Research Article

Structural Analysis of Alkaline Pretreated Rice Straw for Ethanol Production

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Rice straw (RS) is an abundant, readily available agricultural waste, which shows promise as a potential feedstock for Asian ethanol production. To enhance release of glucose by enzymatic hydrolysis, RS was pretreated with aqueous ammonia (27% w/w) at two pretreatment temperatures: room temperature and 60°C. Statistical analysis indicated similarity of enzymatic glucose production at both pretreatment temperatures after 3-day incubation. Chemical composition, FTIR, and EDX analyses confirmed the retention of glucan and xylan in the pretreated solid, but significant reduction of lignin (60.7% removal) and silica. SEM analysis showed the disorganized surfaces and porosity of the pretreated RS fibers, thus improving cellulose accessibility for cellulase. The crystallinity index increased from 40.5 to 52.3%, indicating the higher exposure of cellulose. With 10% (w/v) solid loadings of pretreated RS, simultaneous saccharification and fermentation yielded a final ethanol concentration of 24.6 g/L, corresponding to 98% of maximum theoretical yield. Taken together, aqueous ammonia pretreatment is an effective method to generate highly digestible pretreated RS for bioethanol production and demonstrates potential application in biorefinery industry.

1. Introduction

Ethanol production from lignocellulosic biomass has attracted considerable research attention because of a growing realization of the energy crisis and climate change [1, 2]. Rice straw (RS) is a by-product after rice grain collection. This lignocellulosic waste has been promoted as a potential feedstock for ethanol production in Thailand and other Asian countries due to its ready availability, abundance, and the fact that it is nonfood [3, 4]. This material is an excellent source of sugars for microbial fermentation owing to the presence of carbohydrates that are concentrated in the cell walls [5].

The RS cell walls constitute three biopolymers, namely, cellulose, hemicellulose, and lignin [5]. Cellulose is a homopolymer of cellobiose (two units of glucose), which are linked by β -1,4-glycosidic bonds. The complete hydrolysis of

cellulose yields glucose, which is a preferable carbon source for commonly used fermenting microorganisms in industry [6]. Hemicellulose contains a mixture of polysaccharides by which xylan represents a major portion. Xylan consists of xylose units, which are linked by β -1,4-glycosidic bonds to form xylan main chain. Typically, the main chain is substituted with some sugars (i.e., arabinose, galactose, and glucose), sugar acids (i.e., glucuronic acid), and ferulic and p -coumaric acids, depending on the source of xylans [6]. Lignin is a large complex polymer of unrepeatable phenolic monomers. It significantly contributes to the water conduction and defense systems in plants. However, the hydrophobicity and complex structure (also heterogeneity) of lignin pose challenges for biomass processing and utilization [6].

Production of sugars from RS by enzymatic reaction is of industrial interest because of mild-reaction conditions used and relatively pure product formation [1, 5]. However,

in bioethanol industry, the efficient utilization of lignocellulosic biomass is limited by its recalcitrant nature [5–7]. The chemical components (hemicellulose and lignin) and structural organization restrict the release of glucose from cellulose by enzymes. The rigid, highly ordered organization makes cellulose crystalline and insoluble, thus preventing enzymes and water molecules to access glycan chains [7]. In addition, the high degree of polymerization makes cellulose microfibrils long in length, which impedes enzymatic hydrolysis. Moreover, cellulose is covered with hemicellulose; the cellulose-hemicellulose complex is further sealed with lignin that provides structural integrity and prevention of microbial attack (enzymatic degradation) [6, 7].

Aqueous ammonia pretreatment is a widely studied method to alter the structure and reduce the recalcitrance of biomass for enzymatic hydrolysis [8–13]. This method has potential to be applied in the biorefinery industry because it separates biomass components into two main fractions: carbohydrates and lignin stream owing to the delignification selectivity. Different types of lignocellulosic materials, including Napier grass [8, 10], rye straw [9], poplar [11], corn stover [12], and switchgrass [13], have been tested with aqueous ammonia pretreatment, and those reports have addressed positive correlation of chemical composition change (particularly high rate of lignin removal) and enzymatic digestibility.

Understanding of both chemical components and molecular structure of biomass on enzymatic digestibility is important because these data can give direction of pretreatment efficiency development, enzyme improvement, and enzyme formulation that can overcome the pretreated substrate [7]. In this study, we pretreated RS with aqueous ammonia because this method can be performed at relatively low operating temperature (less than 100°C) and preserve carbohydrates in the pretreated solid. The retention of carbohydrates is one determinant for a successful pretreatment approach due to minimization of sugar loss. The ultrastructure of the pretreated RS was then investigated to reveal structural alteration and to give reasons for such improvements in enzymatic hydrolysis and ethanol production related to the physical changes.

2. Materials and Methods

We followed the methods of Phitsuwan et al. [14], unless otherwise stated.

2.1. Pretreatment. Aqueous ammonia pretreatment was performed as described earlier [14], with minor modifications. RS (*Oryza sativa*), which was collected from rice fields in Thailand, was immediately air-dried prior to any processing. The dried RS was cut into small pieces with a size of 1–2 cm using scissors and blended with a mechanical blender prior to pretreatment. The blended RS were pretreated with aqueous ammonia by immersion in 27% (w/w) ammonium hydroxide at a solid : liquid ratio of 1 : 12 at room temperature ($25 \pm 3^\circ\text{C}$) or $60 \pm 3^\circ\text{C}$ for 7 days. The pretreated solid was then retrieved from the liquid phase and washed with water until neutral to remove inhibitors/impurities. The pretreated solid was dried at 60°C and ground to a particle size of approximately

5 mm using a coffee grinding machine. This material was then used as a substrate for enzymatic hydrolysis and ethanol fermentation.

2.2. Enzymatic Hydrolysis. Enzymatic hydrolysis using commercial cellulase (Cellic Ctec2) and xylanase (Cellic Htec2) cocktails (Novozyme, Bagsværd, Denmark) was conducted as described previously [14]. The cellulase and xylanase doses of 15 FPU and 100 XU/g solid were loaded onto a 1.5% (w/v) solid at a final volume of 10 mL in a 50 mL Falcon tube. The hydrolysis reaction was performed at pH 4.8 using 50 mM citrate buffer, 50°C , and shaken at 200 rpm for 1 h, unless otherwise stated. The hydrolysis reaction was supplemented with sodium azide (0.2%) to prevent microbial growth. Hydrolysates were taken at appropriate times to analyze glucose concentration. The glucose concentration was determined using glucose assay kits (Megazyme).

2.3. Composition Analysis. The carbohydrate and lignin contents were determined according to the National Renewable Laboratory (NREL) Analytical Procedure [15]. In brief, the solid after extractives extraction was subjected to acid hydrolysis in an autoclave and the autoclave hydrolysis medium was vacuum-filtered and analyzed for lignin and structural carbohydrate contents. A sugar-containing solution was centrifuged and filtered through a $0.45\ \mu\text{m}$ syringe filter. This filtrate was then analyzed using a high-performance liquid chromatograph (HPLC; Shimadzu, Japan) equipped with a Bio-Rad Aminex HPX-87P (Bio-Rad Laboratories, CA) and a refractive index detector (Shimadzu RID-10A) to determine the carbohydrate content. The analytical column was operated at 85°C using HPLC-grade water as a mobile phase at a flow rate of $0.6\ \text{mL min}^{-1}$ and a run-time of 35 min. The lignin removal yield was calculated using the following equation [16]:

$$\begin{aligned} &\text{Lignin removal (\%)} \\ &= 1 \\ &\quad - \frac{\text{Lignin in the pretreated RS} \times \text{Recovered solid}}{\text{Lignin in the untreated RS}} \quad (1) \\ &\quad \times 100. \end{aligned}$$

2.4. Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDX). Scanning electron microscope (A JEOL JSM-6610LV, Tokyo, Japan) was used to observe the morphology of RS before and after pretreatment. The samples were gold-coated in a sputter coater prior to imaging.

Elemental analysis was performed using energy dispersive X-ray spectrometer (Oxford INCA350) coupled with SEM.

2.5. Fourier Transform Infrared (FTIR) Spectrometry. FTIR was used to analyze functional groups of the untreated and pretreated RS samples. The FTIR spectra of all samples were run on a Perkin-Elmer UATR Two (Waltham, MA, USA) spectrometer.

2.6. X-Ray Diffractometry. The crystallinity and surface property of untreated and pretreated RS samples were analyzed using an X-ray diffractometer (JEOL, JDX-3530, 2 kW, Tokyo, Japan) at a scan rate of $12^\circ \text{ min}^{-1}$ from 5° to 50° and the generator was operated at 30 kV and 30 mA. The crystallinity index (CrI) was calculated using the following equation:

$$\text{CrI (\%)} = \frac{I_{002} - I_{001}}{I_{002}} \times 100, \quad (2)$$

where I_{002} ($2\theta = 22^\circ$) is the peak intensity of crystalline peak and I_{001} ($2\theta = 16^\circ$) is the peak intensity of the amorphous zone in the chromatogram [17, 18].

2.7. Yeast Strain, Inoculum Preparation, and Fermentation Conditions. In this study, we followed the methods of Phitsuwan et al. [14] to evaluate ethanol fermentation efficiency. *Saccharomyces cerevisiae* Thermosac® DRY (Lallemand, GA) was used for ethanol fermentation. Active inoculums for fermentation were activated by culturing the yeasts in 50 mL of a medium containing 10 g/L yeast extract, 20 g/L peptone (YP medium), and 50 g/L glucose at 37°C and 200 rpm on a rotary shaker overnight. The cultures were centrifuged at $2,720 \times g$ and 4°C for 10 min to collect the cells, and the cell pellets (approximately 1.2–1.4 g wet weight) were resuspended with 10 mL of a fresh YP medium to be used in ethanol fermentation.

Ethanol fermentation by saccharification and fermentation (SSF) approach [14] was performed at a total volume of 200 mL. Pretreated RS was loaded into a 500 mL serum bottle at a concentration of 10% (w/v) solid loadings. The suspension of 1.2 g yeast cells in 10 mL YP medium and cellulase and xylanase enzymes at the dosages of 15 FPU and 100 XU/g solid were added to the reaction medium. The final reaction volume of 200 mL was adjusted with the YP medium. The bottle was then equipped with a rubber stopper, sealed with an aluminium cap, and perforated with a syringe needle for gas release. SSF was performed at 37°C and 200 rpm shaking speed for 96 h. The culture supernatant was periodically taken to determine the glucose and ethanol concentrations. The concentration of ethanol was analyzed using a gas chromatography (model GC-2014; Shimadzu) with a flame-ionization detector (FID).

SSF with prehydrolysis was performed by which the enzymatic hydrolysis was carried out at 50°C for 3, 6, and 16 h. After that, the medium temperature was decreased to 37°C and the suspension of yeasts was added. The time of yeast addition was considered as time 0. The fermentation conditions were conducted as described above.

The fermentation efficiency was calculated using the following equations:

$$\text{FE (\%)} = \frac{\text{Ethanol titer}}{\text{Glucose}_{\text{TG}} \text{ in biomass} \times 0.51} \times 100, \quad (3)$$

where ethanol titer refers to the overall amount of ethanol (g), $\text{glucose}_{\text{TG}}$ is the theoretical glucose in the biomass, respectively, and 0.51 is the conversion factor of glucose to ethanol.

FIGURE 1: Effects of temperature and incubation time on glucose production from pretreated RS after 1 h of enzymatic hydrolysis (hydrolysis conditions: 1.5% solid loading, 15 FPU and 100 XU/g solid, pH 4.8, and 50°C). Different letters within bars indicate significant differences ($p < 0.05$) between the means of glucose concentrations as analyzed by Tukey's test of multiple comparisons after ANOVA test. Each bar is the mean value of three experiments ($n = 3$). Error bars represent standard error.

2.8. Statistical Analysis. Statistical analysis was performed using JMP13 statistical software (SAS Institute Inc.). The one-way analysis of variance (ANOVA) was carried out with a significant level of 95% ($p < 0.05$). For multiple comparisons, one-way ANOVA followed by Tukey's test ($p < 0.05$) was performed to check the difference between treatment means.

3. Results and Discussion

3.1. Aqueous Ammonia Pretreatment and Enzymatic Hydrolysis. In the previous study, we found that aqueous ammonia pretreatment of RS at room temperature was effective for lignin removal and enhanced biomass digestibility [14]. For industrial application, a large-volume pretreatment tank can be constructed and stands without protection and temperature control. As a result, a variation of temperature may occur during the day, and this variation may affect the pretreatment efficiency.

In Thailand, the temperature during day and night times generally ranges from 25 to 35°C . However, during daytime, the temperature inside the pretreatment reactor can reach to 60°C . Thus, the effect of pretreatment temperatures at room temperature (25°C) and 60°C and incubation time were studied and assessed for their impacts on glucose yield. The glucose concentrations by pretreatments at room temperature and 60°C for 3 to 7 days ranged from 1.43 to 1.72 g/L and 1.24 to 1.65 g/L, respectively (Figure 1). Interestingly, the impacts of pretreatment temperatures and incubation times

TABLE 1: Recovery of total solids, glucan, xylan, and residual lignin contents in untreated and pretreated RS.

Pretreatment method	Solid recovery (%)	Glucan (%)		Xylan (%)		Lignin (%)	
		Content	Recovery	Content	Recovery	Content	Removal
Untreated	100	32.2	—	12.4	—	21.1	—
aRS	68.0	44.3	93.6	13.3	72.9	12.2	60.7

FIGURE 2: SEM micrographs: (a) untreated RS and (b) aRS.

did not result in significantly different glucose production ($p > 0.05$) after 3-day incubation, as indicated by Tukey's test. Additionally, time courses for overall hydrolysis, reaching the plateau yield after 3 days, of different pretreated substrates were similar and the sugar yields from different pretreated substrates were comparable (around 3 g/L) (not shown). This phenomenon occurred possibly due to the high concentration of aqueous ammonia used. The similarity of glucose yield at either room temperature or 60°C after 3 days by the pretreatment may imply the homogeneity of biomass properties. To further investigate the structural change induced by aqueous ammonia pretreatment, the pretreated RS at 60°C for 7 day was selected and designated as aRS thereafter, for further topographical characterization.

3.2. Composition and SEM Analyses. In this study, we focused on compositional changes of carbohydrates and lignin because they are represented as the major components in RS. Table 1 shows the compositions between untreated RS and aRS. The untreated RS contained 32.2% cellulose. After pretreatment, the cellulose content increased by 12.1%. Xylan content likely remained unchanged, increasing from 12.4 to 13.3%. The lignin content decreased from 21.1 to 12.2%. The reduction of lignin was due to the cleavage of ester linkages between carbohydrates and lignin, which lead to lignin solubilization [8, 11, 12]. The major loss of lignin contributed to an increase of carbohydrates by gram of the aRS solid.

The actual change of the carbohydrates and lignin was determined on the basis of the untreated initial values. In this study, the recovery of insoluble solid after pretreatment yielded 68.0%. The recoveries of glucan and xylan yielded 93.6% and 72.9% based on initial glucan and xylan contents, respectively. The removal of lignin was 60.7%. These values were in ranged with previous report [19].

We next sought to observe the morphological alteration of aRS using SEM. Figure 2(a) shows the well-organized and intact surface of the untreated RS. The smooth surfaces indicated the lignin coverage on the fibers. After pretreatment, the surface of aRS became rough and disorganized (Figure 2(b)). The highly distorted structure could be the easily hydrolysable lignocellulose parts. The creation of porosity and the disintegration of joined fibrous matrix occurred as a result of lignin solubilization. This modified structure with an increase of external surface area enhances enzymatic hydrolysis of aRS.

3.3. FTIR Analysis. FTIR analysis was performed to investigate the chemical and structural changes in RS after pretreatment. The assignment of FTIR peaks corresponding to the functional groups of lignocellulosic components according to literatures [20–24] is listed in Table 2. It was obvious that the FTIR spectra between the untreated and aRS samples showed clear differences in terms of intensity and shape (Figure 3). The broadband at 3400–3200 and the signal at 2910 cm^{-1} have been assigned as the O-H stretching of hydrogen bonds and C-H bonds, respectively. These bands are sensitive to characteristic features of cellulose [23]. The decrease in absorption of O-H vibration (3350 cm^{-1}) in aRS suggests some rupture of the hydrogen bonds of cellulose structure [24]. The signal around 1720 cm^{-1} corresponding to the C=O functional group is a characteristic peak of ester-linked acetyl, feruloyl, and p -coumaroyl groups between hemicelluloses and lignin [22]. The near absence of this peak indicates removal of lignin through ester bond cleavage by the pretreatment. The signal observed at approximately 1432 cm^{-1} corresponds to CH_2 bending of cellulose [22]. The characteristic lignin peaks at 1320 (C-O of syringyl) and 1268 (C-O of guaiacyl ring) cm^{-1} [12] have significantly reduced.

TABLE 2: Assignment and description of peaks corresponding to functional groups of biomass components by FTIR analysis.

Wave number (cm^{-1})	Functional group assignment	Related biomass component
3400–3200	O-H stretching (hydrogen bond)	Cellulose
2910	C-H stretching	Cellulose
1720	C=O stretching	Hemicellulose/lignin
1650–1515	C=C aromatic skeletal vibration	Lignin
1465	C-H deformation	Lignin
1432	CH_2 bending	Cellulose
1375	C-H deformation	Cellulose
1320	Syringyl ring C-O stretching	Change in lignin monomer
1268	Guaiacyl ring C-O stretching	Change in lignin monomer
900	C-H out of plane	Cellulose

FIGURE 3: FTIR spectra of untreated RS and aRS.

FIGURE 4: XRD patterns of untreated RS and aRS.

The signal at 900 cm^{-1} is also attributed to β -1,4-glycosidic linkages, revealing the typical structure of cellulose [25]. These results are consistent with the chemical composition analysis and confirm the reduction in lignin and the increase in cellulose contents after pretreatment.

3.4. XRD Analysis. CrI is the proportion of crystalline material in lignocellulosic biomass and indicates the exposure of crystalline fraction of biomass surface. The X-ray diffractograms of untreated RS and aRS exhibited two major reflections at 2θ around 22° and 16° , representing crystalline cellulose and amorphous compounds, respectively [17, 18] (Figure 4). Compared to untreated RS, an increase in peak height at 22° was clearly observed in aRS, suggesting the higher exposure of cellulose [18]. A broad hump centered around 16° marginally increased in aRS, possibly due to the presence of amorphous cellulose. The CrIs of untreated RS and aRS were 40.5 and 52.3%, respectively. The increase in CrI is proportional to the increase of crystalline cellulose in the total solid, as a large portion of amorphous lignin was removed. Similar results of the increase in CrI have been reported earlier for pretreatment of lignocellulosic biomass with alkali [12, 22, 26].

3.5. EDX Analysis. Rice plant takes up nutrients and metals from water and soil during its growth. Metal elements, that is, Ca, Mg, and Si, have been shown to have negative or positive effect on enzymatic activity. Therefore, metal ions in the RS samples were analyzed using EDX technique to provide useful information for biomass conversion. The EDX results indicated that untreated RS contained carbon (41.81%), oxygen (49.02%), and silica (9.15%) as dominant compositions (Figure 5). The carbon and oxygen atoms typically originated from the natural fiber. The relatively high content of silica was commonly found in RS, and the value of 9.15% was consistent with other reports [27, 28]. After pretreatment, the EDX spectra showed higher percentage of carbon than oxygen, which may reflect the cellulosic component [29]. The silica content decreased about 3-fold, indicating that the pretreatment affected the silica layers. Possibly, the reduction of silica may be associated with lignin removal by pretreatment, as silica is complexed with lignin moieties in rice plant [28]. The silica-lignin matrix hinders cellulase reaction. Thus, the reduction of this matrix is beneficial for enzymatic hydrolysis because of the more exposure of cellulose surfaces for cellulases to hydrolyze.

FIGURE 5: EDX profiles and elemental compositions of (a) untreated RS and (b) aRS.

3.6. Ethanol Fermentation. Batch fermentation of aRS was studied through SSF with high feedstock loading. The high solid loading is important to increase concentration of sugar and, in turn, ethanol yield. However, in this study, the solid loading was limited to 10% charges due to the bulky property of aRS and low water content in the system that increased the viscosity and reduced enzymatic efficiency. Although the yeast *S. cerevisiae* Thermosac DRY used in the present work is a nonxylose utilizing strain, an excess of xylanases Cellic Htec2 was combined with cellulase Cellic Ctec2 in order to reduce the physical barrier of xylan on cellulose accessibility to cellulase enzymes and avoid limitation of cellulose degrading activity of the enzymes.

With cellulase and xylanase dosages of 15 FPU and 100 XU/g solid, the SSF fermentation using *S. cerevisiae* produced a final ethanol concentration of 24.6 g/L after 48 h, which corresponded to 98% of maximum theoretical yield based on glucan content (Figure 6: nonprehydrolysis). The value of the theoretical ethanol yield in this study is comparable to previous report on SSF with *S. cerevisiae* [30, 31].

Several factors, including substrate feature, pretreatment method, property of yeast, and fermentation process parameter, affect ethanol fermentation yield. In this study, the possible explanations for the high theoretical yield of ethanol are addressed as follows.

(1) **Yeast Culture.** The yeast *S. cerevisiae* was activated to the exponential growth phase prior to SSF and the relatively high amount of yeast cells was used, thus resulting in no

FIGURE 6: Concentrations of glucose and ethanol produced by SSF without/with prehydrolysis at 3, 6, and 16 h (SSF conditions: 10% solid loadings, cellulase and xylanase dosages of 15 FPU and 100 XU/g solid, and 37°C). Glu is short for glucose.

lag phase over 96 h SSF (Figure 6) and improving ethanol yield [30, 32]. In addition, the yeast strain has been reported to tolerate towards fermentation inhibitors and efficiently ferments glucose to ethanol [33].

(2) *Pretreated Substrate and Substrate Loading.* The pretreatment method generated a highly digestible fraction of cellulose as evident in the present study (lower in physicochemical barriers, i.e., reduction in lignin and lignin-silica layers and more exposure of cellulose). The pretreated substrate used for SSF was washed with water for several times after pretreatment, thereby lowering concentration of inhibitors, which can reduce yeast performance. In terms of substrate loading, the 10% loadings of the pretreated substrate allow mass transfer, making the system less viscous [32]. Thus, this substrate loading enables the diffusion of enzymes and hydrolysis products and may not decrease the fermentation capability of the yeast.

(3) *Enzyme Preparation.* The choice of the enzymes used in SSF was cellulase cocktail Cellic CTec2, in a combination with hemicellulase Cellic Htec2. These enzyme cocktails are an advanced mixture of aggressive cellulases, β -glucosidases, and xylanases developed for industrial use [34]. The enzyme cocktails have demonstrated high activity towards lignocellulosic substrates [34]. Therefore, the synergistic interactions of pretreatment, substrate features, enzymes, and yeast culture used could enhance ethanol yield in the SSF system.

Prehydrolysis refers to a period of enzymatic hydrolysis time prior to SSF. This step is to provide optimum conditions for enzymatic reaction to generate sugar supply before yeast inoculation in the SSF system. This step is expected to increase liquid volume in the system, thus improving ethanol production rate by yeast [35, 36]. In this study, the prehydrolysis times of 3, 6, and 16 h were tested to find the suitable time for inoculating yeasts, and their ethanol yields were compared with that by the SSF alone (Figure 6). The initial glucose concentrations increased according to increasing prehydrolysis time. After yeast inoculation, the glucose concentrations substantially decreased from the initial values: 42.0, 49.7, and 54.1 g/L for 3, 6, and 16 h prehydrolysis, respectively, and appeared zero after fermentation for 24 h. On the other hand, the glucose concentration was not observed in the conventional SSF. This was possibly because the yeast fermentation rate was faster than enzymatic hydrolysis rate under these conditions.

The ethanol concentrations increased rapidly within 24 h and remained steady thereafter in any modes tested. The sudden increase of ethanol indicated adequate supply of glucose for the exponential growth phase of yeasts. The final ethanol concentrations after 48 h were 24.6, 25.1, 24.7, and 24.5 g/L for SSF and SSF with prehydrolysis for 3, 6, and 16 h, respectively (Figure 6). Interestingly, the ethanol production rate and the ethanol concentrations from the conventional SSF and the SSFs with different prehydrolysis times were comparable. This phenomenon was consistent with earlier reports by which the prehydrolysis of pretreated barley and corn stover at 10% solid loadings prior to SSF did not affect the final ethanol yields [35, 36]. However, it was clearly observed that prehydrolysis significantly liquefied the insoluble solids, thus reducing the solid volume and viscosity in the reactor (Figure 7). This demonstrated the potential to increase the final ethanol concentration in the SSF by adding

FIGURE 7: Appearance of 10% (w/v) aRS loadings without/with prehydrolysis at 3, 6, and 16 h.

more fermentable sugars (solid loadings) through fed-batch mode system.

4. Conclusion

Preparation of RS for ethanol production with aqueous ammonia pretreatment at room temperature and 60°C gave similar glucose yield. Results of SEM, FTIR, XRD, and EDX indicated the reduction of lignin and silica layers after pretreatment, thus increasing the exposure of cellulose surface for enzymatic hydrolysis. Ethanol production by SSF with 10% (w/v) solid loadings yielded a final ethanol concentration of 24.6 g/L, corresponding to 98% efficiency. Although prehydrolysis prior to SSF did not improve ethanol production rate and overall ethanol yield under conditions studied, it obviously increased free volume in the reactor, thus allowing the addition of more feedstock. Therefore, for further development, prehydrolysis can be combined with fed-batch operation of SSF in order to increase the final ethanol concentration, thus making ethanol process more economic.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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