HIGH-TEMPERATURE HEATING AND MICROWAVE PRETREATMENTS
A new light in bamboo's enzymatic hydrolysis

by

Jiajia FU [a,b,c*], Yu LI [a,b], Qun ZHANG [a,b], Shen SHEN [a,b], Xiao-Ying DU [a,b], Hong-Bo WANG [a,b], and Wei-Dong GAO [a,b]

[a] Jiangsu Engineering Technology Research Center for Functional Textiles, Jiangnan University, Wuxi, China
[b] Key Laboratory of Eco-Textiles, Ministry of Education, Jiangnan University, Wuxi, China
[c] National Engineering Laboratory for Modern Silk, Soochow University, Suzhou, China

Original scientific paper
DOI: 10.2298/TSCI1603999F

A combined technology of high-temperature heating and microwave is proposed to treat bamboo substrates prior to enzymatic hydrolysis to overcome the limits of either technology. The combined technology helps to promote the reducing sugar yield from enzymatic hydrolysis of bamboo. The protein adsorption test indicated that more enzyme proteins could adsorb on the surface of bamboo pretreated by the combined technology. Thus, the possibility of enzymes to hydrolyze bamboo increased. Meanwhile, X-ray diffractometry tested revealed that the combined technology benefited the removal of non-cellulosic substances from bamboo and resulted in a higher crystallinity.

Key words: *bamboo, cellulase, xylanase, high-temperature treating, microwave*

Introduction

In recent years, more and more attentions have been paid on bamboo in the fiber processing due to its excellent properties [1]. Typically fiber processing mainly relies on chemical degradation, which consumes high energy and causes server environmental pollution. It is of utmost importance to develop the eco-friendly processes with limited liberation of pollutions to attain sustainable utilization of bamboo. The implementation of enzymes can help in achieving this objective.

Previous investigations pointed out the possibility for enzymes to hydrolyze the bamboo [1-3]. However, the effect of enzymes is greatly restrained due to bamboo's naturally extra-compacted structure. Great efforts should be paid to seek reliable pretreatments to enhance the enzyme accessibility towards bamboo. In this work, high-temperature heating and microwave were adopted to pretreat the bamboo with the purpose to enhance the enzymatic hydrolysis in subsequence.

Materials and methods

Materials

Bamboo powders were passed through a No. 80 mesh sieve and extracted by toluene and ethanol with a volume ratio of 1:2 for 4~6 hours. Then the powders were washed by the dis-

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

tilled water and dried. Cellusoft 8000L was kindly provided by Novozymes and xylanase from *Streptomyces* sp. FA1 was offered by the State Key Laboratory of Jiangnan University. All the other chemicals were of reagent grade and were purchased from J&K Chemical Ltd., Shanghai.

Methods

High-temperature heating and microwave pretreatments

Erlenmeyer flasks containing a total volume of 10 ml deionized water and 0.15 g of bamboo powders were placed in a high pressure reactor at 120 °C for 30 minutes for high-temperature heating. Microwave treating was carried out at 390 W for 23 minutes.

Influence of pretreatment on enzymatic hydrolysis of bamboo

Bamboo powders with different pretreatments were subjected to combined cellulase (cellusoft L) and xylanase hydrolysis as described in our previous work [2], with a total reaction volume of 25 ml, consisting 15 U of cellusoft 8000 L and 309 U of xylanase as well as 0.15 g of bamboo powders at pH = 7, 50 °C for 12 hours. 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 method [3].

Protein adsorption test

Bamboo powders weighted 0.15g were placed in the Erlenmeyer flasks with 15 U of cellusoft 8000 L and 309 U of xylanase properly diluted with distilled water. The total reaction volume was limited to 25 ml. The incubation was carried out at 4 °C in the refrigerator. Reaction liquid was sampled and the protein content was tested by a modified Bradford method [3].

X-ray diffractometry

Bamboo powders with and without combined pretreating of high-temperature heating and microwave were collected, cleaned, and dried for crystallinity test. The X-ray diffraction patterns was obtained by using a D8 Super Speed Powder X-ray Diffractometer (Bruker Axs GmbH, Karsruhe, Germany) with the following operating conditions: 6-40°, Cu, 40 kV, 40 mA, 0.02°, 4° per minute.

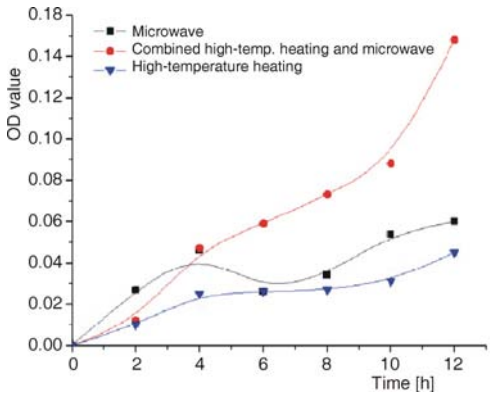

Figure 1. Reducing sugar yield of combined cellulase and xylanase hydrolysis with different pretreatments

Results and discussion

Effects of high-temperature heating and microwave pretreatments on bamboo's enzymatic hydrolysis

Figure 1 showed the reducing sugar yield of combined cellulase and xylanase hydrolysis of bamboo powders with and without pretreatments. As shown in fig. 1, the change of optical displacement (OD) value corresponded to the variation of reducing sugar yield. Highest OD value was obtained from the bamboo powders pretreated in the combination of high-temperature heating and microwave after 4 hours incubation by enzymes, indicating the highest

yield could be achieved under this treating condition. The reducing sugar yield from the bamboo powders pretreated by the combined technology was higher than the sum of yield treated by either technology. The mechanism of microwave heating and high-temperature heating in the pressure reactor is different from each other. As presented in fig. 2, microwave heating is mainly caused by internal heat generation due to absorption of electrical energy from the microwave field. The coupling interaction among the polar molecules in the reaction system results in the microwave heating of materials. High-temperature heating in the pressure reactor can heat the samples by direct or indirect heat source with an opposite heat generation from microwave. Taking the full advantages of these two heating methods, bamboo structure can be loosened and thus become more accessible to enzymes.

Characterization of bamboo powders with different pretreatments

Figure 3 showed the average amount of protein that adsorbed on bamboo powders. It is apparent that with the time prolonging, more protein could be adsorbed on the surface of bamboo. After 10 minutes incubation, bamboo powders pretreated with combined technology showed a higher protein adsorption. It is known that the enzymes only take effects when their protein can properly adsorb on the bamboo surface. The more enzyme protein can be attached on the substrates, the more efficient effort enzyme can provide. Such result indicated that the combined technology helped to swell bamboo structure and break the macromolecular chain of cellulose. Therefore, more chemical bonds with the possibility to bind enzymes were exposed and more active sites with catalytic potential were available for enzymes. The X-ray diffractograms of bamboo powders with different pretreatments were presented in fig. 4. The crystallinity of original bamboo powders was 34%. The crystallinity of bamboo powders reached 48% after treating by the combined technology of high-temperature heating and microwave. The increase of crystallinity proved the re-

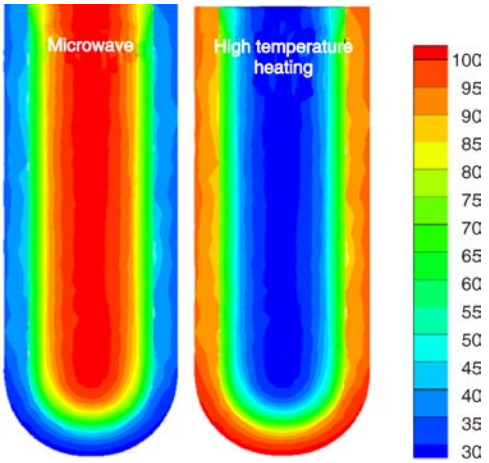

Figure 2. Temperature profile of microwave and high-temperature heating in high pressure reactor *(for color image see journal web-site)*

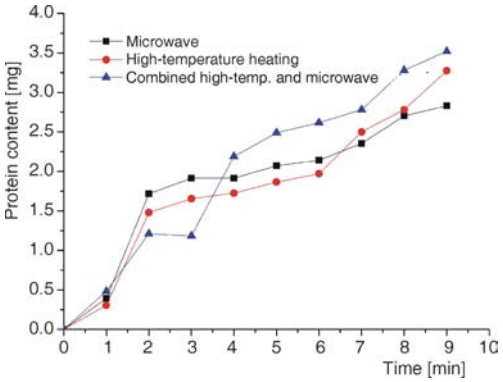

Figure 3. Monitoring of protein content (%) on bamboo powders with different pretreatments

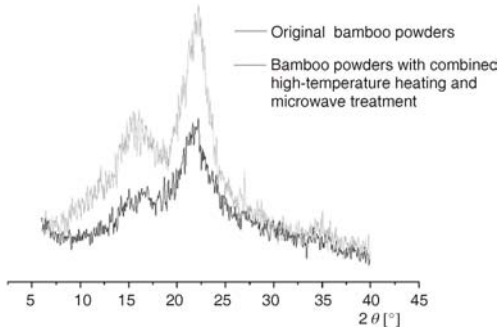

Figure 4. The X-ray diffractograms of bamboo powders with/without pretreatment

moval of hemicellulose, pectin, waxes as well as other substances in amorphous region to some content.

Conclusions

The influence of different pretreatments on cellulase-xylanase hydrolysis of bamboo was investigated. It is concluded that the bamboo powders pretreated with a combined technology of high-temperature heating and microwave showed positive effect to enhance enzymatic hydrolysis in subsequence. The combination of different heating methods can lead to a swollen and loosen structure of bamboo, thus more active sites with catalytic potential were exposed to enzymes and finally benefitted the enzyme performance. Meanwhile, the removal of the substances in amorphous region can help to reduce the burden of enzymatic hydrolysis of bamboo. It is recommended that the combined technology of high-temperature heating and microwave is the preferred method to pretreat bamboo for enzymatic hydrolysis.

Acknowledgment

This work is supported by the National Natural Science Foundation of China (Grant No. 31470509 and 31201134); the Opening Project of National Engineering Laboratory for Modern Silk (Grant No. KJS1312); the Industry-Academic Joint Technological Prospective Fund Project of Jiangsu Province (Grant No. BY2013015-24), the Fundamental Research Funds for the Central Universities (Grant No. JUSRP51622A), and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

References

[1] Fu, J. J., *et al*., Bio-Processing on Bamboo Fiber Extraction for Textile Application: A Mini Review, *Biocatalysis and Biotransformation, 30* (2012), 1, pp. 141-153
[2] Zhang, Q., *et al*., Effect of High Temperature Pretreatment on Xylanase and Cellulase Hydrolysis of Bamboo, *Thermal Science, 19* (2015), 4, pp. 1341-1344
[3] Fu, J. J., *et al*., Bamboo Fiber Processing: Insights Into Hemicellulase and Cellulase Substrate Accessibility, *Biocatalysis and Biotransformation, 30* (2012), 1, pp. 27-37

Paper submitted: December 24, 2015
Paper revised: February 1, 2016
Paper accepted: March 31, 2016