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Original Article

Nitrogen Adsorption Analysis of Wood Saccharification Residues[1]

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ABSTRACT

The objective of this study was to examine changes in the porosity and internal structure of wood as it goes through the process of saccharification (extraction of fermentable sugars). This study also examined the use of different drying methods to prepare samples for characterization of internal pores, with particular emphasis on the partially disrupted cell wall. Aspen wood flour samples after dilute acid pretreatment followed by enzymatic hydrolysis were examined for nitrogen adsorption. The resulting isotherms were analyzed for surface area, pore size distribution, and total pore volume. Results showed that freeze drying (with sample pre-freezing) maintains the cell wall structure, allowing for examination of saccharification effects. Acid pretreatment (hemicellulose removal) doubled the surface area and tripled the total volume of pores, which were mostly 10-20 nm wide. Subsequent enzymatic hydrolysis (cellulose removal) caused a 5-fold increase in the surface area and a ~ 11-fold increase in the total volume of pores, which ranged from 5 to 100 nm in width. These results indicate that nitrogen adsorption analysis is a feasible technique to examine the internal pore structure of lignocellulosic residues after saccharification. The information on the pore structure will be useful when considering value-adding options for utilizing the solid waste for biofuel production.

Keywords : porosity, internal structure, dilute acid pretreatment, enzymatic hydrolysis, nitrogen adsorption

1. INTRODUCTION

Recently, natural fibers are being increasingly favored as a reinforcing material for composites (Marcovich *et al*., 2006) because of their recyclability, relatively high strength, and low density compared to conventional inorganic materials. Renewable fibers from plant sources have been gaining increasing attention as an alternative material to synthetic fibers in various manufacturing sectors including automobile, packaging, and furniture industries (Alemdar and Sain, 2008). These cellulosic fibers have advantages such as biodegradability, low cost, high specific mechanical properties, low toxic by-products, and fewer environmental concerns compared to inorganic reinforcements such as silica, nanoclay, mica, carbon nanofibers, and glass fibers.

In the current era of bioeconomy, plant bio-

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mass is viewed as a feedstock for the sugar platform, from which a range of bio-based chemicals including alcohol-based biofuels can be produced. The process of liberating fermentable sugars from biomass is called saccharification. Two established processes are commonly used for the purpose: acid hydrolysis and enzymatic hydrolysis (Ishizawa *et al*., 2007). Both processes break down cellulose and hemicelluloses, two out of the three primary components of wood and other lignocellulosic materials. As a consequence, there is an emerging need to better understand lignin-rich residual material for current applications if not new industrial applications. These materials are expected to have an altered internal structure due to the removal of constituting components from their cell wall. In this respect, examining cell wall internal pores by analyzing the surface area, pore size, and pore volume has become an important characterization need (Ishizawa *et al*., 2007).

It is essential to determine the appropriate drying procedure while preparing samples for the characterization of porosity and internal structure, representative of the material's state of interest. A limited number of publications (Ass *et al*., 2006; Barthel and Heinze, 2006; Frisoni *et al*., 2001) have described the use of vacuum drying for the lignocellulose sample, while only few studies (e.g. Chandra *et al*., 2008; Sluiter *et al*., 2010) have reported alternative methods such as freeze drying. Moisture removal from semi- or non-rigid porous materials, such as biomass, often induces partial or complete collapse of the internal structure (Ishizawa *et al*., 2007). Therefore, determining the best available/ highly recommended techniques to remove water from pretreated and saccharified biomass samples is the most important issue in the characterization of these materials.

The BET (Brunauer-Emmett-Teller) gas adsorption method can be used to examine the effects of drying and those of saccharification on the internal structure of biomass. This method involves a theory (called BET theory) that explains the multilayer physical adsorption of gas molecules on a solid surface (Brunauer *et al*., 1938); it provides a means to deduce information of monolayer coverage, thus, surface areas of the sample. The gas adsorption experiment is usually conducted using nitrogen as the adsorbate. The experiment has become the most widely used standard procedure for measuring the surface area of finely-divided and/or porous materials (Sing *et al*., 1985). Data from the same experiment also allows the calculation of pore size distribution, for example, through the widely used (Leofanti *et al*., 1998) Barrett-Joyner-Halenda (BJH) method (Barrett *et al*., 1951). The BJH method examines the amount of adsorbed gas in the material, from high relative pressure (condensation pressure) to a low relative pressure. It provides a procedure to calculate the emptying of pores (radius and volume changes) by considering the vaporization of liquid (condensed) gas and reduction of pre-adsorbed gaseous film layer (Murray *et al*., 1999). The information on pore size and distribution is expected to provide supportive

evidence for changes in the porosity and internal structure of biomass after saccharification.

The objective of this study was to examine the effects of a two-step saccharification treatment on the internal structure of woody biomass. Specifically, the study aimed at characterizing the internal pores and the surface area of the solid residue after dilute acid pretreatment, followed by the enzymatic hydrolysis process. This study also assessed different drying methods to prepare samples for BET nitrogen adsorption analysis.

2. MATERIALS and METHODS

2.1. Materials

The woody biomass source for this study is quaking Aspen (*Populus tremuloides*), which is a major pulpwood source in the upper Midwest region of the United States and a close relative of hybrid poplar representative of hardwood energy crops. The saccharified sample in this study was provided by the laboratory of Professor Schilling at the University of Minnesota. It was obtained by first pretreating wood flour of Aspen with 0.5% (dilute) sulfuric acid at 170℃ for 2 h, followed by enzymatic treatment using *Trichoderma reesei cellulases* in Celluclast 1.5 L and β-glucosidase from Aspergillus niger in Novozyme 188 at 50℃ for 9 days at 68 rpm. This two-step treatment, similar to that reported in a published study (Schilling *et al*., 2009), removed hemicelluloses and also converted the most cellulose into glucose. The solid residue, after being air-dried to 10-11 wt.% moisture content (dry basis), was used as the starting material in this study.

2.2. Sample preparation

Prior to BET experiment, the biomass samples were dried either in a forced air oven, vacuum oven (-127 mmHg), or freeze dryer at 105℃, 60℃ and -80℃, respectively. Each type of drying was conducted for different durations up to 3-4 days until the sample reached a constant mass and moisture content (MC) was recorded every day. Prior to freeze drying, the sample was either directly loaded into the dryer without pre-freezing (freeze drying method #1), pre-frozen at a moderate speed in a freezer (-72℃) for a single day (freeze drying method #2), or rapidly pre-frozen in liquid nitrogen for 1-2 min (freeze drying method #3). In all three methods, freeze drying was conducted at a vacuum pressure setting of 0.03 mmHg.

2.3. Test methods

The nitrogen adsorption experiments (also known as BET measurement) were conducted in a surface area and porosity analyzer (Tristar II 3020 V1.03, Micromeritics, Norcross, GA, USA). The analyzer was used to first outgas the sample (around 5 g oven-dry mass equivalent) at room temperature for 16 h under vacuum, and then to record nitrogen adsorption and desorption isotherms at -196℃. From the isotherms, the surface area of pores, pore volume and pore size distribution curve were de-

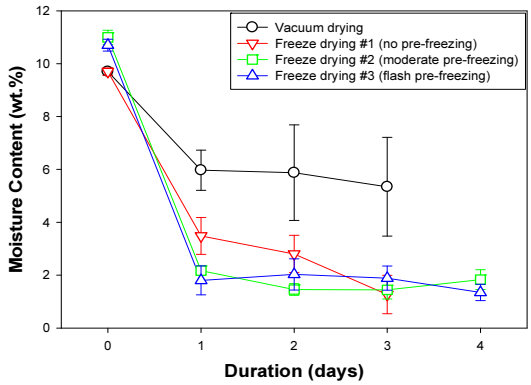


Fig. 1. Moisture removal of biomass sample using different drying methods.

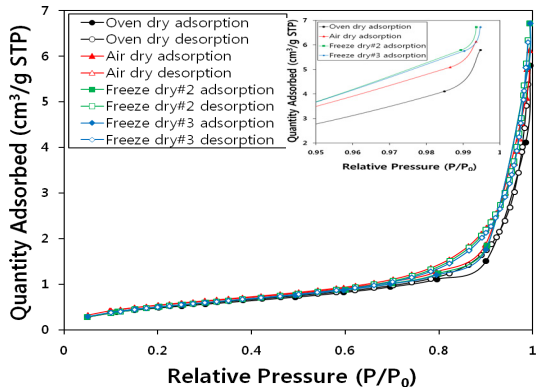


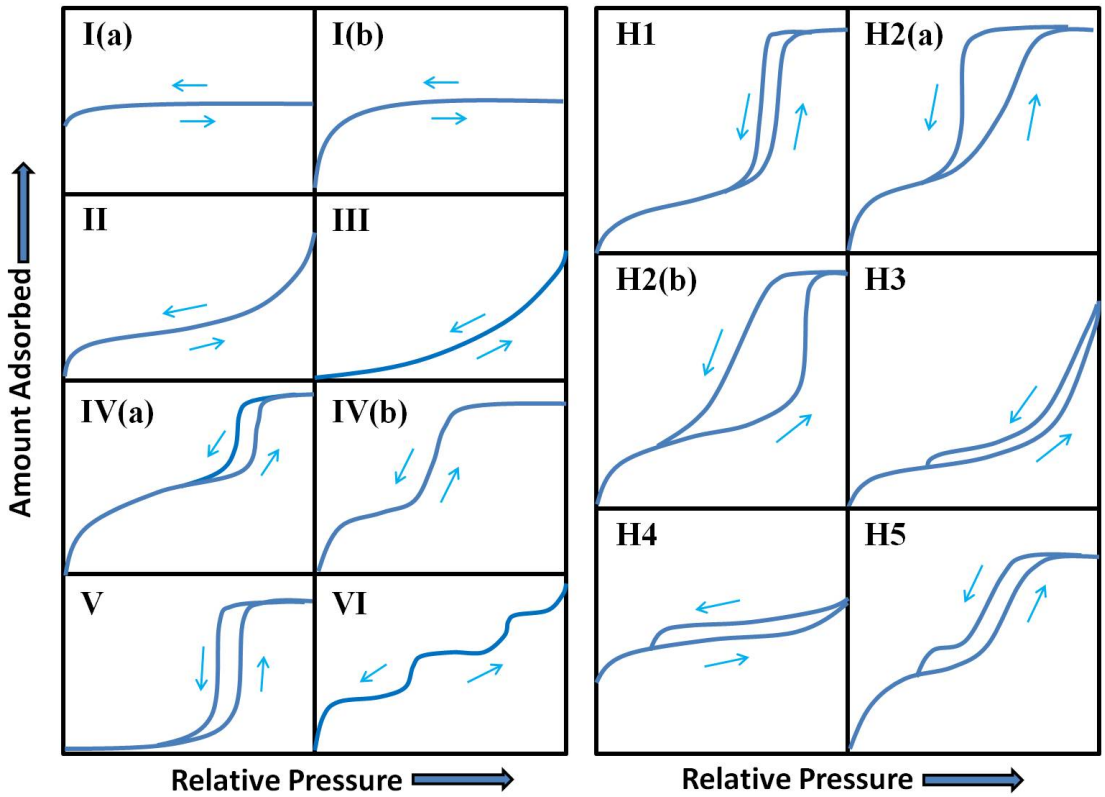
Fig. 2. Nitrogen adsorption-desorption isotherms of acid-pretreated biomass sample dried using different drying methods. The insert shows the amount of nitrogen adsorbed at high relative pressures (P/P_0 0.95-1.0).

termined using the BJH method. The surface area and pore volume data were obtained from one replicate of each sample due to limited test materials.

3. RESULTS and DISCUSSION

The effect of different drying methods on water removal is illustrated in **Fig. 1**, using untreated wood (initial weight ~0.40 g) as an example. After 3 days of vacuum drying, the moisture in the sample was still approximately 6.0% of its oven-dry weight; therefore, it is not quite an effective method (under the conditions tested) to remove water from the wood sample. For freeze drying method #1 (i.e., without pre-freezing), the sample was almost completely dried (< 2 wt.% MC) after 3 days. Samples obtained from freeze drying methods #2 and #3 (both with pre-freezing) exhibited quicker drying, achieving a plateau of MC of 2.0 wt.% within a day. There was no difference between moderate and flash pre-freezing (method #2 vs. method #3) in terms of the final MC of the freeze-dried samples. Based on the results, vacuum drying and freeze drying method #1 were not used in subsequent analyses.

Isotherms of nitrogen adsorption and desorption for differently dried samples are shown in **Fig. 2**, using the acid-pretreated sample as an example. The air-dried sample, which was the starting material in the oven- and freeze drying studies, was also included in the analysis to assess the effects of drying. The adsorption branch of the isotherms (**Fig. 2**) at first glance resembles a type II curve (non-porous or macroporous materials) as per the IUPAC classification (**Fig. 3**); however, contradictory to what is specified for type II, the sorption process is not reversible in our samples. The isotherms of our samples exhibit an adsorption-desorption hysteresis, suggesting capillary condensation typically observed in mesopores (Dąbrowski, 2001). Mesopores are pores measuring 2-50 nm



(a) adsorption isotherms (b) hysteresis loops

Fig. 3. The IUPAC classification for adsorption isotherms (a), and six classifications of hysteresis loops (b) (Thommes *et al*. 2015)(© IUPAC, De Gruyter, 2015).

in width, whose sizes lie between those of the micropores (< 2 nm) and macropores (> 50 nm) (Rouquerol *et al*., 1994). The isotherms have a sharp step increment at a relative pressure (P/P_0) of ~0.9. Similar isotherms were also reported for air-dried bleached kraft pulp fiber (Kimura *et al*., 2014). The volume of nitrogen adsorbed at ~0.96 was estimated (assumed cylindrical pores) to correspond to a pore diameter of 50 nm (Kimura *et al*., 2014). Comparatively, both freeze-dried samples exhibit the highest adsorption at high relative pressures, while the oven-dried sample shows the lowest adsorbed volume (see insert of **Fig. 2**), suggesting differences (albeit small) in mesopore volume, which will be quantified in a later paragraph.

The hysteresis in the present study shows a narrow loop, resembling a type H3 loop under the IUPAC classification (**Fig. 3**). This kind of loop is associated with slit-shaped pores (Sing *et al*., 1985). The presence of slit-like pores in the wall of (either developing or delignified) wood cells has been verified via transmission

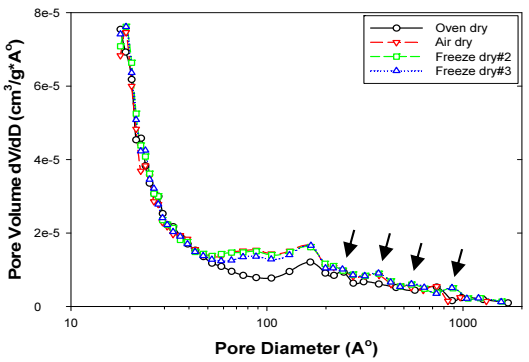


Fig. 4. Pore size distribution plot of acid-pretreated biomass sample dried using different methods. The distribution was calculated according to the BJH equation from the desorption branch of the isotherm (Arrows indicate small peak-like shapes).

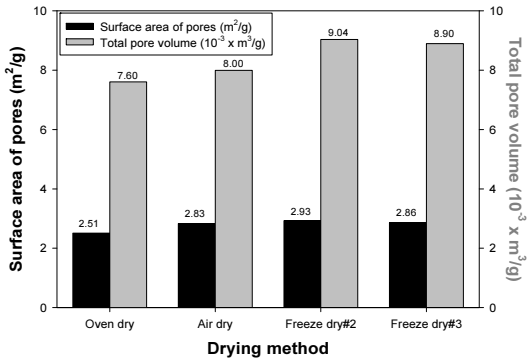


Fig. 5. The BJH desorption cumulative surface area and total pore volume of acid-pretreated biomass sample from different drying methods.
Note: The total pore volume was determined from N_2 desorption isotherm curve at P/P_0 = 0.99 (single point).

electron microscopy (Hefrén *et al*., 1999); the slit shape is due to the (exposed) voids between uni-directionally aligned microfibrils in the S2 layer of the cell wall.

Fig. 4 shows the BJH pore size distribution plots of an acid-pretreated wood sample calculated from the isotherms in **Fig. 2**. The most frequent diameter size is around 100-200 Å (10-20 nm), followed by small populations (peak-like shapes) of pores with larger sizes up to 1000 Å (100 nm). This result suggests inhomogeneous pore sizes in the samples. The disappearance of pores with sizes between 50 and 100 Å (5-10 nm) is noteworthy as the sample was oven-dried. This observation suggests that smaller pores largely collapse during oven drying. Pores that are smaller than 5 nm are not discernible in the size distribution curves of differently dried samples. The BJH method, like other methods that rely on capillary condensation data, has been considered inaccurate for small mesopores that are below 5 nm (Murray *et al*., 1999).

Fig. 5 depicts the surface area and total pore volume based on the BJH analysis of the acid-pretreated samples dried using different methods. Oven-dried samples show the lowest values in terms of both surface area (2.51 m^2/g) and total pore volume (0.0076 m^3/g). These results are in agreement with its adsorption isotherm (insert of **Fig. 2**) and pore size distribution curve (**Fig. 4**) discussed earlier, confirming the occurrence of pore collapse during oven drying. The two freeze-dried samples show surface areas of 2.93 m^2/g and 2.86 m^2/g, and these values are close to that of the air-dried sample (2.83 m^2/g), the starting material for freeze (and also oven) drying. While these outcomes suggest that freeze drying preserved the internal structure of the air-dried sample, the total pore volume of freeze-dried

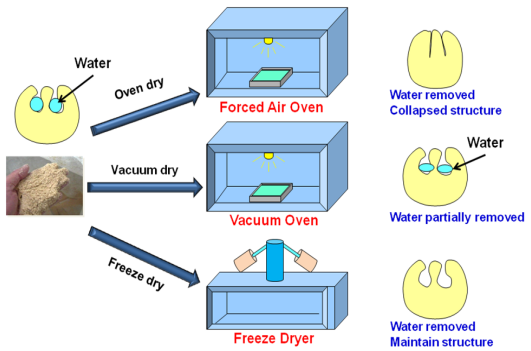


Fig. 6. Internal structures of biomass sample using different drying methods.

samples (~0.0090 m^3/g) turns out to be larger than that (0.0080 m^3/g) of the starting material (air-dried sample). One possible explanation is the partial collapse of pores in the air-dried sample when its 10-11 wt.% of residual moisture had evaporated during sample handling or outgassing (under vacuum; ambient temperature) prior to nitrogen adsorption studies. Indeed, larger pores measuring 80-100 nm in width are missing from its pore size distribution curve (**Fig. 4**), which otherwise looks very similar to those of freeze-dried samples. Nonetheless, the benefit of freeze drying is evident in preservation of the biomass internal pore structure. This benefit would be even more noticeable if freeze drying is conducted on green biomass, i.e. above its cell wall saturation point (MC ~30 wt.% for wood) - the resulting sample would have a higher porosity (fewer or negligible pore collapse) compared to the case of air drying.

Observations from the drying studies discussed so far are summarized in **Fig. 6**. For oven drying, it can be assumed that water was completely removed, resulting in collapse of the cell wall structure through surface tension forces of the liquid water as it evaporated (Rahman, 2001). The measured surface area and porosity therefore would not be representative of the "wet" state (before drying). In the case of vacuum drying, liquid water was removed partially (referring to **Fig. 1**), and the cell wall structure, although not examined, also possibly collapsed albeit to a smaller extent. During freeze drying, water was removed from the solid phase (ice) to the gas phase to avoid surface tension forces; therefore, the cell wall structure could possibly be maintained. Therefore, among the drying techniques examined, freeze drying (with pre-freezing) is recommended as a sample preparation method for surface area and porosity analyses to discern the effects of pretreatment and saccharification on the internal structure of biomass. Between the two types of pre-freezing examined for freeze drying, there is no obvious difference in either the surface area or the total pore volume of the resulting dry samples. For a quicker process, liquid nitrogen pre-freezing (freeze drying method #3) is preferred and it was used in subsequent studies.

Fig. 7 shows the adsorption-desorption isotherms of untreated, acid-pretreated and enzymatically saccharified samples prepared by freeze drying method #3. The untreated biomass shows an adsorption curve (see insert in **Fig. 7**) with a relatively significant uptake of nitrogen at low relative pressures when compared to the total adsorption in the same isotherm; this indicates the pore-filling process in micropores (Thommes *et al.*, 2015). The adsorption-de-

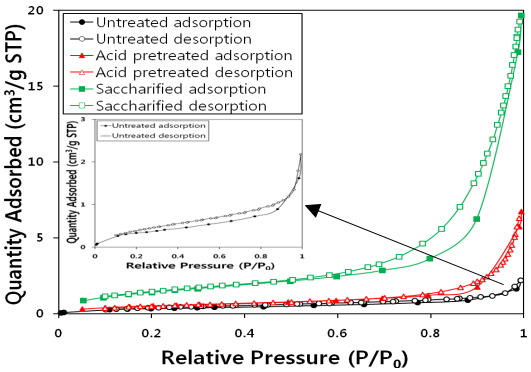


Fig. 7. Nitrogen adsorption-desorption isotherms of untreated, acid-pretreated, and enzymatically saccharified biomass sample prepared from freeze drying. The insert shows isotherm of untreated sample.

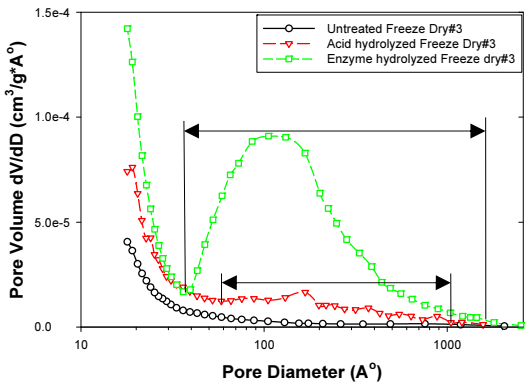


Fig. 8. Pore size distribution comparison of untreated, acid-pretreated and enzymatically saccharified biomass sample. The distribution was calculated according to the BJH method from the desorption branch of the isotherm (Indicated ranges refer to the start and end point within which peaks are apparent).

sorption curve of the untreated sample is quite similar to the type H4 loop under the IUPAC classification (**Fig. 3**), suggesting the presence of micropores having a narrow slit-like shape (Sing *et al*., 1985). Unlike the untreated sample, the acid-pretreated sample (discussed earlier) and enzymatically saccharified samples exhibit the type H3 loop indicative of slit-like pores of larger sizes. The enzymatically saccharified sample was a product of acid pretreatment followed by enzymatic hydrolysis in a typical two-step saccharification process. **Fig. 7** shows an increasingly higher overall adsorption as the biomass sample sequentially underwent acid pretreatment and then enzymatic hydrolysis. This observation is a clear indication of an increasingly larger pore volume as cell wall components were progressively removed from the biomass.

Fig. 8 compares the pore size distribution profiles of untreated, acid-pretreated and enzymatically saccharified samples. No pores are observed for the untreated biomass in the size range of mesopores (2-50 nm) up to 100 nm. This observation reiterates the inference regarding micropores (discussed earlier with respect to **Fig. 7**), and it probably also suggests the presence of narrower mesopores (2-5 nm) that are beyond the accuracy range of the BJH method (Murray *et al*., 1999) used for analysis. The acid-pretreated sample shows a pore size distribution mostly in the mesopore region, with the 10-20 nm pores being the most frequently detected. For the enzymatically saccharified sample, a broad peak expanding to the lower (50-100 nm) macropore region is evident; the considerably larger and broader distribution suggests mixed pore sizes and shapes. These observations combined suggest an increasing exposure and assortment of voids attributable to the progressive removal of cell wall components as a result of acid pretreatment followed

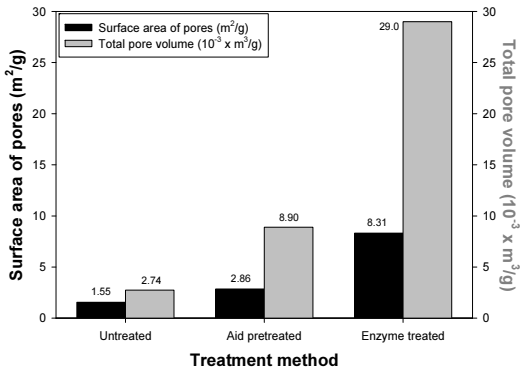


Fig. 9. The BJH desorption cumulative surface area and total pore volume of untreated, acid-pretreated, and enzymatically saccharified biomass sample prepared from freeze drying.
Note: The total pore volume was determined from N_2 desorption isotherm curve at P/P_0 = 0.99 (single point).

by enzymatic hydrolysis.

Fig. 9 presents the surface area and total pore volume of untreated, acid-pretreated, and enzymatically saccharified samples. Acid pretreatment doubled the surface area (from 1.55 m^2/g to 2.86 m^2/g) and tripled the total pore volume (from 0.00274 m^3/g to 0.00890 m^3/g) of the biomass. This result reflects the common goal of pretreatments for disrupting the biomass structure to promote digestibility of polysaccharides (Yin *et al*., 2011). Subsequent enzymatic hydrolysis resulted in an even more remarkable increase in porosity with the surface area increasing five times (from 1.55 m^2/g to 8.31 m^2/g) and the total pore volume increasing 10-11 times (from 0.00274 m^3/g to 0.0290 m^3/g) relative to those of the untreated biomass. Overall, the findings show that upon removal of hemicelluloses by dilute acid pretreatment (Schilling *et al*., 2009) followed by that of cellulose by enzymatic hydrolysis, an increasing amount of voids were generated in the internal structure of the cell wall.

4. CONCLUSIONS

This study first examined the drying technique for sample preparation and then the effects of a two-step hydrolysis treatment on the internal structure of wood. The nitrogen adsorption experiments yielded conclusions that reiterated the expected advantage of freeze drying, and verified that the drying method can preserve the pore structure to allow examination of cell wall disruption such as saccharification. Further analyses revealed that as the wood sample underwent dilute acid pretreatment (hemicellulose removal) and then enzymatic hydrolysis (cellulose removal), higher surface area, larger pores, and more voids were increasingly created in the cell wall. Overall, findings obtained from this study indicate that the nitrogen adsorption analysis is a feasible technique for examining the internal pore structure of lignocellulosic residues after saccharification. The obtained information will be useful when considering value-adding operations for utilizing the solid waste for biofuel production.

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