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Kraft 펄프의 효소표백반응에 미치는 페놀라디칼 전달체의 영향

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The Effects of Phenolic Radical Carriers on the Enzymatic Bleaching of Kraft Pulp

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ABSTRACT

The effects of enzymatic pretreatments on the bleaching of kraft pulp were studied. The kappa number of pulp samples which represents the lignin content of pulp decreased by 25.2% by the pretreatments with xylanase(EC 3.2.1.8, Pulpzyme HB) while it decreased by 13.7% without enzyme pretreatments after the extraction of the pretreated pulp samples in 1N NaOH. To enhance the effects of enzymatic pretreatment on the bleaching of kraft pulp, phenols were used as radical carriers with the simultaneous use of peroxidase(EC 1.11.1.7, Novozyme 502), H_2O_2, and xylanase. Guaiacol (1mM) was most effective by decreasing the kappa number by 29.6% when a low initial concentration of H_2O_2 (0.1mM) was used. The use of either a higher initial concentration of H_2O_2 or phenols lacking electron donating substituents such as phenol and p-chlorophenol, however, decreased the efficiency of enzymatic pretreatment indicating that the production rate and the stability of phenolic radicals are important parameters.

INTRODUCTION

Lignin is the most abundant aromatic polymer in nature and found in the cell wall of most plants being covalently bonded to hemicellulose (1). Lignin is an amorphous polymer having phenyl propane as a monomeric unit and most resistant to biological degradation among natural polymers. To produce bleached pulp from woody plants, most lignin is removed during alkaline pulping process(kraft-process) and the remaining small amount of lignin is further removed from the pulp through the bleaching processes most of which use chlorine or chlorine dioxide(2). Due to the environmental concerns caused by the use of the chlorinated compounds which generates in the effluents chlorinated aromatic compounds recalcitrant to conventional waste treatment processes, alternative bleaching methods which do not require chlorinated compounds are being sought. Among these are the bleaching processes using oxygen, ozone, or hydrogen peroxide as well as microorganisms or enzymes(3).

Using enzymes for the bleaching of pulp causes virtually no environmental problems and is very promising in practical point of view. Xylanases (endo-1,4-β-D-xylanase) which hydrolyze hemicellulose, thereby help to dissociate lignin from cellulose(3) and peroxidases which are known to be involved in the natural degradation of lignin by microorganisms are of great interest(4, 5). Enzymes, however, have poor accessibility to lignin molecules in aqueous solution due to the high molecular weights of enzymes and the low solubility of lignin in water. The reactivity between enzymes and lignin samples which are either isolated from biomass or synthesized *in vitro* was shown to increase by adding veratryl alcohol(6) or organic solvents(7, 8).

In this study, we investigated the use of a xylanase(EC 3.2.1.8, Pulpzyme HB) and a peroxidase(EC 1.11.1.7, Novozyme 502) which are commercially available for the bleaching of kraft pulp which is produced locally. We especially studied the possibility of using various phenolic compounds as radical carriers with the concomitant use of xylanase, peroxidase, and H_2O_2. The production rate and the stability of the phenolic radicals formed by the peroxidase and H_2O_2 were varied by either changing H_2O_2 concentration or using phenols of different substituents, respectively. Furthermore, the effects of enzymatic pretreatments on the efficiency of the subsequent processes including alkali extraction and bleaching with chlorinated compounds were, also, examined.

MATERIALS AND METHODS

Materials

Phenols were purchased from Aldrich Chemical Co.(Milwaukee, WI, U. S. A) and guaiacol from Sigma Chemical Co.(St. Louis, MO, U. S. A). Xylanase (EC 3.2.1.8, Pulpzyme HB) and peroxidase(EC 1.11.1.7, Novozyme 502) were obtained from Novo Nordisk A/S(Basvaerd, Denmark). Xylanase has an activity as an endo-1,4-β-D-xylanase without showing an activity as a cellulase. Other chemicals were purchased from Sigma Chemical Co. and used without further purification.

Measurement of Kappa Number

The lignin content of a pulp sample is represented by a kappa number which is defined as the ml of $KMnO_4$ solution(0.1N) consumed to oxidize 1gr oven-dried(o.d.) pulp samples. The kappa number of a pulp sample was measured following the procedures suggested in TAPPI T 236cm^{-85}. The approximate lignin content of a pulp sample is obtained by dividing the kappa number of the sample by 7(4).

Measurement of Brightness

The brightness of a pulp sample is determined by measuring the reflectance at 457nm of the handsheet made of a pulp sample and representing the reflectance as the percentage of the reflectance of the perfectly reflecting surface. An elrepho 2000(model : datacolor) was used to measure the brightness.

Enzymatic Pretreatments of Pulp

Samples of unbleached kraft pulp after the alkaline pulping of the wood chips were taken from the production line, washed with distilled water, air-dried, then stored at room temperature for further use. For a typical enzymatic pretreatment, 200gr of the stored pulp samples were taken and soaked in a 2 ℓ of a buffer solution. After 15hrs of soaking pulp samples were dewatered by centrifugation. After measuring the water content and kappa number of the pulp sample, 100gr of the dewatered pulp sample was taken into a plastic bag and preheated to 50℃ in a temperature controlled water bath. The enzymatic pretreatment of the pulp sample was started by adding solutions of enzymes and hydrogen peroxide separately. The pulp consistency(solid content) was adjusted to 6% by adding a buffer solution. The pretreatment was continued for 3hrs at 50℃ with frequent kneading. Typically, pretreated pulp samples were washed with distilled water, dewatered, then extracted in 1N NaOH solution at 70℃ for 90min. The pulp consistency at the extraction was adjusted to 10%. After the extraction pulp samples were washed with distilled water, dewatered, then the brightness and the kappa number were measured.

Bleaching of Pulp with Chlorine Dioxide

After enzymatic pretreatment and alkaline extraction as described above, pulp samples were further bleached with chlorine dioxide according to the following procedure. To a 50g of pulp in a plastic bag chlorine dioxide was added, the amount of which varied between 1% to 6%(w/w) of the weight of o.d. pulp sample. The weight of chlorine dioxide was represented by the weight of the active chlorine. Bleaching by chlorine dioxide was performed at 65℃ for 2hrs with the pulp consistency being adjusted to 10% by adding distilled water. After the bleaching, pulp samples were washed with distilled water, dewatered, then extracted in an alkaline solution at 70℃ for 1hr at a pulp consistency of 10%. The amount of NaOH used for the alkaline extraction was 0.4% of the weight of the o.d. pulp sample. After the alkaline extraction the pulp samples were washed with distilled water, dewatered, then the brightness and the kappa number of the final pulp samples were measured.

RESULTS AND DISCUSSION

Enzymatic Pretreatments of Pulp with Xylanase

The optimum conditions for the pretreatment of pulp samples with Pulpzyme HB as suggested by Novo Nordisk A/S are as followings : the amount of enzyme, 300~800 EXU/kg o.d. pulp ; buffer pH, 6.0~8.0 ; temperature, 50℃ ; treatment period, 2~3hrs ; pulp consistency, 10%. Based on these pretreatment conditions, we tested the enzyme for the pretreatments of kraft pulp produced at Donghae Pulp Co. Ltd.(Ulsan, Korea) Table 1 shows the decrease in kappa number of kraft pulp by various pretreatments in distilled water containing no buffering agent. By the enzymatic pretreatment and washing without subsequent alkaline extraction followed, the kappa number of the kraft pulp decreased by 9.1 % from the initial value of 18.6 to the final value of 16.9. This extent of decrease in kappa number was comparable to that of 8.6% for the control experiment without using enzyme. Therefore, the effect of using the enzyme for the pretreatment of pulp seems insignificant. The effect of the enzymatic pretreatment of pulp, however, becomes conspicuous after going through the subsequent alkaline extraction step. After the alkaline extraction, the kappa number of the pretreated pulp samples with xylanase decreased by 18.8% almost the twice of that of 9.7% for the pretreated pulp samples in the absence of enzyme. Xylanase is known to cleave the hemicellulose which connects lignin to cellulose, thereby, enhance the extractability of lignin by alkaline extraction(4). When the pretreatment of pulp was performed in a buffer solution(pH7, 100mM KH_2PO_4) instead of distilled water, the kappa number after alkaline extraction decreased by 25.2% using xylanase while it was 13.7% without using xylanase.

Table 1. Effects of various pretreatments of unbleached kraft pulp in distilled water.

Treatment	Kappa number	% decrease
unbleached kraft pulp	18.6	0.0
distilled water[a]	17.0	8.6
distilled water+extraction[b]	16.8	9.7
pulpzyme HB[a]	16.9	9.1
pulpzyme HB+extraction[b]	15.1	18.8

a : Kraft pulp was treated in a plastic bag at 50℃ for 3hrs at 10% consistency with frequent kneading. Pulpzyme HB(xylanase preparation from NOVO) was used at the loading of 600units/kg o.d. pulp

b : Enzyme-treated kraft pulp was washed with distilled water, dewatered, then extracted with 1% NaOH in distilled water at 70℃ for 90min at 10% consistency.

Next studied were the effects of changes in various pretreatment conditions on the bleaching of kraft pulp by xylanase. These were pH values of buffer solutions, the amount of xylanase used, and pulp consistency. Each enzymatic pretreatment was followed by the alkaline extraction ac-

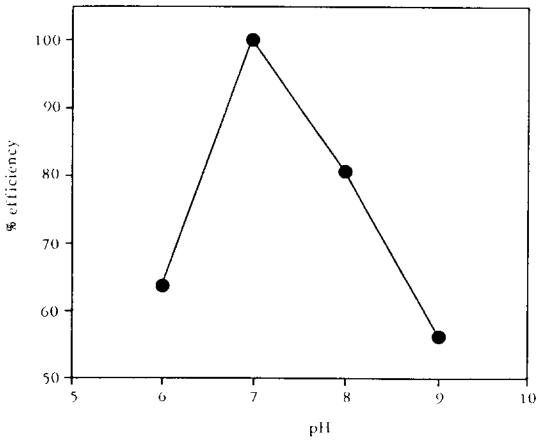

Fig. 1. Effects of solution pH on the efficiency of pretreatments of pulp with xylanase. Buffers used were KH_2PO_4(pH 6, 7, 8) and $NaCO_3$(pH 9) at 100mM each.

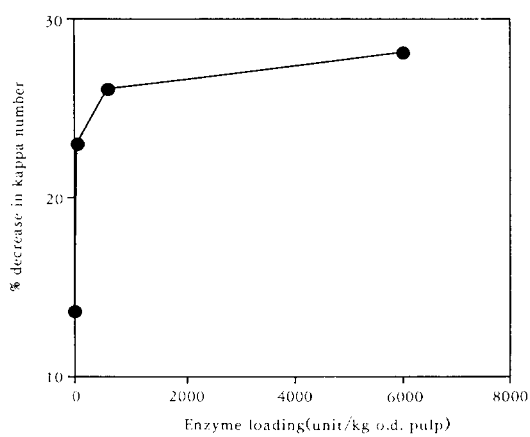

Fig. 2. Effects of xylanase loading on the efficiency of pretreatments of pulp with xylanase.

cording to the procedures as described above. Fig. 1 shows the dependence of the relative efficiency of the pretreatment with xylanase on the pH values of buffer solutions. The bleaching efficiency was maximal in a buffer solution of pH 7. Fig. 2 shows the effects of the amount of xylanase used on the percentage of decease in kappa number of pulp samples after the pretreatment and subse-

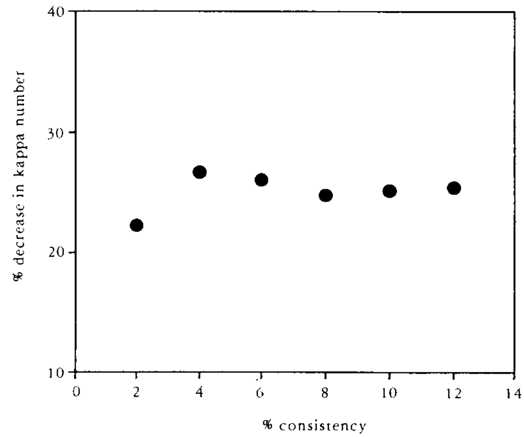

Fig. 3. Effects of pulp consistency on the efficiency of pretreatments of pulp with xylanase.

quent alkaline extraction. When the amount of xylanase used was above 600 EXU per kg o.d. pulp, the efficiency of enzymatic pretreatment was saturated without any significant increase in the percentage of decrease in kappa number as the amount of xylanase used increased. This is possibly due to the heterogeneity of the pretreatment system. The pulp samples are in a solid state while xylanase is in a dissolved state. Therefore not all xylanase molecules present are available for the enzymatic pretreatment. In another word, the efficiency of enzymatic pretreatment is governed by the surface area of the pulp samples accessible by the enzyme molecules. Therefore it is worthwhile to increase the accessible surface area of pulp for the enzymatic action by decreasing the size of pulp through some mechanical methods for example prior to the enzymatic pretreatment. Fig. 3 shows that the effects of pulp consistency on the efficiency of pretreatments of pulp by xylanase is not significant over the consistency range between 2% to 12%. Based on those results, typical enzymatic pretreatments for the subsequent experiments were performed in a buffer solution of pH 7 with the xylanase usage of 600 EXU/kg o.d. pulp of the consistency of 6% for ease of mixing.

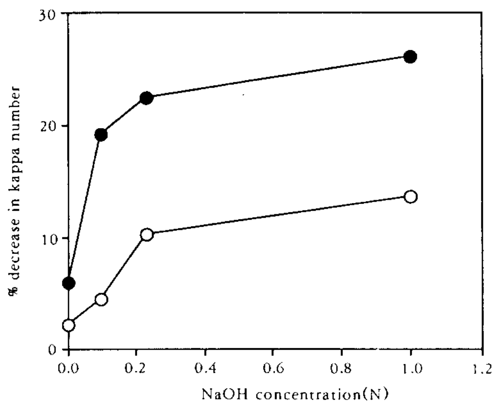

Fig. 4. Effects of pretreatments of pulp with xylanase on the extractability of pulp by sodium hydroxide for the pretreated pulp samples without enzyme(○) and with xylanase(●).

The Effects of Enzymatic Pretreatments on the Efficiency of Alkaline Extraction

The most heavily used chemical for the production of bleached kraft-pulp is sodium hydroxide most of which is recovered from the process effluents. The recovery process of sodium hydroxide, however, requires a large quantity of energy most of which is supplied by burning lignin separated from the wood chips. Reducing the usage of sodium hydroxide in alkaline extraction process will decrease the energy requirement for the recovery process which will in turn render more lignin available for the potential high value usages(9) rather than the current cheapest use of burning as energy supplement. Therefore it was of interest to study how the enzymatic pretreatment of pulp will affect the efficiency of the subsequent alkaline extraction with sodium hydroxide. Fig. 4 compares the percentage of decrease in kappa number of pulp after the alkaline extraction in various concentration of sodium hydroxide over the range between 0 to 1N with or without prior enzymatic pretreatments. The effects of enzymatic pretreatments on the efficiency of alkaline extraction became more pronounced when lower concentrations of sodium hydroxide were used. When the concentration of sodium hydroxide for the extraction was 0.1N, for instance, the percentage of decrease in kappa number of the pretreated pulp samples with xylanase was 19.2% almost 4.4 times of 4.4% of the pretreated pulp samples without xylanase. As the concentration of sodium hydroxide for the extraction increased the effect of enzymatic pretreatment was attenuated. When the concentration of sodium hydroxide was 1N, the enzymatic pretreatment of pulp enhanced the efficiency of the alkaline extraction by twice. Consequently enzymatic pretreatment of pulp by xylanase will result in significant decrease in the usage of sodium hydroxide for the extraction of lignin from the pulp samples.

The Use of Peroxidase and Phenols for Enzymatic Pretreatments

Peroxidase which oxidizes phenolic compounds, structural units of lignin, was used to investigate whether peroxidase will enhance the bleaching efficiency of enzymatic pretreatments. Using peroxidase only in enzymatic pretreatment was shown to have a minor effect on the bleaching of pulp (10). In this study, therefore, peroxidase was used simultaneously with xylanase. The potential of using phenolic radicals which are produced from the oxidation of added phenolic compounds by peroxidase and hydrogen peroxide as radical carriers to enhance the bleaching efficiency was also studied. When using phenolic radicals, two consequences can be expected : One is the degradation of lignin as studied by Hammel and Moen to degrade synthetic lignin by lignin peroxidase (6). The other is the combination of phenolic radicals and lignin molecules to increase molecular weight of lignin as studied by Blinkovsky and Dordick(11) to produce phenolic resin-like polymers from lignin. The possibility of each consequence to occur depends on the properties of phenols used and the production rate of phenolic radicals.

We tested the effects on the bleaching efficien-

Table 2. Effects of various pretreatments of kraft pulp in a buffer solution.

Treatment	% decrease
unbleached kraft pulp	0
buffer	2.2
buffer+extraction	13.7
pulpzyme HB	6
pulpzyme HB+extraction	25.2

Methods of pretreatments and extraction of pulp samples were the same as those in Table 1 except a buffer solution (pH 7, 100mM KH_2PO_4) was used in stead of distilled water.

Table 3. Effects of the addition of phenols as radical carriers.

phenols \ mM H_2O_2	0.2	1	2
phenol	18.0	—	12.0
p-cresol	25.8	22.2	16.5
p-chlorophenol	20.2	—	12.5
guaiacol	29.2	16.7	15.9

Xylanase and peroxidase loadings were 600 unit/kg o.d. pulp and 3×10^5 unit/kg o.d. pulp, respectively. The fixed initial concentration of phenol was 1mM.

cy of kraft pulp of electronic properties of phenols and the production rate of phenols being controlled by the initial concentration of H_2O_2. Table 3 lists the percentage of decrease in kappa number when four phenols(phenol, p-cresol, p-chlorophenol, guaiacol) were used at 1mM with various concentrations of H_2O_2(0.2mM-1mM). When the initial concentration of H_2O_2 was 0.2mM bleaching efficiency was reduced for phenol and p-chlorophenol. The percentage of decrease in kappa number was reduced to 18.0% for phenol and 20.2% for p-chlorophenol for example while it was 25.2% in the absence of the peroxidase system. For p-cresol and guaiacol, however, bleaching efficiency was increased with the percentage of decrease on kappa number of 25.8% and 29.2% respectively. These results imply the importance of the stability of phenolic radicals. p-Cresol and guaiacol which have an electron donating methyl or methoxy group have higher radical stability by localization than phenol or p-chlorophenol. Therefore radicals of p-cresol or guaiacol have longer life time and subsequently more chance to interact with lignin than the radicals of phenol or p-chlorophenol which has shorter life time, therefore, form dimers or oligomers of themselves immediately after the formation of the corresponding radicals.

The local concentration of phenolic radicals is also an important parameter for radical transfer. If it is high, phenolic radicals will react with themselves to form dimer or oligomers rather than transfer radicals to lignin molecules. This rationale is also shown in Table 3. When the concentration of H_2O_2 was as high as 2mM, for example, the percentage of decrease in kappa number was reduced for all phenols. As consequences, producing phenolic radicals of high stability at a low rate enable the radical transfer from phenols to lignin thus enhance the bleaching of kraft pulp. When the concentration of H_2O_2 was lowered to 0.1mM, the percentage of decrease in kappa number was 29.6% for guaiacol.

The Effects of Enzymatic Pretreatments on the Chlorine Bleaching of Pulp

Enzymatic pretreatments of kraft pulp which enhance the extractability of lignin in alkaline extraction can also increase the efficiency of chlorine bleaching of pulp. Three samples of pretreated kraft pulp ; one as a control in the buffer solution in the absence of any enzyme, the second pretreated with xylanase, and the third pretreated in the simultaneous presence of xylanase, peroxidase, 1mM guaiacol and 0.2mM H_2O_2. After the alkaline extraction in 1N NaOH at 50℃ for 3 hours, the three samples of pretreated kraft pulp were bleached by chlorine dioxide. Fig. 5 and Fig. 6 show the dependence of kappa number and brightness, respectively, of the three pulp samples after the bleaching on the changes in the active chlorine charge. When pulp was pretreated by either xylanase or xylanase plus peroxidase, the percentage of decrease in kappa number was greater by 2 as compared to

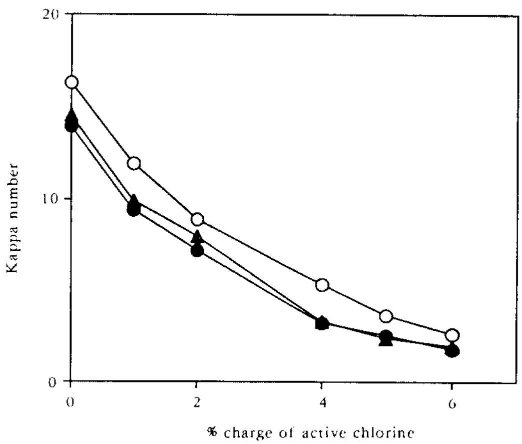

Fig. 5. Dependence of the percentage of decrease in kappa number of bleached pulp on the active chlorine charge for the pretreated pulp samples with xylanase and peroxidase(●), with xylanase(▲), and without enzyme(○).

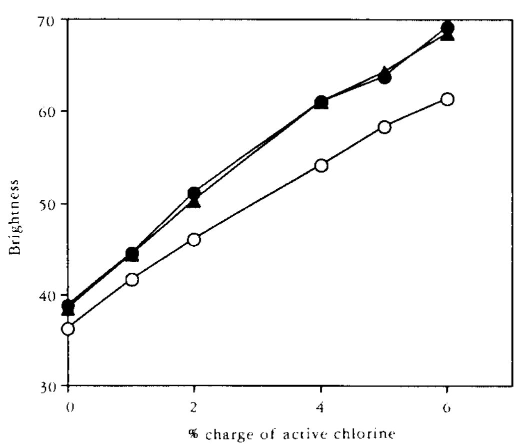

Fig. 6. Dependence of the brightness of bleached pulp on the active chlorine charge for the pretreated pulp samples with xylanase and peroxidase(●), with xylanase(▲), and without enzyme(○).

the case of pretreated pulp without enzyme. The pretreated pulp samples with enzymes also shows greater value of brightness after the bleaching. Another important point is that enzymatic pretreatments of pulp can reduce the use of chlorinated compounds for bleaching, therefore, decrease the formation of chlorinated compounds in the effluents from pulping processes which cause environmental problems.

In conclusion, enzymatic pretreatments of pulp can enhance the efficiencies of subsequent alkaline extraction and chlorine bleaching. The resultant reduced use of sodium hydroxide and chlorinated compounds will alleviate the energy requirement and the environmental problems in the pulping processes. The efficiency of enzymatic pretreatments can be enhanced by using externally added phenolic compounds acting as radical carriers depending on the properties of phenolic compounds and the production rate of radicals. Further studies on the use of various compounds as radical carriers are required.

요 약

효소를 이용한 펄프의 전 처리가 펄프의 표백 효과에 미치는 영향을 조사하였다. 펄프의 kappa number 감소율은, 효소를 사용하지 않고 1N NaOH 용액으로 추출하였을 경우에는 13.7%였으나 xylanase(EC 3.2.1.8, Pulpzyme HB)를 사용하여 전 처리를 하였을 경우에는 1N NaOH 추출 후 25.2%로 증가하였다. Xylanase와 함께 peroxidase(EC 1.11.1.7, Novozyme 502) 및 H_2O_2(0.1mM)를 사용하여 전 처리를 하였을 때, 페놀성 물질을 radical carrier로 사용할 수 있는 가능성을 조사하였다. Radical carrier로서 guaiacol(1mM)을 사용하면 kappa number의 감소율이 29.6%로 증가하였다. 그러나 phenol, p-chlorophenol 등을 radical carrier로서 사용하거나 높은 농도의 H_2O_2(1mM)를 사용할 경우에는 kappa number의 감소율은 줄어들므로 radical의 생성 속도 및 radical의 안정성이 중요한 변수가 됨을 알 수 있었다.

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NOMENCLATURES

EXU : Endo xylanase unit as defined in detail in reference 12

o.d. : oven dried

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