# Sulfamic Acid Combined with Alkaline Hydrogen Peroxide Pretreatment of Bagasse

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## Abstract

**In the present study, the sulfamic acid pretreated bagasse was combined with alkaline hydrogen peroxide delignification at different pH to increase the cellulose conversion. The higher the pH of the alkaline hydrogen peroxide, the more thorough the lignin removal. At pH 13.5, the cellulose conversion reached a maximum. The results of Fourier transform infrared spectroscopy also confirmed that sulfamic acid removed a large amount of hemicellulose from bagasse, and alkaline hydrogen pero xide removed a large amount of lignin from bagasse. Sulfamic acid combined with alkaline hydrogen peroxide can significantly improve the cellulose conversion of bagasse, which is an excellent pretreatment method.**

## Keywords

**Bagasse, pretreatment, cellulose conversion rate.**

## 1. Introduction

With the construction of the “One Belt and One Road” and the rapid development of industry in China, the demand for energy has also risen sharply. Vigorously developing lignocellulose fuel ethanol is a solution to effectively alleviate the energy crisis. China is the third largest sugar cane grower in the world, after Brazil and India [1]. More than 6 million tons of bagasse remains unused every year [2].

Bagasse is a rich, high-yield and renewable lignocellulose. It consists mainly of cellulose (40-60%), hemicellulose (20-40%) and lignin (10-25%).The natural structure of bagasse is dense, which is not conducive to the hydrolysis of cellulose. In order to remove the barriers of hemicellulose and lignin and allow cellulose to better contact cellulose, the choice of pretreatment methods is crucial. Common pretreatment methods are acidic and alkaline pretreatment. Acid pretreatment can dissolve most of the hemicellulose of bagasse and increase the accessibility of the enzyme to cellulose [3].Sulfuric acid is a chemical cleaning agent commonly used for metal decontamination and descaling [4].The aqueous solution has similar acidity to sulfuric acid and hydrochloric acid and is an ideal acid pretreatment reagent. In the alkaline pretreatment process, the ester bond in hemicellulose and lignin is easily decomposed under alkaline conditions. The cleavage of these bonds promotes the dissolution of hemicellulose and lignin, resulting in cellulose being more readily bound to the cellulose. Alkaline hydrogen peroxide (AHP) is a pretreatment method that selectively removes lignin. Hydrogen peroxide decomposes in an alkaline solution to form free radicals such as HO·, HOO·, O2-·, which play an important role in the process of lignin DE polymerization [5].

In this study, sulfuric acid was combined with AHP to pretreat bagasse, which aims to significantly improve the cellulose conversion rate of bagasse.

## 2. Materials and Methods

### 2.1 Bagasse and Cellulase

Bagasse is provided by GanWu Sugar Factory(Zhuhai, China). It was placed in an oven at 60°C and baked to constant weight over 24 h. The dried bagasse is then treated with a low speed and high speed pulverizer, passed through a 200 mesh screen, and sealed and stored in a dry environment. Cellulase Ctec2 is provided by Novozymes (Denmark) and stored at 4°C.

### 2.2 Pretreatment

A certain amount of bagasse was mixed at a solid-liquid ratio of 1:10, added with 3% sulfamic acid, and reacted at 121 °C for 1 hour. The bagasse after the reaction was washed and dried.The sulfamic acid treated bagasse (10 g) was placed in an Erlenmeyer flask. At a solid-liquid ratio of 1:10, 100 mL of a 2% hydrogen peroxide solution of pH 10.5, 11.5, 12.5, 13.5 was added. After thoroughly mixing, it was reacted at 50 °C for 12 h. After the reaction was completed, the pretreated bagasse was placed in a Buchner funnel for solid-liquid separation. The solid bagasse is washed with hot water (about 90 °C) to neutral to remove excess pretreatment liquid. The washed bagasse was placed in an oven and baked at 60 °C to constant weight.

The main components of bagasse were determined by reference to the laboratory analytical procedures (LAP) of the National Renewable Energy Laboratory (NREL) [6].

### 2.3 Enzymatic Hydrolysis

Bagasse (0.5 ± 0.0005 g) was added to 25 mL of citric acid-trisodium citrate buffer (pH 4.8), CTec 2 (18 FPU). It was placed in an incubator at 50 °C and digested at 200 rpm for 96 h. The supernatant was taken every 24 h, and the glucose concentration was measured by High Performance Liquid Chromatography (HPLC, Shimadzu, Japan), and the cellulose conversion rate was calculated according to the following formula.

$$CC = \frac{C_5}{m_5 * X * 1.11} * 100$$

CC—cellulose conversion rate, %;

C5—the amount of glucose produced by enzymatic hydrolysis of bagasse, g/L;

m5—the amount of substrate bagasse,g/L;

X—cellulose content, %;

1.11—The cellulose is converted to the coefficient of glucose.

### 2.4 FTIR Analysis

A certain amount of potassium bromide was placed in an agate mortar and baked in an infrared lamp for 30 min to remove excess water. Place a 1-2 mg sample into the agate mortar and grind it clockwise to thoroughly mix the sample with potassium bromide. The mixed sample was placed in a metal mold for compression, and the sample was placed in a Fourier transform infrared spectrometer (The Nicolet iS10, Thermo Scientific™) with a resolution of 4 cm-1, a scanning range of 4000 to 400 cm-1, and a scan of 32 times.

## 3. Results and Discussion

### 3.1 Effect of AHP Pretreatment with Different Ph on The Composition of Bagasse

Previous studies have found that sulfamic acid can remove large amounts of hemicellulose, but the lignin content is still very high, which will result in lower cellulose conversion.In order to increase the cellulose conversion rate of bagasse, the bagasse after acid treatment (3% sulfamic acid, 121°C, 1.0 h) was subjected to delignification experiments using AHP with different pH, and the components and lignin removal rate of bagasse were determined. As shown in Fig. 1, 10 g of bagasse after acid treatment contained cellulose 5.59 g, hemicellulose 1.03 g, acid-soluble lignin (ASL) 0.32 g, acid-insoluble lignin (AIL) 2.70 g, and other ingredients 0.36 g.

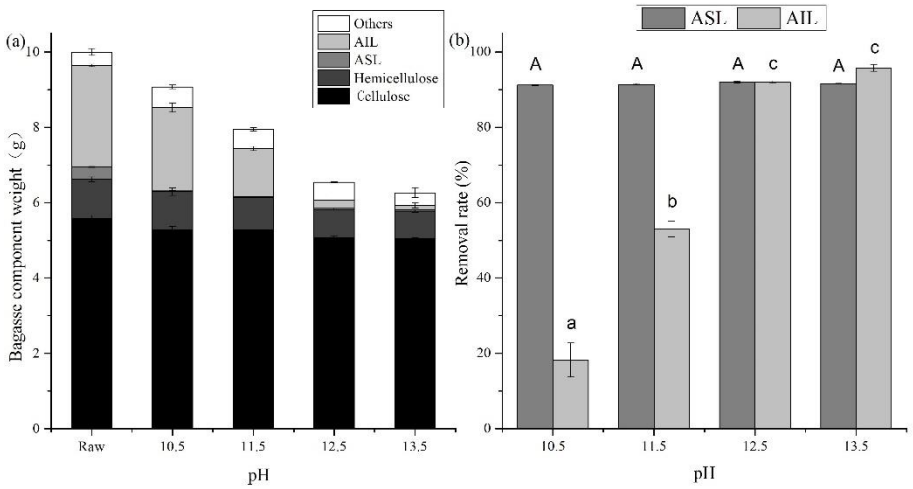


Fig 1. Bagasse compositions (a) and lignin removal rate (b) after AHP pretreatment at different pH values

Note:Capital letters represent the removal rate ofASL, while lowercase letters represent the removal rate of AIL.Different letters are significantly different ($p<0.05$).

The total mass of bagasse decreased with increasing pH (Fig. 1a). When the pH rose from 10.5 to 12.5, the total mass of bagasse rapidly decreased. When the pH is 12.5, the bagasse remains 6.54 g; As the pH increased from 12.5 to 13.5, the total mass of bagasse decreased to a flat level. When the pH is 13.5, the bagasse residue remained at least 6.27 g. With the increase of pH, the cellulose quality gradually decreased, but the decrease was not significant. When the pH rose from 10.5 to 13.5, the loss rate of cellulose increased from 5.38% to 9.84%. The hemicellulose mass also decreased with increasing pH. At pH 13.5, the hemicellulose mass reached a minimum of 0.74 g. ASLwas mostly removed after AHP pretreatment, and only about 0.04 g of ASL remained after treatment with different pH.The quality of AIL is greatly affected by pH changes. When the pH increased from 10.5 to 12.5, the quality of AIL decreased rapidly, and its mass decreased from 2.21 g to 0.22 g.When the pH rose from 12.5 to 13.5, the AIL quality decreased slowly. When the pH is 13.5, the AIL has a minimum mass of 0.12 g.The quality of other ingredients is not affected by pH changes. In an alkaline environment, the ester bonds formed by p-coumaric acid, syringic acid, and p-hydroxycinnamic acid in lignin are easily hydrolyzed, and the stronger the alkalinity, the more thorough the reaction [7]. In addition, hydrogen peroxide will decompose in an alkaline environment to produce highly active molecules such as hydroxyl radicals and peroxy radicals, which oxidize the phenolic structure of lignin and further remove more lignin [5].

The ASL removal rate after treatment with different pH values was about 92% (Fig. 1b), and there was no significant difference ($p>0.05$).The AIL removal rate increases with increasing pH. The AIL removal rate was significantly increased ($p< 0.05$) when the pH was increased from 10.5 to 12.5. However, there was no significant difference in pH between 12.5 and 13.5 ($p>0.05$).

### 3.1.1 FTIR Analysis of Sugarcane Bagasse after Different Pretreatments

In order to compare the functional groups and chemical bond changes of bagasse that have been subjected to different pretreatments. The treated samples were subjected to FTIR analysis, and the results of the spectra are shown in Fig. 2. The pretreated bagasse did not produce a new absorption peak compared to the untreated FTIR spectrum. This indicates that the pretreatment did not produce new functional groups on the bagasse, but only a non-derivatized dissolution occurred on the chemical bond of the bagasse[8].

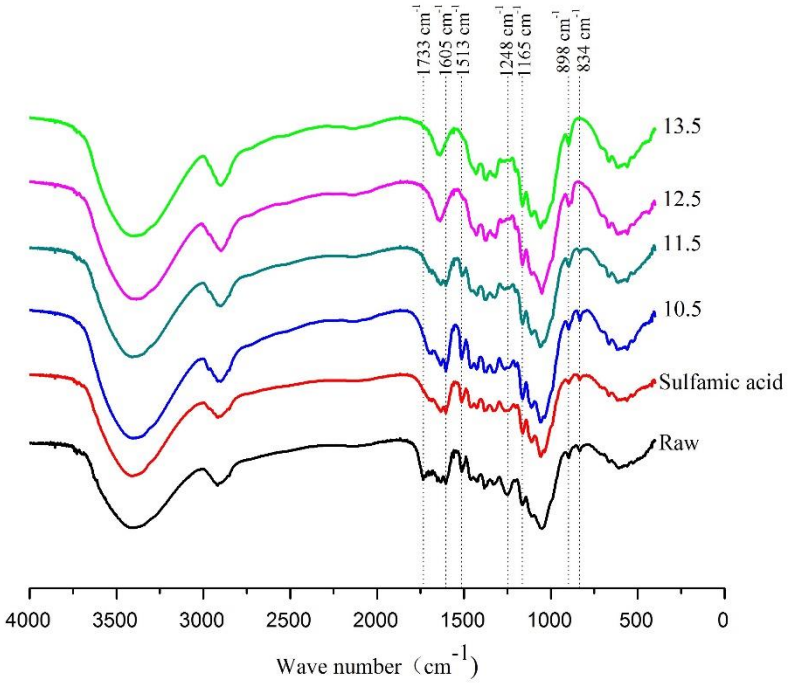


Fig 2. FTIR spectra of differently pretreated bagasse

Untreated bagasse has an absorption peak at 1248 cm-1 (characteristic peak of hemicellulose) and 1733 cm-1 (stretching vibration of C=O of carbonyl or acetyl in xylan) [9]. After the sulfamic acid pretreatment, the absorption peaks at these two sites were significantly weakened. In an acidic environment, the dissolution of a large amount of hemicellulose causes the characteristic peak to weaken, which is similar to the result of Jia Yanxi's pretreatment of bagasse with dilute sulfuric acid [8]. In this study, after pretreatment with AHP with different pH, the two absorption peaks at 1248 cm-1 and 1733 cm-1 were slightly weakened, and the hemicellulose was slightly dissolved after pretreatment with AHP with different pH.The results are consistent with Fig. 1a.

The absorption peak at 834 cm-1 (stretching vibration of syringyl group C-H), 1513 cm-1 (stretching vibration of lignin aromatic ring C=C), and 1605 cm-1 (stretching vibration of lignin benzene ring) in the figure represent characteristic peaks of lignin [10]. Compared with the untreated bagasse, the sulfamic acid-treated bagasse had little change in the characteristic peak of lignin. After the AHP pretreatment, the absorption intensity of the characteristic peaks of lignin decreased with the increase of pH. At pH 13.5, the three characteristic peaks of lignin disappeared, and the lignin was largely removed. Weixing Cao et al. used AHP to pretreat sweet sorghum slag similar to the experimental results [10].

In the spectrum, 898 cm-1 (the stretching vibration of β-glucosidic bond) and 1165 cm-1 (the stretching vibration of cellulose C-O-C) are the characteristic peaks of cellulose [11]. Compared with the untreated bagasse, the sulfamic acid-treated bagasse had a small change in the characteristic peak of cellulose. After AHP pretreatment, the intensity of the characteristic peaks at both sites of cellulose was enhanced. The removal of large amounts of hemicellulose and lignin significantly increases the percentage of cellulose.

### 3.1.2 Effect of AHP Pretreatment with Different Ph on Enzymatic Hydrolysis of Bagasse

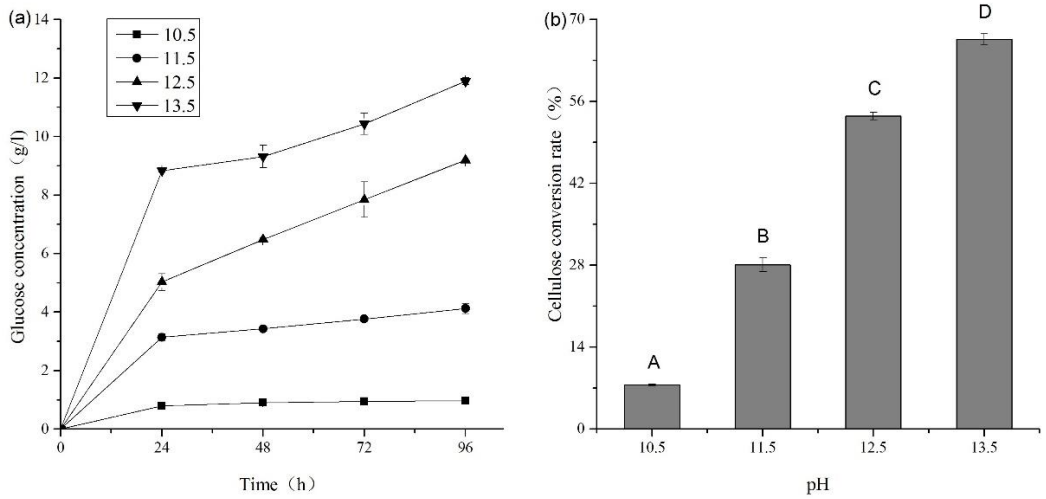


Fig 3. Enzymolysis curve (a) and cellulose conversion rate (b) of bagasse atdifferent pH AHP pretreatment

Note: Columns with different letters are significantly different ($p<0.05$)

The bagasse after pretreatment with different pH of AHP reacted under the same enzymatic conditions, and the enzymatic hydrolysis curve and cellulose conversion rate are shown in Figure 3.During the same enzymatic hydrolysis time, the higher the pH, the higher the glucose concentration (Fig. 3a). This is because the higher the pH, the lower the lignin content of the bagasse after pretreatment (Fig. 1a). The non-productive adsorption and steric hindrance formed by lignin and cellulase are smaller. More cellulase is effectively involved in the cellulose hydrolysis reaction, producing more glucose.When the enzymatic hydrolysis time was from 0 to 24 h, the glucose concentration of each group increased the most. As the enzymatic hydrolysis time increased gradually, the increase of glucose concentration in each group tended to slow down compared with the first 24 h. After 24 h of enzymatic hydrolysis, the glucose concentration at pH 10.5 did not change substantially, and the glucose concentration at pH 12.5 increased the most. After different pretreatments, the surface structure of the bagasse becomes rough, porous and fluffy. This increases the contact area and accessibility of cellulase to cellulose [9]. The amorphous structure of cellulose can be rapidly hydrolyzed in the early stage of enzymatic hydrolysis (0-24 h). With the increase of enzymatic hydrolysis time (>24 h), cellulase further acts on the highly ordered crystal structure of cellulose, and its enzymatic hydrolysis rate also decreases.

In Figure 3b, cellulose conversion was significantly increased with increasing pH ($p<0.05$). Studies have shown that the cellulose conversion rate is positively correlated with the lignin content [12]. At pH 13.5, the cellulose conversion was 66.60%. At the lignin removal rate (Fig. 1b), there was no significant difference in lignin removal rate between pH 12.5 and pH 13.5 ($p>0.05$), but the cellulose conversion at pH 13.5 was significantly higher than pH 12.5 ($p<0.05$). Therefore, pH 13.5 was chosen as the optimal AHP pretreatment condition.

## 4. Conclusion

The bagasse is pretreated with sulfamic acid in combination with AHP. It was found that AHP pretreatment with different pH had little effect on the quality of cellulose and hemicellulose, and significantly removal of lignin (ASL, AIL).When the pH was 13.5, the lignin (ASL, AIL) removal rate of bagasse was the highest. Compared to other pH pretreated bagasse, the cellulose conversion rate reached a maximum at a pH of 13.5. It can be seen from the FTIR results that the bagasse, hemicellulose and lignin which have been pretreated with sulfamic acid and AHP are well removed. sulfamic acid combined with AHP can effectively increase the cellulose conversion rate and is an ideal pretreatment method.

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