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Enhancement of the Enzymatic Digestibility of Waste Newspaper Using Tween

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Abstract

Methods of increasing the enzymatic digestibility of waste newspaper by adding Tween (TW)-20 and 80 surfactants were investigated. Tween-series surfactants were selected because these surfactants increase cellulase activity during enzymatic hydrolysis and do not inhibit cell growth in downstream fermentation processes. When surfactant was used in a pretreatment, a benefic effect was expected in the enzymatic hydrolysis stage owing to surfactant carry-over from the pretreatment stage immediately upstream of the hydrolysis. However, because it was necessary to wash the pretreated substrate with water to remove inhibitors produced during pretreatment, no added benefit was obtained. When surfactant was used in the pretreatment only, it was found that it had a marked effect on digestibility and that this effect was higher at lower enzyme loadings. Also, TW-80 was found to be more effective than TW-20, and the addition of enzyme and TW-80 to substrate at the beginning of enzyme reaction was found to most effectively increase digestibility. When TW-80 was added into either the pretreatment stage or the hydrolysis stage the digestibilities of untreated sample increased by approx 40%, whereas an increase of only 45% was observed when TW-80 was added to both stages. These results show that the addition of surfactant to either the pretreatment or the enzymatic hydrolysis stage is sufficient to increase digestibility.

Index Entries: Pretreatment; newspaper; surfactant; hydrolysis; enzymatic digestibility.

Introduction

Energy supply problems owing to abrupt increases in petroleum prices have accelerated developments on alternative energy sources. One of the promising energy sources is bioethanol, which can be produced from renewable lignocellulosic materials such as forest and agricultural residues, and municipal solid wastes. Municipal solid wastes, such as waste paper, can provide cheap feedstocks for ethanol and meet targets of resource reutilization and environmental compatibility *(1)*. Korea produces annually about 1.3 and 3.4×10^6 t of paper sludge and unrecycled waste

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paper, respectively *(2)*. In the case of organic sludge, landfilling was prohibited by law in 2003. Incineration involves high dewatering costs owing to the high moisture contents of sludge, and also produces secondary air pollution. Wastepaper constitutes half of municipal solid waste, and newspaper, which represents 14% of the waste, is recovered relatively easily *(3)*. As in comparison with wood, waste cellulosic materials have very low associated costs and some of them contain high levels of cellulose, which can provide good feedstocks for ethanol production.

Newspaper is mostly derived from softwoods and exhibits low enzymatic digestibility because of its high lignin content and dense structure *(4)*. However, because newspaper has already received considerable chemical and/or physical treatment, it does not require the extensive pretreatment required for woody and herbaceous materials. But ink and some additives added during the paper-making process can interfere with the enzymatic hydrolysis of wastepaper. The pretreatment methods developed for wastepaper were similar to those developed for woody and herbaceous materials. Of numerous studies on pretreatment, there have been limited studies on wastepaper pretreatment using electron beam irradiation *(5)*, carbon dioxide explosion *(6)*, and ammonia–hydrogen peroxide *(7–9)*. These methods appear to be uneconomical because of high energy and/or chemical cost. To reduce energy consumption, we previously pretreated wastepaper with surfactant at temperature less than 50°C *(10,11)*. Surfactant added to a pretreatment stage can remove many of the components that hinder enzyme access to substrate, and increase digestibility *(8–11)*. Also, surfactant added during enzymatic hydrolysis can increase enzyme stability or positively affect enzyme–substrate interactions, and again have a positive effect on digestibility *(12–15)*. Therefore, two types of experiments were carried out in this study. First, raw newspaper was pretreated using a surfactant to determine pretreatment performance, and the digestibility of this pretreated newspaper was measured without the addition of surfactant in hydrolysis stage. Second, pretreated newspaper was hydrolyzed in the presence of surfactant and again digestibility was measured to determine the effect of surfactant on enzymatic hydrolysis.

Materials and Methods

Materials

A mixture of Korean newspapers was used as substrate. Newspaper was cut into approx 0.5×0.5 cm pieces. Its moisture content was 7.2 wt% and it had the following composition on a dry substrate basis: 59 wt% glucan, 16.2 wt% xylan + mannan + galactan, 12.4 wt% klason lignin, and 6 wt% ash. Tween-series surfactants (Sigma Chemical Co., St. Louis, MO) were used in this study as listed in Table 1. Commercial cellulase and β-glucosidase (Novo Nordisk, Bagvard, Denmark) were supplied from Novozymes Korea Ltd., (Seoul, Korea), and a mixture of Celluclast (80 filter

Table 1
Nonionic Surfactants

	Chemical name	HLB[a]
TW-20	Polyoxyethylene sorbitan monolaurate	16.7
TW-80	Polyoxyethylene sorbitan monooleate	15

[a]HLB, hydrophile-lypophile balance; TW, Tween.

paper units [FPU]/ml) and Novozym 188 (792 cellobiase units [CBU]/mL) was used in the ratio of 4 FPU of Celluclast/CBU Novozym to alleviate end-product inhihition by cellobiose.

Pretreatment

Ten grams of substrate was added to a 500 mL round flask containing 200 g of deionized water. Then, 0–2 wt% of a surfactant was added to this solution and the contents were agitated at 400 rpm and 40°C for 1 h. The concentration of the surfactant was calculated as wt% based on the 10 g of dry substrate. After pretreatment, the wet solid material was washed with 1 L of deionized water, filtered to a moisture content of 70–80%, and then separated into two portions. One was oven dried at 105°C overnight to determine moisture content and weight loss during pretreatment, and analyzed for composition. The other was stored in a refrigerator until required for enzymatic digestibility testing.

Enzymatic Digestibility Test

Enzymatic digestibility of pretreated substrate was performed in duplicate according to National Renewable Energy Laboratory (NREL) standard procedure no. 009. The NREL standard procedures can be obtained from the following website: www.ott.doe.gov/biofuels/analystical_methods.html. An amount of solid required to produce 0.5 g glucan in 50 mL was added to a 250-mL flask. The buffer solution was 0.05 *M* citrate, pH 4.8. Cellulase was then loaded at 30 FPU/g glucan, and 0.5% of the dry substrate weight of surfactant, when needed, was added. The contents of the flask were 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 high-performance liquid chromatography (HPLC). The glucose contents after 24, 48, and 72 h of hydrolysis were used to calculate the enzymatic digestibility.

Analytical Methods

Solid biomass sample was analyzed for sugars, klason lignin, and ash according to NREL standard procedures nos. 002, 003, and 005. Sugars concentrations were measured by HPLC (Thermo Separation Products)

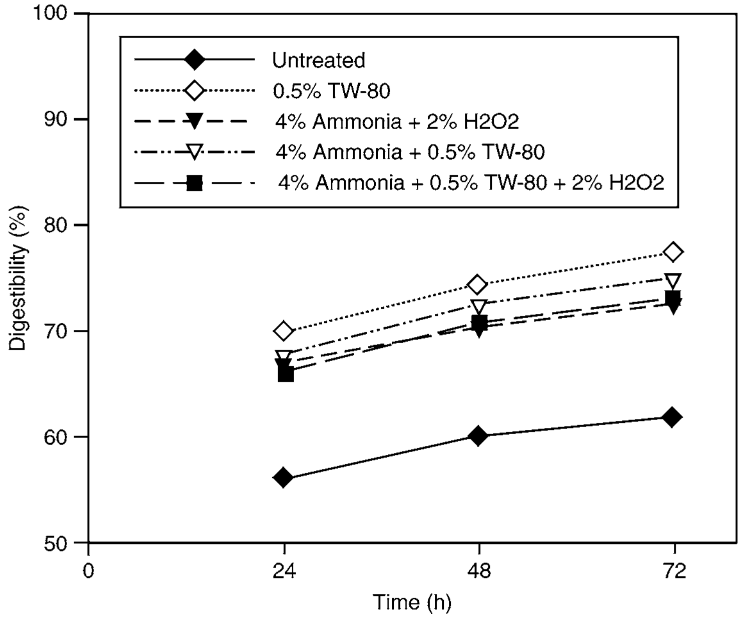

Fig. 1. Effect of composition of pretreatment solution on the enzymatic digestibility of newspaper.

using a Bio-Rad HPX-87H column (condition; 0.6 mL/min, 65°C, 0.005 *M* H_2SO_4). Because this column did not resolve xylose, mannose, and galactose, their combined concentrations were used.

Results and Discussion

Effect of Surfactant on Pretreatment

A mixture of ammonia and hydrogen peroxide was previously found to be highly effective at pretreating lignocellulosic biomasses, such as, corn cobs/stover mixture *(16)*. And the combined use of ammonia, hydrogen peroxide, and surfactant was found to be effective at pretreating wastepaper before hydrolysis *(8,9)*. These studies revealed that newspaper should not be pretreated using the methods developed for wood and herbaceous biomass, because paper is produced from wood using many processing stages, and a multitude of additives and ink are added during the paper producing and printing processes. Thus, wastepaper was pretreated at near room temperatures, not at the high temperatures and pressures usually required to treat other lignocellulosic biomasses. Although ammonia and hydrogen peroxide help to swell cellulosic fibers and remove ink, we investigated methods that require minimal amount of chemicals in the present study, because of the environmental aspects and because these chemicals represent a major fraction of the total pretreatment cost.

As shown in Fig. 1, in this study we examined combinations of ammonia, hydrogen peroxide, and/or surfactant to pretreat newspaper.

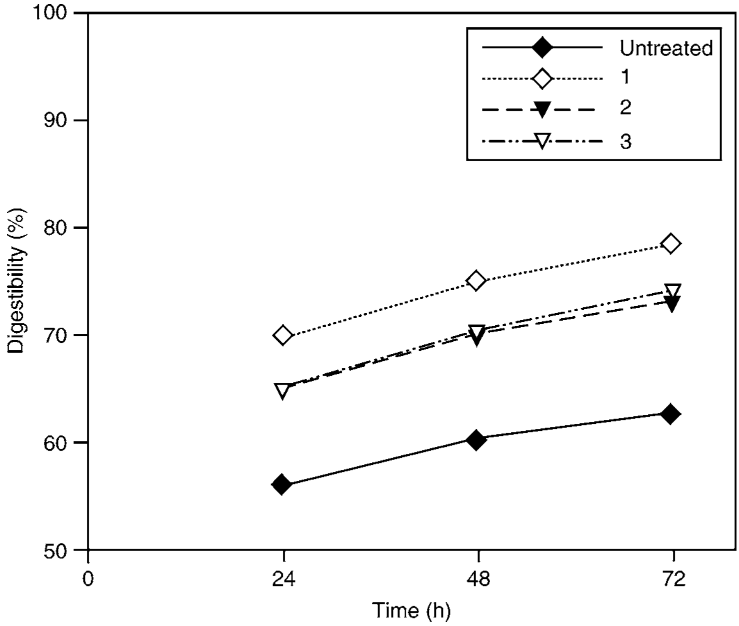

Fig. 2. Effect of treatment method after pretreatment on the enzymatic digestibility of newspaper.

Method	Pretreatment	→	Hydrolysis
1	0.5% TW-80	Washing	No surfactant
2	"	Filtering	No surfactant
3	"	Filtering	Compensate TW-80 to make 0.5% in hydrolysis solution

The digestibility of materials obtained using any combination of these three chemicals was much higher than that of untreated sample. Here, untreated sample is defined as a substrate without any form of treatment, because the digestibility of untreated feedstock at 72 h is only 2% lower than that of feedstock pretreated by soaking in water for an hour (data not shown). The results obtained showed that pretreatment with surfactant only (i.e., containing no ammonia and hydrogen peroxide) proved best. This finding was unexpected, but it does mean that pretreatment using surfactant offers processing economies and environmental benefits.

We selected Tween surfactants because these surfactants increase cellulase activity during enzymatic hydrolysis and do not inhibit cell growth during subsequent fermentation processes *(12,14,15)*. This suggests that Tween can be used in both the pretreatment and hydrolysis processes. When Tween (TW)-80 is used in the pretreatment, the pretreatment solution can be partially removed by filtration without water washing. Because the surfactant left in the pretreated substrate could have a positive effect on enzymatic digestibility, three schemes were devised as shown in Fig. 2. The results

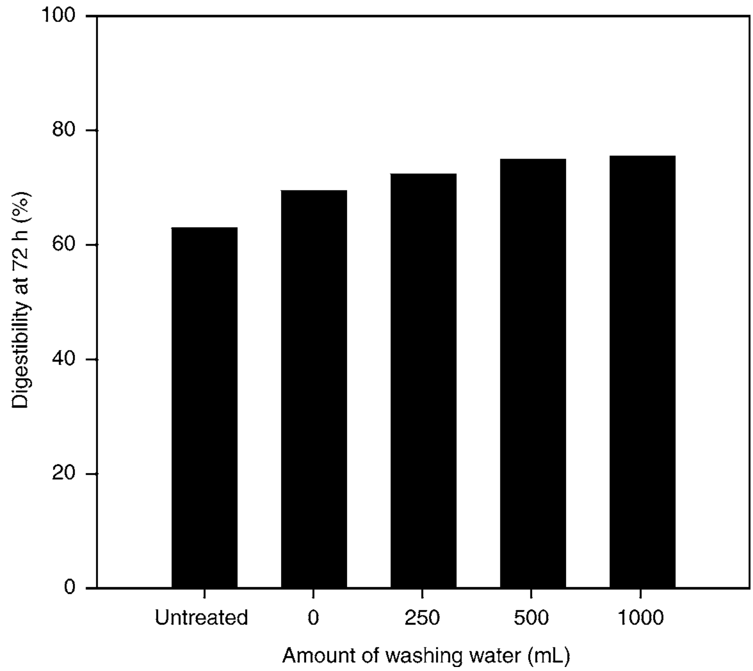

Fig. 3. Effect of amount of washing water used after pretreatment on enzymatic digestibility (pretreatment: 0.5% TW-80).

showed that method 1, in which the pretreated sample was washed with water, had 5% higher digestibility than method 2, in which the pretreatment solution was partly removed by filtration, and than method 3, in which the surfactant was added (to a concentration of 0.5% in the hydrolysis stage) to material obtained using method 2. These results showed that a washing step is required between the pretreatment and hydrolysis stages to obtain high digestibility. This is probably because ink components and other additives detached from paper fibers directly hinder the enzymatic hydrolysis or reattach to the fiber surface and reduce digestibility.

Figure 3 shows the effect of the amount of washing water on 72 h digestibility. The digestibility of the unwashed material was 6% higher than that of untreated sample and digestibility increased with the amount of used water, but this effect was negligible at more than 500 mL. This means that the components that hinder hydrolysis are effectively removed by washing pretreated substrate with 500 mL of water. Figure 4 shows the effect of TW-80 loading in the pretreatment stage on 72 h digestibility. Digestibility increased with surfactant loading, but this increase was negligible above 0.5%, thus 0.5% was used in further experiments.

Table 2 shows the effect of surfactant type and enzyme loading on 72 h digestibility after newspaper was pretreated with 0.5% surfactant. In comparison with digestibility at 30 FPU, digestibilities at 15 FPU, were 14.9%, 7.2%, and 6.4% lower for untreated sample, and TW-20 and TW-80-pretreated

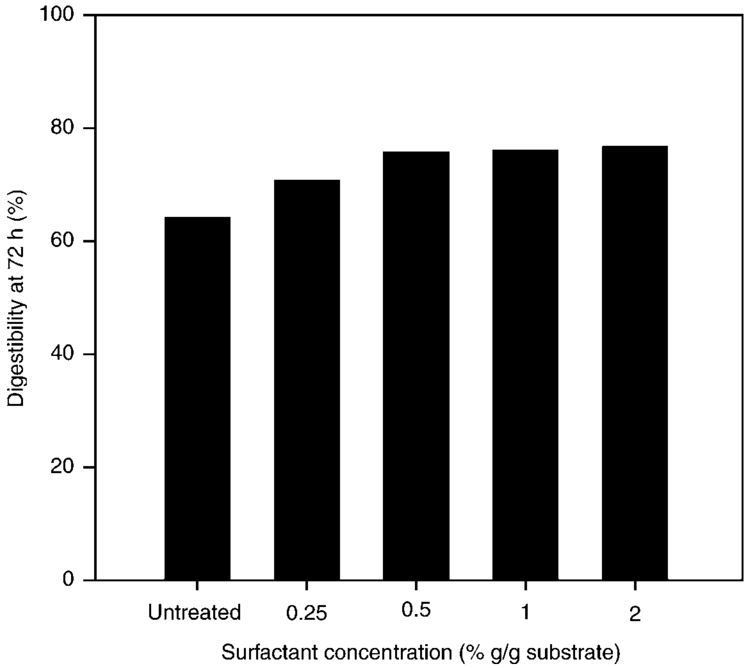

Fig. 4. Effect of TW-80 loading in the pretreatment stage on the enzymatic digestibility of newspaper.

Table 2
Effect of Surfactant Type in the Pretreatment Stage and Enzyme Loading on 72 h Enzymatic Digestibility of Newspaper (Surfactant Concentration = 0.5%)

Enzyme loading	Surfactant		
(FPU/g glucan)	Untreated	TW-20	TW-80
15	49.1	62.9	68.1
30	64	70.1	74.5

samples, respectively. When the enzyme loading was halved, the digestibility of untreated sample was maximally reduced, but surfactants significantly attenuated this reduction. This indicates that surfactants have a greater effect on digestibility when enzyme loading is lowered *(10,13)*. The digestibility of TW-80-pretreated sample at 15 FPU was about 4% higher than that of untreated sample at 30 FPU. Because TW-80 increased digestibility by 4% vs TW-20, we concluded that it is probably more suitable for the pretreatment of newspaper.

Effect of Surfactant on Enzymatic Hydrolysis

Enzyme activity reduces as the reaction proceeds, and this deactivation can be reduced by the addition of surfactants. The mechanism underlying

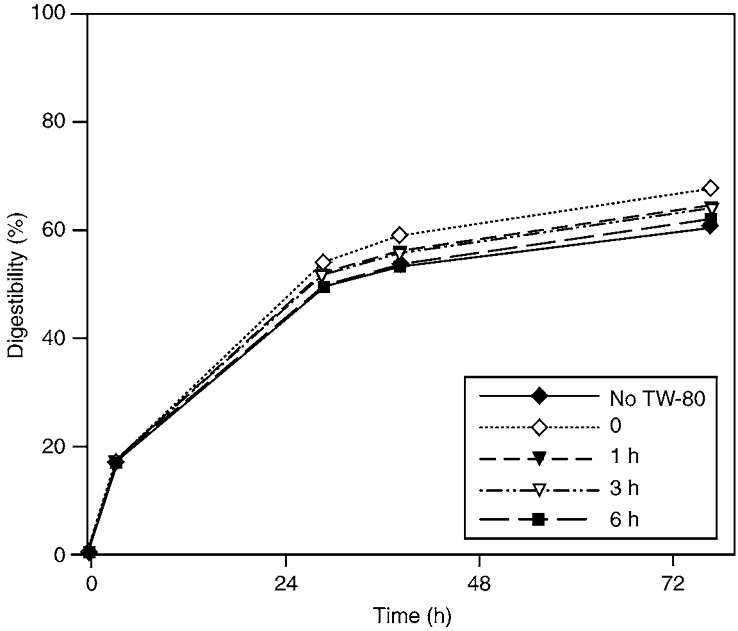

Fig. 5. Effect of time of surfactant addition on enzymatic digestibility (hydrolysis: 0.5% TW-80).

this phenomenon has not been elucidated, but one of the most promising explanations is that surfactant causes cellulase to desorb easily from cellulose surface after hydrolysis *(12–15)*. In order to convert solid cellulose to soluble sugars, enzymes must be adsorbed onto the cellulose surface. Because surfactant could prevent the adsorption of enzyme onto a cellulose surface, we considered that enzyme should be added first and that the surfactant be added later. Figure 5 shows the effect of time of surfactant (TW-80) addition on the digestibility of newspaper. Digestibility was maximized when surfactant was present from the beginning of the hydrolysis and was reduced if the surfactant addition was postponed. This implies that surfactant does not prevent binding between enzyme and substrate. Also this result supports the explanation that the surfactant increases the hydrophilicity of the substrate, and facilitates enzymes access *(13)*, or that it prevents unproductive enzyme adsorption to the lignin part of the substrate during the initial stage of the hydrolysis *(12)*.

The digestibility of newspaper has been reported to be dramatically increased when TW-80 or other surfactants are added to the hydrolysis reaction *(11–15)*. Figure 6 shows the effect of surfactant in the pretreatment and/or in the hydrolysis stage at an enzyme loading of 15 FPU/g glucan. Process 1, in which the surfactant was present in the pretreatment stage only, and process 2, in which surfactant was present in the hydrolysis stage only, showed 20% higher digestibility than the untreated sample. Thus, it can be concluded that the effect of surfactant on either process is similar and

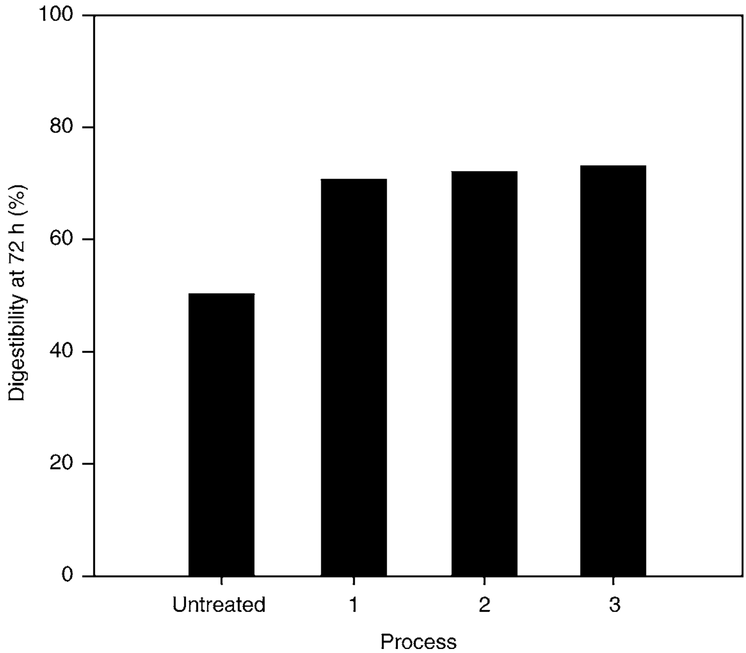

Fig. 6. Effect of process on the enzymatic digestibility of newspaper (enzyme loading = 15 FPU/g glucan).

Process no.	Pretreatment	→	Hydrolysis
1	0.5% TW-80		No surfactant
2	No surfactant	Washing	0.5% TW-80
3	0.5% TW-80		0.5% TW-80

significant. Process 3, in which surfactant was present in both stages, was found to have a 2–3% higher digestibility than processes 1 or 2. This implies that surfactant does not affect the hydrolysis if newspaper is pretreated with surfactant, and that surfactant is needed only once, in the either pretreatment or hydrolysis stage. Surfactant in the pretreatment stage removes components that retard enzymatic hydrolysis, and surfactant in the hydrolysis stage prevents enzyme deactivation and improves enzyme-substrate interactions, both of which would lead to enhanced digestibility. However, we cannot explain why the effect of surfactant on either pretreatment or hydrolysis is significant, whereas the effect of surfactant on the hydrolysis of surfactant-pretreated substrate is almost negligible.

Conclusion

We investigated the possibility of enhancing the enzymatic digestibility of waste newspaper using nonionic surfactants. In order to increase the digestibility of newspaper, pretreated substrate containing surfactant should be washed with water before hydrolysis. Therefore, a surfactant used in the pretreatment stage cannot affect the hydrolysis stage, and thus it is not necessary to use the same surfactant in both stages. Moreover, because surfactant in the hydrolysis stage may inhibit cell growth in a subsequent fermentation, surfactant selection is limited, whereas any surfactant can be used in the pretreatment stage, because of the intervening washing stage before the hydrolysis. Also surfactant and enzyme must be added from the beginning of the hydrolysis reaction to obtain high digestibility. One addition of TW-80 to either the pretreatment or hydrolysis stage was found to be sufficient, because the effect of surfactant addition to both stages found to be only marginally higher than its effect when added to either of the two stages.

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