degradation products increased as pectinase concentration grew higher at same incubation time, the growth multiples of the maximum degradation rate which was used as the starting degradation rate were less than those of initial enzyme concentration. The degradation kinetics of pectins in cotton fibers with a pectinase could be described by modified Ghose–Walseth kinetic empirical equations which had been previously applied to the degradation reaction of cellulose.

Keywords: Degradation; Kinetics; Pectin; Pectinase; Bioscouring; Cotton

1. Introduction

The existence of approximate 6% natural impurities including pectins, fats and waxes, proteins, sugars, ashes as well as other materials make raw cotton fiber having poor wetting property, which will cause some quality problems in subsequent dyeing and finishing processing. The scouring of cotton fabrics is necessary to obtain adequate wettability through removing noncellulosic impurities from the surface of cotton fibers (Perkins, 1996).

Traditionally, the scouring process is performed by boiling with caustic alkali, which could not only remove the noncellulosic impurities but also attack the cellulose, causing deterioration in the strength of the fabric. Furthermore, large volumes of wastewater coming from alkaline scouring process are highly polluted with higher BOD, COD and alkalinity (Traore & Buschle-Diller, 2000).

The enzymatic scouring of cotton is referred as bioscouring or biopreparation and has become a promising eco-friendly alternative to conventional alkaline scouring in cotton preparation. Although several types of enzyme—pectinase (Achwal, 1992; Buschle-Diller, El Mogahzy, Inglesby, & Zeronian, 1998; Li & Hardin, 1997; Röbner, 1993), cellulase (Csiszar, 1998; Li & Hardin, 1997; Röbner, 1993), protease (Buchert, Pere, Puolakka, & Nousiainen, 2000; Lin & Hsieh, 2001; Sangwatanaroj & Choonukulpong, 2003), lipase (Buchert et al., 2000; Sangwatanaroj & Choonukulpong, 2003), xylanase (Csiszar et al., 2001a; Csiszár, Urbánszki, & Szakács, 2001b) and cutinase (Degani, Gepstein, & Dosoretz, 2002)—have been studied, pectinases have proved to be the most effective and suitable for cotton bioscouring. The mechanism of pectinase scouring reportedly assumes that the degradation and elimination of pectins makes the loosened waxes more easily removable with the help of surfactants and mechanical agitation; this allows

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0144-8617/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2006.06.031

the cotton to achieve superior hydrophilicity without fiber deterioration.

Although much of the work in the area of cotton bioscouring has been focused on investigating the utility of various enzymes, the study on degradation kinetics of pectin substances in cotton by pectinases has not been reported yet. In fact, although wettability (determined by drop test or wicking test) is the best evaluation method for scouring effect from practical point of view, some factors such as residual of surfactants and the drying methods of samples before wettability determination, will greatly interfere its measurement accuracy (Lenting, Zwier, & Nierstrasz, 2002; Li & Hardin, 1998; Margarita & Pere, 2004). Establishing empirical kinetic equation and examining the change of degradation productions in scouring bath may be a feasible method to monitor the scouring degree of cotton fabrics quantitatively. The aim of this work was to investigate the kinetic behavior of the bioscouring of cotton fabrics with a commonly used commercial alkaline pectinase named 'Bioprep3000L'. The content of total reducing galacturonic acid (GA) in treatment bath was taken as a control parameter to assess the effects of initial pectinase concentration and bioscouring time on the degradation of pectins in cotton fabrics.

2. Materials and methods

2.1. Materials

Galacturonic acid (GA) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Greige cotton knits (14.5 tex, 150 $g m^{-2}$) were kindly supplied by Wuxi Knitting Factory (Wuxi, China). Other chemicals used were of analytical grade unless particular explanation.

2.2. Enzyme

Bioprep 3000L is a commercial bacterial pectinase with lyase activity supplied by Novozymes (Kalundborg, Denmark). The enzyme activity was 294.69 ± 3.61 U ml^{-1} according to enzymatic reaction with polygalacturonic acid as described by Bruhlmann (Bruhlmann, Kim, Wolfgang, & Fiechter, 1994). Pectinase was stored at 4 °C before the experiments.

2.3. Scouring experiment

The cotton fabrics were preincubated in boiling water bath for 5 min and followed by rinsing with deionized water and line drying. Exposure to boiling water can melt fats and waxes in cotton cuticle which having melting temperatures less than 100 °C so as to form cracks in the hydrophobic surface or redistribute them on the fiber surface. The change of cotton surface will increase the accessibility of pectinase to substrates because more pectins are exposed to enzyme.

Scouring experiments were carried out by agitating the desired (0.625–5.0% owf) pectinase solution at a moderate stirring speed of 90 rpm with 4 g of cotton knits in 500 ml conical flasks at 65 °C and a liquor ratio of 25:1 using a WHYF-2F thermostatic bath (Shanghai Yuejin Co. Ltd, China). The pH values of treatment baths were adjusted to 8.5 with 0.02 mol L^{-1} sodium borate/HCl buffer solution. After different incubation time, one flask was taken out from the thermostatic bath and the treatment solution was filtrated through Xinhua filter papers. The product (total reducing GA) concentrations in the filtrates were quantified for each of the time studied and used as control parameters to reflect the biodegradation process of pectins on cotton surface.

2.4. Determination of the content of total reducing GA

The total reducing GA content in reaction bath was determined by means of carbazole colorimetry method with a slight modification (Jin & Maekawa, 2001). Accurately weighed dried cotton fabrics (4.0000 g) pretreated with boiled water for 5 min were bioscoured with pectinase under condition described as above. After different incubation time, 0.5 ml filtrate of degradation solution was mixed with 3 ml of sulfuric acid (Conc., 95–98%, guaranteed reagent) in a test-tube and cooled with ice water immediately, then boiled for 10 min and cooled with running tap water. After adding 0.25 ml of carbazole ethanol solution used as a developer to above mixture system and standing at room temperature for 30 min, the absorbance at 530 nm (A_{530nm}) was measured with a 721 spectrophotometer (Shanghai Precision & Scientific Instrument Co., Ltd) at room temperature. A standard curve was made by following the A_{530nm} change induced by different concentration of galacturonic acid solutions. Consequently, the content of GA was able to be obtained according to the standard curve. All the experiments were duplicated.

3. Results and discussion

3.1. Effect of initial pectinase concentration on degradation kinetics of pectins in cotton

The result of degradation kinetics of pectins in cotton fibers with a pectinase as a function of time at different initial enzyme concentration (C) was presented in Fig. 1. The change trends of the total reducing GA concentration versus bioscouring time, which are also called process curve, showed that the degradation products increased rapidly at the initial phases and then improved less for different initial enzyme concentration. This is under our expectation because some factors will inhibit biocatalytical degradation of pectins in cotton fibers with pectinase. The accessibility for enzymatic attack of pectin substances will decrease after the outer pectins are eliminated. Additionally, thermal inactivation of pectinase is also unavoidable when bioscouring is carried out.

Fig. 1 also indicated that the degradation products increased during the enzymatic scouring when the

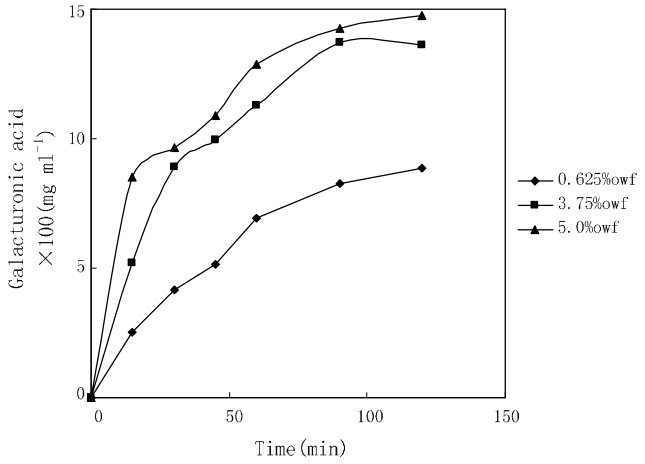

Fig. 1. Reducing GA produced in bioscouring bath as a function of time at different initial pectinase concentrations. Maximal percentage error in total reducing GA concentration is less than 5%.

concentration of pectinase grew higher at same incubation time. This could be attributed to the formation of more contacts between enzymes and substrates when more pectinases were used.

3.2. Effect of initial pectinase concentration on maximum degradation rate

The degradation rate (reaction velocity) can be expressed as the increase in the concentration of GA produced in bioscouring bath per minute ($mg\,ml^{-1}\,min^{-1}$), which can be determined by means of the slope of the tangent to the process curve in Fig. 1. Obviously the slopes of the tangents to the process curve taper over time, which means that the degradation rates drop off accordingly. The maximum degradation rate (V_m) used as the starting degradation rate was tested by means of the experimental data from different initial pectinase concentrations at treatment time of 15 min and presented in Table 1. Although the values of V_m improved as the initial pectinase concentration increased, there was no direct proportion relationship between them. The growth multiples of V_m (V_m/V_{m0}) were less than those of initial enzyme concentration (Table 1). This may be attributed to the complex distribution of pectins in cotton fibers which merely provide limited accessibility of pectinase to the substrates during bioscouring. The experimental results provided such useful information that using additional enzymes to produce higher starting degradation rate may be uneconomical in that unapparent improvement of degradation rate occurred under experimental condition. For example, the degradation effect of pectinase at concentration of 3.75% owf was close to that of 5.0% owf.

Table 1
Effect of initial pectinase concentration (C) on maximum degradation rate (V_m)

C (% owf)	V_m ($mg\,ml^{-1}\,min^{-1}$)	V_m/V_{m0}[a]
0.625	0.00169	1.000
3.75	0.00347	2.053
5.0	0.00566	3.349

[a] $V_{m0} = 0.00169\ mg\,ml^{-1}\ min^{-1}$.

3.3. Kinetic empirical equation

The enzymatic degradation of pectins in cotton is complex because it involves a heterogeneous fiber-pectinase system. The pectin in cotton is a solid substrate and pectinase solution is an aqueous phase. Classic enzymatic reaction kinetic equation—Michaelis-Menten model cannot be used as kinetic equation of cotton bioscouring because it is only suitable for homogeneous reaction system.

To fit the experimental data, the Ghose–Walseth kinetic empirical equation which had been previously applied to the degradation reaction of cellulose materials such as Lyocell, ramie and linen fabrics, was used in this study, expressed as Eq. (1) (Valldeperas, Carrillo, Lis, & Navarro, 2000; Wolf-Dieter, Huber, & Schurz, 1985):

$$P = kt^m, \tag{1}$$

where P was product concentration at time t ($mg\,ml^{-1}$), indicating the extension of enzymatic degradation; t was the enzymatic degradation time (minute), k was the kinetic constant, and m was the characteristic parameter of the substrate–enzyme system.

By means of fitting the process curves in Fig. 1 with the software Windows Excel 7.0, the nonlinear regressions of the experimental plots of total reducing GA in scouring bath versus bioscouring time were performed (Fig. 2). According to these proposal equations, the kinetic parameters and models (Eqs. (2)–(4)) for different initial pectinase concentrations were obtained (Table 2). The values of statistical parameter r^2 (correlation coefficient), which could characterize the correlation of assumed model and the experimental data, showed the reliability and applicability of biodegradation kinetic model for enzymatic scouring

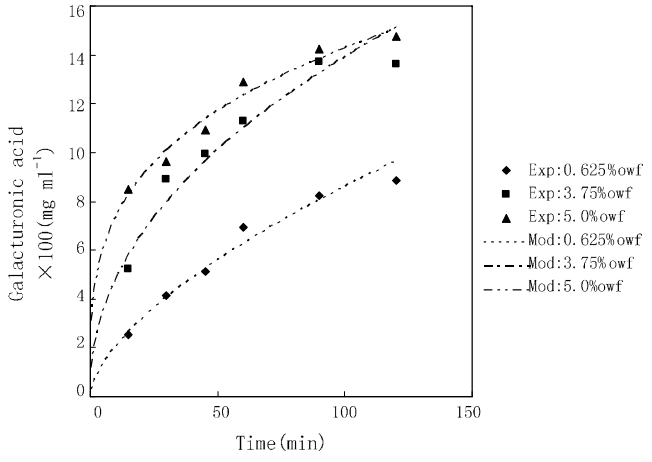

Fig. 2. Modeling biodegradation kinetics of pectins in cotton fiber at different initial pectinase concentrations (Exp and Mod denote experimental data and fitted Ghose–Walseth models, respectively).

Table 2
Kinetic models of cotton bioscouring under different initial pectinase concentrations

C (% owf)	r^2	Model	
0.625	0.9817	$P = 0.4881\,t^{0.6231}$	(2)
3.75	0.9416	$P = 1.6518\,t^{0.4627}$	(3)
5.0	0.9693	$P = 3.7707\,t^{0.2891}$	(4)

of cotton fabrics and its validity to make quantitative prediction during the whole process.

4. Conclusions

The content of total reducing galacturonic acid in treatment bath was successfully taken as a control parameter to quantitatively examine the effect of initial enzyme concentration and bioscouring time on the degradation of pectins in cotton fabrics by a commercial alkaline pectinase "Bioprep 3000L". For different initial enzyme concentration, the degradation products increased rapidly at the initial phases and then tapered over time. This revealed that the maximum degradation rate which was used as the starting degradation rate was not proportional to the initial enzyme concentration. Actually, the growth multiples of V_m were less than those of initial enzyme concentration. This indicated that using additional enzymes to produce higher starting degradation rate may be uneconomical.

Ghose–Walseth kinetic empirical equation, which had been applied to the degradation reaction of cellulose–cellulase system, was also proven to be suitable for describing the degradation of pectins in cotton fibers during bioscouring under different initial pectinase concentrations. In order to characterize the influence of initial enzyme concentration and incubation time on bioscouring effect, modified Ghose–Walseth kinetic empirical equations were successfully established.

Acknowledgements

This work was financially supported by the National High Technology Research and Development Program of China (2003AA322050), Jiangsu Provincial Natural Science Foundation (BK2006020) and SYTU Fund (2005LYY0015). The authors gratefully acknowledge the assistance of Novozymes for their kindly offering the commercial pectinase.

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