

Preparation of Xylanase Loaded Biomass-based Deinking Agents and their Application in Secondary Fiber Recycling

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Recently, biomass-based deinking agents have attracted considerable interest in the pulp and paper industry due to their clean, renewable, and good deinking properties. In this study, the xylanase loaded biomass-based deinking agent (XBD) was prepared with coconut oleic acid, palmitic acid, rosin, and xylanase. The preparation technology of XBD was optimized by an orthogonal test and range analysis. The effects of the moisture content, free alkali, and chelating agent on the enzyme activity of XBD were determined *via* a single factor experiments. Based on the analysis of the optical and physical properties, the optimum xylanase addition into the biomass-based deinking agent was 15 wt.%, the brightness of the secondary fiber after flotation was 60.2% ISO, and the effective residual ink concentration was 223 ppm.

Keywords: Xylanase; Biomass; Deinking agent; Secondary fiber

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INTRODUCTION

The recycling of secondary fiber is an alternative process that can preserve green plants and save the environment in terms of pollution, water, and energy (Pelach *et al.* 2003; Ibarra *et al.* 2012). Recycling of secondary fiber requires the removal of printing ink from the waste paper through the deinking process, and the deinking agent is key to this process (Singh *et al.* 2012; Lee *et al.* 2013). The deinking agent may be comprised of surfactants, as they can increase ink wettability, permeability, swelling, reduce the binding force of the fibers, promote defibering, support ink particle detachment, and help remove the inks from the fibers (Li *et al.* 2007).

At this stage, scholars mostly have researched the use of combinations of surfactants. These types of combinational deinking agents have suitable bubbling effects, but the ink removal efficiencies have been found to be poor and the systems to be expensive (Zhang *et al.* 2016). At the same time, the synthetic surfactants under consideration have been based on petrochemicals as raw materials. With long-term and large-scale use, such surfactants can produce a harmful effect on the eco-environment and people's health (Tian *et al.* 2016). Moreover, the raw materials are non-renewable, and with the increasing exhaustion of raw materials, the production costs will only continue to rise. Therefore, using renewable biomass for non-renewable fossil resources to prepare environmentally friendly and inexpensive deinking agents will be the developmental focus of the future (Brycki *et al.* 2014).

Biomass-based deinking agents are prepared by using renewable biomass resources that not only have good deinking properties but also are clean and decomposable (Liu *et al.* 2009). However, just using the biomass-based deinking agents cannot be expected to result in perfect deinking efficiency, because the inks are tightly bound to fine fibers and therefore cannot be separated from the fiber effectively during flotation deinking (Li *et al.* 2007).

Xylanase is a giant molecule with high specificity (Battan *et al.* 2007). It has excellent properties of high activity and is currently attracting more attention in the pulping and papermaking industries due to its high efficiency of hydrolyzing hemicelluloses (Bim and Franco 2000). Furthermore, to some extent xylanase can also separate the fine fibers and inks. But using just the xylanase has been found not to provide the desired deinking efficiency during the process of waste paper flotation deinking (Ma and Yang 2015). Therefore, if the biomass-based deinking agent can be combined well with the xylanase, then an advantageous combination agent for clean and efficient deinking of secondary fiber for paper recycling will have been prepared.

Renewable rosin, palmitic acid, and coconut fatty acid were used for preparing the biomass-based deinking agent. To get the xylanase loaded biomass-based deinking agent (XBD), xylanase was added to the solution. Finally, to obtain better deinking properties, the preparation process of biomass-based deinking agents was considered and the effects of the moisture content, free alkali, and chelating agents on the enzyme activity of XBD were analyzed. At the same time, the effects of xylanase treatments on the optical and mechanical properties were also investigated.

EXPERIMENTAL

Materials

Rosin, palmitic acid, and coconut fatty acid were provided by Guangzhou Senhua Chemical Industry Co., Ltd. (Guangzhou, China). American waste paper #9 was supplied by Dongying Huatai Co., Ltd. (Dongying, China). The Xylanase-2 was supplied by Novozymes Biotechnology Co., Ltd. (Tianjing, China), and the enzyme activity was 15000 U/mL. All of the other chemicals and reagents were analytical grade, and supplied by Guangzhou Chemical Reagent Company (Guangzhou, China).

Methods

Preparation of XBD

The preparation process of XBD is illustrated in Fig. 1. The rosin was placed into a three-necked, round-bottomed flask and heated to 105 °C to melt the material. Then, the liquid was slowly brought to a certain temperature, and the palmitic acid and coconut fatty acid were added to the flask. An electric mixer was used to effectively mix the solution. The NaOH solution was then added to the well-mixed fatty acids and reacted for a certain time to get a crude product, and a 1:1 mole ratio of the $n_{\text{NaOH}}:n_{\text{plant fatty acid}}$.

The crude product was quickly added to 80 °C saturated salt water, stirred, and allowed to sit for 30 min. Vacuum suction filtration was used to separate the mixture, then the filter cakes were put into a drying oven, and lastly the powdered biomass-based deinking agent was synthesized. A vacuum-rotary evaporation procedure was used to dispose the filtrate and the glycerol was recycled.



Fig. 1. The preparation process of XBD

About 10 g of the biomass-based deinking agent was placed into a flask, heated to 50 °C through a water-bath, and then stirred rapidly during the continuous addition of a 20 mL borate buffer (pH = 8.0). Then, 1.6 g of an enzyme stabilizer was added, which contained 0.4 g borax, 1.2 g glycerin. The contents were mixed well. Citric acid was used to control the content of free alkali. Then, xylanase was added to the mixture, and an ultrasonic dispersion method was adopted to disperse the mixture.

Moisture content influence on enzyme activity of XBD

The 1.5 wt.%, 3.0 wt.%, 4.5 wt.%, and 6.0 wt.% moisture content of XBD was obtained through a constant temperature humidity chamber set to 25 °C, and the free alkali of the agent was approximately 0.10 wt.%. The activity of xylanase was measured every two days for a total of 10 days. The xylanase activity was measured using the 3,5-dinitrosalicylic acid method according to GB/T 23874 (2009). The enzyme activity retention rate (EAR) was calculated using Eq. 1,

$$EAR(\%) = \frac{U_0}{U_n} \times 100 \quad (1)$$

where *EAR* is the enzyme activity retention rate (%), *U*₀ is the initial enzyme activity (U/mL), and *U*_{*n*} is the enzyme activity *n* days later (U/mL).

Free alkali influence on enzyme activity of XBD

The biomass-based deinking agents of 0.05 wt.%, 0.10 wt.%, 0.15 wt.%, and 0.20 wt.% free alkali were prepared, monitored, and then tested *via* ISO 456 (1973). Then, xylanase was added to them and an ultrasonic dispersion method was used to disperse the mixture. The moisture content of the agent was approximately 3.0 wt.%, and the temperature was 25 °C. The xylanase activity was measured every two days for 10 days. The xylanase activity was measured using the 3,5-dinitrosalicylic acid method according to GB/T 23874 (2009). The EAR was calculated using Eq. 1.

Chelating agent influence on enzyme activity of XBD

The 0 wt.%, 0.15 wt.%, 0.30 wt.%, 0.45 wt.%, and 0.60 wt.% ethylene diamine tetraacetic acid tetrasodium (EDTA-4Na) were added into the XBD separately. The

moisture content of the agent was approximately 3.0 wt.%, the temperature was 25 °C, and the free alkali was 0.10 wt.%. The xylanase activity was measured every two days for 10 days. The xylanase activity was measured using the 3,5-dinitrosalicylic acid method according to GB/T 23874 (2009). The EAR was calculated using Eq. (1).

Application in secondary fiber recycling

The secondary fiber recycling process involved the deinking pulp preparation and flotation. Pulp preparation was performed at 10% consistency using 0.5% NaOH, 2% Na₂SiO₃, 0.4% EDTA, 2% H₂O₂, and 0.2% XBD. The treatment was performed at 50 °C for 30 min and the rotation speed was 300 r/min. The flotation process was performed using floatation cells (AMC L-100, 28L), wherein the air flow rate was 2.0 m³/h, the deinking pulp consistency was 0.2%, and the pH was 9. The treatment was performed at 50 °C for 20 min. Then, the deinking pulp was concentrated. Control experiments were also performed using the deinking pulp, which were heat inactivated by boiling for 15 min.

Sheet formation

The handsheets had a target basis weight of 80 g/m² and were made in a handsheet former (Testing Machines Inc., New Castle, USA) as per TAPPI Standard T205 sp-02 (2002).

Analysis of deinked secondary fiber and handsheets properties

After deinking, the secondary fibers' optical properties were examined (brightness and effective residual ink concentration), and the handsheets mechanical properties were examined (tensile and burst). The brightness was measured as described in ISO 2470-2 (2008), using a brightness tester (Technidyne Corporation, New Albany, USA). The effective residual ink concentration was measured with a Technibrite Micro TB-1C (Technidyne Corporation, New Albany, USA) according to TAPPI T567 (2004). The tensile property of the handsheets was examined using ISO 1974 (1990), using a tensile strength tester (ABB, Zurich, Switzerland). The burst property of the handsheets was measured according to ISO 2758 (2001), using a burst strength tester (ABB, Zurich).

RESULTS AND DISCUSSION

Optimization Preparation Process of Biomass-based Deinking Agents

The reaction time and temperature, concentration of NaOH solution, and proportion of plant fatty acids had impacts on the final product. The orthogonal test method was used instead of a comprehensive test to greatly reduce the number of required experiments and achieve reasonable results (Zhu and Chen 2014). It was employed to test the effects of four factors, while the appropriate formula and technologic conditions were determined *via* the orthogonal test and range analysis.

Table 1 shows the results and the analysis of the orthogonal test. Figure 2 shows the effects of various factors on brightness. Table 1 and Fig. 2 show the proportion of plant fatty acids, which had the most important influences on the final product. The concentration of the NaOH solution had secondary most important influences on the final product, and the reaction time and temperature had the least important influences on the final product. Figure 2 shows that when the mass ratio of $m_{\text{rosin}}:m_{\text{coconut fatty acid}}:m_{\text{palmitic acid}}$

= 2.5:3.5:4, the brightness of the secondary fiber showed the biggest increase, and when $m_{\text{rosin}}:m_{\text{coconut fatty acid}}:m_{\text{palmitic acid}} = 2:3:5$, the brightness of the secondary fiber showed the smallest increase. Figure 2 also shows that as the concentration of the NaOH solution increased from 30 wt.% to 40 wt.%, the brightness of the secondary fiber also increased. However, when the concentration of the NaOH solution increased from 40 wt.% to 50 wt.%, the brightness of the secondary fiber started to drop. The major reason for this was that when the concentrations of the NaOH solution were too high, the mixture was most likely to become nodular, resulting in the fatty acids not making contact with the NaOH solution well and the efficiency of the reaction declining. Therefore, the deinking properties also declined. Lastly, Fig. 1 shows that the reaction time and temperature did not have much effect on the brightness of the secondary fiber. Therefore, the optimized preparation conditions for the biomass-based deinking agent was 30 min, a temperature of 80 °C, a 2.5:4.5:4 mass ratio of $m_{\text{rosin}}:m_{\text{coconut fatty acid}}:m_{\text{palmitic acid}}$, and a NaOH concentration of 40 wt.%.

Table 1. Results and Analysis of Orthogonal Test

	Time (min)	Temperature (°C)	Concentration (wt.%)	Proportion*	Value of Brightness (% ISO)
1	30	80	50	2:3:5	3.82
2	40	80	30	2.5:3.5:4	5.62
3	50	80	40	3:4:3	4.85
4	30	90	40	2.5:3.5:4	6.12
5	40	90	50	3:4:3	4.67
6	50	90	30	2:3:5	3.88
7	30	100	30	3:4:3	4.63
8	40	100	40	2:3:5	3.96
9	50	100	50	2.5:3.5:4	5.31
K1	4.86	4.76	4.71	5.68	-
K2	4.75	4.89	4.98	3.89	-
K3	4.68	4.63	4.60	4.72	-
R	0.18	0.26	0.38	1.79	-

Note: *The proportion is $m_{\text{rosin}}:m_{\text{coconut fatty acid}}:m_{\text{palmitic acid}}$

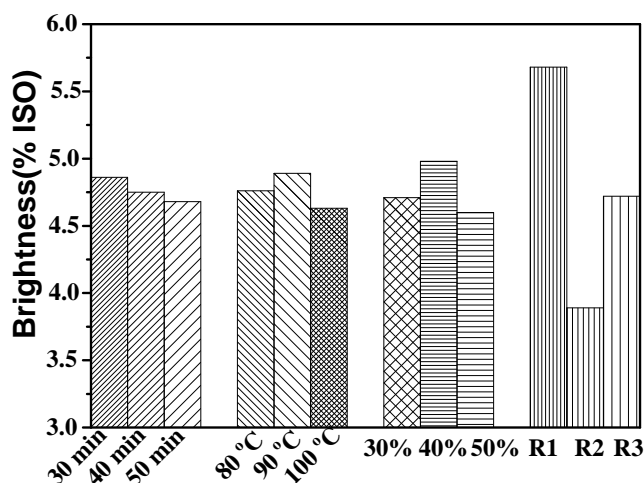


Fig. 2. The effect of various factors on brightness; R1, R2, R3 is $m_{\text{rosin}}:m_{\text{coconut fatty acid}}:m_{\text{palmitic acid}}$, and R1=2.5:3.5:4, R2=2:3:5, R3=3:4:3

Moisture Content Influence on Enzyme Activity Retention Rate of XBD

Figure 3 shows that the moisture content influenced the enzyme activity of XBD. As the moisture content was increased, the enzyme activity decreased faster. But during the preparation process, moisture was inevitable. Thus, in the formula it was essential to reduce the water content of the biomass-based deinking agent with the widest possible margin.

As shown in Fig. 3, when the moisture content was increased from 1.5 wt.% to 3.0 wt.%, the enzyme activity was not changed. By contrast, when the moisture content increased from 3.0 wt.% to 4.5 wt.%, the enzyme activity changed greatly. This was because when the moisture content was low enough, there were almost no free electriferous groups, so the enzyme activity was less affected. But when the moisture content was high, there were abundant free electriferous groups, such as Na^+ . The electriferous groups could be combined with the enzyme molecule by the electrostatic interactions, and it could alter the conformation of enzyme, lead to the loss of enzyme activity (Rubingh 1996; Sun *et al.* 2013). So it could decrease enzyme activity heavily in the course of extended storage time of XBD. At the same time, when the moisture content of the biomass-based deinking agent was too low, it could not mix well with the enzyme and had a bad effect on the products. At the same time, when the moisture content of the biomass-based deinking agent was too low, it could not mix well with the enzyme and had a bad effect on the products. Based on each kind of situation, the optimum moisture content should be limited to 3.0 wt.%.

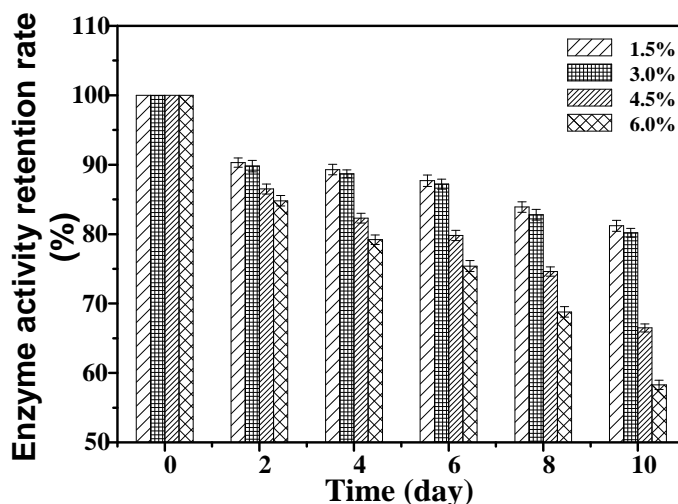


Fig. 3. The influence of moisture content on the enzyme activity retention rate

Free Alkali Influence on Enzyme Activity Retention Rate of XBD

It is clear that the preparation of the biomass-based deinking agent involved the neutralization reaction. After the neutralization reaction, the agent presented alkalinity. Although the xylanase is alkaline xylanase and its stability system should present weak alkalinity. However, because of the presence of free alkali, the pH of the biomass-based deinking agent was higher than the pH of the stabilized system, which made it necessary to discuss the free alkali influence on the enzyme activity of XBD.

Figure 4 shows that when the free alkali was increased from 0.05 wt.% to 0.10 wt.%, the decreasing extent of enzyme activity was not high. Though when the free alkali was higher than 0.10 wt.%, the decreasing extent of the enzyme activity was very high. It was found that the higher the free alkali, the lower the enzyme activity. Considering cost

and the enzyme activity, it follows that the free alkali of the biomass-based deinking agent should be controlled under 0.10 wt.%.

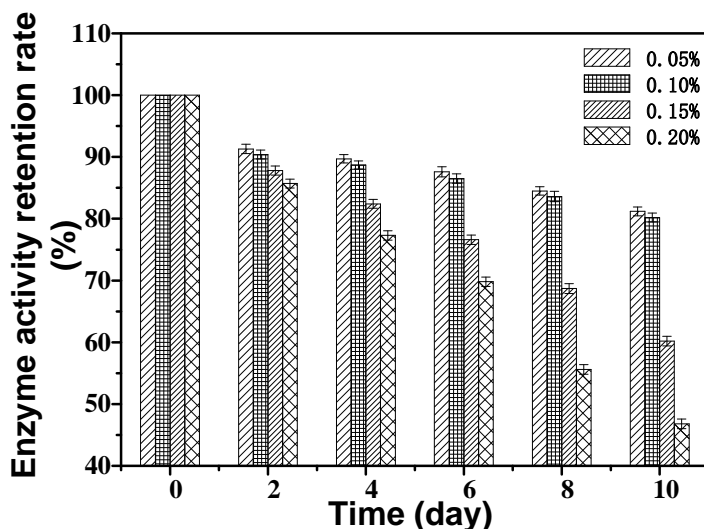


Fig. 4. The influence of free alkali on the enzyme activity retention rate

Chelating Agent Influence on Enzyme Activity Retention Rate of XBD

Because the chelating agent has a certain coordinating ability with some heavy metal ions (Ca^{2+} , Mg^{2+} , *etc.*), it could soften hard tap water by binding with dissolved metal ions and it could also prevent oxidation of the biomass-based deinking agent. In contrast, the EDTA-4Na is one of the chemical auxiliaries that had to be used in the current deinking process, as it could reduce soap scum from forming in the pipe.

Figure 5 shows the influence of the chelating agent on the enzyme activity of XBD. It was found that EDTA-4Na had no noticeable effect on enzyme activity with the same experimental conditions, which indicated that the XBD could be used in the deinking process smoothly.

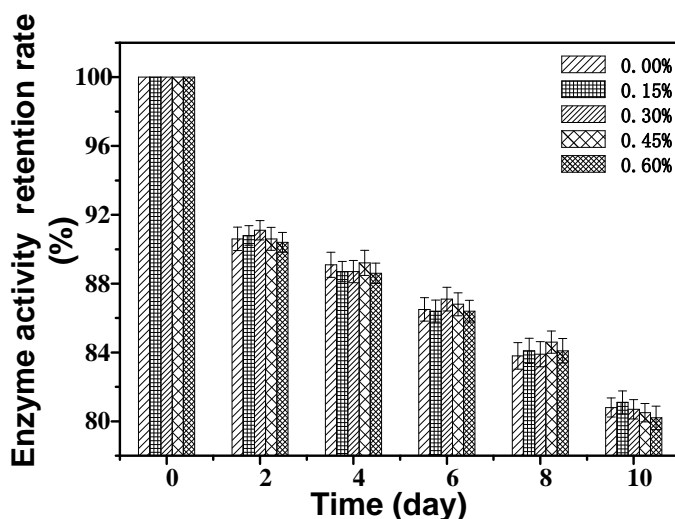


Fig. 5. The influence of EDTA-4Na on the enzyme activity retention rate

Dosage Influence of Xylanase on Secondary Fiber and Handsheet Properties

The relationships between the secondary fiber and handsheet properties with xylanase additions are shown in Figs. 6 through 8.

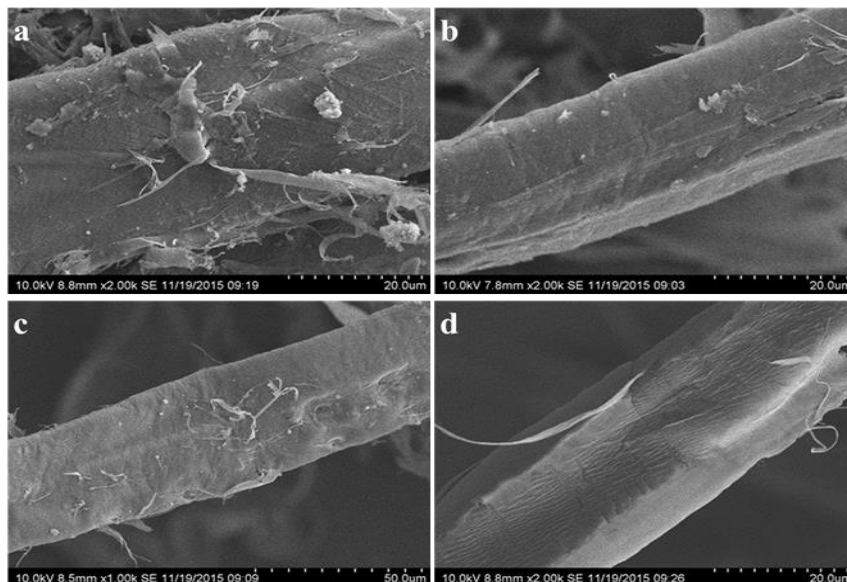


Fig. 6. SEM images of fiber before and after flotation: (a) fiber before flotation, (b) without added xylanase, (c) added 10 wt.% dosage of xylanase, and (d) added 15 wt.% dosage of xylanase

As shown in Figure 6, the effect of the xylanase treatment on the secondary fiber was clear. Figure 6a shows the SEM image of a single fiber before flotation. In this fiber there were lumpy and grainy ink clumps of varying dimensions. Figures 6b, c, and d were the SEM images of single fibers after flotation, and the XBD dosage was 0.2% during the flotation process. Figure 6b shows the single fiber after flotation without added xylanase into the biomass-based deinking agent. There were some lumpy and grainy ink residues in this fiber as well. Figure 6c shows the single fiber after flotation with an added 10 wt.% dosage of xylanase into the biomass-based deinking agent (there still were little grainy inks in the fiber). Figure 6d shows the single fiber after flotation with added 15 wt.% dosage of xylanase, where there were no noticeable lumpy or grainy inks in the fiber surface, but rather a smooth surface.

Figure 7 shows that when the XBD dosage was 0.2% and had an increased xylanase addition into the biomass-based deinking agent after flotation, the effective residual ink concentration of the secondary fiber decreased rapidly at first. It decreased from 274 ppm in the blank sample to 223 ppm. When the addition of xylanase exceeded 15 wt.%, the concentration decreased more slowly and finally became steady. The inks often connected with the fine fiber, and therefore they could not be separated from the fiber effectively during flotation deinking, thus this led to a high residual ink. The xylanase attacked the hemicellulose when used. The xylanase was highly efficient at hydrolyzing hemicelluloses, and therefore created a greater separation between the fiber and inks during flotation deinking that improved the flotation efficiency of flotation deinking effectively, and reduced the effective residual ink concentration. After the xylanase treatment, the brightness was higher than that of the blank, and the variation of the brightness was quite similar to that of one with an effective residual ink

concentration. When the xylanase addition into the biomass-based deinking agent was 15 wt.%, the brightness of the secondary fiber was 60.23% ISO. It was reasonable to infer that the amount of residual inks in the secondary fiber was decreased after the xylanase treatment. As the xylanase reaction substrate was constant, the xylanase dosage did not have a noticeable effect on the secondary fiber's effective residual ink concentration or brightness at concentrations over 15 wt.%.

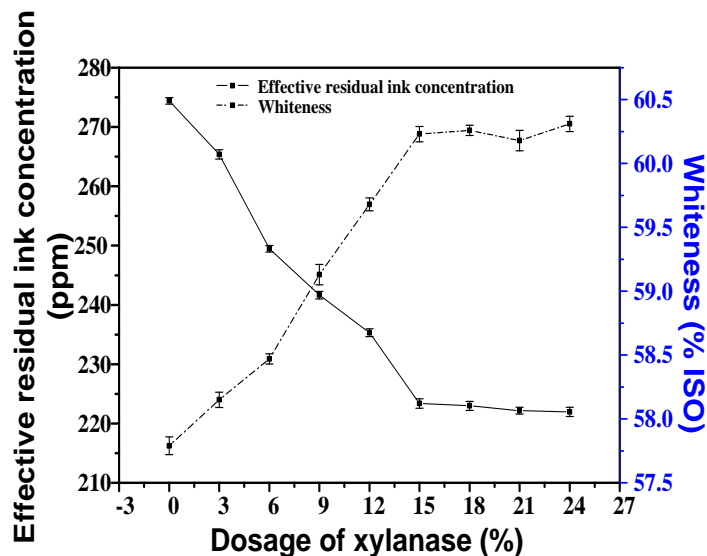


Fig. 7. The dosage influence of xylanase on brightness and effective residual ink concentration

Figure 8 shows that when the XBD dosage was 0.2%, with increased xylanase addition into the biomass-based deinking agent after flotation, the physical properties of handsheets made from the secondary fiber changed. The tensile index and burst index first increased when the dosage of xylanase was 15 wt.%.

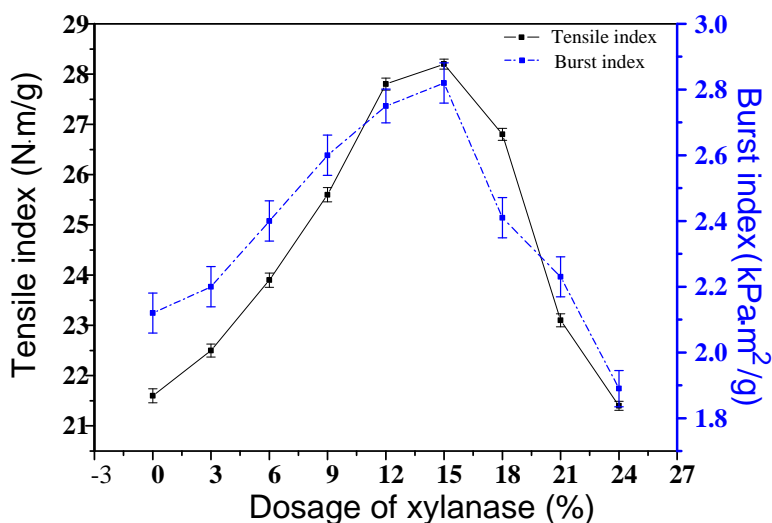


Fig. 8. The dosage influence of xylanase on tensile index and burst index

When the burst index of handsheets was $2.82 \text{ kPa}\cdot\text{m}^2/\text{g}$ and the tensile index of handsheets was $28.4 \text{ N}\cdot\text{m}/\text{g}$, it reached its maximum. When the dosage of xylanase was above 15 wt.%, the tensile index and burst index decreased. This was because the xylanase attacked the hemicellulose of the fiber, which had little to no influence on the cellulose; thus it improved the cellulose content of the fiber. The xylanase treatment process strengthened the binding forces between the fibers, and the average fiber length increased as short chains of hemicellulose and fines were destroyed, which improved the physical properties of the handsheets. However, when there was an excess of xylanase, some of the hemicellulose was degraded and the physical properties of the handsheets were reduced.

Based on a comprehensive analysis of the optical properties and physical strength properties, the optimum xylanase addition into biomass-based deinking agent was 15 wt.%, as well as when the XBD dosage was 0.2% after flotation. The optimum also occurred when the brightness of the secondary fiber was 60.2% ISO, the residual ink concentration was 223 ppm, the burst index of the handsheets was $2.82 \text{ kPa}\cdot\text{m}^2/\text{g}$, and the tensile index of the handsheets was $28.4 \text{ N}\cdot\text{m}/\text{g}$.

CONCLUSIONS

1. The optimized preparation conditions of biomass-based deinking agent was with a duration of 30 min, a temperature of $80 \text{ }^\circ\text{C}$, a 2.5:3.5:4 mass ratio of $m_{\text{rosin}}:m_{\text{coconut fatty acid}}:m_{\text{palmitic acid}}$, and a NaOH concentration of 40 wt.%.
2. Based on each kind of synthesized situation, the optimum moisture content should be limited to 3.0 wt.%. This resulted in the enzyme activity of XBD to be relatively stable for 10 days. With consideration to cost, and the enzyme activity, the free alkali of the biomass-based deinking agent should be controlled under 0.10 wt.%.
3. When the deinking agent dosage was 0.2%, the optimum xylanase addition into the biomass-based deinking agent was 15 wt.%. After flotation, the brightness of the secondary fiber was 60.2% ISO, the residual ink concentration was 223 ppm, the burst index of the handsheets was $2.82 \text{ kPa}\cdot\text{m}^2/\text{g}$, and the tensile index of handsheets was $28.4 \text{ N}\cdot\text{m}/\text{g}$.
4. The chelating agent had no influence on the enzyme activity of XBD, indicating that XBD could be used in the de-inking process smoothly.

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