IMPROVED ENZYMATIC HYDROLYSIS OF TOBACCO STALK BY STEAM EXPLOSION AND THREAD ROLLING PRETREATMENTS

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To improve the efficiency of enzymolysis, tobacco stalk was pretreated by steam explosion, the thread rolling and then it was enzyme hydrolyzed by pectinase. Hydrolysis products yields of 0.139 g g^{-1} and 0.127 g g^{-1} could be obtained by steam explosion and thread rolling, which was much higher than that for the untreated tobacco stalk (0.042 g g^{-1}). Furthermore, SEM showed that the enzymolysis effect was remarkable, especially after pretreatment. However, there was no obvious change in the chemical structure of tobacco stalk, based on FTIR. Therefore, the pretreatment was significant and necessary for enzymolysis.

Keywords: waste tobacco stalk, pretreatment, pectinase, enzymatic hydrolysis, galacturonic acid

INTRODUCTION

At present, waste biomass utilization has become a trend. Lignocellulosic biomass, as an important source for production of bioethanol and bioproducts, has aroused worldwide interest.[1-4] Tobacco stalk is a byproduct of the tobacco industry, obtained after harvesting the tobacco. Every year, large amounts of tobacco stalks are produced. Most of them are generally incorporated into soil or burned in the field, which causes both waste of resources and environmental pollution. Therefore, the proper utilization of tobacco stalk and its exploitation as potential biomass material would be imperative.[5]

Various processes have been developed to make full use of tobacco stalk reasonably, for example, the expanding process for tobacco stems,[6] the papermaking process for reconstituted tobacco sheets,[5] and valorization processes for converting tobacco stalk into a variety of chemicals.[7] Reconstituted tobacco has been accepted by the tobacco industry, due to its advantageous economic impact on the manufacturing cost of cigarettes. Most importantly, reconstituted tobacco has become one of the major components in modern blended cigarettes owing to its great potential for reducing tar and the increasing demand for safer cigarettes.[8,9] Based on our previous research on reconstituted tobacco sheets, we have found a large amount of cell wall biopolymers (i.e. cellulose, hemicelluloses, pectin) still existing in the paper-base.

Pectin plays an important role during the smoking process. Pectin, one of the most important parts in tobacco, undergoes pyrolysis and combustion, forming many pyrolysis gaseous products (including methanol, methanoic acid etc.), which influence the characteristics of smoke greatly.[10,11] Pectins are a family of complex polysaccharides that contain 1,4-linked α-D-galactosyluronic acid residues.[12,13] Pectinase enzymes catalyze the hydrolysis of pectin into sugars. In an earlier investigation, Mark R. Wilkins *et al.*[14] stated that galacturonic acid from pectin, as well as other sugars, could be fermented to produce ethanol and acetic acid,[15] with the optimum pH of 4.8, while pectin and cellulose from grapefruit peel waste could be hydrolyzed by pectinase and cellulase enzymes to monomer sugars. Haikel Garna *et al.*[16] have developed a rapid, accurate and reproducible analysis method for the quantification of galacturonic acid of

pectins.

The enzymatic hydrolysis of lignocellulose is influenced by several factors. Chang *et al.*[17] concluded that the crystallinity of cellulose is just one of the factors. Other researchers pointed out other factors such as the degree of polymerization, moisture content, available surface area and lignin content.[18] Therefore, the pretreatment technology is a key step, allowing to disrupt the fibrous structure. As known, there are many pretreatment methods ranging from thermophysical (e.g. steam explosion and hydrothermal extraction) to thermochemical (e.g. AFEX) and biological approaches.[19-21]

The changes in the surface appearance and physical properties of tobacco stalk were selected as the main evaluation criteria in the experiments. FTIR analysis was also used to complement the results obtained.

EXPERIMENTAL

Materials and chemicals

D-galacturonic acid, sodium citrate buffer and pectin were purchased from Aladdin, the enzymes were manufactured by National Medicine Group Shanghai Chemical Reagent Co., Ltd. Standard solutions of galacturonic acid with concentrations from 1 ppm to 10 ppm were prepared. Tobacco stalks were supplied by China Tobacco Guangdong Industrial Co., Ltd.

Pretreatment methods

Thread rolling pretreatment

Firstly, the tobacco stalk was soaked in 40 °C water for 3~5 s to remove dust. Then, the tobacco stalk was loaded into a rub silk machine (stock inlet consistency 20~90%, screw speed 322 rpm, speed ratio 1:3.5) and water was added in order to prevent clogging.

Steam explosion pretreatment

Tobacco stalk was rapidly heated by high-pressure steam without addition of any chemicals.[19] The pressure inside the digester was 0.5 MPa, and the biomass/steam mixture was held for 20 s.

Enzymatic hydrolysis

Enzymatic hydrolysis was then performed in 250 mL flasks, using 50 mL sodium citrate buffer (pH 3.0) and a certain amount of dry matter at 50 °C on an orbital shaker for 10 h. First, we measured the moisture content of the pretreated solid, and then weighed the pretreated solid on wet basis, which contained a certain amount of dry mass. The weighed pretreated solid was further mixed with sodium citrate buffer with a final volume of 50 mL for enzymatic hydrolysis. The pectinase loading was set at 0.4 U/mL, 0.8 U/mL, 1.2 U/mL, 1.6 U/mL, 2.0 U/mL. The galacturonic acid in generated during the process of enzymatic hydrolysis was analyzed by ion chromatography (Dionex ICS-3000).

Analytical methods

The enzyme solution was filtered using a 0.22 μm microfiltration membrane and then diluted 200 times using deionised water to analyse galacturonic acid production. The galacturonic acid was detected using an ion chromatography system (PA1 Column and Electrochemical Detector, column temperature of 30 °C, flow rate of 0.5 mL/min). Sample images were taken using an EVO 18 (Zeiss) scanning electron microscope (SEM). FTIR analysis was performed (TENSOR27/HYPERION) on both the pretreated tobacco stalk and the pretreated solid residue after enzyme hydrolysis.

RESULTS AND DISCUSSION

Ion chromatography assays

Ion chromatography was employed for the quantification of galacturonic acid, and the specific peak is shown in Fig. 1.

Influence of pretreatment methods on galacturonic acid production

The influence of thread rolling pretreatment and steam explosion on galacturonic acid production from tobacco stalk is shown in Fig. 2.

Galacturonic acid yield is a crucial index in determining the effects of the pretreatment processes. Figure 2 shows that with the increase in the amount of enzyme, the yield of galacturonic acid for all samples increased at first, and then decreased. For untreated tobacco stalk, when the amount of enzyme was raised from 0.4 U/mL to 1.6 U/mL, the corresponding yield of galacturonic acid increased from 0.0196 g g^{-1} to 0.042 g g^{-1}, while with further increase in the amount of enzyme, the yield of galacturonic acid decreased.

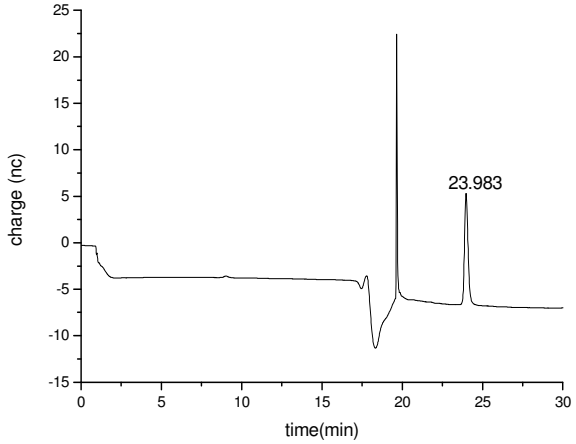


Figure 1: IC chromatogram of a standard solution (4 ppm)

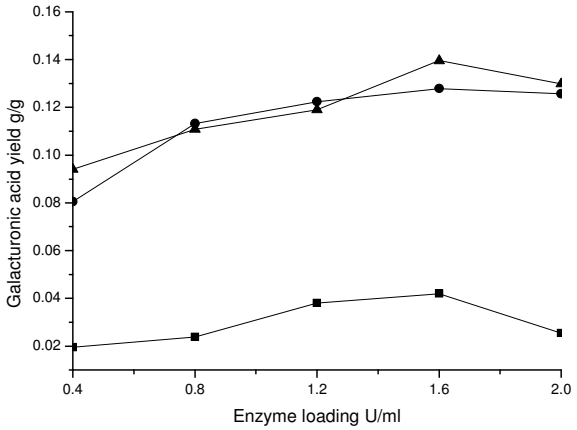


Figure 2: Effect on enzyme amount on galacturonic acid yield during enzymatic hydrolysis, (-●-) thread rolling pretreatment; (-■-) untreated tobacco stalk; (-▲-) steam explosion

For pretreated tobacco stalk, the yield of galacturonic acid was higher than that for the untreated sample, which indicates that the pretreatment had a positive effect. The maximum galacturonic acid yields were of 0.139 g g^{-1} after steam explosion pretreatment and 0.127 g g^{-1} after thread rolling pretreatment.

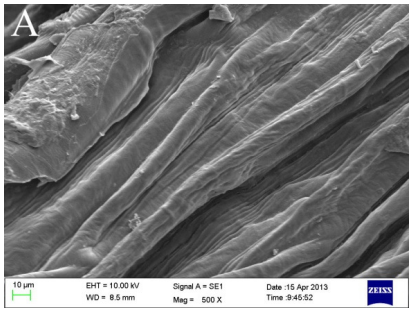
Surface structure analysis of tobacco stalk

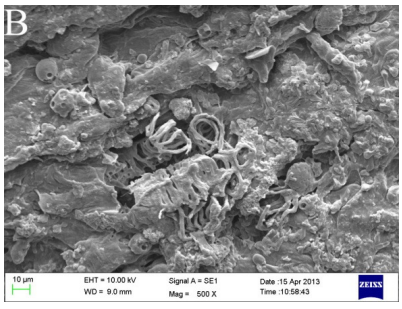
Pretreated and enzymolytic tobacco stalk samples were observed using SEM to detect physical morphology. As shown in Fig. 3A, in the initial samples, microfibrils constitute the skeletal structure of the cell wall, and the texture of tobacco stalk is compact and smooth. However, after enzymatic hydrolysis, the surface structure of tobacco stalk becomes rough, and exhibits holes and pits, and reveals the fiber skeleton (Fig. 3B). From the observation of the samples surface profile we can infer that pectin was degraded in tobacco stalk after enzymatic hydrolysis. The rigid structure fibrils appeared to be loosening after thread rolling pretreatment (Fig. 3E), while for tobacco stalk treated by steam explosion, they became "thinner"[22] (Fig. 3C) and exposed more internal areas. The enzymatic hydrolysis samples are easily recognizable (Fig. 3D, F), the enzymolysis effect being remarkable by the great number of regions presenting fiber skeleton.

The results that we have found indicate that these two pretreatment methods are good options in the industrial manufacture dealing with tobacco stalk before proceeding to the next step.

Infrared spectrum analysis

The infrared spectra for the pretreated and enzymolytic samples are presented in Fig. 4. Comparing the data provided by the infrared spectra (Fig. 4 (I) and (II)), a change in the intensities of the bands can be noted, but the wavenumber of the characteristic peaks did not change significantly for sample (a) and sample (b).





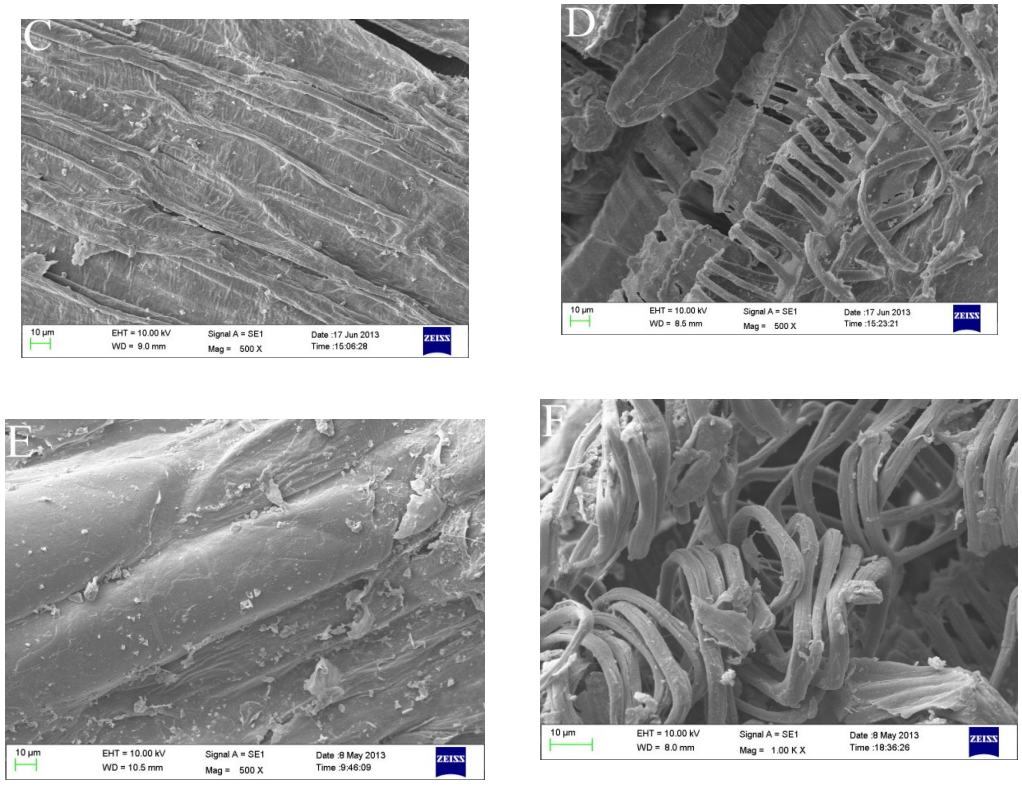


Figure 3: SEM images of (A) untreated tobacco stalk; (B) enzymolytic tobacco stalk; (C) tobacco stalk pretreated by steam explosion; (D) steam explosion coupled with enzymatic hydrolysis; (E) tobacco stalk pretreated by thread rolling; (F) thread rolling coupled with enzymatic hydrolysis (×500)

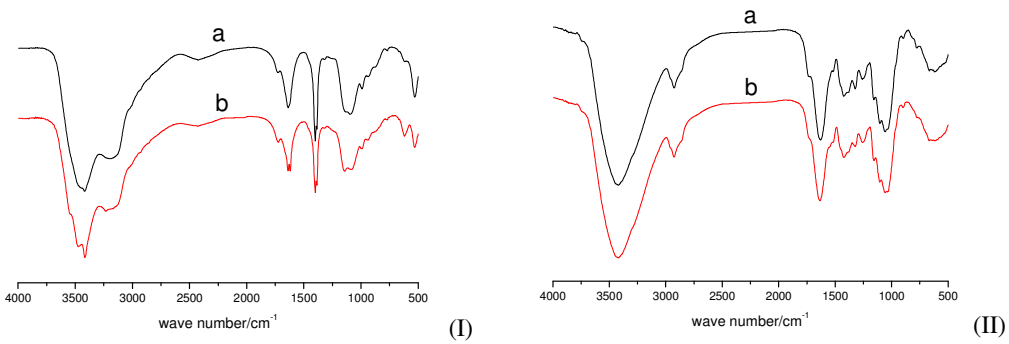


Figure 4: FTIR analysis of (I) tobacco stalk pretreated by (a) steam explosion, (b) steam explosion coupled with enzymatic hydrolysis; (II) tobacco stalk pretreated by (a) thread rolling, (b) thread rolling coupled with enzymatic hydrolysis

This can be explained by the fact that the structure of pectinase is similar to that of cellulose, both belong to the polysaccharide group compounds. Further analysis of the infrared spectra shows some other common peaks. Thus, the broad bands at approximately 3400 cm^{-1} are attributed to the vibration of the OH functional group, the band at 2920 cm^{-1} is characteristic of the elongation of the methylene ($-CH_2$) groups, which is only visible in Fig. 5 (II). Finally, the C–O bond absorption

peaks show the difference between the effects of the two pretreatments.[23]

CONCLUSION

The effect of different pretreatments on the hydrolysis of tobacco stalk was investigated in this paper. Hydrolysis products acid yields of 0.139 g g^{-1} and 0.127 g g^{-1} could be obtained by steam explosion and thread rolling pretreatment, respectively. Also, these pretreatments revealed a higher surface area, which contributed to enzyme hydrolysis. Therefore, both pretreatment methods are suitable for galacturonic acid production from tobacco stalk, leading to reducing the content of pectin, which would be useful in applications in industrial manufacture.

REFERENCES

[1] Z. Merali, J. D. Ho, S. R. A. Collins, G. L. Gall, A. Elliston *et al*., *Bioresour. Technol*., **131**, 226 (2013).

[2] A. F. A. Carvalho, P. O. Neto, D. F. Da Silva, G. M. Pastore, *Food Res. Int*., **51**, 75 (2013).

[3] F. Xu, Y. C. Shi, D. H. Wang, *Carbohyd. Polym*., **88**, 1149 (2012).

[4] E. Bahcegul, H. E. Toraman, N. Ozkan, U. Bakir, *Bioresour. Technol*., **103**, 40 (2012).

[5] D. L. Davis, in "Tobacco: Production, Chemistry and Technology", edited by M. T. Nielsen, 1999, pp. 353-387.

[6] E. H. Theophilus, D. H. Pence, D. R. Meckley, M. A. Higuchi, B. R. Bombick *et al*., *Food Chem. Toxicol*., **42**, 631 (2004).

[7] O. Akpinar, K. Erdogan, U. Bakir, L. Yilmaz, *LWT - Food Sci. Technol*., **43**, 119 (2010).

[8] Y. J. Sung and Y. B. Seo, *Thermochim. Acta*, **486**, 1 (2009).

[9] W. Wang, Y. Wang, L. Yang, B. Liu, M. Lan *et al*., *Thermochim. Acta*, **437**, 7 (2005).

[10] S. Zhou, Y. B. Xu, C. H. Wang, Z. F. Tian, *J. Anal. Appl. Pyrol*., **91**, 232 (2011).

[11] D. H. Zhu, C. X. Li, A. Z. Zhang, R. B. Song, *Tob. Sci. Technol*. (China), **4**, 25 (1999).

[12] R. P. Jolie, T. Duvetter, A. M. Van Loey, M. E. Hendrickx, *Carbohyd. Res*., **345**, 2583 (2010).

[13] B. L. Ridley, M. A. O'Neill, D. Mohnen, *Phytochemistry*, **57**, 929 (2001).

[14] M. R. Wilkins, W. W. Widmer, K. Grohmann, R. G. Cameron, *Bioresour. Technol*., **98**, 1596 (2007).

[15] K. Grohmann, E. A. Baldwin, B. S. Buslig, L. O. Ingram, *Biotechnol. Lett*., **16**, 281 (1994).

[16] H. Garna, N. Mabon, K. Nott, B. Wathelet, M. Paquot, *Food Chem*., **96**, 477 (2006).

[17] V. S. Chang and M. T. Holtzapple, *Appl. Biochem. Biotechnol*., **84**, 5 (2000).

[18] L. Laureano-Perez, F. Teymouri, H. Alizadeh and B. E. Dale, *Appl. Biochem. Biotechnol*., **121**, 1081 (2005).

[19] N. Mosier, C. Wyman, B. Dale, R. Elander, Y. Y. Lee *et al*., *Bioresour. Technol*., **96**, 673 (2005).

[20] C. E. Wyman, B. E. Dale, R. T. Elander, M. Holtzapple, M. R. Ladisch *et al*., *Bioresour. Technol*., **96**, 1959 (2005).

[21] C. E. Wyman, B. E. Dale, R. T. Elander, M. Holtzapple, M. R. Ladish, *Bioresour. Technol*., **96**, 2026 (2005).

[22] Z. H. Hu and Z. Y. Wen, *Biochem. Eng. J*., **38**, 369 (2008).

[23] Z. Marrakchi, R. Khiari, H. Oueslati, E. Mauret, F. Mhenni, *Ind. Crop. Prod*., **34**, 1572 (2011).