Effect of Dilute-acid Hydrolysis Conditions on Sugar and Furfurals Productions from Paragrass

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Abstract. In this study, the productions of sugars as well as inhibitory compounds, e.g. hydroxymethylfurfural (HMF) and furfural, from paragrass (Brachiaria mutica) were investigated using dilute acid hydrolysis. Sulphuric acid concentration of 1.0, 1.2, 1.5 and 2.0 wt% and reaction temperature of 120, 130, 140 and 150 °C for either 30 or 60 min were investigated. Xylose was found to be the major product at a maximum yield of 99±3 mg/g grass using 1.2 wt% H₂SO₄ and reaction temperature of 140 °C for 30 min. Analysis of variance (ANOVA) showed that for the reaction temperature between 120-140 °C, sulphuric acid concentration was the most important factor affecting the yields of hemicellulose sugars from paragrass. However, the temperature ≥ 150 °C caused the marked drop in all sugar compounds. At the optimal condition, the concentration of HMF was 0.16 g/l and furfural 0.09 g/l. The formation of HMF and furfural was almost linearly increased with increasing hydrolysis temperature between 120 and 150 °C. Furthermore, longer reaction times led to higher levels of HMF and furfural. The dilute acid hydrolysis in series with enzyme saccharification of paragrass yielded 122 mg total reducing sugar (TRS) per g grass in the enzyme hydrolysate.

1. Introduction

Lignocellulose is the most abundant renewable biomass which has been considered to be a potential raw material for producing second generation biofuels [1]. The main components of lignocellulose are cellulose, hemicellulose and lignin, forming an integrated structure with high resistance to degradation. Paragrass is an ideal lignocellulosic substrate for producing second generation biofuels due to its abundance and relatively low cost. When compared to energy crops, e.g. napiergrass (Pennisetum purpureum), the lignocellulose required relatively low input of fertilizer and water, and it can adapt to a wide range of soil types. Dry matter yields on fertilized land ranged between 5000-12000 kg/ha a, while dry matter yields on unfertilized land ranged between 2000-4000 kg/ha a [2].

 Pretreatment of lignocellulosic materials is a necessary step for breaking down the integrated structures of lignocellulose. A number of methods have been studied for extracting valuable sugars...
from various grass species including (i) biological pretreatment such as aerobic degradation by white, brown and soft rot-fungi [3] (ii) physical pretreatment such as milling and dextrusion; (iii) chemical pretreatment including alkaline [4-7], acid [4, 8-9] and ionic liquids [10] and (iv) physical–chemical pretreatments includes steam explosion and ammonia fiber explosion [10]. An ideal pretreatment method should yield high amount of sugars with low amounts of inhibitory compounds as well as is economically feasible.

Dilute or weak acid pretreatment has been illustrated to be an effective method for removing hemicellulose especially for biomass with low lignin content. Examples are dilute acid hydrolysis of coastal Bermuda grass using 1.2% (w/v) \( \text{H}_2\text{SO}_4 \) at 140 °C for 30 min [8], dilute acid hydrolysis of coastal switchgrass using 1% (w/v) \( \text{H}_2\text{SO}_4 \) at 140 °C for 40 min [9], dilute acid hydrolysis of napier grass using 2% (w/v) \( \text{H}_2\text{SO}_4 \) at 90 °C for 30 min [4]. The acid pretreatment led to the hydrolysis of hemicellulose, yielding hemicellulose sugars, but hardly to the degradation of cellulose and lignin. The removal of hemicellulose led to the increased porosity of the biomass, and consequently the increased enzymatic digestibility [11]. Dilute acid hydrolysis can be divided into two categories based on the level of hydrolysis temperature: high temperature (> 160 °C) and low temperature (≤160 °C) [11]. Higher temperatures generally will lead to higher amount of hemicellulose released, but also increased the possibility of the conversion of hemicellulose sugars to inhibitory compounds, i.e. HMF and furfural.

The aim of this work was to study the conversion of hemicellulose in paragrass into mono- and di-saccharides using low temperature dilute sulphuric acid. Effects of sulphuric acid concentration, reaction temperature and hydrolysis time on the profiles of sugars as well as inhibitory compounds were investigated. The potential of paragrass as the substrate for producing sugars was discussed.

2. Materials and method

2.1. Biomass handling and storage

Paragrass was collected from Prachinburi province, Thailand (13.8196 °N, 100.5148 °E). Fresh paragrass samples had the humidity content of ca. 84%. The paragrass samples were size reduced to < 2 mm, oven dried at 105°C until the moisture content was reduced to less than 5%, and were stored in a desiccator.

2.2. Dilute acid hydrolysis

3 g of dried paragrass was immersed in 30 ml of 1.0, 1.2, 1.5 and 2.0 wt% \( \text{H}_2\text{SO}_4 \), depending on trials. The acid hydrolysis was performed in a Parr reactor (Amar Equipments PVT, A2101, India) at 120, 130, 140 and 150°C for either 30 or 60 min with the ratio of 1 g dried paragrass to 10 ml \( \text{H}_2\text{SO}_4 \) dilute solution. The reaction pressure ranged between 20-24 psi. After the reaction course, the sample was cooled down to stop the reaction and was vacuum filtered to separate the hydrolysate from the solid fraction. The liquid fraction was stored in a tube and frozen at -20 °C, while the solid fraction was rinsed with deionized water and stored at 4°C.

2.3. Enzymatic saccharification

The enzymatic hydrolysis was carried out with the commercial cellulase enzyme blend of cellulases, \( \beta \)-glucosidases, and hemicellulase (SAE0020, Sigma-Aldrich, USA). The density of the enzyme blend was 1.15 g/ml. 0.5 g of the acid pretreated paragrass (pretreated with 1.2 wt% \( \text{H}_2\text{SO}_4 \) at 120 °C for 30 min) was transferred into a 50 ml test tube and was added with 17 µl of the enzyme blend, 100 µl of 0.3% (w/v) \( \text{NaN}_3 \) and finally 0.05 M sodium citrate buffer. The final volume of the mixture was 15 ml with pH 4.8. The reaction was carried out at 55 °C, 155 rpm for 24, 48 and 72 h. After the reaction course, the sample was vacuum filtered to separate the hydrolysate from the solid fraction. The liquid fraction was stored in a tube and frozen at -20 °C, while the solid fraction was rinsed with deionized water until pH 7. The solid fraction, referred as biomass residual, was oven dried at 105 °C until the moisture content was reduced to less than 5%.
2.4. Measurement of biomethane potential
The method of biomethane potential measurement was followed that of [12].

2.5. Chemical analysis
Cellulose, hemicellulose and lignin content were measured by the detergent method [13]. The hemicellulose content was calculated from the difference between neutral detergent fiber (NDF) and acid detergent fiber (ADF). The lignin content was calculated from the difference between ADF and permanganate lignin (PML). After the PML analysis, the cellulose content was estimated from the weight loss of the sample held at 550 °C for 3 h.

Concentrations of sugars in hydrolysate were analyzed using a high-performance liquid chromatography (HPLC, Waters corporation, e2695, USA) equipped with a Rezex RPM monosaccharide Pb+2 analysis column (Phenomenex, USA) and a refractive index detector. The column temperature was 85 °C and the detector temperature was 40 °C. HPLC-grade water was used as a mobile phase with a flow rate of 0.6 ml/min.

Concentrations of HMF and furfural were analyzed using an HPLC equipped with a C18 HPLC column (Phenomenex, USA) and a refractive index detector. The column temperature was 60 °C and the detector temperature was 40 °C. A mixture of 99.99% (v/v) methanol and 1% (v/v) was used as a mobile phase at a ratio of CH₃OH:CH₃COOH 1:9 (v/v). The flow rate of the mobile phase was 2.5 ml/min.

TS, VS and MLVSS were determined by the Standard Methods [14].

2.6. Analysis of variance
Two-way ANOVA was performed using the Statistics and Machine Learning Toolbox™ function anova2 in Matlab R2018b (MathWorks, USA). The function multcompare was used to analyze the pairwise comparison results from a multiple comparison test.

3. Results and discussion

3.1. Composition of paragrass
Table 1 shows the composition of the native paragrass samples used in this study. The major composition of the paragrass was cellulose, while hemicellulose was the second highest component. Lignin content in the paragrass was lower than 10%, which was less than half of the lignin content in most investigated energy crops. Based on the lower lignin content, paragrass is expected to have higher digestibility than other energy crops.

Table 1 Composition of paragrass in comparison with other grass in the literature.

| Composition (%) | Paragrass<sup>a</sup> (%) | Napiergrass<sup>b</sup> (P. purpureum) | Switchgrass<sup>c</sup> | Bermuda grass<sup>d</sup> | Kans grass<sup>e</sup> | Miscanthus giganteus<sup>f</sup> |
|-----------------|---------------------------|--------------------------------------|------------------------|------------------------|----------------|--------------------------------|
| TS              | 97.26 ± 0.21              | n.a.                                 | n.a.                   | n.a.                   | n.a.           | n.a.                           |
| VS              | 89.37 ± 0.39              | n.a.                                 | 94.9                   | n.a.                   | n.a.           | n.a.                           |
| cellulose       | 43.32 ± 2.03              | 42                                   | n.a.                   | n.a.                   | 44             | 48.3                           |
| hemicellulose   | 28.40 ± 1.82              | 20                                   | n.a.                   | n.a.                   | 24             | 21.3                           |
| lignin          | 7.63 ± 0.89               | 19                                   | 14.8                   | 19.3                   | n.a.           | 28.8                           |
| glucan          | n.a.                      | n.a.                                 | 29.9                   | 25.6                   | n.a.           | n.a.                           |
| xylan           | n.a.                      | n.a.                                 | 15.6                   | 15.9                   | n.a.           | n.a.                           |

<sup>a</sup>this study; <sup>b</sup>[4];<sup>c</sup>[10]; <sup>d</sup>[8]; <sup>e</sup>[15]; <sup>f</sup>[5]; n.a. not available.
3.2. Effect of acid concentration, reaction temperature and time on yields of hemicellulose sugars

Figure 1 shows the yields of hydrolysate xylose obtained by the dilute acid hydrolysis with 1, 1.2, 1.5 and 2 wt% H$_2$SO$_4$. The corresponding H$_2$SO$_4$:biomass ratio was 0.1, 0.12, 0.15 and 0.2 g H$_2$SO$_4$/g dried biomass, respectively. For all investigated temperatures, the lowest values of xylose were obtained by using either 1 or 2 wt% H$_2$SO$_4$ concentration, while a maximum value of xylose was obtained when using either 1.2 or 1.5 wt% H$_2$SO$_4$ concentration, depending on the reaction temperature. The 1 wt% H$_2$SO$_4$ concentration was suggested to be too low to effectively solubilize hemicellulose, while the 2 wt% H$_2$SO$_4$ concentration may be too strong and caused the significant conversion of xylose to other chemicals e.g. furfural. A maximum xylose yield of 99±3 mg/g grass was achieved by using 1.2 wt% H$_2$SO$_4$, reaction temperature of 140 °C and reaction time 30 min.

![Figure 1](image.png)

Figure 1. Effect of H$_2$SO$_4$ concentration on yields of xylose in the hydrolysate
(a) 120 °C (b) 130 °C (c) 140 °C (d) 150 °C.

The yields of hydrolysate glucose obtained by the dilute acid hydrolysis are shown in figure 2. Similar to the xylose yields, the glucose yields were lowest when using either 1 or 2 wt% H$_2$SO$_4$ for all investigated reaction temperatures. A maximum glucose yield of 46 mg/g grass was achieved by using 1.5 wt% H$_2$SO$_4$, reaction temperature of 130 °C and reaction time 30 min.

Similar to the xylose and glucose yields, the yields of arabinose and mannose were maximized when using 1.5 wt% H$_2$SO$_4$ concentration for all investigated reaction temperatures. A maximum yield of arabinose and mannose was 25 mg/g grass was achieved by using 1.5 wt% H$_2$SO$_4$, reaction temperature of 130 °C and reaction time 30 min.
Figure 2. Effect of H$_2$SO$_4$ concentration on yields of glucose in the hydrolysate  
(a) 120°C (b) 130°C (c) 140°C (d) 150°C.

Figure 3 illustrates the effect of hydrolysis temperature on yields of xylose, glucose, arabinose and mannose and the sum of these sugars (referred as total reducing sugars in this study). Cellobiose was nil. Xylose was the major product from the acid hydrolysis, following by glucose and arabinose and mannose. The reaction temperature of 120 °C, 130 °C and 140 °C yielded approximately the same amounts of total reducing sugars. When increasing the reaction temperature to 150 °C, the yields of all types of hemicellulose sugars markedly dropped, while the concentrations of HMF and furfural reached the highest. When increasing the reaction time from 30 min to 60 min, the yields of the hemicellulose sugars were not significantly different ($P > 0.05$) from those reported for 30 min (data not shown). When using 1.2 wt% H$_2$SO$_4$, reaction temperature of 140 °C and reaction time 30 min, the TRS yield was ca. 140 mg/g.

Examining the data, the yields of the hemicellulose sugars were notably affected by the H$_2$SO$_4$:biomass ratio. When using 16 data points with temperature between 120, 130, 140 and 150 °C, sulphuric acid concentrations between 1.0, 1.2, 1.5 and 2.0 wt% H$_2$SO$_4$ and reaction time of 30 min, the two-way analysis of variance using the yield of xylose as the response showed the significance of the H$_2$SO$_4$:biomass ratio ($P < 0.001$) and statistical non-significance of the temperature ($P > 0.05$). The pairwise comparison showed that the xylose yields from the pretreatment with the 1.2 and 1.5 wt% H$_2$SO$_4$ were significantly higher than those with 1.0 and 2.0 wt% H$_2$SO$_4$. It may be expected that the temperature, acid concentration and their interactions should have great influences on xylose yields because the kinetics of the acid hydrolysis of hemicellulose into xylose was previously described by modified Arrhenius equations [8, 16-17].
3.3. Effect of acid concentration, reaction temperature and time on yields of HMF and furfural

Figure 4 illustrates the effect of hydrolys is temperature on yields of xylose and the amounts of HMF and furfural in the hydrolysate after the pretreatment with 1.2 wt% H$_2$SO$_4$ for 30 min. The amount of HMF and furfural consecutively increased with increasing reaction temperature, while the xylose yields markedly dropped with increasing the reaction temperature from 140 °C to 150 °C. Figure 5 shows the effect of hydrolysis time on amounts of HMF and furfural in the hydrolysate from the acid hydrolysis with 1.0, 1.2, 1.5 and 2 wt% H$_2$SO$_4$ at 120, 130, 140 and 150 °C. Regardless of the acid concentration, the amounts of HMF and furfural were always higher with a longer reaction time of 60 min. In addition, the amounts of HMF and furfural were higher with higher reaction temperature.

The dilute sulphuric acid pretreatment of paragrass gave the highest sugar when using 1.2 wt% H$_2$SO$_4$ at 140 °C for 30 min, yielding xylose 8.9 g/l and glucose 3.8 g/l. At this condition, the concentrations of HMF was 0.13 g/l and furfural 0.06 g/l. Yeast inhibition by furfurals may begin when the concentrations of furfurals in the culture reaches 1.0 g/l [8]. The generation of furfurals in this study at the optimal condition was well below the suggested minimum inhibitory concentration.

Figure 3. Effect of hydrolysis temperature on yields of sugars in the hydrolysate after pretreatment with 1.2% (w/v) H$_2$SO$_4$ for 30 min.

Examining the data, the production of furfural was notably affected by the reaction temperature. When using 16 data points with temperature between 120, 130,140 and 150 °C, sulphuric acid concentrations between 1.0, 1.2, 1.5 and 2.0 wt% H$_2$SO$_4$ and reaction time of 30 min, the two-way analysis of variance using the yield of furfural as the response showed the significance of the reaction temperature ($P < 0.001$). The higher the reaction temperature, the higher the amount of the produced furfural. In addition, with fixed values of reaction temperature and sulphuric acid concentration, the reaction time was found to significantly affect the amount of the produced furfural ($P < 0.05$). The longer the reaction time, i.e. prolonged from 30 min to 60 min, the higher the amount of the produced furfural. The data suggested that the kinetics of the xylose conversion to furfural can be explained by a modified Arrhenius equation.

3.4. Yields of TRS from enzyme saccharification of acid-pretreated paragrass

Glucose was the main sugar component in the enzyme hydrolysate. The saccharification time of 72 h was required to reach a substantial amount of TRS (figure 6). The enzyme saccharification of the acid-pretreated paragrass yielded 122 mg total reducing sugar per g grass. In comparison with the elephant grass pretreated with 2 wt% H$_2$SO$_4$ at 90 °C for 90 min and subsequent enzyme saccharification, the
yield of reducing sugars from the paragrass was notably higher than that of the napier grass (ca. 85 mg reducing sugar per g grass) [4].

**Figure 5.** Effect of sulphuric acid concentration, reaction temperature and reaction time on amounts of HMF and furfural obtained by acid hydrolysis with various H$_2$SO$_4$ concentrations (a) 120°C (b) 130°C (c) 140°C (d) 150°C.

**Fig. 6** Effect of reaction time on yields of total reducing sugars (TRS) from saccharification of paragrass pretreated with 1.2 %wt H$_2$SO$_4$ at 120 °C for 30 min

**Fig. 7** Methane production from the residual of paragrass
In addition to being a potential resource for sugar production, the paragrass residual was illustrated to be a potential substrate for the production of biogas with a biomethane potential at day 80 of 60 ml CH₄/g (Fig. 7). Further studies on agrofuel from paragrass including practices, policies and prospects should be performed. Such studies have been illustrated to be important for the promotion of biodiesel in Thailand [18, 19].

4. Conclusion
The dilute sulphuric acid pretreatment of paragrass gave the highest sugar when using 1.2 wt % H₂SO₄ at 140 °C for 30 min, yielding xylose 8.9 g/l and glucose 3.8 g/l. Xylose was found to be the major product in the pretreatment hydrolysate at a maximum yield of 99±3 mg/g grass. At this condition, the concentration of HMF was 0.16 g/l and furfural 0.09 g/l. The generation of furfurals in this study at the optimal condition was well below the suggested minimum inhibitory concentration of 1 g/l. The enzyme saccharification of the acid-pretreated paragrass yielded 122 mg TRS per g grass. ANOVA illustrated that the effect of sulphuric acid concentration on the xylose yields and the effect of reaction temperature and time on the generation of furfural were significant.

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