

Enhancing efficiency for reducing sugar from cassava bagasse by pretreatment

S. Gaewchingduang and P. Pengthemkeerati

Abstract— Cassava bagasse is one of major biomass wastes in Thailand from starch processing industry, which contains high starch content of about 60%. The object of this study was to investigate the optimal condition for hydrothermally pretreating cassava bagasses with or without acid addition. The pretreated samples were measured reducing sugar yield directly or after enzymatic hydrolysis (alpha-amylase). In enzymatic hydrolysis, the highest reducing sugar content was obtained under hydrothermal conditions for at 125°C for 30 min. The result shows that pretreating cassava bagasses increased the efficiency of enzymatic hydrolysis. For acid hydrolysis, pretreating cassava bagasses with sulfuric acid at 120°C for 60 min gave a maximum reducing sugar yield. In this study, sulfuric acid had a greater capacity for hydrolyzing cassava bagasses than phosphoric acid. In comparison, dilute acid hydrolysis to provide a higher yield of reducing sugar than the enzymatic hydrolysis combined hydrothermal pretreatment. However, enzymatic hydrolysis in a combination with hydrothermal pretreatment was an alternative to enhance efficiency reducing sugar production from cassava bagasse.

Keywords— Acid hydrolysis, Cassava bagasse, Enzymatic hydrolysis, Hydrothermal pretreatment

I. INTRODUCTION

In Thailand, cassava bagasses were produced about 340,000 tons annually with an increasing trend [1], Cassava bagasse is a fibrous by-product of the starch processing that contains about 50 to 70% starch on a dry weight and 20% fibers (cellulose hemicellulose and lignin) [2-5]. Due to a high portion of starch, cassava bagasse is of interest to be use as an alternative substrate for ethanol production. To improve ethanol production, cassava bagasse should be pretreated. This is because several advantages of pretreatment are to (1) remove lignin and hemicelluloses, (2) reduce cellulose crystallinity, and (3) increase the porosity of the lignocellulosic materials. In addition, pretreatment could enhance sugar production by reducing the formation of by-products that have an inhibitory to the enzymatic hydrolysis and avoiding the degradation or loss of carbohydrate[6].

Hydrothermal pretreatment is an effective method to

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pretreat lignocellulosic materials [7,8]. It refers to the use of water as liquid or vapor or both and heat to pretreated biomass. After hydrothermally pretreating lignocellulosic materials, the efficiency of enzymatic hydrolysis was commonly enhanced [9,10]. Previous studies indicated that pretreatment should be performed under relatively lower temperature for a longer period of time for preventing the production of inhibitory by products [11,12]. In this study, cassava bagasse had been pretreated by physical or chemical methods before enzymatic (alpha-amylase and glucoamylase) hydrolysis to increase the degradation process of hemicelluloses and cellulose [6,13]. Acid hydrolysis is another pretreatment method of lignocellulosic materials by converting cellulose and hemicelluloses to reducing sugar [14].

The purpose of the present study was to determine the optimal hydrothermally pretreatment condition for maximizing reducing sugar production by using enzymatic or acid hydrolysis.

II. MATERIALS AND METHODS

A. Substrate and enzymes

Cassava bagasses was supplied by the Sonish Starch Technology Company, Chachoengsao, Thailand. The cassava bagasse samples were milled to an average size of 0.5 to 2 mm by a grinder and dried overnight at 60°C in a hot-air oven. The milled cassava bagasse was stored in a desiccator. The commercial enzyme, alpha-amylase from *Bacillus licheniformis* (Liquozyme® SC DS), was used for the enzymatic hydrolysis. The product from enzymatic hydrolysis was measured reducing sugar by the DNS method [15].

B. Hydrothermal treatment of cassava bagasse

Cassava bagasse was suspended in water at a solid content of 2%. The mixture was hydrothermally pretreated by heating with an autoclave at various temperature levels ranging from 115 to 130°C and reaction time from 15 to 90 min. The different pretreated cassava bagasse slurries were obtained and used for further analysis.

C. Enzymatic hydrolysis

The pretreated cassava bagasse slurries were hydrolyzed by

alpha-amylase. Enzymatic hydrolysis was conducted at 55°C for 48 h. The buffer for hydrolysis was 0.05 M acetate buffer (pH5.0). After 48-h of hydrolysis, the mixture was centrifuged at a 6500 rpm for 10 min. The supernatant was then analysed for reducing sugar by using the DNS method.

D. Acid hydrolysis

The 2% cassava bagasse mixture was hydrolyzed with dilute acids (sulfuric acid and phosphoric acid) of different concentrations ranging from 0.05 to 0.5 M. The hydrolysis conditions were performed at 121°C for 30 min. The hydrolysates were separated by a centrifuge at 6500 rpm for 10 min. Each sample was neutralized by 1 M NaOH solution for analysis of reducing sugar with DNS method. The acid concentration that gave a maximum reduce sugar was chosen. The selected acid concentration was applied to determine the optimum temperature for cassava bagasse hydrolysis. A temperature range used in this study was between 115 and 130°C and hydrolysis time was from 15 to 90 min.

III. RESULT AND DISCUSSION

A. Enzymatic hydrolysis

In enzymatic hydrolysis, the reducing sugar increased with increasing pretreatment temperature and time. Hydrothermal pretreatment of cassava bagasses for 30 min gave a maximum reducing sugar yield. After treating for 30 min, the reducing sugar contents were almost constant in all studied temperatures (Fig. 1). Temperature effect was less pronounced in this studied condition. The highest reducing sugar content was obtained under hydrothermal conditions at 125°C for 30 min.

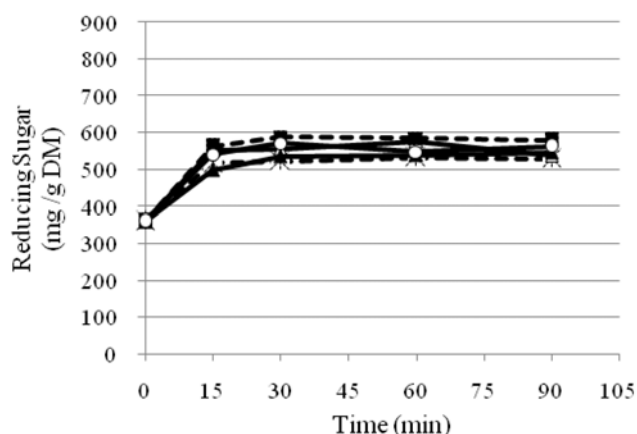


Fig. 1 Effect of temperature and reaction time on the pretreatment of cassava bagasse followed by enzymatic hydrolysis for 48 hr at 55°C and pH 5. (Δ) 115°C; (▲) 120°C; (■) 125°C; (○) 130°C.

B. Effect of acid concentration on acid hydrolysis

The effect of sulfuric and phosphoric acid concentrations on the reducing sugar yield was shown in Figure 2. The

reducing sugar yield was substantially increased at a 0.05 M sulfuric acid or 0.1 M phosphoric acid. The highest reducing sugar obtained in this study condition was 384 mg g⁻¹ when the cassava bagasses were hydrolysed with 0.1 M sulfuric and phosphoric acid. After 0.1 M acid concentration, the reducing sugar yield remained constant. A similar result of acid hydrolysis of cassava peels has been reported by Yoonan and Kongkiattikajorn[16]. Cassava peels was hydrolyzed by 0.1 M sulfuric acid (w/v) at 135 °C for 90 min provided a highest reducing sugar [17]. Previous study observed monomer sugar yields decreased at high acid concentration, due to degradation of product monomers by acid [13]. However, it was not observed in this study.

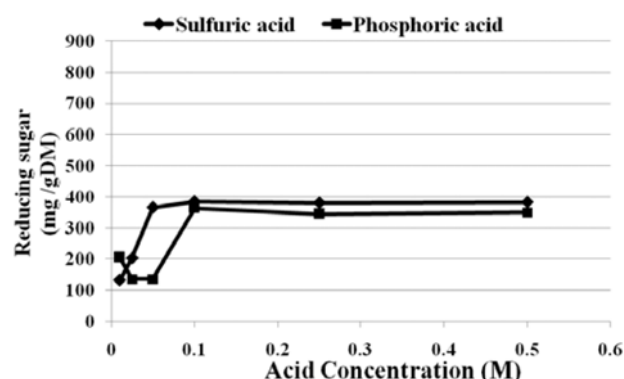


Fig. 2 Reducing sugar yield after hydrolyzing 2% cassava bagasse with various concentrations of dilute acids at 121°C for 30 min. (◆) Sulfuric acid; (▲) Phosphoric acid.

It seems that using 0.1 M sulfuric acid would provide a better hydrolysis of cassava bagasses in comparison with using 0.1 M phosphoric acid (Fig. 2). This observation indicated the effect of acid types on biomass hydrolysis. The reason for this finding may be because sulfuric acid pretreatment has a stronger acid strength that gives a better digestibility of hemicelluloses and cellulose in cassava bagasses than phosphoric acid pretreatment [18].

C. Effect of temperature and time on acid hydrolysis

Cassava bagasse was hydrolyzed in an autoclave at 115 to 130°C with 0.1 M H₂SO₄ or 0.1 M H₃PO₄. Fig. 3a shows the effect of temperature and time on acid hydrolysis with 0.1 M H₂SO₄ on reducing sugar. The results showed that increasing temperature had a positive effect on reducing sugar production. Except for 130°C, all treatment temperatures enhanced the hydrolysis of cassava bagasses with time, as evident by the increased reducing sugar contents with longer treatment time. At 60 min treatment, reducing sugar production was reached a maximum. But, after this time, reducing sugar decreased.

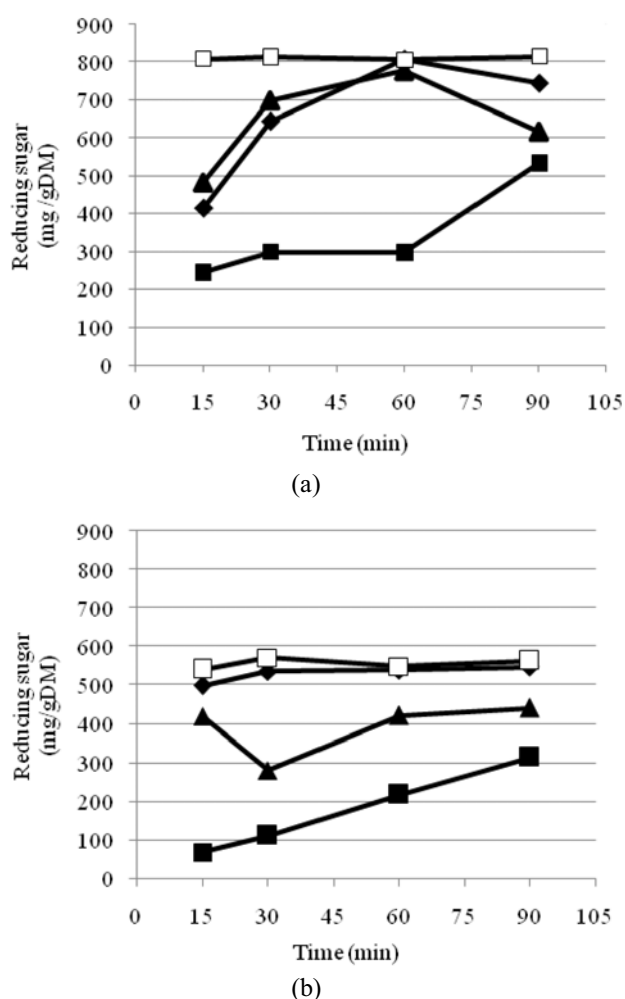


Fig. 3 Effect of temperature on hydrolysis of cassava bagasse to reducing sugar at various times. (a) 0.1 M Sulfuric acid; (b) 0.1 M Phosphoric acid. (Δ) 115°C; (\blacktriangle) 120°C; (\blacksquare) 125°C; (\square) 130°C.

However, effect of treatment time was less pronounced when hydrolyzing cassava bagasse at 130°C. The highest reducing sugar was given when hydrolyzing cassava bagasses with 0.1 M H₂SO₄ at 120°C for 60 min or at 130°C for 30 min. Previous study suggested that the higher treatment temperature (from 130°C) would enhance a formation of by products that might have adverse effects on fermentation process for ethanol production [13]. Hence, treating cassava bagasses with sulfuric acid at 120°C for 60 min may be more appropriate for reducing sugar production in this study.

When treating with phosphoric acid, treatment temperature and time also gave a similar effect on reducing sugar yield, as was observed in sulfuric acid treatment (Fig. 3b). The highest reducing sugar was 535 to 570 mg g⁻¹ when hydrolyzing cassava bagasse at 120°C or 130°C for 30 min.

In comparison, sulfuric acid tended to provide a greater hydrolysis efficiency of cassava bagasses than phosphoric acid.

IV. CONCLUSION

In this experiment, the 2% cassava bagasse pretreated by hydrothermal pretreatment could be hydrolyzed by alpha-amylase to produce reducing sugar. The reducing sugar increased with increasing pretreatment temperature and time. Hydrothermal pretreatment of cassava bagasses for 30 min gave a maximum reducing sugar yield. From this study, pretreating cassava bagasses enhanced the efficiency of enzymatic hydrolysis.

The dilute acids (sulfuric and phosphoric acids) were evaluated for their ability to produce reducing sugars from cassava bagasse. Hydrolyzing cassava bagasse with sulfuric acid pretreatment gave a higher reducing sugar production than using phosphoric acid. The results showed that increasing temperature had a positive effect on reducing sugar production. Except for 130°C, an increased treatment time of acid hydrolysis increased reducing sugar production until 60-min treatment. In this acid hydrolysis, the highest reducing sugar was given when hydrolyzing cassava bagasses with 0.1 M H₂SO₄ at 120°C for 60 min or at 130°C for 30 min. Since higher temperature would enhance a formation of by product, hydrolyzing cassava bagasses with sulfuric at 120°C for 60 min may be more appropriate for reducing sugar production in this study.

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REFERENCES

- [1] Office of Agricultural Economics, "Agricultural Import Export 2552," Ministry of Agriculture and Cooperatives, Bangkok, Thailand, 2553.
- [2] K. Siroth, R. Chollakup, S. Chotineeranat, K. Piyachomkwan and C.G. Oates, "Processing of cassava waste for improved biomass utilization," *Bioresource technology*, vol 71, pp. 63-69, 2000.
- [3] A. Kosugi, A. Kondo, M. Ueda, Y. Murata, P. Vaithanomsat, W. Thanapase, T. Arai and Y. Mori, "Production of ethanol from cassava pulp via fermentation with a surface engineered yeast strain displaying glucoamylase," *Renewable Energy*, vol 34, pp. 1354-1358, 2009.
- [4] U. Rattanachomsri, S. Tanapongpipat, L. Eurwilaichitr and V. Champreda, "Simultaneous non-thermal saccharification of cassava pulp by multi-enzyme activity and ethanol fermentation by *Candida tropicalis*," *Journal of Bioscience and Bioengineering*, vol 107, pp. 488-493, 2009.
- [5] A. Pandey, C.R. Soccol, P. Nigam, V.T. Soccol, L. P.S. Vandenberghe and R. Mohan, "Review paper: Biotechnological potential of agro-industrial residues. II: cassava bagasse," *Bioresource technology*, vol 74, pp. 81-87, 2000.
- [6] H. Inoue, S. Yano, T. Endo, T. Sakaki and S. Sawayama, "Research: Combining hot-compressed water and ball milling pretreatments to improve the efficiency of the enzymatic hydrolysis of eucalyptus," *Biochemistry for Biofuels*, vol 1, Apr. 2008.
- [7] N. Mosier, C. Wyman, B. Dale, R. Elander, Y.Y. Lee, M. Holtzapple and M. Ladisch, "Features of promising technologies for pretreatment of lignocellulosic biomass," *Bioresource Technology*, vol 96, pp. 673-686, Sep. 2005.
- [8] M. Galbe and G. Zacchi, "A review of the production of ethanol from softwood," *Applied microbiology and biotechnology*, vol 59, pp. 618-628, 2002.

- [9] M. Linde, M. Galbe and G. Zacchi, "Bioethanol production from non-starch carbohydrate residues in process streams from a dry-mill ethanol plant," *Bioresource Technology*, vol 99, pp. 6505-6511, Feb. 2008.
- [10] I. Cybulska, H. Lei and J. Julson, "Hydrothermal pretreatment and enzymatic hydrolysis of prairie cord grass," *Energy Fuels*, vol 24, pp. 718-727, 2010.
- [11] M.J. Negro, P. Manzanares, I. Ballesteros, J.M. Oliva, A. Cabanas and M. Ballesteros, "Hydrothermal pretreatment conditions to enhance ethanol production from poplar biomass," *Applied Biochemistry and Biotechnology*, vol 105-108, pp. 87-100, 2003.
- [12] J.D. Wright, "Ethanol from biomass by enzymatic hydrolysis," *Chemical Engineering Progress*, vol 84, Aug. 1988.
- [13] B. Palmarola-Adrados, P. Choteborska, M. Galbe and G. Zacchi, "Ethanol production from non-starch carbohydrates of wheat bran," *Bioresource Technology*, vol 96, pp. 843-850, 2005.
- [14] T. Srinorakutara, L. Kaewvimol and L.-a. Saengow, "Approach of cassava waste pretreatments for fuel ethanol production in Thailand," *The Journal of Scientific Research Chulalongkorn University*, vol 31, 2006.
- [15] L.G. Miller, "Use of Dinitrosalicylic acid reagent for determination of reducing sugar," *Analytical Chemistry*, vol 31, Mar. 1959.
- [16] K. Yoonan and J. Kongkiattikajorn, "A study of optimal condition for reducing sugars production from cassava peels hydrolysis by dilute acid and enzymes," *Kasetsart Journal*, 2005
- [17] K. Yoonan, J. Kongkiattikajorn and K. Rattanakanokchai, "Ethanol production from acid hydrolysates of cassava pulps using fermentation by *Saccharomyces cerevisiae*," *Kasetsart Journal*, 2005.
- [18] B.-H. Um, M.N. Karim and L.L. Henk, "Effect of sulfuric and phosphoric acid pretreatments on enzymatic hydrolysis of corn stover," *Applied Biochemistry and Biotechnology*, vol 105-108, pp. 115-125, 2003.