

Modification of Cellulase with Polyalkylene Oxide Allyl Ester–Maleic Acid Anhydride Copolymer and Its Application to the Pulp and Paper Industry

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ABSTRACT: Functional copolymer-modified cellulase was applied to newspaper reprocessing. The new copolymers, composed of polyalkylene oxide (PAO) and maleic acid anhydride (MA), were synthesized to modify the cellulase. MA was attached to PAO allyl ester, formed by the reaction between PAO allyl alcohol and lauric acid. The modification is produced by the ability of MA to react with the amino acid group of the cellulase for modification. At the maximum degree of modification of 52%, modified cellulase showed more than 65% activity over the unmodified native cellulase. When modified cellulase was applied to the waste paper reprocessing, it was found that the cellulase enhanced the detachment of ink particles from paper. PAO was found to also cause the removal of the detached ink particles by floatation. This process creates a more moderate environment than conventional methods, improves the physical properties of the paper such as freeness and whiteness in comparison to conventional NaOH methods, while maintaining the same ink particle removal ability. © 2002 Wiley Periodicals, Inc. *J Appl Polym Sci* 84: 894–899, 2002; DOI 10.1002/app.10088

Key words: polymerization; enzymes; modification; biological application of

INTRODUCTION

Throughout the world, environmental problems are becoming a major concern for everyone. A major focus to alleviate these environmental concerns is recycling. For instance, waste paper can be collected and treated to be reused in the paper industry, and waste cellulosic materials can be transformed into useful chemicals by enzymatic conversion. Because researchers recognized that some enzymes are able to hydrolyze the cellulose, the application of these enzymes upon natural fibers have been studied not only for sugar production but also in the

pulp and paper field. Some of the applications in the paper industry are enzyme-aided bleaching. Enzymes also have been used to improve deinking, drainage of paper, and pitch removal from sulfite pulp.^{1–3} One of the major problems for enzyme utilization, however, is the high cost and instability of cellulolytic enzyme have been one of the major problems. Inada and Nishio have modified enzymes such as lipase, catalase, chymotrypsin, and peroxidase with copolymer of monomethoxy polyethylene glycol and cyanuric chloride.^{4,5} In our previous report, a new copolymer with polyethylene oxide (PEO) and maleic acid anhydride (MA) was synthesized to modify the enzymes. This modified cellulase resulted in a high stability of activity vs. temperature, pH, and organic solvents.^{6–9} It also demonstrated a greater conversion of the substrate than the

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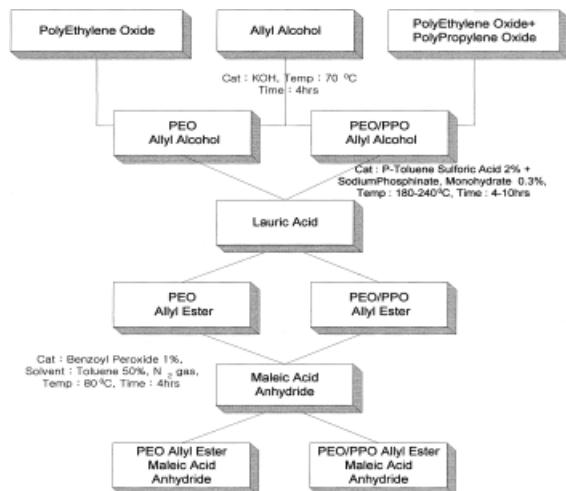


Figure 1 Schematics of PEO/PPO allyl ester synthesis.

native cellulase did. In addition, cellulase gained the additional properties of supporting materials.

In this study, polyalkylene oxide (PAO) allyl ester was synthesized with polyethylene oxide (PEO) and polypropylene oxide (PPO). This synthesis resulted in the creation of new copolymers containing functional groups such as MA and PAO allyl ester. The new copolymer was used to modify the cellulase by a chemical reaction between the amino acid groups of the cellulase and the MA groups of the copolymer. Finally, a modified cellulase was applied to old paper reprocessing. To evaluate the effect of modified cellulase in the deinking process the physical properties such as freeness and whiteness of the reprocessed paper were analyzed.

MATERIALS AND EXPERIMENTAL PROCEDURE

Materials

Crude cellulase Y-NC, from *Aspergillus niger*, was purchased from Yakult Co., Japan. Filter paper hydrolase (FPase) activity was analyzed by using filter paper FP-5C (Toyo Roshi Ltd., Japan) as a standard substrate. FPase activity of cellulase was 0.19 unit/mg, protein at pH 5.2 and 50°C, which was assayed as reported by Mandels and coworkers.¹⁰ A unit of activity is defined by the amount of enzyme, which produce 1.0 μ mol of reducing sugar from the substrate per minute.

Reducing sugar was determined by the dinitrosalicylic acid (DNS) method with glucose used as a standard.¹¹ PAO with varying chain lengths of PEO and PPO were synthesized at the Korea Polyol Co., Korea. All other chemicals used to synthesize the copolymer were all ACE grades and were obtained from Sigma-Aldrich, Korea. To ensure the uniformity of sample for deinking experiments, the old newsprint (ONP) was obtained from Hankook Iibo newspaper printed on October 17, 1998.

Experiment

Synthesis of Copolymer

The synthetic copolymer, which consists of PAO allyl ester MA, was used as a modifier, is shown in Figure 1. First, PAO, which is a mixture of PEO and PPO, was prepared. Allyl alcohol was combined with PEO or PAO at 70°C for 4 h inside of an autoclave with KOH as a catalyst. The number of ethylene oxide (EO) units in one PEO chain was varied from 10 to 40 to see the effect of EO length. The mol ratio of EO and propylene oxide (PO) units was varied to see the effect of PO. Then, PAO allyl alcohol was combined with lauric acid to form ester by esterification. The mixture of PAO allyl alcohol and lauric acid reacted for 4–10 h with a mixture of 2% *p*-toluene sulfonic acid and 0.3% sodium phosphate as catalyst. The temperature increased from 180 to 240°C. Conversion was calculated by the acid value, which is defined as the number of mg of KOH required to neutralize 1 g of the acid sample. The acid value can be found by acid-base titration with phenolphthalein indicator. Finally, MA was added to ester to form the desired copolymer compound. The addition of MA in PAO allyl ester after esterification reaction

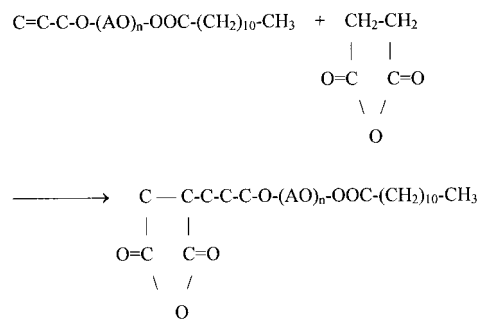


Figure 2 The mechanism of copolymer synthesis with addition of MA in PAO allyl ester AO: alkylene oxide including ethylene oxide (EO) and propylene oxide (PO).

is shown in Figure 2. It is thought that the addition of PAO controls hydrophilicity, while MA gives the functional group necessary to bond with the amino acid groups of the enzyme. Structural analysis was carried out with infrared spectrometry (IR, JASCO IR-700) and Fourier transform nuclear magnetic resonance (FTNMR, Bruker 250 spectrometer).

Modification of Cellulase with Synthesized Copolymer

The synthesized copolymer was combined with cellulase by maleylation to form modified cellulase. Maleylation is one of the chemical modifications of protein with MA.¹² This reaction occurred effectively with the conditions of pH 8.0 and low temperature. However, as the reaction proceeded, pH decreased due to the production of carboxylic acid. It was necessary to control pH value with a base. In the case of modification with copolymer, cellulase reacted easily with MA group of copolymer. Modification of cellulase with the copolymer modifier was carried out as follows: copolymer was added stepwise to the cellulase solution, and the mixture was slowly stirred at 4°C under pH 8.0–8.2, which was controlled with 0.2 M NaOH. The degree of modification (DM) of cellulase with modifiers was defined as follow:

$$DM = 1 - \frac{\text{Unmodified NH}_2 \text{ of modified cellulase}}{\text{Total NH}_2 \text{ of native cellulase}}$$

Amino groups of the cellulase were determined with the trinitrobenzene sulfonic acid (TNBS) method.¹³ The DM was varied by changing the weight ratio of copolymer to cellulase over the range of 0.1–6.0 (w/w).

Application to the Paper Process with Modified Cellulase

Pulping and floatation of the deinking process: Old newsprint (ONP) were cut into 15 × 15-mm square. Fifty-five grams of samples were mixed with appropriate chemicals and diluted to 1.5 L in 2.5 L low-consistency pulper. Then, samples were disintegrated for 20 min. Finally, slurries were transported and diluted to 6.0 L in a 6.5 L capacity floatation cell. Floatation was carried out for 6 min. Air was supplied at 4 L/min for foaming with a rotor speed of 1500 rpm. Slurries were dewatered and stored in the refrigerator for deactivation and for further analysis.

Physical properties of the reprocessed paper: Hand sheets were prepared according to the TAPPI standard T 205 om-88¹⁴ to measure the physical properties of reprocessed paper. Density of the prepared hand sheets ranged from 79.2 to 80.8 g/m². Physical properties of reprocessed paper including freeness whiteness are very important in the paper industry. Freeness, the measurement of the degree of dewatering from the slurry, gives an indication of the amount of energy saved in the dewatering and the drying process. It was measured according to the TAPPI standard method with a freeness tester (L&W, SWEDEN). Whiteness was observed with their tester (Hunter Lab DP-900, USA). A strength tester (Toyo Seiki, Japan) was used to test tensile strength.

RESULTS AND DISCUSSION

Characteristics of Synthesized Copolymer

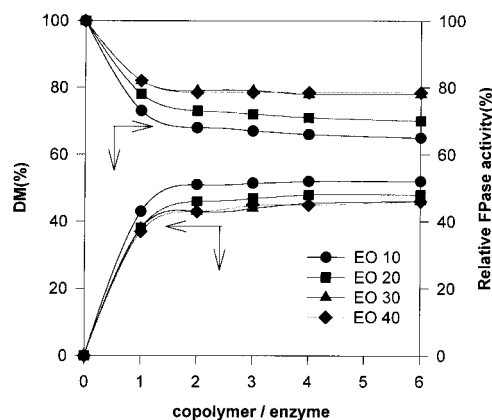
There are three steps to produce the copolymer, which can react with the amino acid of the enzyme. These steps are a PAO addition to the allyl alcohol, esterification reaction between PAO allyl alcohol, and lauric acid and the addition of MA in PAO allyl ester. First, to synthesize PAO allyl alcohol, PAO was added to the oxygen in the OH group of allyl alcohol. Then, PAO allyl alcohol underwent an esterification reaction with lauric acid. This step was necessary to prevent a side reaction for MA addition. The OH group in the allyl alcohol was neutralized producing water (H₂O), with H⁺ coming from the lauric acid while the carboxylate group of lauric acid underwent esterification. Finally, MA was added to the PAO allyl ester for the final product. This mechanism is shown in Figure 2. Before the final reaction, both MA and allyl ester had a carbon double bond. The carbon double bond presented in the MA was broken down into a carbon single bond, and was added to the double bond of PAO allyl ester by breaking it into a carbon single bond.

PAO was added to allyl alcohol at 70°C and mixed for 4 h inside an autoclave with KOH as a catalyst in the first step of synthesis. With the synthesis of PEO, the product is in a liquid state when the EO number is less than 10, and is paste form when the EO number is greater than 10. For the EO series, the hydroxyl value decreased as the EO number of the PEO increased. The hydroxyl value is the amount of KOH needed to neutralize H⁺ from allyl alcohol. The alcohol loses a hydrogen ion when

Table I Acid Value and Conversion of Allyl Ester According to the Reaction Temperature

Copolymer	Initial State	Temperature (°C)	
		200	240
Allyl Ester with 10 E.O.			
Acid Value	72.0	35	3
Conversion (%)	0	51.4	95.8
Allyl Ester with 20 E.O.			
Acid Value	48	25	3.3
Conversion (%)	0	48	93.2
Allyl Ester with 30 E.O.			
Acid Value	35.3	9.8	4.7
Conversion (%)	0	72.2	87.1
Allyl Ester with 40 E.O.			
Acid Value	29.8	7.3	4.4
Conversion (%)	0	75.5	85.3
Allyl Ester with E.O./P.O. (30/10)			
Acid Value	27.7	6.8	2.8
Conversion (%)	0	75.38	89.9
Allyl Ester with E.O./P.O. (20/20)			
Acid Value	26.3	6.3	2.9
Conversion (%)	0	76.12	89
Allyl Ester with E.O./P.O. (10/30)			
Acid Value	25.2	6.0	2.9
Conversion (%)	0	76.11	88.5
Allyl Ester with E.O./P.O. (0/40)			
Acid Value	24.1	5.9	3.4
Conversion (%)	0	75.3	85.9

it is reacted with the PAO chain. PAO allyl alcohol was transformed into ester form to combine with MA without opening its ring structure. It was then combined with lauric acid to form ester, and the conversion could be calculated by measuring the acid value of the ester product. The case of the all PAO including pure PEO and PEO/PPO allyl ester is shown in Table I. As the reaction temperature increased, consumption of acid by the reaction with alcohol increased, and thus, the acid value of the reactant decreased as conversion increased. From these results, it is found that PAO allyl ester with MA can be copolymerized. After PAO allyl ester was combined with MA, the product was analyzed with IR and FTNMR to determine its structure. IR showed functional groups such as ester ($1775, 1745, 1054 \text{ cm}^{-1}$), MA ($1848, 1775, 1745 \text{ cm}^{-1}$), ethylene oxide ($1848, 1775, 1745, 1054 \text{ cm}^{-1}$), and benzene

**Figure 3** Degree of modification and relative Fpase activity of modified cellulase according to the weight ratio of synthesized copolymer (PEO) to cellulase.

($3082, 1465, 948, 888, 848, 722 \text{ cm}^{-1}$), and these coincided with FTNMR. From the FTNMR analysis, 75% of the carbon double bonds in MA ($7.07\text{--}7.27, 7.26 \text{ ppm}$) were converted into carbon single bonds (2.32 ppm). We believed that the synthesis of the copolymer with MA functional group was successfully synthesized with 75% completion.

Modified Cellulase with Synthesized Copolymer

DM and relative FPase activity according to the weight ratio using PEO and PEO/PPO are shown in Figures 3 and 4, respectively. Relative FPase activity is defined as the ratio of the FPase activity of modified cellulase to that of native cellulase. In Figure 3, the characteristics of different EO number on

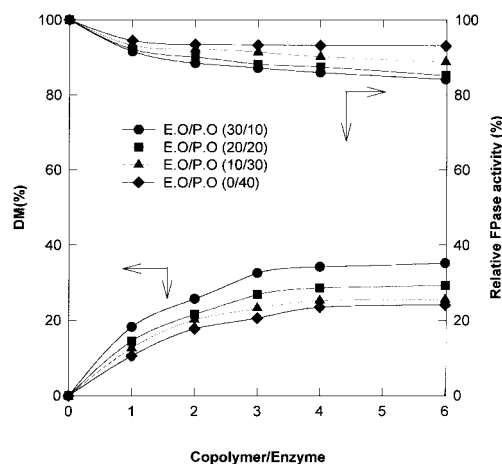
**Figure 4** Degree of modification and relative Fpase activity of modified cellulase according to the weight ratio of synthesized copolymer (PEO/PPO) to cellulase.

Table II Effect of Concentration on Freeness of Reprocessed Paper (unit: mL)

Copolymer	Concentration of Modified Cellulase			
	0.1%	0.2%	0.3%	0.4%
Allyl Ester with 10 E.O.	385	390	390	380
Allyl Ester with 20 E.O.	380	375	375	375
Allyl Ester with 30 E.O.	395	385	375	370
Allyl Ester with 40 E.O.	400	385	380	380
Allyl Ester with E.O./P.O. (30/10)	370	385	370	360
Allyl Ester with E.O./P.O. (20/20)	370	390	370	370
Allyl Ester with E.O./P.O. (10/30)	380	395	385	380
Allyl Ester with E.O./P.O. (0/40)	425	410	410	400

Conventional method: 360 mL.

the PEO chain are shown; as the weight ratio of a copolymer increased, the DM increased, while the relative activity decreased for a weight ratio more than 2, there was scarcely any change for DM and relative FPase activity. As DM increased and the EO number increased, relative FPase activity decreased. From these results, we can find two points. First, the DM value has an inverse relationship with relative activity. Second, the copolymer with a low EO number can react easily with amino acid of enzyme without the steric inhibition caused by the long chains of PEO. For different EO/PO mole ratios (Fig. 4), there was a similar pattern that as the weight ratio of copolymer to enzyme increased the DM increased and relative activity decreased. As EO/PO mol ratio decreased DM decreased, but relative activity increased. The copolymer with a low EO/PO mol ratio is more hydrophobic than with a high mol ratio. This indicates that it is difficult to modify cellulase with a hydrophobic copolymer, and there is an inverse relationship between DM value and relative activity. From the results of Figures 3 and 4, more than 65% of relative activity was retained at the highest degree of modification of 52%. Therefore, it is thought that PEO/PPO chains can be chemically attached to cellulase without great loss of activity, and it is expected that modified cellulase will have additional properties of supporting materials.

Application of the Paper Reprocess with PAO Allyl Ester-Modified Cellulase

The Deinking Process

For the enzymatic paper reprocessing, most of the operation conditions are matched with the opti-

mal conditions for the cellulase and modified cellulase activity. Also, pulping time was fixed at 20 min in the laboratory. Thus, concentration variation of modified cellulase is the only factor tested in this research. Under the same conditions, such as pulping and floatation time, concentration of the modified cellulase was varied from 0.1 to 0.4 wt %, and the products were compared to those obtained by the conventional deinking process of Shinmoorim Paper Co., Korea. In conventional deinking, NaOH and a commercial surfactant are used for the pulping and floatation, and the amount are 1.5% (w/w) and 0.45% (w/w) of paper, respectively. Modified cellulase reprocessing showed almost the same ink particle removal ability in comparison with conventional alkali method (data was not shown). It is thought that the cellulase part of the modified enzyme dissociated the fibers during the pulping stage while the supporting copolymer of the modified cellulase functioned as a surfactant at the floatation stage.

Physical Properties of the Reprocessed Paper

The effect of the modified cellulase on freeness of reprocessed paper is shown in Table II. The values of the cases of modified cellulase with different concentration and different polymer types are always higher than that of conventional method. The data of the concentration variation show that modified cellulase can reprocess ONP without using excessive amounts of enzyme. It is thought that the increase in freeness is due to selective removal of fine fibers by enzymatic hydrolysis. Table III compares the whiteness with variations of the copolymer. Modified cellulase showed

Table III Effect of Modified Cellulase Concentration on Whiteness (unit: %)

Copolymer	Concentration of Modified Cellulase			
	0.1%	0.2%	0.3%	0.4%
Allyl Ester with 10 E.O.	43.03	43.13	42.22	42.88
Allyl Ester with 20 E.O.	41.07	42.62	41.39	40.51
Allyl Ester with 30 E.O.	42.35	42.35	41.6	41.13
Allyl Ester with 40 E.O.	42.25	42.31	42.03	42.11
Allyl Ester with E.O./P.O. (30/10)	40.03	39.94	39.78	39.92
Allyl Ester with E.O./P.O. (20/20)	40.81	40.53	39.58	39.25
Allyl Ester with E.O./P.O. (10/30)	41.51	38.5	39.2	38.62
Allyl Ester with E.O./P.O. (0/40)	41.15	40.87	40.58	39.37

Conventional method: 37.88%.

higher values than the conventional reprocessing method even though there are few differences in the types of copolymer. Modified cellulase reprocessing (copolymer with E.O 10 mol) show 13.9% better whiteness than the conventional method. In the conventional method, NaOH, which makes a strong alkali environment, creates a yellowing phenomena of reprocessed paper that decreases whiteness. From these results, the new copolymer-modified cellulase is expected have an application in paper reprocessing. Moreover, it is expected that the moderate conditions using the modified cellulase method will improve the physical strength of the recycled paper.

CONCLUSIONS

Synthetic copolymer-modified cellulase was applied to waste paper reprocessing. Cellulase was modified with a functional copolymer that included polyalkaline oxide (PAO) and maleic acid anhydride (MA). The hydrophilic properties of the copolymer were altered by varying the mixing ratio of polyethylene oxide (PEO) and polypropylene oxide (PPO). PAO allyl ester was chemically combined with MA to synthesize a copolymer this was confirmed with IR and FTNMR analysis. With cellulase modification, PEO/PPO copolymers showed a lower DM than the PEO copolymer, but they showed higher remaining activity. As the amount of PPO increased, DM decreased while remaining activity increased. However, at a maximum DM of 35%, more than 85% of the remaining activity was retained.

Modified cellulase, when applied to the reprocessing ONP, improved freeness compared to conventional methods that use NaOH. It is thought that the enzymatic hydrolysis removes fine fibers during the pulping process. Also, the whiteness of reprocessed paper using modified cellulase was better than the conventional methods that yellow due to NaOH.

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