EFFECT OF ^{60}CO-γ IRRADIATION ON THE MICROSTRUCTURE AND ENZYMATIC HYDROLYSIS OF RAPESEED STRAW

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Received November 22, 2014

In this study, rapeseed straw was pretreated with ^{60}Co-γ radiation and digested with cellulase to improve sugar production. The compositions of straw were analyzed. Results showed that there were remarkable changes in total reducing sugar and an increase in the degradation of cellulose, hemicellulose and lignin. At a dose of 1200 kGy, 39.3 times more reducing sugar from the irradiated straw was released, as compared to the control (not irradiated) straw (35.34 *vs*. 0.90 mg/g). Furthermore, both the total content and the number of species of sugar compounds increased. Enzymatic digestion of the irradiated straw resulted in the degradation of 79.21% and 75.59% of cellulose and hemicellulose, respectively. The total reducing sugar increased by a factor of 4.6 to 392.50 mg/g in the irradiated straw from 86.18 mg/g in the control, with greater total content and more species of sugar compounds. Analyses with X-ray diffraction, infrared spectroscopy, and scanning electron microscopy showed that the microstructure of the irradiated straw was destroyed.

Keywords: rapeseed straw, γ-ray irradiation, enzymatic hydrolysis, microstructure, total reducing sugar, sugar compounds

INTRODUCTION

Lignocellulosic feedstock is one of the most abundant renewable energy sources on the Earth.[1] However, a large amount of crop straw, such as the straw of rapeseed, maize, and rice, is discarded or burned every year. This is both a waste of resources and a source of pollution.[2,3] In recent years, the application of lignocellulose biomass as raw material for bioenergy production has received greater attention. The production of rapeseed straw is huge in many provinces in China, such as Hunan, Hubei, Anhui, Jiangxi, Henan, Gansu, Xinjiang, Shanxi, Sichuan, and over 100 million hectares of rapeseed are cultivated each year and its total production is estimated at about 1.3×10^7 tons in 2012 in China. Rapeseed straw, an agricultural residue in the process of fuel ethanol production, is an abundant and low-cost lignocellulosic material, which shows a broad prospect for researchers.

The use of lignocellulosic feedstock, such as rapeseed straw, for fuel ethanol production would be a renewable energy solution and would reduce pollution, helping to improve air quality and economy in remote areas. It would also reduce oil dependence and greenhouse gas emissions.[4,5] Lignocellulose materials mainly consist of cellulose, hemicellulose and lignin; cellulose polymers are intricately associated with lignin and hemicellulose, resulting in complex physical and morphological structures. As such, downstream processes, such as enzymatic hydrolysis, have been found difficult. In

order to improve the accessibility of enzymes to cellulose and to increase the yield of fermentable sugar, a pretreatment is absolutely necessary. Various physical, chemical and biological pretreatments for lignocellulose have been studied in the last few decades. For example, the acid or alkaline pretreatment, which enables more than 80% of the theoretical enzyme digestibility of cellulose to be obtained,[6-9] steam explosion, liquid hot-water, and soaking in aqueous ammonia are commonly employed pretreatment methods.[10-12] Irradiation with γ-rays is used as a physical pretreatment process; it does not involve the use of extreme temperature and generates minimum inhibitory substances or not at all.[13,14]

Radiation at higher doses can disrupt the glucosidic bond and can be an effective pretreatment for lignocellulosic biomass for sugar production.[15] The irradiation degradation of various lignocellulosic materials for increasing the accessibility to cellulolytic enzymes has been reported, such as rice straw,[16] chaff,[17] sawdust, [18]jute,[19] wheat straw,[20] empty fruit bunch of oil palm,[21] and cotton.[22]

γ irradiation has been shown to generate long- and short-lived radicals, which induce the secondary degradation of the irradiated materials through a number of chemical reactions, such as chain scission and cross-linking.[19] It has also been shown to reduce the degree of polymerization of cellulose, resulting in increased enzymatic accessibility.[15,23]

In the present study, ^{60}Co-γ irradiation pretreatment was carried out to pretreat rapeseed straw. Our purpose was to study the effect of radiation at different doses on the texture properties and the enzymatic hydrolysis of the irradiated rapeseed straw. By means of X-ray diffraction (XRD), Fourier transform infrared spectrophotometer (FTIR), and scanning electron microscopy (SEM), the radiation-induced changes in the microstructure and morphology of rapeseed straw were investigated and the reasons for the increased sugar production after γ irradiation were analyzed.

EXPERIMENTAL

Materials

Rapeseed straw was collected from the experimental farm of the Oil Crops Research Institute, Hunan Agricultural University. Commercial cellulolytic enzyme was purchased from Wuxi Xuemei Enzyme Preparation Technology Co., Ltd., China. The enzymatic activity was determined to be 30000 U/g. Glucose, xylose, cellobiose, galacturonic acid and glucuronic acid were purchased from Sigma (St. Louis, MO, USA). Other reagents were of analytical grade.

Pretreatment

The straw was dried at 60 °C for 5 days, cut to about 1-2 cm in length and ground to pass through 40-mesh sieves. The powders were irradiated in 500-mL jars (net weight approx. 200 g) with γ irradiation at dosages of 0 (control), 400, 600, 800, 1000, and 1200 kGy at room temperature, the dose rate was 2.0 kGy/h. The irradiation was conducted at Hunan Institute of Atomic Energy for Agricultural Sciences, using a cobalt-60 radiation source of 9.99×10^{15} Bq. The irradiated powders were stored in sealed flasks at room temperature for composition analysis and enzymatic hydrolysis.

Extraction of irradiated sample

The irradiated and unirradiated straw powders were placed into 150-mL Erlenmeyer flasks containing 30 mL of distilled water and shaken on a shaker at 50 °C and 150 rpm for 4 h. The aqueous extract was collected after filtration and diluted properly to analyze the total reducing sugar and sugar compounds.

Enzymatic hydrolysis

The irradiated and unirradiated rapeseed straw powders were enzymatically hydrolyzed in 0.05 M citric acid-sodium citrate buffer (pH 4.8) and the enzyme loading of cellulase was 250 IU per 1 g substrate. Hydrolysis was performed in triplicate in 150-mL flasks on a shaker at 50 °C and 150 rpm for 72 h. The enzymatic hydrolysates were deactivated in a water bath at 100 °C for 10 min, and then were filtered and diluted properly to analyze the total reducing sugar and sugar compounds.

Determination of compositions and sugars

The concentrations of cellulose, hemicellulose and lignin were determined according to previously described methods.[12,24] The degradation ratio of cellulose (DC, %), hemicellulose (DH, %) and lignin (DL, %) was calculated as follows:

$$DRC\ (\%) = (W_{cd}/W_c) \times 100\% \quad (1)$$

$$DRH\ (\%) = (W_{hd}/W_h) \times 100\% \quad (2)$$

$$DRL\ (\%) = (W_{ld}/W_l) \times 100\% \quad (3)$$

where W_{cd}, W_{hd} and W_{ld} are the weight of cellulose, hemicellulose and lignin digested after radiation, respectively; W_c, W_h and W_l are the weight of cellulose, hemicellulose and lignin before the digestion, respectively. Each sample was analyzed in triplicate, and means and standard deviations (SD) were calculated. The total reducing sugar was determined using the DNS (3,5-Dinitrosalicylic acid) method.[25] Sugar compounds were analyzed using high-performance anion exchange

chromatography (HPAEC).[26] A Dionex ICS-3000 ion chromatograph system with a CarboPac PA20 column (150×3 (i.d.) mm, Bio-Rad Labs, USA) was used. The chromatograph was operated at room temperature using a mixture of NaOH and NaOAc as the mobile phase (0.5 mL/min). The supernatant obtained was filtered with 0.2-μm syringe filters before loading onto the column.

X-ray diffraction (XRD) analysis

The degree of crystallinity and crystallite size of the γ-irradiated rapeseed straw were analyzed using X-ray diffraction (XRD) (D/MAX-RB, Rigaku, Japan). The XRD patterns from Cu K_α radiation at 40 kV and 30 mA were recorded in the range of 2θ = 5 to 80°.

The degree of crystallinity was calculated using the formula:[27]

$$CrI = [(I_{002}-I_a) / I_{002}] \times 100\% \quad (4)$$

where CrI is the degree of crystallinity, I_{002} is the maximum intensity of the crystalline plane (002) reflection (2θ = 22.5°), and I_a is the intensity of the scattering for the amorphous component at about 18° in cellulose-I.

The size of sub-micrometer particles, or crystallites, was determined without regard to crystal lattice imperfections using the Scherrer equation:

$$L_{002} = k \lambda /(\beta \cos\theta) \quad (5)$$

where L_{002} is the mean size of the ordered (crystalline) domains perpendicular to the reflective plane, K is a dimensionless shape factor with a value between 0.9 and 1 (the shape factor is 0.94 for the fiber crystallites), λ is the X-ray wavelength (usually 1.54), β is the line broadening at half the maximum intensity (FWHM) (after subtracting the instrumental line broadening, in radians, it is also known as Δ(2θ)), and θ is the Bragg angle.

FT-IR spectroscopy analysis

The structure of the irradiated rapeseed straw was investigated using FT-IR (KBr-disk, 4000 to 400 cm^{-1} scope of scanning, WQF-310/410, Analect, USA).

SEM analysis

The morphology of the γ-irradiated rapeseed straw was examined using SEM (JSM-6380LV, operated at 10 kV accelerating voltage, Electronics Corporation, Japan).

RESULTS AND DISCUSSION

Composition changes due to irradiation and subsequent enzymatic hydrolysis

The biochemical compositions of untreated and irradiated rapeseed straws are shown in Table 1. In the untreated rapeseed straw, the contents of cellulose, hemicellulose and lignin were of 41.37%, 23.63% and 13.03%, respectively. These values were similar to what was reported for cellulose, hemicellulose, and lignin (42.9%, 24.6% and 11.3%) previously.[20]

Table 1
Contents of cellulose, hemicellulose and lignin after irradiation and subsequent enzymatic hydrolysis of rapeseed straw

Pretreatment	Cellulose		Hemicellulose		Lignin	
	Content (%)	DRC (%)	Content (%)	DRH (%)	Content (%)	DRL (%)
0 (Control)	41.37±1.3		23.63±1.3	/	13.03±0.3	/
400 kGy	34.97±1.2	15.47±0.9	21.33±1.2	9.73±0.3	12.52±0.5	3.91±0.1
600 kGy	31.62±1.0	23.57±1.0	17.82±0.9	24.59±1.2	12.49±0.5	4.14±0.1
800 kGy	28.45±0.9	31.23±1.4	12.93±0.7	45.28±1.7	12.46±0.7	4.37±0.2
1000 kGy	25.32±0.9	38.80±1.4	9.65±0.5	59.16±1.8	12.41±0.4	4.76±0.1
1200 kGy	23.76±0.8	42.57±1.6	7.31±0.6	69.06±2.0	12.38±0.4	4.99±0.1
0+E*	36.07±1.2	12.81±0.7	22.43±1.0	5.08±0.1	12.45±0.5	4.45±0.2
400+E*	31.23±1.2	24.51±0.7	17.04±0.8	27.89±1.3	12.07±0.4	7.37±0.2
600+E*	28.47±1.4	31.18±1.2	14.58±0.7	38.30±1.4	11.52±0.3	11.59±0.3
800+E*	22.35±1.3	45.98±1.9	11.02±0.7	53.36±1.5	10.96±0.3	15.89±0.3
1000+E*	13.29±1.2	67.88±2.2	8.94±0.4	62.17±2.1	10.19±0.3	21.80±0.4
1200+E*	8.60±0.3	79.21±2.0	5.77±0.3	75.59±2.0	9.92±0.2	23.87±1.0

Note: E*: enzymatic digestion

Cellulose and hemicellulose were degraded more at higher radiation dosages. After irradiation at a dose of 1200 kGy, the degradation ratio of cellulose and hemicellulose was of 42.57 and 69.06%, respectively, as compared with the contents in the control.

However, the lignin content was 12.38%, only 4.99% less than that in the control, suggesting that

the irradiation resulted in effective degradation of cellulose and hemicellulose, but not lignin. It has been shown that, when irradiated, there were both cleavage and cross-linking reactions in cellulose. At high irradiation dosages, the cleavage reactions dominated, resulting in increased breakage of glycosidic bonds and decreased cross-linkage to lignocellulose, which resulted in direct decomposition of cellulose and hemicellulose.[28-30] Once irradiated, free radicals were formed within lignocellulose molecules.

Table 2
Total reducing sugar and sugar compounds after irradiation and subsequent enzymatic hydrolysis of rapeseed straw

Pretreatment	Total reducing sugar (mg/g)	Glucose (mg/g)	Xylose (mg/g)	Cellobiose (mg/g)	Galacturonic acid (mg/g)	Glucuronic acid (mg/g)
0 (Control)	0.90±0.03	0.18±0.02	0.10±0.01	nd	nd	nd
400 kGy	10.55±0.32	2.08±0.08	1.35±0.03	0.92±0.02	0.19±0.01	0.23±0.06
600 kGy	22.22±0.85	4.31±0.09	3.56±0.09	1.61±0.08	1.12±0.06	1.14±0.07
800 kGy	26.11±0.93	6.76±0.13	4.43±0.12	4.04±0.09	2.08±0.12	1.62±0.09
1000 kGy	34.16±1.18	8.36±0.27	5.52±0.11	4.76±0.23	2.93±0.14	1.99±0.13
1200 kGy	35.34±1.07	9.07±0.45	5.97±0.26	5.53±0.43	3.06±0.23	2.13±0.15
0+E*	86.18±2.16	19.46±0.92	9.83±0.63	7.41±0.46	4.67±0.28	10.21±0.34
400+E*	177.85±4.10	37.99±1.52	25.67±1.03	15.41±0.91	18.4±0. 87	19.35±0.98
600+E*	287.89±5.21	67.52±2.17	45.75±2.21	41.25±0.87	39.58±1.04	21.23±0.76
800+E*	330.85±6.73	85.95±2.02	60.00±1.13	51.48±0.92	40.30±1.12	22.45±0.81
1000+E*	388.98±7.31	101.80±3.03	85.95±1.47	55.60±2.21	42.90±1.18	23.30±0.93
1200+E*	392.50±9.43	107.90±3.04	92.05±2.14	61.73±2.20	44.95±1.21	24.35±0.95

Note: E*: enzymatic digestion; nd: not detected

These radicals could also cause degradation of lignocellulose *via* certain reactions. Lignin, on the other hand, was found to be less sensitive to irradiation, although it could be degraded and decomposed at high irradiation dosages. Von *et al*. found that lignin was embedded in the cellulose and form covalent bonds to some hemicellulose, thereby offering protection against physical and chemical degradation.[23,31]Also, the biochemical compositions of cellulose, hemicellulose, and lignin in the irradiated, unirradiated, and then enzymatically hydrolyzed straws are summarized in Table 1. In the unirradiated sample, the contents of these components were of 36.07, 22.43, and 12.45%, respectively, after enzymatic digestion. These values were by 12.81, 5.08, and 4.45% smaller than those in the unirradiated samples. After irradiation at 1200 kGy, the degradation ratio of these compounds was 79.21, 75.59, and 23.87%, respectively, compared to the unirradiated samples. The results showed that the irradiation dramatically improved hydrolysis for cellulose and hemicellulose, but less so for lignin. The combination of cellulase and irradiation treatments yielded better digestion than the single treatments, suggesting that there was synergy between the treatments.

Total reducing sugar and sugar compounds after irradiation and subsequent enzymatic hydrolysis

The total reducing sugar and sugar compounds in the water extracts from the irradiated and unirradiated straws were measured (Table 2), and these were found to increase dramatically after irradiation as compared with the unirradiated control. Glucose, xylose, cellobiose, galacturonic acid and glucuronic acid were detected, with glucose being the highest (2.08 to 9.07 mg/g), followed by xylose (1.35 to 5.97 mg/g). The regularity of cellobiose production was similar to that of the glucose production. The contents of galacturonic acid and glucuronic acid were relatively low. In the untreated rapeseed straw, the contents of total reducing sugar, glucose, and xylose were of only 0.90, 0.18, 0.10 mg/g respectively, and no other sugar compounds were detected. The level of sugars was found to increase quickly with the dosage. At an irradiation dosage of 1200 kGy, the total reducing sugar, glucose, and xylose were of 35.34, 9.07, and 5.97 mg/g, respectively, which were 39.3, 50.4, and 59.7 times more than the values for the control, respectively. The data showed that the irradiation resulted in the decomposition of cellulose, hemicellulose, and

lignin into various sugars. Previous studies had shown that irradiation can directly degrade fiber components into smaller sugars, resulting in increased total reducing sugar.[19]

The total reducing sugar and sugar compounds after the enzymatic digestion of the irradiated and unirradiated straw are also shown in Table 2. With increasing irradiation dosage, digestion generated more reducing sugar and small amounts of other types of sugar compounds. Glucose was found to be the most abundant (19.46 to 107.90 mg/g), followed by xylose (9.83 to 92.05 mg/g), while the galacturonic acid and glucuronic acid were relatively low. The digestion of the unirradiated straw was inefficient for sugar production. It yielded a total reducing sugar content of only 86.18 mg/g. Of that, 19.46 and 9.83 mg/g were glucose and xylose, respectively. As the radiation dosage increased, the degree of enzymatic digestion also increased significantly. At a dosage of 1200 kGy, the content of total reducing sugar was 392.50 mg/g, 4.6 times higher than that in the unirradiated control. At this irradiation level, the glucose and xylose contents were of 107.90 and 92.05 mg/g, respectively, 8.0 and 11.8 times higher than those of the unirradiated control. There was also an increase in cellobiose. These findings indicated that the irradiation greatly increased the degree of enzymatic digestion. It was believed that irradiation can break linkages between carbohydrates in lignocellulose and lignin, resulting in increased surface area and cellulose reactivity. Such breakage would also result in increased enzyme accessibility to lignocellulose and therefore better digestion.

X-ray diffraction (XRD) analysis

Both the untreated and irradiation-pretreated rapeseed straws were analyzed by X-ray diffraction. The scan strength curve at different degrees of 2θ is shown in Figure 1. All samples exhibited predominantly cellulosic diffraction peaks at 2θ = 22.0°±0.5°, in which the peak corresponds to the 002 crystallographic planes.

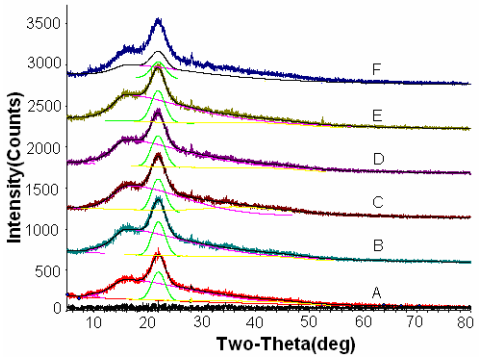

Figure 1: XRD spectra of untreated and irradiation-pretreated rapeseed straw: (A) untreated (control); and pretreated with irradiation dosages of 400 (B), 600 (C), 800 (D), 1000 (E), and 1200 (F) kGy

Table 3
Degree of crystallinity and crystallite sizes in rapeseed straw, as determined by X-ray diffraction (XRD)

Pretreatment	Degree of crystallinity (%)	Crystallite size (Å)
0	17.85 (0.33)	29 (1)
400	16.32 (0.40)	28 (1)
600	15.09 (0.38)	27 (1)
800	13.78 (0.73)	26 (1)
1000	13.63 (0.80)	26 (1)
1200	13.27 (0.34)	25 (1)

The measurements of the degree of crystallinity and crystallite sizes are shown in Table 3. The degree of crystallinity was noted to decrease after irradiation, from 17.85% in the unirradiated straw

to a minimum of 13.27% at an irradiation dosage of 1200 kGy. The crystallite size also decreased (from 29 Å to 25 Å) by the irradiation. These data indicated that the irradiation at the dosages used partially damaged the crystals, reducing the degree of crystallinity and crystallite sizes. It has been hypothesized that the breakage of the crystals would increase the enzyme accessibility into fibers, thus increasing the rate of hydrolysis during the enzymatic treatment. Faster hydrolysis was expected to generate more reducing sugar, as seen in this and other studies.[26]

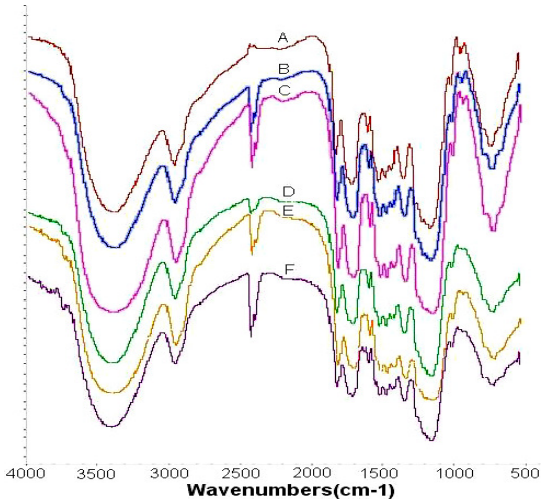

Figure 2: FT-IR spectra of untreated and irradiated rapeseed straw: (A) untreated; pretreated with irradiation dosages of 400 (B), 600 (C), 800 (D), 1000 (E), and 1200 (F) kGy

FT-IR analysis

Infrared spectroscopy has been used for the quantitative analyses of cellulose, hemicellulose and lignin. However, such analysis is very complex. Thus, by a commonly used qualitative analysis, the types of chemical bonds present in a sample can be judged according to their characteristic absorption peaks in the infrared spectrum.[32] For example, the absorption peak for a D-glucoside bond (carbohydrate peak) appears at 897 cm^{-1}. The peaks of lignin appear at 1509 cm^{-1} and near 1618 cm^{-1}. It has two peaks because of carbon-carbon stretching vibrations in its aromatic rings. The absorption peaks near 1739 cm^{-1} are an indication of the presence of hemicellulose. All these peaks could be found in Figure 2, indicating that the rapeseed straw is composed of typical cellulose fibers. The infrared spectra of the irradiated and unirradiated straw are shown in Figure 2.

After irradiation, the oxygen bridge at positions 1 and 4 of glucose rings in the cellulose molecule was cleaved, generating a carbonyl group with weak hydrogen bonding to cellulose molecules. The O-H peak shifts to a higher wavenumber if the hydrogen bond becomes weaker or to a lower wavenumber if the hydrogen bond becomes stronger. As seen in Figure 2, 3355 cm^{-1} was the characteristic location of the O-H stretching vibration absorption peak. The peaks were seen to shift toward higher wavenumbers following irradiation,[33] suggesting that the radiation damaged the intermolecular hydrogen bonds between the fiber molecules, resulting in the destruction of the crystalline structure of the rapeseed straw fibers. In the unirradiated rapeseed straw fibers, the C=O stretching vibration absorption peaks characteristic of cellulose, hemicellulose, and lignin were found at a wavenumber of 1034 cm^{-1}. After the irradiation, the peak disappeared, indicating that the C=O bonds in cellulose, hemicellulose, and lignin were destroyed and that the linkages between them were damaged. Table 4 summarizes the wavenumber changes of peaks corresponding to other major groups found in the rapeseed straw.

SEM analysis

Scanning electron microscope photos of rapeseed straw fibers before and after irradiation are shown in Figure 3. In the unirradiated sample, the surface was smooth, with irregular, raised strips. No remarkable morphological features were seen, except for some mechanical damage.

Table 4
Characteristic FT-IR bands present in rapeseed straw and changes in their positions observed relative to irradiation pretreatment dosages

Characteristic FT-IR bands position (cm^{-1})	Irradiation pretreatment dosage (kGy)					
	0	400	600	800	1000	1200
O-H stretching vibration	3355	3356	3379	3392	3384	3392
C-H stretching vibration (methyl and methylene group)	2921	2923	2922	2922	2923	2922
C＝O stretching vibration (hemicellulose)	1739	1739	1738	1734	1734	1734
C＝O stretching vibration (lignin)	1618	1617	1620	1616	1616	1617
Benzene stretching vibration	1509	1508	1509	1507	1508	1507
CH_2 bending vibration (cellulose) CH_3 bending vibration (lignin)	1424	1424	1424	1423	1423	1419
CH bending vibration (cellulose and hemicellulose)	1375	1374	1376	1374	1374	1374
OH deformation (cellulose)	1326	1330	1330	1321	1321	1338
O-H bending vibration (cellulose and hemicellulose)	1244	1243	1246	1244	1243	1244
C-O-C stretching vibration (cellulose and hemicellulose)	1160	1156	1156	1158	1160	1160
O-H association band (cellulose and hemicellulose)	1104	1100	1104	1108	1108	1108
C=O stretching vibration (cellulose and hemicellulose)	1057	1056	1053	1052	1051	1051
C=O stretching vibration (cellulose, hemicellulose and lignin)	1034	/	/	/	/	/
β-glycosidic bond vibration (carbohydrate)	897	897	897	891	897	895

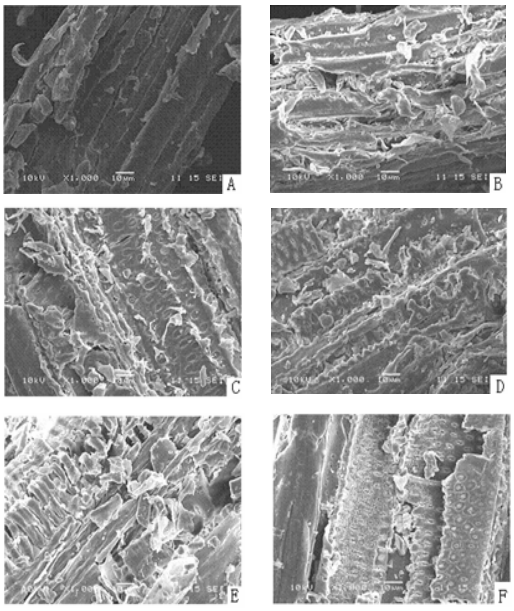

Figure 3: SEM images of untreated and irradiation-pretreated rapeseed straw (× 1000): (A) untreated; pretreated with irradiation dosages of 400 (B), 600 (C), 800 (D), 1000 (E), and 1200 (F) kGy

After irradiation, the surface became increasingly rough, its roughness having increased with the irradiation dosage. Irradiation also formed significant strip cracks in the cell wall structure and

damaged it. It also created small debris and deep grooves. In some areas, honeycomb-like holes were visible. These indicate that radiation damaged the surface structure of the straw, increasing surface area and thereby increasing the accessibility of digestive enzymes. This translated to an increase in digestion efficiency.

CONCLUSION

After enzymatic hydrolysis, the maximum value of total reducing sugar and of glucose in the rapeseed straw at an irradiation dosage of 1200 kGy was attained, which was 392.50 mg/g and 107.90 mg/g respectively. Compared to 0.9 mg/g total reducing sugar and 0.18 mg/g glucose for untreated rapeseed straw, a remarkable increase was observed.

XRD, FT-IR and SEM analyses showed that irradiation can cause significant breakdown of the surface structure of the straw and destroy linkages between cellulose, hemicellulose and lignin. This leads to reduced degree of crystallinity and crystallite size, allowing for the direct decomposition of some components, increased enzyme accessibility and reactivity, resulting in increased digestion efficiency. From the results obtained in this study, it can be concluded that irradiation is an attractive pretreatment for rapeseed straw to be used in ethanol production. More studies are needed to shorten the radiation time and further increase the sugar production to develop a better and applicable protocol for rapeseed straw-based ethanol production.

ACKNOWLEDGEMENT: This study was supported by the National "863" Program of High Technology Research and Development of China (2012AA101804) and the Science and Technology Project of Hunan Province, China (2015NK3009).

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