

Alkaline Xylanase Produced by *Trichoderma reesei*: Application in Waste Paper Pulp Bleaching

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In this work, a facile, green approach to natural biomass bleaching is reported. It was found that *Trichoderma reesei* could produce alkaline xylanase and xylanase is highly active in alkaline environments. It has good environmental adaptability and could be used to reduce the use of bleaching chemicals for the pretreating process and degradation of hemicellulose so as to break the linkages of Lignin-carbohydrate complexes (LCCs). These particular properties benefit the pulp bleaching process and can improve the physical properties of the resulting paper. The optimum bleaching process conditions were as follows: dosage, 12 U/g; pH, 8 to 9; time, 60 minutes; and temperature, 70 °C. The xylanase can decrease the chemical oxygen demand (COD) of bleaching effluent by 41% as compared to the blank. Finally, after xylanase pretreatment, the whiteness, yellow index, tensile index, burst index, and elongation of the resulting paper were 54.8% ISO, 1.73, 33.93 N·m/g, 2.91 KPa·m²/g, and 2.91%, respectively.

Keywords: Xylanase; Waste paper pulp; Bleach; Property

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INTRODUCTION

Trichoderma reesei (*T. reesei*) is an anamorph of the fungus *Hypocrea jecorina*. It is a filamentous fungus and is able to secrete large amounts of cellulolytic enzymes, including both cellulases and hemicellulases (Kumar *et al.* 2008). Microbial hemicellulases have been used in industry, producing hemicelluloses which is a major component of plant biomass, into xylose.

Xylanase is named after a class of enzymes that degrade the linear polysaccharide beta-1,4-xylan into xylose. It includes endo-(1-4)-beta-xylan 4-xylanohydrolase, endo-1,4-xylanase, endo-1,4-beta-xylanase, beta-1,4-xylanase, endo-1,4-beta-D-xylanase, 1,4-beta-xylan xylanohydrolase, beta-xylanase, beta-1,4-xylan xylanohydrolase, beta-D-xylanase (Beg *et al.* 2001). Xylanase plays a major role in breaking down hemicellulose, which is one of the major plant cell walls' components.

Xylanase degrades the plant matter into usable nutrients. Usually, xylanases are produced by bacteria, fungi, yeast, marine algae, snails, protozoans, crustaceans, seeds, insects and others (Beg *et al.* 2001). Mammals do not produce xylanases. Filamentous fungi are used as the source of principal commercial xylanases (Polizeli *et al.* 2005).

Xylanases from soil were first studied by Sørensen (1955). Currently, xylanases are attracting more and more attention in the pulp and paper industry, and some are used in commercial areas. However, the properties of commercial xylanases make them unsuitable for use in the process of pulp bleaching. Many kinds of xylanases are suitable been used in acidic environments (Wang *et al.* 2013). Generally, in alkaline environments, H₂O₂

dissociation of the hydroxyl anion (HOO^-) improves the pulp whiteness (Dence and Reeve 1996). In industrial papermaking, there has been significant interest in xylan and its hydrolytic enzymatic complexes, especially in the bleaching of cellulose pulp. However, there have been few studies of the use of xylanase in waste paper. This paper introduces a process of industrial production of xylanase from *T. reesei* ATCC56765, as well as the properties of xylanase and its application in waste paper bleaching.

The dosage of xylanase, pH, pretreating time, and pretreating temperature affect the process of the H_2O_2 bleaching of waste pulp (Granfeldt *et al.* 1997). These factors are common parameters controlled in industrial production. In this study, it was determined how these factors affect the physical properties of paper and whether xylan decreases the pollution resulting from bleaching. The most suitable application parameters and their application in industrial production were determined.

EXPERIMENTAL

Materials

T. reesei ATCC56765 was obtained from the United States Department of Agriculture. All the chemicals were supplied by the China National Medicine Group (Shanghai Chemical Reagent Co., Ltd) except for Birchwood xylan and xylose were supplied by Sigma-Aldrich Trading Co., Ltd. (Shanghai, China). All aqueous solutions were prepared with ultrapure water ($>18 \text{ M}\Omega \text{ cm}$) from a Milli-Q Plus system (Merck Millipore, USA).

Methods

T. reesei production of xylanase

Through activation, cultivation, and fermentation, *T. reesei* was used to produce the xylanase used in this study.

Table 1. Composition of Fermented Liquid

Substance	Dosage(g)	Substance	Dosage(g)
Ammonium sulfate	4.2	Ferrous sulfate	0.03
Monopotassium phosphate	6	Cobalt chloride	0.01
Calcium chloride	0.9	Manganese sulfate	0.008
Magnesium sulfate	1.0	Zinc sulfate	0.01

All chemical dosages are shown in Table 1. These chemicals were weighed and dissolved in 1000 mL of deionized water. It was then sterilized at 120°C for 20 min. After cooling to room temperature, it was inoculated (the inoculation quantity was 8%). During fermentation, the conditions were maintained at 50°C and 240 rpm. The fermented liquid was centrifuged at 10,000 rpm for 10 min, and the supernatant fluid was collected to determine the activity of the crude xylanase using the 3, 5-dinitrosalicylic acid (DNS) method.

Xylanase dosage influence on xylanase pretreating waste paper pulp bleaching

The xylanase pretreatment conditions were as follows: 65°C ; 1 h; and pH 8.0. The H_2O_2 bleaching conditions were as follows: H_2O_2 concentration 1.5%; alkali-to- H_2O_2 ratio

1:1; magnesium sulfate concentration 1.5%; temperature 70 °C and duration 3 h. All chemical charges used were relative to the oven-dry stockmass.

pH influence on xylanase pretreating waste paper pulp bleaching

Generally, hemicellulose can be produced by fungi at pH values between 4 and 8 (Ye *et al.* 2013). This kind of hemicellulose produced by *T. reesei* can operate across a wide range of pH values. Phosphate buffered saline was used to control the pH. The pH values tested were 6.0, 7.0, 8.0, 9.0, and 10.0, each at a xylanase dosage of 12 U/g. This was done to investigate the effects in pH value on the performance of the pretreatment of xylanase waste paper pulp bleaching. The xylanase pretreatment methods and the conditions of H₂O₂ bleaching were as before. The conditions were as follows: 65 °C, 1 h, and 12 U/g xylanase (to oven-dry stock).

Pretreating time influence on xylanase pretreatment waste paper pulp bleaching

The experimental operation in this case was the same as before. Waste paper pulp was pretreated with xylanase for various durations (0 to 120 min with a gradient of 30 min) to determine the optimal duration. The other pretreatment conditions were as follows: 65 °C, pH 8.0, and xylanase dosage, 12 U/g on oven-dry stock.

Pretreating temperature influence on xylanase pretreatment waste paper pulp bleaching

Waste paper pulp was pretreated with xylanase at various temperatures (40 to 80 °C with a gradient of 10 °C). The strength and optical properties of the as-prepared pulp were addressed. The other conditions remain unchanged.

The COD of the bleaching effluent

The pretreatment conditions were as follows: a dosage of xylanase ranged from 2 to 30 U/g (on oven-dry stock) and the temperature, treatment time, and pH were 70 °C, 1 h, and 8.0, respectively. The experimental bleaching operation was the same as described previously.

RESULTS AND DISCUSSION

Xylanase Activity

The activity of the xylanase produced by *T. reesei* averaged 80 to 100 U/g.

Xylanase Dosage

The relationships between the physical paper properties and xylanase addition are shown in Figs. 1, 2, and 3.

As shown in Fig. 1, the effect of xylanase pretreatment on the waste paper pulp was obvious. It created folds and debris on the treated fiber surface, which would help bleaching chemicals penetrate the fiber. Figure 2 shows that with increased xylanase addition, the whiteness of paper rapid increased at first. It increased from 46.3% ISO in the blank sample to 52.7% ISO, and then increased more slowly, and finally became steady. This is because this xylanase is highly efficient at hydrolyzing hemicelluloses. It attacks hemicellulose and degrades LCC, increasing the contact area of the H₂O₂ bleaching solution and the fiber, improving the whiteness. After xylanase treatment, the yellow indexes were lower than that of the blank. When the xylanase addition was 14 U/g, the yellow index was best (1.89).

Combined with the tendency to increase whiteness, it is reasonable to infer that the amount of residual lignin in the pulp was decreased after the xylanase treatment. As the xylanase reaction substrate concentration was constant, the xylanase dosage did not have an obvious effect on the pulp whiteness or yellow index at over 12 U/g.

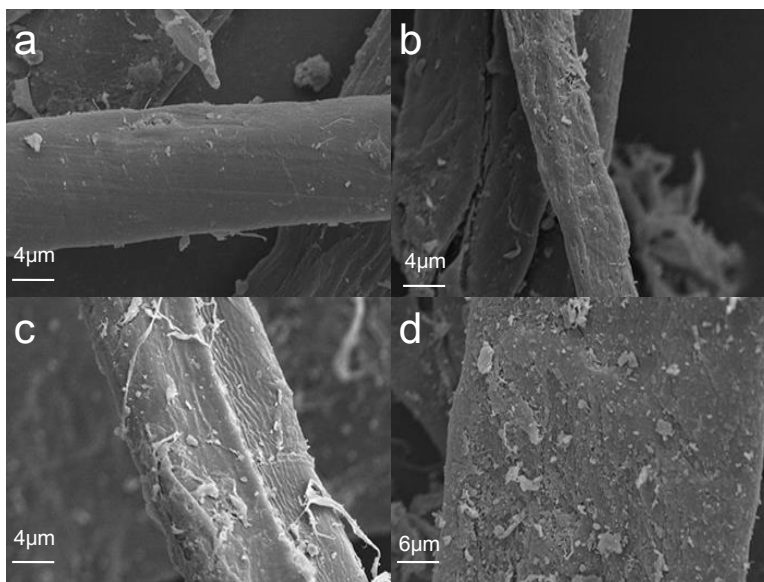


Fig. 1. SEM micrographs of waste paper pulp: (a) blank, (b) 10 U/g xylanase pretreatment, (c) 15 U/g xylanase pretreatment, and (d) 20 U/g xylanase pretreatment

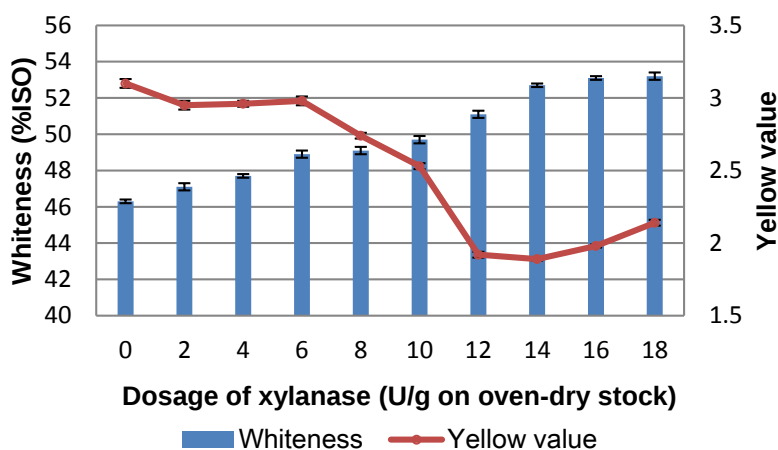


Fig. 2. Effects of xylanase dosage on whiteness and anti-yellow value of paper made via xylanase pretreatment and H₂O₂ bleaching

At the same time, Fig. 2 shows that with increased xylanase addition, the tensile index, burst index, and elongation first increased and then decreased. This is because xylanase attacks the hemicellulose of the fiber, having little to no influence on cellulose, thus improving the cellulose content of the fiber.

The xylanase treatment process strengthened the binding force between the fiber, and the average fiber length increased as short chains of hemicellulose and fines were destroyed, improving the physical properties of the paper. At excessive dosages of xylanase, a large amount of hemicellulose was degraded and the physical properties of the paper were reduced.

The dosage of xylanase plays an important role in the industrial production of paper. The appropriate usage of xylanases can not only improve the properties of the paper, but also decreases the cost of production. Based on a comprehensive analysis of the optical properties, physical strength, cost, and other performance indicators of paper, the best xylanase addition to waste paper pulp was 12 U/g.

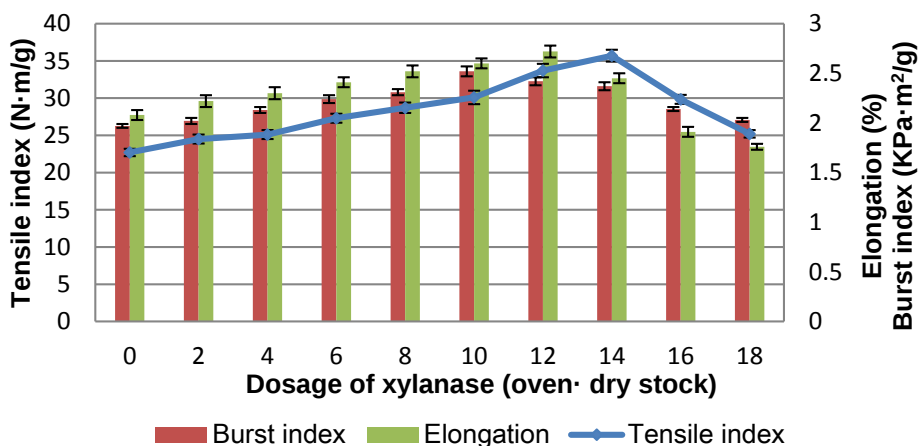


Fig. 3. Effects of xylanase dosage on H_2O_2 bleaching of paper and the physical properties of paper resulting from xylanase pretreatment

pH Influence on Xylanase Pretreating Waste Paper Pulp Bleaching

These results are shown in Figs. 4 and 5. Figure 4 shows that when blank samples without xylanase pretreatment were bleached with 1.5% H_2O_2 , the resulting whiteness of the pulp was only 46.3% ISO. At pH 6.0, 7.0, 8.0, 9.0, and 10.0, additional treatment with 12 U/g xylanase increased the whiteness of the pulp to 51.1%, 52.2%, 52.7%, 53.2%, and 49.7% ISO, respectively. All xylanase-pretreated pulps had higher whiteness as compared to that of the blank sample. When the pH was 9.0, the whiteness of the bleached waste paper pulp was the highest, 6.9% ISO higher than that of the blank sample.

The yellow indexes of samples after xylanase pretreatment were all lower than that of the blank sample. When the pH was 8.0, the xylanase-pretreated sample had the best yellow index, 1.74. This is because under the alkaline conditions, parts of the lignin and hemicellulose fractions are easily dissolved. Thus, the whiteness of the pulp is more stable. At the same time, after xylanase treatment, the hemicellulose in the pulp is partially degraded, more effectively inhibiting the oxidation of hemicellulose end groups, and enhancing the stability of the pulp whiteness. According to comprehensive consideration of both the whiteness and the yellow index, the pH of the xylanase treatment should be controlled to 8.0 to 9.0 to achieve excellent bleaching results, higher whiteness, and an appropriate yellow index.

Figure 5 shows the influence of xylanase treatment at different pH conditions on the physical properties of the paper. As shown in Fig. 5, the untreated sample's tensile index, burst index, and elongation were 22.7 N·m/g, 2.14 KPa·m²/g, and 2.12%, respectively. At pH values of 6.0, 7.0, 8.0, 9.0, and 10.0, the treated pulp exhibited improvement in the tensile index, burst index, and elongation. In addition, the key physical property indicators of the paper first increased and then decreased with increasing pH. The tensile index, burst index, and elongation were excellent at pH 8.0 to 9.0. Compared to those of the blank sample, the tensile index, burst index, and elongation were increased by

1.31%, 1.36%, and 29.51%, respectively. It can be inferred that the xylanase produced in the laboratory is an alkaline xylanase. Under alkaline conditions, the xylanase exhibits the highest activity. The pretreatment process primarily works *via* the adsorption of hemicellulose degradation debris on the fiber surface. It reduces the number of short hemicellulose chains, fines, and increases the binding force between fibers, enhancing the physical properties. To reiterate, at pH values from 8.0 to 9.0, the auxiliary xylanase pretreatment had the best effect on H₂O₂ bleaching.

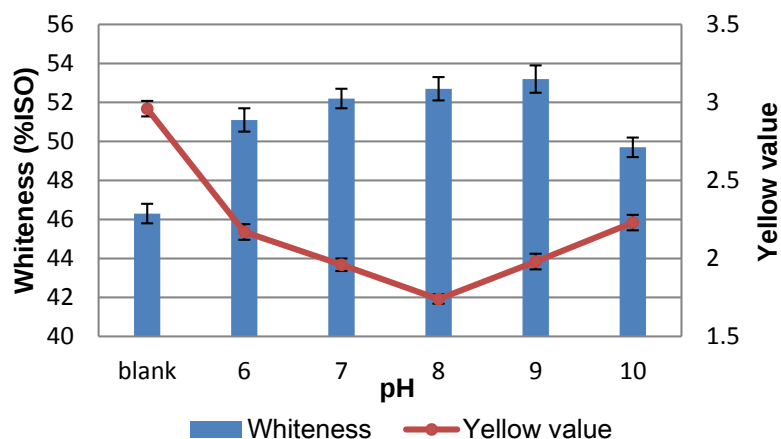


Fig. 4. Effects of pH on whiteness and anti-yellow value of xylanase pretreatment on H₂O₂ bleaching

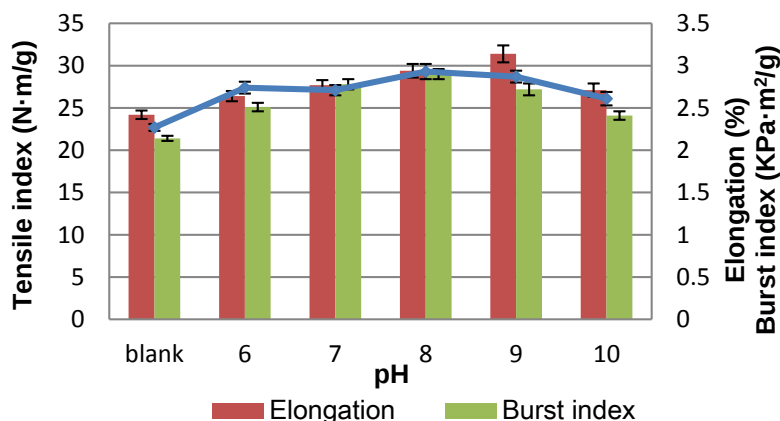


Fig. 5. Effects of pH on xylanase pretreatment on H₂O₂ bleaching and paper physical properties

Pretreating Time

The effects of xylanase pretreatment time on the H₂O₂ bleaching of waste paper are shown in Figs. 6 and 7.

Figure 6 shows that, as compared to that of the blank sample, the whiteness after H₂O₂ bleaching gradually increased with increased xylanase pretreatment time. From 30 to 120 min, the whiteness after bleaching was 51.3%, 52.9%, 53.2%, and 53.8% ISO, respectively. All were higher than the 45.9% ISO of the blank sample. When the pretreatment time exceeded 60 min, the increase in the pulp whiteness was not obvious. This may be because the xylanase solution itself also contained a small amount of pigment,

and with increased xylanase pretreatment time, the release pigment in the reaction system was increased, thus suppressing the promotion of whiteness. The optimum pretreatment time for bleached waste paper pulp was 60 min.

Figure 6 also shows that the xylanase produced in the laboratory had very good ability to inhibit the return of yellow color in the paper. With increasing time, the yellow index of the bleached pulp first increased and then decreased. This indicates that the ability to inhibit the return to yellow first increased and then decreased. As the xylanase and pulp form a heterogeneous system, it takes time for xylanase to make contact with waste paper pulp. When the pretreatment time was 30 min, it was too short for xylanase to adequately contact the waste paper pulp. The reaction was incomplete, so the yellow index was high (2.49).

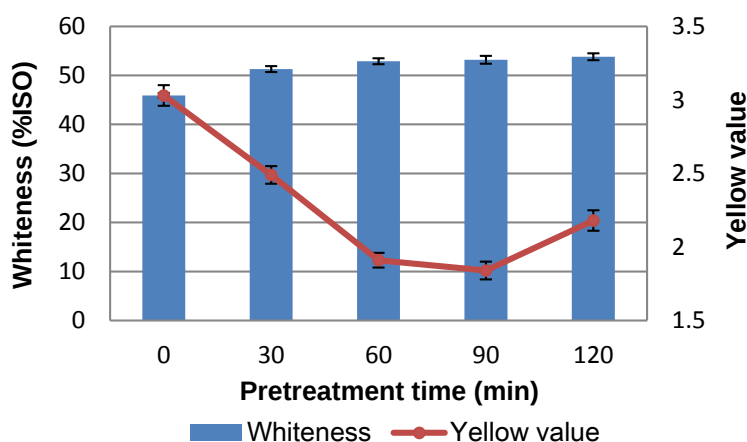


Fig. 6. Effect of duration of xylanase pretreatment on H_2O_2 bleaching on whiteness and yellow index

When the pretreatment time was 60 min, the pulp began to have a lower yellow index (1.91). At the same time, the whiteness increased from 51.3% to 52.9% ISO. When the pretreatment time was 90 min, the yellow index was the lowest (1.84). After that, with increased duration, the yellow index has begun to rise again. It may be that with increased pretreatment time, the hemicellulose pieces were reabsorbed onto the surface of the pulp. These pieces had much more active groups, which were easy to oxidize to chromophoric groups, increasing the yellow index.

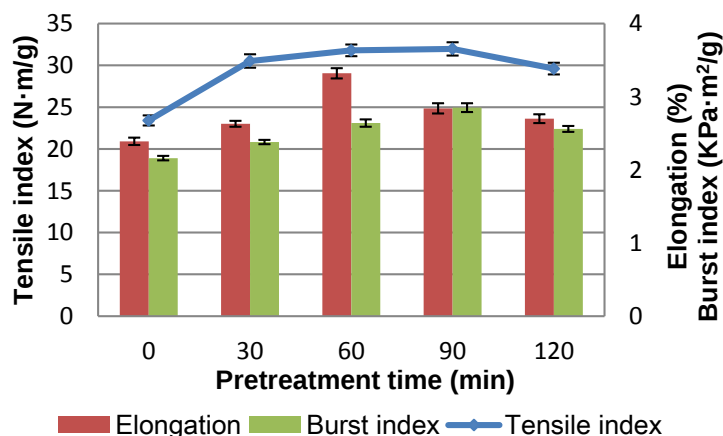


Fig. 7. Effects of the duration of xylanase pretreatment on H_2O_2 bleaching and paper physical properties

Figure 7 shows the effects of the duration of xylanase pretreatment on the bleached paper's physical properties. It indicates that the blank sample's tensile index, elongation, and burst index were 23.14 N·m/g, 2.39%, and 2.16 KPa·m²/g, respectively, lower than those of the xylanase-pretreated samples. When the pretreatment time was 60 min, the tensile index, elongation, and burst index were all ideal. These indexes were 31.79 N·m/g, 3.32%, and 2.64 KPa·m²/g, respectively, increases of 37.38%, 38.91%, and 22.22%, respectively, relative to those of the blank sample. Considering the physical properties, optical properties, and the rationality and efficiency of industrial production, the optimum xylanase pretreatment time was 50 to 70 min.

Pretreating Temperature

As shown in Fig. 8, the brightness of bleached pulp increased then decreased as the temperature was increased. It is noteworthy that when the temperature reached 70 °C from 40 °C, the whiteness of the bleached pulp reached its highest value, 50.9% ISO. Thenceforth, temperatures over 70 °C led to reduced whiteness. This was attributed to the fact that xylanase functioned well at 70 °C, and little change in the whiteness was observed within the range of 40 to 80 °C. Thus, the xylanase is possessed outstanding thermal adaptability.

The same conclusion was reached from how the yellow index of the bleached pulp changed. At temperatures lower than 70 °C, the xylanase became more active as the temperature increased, and higher brightness was obtained as more hemicellulose was degraded. Once it exceeded 70 °C, the xylanase was denatured. Thus, the resulting inactivation of protein overwhelmed the stimulative enzymatic hydrolysis originating from increased temperature, which explains why the brightness of the as-prepared pulp decreased with a higher temperature (over 70 °C).

Figure 9 shows the tensile index, elongation ratio, and burst index measurements. The aforementioned mechanical performances of the pretreated pulp were significantly higher as compared to those of the control group. When the temperature was 70 °C, the tensile index, elongation ratio, and burst index of the bleached pulp were 49%, 20%, and 42% higher than those of the untreated pulp, respectively. The improvement in the mechanical properties of the bleached pulp may be due to the xylanase pretreatment. Based on the above results, the treatment temperature was best in the range of 60 to 80 °C, a range wide enough for use in industrial manufacturing.

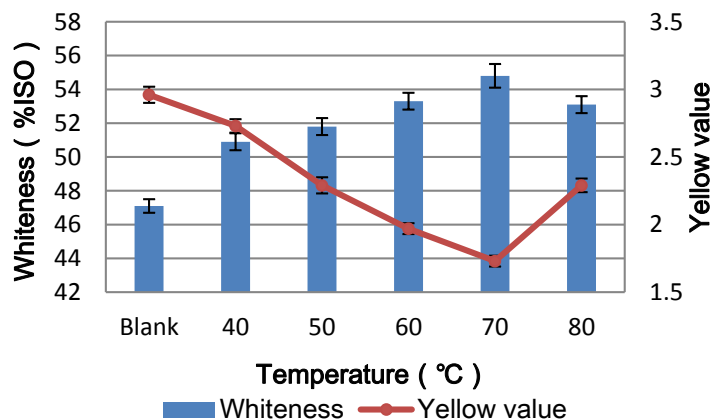


Fig. 8. Effect of temperature on xylanase pretreating H₂O₂ bleached pulp whiteness and yellow index

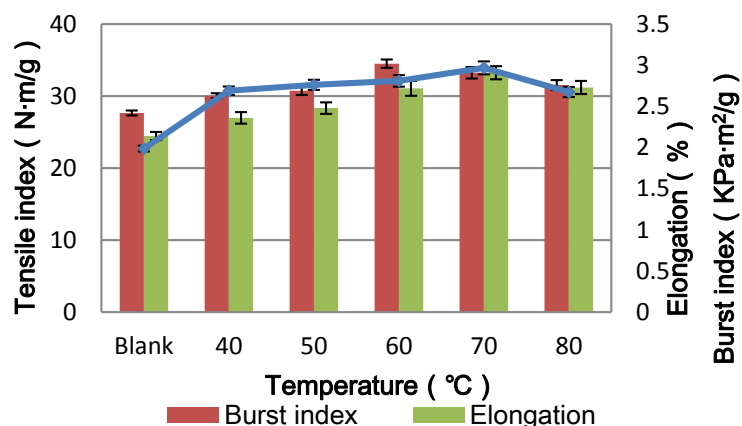


Fig. 9. Effects of the temperature for xylanase pretreating on H_2O_2 bleaching of paper physical properties

The COD of the Bleaching Effluent

As shown in Fig. 10, the COD of the control group was 924.37 mg/L, higher than those of all pretreated pulp samples. When the dosage of xylanase was increased from 0 to 18 U/g, the COD of the effluent dropped rapidly to its lowest value, 547.23 mg/L. However, the downward trend slowed and even reversed if further xylanase was added. These phenomena were assigned to the excessive degradation of the LCC linkages that resulted from higher xylanase addition; consequently, more hemicellulose is contributed to the higher COD of the bleaching wastewater. In this case, to control the cost and optimize the process, the optimal dosage of xylanase was determined to be 18 U/g.

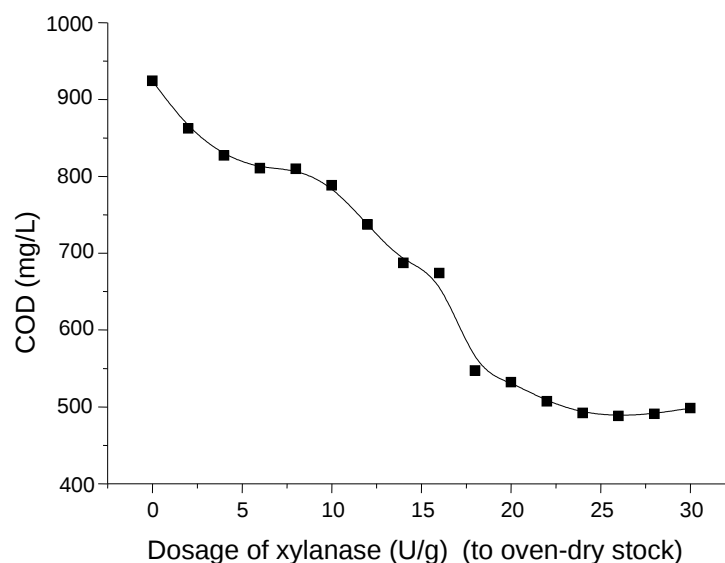


Fig. 10. Effects of the xylanase dosage on the COD of H_2O_2 bleaching effluent

CONCLUSIONS

1. The optimum amount of xylanase was 12 U/g. When the dosage of xylanase passed 16 U/g, the increase in the pulp properties was not obvious.

2. This kind of xylanase produced by *Trichoderma reesei* is alkaline xylanase and the optimum pH is 8.0. When xylanase treatment of waste paper pulp was done at pH 8.0 to 9.0, the results were favorable. When the pH was 8.0, the whiteness, yellow index, tensile index, burst index, and elongation were 52.7% ISO, 1.74, 29.37 N·m/g, 2.90 KPa·m²/g, and 2.94%, respectively. These results show that it was a kind of alkaline xylanase which has very good adaptability and vitality in alkaline environments.
3. The optimum xylanase pretreating time was 60 min and the temperature was 70 °C. When the processing time was 50 to 70 min and temperature was 60 to 80 °C, results were favorable.
4. This type of xylanase pretreatment can reduce the COD content of bleaching effluent. When the dosage of xylanase was 8 to 18 U/g (on oven-dry stock), better results were achieved. When the xylanase dosage was 18 U/g, the COD content was 547.23 mg/L, 41% lower than that of the blank sample.

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