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Importance of acid or alkali concentration on the removal of xylan and lignin for enzymatic cellulose hydrolysis

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ABSTRACT

The effect of hemicellulose and lignin solubilisation by H_2SO_4 and NaOH catalysed pretreatments was correlated to the extent of subsequent enzymatic cellulose hydrolysis. Three different grass-type feedstocks, palm empty fruit bunch, sugarcane bagasse and barley straw, were investigated. Soluble fractions after catalysis were characterised for mono- and oligosaccharides contents, while the residues were analysed for constituent monosaccharides composition.

Alkali pretreatment resulted into extensive lignin removal. This removal resulted in up to 90% (w/w) conversion of glucan into glucose by enzymes. But, the alkaline conditions also provoked up to 50% unwanted xylan losses. Acid pretreatment resulted into solubilisation (70–80% (w/w)) of xylan with almost no losses, while lignin remained. Although moderate xylan solubilisation increased enzymatic cellulose hydrolysis of residual glucan, extensive removal of xylan decreased it. Therefore, under the treatment conditions, the alkali treatments were the most efficient in terms of enzymatic release of xylose and glucose from the insoluble residues.

1. Introduction

Plant biomass based fuels and chemicals are gaining interest due to the rapidly decreasing amounts in fossil fuel resources. Their production comprises four parts. Harvest/storage of lignocellulosic biomass, pretreatment, enzymatic saccharification and fermentation/modification (Himmel et al., 2007). The mechanisms behind various pretreatments and enzymatic saccharification are not fully understood yet. Interestingly, recent microscopic observations have shown (Ding et al., 2012) that extensive hemicellulose removal resulted in a decreased enzymatic cellulose degradability. In addition, lignin removal was suggested to increase this. Nonetheless, quantitative biochemical evidence of the degradation behaviour of lignin or hemicellulose depleted lignocellulosic residues are limited, although they are expected to be complementary to microscopic observations.

Sugarcane (*Saccharum officinurum*) and barley (*Hordeum vulgare*) belong to the *Poaceae* family, while oil palm (*Elaeis guinensis*) belongs to the *Arecaceae* family. All three are monocots or true grasses and are typical lignocellulosic materials composed of around ±25% (w/w) hemicellulose, ±35% (w/w) cellulose and ±25% (w/w) lignin (Escarnot et al., 2011). The hemicelluloses in monocots mainly consist of substituted glucuronoarabinoxylans (GAX). The backbone of GAX consists of β-D-(1-4)-linked xylopyranosyl units that can be substituted with α-L-arabinofuranosyl residues, acetyl residues and/or (4-O-methyl)-α-D-glucuronic acids (Ebringerová et al., 2005). Cellulose consists of β-D-(1-4)-linked glucose units, forming a crystalline polymer. Lignin is an aromatic polymer synthesised from phenylpropanoid precursors.

Chemical pretreatment processes described, resulting in an increased subsequent enzymatic hydrolysis of plant biomass, comprise acid or alkaline based processes.

Acid pretreatment aims at solubilisation of hemicellulose leaving the cellulose accessible for enzymatic saccharification (Lloyd and Wyman, 2005). In these pretreatments, most of the lignin remains in the insoluble cellulosic residue. Alkali pretreatments have been proven to solubilise lignin, while hemicellulose remains in the cellulosic residue. Alkali breaks ester-linkages and may cause swelling of the lignocellulosic material, which increases accessibility for enzymes and thereby increases the accessibility for enzymatic hydrolysis (Park and Kim, 2012; Sun and Cheng, 2002). For both acid and alkali treatments, there is a remarkable effect on

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the composition and structure of the insoluble material. Clearly, the concentration of the acid or alkali is an important factor. At present, comparison of the effect of diluted alkali or acid on enzymatic hydrolysis has been reported (Wilkinson et al., 2014). However, a very limited number of conditions are applied (Giese et al., 2013; Kataria et al., 2013). Or in case of many conditions applied, no detailed quantitative information is given with respect to carbohydrates present in the different fractions obtained.

The present study evaluates for BS, SCB and EFB, the effect of acid and alkali catalysis at elevated temperatures on their enzymatic hydrolysis. Various concentrations of acid (0–6% (w/w) based on dry matter) and alkali (0–12% (w/w) based on dry matter) are applied. Carbohydrate and lignin mass distributions over insoluble residues and soluble hydrolysates after catalysis are determined. In addition, enzymatic saccharification of the insoluble residues is correlated to the ratio of hemicellulose/lignin/cellulose present in these samples.

2. Materials and methods

2.1. Materials used

Sugarcane bagasse (95% (w/w) DM), barley straw (92% (w/w) DM) and empty fruit bunch (37% (w/w) DM) were supplied by Purac Thailand (Banchang, Thailand), Unifarm Wageningen UR (Wageningen, The Netherlands) and Sime Darby (Kuala Lumpur, Malaysia), respectively. Enzyme cocktails CellicCTec2 and CellicHTec were provided by Novozymes (Bagsvaerd, Denmark) and stored at 4 °C.

2.2. Acid and alkali treatments

Stainless steel non-stirred reactors (0.1 L) were loaded with a fixed solid:liquid ratio of 1:10 (w/w), and suspensions were just steerable. Residence time for acid and alkali treatments at 140 °C and 120 °C, were 30 and 60 min, respectively. All reactors were equipped with a controlled thermocouple (Pico Technology, Saint Neots, UK). The reactors were introduced into silicon oil preheated at the desired pretreatment temperature. Reactors removed from the oil after pretreatment were directly submerged in ice-water. The maximum heating and cooling times were 22 and 15 min, respectively. After cooling down to ambient temperature, the pretreated mixture was transferred into 0.25 L polypropylene tubes and centrifuged (10,000 × *g*, 15 min, ambient temperature). The supernatant was decanted and the residue was recovered and washed 3–4 times with water, until the pH of the suspension was neutral. For each raw material, the first supernatant and 3 wash supernatants were combined and freeze dried (denoted as "soluble fraction"). The remaining washed residues were denoted as "residues". All samples obtained, including their pretreatment conditions, are shown in Table 1.

2.3. Enzymatic hydrolysis

Incubations were performed with *CellicCTec2* and with a combination of *CellicCTec2* plus *CellicHTec* (ratio 10:1 (protein (N%x6.25) basis). The activities of the enzyme cocktails were 1000 biomass hydrolysis units (BHU)/g and 2500 fungal xylanase units (FXU)/g, respectively. All residues were milled (<1 mm) (MM 2000, Retsch, Haan, Germany) and incubated in 50 mM sodium acetate buffer pH 5.5. The milled substrate load was 1% (w/w) based on dry matter. The enzyme incubations were performed in duplicate. Residues from selected samples (0, 2, 4, 6% (w/w) sulphuric acid based on dry matter and 0, 4, 8, 12% (w/w) sodium hydroxide based on dry matter (Table 1) were incubated at 55 °C, and rotated head-over-tail for 72 h. Incubation conditions were chosen considering optimum temperature and pH of both enzyme preparations. Incubation time 72 h was considered as end-point incubation. Enzyme dosage was in total 3% (w/w) of protein based on dry matter of the amount of residue/sample loaded. The protein concentration (N%*6.25) of *CellicCTec2* and *CelliCHTec* was 127 mg/mL and 120 mg/mL, respectively. Sodium azide 0.01% (w/v) was used to prevent bacterial growth. After incubation, the digests were heated at 100 °C for 5 min, centrifuged (10,000 × *g*, 5 min, ambient temperature), and analysed for their total carbohydrate content and constituent monosaccharide composition.

2.4. Analytical methods

2.4.1. Neutral constituent monosaccharides content and composition

Neutral carbohydrate content and composition was determined using inositol as an internal standard. Samples were pretreated with 72% (w/w) H_2SO_4 (1 h, 30 °C) followed by hydrolysis with 1 M H_2SO_4 for 3 h at 100 °C. The constituent sugars released were derivatised and analysed as their alditol acetates using gas chromatography (Englyst and Cummings, 1984).

2.4.2. Uronic acid content

Uronic acid content was determined as anhydro-uronic acid by an automated m-hydroxydiphenyl assay (Thibault, 1979), including 0.3% (w/w) tetraborate in the sulphuric acid, with an autoanalyser (Skalar Analytical BV, Breda, The Netherlands). Glucuronic acid was used as reference.

2.4.3. Esterified acetic acid content

Samples (20 mg) were saponified with 1 mL of 0.4 M NaOH in isopropanol/H_2O (1:1 v/v) for 3 h at room temperature. The level of acetic acid substituents was corrected for the free acetic acid present in non-saponified samples. The acetic acid content was determined with an Ultimate system (Thermo Scientific, Sunnyvale, CA, USA) equipped with a Shodex RI detector and an Aminex HPX 87H column (300 mm × 7.8 mm) (Bio-Rad Laboratories, Hercules, CA, USA) plus pre-column. Elution was performed by using 5 mM H_2SO_4 at a flow rate of 0.6 mL min^{-1} at 40 °C.

2.4.4. Lignin content

Selected residues were analysed for acid insoluble (Klason) lignin. To each sample of 300 mg (dry matter) 3 mL of 72% (w/w) H_2SO_4 was added and samples were pre-hydrolysed for 1 h at 30 °C. After this pre-hydrolysis, 37 mL of distilled water was added and samples were put in a boiling water bath for 3 h and shaken every half an hour. Next, suspension was filtered over G4 glass filters (Duran, Mainz, Germany). The residual part was washed until it was free of acid and dried overnight at 105 °C. The weight of the dried residual part was taken as a measure of the acid insoluble lignin content, after correction for the amount of ash in this fraction (Section 2.4.6).

2.4.5. Protein content

Nitrogen content (N%*6.25) was analysed of all raw feedstocks using the combustion (DUMAS) method on a Flash EA 1112 Nitrogen Analyser (Thermo Scientific, Rockford, IL, USA). Methionine (Acros Organics, New Jersey, USA) was used as a standard.

2.4.6. Ash content

Samples (0.5 g) were dried in an oven overnight (105 °C), weighed and put in an oven at 504 °C overnight. Next day, samples were weighed and the difference between the mass at 105 °C and 504 °C was taken as ash content.