EFFECT OF HIGH TEMPERATURE PRETREATMENT ON XYLANASE AND CELLULASE HYDROLYSIS OF BAMBOO

by

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Cellulase and xylanase were applied to hydrolyze bamboo. High temperature treatment was launched to treat bamboo prior to enzymatic hydrolysis. Results indicated that bamboo with a high temperature pretreatment caused a more reduction of sugar yield. It is concluded that the high temperature pretreatment is the most effective method to strengthen enzyme hydrolysis towards bamboo.

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

Introduction

Bamboo is a kind of evergreen plant, mainly grows in East Asia, Southeast Asia, the Indian Ocean, and the Pacific Islands [1]. More and more attentions have been paid on bamboo in the fiber processing due to its properties, such as hygroscopic, biodegradable, resistance to UV radiation, and fast-growing [2].

Bamboo degumming normally consists of physical, chemical, and biological methods. In order to solve the problem of environmental pollution and low degumming efficiency, the above mentioned methods are combined to degrade the bamboo. Previous work indicated that the combination of high temperature pretreatment and cellulase processing presented valid effect on bamboo degradation [3]. In this work, xylanase from *Streptomyces sp. FA1* and commercially available cellulases were applied to hydrolyze bamboo. High temperature pretreatment was employed with the purpose to strengthen the hydrolysis.

Materials and methods

Materials

Two-year-old moso bamboo was grounded into powders and passed through a No. 80 mesh sieve. The bamboo powders were collected for enzyme hydrolysis. Cellusoft 8000L and Suhong Cellish L were kindly offered by Novozymes. Xylanase was produced by State Key Laboratory of Jiangnan University with the activity of 180 U/mL and 270 U/mL at pH 5.5, and pH 7, respectively. All the other chemicals were of reagent grade and were purchased from J&K Chemical Ltd., Shanghai.

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Methods

(1) High temperature pretreatment

Reactions were launched in Erlenmeyer flasks containing a total volume of 10 ml deionized water and 0.15 g of bamboo powders. The Erlenmeyer flasks were placed in a high pressure reactor at 120 °C for 30 minutes.

(2) Influence of pretreatment on cellulase hydrolysis of bamboo

Cellulase hydrolysis was launched in Erlenmeyer flasks with a total reaction volume of 25 ml, consisting of 15 U cellulase and 0.15 g bamboo powders. Cellusoft 8000L was used for neutral processing at pH 7 and Suhong Cellish L was used for acidic processing at pH 4.8. The mixture was incubated at 50 °C. Reaction liquor was sampled at specified times and centrifuged at 10,000 rpm for 10 minutes to obtain the supernatant for sugar yielding test according to the modified dinitrosalicylic acid (DNS) calorimetric method [1].

(3) Influence of pretreatment on combined cellulase and xylanase hydrolysis of bamboo

Combined enzyme hydrolysis was performed in Erlenmeyer flasks. A total reaction volume of 25 ml was selected, containing 15 U of Cellusoft 8000L and 309 U of xylanase for neutral processing at pH 7 as well as 15 U of Suhong Cellish L and 135 U of xylanase for acidic processing at pH 5.5. Bamboo powders with a weight of 0.15 g were applied as substrates. The mixture was incubated at 50 °C. The yield of reducing sugar was measured along with the incubation time by the modified DNS method [1].

Results and discussion

(1) Effects of high temperature pretreatment on cellulase hydrolysis of bamboo

Figure 1 shows cellulase hydrolysis of bamboo powders with and without high temperature pretreatment. Cellusoft 8000L and Suhong Cellish L presented the desired activity under neutral (pH 7) and acidic (pH 5.5) conditions, respectively. As shown in fig. 1, the higher optical displacement (OD) value was detected in the bamboo powders with high temperature pretreatment under both neutral, fig. 1(a), and acidic, fig. 1(b), treating by cellulases, indicating higher reducing sugar yield in the reaction liquid. Reducing sugar yield increased at least 6% after 12 hours' incubation compared with non-pretreated bamboo, revealing that high temperature pretreatment greatly benefited celluase hydrolysis of bamboo. Such result was in accordance with our previous work [3].

(2) Effects of high temperature pretreatment on the hydrolysis of bamboo powders by combined cellulase and xylanase

Cellulase (Cellusoft 8000L and Suhong Cellish L) and xylanase were combined to hydrolyze bamboo powders. As shown in fig. 2, bamboo powders with the high temperature pretreatment at 120 °C for 30 minutes caused more reducing sugar yield. A proper explanation is that part of water changes into steam at high temperature in the pretreating reactor. The formed steam can penetrate the inner part of bamboo and help to further open and loosen bamboo structure. Thus, it was easier for the enzymes to absorb on the bamboo powders and greatly benefitted the reducing sugar yield.

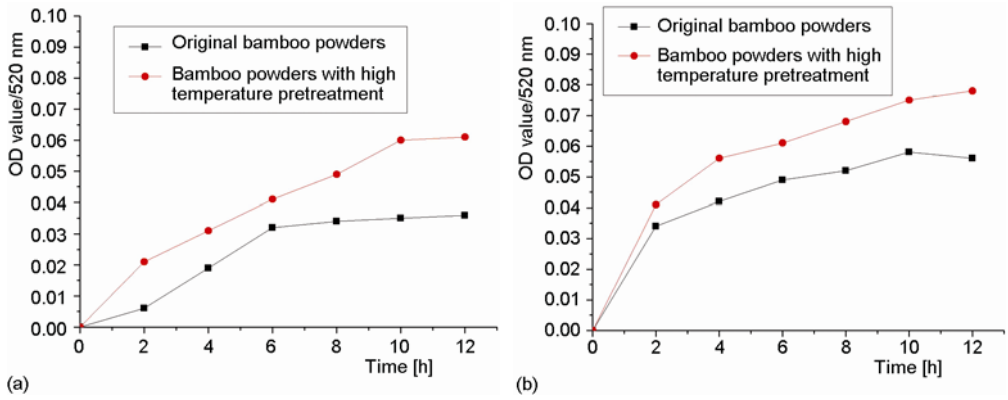

Figure 1. Cellulase hydrolysis on bamboo powders with/without high temperature pretreatment; (a) Cellusoft 8000L, pH 7, 50 °C, and (b) Suhong Cellish L, pH 5.5, 50 °C

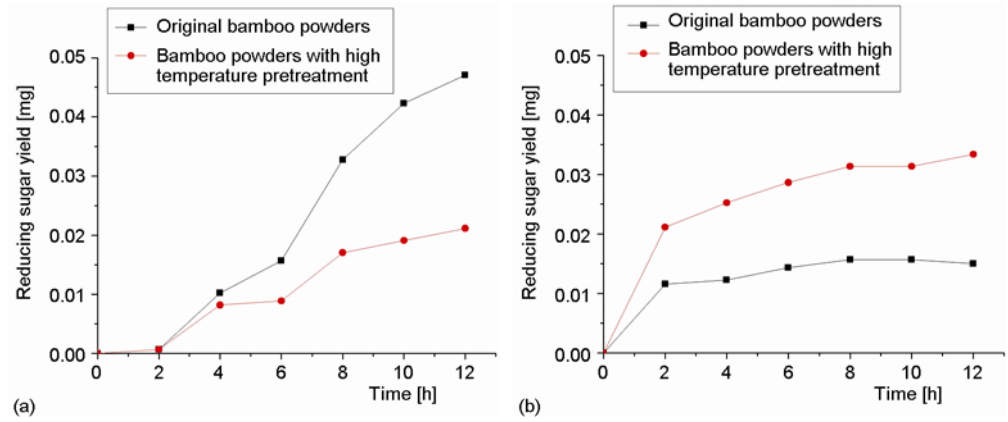

Figure 2. Combined-enzyme hydrolysis on bamboo powders with/without high temperature pretreatment; (a) Cellusoft 8000L and xylanase, pH 7, 50 °C, (b) Suhong Cellish L, and xylanase, pH 5.5, 50 °C

(3) Effect of high temperature pretreatment on bamboo powders

Figure 3(a) shows the change of the solution before and after high temperature treatment. It is obvious that the color of the solution changed from colorless to yellowish after high temperature pretreatment, indicating part of the substances in bamboo have been dissolved in the solution. Reducing sugar content in the yellowish solution reached 0.576 mg/ml, which signified that high temperature treating can hydrolyze bamboo on some level. In fig. 3(b), the weight of bamboo powders in two tubes was the same. However, the bamboo powders with high temperature pretreatments showed larger volume compared with non-pretreated bamboo. It is proved that high temperature treating helps to loosen bamboo structure and make them more swollen. Therefore, more enzymes can attack on pretreated bamboo powders and lead to a better hydrolysis.

Conclusions

We have investigated the influence of high temperature treatment on cellulase hydrolysis and combined cellulase and xylanase hydrolysis of bamboo powders under acidic and

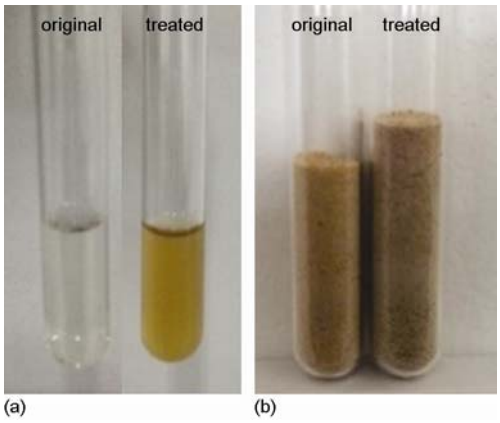

Figure 3. Effect of high temperature pretreatment on the color of the reaction solution (a), and the volume of bamboo powders (b)

neutral conditions. It is indicated that the bamboo powders with high temperature pretreatment at 120 °C for 30 minutes showed more reducing sugar yield compared with non-treated bamboo powders under the same enzyme hydrolysis condition. Bamboo powders become swollen and loosen after the pretreatment, which leads to further penetration of the enzymes and a more effective hydrolysis. It is suggested that high temperature treatment is a desired pretreating method to enhance enzyme hydrolysis of bamboo.

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