Components Analysis of Recycled Alkali Black Liquor Combined with Corn Straw under Ozone Pretreatment

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Abstract

Previous studies showed that the cellulase hydrolysis of corn straw pretreated with circulating alkali black liquor combined with ozone was suppressed. In this paper, the alkali black liquor was sequentially withdrawn for 0–6 times under the optimal pretreatment conditions, and components characterization was analyzed to identify the main factors inhibiting cellulase hydrolysis in recycled alkali black liquor. Through the component analysis, the organic matter and acid precipitation contents increased throughout the cycles. At the fourth cycle, the cellulase hydrolysis rate was decreased significantly, the growth of lignin content in alkali black liquor was slowed down and the total dissolved solid increment was decreased to 8.33mg/mL, 69.52% lower than previous cycle increase. GC-MS results showed that phenols, benzene ring heterocyclic and furans were main degradation products. It indicated that small molecular organics and lignin were inhibitors of cellulase hydrolysis, which accumulated during recycling, reducing alkali utilization and delignification efficiency, resulting in lower enzymatic hydrolysis rate. This study has revealed the components inhibiting the enzymatic hydrolysis of corn straw in recycled alkali black liquor, which is beneficial to the recovery and efficient utilization of recycled alkali black liquor.

1 Introduction

In recent years, with climate changes and the increasing demand for energy, the research on biomass fuels has been focused. Corn straw is a kind of lignocellulosic agricultural waste and clean renewable energy source, which has attracted much attention due to its abundance and availability [1]. As lignocellulosic biomass, corn straw is composed of lignin, cellulose and hemicellulose. The complex three-dimensional polyaromatic matrix of lignin, the crystal structure of cellulose, and the cross-linking between hemicellulose and lignin prevent penetration of solution and enzymes, which makes lignocelluloses degradation difficult [2, 3]. Proper pretreatment can destroy the crystallinity of biomass, reduce the degree of polymerization, increase the accessible surface area of lignocellulose [4], and improve the digestibility of biomass in enzymatic hydrolysis process, which is considered as a critical step [5].

Common biomass pretreatment methods include ultrasounds [6], steam explosion [7], hydrothermal pretreatment [8], alkaline peroxide pretreatment [9], acid pretreatment [10] and so on. Ultrasounds can destory the integrity of the cell wall and cut off the bond between lignin and hemicellulose, but the ultrasonic vibration energy is too low to change the surface conformation of the biomass particles [11]. Steam explosion method has little impact on the environment while a large amount of enzymatic hydrolysis and fermentation inhibitors will formed in the process [12]. Hydrothermal treatment produces less degradation by-products, which avoids equipment corrosion [8]. Alkaline peroxide pretreatment relies on the oxidation of lignin by hydrogen peroxide anion generated in an alkaline environment, and the reaction conditions are relatively mild [9]. Acid pretreatment can effectively dissolve hemicellulose and lignin, but at the same time it will generate degradation by-products and requires corrosion-resistant equipments [13].

Alkali pretreatment is widely used because of its non-toxic, low cost, mild reaction conditions and effective lignin removal ability [14, 15]. During sodium hydroxide (NaOH) pretreatment, hydroxide ions can cause saponification of ester bonds between cross-linking molecules and other molecules such as lignin and hemicellulose [3], attack lignin - hemicellulose bonds in lignocellulose structures effectively, disrupt the ester and carbon-carbon bonds in the lignin molecules, and reduce the porosity of lignocellulose, which result in enhanced the dissolution of hemicellulose and the degradation of lignin [14, 16]. Additionally, alkaline oxygen pretreatment can swell the inner surface of straw, oxidize lignin and hemicellulose, expose the cellulose to the surface, and increase reactivity [17, 18]. Previous

laboratory research found that at relatively low temperature (80℃) alkali combined with ozone treatment can swell the corn straw, break the β-O-4 ether bond between lignin units, condense the β-β and β-5 carbon-carbon bonds, destroy the stable lignin-cellulose-hemicellulose crosslink, degrade the macromolecular lignin and increase the cellulose enzymatic hydrolysis [19].

Alkali has been proved to promote lignin degradation, the formation of phenolics, the decarboxylation and demethoxylation of intermediates, and improve the amount of methoxyl-free phenols by desoxygenation [20]. With hemicelluloses degradation, the soluble toxic chemicals such as furfural and hydroxymethyl furfural are produced [21]. The alkali black liquor that produced by pretreatment of straw contains a large amount of alkali, soluble salt ions and organic chemicals such as lignin, polysaccharides and cross-linked macromolecules composed of many aromatic groups [15, 22]. As a toxic and poisonous waste, it has a significant impact on the environment. Therefore, the recovery and utilization of alkali black liquor is good to resources saving, environment protection and economic benefit improvement. However, studies have shown that the lignin removal and enzymatic hydrolysis efficiency of the recycled alkali black liquor were lower than that of the fresh alkali liquor. Muryanto et al. found that delignification with NaOH solution reached 76.74%, while the use of black liquor only reached 59.30% [3]. Goshadrou treated cogongrass with recycled black liquor for several times, and found that after 24h enzymatic hydrolysis, the hydrolysis yield of fresh NaOH pretreated biomass was 64.90%, which was significantly higher than that of 29.80% in the fourth treatment [15]. Previous laboratory research discovered that the cellulose enzymatic hydrolysis rate of straw treated with recycled alkali black liquor combined with ozone showed a downward trend with the increase of cycle times. When recycled to the fourth time, the cellulase conversion rate decreased significantly from 81.53–76.27%, a decreased of 5.26%, accounting for 32.65% of the overall decline [23].

Regarding the factors contributing to enzymatic hydrolysis yield reduction after pretreatment with circulating alkali black liquor, it might be that high viscosity and high lignin content in black liquor cause lower saccharification yield [24], and it was reported that non-specific adsorption of hydrolytic enzymes to lignin might impede hydrolysis or small phenylpropane units derived from low-molecular-weight lignin as inhibitors rendered enzymes inactive by blocking the active sites [25, 26]. And it was also suggested that the black liquor might contain chemicals inhibiting enzymatic hydrolysis [27]. Thermochemical degradation of lignocellulose can release over 35 inhibitors, which can be classified into weak organic acids, furan derivatives, and phenolic compounds [28]. These three types have been proved to have negative impacts on subsequent enzymatic hydrolysis [29, 30]. However, there are few reports about the components of recycled alkali black liquor that inhibit the hydrolysis rate of lignocellulose at a mild temperature.

In this paper, the alkali black liquor produced in the process of alkali combined with ozone in pretreatment of corn straw was circulated. The material accumulation and composition changes in recycled alkali black liquor were studied by measuring the content of total dissolved solid (TDS), lignin, organic matter (OM) and inorganic matter (IM), acid precipitation and alkali precipitation, combined with GC-MS analysis. Then the relationship between the changes of recycled alkali black liquor components and cellulase hydrolysis rate were analyzed. And the research is aimed to reveal the main components in recycled alkali black liquor that inhibit enzymatic hydrolysis during the circulation process.

2 Materials And Methods

2.1 Materials

Corn straw was collected from a farm in Siping, Northeast China, dried at 50℃ for 48h. Then the raw biomass was crushed, screened through a 60-mesh sieve (particle size: 0.42 mm) and extracted with toluene and ethanol at the ratio of 2: 1 at 95℃ for 3 h. Then washed thoroughly with distilled water and dried to constant weight. The cellulose, hemicellulose and lignin contents of corn straw were measured by two-step acid hydrolysis [31], which were 39.29%, 23.39% and 27.31% respectively.

All chemicals used were of analytical grade.

2.2 Recycled alkali black liquor combined with ozone pretreatment of corn straw

According to the experimental data of Wang et al. [32], corn straw pretreated with 2% (w/w) NaOH at 80 ℃ for 2 h followed by ozone treatment (87mg/mL) for 25 min with an initial pH 9 was found to be the optimal object and the maximum efficiency (91.73%) of cellulose enzymatic hydrolysis was achieved. Therefore, the conditions of recycled alkali black liquor combined with ozone pretreatment referred to the optimal NaOH combined with ozone treatment in previous laboratory research [23, 32].

Alkali black liquor recycle treatment: Two grams of dried corn straw were weighed accurately and reacted with 30 mL of 2% NaOH in a rotating water bath at 80℃ for 2 h and stirred at 150 rpm. After treatment, the mixture was cooled to room temperature and filtered to separate filtrate and solid residue. The residues were washed thoroughly by distilled water to neutrality and dried in an oven at 55℃ for further analysis. The alkali concentration of the filtrate was determined with acid-base titration. The alkali black liquor was supplemented to the initial volume and concentration for new treatment of fresh corn straw.

The treatment of fresh sodium hydroxide treated with the straw was marked as the zeroth cycle, and the first cycle was carried out using the black liquor generated by the zeroth treatment, and so on. The same recycled black liquor pretreatment was repeated for six times [23].

Ozone treatment: Two grams of corn straws treated by NaOH solution or recycled alkali black liquor were weighed and soaked completely for 24 h by adding 30 mL deionized water. The initial pH of the mixture was adjusted to 9. And the mixture was reacted with ozone at a concentration of 78 mg/L for 25 min. After reaction, the treated corn straw was collected and washed to neutrality, then dried in an oven at 55℃ for 24 h.

2.3 Analysis of the content of the TDS in recycled alkali black liquor

After centrifugation at 5000 rpm for 10 minutes, 15 mL of alkali black liquors with different cycles were measured accurately. The alkali black liquors with different cycles were first centrifuged at 5000 rpm for 10 min and then accurately measured 15 mL. The liquors were dried in the oven at 105℃ to constant weight and transferred to a desiccator to cool to room temperature, then weighted to obtain the TDS content. The dried residues were heated to carbonize, then calcinated at 800℃ in the muffle furnace. The masses of the constant solid residues were those of the IM [33]. The contents of OM were the difference between the contents of TDS and IM [33]. All experiments were performed in triplicate and the average was used as the result of the calculation.

2.4 Analysis of the components of alkali and acid precipitations in recycled alkali black liquor

15 mL of centrifuged alkali black liquor of different cycle times were taken accurately and were adjusted pH to 11 by adding NaOH, then centrifuged at 8000 rpm for 10 min [34]. The supernatants were decanted and the residue was dried in the oven to constant weight to obtain the TDS content of alkali precipitation. Then alkali precipitation was carbonized and burned at 800℃ to constant in muffle furnace to determine the IM content the alkali precipitation. The collected supernatant was added HCl until reaching pH 2, then centrifuged at 8000 rpm for 10 min. The obtained residue was acid precipitation, which was treated with the same method as above to get the TDS and IM content [34]. All experiments were performed in triplicate and the average was used as the result of the calculation.

2.5 Determination of lignin content in recycled alkali black liquor

20 mL of alkali black liquors of different cycle times were hydrolyzed with 560 mL of sulfuric acid (3%), which were reacted for 1 h at 121℃. The treated samples were filtered and the filter residue was washed by hot distilled water to neutral, then dried in the oven at 105℃ to constant weight to determine acid insoluble lignin content. The acid-soluble lignin concentration in the filtrate was determined by measuring absorbance at 205 nm using TU-1901 UV spectrophotometer (Leng Guang Technology Co. LTD, Shanghai, China) [35].

$$B = \frac{A \times D}{110}$$

where A requests the absorbance at 205 nm of lignin solution; B requests the concentration of lignin (g/L); D requests filtrate dilution factor; 110 requests absorbance coefficient, L / (g × cm).

2.6 Enzymatic hydrolysis

The cellulase, β-glucosidase and xylanase used in the experiments were purchased from Sigma-Aldrich (St. Louis, MO, USA). The alkali black liquors with different cycle times were complemented to the initial volume and concentration respectively, and combined with ozone to treat fresh corn straw. Then 0.2 g of the treated samples mixed with 60 mL of acetate-sodium acetate buffer (0.1 mol/L, pH 4.8), 30 μL cycloheximide, 40 μL tetracycline hydrochloric acid and 40 μL xylanase (45.8 U/mL) were added into a 100 mL erlenmeyer flask. Then the mixture was incubated in a rotating water bath at 70℃ for 24 h and stirred at 120 rpm. After reaction, the mixture was cooled to room temperature. Then 40 μL cellulase (77.8 FPU/mL) and 30 μL β-glucosidase (690.4 CBU/mL) were added into the mixture and the experiment was carried out at 50℃ in a reciprocating shaker bath at 120 rpm for 72h [32,36,37]. The enzymatic hydrolysate was filtered through 0.22 μm membrane and then analyzed by HPLC, Agilent 1200 (Agilent, Palo Alto, USA) to determine the glucose content to calculate the cellulase hydrolysis degree. All assays were performed in triplicate. The conversion of cellulose to glucose was calculated as follows:

$$\text{Conversion rate of cellulase hydrolysis (\%)} = \frac{C \times V \times 0.90}{m \times W} \times 100$$

where C was glucose concentration (mg/mL); V was the total volume (mL); 0.90 were glucose conversion coefficient; m was quality of corn straw (mg); W was percentage of cellulose content in straw.

2.7 GC-MS Analysis

GC-MS analysis method refers to related research and slightly modified [38]. 300 mL of black liquors with different cycle times were collected, filtrated, and adjusted pH to 2, and mixed with 30 mL absolute ether, then oscillated for 5 minutes under 200 rpm. After stratification, organic phase was taken out. The pH of the aqueous phase was adjusted to 7 and 12 respectively which were treated with the same method as above. The organic phases obtained three times were combined and added with excessive anhydrous sodium sulfate to dehydrate, then filtered by 0.22 μm organic membrane. The filtered organic phase was concentrated to 5 mL under the vacuum of 0.1, 40℃, and nitrogen was blown to 1 mL which was sealed for GC-MS analysis.

The details of GC-MS operational process were as follows: GC-MS was performed on Agilent890A/7000B (Agilent, Palo Alto, CA, USA). The column was a 30 m × 0.25 mm DB-5capillary column. Helium was used as the carrier gas with a constant flow rate of 1 mL/min and a split ratio of 10:1, the temperature of the GC/MS interface was held at 280℃. The column temperature was initially maintained at 40℃ for 2 min, and then increased to 180℃ for 4 min at a heating rate of 10℃/min, 210℃ for 4 min at a heating rate of 5℃/min, 280℃ for 5 min at a heating rate of 10℃/min. The mass spectrometer was operated in electron ionization mode at the ionization energy of 70 eV and the mass spectra were obtained from m/z 33 to 500.

The organic compounds of the recycled alkali black liquor were identified by GC-MS online automatic retrieval, and the relative contents were determined by peak area normalization method.

3 Results And Discussion

3.1 Changes of TDS content in recycled alkali black liquor

Alkali treatment can remove the linkage bonds between lignin, cellulose and hemicellulose, destroy the internal structure of lignin, reduce the crystallinity of cellulose and increase the internal lignocellulose porosity [3]. Changes of TDS content in alkali black liquor circulation at different cycles and the enzymatic hydrolysis rate of pretreated corn straw by recycled alkali black liquor combined with ozone are shown in Fig. 1. It could be seen that the TDS contents increased with circulation times of alkali black liquor. The content of TDS at the sixth cycle was 186.80 mg/mL, which was 2.63 times more than that of the primary cycle. From the overall trend of the TDS content change, it can be observed that the solid content increased sharply from the zeroth to the third cycle, with the increase of 35.33 mg/mL, 38.07 mg/mL and 27.33 mg/mL respectively, whereas the increase was reduced to 8.33 mg/mL at fourth cycle, 69.52% lower than previous cycle.

With the increase of the TDS content, the cellulase hydrolysis rate showed a downward trend, which was most significantly decreased in the fourth cycle. It is indicated that when circulated to the fourth time, the accumulation of degradation compounds has almost reached a threshold, the alkali black liquor was unable to break down the linkages between cellulose, hemicellulose and lignin and internal structure effectively, resulting in the decrease of delignification abilities and limited contact of cellulase with substrate. Or a certain amount of organic substances which inhibit the hydrolysis of cellulase were gradually accumulated during alkali black liquor circulation. The

inhibitors might be attached to corn straw by recycled alkali black liquor pretreatment and could not be completely removed, which led to decreased enzymatic hydrolysis efficiency.

3.2 Changes of the content of OM and IM in recycled alkali black liquor

Alkali black liquor is a mixture of organic and inorganic materials. The contents and proportions of OM and IM in different cycles of alkali black liquor are listed in Table 1. The organic and inorganic compound concentration underwent an upward trend with the increasing cycles of alkali black liquor, which was in consistent with the trend of TDS content. However, the OM and IM content increased slowly at the fourth cycle, indicating that the ability of recycled alkali black liquor to dissolve/remove OM and IM was reduced which was not as effective as fresh NaOH solution.

Table 1 Changes in content of organic matter (OM) and inorganic matter (IM) in different cycles of alkali black liquor

Cycle times	OM			IM		
	Content (g/15mL)	increment	proportion (%)	Content (g/15mL)	increment	proportion (%)
0	0.674 ± 0.07^{e}	-	63.22%	0.392 ± 0.05^{c}	-	36.78%
1	0.959 ± 0.09^{d}	0.285	60.09%	0.637 ± 0.07^{b}	0.245	39.91%
2	1.292 ± 0.10^{c}	0.333	59.64%	0.875 ± 0.08^{a}	0.238	40.36%
3	1.637 ± 0.10^{b}	0.345	63.52%	0.940 ± 0.10^{a}	0.065	36.47%
4	1.739 ± 0.13^{ab}	0.102	64.38%	0.963 ± 0.10^{a}	0.023	35.62%
5	1.822 ± 0.13^{ab}	0.082	65.34%	0.966 ± 0.10^{a}	0.004	34.65%
6	1.834 ± 0.12^{a}	0.012	65.73%	0.956 ± 0.11^{a}	-0.010	34.26%

Note: The OM and IM contents were determined in 15 mL recycled alkali black liquor. The proportion of OM was the ratio of the content of OM to the total content of OM and IM, and so on. The increment was the difference between the content of the current cycle and that of the previous cycle.

There were slight changes in the organic matter proportion (OMP) and inorganic matter proportion (IMP) during the alkali black liquor recycling from zeroth to sixth cycle. The OMP tended to fall then rise, which was contrary to the IMP. And at the second cycle, the OMP reached a minimum value of 59.64%, whereas the maximum value of 40.36% of the inorganic. It can be found that the ability of IM removal in 0-2 cycles was stronger in comparison with that in 3-6 cycles, in contrast, the alkali black liquor recycling with 3-6 cycles had better effect on OM removal than 0-2 cycles. There was no obvious increase in the concentration of OM and IM after the fourth treatment, but the OMP kept rising from the fourth cycle to sixth cycle, indicating that the accumulation of substances in the recycled alkali black liquor had gradually reached saturation, and the OM was related to the decrease of cellulase hydrolysis rate.

3.3 Changes of alkali and acid precipitation in recycled alkali black liquor

3.3.1 Changes in contents of alkali and acid precipitation

The effects of different cycles of alkali black liquor circulation treatment on alkali and acid precipitation contents are presented in Fig. 2. The alkali precipitation content rose first, then descended slightly and reached a maximum of 0.707g at the fourth cycle, an increase by 0.546g was observed compared with the zeroth cycle. Nevertheless, the change of acid precipitation was different from the alkali precipitation, which generally showed an upward trend. The acid precipitation content was 1.626g at the sixth treatment, about an eleven-fold increase from the zeroth treatment. The total precipitation contents was 0.295 g, 1.041 g, 1.411 g, 1.708 g, 1.955 g, 2.175 g and 2.19 g from zeroth to sixth cycle, respectively, showing an overall upward (rising) trend but gradually declining in the increment which was consistent with the trend of TDS content but in contrast to the trend of cellulase hydrolysis yield in 3.1. The decrease of alkali precipitation content after the fourth cycle might be attributed to its deposition on the substrate or reaction and degradation into soluble components in the complex black liquor. In general, compared with alkali precipitation, acid precipitation content was higher and kept increasing indicating that the accumulation of acid insoluble component has effect on the decrease of cellulase hydrolysis.

3.3.2 Changes of alkali precipitation composition in recycled alkali black liquor

The effect of alkali black liquor circulations on the content of OM and IM in alkali precipitation is shown in Fig. 3. The inorganic content gradually rose during 0-4 cycles and then reached stabilized. The organic content showed tendency of first increasing and then descending, which was same as the alkali precipitation and peaked at the fourth cycle. It was indicated that the OM was the major factor to effect the change of alkali precipitation content.

3.3.3 Changes of acid precipitation composition in recycled alkali black liquor

Fig. 4 shows that the content of OM and IM in acid precipitation with different alkali black liquor recycling cycles. The organic content showed the same trend as the acid precipitation, rapid increase at 0-2 cycles and 5-6 cycles and steady growth at 3-4 cycles. Differ from the organics, the inorganic content no longer increased after the fourth time. The results of 3.1 showed that the enzymatic hydrolysis rate of cellulose decreased in the fourth cycle. Combined with the changes in the cellulase hydrolysis rate, it can be seen that with the increase of circulation times of alkali black liquor, the organics of acid precipitation was the main factor inhibiting the enzymatic hydrolysis of cellulose.

3.4 Changes of lignin content in recycled black liquor

The content of lignin in recycled alkali black liquor is presented in Fig. 5. A gradual increase in the lignin content from 2.050 mg/mL to 6.144 mg/mL with increasing alkali black liquor recycling times was observed. At the zeroth cycle, the alkali black liquor showed a good liquidity and light color, which had a tend to present dark color, high viscosity and poor fluidity after several cycle treatment. For all the six cycles, the increment in lignin concentration decreased gradually from 1.049 mg/mL to 0.299 mg/mL. For the 0-3 cycles, the increase in lignin content was

between 35% and 50% of the zeroth cycle, showing that the delignification efficiency maintained the similar levels as the zeroth treatment with fresh NaOH solution [39]. After 4-6 cycles, the growth was 15%-30% compared with the zeroth cycle, indicating that the solubilization power of recycled alkali black liquor reduced and the lignin dissolution reached saturation gradually. The lignin content of circulating black liquor could indirectly reflect the lignin removal ability of straw. The delignification efficiency declined after the six treatment of recycled black liquor, speculating that the accumulation of degradation products reduced the utilization of alkali or high viscosity of black liquor hindered alkali liquor diffusion in lignocellulose [23,27,39].

However, recycling of alkali black liquor for the zeroth time resulted in a maximum cellulase hydrolysis yield of 87.67%, which underwent a downward trend by further recycling. The lower enzymatic hydrolysis rate of corn straw that pretreated with the recycled black liquor can be explained that the residual lignin in recycled black liquor caused reduced delignification efficiency, resulting high lignin content in pretreated straw. And the undegraded lignin was a physical barrier to prevent direct contact of the enzyme with cellulose [15], which reduced the cellulase accessibility.

3.5 GC-MS analysis of recycled alkali black liquor

The identities and the relative abundances of the organic components from recycled alkali black liquor were recognized by using GC-MS, the main identified compounds are listed in Table 2.

53 types of organic compounds were isolated and identified which can be classified into six categories: phenols, benzene ring and heterocyclic, furans, acids, alcohol ketones, and esters. As seen from the table, the total relative content of compounds in alkali black liquor exhibited an overall upward trend with the increasing cycles. And the phenols, benzene ring and heterocyclic, and furans were the three major products with high relative yields, accounting for more than 80% of the total. Among them, 2, 3-dihydrobenzofuran showed the highest relative yield, which was in the range of 31.359% - 34.510%. Then other substances with high relative abundances were 4-vinyl-guaiacol, 2,6-di-tert-butyl-4-methylphenol and 2,4-di-tert-butylphenol, which are typical lignin degradation products.

Lignin is a complex polymer in which guaiacyl, syringyl and p-hydroxyphenyl units are interconnected [40]. Guaiacyl and syringyl units are main composition of corn straw lignin which contain small amounts of p-hydroxyphenyl units. The compounds obtained from degradation of lignin were mainly monomeric aromatic products of which the phenols compounds were the predominant components [41]. Alkali pretreatment can cause scission of linkages between lignin and carbohydrates, expose the cellulose inside the lignocellulose and convert lignin macromolecules into small molecules of aromatic compounds that dissolved or deposited in alkali black liquor. The alkali black liquor mainly contained lignin degradation compounds. It can be speculated that continuous accumulation of small molecular compounds with the increasing cycle numbers resulted in increased concentration of alkali black liquor and reduced the amount of effective alkali, and the infiltration of the refractory organic compounds with alkali liquor to straw limited the swelling effect of alkali and hindered the breaking of the ether and ester bonds between lignin and carbohydrates, thereby reducing the enzymatic hydrolysis rate.

Table 2 Compounds and its relative intensity detected by GC-MS in recycled alkali black liquor from different cycles

Type	Components	Relative content/ area (%)						
		0	1	2	3	4	5	6
Phenols	2,6-Di-tert-butyl-4-methylphenol	4.283	4.596	4.370	4.372	4.355	4.881	4.715
	4-Vinyl-guaiacol	13.922	13.253	12.175	12.236	12.445	11.740	12.495
	2,4-Di-ter-butyl phenol	3.717	4.242	4.765	4.769	4.873	4.580	4.928
	∑	21.922	22.091	21.310	21.377	21.673	21.201	22.138
Benzene ring and heterocyclic	1,2,3,6-Tetrahydrophthalic anhydride	-	2.071	-	0.783	-	0.316	1.099
	1,4-Diisopropylnaphthalene	-	-	1.046	-	1.795	0.804	-
	2,3,4-4a, 8,8a Hexahydropyran [3,2-b] pyran	1.231	-	-	4.958	-	-	-
	4- (2-Methyloctadecyl-4-phenoxymethyl) -2,2-dimethyl-1,3-dioxolane	1.200	3.330	3.481	3.992	4.471	5.450	4.777
	2-Methoxypyrimidin-4-amine	17.080	16.652	15.538	13.517	18.069	17.895	17.441
	Pyridine	2.839	0.919	0.685	1.164	-	-	-
	3-Methyl pyridine	0.572	-	0.634	-	0.570	-	-
	2,5-Dimethylbenzaldehyde	0.472	0.338	0.562	0.551	0.411	0.931	-
	Others	1.841	2.572	2.863	1.082	1.520	1.309	3.317
	∑	25.235	25.882	24.809	26.047	26.836	26.705	26.634
Furans	2,3-Dihydrobenzofuran	31.383	31.359	34.105	34.276	33.760	33.945	34.510
	5-Fluoro-1-α-ribofuranosyl-imidazole-4-carboxylic acid amide	0.209	0.512	0.628	0.697	1.132	1.174	0.976
	∑	31.592	31.871	34.733	34.973	34.892	35.119	35.486
Acids	2-(2- Methoxyethoxy) acetic acid	-	-	-	-	0.200	0.692	0.357
	3,6,9-Trioxaundecanedioic acid	1.263	0.245	0.811	1.360	1.227	-	1.187
	3-	-	0.406	0.865	-	0.772	0.847	-

	(Trimethylsilyl)propionic acid							
	DL-Mandelic acid	-	1.066	0.402	0.582	-	-	-
	Acetic Acid	-	0.534	0.352	0.118	0.404	1.095	0.906
	Others	-	0.182	0.935	1.16	0.396	1.153	0.600
	∑	1.263	2.433	3.365	3.22	2.999	3.787	3.050
Esters	N-allyl-N- [2- (tert-butyldimethylsilyloxy) propyl] methyl carbonate	-	-	-	0.419	0.610	0.345	0.424
	Methyl 2- (2-methoxyethoxy) acetate	-	0.476	0.421	-	0.428	-	-
	5- (2,3,4,5-Tetrahydro-3-hydroxy-4-ureidothiophen-2-yl) valerate	-	-	-	-	0.300	0.310	0.350
	Vanillone lactone	1.227	1.081	0.995	1.048	0.860	0.975	1.080
	1-O-butyl 2-O-octyl benzene-1,2-dicarboxylate	0.439	0.384	0.320	0.534	-	0.355	-
	3,2,4-Tridecyl methoxy acetate	-	0.389	0.580	0.583	1.335	0.825	0.795
	1,3-Diacetoxypropane-2-yldodecanoate	-	-	-	0.397	-	0.557	0.440
	3- Tetradecyl methoxy acetate	-	0.124	0.227	-	-	0.355	0.376
	others	0.398	0.524	0.848	0.906	0.455	-	-
	∑	2.064	2.978	3.391	3.887	3.988	3.722	3.465
Alcohol ketones								
	3-Amino-4, 6-dimethyl-1h-pyridine-2-ketone	-	0.194	0.297	-	0.560	-	-
	3-Methyl-1, 4-oxythio-heterocyclohexane 2-ketone	0.450	1.788	1.900	1.864	1.501	1.759	1.566
	Hexahydro-1,6-pentanedione	0.584	-	-	-	0.242	0.133	-
	others	0.365	0.531	-	0.353	-	0.332	0.650
	∑	1.399	2.513	2.197	2.217	2.303	2.224	2.216

Note: others in each category means some compounds that not listed in Table 2. Other compounds: (acids type) Propyl thioacetic acid, 2- (4-Fluoro-6-oxy-1,6-dihy dropyrimidine-2-imine) -propionic acid, O-acetyl-L-serine; (benzene ring and heterocyclic type)1-(4- Nitrophenyl) ethyl thiosemicarbazide, 5-Isopropyl-2-methyl-1, 3-oxythio-heterocyclic

hexane, 1-Nitroso 3-pyrroline, 2-Cyanoquinoline, 3-Ethyltetrahydrothiophene, 4-Hydroxymethyl-5-methylimidazole, C-(3-methyl-isoquinolin-1-yl) -methylamine, Octahydro-2, 6-cyclothianan [3,2-b] pyran, 1, 4-Diol -2, 3-dimethyl-5-trifluoromethyl benzene, 4,5-Dihydro-2- (4-chlorophenyl) thiazole; (esters type): 2,3,4,6-Tetraacetoxy-5,5-bis (ethylsulfanyl) hexyl acetate, 2- (2-Chloroacetyl) oxypropyl-2-chloroacetate, 2,2,2-Trifluoroethyl methanesulfonate, 7-Dimethoxymethylbic yclo [2.2,1] heptane-1-carboxylic acid methyl ester, Dimethylsilanediol; (alcohol ketones): Mercaptoethanol, 4, 4-Dimethyl-2, 5-cyclohexadiene -1-ketone, 9-Thiabicyclo [3.3.1] non-7-en-2-ol, Aminoacetaldehyde diethanol; 3,6-Dioxa-8-mercaptooctan-1-ol.

4 Conclusions

The compositions of recycled alkali black liquor from zeroth to sixth cycle were analyzed. At the fourth recycle, the contents of total solid, organic matter and lignin in alkali black liquor increased slowly, whereas the cellulase hydrolysis rate decreased significantly, indicating that the capacity of alkali black liquor to degrade straw was reduced. And GC-MS analysis showed that three major components in recycled alkali black liquor were phenols, benzene ring heterocyclic and furans, which accounted more than 80% of the total. Therefore, it can be concluded that organic matter produced by lignin degradation and small molecule lignin are the main inhibitors of cellulase hydrolysis, which prevented alkali from destroying lignocellulosic structure, hindering the contact between cellulase and cellulose.

Declarations

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Conflicts of interest: The authors declare that they have no conflict of interest.

Authors' contributions: Conceptualization: Yiming Li and Shuo Fang; Methodology: Yiming Li, Shuo Fang and Xia Zhou; Software: Yiming Li and Shuo Fang; Visualization: Shuo Fang; Formal analysis: Fei Li, Xiaohong Lu and Yiming Li; Writing - Original draft: Yiming Li, Fei Li and Xiaohong Lu; Writing - Review and Editing: Fei Li, Xiaohong Lu, Xia Zhou, Zhezhen Zhao and Ping Liu; Supervision: Ping Liu. All authors contributed to this work by collaboration.

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Figures

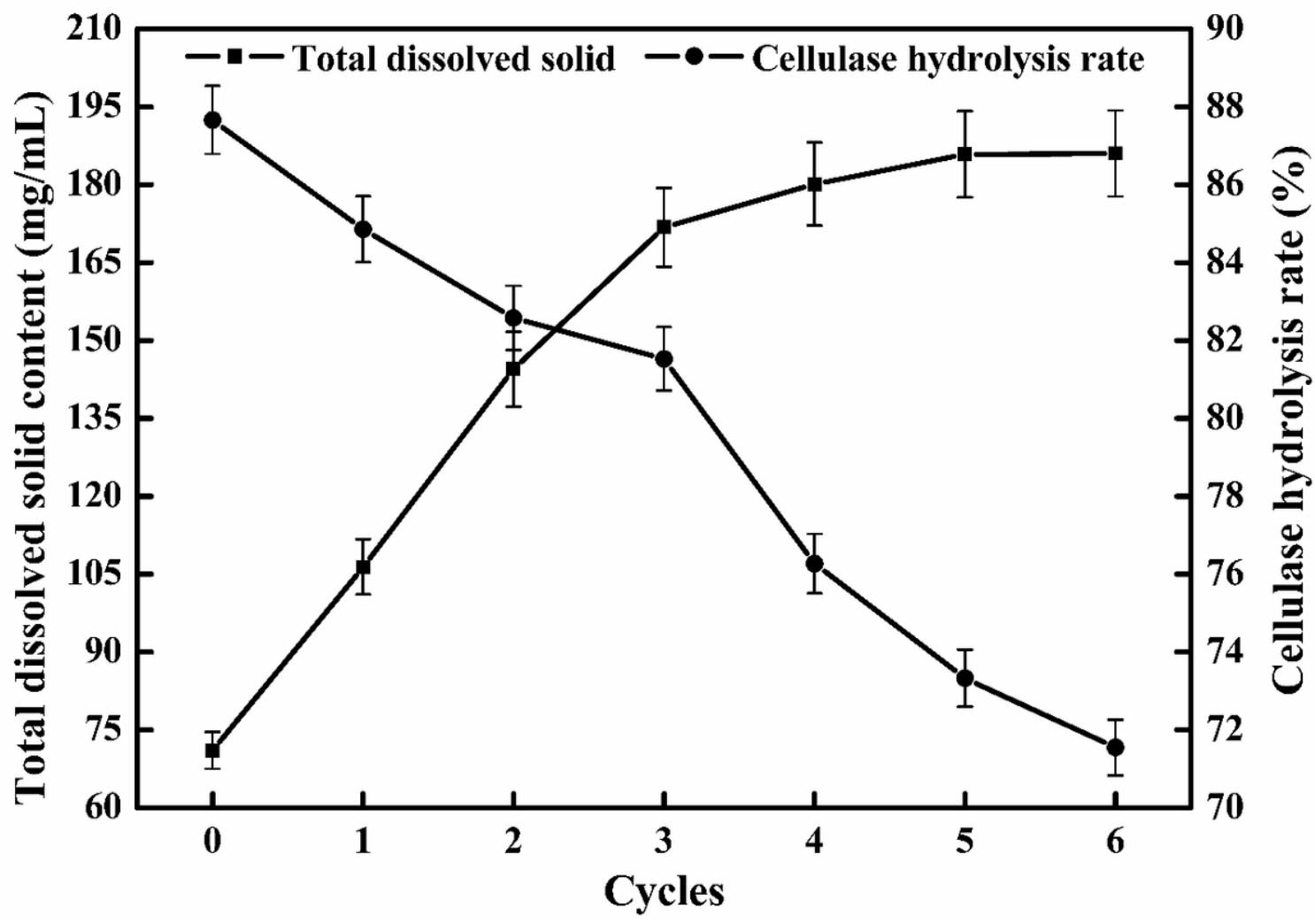

Figure 1

Changes of total dissolved solid content in alkali black liquor circulation and its effect on cellulase hydrolysis rate of pretreated corn straw

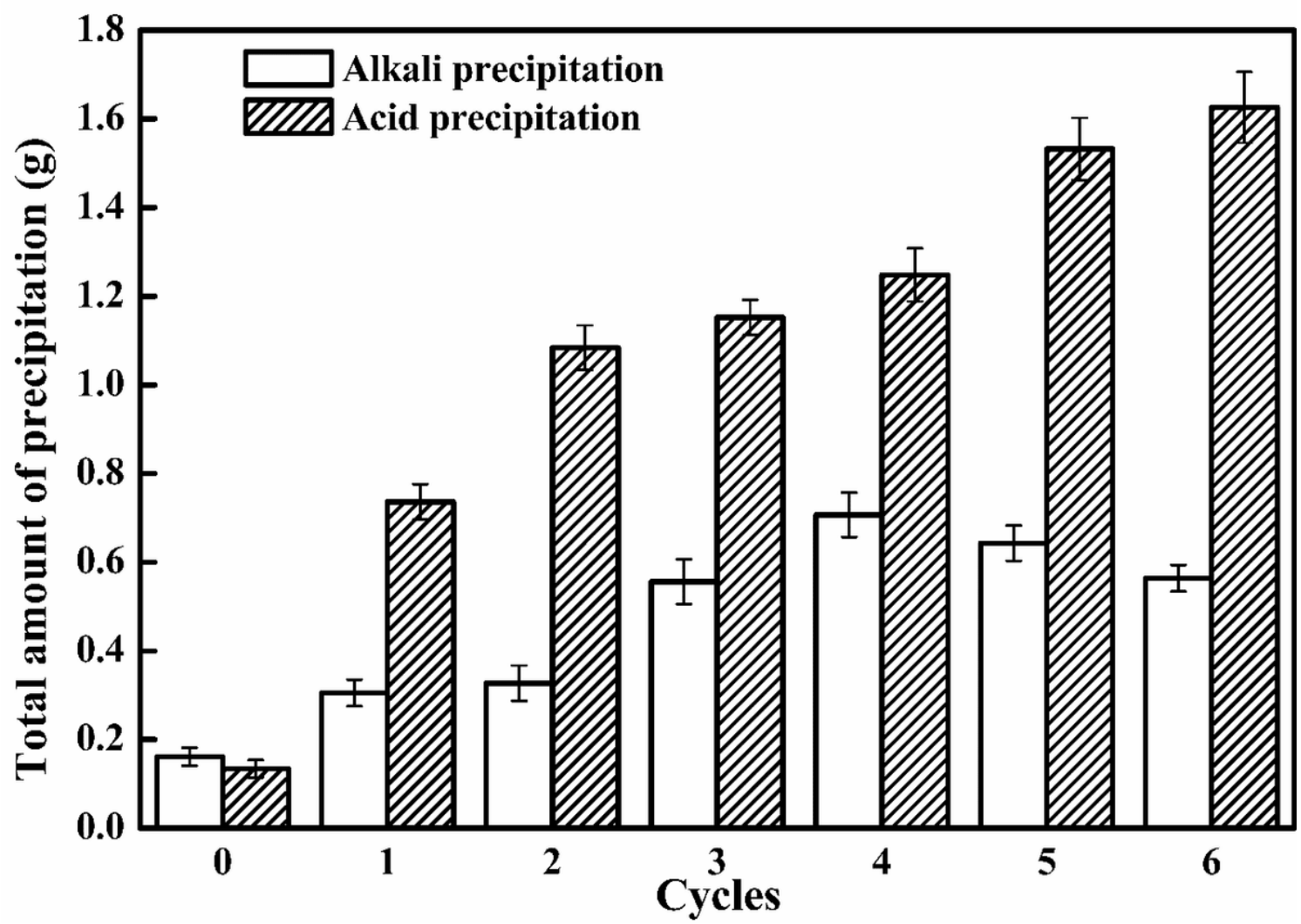

Figure 2

Effect of alkali black liquor circulations on alkali and acid precipitation

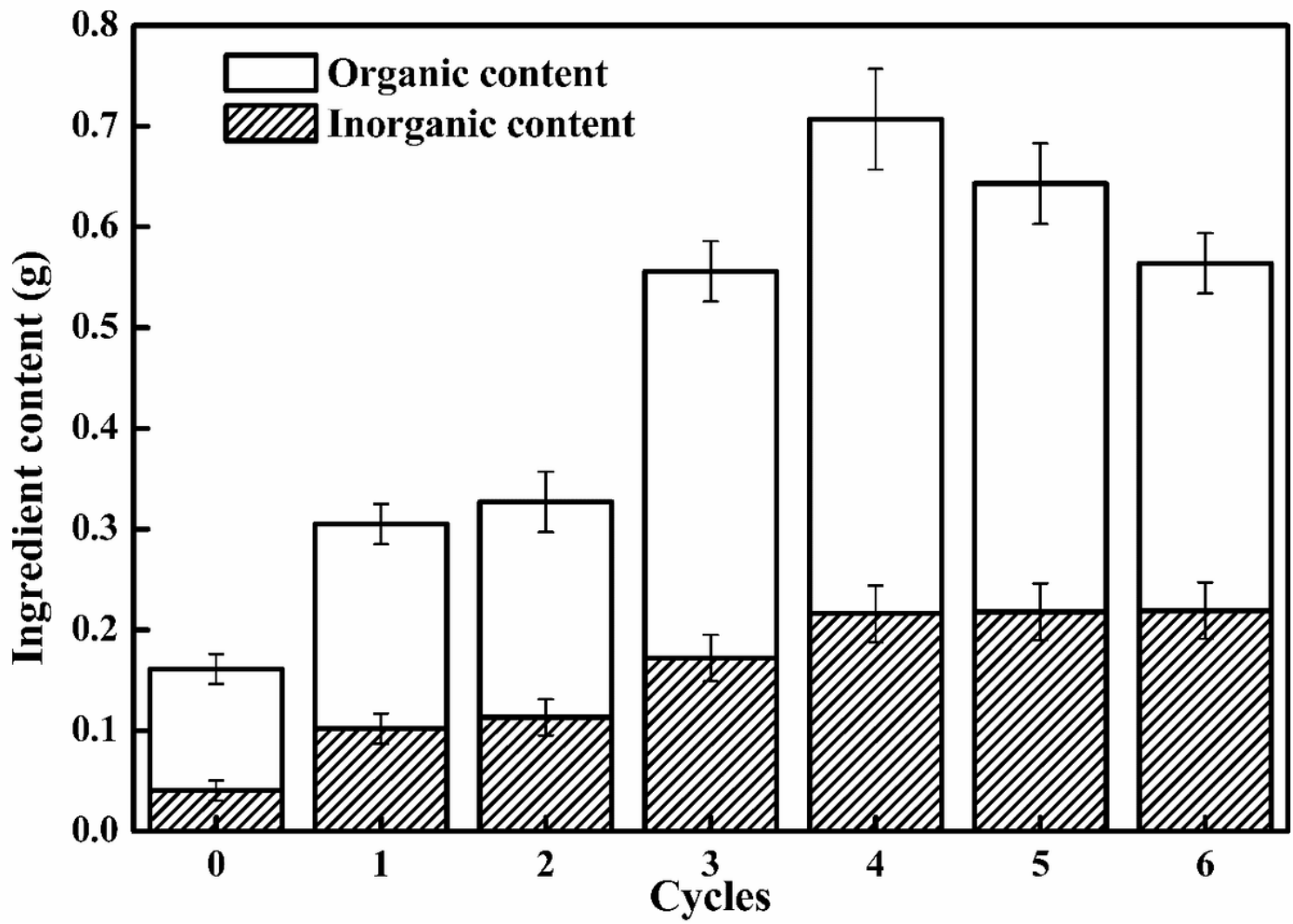

Figure 3

Effect of alkali black liquor circulations on the composition of alkali precipitation

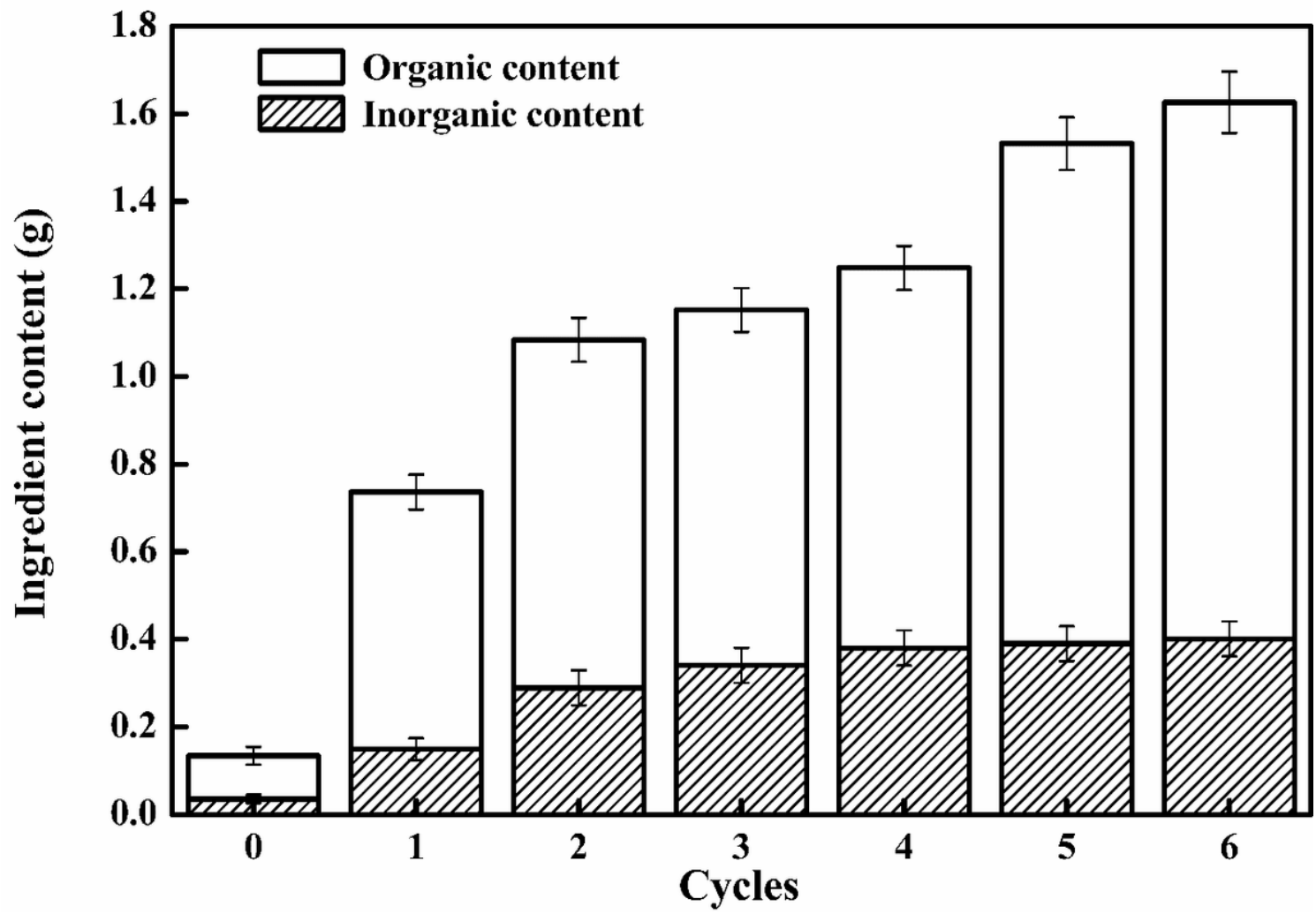

Figure 4

Effect of alkali black liquor circulations on the composition of acid precipitation

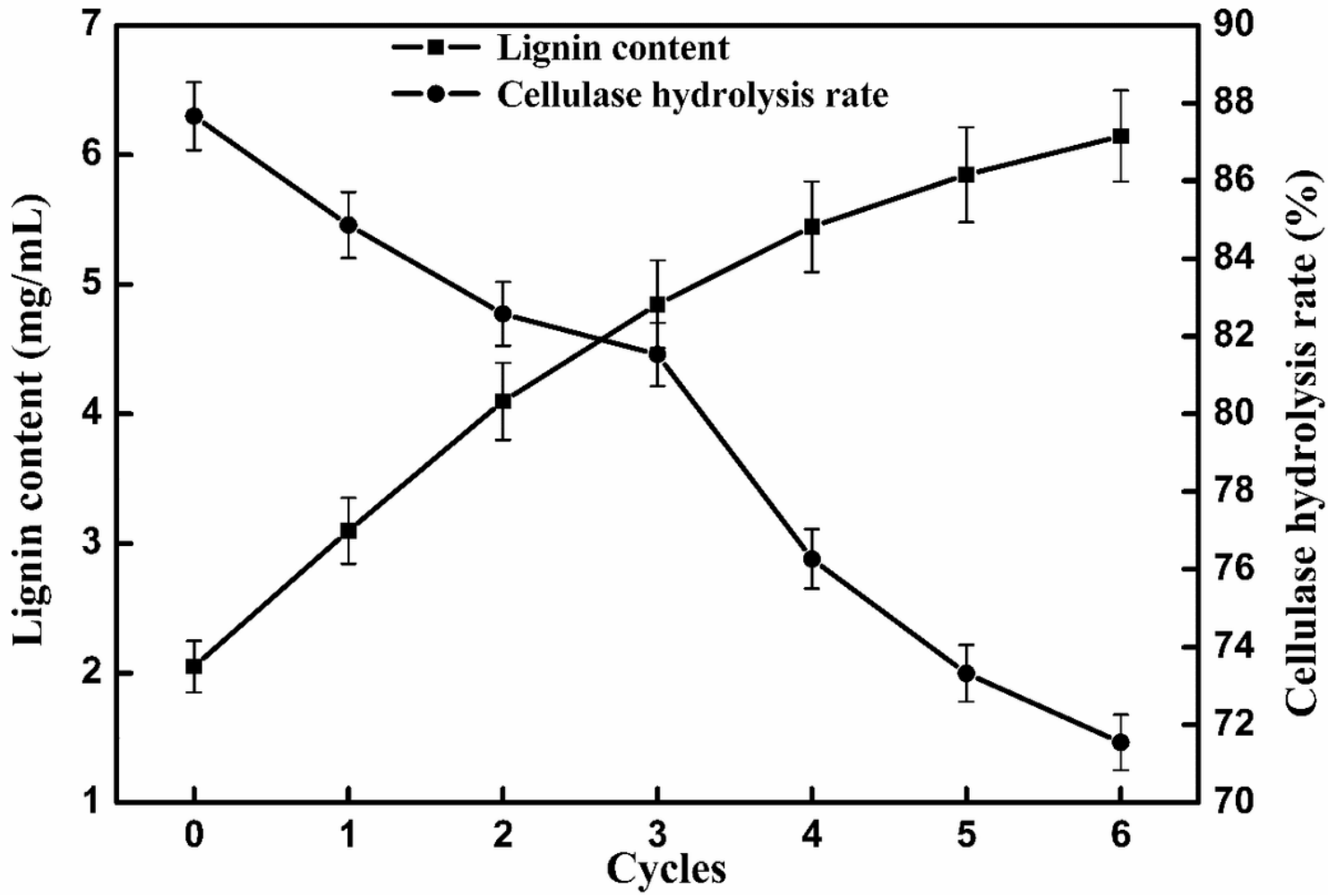

Figure 5

Content of lignin in recycled alkali black liquor and cellulase hydrolysis rate of pretreated corn straw