EFFECT OF GAMMA IRRADIATION ON MICROCRYSTALLINE STRUCTURE OF *PHRAGMITES* CELLULOSE

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Abstract. Effect of ^{60}Co-γ irradiation treatment on cellulose structure originating from *Phragmites communis* trim (PCT) was investigated on the basis of irradiation doses of 0 to 2000 kGy at a dose rate of 2 kGy h^{-1}. Scanning electron micrograph images showed that surface morphologies of PCT cellulose could become fragmented after being treated with ^{60}Co-γ irradiation. Based on X-ray diffraction profiles, crystallinity and amorphous region of microcrystalline cellulose treated by irradiation were obviously changed. Fourier transform IR spectroscopy data indicated that a new characteristic peak corresponding to carbonyl (1734 cm^{-1}) appeared after PCT cellulose was treated with ^{60}Co-γ irradiation, which suggested that PCT cellulose was degraded after irradiation treatment.

Keywords: *Phragmites communis* trim (PCT), microcrystalline cellulose, ^{60}Co-γ irradiation, SEM, XRD, FT-IR.

INTRODUCTION

Bioethanol and biodiesel, which are important alternative bioresource energies, have been attracting increasing attention because of the problem of CO_2 emissions as well as petroleum energy security concerns (Liu et al 2010; Remond et al 2010). Current technologies for bioethanol production mainly rely on starch and sugars, and they have been considerably debated because of poor sustainability. In this context, bioethanol produced from lignocellulosic biomass is an interesting alternative because lignocellulosic raw materials do not compete with food crops and they are also less expensive than conventional agricultural feed stocks. This is especially important for countries with big populations.

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In principle, the major technical problem of ethanol production from lignocellulosic biomass is to hydrolyze lignocellulose into fermentable monosaccharides. Cellulose pretreatment is an essential step for obtaining potentially fermentable sugars in the hydrolysis step. The aim of pretreatment is to break down lignin structures and disrupt crystalline structures of cellulose for enhancing enzyme accessibility to cellulose during the hydrolysis step (Mosier et al 2005). Several recent reviews on pretreatment approaches have been published (Hendriks and Zeeman 2009; Alvira et al 2010). In general, pretreatment can be conducted with chemical methods (alkali pretreatments, acid pretreatments, ozonolysis, organosolvation method, and ionic liquids pretreatment) and physicochemical methods (SO_2 steam explosion, liquid hot water, ammonia fiber explosion, wet oxidation, microwave pretreatment, ultrasound pretreatment, and CO_2 explosion). Nevertheless, no information is yet available on ^{60}Co-γ irradiation treatment effect on structural properties of *Phragmites communis* trim (PCT) cellulose. Therefore, the purposes of this work were to 1) investigate changes in molecular and morphological structure of microcrystalline cellulose before and after ^{60}Co-γ irradiation treatment using scanning electron microscopy (SEM) and X-ray diffraction (XRD); and 2) assess the effect of irradiation treatment on degradation of microcrystalline cellulose using Fourier transform IR spectroscopy (FT-IR). In this study, PCT microcrystalline cellulose was chosen as the raw material because it is one of the most important cellulose resources in China, and PCT can be easily obtained from northeast China plain, inner Mongolia, Xingjiang Province, and northern China plain.

MATERIALS AND METHODS

Materials

PCT cellulose and microcrystalline cellulose made of *Phragmites* were received as gifts from Yuanjiang Paper Mills of Tai Gelin Group in Hunan Province, China. They were both processed before being used in experiments.

PCT cellulose was ground to approximately 500 μm using a cutting mill (Retsch GmbH SM1, Haan, Germany) with a 500-μm stainless steel trapezium-shaped sieve. Then the *Phragmites* cellulose was air-dried, homogenized in separate lots to avoid compositional differences between batches, and stored in polyethylene bags until use. Chemical compositions of PCT were measured using procedures from Ankom Technology (Macedon, NY; protocols available at www.ankom.com). Briefly, lignin was extracted in 72% H_2SO_4 in preweighed filter bags and quantified by weight after rinsing the acid with water at 90°C. Cellulose was quantified as the difference between acid detergent fiber (ADF) and lignin contents, and hemicellulose was quantified as the difference between ADF and neutral detergent fiber contents. With a few modifications, American Oil Chemists' Society methods were used to determine moisture and ash content of PCT (Firestone 1998). Moisture and ash contents were calculated by mass balance after PCT was oven-dried at 105 and 600°C, respectively. Chemical composition of PCT material was determined as follows: 50.15% cellulose, 23.40% hemicellulose, 20.72% lignin, 10.50% moisture, and 2.23% ash.

The isolation method of microcrystalline cellulose was as follows: *Phragmites* cellulose was cooked in the horizontal tube continuous cooking system under conditions of steam pressure 0.7 MPa, 165°C, and duration 35 min. After cooking, the cellulose slurry was obtained. Then 4% NaOH and 0.3% $MgSO_4$ were added to the slurry to delignification under pressure of 0.5 MPa for 90 min. After delignification, beginning pulp was obtained. Then the beginning pulp was oven-dried at 68°C for 48 h and ground to particle size to obtain microcrystalline cellulose.

Rice straw was collected and stored in a storehouse with adequate ventilation, allowing a natural dried condition. After it was stored approximately 3 months, the straw was washed and dried to about 10% moisture content (MC) (untreated sample) before use.

Osmium tetroxide, ethanol, and acetone were bought from Sinopharm Chemical Reagent Co. (Shanghai, China).

^{60}Co-γ Ray Irradiation Treatment

All irradiation treatment experiments were performed using a ^{60}Co-γ ray irradiation device at 1.85 × 1016 Bq in the Hunan Irradiation Center (Changsha, China). A batch of dry PCT cellulose materials in a glass bottle (net weight ≈200 g) was irradiated at room temperature at a ^{60}Co-γ radiation source intensity of 9.99 × 1015 Bq and dose rate of 2.0 kGy h^{-1}. Specific levels of ^{60}Co-γ irradiation doses used were 0 kGy, 400, 800, 1200, 1600, and 2000 kGy.

Surface Morphology Observation by Scanning Electron Microscopy

In the case of SEM examinations, treated cellulose samples were washed with saline solution at room temperature for 10 min, fixed with osmium tetroxide, and dehydrated first with ethanol and then acetone. Then samples were completely dried. Dry samples were mounted on sample holders and coated with gold. SEM images of samples were taken using a JEOL JSM 6360LV SEM microscope (Tokyo, Japan).

Crystalline Analysis by X-Ray Diffraction

X-ray diffraction patterns with Cu Kα radiation (λ = 1.5406 Å) at 36 kV and 30 mA were recorded in the range of 2θ = 10-40° with an XRD diffractometer (Siemens D500-XRD, Karlsruhe, Germany). Samples were mounted on a plastic holder 2 mm thick, and the proportional counter detector was set to collect data at a rate of 2θ = 1° min^{-1} with increments of 0.01° for 2θ values. All samples were cut into particle-like size to erase influence of crystalline orientation. The crystalline structure was attributed according to authoritative data. Total degree of crystallinity (CrI) was estimated by the Segal method (Inagaki et al 2010) using height of the (200) peak (I_{200}, 2θ = 22.7°) and minimum between the (200) and (110) peaks (I_{AM}, 2θ = 16.2°) by the following equation:

$$\mathrm{CrI}\,(\%) = [(I_{200} - I_{AM})/I_{200} \times 100] \quad (1)$$

where I_{200} is intensity of the 200 peak (at about 2θ = 22.7°) and I_{AM} is intensity corresponding to the peak at about 2θ = 16.2°.

Oxidative Degradation Determination by Fourier Transform-IR

FT-IR spectra were recorded with a WQF-310 FTIR spectrometer (Beijing Second Optical Instrument Factory, Beijing, China) and using a liquid nitrogen-cooled mercury–cadmium–tellurium detector in the regions of 4000-400 cm^{-1}. The running conditions were as follows: 4 cm^{-1} spectral resolution, 20 kHz scan speed, 128 scan coadditions, and triangular apodization. By setting the microscope square diaphragm aperture to 100 × 100 μm, an excellent spectrum was collected within a few minutes.

RESULTS AND DISCUSSION

Morphology Changes of ^{60}Co-γ Irradiation-Treated Sample

Effect of ^{60}Co-γ irradiation treatment on morphologies of cellulose was examined, and several representative SEM images are shown in Fig 1a-f.

Before irradiation treatment, the surface of the PCT cellulose was smooth and the conformation was integrated (Fig 1a). However, significant changes in the morphology of PCT cellulose were observed after irradiation treatment (Fig 1b-f). It can be seen that irradiation caused some fragmentation of cellulose fibers, the surface structure of cellulose fibers became loose, and their structure was damaged to increasing degrees as irradiation doses increased. These results confirm that ^{60}Co-γ irradiation would break the structure of cellulose fibers to various extents, depending on the irradiation doses.

To further support these observations, the dry rice straw sample, the crystalline cellulose from

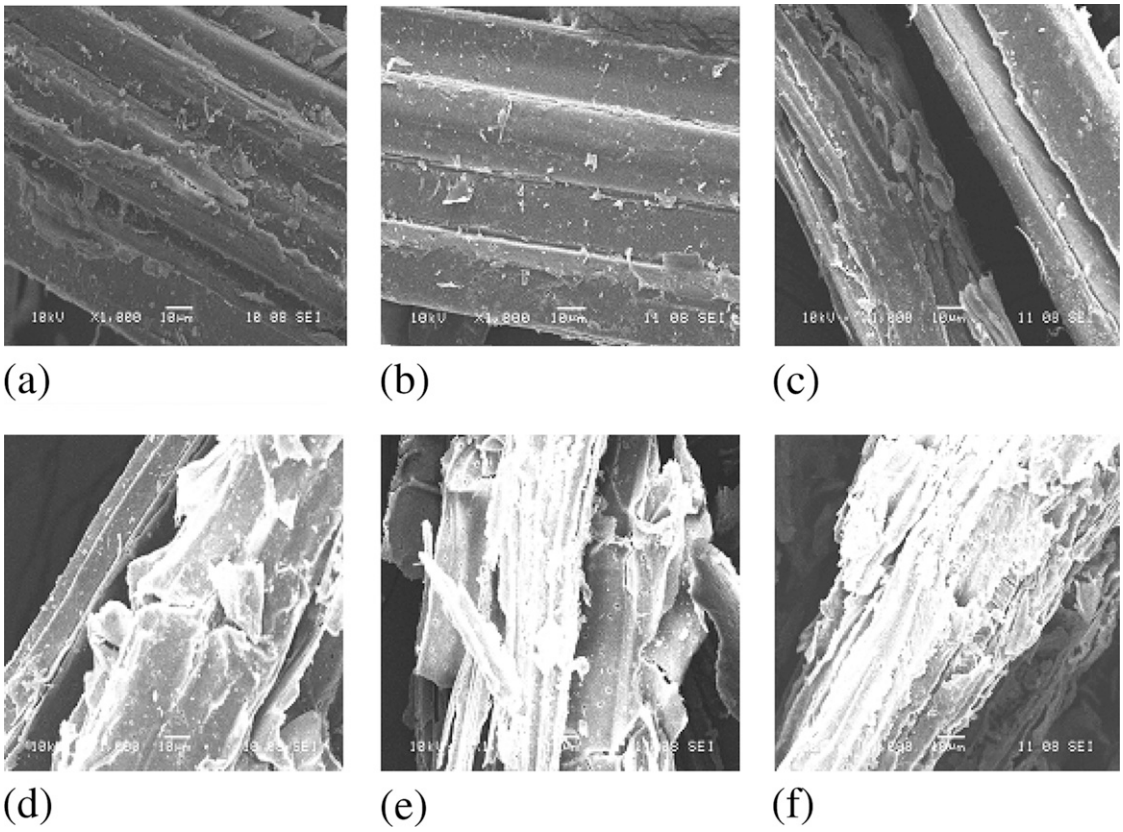

Figure 1. Scanning electron micrograph images of *Phragmites communis* trim (PCT) cellulose sample treated with different irradiation doses ([a] untreated PCT cellulose [0 kGy]; [b] 400 kGy; [c] 800 kGy; [d] 1200 kGy; [e] 1600 kGy; [f] 2000 kGy).

rice straw, and the crystalline cellulose from PCT were also exposed to ^{60}Co-γ irradiation at 2000 kGy. SEM images are shown in Fig 2. The morphology of cellulose samples would be destroyed when treated with ^{60}Co-γ irradiation.

Crystalline Changes of f ^{60}Co-γ Irradiation-Treated Microcrystalline Cellulose From *Phragmites communis* Trim

Cellulose is a complex polymer with crystalline and amorphous areas. XRD was further used to investigate effect of irradiation on the crystalline phase of microcrystalline cellulose. Figure 3 shows XRD patterns of PCT microcrystalline cellulose as a function of diffraction angle as well as irradiation doses changing from 0 to 2000 kGy. Degree of crystallinity was determined by the Segal method from the XRD chart (Fig 4).

After irradiation treatment, the CrI of PCT crystalline cellulose dropped from 78.4 to 40.7% (Fig 4). Hence, XRD indicates that part of crystalline cellulose was disrupted by irradiation. Figure 4 shows that degree of crystallinity obviously decreased with increasing irradiation dose, which may have been caused by degradation of hemicellulose or the amorphous regions in cellulose as observed from SEM spectra (Fig 1). The lower crystallinity of cellulose makes it more accessible to chemical reagents, and thus it is easier to hydrolyze to sugars (Mosier et al 2005; Driscoll et al 2009). Apparently, irradiation more easily cleaves the amorphous region of cellulose than its crystalline region.

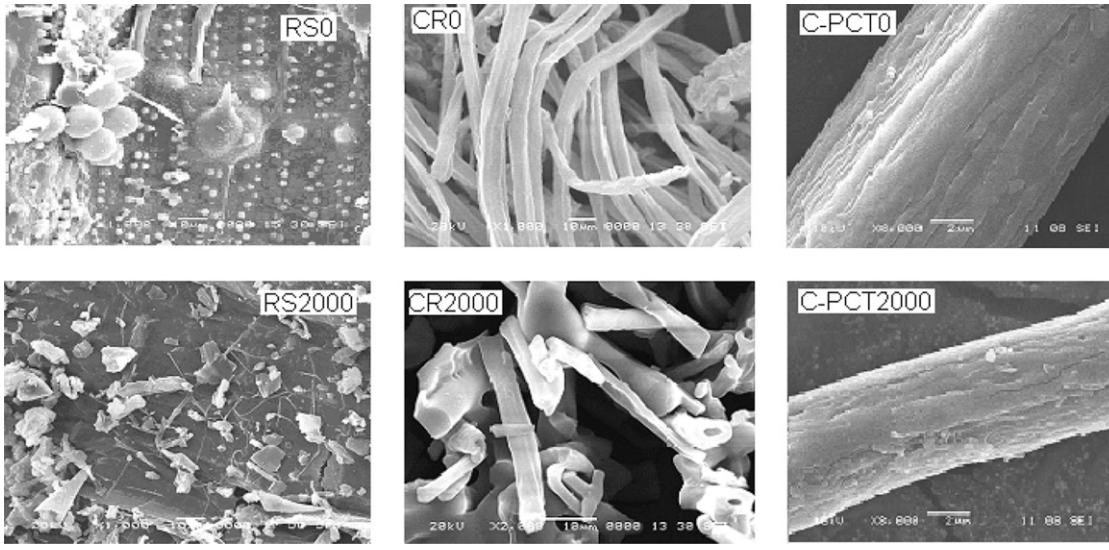

Figure 2. Scanning electron micrograph images of other cellulose samples treated with ^{60}Co-γ irradiation of 2000 kGy (RS0, untreated rice straw sample; RS200, rice straw sample treated with ^{60}Co-γ irradiation of 2000; CR0, untreated crystalline cellulose from rice straw; RS200, crystalline cellulose from rice straw treated with ^{60}Co-γ irradiation of 2000; C-PCT0, untreated crystalline cellulose from *Phragmites communis* trim [PCT]; RS200, crystalline cellulose from PCT treated with ^{60}Co-γ irradiation of 2000).

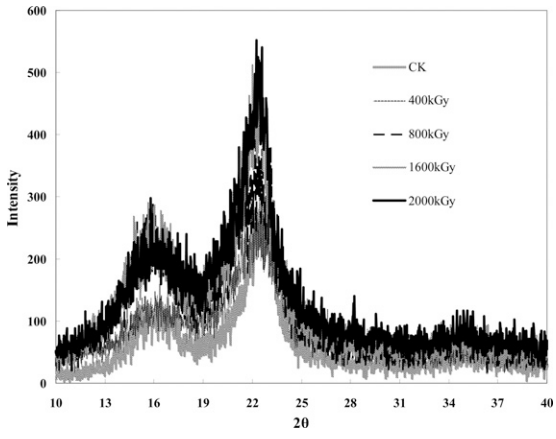

Figure 3. X-ray diffraction (XRD) images of crystalline cellulose of *Phragmites communis* trim (PCT) treated with different irradiation doses (untreated crystalline cellulose of PCT [CK]).

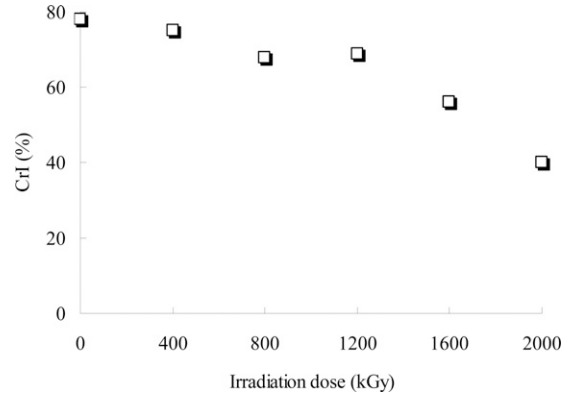

Figure 4. Variation of crystallinity index calculated from X-ray diffraction (XRD) chart.

Effect of ^{60}Co-γ Irradiation on Oxidative Degradation of Cellulose

As shown in Fig 5, additional shoulder appeared at 1734 cm^{-1} (caused by C = O stretching) on the O-H bending band. However, a very small band at 1501 cm^{-1} was noticed for PCT cellulose treated with 800 kGy. This band disappeared with higher irradiation doses (1200, 1600, and 2000 kGy). A reasonable explanation may be the small band at 1501 cm^{-1}, which they assigned to the N-H absorption band of proteinaceous glues, obviously has to be destroyed in higher irradiation doses. This observation is in

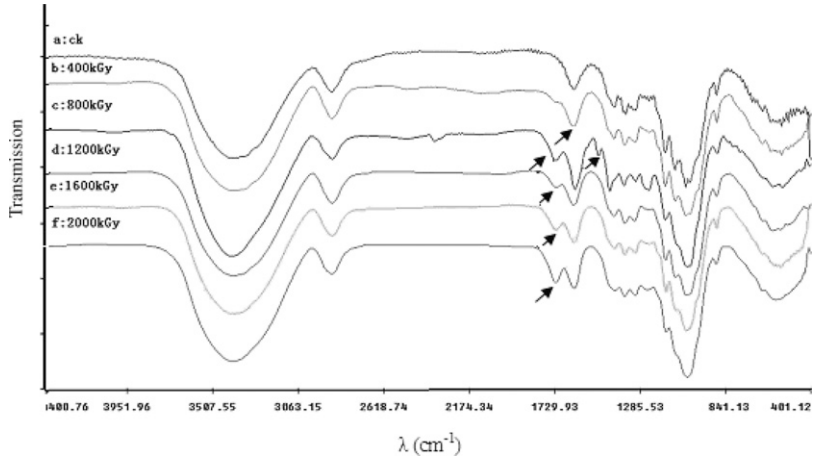

Figure 5. Fourier transform-IR spectra for the oxidative gradation of *Phragmites communis* trim (PCT) microcrystalline cellulose treated with different irradiation doses ([a] untreated microcrystalline cellulose [0 kGy]; [b] 400 kGy; [c] 800 kGy; [d] 1200 kGy; [e] 1600 kGy; [f] 2000 kGy).

good agreement with reported results in which a new characteristic peak of carbonyl groups (1734 cm^{-1}) was observed when the textile was exposed to irradiation (Fōldváry et al 2003). Degradation of cellulose treated with ^{60}Co-γ irradiation was characterized by measurement of the content of carbonyl groups using FT-IR (C = O stretching vibration) at approximately 1740 cm^{-1} (Takács et al 2000). It was shown that PCT cellulose exhibited oxidative degradation after irradiation treatment, which could be attributed to hydrolysis of cellulose.

CONCLUSIONS

Irradiation treatment using high-energy ^{60}Co-γ was found to be an energy-efficient and environmentally benign method to decrease crystallinity of microcrystalline cellulose by partially destroying the morphological structure of cellulose. Irradiation treatments on PCT cellulose could result in oxidative degradation and lead to an increase in carbonyl groups. These changes in structure and properties of PCT cellulose would make it more amenable to treat cellulose instead of using other chemical agents and thus to allow cellulose to be easily used as a raw material for production of sugars, ethanol, and other chemicals.

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