STUDY OF ULTRASONIC TECHNIQUE APPLICATION TO ENHANCE THE PRETREATMENT EFFICIENCY OF BIOETHANOL PRODUCTION FROM SUGARCANE BAGASSE

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Abstract - Lignocellulosic biomass is a potential material source for ethanol production. Particularly, the sugarcane bagasse (SCB) coming from the sugar waste of the refinery is very rich in lignocellulose that can be biochemically transformed into ethanol. However, its recalcitrant structure necessitates a pretreatment step to break up the lignocellulosic matrix, thus improving the accessibility of hydrolytic enzymes to carbohydrates for sugar production. Based on the results of some recent studies, the chemical pretreatment process can be improved further by the application of ultrasound. In this study, ultrasound-assisted alkaline pretreatment of sugarcane bagasse for fermentable sugar production was carried out and the influence of NaOH concentration, sonication temperature and time on the delignification was ascertained by establishing and solving a composite design of experiments. The ultrasound-assisted alkaline pretreatment efficiency was also examined by Scanning Electronic Microscope (SEM), Fourier Transform InfraRed (FTIR) and X-ray Diffraction (XRD) methods.

Key words - sugarcane bagasse; cellulose; lignocellulose; ultrasound-assisted alkaline pretreatment; delignification.

1. Introduction

The steady increase in energy consumption and the depletion of fossil fuels have reawakened the interest in developing alternative energy sources and bioenergy is one of the leading options [1, 2]. In particular, the use of ethanol for blending into gasoline has become increasingly popular around the world and Vietnam is gradually integrated into this overall trend. Today the production of ethanol from raw starch material caused much controversy on the issue of food security. One direction to solve this task is to use biomass derived from lignocellulose of agricultural and alimentary waste in which we are interested in the sugarcane bagasse. Until this time, the sugarcane bagasse (SCB) is used as a internal combustiblesupplying the heatfor the evaporation in sugar refining process. However, it is a resource very rich in lignocellulose that could be biochemically transformed into ethanol [3]. The obtained solid residue after suffering the treatment process for ethanol production will be finally reused as a combustible for sugar refinery.

Some recent researches have proved that lignin is one of important components preventing the attack of enzyme to cellulose [4]. Therefore, the pretreatment step whose purposeto initiate the destruction of lignocellulosic matrix is a key process to permit efficient conversion of lignocellulosic feedstock to ethanol (Figure 1). And alkaline pretreatment is one of the methods which have some potential advantages compared with other pretreatment processes including low operating costs, reducing the degradation of hemicellulose, decreasing significantly the lignin content and safety in the production and use [5, 6, 7].

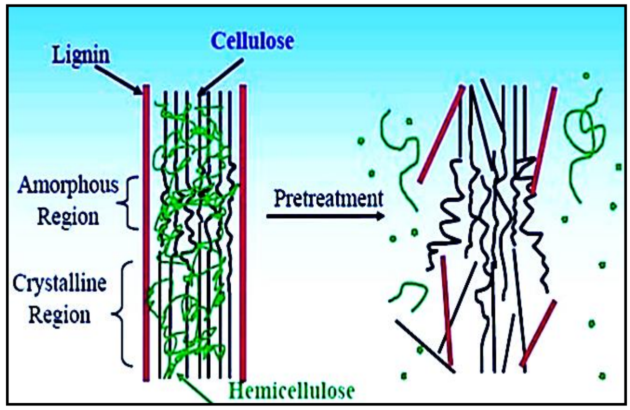

Figure 1. Structures of lignocellulose before and after the pretreatment

In addition, with the rapid development of science and technology, the ultrasound is considered an advanced technique for being efficiently applied to enhance the reaction conversion, decrease significantly the time. The beneficial effect of ultrasound pretreatment on the production of bioethanol has been reported by Filson et al. The application of ultrasound produces cavitation in the aqueous solution and it generates micro bubbles at various nucleation sites in the fluid. The implosion and collapsing of bubbles release violent shock waves that propagate through the medium. The collapse of bubbles produced during cavitation decomposes water into radicals, which helps for the cleavage of lignin linkages [8].

The purpose of this study is determining the optimum conditions for the ultrasound-assisted alkaline pretreatment and simultaneous evaluating the efficiency of ultrasound for the pretreatment of bioethanol production from SCB.

2. Experimental

2.1. Materials and chemicals

Sugarcane bagasse used in this experiment was taken from the Pho Phong sugar refinery in Quang Ngai province. The raw material was dried and stored at room temperature in plastic packet and wasn't washed before pretreatment.

NaOH 98-99wt% is commercial product of China. Distilled deionized (DI) water was used for washing and dilution.

2.2. Ultrasound-assisted alkaline pretreatment

Sugarcane bagasse taken from the Pho Phong sugar refinery was sieved under 18 mesh sieves for collecting an identical dimension about 1 mm. Put into the 250 mL beaker a g these bagasse and b ml NaOH solution whose concentration is 1, 2 and 3% respectively (Liquid/Solid ratio = 25 : 1 (ml/g)).The ultrasonic treatment was carried out within 15, 20 and 25 minutes using an ultrasonic liquid processor (XL-2000 Microson^{TM}, USA). The operating frequency and power of the sonolyzer were 22,.5 kHz and 100 W. The amplitude was maintained at 100% and the temperature was controlled at 30, 40 and 50^{0}C using a water bath. Then, the pulpwas filtered and washed with DI water until the pH of the filtrate reached neutral. Residue obtained was dried at 50^{0}C to constant mass.

Morphology of untreated and pretreated SCB was carried out by using SEM JSM-6010LV (Jeol, Japan) with the maximum magnification is 300,000 times.

Characterization of SCB before and after treatment has been conducted by using X-ray diffraction (Siemens D 5000, Germany) with thestandard Cu X-ray tube(1.5406 A^0); 30kV and scanning range 2-80^o. Furthermore, characterization of functional groups has been performed by Fourier Transform InfraRed (FTIR) spectrometer Nicolet iS10 (Thermo Scientific, USA) by reflection method.

Determination of the main fractions (cellulose, hemicelluloses, and lignin) of SCB was carried out by Chesson method [9].

2.3. Optimization of ultrasound-assisted alkaline pretreatment conditions

The influence on the delignification (y) of three factors: NaOH concentration (Z_1, %), temperature (Z_2, ^{o}C) and sonication time (Z_3, minutes) was ascertained by establishing and solving a composite design of experiments.

The levels and the variable intervals of three factors are showed in Table 1.

Table 1. *Levels and variable intervals of 3 factors*

Factor	Level					Δ
	+ α =1.682	Upper (+)	Mean (0)	Lower (-)	- α =1.682	
Z_1, %	3.68	3	2	1	0.32	1
Z_2, ^{0}C	56.82	50	40	30	23.18	10
Z_3, min	28.41	25	20	15	11.59	5

Note: α - factor level in the suplementery experiments, calculated by using $\alpha = 2^{\frac{k}{4}}$; Δ - Variable interval

The number of experiments for 2 levels, 3 factors design is: $N = 2^k + 2*k + n_0 = 2^3 + 2*3 + 2 = 16$ in which there are two central experiments.

3. Results and discussion

3.1. Examining the effect of ultrasound-assisted alkaline pretreatment on SCB

SCB surface morphology modification effect of ultrasound-assisted alkaline pretreatment was examined using scanning electron microscopy. SEM images of untreated and pretreated SCB (sample N^{0}4 pretreated with 1 wt% NaOH solution at 50^{o}C in 25 minutes) were shown in Figure 2. It was observed that there are not many pores on the untreated SCB (Fig.2.a). After pretreatment, numerous pores were observed in the pretreated SCB structure (Figure 2.b). Besides, the removal of lignin during pretreatmentalso causedthe destruction of lignocellulosic matrix revealing many cellulose fibers, this should favorize the access of cellulase enzymes to hydrolyze cellulose into sugars.

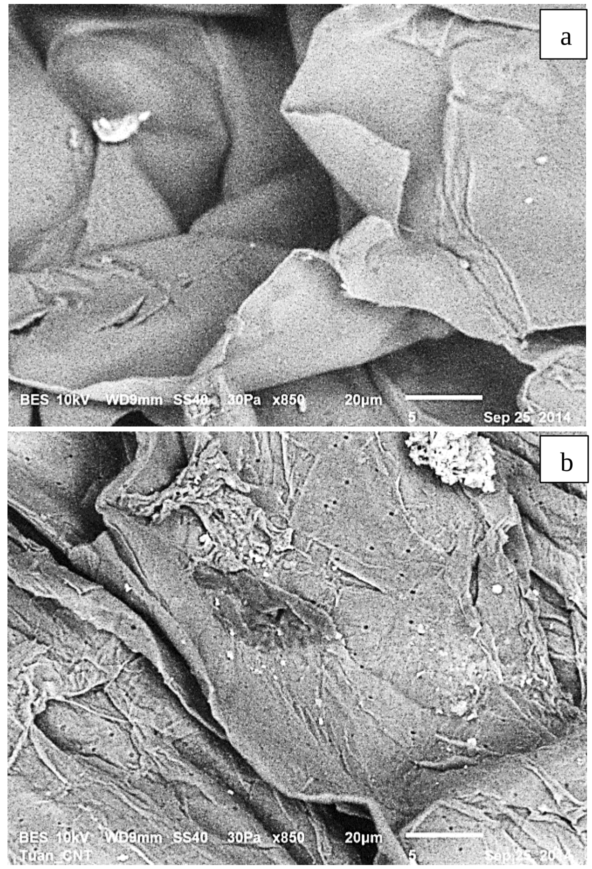

Figure 2. *SEM images of untreated SCB (a) and pretreated SCG (b)*

The crystallinity of untreated SCB and pretreated SCB was investigated using X-ray diffraction and the results were shown in Figure 3. It can be seen that the peaks with $2\theta = 22^o$ and $2\theta = 16^o$ were peaks of cellulose.

The crystallinity index (CrI) was determined using this formula:

$$\mathrm{CrI} = \frac{I_{Crystalline} - I_{Amorphous}}{I_{Crystalline}}$$

Where, $I_{Crystalline}$ = intensity at 22^o and $I_{Amorphous}$ = intensity at 16^o.

With the data of XRD spectra of SCB before and after pretreatment, we could calculate their crystallinity index: The crystallinity index of pretreated SCB was 63.93%, whereas that for raw SCB was 58.12% due to the removal of hemicellulose and lignin fractions which increase the relative content of crystalline cellulose.

Direct information about changes in chemical functionality can be obtained by FTIR spectroscopy. Based on Figure 4, there was a significant difference between the spectra of untreated SCB (Sample N^{o}0) and pretreated SCB (Sample N^{o}1, 2, 3 &5). This difference indicated that there was astructural change because of alkaline treatment. Thebroad peak at 3428 cm^{-1}could be the O-H stretching vibration (i.e. O-H stretching intramolecular hydrogen bonds for cellulose) and a peak at around 2918 cm^{-1} derived from the C-H stretching. The reduction in intensity of the peak at 1514 cm^{-1} (associated with the aromatic ring present in lignin) is due to delignification.

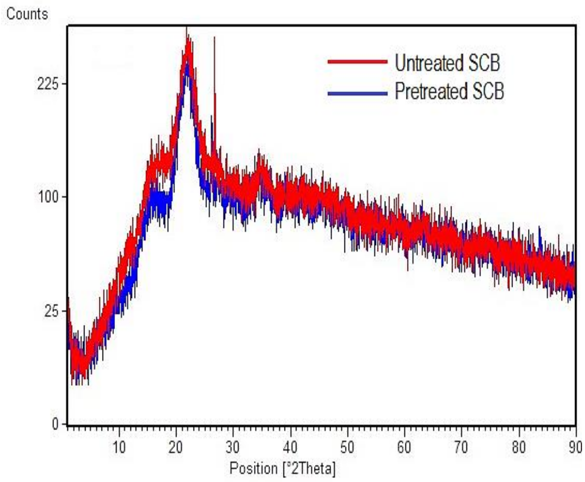

Figure 3. *XRD spectra of SCB before and after pretreatment*

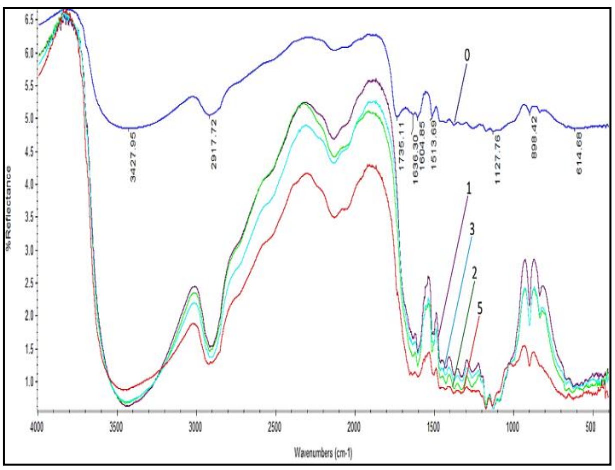

Figure 4. *0: FTIR spectra of untreated SCG; 1, 2, 3, 5: FTIR spectra of pretreated SCB samples*

3.2. Determination of optimal conditions for Ultrasound-assisted alkaline pretreatment

Experiments were conducted according to the levels and variable intervals of 3 factors (Table 1) and collected data of 16 experiments of the composite design was shown in Table 2.

Table 2. *Results of the ultrasound-assisted alkaline pretreatment on SCB*

	N^0	x_1	x_2	x_3	y, %
2^k	1	-1	-1	-1	0.84
	2	-1	-1	1	11.88
	3	-1	1	-1	49.15
	4	-1	1	1	59.69
	5	1	-1	-1	16.88
	6	1	-1	1	39.22
	7	1	1	-1	30.74
	8	1	1	1	55.71
2*k	9	-1.68	0	0	51.78
	10	1.68	0	0	13.56
	11	0	-1.68	0	52.66
	12	0	1.68	0	46.53
	13	0	0	-1.68	20.00
	14	0	0	1.68	61.15
n_0	15	0	0	0	53.89
	16	0	0	0	64.24

After transforming from the variables (Z_1,%; Z_2, ^0C; Z_3, minutes) into the coding variables (x_1, x_2, x_3), the type of the equation of regression is assumed to be:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_{12} + b_{13}x_{13} + b_{23}x_{23} + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 \quad (1)$$

Where y is the predicted response (delignification, %); b_0 is the constant; b_1, b_2, b_3 are linear coefficients; b_{11}, b_{22}, b_{33} are quadratic coefficients; b_{12}, b_{13}, b_{23} are interaction coefficients.

The coefficients within the equation (1) were calculated by these formulas:

$$b_0 = \frac{1}{N}\sum_{u=1}^{N} x_{0u}.y_u$$

$$b_j = \frac{1}{N}\sum_{u=1}^{N} x_{ju}.y_u$$

$$b_{ij} = \frac{1}{N}\sum_{u=1}^{N} x_{iu}.x_{ju}.y_u$$

And the regressionequation was found:

$$y = 59.72 - 3.17x_1 + 8.51x_2 + 10.11x_3 - 8.22x_1x_2 + 3.22x_1x_3 + 0.26x_2x_3 - 10.92x_1^2 - 4.94x_2^2 - 8.13x_3^2 \quad (2)$$

The signification of calculated coefficients of equation (2) is then examined on the basic of the Student standard. The signification test results in removing b_1, b_{13}, b_{23} coefficients.

The regression equation is now rewritten:

$$y = 59.72 + 8.51x_2 + 10.11x_3 - 8.22x_1x_2 - 10.92x_1^2 - 4.94x_2^2 - 8.13x_3^2 \quad (3)$$

By examining the compatibility of the regression using Fisher standard, we could confirm that the regression equation (3) is fully compatible with the experiments and can be used to find out the optimum conditions for ultrasound-assisted alkaline pretreatment by the derivative method.

The optimum conditions of the study is determined by solving the following system of three equations:

$$\begin{cases} -8.22x_2 - 2 \times 10.92x_1 = 0 \\ 8.51 - 8.22x_1 - 2 \times 4.94x_2 = 0 \\ 10.11 - 2 \times 8.13x_3 = 0 \end{cases}$$

Three above equations are in order the partial derivations on x_1, x_2, x_3, as shown below:

$$\left(\frac{dy}{dx_1}\right)_{x_2,x_3} = 0$$

$$\left(\frac{dy}{dx_2}\right)_{x_1,x_3} = 0$$

$$\left(\frac{dy}{dx_3}\right)_{x_1,x_2} = 0$$

The solutions were found: x_1 = -0.47; x_2 = 1.25; x_3 = 0.62 and we could obtain the optimum conditions for delignification after retransforming into the initial variables: Z_1 = 1.53; Z_2 = 52.5; Z_3 = 23.1.

So the maximum delignification occurs at NaOH concentration of 1.53 wt%, sonication time of 23.1 minutes and temperature of 52.5 ^{0}C. The predicted delignification at those values is 68.66%.

Compared these results with those of a domestic study about the SCB pretreatment with NaOH solution without using ultrasound, the lignin content decreased from 21% to 6%, ca. 71.43% delignification, but the processing time lasted 8 time longer (180 minutes) and at a higher temperature (85 ÷ 95^{0}C) [10]. This comparison showed more persuasively the economicefficiency of ultrasound for the pretreatment of bioethanol production from SCB.

4. Conclusions

The efficiency of ultrasound for the alkaline-pretreatment of bioethanol production from sugarcane bagasse has been evaluated by detailed examining the modification of morphology, crystallinity and characterization of functional groups of untreated and pretreated sugarcane bagasse.

The optimum conditions for the ultrasound-assisted alkaline pretreatment of sugarcane bagasse weredetermined by solving a central composite design of experiments. The obtained results show that the maximum delignification value is 68.66% using 1.53 wt % NaOH solution, in sonication time of 23.1 minutes and at temperature of 52.5^{0}C.

REFERENCES

[1] P.S. Nigam, A. Singh, “Production of liquid biofuels from renewable resources”, *Prog. Energy Combust. Sci*, 2011, doi:10.1016/j.pecs.2010.01.003.

[2] Jonathan R. Mielenz, Biofuels: Methods and Protocols, Methods in Molecular Biology, vol. 581, 2009, Preface.

[3] Stefano Macrelli et al, “Techno-economic evaluation of 2nd generation bioethanol production from sugar cane bagasse and leaves integrated with the sugar-based ethanol process”, *Biotechnology for Biofuels* 2012. doi: 10.1186/1754-6834-5-22.

[4] Hetti Palonen, “Role of lignin in the enzymatic hydrolysis of lignocellulose”, *VTT Biotechnology*, 2004, 11-39.

[5] Yu-Shen Cheng, Yi Zheng, Chao Wei Yu, Todd M. Dooley, Bryan M. Jenkins, and Jean S. VanderGheynst, “Evaluation of High Solids Alkaline Pretreatment of Rice Straw”, *Appl Biochem Bioethanol*, 162, 2010, 1768-1784.

[6] Rocio Sierra, Cesar Benigno Granda, and Mark T. Holtzapple, *Lime Pretreatment, Biofuels: Methods and Protocols, Methods in Molecular Biology*, vol. 581, 2009, Chapter 9, 115-124.

[7] Roni Maryana, Dian Ma'rifatun, A. Wheni I, Satriyo K.W., W. Angga Rizal, “Alkaline Pretreatment on Sugarcane Bagasse for Bioethanol Production”, *ScienceDirect*, Energy Procedia, 47, 2014, 250 – 254.

[8] Filson, P.B., Dawson-Andoh, B.E., 2009, “Sono-chemical preparation of cellulose nanocrystals from lignocellulose derived materials”, *Bioresour. Technol*. 100, 2259–2264.

[9] *Biotechnology and Bioengineering*, Vol. XXIII, Pp. 2167-2170 (1981), © 1981 John Wiley & Sons, Inc.

[10] Nguyễn Đình Tiến, 2011, *Nghiên cứu sản xuất ethanol nhiên liệu từ bã mía*, Đồ án tốt nghiệp, Bộ môn Công nghệ sinh học, Khoa Kỹ thuật Hóa học, Trường Đại học Bách khoa TP. HCM.

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