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Effects of Enzyme Mixture and Beating Treatment on the Properties of Pulp Fibers

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ABSTRACT

Pulp fibers were pretreated with the mixture of endoglucanase and endoxylanase to facilitate the nanofibrillation. The effects of the combined pretreatment of the mixed enzymes and beating were investigated in terms of fiber length, fines content, freeness, water retention value (WRV), cupriethylenediamine (CED) viscosity, and the viscosity of cellulose nanofibrils (CNF) suspension. Compared to untreated and pure enzyme-treated pulps, the mixed enzyme-treated pulp showed higher fiber length and lower fines content. The CED viscosity of fibers was mainly affected by endoglucanase. The beating treatment on the enzyme pretreated pulp increased fines content and WRV greatly, which means that fiber structure was sufficiently weakened by enzyme treatment. In particular, when pulp was treated with enzyme mixture and beaten, the WRV and freeness were higher than single component-enzyme treatment. Depending on the enzyme composition and beating treatment, the low shear viscosity of the resultant CNF suspension exhibited different trend. It revealed that the use of endoenzyme mixture is beneficial to decompose pulp fibers and may control the aspect ratio of CNF.

Keywords: *Beating, cellulose nanofibers, combined treatment, enzyme, pulp properties*

1. Introduction

Cellulose nanofibril (CNF) which is prepared by repeated mechanical shearing has drawn a big interest from researchers. Owing to sustainability, biodegradability and unique properties of CNF, diverse applications of CNF has been extensively studied. To expand the use of CNF, the price of CNF should be inexpensive and the miscibility and dispersability should be improved. The production cost

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of CNF can be lowered by reducing the nanofibrillation energy, which can be achieved by pretreatment of pulp fibers prior to nanofibrillation. There are various pulp pretreatment methods such as 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)-mediated oxidation,[1] carboxymethylation,[2,3] and enzyme reaction.[4,5]

Among them, enzyme treatment is recognized as environmentally-friendly pretreatment because it does not require any solvent and produce less waste in the air. Enzymes have been widely used in the pulp and paper industry to hydrolyze materials such as cellulose and hemicellulose[6] for a long time. The enzyme pretreatment with cellulase, hemicellulase and pectinase can help the kraft pulping,[7] and laccase and protease reduce the energy required for the mechanical pulping.[8] Kraft pulp pretreated with cellulase and hemicellulase can be used to reduce the energy consumption required for the refining in papermaking process.[9] Enzymes can be used for removing stickies[10] and deinking[11] in the recovered paper process. In addition, enzymes are also used for the pulp fiber modifications, which change the freeness of pulp fibers and the density and smoothness of the paper. Considering these works of enzymes, enzyme have potential enough to aid the nanofibrillation of the pulp by weakening the structure of the pulp.

In some cases, it is hard to degrade the substrates with single enzyme, and enzyme mixture can be used to hydrolyze the substrates for improving the accessibility of enzymes to the substrates.[12] Such synergistic effects with mixed enzymes have attracted attentions from many researchers.[13]

Advantage of the mixed enzymes treatment is that they can hydrolyze more substrates with limited resources. However, most of researches, using enzyme for preparation of CNF, use single enzyme system,[4,5,14] or use enzymes mixture under the restricted condition with no further considerations of the mixing ratio of enzymes even though each enzyme has different role in the activity.[15] More researches are required to understand the roles of each enzymes in the activity of enzymes mixtures. The aim of this study is to evaluate how the mixed use of endoglucanase and endoxylanase as pretreatment affects the pulp properties prior to production of CNF. In addition, the combined effects of mixed endoenzymes and beating on the pulp properties are investigated. For this purpose, the never-dried bleached kraft pulp was pretreated with mixture enzymes in different ratios. Fiber length, fines, freeness, water retention value (WRV), and cupriethylenediamine (CED) viscosity of each pretreated pulp were evaluated. Beating was used to reveal and promote the effect of enzyme pretreatment on the pulp. In addition, each pretreated pulp nanofibrillation behavior was examined in terms of suspension viscosity.

2. Materials and Methods

2.1 Materials

Never-dried bleached kraft pulp supplied by Moorim P&P Co., Ltd.(Korea) was used as raw material. According to sugar analysis by HPLC, the pulp fibers consist of 75% glucose, 19% xylose, and 0.5% mannose. The contents of lignin and ash were very small, so they are negligible. Endoglucanase (Fibercare R, Novozymes, Denmark) and endoxylanase (Pulpzyme HC 2500, Novozymes, Denmark) were used for pulp treatment in this study. These enzymes were supplied by Buckman Laboratories Korea.

2.2 Pretreatment of pulp fibers with endoenzyme mixture

100 g (oven dried weight) of never-dried bleached kraft pulp was diluted to 10 wt% with deionized (DI) water. The pH of the diluted pulp suspension was

adjusted to 7 using buffer solution. Enzyme mixtures was added by the addition level of 0.5 wt% based on the oven dried weight of the pulp. Enzyme mixtures were prepared by mixing endoglucanase (referred as 'G') and endoxylanase (referred as 'X') with different weight ratios as mentioned in Table 1.

The pulp and enzyme mixtures were placed in a plastic bag and reacted at 50°C for 1 hour in the water bath. During the enzyme reaction, the pulp samples were kneaded at intervals of 15 minutes. For termination of the reaction, the pulp samples were boiled for 15 minutes and then washed to deactivate the enzyme. Each enzyme-treated pulp was beaten with a laboratory Valley beater to reveal the effect of the combined pretreatment of enzyme and beating on the pulp. For beating, the enzyme-treated pulp slurries were diluted to 1% stock with DI water. Each stock was beaten for 15 minutes.

2.3 Evaluation of pulp properties

The pulp properties including fiber length, fines content, freeness, WRV and CED viscosity were measured on the enzyme pretreated pulp samples and the enzyme-beating combined pretreated pulp samples. Fiber length and fines content were measured using a Kajaani FiberLab (Kajaani, Finland). The measurement on each condition was repeated three times and more than 3,000 fibers were measured at each measurement. The fiber length result was suggested as a length-weighted average fiber length. Canadian Standard Freeness (CSF) of each treated pulp was measured in accordance with TAPPI method T 227 om-99.

The WRV of each treated pulp was measured in accordance with Scan-C 62:00. Each sample was centrifuged at 3000 G for 15 minutes. The CED viscosity of the treated pulp fibers was measured in accordance with TAPPI method T 230 om-99. The pulp fibers were dissolved in the CED solution. Then, the viscosity of the cellulose solution was measured using a capillary viscometer.

2.4 Preparation of CNF from enzyme treated pulp

The consistency of each pretreated pulp suspension was adjusted to 1.5 wt%. CNF was prepared using a grinder (Supermasscolloider, Masuko Sangyo, Japan) from each pretreated pulp suspension. The gap distance between the stones was −80 μm and the stone rotation speed was 1500 rpm. Sampling was done after passing 30 times through the grinder, and the low shear viscosity of the CNF suspension was measured. The low shear viscosity of the fiber suspension (1%) was measured at 25±1°C using Brookfield viscometer (Brookfield Engineering Laboratories, USA). The morphology of CNF was observed using field-emission scanning electron microscope (SUPRA 55VP, Carl Zeiss, Germany).

3. Results and Discussion

3.1 Properties of mixed enzyme treated pulp fibers

Pulp fibers were pretreated with the mixture of endoglucanase and endoxylanase to facilitate the nanofibrillation. Also, the effects of the combined pretreatment of the mixed enzymes and beating were investigated in terms of fiber length, fines content, freeness, WRV, and CED viscosity.

Table 1. The composition of enzyme mixture

Condition	G:X 100:0	G:X 80:20	G:X 60:40	G:X 40:60	G:X 20:80	G:X 0:100
Endoglucanase (G)	100	80	60	40	20	0
Endoxylanase (X)	0	20	40	60	80	100

Fig. 1 shows the fiber length of each pretreated pulp depending on the mixing ratio of endoenzymes and beating pretreatment. The 'Con' sample corresponds to untreated pulp fiber sample. When only enzyme pretreatment was done on the pulp fibers, the average fiber length of fibers was rather longer for enzyme pretreated pulp fibers than for the untreated one. The fiber length was increased as the mixing ratio of the endoxylanase was increased up to 60%. The increase in fiber length seemed to be due to the degradation of the fibrils and primary fines in kraft pulp by enzyme. When the endoxylanase content was more than 60%, however, fiber length was decreased. The combined use of endoglucanase and endoxylanase with proper mixing ratio was more effective in increasing the average fiber length. When the enzyme-treated pulp was beaten, the fiber length result shows different trend compared to the case of only enzyme treatment. The fiber length increased by enzyme pretreatment was decreased by beating for all enzyme conditions. In particular, the fiber length was more decreased when the content of endoxylanase in the enzyme mixture was increased. Because endoglucanase attack cellulose chain, it was expected to give greater impact on the fiber length than other conditions. However, endoxylanase boosted the cutting effect rather than endoglucanase. This change in fiber length was likely to be originated from the fines content, as shown in Fig. 2. Generally, the fines content showed opposite trend against fiber length depending on the enzyme composition and beating. With enzyme treatment, fines content was decreased, which resulted in the increase in the fiber length. Endoglucanase and endoxylanase would be more adsorbed on the particles with the large specific surface area, like fibrils or fines, and decomposed them. This result is similar to the work done by Pommier et al.[16] More fines were removed when the enzyme mixture was added to the pulp slurry compared to the use of single component-enzyme. It seemed that accessibility of enzyme to fibers would be improved by use of the mixed enzyme. However, the combined treatment of enzyme reaction and beating generated much more fines in pulp slurry. Contrary to only enzyme treatment, additional beating treatment created fines much more. It was because that some of long fibers were degraded into small particles by enzyme attack. When pulp was treated by endoxylanase with higher dosage and beating, more fines were

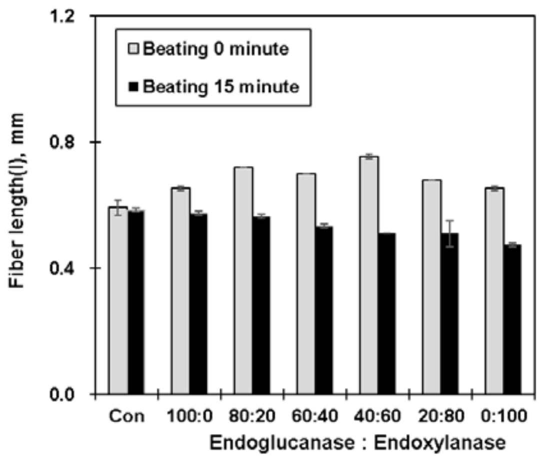

Fig. 1. Fiber length of pulp fibers depending on enzyme composition and beating.

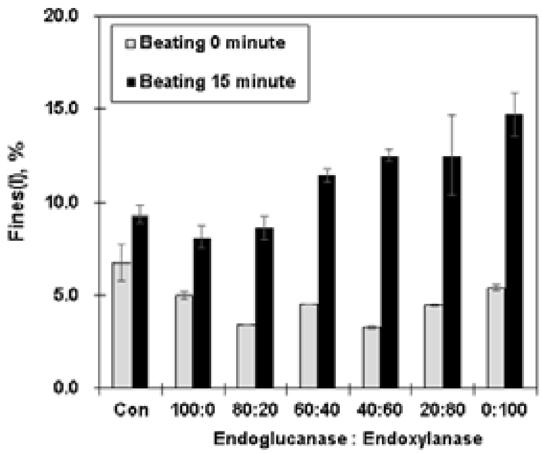

Fig. 2. Fines content of pulp fibers depending on enzyme composition and beating.

produced. Even though endoglucanase decrease the degree of polymerization of cellulose, it doesn't lead to the generation of fines in beating. On the other hand, degradation of hemicellulose(xylose) in fibers is helpful to swelling the fiber wall, and accelerates the beating. It resulted in higher amount of fines.

Fig. 3 showed the WRV of pulp fibers depending on the mixing ratio of the enzymes and beating pretreatment. The WRV of the enzyme-pretreated pulp was slightly higher than that of untreated pulp, and beating treatment increased the WRV independently on the mixing ratio of pulp. Beating treatment was more effective in increasing the WRV compared to enzyme treatment. In addition, beating effect was more remarkable for mixed enzyme pretreated pulp. Abson and Gilbert[17] reported that WRV decreased with removal of fines. In this study, however, WRV is not decreased but slightly increases even though fines are removed by enzyme activity. It means that the enzyme pretreatment has two opposite effects on WRV. The first effect is a decrease in WRV due to the removal of fines. And the second one is an increase in WRV by internal fibrillation which comes from the degradation of fiber component by enzyme mixture. The WRV of the mixed enzyme-treated pulp is a little higher than that of control pulp or single component-enzyme treated pulp, which is due to creation of new surfaces like internal fibrillation by more hydrolysis of pulp component by enzyme mixture. It indicates that the mixed enzyme treatment was more effective in degrading the internal structure of fibers. The change in WRV by beating can be explained by internal and external fibrillation and fines generation as mentioned by Thode et al.[18]

Fig. 4 showed the change in the CSF of the untreated and pretreated pulp fibers with beating. The mixed enzyme-treated pulp has higher CSF than non-treated pulp does, as mentioned in a previous work by Fuentes et al.[19] The CSF of pulp fibers was decreased with beating regardless of enzyme treatment, which was caused by the generation of fines and fibrillation, and fiber swelling. Untreated pulp and single component-enzyme pretreated pulps tended to show a linear decrease in freeness with beating time, whereas the pretreated pulp by the enzyme mixture showed a drastic reduction of the CSF until 7.5 min. and thereafter slow decline in the CSF. According to Clark, too much fines caused a 'negative freeness',

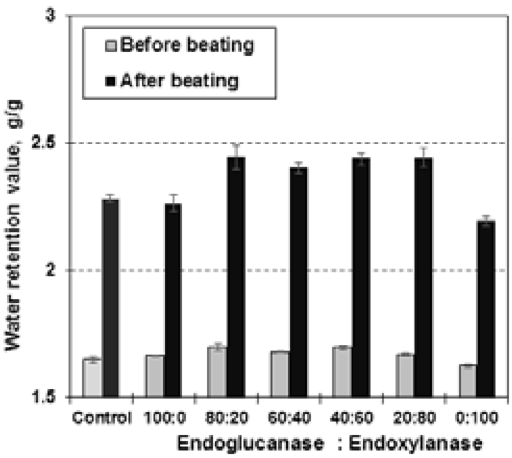

Fig. 3. WRV of pulp fibers depending on enzyme composition and beating.

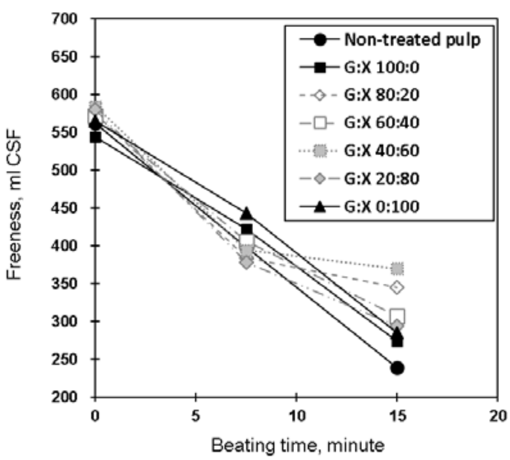

Fig. 4. Freeness of pulp fibers depending on enzyme treatment and beating.

which means an increase in CSF as fines pass through the perforation plate of the CSF freeness tester instead of forming a pulp mat.[20] Therefore, a slight decrease in CSF freeness by 15 min beating might be due to the generation of fines with high amount.

The degree of polymerization (DP) of fibers was evaluated by CED viscosity of cellulose solution. Fig. 5 shows the CED viscosity of cellulose depending on enzyme composition and beating pretreatment. Enzyme treatment decreased the CED viscosity, that is, DP of cellulose in the pulp, but beating did not change the DP of cellulose significantly. The DP of cellulose treated with endoglucanase was reduced by 30% compared to the control untreated pulp in the experimental conditions. The beating did not affect the DP of the single endoglucanase- or endoxylanase-treated pulp, but the DP of the enzyme mixture-treated pulp was reduced to some extent but insignificantly. It means that the DP of cellulose was affected by mainly endoglucanase.

3.2 Effect of enzyme pretreatment on nanofibrillation

CNF was produced using the enzyme-pretreated

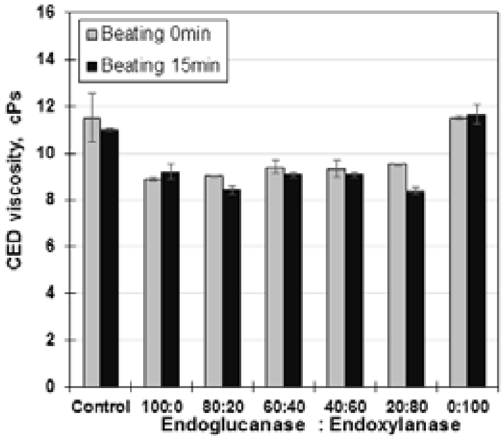

Fig. 5. CED viscosity of pulp fibers depending on enzyme composition and beating.

pulps and the enzyme-beating pretreated pulps by passing through a grinder 30 times. The low shear viscosity of each CNF suspension was measured (Table 2). The low shear viscosity of CNF suspension is one of important properties of CNF because not only it can predict change in morphology such as shape, aspect ratio and surface areas in CNF,[21] but also it can be used to confirm complete nanofibrillation of fibers.[3] Generally, chemical pretreatment like carboxymethylation resulted in CNF suspension with very high viscosity compared to untreated CNF.[3] However, in this study, enzyme-pretreated CNF exhibited relatively lower viscosity compared to untreated CNF. In particular, enzyme mixture-pretreatment created CNF with lowest viscosity. When enzyme pretreatment was followed by beating treatment, the CNF had higher viscosity than those without beating pretreatment. However, enzyme mixture conditions showed lower viscosity compared to untreated CNF or single enzyme case.

The enzyme mixture pretreatment showed a larger change in the CNF suspension viscosity than that of single component-enzyme pretreatment. This seemed be due to the synergistic activity of endoenzymes mixture which reduced the DP of cellulose in the pulp and weakened the strength of pulp structure. It is known that enzyme pretreatment contributes to reduce nanofibrillation energy by decomposing the cell wall components.[4] In this study, grinding pass number to complete nanofibrillation was reduced by enzyme pretreatment. However, the width of enzyme-pretreated CNF is similar to that of untreated CNF. Fig. 6 shows the SEM images on the enzyme-beating pretreated CNFs. Because of the observation magnification, it is hard to measure the length, but the widths of CNFs are similar irrespective of pretreatment condition. Therefore, low viscosity values are attributed to the decrease in the aspect ratio of CNF. Therefore, it is expected that enzyme mixture pretreatment can be utilized to control the aspect ratio of CNF.

4. Conclusions

This study was conducted to investigate the effect of endoglucanase and endoxylanase in the mix usage on the pulp as pretreatment for production of CNF. In addition, the effect of enzyme mixture and beating pretreatment on the properties of pulp and nanofibrillation was evaluated. The enzyme pretreatment removed fines in the pulp and increase fiber length. The DP of cellulose was affected mainly by endoglucanase. With beating treatment, fiber length was decreased with an increase in fines content, and greatly increased WRV. When the mixed enzymes were treated on pulp fibers, WRV and freeness were changed drastically. Enzyme mixture treatment was more effective in changing pulp properties and decompose the pulp fibers compared to control or single-component enzyme treatment. In addition, the mixed enzyme system resulted in CNF suspension with lower Brookfield viscosity. It means the CNF morphology can be affected by enzyme composition. It is expected that enzyme mixture pretreatment can be used to produce CNF with controlled aspect ratio.

Acknowledgments

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Table 2. Low shear viscosity of CNF suspension depending on pretreatment condition (Unit: cPs)

Condition	Control	Endoglucanase : Endoxylanase					
		100:0	80:20	60:40	40:60	20:80	0:100
Without beating	1131	782	414	282	290	370	550
With beating	1600	1498	1262	854	1262	1490	1264

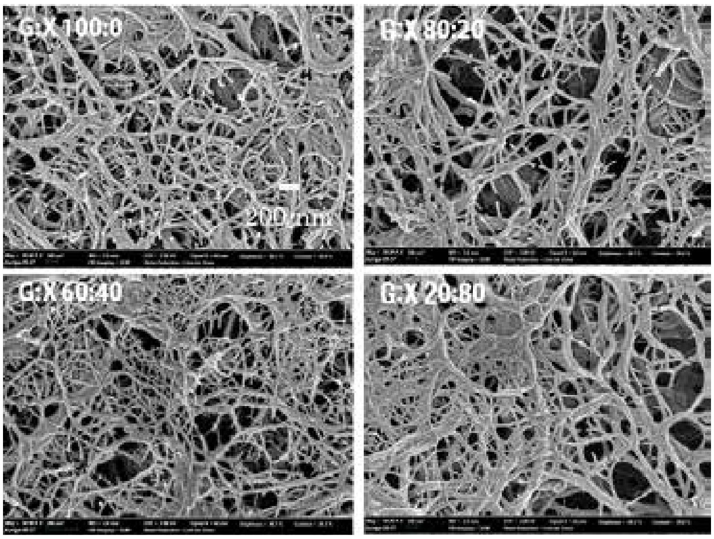

Fig. 6. SEM images of CNF pretreated by enzyme and beating (30 passes).

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