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A comparative study on enzymatic hydrolysis of kenaf from two different harvest time-points, with- and without pretreatment

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ABSTRACT

Kenaf biomass is potentially a valuable feedstock for bio-energy production due to fast growth of this plant, and its high capacity for carbon fixation and accumulation of carbohydrates. In this study, biomass productivity, chemical composition and physical properties of kenaf were investigated at two different harvest times. The average dry weight of early harvested biomass (EH, 109.0 g/plant) was lower than that of late harvested biomass (LH, 132.3 g/plant). However, the daily growth rates for EH and LH were 1.45 and 1.28 g/d, respectively. There was no significant difference in carbohydrate content between EH- and LH-kenaf, while lignin content increased with higher S/G ratio in LH-kenaf. The enzymatic conversion rates of EH- and LH-kenaf with/without popping pretreatment were 90.4%/41.2% and 65.0%/27.5%, respectively. We suggest that early harvest has a positive impact on kenaf biomass, which had a lower lignin content and cellulose crystallinity.

1. Introduction

The depletion of fossil resources, combined with the increased demand for petroleum by emerging economies, and political and environmental issues on fossil fuels are the reasons for the exploration of sustainable, environmentally acceptable sources of liquid fuels (Bilgen, 2014). Biomass obtained from cultivated plants is a renewable and abundant resource for biofuels, valuable chemicals and many other useful materials (Tuck et al., 2006). The first step in the production of the above products is to obtain cheap and abundant biomass with a high productivity of bio-sugar, since the biomass cost affects the economics of biofuel production (Gonzalez-Garcia et al., 2010; Tao et al., 2012). Significant efforts are being made to develop plants with faster growth rates, and which require less energy inputs and fertilizers to produce excellent bioenergy crops cost effectively.

Kenaf, which can serve as a potential bioenergy crop, is an herbaceous annual plant, possessing the C3 photosynthetic pathway, and can be cultivated under a wide range of weather conditions for many and varied applications (pulps, fabrics, textile, building materials, biocomposites, and as bedding and oil absorbing materials) (Saba et al., 2015). Kenaf absorbs nitrogen and phosphorus from the soil (Abe and Ozaki, 1998) and accumulates carbon dioxide at a significantly high rate (Amaducci et al., 2000). It grows fast, more than 3 m in 3 months even under moderate ambient conditions (Sellers and Reichert, 1999). The biomass yield of kenaf varies between 9.45 and 10.22 ton/ha. High yield and low lignin and low ash content of kenaf compared with most other annual and perennial grasses and agricultural crop residues makes this plant a promising feedstock for bioenergy production (Berti et al., 2013).

It is well known that plants have different chemical and physical properties at different developmental stages. Changes in biomass chemical composition can greatly affect the quality of feedstock for biofuel production. Thus, harvest time is an important factor that can affect not only biomass productivity but also chemical composition (Adler et al., 2005; Mambelli and Grandi, 1995). In general, kenaf plants used for paper and composites are harvested at the end of their growing season. The plants are allowed to dry, and then processed to produce fibers. While the yield of early harvest is low, there is an advantage for bioenergy in that there is reduced energy consumption during pretreatment in the bioenergy production process (Rowell and Han, 1999). For example, biomass from early harvested plants contains low amounts of lignin (Morrison et al., 1999), and thus, this biomass is more suitable for bioenergy production, requiring only mild pretreatment with low loadings of hydrolytic enzymes. Because kenaf biomass is used in many and

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Table 1
Phenological dates and productivity of kenaf biomass during the growing season.

	Date of sowing (JD)[a]	Date of flowering (JD)[a]	Growing days (days)	Average weight (dry weight (g)/plant)	Productivity (dry weight (g)/plant day)
EH – kenaf	161	ND	75	109.0 ± 11.2	1.45
LH – kenaf	161	249	103	132.3 ± 14.8	1.28 (0.83)

[a] JD: Julian day.

varied applications, a better understanding of the harvesting time will enable this resource to be utilized more effectively. In our work, kenaf biomass was used as an alternative bioenergy resource, and the effect of harvesting time on enzymatic hydrolysis was studied by analyzing enzymatic saccharification, chemical composition and physical properties. Furthermore, the effect of pretreatment by popping, which has a significantly lower environmental impact and greater enzymatic hydrolysis efficiency over similar methods used conventionally (Wi et al., 2013), was investigated.

2. Materials and methods

2.1. Biomass and pretreatment

Kenaf (*Hibiscus cannabinus*) seeds were sown in rows 20 apart at 25 plants/m^2 on June 10, 2013 in an agricultural plot on the campus of Chonnam National University in Gwangju, South Korea. The dates of sowing and harvest for different growing stages are summarized in Table 1. The plants were harvested on August 24 (early harvesting; EH-kenaf) and October 4 (late harvesting; LH-kenaf). For the late harvest, the 50% of the plants possessed one or more flowers. The average dry weight and growth rate were estimated from 20 plants at two different developing stages. The daily biomass productivity was determined by the number of day in that same growing period. Kenaf samples were milled with a wet-disc mill (particle size: 0.7 ± 0.2 cm), and then stored at −20 °C until use. The popping pretreatment method was applied to each sample for an enzymatic saccharification test. The pressure and reaction time was 1.47 Mpa and 10 min, respectively (Choi et al., 2013; Wi et al., 2011, 2013).

2.2. Histochemistry

Samples were soaked in water and then 20-μm thick sections were cut transversely. The sections were stained using specific reagents (toluidine blue O, phloroglucinol, and potassium permanganate) to microscopically observe tissues and to determine lignin and lignin with syringyl moieties. Lignin was detected by the Wiesner test by soaking the sections in phloroglucinol reagent (1% (w/v) phloroglucinol in 10.1 M HCl-ethanol). Lignin (syringyl moieties) was also detected by the Mäule test (Meshitsuka and Nakano, 1978). For Mäule staining, sections were incubated in 0.5% potassium permanganate solution for 10 min and rinsed with water twice, incubated in 10% (v/v) HCl for 5 min, rinsed again with water twice and mounted in concentrated ammonium hydroxide. All stained cross-sections were mounted on microscope slides and visualized using a Zeiss microscope.

2.3. Chemical composition

The chemical composition (Klason lignin (T 222 om-88), organic solvent extractives (T 204 om-88), and ash (T 211 om-85)) of raw and pretreated kenaf stem was analyzed in accordance with the TAPPI Standard Method (TAPPI committee, 1992).

2.3.1. Carbohydrates

Structural carbohydrates of EH- and LH-kenaf stems were analyzed using gas chromatography (Blakeney et al., 1983; Wi et al., 2009). Each raw and pretreated sample was treated with 72% sulfuric acid (H_2SO_4) for 1 h at room temperature, and hydrolysis was performed at 121 °C for 1 h. Myo-inositol (internal standard) was added to the above solution, and the mixture was neutralized with ammonia water. The carbohydrates were reduced to alditols with sodium tetrahydroborate, and the excess sodium tetrahydroborate was decomposed with acetic acid. Alditols were acetylated with acetic anhydride through methylimidazole catalysis and then extracted with dichloromethane. The dichloromethane was evaporated. This solution was analyzed by GC (GC-2010; Shimadzu, Otsu, Japan) using a DB-225 capillary column (30 m × 0.25 mm ID, 0.25-μm film thickness, J&W Scientific, CA, USA) operated with helium. The operation conditions were as follows: injector temperature 220 °C; flame ionization detector (FID) temperature 250 °C; oven initial temperature 100 °C for 1.5 min; heating rate 5 °C/min up to 220 °C.

2.3.2. Lignin-monomers

The chemical composition of lignin was determined by alkaline nitrobenzene oxidation. 20–30 mg sample was suspended in 2 M sodium hydroxide, added to nitrobenzene, and heated for 2 h at 170 °C. The mixture was immediately placed in ice water, and ethyl-vanillin (internal standard) was added. The mixture was then transferred to a separation funnel and washed with dichloromethane (3 times), after which 4 M HCl was added until a pH of 1 was reached. After extraction with dichloromethane and ether, the two solutions were mixed and washed with distilled water. The organic solvent layer was transferred to a beaker filled with sodium sulfate to remove water and to be evaporated. The dried products were reacted with *N*,*O*-bis(trimethylsilyl)-acetamide at 105 °C for 2–3 min. The trimethylsilyl-derivatized solution was then analyzed by GC (CP-9100, Chrompack, Netherlands) using a CP-Sil 5CB fused silica capillary column (25 m × 0.32 mm ID, 1.2-μm film thickness, Chrompack, Netherlands) operated with helium. The operation conditions were as follows: injector temperature 280 °C; flame ionization detector (FID) temperature 280 °C; initial oven temperature 150 °C for 5 min; heating rate 10 °C/min up to 250 °C.

2.4. Surface analysis and relative crystallinity of biomass

The pore size and volume were measured using BET nitrogen adsorption-desorption isotherms at −196 °C in a surface-area analyzer (ASAP 2020, Micromerits Co., USA) (Wi et al., 2013). The sample was degassed for 1.5 h at 110 °C under vacuum to remove moisture and any other contaminants. The total pore volume was analyzed by converting the amount of nitrogen gas adsorbed to the volume (cm^3/g at STP) of liquid adsorbate.

The relative crystallinity was measured by X-ray diffraction using a diffractometer with Cu Kα radiation at 40 kV and 30 mA (X'Pert PRO MPD, PANalytical, Netherlands) (Wi et al., 2013). The crystallinity of each sample was expressed in terms of a crystallinity index (CrI) (Segal et al., 1959).

2.5. Enzyme assays and enzymatic hydrolysis

Cellulase (celluclast 1.5 L, Novozyme), pectinase (Pectinex SP-L, Novozyme), and xylanase (X2753, Sigma) were used for enzymatic