

Influence of surfactants on the enzymatic hydrolysis of xylan and cellulose

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ABSTRACT: *The influence of surfactants on the activities of xylanase and cellulase was studied. A variety of surfactants representing various ionic natures (anionic, cationic, and nonionic) were used, including detergents, defoamers, flotation aids for deinking, and wetting and washing aids for pulping and deinking. The activity of the enzymes increased with all nonionic and cationic surfactants studied. Circular dichroism studies indicated that interaction with nonionic and anionic surfactants changes the molecular structure of the enzymes. The results suggest that xylanase and cellulase enzymes can be used advantageously with nonionic surfactants during various processes in the pulp and paper industry. The use of cationic surfactants is preferable over anionic surfactants. If an anionic surfactant must be used together with xylanase or cellulase in a process, the lowest addition is indicated. The findings also suggest that the incubation of enzymes with surfactants prior to an enzymatic treatment may lead to higher efficiencies from enzymes.*

KEYWORDS: *Anionic compounds, cationic compounds, cellulase, enzymolysis, substrates, surfactants, xylanase.*

In the pulp and paper industry, enzymes have been applied in biological pulping, chemical pulp bleaching, wastewater treatment, recovered paper modification, upgrading pulps for papermaking, and by-product conversion (1). Enzyme usage can result in processes that

use less energy, raw materials, or hazardous chemicals (2). Surfactants have also been used in the pulp and paper industry as wetting agents for pulping and deinking, detergents for cleaning, dispersants for washing deinking of recovered paper, and collectors for flotation deinking as well

as for defoaming of stock during washing and papermaking (3).

It is necessary to determine whether enzymes and surfactants are compatible to improve process characteristics. Kim *et al.* (4) and Prasad *et al.* (5) suggested that recovered paper deinking can be achieved by enzymatic treatments instead of using conventional alkaline deinking chemicals such as sodium hydroxide, sodium carbonate, sodium silicate, and hydrogen peroxide. Deinking with enzymes may result in reduced wastewater treatment costs and less pollutant discharge. In addition, enzymatic treatments of recovered paper enhance brightness, cleanliness, drainage, and mechanical properties of the resulting pulp. Prasad *et al.* (5) also found that an acidic flotation deinking system, which reduced the need to add conventional deinking chemicals, enhanced the brightness and was more compatible with enzyme use. The chemicals used in their system were enzymes, calcium chloride, and a surfactant as a collector.

Numerous studies have reported on the use of enzymes with surfactants. Although no systematic study has been conducted on the interaction of hemicellulases with surfactants, the interaction of cellulases with various surfactants has been studied. Castanon and Wilke (6) found that a nonionic surfactant, Tween 80, increased the rate and extent of cellulose saccharification. The proposed mechanism for the enhancement of hydrolysis by cellu-

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I. Surfactants

Type	Ionic nature	Function	Chemical family	Supplier
Olinor 1100	Nonionic	Flotation aid for deinking	Alkoxylated glyceride derivative	Henkel Paper Chemicals, Charlotte, NC
DI 600	Nonionic	Flotation aid for deinking	Fatty oil alkylene oxide derivative	Hipchem Products, High Point, NC
Tergitol TP-9	Nonionic	Wetting and washing aid in pulping and deinking	Nonyl phenol	CIBA-Geigy Corp., Greensboro, NC
Tween 80	Nonionic	Surfactant	Polyoxyethylene (20) sorbiton monooleate	Fisher Scientific Co., Fair Lawn, NJ
Discotech 5076	Nonionic	Defoamer	Blend, water-based	Callaway (Exxon) Chemical Co., Columbus, GA
Discotech 5683	Nonionic	Defoamer	Blend, oil-based	Callaway (Exxon) Chemical Co., Columbus, GA
Stearic acid	Anionic	Flotation aid for deinking	Organic acid from animal fat	Fisher Scientific Co., Fair Lawn, NJ
Standapol-A	Anionic	Detergent	Ammonium lauryl sulfate	Henkel Paper Chemicals, Charlotte, NC
Hostapur SAS-30	Anionic	Detergent	Secondary alkanesulfonate, sodium salt	Hoechst Celanese Corp., Charlotte, NC
Trymeen 6606	Cationic	Detergent	Ethoxylated tallow amines	Henkel Paper Chemicals, Charlotte, NC

lase in the presence of surfactants is that the surfactants hinder the immobilization of the enzymes on the substrate by reducing the binding strength. Thus, the enzyme can more easily desorb from the binding site after reaction, and the enzyme can easily move to other binding sites on the substrate (6–8). It was also concluded that the economics of recycling and optimizing the enzyme usage during sugar production from waste cellulose would substantially improve, since the enzyme cost is about 60% of the total process cost (6, 8).

Work by Ooshima *et al.* (9) revealed that nonionic, amphoteric, and cationic surfactants enhanced saccharification by cellulases while anionic surfactants did not. However, both cationic and anionic surfactants denatured cellulase in relatively low concentrations. They also concluded that the nonionic surfactant Tween 20 enhanced the hydrolysis of crystalline cellulose by reducing the binding of endoglucanase on cellulose and by varying the binding equilibrium of endoglucanases and exoglucanases. The hydrolysis enhance-

ment by nonionic surfactants was also reported by Park *et al.* (7). However, one report also indicates that surfactants enhance the hydrolysis of cellulose regardless of the surfactant charge (8).

It has been reported that surfactants can prevent the denaturation of cellulases in agitated systems (10). During agitation, the cellulase is most likely denatured when it is exposed to an air–liquid interface, which causes unfolding of the protein. Agitation continually renews this surface, thus subjecting more of the

II. Influence of surfactants on xylanase activity

Surfactant	Surfactant concentration, %	Hydrolysis pH	Average reducing sugar, mg/mL	Standard deviation	Significant at 0.05 level
Control	0	4.94	0.732	0.0048	-
Olinor 1100	0.05	4.86	0.781	0.0067	Yes
	0.30	-	0.767	0.0034	Yes
	1.50	-	0.744	0.0073	Yes
DI 600	0.05	4.85	0.761	0.0052	Yes
	0.30	-	0.780	0.0063	Yes
	1.50	-	0.839	0.0060	Yes
Tergitol TP-9	0.05	4.96	0.747	0.0051	Yes
	0.30	-	0.757	0.0056	Yes
	1.50	-	0.793	0.0068	Yes
Tween 80	0.05	4.96	0.750	0.0071	Yes
	0.30	-	0.782	0.0073	Yes
	1.50	-	0.799	0.0068	Yes
Discotech 5076	0.05	4.87	0.746	0.0034	Yes
	0.30	-	0.752	0.0042	Yes
	1.50	-	0.792	0.0053	Yes
Discotech 5683	0.05	4.85	0.738	0.0048	No
	0.30	-	0.778	0.0027	Yes
	1.50	-	0.850	0.0069	Yes
Stearic acid	0.05	4.80	0.797	0.0047	Yes
	0.30	-	0.787	0.0012	Yes
	1.50	-	0.192	0.0040	Yes
Standapol-A	0.05	4.85	0.700	0.0035	Yes
	0.30	-	0.666	0.0090	Yes
	1.50	-	0.036	0.0035	Yes
Hostapur SAS-30	0.05	4.94	0.736	0.0048	No
	0.30	-	0.742	0.0051	Yes
	1.50	-	0.069	0.0029	Yes
Trymeen 6606	0.05	4.95	0.773	0.0042	Yes
	0.30	-	0.764	0.0041	Yes
	1.50	-	0.755	0.0016	Yes

protein to the unfolding process, with a concomitant increase in denaturation. The findings indicate that surfactants compete with the protein for the free surface area, thereby reducing the amount of protein exposed to the surface and preventing denaturation (10).

The study reported herein investigated the compatibility of various surfactants, commonly used in the pulp and paper industry, with cellulase and hemicellulase enzymes. Optimization of a process involving both enzymes and surfactants can only be

made by understanding their interaction. The purpose of this work is to assist in the design, control, and optimization of treatments of lignocellulosic fibers in the pulp and paper and other industries.

Materials and methods

Enzymes and substrates

The hemicellulase enzymes used were a partially purified xylanase preparation especially designed to improve the bleachability of hard-

wood and softwood kraft pulps. The enzyme is commercially known as Irgazyme-40S (CIBA-Geigy Corp., Greensboro, NC). The xylanase activity of the preparation was 118 U mL⁻¹ enzyme (U, units of activity, is defined as μ moles of reducing sugar released per minute) using the assay described by Prasad *et al.* (5). The enzyme preparation was charged at 0.5% weight/weight (w/w) based on the weight of xylan. The substrate was 1% oat spelt xylan (Sigma Chemical Co., St. Louis, MO).

III. Influence of surfactants on cellulase activity

Surfactant	Surfactant concentration, %	Hydrolysis pH	Average reducing sugar, mg/mL	Standard deviation	Significant at 0.05 level
Control	0	4.97	0.278	0.0044	-
Olinor 1100	0.05	4.85	0.420	0.0099	Yes
	0.30	-	0.408	0.0031	Yes
	1.50	-	0.384	0.0089	Yes
DI 600	0.05	4.85	0.372	0.0142	Yes
	0.30	-	0.387	0.0048	Yes
	1.50	-	0.399	0.0058	Yes
Tergitol TP-9	0.05	4.97	0.423	0.0030	Yes
	0.30	-	0.433	0.0049	Yes
	1.50	-	0.441	0.0021	Yes
Tween 80	0.05	4.96	0.397	0.0110	Yes
	0.30	-	0.408	0.0045	Yes
	1.50	-	0.418	0.0113	Yes
Discotech 5076	0.05	4.87	0.330	0.0150	Yes
	0.30	-	0.390	0.0030	Yes
	1.50	-	0.431	0.0086	Yes
Discotech 5683	0.05	4.94	0.300	0.0126	Yes
	0.30	-	0.375	0.0076	Yes
	1.50	-	0.412	0.0013	Yes
Stearic acid	0.05	4.65	0.374	0.0133	Yes
	0.30	-	0.286	0.0221	No
	1.50	-	0.258	0.0113	Yes
Standapol-A	0.05	4.87	0.317	0.0122	Yes
	0.30	-	0.284	0.0095	No
	1.50	-	0.236	0.0052	Yes
Hostapur SAS-30	0.05	4.97	0.291	0.0134	No
	0.30	-	0.320	0.0103	Yes
	1.50	-	0.354	0.0097	Yes
Trymeen 6606	0.05	4.98	0.362	0.0152	Yes
	0.30	-	0.332	0.0131	Yes
	1.50	-	0.284	0.0091	No

The cellulase is commercially known as BC Cellulase (Sandoz, Lexington, MA). The CMCase (carboxymethylcellulase) activity of the enzyme was 154 U g⁻¹ enzyme using the assay described by Prasad *et al.* (5). The cellulase was also charged at 0.5% (w/w) based on the substrate weight. The substrate was 0.5% carboxymethylcellulose (Sigma Chemical Co., St. Louis, MO).

Surfactants

A variety of surfactants were used. As described in **Table I**, these in-

clude detergents, defoamers, flotation aids for deinking, and wetting and washing aids for pulping and deinking. These surfactants represent various ionic functionalities, including anionic, cationic, and nonionic. Stearic acid was saponified prior to use in 0.01 N NaOH at pH 11.8; 200 ppm of CaCl₂ (based on the volume of the experimental sample) was then added.

Experimental samples

The 20-mL sample volume included the charge of 0.5% xylanase or cellu-

lase, 15 mL of 1% oat spelt xylan or 0.5% carboxymethylcellulose solutions, surfactants at various concentrations, and citrate buffer (pH 4.8, 50 mM) or deionized water to 20 mL.

Surfactants were charged at concentrations (0.0005%, 0.003%, and 0.015% weight/volume) based on 20 mL of total volume. These surfactant charges also correspond, respectively, to 0.05%, 0.3%, and 1.5% (w/w) based on the 1% oven-dried (o.d.) fiber contained in the total volume. These surfactant charges are representative of industrial use. For ex-

ample, a common surfactant charge for deinking in the paper industry is 0.3% based on o.d. fiber. Although the consistency may vary according to the process, 1% fiber is common and was chosen as the basis for calculations of surfactant charges.

The pH of the experimental samples varied from 4.80 to 4.96 in the experiments where xylanase was used, and from 4.65 to 4.98 when cellulase was used. These differences were apparently due to the type of surfactant used. In experiments where the same surfactant was added to the experimental samples at different concentrations, no pH change was observed.

The experimental sample excluding the substrate (but including enzyme, surfactant, and buffer or deionized water) was incubated in a Lab-Line Incubator-Shaker (Lab-Line Instruments Inc., Melrose Park, IL) at 50°C for 30 min by gently shaking at 180 rpm. The reason for incubating the enzyme with the surfactant prior to adding substrate was to determine the compatibility of surfactants with enzymes. This procedure has also been suggested by Castanon and Wilke (6). Soluble substrates, e.g., oat spelt xylan or carboxymethylcellulose, were used in enzyme assays. After adding the substrate that was preheated to 50°C, the mixture was incubated for an additional 60 min.

The reducing sugar analysis was carried out in a manner similar to xylanase and CMCase activity assays as described by Prasad *et al.* (5): 0.5 mL of dinitrosalicylic acid (DNSA) was added to 1 mL of sample solution and incubated for 5 min at 100°C. The contents were stirred well, and after adding 5 mL of deionized water, the UV-absorption for reducing sugars, expressed in mg/mL, was measured at 540 nm, using xylose and glucose as the standards. Control samples, which excluded the surfactants, were incubated at identical experimental conditions and assayed for reducing sugars. No interference in the activity assays

was observed due to the surfactant additions.

Surfactant addition

The method of adding surfactants to a hydrolysis environment was determined by using the same cellulase (BC Cellulase) and filter paper (Whatman No.1) according to the assay procedures described by Mandels *et al.* (11). These assays were also carried out in 20-mL samples. The 20-mL sample volume included the charge of 1% cellulase (w/w, based on the weight of o.d. fiber), filter paper at 3% consistency (consistency is defined as the weight of o.d. fiber divided by the total weight of fiber and water), 0.3% Tween 80 (w/w, based on o.d. fiber), and citrate buffer (pH 4.8, 50 mM). The 3%-consistency filter paper was chosen to provide more accessible surface area for the enzyme to bind and therefore reduce the time for a detectable activity.

The samples were incubated in a Lab-Line Incubator-Shaker at 50°C with gentle shaking at 180 rpm. The buffer was brought to 50°C in a water bath prior to incubation. This assay determined that the cellulase activity of the controls was 62.3 U g⁻¹ enzyme.

The order of surfactant additions studied was (a) mix the buffer, filter paper, enzyme, and surfactant, and then incubate for 2 hours, (b) incubate the buffer, filter paper, and surfactant for 15 min prior to adding enzyme, and then incubate for an additional 2 hours, and (c) incubate the buffer, enzyme, and surfactant for 15 min prior to adding filter paper, and then incubate for an additional 2 hours. After a 2-hour incubation, a reducing sugars analysis was conducted for glucose. Reducing sugar analysis was carried out in a manner similar to the filter paper activity assay described by Mandels *et al.* (11): 3 mL of DNSA was added to 1.5 mL of sample solution and incubated for 5 min at 100°C. After adding 20 mL of deionized water, the contents were stirred well.

The UV absorption for reducing sugars was measured at 540 nm, using glucose as the standard. Control samples, which excluded the surfactants, were incubated at identical experimental conditions and assayed for reducing sugars. Reducing sugars are expressed as mg/mL.

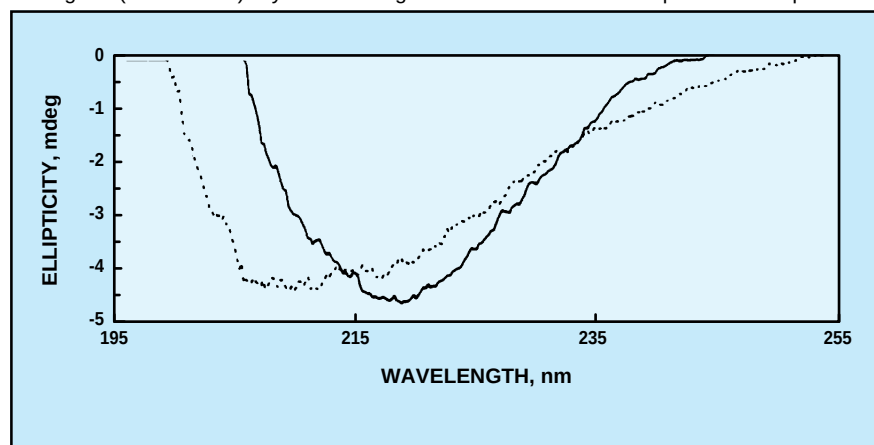
Determination of enzyme-surfactant interaction by circular dichroism

The stability of xylanase and cellulase to a surfactant (Standapol A) was evaluated using circular dichroism (CD) spectropolarimetry. CD allows studying the three-dimensional structure of a protein based on its chiral properties. The CD was measured for either 2 mg/mL of xylanase or 0.6 mg/mL of cellulase and 1.2 mg/mL of surfactant. These amounts enabled the enzymes to be observed on the CD spectrum, and any changes in the molecular structure of the enzymes after interaction with the surfactant could be observed. The surfactant concentrations chosen were eight times higher than the highest concentration used in the hydrolysis experiments, while the xylanase concentration was over 50 times higher and the cellulase concentration was over 30 times higher than the concentrations used in the hydrolysis experiments. Measurements were performed at room temperature in 50-mM sodium acetate buffer at pH 4.8. The CD spectra (254–199 nm for xylanase and 250–199 nm for cellulase) were measured using a Jasco J600 spectropolarimeter; data were recorded on-line using an IBM personal computer. Spectra were recorded at 10 nm/min using silica quartz spectrophotometer cells of 1-mm path length. An instrument range of ± 20 mdeg full scale was used with a 4-second time constant (12).

Results and discussion

The interaction between surfactants and enzymes (xylanase or cellulase)

1. Comparison of the CD spectrum of free xylanase with that of xylanase interacted with the anionic surfactant Standapol-A. Anionic surfactant: 0 mg/mL (solid line—free enzyme) and 1.2 mg/mL (dashed line). Xylanase: 2 mg/mL. Conditions: room temperature and pH 4.8.



and the effect on enzyme activity are presented in **Tables II and III**. The tables indicate the reducing sugar concentrations that were released when various amounts of surfactants were incubated with the particular enzymes prior to the hydrolysis. Each value for the average reducing sugars is the arithmetic mean of four replicates. Values at 0% concentration correspond to the control data for which there was no incubation of enzyme with surfactants.

Influence of surfactants on activity of xylanase

Tables II and III indicate the effects of surfactants on the activities of xylanase and cellulase. As shown in Table II, the xylanase activity improved slightly with increasing concentrations of most nonionic surfactants, except for Olinor 1100. Although Olinor 1100, a nonionic surfactant, improved the xylanase activity at low addition levels, the effect decreased with increasing surfactant concentration. Discotech 5683, a nonionic surfactant, increased the activity most at high addition levels (16% improvement at 1.5% addition) compared to the other nonionic surfactants, while Olinor 1100 increased activity the most at low addition levels (7% improvement at 0.05% addi-

tion). These results suggest that xylanase should be used with nonionic surfactants during various processes in the pulp and paper industry.

The activity of xylanases generally decreased with increasing concentrations of anionic surfactant. All three anionic surfactants studied caused reduced xylanase activity at high addition levels. However, the xylanase activity showed a slight increase at low addition levels of stearic acid compared to the control value. Although the anionic surfactants Hostapur SAS-30 and Standapol-A did not change the xylanase activity substantially at low addition levels, they dramatically reduced xylanase activity at high addition levels as did the stearic acid (average of 86% reduction at 1.5% addition). These results suggest that the use of anionic surfactants with xylanase requires very specific conditions, and if an anionic surfactant must be used with xylanase in a process, that the lowest amount should be used.

The use of cationic surfactants may be preferable to anionic surfactants. As shown in Table II for the cationic surfactant Trymeen 6606, the xylanase activity decreased slightly with increasing surfactant concentration, but the activity remained slightly higher than the control value at all times (6%

improvement at 0.05% surfactant concentration). Therefore, the conditions for using cationic surfactants with xylanase may be less stringent than those for an anionic surfactant.

Influence of surfactants on cellulase activity

The cellulase activity increased with increasing concentrations of all nonionic surfactants except for Olinor 1100 (Table III). Although Olinor 1100, a nonionic surfactant, increased the cellulase activity at all surfactant concentrations, the increase was smaller at the higher concentrations (38% increase at 1.5% surfactant concentration). Nonionic Tergitol TP-9 improved activity more than the other nonionic surfactants, increasing the hydrolysis rate about 60%.

Anionic surfactants did not show a consistent effect. Stearic acid caused a slight increase in activity at low concentrations and a small reduction in enzyme activity at the high levels. Standapol-A behaved similarly, but the increased activity at low concentrations was very small. Hostapur SAS-30 caused an increase in activity at all levels with the best performance at high concentrations, but this was only a 27% rate increase. None of the surfactants caused the large decrease in activity that was observed with the xylanase at high surfactant concentrations.

Results for the cellulase in combination with cationic surfactants were similar to the results for xylanase. Although the cellulase activity decreased with increasing levels of the cationic surfactant Trymeen 6606, the activity remained higher than the ten control values at all times (30% improvement at 0.05% level). This suggests that using this cationic surfactant with cellulase is also feasible, particularly at low levels of surfactant addition. Surfactants can serve as either enzyme accelerators or inhibitors.

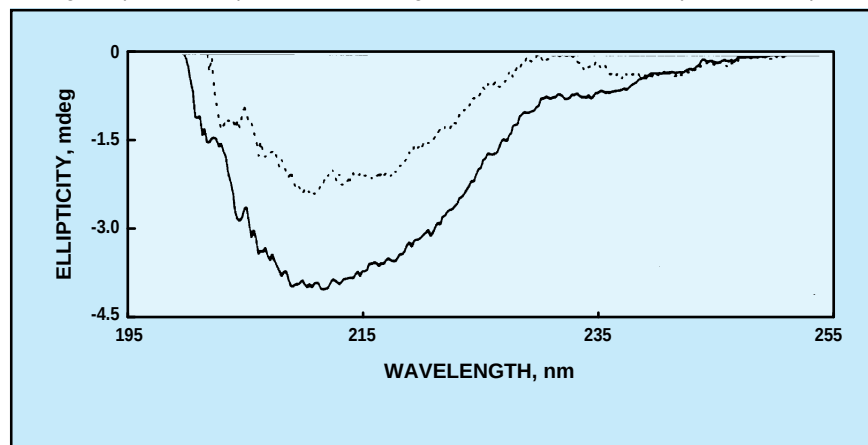
Circular dichroism measurements of xylanase and cellulase in the presence of surfactant

The deactivation of cellulase and xylanase due to interaction with the anionic surfactant Standapol-A was observed using CD spectroscopy to complement the findings of the activity experiments. The anionic surfactant Standapol-A was chosen to determine the interaction between enzyme and surfactant, since it caused the highest deactivation for both xylanase and cellulase. **Figures 1 and 2** indicate the CD spectra of 2 mg/mL of xylanase and 0.6 mg/mL of cellulase, respectively, with 1.2 mg/mL of surfactant concentration. The CD spectra of the free enzymes is indicated by the solid lines. Dashed lines indicate enzymes after interaction with the anionic surfactant. The ellipticities (at 254–199 nm for xylanase and 250–199 nm for cellulase) decreased (in absolute value) upon adding the anionic surfactant to the enzyme solutions, indicating a change in the molecular shape of the enzymes. Ellipticity reduction in the CD spectrum indicates a change in the secondary structure of an enzyme. As in the activity experiments, the influence of an anionic surfactant on xylanase was much more pronounced in the CD spectrum, in which the anionic surfactant changed the wavelength interval for observing xylanase and reduced the ellipticity compared to that of cellulase. These results indicate that the decrease in enzyme activities in the presence of surfactants was due to their denaturation.

Method of surfactant addition

As shown in **Table IV**, hydrolysis of filter paper by cellulase was either inhibited or accelerated, depending on the order of surfactant (Tween 80) addition to the hydrolysis environment. Initial incubating of the buffer, enzyme, and surfactant prior to hydrolysis improved the cellulase activity more compared to mixing the buffer, filter paper, enzyme, and

2. Comparison of the CD spectrum of free cellulase with that of cellulase interacted with the anionic surfactant Standapol-A. Anionic surfactant: 0 mg/mL (solid line—free enzyme) and 1.2 mg/mL (dashed line). Cellulase: 0.6 mg/mL. Conditions: room temperature and pH 4.8.



surfactant at once and incubating. However, initial incubation of the buffer, filter paper, and surfactant prior to hydrolysis inhibited the cellulase activity. The results suggest that incubating the enzyme and surfactant prior to hydrolysis is preferable for both soluble and insoluble substrates (see Table III for Tween 80).

Park *et al.* (7) and Helle *et al.* (8) indicated that surfactants did not affect the hydrolysis of cellulase when water-soluble substrates were used. This may have been due to the type of surfactants used. However, their results could be due to the order of adding surfactants into the hydrolysis environment. We added the surfactant to the enzyme preparation prior to the hydrolysis step. The increase in enzyme activity due to interaction with some nonionic and cationic surfactants may be related to the fact that enzymes are dispersed well during the initial incubation period by the effect of surfactants. This may be because cellulase enzymes are reported to form aggregates in the absence of surfactants, depending on the enzyme concentration in solution (12).

This aggregation can be observed by circular dichroism. In the circular dichroism studies, we also observed the formation of aggregates for both cellulase and xylanase (spec-

tra not shown). This may suggest that nonionic surfactants especially can disperse the enzymes very efficiently in solution, thus preventing stearic inactivation of the enzyme due to aggregation and enabling them to bind to more substrate sites. Alternatively, the improvement in the hydrolysis of filter paper by cellulase that was incubated with nonionic Tween 80 also supports the mechanism proposed by Castanon and Wilke (6), Park *et al.* (7), and Helle *et al.* (8), who reported that surfactants hinder the immobilization of the enzymes on the substrate by reducing the binding strength and make desorption easier from the binding site after reaction. We further propose that a binding energy reduction could allow the enzyme to progress more rapidly along the cellulose chain and thus increase the hydrolysis rate without complete desorption.

In this study, we used a range of surfactant levels near those actually used in industry. The study was not originally designed to investigate the acceleration of enzyme activity due to interaction with surfactants, but rather was intended to investigate the compatibility of the particular enzymes with common surfactants of various ionic natures and functions. However, our findings are also in agreement with those of Castanon

Addition sequence	Surfactant concentration, %	Average reducing sugar, mg/mL	Standard deviation	Significant at 0.05 level
1. Buffer+filter paper+enzyme +surfactant	0	0.510	0.0037	Yes
	0.3	0.534	0.0048	
1. Buffer+filter paper+surfactant (initial incubation for 15 min) 2. +enzyme	0	0.591	0.0100	Yes
	0.3	0.540	0.0058	
1. Buffer+enzyme+surfactant (initial incubation for 15 min) 2. +filter paper	0	0.522	0.0069	Yes
	0.3	0.589	0.0065	

and Wilke (6), Ooshima *et al.* (9), and Park *et al.* (7), in which nonionic surfactants generally improve the enzyme activity while cationic and anionic surfactants inhibit the enzymes, especially at high addition levels.

Conclusions

We drew the following conclusions from our experiments:

- All surfactants studied had some effect on the activities of xylanase and cellulase. Surfactants can act as both accelerators and inhibitors for the enzymes. The enzyme activity improved with all nonionic surfactants studied.
- Although the single cationic surfactant that was tested accelerated the activity of xylanase and cellulase less at increasing addition levels, the activities remained higher than the control value at all times.
- The xylanase activity decreased with increasing concentrations of anionic surfactants. Substantial deactivation was observed at high levels of surfactant addition. However, at low levels of surfactant addition, the anionic surfactants studied caused only a small inhibition of xylanase activity, while stearic acid caused a small accel-

eration. Although the cellulase activity typically decreased with increasing concentrations of anionic surfactants, this reduction was much smaller than that for xylanase. Improvement in the cellulase activity was also observed, especially at low addition levels of anionic surfactants, while only moderate inhibition occurred at high addition levels.

- Circular dichroism studies at high concentrations indicated a change in the molecular structures of the enzymes due to the interaction with an anionic surfactant.
- Xylanase and cellulase can be safely used together with nonionic surfactants during various processes in the pulp and paper industry. The use of cationic surfactants is preferable to that of anionic surfactants. If an anionic surfactant must be used with xylanase and cellulase in a process, the lowest addition is suggested. In this study, severe inhibition of the xylanase occurred at high levels of all anionic surfactants studied.
- The order of combining enzymes, surfactants, and substrates can be important. Incubation of enzymes with surfactants prior to an enzymatic treatment can be beneficial in obtaining higher ef-

ficiencies from enzymes. Surfactant addition to the substrate prior to adding the enzyme may be much less effective. 

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