KMUTNB Int J Appl Sci Technol, Vol. 11, No. 4, pp. 247–256, 2018

Research Article

Selected Paper from the 11th Pure and Applied Chemistry International Conference 2017 (PACCON 2017)

Alkali Pretreatment and Enzyme Hydrolysis to Enhance the Digestibility of Rice Straw Cellulose for Microbial Oil Production

Chayanun Kobkam
Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand
Graduate School, Chiang Mai University, Chiang Mai, Thailand

Jidapha Tinoi* and Sila Kittiwachana
Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand

* Corresponding author. E-mail: jidapha.t@cmu.ac.th

DOI: 10.14416/j.ijast.2018.07.003
Received: 30 June 2017; Accepted: 28 July 2017; Published online: 31 July 2018

Abstract

The alkali and alkali ethanolysis were used for pretreatment and delignification of rice straw. The combined hydrothermal- alkali method presented the high efficiency for removing the lignin. The pretreatment condition was optimized and achieved the highest of delignification (92.5%) under 30% (w/v) NaOH at a ratio of rice straw to alkali solution of 1 : 10 (w/v) at 121°C for 20 min. The highest yield of the remaining cellulose was 81.5% (w/w). The enzymatic hydrolysis of the rice straw cellulose was optimized for enhancement the digestibility via Response Surface Methodology (RSM). The cellulase activity, buffer solution, and hydrolysis time were evaluated. The optimum condition was cellulase activity at 935.18 U, 10 mL of buffer solution 10% (w/v) with hydrolysis time for 120 h, represented the maximum sugar yield with 136.6 g/L (0.54 g/g rice straw). The rice straw hydrolysate was further used as carbon source for microbial oil production by oleaginous yeasts.

Keywords: Rice straw, Alkali pretreatment, Enzymatic hydrolysis, Response Surface Methodology, Delignification, Cellulose

1 Introduction

Rice straw is one of the abundant lignocellulosic waste materials in Thailand. It is annually produced in large quantity reaching about 19 million tons. Rice straw can be generated from the rice production to significant of agricultural residues [1]. In rice producing countries, the common practice of rice straw is open burning after grain harvest, resulting in emissions to air that have the deleterious effect on air quality and human health. Rice straw as an agricultural waste biomass could be a source of alternative energy to substitute fossil energy for reducing greenhouse gas emissions as well as avoid the local pollution problems from open burning. These residues can be recycled as an economical and environmentally friendly renewable resource by converting them to sugar using pretreatment and hydrolysis process. The rice straw is mainly composed of three major components, such as cellulose, hemicelluloses, and lignin. That is the most abundant and renewable organic component and highly attractive feedstock for using as a resource for microorganisms. Rice straw is a lignocellulosic biomass which a cell wall composed mainly of cellulose 32–47%, hemicellulose 19–27%, and lignin 5–24% [2]. The structure of rice straw is very complex due to a lignin which a large molecular structure containing cross-linked polymers of phenolic monomers. It is present in the primary cell

Please cite this article as: C. Kobkam, J. Tinoi, and S. Kittiwachana , "Alkali pretreatment and enzyme hydrolysis to enhance the digestibility of rice straw cellulose for microbial oil production," *KMUTNB Int J Appl Sci Technol*, vol. 11, no. 4, pp. 247–256, Oct.–Dec. 2018.

wall, imparting structural support, impermeability, and resistance to microbial attack [3]. Cellulose is the main fraction of rice straw containing the linear polymer consists of D-glucose subunits linked to each other by β-(1,4)-glycosidic bond. The long –chain cellulose polymers are linked together by hydrogen and van der Waals bonds, cause the cellulose to be packed into microfibrils [4]. Hemicelluloses are the complex heterogeneous polysaccharide with short branches linked by β-(1,4)-glycosidic bond and occasionally β-(1,3)-glycosidic bond [5]. The main feature that the differentiates of hemicellulose from cellulose is that hemicellulose has branches with short lateral chains consisting of different sugars. These monosaccharides include pentoses (xylose, rhamnose and arabinose), hexoses (glucose, mannose and galactose). Therefore, the pretreatment of rice straw is an important step in breaking down the chemical structure and enhancing the conversion efficiency of cellulose and hemicellulose to increase the liberated sugars yield. The advantages of these sugars can be use as carbon sources for high-value-added products production via fermentation. Moreover, the pretreatment process is an essential in order to remove lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the materials [6].

Generally, the pretreatment methods of lignocellulosic material include physical (milling and grinding), physico-chemical (steam pretreatment and hydrothermal lysis), chemical (alkali and dilute acid), biological or a combination of these. Each of pretreatment has its own advantages and disadvantages. For sugar production, have shown a need for delignification stage using sodium hydroxide as catalyst. The delignification is a very important process, due to an increase internal surface area, a decrease in the degree of polymerization, a decrease in cellulose crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure [7]. After delignification, acid and dilute acid pretreatment (H_2SO_4) is a widely employed procedure for depolymerization and solubilization of plant cell wall sturcture. The main sugar, xylose was found in the hemicellulose hydrolysate fraction and the degradable by-products such as 5-hydroxymethylfurfural (5-HMF), furfural and aliphatic acid (formic acid and acetic acid) were also presented [8], [9]. These degradable compounds might have an inhibitory or toxic effect on microorganisms that are used in subsequent bioconversion processes. After pretreatment process, the cellulose fibers become more exposed to subsequent enzymatic hydrolysis. Recently, the advance in enzyme technology for the conversion of cellulosic biomass to sugar have brought significant process in lignocellulosic biomass utilization as carbon sources of oleaginous yeast for lipid production. Enzymatic hydrolysis of cellulose offers the potential for higher yields, higher selectivity, and lower energy costs. Furthermore, lipid production by oleaginous microorganisms demonstrated that the oleaginous yeast has the high ability to convert hexose (glucose) and pentose (xylose), which comprise an integral part of lignocellulosic biomass, into lipid [10]–[12]. Thus, the lignocellulosic biomass has a highly attractive feedstock for microbial oil production [13]. Therefore, in this work aims to pretreat the rice straw for enhancing the delignification by using alkali, alkali ethanolysis and combination of physico-chemical pretreatment. The optimizations of the alkali pretreatment and enzymatic hydrolysis of rice straw cellulose were investigated for enhancing the reducing sugar (glucose) yield. The obtained rice straw hydrolysate could be used as a carbon source in lipid production for further application in biodiesel production.

2 Materials and Methods

2.1 *Material*

Rice straw (*Orysa sativa*) was obtained from a local field at San Sai, Chiang Mai province, Thailand. The air dried rice straw was cut into small pieces (1–2 in). Then, it was dried at 90°C for 6 h in a hot-air oven. The dried rice straw was milled by using a Hammer mill and screened to obtain small particles (less than 1–2 mm). The milled rice straw was collected in a sealed plastic bag and stored at room temperature for further use.

2.2 *The pretreatment of rice straw*

2.2.1 Alkali and alkali ethanolysis pretreatment of rice straw

The alkali (40% w/v NaOH, 15% v/v NH_3) and alkali ethanolysis (40% w/v NaOH in 50% v/v EtOH, 15% v/v NH_3 in 50% v/v EtOH) were selected to delignify of the rice straw (modified of Sakdaronnarong *et al.* [14]) The ratio of rice straw to alkali solution was a 1 : 10 g/mL. The mixture was then treated in a water bath at 90°C for 4 h. After cooling, the mixture was filtered under vacuum and washed repeatedly with deionized water until neutral pH to obtain. Then, the solid residue was characterized the chemical composition.

2.2.2 Combination of physical-alkali pretreatment of rice straw

The ultrasonication, microwave irradiation and hydrothermal were used to improve the rice straw pretreatment by combination with alkali 40% NaOH under the ultrasonic bath, household microwave, and autoclave, respectively. The ultrasonic bath (Sonic system SOMERSET, England) with a frequency of 20 kHz was operated at 60°C for 60 min. Microwave radiation was performed by using household microwave oven (WD700 MG-5062T, LG Electronics Tianjin Appliances CO., Ltd.) at 400 W for 12 min. The hydrothermal pretreatment was treated at 121°C for 15 min using an autoclave. All of the combination pretreatments were reacted in a 250 mL Erlenmeyer flask with a ratio of rice straw to 40% NaOH at 1 : 10, w/v. After that, the solid residue was obtained by filtration and then, washed repeatedly with distilled water until turned to neutral pH. The solid residue (pretreated rice straw) was dried in a hot-air oven at 65°C overnight. The characteristic of pretreated rice straw was investigated.

2.2.3 The optimization of rice straw pretreatment

The combined physico-alkali pretreatment was optimized by varied the sodium hydroxide concentration (10, 20, 30 and 40%), the ratio of rice straw to NaOH solution (1 : 10, 1 : 20 and 1 : 30 w/v), temperature (90, 100, 110 and 121°C) and pretreatment time (5, 10, 15 and 20 min). After that, the solid residue was recovered by filtration and washing with distilled water. The pretreated rice straw residue was dried in a hot-air oven at 65°C overnight for further analysis.

2.3 *The enzymatic hydrolysis of pretreated rice straw*

Enzymatic hydrolysis of 1 g of the pretreated rice straw was conducted in 250 mL Erlenmeyer flasks. 1 g of pretreated rice straw was hydrolyzed by commercial cellulase (iKnowzyme, Reach Biotechnology, Thailand) in 0.1 M sodium citrate buffer (pH 4.8) and incubated at 50°C in a shaking incubator at 150 rpm. The enzymatic hydrolysis was stopped by immediate boiling in a water bath for 10 min and then directly chilling on ice. The mixture was centrifuged at 4°C at 9,000 rpm for 15 min. The total reducing sugar content (the equivalent of glucose) of rice straw hydrolysate was determined. The solid residue was dried to calculate the hydrolysis efficiency of rice straw by enzymatic hydrolysis.

2.3.1 The optimization of pretreated rice straw enzymatic hydrolysis by Response Surface Methodology (RSM)

To further increase the efficiency of the enzyme hydrolysis of rice straw, a number of experiments were conducted according to the experimental designs by using the Central Composite Design (CCD). The CCD is one of the most commonly used response surface designs for optimization the enzyme hydrolysis of pretreated rice straw. Three different parameters, hydrolysis time (0.5–2 h), cellulase activity (50–1,000 U) and the buffer solution (10–100 mL) were chosen and designated as X_1, X_2 and X_3, respectively (Table 1). The experimental design was used to determine optimum of the rice straw enzymatic hydrolysis condition resulting maximum total reducing sugar (glucose) content and the results of the CCD are shown in Table 4. The total number of experiments of enzymatic hydrolysis was 20 and the center point was replicated at least three times. The experimental points, statistical and numerical analysis involving multiple regressions and analysis of variance (ANOVA) were carried out with the software Design Expert version Matlab V7.0 (The Mathworks Inc, Natick).

Table 1: Code and actual values for each variable of the central composite design

Variables	Symbol	Coded Levels of Variable				
		–1.68	**–1**	**0**	**1**	**1.68**
Hydrolysis Time (h)	X_1	0.5	24.68	60.25	95.82	120
Cellulase Activity (U)	X_2	50	242.26	525	807.74	1000
Buffer Solution (mL)	X_3	10	28.21	55	81.79	100

2.4 *Analysis*

The concentration of total reducing sugar in rice straw hydrolysate was investigated by the dinitro salicylic acid (DNS) method [15]. The lignocellulosic compositions (cellulose, hemicelluloses, and lignin) of untreated and pretreated rice straw were determined by the detergent fiber analysis method [16]. The surface of untreated and pretreated rice straw and solid residue after enzymatic hydrolysis were observed with the Scanning Electron Microscope (SEM) (JSM-5910LV, JEOL, Japan).

3 Results and Discussion

The chemical composition of untreated rice straw, especially lignocellulosic composition was evaluated and found that the main component was cellulose (34.40±0.03%, w/w), followed by hemicelluloses (26.68±0.08%, w/w) and lignin (7.3±0.08%, w/w), respectively. Considering for utilization of rice straw as a feedstock in the microbial oil production, the high content of cellulose is beneficial due to can produce more fermentable sugars (mainly glucose) during enzymatic hydrolysis. However, the rice straw lignocellulosic structure is complex, thus a pretreatment of rice straw is needed to convert the lignocellulosic complex into sugars and make it more accessible and usable for the microorganisms.

3.1 *The alkali pretreatments of rice straw and optimization*

The pretreatment of rice straw in this research focus on removal the lignin that surrounds cellulose and hemicellulose which make rice straw lignocellulose highly recalcitrant to enzymatic hydrolysis. Moreover, the pretreatment also decreases the crystallinity of cellulose and increase hemicellulose disruption. Alkali (40% NaOH and 15%NH_3) and alkali ethanolysis (40% NaOH in 50% EtOH and 15% NH3 in 50% EtOH) pretreatments were selected to treat the rice straw and the results are shown in Table 2. For the rice straw delignification, the lignin content was decreased from 7.3 to 1.53% of dry weight the pretreated rice straw with the highest delignification as 78.9% when using 40% NaOH pretreatment at 90°C for 4 h. The high holocellulose (the sum of cellulose and hemicellulose) residue contents (about 85%) in pretreated rice straw were also obtained from the 40% NaOH and 40% NaOH in 50% EtOH. The 15% NH_3 and 15% NH_3 in 50% EtOH at same condition have a slight effect for removing the lignin from rice straw when compared to 40% NaOH. According to Jönsson and Martín [17] reported that NaOH pretreatment can be removed lignin and a part of hemicelluloses, and an increase in the accessibility of the enzymatic hydrolysis. The effect of NaOH on lignocellulosic biomass is delignification by breaking the ester bond which cross-linked between lignin and xylan of hemicellulose, thus increased the porosity of biomass structure [18]. For enhancing the delignification and decreasing the crystallinity of cellulose, the combination of physical and chemical (alkali) pretreatment of rice straw was carried out. The ultrasonication, microwave irradiation and hydrothermal by autoclave combined with 40% NaOH were used and the results present in Table 3. The effect of each physico-alkali pretreatment on lignocellulosic fractionation in termed of lignin, cellulose, and hemicellulose was evaluated. The hydrothermal pretreatment by using autoclave resulted in the higher reduction of lignin than ultrasonic bath and microwave.

Table 2: The lignocellulosic composition and delignification of rice straw by alkali and alkali ethanolysis pretreatments

Samples	Cellulose (%, w/w)	Hemi Cellulose (%, w/w)	Lignin (%, w/w)	Delignification (%, w/w)
Untreated Rice Straw	34.40±0.16	26.68±0.02	7.30±0.24	-
40% NaOH	65.32±0.03	21.84±0.07	1.54±0.03	78.89
40%NaOH in 50% EtOH	62.03±0.38	16.21±0.16	2.63±0.06	63.98
15% NH_3	54.52±0.07	17.34±0.06	5.15±0.06	29.52
15% NH_3 in 50% EtOH	48.36±0.04	20.23±0.19	6.07±0.03	20.18

Table 3: The combination of the physico-alkali pretreatment for enhancing the delignification of rice straw

Samples	Cellulose (%, w/w)	Hemi Cellulose (%, w/w)	Lignin (%, w/w)	Delignification (%, w/w)
Untreated Rice Straw	34.40±0.16	26.68±0.02	7.30±0.24	-
40% NaOH	65.32±0.03	21.84±0.07	1.54±0.03	78.89
Autoclave	71.47±0.06	12.81±0.05	1.47±0.04	79.86
Microwave	71.09±0.05	12.81±0.07	2.49±0.03	65.93
Ultrasonic	62.61±0.07	17.82±0.05	3.21±0.04	56.02

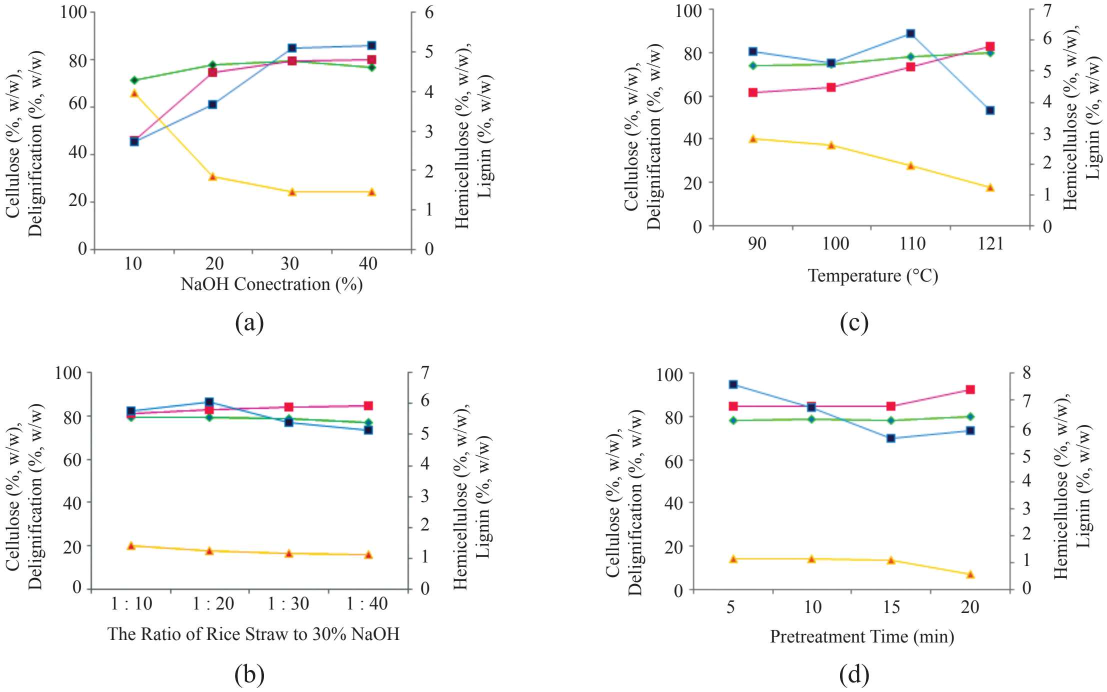


Figure 1: The optimization of rce straw pretreatment using the combined hydrotheral and alkali method. The varied parameters (a) alkali concentration (NaOH), (b) the ratio of rice straw alkali solution, (c) temperature, (d) pretreatment time.

The lignin and hemicellulose content was decreased from 7.30 to 1.47% which presented the highest of delignification about 79.86% by using an autoclave, followed by microwave (65.93%) and ultrasonic bath (56.02%). Moreover, the remaining cellulose of pretreated rice straw was found as a high content of 71.48%. The combined pretreatment (autocalve and 40% NaOH) improved the slightly to remove lignin from rice straw, but the condition was occurred in a short time (15 min) compared to using a water bath for 4 h. Taherzadeh and Keikhosro [19] found that the autoclave pretreatment under a high pressure and temperature that can penetrate into the biomass, soluble cellulose, and removes hemicellulose and lignin. This hydrothermal pretreatment subjects the material to high pressures and temperatures for a short duration of time after which it rapidly depressurizes the system, disrupting the structure of the fibrils. Hydrothermal pretreatment enhances the accessible and susceptible surface area of the cellulose and makes it more accessible to hydrolytic enzymes [20], [21]. The optimization of the rice straw pretreatment by using the combination of autoclave and alkali method was investigated. The NaOH concentration was carried out and resulted in the decreasing of the lignin content while increasing the NaOH concentration [Figure 1(a)]. As, 30% and 40% NaOH presented the highest the lignification about 79.86–80.30% and the remaining cellulose also obtained in a high content as 79.64% and 76.80%, respectively. The ratio of rice straw to alkali solution was varied by 1 : 10, 1 : 20, 1 : 30 and 1 : 40 (w/v) to investigate the effect on the efficiency of pretreated rice straw [Figure 1(b)]. At the 1:10 (w/v) ratio contained the highest content of remaining pretreatment. The lignin content was a highly removed from rice straw when treated from the ratio of 1 : 10 (w/v) to 1 : 40 (w/v) and resulted in a slightly increased the delignification (80.97–84.71%). However, the high ratio of rice straw and alkali solution lead to the higher the loss of cellulose in the pretreated rice straw [Figure 1(b)]. At the 1:10 (w/v) ratio contained the highest content of remaining cellulose of 79.55%. The different operation temperatures had an effect on the pretreatment of rice straw [Figure 1(c)]. The lignification was increased when the temperature increased and at 121°C revealed the highest of removing the lignin (83.06%) and remaining the cellulose (79.93%). The influence of pretreatment time (5–20 min) under autoclaving considerably affected the removal of lignin [Figure 1(d)]. The lignification was 92.52% when treated for 20 min of pretreatment time. The remaining rice straw cellulose was also obtained the highest content of 81.57%. The optimum condition for pretreatment of rice straw by using the combined hydrothermal and alkali method was the ratio of rice straw to 30% NaOH solution of 1 : 10 (w/v) at 121°C for 20 min. The rice straw delignification of this work showed higher than that of Rahnama *et al*. [22]. The results demonstrated that the delignification of rice straw by alkali pretreatment (1% NaOH at 121°C for 20 min) was 35.9%. The results presented that the remaining cellulose content was 59.51% in alkali pretreated rice straw. While as, using 2% NaOH pretreatment with temperature 121°C, 15 psi for 90 min resulted in the highest level of delignification from rice straw about 65.63% [23]. For the effect of each parameter, the NaOH concentration and pretreatment time were statistically significant towards the delignification process as well as the releasing monomeric glucose sugar amount from lignin-carbohydrate complex structure [24], [25]. The high alkali concentration presented more effectively breaks down the lignocellulose matrix and changes the chemical components.

3.2 *Enzyme hydrolysis of pretreated rice straw*

The optimization studies via CCD were conducted to study the interaction effect of each parameter. The results are presented in Table 4. The hydrolysis time, cellulase activity, and buffer solution were the independent variable studies to optimize the enzymatic hydrolysis of rice straw cellulose to obtain the total reducing sugar (glucose) yield. The maximum glucose yield of 123.96 g/L was obtained at the center point condition of the 525 U of cellulase activity with 10 mL of buffer solution for 60.25 h in hydrolysis time. The other center point conditions were used to determine the experimental error and showed the relatively high glucose yields only the maximum one. For the utilized conditions in this study, the model with coded variable expressed in further equation that represents the maximum glucose yield (Y) from the cellulose fraction of rice straw, as a fraction of hydrolysis time (X_1), cellulase activity (X_2) and buffer solution (X_3). A model is regarded significant if its *p*-value (also known as the 'prob > F' value) is lower than 0.01 indicating only a 1% chance that a 'model *F*-value' could occur because of noise [26]. Table 5 showed the model *F*-value of 16.71 with a low *p*-value ($p < 0.05$) implied that this model was statistically significant at 95% confidence level. The significant effect was presented by *p*-values < 0.05 and the study terms; A, B, C, and C_2 also have a significant effect. The obtained model was significant, presenting a determination coefficient of $R^2 = 0.93$ (93%). It was suggested that R^2 value should be at least 0.80 for a good fit of a model [27] The maximum of a predicted glucose yield in enzymatic hydrolysis conditions was about 138 g/L at cellulase activity of 935.18 U with 10 mL of buffer solution (10% solid concentration) for 120 h of hydrolysis time. To confirm the results of the predicted value, the experiments were conducted at the optimal condition, displaying the glucose yield of 136.6±0.35 g/L.

$$Y = -0.30\ X_1^2 - 2.03\ X_2^2 + 16.9\ X_3^2 - 2.47\ X_1X_2 - 2.59\ X_1X_3 - 3.39\ X_2X_3 + 7.76\ X_1 - 5.69\ X_2 - 21.88\ X_3 + 28.44$$

Table 4: Coded level combinations for a three-variable central composite orthogonal and rotatable design (CCD)

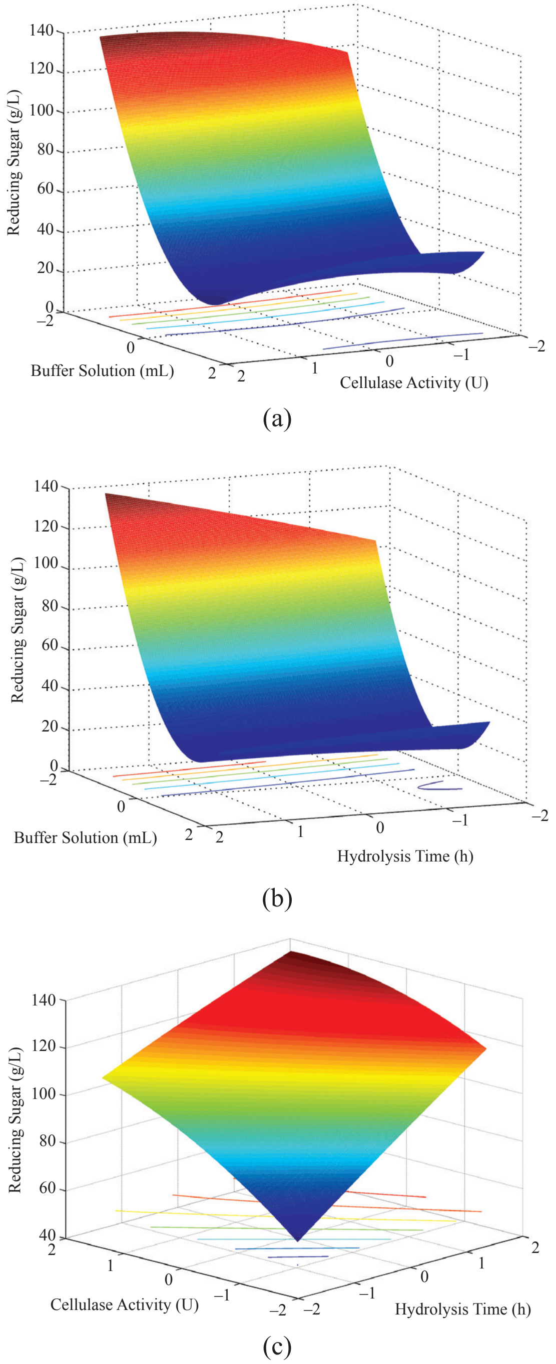
Test Run No.	Coded Level of Valiable			Reducing Sugar Content (g/L)	
	Hydrolysis Time (h)	Cellulase Activity (U)	Buffer Solution (mL)	Predicted	Experimental
1	24.68	242.26	28.21	42.74	37.79
2	24.68	242.26	81.79	10.93	20.08
3	24.68	807.74	28.21	64.23	67.47
4	24.68	807.74	81.79	22.07	20.72
5	95.82	242.26	28.21	69.98	63.68
6	95.82	242.26	81.79	24.60	20.15
7	95.82	807.74	28.21	81.60	68.02
8	95.82	807.74	81.79	25.87	26.39
9	0.50	525.00	55.00	14.91	9.15
10	120.00	525.00	55.00	40.98	53.02
11	60.25	50.00	55.00	13.52	15.28
12	60.25	1000	55.00	32.64	37.16
13	60.25	525.00	10.00	113.25	123.96
14	60.25	525.00	100.00	39.72	35.29
15	60.25	525.00	55.00	38.55	37.43
16	60.25	525.00	55.00	38.55	27.60
17	60.25	525.00	55.00	38.55	26.96
18	60.25	525.00	55.00	38.55	25.45
19	60.25	525.00	55.00	38.55	26.51
20	60.25	525.00	55.00	38.55	25.44

Table 5: ANOVA for response surface the quadratic model

Source	Sum of Squares	df	Mean Squares	F-Value	p-Value prob >F	
Model	12328.52	9	1369.84	16.71	0.0001	Significant
A	821.57	1	821.57	10.02	0.0101	
B	441.99	1	441.99	5.39	0.0426	
C	6533.69	1	6533.69	79.69	0.0000	
AB	48.71	1	48.71	0.59	0.4587	
AC	53.56	1	53.56	0.65	0.4378	
BC	92.07	1	92.07	1.12	0.3142	
A^2	1.33	1	1.33	0.02	0.9013	
B^2	59.03	1	59.03	0.72	0.4160	
C^2	4100.75	1	4100.75	50.02	0.0000	

$R^2 = 0.93$

Response surface and contour line are presented in Figure 2 and the results showed the effect of the hydrolysis parameters on glucose yields. The interactions of cellulase activity and buffer solution, buffer solution and hydrolysis time, cellulase activity and hydrolysis time for liberated the glucose yield were depicted in Figure 2(a), (b) and (c), respectively. The glucose yield increased with increasing the hydrolysis time and cellulase activity. Figure 3 shows the SEM images that revealed the morphology of the rice straw greatly altered after alkali pretreatment and enzymatic hydrolysis. Untreated rice straw exhibited an intact and ordered fibrillar structure [Figure 3(a)]. The lignocellulosic structure was largely destroyed after the alkaline pretreatment, the pretreated rice straw exhibited the rod-like and filament of cellulose structure [Figure 3(b)]. The aggregation of rice straw cellulose was broken down to a great extent after enzymatic hydrolysis by cellulase which can be observed in the SEM micrograph as shown in Figure 3(c). Based on the above xperiments, the rice straw hydrolysate with a high content of reducing sugar as 136.6 g/L or 0.54 g/g of rice straw (54%, w/w) was obtained by using the improved alkali pretreatment with a combination of hydrothermal and sequence the enzymatic hydrolysis. Comparison of the pretreatment and enzymatic hydrolysis efficiency of rice straw by other pretreatment methods, Kim *et al.* [28] liberated the fermentable sugars from rice straw by using two-stage pretreatment with aqueous ammonia and dilute acid. The results demonstrated that the glucose content was found to be 31.9% (w/v). While as, Sakdaronnarong *et al.* [14] reported that the single step pretreatment of rice straw by alkaline fractionation at 40% (w/w) NaOH in an ultrasonic bath at 90°C for



(a)

(b)

(c)

Figure 2: Response surface plots of the CCD for the optimization of the enzymatic hydrolysis of pretreated rice straw. Effect of (a) buffer solution and cellulase activity (b) buffer solution and hydrolysis time (c) cellulase activity and hydrolysis time.

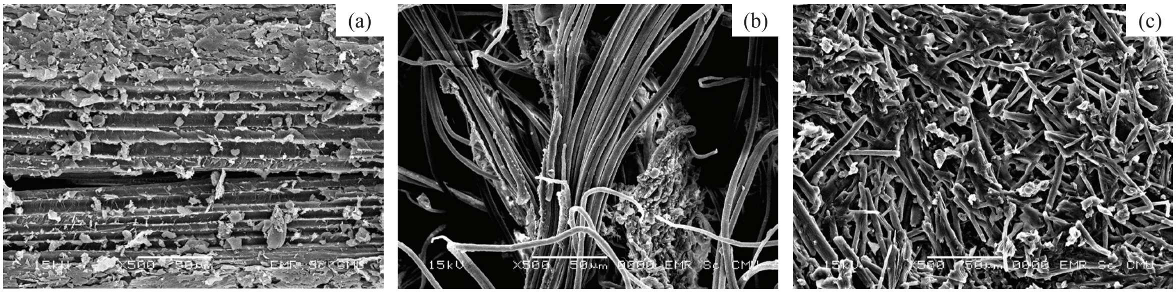


Figure 3: SEM micrographs of (a) Untreated rice straw, (b) Pretreated rice straw, (c) Cellulase hydrolyzed rice straw.

4 h presented the highest of sugar yield (glucose and xylose) as 25.8% (w/w). For the further utilization of the rice straw hydrolysate in microbial lipid production, was promising a carbon source for growth and lipid production by oleaginous yeast. According to several lignocellulosic hydrolysates have been used as feedstock for lipid production such as wheat straw [29], corn cob [30], corn stover [31], paper mill sludge [32] etc.

4 Conclusions

The combination of physico-alkali pretreatment improved the delignification and removal hemicelluloses from rice straw. The optimal condition of the hydrothermal using autoclaving combined alkali (NaOH) pretreatment was achieved the highest delignification of 92.5% and remaining cellulose of 81.5% (w/w). Response surface methodology was generated in order to optimize the enzymatic hydrolysis process of pretreated rice straw. The optimal hydrolysis condition was 935.18 U of cellulase activity with 10 mL of buffer solution (10% rice straw concentration) for 120 h. The highest reducing sugar yield found to be about 136.6 g/L. The rice straw hydrolysate could be used as a carbon source for lipid production of oleaginous yeast.

Acknowledgements

This research was supported by the National Research Council of Thailand (NRCT) and also thanked Department of Chemistry, Faculty of Science, Chiang Mai University, and Graduate School, Chiang Mai University. The authors would like to convey special appreciation to the academic committee of Pure and Applied Chemistry International Conference (PACCON2017) for providing the opportunity for this work to be published in this journal.

References

[1] Department of Alternative Energy Development and Efficiency. (2016, Jan.). Biomass Potential Reporting System in Thailand. Ministry of energy. Bangkok, Thailand [online]. Available: http://biomass.dede.go.th/Symfony/web/map/district?year=2556

[2] R. Zhao, Z. Zhang, R. Zhang, M. Li, Z. Lei, M. Utsumi, and N. Sugiura, "Methane production from rice straw pretreated by a mixture of acetic–propionic acid," *Bioresource Technology*, vol. 101, pp. 990–994, Feb. 2010.

[3] J. Pérez, J. Muñoz-Dorado, T. de la Rubia, and J. Martínez, "Biodegradation and biological treatments of cellulose, hemicellulose and lignin: An overview," *International Microbiology*, vol. 5, pp. 53–63, Jun. 2002.

[4] P. Kumar, D. M. Barrett, M. J. Delwiche, and P. stroeve, "Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production," *Industrial and Engineering Chemistry Research*, vol. 48, pp. 3713–3729, Mar. 2009.

[5] R. C. Kuhad, A. Singh, and K.-E. L. Eriksson, "Microorganisms and enzymes involved in the Degradation of plant fiber cell walls," *Advances in Biochemical Engineering/Biotechnology*, vol. 57, pp. 45–125, 1997.

[6] K. Karimi, S. kheradmandinia, and M. J. Taherzadeh, "Conversion of rice straw to sugars by dilute-acid

hydrolysis," *Biomass and Bioenergy*, vol. 30, pp. 247–253, Mar. 2006.

[7] Y. Sun and J. Cheng, "Hydrolysis of lignocellulosic materials for ethanol production: A review," *Bioresource Technology*, vol. 83, pp. 1–11, May. 2002.

[8] F. Monlau, C. Sambusiti, A. Barakat, M. Quéméneur, E. Trably, J.-P. Steyer, and H. Carrère, "Do furanic and phenolic compounds of lignocellulosic and algae biomass hydrolyzate inhibit anaerobic mixed cultures? A comprehensive review," *Biotechnology Advances*, vol. 32, pp. 934–951, Sep.–Oct. 2014.

[9] B. J. Khawla, M. Sameh, G. Imen, F. Donyes, G. Dhouha, E. G. Raoudha, and N.-E. Oumèma, "Potato peel as feedstock for bioethanol production: A comparison of acidic and enzymatic hydrolysis," *Industrial Crops and Products*, vol. 52, pp. 144–149, Jan. 2014.

[10] E. R. Easterling, W. T. French, R. Hernandez, and M. Licha, "The effect of glycerol as a sole and secondary substrate on the growth and fatty acid composition of Rhodotorula glutinis," *Bioresource Technology*, vol. 100, pp. 356–361, Jan. 2009.

[11] C. Dai, J. Tao, F. Xie, Y. Dai, and M. Zhao, "Biodiesel generation from oleaginous yeast Rhodotorula glutinis with xylose assimilating capacity," *African Journal of Biotechnology*, vol. 6, pp. 2130–2134, Aug. 2007.

[12] T. L. D. Silva, D. Feijão, J. C. Roseiro, and A. Reis, "Monitoring *Rhodotorula glutinis* CCMI 145 physiological response and oil production growing on xylose and glucose using multi-parameter flow cytometry," *Bioresource Technology*, vol. 102, pp. 2998–3006, Feb. 2011.

[13] G. Vicente, L. F. Bautista, R. Rodríguez, F. J. Gutiérrez, I. Sádaba, R. M. Ruiz-Vázquez, S. Torres-Martínez, and V. Garre, "Biodiesel production from biomass of an oleaginous fungus," *Biochemical Engineering Journal*, vol. 48, pp. 22–27, Dec. 2009.

[14] C. Sakdaronnarong, N. Srimarut, N. Lucknakhul, N. Na-songkla, and W. Jonglertjunya, "Two-step acid and alkaline ethanolysis/alkaline peroxide fractionation of sugarcane bagasse and rice straw for production of polylactic acid precursor," *Biochemical Engineering Journal*, vol. 85, pp. 49–62, Apr. 2014.

[15] G. L. Miller, "Use of dinitrosalicylic acid reagent for dertermination of reducing sugar," *Analytical Chemistry*, vol. 31, pp. 426–428, Mar. 1959.

[16] Forage Fiber Analyses (*Apparatus, Reagents, Procedures*, *and Some Applications*), *Agriculture Handbook No. 379*, United States Department of Agriculture (USDA), Washington, DC, 1970, pp. 1–20.

[17] L. J. Jönsson and C. Martín, "Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects," *Bioresource Technology*, vol. 199, pp. 103–112, Jan. 2016.

[18] H. Tarkow and W. C. Feist, "A mechanism for improving the digestibility of lignocellulosic materials with dilute alkali and liquid ammonia," *Cellulases and Their Applications*, vol. 95, pp. 197–218, Jun. 1969.

[19] M. J. Taherzadeh and K. Karimi, "Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review," *International Journal of Molecular Sciences*, vol. 9, pp. 1621–1651, Sep. 2008.

[20] M. Zeng, N. S. Mosier, C.-P. Huang, D. M. Sherman, and M. R. Ladisch, "Microscopic examination of changes of plant cell structure in corn stover due to hot water pretreatment and enzymatic hydrolysis," *Biotechnology and Bioengineering*, vol 97, pp. 265–278, Jun. 2007.

[21] I. Kim and J.-I. Han, "Optimization of alkaline pretreatment conditions for enhancing glucose yield of rice straw by response surface methodology," *Biomass and Bioenergy*, vol. 46, pp. 210–217, Nov. 2012.

[22] N. Rahnama, S. Mamat, U. K. M. Shah, F. H. Ling, N. A. A. Rahman, and A. B. Ariff, "Effect of alkali pretreatment of rice straw on cellulase and xylanase production by local Trichoderma harzianum SNRS3 under solid state fermentation," *Bioresources*, vol. 8, pp. 2881–2896, 2013.

[23] R. A. Silverstein, Y. Chen, and R. R. Sharma-Shivappa, "A comparison of chemical pretreatment methods for improving saccharification of cotton stalks," *Bioresource Technology*, vol. 98, pp. 3000–3011, Nov. 2007.

[24] R. Sun, J. M. Lawther, and W. B. Banks, "Influence of alkaline pre-treatments on the cell wall

components of wheat straw," *Industrial Crops and Products*, vol. 4, pp. 127–145, Jul. 1995.

[25] C. Tengborg, K. Stenberg, M. Galbe, G. Zacchi, S. Larsson, E. Palmqvist, and B. Hahn-Hägerdal, "Comparison of SO_2 and H_2SO_4 impregnation of softwood prior to steam pretreatment on ethanol production," *Applied Biochemistry and Biotechnology*, vol. 70–72, pp. 3–15, Mar. 1998.

[26] H. T. Tan, K. T. Lee, and A. R. Mohamed, "Pretreatment of lignocellulosic palm biomass using a solvent-ionic liquid [BMIM]Cl for glucose recovery: An optimisation study using response surface methodology," *Carbohydrate Polymers*, vol. 83, pp. 1862–1868, Feb. 2011.

[27] A. M. Joglekar and A. T. May, "Product excellence through design of experiments," *Cereal Food World*, vol. 32, pp. 857–868, May 1987.

[28] J.-W. Kim, K. S. Kim, J.-S. Lee, S. M. Park, H.-Y. Cho, J. C. Park, and J. S. Kim, "Two-stage pretreatment of rice straw using aqueous ammonia and dilute acid," *Bioresource Technology*, vol. 102, pp. 8992–8999, Oct. 2011.

[29] X. Yu, Y. Zheng, K. M. Dorgan, and S. Chen, "Oil production by oleaginous yeasts using the hydrolysate from pretreatment of wheat straw with dilute sulfuric acid," *Bioresource Technology*, vol. 102, pp. 6134–6140, May 2011.

[30] X.–F. Chen, C. Huang, X.–Y. Yang, L. Xioang, X.-D. Chen, and L.-L. Ma, "Evaluating the effect of medium composition and fermentation condition on the microbial oil production by Trichosporon cutaneum on corncob acid hydrolysate," *Bioresource Technology*, vol. 143, pp. 18–24, Sep. 2013.

[31] W. Liu, Y. Wang, Z. Yu, and J. Bao, "Simultaneous saccharification and microbial lipid fermentation of corn stover by oleaginous yeast Trichosporon cutaneum," *Bioresource Technology*, vol. 118, pp. 13–18, Aug. 2012.

[32] F. Deeba, V. Pruthi, and Y. S. Negi, "Converting paper mill sludge into neutral lipids by oleaginous yeast Cryptococcus vishniaccii for biodiesel production," *Bioresource Technology*, vol. 213, pp. 96–102, Aug. 2016.