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Two Stage Acid Hydrolysis of Corn Stalks for Production of Reducing Sugars

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Abstract: *Acid hydrolysis of corn stalks offers enormous opportunities to convert abundant agricultural residues to valuable chemicals. This paper investigates the effect of using dilute acids to produce reducing sugars. 5% w/v corn stalk was hydrolyzed with Acetic acid, HCl and H_2S0_4 at different concentrations of 1% v/v to 2.5% v/v. Results show that maximum percentage reducing sugar of 36.11% was obtained for 1.5% v/v sulfuric acid while maximum reducing sugar of 32.23% and 23.32% were obtained for 1.5% v/v HCl and 2% v/v acetic acid respectively. A second stage acid hydrolysis that involves the re-suspension of residues after an initial hydrolysis with another acid was also studied by combining different acids. A Sulfuric acid-sulfuric acid double step hydrolysis gave a maximum reducing sugar of 49% while other combinations of acids led to increased sugar yields of 40% and above.*

Keywords: *Acid Hydrolysis, Corn stalks, Reducing sugars*

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I. I Introduction

Lignocellulose materials like agricultural residues provide abundant natural resources that can be harnessed to produce biomaterials that are used as raw materials for the production of biofuels and other essential products.[1,2]Corn stalks and stover are agricultural residues with a huge potential for use as value added bio-resources. However, corn stalks like other lignocellulose materials have a durable plant cell wall that contains cellulose, hemicellulose and lignin which contribute to a recalcitrance structure. Cellulose and hemicellulose are polysaccharides and can be extracted to produce simple sugars by undergoing pretreatment and hydrolysis. Pretreatment is required to improve digestibility and conversion efficiency[3-5] Common pretreatment methods include acid treatment, alkali treatment, steam explosion and oxidative treatments[6] (Oliveira, 2017). The use of acids and alkaline hydrolysis represents one of the most common treatment methods utilized although one of the drawbacks of this is that it requires additional treatment like neutralization before either enzyme hydrolysis or fermentation can take place[7-8]. Dilute acid hydrolysis has been studied extensively and it is affected by residence time, particle size, acid concentration amongst others[9-12].

This study will investigate the effect of varying concentrations of dilute HCl, H_2SO_4 and CH_3COOH acid on the production of reducing sugar from corn stalks. It will study the effect of residence time on the hydrolysis yield. This paper will also investigate a two stage acid hydrolysis by combining different acids after an initial single stage hydrolysis to observe whether acid hydrolysis can be used as a stand-alone treatment.

II. Materials and Methods

Corn stalks were collected from a local farm in University of Agriculture Makurdi, Nigeria. There were washed to remove impurities. The stalks were then air dried in the sun and ground with a table blender into small particles in the range of 0.36 mm to 1.50 mm. The corn stalks were then stored in a tight container at room temperature until use.

The dilute acid hydrolysis was carried out on 5% w/v straw (5g in 100 ml). Different concentrations of dilute acids were prepared by dissolving the acids with distilled water. 1M HCl, H_2SO_4 and HNO_3 were used) with serial dilutions of 1% v/v, 1.5 % v/v, 2% v/v, and 2.5% v/v for the reactions. The first stage acid hydrolysis assays were performed in an autoclave at 120°C in 250 mL flasks with H_2SO_4, HCl, or CH_3COOH at different residence times from 30 minutes to 150 minutes. The best conditions that produced the maximum reducing sugar yields for each acid were used for the second stage hydrolysis. The hydrolysate from the first stage hydrolysis was drained and the residue was then re-suspended for the second stage hydrolysis. Samples taken at different times were then neutralized with $Ca(OH)_2$ by adjusting the pH to between 6 and 7. Samples were then analyzed for reducing sugars. The reducing sugar produced from the acid treatment was measured by DNS method. This involves mixing 3mL of diluted hydrolysate collected at different times and mixing with 3ml

of DNS reagent. This mixture is then heated in a test tube for 5 minutes and allowed to cool. The absorbance was then read by spectrum UV spectrophotometer at a wavelength 540 nm[13] (Milner 1959). The concentration of reducing sugars was estimated from the calibration curve produced for glucose of concentration of between 0.1 g/l and 0.5g/l. Experiments were done in triplicates.
The percentage reducing sugar was calculated as

$$\% \text{ Reducing sugar} = \frac{\text{Concentration of sugar in solution (g/l)}}{\text{Concentration of rice straw (g/l)}} \times 100$$

III. Results and Discussion

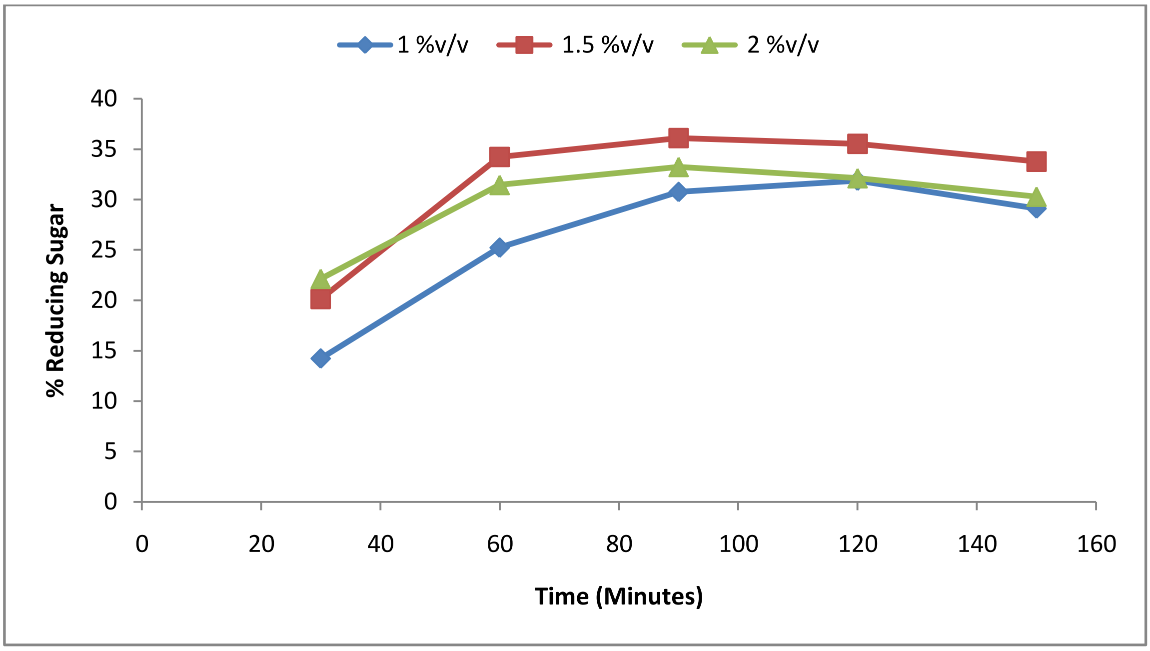

Figure 1: First Stage Acid hydrolysis of 5%w/v corn stalk at different sulfuric acid concentrations.

Acid hydrolysis of corn stalks using different concentrations of sulfuric acids are shown in Figure. It can be observed that within the first 30 minutes of reaction, 2% v/v has the highest percentage reducing sugar while 1% v/v H_2SO_4 had the lowest percentage reducing sugar. This shows that at higher acid concentration, initial rate of hydrolysis is higher however as the reaction progresses to 60 minutes, 1.5% v/v gave the higher hydrolysis yield when compared to 1% v/v or 2% v/v. The maximum percentage hydrolysis yield of 36.11% is achieved for 1.5% v/v H_2SO_4 in 90 minutes. This was closely followed by reactions carried out at 2% v/v with 33.23% in 90 minutes as well. However, reactions at 1% v/v gave a maximum reducing sugar of 31.12% at 2 hours of reaction. There is a reduction in sugar produced after 2 hours for 1.5% v/v and 2% v/v and a reduction after 2.5 hours for 1% v/v sulfuric acid. Results in literature show that most reactions carried out at 1% v/v sulfuric acid reported higher sugar yields. [14]Satarn et al. 2014 reported that reactions carried out at 1% v/v and 2% v/v gave percentage reducing sugar yields of 50% and 46% respectively in 60 minutes. [15]El Tayeb et al. 2012 reported similar results for hydrolysis carried out with 1% v/v sulfuric acid. They observed that percentage reducing sugar was 50.20% and 54.00% at 60 minutes and 90 minutes respectively. These results from literature are significantly higher than those reported in this study. In both studies, only 1g of corn stalks is hydrolyzed in 20 ml of acid however 5g of acid was used for this study. It is possible that the accumulation of higher concentration of sugars might have resulted in lower sugar yields. Degradation of sugars might also contribute to the lower sugar yields observed.

Similar trends are observed for the acid hydrolysis of HCl acid as shown in Figure 2. Although results show that compared with hydrolysis by sulfuric acid, HCl produces lower reducing sugars. The maximum reducing sugar yield of 32.23% was obtained in 90 minutes for reactions at 1.5% v/v HCl. This was followed by the acid hydrolysis carried out at 2 %v/v and 1% v/v HCl. Reports also show that at 30 minutes to 60 minutes, 2% v/v has the highest amount of sugars produced however at 90 minutes 1.5% v/v HCl had produced the highest reducing sugar yield. Reactions at 1% v/v HCl showed a gradual increase in sugar yields until a maximum is reached at 120 minutes of reaction. It is also observed that after a maximum reducing sugar is reached. There is a gradual decrease in sugars. This is probably due to the degradation of the monosaccharaides. El-Tayeb et al. 2012 reported a maximum reducing sugar of 44%, 48% and 42% at 60, 90 and 120 minutes respectively when 1% v/v HCl was used to treat 1g/20ml corn stalks. Again, this report shows a significantly higher sugar yields when compared to results obtained in this study. However differences in the reducing sugars obtained can be attributed to various factors (particle size, liquid to solid ratio, type and concentration of acid used, temperature, and reaction time.[16,17] This is besides the fact that biomass in these experiments are five times the size of that reported in literature.

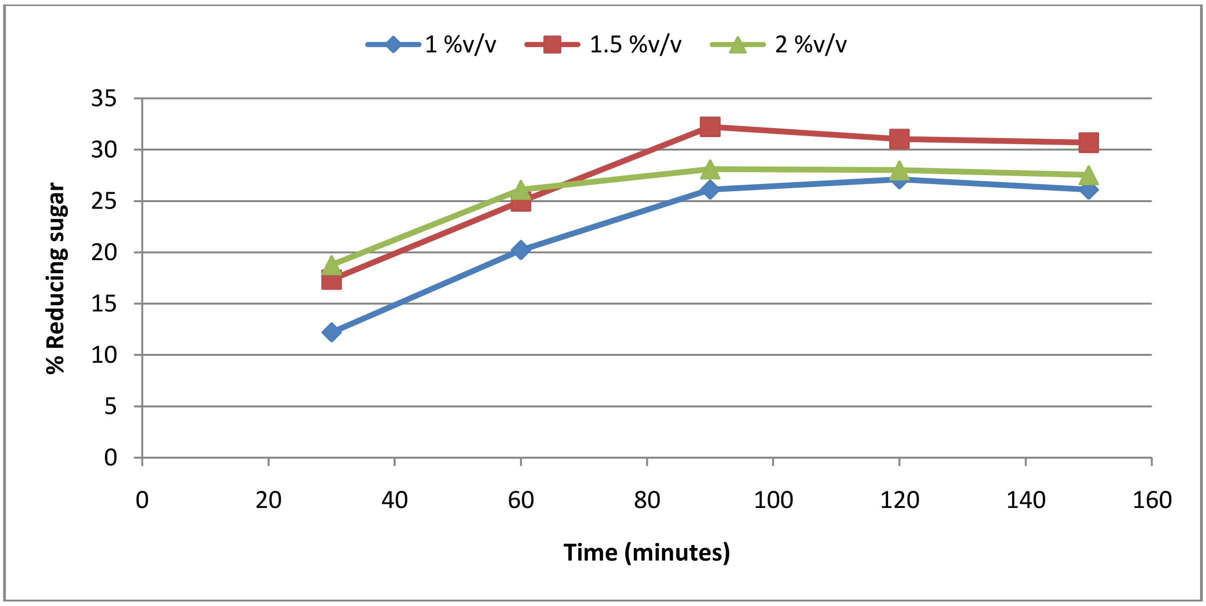

Figure 2: First Stage Acid hydrolysis of 5%w/v corn stalk at different Hydrochloric acid concentrations

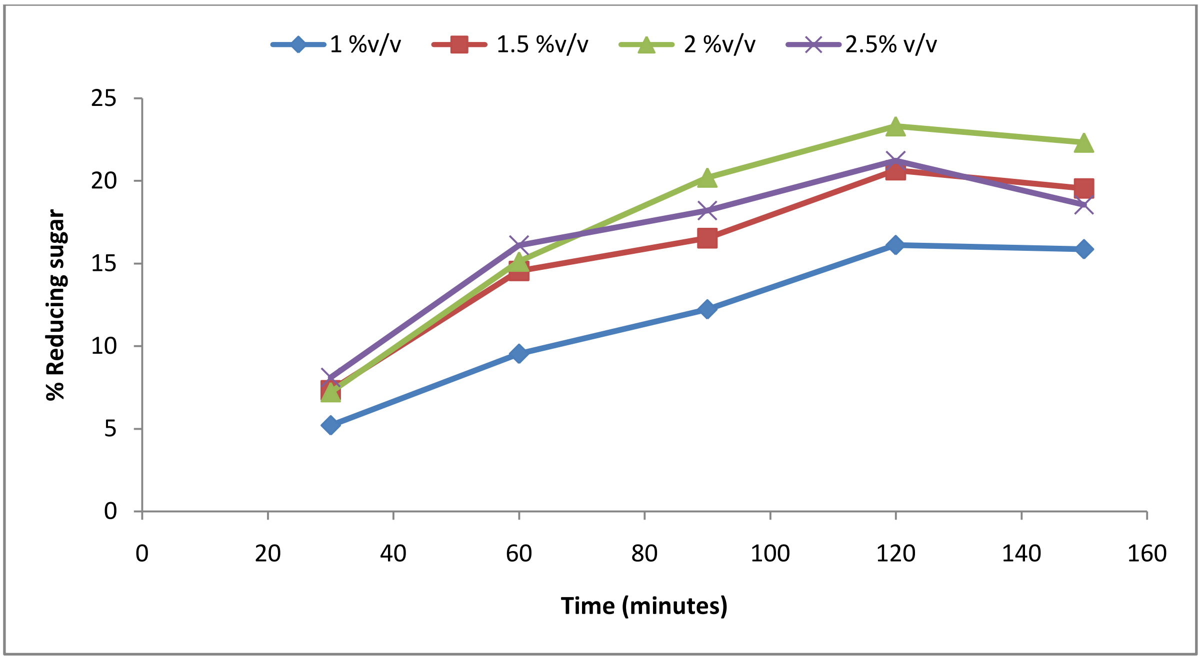

Figure 3: First Stage Acid hydrolysis of 5%w/v corn stalk at different Acetic acid concentrations

Figure 3 shows the results of acetic acid hydrolysis carried out for 150 minutes. Unlike hydrolysis by HCl and H_2SO_4, the percentage reducing sugar yield is much lower. The maximum reducing sugar is also reached in 120 minutes after which the degradation of the monosaccharide begins. The highest reducing sugar of 23.32% is produced for the acetic acid hydrolysis using 2% v/v CH_3COOH. This is followed by reactions carried out at 2.5%, 1.5% and 1% v/v CH_3COOH concentrations respectively. A comparison of the maximum sugars produced from each of the acids is shown in Figure 4.

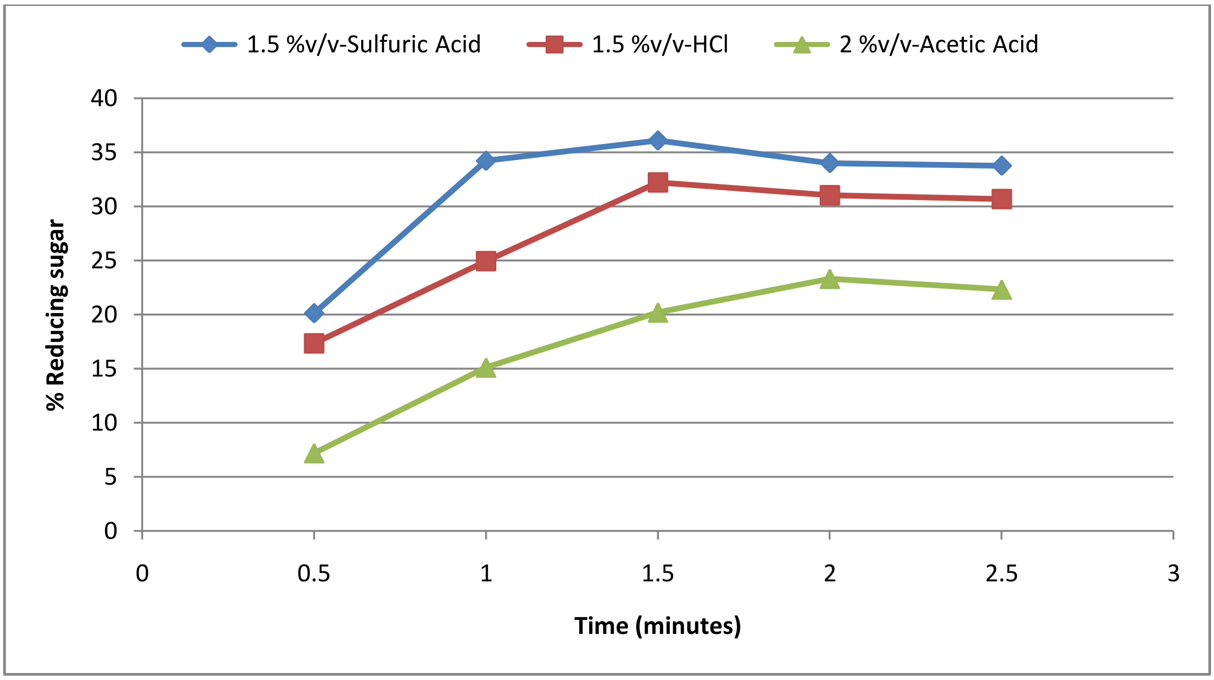

Figure 4: Comparison of the maximum sugar yields produced from Acetic acid, HCl and sulfuric acid

It is shown from figure 4 that 1.5% v/v sulfuric acid produced the highest sugar yield while 2% v/v acetic acid produced the lowest sugar yield. The lower results observed is because acetic acid is an organic acid and a much weaker acid compared to sulfuric acid or hydrochloric acid. To maximize sugar yields, further hydrolysis was investigated after washing the hydrolysate and re-suspending the residues as shown in Figure 5. In this study, the acid concentrations that produced the maximum reducing sugars were utilized for the second stage hydrolysis. Different combinations of 1.5% v/v sulfuric acid, 1.5 % v/v HCl and 2% v/v Acetic Acid were studied to see how much additional sugars can be produced from the residues.

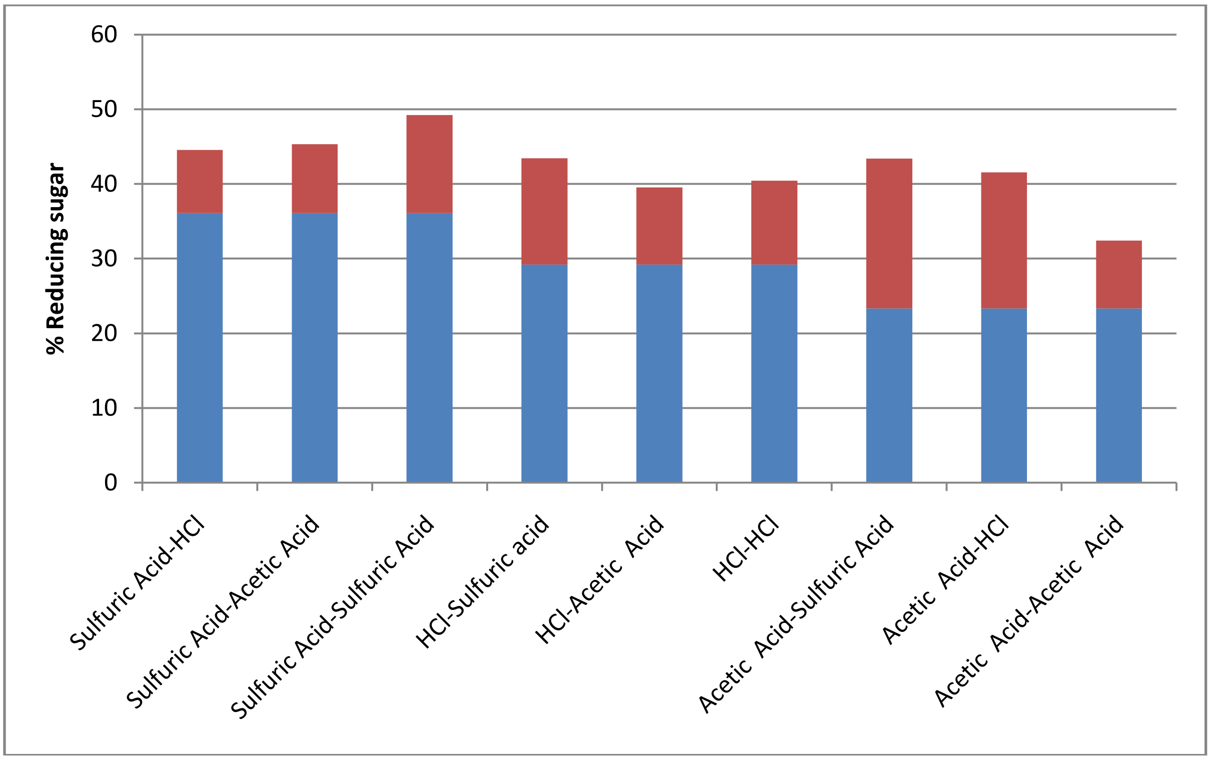

Figure 5: Hydrolysis yield of second stage hydrolysis using a combination of acids.

These results show that an initial hydrolysis of sulfuric acid combined with 1 hr of a second stage consecutive hydrolysis by sulfuric acid produced the highest amount of 49.22% reducing sugar. Sulfuric acid-Acetic acid produced lower yields of 44.56%. Reactions that involve an initial hydrolysis with 1.5% v/v HCl followed by a subsequent hydrolysis by sulfuric acid, acetic acid or hydrochloric acid also showed high reducing sugar yields. HCl-Sulfuric Acid hydrolysis produced a yield of 43.42% while HCl-Acetic acid and HCl-HCl produced 39.55% and 40.44% respectively. It can be observed that after the first stage acid hydrolysis that the difference between HCl-HCl and HCl-Acetic Acid yields is not significantly different. This might be due to the reduced effect of the decomposition of simple sugars when the corn stalks are washed and re-suspended. The reverse hydrolysis that involves the hydrolysis of corn stalks with 2% v/v acetic acid followed by a subsequent hydrolysis with 1.5% v/v HCl or H_2SO_4 also gave high reducing sugar yield. Hydrolysis of acetic acid-sulfuric acid gave 43.42% reducing sugar while acetic acid-HCl gave 41.55%. It can be observed that a two-step acid hydrolysis improves sugar yields significantly. While combining acetic acid hydrolysis with a second hydrolysis with acetic acid, the lowest sugar yields of 32.43% was observed however all other two step hydrolysis gave approximately 40% reducing sugars or higher yields. These results agree with those reported in literature for hydrolysis carried out between 1% v/v and 4% v/v HCl and H_2SO_4 acids (Satarn et al. 2014, El Tayeb et al. 2012, Amenaghawon et al. 2013). This high yield of sugars can be attributed to the effect of the first step hydrolysis that acts as a pretreatment on the corn straw. This first stage removes most of the hemicellulose from the corn straw and causes significant changes in the structure of the lignocellulose. This can contribute to more easily digestible corn stalk in the second stage hydrolysis. Acid hydrolysis is known to cause the delignification of the biomass to make the cellulosic component amenable to saccharification.[18]

IV. Conclusion

A two step dilute acid hydrolysis provides an efficient strategy for converting of corn stalks into simple sugars. This paper has shown that a combination of mineral acids like sulfuric acid and hydrochloric acid can be used to effectively produce simple sugars within 3 hours. The removal of the sugars produced in the first stage and a suspension of the biomass for continual hydrolysis improves the sugar yields significantly. Results also show that sulfuric acid produced the highest reducing sugar after 90 minutes while acetic acid produced the lowest sugars in 2 hours.2009;7(3):221–230

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