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Enzymatic Digestibility of Used Newspaper Treated with Aqueous Ammonia–Hydrogen Peroxide Solution

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Abstract

Wastepaper constitutes approximately half of municipal solid waste, making it a potential source of bioenergy. Newspaper was pretreated with an ammonia-hydrogen peroxide (H_2O_2) mixture in a shaking bath from room temperature to 80°C, and then its enzymatic digestibility was measured. A significant amount of ink was removed from the newspaper slurry by the reciprocating movement of the shaking bath. In addition, the ammonia-H_2O_2 significantly swelled the substrate, thereby greatly increasing its susceptibility to enzymatic digestion. After pretreating the newspaper with conditions of 40°C, 3 h, 130 strokes/min, and 4 wt% ammonia–2 wt% H_2O_2, the enzymatic digestibility was almost 90% of theoretical, or about 25% higher than that of untreated substrate. Digestibility was also investigated as a function of ammonia concentration, H_2O_2 concentration, shaking speed, pretreatment temperature, and time.

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

Introduction

Lignocellulosic materials have considerable promise as a source of future energy. Wastepaper is a plentiful and low-cost feedstock for making bioethanol *(1)*. Typically, wastepaper constitutes half of municipal solid waste, and newspaper alone 14% of the waste *(2)*. In the past, most of this material was used only once and then was landfilled or incinerated. Even recycled wastepaper can be used only two to three times before the fibers become unacceptably short.

Lignocellulosic biomass generally resists enzymatic hydrolysis; thus, effective pretreatment is an essential prerequisite to enhance the digestibil-

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ity of lignocellulosic residues. Of a number of studies on the pretreatment of lignocellulosic materials, there have been limited studies on wastepaper pretreatment using electron beam irradiation *(3)*, carbon dioxide *(4)*, steam explosion *(5)*, ammonia fiber explosion *(6)*, and ammonia-hydrogen peroxide (H_2O_2) in a percolation reactor system *(7)*. The pretreatment methods used in most of these studies, however, were the same as those used in woody and herbaceous materials. Because paper has already had considerable chemical and/or physical treatment, wastepaper does not require the extensive pretreatment developed for woody and herbaceous materials. Another difference between wastepaper and raw lignocellulosic material is ink and fillers added in the paper-making process. These chemicals could potentially interfere with the enzymatic hydrolysis of wastepaper.

H_2O_2 in aqueous ammonia solution is very effective for pretreating lignocellulosic materials *(8,9)*. It also has been used to pretreat wastepaper *(6,7)*. However, the reported methods appear to be uneconomical because of high chemical costs. To increase enzymatic digestibility without severe pretreatment, a pretreatment process was devised that can remove ink and swell the substrate easily on a shaking bath. In our previous study *(10)*, this process proved to be extremely effective at removing ink from newspaper. The main purpose of the present study was to investigate parameters that affect enzymatic digestibility when treating newspaper in an ammonia-H_2O_2 mixture on a shaking bath. The pretreatment temperatures used were <80°C, which are very mild compared to conventional pretreatment conditions.

Material and Methods

Newspaper and Chemicals

A mixture of four newspapers issued in Korea was used as substrate. Newspaper was cut into approx 1 × 1 cm pieces. The moisture of this paper was 7.9% with the following composition: 61.3 wt% glucan, 9.8 wt% xylan + mannan + galactan (XMG), 12.0 wt% klason lignin, 5.7 wt% ash, and 11.3 wt% unaccounted for components. Commercial cellulase and β-glucosidase (Novo Nordisk, Bagvard, Denmark) 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 Novozym to alleviate end-product inhibition by cellobiose.

Pretreatment

Pretreatment was performed on a reciprocating shaking water bath. Ten grams of substrate was added to a 1-L autoclave bottle with 200 g of ammonia or ammonia-H_2O_2 solution. The concentration of each component was expressed as wt% based on the total amount of ammonia-H_2O_2 solution. Then the bottle was placed for 1–3 h on a shaker operating at 20–80°C and 70–130 strokes/min. After pretreatment, the wet solid was

washed with deionized water and separated into two portions. One was oven dried at 105°C overnight to measure weight loss and was further subjected to composition analysis. The other was used in the enzymatic digestibility test.

Digestibility Test

Enzymatic digestibility of pretreated substrate was performed according to National Renewable Energy Laboratory (NREL) standard procedure no. 009 *(11)*. 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 contents of the flask were 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. Using the same method, untreated substrate was placed in the bath as a control. Samples were taken periodically and analyzed for glucose using high-performance liquid chromatography (HPLC). The glucose content after 72 h of hydrolysis was used to calculate enzymatic digestibility.

Analytical Methods

The solid biomass sample was analyzed for moisture, sugars, klason lignin, and ash by NREL standard procedures (no. 001–005) *(11)*. Sugars were measured by HPLC (Thermo Separation Products) using a Bio-Rad HPX-87C column (conditions; 0.6 mL/min, 85°C, water). Because this column does not resolve xylose, mannose, and galactose, the combined value of XMG is used in this article.

Results and Discussion

Newspaper is mostly derived from softwood and exhibits low enzymatic digestibility because of its high lignin content and dense structure. Additionally, chemicals such as fillers, ink, and other additives make it difficult to hydrolyze enzymatically. Our previous study *(10)* investigated factors that affect newspaper hydrolysis, such as ash content, substrate size, and ink. We found that ink had a significant effect on digestibility, whereas ash content and substrate size had a very small effect. The basic components of most water-based newsprint inks—constituting 1 to 2 wt% of newspaper—are carbon black pigment; acrylic resin binder; and various additives such as defoamers, plasticizers, and lubricants *(13)*. Most conventional de-inking of wastepaper is done with alkali and other chemicals. Alkali has two purposes: (1) to remove rosin sizing from the paper and (2) to saponify the ink vehicle and release the pigment in the ink *(14)*. The ink particles released from the fiber surface are removed from the slurry by either washing or flotation.

Ammonia is a proven alkaline reagent for pretreating lignocellulosic materials. Previous studies *(8,9)* showed that ammonia alone was not effective in pretreating lignocellulosic materials; thus, H_2O_2 was added to the

Table 1
Effect of H_2O_2 and Ammonia Concentration on the Composition of Solid Residue of Newspaper[a]

Pretreatment	Solids remaining (%)	Glucan (%)	XMG (%)	Klason lignin (%)	Ash (%)
Untreated	100.0	61.3	9.8	12.0	5.7
4 wt% Ammonia	95.4	54.7	8.7	11.5	5.1
4 wt% Ammonia + 2 wt% H_2O_2	94.7	55.5	8.1	11.5	4.9
8 wt% Ammonia	95.0	53.9	8.6	11.2	5.0
8 wt% Ammonia + 2 wt% H_2O_2	93.3	55.3	8.1	11.5	5.0

[a]All sugar contents are based on the original oven-dried untreated biomass and expressed as glucan, xylan, mannan, and galactan equivalents. Pretreatment condition: 3 h, 40°C, 130 strokes/min; mass ratio of solid to liquid = 1/20.

ammonia solution as an oxidant. H_2O_2 in alkaline solution promotes rapid oxidative depolymerization of lignin. In addition, H_2O_2 can aid de-inking operations *(14)*.

The appearance of the ammonia- H_2O_2-treated sample in the present experiment was very different from the ammonia-treated sample. The volume of ammonia- H_2O_2-treated sample was about 1.5 times bigger than that of the ammonia-treated sample. Furthermore, a significant amount of dark-colored ink components, which separated from cellulose fibers, was observed in the upper portion of the bottle. In the case of the ammonia-treated sample, only a very small amount of ink components was observed. Therefore, H_2O_2 is very effective in swelling fibers and removing ink components from fibers.

Table 1 shows the effect of H_2O_2 and ammonia concentration on the composition of the solid residue of newspaper. The percentage of solid remaining was 93.3–95.4%, which indicates that only a small portion of newspaper was solubilized by H_2O_2 and/or ammonia concentration. The percentage of glucan, XMG, klason lignin, and ash were almost the same, regardless of the different pretreatment conditions. However, the digestibility for each condition was significantly different (Fig. 1). The digestibilities of substrates treated with 4 and 8 wt% ammonia were the same as the untreated ones, which was almost 25% less than those treated with ammonia-H_2O_2. (Note that here, untreated sample means a substrate that is soaked in water for 3 h at room temperature.) Such an increase in digestibility, despite no significant change in composition after pretreatment, probably depends on ink removal and substrate swelling, not the removal of individual component. However, in hardwood, digestibility usually depends on removing hemicellulose and/or lignin *(8)*.

Another consideration in this pretreatment is the physical shock provided by the shaking bath. In a conventional de-inking process, ink compo-

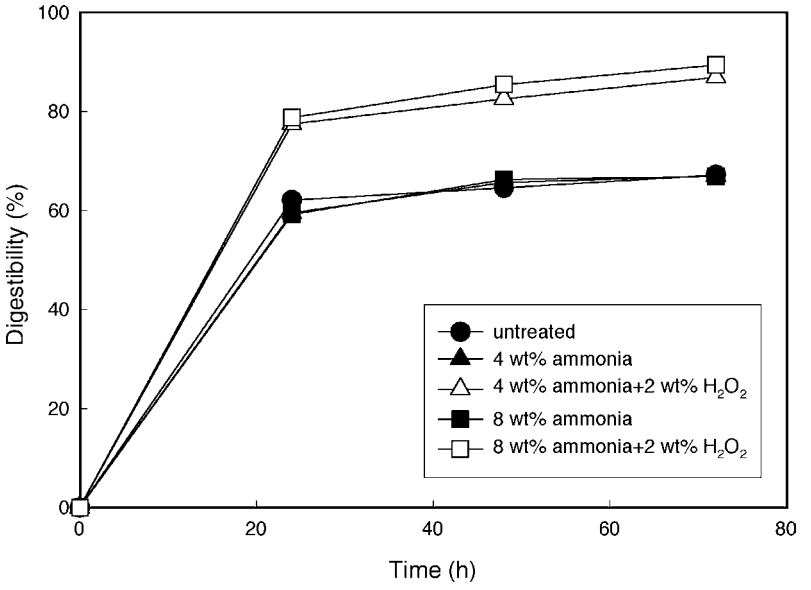

Fig. 1. Effect of H_2O_2 in ammonia solution on enzymatic digestibility (pretreatment conditions: 3h, 40°C, 130 strokes/min).

nents must be removed from fibers by either washing or flotation; otherwise ink is left in the space between fibers and interferes with enzymatic hydrolysis. Figure 2 shows the effect of shaking speed on digestibility. As the speed increased, digestibility increased. This can be explained from experimental observation; as the speed increased, a greater amount of ink components was observed in the upper portion of the bottle and the substrate was more swollen. This means that the physical shock provided by the shaking bath gives an effect equivalent to flotation and increases the contact between substrate and ammonia- either. Experiments above 130 strokes/min were not done because of equipment limitations.

Figure3 shows the effect of ammonia concentration on enzymatic digestibility at 2 wt% H_2O_2. The digestibilities of substrates treated with >4 wt% were almost the same, so 4 wt% ammonia concentration was enough for pretreatment.

H_2O_2 is almost 10 times more expensive than ammonia, so the H_2O_2 concentration was decreased to 0.5 and 1 wt%. As shown in Fig. 4, the digestibility decreased as the H_2O_2 concentration decreased; therefore, the H_2O_2 concentration increased to 3 wt%, but the digestibility was marginally increased. The optimum H_2O_2 concentration seems to be 2 wt%.

The effect of pretreatment temperature was investigated using 4 wt% ammonia and a mixture of 4 wt% ammonia and 2 wt% H_2O_2 (Fig. 5). When only ammonia was used, digestibility increased from 68% to 78% as temperature increased from 20 to 80°C. But when the substrate was treated with ammonia-H_2O_2, the digestibility only slightly increased as the tem-

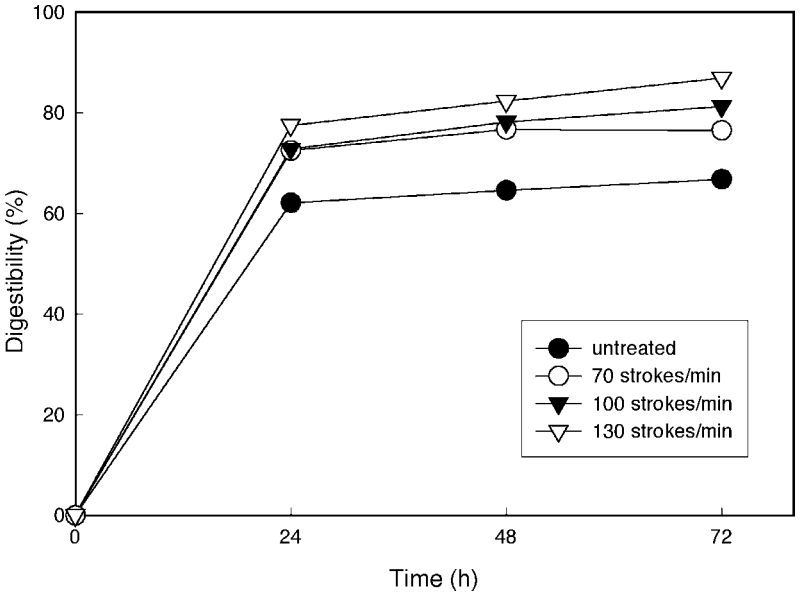

Fig. 2. Effect of shaking speed on enzymatic digestibility (pretreatment conditions: 3h, 40°C, 4 wt% ammonia + H_2O_2).

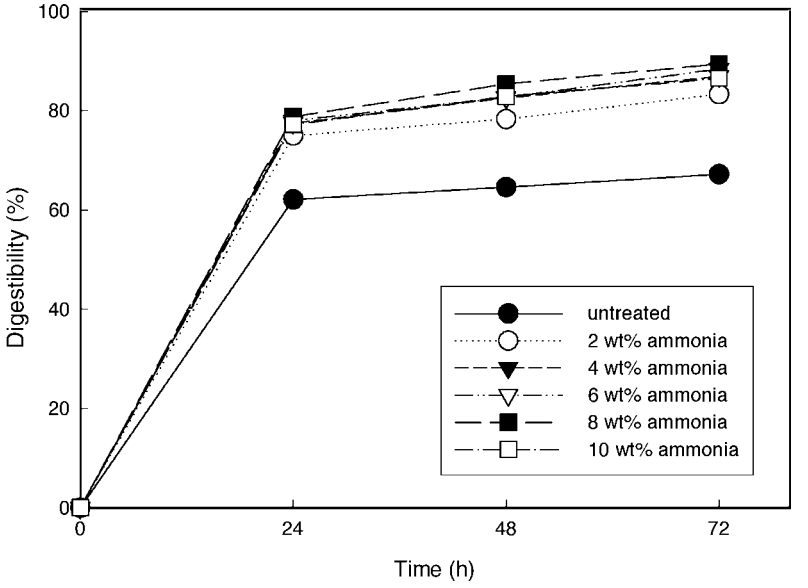

Fig. 3. Effect of ammonia concentration on enzymatic digestibility at 2 wt% H_2O_2 concentration (pretreatment conditions: 3h, 40°C, 130 strokes/min).

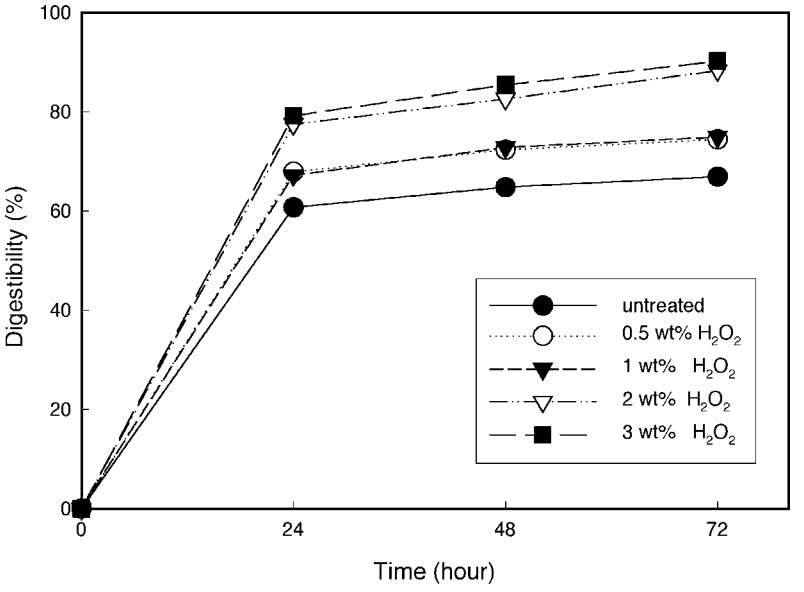

Fig. 4. Effect of H_2O_2 concentration in 4 wt% ammonia solution on enzymatic digestibility (pretreatment conditions: 3h, 40°C, 130 strokes/min).

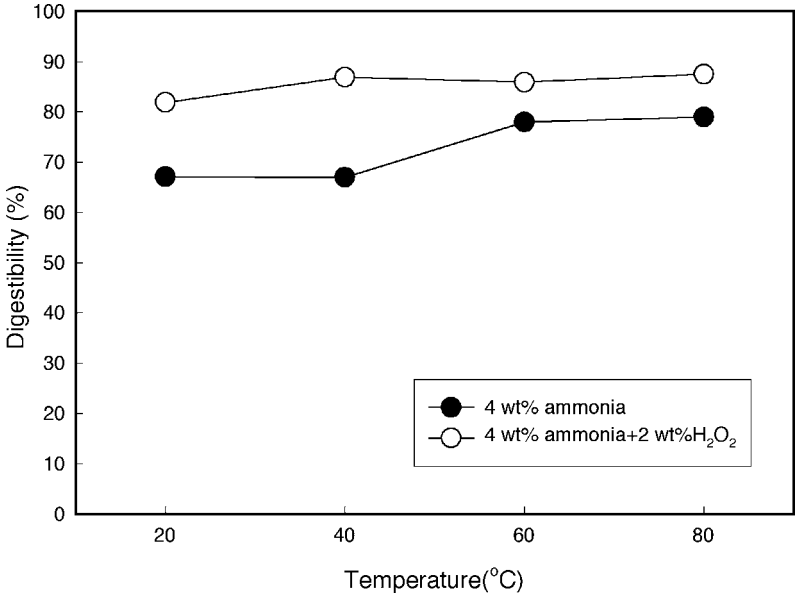

Fig. 5. Effect of pretreatment solution on enzymatic digestibility (pretreatment conditions: 3h, 40°C, 130 strokes/min).

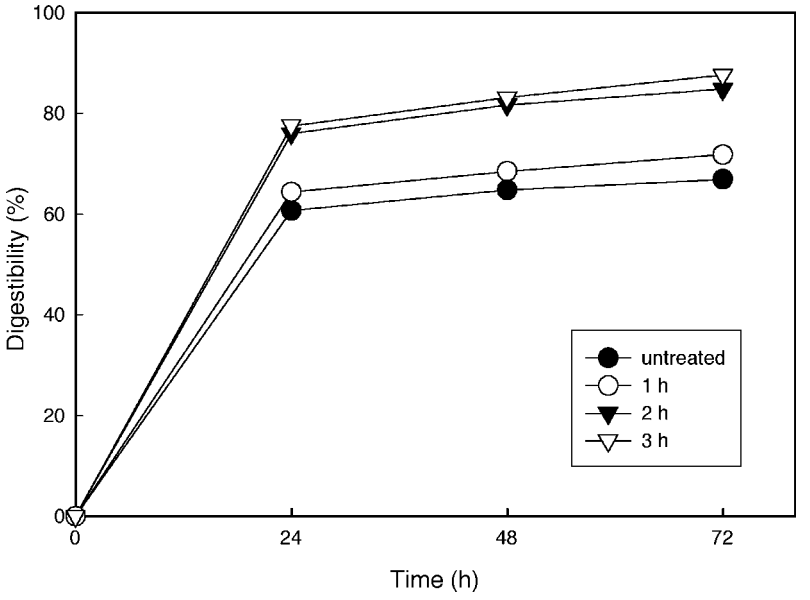

Fig. 6. Effect of pretreatment time on enzymatic digestibility (pretreatment conditions: 4 wt% ammonia + 2 wt% H_2O_2, 40°C, 130 strokes/min).

perature increased from 20 to 40°C. Further increases in temperature did not affect digestibility.

Figure 6 shows the effect of pretreatment time on digestibility. As time increased, digestibility increased. As can be seen at least 2 h were required to pretreat newspaper.

Acknowledgments

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