



Effects of two different enzyme treatments on the microstructure of outer surface of wheat straw

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Received: 24 October 2021 / Revised: 22 November 2021 / Accepted: 24 November 2021 / Published online: 4 January 2022
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Abstract

The outer surface of wheat straw (WS) plays a critical role for the penetration of chemicals in the pulping production. In this study, we characterized the changes of outer surface before and after enzyme treatment. The results showed that neutral cellulase and lipase had different effects on the microscopic morphology of the outer surface of WS. Both enzyme treatments reduced the silicon content on the outer surface of WS, and the effects of neutral cellulase treatment were more obvious. Neutral cellulase mainly destroyed the vascular bundles on the outer surface of WS, degraded part of the cellulose, and then promoted the shedding of the siliceous layer connected to the cellulose, while lipase mainly destroyed the cuticle on the outer surface of WS. Furthermore, neutral cellulase promoted the dissolution of various sugar components, especially the dissolution of glucose. The dissolution of glucose had increased by 7 times with the addition of neutral cellulase. Therefore, adding a process of enzyme treatment before pulping production could destroy the microstructure of the outer surface of WS, provide favorable conditions for the penetration of chemicals in the subsequent pulping process, and reduce the use of chemicals.

Keywords Wheat straw · Enzyme · Microstructure · Surface

1 Introduction

The background of increased fossil fuel consumption has sown the seeds of environmental pollution, global warming, shortage of forest land, and resource supply/demand imbalance, which have caused a high cost of wood and papermaking [1–7]. Searching for alternative energy sources from renewable resources, agricultural waste

biomass has become an important field in the global economy [8–11]. China is a predominantly agricultural country with rich grass resources, among which the annual yield of wheat straw (WS), rice straw, and reed is high [12–18], while traditional incineration of agricultural waste leads to air pollution and waste of resources. Green ecological development will be achieved by converting large amounts of available, renewable, and inedible agricultural waste biomass into usable energy sources, fuels, and chemicals [19–31].

WS is an annual grass plant of the *Gramineae* with high environmental tolerance and is widely distributed in the northern land of China. WS has the characteristics of high cellulose content, loose structure, and thin cell wall, so it has great potential for papermaking among the primary agricultural wastes [32–35]. However, the market occupation rate of traditional soda-AQ pulp is still low in the papermaking market. Furthermore, it has many disadvantages, such as low pulping yield, high pollution, and difficult alkali recovery of black liquor due to the short growth cycle and the high content of non-fibrous cells [36–38]. On the other hand, China has completely banned the imports of solid waste from 2021, resulting in

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a shortage of mainstream sources such as secondary fiber resources of papermaking [39]. According to the above background, developing new technologies of replacing waste paper pulp with WS of high yield, less resin, and low energy consumption will be an effective way to solve the current shortage of raw materials for papermaking.

In recent years, more and more studies on the chemical recalcitrance of lignocellulosic biomass have been investigated [40, 41]. The adequate degradation of hemicellulose and lignin can be overcome by eco-friendly, time-saving, and large-scale operational pretreatment methods [42–54]. It should be noted that pretreatment is performed without affecting the subsequent efficiency of cellulase hydrolysis or the quality of pulp production [55]. Currently, pretreatment methods, such as chemical pretreatment or physicochemical pretreatment processes, have not yet achieved optimal industrial output in a sustainable and eco-friendly way [56–70]. In consequence, people pay more and more attention to biological enzyme technology. It can reduce energy consumption and the use of chemicals in the pulping process. At the same time, it is easy to manage waste liquid [71]. Biological enzyme technology has great potential in reducing environmental pollution, improving pulp performance, and comprehensive utilization of lignocellulosic biomass [72–74]. Currently, three enzymes are used to treat lignocellulosic biomass, namely cellulase, hemicellulose, and ligninase [75–78]. Cellulase can be utilized to improve the performance of dissolving pulp [79] and reduce the energy consumption of pulp refining [80]. Hemicellulase is mainly used to assist in the pulp bleaching and reduce environmental pollution load [81, 82]. Main ligninases such as laccase can be used to treat lignocellulosic biomass and reduce pollution [83]. For example, Saleem et al. had demonstrated that enzyme treatment could hydrolyze the xylan in the fiber, thereby enhancing the permeability of the pulp [84]. Meighan et al. had proved that biological enzyme technology had great potential in reducing environmental pollution and the comprehensive utilization of lignocellulosic biomass [85]. Varghese et al. had demonstrated that enzyme pretreatment successfully reduced chemical loading during alkaline pulping along with the production of higher quality paper [86]. Nagpal et al. had reported that enzymes played a crucial role in regulating the permeability of pulping chemicals along with enhancing the WS pulp yield and quality [87].

In the paper industry, the previous researches of biological enzyme technology were basically focused on slurry and biofuels. This article mainly explored the effects of neutral cellulase and lipase treatments on the outer surface of WS [88–90]. Under relatively mild conditions, the cuticle and siliceous layers on the surface of WS were separated and degraded by enzymes, which could provide favorable

conditions for the later pulping process. Modern instrumental analyses such as ion chromatography (IC), scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), and micro-CT were employed to characterize changes in the microstructure of WS and the composition of the pre-hydrolyzed solution. A single-factor experiment was used to optimize the enzyme pretreatment conditions of WS and hoped to provide technical support and theoretical basis for the combination of biomass refining and pulp and paper technology.

2 Materials and methods

2.1 Materials

Neutral cellulase and lipase are produced by Shandong Longkete Enzyme Preparation Co., Ltd. Acetic acid, sodium acetate, potassium dihydrogen phosphate, and disodium hydrogen phosphate dodecahydrate are produced by China National Pharmaceutical Group Corporation. Fifty percent NaOH solution is purchased from Shanghai Macklin Biochemical Co., Ltd. Glucose, arabinose, galactose, xylose, and mannose were all purchased from Shanghai Macklin Biochemical Co., Ltd. All chemical reagents are of analytical grade and no further purification is required. The rolled WS is taken from a paper mill in Weifang City, Shandong Province, and used as raw material after being washed and dried.

2.2 Methods

2.2.1 Enzyme treatment of wheat straw

Two different enzymes were used to process WS. After balancing the moisture content of the raw materials, 20 g (calculated on the absolute dry basis) of WS was taken and placed in a polyethylene bag. Acetic acid-sodium acetate buffer at pH 5.5 was used for cellulase and phosphate buffer at pH 7.5 was used for lipase and adjusted the solid–liquid ratio to 1:6. The mixed slurry was put in a constant-temperature water bath and treated for a while at the optimum temperature of enzyme (the optimum temperatures of neutral cellulase and lipase are 55 °C and 45 °C, respectively). Then, part of the solid sample was taken, washed, and freeze-dried. The remaining sample was squeezed out of the reaction solution and stored at 4 °C. The specific experimental parameters are shown in Table 1.

2.2.2 Characterization

The SEM images of the outer surface of the untreated and treated WS were obtained using a scanning electron

Table 1 Experimental conditions with different samples

Sample name	Enzyme type	Enzyme dosage (U/g)	Processing time (min)	Processing temperature °C
BWS ₁	Acetic acid-sodium acetate buffer with a pH of 5.5	0	30	55
BWS ₂		0	60	
BWS ₃		0	90	
NCWS ₁	Neutral cellulase	10	30	
NCWS ₂		20	30	
NCWS ₃		30	30	
NCWS ₄		10	60	
NCWS ₅		20	60	
NCWS ₆		30	60	
NCWS ₇		10	90	
NCWS ₈		20	90	
NCWS ₉		30	90	
BWS ₄	Phosphate buffer with pH 7.5	0	30	45
BWS ₅		0	60	
BWS ₆		0	90	
LWS ₁	Lipase	10	30	
LWS ₂		20	30	
LWS ₃		30	30	
LWS ₄		10	60	
LWS ₅		20	60	
LWS ₆		30	60	
LWS ₇		10	90	
LWS ₈		20	90	
LWS ₉		30	90	

microscope (TM4000Plus, Hitachi, Japan) at an accelerating voltage of 15 kV. The Model 550i EDS Detector Power coupled with the TM4000Plus scanning electron microscope was used to analyze the elements on the outer surface of WS to determine the content and distribution of the elements.

The WS sample was cut into 1 mm × 1 mm × 1 cm size and fixed to the sample rod. Then the WS sample was scanned by 15C13014 microscopic CT at 300 nm to observe the internal structure of WS. The scanner model is Sky-Scan2211, the power supply voltage is 50 kV, the source current is 350 μA, the source focus mode is micro-focus, the camera model is MX11002, the exposure time is 500 ms, and the image pixel size is 0.3 μm.

A certain amount of reaction solution was taken, and the pH of the impregnating solution was adjusted to 3.5 with dilute sulfuric acid. After centrifugation, 1 mL of supernatant was taken and placed in a 10-mL digestion tube. In total, 70 μL 72% H₂SO₄ and 0.93 mL deionized water were placed in a multifunctional intelligent digestion apparatus (GL-16, Greencare Company, China) at 121 °C for 60 min [91]. Dilute the supernatant to an appropriate multiple, prepare a 1000 mg/L standard sugar solution (arabinose, galactose, glucose, xylose, mannose), and dilute to 0.5, 1.0, 2.0, 5.0, 10.0 mg/L. The samples and the sugar standard solutions

were filtered into the sample bottles by 0.22-μL inorganic syringe filters. The composition and content of monosaccharides in the WS treatment solution were determined by an ion chromatograph (ICS-5000+, Thermo Fisher Scientific, USA), Carbo Pac PA20 series column (3 mm 150 mm), guard column (3 mm 30 mm), and EC detector (working electrode is Au electrode). The sample volume is 25 μL. The column temperature is 30 °C. The mobile phase was eluted with 250 mmol/L NaOH and distilled water in a ratio of 96% and 4% at a flow rate of 0.4 mL/min and a time of 55 min.

3 Results and discussion

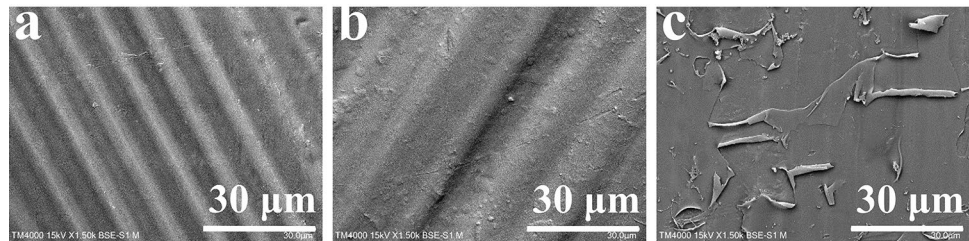
3.1 The effects of neutral cellulase treatment on wheat straw

3.1.1 SEM images

The SEM was used to explore the influence of neutral cellulase treatment on the outer surface of WS, as shown in Figs. 1 and 2.

It can be clearly observed that the outer surface of the untreated WS was relatively smooth, and the epidermal cells

Fig. 1 SEM images of WS outer surface, **a** untreated, **b** acetic acid-sodium acetate buffer 90-min treatment, **c** neutral cellulase 30 U/g, 90-min treatment

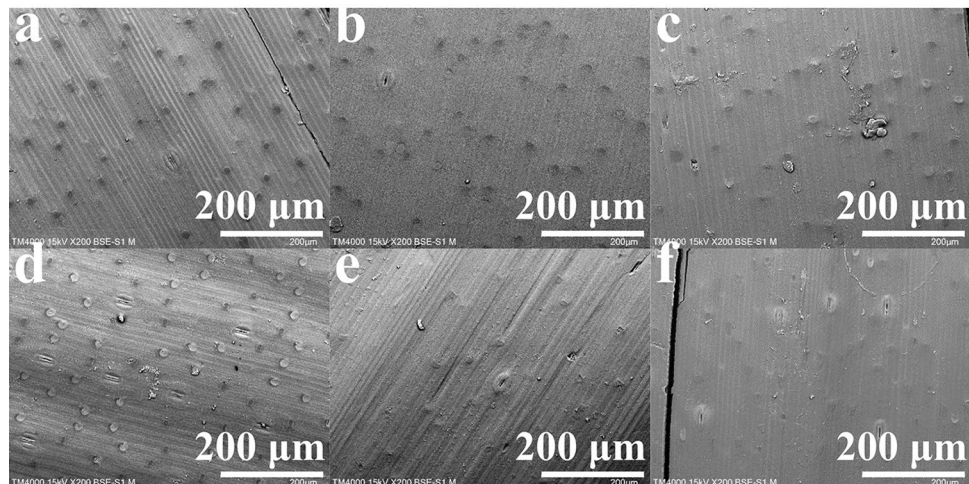


were covered with a dense and thick membrane (Fig. 1a). This membrane was the cuticle and siliceous layers on the outer surface of WS. It had a certain hardness and could protect the internal fibers of WS. However, it would also hinder the impregnation effect of the solution in the pretreatment process [92–95]. At the same time, there were some damages on the outer surface caused by mechanical external forces, which might be caused by harvesting or making samples. It can be observed from Fig. 1b that only some tiny protrusions appeared on the outer surface of WS after 90 min of buffer treatment. There was no new damage except those caused by external mechanical forces. The results showed that the buffer treatment had little effect on the cuticle and siliceous layers and did not affect the final result. Figure 1c displays that after treatment with 30 U/g neutral cellulase for 90 min, the membranes on the outer surface of WS were obviously damaged and fallen off, indicating that neutral cellulase destroyed the membranes on the outer surface of WS, and part of the cuticle and siliceous layers were removed.

The outer surface of WS was observed with $\times 200$ magnification by SEM. It can be clearly noticed that the outer surface of untreated WS was relatively smooth, and the outer surface of WS was not damaged (Fig. 2a). From Fig. 2b, it can be found that the outer surface of WS treated with buffer for 30 min was similar to that of untreated WS, and there was no change, indicating that the short-term warm water immersion would not affect the outer surface of WS. From Fig. 2c, it can be found that protrusions obviously began

to appear on the outer surface of WS after the neutral cellulase treatment. The reason for this phenomenon was that the neutral cellulase entered the WS through the breathing holes and small damages on the outer surface of the WS and decomposed the fibers in the vascular bundle tissue, parenchyma, and fibrous tissue band. Then the dissociation of the tissue resulted in exfoliation of cuticle and siliceous layers [96]. Moreover, the protrusions on the outer surface of WS gradually increased, and some areas were damaged with the amount of neutral cellulase increasing (Fig. 2d). The reason for this phenomenon was that more neutral cellulase had entered the vascular tissue on the outer surface of WS. The degraded fibers increased, and the damage to the microstructure of WS was greater. As a result, the damage was more obvious. There were some small protrusions on the outer surface of WS in Fig. 2e. It indicated that long-time warm water soaking had a certain impact on the cuticle and siliceous layers. However, the impact on the cuticle and the siliceous layer was very small and would not affect the experiment. Part of the fibers of internal vascular bundle tissue were hydrolyzed, which affected the outer surface of WS. Compared with Fig. 2c, it showed that the neutral cellulase promoted the degradation of the fibers of the vascular bundle tissue and made the outer surface of the WS change more obviously. It can be seen from Fig. 2d and f that the damage degree of WS surface gradually increased, broke, or fell off, with the extension of neutral cellulase treatment time. The reason for this phenomenon was that with the

Fig. 2 SEM images of WS outer surface: **a** untreated; **b** acetic acid-sodium acetate buffer 30-min treatment; **c** neutral cellulase 10 U/g, 30-min treatment; **d** neutral cellulase 30 U/g, 30-min treatment; **e** buffer 90-min treatment; **f** neutral cellulase 30 U/g, 90-min treatment



treatment time of neutral cellulase prolonged, more vascular bundle tissue fibers were degraded. Then the microstructure of WS surface had changed by damaging the organizational structures of WS, which gradually increased the damage on the outer surface of WS [97].

As shown in Fig. 2, the neutral cellulase caused a certain degree of damage to the cuticle and siliceous layers. The cuticle and siliceous layers on the outer surface of WS were further destroyed by increasing the amount of neutral cellulase and enzyme treatment time. It is preliminarily believed that these protrusions were aggregates of silicon elements after neutral cellulase treatment. With increasing the amount of neutral cellulase and enzyme treatment time, these protrusions increased, then broke and fell off.

3.1.2 SEM-EDS analysis

In order to further analyze the effect of neutral cellulase treatment on the outer surface element content of WS, the outer surface of WS was observed by EDS Detector Power. The results are shown in Table 2. In Table 2, a is the untreated WS, b is the sample NCWS₁, c is the sample NCWS₃, and d is the sample NCWS₉. The outer surface silicon content of untreated WS was extremely high, while the silicon content decreased after adding neutral cellulase. After 30 U/g neutral cellulase treatment for 90 min, the content of silicon reached the lowest.

Therefore, with the increase of the amount of neutral cellulase, the content of silicon decreased. This may be because the neutral cellulase entered the vascular bundles, parenchyma, and fibrous tissues through the breathing holes and tiny broken gaps on the outer surface of the WS. It degraded part of the fibers, and the cellulose was degraded to glucose, which reduced the bonding force between the siliceous layer attached to this part of cellulose fiber and the fiber; thus, the siliceous layers were broken and exfoliated. Besides, with the extension of the treatment time, the neutral cellulase promoted the degradation of internal tissue fibers on the outer surface of the WS more fully, and the effect of destroying the microstructure was more obvious. Then, the content of

silicon element decreased, along with more outer surface film falling off. The results of element content analysis are consistent with the abovementioned SEM.

Figure 3 are all elements analysis of WS outer surface. From Fig. 3a–d, it can be clearly observed that after treatment with neutral cellulase, the distribution of silicon became sparse, especially after 30 U/g, 90-min treatment with neutral cellulase. It can be further proved that after neutral cellulase treatment, the silicon content on the outer surface of WS had reduced. As can be seen from Fig. 3c, the distribution of silicon element was obviously dense, where there are protrusions on the outer surface of WS. Therefore, it can be proved that the abovementioned conjecture in SEM was correct. The protrusions on the surface of WS in Fig. 2c were aggregates of silicon. With the extension of the neutral cellulase treatment time, the distribution of silicon became sparser. Because with the extension of the enzyme treatment time, the number of protrusions gradually increased, and then they were easily damaged and removed, thereby part of the silicon was removed from the outer surface of the WS. The above results proved that the amount of neutral cellulase and enzyme treatment time had effect on the removal of silicon.

3.1.3 Micro-CT analysis

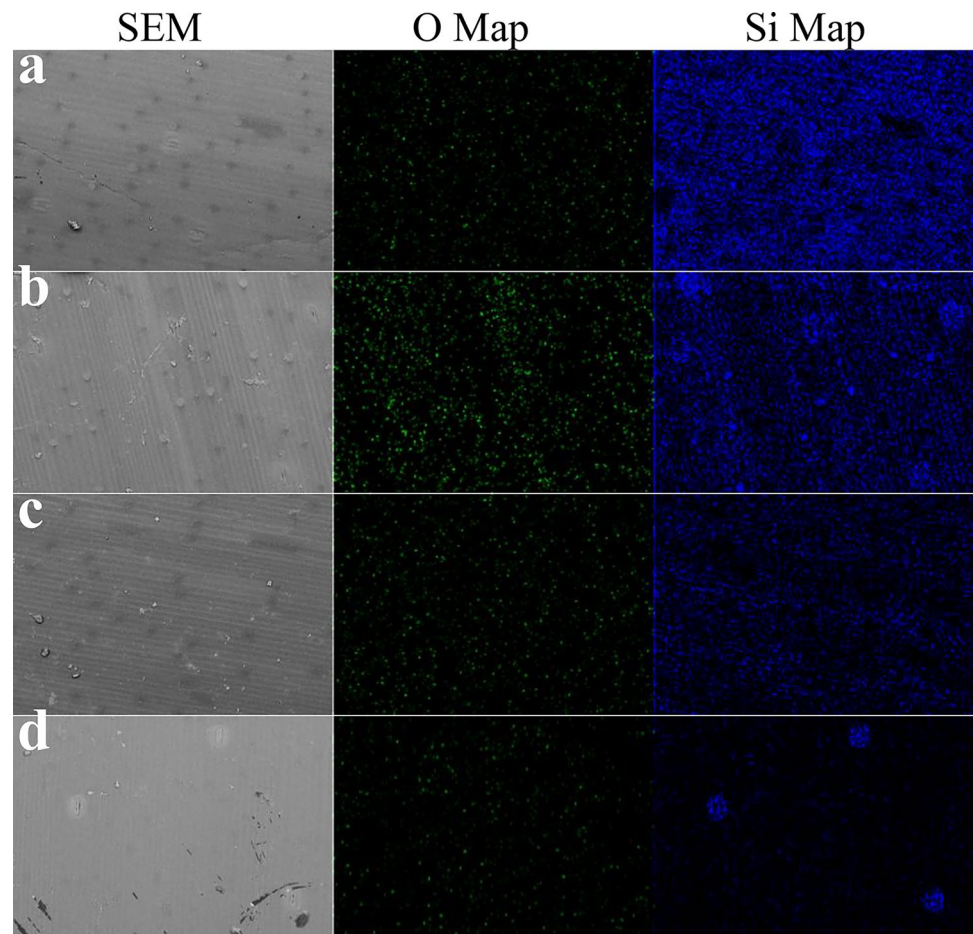
Figure 4 shows the changes in the fiber morphology and structure of the WS before and after the neutral cellulase treatment by micro-CT. There was a relatively smooth, hard-textured cuticle on the outer surface of the untreated WS in Fig. 4a. Figure 4d displays that there were indeed protrusions on the outer surface of WS, after being treated with 10 U/g neutral cellulase for 30 min. These protrusions should be mainly located at the pores covered by the cuticle and siliceous layers. It should be the neutral cellulase that caused a certain degree of degradation to the outer vascular tissue of WS. As a result, the bonding force between the keratinous and siliceous layers and the fibers was reduced, thereby forming these protrusions [96], which was consistent with the SEM results.

It can be seen that the skeleton structure of untreated WS fiber was complete in Fig. 4c. The outer side was relatively dense

Table 2 The effect of neutral cellulase treatment on the outer surface element content of WS

Sample name	Elt	Line	Intensity (c/s)	Atomic %	Atomic ratio	Conc.	Units
a	O	Ka	1.93	8.661	1.0000	5.125	wt.%
	Si	Ka	161.62	91.339	10.5459	94.875	wt.%
b	O	Ka	1.42	16.613	1.0000	10.192	wt.%
	Si	Ka	53.76	83.387	5.0196	89.808	wt.%
c	O	Ka	2.26	25.179	1.0000	16.087	wt.%
	Si	Ka	47.42	74.821	2.9715	83.913	wt.%
d	O	Ka	1.42	48.546	1.0000	34.958	wt.%
	Si	Ka	8.49	51.454	1.0599	65.042	wt.%

Fig. 3 Distribution maps of elements on the outer surface of WS: **a** untreated; **b** neutral cellulase 10 U/g, 30-min treatment; **c** neutral cellulase 30 U/g, 30-min treatment; **d** neutral cellulase 30 U/g, 90-min treatment



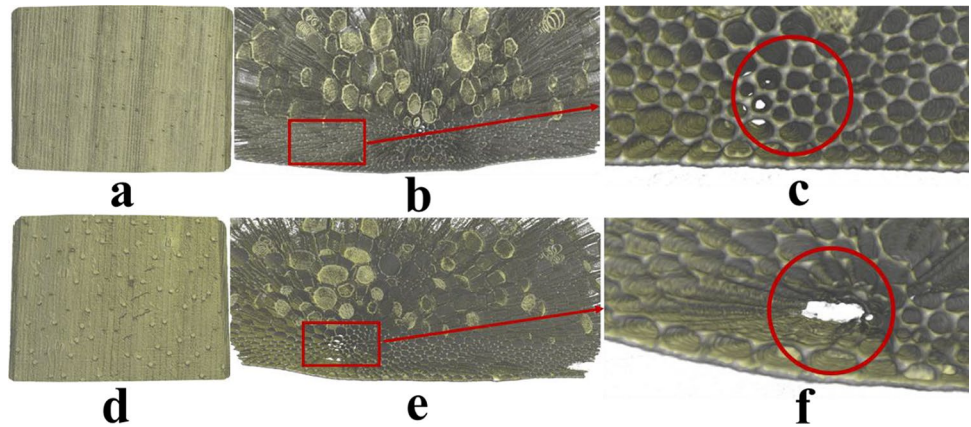
vascular bundle cells. The vascular bundle tissue composed of untreated WS vascular bundle cells had a smaller diameter and was thicker. The middle part of the inner test vascular bundle cells were parenchyma cells, which were large in volume and relatively small in density. After treatment with neutral cellulase, the internal microstructure of WS was changed (Fig. 4e). The diameter of vascular bundles outside the WS increased and the wall became relatively thin. At the same time, it can be seen that the vascular bundle tissue was partially degraded in Fig. 4f, indicating that the neutral cellulase had a degradation effect on the fiber in vascular bundle tissue of WS, while there was basically no change in the internal tissue skeleton structure of WS (Fig. 4e). It showed that when the neutral cellulase entered the inside of WS from the back, it might only damage the small fibers, and did not cause obvious damage to the inside of WS.

3.1.4 Analysis of the dissolution content of monosaccharides

Figure 5 shows the effect of neutral cellulase treatment on sugar dissolution. It can be seen that with the addition of neutral cellulase in the pretreatment process, the dissolution of various sugar components was significantly increased.

And the dissolution of glucose was particularly obvious, as shown in Fig. 5d. From Fig. 5a, it can be found that the dissolution amount of arabinose, galactose, xylose, and mannose increased with the increase of amount of neutral cellulase. The reason is that cellulase was difficult to be purified, and it usually contained some hemicellulase and other related enzymes in practical applications. A small amount of hemicellulase would also degrade part of the exposed hemicellulose, promoting the dissolution of arabinose, galactose, xylose, and mannose. Among them, the dissolution of xylose and mannose increased more obviously than other sugars. It could be because the neutral cellulase contained more xylanase and mannase. In addition, with the addition of neutral cellulase, the dissolution of glucose increased dramatically, which was obviously increased by several times (Fig. 5d). Because of the selectivity of enzyme, after adding neutral cellulase, the neutral cellulase entered WS from both front and back sides simultaneously. Specifically, neutral cellulase entered the inner fibrous tissue of WS from the back, destroyed its microstructure, and degraded part of the cellulose. On the other hand, the neutral cellulase entered the vascular bundle tissue from the breathing holes and some tiny broken gaps in the front side, destroying the fiber cells

Fig. 4 Micro-CT images of **a, b, c** WS untreated; **d, e, f** neutral cellulase 10 U/g, 30-min treatment



and degrading part of the cellulose. When the neutral cellulase was added in the beginning, the first degradation should

be the amorphous region on the cellulose macromolecular chain. Because there were many glycosidic bonds exposed

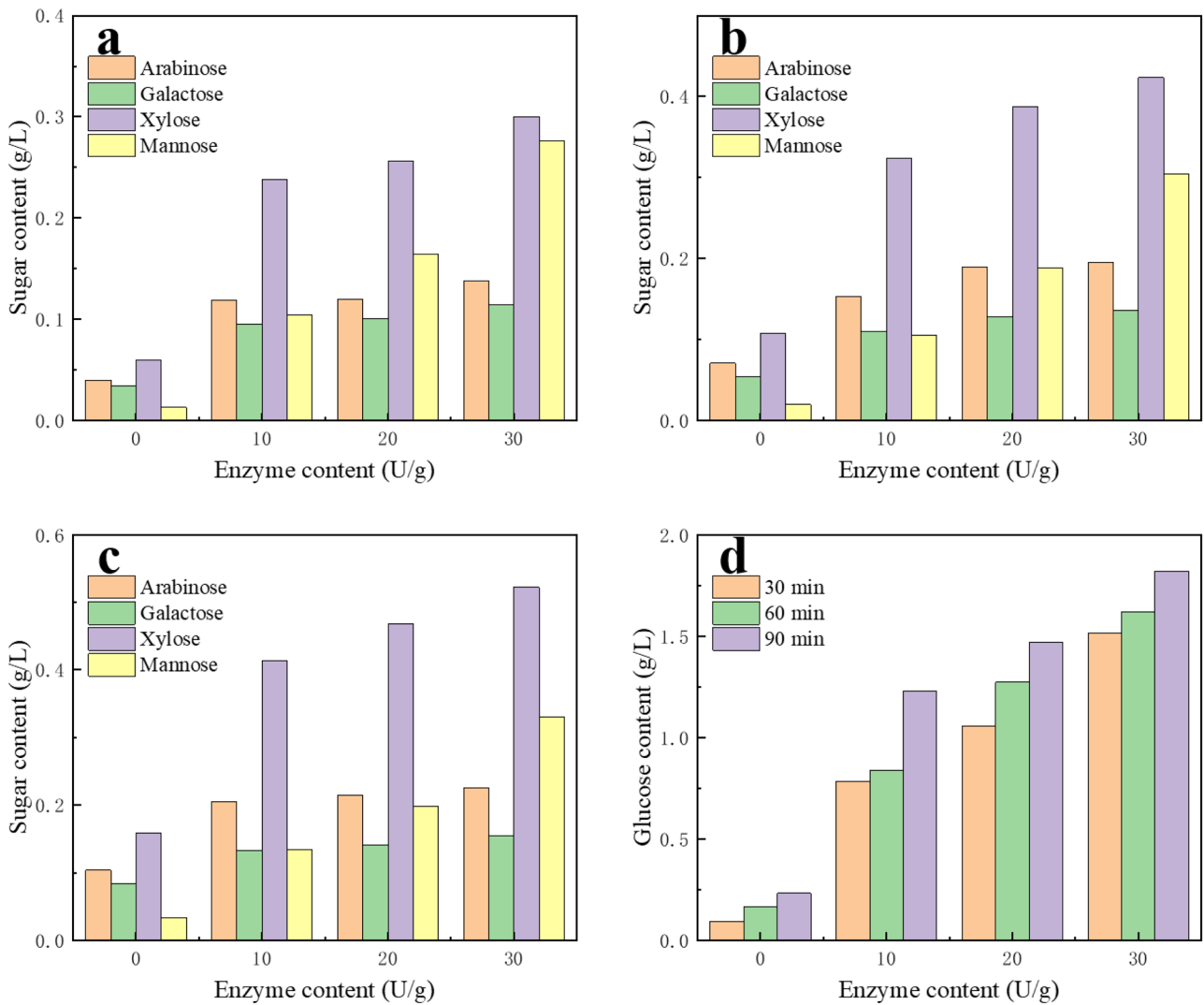


Fig. 5 The influence of neutral cellulase treatment on the dissolution of sugar: **a** 30 min; **b** 60 min; **c** 90 min; **d** the dissolution of glucose

in the amorphous region of cellulose, the regularity of molecular chain arrangement and orientation was poor, and there were many lattice defects. The formed intermolecular and intramolecular hydrogen bonds had weak force, relatively high accessibility, and fast hydrolysis rate [98, 99]. Through the action of neutral cellulase, glycosidic bonds were degraded to produce long-chain oligosaccharides, which were cleaved into short-chain oligosaccharides, and cellobiose was released from the reducing or non-reducing ends, which were then hydrolyzed into glucose. As a consequence, it promoted the dissolution of glucose, so the degradation effect of cellulose was more obvious.

At the same time, it can be seen from Fig. 5 that with the extension of the neutral cellulase treatment time, the dissolution of various sugars had also increased. The reason is that with the extension of the treatment time, more cellulose and hemicellulose were degraded, and some crystalline regions on the cellulose macromolecular chain were also broken, thereby dissolving more sugar components.

3.2 Lipase treatment

3.2.1 SEM images

From Fig. 6b, it can be clearly observed that after 30 U/g, 90-min treatment with lipase, the outer surface of WS was damaged, which was more serious than that of neutral cellulase treatment. In addition, compared with the neutral cellulase treatment, the damages caused by lipase were different. The material from the damaged outer surface of WS was similar to gelatinous material after treatment with lipase. It is speculated that after lipase treatment, the damaged parts of the outer surface of WS might be mainly the cuticle on the outer surface. The reason is that part of the membrane covered on the outer surface of WS was cuticle, and the chemical composition was mainly cutin and waxy. Both cutin and waxy contained a large number of fatty compounds. When WS was treated with lipase, lipase would accelerate the decomposition of cutin and wax, where the cuticle on the outer surface of WS was destroyed [100–102].

From Figs. 6 and 7, it can be observed that the outer surface of WS was damaged with the treatment of lipase. While compared with neutral cellulase, the damage was slightly different. Under the same experimental conditions, the outer surface of WS was damaged obviously and a large amount of gelatinous material fell off with the treatment of lipase, whereas only a thin layer of film was removed from the outer surface treated with neutral cellulase. Therefore, after lipase treatment, the damaged part of the WS outer surface should mainly be the cuticle [90]. With the increase of lipase dosage, more cutin and wax were destroyed. The treatment effect of the WS outer surface was more obvious. With the increase of lipase treatment time, the decomposition of cutin and wax was more complete, and more gelatinous substances appeared on the outer surface of WS, then more damaged locations and larger areas have appeared, resulting in better treatment effects. It can be seen that with the increase of lipase amount and treatment time, the damage degree of WS surface was aggravated.

3.2.2 SEM-EDS analysis

In order to further analyze the influence of lipase treatment on the outer surface element content of WS, the surface morphologies were observed with $\times 200$ magnification by EDS. The results are shown in Table 3.

In Table 3, a is the untreated raw material, b is the sample LWS₁, c is the sample LWS₃, and d is the sample LWS₉. It can be seen from Table 3 that the outer surface silicon content of the untreated WS was extremely high. With the addition of lipase, the silicon content on the outer surface of the WS had been reduced to a certain extent. After lipase 30 U/g and 90-min treatment, the silicon content on the outer surface of WS reached the lowest. However, compared with neutral cellulase, there was still a gap in the removal effect of lipase treatment on silicon. The reason is that lipase acted on the cuticle on the outer surface of WS. With the increased amount of lipase, the destructive effect of the cuticle on the outer surface of WS became more obvious. With the removal of the cuticle, part of the siliceous layer connected to the cuticle should also fall off as the cuticle fell off, thereby

Fig. 6 SEM images of WS outer surface: **a** untreated; **b** lipase 30 U/g, 90-min treatment

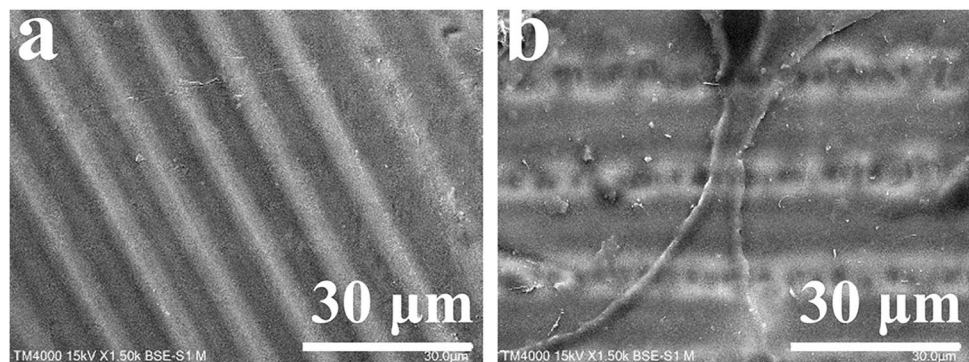
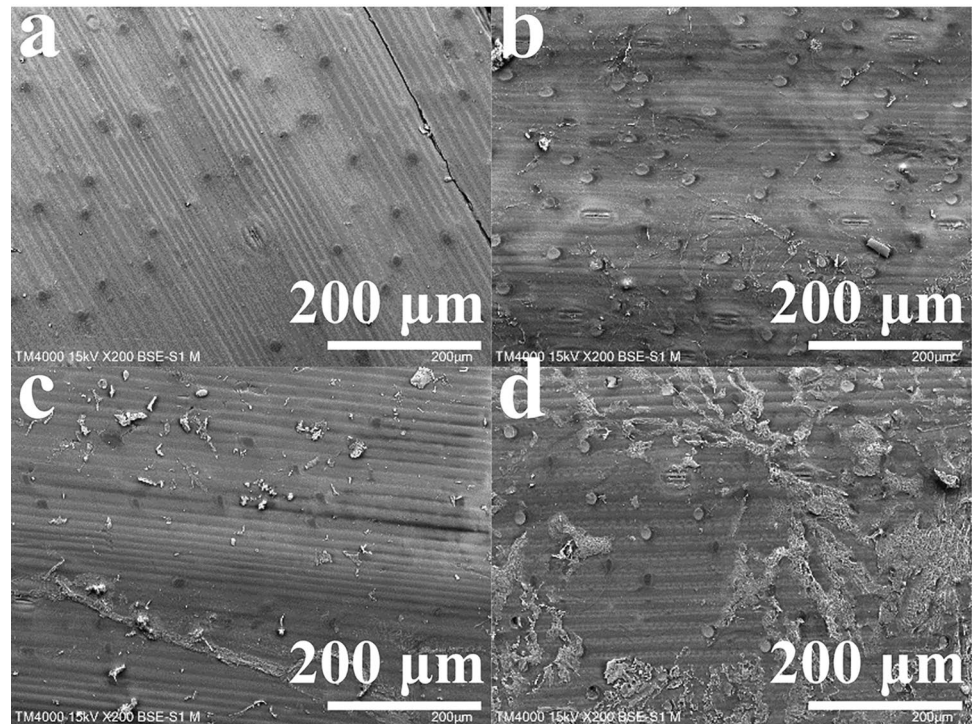


Fig. 7 SEM images of WS outer surface: **a** untreated; **b** lipase 10 U/g, 30-min treatment; **c** lipase 30 U/g, 30-min treatment; **d** lipase 30 U/g, 90-min treatment



reducing the silicon content on the outer surface of the WS. But part of the siliceous layer should be connected to the vascular tissue and would not fall off. Therefore, the silicon content on the outer surface of WS treated with lipase is higher than that after treatment with neutral cellulase. With the prolongation of lipase treatment time, the degradation effect of lipase on cutin and wax was more sufficient, and the damaging effect on the outer surface of WS was more significant. More cuticles were removed, and more siliceous layers would fall off at the same time. The silicon content on the outer surface of the WS was gradually reduced. It could be seen that the treatment effect of lipase treatment on the outer surface of WS was mainly manifested in the cuticle on the outer surface, and part of the siliceous layer would fall off as the cuticle was destroyed.

Figure 8a–d are all elements analysis of WS outer surface magnified 200 times. It can be seen from Fig. 8 that after the

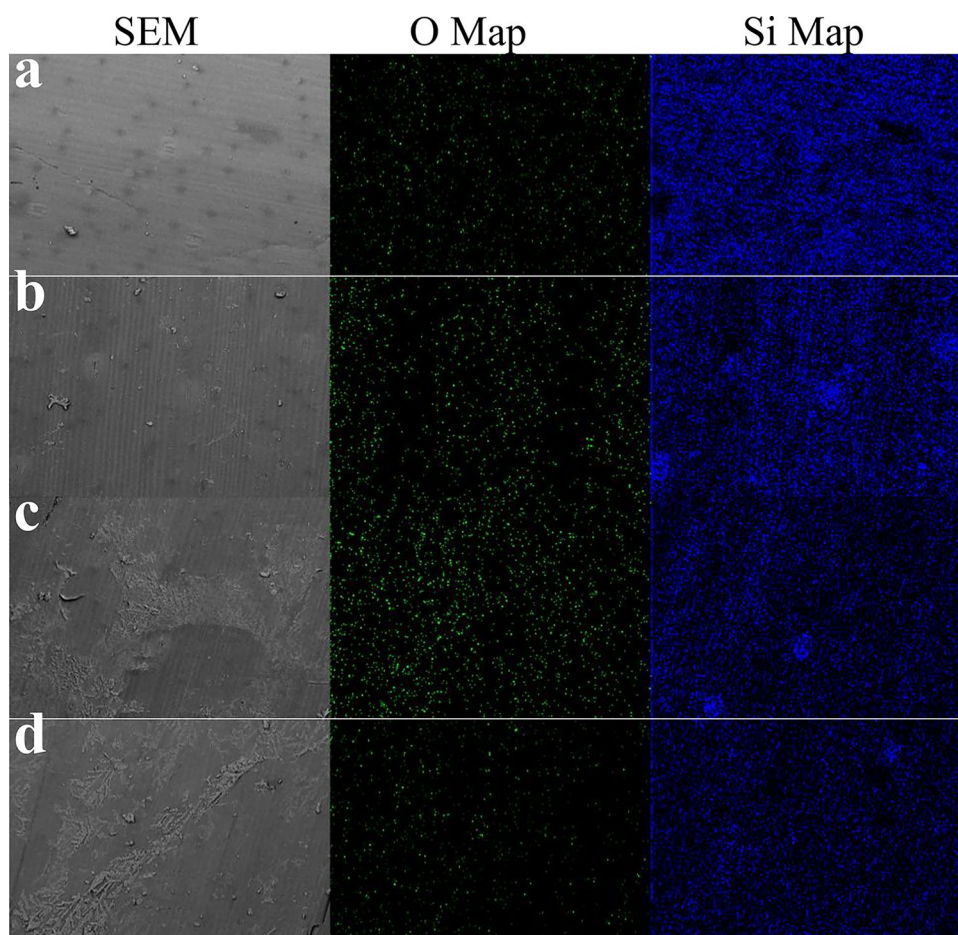
lipase treatment, the distribution of silicon on the outer surface of the WS became relatively sparse, indicating that the siliceous layer on the outer surface of the WS was destroyed to a certain extent after the lipase treatment. The reason for this change should be that the lipase decomposed cutin and wax and destroyed the cuticle by adding lipase. The outer surface of WS was composed of cuticle and siliceous layers, destroying the cuticle, and part of siliceous layers connected to the cuticle fell off, thereby reducing the silicon content on the outer surface of the WS.

From the comparison of Figs. 3 and 8, it can be seen that under the same enzyme dosage and enzyme treatment time, the silicon content on the outer surface of WS treated with neutral cellulase was significantly lower than that of WS treated with lipase. The lower silicon content indicates that compared with lipase treatment, neutral cellulase had a better treatment effect on the siliceous layer on the outer surface

Table 3 The effect of lipase treatment on the outer surface element content of WS

Sample name	Elt	Line	Intensity (c/s)	Atomic %	Atomic ratio	Conc.	Units
a	O	Ka	1.93	8.661	1.0000	5.125	wt.%
	Si	Ka	161.62	91.339	10.5459	94.875	wt.%
b	O	Ka	1.76	11.271	1.0000	6.748	wt.%
	Si	Ka	108.41	88.729	7.8727	93.252	wt.%
c	O	Ka	3.91	22.628	1.0000	14.281	wt.%
	Si	Ka	96.30	77.372	3.4193	85.719	wt.%
d	O	Ka	1.62	32.097	1.0000	21.215	wt.%
	Si	Ka	22.90	67.903	2.1155	78.785	wt.%

Fig. 8 Distribution maps of elements on the outer surface of WS: **a** untreated; **b** lipase 10 U/g, 30-min treatment; **c** lipase 30 U/g, 30-min treatment; **d** lipase 30 U/g, 90-min treatment



of WS. It can be found that lipase had a relatively better effect on the treatment of the cuticle on the outer surface of WS by comparing Figs. 1, 2, 6, and 7.

4 Conclusions

In this study, the effects of neutral cellulase and lipase treatments on the outer surface of WS were demonstrated. The results showed that the outer surface damage of two enzymes on WS was different. Neutral cellulase treatment of WS had more remarkable destruction on the outer surface silicon layer than lipase. Besides, it is demonstrated that the damage of part of vascular tissues and the outer surface of WS increased with increasing amount of neutral cellulase and enzyme treatment time. Then film of the outer surface was fallen off and the content of silicon was reduced. With regard to lipase, the major damage was the outer surface cuticle of WS. What's more, the degradation of cutin and wax aggravated with increased lipase dosage and enzyme treatment time. As a consequence, the cuticle was broken

severely, leading to the exfoliation of the cuticle and siliceous layers.

Above all, this research proved that the neutral cellulase and lipase treatment removed the cuticle and siliceous layers on the outer surface of WS. It is expected that enzyme pretreatment before pulping production will promote the impregnation of chemical liquids in subsequent pulping production; at the same time, it can reduce the number of chemicals and pollution of the environment.

Funding The authors received supports from National Key R&D Program of China, Grant No. 2019YFC1905900; National Natural Science Foundation of China, Grant No. 31870566; Taishan Scholars Program and Yuandu Leading Talents Program, and Foundation of State Key Laboratory of Biobased Material and Green Papermaking, Qilu University of Technology, Shandong Academy of Sciences (No. GZKF202001).

Declarations

Conflict of interest The authors declare no competing interests.

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