The Effects of Nonionic Surfactants on the Pretreatment and Enzymatic Hydrolysis of Recycled Newspaper

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Abstract The effects of surfactants on the pretreatment and enzymatic hydrolysis stages of recycled newspaper processing were examined. Newspaper substrate was pretreated with surfactants at 40°C and 400 rpm for 1 h, and the enzymatic digestibilities of the pretreated substrate were compared. NP-20 was 10~20% more effective as a surfactant than Tween-20 and Tween-80. To investigate the effects of the surfactants on the subsequent enzymatic hydrolysis stage, the newspaper was pretreated with NP-20 and then hydrolyzed in the presence of TW-20 or TW-80. TW-80 showed an approximate 7% higher digestibility than TW-20. The surfactant effect on the hydrolysis of the untreated newspaper was significant, whereas the surfactant effect on the hydrolysis of the surfactant-pretreated newspaper was marginal. When the digestibilities of the pure cellulose substrates (α-cellulose and filter paper) were examined, markedly different surfactant effects were observed. In contrast to the newspaper substrate, the surfactant-pretreated pure cellulose substrates had a significant effect on digestibility when they were hydrolyzed in the presence of a surfactant, indicating that the surfactant effect on digestibility is highly dependent on substrate type.

Keywords: newspaper, pretreatment, enzymatic hydrolysis, surfactant, digestibility, α-cellulose

INTRODUCTION

Due to the ongoing pressures that are placed on petroleum oil stocks, lignocellulosic materials are being studied as renewable energy feedstocks. The utilization of waste cellulosics such as the waste paper and sludge from pulp and paper plants has received extensive attention [1-3]. Annually Korea produces about 1.3 and 3.4 million tons of paper sludge and unrecycled waste paper, respectively [4]. Currently, most of this waste is landfilled or incinerated; however, both of these methods are of growing environmental concern. Therefore, the conversion of waste biomass to energy serves both energy and environmental interests.

Cellulose, the major component of wastepaper, can be converted into fermentable sugars by enzymatic hydrolysis. However, inks and certain additives used in paper production can hinder enzyme access to the substrate. Thus, an effective pretreatment is an essential step to increasing the enzymatic digestibility of waste paper and reducing enzyme consumption. In our previous studies [5,6], we have shown that wastepaper requires much less pretreatment for enzymatic hydrolysis than woody and herbaceous materials. In these studies ammonia/hydrogen peroxide solutions, with or without a surfactant, were used as pretreatment reagents at 40°C and proved to be highly effective at increasing the enzymatic digestibility of newspaper. It is believed that surfactants help remove the ink and other components that physically interfere with enzymatic hydrolysis. Studies of pretreatments that were based on surfactants alone are very limited. Kurakake *et al.* [7] examined the potential use of nonionic surfactants in the pretreatment of bagasse at high temperatures 170~190°C. They compared the enzymatic hydrolysis rates of surfactant-pretreated substrates with those of a water-pretreated substrate and concluded that the surfactant's pretreatment effect was due to its ability to extract hydrophobic degradation products that had been produced during the pretreatment.

Furthermore, the addition of a surfactant to the enzymatic hydrolysis stage of lignocelluloses increases the conversion of cellulose to glucose [8-14]. The mechanism for this phenomenon has not been established, but the effect of surfactants on cellulose hydrolysis may be explained by the following three factors: enzyme stability, substrate struc-

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ture, and enzyme-substrate interactions [9].

The main purpose of this study was to investigate the effects of surfactants in the pretreatment and/or enzymatic hydrolysis stages of newspaper hydrolysis. This approach may be useful to maximize enzymatic digestibility because surfactants could increase the digestibility of newspaper at each stage if they were added to both the pretreatment and hydrolysis stages. To investigate such possible effects, raw newspaper substrate was pretreated with a surfactant and the digestibility for this pretreated substrate was determined. To investigate the effects of surfactants on enzymatic hydrolysis, untreated or pretreated substrates were hydrolyzed in the presence of a surfactant and the substrate digestibilities were measured.

MATERIALS AND METHODS

Newspaper and Surfactants

A mixture of three newspapers (Gyungnam Ilbo, Maeil Business Newspaper, and Donga Ilbo) was used as the substrate. Its moisture was 7.2% (w/w) with the following composition based on the dry substrate: 59.0% glucan, 16.2% xylan + mannan + galactan, 12.4% klason lignin, and 6.0% ash. NP-5, 10, and 20 surfactants were purchased from TCI (Tokyo Kasei Kogyo Co., Japan). TW-20 and TW-80 surfactants and α-cellulose were obtained from Sigma Chemical Co. (St. Louis, MO, USA), and Whatman filter paper No. 1 from Whatman International Ltd. (Maidstone, England). The characteristics of the surfactants used are listed in Table 1. Newspaper and filter paper were cut into approximate 0.5 × 0.5 cm pieces.

Table 1. The characteristics of the surfactants

Name	Composition	EO[a] (mol)	HLB[b]
NP-5	Polyethylene glycol	5	10.0
NP-10	mono-4-nonylphenyl	10	13.3
NP-20	ether	20	16.0
TW-20	Polyoxyethylene sorbitan monolaurate		16.7
TW-80	Polyoxyethylene sorbitan monooleate		15.0

[a]EO: ethylene oxide, [b]HLB: hydrophile-lypophile balance.

Pretreatment

Ten grams of substrate was added to a 500 mL round flask with 200 g of deionized water or aqueous ammonia solution. Then 0.5% (w/w) of a surfactant was added to this solution and the content of the flask was agitated at 400 rpm and 40°C for 1 h. The concentration of the surfactant was calculated as % (w/w) based on the 10 g of dry substrate. After pretreatment the wet solid was washed with 1 L of deionized water, filtered to a moisture content of 70~80%, and then separated into two portions. One portion was oven dried at 105°C overnight to determine its moisture content and the weight loss that occurred during pretreatment. It was then further subjected to compositional analysis. The other portion was stored in a refrigerator until it was needed for the enzymatic digestibility testing.

Enzyme and Digestibility Tests

Commercial cellulase and β-glucosidase (Novo Nordisk, Bagvard, Denmark) enzymes were supplied from Novozymes Korea Ltd. A mixture of Celluclast (60 filter paper units [FPU]/mL) and Novozym 188 (792 cellobiase units [CBU]/mL) was used in the ratio of 4 FPU Celluclast/CBU Novozym to alleviate end-product inhibition by cellobiose.

Enzymatic digestibility testing of the pretreated substrate was performed in duplicate according to the National Renewable Energy Laboratory (NREL) standard procedure No. 009 [15], and the results were presented as the averages of two experiments. An amount of solid required to give 0.5 g of glucan in 50 mL was added to a 250-mL flask. The buffer solution was 0.05 M citrate, pH 4.8. The cellulase enzyme was loaded at 15 FPU/g glucan, or as otherwise specified. The surfactant was added at 0.5% based on the dry substrate, when needed. The content of the flask was preheated to 50°C before the enzyme was added. The flask was then placed on a shaking bath at 50°C and 90 strokes/min. Samples were taken periodically and analyzed for glucose using HPLC. The glucose contents after 24, 48, and 72 h of hydrolysis were used to calculate the enzymatic digestibility as follows: % digestibility = (g glucan hydrolyzed/g glucan added) × 100.

Analytical Methods

The solid biomass sample was analyzed for sugars, klason lignin, and ash according to the NREL standard procedures [16-18]. Sugars were measured by HPLC (Thermo Separation Products) using a Bio-Rad HPX-87H column (conditions; 0.6 mL/min, 65°C, 0.005 M H_2SO_4). Because this column does not resolve xylose, mannose, and galactose, their combined value was used.

RESULTS AND DISCUSSION

The Surfactant Effect on Pretreatment

In our previous study [19], when newspaper was pretreated to increase its enzymatic digestibility the pretreatments based on a surfactant alone showed higher digestibilities than the pretreatments based on Tween-series surfactants, ammonia, and/or hydrogen peroxide. In this study NP series and TW-80 surfactants were selected to investigate the effects of surfactant types on pretreatment. In our preliminary experiments NP-20 showed slightly better performance than NP-5 and NP-10, and therefore, NP-20 was selected for further experimentation (data not shown). NP

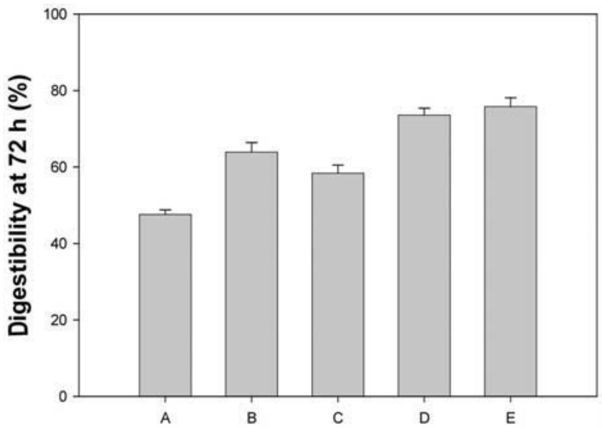

Fig. 1. The effect of the pretreatment solution composition on the enzymatic digestibility of newspaper. (A: untreated, B: 0.5% TW-80, C: 0.5% TW-80 + 4% ammonia, D: 0.5% NP-20, E: 0.5% NP-20 + 4% ammonia).

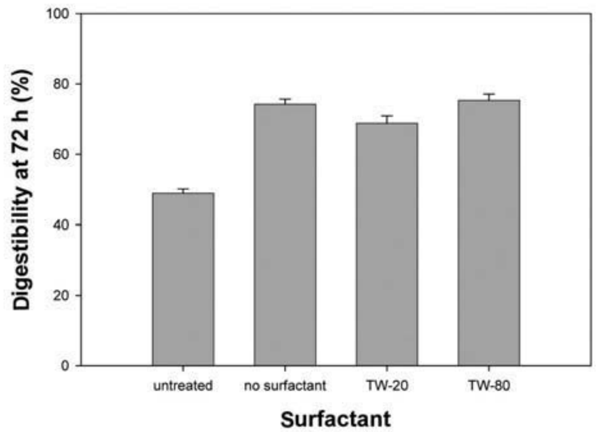

Fig. 2. The effects of surfactants on the enzymatic digestibility of pretreated newspaper. (pretreatment: 0.5% NP-20).

series surfactants could have been used in the enzymatic hydrolysis; however, we chose to use them in the pretreatment only because the toxic phenols that are formed during their biodegradation could inhibit cell growth in any subsequent fermentation.

Fig. 1 shows the effects of NP-20 and TW-80 on pretreatment performance. The digestibility of the NP-20-pretreated substrate was approximately 10% higher than that of TW-80-pretreated substrate. Here, an untreated sample was defined as a substrate without any treatment since its digestibility at 72 h was only 2% lower than that of the substrate pretreated with water for one hour at 15 FPU (see Table 2). The digestibility of the substrate pretreated with ammonia and TW-80 was 5% lower than that pretreated with only TW-80, whereas the digestibility of substrate pretreated with ammonia and NP-20 was about 2% higher than that pretreated with only NP-20. Given the high pH (pH 11.8) of the 4% ammonia solution, the pH in itself could have modified the effects of both surfactants. In the case of NP-20, we believed ammonia was not needed in the pretreatment due to its marginal effect on digestibility and the extra chemical cost.

The Surfactant Effect on Enzymatic Hydrolysis

It is a well-known fact that the addition of a surfactant into the enzymatic hydrolysis of lignocellulosic biomass increases its digestibility [8-14]. However, previous studies have used surfactants in the hydrolysis stage only. Since the use of surfactants in the pretreatment stage was found to have a significant effect on digestibility, surfactant-pretreated substrates may be used in enzymatic hydrolysis to maximize digestibility. In this study a substrate was pretreated with NP-20 and then hydrolyzed by adding Tween-series surfactants. Fig. 2 shows the effect of the surfactants on the hydrolysis of surfactant-pretreated newspaper. Compared to the digestibility with no addition of surfactant, TW-20 showed a 5% lower digestibility, but TW-80 showed a marginal increase in digestibility. This result indicated that the Tween-series surfactants could hinder or slightly enhance the enzyme reaction using surfactant-pretreated newspaper. We considered that the surfactant loading might have been insufficient for enzyme hydrolysis to occur, therefore, the surfactant loading effect was examined in the range of 0.25~2.0% based on the dry substrate (in actual solution: 0.0045~0.036%). However, the surfactants had almost no effect on digestibility over the above range (data not shown). The reason why TW-20 hindered enzymatic hydrolysis is not clear, nevertheless, we chose to use TW-80 in further experiments.

The digestibility of newspaper was previously reported to dramatically increase when TW-80 or other surfactants were added at the enzymatic hydrolysis stage [8,13,14]. However, Duff *et al.* [20] reported that TW-80 only marginally increased sugar production when disposal sludge from de-inking mills was hydrolyzed with cellulase. A reason they gave for this was that surfactants added during the de-inking process may have remained in the sludge, reducing the effect of the TW-80 surfactant on hydrolysis. As discussed above the effect of TW-80 in the hydrolysis of NP-20-pretreated substrate was marginal. Thus, we suspected that some surfactant left in the pretreated substrate after washing may have affected the hydrolysis. Assuming that the surfactant concentrations in the pretreatment reagent mixture and washing water were uniform, the ratio of surfactant in the pretreated substrate versus that in the original pretreatment could be calculated. Our calculations showed that the pretreated substrate probably contained only 2% of the surfactant that was initially added. Fig. 3 shows the effect on digestibility from the remaining surfactant following pretreatment. To remove the surfactant completely from the pretreated substrate, 10 g of NP-20-pretreated substrate was washed with 2 L of water (twice the amount used in regular experiments). We observed almost no increase in the digestibility, even if all the surfacetant that was used in the pretreatment was left in the hy-

Table 2. The enzymatic digestibility of newspaper, α-cellulose, and filter paper in four differently devised processes

Substrate	Enzyme loading (FPU/g glucan)	72 h Digestibility					
		Untreated	Process				
			Pretreatment Hydrolysis	1 Water No surfactant	2 0.5% NP-20 No surfactant	3 Water 0.5% TW-80	4 0.5% NP-20 0.5% TW-80
Newspaper	7.5	40.5 ± 1.5			64.8 ± 3.0		65.6 ± 2.1
	15	49.5 ± 0.5		51.8 ± 0.8	74.0 ± 2.1	70.6 ± 1.7	75.7 ± 2.4
	30	62.4 ± 1.7			80.7 ± 2.0		81.1 ± 2.7
α-Cellulose	15	59.4 ± 1.2		62.1 ± 1.5	71.7 ± 2.7	81.3 ± 1.8	84.9 ± 2.5
Filter paper	15	69.2 ± 0.9		74.7 ± 1.2	79.5 ± 2.1	91.2 ± 1.5	95.9 ± 1.9

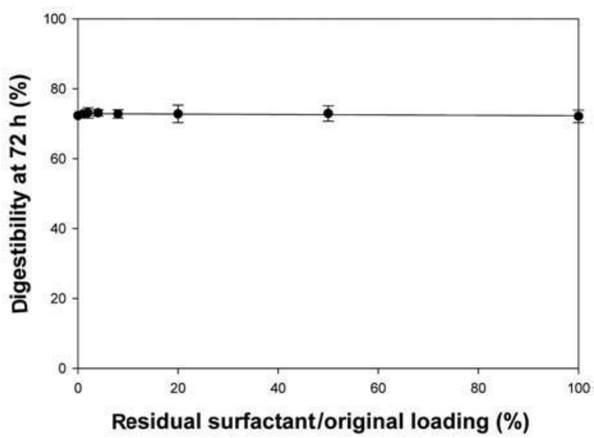

Fig. 3. The effect of residual surfactant in pretreated newspaper on enzymatic digestibility. (original NP-20 loading = 0.5% of dry untreated substrate weight).

drolysis solution. This suggests that the surfactant carry-over in the pretreated substrate was not responsible for the lack of a surfactant effect in the enzymatic hydrolysis stage of an NP-20-pretreated substrate.

The Overall Surfactant Effect on Enzymatic Digestibility

To investigate the overall effect of the surfactant on both pretreatment and hydrolysis, three kinds of substrates were selected and four kinds of processes were devised (Table 2). In the case of newspaper, the digestibility was directly related to the enzyme loading. In process 1 where the substrate was pretreated with water, an approximately 2% higher digestibility resulted compared to the untreated sample. For process 2, in which the substrate was pretreated with NP-20, there was an approximately 24% higher digestibility than that for the untreated sample, and the surfactant effect in the pretreatment was marked. In process 4, where NP-20 and TW-80 were added into the pretreatment and hydrolysis stages, respectively, there was a less than 2% digestibility enhancement versus process 2. This suggests there was little surfactant effect on hydrolysis when the substrate had been pretreated with NP-20, as discussed above. In process 3 TW-80 was added to the hydrolysis stage of a water-pretreated substrate. Here there was a 21% higher digestibility compared with the untreated sample, and again the surfactant effect was marked. This large increase in process 3 was consistent with previous findings [8-14]. According to the above results, a surfactant addition at the pretreatment stage was slightly more effective than a surfactant addition at the hydrolysis stage.

By acting as a physical barrier to enzyme, lignin is clearly the most recognized factor limiting cellulose hydrolysis; many studies have reported that the unspecific adsorption of cellulase on lignin can decrease the hydrolysis rate of lignocellulosic substrates [8-11]. To determine the effects of lignin and other components contained in newspaper on hydrolysis, we examined the hydrolysis of two pure cellulose substrates, α-cellulose and filter paper. As shown in Table 2, noticeably different results were obtained. A surfactant addition to the hydrolysis stage (process 3) was more effective in terms of digestibility than a surfactant addition to the pretreatment stage (process 2). Also, process 4 showed a significantly higher digestibility than process 2. These findings imply that a significant surfactant effect occurred with the addition of TW-80 to the hydrolysis stage of a NP-20-pretreated substrate. This significant effect on the digestibility of α-cellulose and filter paper was in contrast to the small surfactant effect that was seen with newspaper. Such differences in the digestibilities of the substrates were probably due to the residual components of the pretreated substrates, such as lignin, additives, ink, *etc.*, which hindered the enzyme reaction.

The results of processes 1 and 3 show that TW-80 had almost the same effect on the hydrolysis of all the substrates, regardless if the substrates contained lignin or not. This is not consistent with the results from Eriksson *et al.* [9] who reported that TW-20, added in the hydrolysis stage, increased the enzyme conversion of lignocellulose (steam-pretreated spruce) much more than the conversions of pure cellulose substrates (Avicel and delignified steam-pretreated spruce). Therefore, they proposed that the surfactant adsorption to lignin prevents the unproductive binding of the enzyme to lignin, resulting in increased hydrolysis. In our case, it appeared that lignin did not affect the hydrolysis of the substrates when a surfactant was only added to the hydrolysis stage, but the surfactant effect on the digestibility was highly dependent on the substrate type.

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