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Pretreatment Characteristics of Waste Oak Wood by Ammonia Percolation

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Abstract A log of waste oak wood collected from a Korean mushroom farm has been tested for ammonia percolation pretreatment. The waste log has different physical characteristics from that of virgin oak wood. The density of the waste wood was 30% lower than that of virgin oak wood. However, there is little difference in the chemical compositions between the woods. Due to the difference in physical characteristics, the optimal pretreatment conditions were also quite different. While for waste oak the optimum temperature was determined to be 130°C, for virgin oak wood the optimum pretreatment was only achieved at 170°C. Presoaking for 12 h with ammonia solution before pretreatment was helpful to increase the delignification efficiency.

Keywords Waste oak wood · Pretreatment · Ammonia · Enzymatic hydrolysis · Lignin

Introduction

The oil crisis and global warming are the major threats to the sustainable developments of our society. Biofuels are emerging as a promising solution to overcome the barriers. However, now the biofuels are mainly produced from edible materials so that the shortage of the raw materials should be encountered in near future as the implementation of biofuels is getting more active.

Now the bioethanol is a dominant biofuel and the demand for the bioethanol continues to increase steadily for several decades [1]. To overcome the shortage of raw material, the technology developments for bioethanol production from lignocellulosic biomass have been carried out intensively. Since the collection cost of the wood biomass is too high in Korea,

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it will not be economically feasible even though a very highly efficient technology is developed [2]. An alternative is to focus on lignocellulosic biomass which does not require too much collection cost. One of the promising candidates is the mushroom bed which can be easily collected from the mushroom farms. Currently, about 40,000 tons of oak-wood logs are used annually for growing the mushrooms, and about same amount of the waste log should be generated annually. The waste log woods are being powdered and used as soil conditioner [2].

Since the direct conversion efficiency of the lignocellulosic biomass into the fermentable sugars is not high, various pretreatment technologies have been investigated to produce sugars more economically [3]. As a pretreatment reagent, ammonia has a number of desirable characteristics: swelling of cellulosic materials, highly selective delignification reaction, low interaction with carbohydrates, and high volatility. One of the known reactions of aqueous ammonia with lignin is the cleavage of C–O–C bonding in lignin as well as ether and ester bonding in lignin carbohydrate complex. There are many advantages of removing lignin at the early phase of the conversion process before it is subjected to the biological processing. Lignin is believed to be one of the major hindering factors in the enzymatic reaction [4–6].

Previous investigations have been conducted on various pretreatment processes using a flow-through (percolation) reactor system. Among them is the ammonia recycled percolation (ARP) process that has been studied for pretreatment of various lignocellulosic biomass feedstocks including hardwood [7], corn stover [8, 9], and pulp mill sludges [10]. The recent research includes development of a novel pretreatment/delignification of biomass by soaking in aqueous ammonia for enzymatic saccharification [11]. The purpose of this investigation is to assess the effectiveness of the ARP treatment as a pretreatment process specifically for waste oak wood.

Materials and Methods

Materials

Virgin oak wood and waste oak wood were supplied from a Korean local mushroom farm. They were ground and screened. The fraction collected between 2 mm~20mesh was used in all experiments. The initial composition of virgin oak wood was determined to be: 43.3% glucan, 24.6% xylan, 2.1% arabinan, 1.0% mannan, 1.5% galactan, 19.2% Klason lignin, 2.3% acid-soluble lignin, 3.3% ash, 2.6% acetyl group, and 3.4% protein. The initial composition of waste oak wood was determined to be: 43.7% glucan, 25.5% xylan, and 17.5% Klason lignin that has similar component with virgin oak wood. The density of the waste wood was 30% lower than that of virgin oak wood. The cellulase enzyme, Celluclast 1.5L, was purchased from Novo. The average activity of the enzymes was 89.6 FPU/ml. The β-glucosidase, Novozyme 188, was also purchased from Novo.

Experimental Setup and Operation of ARP

The ARP apparatus was used in the conduction of pretreatment of virgin oak wood and waste oak wood [12]. The system consists of a stock solution reservoir, pump, temperature-programmable oven, SS-316 column reactor (9/10 in. ID $\times$ 10 in. L, internal volume of 101.9 cm^3), and liquid holding tank. The reactor was operated in a flow-through mode, the liquid flowing through the reactor column packed with biomass. Aqueous ammonia concentration was 15 wt%, and flow rate was 5.0 ml/min. The reactor system was pressurized by nitrogen at 325 psig to prevent vaporization. In a typical ARP experiment, 18 g of biomass sample were packed into the reactor. The reactor temperature was controlled in a forced-air convection oven. About 15 min of preheating time was necessary to reach the desired temperature. The reaction time was counted after the desired temperature was attained. The wet solids discharged from the reactor were separated into two portions. One was oven-dried at 105°C overnight for measurement of weight loss and further subjected to composition analysis. The other was used in the enzymatic digestibility test.

Digestibility Test

The enzymatic digestibility of oak wood biomass was determined according to the National Renewable Energy Laboratory (NREL) Chemical Analysis and Testing Standard Procedure no. 009 [13]. Enzymatic digestibility of pretreatment feedstocks was performed at 50°C and pH 4.8 (0.05 M sodium citrate buffer) on a shake bath agitated at 150 rpm. The digestibility is defined as percent of theoretical glucose released after 72 h of incubation with cellulase enzyme. The cellulase enzyme loading was at 10–60 FPU/g glucan. Samples were taken periodically and analyzed for glucose and cellobiose. Total glucose content after 72 h of hydrolysis was taken for calculation of the enzymatic digestibility. Untreated feedstocks and α-cellulose were subjected to the same digestibility test as a control and as a reference.

Analytical Methods

The samples of virgin oak wood and waste oak wood were analyzed for glucan, xylan, Klason lignin, and acid-soluble lignin following the procedures of NREL Chemical Analysis and Testing Standard Procedures no. 001~004 [13]. Sugars were determined by high-performance liquid chromatography using a Bio-Rad Aminex HPX-87P column for carbohydrate analysis of solids. For enzymatic digestibility, the glucose content was measured by HPX-87H column.

Results and Discussion

The density of the waste wood was 30% lower than that of virgin oak wood. The effects of ARP and pre-soaking were investigated on enzymatic digestibility for virgin oak wood and waste oak wood.

Ammonia Recycled Percolation

The feedstocks (oak wood and waste oak wood) were first put through a series of ARP processes using 15 wt% aqueous ammonia solution. Based on the previous investigations and the results of preliminary experiments of this work, 15 wt% of ammonia concentration was chosen for the ARP operation [14]. The temperature was varied over the range of 100–210°C. The weight remaining after the treatment varied from 77% to 43% for oak wood and from 76% to 38% for waste oak wood. The treated solid samples were then analyzed for sugars and Klason lignin. The results are summarized in Table 1. The glucan fraction after the ARP treatment decreased slightly as temperature was raised from 130°C to 170°C. The glucan retention at these temperatures was near 80% for oak wood and 75% for waste oak wood based on initial solid content. The xylan content and Klason lignin decreased

Table 1 Composition of oak wood (O) and waste oak wood (W) after ARP Treatment.

Reaction (°C)	% Weight remaining	Composition (%)		
		Glucan	Xylan	Klason lignin
Untreated	O 100	43.3	24.6	19.2
	W 100	43.7	25.5	17.5
ARP 100	O 77.7	42.4	20.5	15.9
	W 75.9	42.8	17.1	13.3
ARP 130	O 70.1	39.9	17.8	10.3
	W 68.3	39.4	14.5	6.6
ARP 150	O 62.5	38.1	12.2	8.9
	W 63.3	37.5	11.1	5.1
ARP 170	O 58.5	38.6	11.9	3.6
	W 55.1	29.2	4.8	2.1
ARP 190	O 50.3	32.2	6.4	2.4
	W 45.5	28.9	4.7	2.0
ARP 210	O 42.9	32.2	2.2	2.2
	W 37.7	27.7	2.0	1.5

Contents of all sugar and Klason lignin are based on untreated oven-dried biomass. Reaction condition: flow rate of ammonia (15 wt%)=5.0 ml/min

highly as temperature was raised up to 210°C. About 30–80% of the lignin and xylan were removed form feedstocks by the ARP process at various temperature conditions. Because of high retention of glucan and relatively high delignification, 170°C is deemed as the optimum operating temperature of ARP for virgin oak wood. The compositional changes in solid and liquid samples of virgin oak wood during the ARP at 170°C are summarized in Table 2. The most significant change of the composition is in the lignin. The ARP process removed 66–85% of the total lignin of virgin oak wood feedstock. The delignification reaction is rapid to the extent that 66% of lignin is removed within 10 min of treatment. About half of xylan (main component of hemicellulose) is also solubilized. The glucan content, however, remains relatively intact. The overall reduction of solid mass (expressed as solid remaining, SR) by the ARP is slightly less than half (53.3–64.4%). As indicated in Table 2 by the total of glucan and xylan (amount of carbohydrates in the solid plus that in

Table 2 Effect of reaction time on the compositions of oak wood after ARP treatment.

Time (min)	Solid (%)					Liquid (%)		Total (%)		Delignification (%)
	SR	Klason lignin	ASL	Glucan	Xylan	Glucan	Xylan	Glucan	Xylan	
Untreated	100	19.2	2.30	43.3	24.6	0.0	0.0	43.3	24.6	0.00
10	64.4	7.9	1.1	39.9	14.7	0.8	8.6	40.7	23.6	65.9
20	58.5	3.6	1.0	38.6	11.9	1.2	11.2	39.8	23.1	80.1
40	56.1	3.3	0.9	38.1	10.1	1.5	11.9	39.6	22.0	82.3
60	54.4	2.9	0.8	37.8	9.3	1.6	12.0	39.4	21.3	83.2
90	53.3	2.9	0.7	36.5	9.2	1.6	12.2	38.1	21.4	84.7

All sugar and lignin content based on the oven-dry untreated biomass. Pretreatment conditions: 15wt% of ammonia, 170°C, 5 ml/min of flow rate, 325 psig

SR Solid remaining after reaction, *ASL* acid-soluble lignin during analysis liquid), the accountability of sugars is above 91% for glucan and 89% for xylan with up to 40 min of treatment. The carbohydrates in the biomass are thus well preserved in the ARP process, a very important benefit as a pretreatment process.

For the waste oak wood feedstock, 130°C of reaction temperature for ARP was optimum considering high retention of glucan and relatively high delignification. This optimal temperature for ARP treatment decreased for the waste oak wood feedstock presumably due to the biodegradation of this material that occurs during the mushroom cultivation. The compositional changes in solid and liquid samples of waste wood during the ARP at 130°C are summarized in Table 3. The delignification reaction is rapid to the extent that 79% of lignin is removed within 20 min of treatment.

Pre-soaking Effect

The pre-soaking effect on enzyme digestibility was investigated for waste oak wood. This work was done to achieve the higher delignification than typical ARP treatment. The waste oak wood was packed into the reactor and soaked with aqueous ammonia (15 wt%). The pre-soaking time was 0 to 48 h at room temperature. After the certain time, the pre-soaked biomass was submitted to an ARP treatment at the conditions previously optimized. The results are summarized in Table 4. The optimum pre-soaking time was selected to 24 h due to the high retention of glucan and relatively high delignification.

Enzymatic Digestibility

The data presented to this point dealt with the changes in the composition of biomass brought about by various pretreatments. The composition of biomass is an important factor in the process economics. It can also serve as an indirect index for the digestibility, the lignin content, for example. However, the true yardstick for a pretreatment must come from the direct measurement of digestibility. All of the solid samples obtained from the ARP treatment were therefore subjected to the standard enzymatic digestibility test. The digestibility data after ARP treatment for both feedstocks with pre-soaking are presented in Figs. 1 and 2. The digestibility was measured with loading of cellulose, 60 FPU/g glucan. Regardless of the treatment conditions, the digestibility of the pretreated biomass

Table 3 Effect of reaction time on the compositions of waste oak wood after ARP treatment.

Time (min)	Solid (%)					Liquid (%)		Total (%)		Delignification (%)
	SR	Klason lignin	ASL	Glucan	Xylan	Glucan	Xylan	Glucan	Xylan	
Untreated	100	17.5	2.3	43.7	25.5	0.0	0.0	43.7	25.5	0.00
10	70.2	10.1	1.1	41.8	17.5	0.5	3.6	42.3	21.1	60.4
20	68.3	6.6	1.1	39.4	14.5	0.9	9.7	40.3	23.2	78.7
40	63. 8	4.5	1.1	39.1	10.0	1.2	10.1	40.3	20.1	80.1
60	61.5	3.9	1.0	38.9	9.5	1.5	12.5	40.4	22.0	80.4
90	60.2	3.3	1.0	38.8	9.0	1.5	12.9	40.3	21.9	81.9

All sugar and lignin content based on the oven-dry untreated biomass. Pretreatment conditions: 15 wt% of ammonia, 130°C, 5 ml/min of flow rate, 300 psig

SR Solid remaining after reaction, *ASL* acid-soluble lignin during analysis

Table 4 Composition of pre-soaked waste oak wood after ARP treatment.

Pre-soaking Time (h)	% Weight remaining	Solid composition (%)		
		Glucan	Xylan	Klason lignin
0	68.3	39.4	17.5	6.6
6	68.0	38.9	16.2	5.8
12	64.8	37.6	15.4	3.1
24	63.4	38.1	15.5	3.0
48	64.6	37.7	14.9	2.9

Contents of all sugar and Klason lignin are based on untreated oven-dried biomass. Reaction temperature, 130°C; flow rate of ammonia (15 wt%), 5.0 ml/min

has significantly improved from that of the control (untreated biomass). After ARP treatment, the digestibility of virgin oak wood and waste wood is improved by about 64% (from 22.5% to 87.4% for the former feedstock and from 23.0% to 86.1% for the latter feedstock). Apparently, the lignin content is a prime factor controlling the digestibility for these feedstocks. For virgin oak wood, the enzyme digestibility was increased up to 87.4% at 170°C of ARP as shown in Fig. 1. Further increase in temperature did not have an effect on enzymatic digestibility. For waste oak wood, the enzyme digestibility was 86.1% at 130°C of ARP and had similar values of these at higher than 130°C in Fig. 2. These results provide further support that the optimum temperature of ARP for waste oak wood is lower than that for virgin oak wood.

The enzymatic digestibility data on the ARP-treated various wood feedstocks (virgin oak wood, waste oak wood, and pre-soaked waste oak wood) are shown in Fig. 3. The digestibilities were measured with three different loadings of cellulase: 60 FPU/g glucan, 30 FPU/g glucan, and 10 FPU/g glucan. With 60 FPU/g glucan enzyme loading, the digestibilities were all above 85%, the highest being near quantitative, 90.3%, which is observed with pre-soaked waste oak wood. With 10 FPU/g glucan enzyme loading, the digestibilities were about 85%. Whether this level is acceptable from a process viewpoint is debatable.

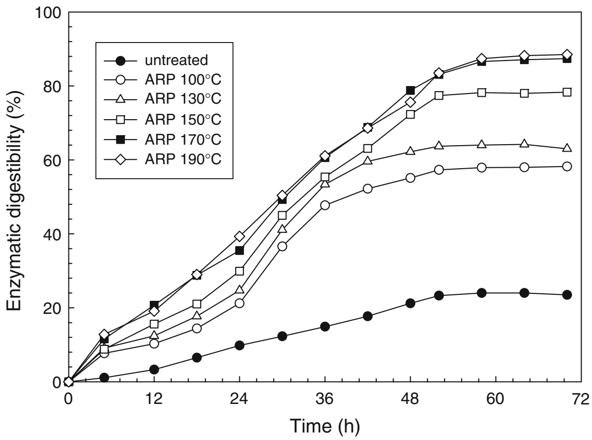

Fig. 1 Enzymatic digestibility of oak wood after ARP at various temperatures (enzyme loading, 60 FPU)

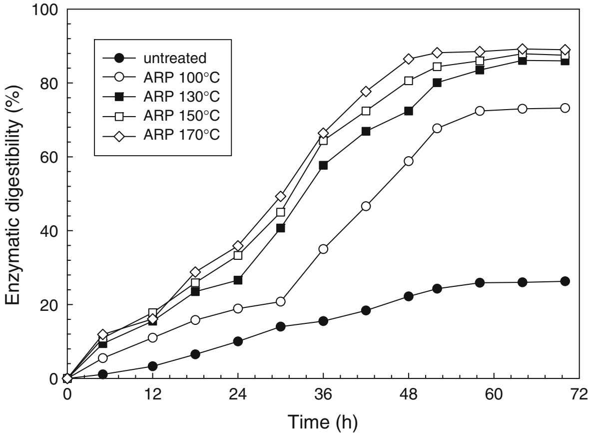

Fig. 2 Enzymatic digestibility of waste oak wood after ARP at various temperatures (enzyme loading, 60 FPU)

Conclusions

Pretreatment of oak wood and waste oak wood, especially by aqueous ammonia, is highly effective in enhancing the enzymatic digestibility and reducing lignin content. The ARP treatment removes 60–85% of the total lignin but retains more than 90% of the glucan content. Pre-soaking and ARP treatment resulted in higher extent of delignifcation as well as enzymatic digestibility. The enzymatic digestibilities of ARP-treated waste oak wood yielded 86.1% with 60 FPU/g glucan and 82.3% with 10 FPU/g glucan. The ARP process could achieve higher enzyme digestibility as well as low enzyme loading.

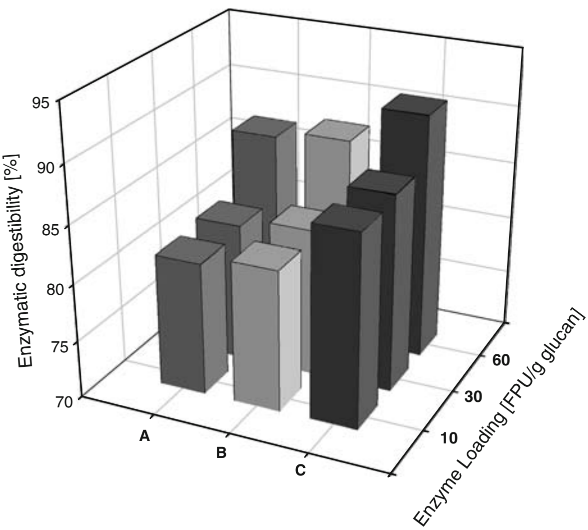

Fig. 3 Enzymatic digestibility of oak wood feedstocks (*A*, *B*, *C*) after ARP at various enzyme loadings. *A* Oak wood after ARP; *B* waste oak wood after ARP; *C* pre-soaked waste oak wood after ARP

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