Biological Reprocessing of Mixed Office Waste (MOW) Using Modified Cellulase by Production of Functional Copolymer

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Abstract–Copolymer containing functional groups such as polyethylene oxide (PEO) and maleic anhydride (MA) was synthesized to modify the cellulase. MA was attached to PEO allyl ester, which was the product of the reaction between PEO allyl alcohol and lauric acid. The number of ethylene oxide (EO) units in one PEO chain was varied from 10 to 40. MA groups of the copolymer formed the chemical bond with the amino acid groups of the cellulase in the modification reaction. When cellulase was modified with synthesized copolymer, modified enzyme showed high relative activity regardless of high degree of modification compared with other modification methods. In mixed office waste reprocessing, modified cellulase improved many physical properties of the paper including freeness, optical properties, and strengths compared to the conventional process. Even though native cellulase improved the physical properties, modified cellulase showed an increased tensile strength and internal bond over those of unmodified cellulase. From these results, modified cellulase method is an effective biological treatment that would save pulp resources in the reprocessing.

Key words: Modification, Stability of Enzyme Activity, Degree of Modification (DM), Mixed Office Waste (MOW), Reprocessing

INTRODUCTION

Environmental problems are becoming a major concern for all people living on the earth. One solution to alleviate this problem would be to recycle useful waste. For instance, waste papers can be collected and treated for recycle, and waste cellulosic materials can be transformed into useful chemicals by enzymatic conversion. For enzymatic conversion, high cost and unstable cellulolytic enzyme have been one of the major problems associated with this process [Clanet et al., 1988; Rivers and Emert, 1988; Tanaka and Matsuno, 1985]. Kajiuchi et al. [1992] showed that non-ionic surfactant increased the rate and extent of saccharification of the substrate and thereby improved the recovery of enzyme. In that case, the surfactant produces hydrophilic environments that played an important role in the control of adsorption and desorption processes of cellulase on the cellulose surface and enhanced saccharification of the substrate. Also, those free enzymes are easier to recover than the enzymes attached to the substrates. Inada et al. [1986] and Nishio et al. [1987] have modified enzymes such as lipase, catalase, chymotripsin and peroxidase with copolymer of monomethoxy polyethylene glycol and cyanuric chloride. Park [1995] modified cellulase with amphiphilic copolymer, and the modified cellulase was soluble in organic solvents and showed enzymatic activity in the solvents. Moon et al. [1993] demonstrated two-phase separation of modified cellulase with amphiphilic copolymer and dextran.

In this study, new copolymers containing functional groups such as maleic acid anhydride (MA) and polyethylene oxide (PEO) allyl ester were used to modify the amino acid groups of the cellulase.

Cellulase was modified to control the hydrophilic property and stability against pH, temperature, and organic solvent variation. Modified cellulase also has surfactant property of an added copolymer. The remaining activity of modified cellulase was determined. Then, modified cellulase was applied in the deinking process, and the physical properties including strength, optical properties of the hand sheets were measured.

MATERIALS AND EXPERIMENTAL PROCEDURE

1. Materials

Crude cellulase Y-NC from *Aspergillus Niger* was purchased from Yacult Co. Japan. FPase activity was analyzed by using filter paper FP-5C (Toyo Roshi Ltd. Japan) as a standard substrate. FPase activity of the cellulase was 0.19 unit/mg protein at pH 5.2 and 50 °C, which was assayed as reported by Mandels and co-workers. A unit of activity was defined as the amount of enzymes, which produced 1.0 μmol of reducing sugar from the substrate per minute. Reducing sugar was determined by dinitrosalicylic acid (DNS) method with glucose as standard [Habeeb, 1966]. PEO with different chain length was obtained from Korea Polyol Co., Korea. The rest of the chemicals used to synthesize the copolymer were all ACE grades and were obtained from Sigma-Aldrich, Korea.

2. Experiments

2-1. Synthesis of Copolymer

Copolymer, which consists of polyethylene oxide (PEO) and maleic acid anhydride (MA), was used as a modifier (Fig. 1). Reaction mechanism of polymer synthesis is shown in Table 1. First, allyl alcohol was combined with PEO at 70 °C for 4 hrs in an autoclave using KOH as a catalyst. The number of EO units in one PEO chain was varied from 10 to 40 to see the effect of EO length. Then, PEO
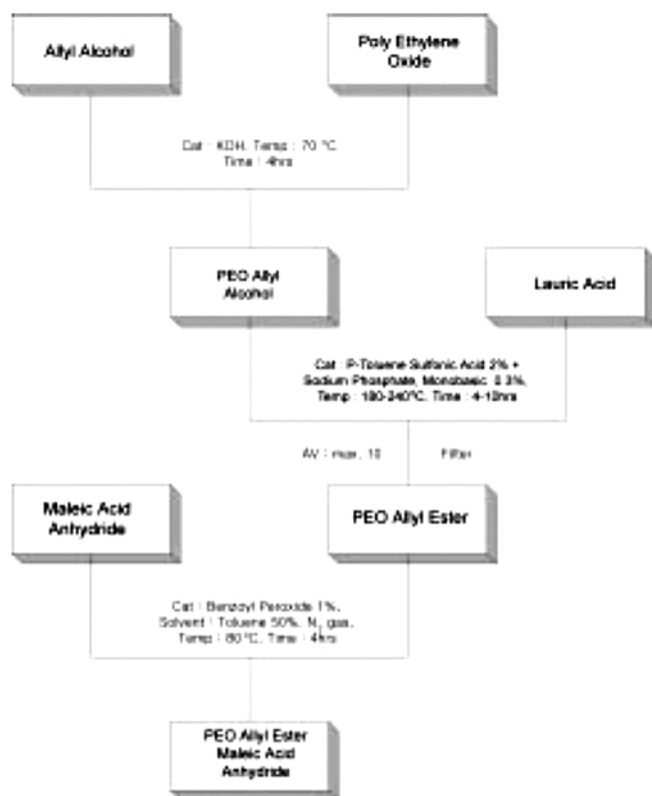

Fig. 1. Schematics of the copolymer synthesis.

allyl alcohol was combined with lauric acid to form ester by esterification reaction. The mixture of PEO allyl alcohol and lauric acid was reacted for 4-10 hrs under 2% p-toluene sulfonic acid and 0.3% sodium phosphate catalyst at 180-240 °C. Conversion was calculated by the acid value, which was defined as the number of mg of KOH to neutralize 1 g of the acid sample. Acid value can be found by the acid-base titration with phenolphthalein indicator. Finally, MA was added to ester to form the desired copolymer compound. It is thought that PEO makes copolymer a hydrophilic environment in water while MA gives the functional group necessary to bond with amino acid groups of enzyme. Structural analysis was carried

out by infrared spectrometry (IR, JASCO IR-700) and Fourier transform nuclear magnetic resonance (FTNMR, Bruker 250 spectrometer).

2-2. Modification

Synthesized copolymer was combined with cellulase by maleylation reaction [Butler et al., 1969] to form modified cellulase. Maleylation is a method of the chemical modifications of protein with MA [Park and Park, 1999]. This reaction occurs effectively under the condition of pH 8.0 and low temperature. However, as the reaction proceeds, pH decreases due to the production of carboxylic acid, and it is thus necessary to control pH with a base. In case of modification with copolymer, cellulase reacted easily with MA group of copolymer. Modification of cellulase with copolymer modifier was carried out as follows. Copolymer was added stepwise to cellulase solution, and the mixture was slowly stirred at 4 °C under pH 8.0-8.2, which was controlled with 0.2 N NaOH. The degree of modification (DM) of cellulase with modifiers was defined as follows:

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

Amino groups of the cellulase were determined with trinitrobenzene sulfonic acid [Miller, 1959]. The DM was varied by changing the weight ratio of copolymer to cellulase over the range of 0.1-4.0 (w/w), and by using different PEO chain length of copolymer. PEO chain length was varied from 10 to 40. Relative activity is the measurement of stability, and it was calculated from reducing sugar analysis [Mandels et al., 1976].

2-3. Pulping and Flotation

White ledger mixed office waste (MOW) printed by laser and xerographic was used as the waste paper sample. MOW was cut into approximately 2-cm squares. The waste paper was disintegrated to a 5% consistency with distilled water and a deinking aid in the pulping mixer (2.0 L capacity). The pulped sample was transferred to a flotation cell (6.5 L capacity) and diluted to 6 L in water. Air was supplied at 4 L/min for foaming, and the slurry was agitated at a rotor speed of 1500 rotations per minute for 6 min. Hand sheets were made with deinked slurry. Density of the prepared hand sheets ranged from 79.2 to 80.8 g/m^2. Four hand sheets were prepared for each experiment. Physical properties such as freeness, optical pro-

Table 1. Reaction mechanism of polymer synthesis

	Reaction path	Remarks
Allyl alcohol	$C=C-C-OH+(CH_2CH_2O)_n \longrightarrow$ $C=C-C-O-(CH_2CH_2O)_n-H$	Addition
Allyl ester	$C=C-C-O-(CH_2CH_2O)_{n-1}-CH_2-CH_2-OH+CH_3-(CH_2)_{10}-C-OOH$ $\longrightarrow \quad C=C-C-O-(CH_2CH_2O)_n-OOC-(CH_2)_{10}-CH_3+H_2O$	Esterification
Copolymer	$C=C-C-O-(CH_2CH_2O)_n-OOC-(CH_2)_{10}-CH_3+CH_2-CH_2$ $O=C \quad C=O$ $\diagdown O \diagup$ $\longrightarrow \quad C—C-C-C-C-O-(CH_2CH_2O)_n-OOC-(CH_2)_{10}-CH_3$ $O=C \quad C=O$ $\diagdown O \diagup$	Addition Reaction

Table 2. Characteristics of PEO allyl alcohol/ester

	Allyl alcohol				Allyl ester			
	10	20	30	40	10	20	30	40
EO mol number	11	21.9	30.8	39.6	-	-	-	-
Molecular weight	544.8	1020.6	1413.8	1798.1	-	-	-	-
Hydroxyl value	103.0	55.0	39.7	31.2	-	-	-	-
Acid value	-	-	-	-	3.3	3.3	4.6	4.4
Saponification value	-	-	-	-	75.1	51.0	37.9	29.7
Conversion	-	-	-	-	95.8	93.2	87.1	85.3

perties, and tensile strength were measured six times for each sheet. Freeness was measured according to the Tappi standard method [1993, 1994] with a freeness tester (L&W, SWEDEN). Optical properties were measured by the optic tester (Hunter Lab DP-900, U.S.A.). A Toyo Seiki (Japan) was used to measure the tensile strength.

RESULTS AND DISCUSSION

1. Characteristics of Synthesized Copolymer

Copolymer was synthesized, and its reaction mechanism follows the reaction path described in the following. PEO was added to allyl alcohol at 70 °C and mixed for 4 hrs in an autoclave with KOH as a catalyst in the first step of the synthesis. Characteristics of PEO allyl alcohol is shown in Table 2. The products showed different physical status depending on the mole number of added PEO. The product was at liquid state when the PEO with 10 EO number was used, but other products were paste forms. The hydroxyl value decreased as EO number of PEO increased. The hydroxyl value is the amount of KOH needed to neutralize H⁺ from allyl alcohol. Allyl alcohol loses a hydrogen ion when it is reacted with PEO chain. PEO allyl alcohol was transformed into ester form to combine with MA without opening its ring structure. PEO allyl alcohol was combined with lauric acid to form ester, and the conversion could be calculated by measuring the acid value of the ester product. As the reaction temperature increased, consumption of acid by the reaction with alcohol increased and thus acid value of the reactant decreased while conversion increased. Acid value and conversion were 3.3 and 95.8% at 240 °C respectively. Allyl esters of different EO numbers were manufactured and were shown in Table 2. As EO number increased, the saponification value and temperature which

Table 3. Summary of the functional group and wavelength of synthesized copolymer, polyethylene oxide ether maleic acid anhydride

Functional groups	Wavelength (cm⁻¹)
-RCH=CHR'-	1645, 722, 697
-Benzene-	3082, 1465, 722, 848, 888, 948
-R₂C=O-	1775, 1745, 1054
-C=C-CO-	1645, 1054
5-MEM. RING, -C=O-	1745
-R-CH-O-	2840, 1745, 1358, 948, 888, 848
-MAA-	1848, 1775
-CO-O-CO-	1848, 1775, 1745, 1054

completes the reaction, decreased. Conversion for each EO number of 10, 20, 30, and 40 were 95.8, 93.2, 87.1, and 85.3%, respectively. PEO allyl ester was combined with MA, and the product was analyzed with IR and FTNMR for its structure. IR (Table 3) showed functional groups such as ester (1,775, 1,745, 1,054 cm⁻¹), MA (1,848, 1,775, 1,745 cm⁻¹), ethylene oxide (1,848, 1,775, 1,745, 1,054 cm⁻¹), and benzene (3,082, 1,465, 948, 888, 848, 722 cm⁻¹), and these coincided with FTNMR. From the FTNMR analysis (Table

Table 4. Summary of peaks and identification of functional groups in the synthesized copolymer, polyethylene oxide allyl alcohol maleic acid anhydride

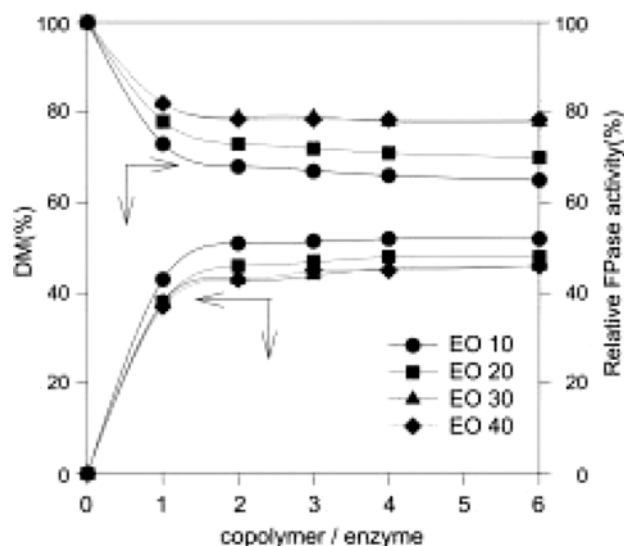| Compounds	Peaks (ppm)	Areas	Identity
Maleic acid	7.01-7.07	-	Cis
anhydride	7.26	-	Trans
Allyl alcohol	2.01	1	OH
	4.12-4.14	2	C-C-OH
	5.1-5.3	2	C=C-C
	5.9-6.0	1	CH₂=C
Ally alcohol	2.92	2	COC-H
(EO 10)	3.5-3.65	42	-COC-
	3.93-3.96	2	C-C-COC-
	5.08-5.23	1	C=HC-C
	5.78-5.85	2	HC=C
Allyl ether	0.82-0.87	1	-CH₃
(EO 10)	1.22	5.43	-CH2-
	1.55-1.61	0.815	CO₂-CH₂-C
	2.26-2.32	0.673	C-C-CO₂
	3.56-3.68	14.5	-EO-
	3.98-4.0	0.510	C-EO-(EO)₉-
	4.17-4.2	0.562	C-CH₂-EO
	5.16-5.28	0.493	C=CH-C
	5.83-5.89	0.239	CH2=C
Copolymer	1.256	2.46	Ether
	1.900	4.32	
	4.020	0.82	
	1.700	8.19	CH₃-, CH₂
	2.320	1.26	MAA (-C-C-)
	3.650	15.61	-EO-
	4.250	1.00	
	7.000	0.43	MAA (-C=C-)
	7.230	0.42	Benzene (-C=C-)

Fig. 2. Degree of modification and relative FPase activity of modified cellulase according to the weight ratio of copolymer to cellulae.

4), 75% of the carbon double bonds in MA (7.07-7.27, 7.26 ppm) were converted into the carbon single bonds (2.32 ppm). We thought that the copolymer with MA functional group was successfully accomplished the synthesis with 75% completion.

2. Modified Cellulase with Synthesized Copolymer

Degree of modification (DM) and relative FPase activity according to the weight ratio of copolymer to enzyme are shown in Fig. 2. Relative FPase activity is defined as the ratio of the FPase activity of modified cellulase to that of native cellulase. As the weight ratio of copolymer increased, DM increased, and the relative activity decreased. However, DM and relative FPase activity for a weight ratio more than 2 almost stayed at the constant value. For a different PEO number, relative activity increased as PEO number increased, but DM decreased. More than 65% of relative activity was retained at the highest degree of modification of 52%. It is thought that PEO chains can be chemically attached to cellulase without great loss of activity and modified cellulase will have additional properties of

the supporting materials.

3. Waste Paper Reprocessing with Modified Cellulase

Throughout the entire process of paper making, pulping is the most important stage since most of the chemical treatments are completed in this stage. Physical properties of the reprocessed paper with respect to the pulping time are shown in Table 5. The values of the modified cellulase treatments were compared to those of the conventional method used by Shinmoorim Paper Co., Korea. In the conventional process, NaOH and a commercial surfactant were used for the pulping and flotation with quantities of 0.5 wt% and 0.7 wt% of paper respectively. Both conventional and modified cellulase processes indicated improvement of physical properties with increased pulping time. The modified cellulase method exhibited greater increments of whiteness over conventional method. Biological paper reprocessing with modified cellulase removed the yellowness of paper, which appeared when NaOH was used in the conventional process. In the case of tensile strength and internal bond, the values of modified cellulase method were always higher than those of conventional methods. Freeness is obtained by measuring the dehydration rate, and it determines the drying time and energy consumption during the drying process. In general, strength has inverse relation to freeness in conventional treatment, but modified cellulase improved both freeness and tensile strength. It is thought that the increase in freeness is due to the selective removal of fine fibers by enzymatic hydrolysis while strength increased due to the enhancement of hydrogen bonding due to the fibrillation of the fiber. It is expected that modified cellulase would maintain a mild deinking condition, and increase the strength of the reprocessed paper while it also increased the freeness.

4. Comparison of Modified Cellulase with Unmodified Cellulase

During mechanical pulping, the cellulase enhanced the detachment of the ink particles from the paper by partial hydrolysis of the fiber. PEO acted as a surfactant, and it generated foams and removed the detached ink particles during the flotation process. But these effects could be expected by unmodified cellulase with copolymer surfactant as well. To consider the effects of modification, the physical properties of reprocessed paper were examined for both modified and unmodified cellulase (Table 6). As modified cellulase con-

Table 5. Effect of the pulping time on the physical properties of the reprocessed paper (cellulase to copolymer weight ratio 1 : 4; DM 55%; pulping temperature 55 °C; pH not controlled; concentration of modified cellulase 1.0%)

	Conventional pulping					Modified cellulase				
Pulping time (min)	5	10	15	20	30	5	10	15	20	30
Brightness (%)	70.4	71.5	72.6	73.6	74.3	68.6	70.8	72.5	73.5	74.3
	(1.2)	(1.2)	(1.2)	(1.3)	(1.3)	(1.1)	(1.2)	(1.2)	(1.2)	(1.3)
Whiteness (%)	74.0	75.6	77.1	77.7	78.4	75.0	79.5	83.0	85.0	86.5
	(1.2)	(1.3)	(1.4)	(1.4)	(1.5)	(1.3)	(1.5)	(1.8)	(1.8)	(1.8)
Tensile strength (Kg/m)	3.5	3.7	3.9	4.1	4.2	3.9	4.2	4.5	4.6	4.8
	(0.1)	(0.1)	(0.1)	(0.1)	(0.2)	(0.1)	(0.1)	(0.2)	(0.2)	(0.2)
Internal bond (N/m²)	38.4	43.0	48.0	52.5	57.8	55.3	60.4	65.8	68.5	70.2
	(1.5)	(0.5)	(0.7)	(0.6)	(0.7)	(1.7)	(0.8)	(0.7)	(0.8)	(0.7)
Freeness (ml)	360	360	350	365	375	480	485	480	485	485
	(10)	(15)	(15)	(15)	(20)	(20)	(15)	(20)	(15)	(20)

The numbers of the parenthesis mean the values of the standard deviation.

Table 6. Effect of the cellulase concentration on the physical properties of the reprocessed paper (cellulase to copolymer weight ratio 1 : 4; DM 55%; pulping temperature 55 °C; pulping time 30 min; pulping pH not controlled)

	Unmodified cellulase					Modified cellulase (DM 55%)					Control
Concentration (%)	0.2	0.4	0.6	0.8	1.0	0.2	0.4	0.6	0.8	1.0	1.2
Brightness (%)	73.1	73.8	74.9	75.3	74.7	72.7	73.7	74.0	74.5	74.3	74.3
	(0.5)	(0.5)	(0.7)	(0.5)	(0.5)	(0.5)	(0.5)	(0.7)	(0.5	(0.5)	(0.5)
Whiteness (%)	81.2	83.1	84.1	84.9	85.4	79.4	81.4	83.7	84.9	86.5	78.4
	(0.7)	(0.6)	(0.5)	(0.6)	(0.7)	(0.5)	(0.6)	(0.5)	(0.7)	(0.7)	(0.7)
Tensile strength (Kg/m)	4.4	4.9	5.0	4.9	4.5	5.4	5.3	5.3	5.1	4.8	4.2
	(0.2)	(0.1)	(0.1)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.1)	(0.1)	(0.2)
Internal bond (N/m^2)	66.0	67.6	69.2	68.8	68.0	71.6	71.8	72.0	70.6	70.2	57.8
	(0.7)	(0.7)	(1)	(0.9)	(1.2)	(0.9)	(0.9)	(1.2)	(0.5)	(0.6)	(0.8)
Freeness (ml)	475	490	475	480	470	480	470	460	480	485	375
	(20)	(25)	(20)	(20)	(10)	(25)	(20)	(15)	(20)	(25)	(25)

The numbers of the parenthesis mean the values of the standard deviation.

centration increased, the whiteness increased slightly. However, tensile strength and internal bond were slightly decreased with an increase of concentration. It is thought that large amounts of modified cellulase weaken the strengths of the paper by the excessive relaxation and hydrolysis of the fiber. Unmodified cellulase also exhibited a similar pattern of paper properties associated with its concentration. In the comparison of modified cellulase with unmodified cellulase, the values of optical properties and freeness were nearly identical, but the tensile strength and internal bond of the modified cellulase were higher than those of unmodified cellulase at the same concentration.

CONCLUSION

Modification of cellulase by synthesized copolymer with polyethylene oxide and maleic acid anhydride was carried out, and modified cellulase was applied in reprocessing (deinking) of mixed office waste.

PEO Allyl Ester was chemically combined with MA to form a copolymer, and this was confirmed with IR and FTNMR analysis. When synthesized copolymer was used to modify the cellulase, modified cellulase displayed a high stability of activity against temperature, pH, and in organic solvents. Therefore, it is thought that modified cellulase does possess some advantages over unmodified cellulase. Firstly, modified cellulase is a combination of cellulase and copolymer, thus, it does not require separate stages to put the chemical into the deinking process. Secondly, unlike unmodified cellulase, modified cellulase is stable against various pH, temperature, and storage time, thus, it can be prepared and stored for a long time. Finally, modified cellulase deinking produced a reprocessed paper with better tensile strength than the unmodified cellulase or conventional methods.

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