The Effects of UV-A on Dry Rice Straw Decomposition under Controlled Laboratory Conditions

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In arid and semi-arid areas, organic matter decomposition is stimulated by ultraviolet radiation. In this paper, the association between straw decomposition and UV-A exposure was evaluated. Oven-dried rice straw samples were chronically exposed to UV-A radiation and examined periodically for up to 90 days at room temperature. Scanning electron microscopy (SEM) showed that noticeable disintegration of the fiber structure occurred on the irradiated sample surface in comparison to the control. At the end of the UV-A treatment period, straw mass had decreased by 5%, and dissolved organic carbon (DOC) increased by 18%. The content of cellulose, hemicellulose, and lignin of the irradiated straw decreased by 29.3%, 14.4% and 49.3%, respectively. The marked loss of nitrogen and potassium in the exposed straw were also observed. Thermogravimetric analysis (TGA) showed that treatment with UV-A radiation tended to decrease the mass loss rate and the thermal degradation temperature of the straw biomass from 220 °C to 208 °C. Infrared spectrometric analysis (ATR-FTIR) showed that functional groups, *e.g.*, C–OH and C–O–C, were disrupted obviously due to UV-A exposure. These results suggest that ultraviolet-A irradiation facilitates straw decomposition by direct photochemical degradation.

Keywords: Ultraviolet-A irradiation; Rice straw photodegradation; Straw chemical composition; TGA; ATR-FTIR

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INTRODUCTION

Because ultraviolet irradiation accelerates organic matter decomposition, different wavelengths of ultraviolet light have been tested in practice (Moorhead and Reynolds 1989; Schade *et al.* 1999; Day *et al.* 2007; Brandt *et al.* 2010). Due to its long wavelengths and ability to penetrate the ozonosphere, UV-A radiation (315 to 400 nm) has an important effect on photodecomposition in the biosphere (Brandt *et al.* 2007). Although straw decomposition rates are accelerated by UV radiation in dry climates where precipitation is infrequent, UV-A facilitation of straw photodegradation has not been totally explained.

Under normal circumstances, exposure to ultraviolet irradiation in a dry environment promotes organic material decomposition and changes the microbial community structure and activity, making the non-biological factors the main factor in straw degradation (Austin and Vivanco 2006). In areas where precipitation is rare, ultraviolet irradiation promotes straw photodegradation (Grote *et al.* 2010). Degradation is even accelerated by UV in the absence of microbial enzymes. This effect is due to direct

photodegradation, which is the decomposition of complex organic matter into simple compounds that are more easily utilized by microorganisms (Parton *et al.* 2007).

Recently, the efficacy of ultraviolet irradiation on straw decomposition has been questioned. The effects of continuous exposure to ultraviolet irradiation on straw degradation become trivial with increased precipitation (Gallo *et al.* 2006; Uselman *et al.* 2011). However, the opposite effect has also been observed (Smith *et al.* 2010). This phenomenon may be attributed to interactions between microorganisms and ultraviolet radiation. In humid environments, microbial activity may be the primary driver of straw decomposition. Conversely, UV irradiation plays the major role in dry conditions (Foereid *et al.* 2010).

Crop residue decomposition plays a significant role in the biogeochemical circulation, especially in the carbon cycles. Compared with the mechanism of crop residue biodegradation that is mainly driven by microbes, the effect of UV-A irradiation on straw photodegradation has not been clearly expounded. The research on it is very meaningful in affirming independent survey observations from the field, and describing the related acting spectra that may be involved in direct photodegradation (Kirschbaum *et al.* 2011). This study evaluated the influence of UV-A radiation on the decomposition of dry straw. Straw samples were chronically exposed to UV-A radiation and examined periodically for 90 days at room temperature. The aims were: (i) to ascertain whether the tendency and the magnitude of straw decomposition is dependent on UV-A radiation, and (ii) to explain the mechanism of these phenomena with morphological and molecular observations.

EXPERIMENTAL

Sample Collection and Preparation

Rice straw was collected from an experimental station (32°2'16"N, 118°51'58"E) of Jiangsu Academy of Agricultural Sciences, Nanjing, China, in August 2014. The paddy rice cultivar was Nanjing 46 (*Oryza saliva* L.), and its growth period was 180 days. After collection, the straw was immediately dried in a drying oven at 55 °C for 48 hours. The composition of the straw was determined by physical and chemical analysis methods.

Methods

Experimental treatments

UV-A radiation was administered in a UV testing machine (Yishi Co., Shanghai, China) for 90 days from August to October of 2014. Straw was cut to a length of 20 cm, and the straw stems were affixed vertically by springs and fixed on an iron plate. Three plates were tied together. The weight of straw on each plate was 20.0 ± 0.89 g. Next, 9 replicate plates were placed at a 45° angle against the ground plane in the UV testing machine. A temperature of 26 °C and 20% relative humidity were maintained in the machine throughout the experiment. The radiation dosage was set to 1.5 Kw/m^2 of UV-A (315 nm) and continuously monitored using a broadband UV-A pyranometer (DSCUV, Yishi Co., Shanghai, China). Based on the transmission properties of UV-A, it was assumed that it radiated uniformly to all straw samples under the screen. One straw plate was taken out at day 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90. Photographs of the setup used for exposure of the specimens are given in Fig.1. For the control condition some straw stems, which were dried to constant weight, were put into the valve bags and then kept in the dark for 90 days at the beginning of the experiment. A temperature of 26 °C and 20%

relative humidity were maintained in another UV testing machine throughout the experiment, and they were analyzed at the end of the experiment.

Fig. 1. Photographs of the UV-A exposure setup

Scanning electron microscopy (SEM)

Stem surface of sample was examined by SEM (model KH-7700, Questar China Limited, China). Samples were gold-sputtered.

Mass measurements

Irradiated straw degrades and partially loses chemical components in the form of volatile compounds (McCulley *et al.* 2005). Straw mass was monitored to measure these changes. The percent remaining mass (r) was calculated as,

$$r = m_t/m_0 \times 100 \quad (1)$$

where the initial weight was m_0 and the mass of treated samples was m_t (t = 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 d).

Photo chemical analysis

If UV-A irradiation reaches a certain cumulative dosage, it breaks down bio-macromolecules such as cellulose, hemicellulose, lignin, protein, and lipids (Moorhead and Sinsabaugh 2006). Dissolved organic carbon (DOC) was extracted from straw as follows. A total of 1 g straw powder and 50 mL deionized water were placed into tubes. These tubes were shaken at 220 rpm for up to 5 hours and centrifuged for 15 minutes at 4000 rpm. Liquid was separated from precipitate with a 0.50-mm film filter. The DOC of the supernatant was analyzed by a DOC analysis apparatus (Multi N/C 3100 Analyzer, Analytik-Jena Group, Germany).

For elemental analyses, rice straw was ground to powder. Holocellulose content was detected *via* the Van-Soest method (Brandt *et al.* 2007). Nitrogen (N) content was determined by an elemental analyzer (Multi N/C 3100 Analyzer, Analytik-Jena Group, Germany). Phosphorus (P) content was detected by the Kjeldahl method and recorded using photometric analysis (Song *et al.* 2014). The nutrient potassium (K) was measured using a flame photometer (model FP6410, Shanghai Xin Yi Instruments, China). The nutrient concentration was calculated using Eq. 2,

$$E = (M_t \times C_t)/(M_0 \times C_0) \times 100 \quad (2)$$

where E is the nutrient concentration of the original sample value (%), M_0 is the original dried mass (g), C_0 is the original nutrient content (mg/g), M_t is dried mass at time t, and C_t is nutrient content at time t.

Thermogravimetric analysis (TGA)

Straw decomposition by UV-A radiation was determined by thermogravimetric analysis (SII-7200, Hitachi Limited, Japan) of rice straw powder samples of 6 to 8 mg. The selected temperature rate was 25 °C/min, and the experiments were conducted in a nitrogen atmosphere (rate of flow, 20 mL/min).

Fourier transform infrared analysis (FTIR)

The original straw and samples treated for 90 days were milled, sieved, and dried for 48 h at 60 °C. Spectra were collected in the range of 4000 to 500 cm^{-1} (model J200, Thermo Fisher Scientific, USA). Thirty two scans were accumulated at a resolution of 4 cm^{-1}.

Data analyses

The influence of UV-A irradiation time on straw mass loss, straw nutrient content, or DOC was fitted to a general linear model (GLM) over the course of the 90-day irradiation cycle. The model contained the prime factors between UV-A irradiation and experiment time. For FTIR and derivative thermogravimetry (DTG) data, multivariate analysis of variance (MANOVA) was applied to evaluate exposure effects, by contrasting the main peak areas for significant functional groups. These analyses were performed using the Software Statistical Package for the Origin Pro (Version 8.0, OriginLab, USA).

RESULTS AND DISCUSSION

Initial and Final Analysis of Control

Table 1 showed the changes of mass remaining and chemical composition of straw samples under dark condition with 90 days. Straw degradation was at a very low level during the whole experiment in the condition. The biodegradation and photodegradation were negligible in this study.

Table 1. Initial and Final Analysis (mean ± SD) of Samples under Dry and Dark Condition (n=3)

Parameter (%)	Initial	Shade
Mass Remaining	100 ± 0	99.39 ± 0.13
DOC	5.63 ± 0.44	5.68 ± 0.56
Nitrogen	0.76 ± 0.04	0.75 ± 0.06
Phosphorus	0.18 ± 0.01	0.17 ± 0.01
Potassium	1.89 ± 0.08	1.88 ± 0.04
Cellulose	31.22 ± 0.81	31.16 ± 0.83
Hemicellulose	28.85 ± 0.82	28.41 ± 1.38
Lignin	18.30 ± 0.30	18.22 ± 0.36

Scanning Electron Microscopy

Morphological changes of sample surface in the controlled and treated samples were observed by scanning electron microscopy (Fig. 2). Figures 2a and 2b showed the sample surface of initial and control, respectively. Sample surface were covered with large quantities of silicon and cork cells. Obvious histological changes in the straw surface were observed in Fig. 2c and Fig. 2d, which represented the irradiated sample at day 90. The fiber structure was disintegrated obviously after the straw samples were treated with UV-A. And the straw surface mulch was partially disrupted with the disintegration of the fiber structure, which caused by the elevated UV-A irradiation. Apparent morphological changes of sample surface provided strong evidence that the effect of UV-A on straw degradation was substantial.

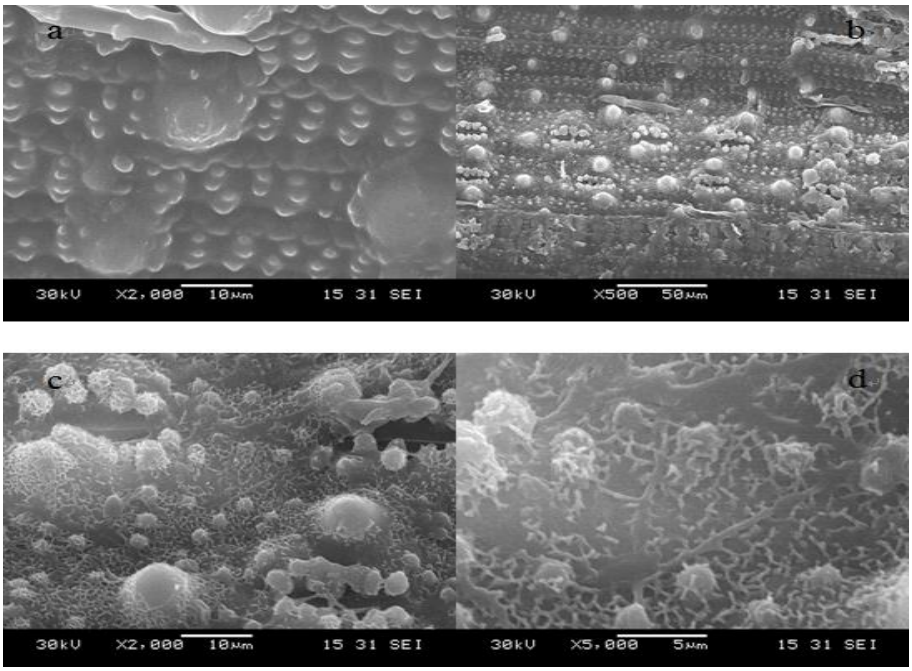

Fig. 2. SEM of rice straw surface. (a): initial; (b): sample surface of control at day 90; (c) and (d) refer to irradiated straw surface at day 90

Mass Remaining

The mass change in rice straw treated by UV-A radiation in dry conditions is shown in Fig. 3. Compared with the initial (Table 1), the total straw mass loss of irradiated samples was 5% during the entire experiment. The maximal rate of decomposition occurred during the initial 30 days and the last 20 days. The mass decreased by 2.66% in the first 30 days, 0.42% in the next 40 days, and 1.92% during the last 20 days.

UV light accelerates lignocellulosic material decomposition (Rutledge *et al.* 2010). A dramatic increase of more than 20% straw mass loss occurs after UV-B irradiation for up to 1.8 years. However, elevated UV radiation can also decrease straw mass loss. The differences in reported findings may be due to straw age, sample composition, differences in UV dose, and experiment time (Moorhead and Callaghan 1994; Verhoef *et al.* 2000). Because the experimental conditions and substrates affect straw degradation by ultraviolet irradiation, it is unreasonable to compare studies that use different exposure methods, UV wavelengths or intensities, or chemical composition analyses.

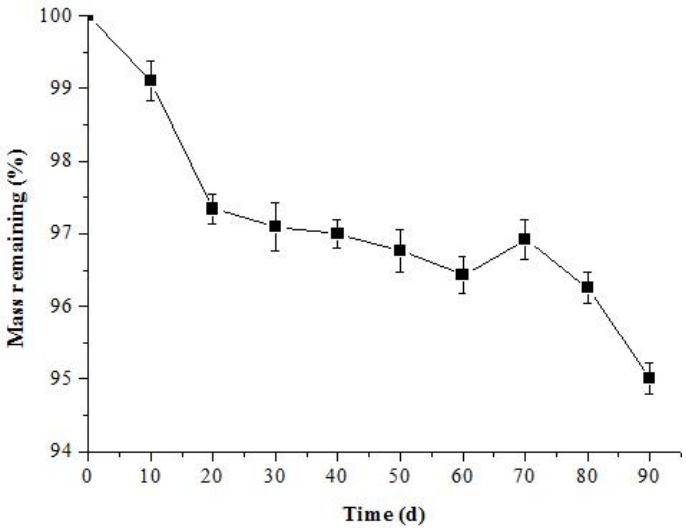

Fig. 3. Mass remaining in samples irradiated with UV-A in dry conditions for 90 days. Data points indicate means, and vertical bars refer to the standard deviation (n = 3).

In this study, dried rice straw sample was exposed continuously to ultraviolet irradiation under controlled laboratory conditions. The measured decomposition of straw was consistent with published values (Liu *et al.* 2014). Total straw mass loss was 5% during the entire treatment period (90 d). The maximum decomposition rate appeared first during the initial 20 days of irradiation, which was mainly caused by the photodegradation of lignin and partially because of the loss of combined water. Mass loss was not obvious from day 30 to day 70. During the 20 days before the end of experiment, the decomposition rate increased with the amount of radiation employed (Brandt *et al.* 2010). While it was hypothesized that mass loss would change dramatically under the elevated UV-A, this was not the case. Relatively low mass remaining in this study was in line with the previous study (Liu *et al*. 2014), which involved exposure of the straw to UV radiation. Their observed enhancement was small, amounting to a weight loss of less than 10% over 228 days of continuous exposure, but they showed that the straw's DOC and CO_2 emission rate changed obviously in the course of UV irradiation.

In the present paper, morphological changes of sample surface in the controlled and treated samples were observed by scanning electron microscopy. The fiber structure was disintegrated obviously after the straw samples were treated with UV-A. This further demonstrated that straw photodegradation was accelerated by UV-A radiation. Advanced decomposition with irradiation mainly depends on decreases in lignocellulose, nitrogen (N) content, and potassium (K) immobilization. In this study, straw DOC increased during the experiment, and the disintegration of lignocellulose in irradiated samples occurred at the same time (Fig. 2, Fig. 6).

Earlier research suggested that complex organic matter is broken into oxynitride, carbon oxides, *etc*., by direct photodegradation (Gallo *et al.* 2006; Liu *et al.* 2014). Also, the release of these gases has been stated as the main cause of mass loss (King *et al.* 2012). Unfortunately, gas emissions from straw were not measured in this experiment, which could have determined mass loss directly. Taking the experiment period into consideration, the short-term irradiation (3 months) may be the main explanation for the low mass loss. Compared with straw in dark, the total mass loss was more than 5% of irradiated straw which indicated that elevated UV-A radiation had a certain influence on straw degradation.

Straw Chemical Composition

The ability of UV-A to split large and complex lignocellulosic compounds into simple, more easily utilized materials was investigated. In general, lignin has a high resistance to microbial enzymes, but it is sensitive to different wavelengths of ultraviolet radiation (Rozema *et al*. 1997). It seemed possible that lignin decomposed into water-soluble species without relevant gas emission (Gould 1982; Lanzalunga and Bietti 2000; Henry *et al.* 2008).

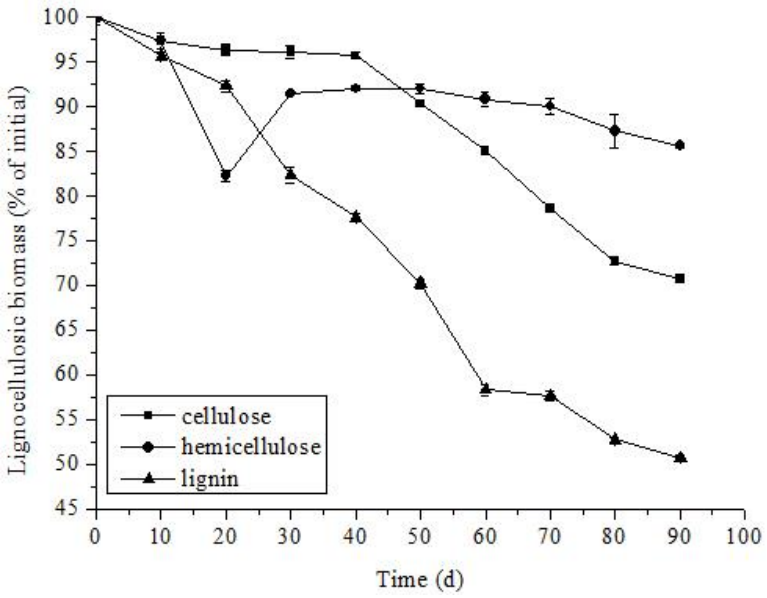

Fig. 4. Holocellulose remaining of irradiated straw. The data points show the mean values (n = 3) of cellulose, hemicellulose, and lignin in treated samples compared with the initial content (%). Standard deviation is shown by the vertical bars.

During UV-A exposure, straw lignocellulosic components decreased to different extents (Fig. 4). Compared with their initial biomass, cellulose, hemicellulose, and lignin decreased by 29.3%, 14.4%, and 49.3% at the end of the UV-A exposure experiment, respectively. Most studies, involving factors other than ultraviolet irradiation, tend to indicate more rapid degradation of hemicellulose by a variety of degradative pathways in comparison with cellulose. However, the mass loss of cellulose was obviously higher than hemicellulose in the present study. The decomposition rate of cellulose and hemicellulose was 29.3% and 14.4%, respectively. It was noteworthy that the decomposition rate of cellulose and hemicellulose was 4.3% and 8.6% during the first 40 days of UV-A irradiation, which account for 14.7% and 59.8% of total mass loss, respectively. This showed that hemicellulose was degraded at a higher rate in comparison to cellulose in this period. However, in the last 50 days, the decomposition rate of cellulose was 70.7% of total mass loss, which was about 1.8 times of the mass loss of hemicellulose during the same period. Most of the degradation of cellulose occurred in this period. Increased degradation of cellulose in the present study is in agreement with the previous research showing that cellulose is susceptible to UV-A irradiation (Schade *et al.* 1999).

However, their observed results (Shade *et al.* 1999) could not explain the mass loss of cellulose in this study was higher than that of hemicellulose. A photodegradation mechanism to explain the results in this paper can be proposed, based on the composition, the molecular arrangements of the components, and characteristics of lignocellulose. In an

intact lignocellulosic structure, lignin plays an important role as the protective layer. In this study, lignin was degraded at a high rate throughout the entire experiment. The degradation rate of lignin was 22.4%, which accounted for 45.4% of the total mass loss. As a result of the photodegradation of lignin, cellulose and hemicellulose were exposed directly to the UV-A irradiation. Cellulose in lignocellulosic materials was made up of 7,000 to 10,000 glucose units. In contrast to cellulose, hemicellulose is a heterogeneous polymer that is made up of 500 to 3,000 monosaccharide units. Therefore, the degradation of lignin led to the increasing of UV-contact area about cellulose, which was much higher than that of hemicellulose. Furthermore, along with the degradation of cellulose, long-chain cellulose was decomposed into many shorter molecules. Compared to cellulose, hemicellulose is generally believed to be more closely associated with lignin. It follows that the remnants of lignin within the straw would be more effective in protecting hemicellulose molecules from the incidence of UV rays, compared to cellulose. In the present study, the direction of incidence of the light did not change. When hemicellulose was sheltered from the ultraviolet irradiation by the remnants of lignin molecules, the rate of degradation of hemicellulose would be decreased. This is the reason why the degradation rate of hemicellulose was very slow in the last 50 days. A schematic representation of the effect of UV-A irradiation on lignocellulose at different stage is given in Fig. 5.

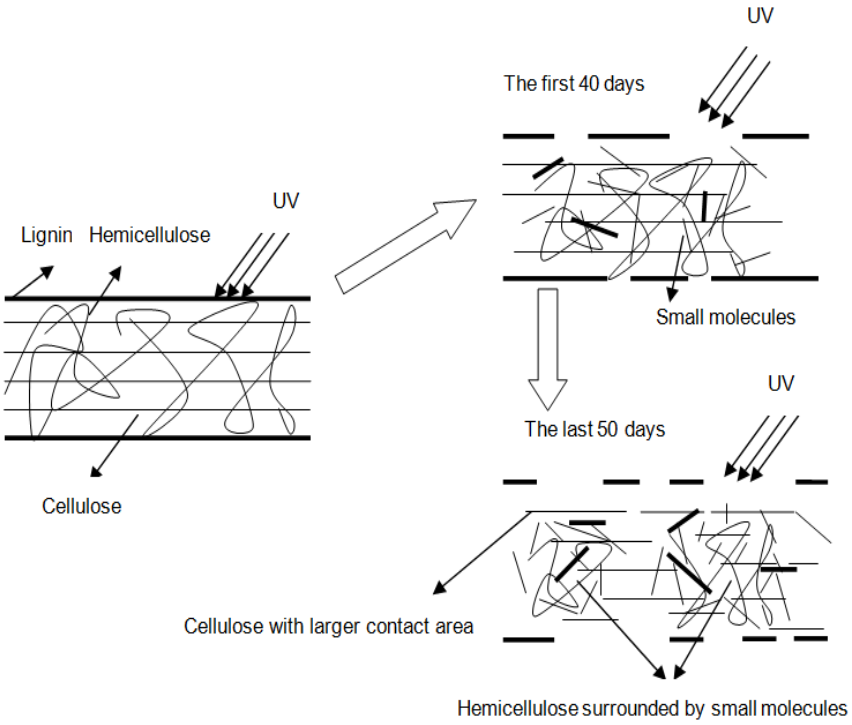

Fig. 5. A schematic representation of the effect of UV-A irradiation on lignocellulose at different stage.

To verify biological macromolecule degradation, dissolved organic carbon was also monitored during the UV treatment period (Fig. 6). The extractable DOC rapidly increased by 19.8% in the first 30 days, compared with the initial content (56.3 mg/g). DOC slightly decreased during days 30 through 70 and increased again during the last 20 days. After the 90-day treatment, straw DOC had increased by 18%. What caused the increase of the content of DOC during the last 20 days was complicated. One of the main reason was that

along with the destruction of the protective barrier in intact cellular structure, other carbon-containing compounds such as cellulose and hemicellulose were exposed to the UV-A irradiation directly and further degraded by free radical, oxygen and enzymes, *etc*.; such free radicals would be generated during the UV-A irradiation (Liu *et al.* 2014). This change implied that UV-A radiation broke down biological macromolecules into soluble organic small molecules in dry conditions. Phosphorus content was not noticeably different during the entire treatment period (data not shown). N and K content were markedly reduced to 70.0% and 62.8%, respectively, of the initial content (Fig. 6). The content of DOC, nitrogen, and potassium in irradiated straw was reduced notably in comparison with the control (Table. 1). Therefore, photodegradation played an important role in straw decomposition.

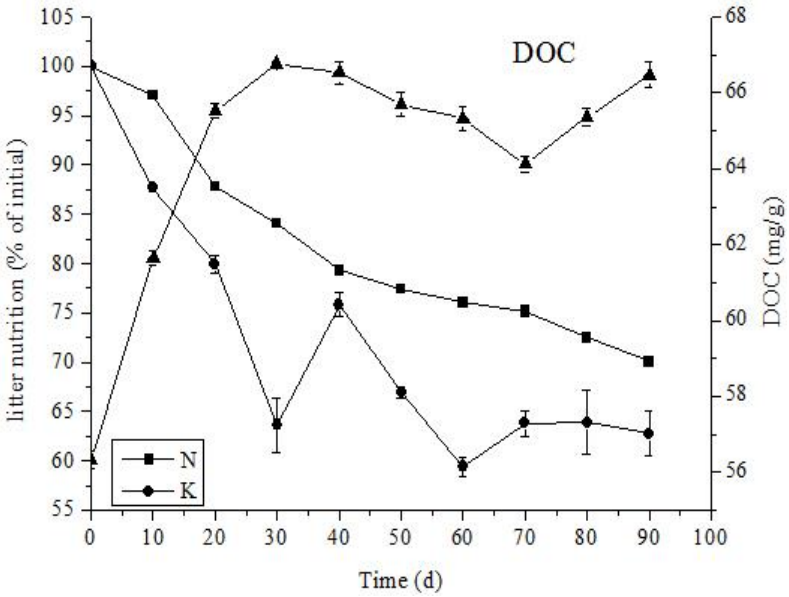

Fig. 6. The content of N, K and DOC in irradiated straw samples. Data points indicate means, and vertical bars refer to the standard deviation (n = 3).

Straw N release is partially driven by initial N concentration (Smith *et al.* 2010). N release occurs when the initial N concentration is between 0.6% and 2.8%. In this study, the initial straw N concentration was in the lower range, leading to N release during the experiment. UV radiation suppresses the growth and activity of microbial decomposers, resulting in slower N release (Pancotto *et al.* 2005; Jeffery *et al.* 2009). However, increased ultraviolet irradiation has no obvious impact on N release in subtropical climates (Song *et al.* 2011; Song *et al.* 2012). These conflicting findings could be a result of interspecific differences and the complexities of straw N release during elevated UV radiation.

Unlike N and C, potassium is not released in gaseous form. In the present study, K was the nutrient most rapidly lost from the decomposing straw, and approximately 38% of the initial K was lost. SEM images showed that complete sample surface was covered with tiny particles of silicon, cork cells and wax layer at the beginning of the experiment (Fig. 2a). However, the wax layer was disrupted at the end of the exposure experiment (Fig. 2c; Fig. 2d). The silicon and cork cells on the surface decreased notably while the intact fiber structure was being disintegrated by the prolonged UV-A irradiation. In general, the loss of potassium in straw was due to the volatilization during the pyrolysis process and the disintegration of intact lignocellulosic structure. Compared with control (Fig. 2b), apparent

decrease of the content of potassium proved indirectly that UV-A irradiation accelerated the photodegradation of straw. However, the total mass loss was 5% of irradiated straw. The relative low mass loss did not match the high potassium loss rate. The authors were not able to find any other studies that investigated such effects of ultraviolet-A irradiation on potassium loss. This is a brand new part of research which is currently under active development.

TGA and DTG of UV-Treated Rice Straw

Straw degradation can be described by thermogravimetric analysis (Fisher *et al.* 2002). TG and DTG curves were calculated for the initial, control and treated rice straw samples. Irradiated samples were collected on day 30 and day 90 (Fig. 7). Two decomposition stages were observed. Stage one (< 150 °C) reflected the volatilization of adsorbed water and bound water (Qu *et al.* 2015). Stage two (150 to 400 °C) comprised the decomposition of holocellulose. The main peak in DTG curves during this period is caused by the pyrolysis of straw cellulose. Lignocellulosic materials revealed a shoulder at left, which is due to the thermal decomposition of hemicellulose and lignin (Li *et al.* 2008).

The mass loss of initial, control, 30-day-treated sample, and 90-day treated sample during the pyrolysis process increased successively. The initial temperature of pyrolysis of the control was basically the same as for specimens that had not been exposed to UV light. The initial temperature of straw thermal decomposition was lowered from 220 °C in the initial to 208 °C in the 90-day-treated sample, and the mass lost during TG process was reduced from 76% to 68%, respectively. The lignin was decomposed by degrees along with UV-A radiation. This result is consistent with the variation in lignocellulose (Fig. 4).

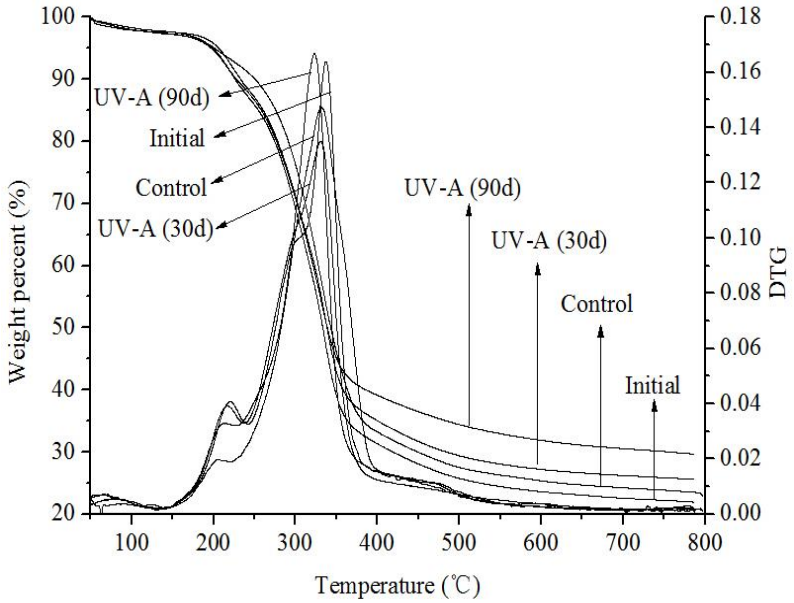

Fig. 7. Thermogravimetric analysis of initial, control, and irradiated rice straw. The temperature rate was 25 °C/min.

Lignin acts as a protective layer to the cellulose and as an antioxidant, which enhances the oxidation stability of cellulose (Johnson 2003; Yang *et al.* 2010). Using the temperature rate of 25 °C/min, DTG curves of UV-A treated rice straw showed a shoulder at the lower temperature, which contrasted with the control sample. This result was attributed to the loss of lignin and the decomposition of cellulose (Zeng *et al.* 2011). Large

organic matter in straw decomposed into plentiful dissolved organic matter, for example, dissolved organic carbon and water-soluble phenolic acid (Fig. 6). Hence, as anticipated in the course of lignin decomposition, DOC was dramatically associated with lignin in the sample.

Fourier Transform Infrared Analysis

Infrared spectroscopy analysis of straw samples is shown in Fig. 8. The characteristic peak from 3000 to 3750 cm^{-1} was caused by the C-OH, amine stretching vibration, and hydrogen bonds between molecules (Guan *et al.* 2006; Nanda *et al.* 2007). The length extension vibration mode detected at 2906 cm^{-1} was mainly due to C-H bonds. The characteristic absorption peaks at 1600 cm^{-1}, 1580 cm^{-1}, 1500 cm^{-1}, and 1450 cm^{-1} were attributed to benzene skeleton vibration in lignin. The absorption bands at 1036 and 830 cm^{-1} referred to the C-C and C-O length extension vibration, respectively. Results in this study showed that the degradation of the functional groups of samples in the dark condition was negligible in comparison with the initial. The FTIR spectrum of control was basically the same as the initial state of the material.

The relationship between straw chemical properties and UV-A exposure is illustrated in Fig. 8. Compared with the initial, the peaks in the 90-day radiated sample were shifted from 2897, 1027, and 823 cm^{-1} to 2906, 1036, and 830 cm^{-1}, respectively, which indicated that the C-H, C-C, and C-O bonds were affected by the UV-A. Furthermore, the absorption bands of the -OH, -CH, and C-C were weaker. UV-A radiation decreases the amount of C-O-C in benzene, which is consistent with the TGA results.

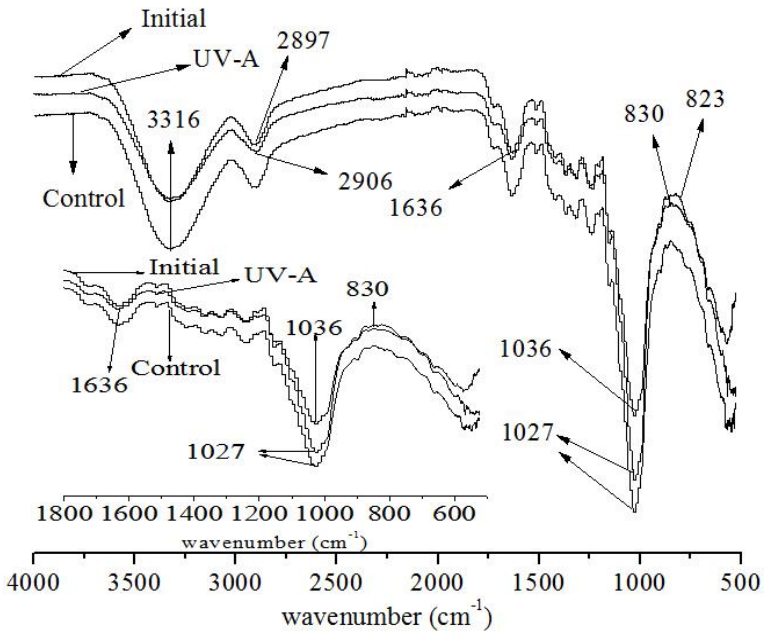

Fig. 8. Infrared spectroscopy analysis of initial, control, and treated rice straw samples at day 90

UV-A degradation of the rice straw resulted in the decomposition of lignin and a corresponding increase in DOC (Fig. 6). This result was in accord with previous studies, which found that UV irradiation accelerates the decomposition of the lignin, and many lignin monomers are detected after the experiment (Yuan *et al.* 1998). The role of UV-A in the mass loss of straw was largely caused by lignin photodecomposition, as UV light destroys lignin cell wall structures and releases compounds for microbial consumption.

CONCLUSIONS

1. In this study, the total mass loss of irradiated straw was 5%. Compared with the control, intact lignocellulosic structure of irradiated straw was disintegrated at the end of the experiment. Therefore, ultraviolet irradiation played an important role in the decomposition of rice straw.
2. Decomposition was accompanied by an increase in straw DOC content and a decrease in nitrogen and lignin. In addition, TG and FTIR analysis were consistent with variations in lignin. Therefore, lignin appears to be the most important factor in straw photodecomposition.
3. UV-A irradiation accelerated the decomposition of lignocellulosic materials directly by photodegradation under dry conditions. Large molecular substances were mainly broken into diverse dissolved organic matters. The increase in DOC content offset the photodecomposition of cellulose, hemicellulose, and lignin; thus, mass loss was relatively small.

ACKNOWLEDGMENTS

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