Microwave-assisted alkali extraction of bagasse hemicellulose enhanced by an enzymatic pretreatment process

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ABSTRACT

Extraction of hemicelluloses is a primary step in the conversion of plant biomass to valuable biofuels and chemicals. Hemicellulose is distributed in the fiber cell wall, and the compact structure of the fiber cell wall barricades the transport of hemicelluloses in the extraction process. In this paper we presented a novel microwave-assisted extraction process enhanced with an enzymatic pretreatment to improve the hemicelluloses extraction rate from bagasse. Experimental results showed that the enzyme dosage, alkali concentration and microwave irradiation time were main factors governing the yield of hemicellulose. The optimal conditions of the enzymatic pretreatment were: enzyme dosage of 138 IU/g bagasse and irradiation time of 30 min. Under these conditions, 86% of the hemicellulose in bagasse was extracted. After the enzymatic pretreatment the surface area of bagasse increased significantly which facilitated the subsequent microwave-assisted extraction process.

Keywords: Bagasse; Extraction; Hemicellulose; Microwave; Enzyme

1. INTRODUCTION

The gradual depletion of fossil-based materials made necessary the investigation of renewable alternatives as a source of materials and energy. In this context, utilization of non-wood biomass resources, such as agricultural wastes, is becoming an interesting alternative owing to their renewable origin and the biodegradability of their components. According to the concept of the full utilization of the whole components of biomass, cellulose and lignin can be used to produce bio-ethanol, energy and chemicals. However, hemicellulose, as the second abundant natural polysaccharide, is not efficiently utilized due to the complexity of its chemical properties and the extraction process.

Hemicelluloses are heteroglycans with various different sugar units, such as xylans, xyloglucans, mannans, glucomannans, and glucans, where xylans is one of the major components. The composition of hemicelluloses depends on the biomass source and the extraction method. Various multistage procedures have been proposed for the extraction of hemicelluloses which usually include a steam explosion, organic solvents, dilutes acid and alkaline treatment. However, it has been difficult to extract the majority of the hemicelluloses without severe degradation due to the strong interaction between hemicelluloses and lignin in plant cell walls. Microwave-assisted technique has been postulated as an alternative heating technology to accelerate hemicelluloses extraction since it reduces the reaction time and improve the extract efficiency as compared with conventional extraction modes. However, the extraction yield of hemicellulose is still in need of improvement. Raising the alkali concentration increased the hemicelluloses yield, but the hydrolysis rate also increased.

In this paper, microwave irradiation was combined with enzymatic pretreatment to improve the alkali extraction of hemicelluloses from bagasse, as shown in Fig. 1. Many literatures have been directed to the lignocellulose complex is made up of a matrix of cellulose and lignin bound by hemicellulose chains, and lignin cause hindrance in microbial attack, so pretreatment is required to extract hemicelluloses. In this work, the combination of pretreatment with enzyme in a first step and microwave-alkali in a second step was expected to overcome the barriers that hinder hemicellulose extraction. The enzyme was devoted to break chemical bond between hemicelluloses and lignin, and thus increase the pore size, pore volume and specific surface area of the biomass. In the second step, under the vibrating action of microwave in the molecular level, the exposed hemicellulose was dissolved more readily in the alkali solution. The objectives of this study were: (1) to investigate the effect of enzyme pretreatment on the extraction yield of hemicelluloses, (2) to optimize process parameters (dosage of enzyme, sodium hydroxide concentration and irradiation time) on the extraction of hemicelluloses and (3) to explore the underlining mechanism that leads to the improved hemicellulose extraction in the combined enzymatic and microwave assisted process.

2. EXPERIMENTAL

2.1 Materials
The bagasse was obtained from Nan’en Sugar and Paper Co. Ltd in Yunnan province, China. The sugarcane bagasse was air-dried and de-pithed. The chemical contents of the de-pithed bagasse were determined as follows: 51.52% cellulose, 29.03% hemicelluloses, 19.07% lignin, 0.06% ash and 4.58% moisture content. Laccase OMNO 7015 (activity 800 UI/g) were procured from Novozymes, Tian Jin, China. All other chemicals were analytic grades and used without further purification, unless noted.

![Scheme of the enzymatic pretreatment and microwave-assisted extraction process](image)

Fig. 1. Scheme of the enzymatic pretreatment and microwave-assisted extraction process

### 2.2 Enzyme pretreatment

Enzyme pretreatment experiments were carried out in glass bottles of 1 L capacity immersed in a laboratory-scale water bath with temperature control. 50 g of air dried de-pithed bagasse and a pre-determined amount of enzyme solution were mixed at 10% solid content, and were continuously stirred gently for 4 h at 45°C. Then the bagasse was separated from the liquor through filtration, and washed thoroughly with deionized water until the bagasse was neutral, and then dried in the oven at 50°C for 16 h. The pretreated bagasse was then subjected to alkali extraction under microwave irradiation. The hemicelluloses yield was calculated according to Eq. 1.

\[
\text{Hemicelluloses yield (\%)} = \frac{A}{B \times C} \times 100
\]

Where \(A\) was the weight of the amount of extracted hemicelluloses (g), which was precipitated and collected from the extract, and \(B\) was the weight of de-pithed bagasse raw material (g), and \(C\) was the total content of hemicelluloses in the de-pithed bagasse (%).

### 2.3 Alkali extraction under microwave irradiation

After the enzyme pretreatment, the bagasse was mixed with alkali solution and heated to 80 °C in a microwave reactor of 900 W power. The liquid-to-bagasse ratio was kept constant at 20:1 (L/kg), and the alkali (sodium hydroxide) concentration was varied from 2% to 10%. The microwave irradiation times varied from 15 to 60 min, with the temperature kept constant at 80 °C through the whole extraction period. At the end of the desired reaction time, the content in the reactor was rapidly cooled down to the room temperature and the liquor was separated by filtration. The solubilized hemicelluloses were neutralized to pH 5.5 with 6 M HCl and concentrated by evaporation under reduced pressure, and then mixed with four volumes of 95% ethanol to precipitate the dissolved hemicellulose. The precipitated hemicelluloses were recovered by centrifugation and freeze-dried.

### 2.4 Specific surface area

The specific surface area (SSA), total pore volume (TPV) and average pore size (APS) of bagasse samples were measured with an ASAP 2020 micropore apparatus (Micromeritics Co., USA). High-purity N\(_2\) was used as the adsorbate. The absorption-desorption of N\(_2\) was conducted at 77 K with a liquid nitrogen trap according to the principle of static volumetric method.

### 3. RESULTS AND DISCUSSION

#### 3.1 Effect of Enzymatic treatment on the specific surface area and porosity of bagasse

The swelling ability of biomass and its accessibility to chemicals can be characterized by the specific surface area (SSA), total pore volume (TPV) and average pore size (APS). As shown in Figure 2, the SSA and TPV of bagasse increased 250.87% and 11.74%, respectively, after the enzymatic pretreatment, in comparison to the untreated samples. The enzyme can cleavage some of the inter-unit bonds and dissolve part of the hemicelluloses and lignin, thus...
opening up the fiber cell wall. It is interesting to note that the APS decreased after the enzyme pretreatment, indicating that the pores created by enzyme had a size smaller than 231 nm.\textsuperscript{16,17}

**Table 1.** Effect of enzymatic treatment on the orosity of bagasse

<table>
<thead>
<tr>
<th>Samples</th>
<th>SSA(BET) /m$^2$/g$^{-1}$</th>
<th>TPV(BJH) /cm$^3$/g$^{-1}$</th>
<th>APS(BJH) /nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.057</td>
<td>$1.508\times10^{-3}$</td>
<td>$2.311\times10^2$</td>
</tr>
<tr>
<td>Enzyme pretreatment</td>
<td>0.200</td>
<td>$1.685\times10^{-3}$</td>
<td>$1.508\times10^2$</td>
</tr>
</tbody>
</table>

### 3.2 Effect of enzyme pretreatment on the hemicelluloses yield

A typical hemicelluloses extraction process is operated at a NaOH concentration of 8-10\%\textsuperscript{18}. It is a compromise between the extraction yield and degradation of hemicellulose. To increase the hemicellulose yield without compromising its qualities, we applied a lower NaOH concentration in the extraction process. The key was to combine the enzymatic pretreatment and microwave irradiation in the alkali extraction process. Results in Fig. 2 show that with the enzymatic pretreatment with 85 IU/g enzyme at 45$^\circ$C for 4 hours the hemicellulose yield reached 62.61\%, which was about 55\% higher than the control (without enzymatic pretreatment), under the same alkali extraction at 4\% NaOH. Without the enzymatic pretreatment, the caustic concentration had to be raised to 6\% or higher to reach the similar extraction yield of hemicellulose. These results demonstrated that the enzyme pretreatment was critically important in promoting the hemicelluloses yield in the extraction process operated at a lower caustic concentration to preserve the qualities of the hemicellulose.

![Fig. 2. Effect of enzyme pretreatment and caustic concentration on the extraction yield of hemicellulose (Other conditions: 85 IU/g enzyme dosage in the pretreatment; 30 min of microwave irradiation time at 80$^\circ$C)](image)

### 3.3 Effect of enzyme dosage on the hemicelluloses yield

![Fig. 3. Effect of enzyme dosage in the enzymatic pretreatment on the hemicelluloses yield (extraction conditions: 6\% NaOH concentration and 30 min of microwave irradiation time at 80$^\circ$C)](image)

As shown in Fig. 3, in the enzyme pretreatment step, with the liquid to solid (L/S) ratio of 6:1, and reaction time of 30 minutes at 80 $^\circ$C under microwave irradiation, the extraction yield of hemicelluloses increased with the increase of the enzyme dosage from 80 to 135 IU/g, and the highest hemicellulose yield of about 86.8\% was achieved with 138 U/g of enzyme in the pretreatment. The enzyme could break the chemical bonds between cellulose and lignin,\textsuperscript{19} and expose the hemicellulose to alkali for easy extraction.

### 3.4 Effect of NaOH concentration on the hemicelluloses yield

![Fig. 4. Effect of NaOH concentration on the extraction yield of hemicelluloses (Other conditions: 135 IU/g enzyme dosage in the pretreatment; 30 min of microwave irradiation time at 80$^\circ$C in the extraction)](image)

In the microwave-assisted caustic extraction step, the effect of NaOH concentration on the hemicelluloses yield is shown in Fig. 4. Under the conditions of 135 IU/g enzyme dosage in the enzymatic pretreatment, microwave irradiation at 80 $^\circ$C for 30 min, about 58.2\% hemicelluloses was
extracted at 3% NaOH concentration. The hemicelluloses yield increased with the increase of the caustic concentration, and the maximum hemicelluloses yield of 79.32% was achieved at 6% NaOH concentration. Further increasing the caustic concentration caused the hemicellulose yield to decrease. The solubility of hemicellulose in the caustic solution increased with the caustic concentration, but too high caustic concentration caused more alkaline hydrolysis of hemicelluloses.\(^2\)

3.5 Effect of irradiation time on the hemicelluloses yield

![Fig. 5. Effect of microwave irradiation time on the extraction yield of hemicelluloses (Other conditions: 135 IU/g enzyme in the pretreatment; 6% NaOH and 30 min of microwave irradiation time at 90 °C in the extraction)](image)

The molecular vibration caused by microwave irradiation can accelerate the dissolution process of hemicellulose and thus increase the extraction yield of hemicellulose. As showed in Fig. 5, over 74% hemicelluloses could be separated when the irradiation was 30 min at 90 °C. However, if the irradiation time was further prolonged to 60 min, the hemicelluloses yield decreased to 66.3%. It was found that extensive microwave irradiation caused a decrease of the pH which led to re-deposition of the dissolved hemicelluloses fiber surface by electrostatic interaction.\(^2\)

4. CONCLUSIONS

Up to 87% of the hemicellulose in bagasse was extracted by the novel microwave-assisted alkali extraction process enhanced with an enzymatic pretreatment. The extraction was performed at a low caustic concentration to preserve the qualities of the hemicellulose. Experimental results showed that the enzyme dosage, alkali concentration and microwave irradiation time were main factors governing the yield of hemicellulose. The optimal conditions of the enzymatic pretreatment were: enzyme dosage of 135 IU/g bagasse and irradiation time of 30 min. Under these conditions, 86.8% of the hemicellulose in the de-pithed bagasse was extracted. After the enzymatic pretreatment the surface area of bagasse increased significantly which facilitated the subsequent microwave-assisted extraction process.

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