Songklanakarin J. Sci. Technol.
40 (3), 555-562, May - Jun. 2018

http://www.sjst.psu.ac.th

Original Article

Optimization of glucose production from corncob by microwave-assisted alkali pretreatment and acid hydrolysis

Sininart Chongkhong* and Chakrit Tongurai

Department of Chemical Engineering, Faculty of Engineering,
Prince of Songkla University, Hat Yai, Songkhla, 90112 Thailand

Received: 31 July 2016; Revised: 6 January 2017; Accepted: 13 February 2017

Abstract

The production of reducing sugar is the most crucial process in the production of cellulosic ethanol and can be divided into two steps. First, pretreatment removes the unwanted constituents of lignocellulose to produce cellulose; then the cellulose is hydrolyzed to glucose (a reducing sugar) in the hydrolysis step. In this work, microwave heating was applied for both alkali pretreatment and sequential acid hydrolysis of corncob in which the conditions were optimized by response surface methodology. The optimal cellulose content in the pretreated corncob was 52.9 % w/w obtained with 1 M sodium hydroxide using a 1:12 weight ratio of corncob to solution at 900 W microwave power for 20 min, which was used to optimize the glucose yield in the following step. The highest yield of 14.8% glucose was achieved with 1.3% w/w sulfuric acid solution at 900 W for 16 min.

Keywords: corncob, microwave, alkali pretreatment, acid hydrolysis, reducing sugar

1. Introduction

Demand for fuel for transportation is increasing worldwide and environmental concerns make biofuels an attractive option to traditional petrochemical based fuels. The most suitable feedstock for biofuel production is lignocellulosic biomass, a waste product from agricultural activities. Using agricultural residues or agricultural byproducts can reduce the accumulation of bio-waste and the emission of carbon dioxide (Goldemberg, 2007; Tilman *et al.,* 2006). In Thailand and other tropical countries, one of the major agricultural residues are corncobs, which are what remains of corn once its kernels have been removed, and are a source of both lignocellulose and carbohydrate. Thailand is the third largest producer of sweet corn in the world. In 2010, Thailand exported 128.6 thousand tons, worth 3,834 million baht, mostly in the form of canned kernels, and exports are likely to have increased by18–19% per year over the last five years, with an average growth of 7–8% per year in the global market for corn (MGR Online, 2010). The corncob is the main waste from the processing of corn and represents 40–50% by weight of the corn fruit (Richana *et al.,* 2007). Corncobs are composed mainly of carbohydrates with high starch, cellulose, hemicellulose and glucose contents, which can be used in the production of bioethanol through fermentation (Girio *et al.,* 2010). However, in order to efficiently utilize lignocellulosic biomass for ethanol production the biomass has to be treated to separate the cellulose, hemicellulose, and lignin (Hahn-Hagerdal *et al.,* 2006). Pretreatment is necessary in order to remove the lignin and hemicellulose thus improving the availability of the cellulose in the biomass, as well as disrupting the crystalline structure of the cellulose (Chen *et al.,* 2008; Zheng *et al.,* 2009).

Pretreatment processes by various methods have been developed to enhance the hydrolysis of polysaccharides including starch, cellulose, and hemicellulose. These include the use of aqueous ammonia (Chen *et al.,* 2012; Kim & Lee, 2007; Ko *et al.,* 2009), alkali (Ruangmee & Sangwichien, 2013; Zhu *et al.,* 2006), acid (Ballesteros *et al.,* 2008; Mohon *et al.,* 2013; Sun & Cheng, 2005; Wyman *et al.,* 2005), acid/alkali (Gabhane *et al.,* 2014; Kim & Kim-Ho, 2013; Zhang *et al.,* 2010), steam explosion (Ballesteros *et al.,* 2006), and oxidative (Hendriks & Zeeman, 2009) and hydrothermal treatment (Thomsen *et al.,* 2006).

---

*Corresponding author
Email address: sininart.c@psu.ac.th

Another crucial step in obtaining a high concentration of ethanol is increasing the concentration of fermentable sugars by hydrolysis of the starch or cellulose or both. The general methods used are acid hydrolysis (Hermiati *et al.,* 2012; Marija *et al.,* 2009; Tsubaki *et al.,* 2013), enzymatic hydrolysis (Kongkiattikajorn & Yoonan, 2004; Rattanachomsri *et al.,* 2009), and hydrothermal hydrolysis (Kosugi *et al.,* 2009). Although the enzyme method is well known for giving a high sugar yield, it entails some disadvantages including the high cost of the raw pretreatment and the high price of the enzymes utilized. Therefore, a combination of alkali and acid pretreatment has been developed to decrease unwanted constituents and increase fermentable sugars (Alvira *et al.,* 2010; Da Costa Sousa *et al.,* 2009; FitzPatrick *et al.,* 2010).

Microwave heating is an option which can be employed to accelerate pretreatment (Azuma *et al.,* 1984; Chen *et al.,* 2012; Gabhane *et al.,* 2014; Zhu *et al.,* 2006) and the hydrolysis of the carbohydrate or cellulose or both (Suet-Pin *et al.,* 2014). Microwave irradiation promotes vibration of the water molecules and converts the molecular motion into heat. Microwave heating has been employed to make both organic and inorganic chemical reactions efficient (Kappe, 2008). It can also decrease the consumption of chemicals and reduce the reaction time (Yoshida *et al.,* 2010).

The study reported in this paper investigated the alkali pretreatment and sequential acid hydrolysis of corncob using microwave heating for the production of reducing sugar. Response surface methodology (RSM) was used to determine the optimum conditions in order to minimize the number of experiments and the time required to carry them out.

2. Materials and Methods

2.1 Materials

Corncobs were obtained from a local farmer in Hat Yai, Songkhla Province, Thailand and were initially crushed into 2–3 mm pieces. The corncobs used in this study were only the sweet corn cores without the husks and silk of the corn. The whole corn core was used without separation from its corn fiber. The chemical composition of the untreated corncob on a dry basis (i.e. initial content of the corncob) was 91.7% w/w total carbohydrate, 1.5% w/w crude fat, 5.1% w/w protein, and 1.7% w/w ash determined by the Agro-Industry Development Center for Export (ADCET), Prince of Songkla University. The total carbohydrate was composed of 52.3% w/w starch (amylose and amylopectin or polysaccharides), 1.7% w/w total sugars (sum of reducing and non-reducing sugars), 22.1% w/w cellulose, 9.6% w/w hemicellulose and 6.0% w/w lignin.

All chemicals used in this experiment were reagent grade and were purchased from CEG Science & Service Limited Partnership, Hat Yai, Songkhla, Thailand.

2.2 Microwave-assisted production of glucose (Reducing sugar)

A microwave reactor (Scientific Equipment Center, Prince of Songkla University, Thailand) was used to treat the corncob pieces. The maximum output power of the microwave reactor was 900 W. The pretreatment and hydrolysis were implemented in a 5.0 L glass three-necked round-bottom flask placed in the microwave oven and equipped with a reflux condenser set (Figure 1). A condenser was installed to prevent corrosion or damage to the equipment caused by the vapor of the alkali/acid solution. This equipment was able to maintain the solution held in it without loss of steam during the pretreatment and hydrolysis, which were conducted under atmospheric pressure at a saturated temperature of the substrate of 95 °C. After 10 min of microwave heating, vaporization and condensation of the substrate occurred continuously and the condensate was recycled into the pretreating or hydrolyzing flask.

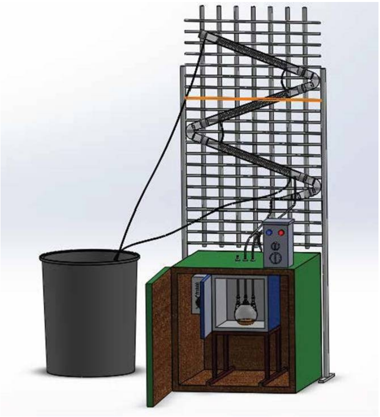


Figure 1. Microwave equipment set for pretreatment and hydrolysis.

The production of glucose consisted of two steps, alkali pretreatment and acid hydrolysis. For the pretreatment step, a quantity of 1000 g of crushed corncob was put in the flask and pretreated with 1.0–2.5 M sodium hydroxide (NaOH) solution at various weight ratios of corncob to solution between 1:5 and 1:12. The microwave power for all the experiments was set at a 900 W for heating times of between 2 and 20 min. The pretreatment conditions were varied (Table 1). After each pretreatment, the slurry was filtered and the solid material was washed to render it neutral. After that, it was dried at 105 °C for 24 h until its moisture content was <10% w/w. The dried pretreated-solid was sampled to analyze its cellulose content before hydrolysis. A near optimal set of pretreatment conditions was determined and then used as the starting point to optimize the glucose yield in the hydrolysis step which followed.

In the hydrolysis step, the dried solid that was pretreated at the optimum conditions was then hydrolyzed by heating it with 0.5–2.0% w/w sulfuric acid (H_2SO_4) solution at 900 W microwave power for 2–20 min. The experimental conditions are presented in Table 2. The hydrolysate was filtered to achieve a clear liquid for the determination of the glucose and total sugar content.

2.3 Analytical methods

After the optimal pretreatment and the optimal hydrolysis, the pretreated product and the hydrolysate were filtered. The solid fractions were analyzed using scanning electron microscopy (SEM) to observe the physical structure. The AOAC method (AOAC, 1990) was used to determine the cellulose content in the dried solid. The liquid fractions were

Table 1. Experimental conditions and results for alkali pretreatment.

Exp. No.	NaOH solution (M)	Weight ratio of corncob to solution	Time (min)	Glucose yield (%)	Total sugar yield (%)	Cellulose content (% w/w)
1	1	8.5	11	0.3	41.5	45.8
2	1.3	10.6	6	1.0	29.4	35.2
3	1.3	6.4	16	0.5	62.2	34.1
4	1.3	6.4	6	0.7	28.7	34.5
5	1.3	10.6	16	0.7	40.0	38.9
6	1.75	5.0	11	0.7	38.5	34.8
7	1.75	8.5	20	0.6	34.9	30.7
8	1.75	12.0	11	0.5	57.1	32.9
9	1.75	8.5	11	0.5	35.5	33.8
10	1.75	8.5	11	0.4	36.8	33.7
11	1.75	8.5	11	0.3	42.8	33.7
12	1.75	8.5	2	1.1	27.1	34.7
13	2.2	6.4	6	0.6	28.7	43.2
14	2.2	10.6	16	0.4	36.2	32.8
15	2.2	6.4	16	0.7	32.4	34.9
16	2.2	10.6	6	0.4	42.9	34.8
17	2.5	8.5	11	0.2	36.7	40.3

Table 2. Experimental conditions and results for acid hydrolysis.

Exp. No.	Sulfuric acid (%w)	Time (min)	Glucose yield (%)
1	0.5	11.0	9.8
2	0.7	17.4	13.7
3	0.7	4.6	9.4
4	1.3	11.0	14.6
5	1.3	11.0	13.4
6	1.3	11.0	14.3
7	1.3	2.0	10.7
8	1.3	20.0	13.7
9	1.8	17.4	14.4
10	1.8	4.6	11.5
11	2.0	11.0	11.7

analyzed using a UV-Vis spectrophotometer to establish the glucose (reducing sugar) content by the 3, 5-dinitrosalicylic acid method (Miller, 1959) and the total sugar content by the modified phenol sulfuric method (Dubois *et al.,* 1956). The glucose or total sugar yield was calculated by Equation 1.

$$\text{Glucose or total sugar yield (\%)} = \frac{\text{Sugar content (g/L)} \times \text{Volume (L)}}{\text{Corncob substrate (g)}} \times 100 \qquad (1)$$

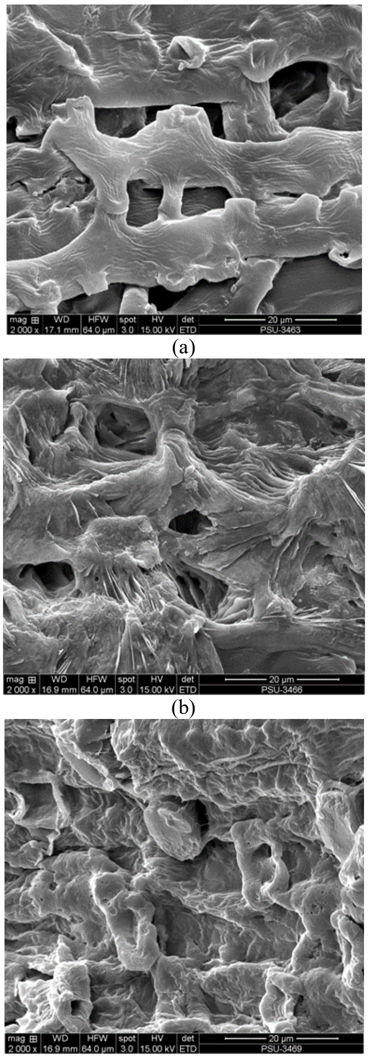
2.4 Statistical analysis

For both alkali pretreatment and acid hydrolysis with microwave heating, the individual and interactive effects were evaluated by analysis of variance (ANOVA) for statistical effects and RSM to optimize the pretreatment and hydrolysis conditions, using a central composite design. The design conditions are shown for the pretreatment in Table 1 and for the hydrolysis in Table 2.

3. Results and Discussion

3.1 Morphological changes

SEM images of the raw corncob following microwave-assisted alkali pretreatment and acid hydrolysis are shown in Figure 2. Untreated corncob presents rigid and compact structures (Figure 2a). Figure 2b demonstrates that the surface of pretreated corncob shows weak erosion. The cellulose structure chain was broken when the lignin was dissolved in the alkali solution. The cellulose swelled slightly which led to an increase in the reactive surface area and an improvement in the hydrolysis reaction. Figure 2c shows the surface of the sequential solid hydrolysate which became loose and clearly swollen and was composed of irregular, rough, and porous fibrils.



(a)

(b)

(c)

Figure 2. SEM images: (a) untreated corncob, (b) pretreated corncob, and (c) sequential solid hydrolysate.

3.2 Effect of alkali pretreatment on the corncob

The effects of the alkali pretreatment with microwave heating on the corncob are shown in Table 1. The pretreatment results indicated that the pretreatment and prehydrolysis of the corncob occurred in a single step due to the combination of microwave irradiation which instantaneously promoted vibration of the water molecules and converted the molecular motion into heat. The use of a NaOH-water solution caused an increase in the internal surface area and a decrease in the degree of polymerization of the lignocellulose. This facilitated both the pretreatment and the hydrolysis which involved a reaction that breaks bonds in a molecule using water. This can be observed from the high yields of total sugars obtained from the partial hydrolysis of the cellulose or starch or both which are the major components of the corncob (Table 1).

The total sugars are the sum of the reducing and non-reducing sugars including several types of saccharides: monosaccharides (i.e. glucose and fructose), disaccharides (i.e. sucrose and cellobiose), and trisaccharides (i.e. maltotriose). The pretreatment results gave lower yields of glucose but higher yields of total sugars. The high yield of total sugars showed that the pretreated corncob (pre-hydrolysate) was high in di- and tri- (or bigger) saccharides which can be converted to smaller sugars or glucose by acid/enzymatic hydrolysis. Alternatively, the amounts of glucose, total sugars, and cellulose suggest that the pretreated corncob would be appropriate as the substrates in a simultaneous saccharification and fermentation process for ethanol production (Table 1).

During pretreatment under alkaline conditions, the lignin was removed from the lignocellulose, the crystalline structure of the cellulose was disrupted, the porosity of the lignocellulose increased (Figure 2b); therefore, the cellulose content increased. A regression analysis of the experimental data (Table 1) was conducted for the cellulose content using alkali pretreatment based on the following quadratic equation:

$$Y_1 = 49.680 - 30.210X_1 + 2.627X_2 + 0.457X_3 + 15.750X_1X_1 - 0.027X_2X_2 - 0.018X_3X_3 - 2.137X_1X_2 - 0.706X_1X_3 + 0.117X_2X_3 \quad (2)$$

where Y_1 is the content of cellulose (% w/w: w of cellulose/w of dried pretreated solid), X_1 is the NaOH concentration (M), X_2 is the weight ratio of corncob to NaOH solution (w/w) and X_3 is the pretreatment time (min).

Figures 3a–3c show the RSM plots received from the regression model based on Equation 2. It can be seen that the ratio (solid to liquid) and time had minor effects on the cellulose contents, whereas the alkali concentration had a major effect on the cellulose content. The pretreatment was efficient with a dilute alkali solution (Figures 3a and 3b). The influence of the solid to liquid ratio on the increase in cellulose is shown in Figures 3a and 3c.

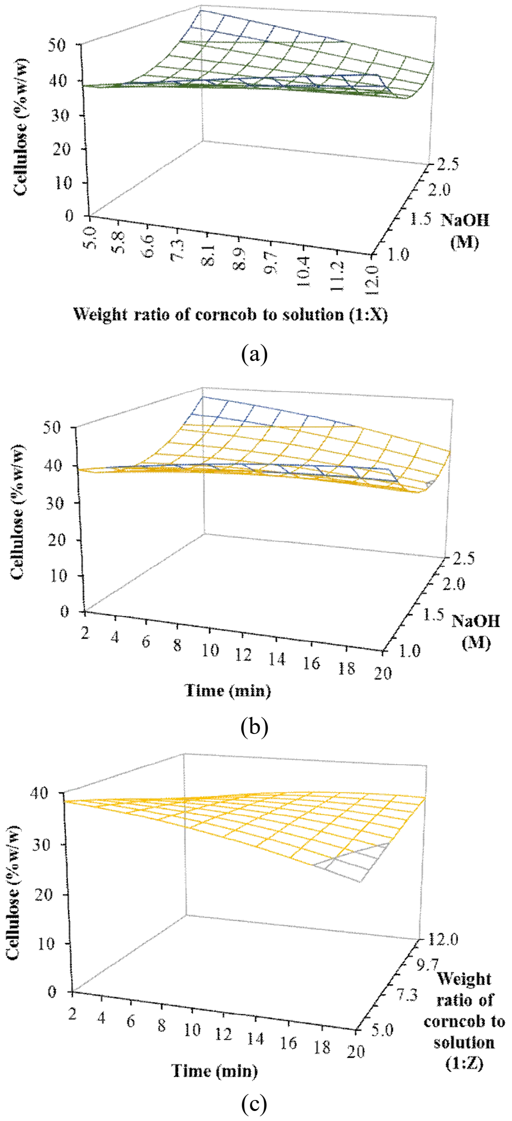


Figure 3. Cellulose content in alkali pretreatment as a function of (a) NaOH concentration and corncob to solution ratio for 11 min, (b) NaOH concentration and time with 1:8.5 corncob to solution, and (c) corncob to solution ratio and time with 1.75 M NaOH.

Cellulose dissolution (i.e. the destruction of the hydrogen bonds between cellulose molecules) is difficult due to the rigidity of the cellulose chain and the substantial network connecting the hydrogen bonds. However, the alkali/NaOH solution could fully dissolve cellulose with a low molecular weight and partially dissolve cellulose with a high molecular weight. An increase in the NaOH concentration may increase the cellulose dissolution although the cellulose can only be dissolved in a narrow range of alkali concentrations. Too high a concentration causes the hydration of NaOH ions to be insufficient to break the hydrogen bonding while too low a concentration causes the hydrodynamic diameter of the NaOH ions to be too large to penetrate into the crystalline region of the cellulose (Wang, 2008). Consequently, the NaOH concentration is critical for the pretreatment of lignocellulose.

The results indicated that the optimal cellulose content (40–50% w/w) under a microwave power of 900 W for an irradiation time of 11 min was obtained using NaOH concentrations of 1 M (4.1% w/w [i.e. 4.1 g NaOH in 100 g solution]) or 2.5 M (10.5% w/w) when both pretreatments were carried out with the same weight ratio of corncob to NaOH, i.e. 1 to 0.5 (Figure 3a). This implied that the optimum conditions could be achieved with a weight ratio of 1 to 0.5 of corncob to NaOH (solid) which provided two alternative pretreatments: a high ratio of 1 to 12 of corncob to NaOH solution with the low NaOH concentration of 1.0 M or a low ratio of 1 to 5 with a high concentration of 2.5 M, i.e. a lower concentration of NaOH solution had to be used in larger quantities than the more concentrated solution.

The time required for pretreatment also depended on the NaOH concentration as well as the solid to liquid ratio (or water amount). An increase in the concentration increases the alkali ions which accelerates the reaction. With a sufficiently high concentration the pretreatment time could be reduced, whereas a longer heating time may lead to a higher lignin concentration in the solution which encourages the re-adsorption of lignin on the surface (Gong *et al.,* 2010). These effects can be observed in Figures 3b and 3c. With inadequate liquid in the solution, the cellulose decreased when the heating time was increased. Conversely, with sufficient liquid in the solution the cellulose content increased with increasing time. Increased liquid led to an increase in the dissolution of the lignin in the NaOH solution which would tend to increase the cellulose content with increasing time. In addition, the lignin remaining on the solid biomass was able to be displaced during the washing step.

3.3 Effect of acid hydrolysis on the pretreated corncob

The pretreated corncob obtained at optimal pretreatment conditions, as described in Optimization (below), was used as the substrate for glucose production. The cellulose in the pretreated corncob can be hydrolyzed with acid solution into glucose or reducing sugars. The regression equation model for the glucose yield is represented by Equation 3:

$$Y_2 = -1.930 + 15.680X_4 + 0.764X_5 - 5.310X_4X_4 - 0.019X_5X_5 - 0.098X_4X_5 \qquad (3)$$

where, Y_2 is the yield of glucose (%), X_4 and X_5 are the H_2SO_4 concentrations (% w/w) and hydrolysis time (min), respectively.

The RSM plot of the effect of the H_2SO_4 concentration and time on the glucose yield is shown in Figure 4. For all concentrations of H_2SO_4, the glucose yield increased with time ranging from 2 to 16 min. However, with further increases in time, there was no improvement in glucose yield which suggested that the optimal range of H_2SO_4 concentration was 1.2–1.4% w/w.

H_2SO_4 is a powerful agent for cellulose hydrolysis (Sun & Cheng, 2002) which can be used to break the glycosidic (ether) bond of the cellulose polymer to produce sugar monomers. The strong acid performs as a catalyst in which the actual catalyst is the hydroxonium ions (H_3O^+) present in a solution of acid in water. However, the hydrolysis reaction is reversible and in order to gain as much hydrolysis as possible, an excess of water needs to be used. The dilute acid can thus provide both the acid catalyst and the water for the reaction (Harmsen *et al.,* 2010). The appropriate amount of H_2SO_4 for the hydrolysis of the pretreated corncob was found to be approximately 1.3% w/w which produced the maximum amount of glucose. There was no improvement of glucose content when the amount of acid was increased above 1.4% w/w (Figure 4).

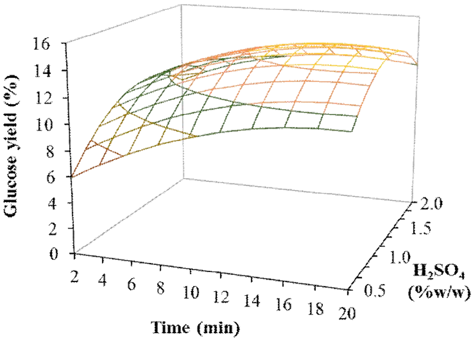


Figure 4. Yield of glucose in acid hydrolysis as a function of H_2SO_4 concentration and time.

3.4 Optimization

The determination coefficients of both alkali pretreatment ($R^2 = 0.936$) and acid hydrolysis ($R^2 = 0.915$) were satisfactory indicating the accuracy of the second order polynomial models, Equation 2 and Equation 3.

The results of the ANOVA on the variables for both alkali and acid treatments are shown in Table 3. The results of the ANOVA for the pretreatment implied that the linear effect of the NaOH concentration was significant as well as its quadratic and interactive effects ($P < 0.05$) which indicated that the alkali concentration was the most significant factor for the pretreatment of the lignocellulose. Similarly, the acid concentration was the most important factor for the hydrolysis. Even if the individual effects of both the H_2SO_4 concentration and the hydrolysis time were significant, the quadratic effect of the H_2SO_4 concentration was more clearly significant. This suggests that the concentrations of the alkali and acid solutions had been optimized for the efficient sugar production.

The pretreatment aims were to remove the non-cellulose components (lignin and/or hemicellulose) and improve the cellulose hydrolysis. Thus an increase in cellulose content is the desired outcome (Li *et al.,* 2014). Based on the regression model shown in Equation 2, the optimal pretreatment conditions using 1 M NaOH with 1:12 corncob to solution ratio at 900 W microwave power for 20 min were able to provide the highest level of 52.9% w/w cellulose content which represents an increase of 139.4% (from 22.1 to 52.9% w/w). This percentage increase in cellulose content is higher than that achieved in prior work by Li *et al.* (2014) using microwave-assisted NaOH pretreatment of corncob, which achieved an increase of 114.6% (from 40.2 to 86.2% w/w).

In addition, the microwave heating has some advantages over conventional heating; for instance, it was able to convert starch/cellulose to glucose/sugars in a relatively short time without the use of expensive enzymes and it was also useful for lignocellulose pretreatment, which increased the susceptibility of the hydrolytic enzyme/acid (Hermiati *et al.,* 2010). From the regression models, Equation 3, the optimal conditions which involved the optimally pretreated corncob being hydrolyzed using 1.3% w/w H_2SO_4 at 900 W for 16 min were able to provide a maximum glucose yield of 14.8% in the experiment. This maximal yield was achieved with a shorter operating time (36 min in total: 20 min for pretreatment and 16 min for hydrolysis) and was higher than previous work with oil bath heating which provided a 12.4% glucose yield by the pretreatment of corncob using 2.5 M NaOH solution at 60 °C for 18 h and sequential hydrolysis using 0.5% w/w H_2SO_4 solution at 100 °C for 3 h (Kongjindamunee, 2013). Moreover, this microwave heating was carried out in a container made of glass equipped with a condenser set that was not susceptible to corrosion or loss of steam during the treatment and was conducted under atmospheric pressure.

Consequently, the production of glucose using microwave-assisted alkali pretreatment to remove lignin and hemicellulose in order to increase the cellulose content, followed by microwave-assisted acid hydrolysis of the cellulose/starch is an attractive alternative to other methods because the chemicals used were cheaper than enzymes, no high pressure equipment was required, and the treatment time was shorter than a conventional heating method.

Table 3. Analysis of variance (ANOVA) of models.

Terms	Coefficient	*P*
Alkali pretreatment		
Constant	49.680	0.017
NaOH concentration (X_1)	-30.210	0.016
Weight ratio of corncob to solution (X_2)	2.627	0.252
Pretreatment time (X_3)	0.457	0.514
NaOH* NaOH(X_1*X_1)	15.750	0.000
Ratio*Ratio (X_2*X_2)	-0.027	0.802
Time*time (X_3*X_3)	-0.018	0.282
NaOH*ratio (X_1*X_2)	-2.137	0.007
NaOH*time (X_1*X_3)	-0.706	0.015
Ratio*time (X_2*X_3)	0.117	0.045
|R| = 0.967, R^2 = 0.936, R^2 adjusted = 0.854, *F* = 11.360, *F Signif* = 0.002		
Acid hydrolysis		
Constant	-1.930	0.504
H_2SO_4 concentration (X_4)	15.680	0.005
Hydrolysis time (X_5)	0.764	0.023
H_2SO_4* H_2SO_4 (X_4*X_4)	-5.310	0.007
Time*time (X_5*X_5)	-0.019	0.069
H_2SO_4*time (X_4*X_5)	-0.098	0.441
|R| = 0.956, R^2 = 0.915, R^2 adjusted = 0.830, *F* = 10.750, *F Signif* = 0.010		

4. Conclusions

The sequence of alkali pretreatment and acid hydrolysis was effective in producing glucose from corncob for ethanol production. Microwave-assisted NaOH pretreatment improved the susceptibility of the corncob to H_2SO_4 hydrolysis. The advantages of microwave technology to produce fermentable sugar from lignocellulose are 1) no need to use expensive enzymes, 2) low energy consumption, and 3) a short reaction time.

Acknowledgements

The research was supported by a grant from Prince of Songkla University, contract no. ENG550365S. The authors thank Mr. Michael Currie and the RDO service (Research and Development Office), Prince of Songkla University for assistance with proofing the English.

References

Alvira, P., Tomas-Pejo, E., Ballesteros, M., & Negro, M. J. (2010). Pretreatment technology for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresource Technology, 101*, 4851–4861.

Association of Official Analytical Chemists. (1990). AOAC Method No. 973.18-Fiber (Acid Detergent) and lignin in Animal Feeds. In K. Helrick (Ed.), *Official method of analysis* (15th ed.). Arlington, VA: Author.

Azuma, J., Tanaka, F., & Koshijima, T. (1984). Enhancement of enzymatic susceptibility of lignocellulose waste by microwave irradiation. *Journal of Fermentation Technology, 62*, 377–384.

Ballesteros, I., Ballesteros, M., Manzanares, P., Negro, M. J., Oliva, J. M., & Saez, F. (2008). Dilute sulfuric acid pretreatment of cardoon for ethanol production. *Biochemical Engineering Journal, 42*, 84–91.

Ballesteros, I., Negro, M. J., Oliva, J. M., Cabanas, A., Manzanares, P., & Ballesteros, M. (2006). Ethanol production from steam-explosion pretreated wheat straw. *Applied Biochemistry and Biotechnology, 130*, 496–508.

Chen, C., Boldor, D., Aita, G., & Walker, M. (2012). Ethanol production from sorghum by a microwave-assisted dilute ammonia pretreatment. *Bioresource Technology, 110*, 190–197.

Chen, H. Z., Han, Y. J., & Xu, J. (2008). Simultaneous saccharification and fermentation of steam exploded wheat straw pretreated with alkaline peroxide. *Process Biochemistry, 43*, 1462–1466.

Da Costa Sousa, L., Chundawat, S. P., Balan, V., & Dale, V. E. (2009). Cradle-to-grave assessment of existing lignocellulose pretreatment technologies. *Current Opinion in Biotechnology, 20*, 339–347.

Dubois, M., Gilles, K. A., Atmelton, G. K., Rabers, P. A., & Smith, F. (1956). Calorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*, 50-56.

FitzPatrick, M., Champagne, P., Cunningham, M. F., & Whitney, R. A. (2010). A biorefinery processing perspective: treatment of lignocellulose materials for the production of value-added products. *Bioresource Technology, 101*, 8915–8922.

Gabhane, J., William, S. P. M. P., Gadhe, A., Rath, R., Vaidya, A. N., & Wate, S. (2014). Pretreatment of banana agricultural waste for bio-ethanol production: Individual and interactive effects of acid and alkali pretreatments with autoclaving, microwave heating and ultrasonication. *Waste Management, 34*, 498–503.

Girio, F. M., Fonseca, C., Carvalheiro, F., Durate, L. C., Marques, S., & Bogel-Lukasik, R. (2010). Hemicelluloses for fuel ethanol: A review. *Bioresource Technology*, *101*, 4775–4800.

Goldemberg, J. (2007). Ethanol for a sustainable energy future. *Science, 315*, 808–810.

Gong, G., Liu, D., & Huang, Y. (2010). Microwave-assisted organic acid pretreatment for enzymatic hydrolysis of rice straw. *Biosystems Engineering, 107*, 67–73.

Hahn-Hagerdal, B., Galbe, M., Gorwa-Grauslund, M.F., Liden, G., & Zacchi, G. (2006). Bio-ethanol – the fuel of tomorrow from the residues of today. *Trends in Biotechnology, 24*, 549–556.

Harmsen, P. F. H., Huijgen, W. J. J., Bermúdez López, L. M., & Bakker, R. R. C. (2010). Literature review of physical and chemical pretreatment processes for lignocellulosic biomass. *Energy Research Centre of the Netherlands, 10*(13), 1-49.

Hendrisks, A. T. W. M., & Zeeman, G. (2009). Pretreatment to enhance the digestibility of lignocellulose biomass. *Bioresource Technology, 100*, 10–18.

Hermiati, E., Mangunwidjaja, D., Sunarti, T. C., Suparno, O., & Prasetya, B. (2012). Microwave-assisted acid hydrolysis of starch polymer in cassava pulp in the presence of activated carbon. *Procedia Chemistry, 4*, 238–244.

Hermiati, E., Mangunwidjaja, D., Sunarti, T.C., Suparno, O., & Prasetya, B. (2010). Application of microwave heating in biomass hydrolysis and pretreatment for ethanol production. *Annales Bogorienses, 14*(1), 1-9.

Kappe, O. C. (2008). Microwave dielectric heating in synthetic organic chemistry. *Chemistry Society Reviews, 37*, 1127–1139.

Kim, S., & Kim, C. H. (2013). Bioethanol production using the sequential acid/alkali-pretreated empty palm fruit bunch fiber. *Renewable Energy, 54*, 150–155.

Kim, T. H., & Lee, Y. Y. (2007). Pretreatment of corn stover by soaking in aqueous ammonia at moderate temperatures. *Applied Biochemistry and Biotechnology. 137*, 81–92.

Ko, J. K., Bak, J. S., Jung, M. W., Lee, H. J, In-Geol, C., Kim, T. H., & Kim, K. H. (2009). Ethanol production from rice straw using optimized aqueous-ammonia soaking pretreatment and simultaneous saccharification and fermentation processes. *Bioresource Technology, 100*, 4374–4380.

Kongjindamunee, W. (2013). *Ethanol production from corncob* (Master's dissertation, Prince of Songkla University, Songkhla, Thailand).

Kongkiattikajorn, J., & Yoonan, K. (2004). A study of optimal condition for reducing sugars production from cassava peels by dilute acid and enzymes. *Kasetsart Journal (Natural Science), 38*, 29–35.

Kosugi, A., Kondo, A., Ueda, M., Murata, Y., Vaithanomsat, P., Thanapase, W., . . . Mori, Y. (2009). Production of ethanol from cassava pulp via fermentation with a surface-engineered yeast strain displaying glucoamylase. *Renewable Energy, 34*, 1354–1358.

Li, M., Cheng, Y. L., Fu, N., Adhikari, B., & Chen, X. D. (2014). Isolation and characterization of corncob cellulose fibers using microwave-assisted chemical treatments. *International Journal of Food Engineering, 10*(3), 1-17.

MGR Online. (2010). "Thailand's sweet corn" future further in the global market. Retrieved from: http://www.manager.co.th/iBizchannel/ViewNews.aspx?NewsID=9530000165014.

Mariji, B., Tasic, B. V., Konstantinvic, M. L., & Lazic, V. B. V. (2009). The acid hydrolysis of potato tuber mash in bioethanol production. *Biochemical Engineering Journal, 43*, 208–211.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagen for determination of reducing sugar. *Analytical Chemistry, 31*, 420–428.

Mohan, P. R., Kumar, B. V., & Reddy, O. V. S. (2013). Optimization of pretreatment conditions for increased cellulose conversion of sugarcane bagasse using peracetic acid employing central composite design. *Songklanakarin Journal of Science and Technology, 35*(2), 177–185.

Rattanachomsri, U., Tanapongpipat, S., Eurwilaichitr, L., & Champreda, V. (2009). Simultaneous non-thermal saccharification of cassava pulp by multi-enzyme activity and ethanol fermentation by Candida tropicalis. *Journal of Bioscience and Bioengineering, 107*(5), 488–493.

Richana, N., Irawadi, T. T., & Nur, A. M. (2007). The extraction of hemicellulose from corn cobs. *Past harvest Journal, 4*(1), 38-43.

Ruangmee, A., & Sangwichien, C. (2013). Statistical optimization for alkali pretreatment conditions of narrows-leaf cattail by response surface methodology. *Songklanakarin Journal of Science and Technology, 35*(4), 443–450.

Suet-Pin, F., Li-Qun, J., Chin-Hua, C., Zhen, F., Sarani, Z., & Kah-Leong, C. (2014). High yield production of sugars from deproteinated palm kernel cake under microwave irradiation via dilute sulfuric acid hydrolysis. *Bioresource Technology, 153*, 69–78.

Sun, Y., & Cheng, J. J. (2005). Dilute acid pretreatment of rye straw and bermudagrass for ethanol production. *Bioresource Technology, 96*, 1599–1606.

Sun, Y., & Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresource Technology, 83*(1), 1-11.

Thomsen, M. H., Thygesen, A., Jorgensen, H., Larsen, J., Christensen, B. H., & Thomsen, A. B. (2006). Preliminary results on optimization of pilot scale pretreatment of wheat straw used in coproduction of bioethanol and electricity. *Applied Biochemistry and Biotechnology, 130*, 448–460.

Tilman, D., Hill, J., & Lehman, C. (2006). Carbon-negative biofuel from low-input high diversity grassland biomass. *Science, 314*, 1598–1600.

Tsubaki, S., Oono, K., Ueda, T., Onda, A., Yanagisawa, K., Mitani, T., & Azuma, J. (2013). Microwave-assisted hydrolysis of polysaccharides over polyoxometalate clusters. *Bioresource Technology, 144*, 67–73.

Wang, Y. (2008). *Cellulose fiber dissolution in sodium hydroxide solution at low temperature: Dissolution kinetics and solubility improvement* (Doctoral dissertation, Georgia Institute of Technology, Atlanta, GA)

Wyman, C. E., Dale, B. D., Elander, R. T., Holtzapple, M., Ladish, M. R., & Lee, Y. Y. (2005). Coordinated development of leading biomass pretreatment technologies. *Bioresource Technology*, *96*, 1959–1966.

Yoshida, T., Tsubaki, S., Teramoto, Y., & Azuma, J. (2010). Optimization of microwave-assisted extraction of carbohydrate from industrial waste of corn starch production using response surface methodology. *Bioresource Technology*, *101*, 7820–7826.

Zhang, M., Wang, F., Su, R., Qi, W., & He, Z. (2010). Ethanol production from high dry matter corncob using fed-batch simultaneous saccharification and fermentation after combined pretreatment. *Bioresource Technology, 101*, 4959–4964.

Zheng, Y., Pan, Z., & Zhang, R. (2009). Overview of biomass pretreatment for cellulosic ethanol production. *International Journal of Agricultural and Biological Engineering*, *2*, 51–68.

Zhu, S., Wu, Y., Yu, Z., Zhang, X., Wang, C., Yu, F., & Jin, S. (2006). Production of ethanol from microwave-assisted alkali pretreatment wheat straw. *Process Biochemistry, 41*, 869–873.