Effect of Acid-Alkaline Pretreatment on Reducing Sugar Yield and Lignocellulosic Compositions of Rice (*Oryza sativa* L.) Residues

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Abstract:

Rice residues (RRs) are classified as an agricultural waste, which refer to rice bran, rice husk and rice straw. In the present study, the chemical compositions of each RRs were analyzed. The determination of lignocellulosic compositions of rice bran was revealed and represented as an outstanding biomass that consisted of high cellulose and low hemicellulose and lignin. While the compositions of rice husk and rice straw was obviously contrasted from rice bran. Furthermore, the acid-alkaline pretreatment of RRs under autoclaving at 121 ºC for 90 min was investigated for determination of the reducing sugar yield and lignocellulosic compositions change. The study consists of four pretreatment conditions for disruption of RRs structure with different solutions, including the condition (1): 1%(v/v) H_2SO_4, (2): 1%(w/v) NaOH, (3): 1%(w/v) NaOH following by 1%(v/v) H_2SO_4, and (4): distilled water (control). The result showed that the acid pretreatment with 1%(v/v) H_2SO_4 solution (condition 1) was the best condition that provided the highest reducing sugar yield from all RRs kinds. The dilute acid and alkaline pretreatment of RRs under 121 ºC for 90 min illustrated a clearly variation of its lignocellulosic compositions. The acid pretreated rice bran showed that cellulose reduction which correlated with concentration of reducing sugar yield released in the same experiment condition.

Keywords: Acid-alkaline pretreatment, Rice (*Oryza sativa* L.), Lignocellulosic biomass, Reducing sugar

Introduction

Lignocellulosic biomass such as agricultural and forest residues are considered as an alternative, inexpensive, renewable, and abundant source for fuel ethanol production [1]. This biomass contains polymers of cellulose, hemicellulose, and lignin, bound together in a complex structure. Liquid biofuels, such as ethanol, can be produced from biomass via fermentation of sugars derived from the cellulose and hemicellulose within lignocellulosic materials.

In 2018, production of rice (*Oriza sativa L.*) in Thailand is approximately amounts to 24.22 million tons [2]. The residues of rice are one of alternative and potential crops that can use as lignocellulosic biomass to produce biofuel. The main by-products of rice called rice residues (RRs) includes rice bran, rice husk and rice straw, are an abundant, readily available agricultural waste, which shows as a potential feedstock for bioenergy production.

The RRs cell walls consists of three main biopolymers, namely, cellulose, hemicellulose, and lignin. Cellulose is a homopolymer of cellobiose (two units of glucose), which are linked by □-1,4-glycosidic bonds. The complete hydrolysis of cellulose yields glucose, which is a preferable carbon source for commonly used fermenting microorganisms in industry [3]. Hemicelluloses are branched, heterogenous polymers of pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose) and acetylated sugars [4]. Lignin is a large complex polymer of unrepeated phenolic monomers. It significantly contributes to the water conduction and defense systems in plants [3].

These residues can be recovered into a cheap and environmentally friendly renewable resource by converting them to sugars using pretreatment processes. The aim of pretreatment is to disrupt the crystalline structure of lignocellulosic materials and to remove or reduce hemicelluloses and/or lignin. Pretreatment is the most important and costly steps, which has a major influence on the cost of bioenergy production from lignocellulosic biomass [5]. Normally, pretreatment methods of lignocellulosic material include physical (milling and grinding), physico-chemical (steam pretreatment and hydrothermal lysis), chemical (alkaline and dilute acid) and biological (enzyme hydrolysis) or combination of these [6].

Among these pretreatment methods, dilute sulfuric acid pretreatment has been found to be cheap, and to produce high hemicellulose recoveries and cellulose digestibility [7]. The optimization of acid pretreatment of rice husks has been studied previously [8]. Whereas, alkaline pretreatment is well-known that improves cellulose digestibility by providing the effective delignification and chemical swelling of fibrous cellulose [9]. In the biological pretreatment, is provided a high biodegradability rate which realized at exorbitant cellulolytic enzyme **costs** and enzymes activity during biomass decomposition is very slow requiring long duration [9]. Therefore, the main purpose of this research was to compare the effectiveness of dilute acid-alkaline pretreatments on chemical composition and reducing sugar production of rice bran, rice husk and rice straw.

Materials and methods

Raw materials preparation

Rice bran, rice husk and rice straw were collected in 2018 from agriculture field of Sakon Nakhon Province, Thailand. These RRs were mashed into small pieces and oven-dried overnight at 70 ºC. All RRs samples were stored in a sealed plastic bag at room temperature until further use.

Determination of chemical compositions

The chemical compositions (such as cellulose, hemicellulose, lignin and ash) of dried RRs were analyzed both before and after pretreatment, and described the method by Serechodchawong and Sangkharak (2014) [11].

Acid-alkaline pretreatment

The dilute acid-alkaline pretreatment of dried RRs was modified from Sotthisawad et al. (2017) [12]. 5 g of each RRs (rice bran, rice husk and rice straw) **were treated** and added as a substrate to solution at a ratio of 1:10, prior to autoclaving at 121 ºC for 90 min. The pretreatment was performed **by** four different pretreatment conditions including:

(1)-Acid pretreatment with 1%(v/v) H_2SO_4 solution

(2)-Alkaline pretreatment with 1%(w/v) NaOH solution

(3)-Acid-alkaline pretreatment with 1%(w/v) NaOH and following by 1%(v/v) H_2SO_4 solution

(4)- Pretreatment with distilled water (DW) and served as controls

Reducing sugar assay

The amount of reducing sugar released after the pretreatment process was assayed by 3,5-di nitrosalicylic acid (DNS) analysis method of Miller (1959) [13] using spectrophotometer at 540 nm.

Results and discussion

Chemical compositions of RRs

Chemical characteristics of rice bran, rice husk and rice straw are presented in Table 1. The carbohydrate composition refer as lignocellulosic compositions of rice husk and rice straw were quite similar, while rice bran was clearly differed from them. The composition analysis revealed that rice bran is wealthy of cellulose that can be hydrolyzed to produce glucose, was found to be 77.55±2.19%, (w/w). While, hemicellulose content was found that higher than other components in rice husk and rice straw to 42.67±1.15 and 53.33±1.15 %, (w/w), respectively. The results obtained in the present study are in agreement with Sridevi et al. (2015) [14] who also reported that rice bran contained highest cellulose content.

Table 1 Chemical compositions of raw rice residues

Components	Contents (%, w/w)		
	Rice bran	Rice husk	Rice straw
Cellulose	77.55±2.19	12.67±1.15	10.00±2.00
Hemicellulose	3.00±1.41	42.67±1.15	53.33±1.15
Lignin	16.67±0.58	41.75±0.95	35.17±2.98
Ash	2.78±0.20	2.92±0.37	1.50±0.07

Reducing sugar released from pretreatment

Pretreatment processes are vital for efficient separation of the complex interlinked components and enhance the availability of every component, i.e., cellulose and hemicellulose [15]. The pretreatment of RRs with dilute acid and alkaline was carried out in this research. The yield of reducing sugar in the liquid fraction resulting from 5 g of each RRs after acid/alkaline pretreatments at 121 ºC for 90 min in four conditions of pretreatment was analyzed (Fig. 1).

It was noted that reducing sugar yields from acid/alkaline pretreated RRs were higher than those from the control, indicating that pretreatment is critical for increasing the saccharification of RRs. Especially, the acid pretreatment with 1%(v/v) H_2SO_4 resulted in the highest reducing sugar yield from all RRs kinds. The maximum amount of reducing sugar liberated from acid pretreated rice bran was 20.04±0.49 g/L, followed by rice husk (18.01±0.91 g/L) and rice straw (9.76±0.16 g/L).

Based on the findings in this study, pretreatment of RRs with 1%(v/v) H_2SO_4 was the best suited for enhancing the reducing sugar yield. Similarity, Deejing and Ketkorn (2009) [16] found the maximum reducing sugar concentration released from rice bran was obtained by hydrolysis with 1.0% (v/v) H_2SO_4 under 111 ºC for 30 min. Besides, the findings of the experiments carried were supported by recent reports about pretreatment of mushroom cultivation waste material with 1% (v/v) H_2SO_4 for 90 min resulted in the highest yield of reducing sugar of 14.09±0.38 g/L [12].

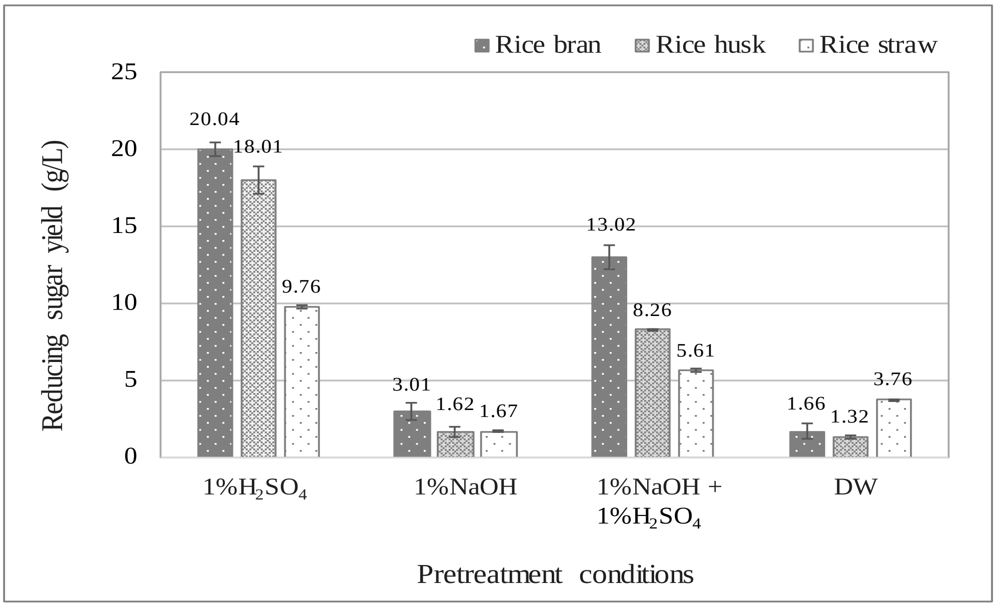

Figure 1 Reducing sugar contents released after acid-alkaline pretreatment of RRs. Bars represent the means of three replicates, and error bars indicate the standard deviation.

Effect of pretreatments on lignocellulosic compositions of RRs

Some chemicals such as acids, alkali, organic solvents, and ionic liquids have been reported to have significant effect on the native structure of lignocellulosic biomass [17]. Compositional analysis of lignocellulose, before and after pretreatment, is used in almost all second-generation biofuel production studies [18].

The effect of RRs pretreatment with dilute acid and alkaline under 121 ºC for 90 min showed a variation of lignocellulosic compositions of RRs (Table 2). All conditions of rice bran pretreatment found that caused of decreasing of cellulose content whereas hemicellulose and lignin increased. The rice bran pretreatment with 1%(v/v) H_2SO_4 (condition 1), revealed a lowest cellulose content which may be resulted by the breakdown of cellulose complex into glucose or other carbohydrates. Results of the present study on dilute sulfuric acid pretreatment has been studied for a wide range of lignocellulosic biomass [3]. It results in high recovery of the hemicellulosic sugars in the pretreatment liquid, and in a solid cellulose fraction [19].

Table 2 The lignocellulosic composition changes of RRs after acid-alkaline pretreatment

Rice residues	**Pretreatment conditions***	**Contents (%, w/w)**			
		Cellulose	**Hemicellulose**	**Lignin**	**Ash**
Rice bran	***untreated***	77.55±2.19	3.00±1.41	16.67±0.58	2.78±0.20
	1	17.00±12.73	37.00±1.41	43.75±10.82	2.25±0.49
	2	46.00±8.49	29.00±9.90	23.37±1.23	1.63±0.18
	3	36.00±2.83	11.00±1.41	49.15±0.89	3.85±2.31
	4	50.00±2.83	12.00±5.66	35.66±2.63	2.34±0.20
Rice husk	***untreated***	12.67±1.15	42.67±1.15	41.75±0.95	2.92±0.37
	1	22.00±8.49	32.00±2.83	43.65±5.64	2.35±0.01
	2	36.00±2.83	2.00±0.00	41.70±2.83	2.30±0.00
	3	30.00±2.83	16.00±0.00	52.11±4.23	1.89±1.40
	4	31.00±1.41	34.00±2.83	31.71±1.60	3.29±0.18
Rice straw	***untreated***	10.00±2.00	53.33±1.15	35.17±2.98	1.50±0.07
	1	46.00±19.80	42.00±11.31	21.60±16.46	0.40±0.51
	2	67.00±7.07	8.00±2.83	23.75±3.86	1.25±0.38
	3	40.00±0.00	11.00±4.24	47.88±5.43	1.12±1.19
	4	34.00±0.00	43.00±1.41	22.12±1.30	0.88±0.11

*Rice residues are both untreated and pretreated with *i*: 1%(v/v) H_2SO_4; *ii*: 1%(w/v) NaOH; *iii*: 1%(w/v) NaOH following by 1%(v/v) H_2SO_4; and *iv*: distilled water (served as control).

On the other hand, the dilute acid and alkaline pretreatment of rice husk and rice straw effected to cellulose content expanding while hemicellulose decrease. After pretreatment with 1%(w/v) NaOH following by 1%(v/v) H_2SO_4 (condition 3) found that lignin content of rice husk and rice straw were higher than other conditions (52.11±4.23 and 47.88±5.43%(w/w), respectively).

Moreover, the pretreatment of rice straw and rice husk with 1%(w/v) NaOH (condition 2) illustrated the highest cellulose content of 67.00±7.07 and 36.00±2.83 %(w/w), respectively. This result correlated with Kobkam et al. (2018) [**6**] discussed that the alkaline pretreatment of rice straw affects the crystallinity decreasing of cellulose and increases hemicellulose disruption. The effect of alkali pretreatment (such as NaOH, KOH and anhydrous ammonia) cause swelling of biomass, which increases the internal surface area of the biomass, and decreases both the degree of polymerization, and cellulose crystallinity. Alkaline pretreatment disrupts the lignin structure and breaks the linkage between lignin and the other carbohydrate fractions in lignocellulosic biomass, thus making the carbohydrates in the heteromatrix more accessible [20].

Conclusions

The lignocellulosic compositional analysis of each RRs, both before and after pretreatment, is an important step for development of biofuel production studies. The biomass provided a high cellulose content will be considered and chosen to use as a raw material for biofuel generation. The acid-alkaline pretreatment of lignocellulosic biomasses is also essential process for alter the structure of biomass residues to enhance the reducing sugars yield. It is clear from this study that rice bran represents as a distinctive biomass provided high cellulose and low hemicellulose and lignin. The pretreatment of rice bran with 1%(v/v) H_2SO_4 at 121 ºC for 90 min was the optimal condition for liberation of reducing sugar yield from this substrate.

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