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Effects of Surfactants on the Enzymatic Bleaching of Kraft Pulp by Xylanase

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A xylanase was purified from a commercial crude xylanase, Pulpzyme HC, and used for the bleaching of kraft pulp in the absence or in the presence of nonionic surfactants, Tween 20, Tween 80, and Igepal C930. The purified xylanase has a molecular weight of 23,500 as determined by a reducing SDS-PAGE. Tween 20 was most effective to enhance the efficiency of the enzymatic bleaching of kraft pulp by xylanase.

Key words: kraft pulp, xylanase, bleaching, surfactants

INTRODUCTION

Bleaching of kraft pulp which is prepared by removing most lignin contained in wood chips by alkaline extraction has been performed by using chlorine and/or chlorine dioxide. The effluents from conventional bleaching processes of kraft pulp, however, contain various chlorinated organic compounds which are known to influence toxic effects on environment. Growing concerns regarding the environmental influence of these chlorinated organic compounds has driven pulp industry to develop alternative bleaching processes which can reduce or eliminate the requirement of chlorine compounds in kraft pulp bleaching. The use of xylanase which catalyzes the hydrolysis of xylan has shown promise for these purposes [1, 2]. Xylan is covalently bridging cellulose and lignin in natural pulps, therefore, the enzymatic hydrolysis of xylan by xylanase can facilitate the release of lignin from pulp during alkaline extraction. Despite the extensive efforts, however, the use of xylanase only cannot bring about the bleaching efficiency sufficient enough to totally eliminate the need of chlorine compounds for bleaching but reduces chlorine use by 25-30% [1]. The upper limitation in the enzymatic bleaching efficiency of kraft pulp by xylanase may be due to various reasons; xylanase may adsorb onto pulp components thereby lose parts of its activity as observed for cellulases for the hydrolysis of cellulose [3], xylanase may form aggregates in solutions which reduce the availablity of the enzyme for catalysis [4], or xylanase may have restricted accessibility toward the xylan due to the pulp structure.

Various ionic and nonionic surfactants were tested for the compatability with xylanase. Kaya *et al.* (1995) suggested that xylanase can be used with nonionic surfactants for various processes in the pulp and paper industry since xylanase activity was improved slightly with nonionic surfactants [4]. In this study, we tested their hypothesis by investigating the effects of nonionic surfactants on the bleaching of kraft pulp by xylanase using Tween 20, Tween 80, and Igepal C930 and reports the results here. We purified and employed a xylanase from the commercial preparation of a xylanase, Pulpzyme HC, which was developed for the kraft pulp bleaching.

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MATERIALS AND METHODS

Materials

Pulpzyme HC as a brown liquid preparation was kindly gifted by Novo Nordick A/S (Denmark). Nonionic surfactants Tween 20 and Tween 80 were purchased from Sigma Chemical Company and Igepal C930 from Aldrich. DEAE Sephadex A-50 and Sephacryl S-300 were from Pharmacia. Air-dried unbleached kraft pulp was obtained from Donghae Pulp Co. (Ulsan, Korea).

Analysis

Xylanase activity was determined as follows. Typically 20 μL of xylanase solution was added to 2 mL of 1% (w/w) xylan solution. This mixture was incubated for 30 min at 50°C. The increase in reducing sugars in the mixture during the incubation period was measured by dinitrosalicyclic acid method [5] with D-xylose as a standard for calibration. One unit of xylanase activity was defined as the amount of enzyme liberating one micromole of xylose in one minute. The activity of Pulpzyme HC and purified xylanase were 236 units/mL and 205 units/mg, respectively. The bleaching effects were determined by measuring the decrease of kappa number of pulp samples. Kappa number is defined as the amount (mL) of 0.1 N potassium permanganate solution consumed by one gram of oven-dried pulp and used as a measure of lignin content of pulp samples (approximate lignin content of pulp is obtained by dividing the kappa number by 7 [6]). The details of kappa number measurement followed the method as described in TAPPI T 236 cm-85.

Enzymatic Bleaching of Kraft Pulp

Typical enzymatic bleaching of kraft pulp was performed as follows. Air-dried pulp was added to an appropriate buffer solution (usually 100 mM Na_2CO_3, pH 9.0) and disintegrated by agitation with a motorized stirrer at 250 rpm for 30 min at 40°C. After disintegration pulp sample was dewatered by vacuum filtration. For enzymatic treatment, 15 g of the dewatered pulp sample (25% consistency) was added to a buffer solution (usually 100 mM Na_2CO_3, pH 9.0) which contained xylanase and/or a surfactant. The mixture was agitated in a constant-temperature water bath for 3 h at 40 °C. The treated pulp was dewatered by vacuum filtration and subjected to an alkaline extraction in a 0.05 N NaOH solution for 90 min at 70°C with agitation. The bleaching efficiency was determined by the percent decrease in kappa number of pulp samples during the enzymatic treatment and alkaline extraction. The initial kappa number of disintegrated and dewatered pulp sample was 13.82 ($\pm$0.1). The dry pulp content (consistency) in solutions for disintegration, enzymatic treatment, and alkaline extraction steps was 4% (w/w).

RESULTS AND DISCUSSION

Purification and Characterization of Xylanase

In order to eliminate the possible biases to our study due to the various formulating agents present in Pulpzyme HC, we purified and used for our experiment a xylanase which composes the major xylanase activity in Pulpzyme HC as followings. A mixture of 50 mL Pulpzyme HC and 50 mL of a Tris-HCl buffer (10 mM, pH 8.1) was applied to a DEAE-Sephadex A-50 column (4.8$\times$10 cm) which was equilibriated with the same Tris-HCl buffer. Stepwise elutions were performed on order of 10 mM Tris-HCl (pH 8.1) and 10 mM, 100 mM, and 200 mM sodium phosphate (pH 7.0). As shown in Fig. 1, a peak containing the major xylanase activity was eluted with the Tris-HCl buffer.

Fractions containing the major xylanase activity were collected, concentrated, then applied to a Sephacryl S-300 column (2.6$\times$90 cm) which was subsequently eluted with distilled water. A peak of xylanase activity was collected, concentrated, then freeze dried for further experiments. Reducing SDS-PAGE of the purified xylanase with a slab gel consisting of 4% PAA stacking gel and 12% PAA resolving gel showed a single band of the molecular weight of 23,500 which is similar to those of *Trichoderma* xylanases [7, 8].

The xylanase showed optimal activity in alkaline conditions of pH values greater than 7.0. The stability of xylanase was good in most solutions of pH values greater than 4.0 retaining more than 80% of its initial activity after being stored in solutions of corresponding pH values for 24 h at room temperature.

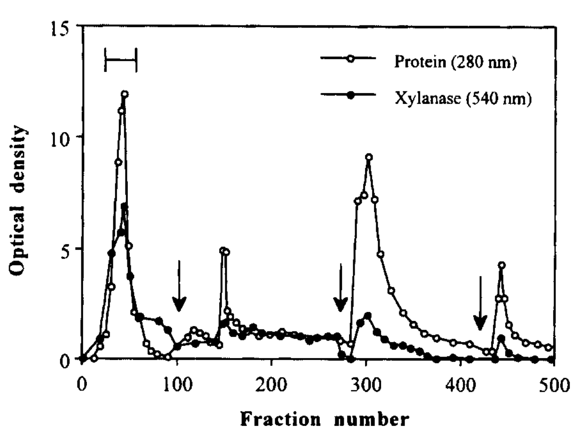

Fig. 1. DEAE-Sephadex A-50 column chromatography of Pulpzyme HC. Stepwise elutions were performed with 0.01 M Tris-HCl (pH 8.1) and phosphate buffers (pH 7.0) of increasing concentrations of 0.01 M, 0.1 M, and 0.2 M. The arrows indicate changes in the eluting buffers. Active fractions were collected as indicated by a bar.

Influence of Nonionic Surfactants on the Enzymatic Bleaching of Kraft Pulp

We tested how nonionic surfactants affect the bleaching efficiency of kraft pulp by the purified xylanase. As shown in Fig. 2, Tween 20 enhanced the enzymatic bleaching efficiency most; the kappa number of the initial pulp sample (pulp after disintegration but before enzyme treatment) decreased by 24.58% when both Tween 20 (0.8% w/w of dry pulp) and xylanase (16.4 units/g dry pulp) were used while it was reduced by 17.85% when only xylanase (16.4 units/g dry pulp) was used. Increasing Tween 20 content from 0.8% to 1.3%, however, did not enhanced bleaching efficiecy further.

Enhancement of bleaching efficiency by Tween 20 (0.8% w/w of dry pulp) was more pronounced as more enzyme was used. As shown in Fig. 3, increasing enzyme loading over 20 units per gram dry pulp reduced the kappa number by about 22% in the absence of Tween 20 and by 25% in the presence of Tween 20 (0.8% w/w). The enhancement of bleaching efficiency in the presence of Tween 20, however, seems to result from some direct interactions between pulp and Tween 20 rather than from the enhanced activity of xylanase by Tween 20. In the absence of xylanase, the percent decrease of kappa number increased from 13.67% without Tween 20 to 17.08% with Tween 20 (0.8% w/w). One distinguishing feature in the presence of Tween 20 is that more xylanase was required to saturate the bleaching efficiency. This implies that Tween 20 may interfere with the interactions between xylanase and pulp.

We examined the effects of nonionic surfactants on the bleaching of kraft pulp with crude xylanase (Pulpzyme HC) also. As shown in Fig. 4. Tween 20 at 0.8% w/w of dry pulp was most effective as was observed with purified xylanase; initial kappa number decreased by 26.08% in the presence of Tween 20 (0.8% w/w of dry pulp) while it was decreased by 21.85% in the absence

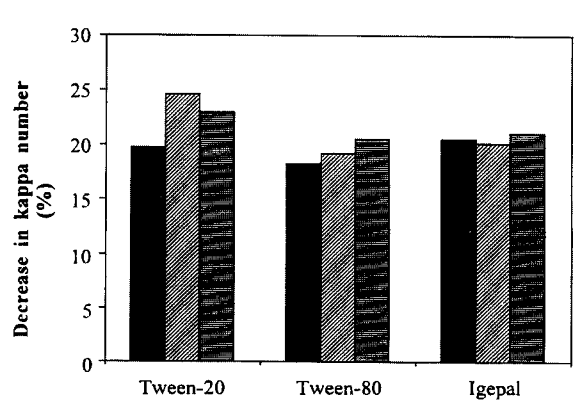

Fig. 2. Influence of nonionic surfactants on the bleaching efficiency of kraft pulp by purified xylanase (16.4 units/g dry pulp). Surfactant content, ■ ; 0.4%, ▨ ; 0.8%, ▤ ; 1.3%.

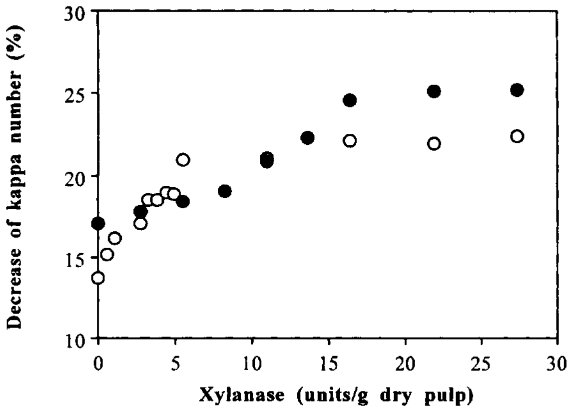

Fig. 3. Effects of enzyme loading on the bleaching efficiency of kraft pulp in the absence (○) and the presence (●) of Tween 20 (0.8% w/w of dry pulp).

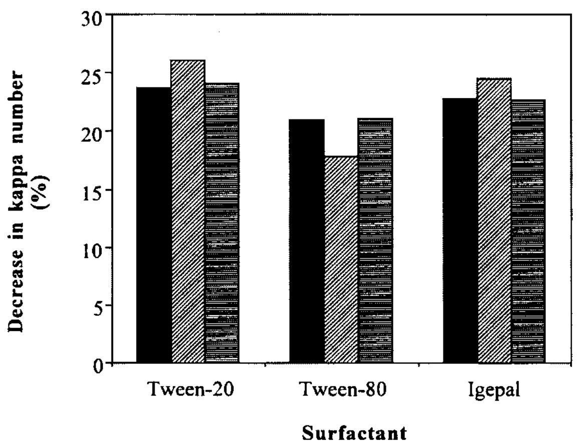

Fig. 4. Influence of nonionic surfactants on the bleaching efficiency of kraft pulp by crude xylanase. Pulpzyme HC content was 6.29 units/g dry pulp. Surfactant content, ■; 0.4%, ▨; 0.8%, ▤; 1.3%.

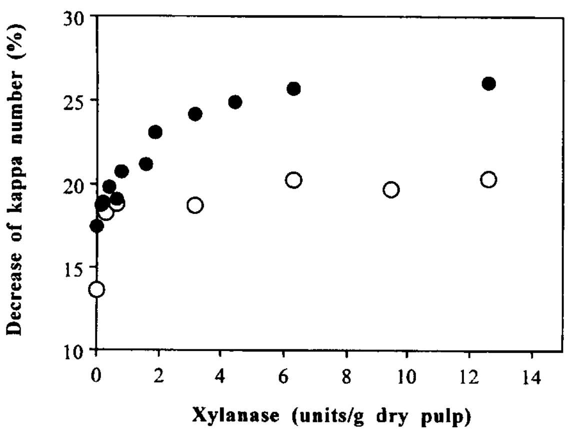

Fig. 5. Effects of enzyme loading on the bleaching efficiency of kraft pulp in the absence (○) and presence (●) of Tween 20 (0.8% w/w of dry pulp) for Pulpzyme HC.

of Tween 20. As shown in Fig. 5, increasing enzyme loading of Pulpzyme HC further enhanced bleaching efficiency. The maximal decrease of the initial kappa number was about 26% in the presence of Tween 20 (0.8% w/w).

CONCLUSIONS

A nonionic surfactant, Tween 20, enhanced the bleaching efficiency of kraft pulp by either a crude enzyme, Pulpzyme HC, and a purified xylanase which composes the major xylanase activity of Pulpzyme HC. The optimum content of Tween 20 was 0.8% (w/w) of dry pulp. The enhancement of bleaching efficiency by Tween 20 was, however, resulted from the direct interaction between the surfactant and kraft pulp rather than from the increased activity of xylanase for the bleaching. Tween 20 seems to diminish the accessibility of xylanase onto pulp by some mechanisms unidedified yet in this study: the kraft-pulp bleaching efficiency by purified xylanase was saturated close to a maximum when xylanase content was above 5 xylanase units/g dry pulp in the absence of Tween 20 while it was above 15 xylanase units/g dry pulp in the presence of Tween 20 at 0.8% w/w.

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