

## PRETREATMENT OF LIGNOCELLULOSIC BIOMASS BY AUTOHYDROLYSIS AND AQUEOUS AMMONIA PERCOLATION

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**Abstract** – A two-stage biomass pretreatment process—a combination of autohydrolysis and aqueous ammonia percolation—was experimentally studied as a method to remove and recover hemicellulose from lignocellulosic biomass. Hemicellulose was completely separated from the biomass after 1 hr of autohydrolysis at 200 °C. As reaction temperature and/or time of autohydrolysis was increased in the range of 170–200 °C and 1–2.5 hr, respectively, the amount of hemicellulose solubilization was increased; however, more sugars were decomposed. Most of the extracted hemicellulose was recovered as xylose oligomer. Hemicellulose was found to inhibit the enzymatic hydrolysis of cellulose. When the biomass was consecutively pretreated with pure water at 180 °C for 30 min and with 10 wt% ammonia solution at 180 °C for 30 min, about 62% of the hemicellulose was extracted. The enzymatic digestibility of the pretreated biomass was as high as 95%.

Key words: Autohydrolysis, Cellulose, Hemicellulose, Enzymatic Hydrolysis, Ammonia

### INTRODUCTION

Pretreatment is a necessary element in bioconversion of lignocellulosic materials to fuels and chemicals. The primary purpose of pretreatment is to make the lignocellulosic biomass accessible and reactive to allow high rates and yields on enzymatic hydrolysis. To date, a number of pretreatment methods have been proposed and investigated, including dilute-acid pretreatment [Grethlin et al., 1984; Lee et al., 1994; Torget et al., 1991; Schell et al., 1991], steam explosion [Brwonell and Saddler, 1987; Grous et al., 1986; Beltrame et al., 1991], organosolv pretreatment [Chum et al., 1989; Stockberger, 1993], and use of supercritical fluids [McMillan, 1994], oxidizing agents [Thomson et al., 1992], and alkali [Koullas et al., 1992]. Interesting claims have been made with regard to the effectiveness of these pretreatment methods. The economic and environmental constraints, however, limit the applicability of these known methods. For the production of ethanol, product yield has a strong influence on process economics [Lynd, 1990]. With the increasing availability of organisms capable of converting pentoses to ethanol [Beall et al., 1991; Zhang et al., 1995], high recovery of hemicellulose sugars is a key feature of leading pretreatment methods.

Recently, a novel pretreatment method based on aqueous ammonia was developed in our laboratory [Yoon et al., 1995]. This process was termed the ammonia recycled percolation (ARP) process. The ARP when applied to woody biomass brought about not only pretreatment effects, but also a partial fractionation of biomass into cellulose, hemicellulose, and lignin. In this study, the ARP was incorporated with an autohydrolysis to completely fractionate hemicellulose. When biomass

is heated to a high temperature (above 150 °C) with pressurized water, acids are formed by solubilization of acidic components and de-esterification of ester groups (like acetate groups) in hemicellulose. These acids catalyze the hydrolysis of the hemicellulose [Lora and Wayman, 1978], and thus pretreatment of biomass with hot water is called autohydrolysis. The acids formed in autohydrolysis are weak acids (such as formic acid, acetic acid, and glucuronic acid etc.) [McGinnis et al., 1983] and are not strong enough to hydrolyze cellulose; therefore, autohydrolysis can selectively extract hemicellulose from the biomass [Mok and Antal, 1992; Walch et al., 1992]. Hemicellulose in biomass could be an important barrier to enzymatic hydrolysis of cellulose. The removal of hemicellulose from biomass, therefore, would be a prerequisite for complete hydrolysis of cellulose and recovery of hemicellulose.

The overall objective was to evaluate the combined process of ARP and autohydrolysis as a pretreatment method. The optimum conditions for autohydrolysis were investigated in terms of maximizing the solubilization and recovery of hemicellulose sugars. The effect of hemicellulose on enzymatic digestibility was also investigated.

### MATERIALS AND METHODS

#### 1. Materials

Hybrid poplar milled to a nominal size of 80 to 60 mesh was used as feedstock. Filter paper (Whatman No.1) and  $\alpha$ -cellulose (Aldrich) were used as reference substrate. The cellulase enzyme, Spezyme-CP, was obtained from Environmental Biotechnology, Inc. The specific activity of the enzyme was determined by the supplier as follows: filter paper activity=64.5 FPU/mL,  $\beta$ -glucosidase activity=57.6 p-NPGU/mL. Birch wood hemicellulose (Sigma) was used in hydrolysis experiment.

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## 2. Experimental Setup and Operation

A schematic diagram of the experimental apparatus for the pretreatment is shown in Fig. 1. The details of the experimental setup are described elsewhere [Yoon et al., 1995]. The process involves treating biomass with water and/or an ammonia hydroxide solution at temperature above 150°C and pressure around 325 psi. In the pretreatment operation, 5-7 g of biomass sample was packed into the reactor. For the autohydrolysis, two types of operation mode were employed, batch and percolation process. In batch operation, water was pumped into the reactor prepacked with biomass and pumping stopped during reaction. The liquid to solid weight ratio in the reactor was about 7-10. In the percolation process, a constant flow of water (1.0 mL/min) was applied during reaction. After the autohydrolysis step, the biomass was then treated by ARP process, if necessary. In operation of ARP, an aqueous ammonia solution (10 wt% NH<sub>3</sub>, 1.0 mL/min) was pumped to the reactor. After the ARP reaction, water was pumped to remove residual sugars and ammonia trapped in biomass. The reactor was then flushed with nitrogen to remove the excess water. A portion of the wet solid residue was oven-dried at 105°C overnight to measure the weight loss on pretreatment and further subjected to chemical compositional analysis. The remaining portion of the solid residues was stored in a refrigerator under wet condition for carrying out the enzymatic digestibility test. The effluent from the reactor collected in a holding tank was transferred into an air-tight sample bottle and stored in the refrigerator for further composition analysis.

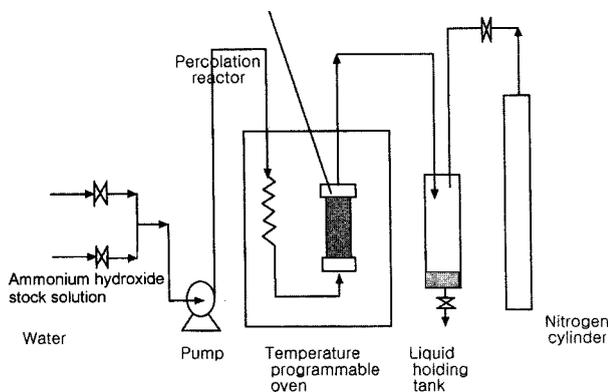


Fig. 1. Schematic diagram of ammonia recycled percolation process.

## 3. Digestibility Test

Enzymatic hydrolysis of pretreated substrates was performed in a 250 mL glass bottle at 50°C and pH 4.8 (0.05 M sodium citrate buffer) on a shaker bath agitated at 150 rpm. The enzyme loading was 30 IFPU/g cellulose, and the initial glucan concentration was 1% (v/v). Samples were taken periodically and analyzed for glucose content. The total glucose content after 72 hr of hydrolysis was taken to calculate the enzymatic digestibility. The enzymatic digestibility is defined as (total amount of glucose released) × 0.9 / (total cellulose). A dehydration factor of 0.9 is used to convert the glucose to cellulose (glucan).

## 4. Analytical Methods

The solid biomass samples were analyzed for sugar and Klason lignin contents following the procedure described in NREL-CAT Standard Procedures. Since most of the sugars contained in the liquid effluents were oligomers, a secondary acid hydrolysis was carried out at 121°C with 4 wt% sulfuric acid for 1 hr of reaction time to convert oligomers into monomers. Sugars and decomposition products were measured by HPLC using an Aminex HPX-87H (Bio-Rad) column.

The percent hemicellulose solubilization and recovery were calculated as follows:

$$\text{Percent hemicellulose solubilized} = (1 - C/C_o) \times 100$$

$$\text{Percent hemicellulose recovered} = [X/(C_o - C)] \times 100$$

To calculate these values, the following terms must be measured:

- C<sub>o</sub> = hemicellulose content of initial biomass (wt%)
- C = hemicellulose content of treated biomass sample based on initial biomass (wt%)
- X = xylose content in liquid effluent based on initial biomass (wt%)

The amount of xylan was used to represent the hemicellulose content in this study.

## RESULTS AND DISCUSSION

### 1. Effect of Temperature in Autohydrolysis

The effect of reaction temperature in autohydrolysis of hybrid poplar was investigated over 170-200°C. An autohydrolysis experiment was carried out under batch and percolation mode. Reaction time was kept for 1 hr for all runs. Table 1

Table 1. Effect of reaction temperature on composition of solid residues in autohydrolysis operated under percolation and batch modes<sup>a</sup>

Temperature, °C	Percolation mode				Batch mode			
	% Solid remaining	% Lignin	% Glucan	% Xylan	% Solid remaining	% Lignin	% Glucan	% Xylan
Untreated	100	26.73	43.52	14.43	100	26.73	43.52	14.43
170	73.70	16.80	42.50	6.60	79.40	26.40	42.90	6.10
180	66.10	18.30	42.30	3.40	71.20	22.90	40.20	3.10
190	59.60	15.70	38.55	2.20	70.40	25.40	38.60	1.50
200	61.50	18.50	39.36	1.80	67.40	26.70	35.50	trace

<sup>a</sup>All sugars and lignin content in the table based on oven-dry untreated biomass. Autohydrolysis conditions: de-ionized water, 1 h, 325 psi, water flow rate=1.0 mL/min (for percolation mode).

summarizes the composition data for the remaining solids after autohydrolysis. As shown in Table 1, hemicellulose was effectively solubilized (or removed) by the autohydrolysis pretreatment. A higher hemicellulose solubilization performance was observed in the batch process than in the percolation process, probably due to the more acidic environment in the batch process. The accumulation of acidic components during batch operation should result in the more acidic environment in the batch reactor than in the percolation reactor. The results collectively indicate that the reaction temperature is an important operating variable in autohydrolysis. As reaction temperature increased, hemicellulose remaining in the treated solid decreased, that is, the amount of hemicellulose solubilized increased. At 200 °C, the extent of hemicellulose solubilized in the percolation and batch process was 88% and about 100%, respectively. During the autohydrolysis, some of lignin was also removed from the biomass. As much as 41% of lignin was removed by the percolation process. However, no significant amount of lignin was removed by the batch process. Due to the nature of the percolation process, the solubilized lignin was removed immediately from the reactor and reprecipitating on the substrate was prevented.

Fig. 2 shows the enzymatic digestibility of biomass pretreated by autohydrolysis. The biomass samples pretreated by auto-

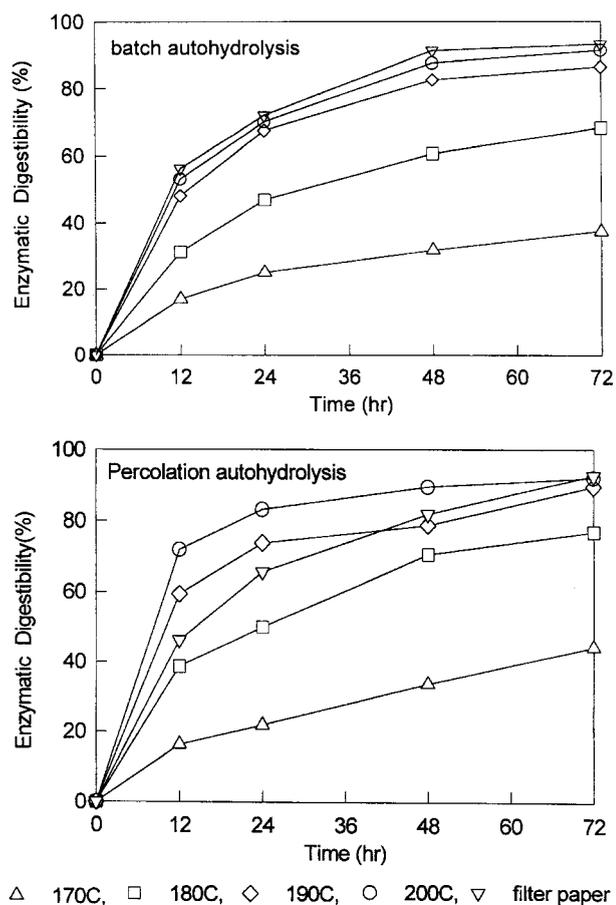


Fig. 2. Enzymatic digestibility of hybrid poplar treated by autohydrolysis under batch and percolation operation mode at various temperatures.

hydrolysis in either the batch or percolation process exhibit enhanced enzymatic digestibility of cellulose. The cellulose digestibility increased with the reaction temperature of autohydrolysis. The digestibility at 72 hr of enzymatic hydrolysis increased from 41.5 to 92.5% as the autohydrolysis temperature increased from 170 to 200 °C. A similar result was observed for samples treated by batch autohydrolysis. An important observation is that when the pretreatment temperature was above 190 °C in the batch process, almost no lignin was removed from the biomass, but the treated substrates exhibited very high digestibility close to that of the filter paper. These results indicate that thermal deformation of lignin, and not necessarily removal, may be a significant factor affecting the digestibility of biomass. The overall results indicate that the pretreatment temperature is a very important factor in autohydrolysis.

The liquid collected from the pretreatment reactor was analyzed for its composition. Most of the hemicellulose extracted into the liquid was found to exist as xylose oligomer, with only a small amount of free xylose and mannose. Since most of the sugars contained in the liquid sample were oligomers, a secondary acid hydrolysis was carried out at 121 °C with 4 wt% sulfuric acid for 1 hr of reaction time to convert oligomers into monomers. Table 2 summarizes the composition data for liquid samples (hydrolysates) obtained after batch autohydrolysis. Among the identifiable components were acetic acid, formic acid, hydroxymethyl furfural (HMF), and furfural. The concentrations of these acidic components increased with autohydrolysis temperature. The increase of the acid components was also indicated by a decrease in pH of the liquid sample. The pH's of the liquids produced after autohydrolysis at 170 °C and 200 °C were observed to be 5.0 and 3.2, respectively. The furfural and HMF content in the primary hydrolysate dramatically increased at temperatures above 180 °C, indicating the decomposition of sugars.

The effect of reaction time on hemicellulose solubilization and recovery was investigated over the range of 1 to 2 hr at 170 °C and 180 °C in batch autohydrolysis, as shown in Table 3. The percent recovery was calculated from the amount of xylose in the liquid stream based on the amount of hemicellulose solubilized. As expected, the hemicellulose solubilization increased as reaction time increased from 1 to 2.5 hr. The recovery of hemicellulose, however, decreased with reaction time. Within the scope of this experiment, the solubilization of cellulose and hemicellulose increased with the reaction temperature and time, and the recovery of sugars decreased with the temperature and time. For example, at 170 °C and reaction time of 1 hr, about 60% of the original hemicellulose was solubilized and 92% of the solubilized hemicellulose was recovered as xylose oligomer. When the reaction temperature was increased to 180 °C and the time to 2 hr, the solubilization of hemicellulose was increased to 86%, but only 11% of the solubilized hemicellulose was recovered. Since maximizing hemicellulose sugar yield is one of the pretreatment objectives, temperatures in the range of 170-180 °C and reaction times of less than 1 hour are considered to be optimal for autohydrolysis, although no further attempt was made to optimize the autohydrolysis process.

**Table 2. Effect of reaction temperature on composition of hydrolysate after batch autohydrolysis<sup>a</sup>**

Pretreatment conditions	%wt Glucan	%wt Xylan	%wt Mannan	%wt Formic acid	%wt Acetic acid	%wt HMF	%wt Furfural	pH
Primary hydrolysate								
170 °C, 1 h	trace	0.26	0.37	0.45	1.86	trace	0.64	5.0
180 °C, 1 h	trace	0.98	0.45	0.92	2.93	0.15	1.72	3.2
190 °C, 1 h	trace	0.56	0.25	0.83	3.68	0.55	2.87	3.0
200 °C, 1 h	trace	trace	trace	0.85	4.20	1.18	3.29	3.2
Hydrolysate after 2nd hydrolysis								
170 °C, 1 h	0.37	5.88	1.40	0.49	2.83	trace	0.8	-
180 °C, 1 h	0.70	6.12	1.43	0.936	3.16	0.20	2.06	-
190 °C, 1 h	1.03	1.87	0.79	0.85	4.28	0.28	3.23	-
200 °C, 1 h	1.08	0.31	0.26	1.56	4.79	0.32	3.28	-

<sup>a</sup>Data in the table based on oven-dry untreated biomass.

**Table 3. Effect of reaction time and temperature on sugar solubilization and recovery in the batch autohydrolysis**

Pretreatment conditions	% Solubilization <sup>a</sup>				% Recovery <sup>b</sup>
	Biomass	Lignin	Glucan	Xylan	Xylan
170 °C, 1.0 h	19.4	5.30	2.77	58.8	92.2
170 °C, 1.5 h	21.2	2.65	3.40	63.7	74.3
170 °C, 2.0 h	22.7	3.41	8.39	70.0	66.4
180 °C, 1.0 h	31.1	15.1	6.28	63.4	88.5
180 °C, 1.5 h	31.1	15.1	-	81.8	23.6
180 °C, 2.0 h	31.5	8.33	10.2	86.2	10.9
180 °C, 2.5 h	30.6	3.41	20.1	95.3	5.45

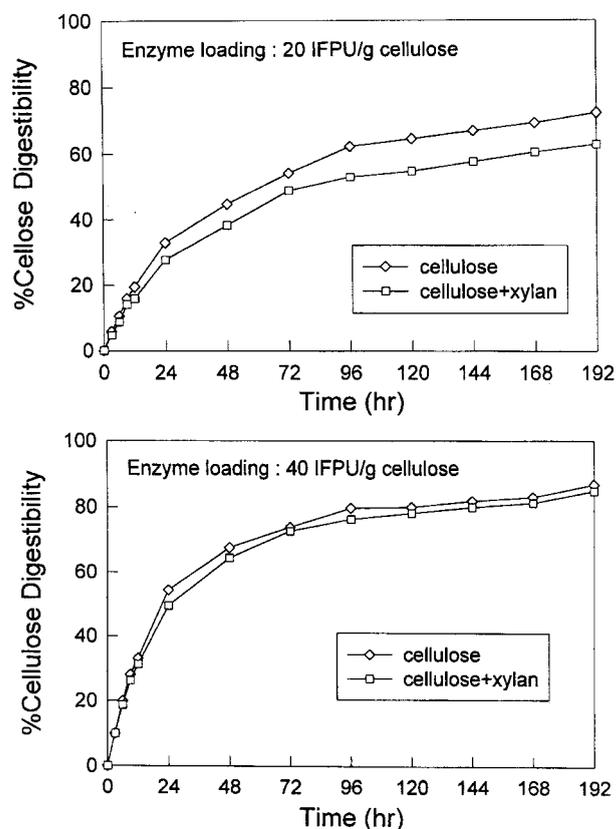
<sup>a</sup>based on original content in over-dry untreated biomass.

<sup>b</sup>based on the amount solubilized.

## 2. Effect of Hemicellulose on Cellulose Hydrolysis

In a previous study on ARP, it was found that the enzymatic digestibility of lignocellulosic biomass was greatly enhanced by removal of hemicellulose and lignin [Yoon et al., 1995]. Since the hemicellulose and lignin were removed simultaneously in the ARP process, their isolated effect could not be identified. The role of lignin in the enzymatic hydrolysis of lignocellulosic biomass has long been recognized. However, the role of hemicellulose is still uncertain. In this work, the effect of hemicellulose on enzymatic digestibility was investigated. The enzymatic hydrolysis of cellulose was conducted at various levels of enzyme loading with and without hemicellulose addition. As shown in Fig. 3, the cellulose hydrolysis under the enzyme loading of 20 IFPU/g cellulose was suppressed by the presence of hemicellulose. As the enzyme loading was increased to 40 IFPU/g cellulose, the effect of hemicellulose diminished. The cellulase enzyme, most likely the endoglucanase, has active sites for hemicellulose. It seems that hemicellulose adsorbs cellulase enzyme and the adsorbed enzyme is unavailable for cellulose hydrolysis. Hemicellulose in a lignocellulosic substrate physically is also known to block the contact between cellulase enzyme and cellulose. For conventional SSF processes, the enzyme loading is limited to 10-20 IFPU/g cellulose because of the high cost of cellulase enzyme. It is therefore concluded that hemicellulose could be an important barrier to enzymatic hydrolysis of cellulose and its removal is a prerequisite for complete hydrolysis of cellulose in biomass.

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**Fig. 3. Effect of hemicellulose on enzymatic digestibility of cellulose at different enzyme loading. weight ratio of hemicellulose to cellulose=0.4 : 1.**

## 3. Combined Pretreatment of Autohydrolysis and ARP

A primary result of the autohydrolysis is hemicellulose recovery; and the ARP was proven to increase digestibility. Hence a two-stage pretreatment process—a combination of autohydrolysis and ARP—was experimentally studied as a new method of biomass pretreatment. The biomass feed was pretreated with the following liquids: pure water (autohydrolysis) for 1 hr, 10 wt% ammonia solution for 1 hr, pure water for 30 min followed by 10 wt% ammonia solution for 30 min, and finally 10 wt% ammonia solution for 30 min followed by pure water for 30 min. The pretreated biomass samples were enzymati-

**Table 4. Effect of autohydrolysis and aqueous ammonia pretreatment on the composition of solid residues and enzymatic digestibility<sup>a</sup>**

Pretreatment condition	%wt remaining	%Lignin content	%Glucan content	%Xylan content	%Digestibility @72 hr
Untreated biomass	100.0	26.0	42.9	14.8	7.8
ARP (10 wt% NH <sub>3</sub> , 180 °C, 1 h)	68.9	13.1	40.0	7.7	95.2
AHP (Pure water, 180 °C, 1 h)	66.1	18.3	41.7	3.4	69.8
AHP+ARP (180 °C, 0.5 h each)	62.8	15.7	37.9	5.7	95.0
ARP+AHP (180 °C, 0.5 h each)	67	14.6	40.6	8.0	84.3
AHB (pure water, 180 °C, 1 h)	71.1	20.4	40.7	2.2	71.7

<sup>a</sup>All sugars and lignin content in the table based on oven-dry untreated biomass.

ARP: ammonia recycled percolation. AHP: autohydrolysis run in a percolation mode.

cally hydrolyzed. Table 4 summarizes the composition and digestibility data after these pretreatments. The results indicate that the autohydrolysis (water) treatment increases hemicellulose extraction from the biomass. The major role of ARP, on the other hand, appears to be delignification and subsequent increase of digestibility. When the biomass was pretreated with pure water at 180 °C for 1 hr, about 85 % of the hemicellulose was extracted. Without the ARP treatment, however, the autohydrolysis sample had an enzymatic digestibility of only about 70 %. The enzymatic digestibility of the biomass sample treated by a single ARP process was 95 %; however, only 48 % of the hemicellulose was removed during the ARP process. When the biomass was pretreated with pure water at 180 °C for 30 min followed by 10 wt% ammonia solution for 30 min, about 62 % of the hemicellulose was extracted, and the enzymatic digestibility was as high as 95 %. From these results, it was thought that autohydrolysis followed by the ARP treatment would eventually prove to be an ideal pretreatment method, one that ensures the selective separation of hemicellulose from the biomass as well as sufficient delignification to give acceptable enzymatic digestibility.

### CONCLUSION

A combination pretreatment of the autohydrolysis and the ARP was proven to be an ideal pretreatment method, ensuring the selective separation of hemicellulose from the biomass as well as sufficient delignification to give acceptable enzymatic digestibility. Hemicellulose was completely separated from the biomass after 1 hr of autohydrolysis at 200 °C. As reaction temperature and/or time of autohydrolysis was increased in the range of 170-200 °C and 1-2.5 hr, respectively, the amount of hemicellulose solubilization was increased; however, more sugars were decomposed. Most of the extracted hemicellulose was recovered as xylose oligomer. For the autohydrolysis process, a batch reactor operation mode was observed to be more effective than a percolation mode. When the biomass was consecutively pretreated with pure water at 180 °C for 30 min and with 10 wt% ammonia solution at 180

°C for 30 min, about 62 % of the hemicellulose was extracted. The enzymatic digestibility of the pretreated biomass was as high as 95 %. It was also found that hemicellulose inhibited cellulose hydrolysis. Fractionation of hemicellulose from biomass is, therefore, a prerequisite for the improvement of the enzymatic digestibility of the biomass and for the hemicellulose recovery.

### ACKNOWLEDGEMENT

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