Hydrolyzability of Pectic Anionic Substances in Process Waters by Pectinases

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In this paper, the efficiency and mechanisms of two pectinases (pectate lyase (PL) and alkaline pectinase (AL)) to hydrolyze model pectic substances and dissolved and colloidal substances (DCS) of Masson pine bleached chemithermomechanical pulp (BCTMP) were investigated. The cationic demand values of model polygalacturonic acid and DCS could be reduced to about 20% and 60 to 70% by these two pectinases, respectively. However, due to the unmethylated form of the pectic substances in DCS of BCTMP, PL is more efficient than AL. The hydrolysis mechanism of polygalacturonic acid with PL was investigated. The results showed that there was no need to hydrolyze the polymeric pectic substance to their monomers, since a minimum average degree of polymerization (DP) of 6.0 was required for pectic acid to interact strongly with cationic polymers and reduce the efficiency of the latter.

Keywords: Pectinase; Pectic substances; Dissolved and colloidal substances; Cationic demand; Hydrolysis

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INTRODUCTION

During the production of chemi-mechanical pulp, dissolved and colloidal substances (DCS) are released from the fibers into the process water (Thornton *et al*. 1994). Nowadays, white water systems are increasingly closed, which results in the accumulation of DCS in water systems. The increased amounts of DCS will affect the pulp and papermaking process and the paper quality (Francis and Ouchi 2001; Nurmi *et al*. 2004). The major anionic substance released during the mechanical pulping processes is in a polygalacturonic form. Pectinase treatment of the DCS from peroxide bleaching process waters could degrade the dissolved polygalacturonic acid, thus showing promise as a means of preventing potentially detrimental polygalacturonic acids from complexing with cationic polymers (Thornton 1994).

However until now, most research of the enzymatic hydrolysis of anionic substances in process water has been focused on the reduction of functional cationic polymers used in the process (Reid and Ricard 2000; Peng *et al*. 2003; Ricard *et al*. 2005). Little attention has been paid to the mechanism and hydrolyzability of DCS substances in process water by pectinases. In this paper, the treatment conditions of two pectinases, pectate lyase (PL) and alkaline pectinase (AL), to reduce the cationic demand of model pectic substances were optimized at first. Then, the efficiency and mechanism of these two pectinases to hydrolyze model pectic substances and DCS were investigated.

Pectate lyase and polygalacturonase both degrade pectin. Pectate lyase carries out a non-hydrolytic breakdown of polygalacturonic acid and pectin (methyl esterified PGA)

via a β-elimination reaction, yielding oligosaccharides with 4-deoxy-α-D-mann-4-enuronosyl groups at their non-reducing ends that absorbs light at 230 to 235 nm. On the other hand, the alkaline pectinase (AL, also known as polygalacturonase) used in this paper is able to break down or to transform pectins by random hydrolysis of the glycosidic bonds (1,4-alpha-D-galactosiduronic) that link galacturonic acid residues.

EXPERIMENTAL

Enzyme Preparations and Assays

Pectate lyase (PL) was supplied by Novozymes (Tianjin, China). Alkaline pectinase (AL) was bought from the EDT Company (Norcross, Georgia, USA). The determination of the activity of AL was based on the 3,5-dinitrosalicylic acid (DNS) color reaction and use of galacturonic acid as the substrate for the calibration curve plot. One unit of AL activity was defined as 1 mL enzyme preparation releasing 1 mg galacturonic acid per hour under the given conditions (50 °C, pH 8.0). PL activity was assayed by measuring the formation of unsaturated oligo-galacturonates at 235 nm (Gummadi and Kumar 2006). One unit of enzyme activity was defined as the amount of enzyme that produced an increase of 1 unit of A_{235} of the reaction mixture per minute.

Sampling of DCS from Mechanical Pulp

The pulp was suspended in distilled water and gently agitated for 3 h at 60 °C in order to ensure equilibrium release and sorption between the fibers and the water phase. To remove fibers and other non-colloidal substances, the suspensions were centrifuged at 500 g for 30 min, and the resulting upper supernatant was pipetted off carefully and collected as DCS samples.

Determination of Cationic Demand (CD) Values

The CD values were measured using a MUTEK particle charge detector (PCD-03). The titrant employed was Poly-DADMAC (poly-diallyl-dimethyl-ammonium-chloride, 0.001N).

RESULTS AND DISCUSSION

Optimal Conditions for Pectinase Treatment

Pectinase is a general term for enzymes that break down pectin, a polysaccharide substrate that is found in the cell walls of plants. Among pectinases, pectate lyase (PGL, EC 4.2.2.2) is an enzyme that catalyzes the chemical reaction. It can cleave (1-4)-D-galacturonan into oligosaccharides with 4-deoxy-alpha-D-galact-4-enuronosyl groups at their non-reducing ends.

Two pectinases, alkaline pectinase (AL) and pectate lyase (PL) were evaluated for their ability to reduce the detrimental effects of anionic trash, such as polygalacturonic acid or pectic substances. Emphasis was put on the enzyme PL, with the results of the enzyme AL just listed. The efficiency of the enzyme PL to degrade or hydrolyze pectic acid (PGA; Sigma-Aldrich), as well as the dissolved and colloidal substances (DCS) from bleached chemithermomechanical pulp (BCTMP), was investigated. Efficiency was expressed as the cationic demand changes in the substrate after treatment by PL. Two

parameters, temperature and pH, were considered. Based on the experimental results, the optimal conditions for PL treatment could be deduced as follows: temperature 60 °C, pH 9.0 (as shown in Figs. 1 and 2). Similarly, the optimal conditions for AL treatment were 70 °C and pH 9.0.

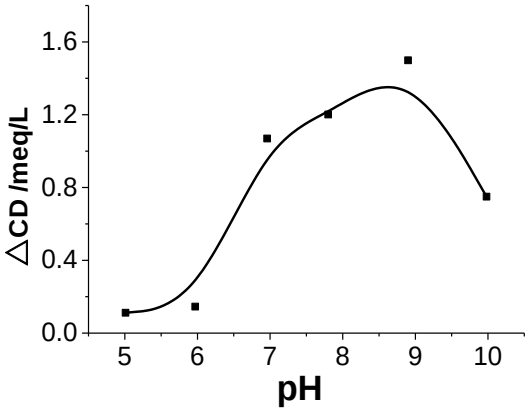

Fig. 1. Effect of pH on the delta CD value of reaction products

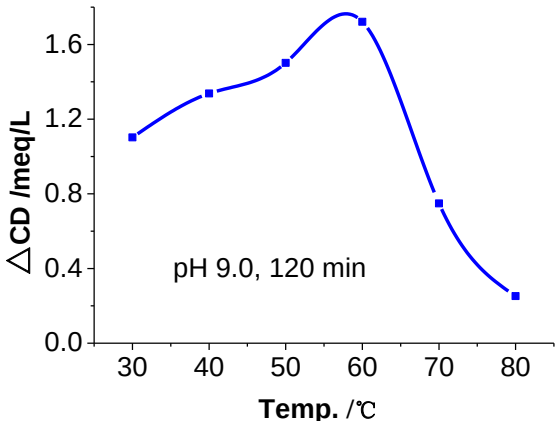

Fig. 2. Effect of temperature on the delta CD value of reaction products

Mechanism of Hydrolysis by Pectinase

The CD values and absorbance at 235 nm of the treatment products at different PL dosages under optimal conditions were determined (PGA 12 mg). The results are shown in Fig. 3. The CD values decreased rapidly with increasing PL dosage and reached a platform of about 0.4 meq/L.

The actual CD decreased to less than 20%. It can be seen from Figs. 3 and 4 that the absorbance of the products at 235 nm increased with PL dosage, and even when the CD values reached a platform, the absorbance could still be increased. This indicated that more unsaturated oligogalacturonates were being produced. However, the formed oligogalacturonates no longer consumed the titratant Poly-DADMAC, which meant that a minimum DP (degree of polymerization) might still be present for PGA to consume cationic polymers to some extent.

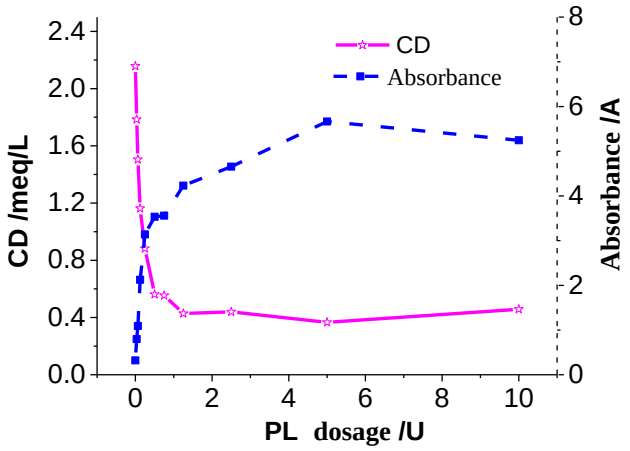

Fig. 3. CD values and absorbance of products at different PL dosages

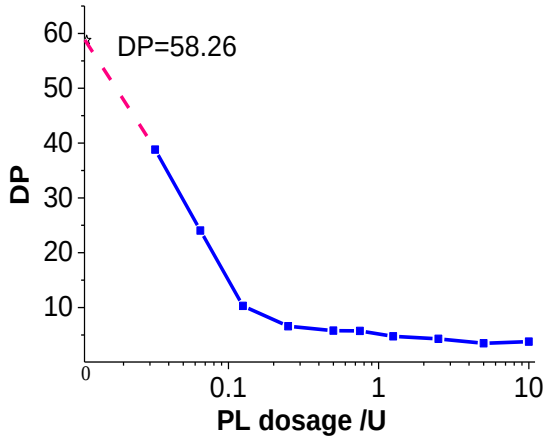

Fig. 4. Effect of PL dosage on the DP of PGA

According to Lambert-Beer's law,

$$A = \varepsilon * b * C$$

$$\triangle C = \triangle A/4.6 \text{ mmol/L},$$

where ε is 4600 $M^{-1}cm^{-1}$, *b*= 1 cm (thickness of cuvette), *C* stands for the concentration of unsaturated oligo-galacturonates. △A is the absorbance change at 235 nm.

The original concentration of PGA was 0.8 g/L, *i.e.*, 4.04 mmol/L. The average degree of polymerization (DP) of the substrate (PDA) decreased rapidly as the dosage of PL increased (Fig. 3), which meant that PL could effectively hydrolyze/degrade the polymeric pectic substances to oligomers or even monomers under the optimal conditions.

Meanwhile, to investigate the effect of DP on the complex reaction between anionic poly-galacturonic acid and the cationic polymers used in the wet end of the papermaking process, the relationship between the CD values and DP of PGA was deduced, as shown in Fig. 5. Results showed that when the average DP of PGA was less

than about 6.0, PGA could not consume cationic polymers enough to disturb the wet-end operation. That meant there was no need to hydrolyze the polymeric pectics to their monomers, since a minimum average DP of 6.0 was required for pectic acid to interact strongly with cationic polymers and reduce the efficiency of the latter. Then a fundamental understanding of the reaction between anionic poly-galacturonic acid (or anionic trash) and added cationic polymers is gained.

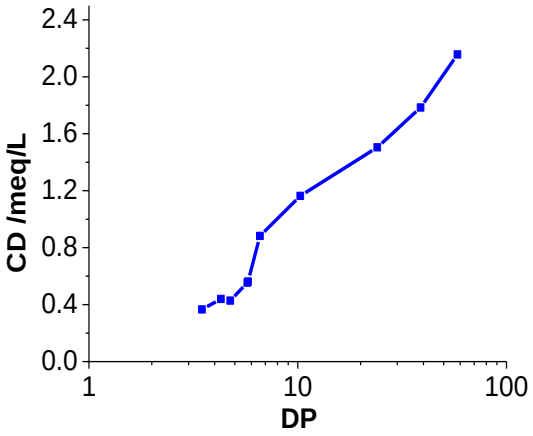

Fig. 5 Correlation between CD value and DP of PGA

Hydrolyzability of DCS Substances by Pectinase

To evaluate the hydrolyzability of DCS substances in process water by these two pectinases (PL and AL), their effectiveness in reducing the CD value of DCS from Masson pine BCTMP was investigated (Figs. 6 and 7). Results showed that PL and AL pectinases could effectively reduce to 60% and 70% of the CD value of DCS from Masson pine BCTMP, respectively. This indicated that both pectinases can hydrolyze the pectic substances in the DCS samples. However, not all the anionic trash or cationic polymers consumed originated from the anionic pectic substances (Thornton 1994). Other substances, like fatty and resin acids or carboxyl group enriched substances, might consume a certain amount of cationic polymers (Holmbom *et al*. 2000). As expected, the two pectinases used could not work on these substances. The results suggested that the pectinase should not be completely relied on to solve anionic-induced problems.

The differences in their mode of action can be expected to have led to their different efficiency in breaking down pectins into pieces with low DPs. Based on the dosage and CD values, PL (pectate lyase) that degrades polygalacturonic acid and pectin *via* a β-elimination reaction seemed more efficient than AL in yielding oligosaccharides with DP of less than 6.0, as deduced above. On the contrary, the alkaline pectinase (AL) used in this study is able to break down or to transform pectins by random hydrolysis of the glycosidic bonds that link galacturonic acid residues. The random hydrolysis produced oligosaccharides with different length of chains, even monomers. It is believed that a portion of AL might act on oligosaccharides of short chains, which do not have any contribution to lowering the CD value of DCS samples.

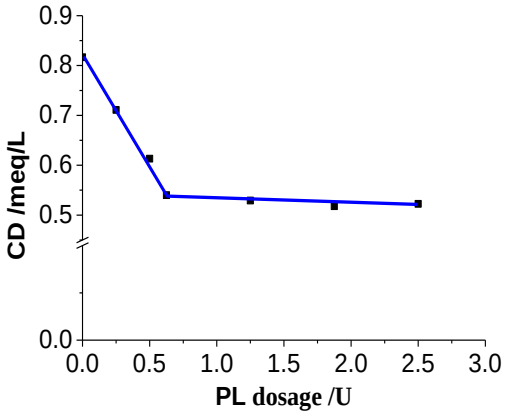

Fig. 6. PL treatment on the CD value of DCS

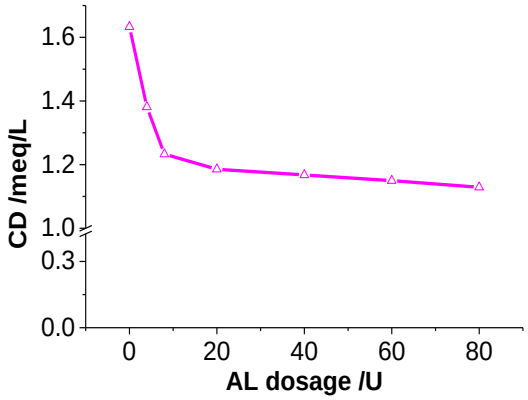

Fig. 7. AL treatment on the CD value of DCS

CONCLUSIONS

1. The optimal conditions for two pectinases were as follows: pH 9.0, temperature 60 to 70 °C. Under optimal conditions, both pectinases, alkaline pectinase (AL) and pectate lyase (PL), could effectively degrade the model pectics and DCS. The results also suggested that the pectinase treatment should not be completely relied upon to solve anionic-induced problems.
2. Investigation into the mechanism indicated that a minimum average DP of 6.0 was required for pectic acid to interact strongly with cationic polymers. PL seemed more efficient than AL to break down the chains of pectin *via* a β-elimination reaction, yielding oligosaccharides whose DP values were less than 6.0. This helped garner a better understanding of the reaction between anionic polygalacturonic acid (or anionic trash) and cationic polymers that were added.

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