Carbohydrate Polymers 83 (2011) 720–726

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Comparison of alkaline pulping with steam explosion for glucose production from rice straw

Maha M. Ibrahim[a,*], Waleed K. El-Zawawy[a], Yasser R. Abdel-Fattah[b], Nadia A. Soliman[b], Foster A. Agblevor[c]

[a] National Research Center, Cellulose and Paper Department, El-Tahrir St., Dokki, Cairo, Egypt
[b] Genetic Engineering and Biotechnology Research Institute, Mubarak City for Scientific Research and Technology Applications, Universities and Research Institutes Zone, 21934 New Burg El-Arab City, Alexandria, Egypt
[c] Virginia Polytechnic Institute and State University, Department of Biological Systems Engineering, 213 Seitz Hall, Blacksburg, VA 24061, USA

ARTICLE INFO

Article history:
Received 5 August 2010
Accepted 17 August 2010
Available online 24 August 2010

Keywords:
Agricultural residues
Rice straw
Pretreatment process
Alkaline pulping
Steam explosion
Glucose production

ABSTRACT

Agricultural residues, such as rice straw, are renewable, largely unused, and abundantly available resources. They contain cellulose and hemicellulose, which could be used to produce ethanol and many other value-added products. The current research investigates the utilization of rice straw as a lignocellulosic biomass feedstock to produce a value-added product. Investigation was carried to convert the rice straw into glucose which can be further fermented to produce ethanol. Different pretreatment methods, such as chemical pretreatment process using alkaline pulping and steam explosion were applied in this study to pretreat the lignocellulosic biomass. A Spezyme CP® cellulase enzyme was used in the experiment to hydrolyze the pretreated material into glucose. The total reducing sugars produced from the enzymatic hydrolysis of cellulose was measured by the dinitrosalicylic acid (DNS) method. The data from the enzyme hydrolysis time study were analyzed to provide information on enzyme hydrolysis rates. The results indicated that 28.9–58.4 g/L of glucose can be produced from rice straw depending on the pretreatment method.

1. Introduction

Research in utilization of lignocellulosic plant materials for bioethanol production has grown significantly over the last few decades as the depletion of non-renewable fuels and increasing greenhouse gas emissions continue to create an increasing need for an alternative non-fossil transportation fuel. Agricultural residues, such as rice straw, are natural lignocellulosic materials. Lignocellulosic materials are renewable, largely unused, and abundantly available sources of raw materials for the production of fuel ethanol. They can be obtained at low cost from a variety of resources, e.g. forest residues, municipal solid waste, waste paper, and crop residue resources (Cheng & Zhu, 2008). These materials predominantly contain a mixture of carbohydrate polymers (cellulose and hemicellulose), lignin, extractives, and ashes. The term "holocellulose" is often used to describe the total carbohydrate contained in a plant or microbial cell. Holocellulose is therefore comprised of cellulose and hemicellulose in lignocellulosic materials. Sugars polymerized in form of cellulose and hemicellulose can be liberated by hydrolysis and subsequently fermented to ethanol by microorganisms (Millati, Niklasson, & Taherzadeh, 2002; Palmqvist & Hahn-Hägerdal, 2000).

Pretreatment of lignocelluloses is intended to disorganize the crystalline structure of macro- and microfibrils, in order to release the polymer chains of cellulose and hemicellulose, and/or modify the pores in the material to allow the enzymes to penetrate into the fibers to render them amenable to enzymatic hydrolysis (Galbe & Zacchi, 2002). Pretreatment should be effective to achieve this goal, avoid degradation or loss of carbohydrate, and avoid formation of inhibitory by-products for the subsequent hydrolysis and fermentation; obviously, it must be cost-effective (Sun & Cheng, 2002).

Steaming with or without explosion (autohydrolysis) is one of the popular pretreatment methods of lignocellulosic materials. Steam pretreatment removes the major part of the hemicellulose from the solid material and makes the cellulose more susceptible to enzymatic digestion. In this method the biomass is treated with high pressure steam. The pressure is then swiftly reduced, in steam explosion, which makes the materials undergo an explosive decompression. Steam explosion is typically initiated at a temperature of 160–260 °C for several seconds to a few minutes before the material is exposed to atmospheric pressure (Kurabi et al., 2005; Sun & Cheng, 2002; Varga, Reczey, & Zacchi, 2004).

* Corresponding author. Tel.: +20 124436805.
E-mail address: mwakleed@hotmail.com (M.M. Ibrahim).

0144-8617/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2010.08.046

On the other hand, chemical pulping methods attempt to dissolve the lignin with a minimum of dissolution or degradation of the cellulose and hemicellulose components of the fibers under high temperature and pressure. Historically, the most successful approaches to achieve these goals have employed NaOH (the soda process), SO_3^{2-} (the sulfite process), and alkaline S^{2-} (the kraft process) (Rojas & Hubbe, 2004).

The present study was conducted to compare alkaline pulping (soda process), as the one used in Egyptian pulp mills for rice straw, and steam explosion as alternative pretreatment methods for ethanol production from starchy materials. The agricultural residue was subjected to the pretreatment then followed by enzymatic hydrolysis. The results of the glucose concentration in the culture sample were determined using enzymatic colorimetric method.

2. Experimental

2.1. Materials and chemicals

Rice straw, as an Egyptian agricultural residue, was used as a source for the lignocellulosic material. A Spezyme CP® cellulase enzyme preparation (Lot #301-03195-109, Genencor, International, Rochester, NY) was used for the enzymatic hydrolysis for glucose production.

2.2. Pretreatment

2.2.1. Steam explosion and pulping process

The steam explosion and alkaline pulping of the rice straw were carried out as a pretreatment process. Steam explosion of the agricultural residue sample was carried out in a 25-L batch reactor located at the Thomas M. Books Forest Products Center, Blacksburg, VA. The procedure reported by Jeoh and Agbelvor (2001) was used in this study. About 1 kg of agricultural waste sample were weighed and loaded into a 25-L batch steam explosion chamber and the ball valve was closed. Saturated steam was admitted into the chamber, and the biomass temperature was raised to 220 °C. When the material attained the reaction temperature, timing of the reaction was started. For this experiment, 240 s was used for the reaction. At the end of the allotted steaming time, the valve was opened for the "explosive depressurization" to occur. The steam-exploded material shot through the connecting piping and collected in the collection bin. The product came out in a sludge form and the fibers were bagged, weighed and stored in a cold room.

The pulping was done to the same waste, i.e. rice straw, on an electrically heated rotatory autoclave located at the National Research Center, Egypt, and the conditions were as follows: total chemical as NaOH was 10% (wt/wt), and liquor to fiber ratio was 6:1. The fibers were cooked at 170 °C for 2 h. After cooking, the pressure was released to atmosphere. The pulped fiber was washed with water until neutrality, then air-dried.

2.2.2. Water and alkali extraction

The steam-exploded fiber (10 g, oven dry fiber, odf) was extracted with water at 80 °C for 1 h using fiber to water ratio of 1:10. The mixture was vacuum filtered in a Buchner funnel and then washed with 500 mL water. The water-extracted fiber was further extracted with 20 wt.% sodium hydroxide (NaOH) at 80 °C for 1 h using a fiber to liquor ratio of 1:10. The mixture was filtered and the alkali-extracted water was collected and the residue was washed with water until the pH was neutral. The alkali-extracted fiber was air-dried at ambient laboratory conditions and stored in a cold room.

2.3. Compositional analysis of the steam exploded and pulped material

The holocellulose (TAPPI T257 om-85), Klason lignin (TAPPI T222 om-88), and ash content (TAPPI T211 om-85) of the steam-exploded and pulped fiber samples were determined and collected in Table 1.

2.4. Fiber characterization

2.4.1. FTIR spectra

Fourier transform infrared (FTIR) spectroscopy was used to evaluate the fiber resulting from alkaline pulping and steam explosion. Also, it was used to assess the crystallinity index (C.I.) of the fibers according to Hulleman, Vanhazendonk, and Vandam (1994) and Wistara, Zhang, and Young (1999), which is based on the ratio of the absorbance of the bands at 1429 (CH_2 scissoring) and 893 cm^{-1} ($C_{(1)}$ group vibration).

The FTIR spectra of steam exploded and alkaline pulping rice straw were performed using a Thermo-Nicolet Model 670 Instrument (Thermo Electron, Inc., Madison, WI).

2.4.2. Scanning electron microscopy

Scanning electron microscopy (SEM) was used to investigate the morphology of the different types of fibers, i.e. steam exploded and alkaline pulped rice straw using a JEOL JXA-840 A electron microprobe analyzer (JEOL USA Inc., Peabody, MA). The specimens for the fibers were coated with gold/palladium and observed using an applied tension of 30 kV.

2.5. Hydrolysis

Enzymatic hydrolysis is the most promising alternative to the use of dilute acid, but it is certainly not a replacement process. We aimed to develop an optimum condition of using the enzyme for the pretreated agricultural residues to convert the resulted cellulose into glucose. The condition is to adjust the temperature, pH, and time of incubation. The total reducing sugars produced from the enzymatic hydrolysis of cellulose was measured by the dinitrosalicylic acid (DNS) method.

Table 1
Chemical analysis for raw material and both steam-exploded and pulped rice straw fibers.

Raw material and unbleached steam exploded and pulped fibers	Severity parameter[a]	Temperature (°C)	Retention time (min)	Holocellulose (%)	α-Cellulose (%)	Lignin (%)	Ash (%)	C.I.
Rice straw raw material	–	–	–	68.09	38.83	14.55	16.34	–
Steam rice straw	4.15	220	4	50.74	44.95	23.84	22.75	2.33
Alkali-extracted steam-exploded rice straw	–	80	120	86.90	78.37	4.20	12.00	–
Pulped rice straw	–	170	120	71.61	58.37	10.32	13.70	2.00

[a] The severity parameter is a relation between the temperature and the retention time.

2.5.1. Enzyme hydrolysis time study

Samples of rice straw, at different pretreatments, i.e. steam explosion, alkaline pulping, and steam explosion-alkali extraction, were selected for an initial study of enzyme hydrolysis. The materials were either steam-exploded or pulped according to the same experiment as described above.

A Spezyme CP® cellulase enzyme preparation (Lot #301-03195-109, Genencor, International, Rochester, NY) was used for the hydrolysis. The pretreated fibers (10 g odf) in 100 mL sodium acetate buffer at pH 4.7 were sterilized first at 121 °C for 20 min. 2.0 mL of Spezyme® (64 FPU/mL) was added after cooling to the above samples. The mixture was incubated in a shaker bath at 50 °C and 75 rpm for 4 days. After a reaction time of 2, 3 and 4 days, the reaction was stopped by placing the sample in a boiling beaker of water for 5 min. The mixture was vacuum filtered and the glucose concentration was analyzed using the enzymatic colorimetric method. A reference for pure cellulose was used to compare for the waste material.

2.5.2. Enzyme hydrolysis calculations

Data from the enzyme hydrolysis time study were analyzed to provide information on enzyme hydrolysis rates. Enzyme hydrolysis rates were computed as concentration of glucose released per hydrolysis time:

$$v = \frac{dS}{dt} = \frac{\mathrm{Glu}_t - \mathrm{Glu}_0}{t - t_0} \qquad (1)$$

where v = enzyme hydrolysis rate (mg/mL glucose per hour), Glu_t = concentration of glucose at time t (mg/mL), Glu_0 = initial glucose concentration at time = 0 h (mg/mL), t = hydrolysis time (h), and t_0 = time = 0 h.

3. Results and discussion

3.1. Fiber composition and SEM

A common feature of the enzymatic hydrolysis step is the need for pretreatment of the lignocellulosic material resulting in a more efficient reaction despite the recalcitrant nature of the plant cell wall (Himmel et al., 2007). The effect of the pretreatment has been described as a disruption of the cell wall matrix including the connection between carbohydrates and lignin, as well as depolymerizing and solubilising hemicellulose polymers (Ramos, 2003). Pretreatment is also able to change the degree of cellulose crystallinity (Chang & Holtzapple, 2000).

Steam explosion was selected as the processing technology because it requires little or no chemical input and thus is environmentally benign relative to other technologies. Both the steam exploded and the pulped rice straw materials were coarse, powdery, and had dark-brown color. These color changes during the steam and pulping pretreatment could have resulted from the degradation of the cell wall components and extractives (Sun, Xu, Sun, Fowler, & Baird, 2005).

The chemical characterization revealed the proportion of each component of the fibers from agricultural residues. As seen in Table 1, the alkaline pulping treatment results in a higher holocellulose and lower lignin compared to the raw material, while for steam explosion the holocellulose is lower compared to the raw material and this can be explained due to the higher lignin content after steam explosion which may be due to the re-condensation of the lignin during the steam explosion process. On the other hand, the comparison between the two pretreatment methods indicated that the main effect of the hydrothermal pretreatment on the composition of the biomass is the partial but substantial removal of hemicelluloses, where the results seen in Table 1 indicated that the holocellulose for the unbleached alkaline pulping rice straw was higher than those of the steam-exploded rice straw. The steam explosion technology involves short time cooking of biomass feedstock at high temperature and pressure in saturated steam. Following the cooking, the digester is discharged by a fast decompression. This process can be characterized as a thermo-mechanochemical process in which hydrolytic reactions as well as mechanical forces acting in the decompression step lead to the massive liberation of fibers. The main chemical reactions that occur during the process are the cleavage of easily hydrolyzed glycosidic bonds, cleavage of some ether bonds in lignin, and cleavage of lignin–carbohydrate linkages. In addition, the steam explosion process induces, as mentioned above, a re-condensation of the lignin onto the fibers, as shown from the higher lignin content for the steam-exploded rice straw (Table 1), and hence a higher ash content. During the alkaline pulping, the lignin dissolved and separated in the black liquor.

This means that, for steam explosion, the treatment depolymerizes and degrades hemicellulose while most of the crystalline structure of the cellulose is preserved. After delignification of the pretreated material, less Klason lignin can be detected.

Steam-exploded rice straw was also investigated microscopically. Steam explosion is considered one of the most promising pretreatment technologies. Fig. 1A and B shows the SEM images of steam-exploded rice straw under various magnifications. Fig. 1A clearly shows the shape and size distribution of the fibers in steam-exploded rice straw at severity of 4.15. Well-separated fibers can be seen with an average diameter of 5.114 μm. The SEM image (Fig. 1B) of one individual fiber at larger magnification shows many holes on the surface of steam-exploded rice straw. This is probably due to the removal of some fibrous layers. In a previous work (Ibrahim, Agblevor, & El-Zawawy, 2010) we proposed that these changes to the steam-exploded fibers result from the removal of very reactive amorphous cellulose on the surface.

Moreover, the most apparent effect of the alkaline pulping pretreatment apart from a color change from yellow into dark brown is the defibration, or separation of individual fibers and cell types of rice straw. The pretreated material is quite heterogeneous and contains larger pieces, which are easily recognized (Fig. 1C). When looking more closely at the pretreated fibers it becomes apparent that the surface appeared smooth and was covered with deposits (Fig. 1D). No holes or cracks were seen in the fibers, but semispherical formations appear to be emerging from the cell wall (Fig. 1D). Selig et al. (2007) hypothesized that the observed droplet formations evolve from the lignocellulosic matrix during pretreatment, potentially depositing back onto the cell wall surface. In addition, Simola, Malkavaara, Alen, and Peltonen (2000) and Simola-Gustafsson, Hortling, and Peltonen (2001) mentioned that on the basis of previous guidance from the pulp and paper industry it can be hypothesized that these droplets originate from lignin present in the untreated biomass.

Generally, pretreatment had an effect on the overall structure of the pretreated material apart from a change in color; the fibers were partially defibrated in the alkaline pulping (Fig. 1C), presumably due to the hemicellulose content of the middle lamella (Donaldson, Hague, & Snell, 2001), and well separated in the steam explosion (Fig. 1A). However, upon closer observation, the surface of the individual fibers had changed drastically. The uneven surface now appeared smooth after alkaline pulping, while it showed holes and cracks upon steam explosion.

3.2. FTIR spectroscopic analysis

FTIR spectroscopy was used as an analytical tool to qualitatively determine the chemical changes in the surface of pretreated agri-

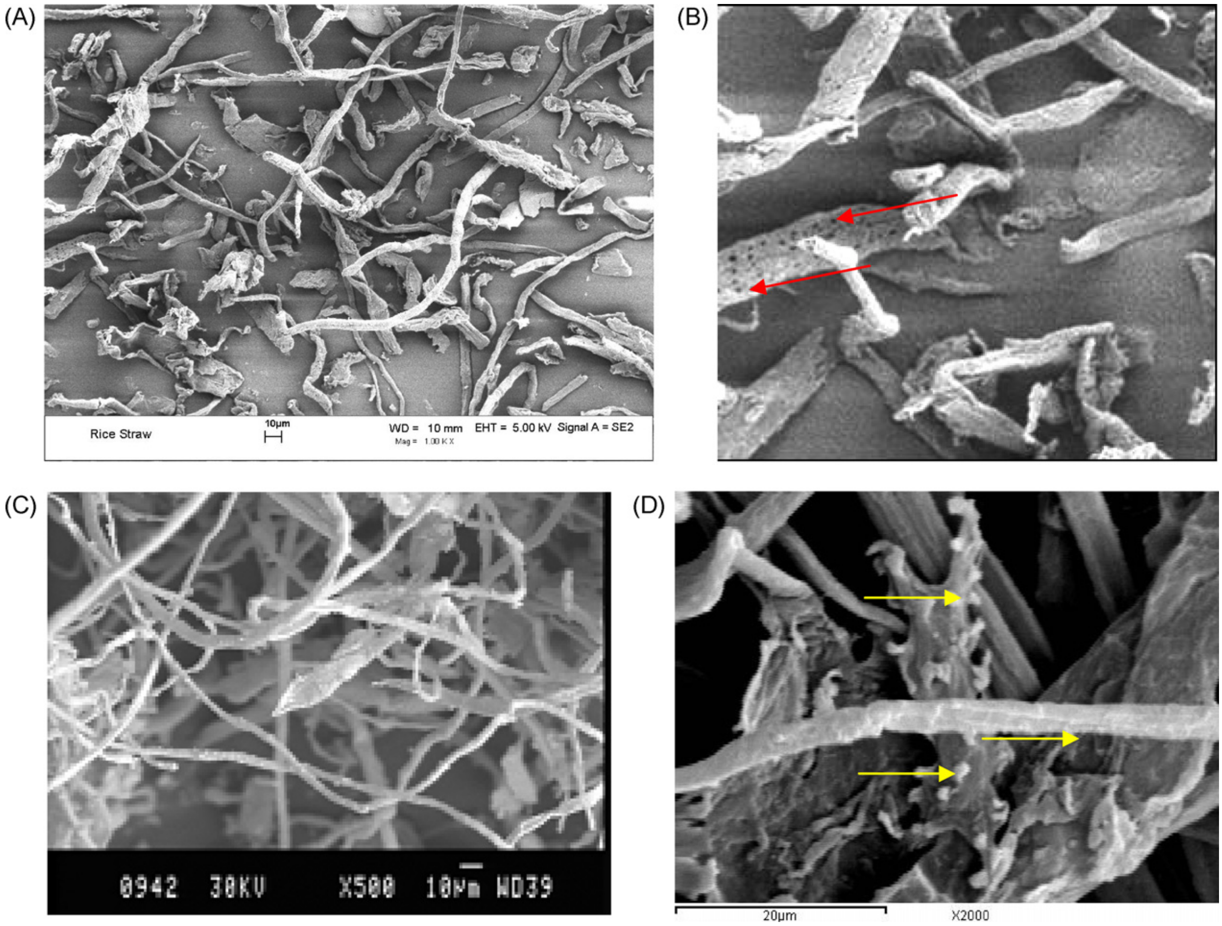

Fig. 1. Microscopic images. SEM images of steam exploded (A and B) and alkaline pulped rice straw (C and D). Steam explosion causes fiber separation (A) and a surface layer with holes (B). Holes are indicated with arrows. In alkaline pulping pretreated rice straw, the defibrating effect of the pretreatment causes the individual fibers to partially separate, as can be seen in (C). The pretreatment leaves a surface layer of droplets on the individual fibers (D). Droplets are indicated with arrows.

cultural residue to complement and understand the microscopic investigations. The FTIR spectra of delignified alkaline pulping pretreated and steam-exploded rice straw samples are shown in Fig. 2A. Excerpts of the spectra are presented in Fig. 2B.

The infrared spectra in the 400–4000 cm^{-1} region for steam-exploded and alkaline pulped rice straw are shown in Fig. 2A. Kristensen, Thygesen, Felby, Jørgensen, and Elder (2008) have mentioned that one of the effects of the pretreatment is the removal of wax from the straw, where the CH_2– stretching bands at approximately 2850 and 2920 cm^{-1} reduced for the pretreated sample, signifying a reduction in the amount of the aliphatic fractions of waxes. The same observation can be noticed for the raw material of rice straw, Fig. 2A, where a reduction in the CH_2 band can be investigated after alkaline pulping and steam explosion. On the other hand, absorption bands for different pretreatments appeared similarly. The absorption band at the region of 3422 and 3345 cm^{-1}, may be due to various hydroxyl (OH) stretching vibrations. The OH compounds may include absorbed water, aliphatic primary and secondary alcohols found in cellulose, hemicellulose, lignin, extractives; and carboxylic acids in extractives (Khan, Idriss Ali, & Basu, 1993; Kolboe & Ellefsen, 1962). The absorption band near the OH stretching vibrations, at 2918 and 2904 cm^{-1} may be associated with CH stretching vibrations. Kolboe and Ellefsen (1962) suggested that the bands in the 1644 cm^{-1} region for cellulose may be attributed to C=O stretching vibration of the alpha-keto carbonyl. The presence of the bands at the region of 1323 and 1318 cm^{-1} may be attributed to the lignin (syringic group). According to the literature (Ibrahim, 2000), the peaks at 1170–1040 cm^{-1} arise from the ether linkage. The absorbances at 1431, 1372, 1319, 1165, 1059 and 896 cm^{-1} which are typical of pure cellulose can be seen in the FTIR spectra.

Two interesting features are revealed in Fig. 2B. It can be seen that the carbonyl band at 1735 cm^{-1}, which has been ascribed to hemicelluloses and celluloses, is changed according to the treatment used. This is expected, as the pretreatment is known to affect the hemicelluloses and the celluloses in this region. Second, lignin bands at approximately 1595 and, in particular, 1510 cm^{-1} (aromatic ring stretch) (Stewart, Wilson, Hendra, & Morrison, 1995) are strongly enhanced in the alkaline pulping pretreated samples compared with steam-exploded samples, where these peaks are reduced (Fig. 2B). One explanation for this could be a relative increase in the amount of lignin in the steam explosion pretreatment due to the removal of hemicelluloses. Another reason could be that lignin is released and re-deposited on the surface. The increase in lignin is believed to be too significant to be only due to the hemicellulose removal.

One of the strategies employed in increasing convertibility is to change the cellulose crystallinity. Differences between samples with regard to the relative amounts of amorphous and crystalline cellulose have earlier been described through infrared peak ratios. At least four different peak pairs have been proposed (Hulleman et al., 1994; Wistara et al., 1999). Of these, only the peak pair 1429 cm^{-1} (crystalline) and 893 cm^{-1} (amorphous) is seen for the samples of the present study. The peak ratios for the steam

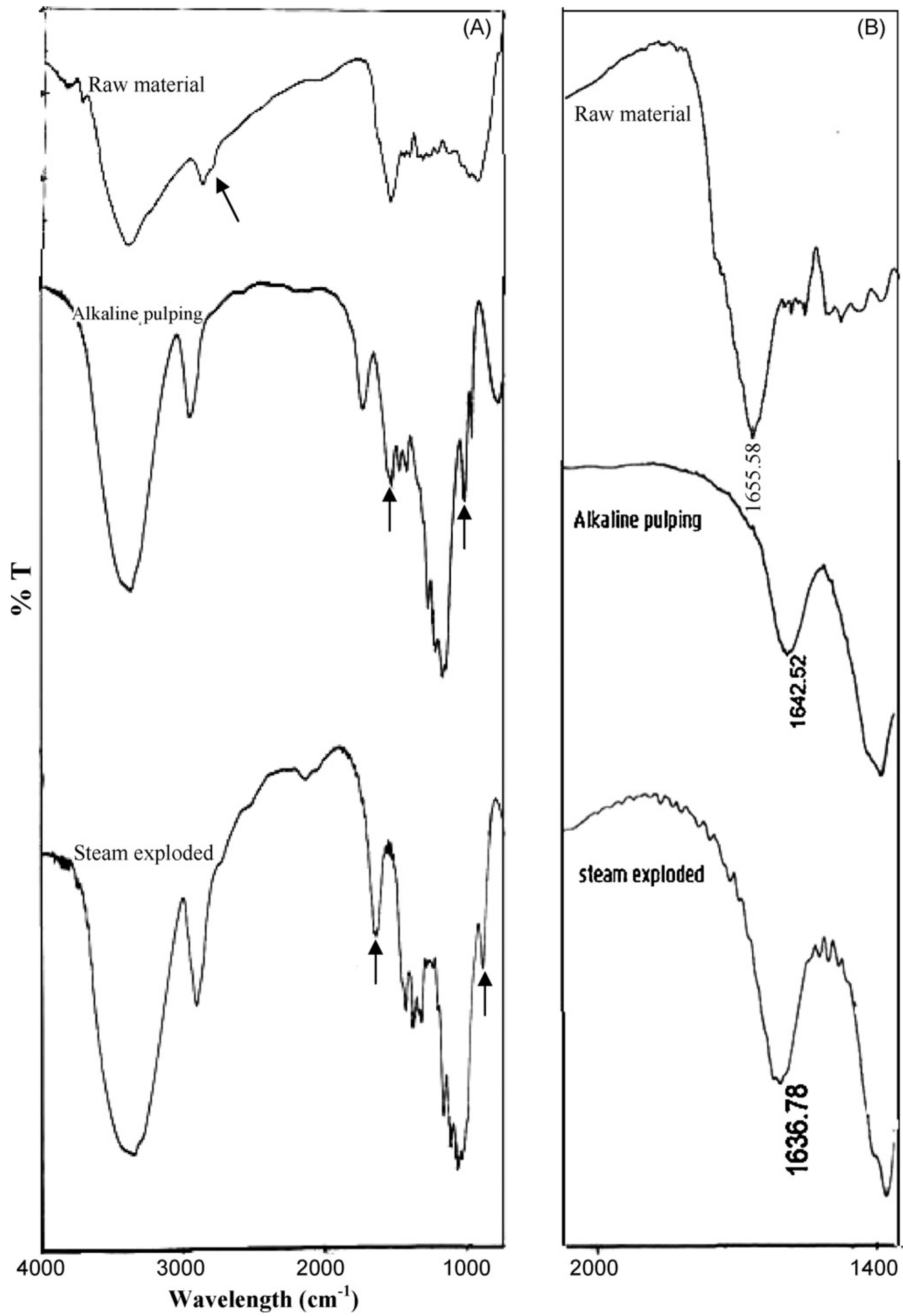

Fig. 2. FTIR spectra for steam exploded and alkaline pulping pretreatment rice straw (the arrows in (A) indicate the peaks at 1429 and 893 cm^{-1} for the pretreated fibers and at 2850 cm^{-1} for the raw material).

exploded and pulped raw materials were gathered in Table 1. The peak ratio for the alkaline pulped rice straw was 2.0, while it was 2.33 for the steam-exploded one. When comparing the results, it appears that the crystallinity index showed higher value for the steam-exploded fibers compared to the alkaline pulped fibers. This means that, the steam explosion raises the crystallinity of the cellulose component because the main reactions that occur during the process are the cleavage of the amorphous fractions of the cellulose, while the crystalline fraction is more resistant to cleavage under these conditions.

3.3. Glucose production

3.3.1. Effect of pretreatment on sugar content

The data presented in Table 2 show that the glucose concentration resulting from the enzyme hydrolysis of the pretreated rice straw depended on the pretreatment method. Glucose data in Table 2 is graphically represented in Fig. 3. The graph clearly shows that the glucose concentration was higher for alkaline pulped rice straw compared to steam-exploded material. On the other hand, the further treatment for the steam-exploded fibers with alkali

Table 2
Glucose and theoretical ethanol concentrations (g/L) for the pretreated samples.

Samples	Glucose concentration (g/L)	Theoretical ethanol concentration (g/L)
Pure cellulose	45.8	23.41
Alkaline pulped rice straw	47.8	14.77
Steam-exploded rice straw	28.9	24.43
Alkali-extracted-steam-exploded rice straw	58.4	29.84

extraction resulted in higher glucose concentration after enzyme hydrolysis for four days compared to both steam explosion and alkaline pulped pretreatments.

From the above observations the product of steam explosion pretreatment has been shown to be easily digestible by enzymes after alkali extraction. This is may be due to the fact that lignin is re-localized on the fiber by steam explosion, resulting in higher lignin content, as can be seen from Table 1, and by further alkali treatment it can be removed. It is known that lignin is responsible for unproductive adsorption of cellulases (Eriksson, Börjesson, & Tjerneld, 2002; Kristensen, Börjesson, Bruun, Tjerneld, & Jørgensen, 2007), where it encases the cellulose in the cell wall matrix, hindering cellulases from reaching cellulose fibrils. We hypothesize that the migration of lignin to the outer surface exposes internal cellulose surfaces. Selig et al. (2007) have also observed the formation and migration of spherical lignin deposits onto the surface of fibers as a result of pretreatment. They also suggest that the deposited lignin can have a negative impact on the enzymatic cellulose hydrolysis. It is possible, however, that the surface lignin layer is easily removed by mild conditions of alkali extraction for the steam-exploded fibers, due to lignin being less strongly bound to carbohydrate polymers, compared with its native linkages. Moreover, we theorize that the re-located lignin has exposed cellulose inside the cell wall, thus increasing the enzyme accessibility.

Based on these observations, we therefore propose that the re-localization of lignin as well as partial hemicellulose removal are likely to be important factors in increasing the enzymatic digestibility through pretreatment. It seems that exposing cellulose through manipulation of hemicelluloses and lignin are equally as important as altering the crystallinity and rupture of the skeletal structure of the cell wall.

From the glucose concentration, one can calculate the theoretical yield for ethanol production. For the theoretical calculation, it is known that each mole of glucose will produce two moles of ethanol. Glucose has a molar mass of 180 g/mol. The molar mass of ethanol is 46 g/mol. The other product of fermentation is carbon dioxide, which is not considered in the calculations. The equation for this

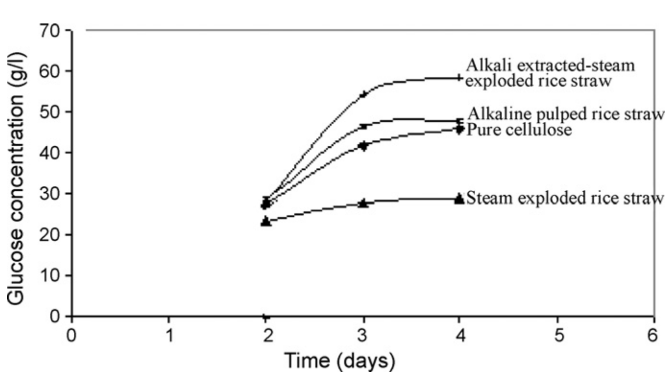

Fig. 3. Glucose concentration upon enzyme hydrolysis of pure cellulose and pretreated rice straw.

Table 3
Rate of enzyme hydrolysis (mg/mL glucose per hour) for pretreated materials.

Samples	Rate of enzyme hydrolysis		
	2nd day	3rd day	4th day
Pure cellulose	0.575	0.581	0.477
Alkaline pulped rice straw	0.592	0.649	0.498
Steam-exploded rice straw	0.491	0.387	0.301
Alkali-extracted-steam-exploded rice straw	0.589	0.753	0.608

reaction is as follows:

$$\underset{\substack{\text{glucose}\\ 180\ \text{g/mol}}}{C_6H_{12}O_6} \xrightarrow{\text{Fermentation}} \underset{\substack{\text{ethanol}\\ 46\ \text{g/mol}}}{2C_2H_5OH} + \underset{\substack{\text{carbon dioxide}\\ 44\ \text{g/mol}}}{2CO_2}$$

Based on the balanced equation, the alkali-extracted steam-exploded rice straw should produce 29.84 g/L ethanol compared to 23.41 g/L for pure cellulose, 24.43 g/L for steam-exploded rice straw and 14.77 g/L for alkaline pulped rice straw (Table 2). From the theoretical ethanol concentration, one can notice that the production of ethanol depends on the pretreatment method according to the glucose formation. From the data given here, it is evident that steam explosion treatment, after alkali extraction, can improve the potential for pretreated rice straw to glucose conversion.

Furthermore, the calculated rates of enzyme hydrolysis are gathered together in Table 3, where one can notice that the higher rate of enzyme hydrolysis was obtained for the alkali-extracted steam-exploded rice straw. The rate of the enzyme hydrolysis was rapid for the first days, where it depends on the action of the enzyme on converting the cellulosic products into glucose. It is known that, enzymatic degradation of cellulose to glucose is generally accomplished by synergistic action of at least three major classes of enzymes: endoglucanases, exoglucanases, and β-glucosidases. These enzymes are usually called together cellulase or cellulolytic enzymes (Wyman, 1996, chap. 1). According to that the enzyme plays an action where the endoglucanases attack the low-crystallinity regions of the cellulose fiber and create free chain-ends. The exoglucanases further degrade the sugar chain by removing cellobiose units (dimers of glucose) from the free chain-ends. The produced cellobiose is then cleaved to glucose by β-glucosidase. This action is very important to complete depolymerization of cellulose to glucose.

4. Conclusions

The results indicated that the higher glucose concentration can be obtained from the alkali-extracted steam-exploded rice straw. This was noticed compared to the results obtained from alkaline pulping and steam explosion of the waste material as a pretreatment process. This can be attributed to the fact that the steam explosion affects the cellulosic region and the further alkali extraction facilitates removing of the re-localized lignin and hence facilitates the enzyme treatment. Furthermore, the results indicated that the rate of hydrolysis differs according to the different pretreatments applied to the raw material. Also, the results indicate that via enzymatic hydrolysis, rice straw can be used as a cellulosic source after pretreatment for glucose production, where it can produces glucose higher than that resulted from pure cellulose.

Acknowledgments

The authors acknowledge the USA-Egypt Science and Technology Program, USDA, and NSF for funding the project (ENV8-004-002). Also, the authors acknowledge Bob Wright for conducting the steam explosion experiments.

References

Chang, V. S., & Holtzapple, M. T. (2000). Fundamental factors affecting biomass enzymatic reactivity. *Applied Biochemistry and Biotechnology*, *84*, 5–37.

Cheng, S., & Zhu, S. (2008). Use of lignocellulosic materials for a sustainable chemical industry. *BioResources*, *3*, 666–667.

Donaldson, L., Hague, J., & Snell, R. (2001). Lignin distribution in coppice poplar, linseed and wheat straw. *Holzforschung*, *55*, 379–385.

Eriksson, T., Börjesson, J., & Tjerneld, F. (2002). Mechanism of surfactant effect in enzymatic hydrolysis of lignocellulose. *Enzyme and Microbial Technology*, *31*, 353–364.

Galbe, M., & Zacchi, G. (2002). A review of the production of ethanol from softwood. *Applied Microbiology and Biotechnology*, *59*, 618–628.

Himmel, M. E., Ding, S. Y., Johnson, D. K., Adney, W. S., Nimlos, M. R., Brady, J. W., et al. (2007). Biomass recalcitrance: Engineering plants and enzymes for biofuels production. *Science*, *315*, 804–807.

Hulleman, S. H. D., Vanhazendonk, J. M., & Vandam, J. E. G. (1994). Determination of crystallinity in native cellulose from higher-plants with diffuse-reflectance Fourier-transform infrared-spectroscopy. *Carbohydrate Research*, *261*, 163–172.

Ibrahim, M. M. (2000). Preparation of cellulose and cellulose derivative azo compounds. *Cellulose*, *9*, 337–349.

Ibrahim, M. M., Agblevor, F. A., & El-Zawawy, W. K. (2010). Isolation and characterization of cellulose and lignin from steam-exploded lignocellulosic biomass. *BioResources*, *5*, 397–418.

Jeoh, J., & Agbelvor, F. A. (2001). Characterization and fermentation of steam exploded cotton gin waste. *Biomass and Bioenergy*, *21*, 109–120.

Khan, M. A., Idriss Ali, K. M., & Basu, S. C. (1993). IR studies of wood plastic composites. *Journal of Applied Polymer Science*, *49*, 1547–1551.

Kolboe, S., & Ellefsen, O. (1962). Infrared investigatigations of lignin. A discussion of some recent results. *Tappi*, *45*, 163–166.

Kristensen, J. B., Börjesson, J., Bruun, M. H., Tjerneld, F., & Jørgensen, H. (2007). Use of surface active additives in enzymatic hydrolysis of wheat straw lignocellulose. *Enzyme and Microbial Technology*, *40*, 888–895.

Kristensen, J. B., Thygesen, L. G., Felby, C., Jørgensen, H., & Elder, T. (2008). Cell-wall structural changes in wheat straw pretreated for bioethanol production. *Biotechnology for Biofuels*, *1*(5), 1–9.

Kurabi, A., Berlin, A., Gilkes, N., Kilburn, D., Bura, R., Robinson, J., et al. (2005). Enzymatic hydrolysis of steam-exploded and ethanol organosolv-pretreated Douglas–Fir by novel and commercial fungal cellulases. *Applied Biochemistry and Biotechnology*, *121–124*, 219–230.

Millati, R., Niklasson, C., & Taherzadeh, M. J. (2002). Effect of pH, time and temperature of overliming on detoxification of dilute-acid hydrolyzates for fermentation by *Saccharomyces cerevisiae*. *Process Biochemistry*, *38*, 515–522.

Palmqvist, E., & Hahn-Hägerdal, B. (2000). Fermentation of lignocellulosic hydrolysates. II. Inhibitors and mechanisms of inhibition. *Bioresource Technology*, *74*, 25–33.

Ramos, L. P. (2003). The chemistry involved in the steam treatment of lignocellulosic materials. *Quim Nova*, *26*, 863–871.

Rojas, O. J., & Hubbe, M. A. (2004). The dispersion science of papermaking. *Journal of Dispersion Science and Technology*, *25*, 713–732.

Selig, M. J., Viamajala, S., Decker, S. R., Tucker, M. P., Himmel, M. E., & Vinzant, T. B. (2007). Deposition of lignin droplets produced during dilute acid pretreatment of maize stems retards enzymatic hydrolysis of cellulose. *Biotechnology Progress*, *23*, 1333–1339.

Simola-Gustafsson, J., Hortling, B., & Peltonen, J. (2001). Scanning probe microscopy and enhanced data analysis on lignin and elemental chlorine free or oxygen delignified pine kraft pulp. *Colloid and Polymer Science*, *279*, 221–231.

Simola, J., Malkavaara, P., Alen, R., & Peltonen, J. (2000). Scanning probe microscopy of pine and birch kraft pulp fibres. *Polymer*, *41*, 2121–2126.

Stewart, D., Wilson, H. M., Hendra, P. J., & Morrison, I. M. (1995). Fourier-transform infrared and Raman-spectroscopic study of biochemical and chemical treatments of oak wood (*Quercus rubra*) and barley (*Hordeum vulgare*) straw. *Journal of Agricultural and Food Chemistry*, *43*, 2219–2225.

Sun, X. F., Xu, F., Sun, R. C., Fowler, P., & Baird, M. S. (2005). Characteristics of degraded cellulose obtained from steam-exploded wheat straw. *Carbohydrate Research*, *340*, 97–106.

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

Varga, E., Reczey, K., & Zacchi, G. (2004). Optimization of steam pretreatment of corn stover to enhance enzymatic digestibility. *Applied Biochemistry and Biotechnology*, *113–116*, 509–523.

Wistara, N., Zhang, X., & Young, R. A. (1999). Properties and treatments of pulps from recycled paper. II. Surface properties and crystallinity of fibers and fines. *Cellulose*, *6*, 325–348.

Wyman, C. E. (1996). Ethanol production from lignocellulosic biomass: Overview. In C. E. Wyman (Ed.), *Handbook on bioethanol: Production and utilization* (pp. 11–12). Washington, DC: Taylor & Francis.