The Optimum Process of Wheat-straw Fiber Treated By Enzyme

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Abstract

It was investigated that the optimum process of wheat-straw fiber treated by enzyme. Being boiled with 12% NaOH in 150°C for 4h and bleached with 5% H₂O₂ in 70°C for 6 h, the wheat-straw material used for enzyme treatment was composed of 88.32% cellulose, 1.43% hemicellulose and 2.53% lignin. From the glucose amount and enzyme dose of different samples, enzyme activity measurement formula was attained, and the filter paper enzyme activity and corresponding solid enzyme vitality was got: 0.2969 IU/mL of 2g/L liquid enzyme fibrils and 148.45IU/g. Single-factor experiment was applied in order to get the optimum enzyme treatment condition: water bath in the oscillation trough at the frequency of 80 in 50°C with enzyme usage 35IU/g for 48h. The wheat-straw micro/nano fibrils suspension was got with disposal to mixture system of enzyme decomposing production after enzyme treatment.

Keywords: enzyme treatment; wheat-straw; micro/nano fibrils
Introduction

China is lack of forestry resources, with percentage of forest cover 20.36%, taking up 67% or so of world forest acreage. And forest cover possession per capita is 0.145hm², not reaching to 1/4 of world possession per capita, while forest cumulation per capita 10.151m³, only 14.3% of world cumulation per capita. Therefore, making full use of non-wooden materials is an effective way to make up for the shortage of wood resources in China.

Wheat-straw is a kind of price moderate, fast grown and rich raw material, which can also be applied in wood-based panel. It contains about 65-68% holocellulose and 20-21% acid-insoluble lignin.

Cellulose micro/nano fibrils has many advantages, promising a broad future in application. At present, there are three ways to prepare cellulose micro/nano fibrils, chemical, mechanical and biological methods. Preparing cellulose micro/nano fibrils with enzyme treatment is placid and concentrated, but has a special requirement to enzyme and reaction process.

The aim of the paper is to investigate the optimum preparation process of the micro/nano fibrils from the wheat-straw treated by enzyme.

Materials and Methods

Materials. Wheat-straw material, being boiled with 12% NaOH in 150°C for 4h and bleached with 5% H₂O₂ in 70°C for 6 h; composed of 88.32% cellulose, 1.43% hemicelluloses and 2.53% lignin.

Enzyme treatment process

Enzyme activity measurement

According to the standard measurement commented by International Union of Pure and Applied Chemistry (IUPAC), an international unit (FPU) of filter paper enzyme energy equals to the enzyme amount that created 1 mol glucose in standard condition in one minute, as following:

(1) In 5 of the 6 cuvettes, add 50 mg rotary filter paper (1×6 cm) to each cuvette and dilute enzyme liquor relevantly. Manipulate as table 1. (No.5 with substrate without enzyme, No.6 with enzyme without substrate)

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
</table>

Tab. 1 different adding amount of diluted enzyme and 0.05M citric acid buffer solution

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(2) Seal the cuvettes with plastic cloth and tie with elastics. Bath in water in 50°C at the frequency of 80 for 60 minutes. Add 3 ml DNS solution rapidly after taking it out and boil it in 100 °C for 5 minutes. Afterwards, cool it down in ice water and make it constant volume 50 ml. Shake up and measure absorbency OD in 550 nm, and get the glucose amount in reaction according to the glucose standard curve.

(3) Chart the glucose amount produced by 0.2, 0.3, 0.4, 0.5 ml enzyme separately as abscissa, and the logarithm of enzyme dose as ordinate. The enzyme needed to product 2 mg glucose was attained from the chart, and then the filter paper enzyme energy of sample can be calculated with formula.

**Optimum enzyme amount**

(1) To ensure reaction process equally in the water bath oscillation trough, control the buffer solution volume 25ml and substrate quality 0.5g on the basis of reaction container. Mark 5, 15, 25, 35, 50 IU/g substrate separately with sample number 1-5 to ascertain the enzyme amount( the substrate is production apart from the hemicelluloses and lignin which contains 88.32% cellulose), as tab. 2.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Substrate quality (g)</th>
<th>Enzyme usage (IU/g substrate)</th>
<th>Enzyme quality needed (mg)</th>
<th>Buffer solution volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>5</td>
<td>16.84</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>15</td>
<td>50.52</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>25</td>
<td>84.20</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>35</td>
<td>117.88</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>50</td>
<td>168.41</td>
<td>25</td>
</tr>
</tbody>
</table>

(2) Decompose the samples in water bath oscillation trough at the frequency of 80 times each minute in 50°C. After centrifuge treatment, take clear solution of the enzyme decomposing production to measure the glucose concentration with DNS method.

**Optimum enzyme decomposing time**

Choose enzyme usage as 35IU/g, and enzyme decompose in 50°C in water bath oscillation trough at the frequency of 80 for 12h, 24h, 48h, 72h separately. After centrifuge treatment, take clear solution of the enzyme decomposing production to measure the glucose
concentration with DNS method.

**Disposal to mixture system of enzyme decomposing production**

(1) To release the enzyme activity, cook the flask of mixture with enzyme decomposing production in boiling water for 5 min.
(2) Wash in centrifuge to wipe out the soluble materials of the mixture system such as glucose, and repeat it 3 to 5 times.
(3) After centrifuge washing, disperse the fibrils with trifle enzyme in distilled water. Add disperse system and chloroform in a cuvette in 1:4 volume proportion. Shake up and then keep static for 30min±5min. Finally, the mixture system was delaminated. The upper-layer was chloroform, under-layer water, and the middle-layer was fibrils and deposition which was denaturalized protein in chloroform.
(4) In the cuvette, protein deposited in the bottom of water while the fibrils suspension separated out. Extracting the upper suspension with tubularis, and fibrils without protein was attained. In avoid of protein, 5-6 cm bottom of water cannot be extracted.

**Results And Discussion**

**Enzyme activity mesuration**

The absorbency of sample is listed in tab. 3:

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>absorbency</td>
<td>0.432</td>
<td>0.537</td>
<td>0.601</td>
<td>0.685</td>
<td>0.055</td>
<td>0.204</td>
</tr>
</tbody>
</table>

Substitute the absorbency to the standard curve formula \( y=12.85x-0.0377 \), the glucose amount of sample 1-4 was attained, as in tab. 4:

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose amount (mg)</td>
<td>0.820</td>
<td>1.228</td>
<td>1.477</td>
<td>1.804</td>
</tr>
<tr>
<td>logarithm of enzyme dose</td>
<td>-0.6990</td>
<td>-0.5229</td>
<td>-0.3979</td>
<td>-0.3010</td>
</tr>
</tbody>
</table>

Charting the glucose amount as abscissa, and the logarithm of enzyme dose as ordinate, the enzyme activity formula can be attained: \( y=0.412x-1.029 \) \((R^2=0.991)\), as fig. 1 shows.
Fig. 1  the enzyme activity measurement formula

According to the above formula, the enzyme needed to produce 2ml glucose is 0.6237mL.
Choosing the testing liquor concentration as 2g/L, then calculate the enzyme activity with the formula:

\[
\text{Filter paper enzyme activity} = \frac{2 \text{mg glu cos e}}{60 \text{min} \times 0.18(\text{mg/μmol}) \times \text{the enzyme needed to produce 2mg glu cos e (mL})}
\]

So the filter paper enzyme activity of 2g/L liquid enzyme fibrils is 0.2969 IU/mL, and its corresponding solid enzyme vitality is 148.45IU/g.

**Optimum enzyme amount**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose concentration after 24h enzyme decomposing (g/L)</td>
<td>0.107</td>
<td>0.200</td>
<td>0.249</td>
<td>0.320</td>
<td>0.324</td>
</tr>
<tr>
<td>Glucose concentration after 72h enzyme decomposing (g/L)</td>
<td>0.215</td>
<td>0.388</td>
<td>0.454</td>
<td>0.481</td>
<td>0.492</td>
</tr>
</tbody>
</table>

From tab. 5 we can conclude that productions of sample 4 and 5 contained highest glucose amount. In the view of economy, choose sample 4 as the optimum enzyme amount, namely the enzyme usage is 35IU/g.

**Optimum enzyme decomposing time**

Tab. 6 the glucose concentration of different decomposing time
<table>
<thead>
<tr>
<th>Enzyme decomposing time (h)</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose concentration (g/L)</td>
<td>0.222</td>
<td>0.323</td>
<td>0.450</td>
<td>0.481</td>
</tr>
</tbody>
</table>

From tab.6 we can choose 48h as optimum decomposing time, with the glucose concentration 0.45 g/L, as decomposing efficiency decreased with time increasing.

**Conclusions**

The filter paper enzyme activity of 2g/L liquid enzyme fibrils is 0.2969 IU/mL, and its corresponding solid enzyme vitality is 148.45IU/g. The optimum enzyme treatment condition was water bath in the oscillation trough at the frequency of 80 in 50℃ with enzyme usage 35IU/g for 48h. With enzyme treatment above, micro/nano fibrils can be attained from wheat-straw fiber. Preparing micro/nano fibrils with enzyme is complicated to control the reaction, so it’s still called for further research before put into manufacturing.

**Acknowledgements**

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**References**

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