Research Article

Effect of Organic Acid Pretreatment on Napier Grass (*Pennisetum purpureum*) Straw Biomass Conversion

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Abstract
Production of specialty chemicals or bioproducts from lignocellulosic biomass has a major bottleneck at hydrolysis step due to recalcitrant structures of lignocellulose fibrils. Pretreatment is necessary to be executed to enhance saccharification efficiency. This study, the effect of acid pretreatment on lignocellulosic conversion of Napier grass was evaluated based on Response Surface Methodology (RSM). Pretreatment conditions were optimized for oxalic, citric, acetic, and hydrochloric acids. Validation indicated that the models were predictive to magnify the conversion. Hydrochloric acid exhibited highest effectiveness, followed by oxalic, acetic, and citric acids. The optimal conditions were using 0.7% (w/v) hydrochloric acid at 105°C for 60.18 min and using 5.72% (w/v) oxalic acid at 104.66°C for 76.94 min. The obtained sugar yields were low compared to those from rice straws pretreated with the optimal, same acid conditions. The results here suggested the requirement of optimization study before choosing organic acid pretreatment to different types of lignocellulosic biomass.

Keywords: Organic acid pretreatment, Lignocellulose, Napier grass, Saccharification, Response surface methodology

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1 Introduction

Plant-based biomasses provide the most abundant organic substances on Earth and thus are potential substrates for various biorefinery applications [1]. To make plant carbohydrates readily available for subsequent production of biofuels, the lignin-polysaccharide matrix must first be broken down into lignin, cellulose, and hemicellulose, then the two obtained polysaccharides must be further broken down into simple, fermentable sugars. Such the upstream biomass conversion generally consists of two main steps, pretreatment and saccharification. Pretreatment of lignocellulosic biomass aims at disrupting the lignin-polysaccharides network [2], and at reducing the crystalline structure of cellulose [3], both ultimately lead to increase in cellulose availability and accessibility for subsequent hydrolysis of the plant polysaccharides. Various pretreatments (physical, chemical, biological, and combinational) have been implemented to locate the best operational and cost-effective conditions [4]–[6]. Catalysis using dilute acid at high temperature for an extended period of time is a promising condition that has been shown to effectively degenerate lignocellulosic biomass from various plant sources [7]–[9]. Nevertheless, such the harsh pretreatment condition normally results in by-products that are inhibitory to microorganisms as well as to functioning of enzymes used in downstream processes of biofuel production.

Three major inhibitory by-products are 1) small acids formed from degraded hemicellulose, lignin, and sugars; 2) furan derivatives of dehydrated monosaccharides; and 3) phenolic compounds degraded from lignin polymers [10]. Hot acid hydrolysis of lignocellulose generally leads to accumulation of the acid used for pretreatment, acetic acid converted from acetate groups of hemicellulose and lignin, as well as formic, levulinic, and other organic acids which are degradation products of sugar monomers [11]. All these weak acids mainly affect the growth of fermenting microorganisms by disrupting cytoplasmic pH homeostasis [12]. During hot acid pretreatment, pentoses and uronic acid released from the polysaccharides, mainly hemicellulose, can be further degraded into furfural whereas hexoses released from the polysaccharides can be further degraded into 5-hydroxymethylfurfural (HMF) [13]. Inhibitory effects of the two aldehydes include inhibiting the growth of fermenting microorganisms [14], [15], and, since the furans are relatively unstable, prolonged hot acidic hydrolysis can lead to formation of inhibitory acids, formic and levulinic acids from HMF and formic acid from furfural [11], [16]. Last but very potent inhibitory by-products are lignin and phenolic compounds. While insoluble lignin appears to inhibit enzymatic saccharification by binding with and hence limiting the availability of cellulase [17], their soluble, degradation phenolic products such as dihydroconiferyl alcohol, *p*-hydroxybenzoic acid (PHBA), syringic acid, *p*-coumaric acid, ferulic acid, vanillin, coniferyl aldehyde, syringaldehyde, and Hibbert ketone can not only deactivate cellulase [18], but can also cause disruption of plasma membrane of fermenting microorganisms which eventually lead to cell death [19].

Similar to pretreatment efficiency, formation (type and concentration) of the inhibitory by-products varies depending on properties of the biomass substrate (such as composition of lignin, cellulose, and hemicellulose and degree of polymerization and crystallinity) as well as parameters used for the pretreatment condition (such as particle size of substrate, type of acidic catalyst, acidity (pH) of solvent, substrate to solvent ratio, mixing of substrate to solvent, treatment time, and treatment temperature) [20]. As a result, efficiency of lignocellulosic biomass conversion could be improved by minimizing formation of the inhibitory by-products which could be attained through using starting biomass materials with lower lignin and/or hemicellulose contents and through optimizing/refining the pretreatment condition such as using weak acids instead of strong acids to pretreat lignocellulose biomass.

Dilute organic acid pretreatments appear to be less severe and have potential to be optimized to obtain cost-effective pretreatment conditions. In our previous study, effectiveness of pretreating rice straw was compared using three different organic acids (acetic, citric, and oxalic acids) and one inorganic acid (hydrochloric acid) as the catalysts. The results indicated that high-temperature, long-time pretreatment with dilute oxalic acid (5.01% (w/v) acid concentration at 135.91°C for 30.86 min) significantly enhanced saccharification of rice straw when compared to the other three acid pretreatments [9]. In this study, we investigated effect of the four acid pretreatments on Napier grass straw, differing from rice straw in lignin-polysaccharide composition, to determine effectiveness

and reproducibility of the pretreatments and to determine effect of type of biomass substrate on pretreatment and enzymatic saccharification. Pretreatment optimization was conducted using Response Surface Methodology (RSM) and effectiveness of the pretreatment conditions was evaluated in term of sugar yield.

2 Materials and Methods

2.1 *Lignocellulosic biomass, chemicals, and enzymes*

Napier grass (*Pennisetum purpureum*) straws were harvested from Ratchaburi province, western part of Thailand. The obtained straws were immediately dried in hot air oven at 80°C to a constant weight, then milled to particle size between 10–20 mesh using aluminium sieve and stored in sealed plastic bags at room temperature until use. The lignocellulosic composition of the grass was determined in triplicate according to the method described by Van Soest and Wine, 1967 [21]. Oxalic acid ($C_2H_2O_4$), acetic acid ($C_2H_4O_2$), citric acid ($C_6H_8O_7$), hydrochloric acid (HCl), sodium citrate and sodium azide were purchased from Ajax Finechem (New South Wales, Australia). 3,5-dinitrosalicylic acid, Celluclast® 1.5L and Novozyme 188 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Beta-glucosidase was purchased from Megazyme (Wicklow, Ireland).

2.2 *Preliminary acid pretreatments of Napier grass straw*

Since lignocellulosic hydrolysis is generally affected by particle size of substrate, substrate to solvent ratio, mixing of substrate to solvent, acidity (pH) of solvent, temperature, and time, we fixed the former three parameters and varied the later three parameters to obtain optimal parameter combination process for each of the acids. Prior to optimizing the pretreatment conditions using RSM, preliminary pretreatments were conducted in duplicate to locate range for each of the three parameters. Based on our previous study [9], the pretreatment temperatures were trailed at 100, 120, 140, and 160°C (using 2% (w/v) HCl for 30 min) whereas the pretreatment durations were trailed for 30, 60, 90, and 120 min (using 2% (w/v) HCl at the identified optimal temperature). The pretreatments of hydrochloric acid were trailed using the concentration of 0.5, 1, 1.5, and 2% (w/v) while of the organic acids were trailed using the concentration of 2.5, 5, 10, and 15% (w/v) (at the identified optimal temperature and time).

Each preliminary pretreatment trail was conducted by immersing and vortex-mixing five grams of milled straw in 45 mL of acid solvent at concentration specified above in a screw-capped bottle. The pretreatments were carried out in a hot air oven using temperature-time conditions specified above. Each pretreated sample was collected by filtering through a fritted-glass filter and washed with copious amount of deionized water to remove acid solvent and residuals that are inhibitory to enzymatic saccharification. The sample was then immediately dried in hot air oven at 80°C to a constant weight and stored in sealed plastic bags at room temperature until use for enzymatic saccharification.

2.3 *Enzymatic saccharification*

The pretreated samples were hydrolyzed using a cellulase-cellobiase mixture containing 20 FPU/g-substrate of Celluclast® 1.5L and 100 CBU/g-substrate of Novozyme 188 [22], [23]. Each hydrolysis was conducted by immersing and vortex-mixing 0.5 gram of sample in 20 mL of 50 mM sodium citrate buffer (pH 4.7) containing 200 µl of 2 M sodium azide in a screw-capped plastic tube. Hydrolysis was carried out in a 200 rpm shaking incubator at 45°C for 72 hours. The released reducing sugars were measured using the 3,5-dinitrosalicylic acid (DNS) method [24] using spectrophotometer.

2.4 *Response Surface Methodology (RSM) optimization*

The Box-behnken experimental design of RSM, regression analysis, and estimation of the coefficients were conducted using Design-Expert software version 7.0.0 (Stat-Ease, Inc., Minneapolis, MN, USA) [25]. Minimum (–1) and maximum (+1) coded values of the three independent variables derived from the preliminary trials were 30 and 90 min for the reaction time (X_1), 100 and 140°C for the treatment temperature (X_2), 2 and 12% (w/v) for the organic acid concentration (X_3), and 0.5 and 2% (w/v) for the hydrochloric acid concentration (X_3). The second order polynomial

regression model was generated to enhance the response by optimizing the three pretreatment parameters, as followed:

$$Y = \beta_0 + \sum_{i-1}^{3} \beta_i X_i + \sum_{i-1}^{3} \beta_{ii} X_i^2 + \sum_{i-1}^{2} \sum_{i-1}^{3} \beta_{ii} X_i X_j$$

The dependent variable was reducing sugar yield (Y), i and j are linear and quadratic coefficients, and β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients. A total of 17 pretreatment experimental runs with varied input parameters were carried out in duplicate for each of the acids. Afterwards, enzymatic saccharification was conducted for each of the preatreated samples and the obtained sugar yields were compared to determine effectiveness of the RSM optimal conditions. The four RSM-optimized mathematical models were performed accordingly to validate the models.

2.5 *Statistical analysis*

Enzymatic saccharification was expressed as yield of reducing sugars released from each of the pretreated biomass. Statistical significance of differences among experimental variables were conducted using analysis of variance (ANOVA) using Design-Expert software version 7.0.0 (Stat-Ease, Inc., Minneapolis, MN, USA). Probability of $p < 0.05$ was used to indicate statistical significance.

3 Results and Discussions

3.1 *Lignocellulosic biomass content of the Napier grass straw*

As shown in Table 1, the cellulose, hemicellulose, lignin, and ash contents of the Napier straw substrate were 41.18%, 30.15%, 6.68%, and 2.90%, respectively. The Napier straw used in this study had as high hemicellulose content as rice straw used in our previous study [9], but had slightly higher cellulose content and lower lignin content when compared to other grasses in the same family [9], [27]. This is similar to what reported by Yasuda *et al*., [26]. As a result, it is likely that this Napier straw would provide less phenolic inhibitory by-products degraded from lignin and hence would be a suitable substrate for enzymatic saccharification and microbial fermentation.

Table 1: Composition of lignocellulose of different grasses in the Poaceae family

Type of Grass	% Composition on a Dry Weight Basis			
	Cellulose	Hemicellulose	Lignin	Ash
Napier Grass Straw	41.18	30.15	6.68	2.90
Wheat Straw [27]	38–48	23–29	13–19	5–9
Bagasse [27]	36–45	25–28	17–20	1–3
Corn Stover [27]	36–41	26–30	16–21	2–6
Rice Straw [9]	34.63	29.74	15.34	11.02

3.2 *Effect of pretreatment temperature, time, and acid concentration on the enzymatic saccharification of Napier grass straw*

The effects of pretreatment temperature on the enzymatic saccharification of Napier grass straw were investigated at 100, 120, 140, and 160°C using 2% (w/v) HCl for 30 min. As shown in Figure 1 (a), increase in pretreatment temperature from 100°C to 120°C and further to 140°C resulted in higher enzymatic hydrolysis yield from 21.86 mg to 35.19 mg and to 40.69 mg, respectively. This indicated that increasing the temperature increased the reaction rates. However, the higher pretreatment temperature led to more degradation of the lignocellulosic biomass, as illustrated by the % remaining biomass weight. The pretreatment of Napier grass straw at 140°C led to the greatest enzymatic hydrolysis yield, we therefore used this optimal temperature to locate the range of pretreatment time. We noted that the pretreatment at 140°C was relatively potent which led to more than 50% solid loss while other thermal pretreatments of Napier grass including pretreating with $CaOH_2$, with 27% aqueous ammonia, and with 1% H_2O_2 plus 0.8% NaOH led to less than 40% solid loss [28].

The 30-min pretreatment resulted in relatively similar sugar yield to the 60-min pretreatment which was 40.28 mg and 39.44 mg, respectively, Figure 1 (b). Nevertheless, the % remaining biomass weight indicated that more degradation of the lignocellulose biomass occurred when using the 60-min pretreatment time (43% vs. 47.02% remaining weight) and the 30-min holding time was the most favourable in term of sugar yield and cost. On the same hand, longer pretreatment time did not enhance enzymatic hydrolysis but led to more reduction in the obtained sugars, yielding

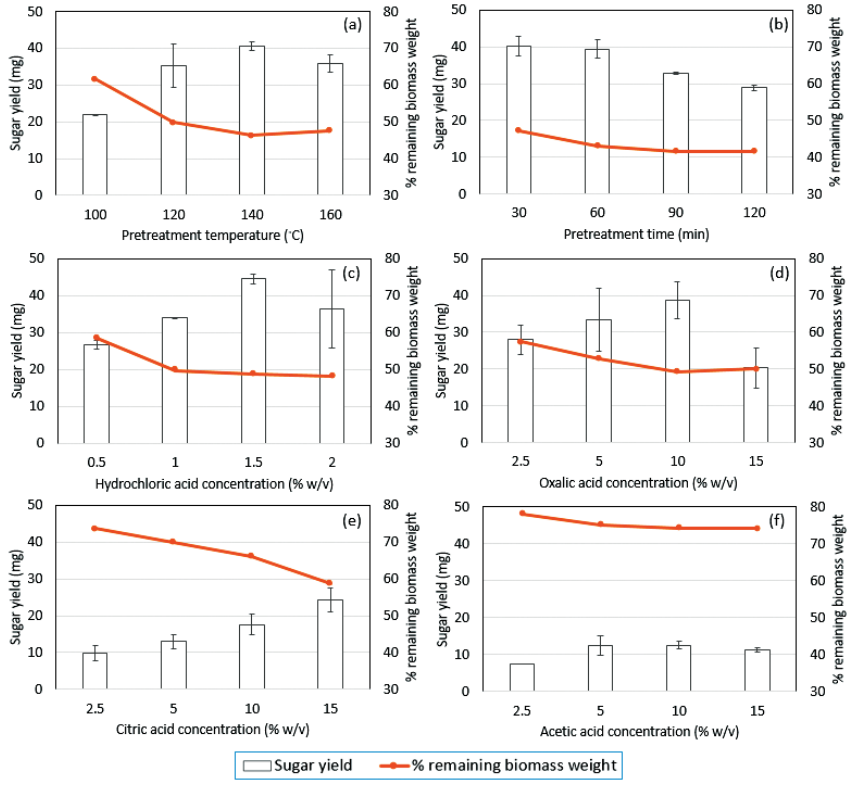

Figure 1: Effect of pretreatment temperature, time, and acid concentration on the enzymatic saccharification of Napier grass straw. (a) The pretreatments were conducted using 2% (w/v) HCl for 30 min, (b) The pretreatments were conducted using 2% (w/v) at 140°C, (c)–(f) The pretreatments were conducted at 140◦C for 30 min.

32.72 mg for 90-min holding time and 28.90 mg for 90-min holding time, respectively. In conclusion, for this somewhat harsh condition, the shorter the holding time, the less the degradation of the lignocellulosic biomass. The 30-min holding time was the optimal, we therefore used this holding temperature to locate the range of acid concentration for each of the acids.

The optimal hydrochloric acid concentration to pretreat Napier grass straw was trailed using the concentration of 0.5, 1, 1.5, and 2% (w/v) while the optimal organic acid concentrations for the pretreatments were trailed using the concentration of 2.5, 5, 10, and 15% (w/v). All the trails were conducted at 140°C for the holding time of 30 min. The inorganic and organic acids catalyzed the lignocelluloses which results in releasing the reducing sugars as well as biomass weight loss. The results for hydrochloric, citric, and acetic acids were in consistent with our previous study pretreating rice straws [9], in that highest enzymatic saccharification yields were obtained when using the hydrochloric acid concentration of 1.5% (w/v), Figure 1 (c), and when using the citric acid concentration of 15% (w/v), Figure 1 (e). As illustrated by the % remaining biomass weight, at 140°C for the holding time of 30 min, citric acid was not very effective in pretreating the Napier biomass when compared to hydrochloric and oxalic acids and as high as 15% (w/v) concentration of citric acid was needed to obtain an elevated sugar yield of 24.30 mg. Likewise, for this temperature-time condition, even the highest acetic acid of 15% (w/v) was inefficient in pretreating the lignocellulose, did not greatly affect the lignocellulose and did not lead to enhancement of the enzymatic saccharification. Another reason to explain this phenomenon is the fact that acetic acid is a major inhibitory by-product of ultra-high-temperature, acid hydrolysis of lignocellulose, increasing the dose of acetic acid to thermally hydrolyze the biomass therefore

is increasing the concentration of the inhibitor [11].

Oxalic acid is a reducing agent and exhibits greater acid strength than citric and acetic acids [29]. The highest enzymatic saccharification yield of 38.66 mg was obtain when using the oxalic acid concentration of 10% (w/v), Figure 1 (d), which was slightly lower than the highest yield of 44.44 mg obtained when using the hydrochloric acid concentration of 1.5% (w/v), Figure 1 (c). The hot pretreatments using the two acids resulted in similar % solid loss. This suggested that oxalic acid has potential to be used to substitute hydrochloric acid which would be advantageous in term of process severity and cost. Hot pretreatment conditions using dilute oxalic acid exhibited superior potential compared to many other organic and inorganic acids. This was substantiated by a number of studies. For example, Zhang et al., 2013 reported that hot oxalic acid pretreatment led to greatest sugar yields from maple wood when compared to the pretreatments using sulfuric and hydrochloric acids [30].

3.3 *RSM optimization of acid pretreatment of Napier grass straw*

The Box-behnken experimental design of RSM was optimized for each of the two types of acid. A total of 17 pretreatment experimental runs with varied input parameters were carried out in duplicate for each of the four acids. The coded values of the three independent variables together with the dependent variable, the reducing sugar yield, quantified using the DNS method were shown in Table 2. The yields of the released reducing sugars were different for different acids at different conditions. The inorganic acid resulted in the highest sugar yield of 42.11 mg when pretreated using the moderate pretreatment temperature, time, and acid concentration (1.25% (w/v) acid concentration at 120°C for 60 min), indicating that moderate conditions should be used for such the strong acid. While, the most effective oxalic and citric acid pretreatments happened similarly when pretreated using the moderate acid concentration (7% (w/v)) along with the extreme pretreatment temperature and time (at 140°C for 90 min). On the other hand, the most effective acetic acid pretreatment happened when using the extreme acid concentration (12% (w/v)) at the moderate temperature and for the short treatment time (at 120°C for 30 min). The reducing sugar yields were relatively high when pretreated using the extreme strength of treatment time and acid concentration (condition 13) and when pretreated using the

Table 2: Box-behnken experimental design of RSM with values of the three independent variables and the measured dependent variable, reducing sugar yield, for each acid pretreatment

Condition	X_1 (time, min)	X_2 (temperature, °C)	X_2 (% acid (w/v))		Sugar yield (average ± SD, mg)			
			Inorganic acid	Organic acid	Hydrochloric acid	Oxalic acid	Citric acid	Acetic acid
1	60	100	0.5	2	10.21 ± 0.411	11.71 ± 0.103	15.10 ± 1.917	5.83 ± 0.582
2	30	100	1.25	7	10.70 ± 0.068	17.81 ± 0.770	11.52 ± 0.051	7.50 ± 0.205
3	90	100	1.25	7	18.52 ± 0.171	29.82 ± 1.490	14.62 ± 1.643	7.42 ± 0.667
4	60	100	2	12	15.66 ± 0.171	29.50 ± 0.479	16.22 ± 1.917	7.79 ± 0.548
5	30	120	0.5	2	10.09 ± 0.034	6.68 ± 0.103	7.89 ± 0.826	8.57 ± 1.335
6	90	120	0.5	2	38.10 ± 2.738	13.84 ± 2.330	22.17 ± 0.952	9.92 ± 0.565
7	60	120	1.25	7	36.72 ± 4.895	20.94 ± 1.164	11.09 ± 0.565	10.04 ± 0.753
8	60	120	1.25	7	36.29 ± 4.039	21.64 ± 1.130	10.23 ± 0.650	17.77 ± 2.470
9	60	120	1.25	7	37.09 ± 3.851	24.28 ± 1.130	14.28 ± 0.967	21.35 ± 0.587
10	60	120	1.25	7	42.11 ± 2.105	23.09 ± 0.257	12.70 ± 0.359	19.81 ± 9.704
11	60	120	1.25	7	32.70 ± 2.122	28.80 ± 3.122	13.75 ± 0.257	20.65 ± 0.342
12	30	120	2	12	33.88 ± 0.976	16.22 ± 1.780	5.81 ± 0.205	35.09 ± 1.743
13	90	120	2	12	31.14 ± 0.359	31.54 ± 2.259	14.62 ± 1.539	27.06 ± 0.851
14	60	140	0.5	2	36.98 ± 1.232	20.57 ± 4.826	11.64 ± 0.753	23.96 ± 0.581
15	30	140	1.25	7	24.64 ± 0.616	19.60 ± 0.411	9.68 ± 1.386	24.59 ± 1.469
16	90	140	1.25	7	33.32 ± 1.951	33.50 ± 1.198	23.04 ± 1.078	26.48 ± 0.365
17	60	140	2	12	30.45 ± 4.005	32.43 ± 0.976	21.44 ± 1.597	22.91 ± 0.479

extreme strength of treatment temperature and acid concentration (condition 17). This indicated that such the intense conditions enhance the subsequent saccharification [11], [13]. The results suggested that the saccharification yields were considerably influenced by the three pretreatment parameters and the RSM optimization could provide optimal pretreatment condition of each of the four acids.

RSM was used to assess effects of the three independent parameters and their interactions on the dependent variable, the sugar yield. Significance of each independent parameter effect was determined using ANOVA and expressed as F value (Table 3). Quadratic mathematic models were suggested to fit the optimal parameter combination process for hydrochloric and oxalic acid with high coefficient of determination (R^2) values of 0.9392 and 0.9526, respectively. The significant model was indicated with the *p*-value (Prob. > F) of less than 0.05 while a cut-off *p*-value of less than 0.1 was applied to each of the model terms (Table 3). According to the specified criteria, the significant model terms of hydrochloric acid pretreatment were 0.0003, of oxalic acid pretreatment were <0.0001, of citric acid pretreatment were 0.0009, and of acetic acid pretreatment were 0.0004. Correspondingly, four predicted mathematical models giving the optimal saccharification yield (mg) as a function of pretreatment time (min), temperature (°C), and acid concentration (% (w/v)) and four optimal conditions were generated for the four acid pretreatments, Table 4.

Table 3: ANOVA of the fitted model of sugar yield obtained from saccharification of the pretreated Napier grass straws

Source	df	Mean square	F-value	p-value
Hydrochloric acid				
Model	6	282.36	13.75	0.0003
Conc	1	30.98	1.51	0.0474
Time	1	217.89	10.61	0.0086
Temp	1	617.72	30.08	0.0003
Conc x Time	1	236.39	11.51	0.0069
Time2	1	118.37	5.76	0.0373
Temp2	1	445.47	21.69	0.0009
Residual	10	20.53		
Oxalic acid				
Model	6	148.87	20.65	<0.0001
Conc	1	404.38	56.08	<0.0001
Time	1	292.60	40.58	<0.0001
Temp	1	37.22	5.16	0.0464
Conc2	1	72.63	10.07	0.0099
Time2	1	26.86	3.72	0.0824
Temp2	1	66.06	9.16	0.0127
Residual	10	7.21		
Citric acid				
Model	1	195.5	16.82	0.0009
Time	1	195.5	16.82	0.0009
Residual	15	11.63		
Acetic acid				
Model	2	425.98	14.68	0.0004
Conc	1	248.99	8.58	0.0110
Temp	1	602.97	20.77	0.0004
Residual	14	29.02		

Table 4: Mathematical models giving the saccharification yield (mg) as a function of pretreatment time (min), temperature (°C), and acid concentration (% (w/v)) and optimized saccharification yields for each of the acids

Catalyst	Mathematical Models	Optimal Pretreatment Parameter			Sugar Yield (mg)	
		Time (min)	Temperature (°C)	Acid Concentration (% (w/v))	Predicted	Experimental
Hydrochloric acid	Reducing sugar = -447.54743 + 23.12405*Conc + 1.30703*Time + 6.60236*Temp – 0.34167*Conc*Time – 5.88323*10-3*Time^2 – 0.025679*Temp^2	60.18	105.14	0.70	42.12	38.29 ± 0.171
Oxalic acid	Reducing sugar = 113.10435 + 3.74775*Conc + 0.53834*Time – 2.26865*Temp – 0.16613*Conc^2 – 2.80622*10^{-3}*Time^2 + 9.90210*10^{-3}*Temp^2	76.94	104.66	5.72	33.9	30.35 ± 4.004
Citric acid	Reducing sugar = 3.98332 + 0.16478*Time	81.98	105	9.94	12.81	10.84 ± 1.592
Acetic acid	Reducing sugar = –42.44094 + 1.11576*Conc + 0.43408*Temp	54.34	105.08	4.05	31.72	27.06 ± 1.130

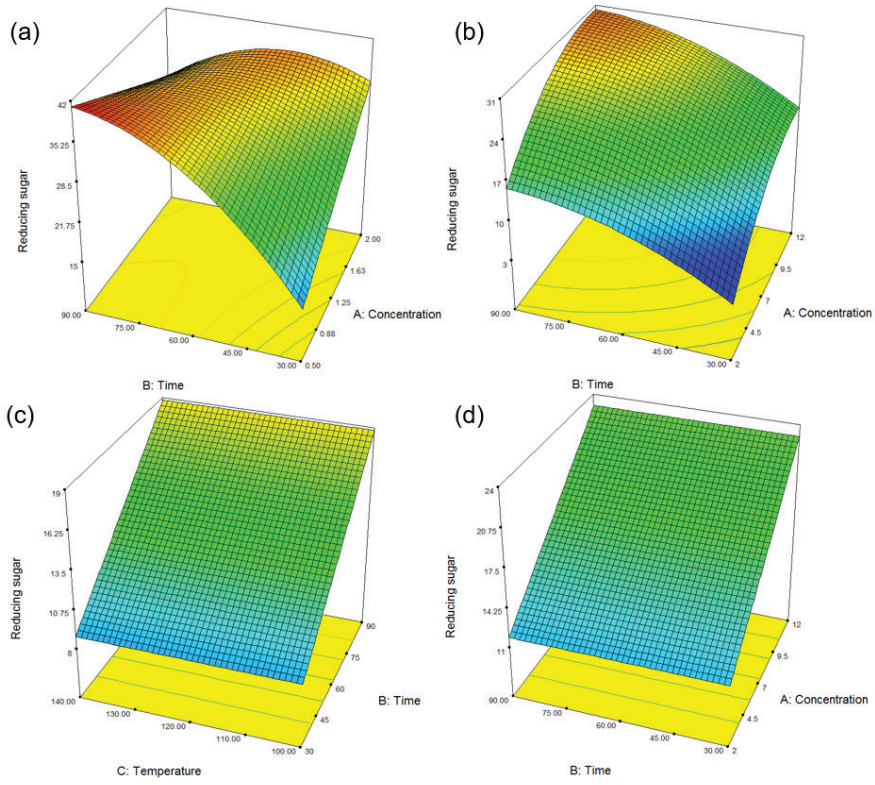

Figure 2: Response surface plots representing interaction effect of the independent variables on saccharification yield from pretreatments with hydrochloric acid (a), oxalic acid (b), citric acid (c), and acetic acid (d).

Response surface plots were generated to visualize the interactive effects of the pretreatment parameters on the obtained sugar yields, Figure 2. As depicted in the plots, pretreatment time and concentration significantly affect the saccharification yield for hydrochloric and oxalic acid.

Interestingly, the maximum sugar yield was obtained when using lower concentration of hydrochloric acid, while higher concentration of oxalic acid was preferred condition, Figure 2 (a and b). On the other hand, RSM models of citric and acetic acid pretreatment were fitted with linear regression, which time and concentration were the major factors affecting the sugar yields in citric and acetic acid, respectively Figure 2 (c and d). These observations suggested that the mechanisms of acid pretreatments of different types of acids were different and highlighted the importance of optimization experiments.

The RSM-predicted, optimal sugar yields were 42.12 mg, 33.9 mg, 12.81 mg, and 31.72 mg for the models of hydrochloric, oxalic, citric, and acetic acids, respectively. The RSM-optimized mathematical model for each of the acids were performed accordingly to validate the models. The experimental results were 38.29 mg, 30.35 mg, 10.84 mg, and 27.06 mg for the models of hydrochloric, oxalic, citric, and acetic acids, respectively, Table 4. While the pretreatment with citric and acetic acids were less promising in enhancing the saccharification, the hydrochloric acid pretreatment provided the greatest yield followed by the oxalic acid pretreatment. This supported our findings in the preliminary step that hot pretreatments using the two acids resulted in similar % solid loss and thus suggesting that they were effective in converting the Napier grass biomass. The two acids were found to be highly effective in pretreating lignocellulose biomass of various sources. Similar to our finding, 1% (w/v) hydrochloric acid pretreatment at 120°C for 60 min led to the highest sugar yield from eucalyptus bark [31], while 82 mM oxalic acid pretreatment at 160°C for 58 min was the optimal condition to pretreat yellow

poplar biomass [32]. Nevertheless, our previous study pretreating rice straw using the oxalic and hydrochloric acids resulted in about 7 and 4.5 times higher reducing sugar yield, respectively [9]. By comparing the % remaining biomass weight, it was found that about 60–65% remaining biomass weight of the pretreated rice straws were retained while about 50% remaining biomass weight of the pretreated Napier straws were retained which indicated that more and about half of the Napier straw biomass was degraded, released, and washed out during pretreatment implementation. To this end, saccharification yield would likely to be improved by using neutralized, whole pretreatment slurry as saccharification substrate, as demonstrated in the maleic acid pretreatment of rice straw by Jung et al., 2015 [33]. Another possibility is formation of inhibitory by-products during hot acidic hydrolysis [34]. The pretreatments of rice straws might have led to less formation of inhibitory by-products, especially lignin, that interfere with the functioning of the cellulase enzymes used in the saccharification step. In the future, we plan to determine the inhibitory by-products generated from pretreating the rice and Napier grass straws using the optimized acid pretreatment conditions to investigate their possible effects on the saccharification yield.

In summary, the sugar yields obtained from the validating experiments were relatively close to the predicted yields. For instance, the predicted and experimental yields for the hydrochloric acid were 42.12 mg and 38.29 mg, respectively, which is about 9.09% different, Table 4. Together, this indicated that the four models were predictive and could be used for the pretreatments to magnify lignocellulosic biomass conversion.

4 Conclusions

Conversion of Napier grass lignocellulosic biomass could be conducted in two sequential steps, hot acid pretreatment and enzymatic saccharification. Our finding demonstrated that the Box-behnken experimental design of RSM was useful to predict the optimal hot acid pretreatment conditions and to magnify the enzymatic saccharification. Pretreatment using dilute hydrochloric (0.70% w/v) and oxalic (5.72% w/v) acids at about 105°C for nearly 60 min and 77 min, respectively, were optimal to enhance the subsequent enzymatic hydrolysis of the available polysaccharides. The two lower acid-consuming conditions are more advantageous in term of process severity and cost, yet the sugar yields were not high when compared to those obtained from the optimal pretreated rice straws and further study is needed to improve the conversion efficiency.

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