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The green hydrolysis technology of hemicellulose in corncob by the repeated use of hydrolysate☆

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ABSTRACT

To achieve green hydrolysis technology of hemicellulose through repeated using hydrolysate, the hydrolysis of hemicellulose in corncob was studied. The influence of repeated use of corncob hydrolysate on concentrations of D-xylose and L-arabinose was investigated. The loss rates of D-xylose in the prepared D-xylose solutions both with and without corncob, and repeated using corncob hydrolysate under identical acidity condition were discussed. The result shows that D-xylose concentration and L-arabinose concentration are all gradually increasing with the growing time of repeated use of corncob hydrolysate. After the fifth repetition, the concentrations of D-xylose and L-arabinose are $196.7 \text{ g} \cdot \text{L}^{-1}$ and $22.0 \text{ g} \cdot \text{L}^{-1}$, respectively. Substance inhibiting the degradation of D-xylose is generated during repeated use of corncob hydrolysate, and the production is further proved by the change of D-xylose concentration and the loss rate of D-xylose over heating time.

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1. Introduction

Biomass resources are the most abundant renewable resources on earth and the annual output of corncob has reached about 50 million tons in China [1]. More importantly, the main raw material for D-xylose production is hemicellulose. Hemicellulose takes up a large proportion in corncob as the structure of which is mainly composed of macromolecule xylan [2,3]. Aside from producing furfural [4,5], xylitol [6,7], fumaric acid [8] and other products as an important raw material, D-xylose is also widely used in baked food, top grade soy sauce and zero-calorie sweeteners [9].

Acid hydrolysis and enzymatic hydrolysis are main methods for D-xylose production in hemicellulose degradation. The process of using enzymatic hydrolysis to produce D-xylose is characterized by mild reaction and long-cycle [10,11]. For instance, Hang *et al.* [10] took corncob as the raw material, the corncob was pretreated by $1.25 \text{ mol} \cdot \text{L}^{-1}$ sodium hydroxide firstly, and then it was enzymolyzed by the enzyme of Rapidase Pomaliq at 50°C for 48 h under conditions of the solid/liquid ratio (by mass) of 1:21.2 and the pH of 5.0, respectively. The concentration of D-xylose in the hydrolysate turned to $10.4 \text{ g} \cdot \text{L}^{-1}$, while the ratio of D-xylose to water (by mass) was approx. 1:96. The utilization of acid hydrolysis to produce D-xylose has fast and short-cycle characteristics [12,13]. Sulfuric acid [14–16], hydrochloric acid [17], formic acid [18],

phosphoric acid [19] and sulfurous acid [20] can be used as the acid medium in the course of acid hydrolysis of hemicellulose. In general, the concentration of acid is usually 3%–6%, the solid/liquid ratio (by mass) is 1:7–1:12; the concentration of D-xylose in hydrolysate is $17 \text{ g} \cdot \text{L}^{-1}$ – $32 \text{ g} \cdot \text{L}^{-1}$, the ratio of D-xylose to water (by mass) is 1:31–1:58, the ratio of D-xylose to acid (by mass) is 1:5–1:18 when the acid solutions are used to hydrolyze hemicellulose. For example, Rafiqul *et al.* [16] used sulfuric acid solution to hydrolyze wood chips to produce D-xylose. The wood chips were hydrolyzed for 1 h at 125°C under conditions of the concentration of 4% and the solid/liquid ratio (by mass) of 1:8, respectively. After the hydrolysis, the D-xylose concentration in the hydrolysate was $17.9 \text{ g} \cdot \text{L}^{-1}$, the ratio of D-xylose to water (by mass) was 1:55.9 and the ratio of D-xylose to sulfuric acid (by mass) was 1:13.5, respectively. According to the ratios of D-xylose to water and D-xylose to acid, it is concluded that water and acid are in great demand in producing D-xylose. In the industrial production of D-xylose, the burden of the treatment of acid solution will be comparatively high if the acid is in high demand. The D-xylose is produced through the crystallization of the D-xylose mother liquor in which the sugar content has condensed to about 80 wt%. Therefore, the more the water contains, the higher consumption it produces during the concentration process. Consequently, it is of great significance to develop a new technology in hydrolyzing hemicellulose with less acid consumption and high concentration of D-xylose.

In this paper, a new technology in producing hydrolysate with high concentration of D-xylose by the repeated use of corncob hydrolysate was studied at atmospheric pressure by taking corncob as the raw material and sulfuric acid as the catalyzer. Our research has evident effects on reducing the amount of acid and water used in D-xylose production.

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2. Materials and Methods

2.1. Materials

Corncob was provided by a farm at Zhang Jialou town, Shandong, China. This material was ground and passed through a 120 mesh sieve to separate particles. Undersize product was dried to constant weight and used in this study. Detailed information of chemical reagents are shown in Table 1.

Table 1

Detailed information of chemical reagents used in this study

Reagent	Sources	Mass fraction Purity/%
Methylglyoxal	Macklin	40
Furfural	Aladdin	99
Glycolaldehyde dimer	Sigma	98
Humic acid	Aladdin	–

2.2. Analytical methods

The content of D-xylose in solution was analyzed by HPLC (L-2000, Hitachi Limited, Japan) with a refractive index detector and a Aminex HPX-87H column (300 mm × 7.8 mm, Bio-Rad, America). The mobile phase was an aqueous solution of sulfuric acid (0.005 mol·L⁻¹), while the flow rate was 0.55 ml·min⁻¹. The column temperature was 333.15 K, and the temperature of the refractive index detector was 308.15 K.

2.3. Experimental procedure

Corncob was pretreated by deionized water for 0.5 h at the condition that the solid/liquid ratio was 1:10 at 100 °C. then adjusted the solution acidity to 0.1 wt% using sulfuric acid, and continue to pretreat for 1 h. The solution was stirred continuously by an electric stirring in the process of pretreatment. Corncob was separated by filtration under diminished pressure after pretreatment and was washed with deionized water, the amount of deionized water was twice that of dry corncob. The corncob was eventually dry to constant mass for hydrolysis.

Combined 30.0 g corncob with 2 wt% sulfuric acid aqueous solution (H⁺: 0.408 mol·L⁻¹) and the solid–liquid ratio was 1:10. The system reacted for 3 h at 103 °C and was stirred simultaneously, the process was known as the first use of corncob hydrolysate. Then the hydrolysate was separated and the volume of it was measured, while the filter residue was washed for 3 times using 50–60 ml deionized water each time and the lotion was collected and measured for the volume of it. The right amount of lotion and sulfuric acid was added to adjust the volume of the hydrolysate and concentration of sulfuric acid equal to that before reaction. Continue to hydrolyze the corncob for 3 h using this hydrolysate with the solid/liquid ratio of 1:10 at 103 °C. The process was known as the second repeated use of corncob hydrolysate. Based on this method, one can repeatedly use the hydrolysate of corncob to hydrolyze corncob using the repeated using. The concentration of D-xylose and L-arabinose in hydrolysate and lotion was analyzed by HPLC after each reaction. To minimize the experimental error, each experimental solution was measured at least three times, and expressed by the mean values.

3. Results and Discussion

3.1. The study of production technology of D-xylose using repeated-using corncob hydrolysate

3.1.1. The effects of times of repeated use of corncob hydrolysate on the concentration of D-xylose and L-arabinose

The 2 wt% sulfuric acid aqueous solution (H⁺: 0.408 mol·L⁻¹) was used to hydrolyze corncob at 103 °C. The concentrations of D-xylose and

L-arabinose in the solution after repeated use of the hydrolysate are listed in Table 2. As shown in Table 2, the concentrations of D-xylose and L-arabinose increase with growing times of using corncob hydrolysate, which indicates the accumulation of D-xylose and L-arabinose in solution.

Table 2

The concentrations of D-xylose and L-arabinose in the solution after repeated use of the hydrolysate

Number of times	Concentration of D-xylose/g·L ⁻¹	Concentration of L-arabinose/g·L ⁻¹
1	28.0	3.1
2	52.7	6.1
3	76.0	8.5
4	91.9	10.3
5	110.2	12.4
6	122.3	14.3
7	134.2	15.3
8	150.5	16.9
9	160.2	17.1
10	170.3	18.9
11	177.1	19.7
12	184.5	20.4
13	187.0	20.9
14	190.7	21.2
15	196.7	22.0

3.1.2. The effects of times of repeated use of corncob hydrolysate on the D-xylose yield

Table 2 shows that the D-xylose content in hydrolysate is the highest, which accounts for almost 90% of the total pentose. Hence, we focus on discussing the production technology of D-xylose using repeated use of corncob hydrolysate.

The D-xylose is 8.4 g in the solution after the first time use of corncob hydrolysate. This study takes the mass of D-xylose as its theoretical increase with the repeated use of hydrolysate each time, while the method for calculating the D-xylose yield after repeatedly using hydrolysis is shown in Eq. (1):

$$\eta = \frac{\Delta m_N}{m_1} \times 100\% \quad (1)$$

where η is the yield of D-xylose, %. Δm_N is the increase of D-xylose when the hydrolysate was repeated used for the N th time, g. N is the number of times of repeated use of hydrolysate. m_1 is theoretical increase in the repeatedly-using hydrolysate, $m_1 = 8.4$ g.

The variation of D-xylose yield and the initial concentrations of the D-xylose in solution when hydrolysate was repeatedly used over the times of repeated use of hydrolysate are shown in Fig. 1. It can be seen

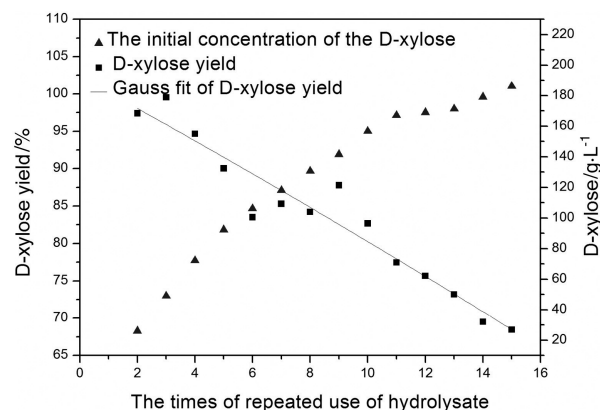


Fig. 1. The variation of D-xylose yield and the initial concentrations of the D-xylose in solution when hydrolysate was repeatedly used over the times of repeated use of hydrolysate.

from Fig. 1 that the yield of D-xylose decreases with the increase of initial concentrations of the D-xylose in solution when hydrolysate was repeatedly used. When the hydrolysate is repeatedly used for two or three times, the increases of D-xylose are 8.2 g and 8.4 g, respectively, while the yield of D-xylose is 97.4% and 99.5%, respectively. When it comes to the fifteenth time, the concentration of D-xylose in hydrolysate is $196.7 \text{ g} \cdot \text{L}^{-1}$, the D-xylose increases by 5.8 g and the yield of D-xylose is 68.4%.

3.1.3. The effects of times of repeated use of corncob hydrolysate on byproduct acetic acid

Acetic acid is the byproduct in the process of producing D-xylose by the acidolysis of corncob. The variation of concentrations of acetic acid in solution over the times of repeated use of hydrolysate is shown in Fig. 2. It can be seen that the concentration of acetic acid increases with growing times of repeated use of corncob hydrolysis. The variation of increase of acetic acid and the initial concentrations of D-xylose and acetic acid in solution when hydrolysate was repeatedly used over the times of repeated use of hydrolysate are shown in Figs. 3 and 4, respectively. From Figs. 3 and 4, the increase of acetic acid fluctuate is between 0.8 g and 1.5 g, and it has no relations with the initial concentrations of D-xylose and acetic acid. It demonstrates that the main source of acetic acid in the solution is not derived from the degradation of D-xylose. There are branched chains of xylosyl and acetyl in graminaceous hemicellulose, while the main source of acetic acid in hydrolysate is derived from the hydrolysis of graminaceous hemicellulose.

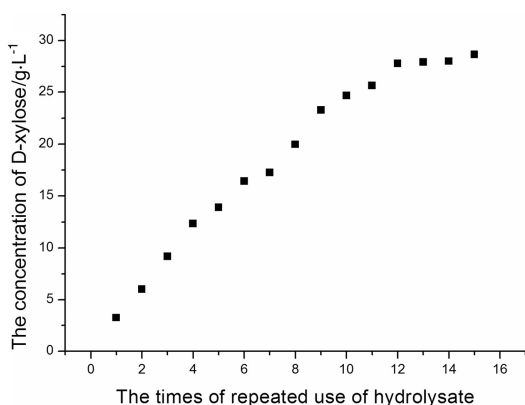


Fig. 2. The variation of concentrations of acetic acid in solution versus the times of repeated use of hydrolysate.

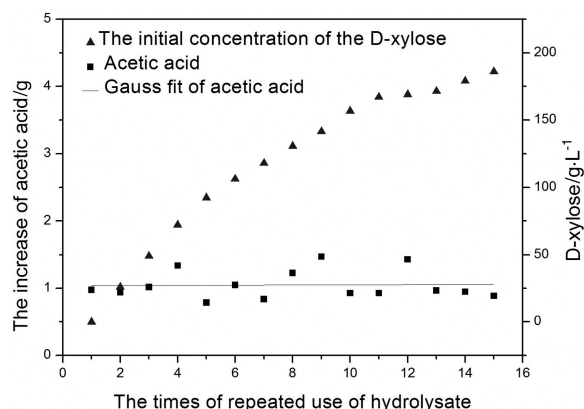


Fig. 3. The variation of increase of acetic acid and the initial concentrations of D-xylose in solution when hydrolysate is repeatedly used versus the times of repeated use of hydrolysate.

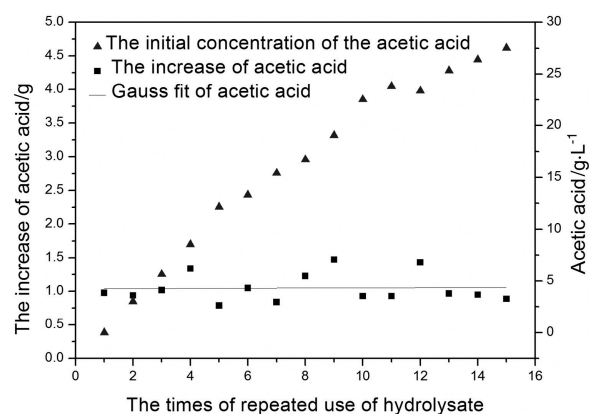


Fig. 4. The variation of increase of acetic acid and the initial concentrations of the acetic acid in solution when hydrolysate is repeatedly used versus the times of repeated use of hydrolysate.

3.2. The comparison of acidolysis of D-xylose solutions both with and without corncob

According to the study on repeatedly-using corncob hydrolysate, when the hydrolysate is repeatedly used, the yield of D-xylose decreases with the increase of the initial D-xylose concentration. We study the variation of D-xylose loss rates in acid solution over different initial concentrations. D-xylose solutions with different concentrations have two sources, one is the repeatedly-using corncob hydrolysate, the other is the prepared solution of pure D-xylose. The acidity of the prepared solutions is the same as the repeatedly-using corncob hydrolysate (H^+ : $0.408 \text{ mol} \cdot \text{L}^{-1}$, H_2SO_4). The concentrations of D-xylose in prepared solutions without corncob are 100, 150 and $200 \text{ g} \cdot \text{L}^{-1}$, respectively. The concentrations of D-xylose in prepared solutions with corncob (the solid to liquid ratio is 1:10, the same as the repeated use of corncob hydrolysate) are 96.0, 142.0 and $193.0 \text{ g} \cdot \text{L}^{-1}$, respectively. D-xylose solutions with different initial concentrations both with and without corncob were heated at 103°C for 3 h, then the D-xylose loss rates were investigated. The method of calculating D-xylose loss rate is shown in Eq. (2):

$$\lambda = \frac{(m' + m_1) - m}{m' + m_1} \times 100\% \quad (2)$$

where λ is the loss rate of D-xylose, %; m' is the initial mass of D-xylose at the N th time of repeatedly using of the hydrolysate; m_1 is the theoretical increase in the repeatedly-using hydrolysate, $m_1 = 8.4 \text{ g}$, if the corncob is not added, then $m_1 = 0$; m is the mass of D-xylose in the solution after repeatedly using of the hydrolysate, g.

The loss rates of D-xylose in solutions with different initial concentrations with and without corncob at 103°C for 3 h are shown in Fig. 5. It can be seen from Fig. 5 that the D-xylose loss rate in repeatedly-using hydrolysate with corncob is the lowest; the D-xylose loss rate in D-xylose preparation solution with corncob, is higher after being heated; while the D-xylose loss rate in D-xylose preparation solution without corncob is the highest.

According to Fig. 5, the D-xylose loss rate in D-xylose preparation solution with corncob is lower than that without corncob. It demonstrates that, by adding corncob to solution, the loss rate of D-xylose can be significantly reduced. This can be explained that the crude fiber, which owns negative electricity, is apt to combine with H^+ in solution to reduce the concentration of H^+ . To verify the effect of corncob on the concentration of H^+ , 30 g of corncob was added into the solution with the concentration of H^+ of $0.408 \text{ mol} \cdot \text{L}^{-1}$ and the concentration of D-xylose of $100.0 \text{ g} \cdot \text{L}^{-1}$. When the solution was heated up to 103°C , the concentration of H^+ decreases to $0.371 \text{ mol} \cdot \text{L}^{-1}$. Therefore, corncob can reduce the concentration of H^+ in solution. The effect of the

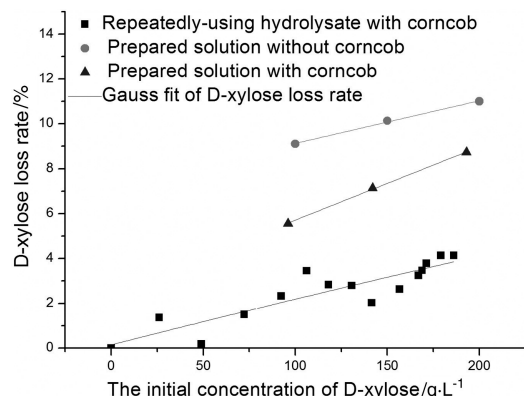


Fig. 5. The loss rates of D-xylose in solutions with and without corncob in it at 103 °C for 3 h: prepared solution without corncob; prepared solution with corncob; repeatedly-using hydrolysate with corncob.

concentration H^+ on the D-xylose loss rate was studied. The loss rates of D-xylose in solutions with different mass fractions of sulfuric acid at 103 °C for 3 h are shown in Fig. 6. It can be seen from Fig. 6 that, under the experimental heating of D-xylose solutions with different concentrations of H^+ at 103 °C for 3 h, the higher the concentration of H^+ , the higher the loss rates of D-xylose it reached. To conclude, the concentration of H^+ decreases by adding the corncob into the D-xylose solution, which brings about a decrease of the loss rate of D-xylose. Hence the loss rate of D-xylose in D-xylose solutions with corncob is lower than that without corncob.

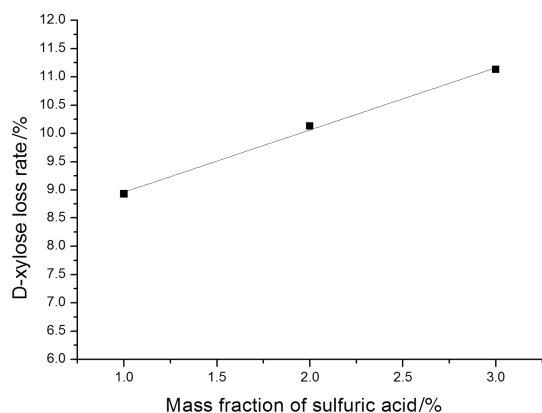


Fig. 6. The loss rates of D-xylose in solutions with different mass fraction of sulfuric acid at 103 °C for 3 h.

Fig. 5 shows that the D-xylose loss rate in repeated-using corncob hydrolysate is lower than that of D-xylose preparation solution with corncob. Therefore, it can be deduced that the appearance of a certain substance inhibits the degradation of D-xylose in the repeated-using process of corncob hydrolysate. To verify this, the relations between D-xylose loss rate and reaction time under acidic conditions were studied. The solution with D-xylose ($150 \text{ g} \cdot \text{L}^{-1}$) and sulfuric acid (2 wt%) was heated at 103 °C, the variation of both the concentration and the loss rate of D-xylose over the reaction time is shown in Fig. 7. It can be seen from Fig. 7 that the concentration of the D-xylose gradually decreases as the solution starts to be heated. However, as the reaction time goes on, the D-xylose loss rate tends to be stable. This reflects that a certain kind of substance which inhibits the degradation of the D-xylose is generated during the process. Also, it demonstrates that a

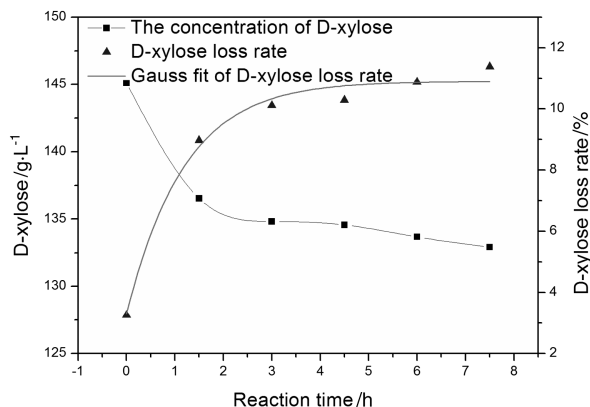


Fig. 7. The solution with D-xylose ($150 \text{ g} \cdot \text{L}^{-1}$) and sulfuric acid (2 wt%) was heated at 103 °C, the variation of concentrations of D-xylose and D-xylose loss rate of the solution versus the reaction time.

certain substance which helps inhibit the degradation of D-xylose arises in the repeated-using process of corncob hydrolysate.

3.3. The analysis of D-xylose degradation

A certain inhibitor of D-xylose degradation has been made in the process of repeated using corncob hydrolysate (see Section 3.2). The D-xylose loss rate tends to be stable with the increase of the heating time under acidic condition. In our analysis, the main reasons for that change can be divided into two aspects: (1) the dehydration process is accompanied in the degradation of D-xylose. The degradation products of D-xylose may increase water activity of the solution, the dehydration of D-xylose is thereby inhibiting. (2) The degradation product of D-xylose may be combined with D-xylose molecules, which makes protonation of hydroxyl and intramolecular isomerization of D-xylose more difficult.

3.4. The additives effects to the D-xylose degradation

Conclusion can be made from Section 3.2 that the degradation of D-xylose will generate a substance which inhibits its degradation under acidic conditions. As the ingredients of D-xylose degradation products are complex, the effects of parts of degradation products on the D-xylose degradation were studied. The products include methylglyoxal, furfural, glycolaldehyde dimer, humic acid, etc. [21,22]. Put different additives into D-xylose solutions with $134.0 \text{ g} \cdot \text{L}^{-1}$ D-xylose and 2 wt% sulfuric acid (H^+ : $0.408 \text{ mol} \cdot \text{L}^{-1}$) respectively, and heated up for 1.5 h at 103 °C. Final concentrations and loss rates of D-xylose are listed in Table 3. The results indicate that there are no changes when methylglyoxal, furfural, glycolaldehyde dimer or humic acid was added into the solution, as compared to no added additive. Thus, it has little influence on D-xylose degradation. Accordingly, the aforementioned substances are not D-xylose degradation inhibitors, i.e., playing no significant role in inhibiting D-xylose degradation.

Table 3

The effect of additive on the concentration and loss rate of D-xylose in solution heated for 1.5 h at 103 °C

Reagent	Additive amount	D-Xylose concentration/ $\text{g} \cdot \text{L}^{-1}$	D-Xylose loss rate/%
–	–	123.5	7.84
Methyl-glyoxal	2 ml	123.3	7.99
Furfural	2 ml	123.4	7.91
Glycolaldehyde dimer	0.50 g	123.9	7.54
Humic acid	1.89 g	124.1	7.39

4. Conclusions

- The new technology of producing D-xylose by repeatedly using corncob hydrolysate was established. The hydrolysate was separated after the hydrolyzed corncob with 2 wt% sulfuric acid aqueous solution. The acidity and volume of the hydrolysate were adjusted to be equal to the initial sulfuric acid aqueous solution, then it was used to hydrolyze corncob. The hydrolysate was repeatedly used for several times by this method. After the first hydrolysis, the concentration of D-xylose was $28.0 \text{ g} \cdot \text{L}^{-1}$, and the concentrations in the third, seventh, twelfth and fifteenth experiment were 76.0, 134.2, 184.5, and $196.7 \text{ g} \cdot \text{L}^{-1}$, respectively. The concentrations of acetic acid, i.e., the by-product of the experiment, were 9.2, 17.3, 27.8, and $28.7 \text{ g} \cdot \text{L}^{-1}$, respectively. From the experiments, the conclusion can be made that repeated use of corncob hydrolysate contributes to an increase of D-xylose content in hydrolysate significantly.
- The loss rates of D-xylose in different solutions with and without corncob at 103°C for 3 h were compared. The results show that the loss rate of D-xylose in repeated-using hydrolysate with corncob is the lowest; while it goes higher after being heated; the loss rate of D-xylose in D-xylose preparation solution without corncob is the highest. Under acidic conditions, the concentration of the D-xylose decreases gradually as the solution starts to be heated; whereas the loss rate of D-xylose tends to be stable as the reaction time goes on. Considering these, the certain inhibitor of D-xylose degradation was generated in the repeated-using process of corncob hydrolysate.
- The effects of methyl-glyoxal, furfural, glycolaldehyde dimer and humic acid on D-xylose degradation under acidic conditions were investigated. The experiments prove that none of them play significant role on D-xylose degradation.

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