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# Hydrolysis of Wheat Straw Hemicellulose

**Sarote Sirisansaneeyakul[1] and Manfred Rizzi[2]**


## ABSTRACT

The dilute acid hydrolysis of 1.5% $H_2SO_4$, solid ratio of 1:15, particle size 0.5-1.4 mm, and 121° C for 30 min. was found as the better alternative for wheat straw hydrolysate preparation than those of the alkaline and enzymatic treatment under the conditions in this study. From scaling up to using 250 g wheat straw as substrate, an average xylose yield of 63.7% or 11.5 g xylose/100 g wheat straw (dry basis) was obtained. Also the inhibitory acetic acid was preferably removed by vacuum evaporation during the concentration of the hydrolysate. At 93% removal of water, only 78% loss of acetic acid was obtained with the remaining concentration of acetic acid 4.8 g/l in hydrolysate concentrate.


**Key words :** acid hydrolysis, alkaline hydrolysis, enzymatic hydrolysis, hemicellulose, wheat straw, monosaccharides, hydrolysate, lignocellulosics

## INTRODUCTION

Wood or agricultural wastes, as renewable resource, still represents an important source of organic chemicals and biotechnological derived products for the future. The well known three major components of these residues are cellulose, hemicellulose and lignin. Among them, the importance of hemicellulose fraction has been considered obviously as an inexpensive raw material for bioconversion to many useful products (Gong *et al.*, 1981; Tsao *et al.*, 1987; Kennedy and Paterson, 1989; Parisi, 1989). The pentoses, mainly D-xylose, derived from hemicellulose carbohydrates can be used by bacteria and yeasts with different conversion pathways (Magee and Kosaric, 1985).

There are plenty of methods reported to treat lignocellulosics, which are mentioned as physical, chemical treatment or combination of them. However, only three considerable pretreatments will be discussed here; namely steam, dilute acid and alkaline treatments.

**Steam pretreatment** This physical primary pretreatment can disrupt the texture and increase the surface area for enzymatic accessibility, as well as highly separate hemicellulose fraction containing mainly xylose. The conditions used, temperature and processing time depend on specified process varying from 170 to 240°C and from 1-30 min, respectively. Some interesting results by steam prehydrolysis have been reported by Kling *et al.* (1987).

**Dilute acid pretreatment** Jeffries (1983) and Ackerson *et al.* (1981) have discussed intensively about the recovery of hemicellulosic sugars by using dilute sulfuric acid hydrolysis in a two-stage process. The disadvantage of the process is the production of furfural and hydroxyfurfural from

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xylose and glucose, respectively. In general, under the following conditions; $H_2SO_4$ concentration 0.5-3.0 % (w/v), temperature 121°C or 140-160°C, processing time 20-60 min and straw concentration 10-15 % (w/v), hemicellulose can be extracted more than 80% of the original wheat straw. Other works have been also reported (Detroy *et al.*, 1981; Cunningham and Carr, 1984; Fanta *et al.*, 1984; Ghohmann *et al.*, 1984) concerning wheat straw hydrolysis.

**Alkaline pretreatment** Several workers have summarized the results from alkaline pretreatment using wheat straw as raw material (Detroy *et al.*, 1981, Cunningham *et al.*, 1985, 1986; Puri and Pearce, 1984). Both NaOH concentration and temperature play an important role in the pretreatment condition to enhance enzymatic hydrolysis of cellulosic residues. Moreover, a combination with acid (Cunningham *et al.*, 1985) or peroxide (Gould, 1984) may complete the enzymatic saccharification. After alkaline pretreatment, the hydrolysates containing lignin fraction (upto 80%) require the removal of soluble lignin before fermentation of monosaccharides by yeasts.

## MATERIALS AND METHODS

### Experimental procedures

The experimental design for xylose extraction from wheat straw has been carried out as follows:

**Acid hydrolysis** The ground wheat straw was prepared in a Moulinex mixer (Type 530, Modell Depose, France) and screened to obtain particle size < 0.5 mm (moisture content = 7.6%). The experiments were carried out in a screw-capped test tube (1.5 × 10 cm) with total weight of 5 g mixture (wheat straw + acid solution)/tube under the specified conditions. After treatment the mixtures were centrifuged and filtered to get clear supernatant for analysis of sugar content by HPLC. The experiments were scaled up in 2-l Erlenmeyer flask for studying the effect of particle sizes on release of monosaccharides, using 50 g ground wheat straw (6.3-6.9% MC) and 700 ml of 1.5 % $H_2SO_4$ (solid ratio = 1:15) at 121°C for 30 minutes. The resulting hydrolysates were collected and filtered to get clear supernatant for analysis of sugar content.

**Alkaline hydrolysis** The ground wheat straw (prepared as mentioned above) was treated with NaOH solution of various concentrations (2-12% w/v and control) in a screw-capped test tube (1.5 x 10 cm) with total weight of 5 g mixture (wheat straw + alkaline solution)/tube with the following condition; temperature 30°C, processing time 24 h, solid ratio 1:15 and 175 rpm shaking. The supernatants collected by centrifugation and filtration were neutralized before using for sugar determination. Xylose and glucose were analyzed by HPLC.

**Enzymatic hydrolysis** Enzymatic digests contained substrate 100 mg wheat straw (7.8% MC and particle size < 1.0 mm) and various amount of pulpzyme HA (Novo Norddisk Ferment AG, CH-4243 Dittingen) from 0.25 to 1.0 ml in 50 mM citrate-phosphate buffer pH 6.5 to obtain final volume of 5 ml (calculated as 2% substrate). The mixtures were incubated at 45°C for 24 h. A steam pretreated wheat straw (121°C for 30 min.) was also conducted under above mentioned condition to compare the degree of hydrolysis. The supernatants were obtained by centrifugation. After removal of solid particles, the crude wheat straw hydrolysates were analyzed for sugar content by HPLC using a HPX-87P column (Biorad, Richmond,CA) and for reducing sugar by dinitrosalisalic acid reagent using xylose as standard (Miller, 1959). The hydrolysis of wheat straw was calculated in percentage of xylose extraction :

$$\% \text{ hydrolysis} = \frac{\text{amount of xylose in hydrolysates (g)} \times 100}{\text{proposed xylose in dry basis of wheat straw used (g)}}$$

Estimated xylose content in wheat straw (dry basis) = 18% (Magee and Kosaric, 1985).

**Preparation of wheat straw hydrolysates**

The preparation was modified from Chen (1985). A 10-l bottle containing 250 g of wheat straw (< 1.0 mm, 6.9% MC) was filled with 3,500 ml of 1.5% $H_2SO_4$ and heated at 121°C for 30 min. The crude hydrolysates were collected after vacuum filtration and neutralized with CaO to pH 10. After neutralization, the supernatants were adjusted to pH 4.0 with concentrated $H_3PO_4$. The supernatants were obtained again after centrifugation and neutralized to pH 6.0 with KOH. If necessary, inhibitory acetic acid was removed by vacuum evaporation (Rotavapor: Bucchi 011 & 461, Switzerland) just after only neutralization with CaO to pH 2.0. The resulting syrup was adjusted to pH 6.0 for use as substrate in fermentation. The overall preparation has been shown in Figure 1. The removal of acetic acid was expressed as percentage of water removal :

$$\% \text{ water removal} = \frac{\text{amount of water removal (ml)}}{\text{total volume of wheat straw hydrolysate used (ml)}}$$

The experiment for acetic acid removal was carried out in 1-l round flask containing 500 ml wheat straw hydrolysate (pH 2.0 with CaO). The evaporation was conducted at 30-35°C under vacuum. At desired intervals, the evaporated water was measured and samples from remaining hydrolysate were taken for analysis of acetic acid by a enzymatic method (Cat. No. 148261, Boehringer, Mannheim).

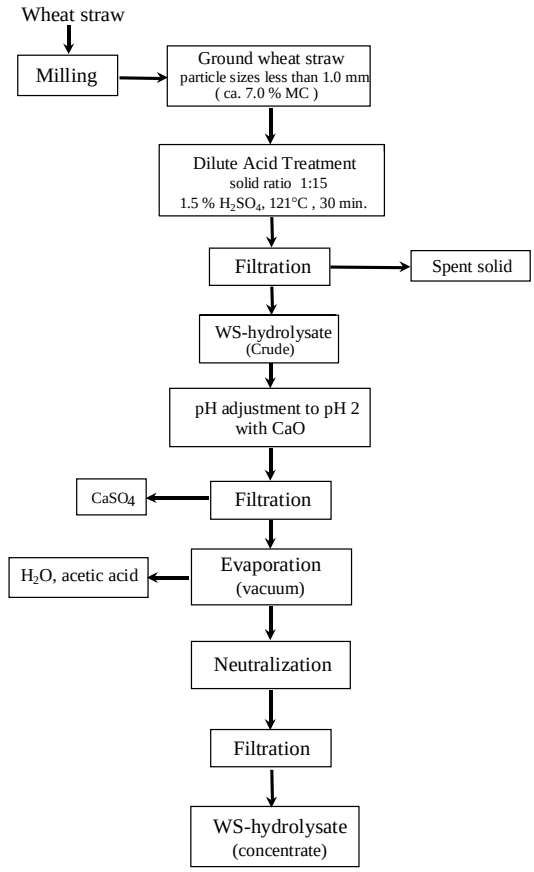


**Figure 1** The preparation of wheat straw hydrolysates.

**Analytical methods**

The sugars were analyzed by high performance liquid chromatography (HPLC) using HPX-87P column (Biorad, Richmond, USA) at 80°C with deionized water flow rate of 0.6 ml/min and 20 ml inject sample volume. Acetic acid was determinded by a enzymatic method using test combination kit (Cat. No. 148261, Boehringer, Mannheim GmbH).

## RESULTS AND DISCUSSION

### Acid hydrolysis

In the present report, acid hydrolysis of wheat straw has been investigated in the following factors:

**Effect of solid ratios** (wheat straw : acid solution) Based on the specified condition (0.5% $H_2SO_4$, 121°C and 30 min.), the solid ratios of 1:10, 1:15 and 1:20 were examined. The ratio of 1:15 has been selected as optimal wheat straw concentration resulting in xylose and glucose concentrations of 4.7 and 0.7 g/l, respectively (Figure 2).

**Effect of sulfuric acid concentrations** The $H_2SO_4$ concentrations of 0.5-3.0% and control (without acid) with 1:15 solid ratio were tested under steaming at 121°C for 30 min. The higher acid concentrations showed seemly the better yield of xylose (Figure 3). In order to minimize acid consumption and alkaline used for neutralization, 1.5% $H_2SO_4$ has been chosen with xylose and glucose concentrations of 11.4 and 1.9 g/l, respectively.

**Effect of processing times** Under the specified condition (1.5% $H_2SO_4$, solid ratio of 1:15 and 121°C) the longer processing time (more than 30 min.) gave higher degradation of xylose. The 30 min. of processing time indicated the best extraction for xylose and glucose resulting in concentrations of 11.8 and 3.1 g/l, respectively (Figure 4).

**Effect of particle sizes** Three ranges of particle sizes, < 0.5, 0.5-1.0 and 1.0-1.4 mm have been investigated for optimal xylose extraction under above obtained conditions. The particle size of ground wheat straw less than 0.5 mm gave no advantage for acid hydrolysis as compared to the others (Figure 5). The particle sizes between 0.5-1.4 mm were considered for ground wheat straw preparation by milling with the expected xylose and glucose concentrations of 10.5-12.1 and 1.1-1.5 g/l, respectively.

### Alkaline hydrolysis

This experiment was carried out under those conditions used in the dilute acid treatment but at 30°C and 24 h. The NaOH concentrations up to

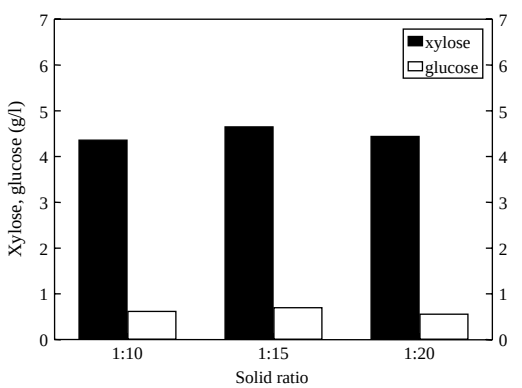


**Figure 2** Effect of solid ratios on xylose extraction from wheat straw by dilute acid hydrolysis with 0.5% sulfuric acid at 121°C for 30 min.

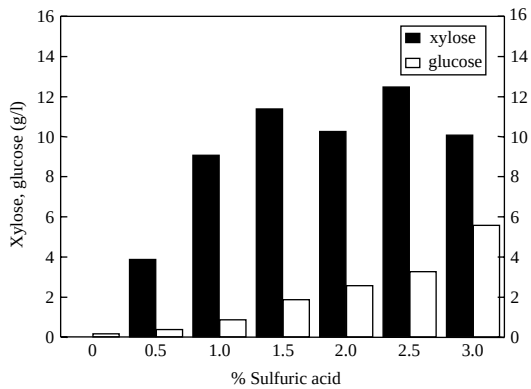


**Figure 3** Effect of sulfuric acid concentrations on xylose extraction from wheat straw at 121°C for 30 min. using solid ratio of 1:15 (0.33 g : 4.67 ml).

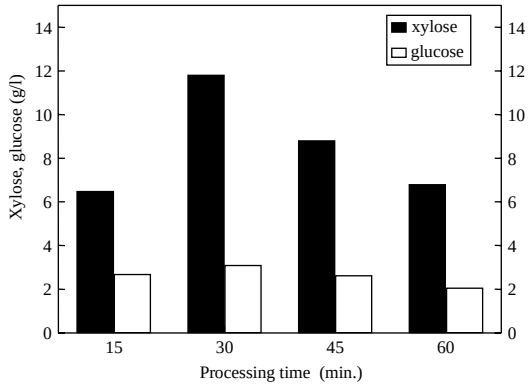


**Figure 4** Effect of processing times on xylose extraction from wheat straw by dilute acid hydrolysis with 1.5% sulfuric acid at 121°C using solid ratio of 1:15 (0.33 g : 4.67 ml).

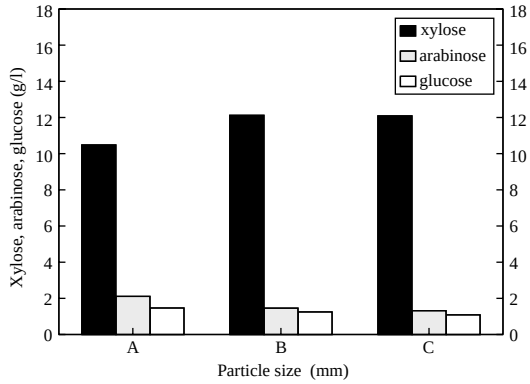


**Figure 5** Effect of particle sizes on xylose extraction from wheat straw by dilute acid hydrolysis with 1.5% sulfuric acid at 121°C for 30 min. using solid ratio of 1:15 (50 g : 700 ml).
A : < 0.5 mm, B : 0.5-1.0 mm, C : 1.0-1.4 mm

12% were studied and found that less extraction of xylose was obtained (Figure 6). This might be the effect of time requirement for xylose extraction. As compared to dilute acid treatment, this alkaline treatment under the above specified condition was not suitable for xylose extraction from wheat straw. On the other hand, glucose extraction has been fairly good, while increasing the NaOH concentration. At 12% NaOH, the concentrations of xylose and glucose were 0.9 and 189.5 g/l, respectively.

**Enzymatic hydrolysis**

The enzyme used, pulpzyme HA contained a mixture of fungal enzymes like xylanases, α-xylosidase and cellulases (Novo Norddisk, 1991). The saccharification of this commercial derived enzymes showed that the degree of hydrolysis (release of xylose) has been improved to 7.7 % from 4.4 % with heat pretreatment of substrate, while increasing the amount of enzyme used to 1.0 ml (Table 1). With heat treatment both xylose and glucose were produced from wheat straw, but only a small amout of xylose was found without heat treatment (Figure 7). This indicates that firstly the heat treatment (121°C, 30 min.) might disrupt the straw texture for better enzymatic accessibility, especially for cellulases, resulting in glucose formation. And secondly it enhanced to produce more reducing sugar with higher amount of enzyme used, showing poor activity of α-xylosidase.

The ratios of wheat straw and enzyme used (1:2.5 to 1:10; g/ml) showed very poor application in a large-scale production due to the low content of xylanases (22 IU/ml; pH 3.8, 30°C, 20 min. incubation). Both pretreatment and better source of xylanases, as well as working under optimal conditions should be obviously considered for enzymatic hydrolysis of wheat straw for xylose production.

**Preparation of wheat straw hydrolysates**

Figure 8 and Table 2 showed the degree of dilute acid hydrolysis from 8 different batches of wheat straw hydrolysate preparation under the specified optimal conditions with the scaling up of 750 times. The recovery of xylose from spent solid (wastes) were neglected from calculations. Therefore, the maximum value obtained for acid hydrolysis and xylose yield were only 73.8% and 13.3 g xylose/100 g wheat straw (dry basis), respectively.

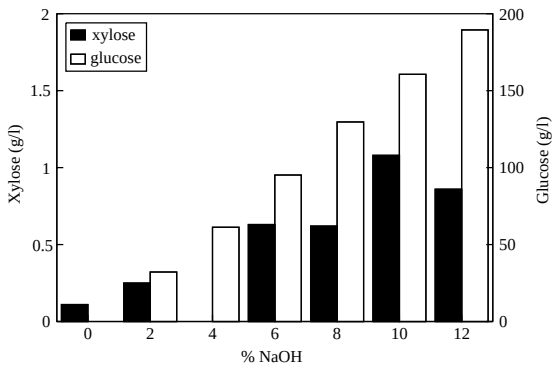


**Figure 6** Effect of NaOH concentrations on xylose extraction from wheat straw by alkali treatment at 121°C for 30 min. using solid ratio of 1:15 (0.33 g : 4.67 ml).

In order to remove acetic acid and concentrate the hydrolysate, vacuum evaporation (ca. 30-35° C) was applied to the preparation process. Figure 9 and Table 3 showed that 78% loss of acetic acid was obtained at 93% removal of water from crude hydrolysate (containing an original concentration of 1.6 g/l acetic acid), resulting in a final concentration of acetic acid 4.8 g/l. This concentration was still higher than the critical acetic acid concentration for yeast fermentation (4.0 g/l) (du Preez *et al.*, 1991; Sirisansaneeyakul, 1993).

**Table 1** Comparison of enzymatic hydrolysis of wheat straw with and without heat treatment (121°C for 30 min.).

| Amount of enzyme (ml) | (a) Without heat treatment | | (b) With heat treatment (121°C, 30 min.) | |
|---|---|---|---|---|
| | Xylose (g/l) | Hydrolysis (%) | Xylose (g/l) | Hydrolysis (%) |
| 0 | 0 | 0 | 0 | 0 |
| 0.25 | 0.51 | 2.7 | 1.02 | 5.5 |
| 0.50 | 0.66 | 3.6 | 0.96 | 5.2 |
| 0.75 | 0.64 | 3.5 | 1.16 | 6.3 |
| 1.00 | 0.82 | 4.4 | 1.42 | 7.7 |

**Note** : (1) Enzymic digests contained 100 mg/5 ml wheat straw in 50 mM citrate-phosphate buffer pH 6.5 plus desired amount of pulpzyme HA (0-1.0 ml) and were incubated at 45°C for 24 h.

(2) Pulpzyme HA (Novo Norddisk Ferment AG) showed xylanase activity approximately 22 IU/ml under defined condition (pH 3.8, 30°C and 20 min. incubation) and using 1 % xylan in 50 mM citrate-phosphate buffer as substrate.

(3) Degree of hydrolysis

$$\% \text{ Hydrolysis} = \frac{\text{Amount of xylose in hydrolysate (g)} \times 100}{\text{Xylose contained in wheat straw used (g)}}$$

(estimated xylose content in wheat straw based on dry basis = 18 %)

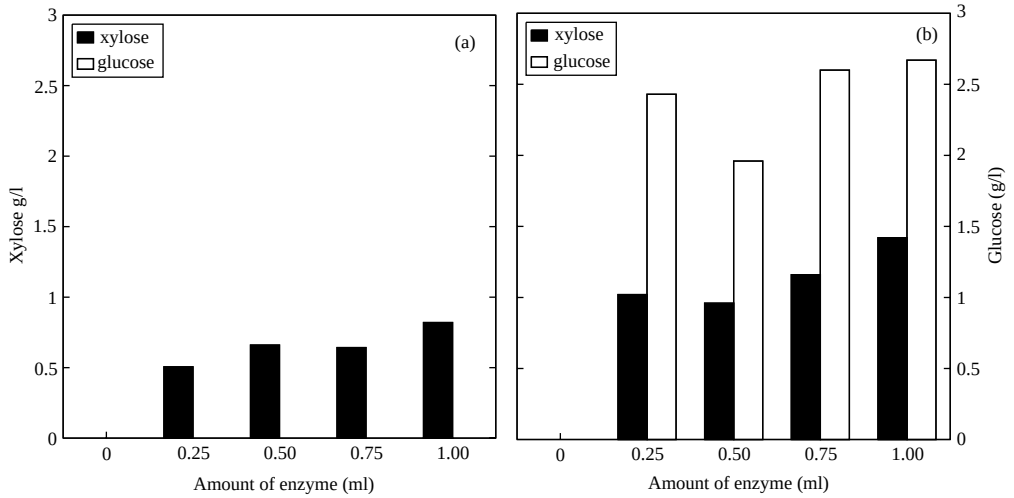


**Figure 7** Effect of enzyme concentrations on saccharification of wheat straw hydrolysing at 45°C for 24 h Digestion mixtures contained 100 mg ground wheat straw and amount of desired pulpzyme in σ 5 ml final volume of 50 mM citrate-phosphate buffer pH 6.5. (a) without heat treatment (b) with heat treatment (121°C for 30 min)

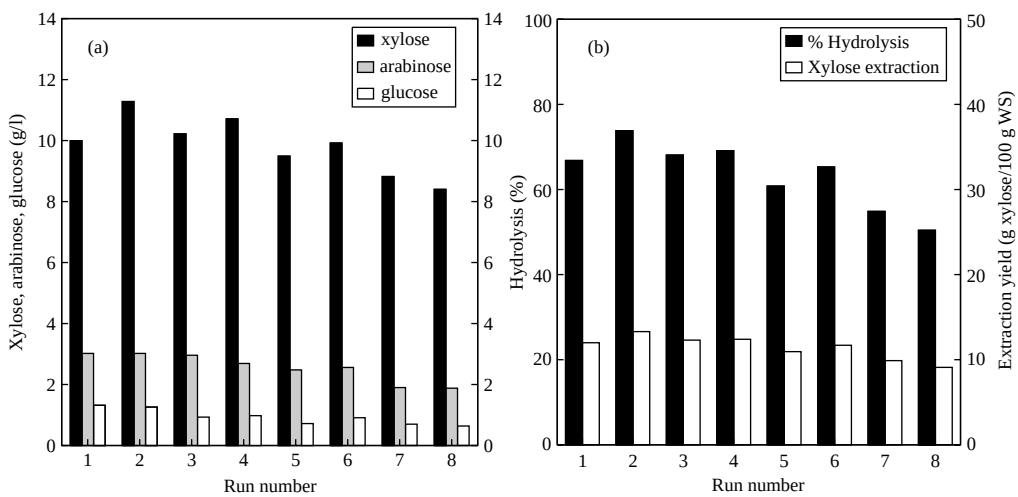


**Figure 8** Batch treatment of wheat straw by dilute acid hydrolysis with 1.5% sulfuric acid at 121°C for 30 min (a) sugar content in WS hydrolysates (b) degree of acid hydrolysis and extraction yield The mixtures contained 250 g ground wheat straw (7.8% MC and particle size less than 1.0 mm) and 3.5 l of 1.5% sulfuric acid based on solid ratio of 1:15.

**Table 2** The main sugar composition of wheat straw hydrolysates showing with degree of acid hydrolysis and xylose extraction yields in 8 batches.

| Batch no. | Sugars in WS-hydrolysis (g/l) | | | Hydrolysis | Xylose yield |
|---|---|---|---|---|---|
| | Xylose | Arabinose | Glucose | (%) | (g xylose/100 g WS) |
| 1 | 9.99 | 3.02 | 1.32 | 66.8 | 12.0 |
| 2 | 11.28 | 3.02 | 1.26 | 73.8 | 13.3 |
| 3 | 10.22 | 2.96 | 0.93 | 68.1 | 12.3 |
| 4 | 10.71 | 2.69 | 0.98 | 69.1 | 12.4 |
| 5 | 9.49 | 2.48 | 0.72 | 60.8 | 11.0 |
| 6 | 9.92 | 2.56 | 0.91 | 65.3 | 11.7 |
| 7 | 8.82 | 1.90 | 0.70 | 54.9 | 9.9 |
| 8 | 8.40 | 1.88 | 0.64 | 50.4 | 9.1 |
| Average | 9.85 | 2.56 | 0.93 | 63.7 | 11.5 |

**Note** : (1) Each batch treatment contained 250 g of ground wheat straw (7.8 % moisture content and particle size less than 1.0 mm) and 3.5 l of 1.5 % sulfuric acid based on solid ratio of 1:15. The mixtures in 10-l bottle were treated at 121°C for 30 min.
(2) Xylose yields were calculated from the real amount of WS-hydrolysates obtained without including that from the xylose remained in spent solid wastes.

**Table 3** The removal of acetic acid from wheat straw hydrolysates by vacuum evaporation.

| Concentrate | Water | | Acetic acid | |
|---|---|---|---|---|
| (ml) | Removal (ml) | Removal (%) | Real concentration (g/l) | Removal (%) |
| 500 | 0 | 0 | 1.6 | 0 |
| 375 | 125 | 25 | 1.8 | 14.3 |
| 250 | 250 | 50 | 2.4 | 24.1 |
| 145 | 355 | 71 | 2.8 | 49.1 |
| 75 | 425 | 85 | 3.4 | 68.3 |
| 37 | 463 | 93 | 4.8 | 77.8 |

**Note** : (1) Degree of water removal

$$\% \text{ Water removal} = \frac{\text{Amount of water removed (ml)} \times 100}{\text{Total volume of WS-hydrolysate used (ml)}}$$

(2) Degree of acetic acid removal

$$\% \text{ Acetic acid removal} = \frac{\text{Expected acetic acid (by calculation)-Real conc. in concentrate} \times 100}{\text{Expected acetic acid (g/l)}}$$

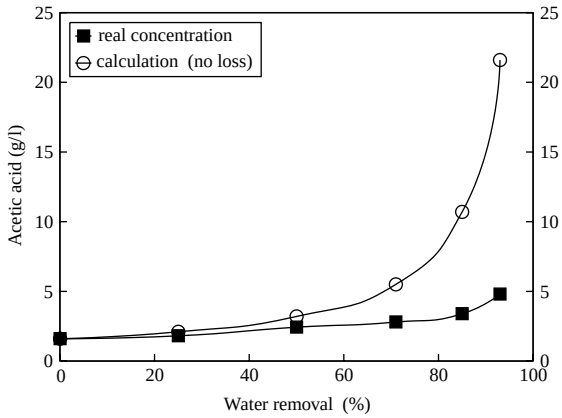


**Figure 9** Removal of acetic acid from wheat straw hydrolysates by vacuum evaporation.

$$\% \text{ Water removal} = \frac{\text{Amount of water removed (ml)} \times 100}{\text{Total volume of WS-hydrolysate used (ml)}}$$

## CONCLUSION

From 3 different treatments of wheat straw for xylose extraction, dilute acid (1.5% $H_2SO_4$, solid ratio of 1:15, particle size 0.5-1.4 mm, and 121°C for 30 min.) was found to be the best alternative for use in wheat straw hydrolysate preparation (Figure 1). The alkaline treatment as well as the enzymatic hydrolysis under this study were not be suitable for xylose hydrolysate preparation, as compared with the dilute acid hydrolysis. Similar results have been previously reported for dilute acid hydrolysis of rice straw (Sornprajak *et al.*, 1995). The maximum xylose concentration of 18.0 g/l was obtained under the following conditions : 1.5% $H_2SO_4$, 7.5% solid concentration, 1.0-2.0 mm rice straw particle size and at 121°C for 30 min. Moreover, the xylose yield of 13.11 g/100 g rice straw was obtained at 200 g scale of rice straw hydrolysis with an increasing time to 70 min. Therefore, the degree of hydrolysis of rice straw (ca. 94%) was quite higher than wheat straw hydrolysis (Table 2).

From 8 different batches of wheat straw hydrolysate preparation, the average value obtained for acid hydrolysis and xylose yield were only 63.7% and 11.5 g xylose/100 g wheat straw (dry basis), respectively. This xylose extraction yield of ca. 64% clearly showed problematic evidence of the large scale production. The suitable processing time may be prolonged to enhance better hydrolysis, as found in rice straw hydrolysis (Sornprajak *et al.*, 1995). However, the need of appropriate reactor design with rheological consideration is highly recommended for higher xylose extraction under the optimal hydrolysis conditions. Because of an inhibitory effect on yeast growth, the appropriate technique for acetic acid removal should be obviously considered at hydrolysate concentration step.

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