INSIGHTS INTO HIGH TEMPERATURE PRETREATMENT ON CELLULASE PROCESSING OF BAMBOO

by

Li-Na FU, Mei-Ying TAN, Ru-Kun SHI, Hong-Bo WANG, and Jia-Jia FU*

Key Laboratory of Science & Technology of
Eco-Textiles Ministry of Education,
Jiangnan University, Wuxi, China

Short paper
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Bamboo processing was performed with commercial cellulase. The properties of cellulase and the effect of high temperature pretreatment on cellulase hydrolysis of bamboo were investigated. Results indicated that cellulase hydrolysis performed fast and dramatically within 30 minutes, and then gradually reached its balance. It was found that pretreatment played an active role in cellulase processing, which enhanced the saccharification of bamboo and benefited high-molecular-weight lignin degradation and removal. Additionally, a better performance of bamboo processing was achieved under the cellulase concentration of 15IU in total reaction system of 100 ml at 50 °C, pH 4.8, together with the high temperature pretreatment of 120 °C for 15 minutes.

Key words: *bamboo, cellulase, high temperature pretreatment*

Introduction

Bamboo is a giant woody, perennial evergreen, fast-growing and high-yield renewable resource [1]. Its attractive properties (*i. e*. antimicrobial property, exceptionally durable, green and eco-friendly, *etc*.) make bamboo a new type of primary textile fiber material [2].

Traditional chemical degumming, mainly processing the bamboo with hot concentrated alkaline solutions, has limits in obtaining fibers with high quality and produces hazardous waste with the use of chemicals [3]. Therefore, more and more attentions have been paid on the application of biotechnology for natural fiber extraction which is recognized as enzymatic and microbial degumming. Previous researches have reported the positive effect of enzymes in bamboo processing [4]. In this article, enzymatic treatment was studied, focusing on the effect of high temperature pretreatment on cellulase processing of bamboo.

* Corresponding author; e-mail: kathyfjj@126.com

Materials and methods

Materials

One-year-old moso bamboo (*Phyllostachys*) was used here. After removing the epidermis, the remaining bamboo culm was crushed into powders. Bamboo powders passing through a No. 60 mesh sieve but retained on a No. 80 mesh sieve were collected as substrates for cellulase processing. Suhong Cellish L was kindly offered by Novozymes. All the other chemicals were procured from China National Pharmaceutical Group Chemical Reagent Co., Ltd.

Properties of cellulase

Assay for cellulase

Cellulase activity was measured with an improved dinitrosalicylic acid (DNS) method [1] and expressed as U ml^{-1}, where one unit (IU) of cellulase is defined as the amount of cellulase required to liberate 1 μmol of glucose from carboxymethyl-cellulose (CMC) during 1 minute under the optimum assay condition. The yield of glucose was measured by the spectrophotometer with the optical density at 540 nm. To achieve pH and temperature profile for cellulase activity, the pH (sodium acetate buffer, 50 mM) ranged from 4.0 to 7.0 at 50 °C and the temperature varied from 30 °C to 70 °C at desired pH, respectively.

Determination of protein content

Protein quantification was carried out by a modified Bradford method [1]. 1 ml of standard bovine serum albumin (BSA) solution was mixed with 3 ml of the Coomassie reagent and the solution was placed in a plate shaker for 10 seconds. After incubating at room temperature for 20 minutes, the absorbance at 595 nm was measured.

High temperature pretreatment

Reactions were launched in test tubes containing a total volume of 10 ml deionized water and 0.5 g of bamboo powders. The tubes were placed in a high pressure reactor at 120 °C with different incubation times.

Properties of enzymatic adsorption

Enzymatic reaction was conducted in 250 ml Erlenmeyer flasks. A total reaction volume of 100 ml was applied, containing 15 IU Suhong Cellish L (properly diluted with sodium acetate buffer, 50 mM). 0.5 g of high temperature pretreated (120 °C, 90 minutes) bamboo powders and original bamboo powers were incubated in the reaction system mentioned previously, respectively. Reaction liquor (3 ml) was sampled at specified times and centrifuged at 10,000 rpm for 10 minutes. The supernatant was kept for protein analysis in order to calculate the amount of cellulase adsorption on bamboo powders.

Results and discussion

Properties of Suhong Cellish L

The effect of pH on Suhong Cellish L activity was shown in fig. 1. The highest outer diameter (OD) value was obtained when pH reached around 5 and then the OD value maintained stable as the pH ranging from 5 to 6.5. When pH was higher than 6.5, the OD value started to decrease dramatically.

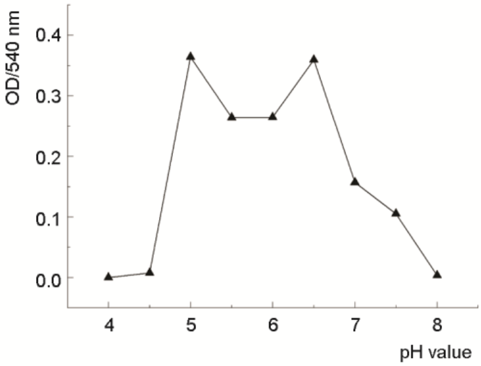

Figure 1. Effect of pH on activities of cellulase

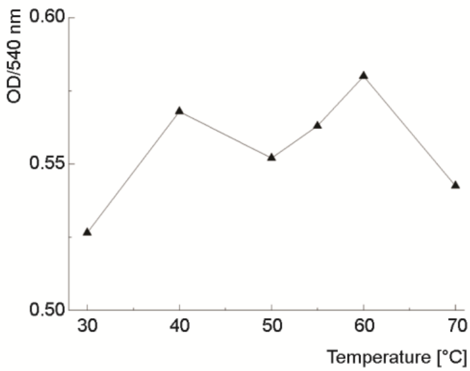

Figure 2. Effect of temperature on activities of cellulase

Figure 2 presents the influence of temperature on Suhong Cellish L activity. When the temperature was in the range of 40 °C to 60 °C, the OD value was stable indicating that the glucose yield was stable. Below 30 °C or above 60 °C, the OD value declined. Generally, 50 °C was chosen. Calculating the optimum activity at pH 4.8, 50 °C, Suhong Cellish L activity was 39 IU/ml and the protein content was 353 μg/ml.

Mechanism of high temperature pretreatment on cellulase processing of bamboo

Figure 3 shows the cellulase adsorption on original and high temperature pretreated (120 °C, 90 minutes) bamboo powders. Samples obtained at the starting time (*i. e.* 0 minute) were set as reference. It was clear that cellulase adsorption on original and pretreated bamboo powders improved with the increase of the incubation time and reached the highest adsorption rate at 30 minutes. However, with the continuous increase of the incubation time, cellulase adsorption rate decreased. A proper explanation was that cellulase, originally adsorbed on bamboo powders, scattered in the solution with the hydrolysis of bamboo components. As shown in fig. 3, the cellulase adsorption rate on original bamboo powders was generally lower than that on high temperature pretreated bamboo powders, indicating that more cellulase could attack on pretreated bamboo and thus might led to a better hydrolysis of the powders.

Reducing sugar yield of enzymatic hydrolysis on bamboo was in accordance with the cellulase adsorption results. As indicated in fig. 4, samples obtained at the beginning time (*i. e.* 0 hour) were set as reference.

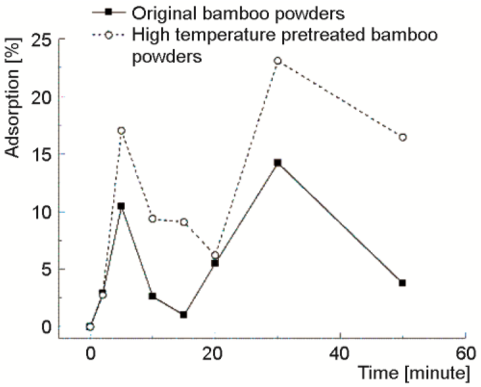

Figure 3. The comparation of cellulase adsorption

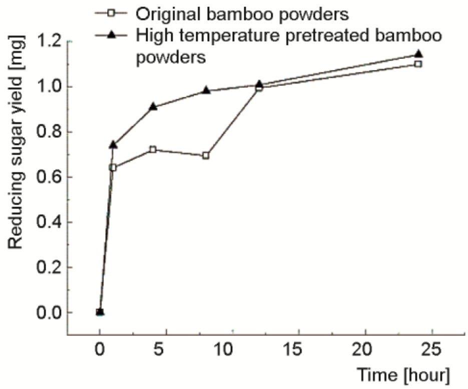

Figure 4. Reducing sugar yield of enzymatic hydrolysis on bamboo of different pretreatment

It seemed that bamboo powders with high temperature pretreatment liberated more reducing sugar under the same cellulase treating condition during the first 10 hours' incubation. However, with the time prolonging, the sugar yield for both reaction systems reached the similar saturation. It meant that high temperature pretreatment helped to boosting cellulase hydrolysis of bamboo. Figure 5 shows the status of bamboo powders with and without high temperature pretreatment. Normally, bamboo powders float on the surface of the water. After the high temperature treatment, bamboo powders can precipitate into the water since water molecules can insert into the bamboo structure and make the powders more swollen and easier to be processed.

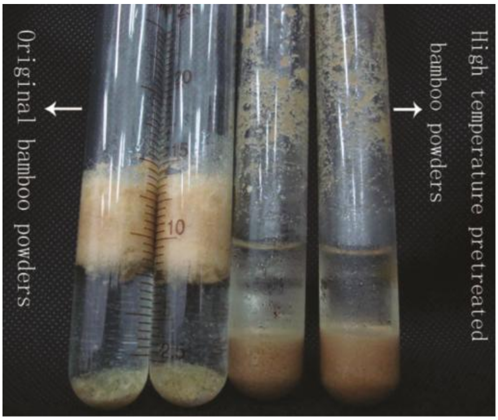

Figure 5. Bamboo powders with and without high temperature treatment

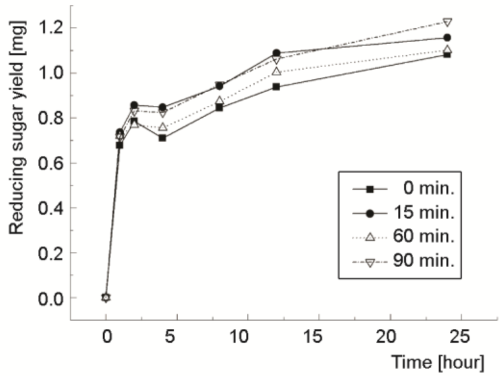

Figure 6. Reducing sugar yield of enzymatic hydrolysis with different processing time

Influence of pretreatment time on the enzymatic hydrolysis

Figure 6 presents the reducing sugar yield of enzymatic hydrolysis with different high temperature pretreatment time. It can be seen that high temperature pretreatment for 15 minutes resulted in a predominant effect, which showed higher reducing sugar yield. The reducing sugar yield of 60-minutes high temperature pretreated bamboo powders was slightly lower than that of 15-minutes pretreated bamboo powders. And the reducing sugar yield of 90-minutes high temperature pretreated bamboo powders was similar with that of 15-minutes pretreated bamboo powders. Therefore, the suitable time for high temperature pretreatment was around 15 minutes.

Conclusions

We have investigated the performance of high temperature pretreatment on cellulase processing of bamboo. It is obtained that Suhong Cellish L can be applied in bamboo processing. High temperature pretreatment was conducive to enhance cellulose processing of bamboo by means of fast increasing the cellulose adsorption on the bamboo in short time and swelling the bamboo powders to loosen the structure. High temperature pretreatment of 120 °C, 15 minutes was suitable for bamboo processing.

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