IJE TRANSACTIONS B: Applications Vol. 26, No. 5, (May 2013) 455-464

International Journal of Engineering

Journal Homepage: www.ije.ir

Comparative Studies on the Effect of Pretreatment of Rice Husk on Enzymatic Digestibility and Bioethanol Production

M. Nikzad, K. Movagharnejad, G. D. Najafpour*, F. Talebnia

Faculty of Chemical Engineering, Noshirvani University of Technology, P.O. Box 484, Babol, Iran.

PAPER INFO

Paper history:
Received 06 November 2012
Recivede in revised form 28 November 2012
Accepted 13 December 2012

Keywords:
Rice Husk
Pretreatment
Enzymatic Hydrolysis
Bioethanol

ABSTRACT

Three different pretreatment methods for rice husk were investigated. In order to determine how each method affects on the composition of the rice husk, the digestibility of the rice husk in enzymatic hydrolysis and ethanol production, dilute-H_2SO_4 (1%v/v, 121°C, 30 min), dilute-NaOH (3% w/v, 121°C, 30 min) and heat treatment (121°C, 30 min) were employed. Among them, the best results were obtained when the pretreatment of rice husk was carried out with 3% NaOH solution. Pretreatment of rice husk with NaOH substantially increased the lignin removal, enzymatic digestibility of cellulose, accessibility of cellulose and fermentable sugar production. The highest glucose concentration, glucose yield and ethanol concentration were 14.54 g/L, 59.6% and 6.22 g/L, which were 5.44, 3.77 and 6.15 times higher than the untreated control samples, respectively. Moreover, the SEM analysis of the pretreated sample illustrated significant physical changes of the rice husk after NaOH pretreatment.

doi: *10.5829/idosi.ije.2013.26.05b.01*

1. INTRODUCTION

Lignocellulosic materials, the most abundant and low cost biomass have been identified as promising feedstock for fuel-ethanol production [1]. Bioethanol production from lignocellulosic biomass is very challenging due to heterogeneous structure of lignocellulose [2]. Lignocellulosic materials contain cellulose, hemicellulose and lignin in a complex crystalline structure which severely restricts the enzymatic hydrolysis [3]. In order to improve the accessibility of the enzyme to cellulose, an efficient pretreatment is needed [4]. Pretreatment has been considered as one of the most expensive steps in bioethanol production from lignocellulose and can make a contribution as much as 30% of the total cost [5]. Improvement in pretreatment efficiency may significantly lower the cost of lignocellulosic ethanol process. An effective pretreatment could be an inexpensive process. Use of simple equipment may avoid losses of carbohydrates, preserve pentoses, and avoid formation of any inhibitory by-product [6]. Several pretreatment methods have been studied to facilitate the enzymatic hydrolysis of lignocellulosic materials [7-10]. NaOH, H_2SO_4 and thermal pretreatment are routinely used in paper and pulp industry to pretreat raw materials. Moreover, large scale units of these processes are commonly used in paper industry. Hence, if these methods are effective, it is anticipated that the technical issues regarding the large scale design of these processes for the rice husk pretreatment would be minimal.

Among different pretreatment methods, acid pretreatment is known to separate pentoses and hexoses: thermal pretreatment such as hot water and autoclaving is known to remove most of the hemicelluloses [11]; while alkali pretreatment is known to separate lignin from lignocellulosic biomass [2]. The pretreatment with dilute-H_2SO_4 solution is a promising method for the production of lignocellulosic bioethanol [12]; but this method promotes hydrolysis of hemicelluloses and part of amorphous cellulose that is resulted in high content of hemicelluloses as monomers in the liquid fraction and high cellulose content in the solid fraction [13, 14]. High temperature and acid condition of the pretreatment cause the released monomeric sugar through hydrolysis to be degraded to furfural and 5-hydroxymethylfurfural (HMF). In addition, aliphatic acids, especially acetic acid is generated from hydrolysis of the hemicellulose acetyl group [3]. These degraded compounds decrease the total yield of sugars and also act as inhibitors in the fermentation process [15]. NaOH pretreatment is an effective process for pretreating lignocellulosic materials. It can remove partially lignin and

*Corresponding Author Email: najafpour@nit.ac.ir (G. D. Najafpour)

hemicellulose in the biomass by breaking the ester bonds [16]. Different studies showed that NaOH pretreatment is one of the most effective methods for improving the enzymatic hydrolysis [17-20]. In addition, no measurable 5-(hydroxymethyl)furfural (HMF) and furfural that are risky for yeast were detected in this process. It is also effective for improving the enzymatic hydrolysis using thermal pretreatment such as hot water and autoclaving which are able to remove most of the hemicellulose [21]. The conditions for hot water pretreatment are high temperature and pressure. Generally, these pretreatment methods are energy intensive. Therefore, it is very important to use an effective pretreatment prior to enzymatic hydrolysis. Chen et al. [22] have compared different chemical pretreatments of corn stover for enhancing enzymatic digestibility. They have reported that the enzymatic hydrolyzate from NaOH pretreated corn stover contained higher content of fermentable sugars. In another investigation carried out by Cao et al. [11], the effects of five pretreatment methods on enhancement of the enzymatic digestibility and ethanol production from sweet sorghum were compared. The best results were achieved with sweet sorghum pretreated by dilute-NaOH solution autoclaved in H_2O_2 immersion. Although several pretreatment methods showed the effectiveness of number of pretreatments methods [4], the combination of two pretreatment methods may be more effective than separate single one.

North of Iran is devoted to rice paddy fields. Rice is one of the most important agricultural crops in Iran. Rice husk is a by-product of the rice milling industry which contains significant amount of sugars as holocellulose (57-61%). Rice husk may be a potential alternative substrate for bioethanol production [17]. However, like other lignocellulosic materials, the use of rice husk as feedstock for bioethanol production has been limited because the chemical structure of rice husk makes it recalcitrant to enzymatic hydrolysis unless it is pretreated to a more accessible form. Very little literature is available on pretreatment of rice husk. The conversion of rice husk into fermentable sugars has been studied as feedstock for ethanol [17, 23-25]. Saha et al. [25] used alkaline peroxide for pretreatment of rice hulls and evaluated the conversion of rice hull cellulose and hemicellulose to simple sugars. An investigation was conducted by Ang et al. [20] for potential application of three ionic liquids. Banerjee et al. [26] evaluated wet air oxidation as a pretreatment method for bioethanol production from rice husk. Moreover, they optimized wet air oxidation pretreatment conditions. However, previous pretreatment methods of rice husk have resulted various outcome due to different sources of biomass, different supply of enzyme and dissimilar analytical method. Lack of commercial applications of the pretreatment methods lead to investigate and obtain comparative data for the selection of suitable pretreatment method.

In this study, to provide comparative information for the pretreatment of rice husk, several pretreatments based on dilute-H_2SO_4, dilute-NaOH and heat treatment (autoclaving) were evaluated using a single source of rice husk; same cellulase enzyme, shared analytical methods that will assist readers to understand the unique features and performances of leading options for releasing sugar from rice husk. The present investigation compares the effects of three different pretreatment methods on improvement of enzymatic digestibility of rice husk and ethanol production from the hydrolyzate. In addition, the compositional and physical changes of the rice husk were investigated.

2. MATERIALS AND METHODS

2. 1. Raw Material The rice husk (obtained from 'Tarom Hashemi', that was harvested in early August 2011), was collected from local milling center in Amol (Mazandaran, Iran). The fresh raw material was oven dried at 50°C for 24 h to a dry matter content of 89.66%. The rice husk was milled with a food homogenizer (Black & Decker, Model No. FX350, England) and then screened to obtain the particle size ranged 0.42-0.6 mm with a sieve shaker. The screened materials were stored in tightly sealed plastic bags at room temperature under dry condition for future use. The commercial enzymes, cellulase (Celluclast 1.5L) and β-glucosidase (Novozyme 188) were purchased from Novozymes A/S Bagsvaerd (Denmark) and Sigma-Aldrich Co. (St. Louis, USA), respectively.

The activity of cellulose-Celluclast 1.5L was measured as 45 FPU/mL. The enzyme activity of Novozyme 188 reported by supplier was 250 IU/mL. All chemicals used in this study were purchased from Merck company (Darmstadt, Germany).

2. 2. Pretreatment Process In the present study, three different methods of pretreatment of rice husk were investigated. In the following experiments, untreated rice husk was considered as the control case. Each experiment was performed in triplicate and the average values were reported.

2. 2. 1. Dilute- H_2SO_4 Solution Pretreatment A 5 g of rice husk sample was mixed with 95 mL of 0.5-1.0% (v/v) H_2SO_4 solution in a 250 mL flask with a stopple and then autoclaved at 121°C, 15 psig for 15-45 min. The mixture was filtered through a Whatman filter paper to separate the solid residue. The residue was washed with distilled water until neutral pH. The sample was air dried and stored in tightly sealed plastic bag at refrigerator for further use.

2. 2. 2. Dilute-NaOH Solution Pretreatment Rice husk sample (5 g) was soaked in 95 mL of 1-3% (w/v) NaOH aqueous solution in a 250 mL flask and then treated in an autoclave at 121°C, 15 psig for 15-45 min. The solid residue was separated from the mixture by filtration and thoroughly washed with distilled water to neutralize its pH. Finally, the filtrate was dried and stored as above.

2. 2. 3. Heat Pretreatment (Autoclaving) A 5 g dry rice husk was mixed with 95 mL distilled water in a 250 mL flask. Pretreatment was done in an autoclave at 121°C, 15 psig for 30 min. After autoclaving, the sample was filtered and the solid residue air dried and stored for further use.

2. 3. Enzymatic Hydrolysis The solid residue of pretreated rice husk was soaked in citrate buffer (50 mM, pH=4.8) to obtain a substrate loading of 4.5% and then incubated for half an hour at 50°C and then enzymes were added. The rice husk samples were hydrolyzed by cellulase and β-glucosidase. Cellulase was supplemented with β-glucosidase to avoid product inhibition made by cellobiose accumulation. The enzyme loadings of Celluclast 1.5L and β-glucosidase were 20 FPU/g dry biomass and 90 U/g dry biomass, respectively. The hydrolysis was carried out at 50°C and 150 rpm in an incubator shaker (IKA, Japan). Samples (1mL) were taken from the reaction mixture periodically to evaluate the effect of reaction time on different pretreated rice husk. The liquid phase was separated from the solid residue by centrifugation at 10000g for 5 min and then stored at -20°C until it was used for sugar analysis.

2. 4. Microorganism and Batch Fermentation The pure stock culture of *Saccharomyces cerevisiae* was used for ethanol fermentation. The strain was originated from Persian type culture collection (PTCC 24860), supplied by Iranian Research Organization for Science and Technology (IROST). The medium was used for seed culture contained, glucose, peptone, NH_4Cl and yeast: 50, 20, 0.45 and 10 g/L, respectively. The medium was autoclaved at 121°C, 15 psig for 20 min. The sterilized medium was inoculated with 5% of pure seed culture of the microorganism and then the culture was cultivated in an incubator at 30°C for 24h. The hydrolyzate was sterilized by autoclaving at 121°C, 15 psig for 20 min before it was inoculated with the yeast medium at the volumetric ratio of 3:100 of the fermentation broth aseptically. The fermentation experiments were conducted at 37±0.5°C in an incubator shaker at 150 rpm. Fermentation yield (g ethanol/g dry substrate) was calculated by the following relation:

$$Y = \frac{EV}{M} \times 100 \quad (1)$$

where E is the ethanol concentration (g/L), V is volume of the reactor (L), and M is the substrate mass in the culture (g). The theoretical ethanol yield was calculated using the following equation:

$$Y_{th} = \frac{0.9Y}{0.55G_r} \times 100 \quad (2)$$

where G_r is the glucan fraction in the raw mixture [27].

2. 5. Analytical Methods The chemical composition of the rice husk was determined by the procedures outlined by National Renewable Energy Laboratory (NREL) [28]. The moisture was measured as the weight loss of rice husk dried in an oven at 105°C for 24 h. The glucose, xylose, acetic acid, furfural, hydroxymethyl furfural (HMF) and ethanol were measured with a HPLC system (Knauer, Germany) equipped with refractive index (RI) detector (Knauer, Smartline RI Detector 2400, Germany) and UV absorbance detector at 275 nm (Knauer, Smartline UV Detector 2500, Germany). A Eurokat H (10μm) column 8×300 mm kept at 75°C and eluted with 0.01 N acid sulfuric at a flow rate of 0.4 mL/min was used for determination of sugar and ethanol concentrations and a Eurospher II (100-5 C18 P, 150×4.0 mm ID) column fixed at 25 °C with water and methanol (20 and 80%) as eluent at flow rate of 1 mL min-1 was used for determination furfural and hydroxymethyl furfural (HMF). The filter-paper activity unit (FPU) of the cellulase enzyme was measured according to the standard procedure recommended by NREL. Physical changes in the native and pretreated rice husk structure were observed by scanning electron microscope (SEM). Images of the native and pretreated husk were taken using a KYKY-EM 3200 scanning electron microscope (China). The specimens to be coated were mounted on a conductive tape and coated with a gold palladium using a SCD 005 sputter coater (BAL-TEC, Switzerland) and observed using a voltage of 26 kV.

3. RESULTS AND DISCUSSION

3. 1. Preliminary Experiments Primarily, several sets of experiments were conducted to investigate the effect of concentration of NaOH and H_2SO_4 and time on the concentration of released glucose from the pretreated rice husk. The first experiment was carried out with dilute- H_2SO_4 for the pretreatment of rice husk (5% solid loading). The experiments were conducted with 0.5, 0.75 and 1.5% (v/v) H_2SO_4 for 15, 30 and 45 min at 121°C, after which the solid radiuses were prepared for enzymatic hydrolysis. As shown in Figure 1a with various dilute- H_2SO_4 pretreatment conditions, 1% concentration of H_2SO_4 gave higher glucose concentration than other acid concentrations. In fact for

the effect of time, there was no significant difference between various pretreatment times. The husk pretreated with 1% H_2SO_4 for 30 min produced higher glucose concentration than other dilute-H_2SO_4 pretreated rice husk. The second sets of preliminary experiments were similar to first set of experiments, but in this set of experiment dilute-NaOH were utilized with 1, 2 and 3% of NaOH (w/v) for 15, 30 and 45 min at 121°C. As Figure 1b depicts, the pretreated rice husk with high concentration of NaOH resulted in high glucose concentration. This finding was in agreement with similar work done by Zhang et al. [16]. It was observed that residence times of 30 and 45 min also yielded in high glucose concentration that was probably due to high lignin removal. Maximum glucose concentration was obtained from the hydrolysis of pretreated rice husk with 3% NaOH for 30 min at 121°C. Based on the obtained data from the above sets of experiments, more strategized experiments were performed.

3. 2. Characterization of Raw and Pretreated Rice Husk Prior to enzymatic hydrolysis, raw rice husk was subjected to three pretreatment methods with dilute-H_2SO_4, dilute-NaOH solution and heat treatment (autoclaving). In order to compare the efficiency and the effectiveness of the different pretreatment processes, the solid residue was analyzed for its composition.

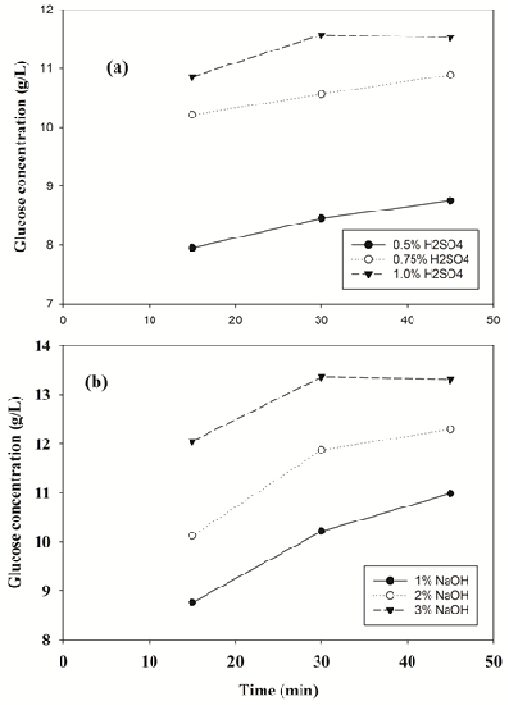

Figure 1. The glucose concentration produced from hydrolysis of pretreated rice husk at different pretreatment conditions (concentration and time) of (a) dilute-H_2SO_4 and (b) dilute-NaOH pretreatments.

Chemical composition, hemicellulose dissolution, lignin removal, and dry matter loss of the pretreated rice husk are summarized in Table 1. Different pretreatment methods resulted in different composition changes of rice husk, reflecting the effectiveness of each pretreatment process. The cellulose fraction was found to be the major component present in the pretreated solid residue. In each applied pretreatment, the recovery of cellulose fraction was higher than 75%, but the removal of hemicellulose and lignin was different. Table 1 shows that the highest cellulose content was obtained (54.31%) and occurred when the rice husk was pretreated by dilute-NaOH, while its lignin content (9.61%) was the lowest one. Dilute-NaOH pretreatment of lignocellulosic biomass has been found to cause swelling, leading to an increase in internal surface area and disruption of the lignin structure [11]. Silverstein et al. [29] reported that 2% NaOH in 90 min at 121°C was the best pretreatment condition, resulted in 65% of delignification. In this work, dilute-NaOH pretreatment was conducted at 121°C for 30 min, which resulted in more than 69%. Comparing dilute- H_2SO_4 and heat pretreatment to dilute-NaOH pretreatment; NaOH removed more lignin fraction from raw material because of the dissolution of lignin in alkaline solution. The lignin removal increases enzyme effectiveness by eliminating nonproductive adsorption sites and by increasing access to cellulose and hemicellulose [12]. Pretreatment with dilute-H_2SO_4 effectively hydrolyzed 73.04% of hemicellulose to soluble sugars but removed only 19.13% of lignin from raw rice husk. When dilute-H_2SO_4, dilute-NaOH and heat pretreatments are compared, it can be deduced that dilute acid pretreatment is more effective than other pretreatment methods in removing hemicellulose. Although, dilute-NaOH pretreatment of sample resulted in 43.80% hemicellulose dissolution. With regard to lignin, most of the lignin was not removed from the rice husk by dilute-H_2SO_4 pretreatment. The lowest weight loss appeared in autoclaving pretreatment, but compared with native rice husk, there was no significant lignin removal. This pretreatment is an environmental friendly pretreatment method with no addition of chemicals. Different studies reported high temperatures e.g. 220°C can dissolve hemicellulose completely and remove lignin partially [30-32]. In this study, heat pretreatment autoclaving was done at 121°C, 15 psig, which is not adequate to dissolve the hemicellulose and remove the lignin. Consequently, cellulose and hemicellulose might still be bundled by a great amount of lignin, that would harm the enzymatic hydrolysis and ethanol fermentation [11].

When different pretreatment methods are compared; it can be deduced that dilute-NaOH pretreatment method is more effective than other methods in retaining cellulose and removing lignin. It was found that NaOH pretreatment successfully removed lignin

from the rice husk under the stated conditions of present work.

TABLE 1. Rice husk chemical composition and effect of various pretreatment methods

	Raw RH[a]	Dilute H_2SO_4	Dilute NaOH	Autoclave
Cellulose[b]	37.55	51.89	54.21	40.38
Hemicellulose[c]	15.24	6.68	14.18	13.55
Lignin	19.22	25.31	9.61	26.18
Hemicellulos dissolution (%)	-	73.04	43.80	37.40
Lignin removal (%)	-	19.13	69.80	39.60
Dry matter loss (%)	-	38.50	4.10	29.60

[a] RH: Rice husk,[b] Based on total glucan,[c] Based on total Xylan.

TABLE 2. The concentration of sugars in the pretreatement liquid fraction and the concentration of inhibitors in the hydrlolzate

Pretreatment methods	Sugars concentration in the pretreatment liquor		Inhibitors concentration	
	Glucose (g/L)	Xylose (g/L)	Furfural (g/L)	Acetic acid (g/L)
Dilute-H_2SO_4	1.60	4.86	0.02	0.32
Dilute-NaOH	1.18	2.42	-	0.17
Autoclave	0.69	2.14	-	0.08

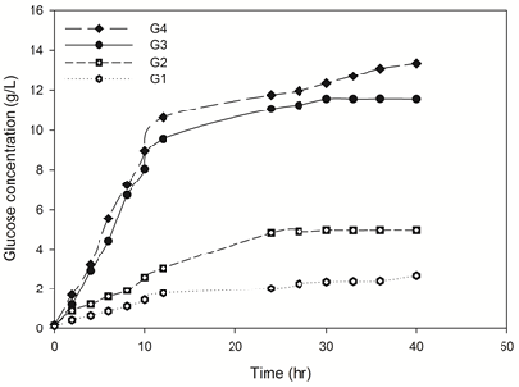

Figure 2. The effects of different pretreatment methods on glucose concentration in the course of enzymatic hydrolysis; G1: Control, G2: Heat pretreatment (Autoclave), G3: Dilute-H_2SO_4 pretreatment, G4: Dilute-NaOH pretreatment.

3. 3. Effects of different pretreatment methods on sugar concentration Key factors for evaluating effective pretreatment of lignocellulosic biomass are highly digestible pretreated solid fractions, high lignin recoveries and high sugar concentrations [33]. The effects of different pretreatment methods on glucose concentration during the enzymatic hydrolysis are shown in Figure 2.

As data illustrate in Figure 2, the glucose concentration in the hydrolyzate increased as the enzymatic hydrolysis time continued and reached a plateau within 40 h. Therefore, it can be concluded that all the glucose production potential had been achieved in this time. With autoclaving pretreatment at 121°C for 30 min followed by enzymatic hydrolysis for 40 h, the glucose concentration was 4.95 g/L, while with 1% H_2SO_4 and 3% NaOH pretreatment at the same condition the glucose concentration increased to 11.57 and 13.36 g/L. As results show, there was significant difference in the glucose concentration between 1% H_2SO_4 and 3% NaOH pretreated and untreated rice husks after hydrolysis for 40 h. According to Figure 2, dilute-NaOH and dilute-H_2SO_4 pretreatment released glucose much faster than thermal pretreatment. Lignin has phenolic skeletal structure; thus, it is very hydrophobic. It has a strong affinity for the hydrophobic patches within the structure of enzymatic proteins [34]. Thus, lignin removal should improve the hydrolysis performance. A relation between cellulose content, lignin removal and released glucose concentration from the pretreated rice husk was observed. It was expected that dilute-NaOH pretreated rice husk generate high concentration of glucose due to high lignin removal. But, in some cases there was no significant difference between the glucose concentrations which were produced from dilute-NaOH and dilute-H_2SO_4 pretreated rice husk. It seemed that the removed lignin from dilute-NaOH pretreated husk generated toxic phenolic compounds and these compounds might inhibit and hindere the enzyme hydrolysis. However, the mechanism of inhibiting effect of phenolic compounds was not elucidated, largely due to the lack of accurate qualitative and quantitative analyses [13].

Limited amounts of glucose were also released from cellulose into liquid fraction during three different pretreatment methods, because the crystalline structure of the cellulose makes its hydrolysis impossible during the pretreatment process [23]. Table 2 gives the glucose and xylose concentrations of liquid fraction after different pretreatment steps.

As stated in Table 2, cellulose is more resistance to harsh conditions than hemicellulose, so only a small amount of glucose was released during different pretreatment methods [35]. In this work, total glucose concentration was considered as the sum of the obtained glucose concentration from the enzymatic hydrolysis and glucose concentration in liquid fraction of pretreated rice husk. Therefore, total glucose concentrations were calculated as 13.17, 14.54 and 5.64 g/L for dilute-H_2SO_4, dilute-NaOH and autoclave pretreatments, respectively, which were 4.93, 5.44 and

2.11 times more than the control, respectively. With regard to 3% NaOH pretreatment, the glucose concentration was the highest one among all methods, which indicates that this pretreatment was the most effective one in converting the cellulose in rice husk to glucose in these three pretreatment methods.

Figure 3 illustrates the effects of different pretreatment methods on the xylose concentration during the enzymatic hydrolysis. As illustrated in Figure 3, the trend of the xylose concentration in the hydrolyzate was similar to that of glucose concentration, but the xylose concentration was much lower than glucose concentration in all the pretreatment methods. This was reasonable because hemicellulose content in both pretreated and untreated rice husk was lower than the cellulose content. Also, hemicellulose is a sensitive compound under harsh conditions and high temperatures; it is rapidly converted to furfural, HMF and acetic acid which acts as an inhibitor and severely decrease enzymatic hydrolysis [8, 15, 35]. Generally, the majority of hydrolyzate of hemicellulose in lignocellulosic material is xylose, and there are minor amounts of other pentose in the hydrolyzate [4].

According to Table 1, there was more hemicellulose in dilute-NaOH and autoclave pretreated rice husks than dilute-H_2SO_4 pretreated rice husk. As data show in Figure 3, the xylose concentration in the hydrolyzate with dilute-NaOH pretreated rice husk was the highest one. Therefore, the amount of lignin left after pretreatment is the key factor for degrading during the enzymatic hydrolysis. The hemicellulose content in dilute-NaOH and autoclave pretreated rice husks were close to the control, but autoclave pretreated and untreated rice husks had lower xylose in the hydrolyzate. This indicated that less amount of hemicellulose in the autoclave pretreated and raw rice husks was hydrolyzed, because much of the hemicellulose is inaccessible for enzyme under the wrap of remained lignin [36].

The dilute acid pretreated rice husk had less xylose concentration in the hydrolyzate, due to the low content of hemicellulose and high content of lignin. Data listed in Table 2 show that the xylose concentration in the pretreatment liquid fraction was 4.86, 2.32 and 2.04 g/L for the dilute-H_2SO_4, dilute-NaOH and autoclave pretreatments, respectively. Similar to total glucose concentration, total concentration of xylose was considered as the sum of xylose concentration from the enzymatic hydrolysis and xylose concentration in liquid fraction of pretreated rice husk. Consequently, total xylose concentrations were calculated as 6.57, 6.38 and 5.04 g/L for dilute-H_2SO_4; dilute-NaOH and autoclave pretreatments, respectively. It was found that dilute-H_2SO_4 released highest content of the xylose concentration among all methods.

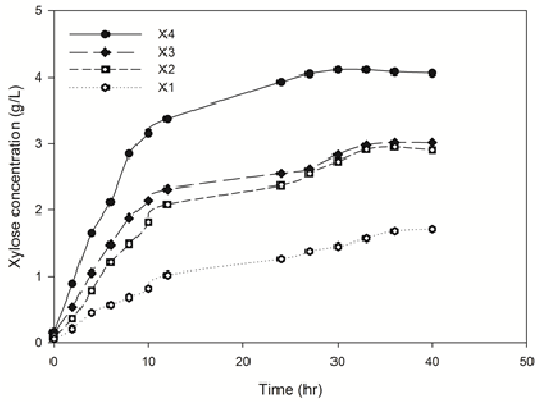

Figure 3. The effects of different pretreatment methods on xylose concentration in the course of enzymatic hydrolysis; X1: Dilute-H_2SO_4, X2: Control, X3: Autoclave pretreatment, X4: Dilute-NaOH pretreatment.

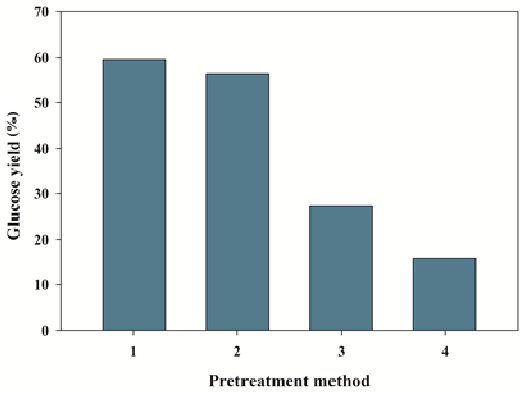

Figure 4. The effects of different pretreatment methods on glucose yield; 1: Dilute-NaOH, 2: Dilute-H_2SO_4, 3: Autoclave, 4: control

3. 4. Effects of Different Pretreatment Methods on Glucose Yield and Ethanol Concentration The performance of the enzymatic hydrolysis was determined by measuring glucose yield from conversion of cellulose. This was expressed as the percentage of glucose in the solid released in relation with the total amount of glucan in the solid residue. Figure 4 shows the effect of various pretreatment methods on glucose yield obtained from enzymatic hydrolysis of rice husk.

The three different pretreatment methods showed different glucose yields. The total glucose yield for dilute-NaOH, dilute-H_2SO_4 and autoclave pretreated samples reached 59.6%, 56.4% and 27.24%, respectively. It indicated that dilute-NaOH pretreatment was good for improving the cellulose hydrolysis. The glucose yield of dilute-NaOH was 59.6%, which was 3.77 times higher than that of control. That may be due to the reason that the content of lignin was lowest in the pretreated rice husk by dilute-NaOH. Previous studies

reported that the cellulose conversion improved with increasing lignin removal [22, 37]. Lignin can adsorb protein from aqueous solutions, and then lignin removal should improve the hydrolysis of cellulose by reducing nonspecific adsorption of cellulase enzymes [22]. The results showed that there was no significant difference in the glucose yield between heat pretreated (autoclaving) and untreated rice husk, because the pretreatment temperature (121°C) of this method was not high enough to dissolve the hemicellulose and remove the lignin for improvement of the cellulose conversion [31]. The pretreatment with dilute-NaOH was the most effective one on increasing the glucose yield in three pretreatment methods.

Acetic acid, HMF and furfural are three main inhibitors to yeast cells in subsequent fermentation, which are formed during the pretreatment and hydrolysis processes [38]. Acetic acid is derived from acetyl degradation in hemicellulose. Under the high temperature of pretreatment, xylose is degraded to furfural. Based on reported data in the literature, the increase in furfural could decrease cellulose conversion due to the furfural depress enzyme activity [15]. HMF is generated from degradation of glucose, mannose and galactose and formic acid is formed when furfural and HMF are broken down. In this study, HMF as inhibitor for the subsequent fermentation step was not detected in substantial level in any sample. In Table 2, the concentrations of inhibitors in the enzymatic hydrolyzate from pretreated rice husk were listed for different pretreatment methods. It was found that the enzymatic hydrolyzate from autoclave and NaOH pretreated rice husk contained low inhibitors, which are more suitable for subsequent fermentation process.

After the pretreatment and the enzyme saccharification of the pretreated rice husk, the fermentation of the hydrolyzate was performed. Maximum ethanol concentrations for dilute-NaOH, dilute-H_2SO_4 and autoclave pretreated rice husk were 6.22, 5.8 and 1.98 g/L, respectively. Maximum ethanol yields were 13.82, 12.88 and 4.4, for dilute-NaOH, dilute-H_2SO_4 and autoclave pretreated rice husk, respectively. These yields were equal to theoretical ethanol yields of 64.8, 60.41 and 20.63%, respectively. Maximum ethanol concentration, ethanol yield and theoretical ethanol yield of untreated rice husk were 1.01 g/L, 2.24 and 10.5%, respectively. These results showed that the highest ethanol concentration, ethanol yield and ethanol theoretical yield were obtained from NaOH pretreated rice husk. These values were 6.16 times higher than the control.

Table 3 gives ethanol yields as a ratio of gram ethanol to gram substrate for different pretreated substrates. In this work, according to results summarized in Table 3, high ethanol yield was obtained for pretreated rice husk. Moreover, several studies which applied alkali pretreatment for other substrates

TABLE 3. Effects of different pretreatment methods on the concentrations, yields and theoretical yields of ethanol for rice husk

Substrate	Pretreatment conditions			Yield (g eth/g sub)	Ref
	Concentration (%)	Time (min)	T (°C)		
Rice husk	3.0% NaOH	30	121	0.138	This study
Rice husk	0.3% H_2SO_4	33	152	0.060	[21]
Rice husk	1.0% H_2SO_4	15	120	0.11	[23]
Birch	7.0% NaOH	120	100	0.170	[39]
Wheat straw	2.15% H_2O_2	1440	35	0.230	[23]

obtained higher ethanol yields than that one obtained in this work (As shown in Table 3). It is self-explaining as the composition of substrate, chemical reagent and the pretreatment conditions were different.

The objectives of rice husk pretreatment were to improve the cellulose content, lignin partial removal and total glucose yield. The aim of pretreatment was to produce more glucose and xylose for the follow-up ethanol fermentation with low inhibitors. Totally, the dilute-NaOH pretreatment method was the most effective one due to high cellulose content, total glucose yield, lignin removal, glucose concentration, ethanol concentration and very low concentration of inhibitors. The total xylose concentration released from pretreated rice husk by dilute-NaOH was much lower than that one from the pretreated rice husk by dilute-H_2SO_4. In this study, for ethanol fermentation *Saccharomyces cerevisiae* was used which was unable to utilize pentose, the amount of xylose concentration was not important in this case. Results in the present study indicate that NaOH pretreated rice husk was efficiently used for ethanol production.

The dilute-NaOH solution effectively enhanced lignocellulosic digestibility by increasing internal surface area, decreasing the degree of polymerization and crystallinity of cellulose and separating structural linkages between lignin and carbohydrates [40]. Milet and his coworkers [41] reported that the digestibility of NaOH-treated hardwood increased with a decrease in lignin content. In another investigation carried out by Bjerre et al. [17], the possibility of enhancing the digestibility of wheat straw was assessed through application of NaOH pretreatment. Wang [19] studied the effect of NaOH pretreatment on the coastal Bermuda grass for enhancing reduced sugar recovery. He reported the highest reducing sugar yield reached up to 86% of theoretical yield for NaOH pretreatment [19]. Xu and his coworkers [42] evaluated different pretreatment methods on swichgrass and coastal Bermuda grass; they concluded that alkaline pretreatments resulted in 56.8 to

129% more sugar yields than acid pretreatments [42]. Zhang et al. [16] investigated the feasibility of converting NaOH-pretreated cattails into cellulosic ethanol. They found that the alkali pretreatment was effectively able to increase enzymatic digestibility of cattail cellulose; nearly 78% of the cellulose from raw cattails; which was converted to fermentable glucose. Also, they reported that about 55.9% of the lignin was removed with pretreatment in 4% NaOH [18].

3. 5. SEM Observation In addition to the changes in the composition of the pretreated solid residue, the physical structures present in the solid residue changed after pretreatment. In this study, the physical changes of pretreated rice husk after dilute-NaOH pretreatment was investigated. SEM images of raw rice husk and the pretreated rice husk are shown in Figure 5. For the raw rice husk, a smooth surface and compact structure is clearly observed in Figure 5a. The structure of rice husk after undergoing the pretreatment with diluted sodium hydroxide was significantly destroyed (Figure 5b). SEM observations indicates that the pretreatment include physical changes in biomass and removed external fibers so that cellulose becomes more accessible to enzymes.

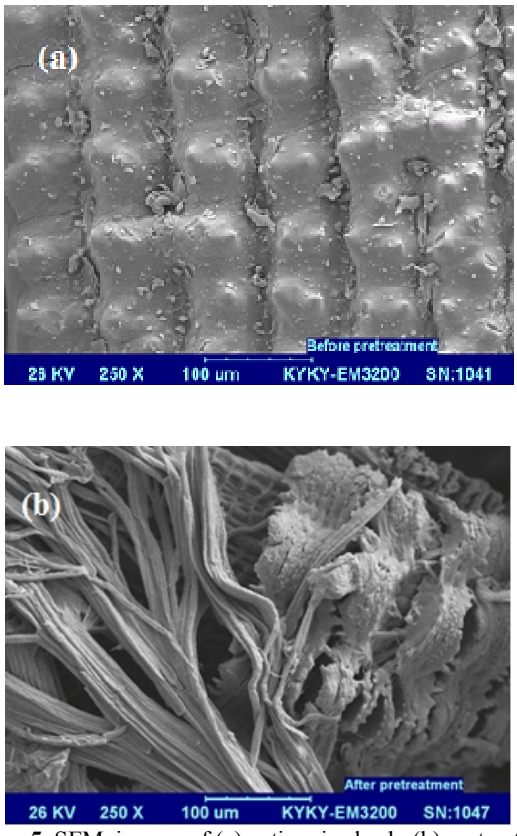

Figure 5. SEM images of (a) native rice husk: (b) pretreated rice husk

4. CONCLUSION

In the present work, rice husk pretreatment was successfully carried out for the enzymatic hydrolysis. Significant improvement in cellulose content, partial removal of lignin and total glucose yield were obtained. The aim of pretreatment was to produce more glucose and xylose for ethanol fermentation while minimizing formation of inhibitors. The dilute-NaOH pretreatment method was the most effective method due to high cellulose content, total glucose yield, lignin removal, glucose concentration, ethanol concentration and very low concentration of inhibitors. The total five carbon sugar concentration released from pretreated rice husk by dilute-NaOH was much lower than that of dilute-H_2SO_4 pretreated rice husk. Ethanol fermentation with *Saccharomyces cerevisiae* was successfully carried with hydrolyzed sugar. The maximum glucose concentration, glucose yield, ethanol concentration were 14.54 g/L, 59.6% and 6.22 g/L, which were 5.44, 3.77 and 6.15 times higher than the control, respectively. With suitable pretreatment method, some significant delignification, compositional and structural changes were found.

5. AKNOWLEDGEMENTS

Authors gratefully acknowledge Biotechnology Research Center, Babol Noshirvani University of Technology for facilitating the present research.

6. REFERENCES

1. Dalgaard, T., Jorgensen, U., Olesen, J. E., Jensen, E. S. and Kristensen, E.S., "Looking at biofuels and bioenergy", ***Science,*** Vol. 312, No. 5781, (2006), 1743-1744.
2. Binod, P., Satyanagalakshmi, K., Sindhu, R., Janu, K.U., Sukumaran, R. K. and Pandey, A., "Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse", ***Renewable Energy,*** Vol. 37, No. 1, (2012), 109-116.
3. Talebnia, F., Karakashev, D. and Angelidaki, I., "Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation", ***Bioresource Technology,*** Vol. 101, No. 13, (2010), 4744-4753.
4. Taherzadeh, M. J. and Karimi, K., "Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review", ***International Journal of Molecular Sciences,*** Vol. 9, No. 9, (2008), 1621-1651.
5. Yang, B. and Wyman, C. E., "Pretreatment: the key to unlocking low cost cellulosic ethanol", ***Biofuels, Bioproducts and Biorefining,*** Vol. 2, No. 1, (2007), 26-40.
6. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. and Crocker, D., "Determination of structural carbohydrates and lignin in biomass", ***Laboratory Analytical Procedure*** , No. NREL/TP-510e42618, (2008).

7. Talebnia, F., Pourbafrani, M., Lundin, M. and Taherzadeh, M., "Optimization study of citrus wastes saccharification by dilute acid hydrolysis". ***BioResources,*** Vol. 3, No. 1, (2008), 108-122.
8. Satari Baboukani, B., Vossoughi, M. and Alemzadeh, I., "Optimisation of dilute-acid pretreatment conditions for enhancement sugar recovery and enzymatic hydrolysis of wheat straw", ***Biosystems Engineering,*** Vol. 111, No. 2, (2011), 166-174.
9. Yu, Q., "Two-step liquid hot water pretreatment of- Eucalyptus grandis- to enhance sugar recovery and enzymatic digestibility of cellulose", ***Bioresource Technology,*** Vol. 101, No. 13, (2010), 4895-4899.
10. Carrillo, F., Lis, M., Colom, X., Lopez-Mesas, M. and Valldeperas, J., "Effect of alkali pretreatment on cellulase hydrolysis of wheat straw: Kinetic study", ***Process Biochemistry,*** Vol. 40, No. 10, (2005), 3360-3364.
11. Cao, W., Sun, C., Liu, R., Yin, R. and Wu, X., "Comparison of the effects of five pretreatment methods on enhancing the enzymatic digestibility and ethanol production from sweet sorghum bagasse", ***Bioresource Technology,*** (2012).
12. Kumar, P., Barrett, D. M., Delwiche, M. J. and Stroeve, P., "Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production", ***Industrial & Engineering Chemistry Research,*** Vol. 48, No. 8, (2009), 3713-3729.
13. Hendriks, A. and Zeeman, G., "Pretreatments to enhance the digestibility of lignocellulosic biomass", ***Bioresource Technology,*** Vol. 100, No. 1, (2009), 10-15.
14. Yang, B. and Wyman, C.E., "Pretreatment: the key to unlocking low cost cellulosic ethanol", ***Biofuels, Bioproducts and Biorefining,*** Vol. 2, No. 1, (2007), 26-40.
15. Feng, Y., Qi, X., Jian, H., Sun, R. and Jiang, J., " Effect of inhibitors on enzymatic hydrolysis and simultaneous saccharification fermentation for lactic acid production from steam explosion pretreated lespedeza stalks", ***BioResources,*** Vol. 7, No. 3, (2012), 3755-3766.
16. Zhang, J., Ma, X., Yu, J., Zhang, X. and Tan, T., "The effects of four different pretreatments on enzymatic hydrolysis of sweet sorghum bagasse", ***Bioresource Technology,*** Vol. 102, No. 6, (2011), 4585-4589.
17. Bjerre, A. B., Olesen, A. B., Fernqvist, T., Ploger, A. and Schmidt, A. S., "Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose", ***Biotechnology and Bioengineering,*** Vol. 49, No. 5, (2000), 568-577.
18. Zhang, B., Shahbazi, A., Wang, L., Diallo, O. and Whitmore, A., "Alkali pretreatment and enzymatic hydrolysis of cattails from constructed wetlands", ***American Journal of Engineering and Applied Sciences,*** Vol. 3, No. 2, (2010), 328-332.
19. Wang, Z., "Alkaline pretreatment of coastal bermudagrass for bioethanolproduction", ***Thesis,www.lib.ncsu.edu/resolver/1840.16/2836,*** (2009).
20. Ang, T. N., Yoon, L. W., Lee, K. M., Ngoh, G. C., Chua, A. S. M. and Lee, M. G., "Efficiency of ionic liquids in the dissolution of rice husk", ***BioResources,*** Vol. 6, No. 4, (2011), 4790-4800.
21. Shen, F., et al., "Evaluation of steam pretreatment on sweet sorghum bagasse for enzymatic hydrolysis and bioethanol production". ***Carbohydrate Polymers,*** Vol. 86, No. 4, (2011), 1542-1548.
22. Chen, M., Zhao, J. and Xia, L., "Comparison of four different chemical pretreatments of corn stover for enhancing enzymatic digestibility", ***Biomass and Bioenergy,*** Vol. 33, No. 10, (2009), 1381-1385.
23. Dagnino, E., Chamorro, E., Romano, S., Felissia, F. and Area, M., "Optimization of the acid pretreatment of rice hulls to obtain fermentable sugars for bioethanol production", ***Industrial Crops and Products,*** Vol. 42, No. (2013), 363-368.
24. Rabah, A., Oyeleke, S., Manga, S. and Hassan, L., "Microbial pretreatment of rice husk and groundnut shell for bioethanol production" (2011).
25. Saha, B. C. and Cotta, M. A., "Enzymatic saccharification and fermentation of alkaline peroxide pretreated rice hulls to ethanol", ***Enzyme and Microbial Technology,*** Vol. 41, No. 4, (2007), 528-532.
26. Banerjee, S., Sen, R., Pandey, R., Chakrabarti, T., Satpute, D., Giri, B.S. and Mudliar, S., "Evaluation of wet air oxidation as a pretreatment strategy for bioethanol production from rice husk and process optimization", ***Biomass and Bioenergy,*** Vol. 33, No. 12, (2009), 1680-1686.
27. Shen, J. and Agblevor, F. A., "Ethanol production of semi-simultaneous saccharification and fermentation from mixture of cotton gin waste and recycled paper sludge", ***Bioprocess and Biosystems Engineering,*** Vol. 34, No. 1, (2011), 33-43.
28. Sun, Y. and Cheng, J., "Hydrolysis of lignocellulosic materials for ethanol production: a review", ***Bioresource Technology,*** Vol. 83, No. 1, (2002), 1-11.
29. Silverstein, R. A., Chen, Y., Sharma-Shivappa, R. R., Boyette, M. D. and Osborne, J., "A comparison of chemical pretreatment methods for improving saccharification of cotton stalks", ***Bioresource Technology,*** Vol. 98, No. 16, (2007), 3000-3011.
30. Laser, M., Schulman, D., Allen, S. G., Lichwa, J., Antal Jr, M. J. and Lynd, L.R., "A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol", ***Bioresource Technology,*** Vol. 81, No. 1, (2002), 33-44.
31. Sreenath, H. K., Koegel, R. G., Moldes, A. B., Jeffries, T. W. and Straub, R. J., "Enzymic saccharification of alfalfa fibre after liquid hot water pretreatment", ***Process Biochemistry,*** Vol. 35, No. 1, (1999), 33-41.
32. Dien, B., Li, X. L., Iten, L., Jordan, D., Nichols, N., O™Bryan, P. and Cotta, M., "Enzymatic saccharification of hot-water pretreated corn fiber for production of monosaccharides", ***Enzyme and Microbial Technology,*** Vol. 39, No. 5, (2006), 1137-1144.
33. Alvira, P., Tomqs-Pejo, E., Ballesteros, M. and Negro, M., "Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review", ***Bioresource Technology,*** Vol. 101, No. 13, (2010), 4851-4861.
34. Palonen, H., Tjerneld, F., Zacchi, G. and Tenkanen, M., "Adsorption of *Trichoderma reesei* CBH I and EG II and their catalytic domains on steam pretreated softwood and isolated lignin", ***Journal of Biotechnology,*** Vol. 107, No. 1, (2004), 65-72.
35. Taherzadeh, M.J. and Karimi, K., "Acid-based hydrolysis processes for ethanol from lignocellulosic materials: A review", ***BioResources,*** Vol. 2, No. 3, (2007), 472-499.
36. Beukes, N. and Pletschke, B. I., "Effect of alkaline pre-treatment on enzyme synergy for efficient hemicellulose hydrolysis in sugarcane bagasse", ***Bioresource Technology,*** Vol. 102, No. 8, (2011), 5207-5213.
37. Lu, Y., Yang, B., Gregg, D., Saddler, J. N. and Mansfield, S. D., "Cellulase adsorption and an evaluation of enzyme recycle during hydrolysis of steam-exploded softwood residues", ***Applied Biochemistry and Biotechnology,*** Vol. 98, No. 1, (2002), 641-654.
38. Martin, C., Galbe, M., Nilvebrant, N. O. and Jonsson, L. J., "Comparison of the fermentability of enzymatic hydrolyzates of sugarcane bagasse pretreated by steam explosion using different impregnating agents", ***Applied Biochemistry and Biotechnology,*** Vol. 98, No. 1, (2002), 699-716.

39. Mirahmadi, K., Kabir, M. M., Jeihanipour, A., Karimi, K. and Taherzadeh, M. J., "Alkaline pretreatment of spruce and birch to improve bioethanol and biogas production", ***BioResources,*** Vol. 5, No. 2, (2010), 928-938.

40. Fang, L., Gharpuray, M. and Lee, Y., Cellulose hydrolysis biotechnology monographs. Berlin: Springer, (1987).

41. Millet, M. A., Baker, A. J., Scatter, L. D., "Physical and chemical pretreatment for enhancing cellulose saccharification", ***Biotechnology and Bioengineering Symposium,*** Vol. 6, No. (1976), 125-153.

42. Xu, J., Wang, Z., Sharma-Shivappa, R. R. and Cheng, J. J., "Enzymatic hydrolysis of switchgrass and coastal Bermuda grass pretreated using different chemical methods", ***BioResources,*** Vol. 6, No. 3, (2011), 2990-3003.

Comparative Studies on the Effect of Pretreatment of Rice Husk on Enzymatic Digestibility and Bioethanol Production

M. Nikzad, K. Movagharnejad, G. D. Najafpour, F. Talebnia

Faculty of Chemical Engineering, Noshirvani University of Technology, P.O. Box 484, Babol, Iran.

P A P E R I N F O

Paper history:
Received 06 November 2012
Recivede in revised form 28 November 2012
Accepted 13 December 2012

Keywords:
Rice Husk
Pretreatment
Enzymatic Hydrolysis
Bioethanol

چکیده

سه روش متفاوت پیش تیماری بر روی شلتوک برنج مورد بررسی قرار گرفته است. برای تعیین تأثیر هر یک از روش ها بر روی ترکیبات شلتوک برنج، هضم شلتوک برنج در آبکافت آنزیمی و میزان اتانول تولیدی، پیش تیماری با اسید سولفوریک رقیق (۱٪ حجمی، ۱۲۱ °C، ۳۰ دقیقه)، سود رقیق (۳٪ وزنی، 121 °C، 30 دقیقه)و پیش تیماری گرمایی (۱۲۱ °C، ۳۰ دقیقه) بر روی آن اعمال گردیدند. در این میان، بهترین نتیجه زمانی بدست آمد که پیش تیماری شلتوک برنج با محلول سود ۳٪ انجام شد. پیش تیماری شلتوک برنج با سود توانست بطور قابل ملاحظه ای حذف لیگنین، هضم آنزیمی سلولز، دسترسی به سلولز و تولید قندهای قابل تخمیر را افزایش دهد. بیشترین غلظت گلوکز، بازده گلوکز و غلظت اتانول به ترتیب ۱٤/٥٤ g/L، ٥۹/٦٪ و ٦/۲۲ g/L بدست آمدند که این مقادیر ٥/٤٤، ۳/۷۷ و ٦/۱٥ برابر بیشتر از مقادیر بدست آمده برای شلتوک برنج بدون پیش تیماری می باشند. علاوه بر این،آنالیز SEM از نمونه های پیش تیماری شده و بدون پیش تیماری، تغییرات فیزیکی قابل توجهی را در اثر پیش تیماری با سود نشان داده است

doi: *10.5829/idosi.ije.2013.26.05b.01*