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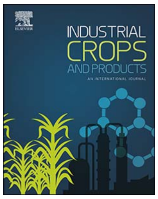

Comparison of a two-stage and a combined single stage salt-acid based lignocellulosic pretreatment for enhancing enzymatic saccharification

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ABSTRACT

This study compares the optimization of combined and two-stage salt-acid pretreatment technique for efficient delignification, hemicellulose removal and enhancement of sugar yield from sugarcane leaf waste. The effect of process parameters $ZnCl_2$ concentration (1 M–5 M), H_2SO_4 concentration (0.10–2.0%, v/v) and solid loading (5–15%, w/v) on the reducing sugar yield were investigated. The developed models showed coefficients of determination (R^2) > 0.94. The two-stage pretreatment under optimal conditions of 3.32 M $ZnCl_2$, 1.84% (v/v) H_2SO_4, and 9.26% (w/v) solid loading gave 0.293 g/g reducing sugar, a 9% improvement compared to the optimized combined pretreatment. SEM and FTIR analysis showed major structural changes in the pretreated biomass. Significant lignin and hemicellulose removal was observed with the two-stage pretreatment. The optimized two-stage pretreatment also showed 66% and 194% yield improvement on sorghum leaves and Napier grass respectively. These results indicate that a two-stage $ZnCl_2$-H_2SO_4 pretreatment can significantly enhance enzymatic saccharification of lignocellulosic waste.

1. Introduction

Lignocellulosic biomass is an abundant non-food feedstock that can sustain alternative fuel production thereby alleviating conventional fuel concerns (Behera et al., 2014). However, the major bottleneck in converting lignocellulosic biomass to monomeric sugars lies in the inherent recalcitrance of the material toward enzymatic and microbial degradation (Qing et al., 2016). Native biomass is composed of cellulose (38–50%) and hemicellulose (23–32%) bound together by an impermeable lignin layer (15–25%) preventing effective degradation (Kim et al., 2008; Sindhu et al., 2016a). Thus, the pretreatment of lignocellulosic biomass is a crucial step that disrupts the crystalline structure, removes lignin and hemicellulose allowing the enzymatic conversion of cellulose to glucose.

Pretreatment can be separated into either physical, chemical or biological methods (Taherzadeh and Karimi, 2008). Commonly investigated pretreatments include acid (Bouza et al., 2016; Moodley and Kana, 2015), alkali (Zhu et al., 2016), inorganic salts (Kang et al., 2013), hot water (Kim et al., 2016), microwave (Jin et al., 2016), ionic liquid (Asakawa et al., 2016; Wang et al., 2015) and glycerol (Zhang et al., 2016) among others. A major drawback to most pretreatment technologies are still high cost, toxicity and energy demand. Acids such as hydrochloric, sulfuric, phosphoric and nitric acid are effective in removing lignin and hemicellulose which explains their extensive use (Zhu et al., 2016). Sulfuric acid is most commonly employed because of its high catabolic activity (Behera et al., 2014). Acid pretreatment, however, tends to produce compounds that are inhibitory to enzymatic activity. In addition, higher process costs can be incurred to acquire corrosion resistant reactors. Thus, lower concentration acids are preferred although it hampers sugar yields (Kim et al., 2016). Conversely, the performance of dilute acid pretreatment could be enhanced when combined with salts (Ramadoss and Muthukumar, 2015). Pretreatment with inorganic salts have recently been reported to increase hemicellulose degradation thus increasing cellulose conversion rates (Kang et al., 2013). Banerjee et al. (2016) reported an 82% saccharification efficiency using NaCl in mustard stalk and straw pretreatment. A combined sodium phosphate and sodium sulfide pretreatment strategy for corn stover was found to yield 91.11% reducing sugar (Qing et al., 2016). However, there are very few reports on lignocellulosic pretreatment using a combination of salt and acid. Li et al. (2014) examined the effect of $FeCl_3$ and HCl on the crystallinity of cellulose. These authors also studied the effect of ultrasonic waves on HCl-$FeCl_3$ pretreatment (Li et al., 2015). The combined pretreatment of salt and acid on corn stover and sugarcane baggase have been reported in some studies (Degenstein et al., 2013; Miranda et al., 2015). However, a combination of salt and acid can result in a double replacement reaction which renders the pretreatment ineffective (Helmenstine, 2016). Double replacement reactions or metathesis is the biomolecular process

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where molecules possessing counter ions are exchanged (IUPAC, 1997). For instance, H_2SO_4 co-catalysed with $FeCl_2$ is routinely employed as a combined salt-pretreatment. In the presence of water, H_2SO_4 can react with $FeCl_2$ to form HCl and $FeSO_4$ thus implying HCl as the net pretreatment effect. Therefore, a two-stage salt-acid pretreatment was explored in this study and compared to a combined regime. There is a knowledge gap on the effects of a two stage pretreatment where biomass is pretreated with salt and acid separately. Moreover, knowledge on the interaction of salt and acid on the degradation of lignocellulosic compounds is scanty.

Sugarcane is a major economic crop cultivated globally with an annual yield of 328 Tg. Among its advantages are high biomass yield and sucrose content (Sindhu et al., 2016b). Sugarcane leaves constitute 40% of the plant and are considered waste. These are either burnt in the field or dumped in landfill sites (Nguyen et al., 2010; Smithers, 2014). Native sugarcane leaves contain 44% cellulose and 28% hemicellulose (Moodley and Kana, 2015), indicating its potential as a feedstock for biofuel production. Very few studies have focused on the utilization of sugarcane leaves for biofuel production (Moodley and Kana, 2015; Jutakanoke et al., 2012).

In this study, the efficiency of two-stage and combined pretreatment with $ZnCl_2$ and H_2SO_4 on the enzymatic saccharification of SLW was modelled, optimized and compared. The optimal operational process conditions of salt concentration, acid concentration and solid loading on reducing sugar yield were elucidated. In addition, structural properties of native and pretreated biomass were investigated by scanning electron microscopy (SEM) and Fourier Transform Infrared analysis (FTIR).

2. Methods

2.1. Materials

Sugarcane leaf wastes (SLW) were collected from a sugarcane plantation located on the North Coast of South Africa (29° 42′ 18″ S, 31° 02′ 44″ E). Samples were dried at 60 °C for 72 h and subsequently milled to a particle size of ≤ 1 mm. Native and pretreated sample composition was determined by the NREL method (Sluiter et al., 2008). The commercial cellulase enzyme Cellic CTec 2 was generously provided by Novozymes (Novozymes A/S, Denmark).

2.2. Preliminary screening

Pretreatment was carried out in 100 ml Erlenmeyer flasks with a solid loading of 10% w/v. Varied concentrations of NH_4Cl, NaCl and $ZnCl_2$ (1 M, 3 M and 5 M) were evaluated with 1.5% (v/v) H_2SO_4 in different combinations and autoclaved at 121 °C for 60 min. Samples were then washed and dried prior to enzymatic hydrolysis. The efficiency of each salt was measured based on the glucose yield.

2.3. Optimization of process parameters affecting salt-acid pretreatment of SLW

Optimization of key process parameters affecting sugar yield from salt-acid pretreatment of SLW was carried out using the Response Surface Box-Behnken design. A total of 17 runs were generated and these were conducted in replicate. The parameters were selected at three levels and these included acid concentration (0.1%, 1.05%, 2.0%, v/v), salt concentration (1 M, 3 M, 5 M) and solid loading (5%, 10%, 15%, w/v). For the two stage pretreatment, SLW was pretreated with salt for 30 min at 121 °C thereafter it was washed with deionized water and dried at 60 °C followed by acid pretreatment for 30 min at 121 °C. For the combined pretreatment, SLW was pretreatment with a salt-acid solution in a single stage at 121 °C for 60 min as set out in the design. Design Expert 7.0 (Stat Ease Inc, USA) was employed for generating the experimental design, statistical analysis and polynomial model development. In addition, response surface plots were generated to illustrate the interactive effect of process parameters on reducing sugar yield. The experimental design is presented in Table 1.

Table 1
Box-Behnken design for optimization of various input parameters affecting salt-acid pretreatment of SLW.

Run Order	H_2SO_4 conc. (%, v/v)	$ZnCl_2$ conc. (M)	Solid loading (%, w/v)	Reducing sugar (g/g)	
				Combined	Two stage
1	2.00	5	10	0.205	0.294
2	1.05	1	5	0.0835	0.143
3	1.05	5	15	0.177	0.189
4	0.10	3	5	0.0661	0.105
5	1.05	3	10	0.226	0.256
6	0.10	1	10	0.0297	0.054
7	1.05	5	5	0.116	0.184
8	1.05	1	15	0.111	0.128
9	0.10	3	15	0.115	0.157
10	1.05	3	10	0.24	0.287
11	2.00	3	5	0.102	0.222
12	2.00	1	10	0.148	0.201
13	1.05	3	10	0.25	0.280
14	2.00	3	15	0.144	0.216
15	1.05	3	10	0.238	0.290
16	0.10	5	10	0.102	0.207
17	1.05	3	10	0.277	0.266

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis of pretreated or untreated SLW was carried out in 100 ml Erlenmeyer flasks by mixing the SLW biomass in 10 ml sodium citrate buffer (pH 4.8, 0.05 M) at a solid and enzyme loading of 10% (w/v) and 10 FPU/g respectively. Hydrolysis was conducted at 50 °C, 120 rpm for 72 h in a shaking waterbath. After enzymatic treatment, the hydrolysate was centrifuged and the supernatant analysed. The total reducing sugar and glucose quantification were obtained using the 3,5-dinitrosalicylic acid method (Miller, 1959) and the YSI 2700 Sugar Analyzer (YSI, USA) respectively.

2.5. Characterization of native and pretreated SLW

2.5.1. Scanning electron analysis

Surface characteristics of pretreated and native SLW was analysed using a ZEISS EVO LS 15 scanning electron microscope. Samples were gold putter coated (Eiko IB-3 Ion Coater) prior to analysis and images were taken at a magnification of 1000 × .

2.5.2. FTIR analysis

Fourier transform infrared spectroscopy (FTIR) was performed on pretreated and native SLW using a Perkin Elmer 100 (Waltham, MA, USA). Samples were mixed with spectroscopic grade KBr and pressed into disks. FTIR spectra were recorded between 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1} with an average of 25 scans.

3. Results and discussion

3.1. Composition of native and pretreated SLW

Native SLW was composed of 43.44% cellulose, 30.98% hemicellulose and 9.16% lignin while combined salt-acid pretreated biomass contained 69.62% cellulose, 4.33% hemicellulose and 22.82% lignin. Fibre composition for the two-stage salt-acid pretreated sample was made up of 60.98% cellulose, 4.25% hemicellulose and 19.06% lignin. Thus, hemicellulose solubilization for the combined and two-stage samples were 86% and 90% respectively. Therefore, a 4% enhancement in hemicellulose solubilization using the two-stage pretreatment was