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Screening conditions for acid pretreatment and enzymatic hydrolysis of empty fruit bunches

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ABSTRACT

Empty fruit bunches were received from Teck Guan, Malaysia and were pretreated and enzymatically hydrolyzed to determine the possible sugar recovery from the biomass. Several different conditions were explored in a screening study. Temperature ranged from 100 °C to 150 °C, time ranged from 30 to 90 min, and acid loading ranged from 0 to 1.3% weight acid/weight liquid. The material was then enzymatically hydrolyzed at three different enzyme loadings 1.67%, 3.33%, and 6.66% (g enzyme/g glucan × 100) and total sugar recovery was calculated for both pretreatment and enzymatic hydrolysis. Best pretreatment conditions yielded 81.4% recovery of hydrolyzed xylan. Best glucan conversions in enzymatic hydrolysis were 74.8%. These conversions and recoveries make empty fruit bunches a good potential feedstock for cellulosic ethanol.

1. Introduction

Ethanol is an important fuel alternative for use in the transportation sector. Ethanol can be derived from many different sugar sources, including starches from corn. As ethanol is becoming more prevalent and widely available, new sources are being sought to replace corn as one of the most used feedstock. Cellulosic ethanol is derived from fermentation of sugars hydrolyzed from cellulose and hemicellulose in plant material, such as agricultural waste and residues. One such feedstock is the lignocellulosic residue that is left over from processing the oil from palm.

Oil palm, *Elaeis guineensis*, is an important oil producing crop for many countries such as Malaysia and tropical regions such as Southeast Asia. The empty fruit bunches (EFB) that are produced after processing the oil from palm are currently used as a substrate for the cultivation of mushrooms as a manure (Piarpuzán et al., 2011) or burned for the BTU value (Zhang et al., 2012). EFB are a fibrous material that is generated after the palm fruit is processed to extract the oil. Fibers are primarily composed of cellulose and hemicellulose, two compounds that can be hydrolyzed into glucose and xylose, which in turn can be fermented into ethanol, and are comparable to a more common cellulosic feedstock, such as corn stover (Table 1). The hemicellulose is composed primarily of xylan with less arabinan making up the composition (Table 1). This is a lower ratio than that of stover.

Several pretreatment conditions have been suggested previously (Rahman et al., 2007; Zhang et al., 2012). Sulfuric acid will be used in this study. Its benefits have been described before (Harmsen et al., 2010). The goal of pretreatment and enzymatic hydrolysis is to maximize the conversion of the polysaccharide components (glucan and xylan) to monomeric sugars (glucose and xylose) for use in fermentation.

During pretreatment the goal is to have conditions severe enough to hydrolyze hemicellulose and cellulose and open the crystalline structures for enzymes to access without being so severe as to create enzymatic hydrolysis and fermentation inhibitors such as hydroxymethylfurfural (HMF) and furfural. HMF is formed from the dehydration of glucose and furfural from xylose (Larsson et al., 1999). This study was carried out as a screening study to observe the effect of different pretreatment conditions of EFB and use corn stover as a benchmark. It also focuses on how recalcitrant EFB are in enzymatic hydrolysis under this study's pretreatment conditions.

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Table 1
Compositional analysis of raw EFB and corn stover. All values are listed as a percentage of total mass and are averages of 3 samples.

Sample	Structural inorganics	Non-structural inorganics	Water extractives	Ethanol extractives	Lignin	Glucan	Xylan	Arabinan	Acetyl	Mass closure
EFB sample	2.61	2.42	3.87	4.79	20.4	33.5	21.5	1.11	4.58	94.8
Corn stover sample	5.44	1.33	10.8	2.38	12.8	34.8	23.7	3.34	2.91	97.5

Table 2
Pretreatment conditions and combined severity (CS) factors for EFB. CS values are an average of two pretreatments ($n = 2$).

Condition	Target time (min)	Target temperature (°C)	Target acid loading (%)	Target solid/liquid ratio (%)	Target total mass (g)	Combined severity
100 °C/90 min/0%H+	90	100	0	12.5	700	−4.39
150 °C/30 min/0%H+	30	150	0	12.5	700	−2.55
100 °C/30 min/1.3%H+	30	100	1.3	12.5	700	0.20
125 °C/60 min/0.65%H+	60	125	0.65	12.5	700	0.80
150 °C/90 min/1.3%H+	90	150	1.3	12.5	700	2.10

Table 3
Yields (amount recovered as a percentage from starting biomass) for each biomass component solubilized in pretreatment $n = 2$.

CS	Lignin	Monomeric xylose	Soluble xylan	Furfural	Monomeric glucose	Soluble glucan	HMF	Acetic acid
−4.39	1.24	0.900	2.32	0.00	0.280	0.640	0.0100	12.7
−2.55	1.42	0.790	4.82	0.0300	0.560	0.680	0.00	24.7
0.20	1.74	8.82	26.3	0.0200	0.430	1.68	0.00	29.3
0.80	1.77	10.4	32.8	0.110	0.390	1.66	0.0100	33.9
2.10	4.94	62.8	1.69	16.9	7.07	0.750	0.430	77.5

2. Materials and methods

2.1. Material

2.1.1. Feedstock

Empty fruit bunches were obtained from Teck Guan, Tawau, Sabah, Malaysia. The feedstock was stored in a cooler at 3 °C. Samples were dried in a 40 °C oven and milled using a knife mill fitted with a 1 mm screen. Compositional analyses were done on three sub-samples and are listed in Table 1. The samples were then used in pretreatment.

2.1.2. Pretreatment

Pretreatments were carried out in two Parr 5100 reactors fitted with two stainless steel 1 L jacketed reactor vessels. The sulfuric acid used was 91.2% sulfuric acid used for Babcock test (Fisher Scientific).

2.2. Methods

2.2.1. Pretreatment

Ten pretreatments were performed using two, 1 L Parr jacketed reactors. Five conditions were explored as part of this screening study, done pairwise in the two reactors. All reactions were completed with a solids loading of 12.5% weight of biomass/weight of liquid (w/w). A working mass was kept constant at 700 g. Several conditions were screened and can be found in Table 2. Due to the limitations of the reactors, higher severity conditions were not able to be explored. The acid concentrations were loaded as a percentage of the total mass of liquid in the reactor. The combined severity (CS) was calculated using time, temperature and pH (Lloyd and Wyman, 2005):

$$\log CS = \log R_O - pH$$

R_O is defined as:

$$R_O = t \times \left[\frac{(T_H - T_R)}{14.75}\right],$$

where t is the time in minutes, T_H is the hydrolysis temperature in °C, and T_R is the reference temperature 100 °C. Once the reactor was loaded and secured, the slurry was brought to the target temperature using steam to heat the jacket of the reactor vessel. The reactor was held at temperature for the target time and cooled to 35 °C in 2–3 min by running water through the vessel jacket. The slurry was then loaded into 1 L centrifuge tubes and centrifuged for 20 min at 4800 × g. The liquor was decanted and sampled in duplicate for analysis. The solids were also sampled for analysis. Both the liquor and solids were retained for enzymatic hydrolysis.

Duplicate samples of the liquor were assayed for sugars, acetic acid, and HMF/furfural concentrations by HPLC analysis. Total solids, total dissolved solids, total suspended solids, and density were done on the liquor. The total solids analysis of the solids was determined. Both liquor and solid samples were analyzed for composition. The liquor compositional analyses were used to determine percent of soluble xylan, glucan, and arabinan, monomeric glucose, xylose and arabinose and to determine mass closures around pretreatment. The composition of the raw (starting) biomass feedstock was also determined and is reported in Table 1.

2.2.2. Enzymatic hydrolysis

Enzymatic hydrolysis was carried out in a BD Falcon 35-1143 Multiwell 12 well plate using NS22146 enzymes (Novozymes). The plates were incubated in a New Brunswick Innova Anova 4300 digital incubator shaker set at 50 °C and 150 rpm. The pH was adjusted to 5.5 in the pretreated samples before enzyme addition. Each pretreatment condition was enzymatically hydrolyzed in duplicate using NS22146 dosed at 1.67%, 3.33%, and 6.66% (g enzyme/g glucan of the pretreated solids × 100). The liquor that was separated by centrifugation was used for make-up water. The enzymatic hydrolysis was carried out in 12 well plates with a volume of 8 mL at 17% solids loading. The enzymatic hydrolysis was 120 h. At the end of the enzymatic hydrolysis the samples were filtered and sugar concentrations were determined by HPLC. Glucose and xylose yields are calculated as a percentage of measured mass over calculated theoretical maximum mass.

2.2.3. Analytical testing

HPLC–Liquid samples were loaded into 1 mL HPLC vials after being filtered through a 0.2 μm filter. The vials were loaded onto a carousel which fits into an autosampler (either 717 plus or 2695 separations module from Waters). An aliquot (5 μL) of the sam-