Enzymatically Modified Cellulase and Its Application in Papermaking

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Abstract

The optimal dosage of TG enzymatically modified cellulase and the change of its heat resistance were determined by measuring the enzyme activity of filter paper. Ninhydrin colorimetric method and BCA method explore the changes of free amino group and protein content during enzymatic modification. Enzymatically modified cellulase treated OCC pulp to explore its influence on pulp fiber quality and paper quality. The effect of TG enzymatic modification of cellulase is the best when the TG enzyme is added at 10 u/mL, and the heat resistance at 60°C is increased by 20% compared with the original enzyme. The free amino group of the enzymatic cross-linked cellulase reaction was reduced by 0.3 μmol/mL, and the protein content was reduced by 500 mg/mL. The fiber quality of OCC pulp treated with enzymatically modified cellulase has been improved, and the break length of the paper increased, and the ring pressure index increased.

Keywords

Papermaking; Cellulase; Filter Paper.

1. Introduction

In the pulp and paper industry, the addition of cellulase can help reduce the chemical substances and energy required for fiber modification [1], which can improve the economics of the papermaking process and reduce the impact on the environment. Suitable cellulase mainly acts on fissures with large accessibility and large specific surface area, free fine fibers and fine fibers in the non-crystalline area of the fiber surface, reducing the content of fine fibers and improving the physical properties of paper.

The protein space structure of the cellulase that degrades cellulose is divided into three parts: the protein catalytic region (CD or CP), the linker, and the cellulose binding region (CBD) with cellulose adsorption capacity [2], or the carbon water at the C terminal. The compound binding module (CBM) consists of three parts. Cellulase from different sources has different specific cellulose binding regions [3]. Excessive cellulase will react excessively from the voids and cracks of the fiber, which will deepen the voids and cracks of the fiber [4], reduce the quality of the fiber, and peel off the fine fibers from the outer surface of the long fiber, which affects the quality of the pulp. The binding domain of cellulase is irreversibly adsorbed on the surface of lignin, which affects the treatment of papermaking fibers by cellulase [5] Based on the above ideas, TGase is used to catalyze the acyl transfer reaction of the cellulase itself to realize the cross-linking of the cellulase itself. The average free amino group modification rate on the enzyme protein molecule, cellulase activity before and after modification, and protein content before and after modification were used as indicators to investigate the influence of different modification conditions on the modification reaction. Explore the mechanism of the enzymatic modification of transglutaminase (TGase) on the enzymatic hydrolysis of cellulase, and the effect of enzyme treatment on pulp drainage, water retention, fiber length distribution and paper properties.

2. Experimental materials

BCA protein quantitative determination kit—Dalian Meilun Biotechnology Co., Ltd.; Xinxing 12.5CM medium-speed qualitative filter paper; waste corrugated box (OCC); cellulase (200 u/mL)

IMT-VL01 Valley Beater; IMT-DJD-DDJ Beating Degree Tester; IMT-SJ01 Standard Fiber Breaker; IMT-CP01B Paper Former; IMT-Burst01P Burst Strength Tester; IMT-202F Type Microcomputer Tensile Strength Tester; IMT-Compress01 Computer Controlled Compressive Strength Tester; MB100-4A Enzyme Orifice Plate Constant Temperature Oscillator; Biotek Microplate Reader.

3. Experimental method

3.1 Determination method of cellulase activity

Put a piece of 1.0 cm×6.0 cm filter paper folded short side into the colorimetric tube vertically, take 2 mL of the test solution preheated at 50 ℃ in advance, and place it in the test tube water bath at constant temperature for 60 min, add 3 mL of DNS reagent, and place it in a boiling water bath. Color for 10 minutes, and cool in a cold water bath quickly. Add distilled water to make up and shake well. The absorbance was measured at 540 nm [6] with a blank tube to zero, and the glucose standard curve was obtained as $y=0.1807x+0.0018$, $R^2=0.9993$. Calculate the enzyme activity against the glucose standard curve, and multiply the measurement result by the dilution factor.

Filter paper activity definition: 1 mL of liquid enzyme is hydrolyzed on the filter paper substrate at 50℃ and specified pH for 1 h to produce reducing sugar equivalent to 1 mg of glucose, which is 1 unit of enzyme activity. Expressed in u/mL.

3.2 The content of free amino groups in the ninhydrin method solution

Draw a standard curve with glycine as the standard, as shown in Table 1 in Figure 1: The glycine concentration and absorbance have a good linear relationship. The linear equation is $y=0.0956x-0.0079$, and the correlation $R^2=0.9991$. Take 1 mL of the reaction solution into a colorimetric tube, then add 1 mL of pH 6.8 sodium acetate-acetic acid buffer, 1 mL of ninhydrin solution, seal it and shake it well, heat it in a boiling water bath for 15 minutes, and then Take it out and put it in cold water to cool for 15 minutes. After the above process, add 5 mL potassium iodate solution to the colorimetric tube and dilute to 10 mL. Adjust the wavelength of the UV-Vis spectrophotometer to 568 nm, adjust the absorbance of the blank sample in the colorimetric tube with distilled water to 0, and then measure the absorbance of other samples in sequence.

3.3 Protein content before and after the enzymatic crosslinking reaction of BCA method

Take 20μL of protein standard solutions and samples of different concentrations into a 96-well microtiter plate, add 200μL of working solution, use the enzyme well plate thermostatic shaker to shake for 30s, place in a 37℃ incubator oven for 30min, use a microplate reader to measure the results at 562nm The absorbance value of the blank control should be subtracted. Draw a standard curve with the concentration and absorbance of the protein standard, and put the absorbance of the sample into the linear formula to obtain the protein content.

3.4 Enzymatic treatment of OCC pulp

Take an appropriate amount of waste corrugated cardboard boxes (OCC), weigh a certain quality of OCC and soak for more than 12 hours. The laboratory Valley beater is slightly beaten, and the beating degree is controlled at about 37 °SR. Add water to the slurry concentration to 1% (mass percentage), adjust the pH to 6.0 after dissolving 30,000 revolutions, then add the diluted enzyme solution, and react at a constant temperature in a water bath at 50 ℃. Knead at intervals to mix the slurry with the enzyme. When the reaction reaches 1.5 h, adjust the pH to 12, inactivate for 10 min, wash to neutral, and balance the moisture to be measured.

3.5 Analysis of pulp fiber by fiber quality analyzer

The measured parameters include length, thickness, fine fiber content, crimp, kinking index, brooming rate, etc.

3.6 Papermaking and paper physical performance testing

The basis weight of the paper is 120 g/m2, and it is dried under negative pressure at 105 ℃ for 5 min. Treat the handsheets under constant temperature and humidity conditions for more than 4 hours, and

then determine the basis weight of the paper (GB/T 451.2-2002), ring compression strength (GB/T 2679.8-1995), and tensile strength (GB/T 453- 2002) and burst resistance (GB/T 454-2002) for testing.

4. Results and discussion of the effect of TG enzymatic modification

4.1 The amount of TG enzyme added and its effect on the thermostability of modified cellulase

Take 10 mL of 10% neutral cellulase solution and add a certain amount of TG enzyme. The amount of TG enzyme (u/mL) is (5, 7.5, 10, 12.5, 15) and react in a water bath at 40°C for 2 h. Determine fiber Enzyme activity. To determine the change in thermal stability, the enzymatically modified neutral cellulase solution was kept in a water bath at 40°C, 50°C, and 60°C for two hours to determine the cellulase activity.

When the amount of TG enzyme is 10 u/mL, the effect of TG enzymatic modification of cellulase is the best, and its heat resistance is the best. At the end of the TG enzymatic reaction, the enzymatic activity of the cellulase modified by TG enzymatically increased by 12.3% compared to the control group. For thermal stability, it increased by 16% at 40 ℃ and 4% at 50 ℃. , It increased by 20% at 60 ℃.

Table 1. The effect of the amount of TG enzyme on the enzyme activity and heat resistance of enzymatically modified cellulase (u/mL)

TGase addition amount	Enzymatically modified cellulase	Heat resistance		
		40℃	50℃	60℃
0	53.85±0.05	36.95±0.35	39.35±0.35	33.15±0.15
5	51.25±0.15	37.6±0.3	40.6±0.3	35.05±0.55
7.5	60.5±0.4	42.15±0.45	39.95±0.15	35.9±0.3
10	60.4±0.1	42.9±0.1	40.85±0.55	39.6±0.2
12.5	60.1±0.1	40.8±0.05	38.85±0.15	35.1±0.1
15	58.45±0.6	39.6±0.1	42.1±0.5	36.8±0.2

4.2 The change of free amino group content before and after the enzymatic modification by the ninhydrin method

It can be seen from Table 2 that after the TG enzyme enzymatically catalyzed cross-linked cellulase reaction, the free amino groups were reduced by 0.3 μmol/mL and the total amount of amino groups decreased, which confirmed that the enzymatic TG enzyme reacted with cellulase. Comparing the cellulase + inactivated TGaes group, the free amino group did not change significantly.

Table 2. Free amino content before and after the enzymatic crosslinking reaction/(μmol/mL)

	Before reaction	After the reaction
cellulase	0.99±0.02	1.19±0.01
cellulase +TGase	3.62±0.04	3.35±0.01
cellulase + inactivated TGase	2.92±0.02	2.95±0.03
inactivated cellulase +TGase	4.33±0.05	3.56±0.05

4.3 Changes in protein content before and after the enzymatic crosslinking reaction

Table 3 shows that TGase enzymatic cellulase has a cross-linking reaction, and the protein content after the reaction is 500 mg/mL less than that after the reaction. In the control group, the protein content of fiber + inactivated TGase histone did not change significantly, and the protein content of inactivated fiber + TGase histone would increase.

Table 3. Changes of protein content before and after the enzymatic crosslinking reaction/(mg/mL)

	Before reaction	After the reaction
cellulase	5702±25	6381±31
TGase	2678±28	2296±31
cellulase +TGase	7445±6	6943±31

4.4 The influence of cellulase and modified cellulase on pulp fiber quality

Table 4 shows the changes of fiber morphological parameters of OCC pulp after cellulase and enzymatic cellulase treatment. Enzymatic cellulase has obvious changes in fiber length, width and fine fiber content than cellulase, indicating that the enzymatic cellulase has greater freeness and preferentially degrades fine fibers during the treatment process, and the fiber thickness is compared with the blank sample. The increase indicates that the laccase-catalyzed cellulase treatment not only occurs on the surface of the fiber, but also involves the inside of the fiber. Water molecules enter the inside of the fiber to swell and cause the fiber mass to increase, which leads to the increase of fiber thickness; Cellulase promotes fiber swelling and makes the fiber tend to be straight, resulting in a decrease in fiber kink index.

Table 4. OCC fiber morphology with Enzymatically modified cellulase treatment

system	length/mm		fiber width/μm	fines/%	curl index/%	kink index/m^{-1}
	L_l	L_w				
untreated	0.994	1.560	19.865	84.05	9.79	827.3
cellulase	0.993	1.567	19.965	83.405	8.89	801.85
Enzymatically modified cellulase	1.018	1.603	21.055	81.84	10.04	761.95

1) L_l: weightedaverage fiber length; L_w: weight-weightedaverage fiber length

4.5 The effect of cellulase and modified cellulase on the physical strength of paper

At a certain slurry concentration, the enzyme binds to the substrate, especially the small fibers with a large specific surface area, which is mainly manifested in the enzymatic hydrolysis of free small fibers by the enzyme. The content of free fine fibers that are preferentially combined with enzymes is significantly reduced. At this time, the filtering effect is best and the beating degree drops from 33°SR to 26°SR. Because the enzymatically modified cellulase increases the fiber length, width, and curl index, it increases the break length of the paper and the ring pressure index.

Table 5. The effect of enzymatically modified cellulase on the beating degree of pulp and the physical strength of paper

system	Percussion /°SR	Tensile index /(N•m/g)	Crack length /(km)	Folding endurance/time	Ring pressure index /(N•m/g)
untreated	33	34.9	3.5563	5/5/4/4	9.84
cellulase	29	34.9	3.5643	3/4/4/4	9.93
Enzymatically modifid cellulase	26	35.4	3.7953	5/4/5/4	11.01

5. Conclusion

When the addition amount of TGase in the 10% neutral cellulase solution is 10 u/mL, the effect of TGase enzymatic modification of cellulase is the best, and its heat resistance is the best. At 60 ℃, the control group without TG enzyme increased by 20%. Although the cellulase activity is lost during the enzymatic modification of cellulase by TGase, it may be that cellulase is cross-linked to form cellulase aggregates, and part of the enzyme activity center is consumed by cross-linking. Enzymatic cross-linking modification can effectively improve the functional properties of cellulase.

The experiment was confirmed that the enzymatic TG enzyme reacted with cellulase, and the total amount of amino groups decreased by 0.3 μmol/mL. After the TG enzyme enzymatic cellulase reaction, the protein content was reduced by 500 mg/mL compared with that after the reaction. The mechanism of the effect of TG enzymatic modification on the enzymatic hydrolysis of cellulase, the effect of enzyme treatment on pulp drainage, fiber length distribution, and paper tensile index and

ring pressure index indicate that the type of enzyme components may be very important. In addition, cellulose The presence or absence of the binding domain may play a decisive role.

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