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Enhancement of Enzymatic Digestibility of Recycled Newspaper by Addition of Surfactant in Ammonia–Hydrogen Peroxide Pretreatment

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Abstract

The effect of surfactant on enzymatic digestibility was investigated during the pretreatment stage. Newspaper was pretreated with an ammonia–hydrogen peroxide mixture on a shaking bath at 40°C and 130 strokes/min for 3 h. Two kinds of nonionic surfactants, NP series and Tween series, were utilized. The effect of hydrophile-lipophile balance (HLB) value of both series surfactants on digestibility was found to be negligible, even though de-inking efficiency was improved as HLB value was increased. The effect of surfactant loading on digestibility was small, below 0.5 wt%, and negligible above 0.5 wt% at 60 international filter paper units (IFPU). The percentage improvement in digestibility increased as enzyme loading decreased. Digestibility of NP-5-added sample relative to control sample, increased significantly at an enzyme loading <60 IFPU, i.e., 19 and 13% at 15 and 30 IFPU, respectively. Such an increase in digestibility was not explained clearly from the experimental results. It was also found that ink removal before enzymatic hydrolysis is very important to enhance digestibility.

Index Entries: Pretreatment; newspaper; ammonia; hydrogen peroxide; enzymatic digestibility; surfactant.

Introduction

The widespread use of papers has created an enormous amount of wastepaper; however, it is not easy to recycle this resource because of the high cost of its utilization process. In the past, recycled wastepaper was used only two to three times before the fibers became unacceptably short *(1)*. This wastepaper can be used in the bioethanol process as inexpensive

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carbohydrate substrate *(2)*. Cellulose, the major component of wastepaper, can be converted into fermentable sugars by enzymatic hydrolysis. However, raw biomass generally resists enzymatic hydrolysis because sites available for enzyme attack are limited *(3)*. Thus, effective pretreatment is an essential prerequisite to enhance the digestibility of lignocellulosic materials *(4,5)*.

Numerous pretreatment methods have been developed to improve cellulose hydrolysis by physical, chemical, or biochemical methods *(6)*. In a number of pretreatment studies, there have been very limited examinations of already delignified biomass such as wastepaper *(7–11)*. However, pretreatment methods used in most of these studies were the same as those used in conventional woody and herbaceous materials. Recent work *(5,12)* has shown that wastepaper did not require such an extensive pretreatment as developed for lignocellulosic materials.Researchers used much milder conditions than conventional conditions, such as 4% ammonia and 2% hydrogen peroxide (H_2O_2) mixture at 40°C *(5)* and 0.25% H_3PO_4 at 20°C *(12)*. According to these researchers, the ink components and some additives used in paper production hindered enzyme access to substrate. Previous studies *(4,5,13,14)* revealed that an ammonia-H_2O_2 mixture proved to be very effective in pretreating lignocellulosic materials. Ammonia helps to separate ink from cellulosic fibers and H_2O_2 to swell fibers. Thus, the enzymatic digestibility of ammonia-H_2O_2- treated substrate was much higher than that of untreated substrate. It was also found that ink had a significant effect on enzymatic digestibility.

In addition to ammonia and H_2O_2, surfactant can be added in a pretreatment process to improve de-inking, which also enhances enzymatic hydrolysis. In a de-inking process, surfactant can aid in reducing the adhesion of the ink to the fibers *(15)*. Furthermore, cellulose hydrolysis is improved when surfactants are present, because they help cellulase to desorb easily from the cellulose surface after hydrolysis reaction *(16)*. Therefore, it was deduced that surfactants could enhance the enzymatic digestibility if they were added in our pretreatment system. The main purpose of our study was to investigate the effect of surfactant on enzymatic digestibility when it was added in our pretreatment stage. Pretreatment was conducted in an ammonia-H_2O_2 mixture on a shaking bath at 40°C and 130 strokes/min for 3 h.

Materials and Methods

Newspaper and Surfactants

A mixture of three newspapers issued in Korea was used as substrate. Newspaper was cut into approx 0.5 × 0.5 cm pieces. The moisture of the paper was 6.8 wt% with the following composition: 56.2 wt% glucan, 13.9 wt% xylan + mannan + galactan (XMG), 15.0 wt% klason lignin, and 7.4 wt% ash. The nonionic surfactants (TCI; Tokyo Kasei Kogyo, Tokyo, Japan) used are listed in Table 1.

Table 1
Nonionic Surfactants[a]

Surfactant	*Chemical composition*	*Appearance*	*EO content (mol)*	*HLB*
NP-5	Polyethylene glycol	Oily liquid	5	10.0
NP-10	Mono-4-nonylphenyl	Oily liquid	10	13.3
NP-20	Ether	White solid	20	16.0
TW-80 (n=1)	Polyoxyethylene	Light yellow liquid		15.0
TW-85 (n=3)	Sorbitan n-oleate	Light yellow liquid		11.0

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

Pretreatment

Petreatment was performed on a reciprocating shaking water bath. Five grams of substrate was added to a 500-mL autoclavable bottle with 100 g of 4 wt% ammonia–2 wt% H_2O_2 solution. The concentration of each component was expressed as wt% based on the total amount of the solution. Then 0.1–1.5 wt% of a surfactant was added to this solution. The concentration of the surfactant was calculated as wt% based on the 5 g of dry substrate. The bottle was placed for 3 h on a shaker operating at 40°C and 130 strokes/min. After pretreatment, the wet solid was washed with deionized water until neutral and then separated into two portions. One was oven dried at 105°C overnight to measure moisture content, and subsequently, the weight loss on pretreatment. It was further subjected to composition analysis. The other was to be used in carrying out the enzymatic digestibility test stored in a refrigerator.

Enzyme and Digestibility Test

Commercial cellulase and β-glucosidase (Novo Nordisk, Bagvaerd, Denmark) supplied from Novozymes Korea were used. A mixture of Celluclast (80 IU or international filter paper units [IFPU]/mL) and Novozym 188 (792 cellobiase units [CBU]/mL) was used with a ratio of 4 IU of Celluclast/CBU of Novozym to alleviate end-product inhibition by cellobiose.

Enzymatic digestibility of pretreated substrate was performed in duplicate according to National Renewable Energy Laboratory (NREL) standard procedure no. 009 *(17)*. The 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, and the cellulase enzyme loading was 60 IFPU/g of glucan. The content of the flask was preheated to 50°C before the enzyme was added. The flask was placed on a shaking bath operating at 50°C and 90 strokes/min. A sample was taken periodically and analyzed for glucose using high-performance liquid chromatography (HPLC). The glucose content after 72 h of hydrolysis was used to calculate the enzymatic digestibility.

Analytical Methods

The solid biomass sample was analyzed for moisture, sugars, klason lignin, and ash by NREL standard procedures no. 001–005 *(17)*. 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, the combined value of XMG is used in this article.

Results and Discussion

Newspaper exhibits low enzymatic digestibility because of its high lignin content. Additionally, chemicals such as ink, fillers, and other additives make it difficult to hydrolyze enzymatically. Our previous studies *(5,14)* showed that ink had a significant effect on digestibility when newspaper was pretreated with ammonia-H_2O_2. Furthermore, Nikolov et al. *(12)* reported that fillers and other additives used in the process of paper production made an adhesive "envelope" around the cellulose fibers, and this envelope was effectively removed with 0.25% H_3PO_4. In their study, the deliginified waste-cellulose fibers left from the processing of paper product, which was not printed, were used as substrate. Therefore, in the pretreatment of lignocellulosic materials, it is important to develop a proper pretreatment method suitable to a specific substrate.

Effect of Hydrophile-Lipophile Balance Value on Digestibility

The hydrophile-lipophile balance (HLB) represents the relative affinity of an emulsifier for water and for oil. The HLB scale goes from 0 to 20. Generally, values below 7 refer to hydrophobic agents and values above 7 to hydrophilic ones. In a de-inking process, the ink particles released from the fiber surface are removed from the slurry either by washing or flotation. The efficiency of de-inking depends on the ink properties and the paper quality used *(15)*. Surfactants are added in the de-inking process to decrease adhesion of the printing ink to the fibers at approx pH 9.0–10.0. The addition of H_2O_2 also helps ink removal by breaking chemical crosslinkages between the binders of the ink.

The optimum HLB value is dependent on ink composition *(18)*. For a washing de-inking, the values are usually above 10, and typical surfactant loadings are 0.25–1.5% relative to dry paper weight. Since nonionic surfactants are most common in de-inking *(15)*, two kinds of nonionic surfactants, NP series and Tween series were selected, as listed in Table 1.

Figure 1A shows the effect of HLB value of NP series surfactant on digestibility at a surfactant loading of 0.5 wt%. The HLB values of NP-5, -10, and -20 are 10.0, 13.3, and 16.0, respectively (Table 1). Here, control sample means a substrate pretreated without surfactant. After pretreatment, a dark-colored band consisting of ink components was observed in the upper portion of the bottle in the control sample, whereas no dark-colored band was observed in the NP-added samples (not shown). It was

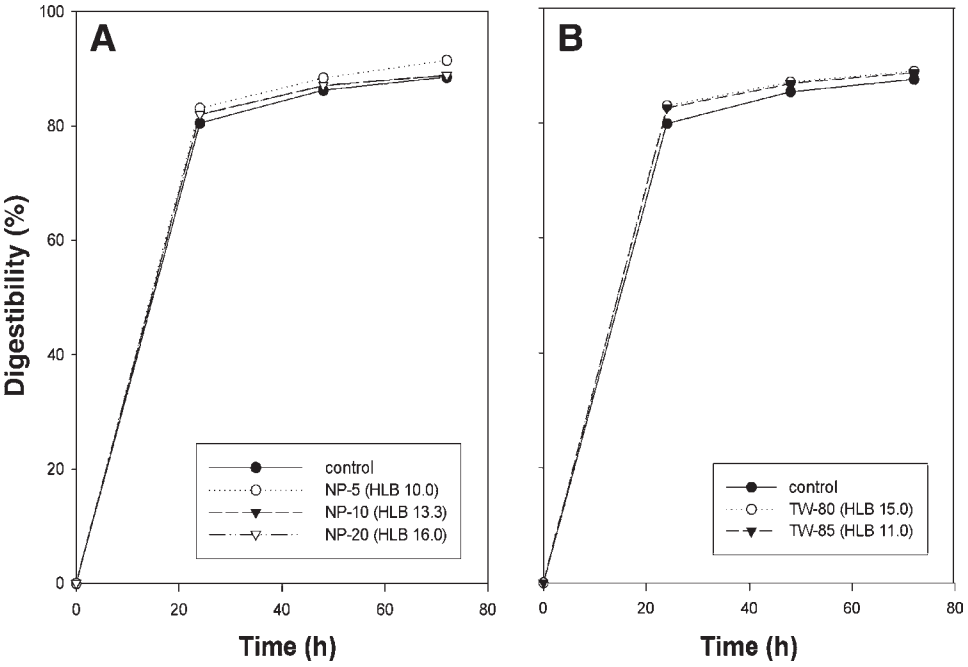

Fig. 1. Effect of HLB value on enzymatic digestibility at surfactant loadings of 0.5 wt%: **(A)** NP series; **(B)** TW series.

apparent that the dark-colored components were emulsified by the addition of surfactant, so these components were evenly distributed among the fibers. After washing pretreated samples, it was also observed that the higher the HLB value used, the whiter the swollen sample obtained. This means that de-inking efficiency is improved with this surfactant. In spite of such improvement, Fig. 1 shows that the digestibilities of NP-5-added samples were almost the same as those of the control one, resulting in the conclusion that digestibility did not depend on the HLB value using a surfactant loading of 0.5 wt%.

Figure 1B shows the effect of HLB value of the two Tween (TW) series surfactants on digestibility using a surfactant loading of 0.5 wt%. The HLB values of TW-85 and TW-80 are 11.0 and 15.0, respectively (Table 1). Unlike NP series surfactants, the dark-colored components were not distributed among the fibers (not shown). However, a slightly thicker band than the one formed in the control sample was observed in the upper portion of the bottle. As in NP series surfactant, digestibility did not depend on the HLB value. From the results shown in Fig. 1, it can be said that surfactants can improve ink removal efficiency, but not enzymatic digestibility.

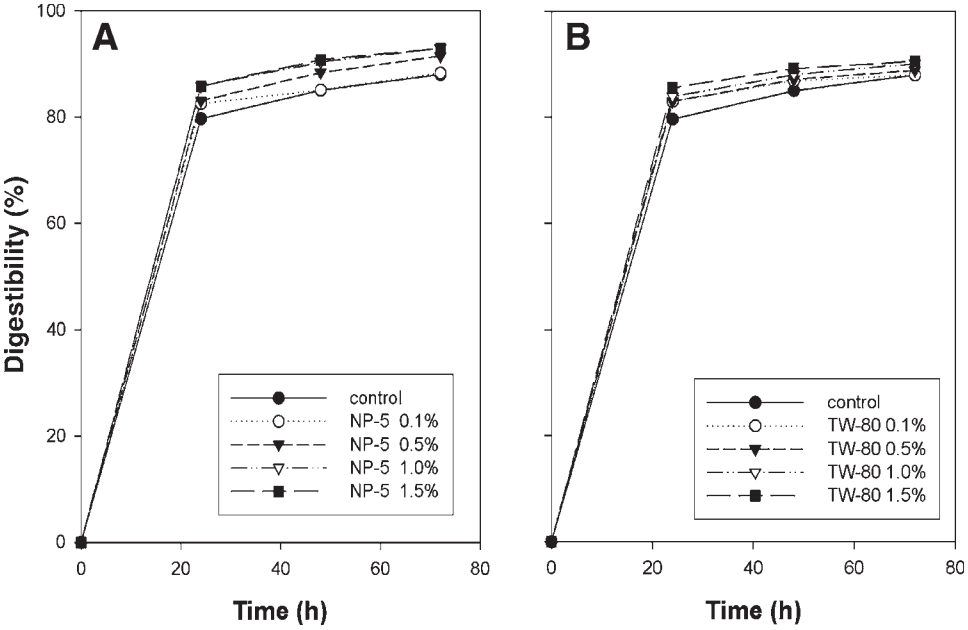

Fig. 2. Effect of surfactant loading on enzymatic digestibility: **(A)** NP-5; **(B)** TW-80.

Effect of Surfactant Loading on Digestibility

NP-5 and TW-80 were selected to examine the effect of surfactant loading on digestibility. As shown in Fig. 2, the increase in surfactant loading from 0.1 to 1.5 wt% caused a small increase in digestibility. With both surfactants, digestibilities were almost the same above 0.5 wt%, even though de-inking efficiency increased as surfactant loading increased. Surfactant is beneficial to enzymatic hydrolysis, but excess surfactant can create excessive foam and inhibit cell growth in the later fermentation process *(19)*. Therefore, an optimum surfactant loading of 0.5 wt% was selected for further experiments.

Effect of Enzyme Loading on Digestibility

As already revealed, the enzymatic digestibility was not increased as much as the de-inking efficiency. This difference may be explained by the fact that there were already detached ink re-deposits on the fiber surface after washing, which interfered with enzyme access to the substrate. Nevertheless, detached ink particles were observed in the control experiment that did not include any surfactant. It has also been reported that liquid-phase ink does not inhibit cellulase activity when ink is added to de-inking newspaper sludge at a concentration up to four times the average ink content *(20)*. Thus, the enzyme loading based on 1 g of glucan was changed from 60 to 15 IFPU and 30 IFPU because it was thought that digestibility was too high to compare the surfactant effect at 60 IFPU.

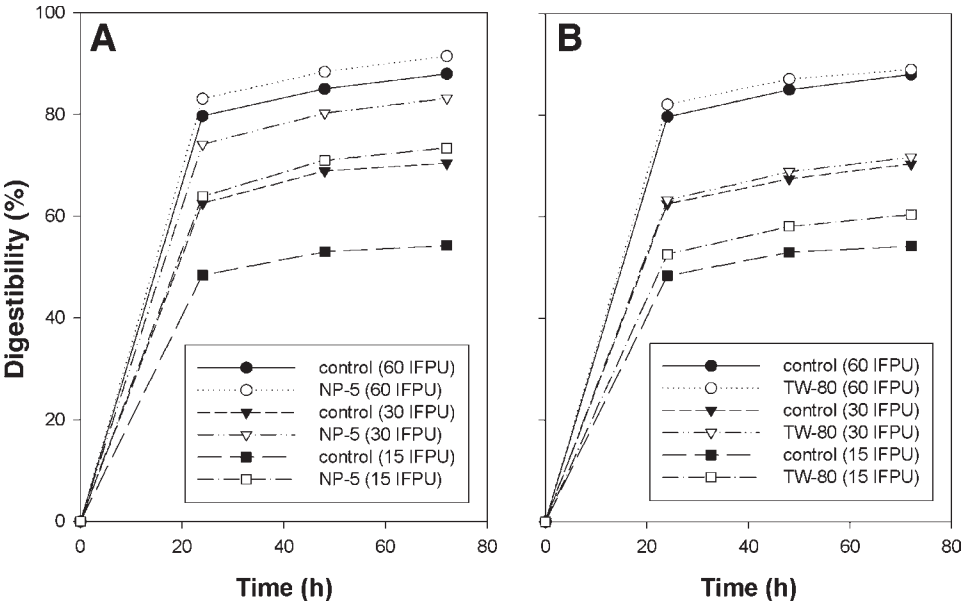

Fig. 3. Effect of enzyme loading on enzymatic digestibility at surfactant loadings of 0.5 wt%: **(A)** NP-5; **(B)** TW-80.

Figure 3 shows the effect of enzyme loading on digestibility at surfactant loadings of 0.5 wt%. As seen in Fig. 3A, the digestibility of NP-5-added sample increased more significantly than that of the control sample—from 54 to 73% for 15 IFPU and from 70 to 83% for 30 IFPU. In the case of TW-80, digestibility remained almost the same except for the lowest enzyme loading, 15 IFPU. This means that a small amount of NP-5 can reduce enzyme loading significantly and digestibility depends on the type of nonionic surfactant. Since the cost of cellulase enzyme accounts for a major portion of total cost in bioethanol production, the use of surfactant can reduce a substantial portion of production cost *(21)*. For both surfactants, the percentage improvement in digestibility increased as enzyme loading decreased. This coincides with the fact that surfactant was much more effective at low enzyme loading *(16)*.

Effect of Residual Surfactant on Digestibility After Pretreatment

Pretreated samples in our study were washed with water until neutralized to pH 7.0, but this step required a lot of water, about 300 times the weight of substrate used, because the pretreated sample was sticky and swollen. It was theorized that there was still a small amount of surfactant left in the washed fibers, and that residual surfactant may help enzymatic hydrolysis. Figure 4 shows the effect of residual surfactant after pretreatment on digestibility at 72 h. To see this effect, newspaper was pretreated without surfactant, and then a given amount of surfactant was added to

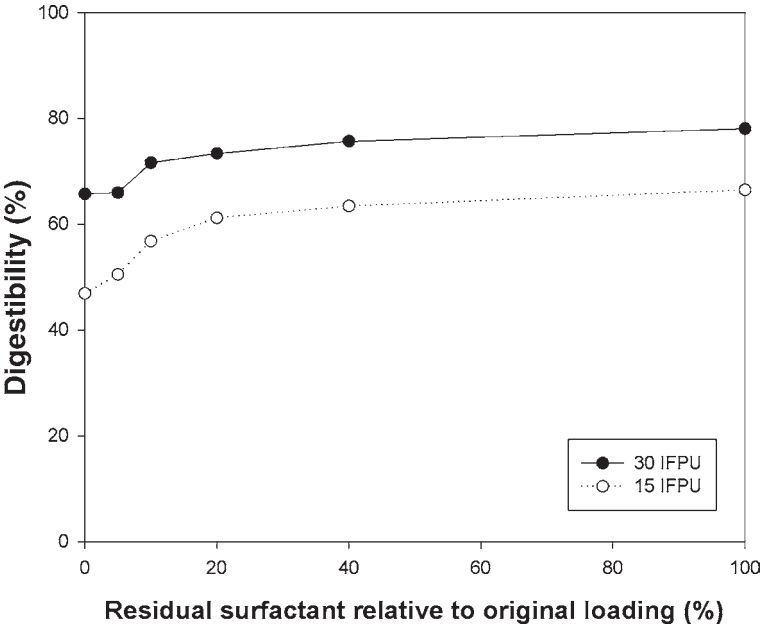

Fig. 4. Effect of residual surfactant on enzymatic digestibility after pretreatment (original NP-5 loading = 0.5 wt% of dry paper weight).

the pretreated sample at enzymatic hydrolysis. The *x*-axis value in Fig. 4 means the surfactant percentage left in pretreated sample relative to original surfactant loading (0.5 wt% of dry paper weight). As expected, digestibility increased significantly at residual surfactant levels below 20%, but marginally above 20%. Since surfactant was mostly removed in our pretreated sample, it can be expected that residual surfactant is not solely responsible for enhancement of digestibility at lower enzyme loadings.

One thing to be noted here is that digestibility depends on which stage of surfactant is added, i.e., pretreatment or enzymatic hydrolysis. As already discussed, the digestibility when surfactant was added in the pretreatment stage was 73% for 15 IFPU and 83% for 30 IFPU. When the same amount of NP-5 was added in the enzymatic hydrolysis stage, 100% in Fig. 4, digestibility was 67% for 15 IFPU and 78% for 30 IFPU. This means that the addition of surfactant in pretreatment stage is more effective on cellulosic hydrolysis. Therefore, the results confirm that ink removal by adding surfactant to pretreatment before enzymatic hydrolysis is very important to enhance digestibility at an enzyme loading <60 IFPU.

Conclusion

The effect of surfactant on enzymatic digestibility was investigated when it was added in ammonia-H_2O_2 pretreatment. The impact of digestibil-

ity depended on the type of nonionic surfactant. A small amount of NP-5 can reduce enzyme loading, resulting in substantial cost reduction in the bioethanol process. Ink removal before enzymatic hydrolysis was shown to be very important to enhance digestibility at low enzyme loading.

Acknowledgments

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