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Efficient removal of lignin with the maintenance of hemicellulose from kenaf by two-stage pretreatment process

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ABSTRACT

The enhancement of lignocellulose hydrolysis using enzyme complexes requires an efficient pretreatment process to obtain susceptible conditions for the enzyme attack. This study focuses on removing a major part of the lignin layer from kenaf (*Hibiscus cannabinus*) while simultaneously maintaining most of the hemicellulose. A two-stage pretreatment process is adopted using calcium hydroxide, $Ca(OH)_2$, and peracetic acid, PPA, to break the recalcitrant lignin layer from other structural polysaccharides. An experimental screening of several pretreatment chemicals, concentrations, temperatures and solid–liquid ratios enabled the production of an optimally designed pretreatment process for kenaf. Our results showed that the pretreatment process has provide 59.25% lignin removal while maintaining 87.72% and 96.17% hemicellulose and cellulose, respectively, using 1 g of $Ca(OH)_2$/L and a 8:1 (mL:g) ratio of liquid–$Ca(OH)_2$ at 50 °C for 1.5 h followed by 20% peracetic acid pretreatment at 75 °C for 2 h. These results validate this mild approach for aiding future enzymatic hydrolysis.

1. Introduction

Lignocellulosic biomass refers to plant biomass with an outer recalcitrant lignin layer and lignin tightly bound with complex carbohydrate polymers (hemicellulose and cellulose). Hemicellulose and cellulose can be further hydrolysed to glucose and various other sugars that are valuable for subsequent fuel ethanol production via fermentation. Plant biodegradation in nature is a very slow process because of the inaccessibility of hydrolytic enzymes to the carbohydrate polymers due to differences in lignin and substrate crystallinity in various biomass materials. Therefore, a suitable pretreatment process for different types of biomass to partially fractionate polymer complexes must be optimally developed (Yang & Wyman, 2004; Yu, Jameel, Chang, & Park, 2011) to enhance the degree of delignification of the substrate. Such pretreatment processes typically require elevated temperature and pressure combined with an acid or base catalysis to yield lignocellulosic materials that are more susceptible to enzyme attack (Berlin, Maximenko, Gilkes, & Saddler, 2007; Kim & Lee, 2005).

A wide variety of pretreatment processes that include enzymatic hydrolysis of the crystalline cellulose layer of the carbohydrate polymers have been studied over the past 30 years. These pretreatments include steam explosion, ammonia fibre explosion (AFEX), harsh acid–alkali and organosolv pretreatment (Mosier et al., 2005), ozone delignification (García-Cubero, González-Benito, Indacoechea, Coca, & Bolado, 2009), sodium chlorite delignification (Hubbell & Ragauskas, 2010) and oxygen delignification (Sierra-Ramírez, Garcia, & Holtzapple, 2011). However, most of these treatments involve the removal of both lignin and hemicellulose and only maintain cellulose content for further enzymatic saccharification into simple sugars. Most of these processes suffer from slow cellulose digestion, low sugar yields and severe reaction conditions (high temperature and/or high pressure), which require the use of expensive equipment. These pretreatments thus have high processing cost, have limited effectiveness, and may generate side products that can inhibit subsequent fermentations (Lau & Dale, 2010). Recent attention was directed to degrading hemicellulose into several valuable products, such as animal feed, xylitol for sugar alternatives, and prebiotics. The partial removal of lignin from lignocellulose significantly improves the subsequent enzymatic hydrolysis of cellulose and hemicelluloses to fermentable sugars (Duncan et al., 2010). Therefore, an efficient lignocellulose pretreatment process with modest reaction conditions is greatly needed to

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reduce sugar degradation, improve cellulose-hemicellulose hydrolysis, decrease operating cost and initial capital investments (Zhang et al., 2007), and increase the efficiency of pretreatment methods for industrial use (Yin et al., 2011).

Kenaf (*Hibiscus cannabinus*) is an annual plant with a woody base that grows up to 1.5–3.5 m tall. The stems are 1–2 cm in diameter. In Malaysia, the cultivation of the kenaf tree is expanding due to its short time to harvest (2–6 months). The development of agricultural practices for sustainable production holds high potential in diverse industrial areas, including paper, biofuels, automobile parts, construction and packaging materials, animal feed and environmental cleaners. Kenaf correspondingly serves as quality animal feed because of its high content of structural carbohydrate (≈89%).

In this study, we proposed a two-stage process of alkaline–acid pretreatment to improve delignification progression in order to facilitate the enzymatic hydrolysis of hemicellulose in kenaf. The effect of the pretreatment conditions on the percentage of lignin removal was screened and investigated to achieve a high percentage of cellulose-hemicellulose polymers retained in the pretreated-kenaf substrate. To the best of our knowledge, no such work has ever been reported on lignocellulosic kenaf.

2. Materials and methods

2.1. Substrate preparation

Fresh whole kenaf stem (core + bast, 3–4 months old) was kindly supplied by a kenaf processing company in Bachok, Kelantan (North-East Malaysia), and was oven dried for 24 h (105 °C) until it was a constant weight. The moisture content of the kenaf stem was approximately 10% (w/w). Dried kenaf stem with a moisture content of 10% (w/w) was then hand chopped into small pieces and ground using a mechanical grinder to obtain particle sizes of 40–60 mesh. The dried-ground kenaf stem was eventually kept in a sealed plastic container prior the pretreatment processes described below.

2.2. Chemical analysis of substrate

The initial chemical content of the kenaf stem was characterised starting with the extraction method prior to holocellulose (defined as the sum of cellulose and hemicellulose contents), alpha-cellulose, hemicellulose determination and the Klason lignin test. Ash and pectin content was determined. Dry weights were determined by oven drying a sample of the feedstock for 48 h at 100 °C, according to the NREL protocol for determining total solids in biomass, LAP-001 (Sluiter & National Renewable Energy, 2008). Acid insoluble lignin content (Klason Lignin) was determined by a modified version of the method described in TAPPI T222, for acid-insoluble lignin in wood and pulp (TAPPI, T-222, 1988), by two-stage sulphuric acid hydrolysis. The chemical composition of the kenaf stem was determined before and after each pretreatment process. All analyses were carried out on a dry weight basis (Sluiter, Ruiz, Scarlata, Sluiter, & Templeton, 2010).

2.3. Screening and optimisation of 1st-stage pretreatment conditions

The dried, ground whole kenaf stem was weighed into several different shake flasks for the screening of different types of chemicals, chemical loadings, liquid-to-solid ratios, and temperatures. In the first step, eight different types of chemicals and their concentrations were screened. One-gram dry weight of solid kenaf and 3 mL of aqueous solution tested were mixed together, which was equivalent to an initial dry solid material concentration of 0.33 wt.%. The chemical pretreatments were performed at 60 °C in a water bath shaker for 1.5 h. After the reaction ended, the solid residues were washed thoroughly with distilled water several times using a sieve until neutral pH and were pressed to remove excess water. The effect of chemical pretreatment on the removal of lignin was investigated using both acid and alkaline chemicals. All of the screening processes were carried out one factor at a time (OFAT) in duplicate. The range of type of chemicals (NaOH, $CaCO_3$, $Ca(OH)_2$, HCL, H_2SO_4); chemical loading (w/v)% (0.5, 0.75, 0.87, 1.0, 6.0, 10.0); liquid-to-solid ratio (mL:g) (4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1); temperature, °C (40, 50, 60, 80, 100) were screened. The best-chosen value of the initial parameter was used for subsequent parameter screening. The time was fixed at 1.5 h for all the experiments. After every screening of each parameter, the kenaf substrate was washed thoroughly with distilled water through a sieve to remove the excess chemical bound to the pretreated substrate, and the substrate was then pressed to remove excess water.

2.4. Preparation of peracetic acid (PPA)

PPA was prepared by the reaction of acetic acid and 30% hydrogen peroxide, with a volume ratio of 1.5:1 at room temperature for 72 h. To hasten the reaction, 1.5% (w/w) of sulphuric acid was added as a catalyst (Zhao, Peng, Cheng, & Liu, 2009).

2.5. 2nd-stage pretreatment

The first stage pretreated kenaf substrate was treated with 20% (v/v) PPA in a water bath shaker at 75 °C for 2 h with a liquid-to-solid ratio of 1.1 (mL:g). The substrate was then thoroughly washed with distilled water several times until neutral pH and oven dried to a constant weight. Finally, further chemical analyses were performed on the lignin, cellulose and hemicellulose remaining after the pretreatment.

The electron micrograph of the pretreated and unpretreated kenaf samples was taken by a scanning electron microscope (SEM). All the samples were initially pre-coated with a thin layer of nano-sized gold powder to enhance the contrast of the images.

2.6. Enzyme saccharification

The solid kenaf biomass was hydrolysed with xylanase at an initial sample concentration of 3% (w/v) in 20 mL of 50 mM sodium acetate buffer (pH 5.0). Recombinant xylanase from *Trichoderma reesei* expressed in *Pichia pastoris* was used for the hydrolysis (400 U enzyme loading). The enzymatic reactions were incubated in a reciprocating water bath shaker at 200 rpm for 24 h at 50 °C. The supernatants were centrifuged and removed for sugar content analysis. The hydrolysis of hemicellulose and the enzymatic saccharification were calculated (Sharma, Kalra, & Kocher, 2004):

$$\text{Hemicellulose hydrolysis (\%)} = \frac{\text{The amount of xylose produced} \times 0.88}{\text{The amount of hemicellulose in raw kenaf}} \times 100$$

where 0.88 is the correction factor to compensate for the addition of a water molecule during hydrolysis.

2.7. Analytical methods

Total cellulose and hemicellulose in raw and various pretreated samples were determined using the American Society for Testing and Materials (ASTM) standard method. Sample recoveries after various pretreatments were determined based on dry weight. Enzymatic hydrolysis products (glucose and xylose) were determined using high performance liquid chromatography (HPLC), equipped with a Rezex RSO-Oligosaccharide Ag+ 4% guard column (60 mm × 10.00 mm, Phenomenex) in line with a