

Comparison of the Thermal Degradation Properties of Crystalline and Amorphous Cellulose, as well as Treated Lignocellulosic Biomass

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Thermo-gravimetric analyses of three cellulosic substances, namely, microcrystalline and amorphous cellulose, and treated Japanese cypress (JC) sawdust were carried out in this study. The thermal degradation temperature of crystalline cellulose decreased with increasing ball-milling time, while that of amorphous cellulose barely changed. However, small differences in the derivative thermo-gravimetric (DTG) curves between crystalline cellulose (*i.e.*, before ball milling) and amorphous cellulose (*i.e.*, after ball milling) were observed. The DTG curves of high-crystalline cellulose were sharp and similar to those of low-crystalline samples. The thermal degradation temperature of JC was decreased by ball milling, and its DTG peak shape became broad and low. These effects could be caused by the denaturing of non-cellulosic substances such as hemicellulose and lignin. The thermal degradation behaviors revealed by the DTG curves may serve as indicators of crystalline cellulose purity and other physical properties of lignocellulosic biomass.

Keywords: Thermal gravimetric analysis; Cellulose; Lignocellulose; Crystallinity; Ball milling

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INTRODUCTION

Cellulose, whose annual worldwide production ranges between 10^{10} to 10^{11} tons, is the most abundant natural organic material on Earth, and it is both renewable and biodegradable (Hon 1994). Almost all native cellulose is covered with hemicellulose, pectin, and lignin. The lignin has a 3D structure composed of phenolic units. These complexes are called "lignocellulose" or "lignocellulosic biomass". Lignocellulose has various structures and compositions, which make it robust or "recalcitrant", meaning that it resists biological degradation. To use cellulose effectively, a facile method for the extraction or separation of cellulose from lignocellulose and simple techniques for its evaluation are required.

Various pretreatments for the enzymatic hydrolysis of lignocellulose have been reported (Sun and Chen 2002; Mosier *et al.* 2005). Previous studies have investigated the pretreatments of lignocellulosic biomass such as Japanese cypress (JC), *Miscanthus* sp., and rice straw (Hideno *et al.* 2009, 2013a,b). Different causes and combinations thereof have been proposed for the increase in enzymatic digestibility, such as increased surface area, cellulose exposure, removal of hemicellulose and lignin, and decreased crystallinity. For example, ball milling has been applied in the pretreatment procedure for the enzymatic hydrolysis of lignocellulosic biomass (Sun and Chen 2002). In general, the increased enzymatic digestibility attained by ball milling is explained by the reduction in crystallinity, which is derived from the cellulose in lignocellulose. However, the effects of ball milling

on hemicellulose and lignin are not fully understood. Hence, multiple measurements, which require large amounts of labor and time, must be performed for the subsequent analysis of lignocellulosic biomass after pretreatment and enzymatic hydrolysis. A simple evaluation method needs to be developed to simplify the overall analysis process.

Thermo-gravimetric analysis (TGA) is considered to be one of the most suitable evaluation methods for characterizing heterogeneous organic materials, such as lignocellulosic biomass (Negro *et al.* 2003). Moreover, TGA is convenient and is reproducible. The different components of a lignocellulosic biomass exhibit thermal behaviors. Antal and Varhegyi (1995) have summarized the kinetics of cellulose pyrolysis based on results obtained from the thermal degradation of cellulose and lignocellulose. TGA has also been used as an alternative method for the determination of α -cellulose and hemicellulose contents (Carrier *et al.* 2011). Highly detailed mechanisms for the thermal degradation of cellulose and generation of levoglucosan, and the interactions between cellulose and other components in wood pyrolysis have been proposed (Hosoya *et al.* 2007; Matsuoka *et al.* 2014; Kawamoto 2015). The effect of cellulose crystallinity on TGA results has also been reported, revealing that low-crystalline cellulose begins to degrade at a lower temperature, with sharper derivative thermo-gravimetric (DTG) curves and lower activation energies (Wang *et al.* 2013). DTG curves also indicated that the thermal degradation temperatures are elevated by interactions between crystalline cellulose and lignin (Hilbers *et al.* 2015). Furthermore, TGA has been used to study the effects of ball milling on the thermal stability of cellulose fiber (Avolio *et al.* 2012) and the effects of ammonia fiber expansion treatment on the thermochemical properties of corn stover (Singh *et al.* 2015). Thus, many such studies have been conducted on the thermal decomposition of cellulose and lignocellulose. However, the effects of crystallinity on the thermal degradation of lignocellulose have not been fully investigated. Moreover, the effects of non-cellulosic organic substances in the pretreated lignocellulose, such as hemicellulose and lignin, on the thermal decomposition are not well known.

To research the possible application of TGA as a simple means of evaluating pretreated lignocellulose for enzymatic hydrolysis, the thermal degradation properties of crystalline and amorphous cellulose, as well as treated lignocellulosic biomass, were analyzed and compared in this study. First, the effects of ball milling on the thermal decomposition of microcrystalline cellulose was investigated. Second, the TGA of various pretreated Japanese cypress with high enzymatic digestibility was conducted *via* curve fitting and peak separation. Third, the relationships between the cellulose contents and crystallinity in lignocellulose, and thermal degradation behavior were investigated.

EXPERIMENTAL

Materials and their Sample Preparation

Avicel PH-101 (Fluka Analytical Co., USA) was used as a standard microcrystalline cellulose and milled using a ball mill to prepare amorphous cellulose. Japanese cypress (JC) and two pretreated materials (*i.e.*, ball-milled JC and organosolv-treated JC) were used to investigate the thermal degradation properties of cellulose in lignocellulosic biomass. The samples from JC and treated JC were prepared as described in previous reports (Hideno *et al.* 2013a). The JC sample of approximately 15 g was soaked in 80 mL of mixed solvent (ethanol: water: hydrochloric acid = 50: 50: 0.4) and autoclaved at 170 °C for 45 min using a portable reactor. Approximately 1 g of Avicel PH-101 or JC

sample was milled using the planetary ball mill (Fritch Japan Co., Japan) with a total of 118 g of stainless-steel balls for approximately 5 to 60 min. The main chemical contents of the samples are summarized in Table 1.

Thermal Gravimetric Analyses

Approximately 5 to 10 mg of the sample was formed into a tablet (Φ 4.5 mm) using a custom-made hand-press machine. The TGA was performed using a TGA instrument (TG/DTA6200, Seiko Instrument Co., Japan) under a nitrogen atmosphere, at a flow rate of 100 mL/min based on the conditions reported by Uetani *et al.* (2014): room temperature to 110 °C (40 °C/min), 110 °C for 10 min, 110 to 550 °C (10 °C/min), and 550 °C for 10 min. The TG and DTG curves were plotted by calculation using the following equations:

$$\text{TG (\%)} = (\text{weight loss due to thermal decomposition} / \text{original weight}) \times 100 \quad (1)$$

$$\text{DTG (\%/}^\circ\text{C)} = \text{TG (\%)} / \text{increase in temperature (}^\circ\text{C)} \quad (2)$$

The weight of the sample at 120 °C was set as the "zero point" of weight loss, defined as 100% dry weight.

Curve fitting and peak separation of the DTG peaks were carried out by using TA7000 software (Hitachi Co., Japan) combined with Fityk (ver. 0.9.4: Wojdyr 2010), which is an open-source software for non-linear curve fitting and analysis. The DTG peaks were separated by the split Gaussian method and fitted with the Levenberg-Marquardt algorithm based on the method of Nyon *et al.* (2015).

X-ray Diffraction Analyses

X-ray diffraction (XRD) analyses were carried out using an Ultima IV XRD instrument (Rigaku Co., Japan) with Cu K α radiation at 40 kV and 300 mA based on the method reported by Abe and Yano (2009). Samples were scanned over the range of $2\theta = 5$ to 40° at a rate of $1^\circ/\text{min}$. The crystallinity index (CrI) was calculated by Eq. 3, based on the method of Segal *et al.* (1959) using the height of the 002 peak (I_{002} , $2\theta = 22.5^\circ$) and the minimum between the 002 and 110 peaks (I_{am} , $2\theta = 18.7^\circ$),

$$\text{Crystallinity Index (\%)} = [(I_{002} - I_{am}) / I_{002}] \times 100 \quad (3)$$

where I_{002} is the peak intensity corresponding to crystalline cellulose I, and I_{am} is the peak intensity of the amorphous fraction.

Table 1. Main Chemical Components of the Cellulosic and Lignocellulosic Samples

Sample	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reference
Avicel PH-101	95>	-	-	Park <i>et al.</i> 2010
Raw Japanese cypress	40	19	31	Hideno <i>et al.</i> 2013a
Organosolv treated Japanese cypress	61	N.D.	23	Hideno <i>et al.</i> 2013a
Alkaline peroxide treated Japanese cypress	41	3	22	Hideno <i>et al.</i> 2013b

RESULTS AND DISCUSSION

The crystallinity index and thermal degradation properties of ball-milled Avicel PH-101 are shown in Table 2. Increasing the ball milling time by 20 min decreased CrI, the thermal degradation temperatures at 5% weight loss, and the height of the DTG peak. These results indicate that the crystalline cellulose was broken into small crystals and amorphous cellulose, and it was fully destroyed by ball milling. The thermal degradation temperature decreased at a lower rate than did the CrI. These findings suggest that the thermal degradation properties are different between crystalline and amorphous cellulose. After the crystallinity of the cellulose was lost and most of it was converted into amorphous cellulose, increase in the milling duration no longer decreased the thermal degradation temperature.

As shown in Fig. 1, thermal degradation temperatures gradually continued to shrink by 10 min of ball milling, though no crystallinity was detected after 20 min of ball milling. Considering the CrI results from the XRD analyses, almost all of the cellulose is thought to change from high-crystalline cellulose to amorphous cellulose after 10 min of ball milling. The trends of the thermal degradation temperatures of cellulose revealed completely different behaviors for crystalline and amorphous cellulose. The present results indicate that ball milling had little effect on the thermal degradation temperatures of amorphous cellulose and that the thermal degradation behaviors were correlated with the cellulose crystallinity under limited conditions (*i.e.*, when detecting crystalline cellulose).

The height and shape of the amorphous and microcrystalline cellulose DTG peaks were almost the same, although the DTG peaks of ball-milled Avicel (amorphous) were slightly higher than those of Avicel (microcrystalline cellulose) (Fig. 2). Meanwhile, the thermal degradation temperature of ball-milled Avicel was lower than that of untreated Avicel. For example, the temperatures for 15% weight loss and DTG peak maximum were approximately 310 °C (amorphous) *vs.* 319 °C (crystalline), and 331 °C (amorphous) *vs.* 339 °C (crystalline), respectively. The present results show that amorphous cellulose does not affect the DTG-peak height but does affect the thermal-degradation temperature, in agreement with a previously report (Wang *et al.* 2013).

Table 2. Crystallinity Index and Thermal Degradation Properties of Ball-Milled Avicel PH-101

Ball-milling duration (min)	CrI (%)	Temperature (°C)		
		at 1% weight loss	at 5% weight loss	at DTG maximum
0	88.0	276.3	305.4	335.1
5	44.6	281.4	302.5	331.0
10	9.8	275.4	297.8	326.5
20	N.D.	273.6	296.5	325.6
30	N.D.	271.8	295.4	326.5
60	N.D.	271.0	295.6	328.6

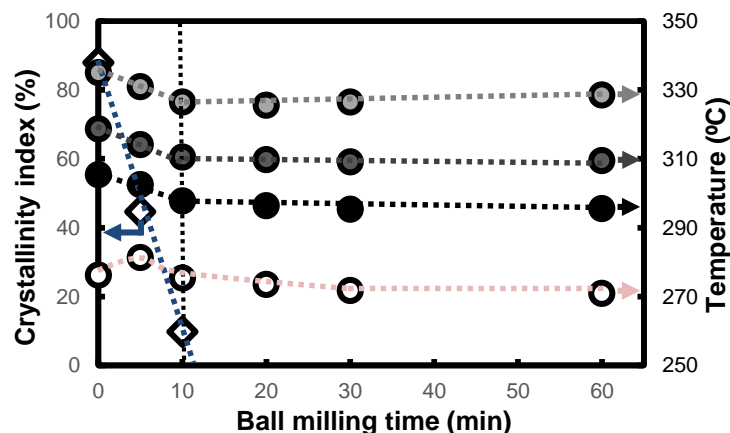


Fig. 1. Effects of ball milling time on crystallinity and thermal degradation temperature. Open, closed, and gray circles represent the thermal-degradation temperatures at 1% weight loss, 5% weight loss, and DTG peak maximum, respectively. Open diamonds indicates the crystallinity index.

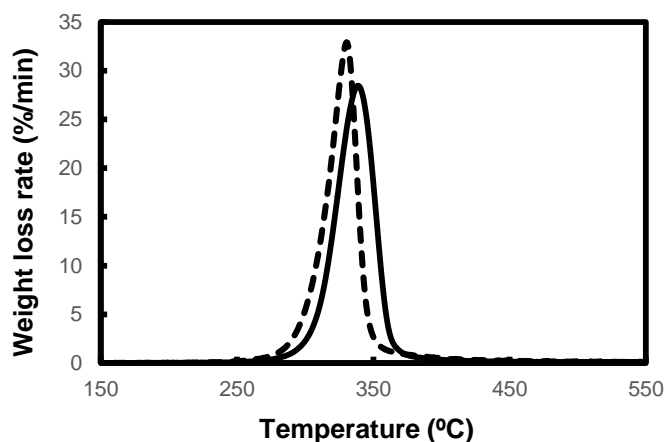


Fig. 2. Comparison of DTG peaks between crystalline cellulose and amorphous cellulose. Solid and dotted lines indicate the DTG curves of crystalline cellulose (without ball milling) and amorphous cellulose (with ball milling for 60 min), respectively.

The experimentally recorded and theoretically fitted and separated DTG curves for the four kinds of JC samples (raw, ball-milled, alkaline hydrogen peroxide-treated, and organosolv-treated JC), are shown in Fig. 3. Table 3 shows the crystallinities and representative thermal degradation properties of the treated JCs. All samples were prepared to contain 5 mg of cellulose content for these TG analyses. The chemical composition of ball-milled JC was basically the same as that of raw JC, but their DTG curves were clearly different. The thermal degradation temperature of ball-milled JC was lower than that of raw JC. In particular, the difference in the DTG-peak temperatures of the raw and ball-milled JC samples was approximately 37 °C (Table 2), which is substantial. Moreover, the DTG curve for ball-milled JC was broad and had lower peak heights than the other DTG curves. This result was clearly different from the result for the ball-milled Avicel PH-101, which is an amorphous cellulose. These results suggest that the effects of ball milling on the thermal degradation of JC were not due to the disappearance of crystalline cellulose;

rather, they were due to the denaturing by the hemicellulose and lignin present in the JC sample. Hosoya *et al.* (2007) have reported that the chemical interaction between woody poly-sugars (cellulose and hemicellulose) and lignin strongly affects their individual thermal degradation behaviors. These chemical interactions in the JC sample may also be affected by ball milling. Ball milling has been widely applied as a pretreatment technique for enzymatic hydrolysis (Hideno *et al.* 2009) and the structural analysis of lignin and hemicellulose using nuclear magnetic resonance (NMR) (Samuel *et al.* 2011). Hence, it has been assumed that mild ball milling does not change the lignin structure (Ikeda *et al.* 2002). However, there are reports that long ball-milling times increase the total C=O fractions in lignin (Mao *et al.* 2006). The present TGA results indicate that not only cellulose but also hemicellulose and/or lignin are damaged by dry ball milling. Especially, it is highly possible that hemicellulose has been damaged by dry ball milling, judging from the increased thermal degradation below 300 °C (Fig. 3 (a) vs. (b)), the slight difference in the DTG curves between the Avicel and ball-milled sample (Fig. 2), and the non-significant interaction between cellulose and hemicellulose during pyrolysis in nitrogen (Hosoya *et al.* 2007). Traditional analyses, such as the monomeric sugars analyses using sulfuric acid, are incapable of detecting these changes in hemicellulose because they detect soluble sugars, including monomeric and oligomeric sugars, after fully hydrolyzing the cellulose and hemicellulose in lignocellulose.

The DTG curve of organosolv-treated JC was sharper and exhibited higher peaks than the other DTG curves. The curve shape was similar to that of the microcrystalline cellulose. In fact, the cellulose content and crystallinity of the organosolv-treated JC were the highest of all the JC samples studied. It is possible that the disappearance of hemicellulose and lignin denaturation and reduction inhibit the thermal degradation of organosolv-treated JC below 250 °C. However, the thermal degradation of organosolv-treated JC began to accelerate above approximately 300 °C. Accordingly, the DTG peak temperature of organosolv-treated JC was lower than that of the raw JC sample. The cellulose in the treated-JC might have been damaged by the organosolv treatment. The present results indicate that the DTG curves reflect the cellulose content and denaturing of other components in lignocellulose.

All DTG curves were separated into 4 to 5 peaks and then fitted. Considering the range of thermal degradation temperatures, the main separated peaks most likely indicate the thermal degradation of cellulose. In particular, the main peak in 3(d) was the sharpest and had the highest peak height. These findings are related to the high cellulose content and crystallinity of the organosolv-treated JC sample. Judging from Figs. 1 and 2, the height of and area under the DTG peaks were substantially related to the cellulose content but only weakly related to the crystallinities. The other separated peaks could not be identified with certainty. These peaks could be attributed to hemicellulose and lignin, or to a hemicellulose-lignin complex in the biomass sample. Alternatively, these peaks may not indicate the actual composition of the biomass, only the expediently calculated signals. The present results suggest that DTG curves reflect the characteristics of the biomass as a whole, and the main separated peaks in these curves are attributed to cellulose.

In Fig. 3, the three samples ((b)-(d)) had exhibited higher enzymatic digestibility than (a) (Hideno *et al.* 2013a,b). The high enzymatic-digestibility sample contained two characteristic DTG peaks: a broad peak at a low temperature and a sharp single peak at approximately 350 °C. The former DTG peak indicates the cellulose, hemicellulose, and lignin damage, whereas the latter indicates the increase in the cellulose content upon lignin and hemicellulose removal. Both phenomena were strongly related to the increase in the

enzymatic digestibility of lignocellulose. Generally, the main component analysis (the detergent method) and XRD analysis have been used to evaluate pretreatment procedures for enzymatic hydrolysis (Mosier *et al.* 2005; Inoue *et al.* 2008; Hiden *et al.* 2009; Karimi and Taherzadeh 2016). However, the main component analysis cannot analyze the denaturing of macromolecules because the analyzed sample is fully hydrolyzed to the monomers by sulfuric acid. Meanwhile, although XRD analysis can determine the crystallinities of cellulose in lignocellulosic samples, it cannot be applied to amorphous materials, such as hemicellulose and lignin. Considering the results of this study and previous works, DTG curves are a candidate for the simple evaluation of pretreatment techniques for the enzymatic hydrolysis of lignocellulose.

Table 3. Crystallinity Index and Thermal Degradation Properties of Treated Japanese Cypress

Sample	CrI (%)	Thermal degradation temperature (°C)		
		at 1% weight loss	at 5% weight loss	at maximum DTG (%/min)
Raw JC	59.5	232.9	272.5	365.1
BM JC	N.D.	214.3	255.3	327.6
AHP-JC	63.4	207.5	239.4	313.2
OS-JC	77.4	210.1	293.8	348.6

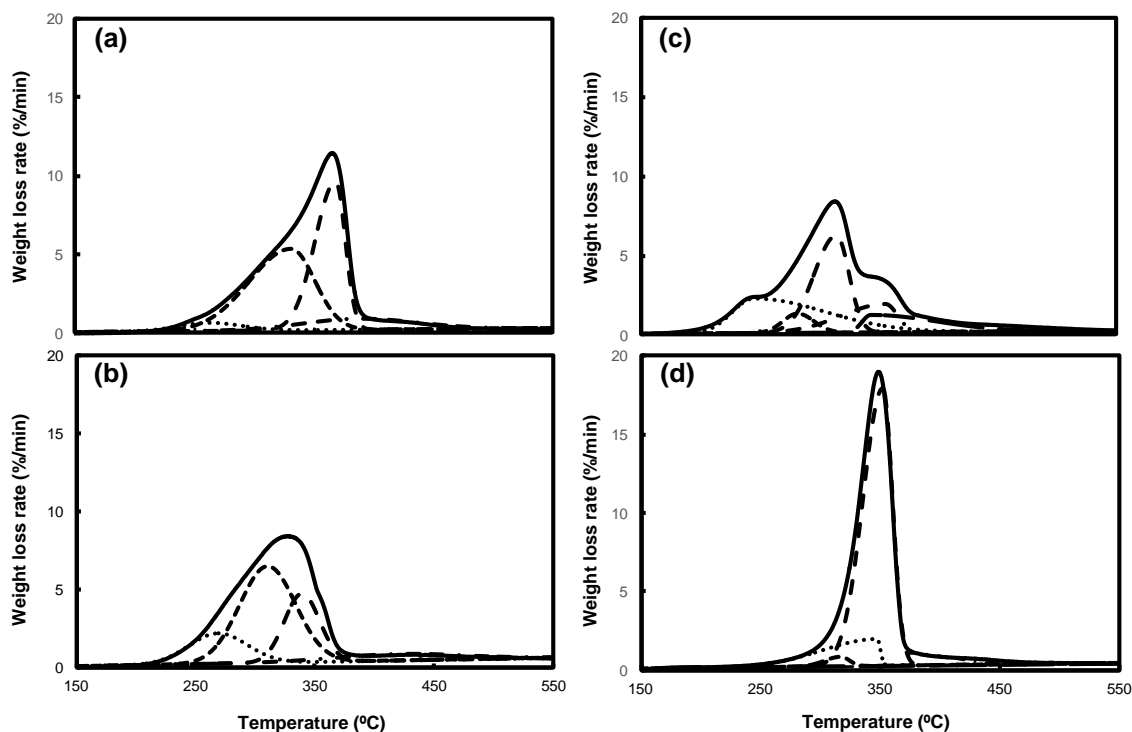


Fig. 3. Thermal degradation behaviors and fitted curves of DTG peaks of four kinds of JC samples as a model of lignocellulosic biomass. (a)-(d) Thermal degradation behaviors of raw, ball-milled, alkaline hydrogen-peroxide treated, and organosolv-treated JC, respectively. Solid lines are the original DTG data. Other peaks are the fitted curve and the separated DTG peaks.

The relationships between the cellulose content and DTG peak height and area are shown in Fig. 4. All of the correlation factors obtained for the three possible relationships

were higher than 0.9. The correlation factor between the peak height obtained for the curve-fitted DTG peaks and cellulose contents was 0.91 (t : 3.11; p : 0.027), slightly higher than that for the peak height obtained from the original DTG peak (0.90; t : 2.97; p : 0.031, Fig. 4 (a)). Moreover, the correlation factor for the area obtained from the curve-fitted DTG peak was 0.95, exceeding those obtained for the DTG peak heights (Fig. 4 (b)). Carrier *et al.* (2011) have reported that TGA can be used for the determination α -cellulose content but not lignin content. The present results partially support their conclusions. However, it is possible to indirectly determine the reduction in lignin content based on the cellulose-purification increase, which can be assessed from the height and sharpness of the DTG peaks. Moreover, the present results show that the correlation factors were higher after the curve fitting and peak separation.

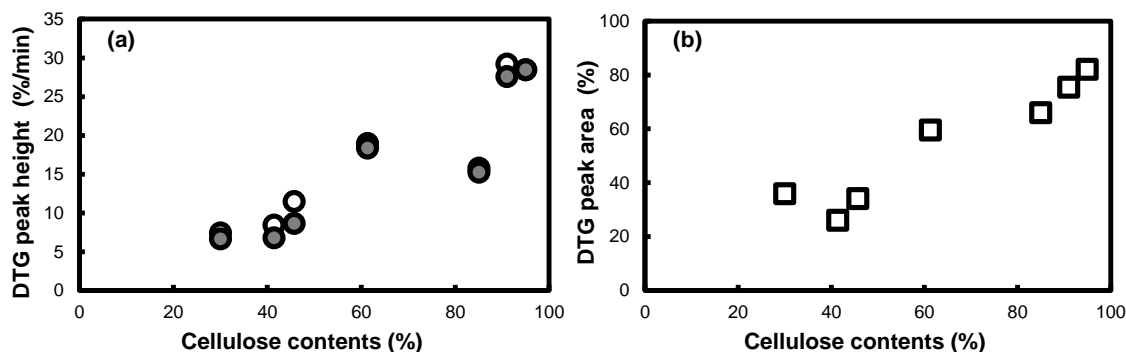


Fig. 4. Relationships between cellulose contents and DTG peak heights (a), and fitted-curve and separated DTG peak area (b). (a) Relationships between the cellulose contents and the original DTG peaks and separated main DTG peaks for various cellulosic biomasses. In (a), open and closed circles indicate the values calculated from the DTG peak height and the separated main DTG peak height, respectively. In (b), open squares indicate the values calculated from the separated main DTG peak area.

Next, the relationships between the cellulose content and crystallinity, and the thermal-degradation temperature were investigated (Fig. 5). In ball-milled Avicel, the correlation factors between the crystallinities and thermal-degradation temperature increased strongly up to 2% weight loss, increased gradually from 2 to 10% weight loss, peaked at 10% weight loss, and then remained stable above 10% weight loss (Fig. 5 (a)).

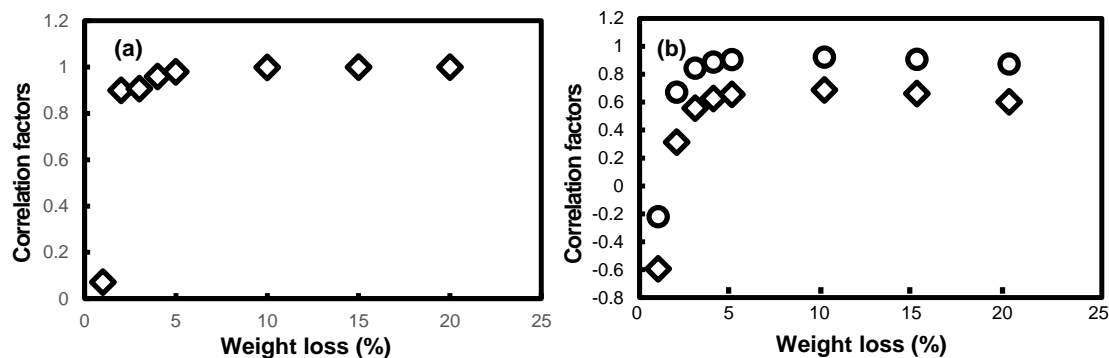


Fig. 5. Relationships between the correlation factors and percentages of weight loss in thermal degradation for (a) ball-milled Avicel and (b) treated JC. Diamonds indicates the correlation factors between the crystallinities and thermal-degradation temperatures. Open circles indicate the correlation factors between the cellulose contents and thermal-degradation temperatures.

In the treated JC sample, the correlation factor significantly increased from 1 to 3% weight loss, slightly increased from 3 to 10%, and gradually decreased above 10% weight loss (Fig. 5 (b)). The trends of the correlation factor between the cellulose crystallinities and contents and the thermal-degradation temperatures were almost the same. The cellulose crystallinity in lignocellulose is affected by non-cellulosic amorphous substances, and the amount and types of crystalline cellulose. Considering the present results, the thermal degradation behaviors are also affected by these characteristics and have the same tendency as the cellulose crystallinity within the limited temperature ranges studied here. In other words, our results show that the TGA results, including the DTG curve and thermal-degradation temperature, are potential indicators of cellulose crystallinity and purity in lignocellulose within the limited temperature range studied here.

CONCLUSIONS

1. The TGA can be used as a simple means of evaluating pretreatment techniques for the enzymatic hydrolysis of lignocellulose.
2. The difference in the thermal degradation behaviors of crystalline and amorphous cellulose was smaller than their crystallinity index.
3. The thermal degradation properties of lignocellulose were significantly affected by ball milling.
4. The thermal degradation behavior changed markedly due to the denaturing and removal of hemicellulose and cellulose purification.
5. TGA can also be used to assess cellulose purity, as well as to detect some damages of hemicellulose in lignocellulosic biomass.

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