Comparative Study of Enzymatic Hydrolysis Properties of Pulp Fractions from Waste Paper

Xiaoli Li,[a] Hailong Yu,[a] Dafeng Sun,[b] Jianxin Jiang,[a,*] and Liwei Zhu [a,*]

As a lignocellulosic material, wastepaper is a potential material for ethanol production. However, little research on the enzymatic hydrolysis of wastepaper pulp has been conducted. In this study, the enzymatic hydrolysis of different waste pulp fractions (R_{80} represents greater than 80-mesh wastepaper pulp, $R_{80\text{-}180}$ represents the range of 80- to 180-mesh wastepaper pulp, and R_{180} represents smaller than 180-mesh waste paper pulp) were carried out at 50 °C, pH 4.8, for 96 h, with a substrate concentration of 5% (w/v) and cellulase loading of 18 FPU/g cellulose. In terms of the specific surface area, fiber structure, and surface morphology, $R_{80\text{-}180}$ had the highest affinity to cellulase and therefore the highest glucose yield of 80.33%. R_{180} had the lowest glucose yield (55.36%) because of its high ash content (21.36%), which reduced the adsorption of cellulase to cellulose. The enzymatic hydrolysis of R_{80} mixed with R_{80} or $R_{80\text{-}180}$ was also studied. Results indicated that adding $R_{80\text{-}180}$ increased the glucose yield of R_{80}. The highest glucose yield (82.57%) was obtained when 15% $R_{80\text{-}180}$ was mixed with R_{80}. However, the glucose content decreased when R_{180} was mixed with R_{80} because of its high ash content.

Keywords: Waste paper pulp fraction; Enzymatic hydrolysis; Cellulose treatment; Glucose yield

Contact information: a: Department of Chemistry and Chemical Engineering, MOE Engineering Research Center of Forestry Biomass Materials and Bioenergy, Beijing Forestry University, Beijing 100083, China; b: Nanjing Institute for the Comprehensive Utilization of Wild Plant, Nanjing 210042, China;
** Corresponding authors: jiangjx2004@hotmail.com; 13683064890@163.com*

INTRODUCTION

With ever-increasing transportation needs, vehicle fuel consumption is also increasing. Transportation accounts for more than 30% of the world's energy consumption, and its energy impact is constantly growing. To reduce carbon dioxide emissions, the application of biological liquid fuels is becoming an increasingly interesting option. By 2020, the proportion of transportation needs being met by bio-fuels is projected to be 10% (Brummer *et al*. 2014). Increasing global energy demands and the potential for significant climate change have led to a burgeoning interest in alternative fuels that are sustainable and environmentally friendly. In consideration of both the environment and economics, it is suitable to use waste materials as raw materials to produce bio-liquid fuel, especially bioethanol from renewable biomass (Yu *et al*. 2013).

Wastepaper is an abandoned renewable resource common in everyday life and in paper production. This includes all kinds of high-grade paper, yellow paperboard, paper boxes, cutting paper, packing paper, business unit paper, engineering paper, books, and newspaper. In China, the degree of waste paper recycling is relatively low compared with that of other countries (Dubey *et al*. 2012). Waste paper pulp is commonly used to make packaging materials and is used in newspaper and other print products (Chen *et al*. 2012). It has been reported that the acid-insoluble lignin content is 17.1% in newspaper, 4.7% in

office paper, 13.9% in magazines, and 14.2% in cardboard (Wang *et al*. 2013). When paper materials are recycled, they are usually used to produce lower-grade paper products. Waste papers can be reutilized several times through the manufacturing of recycled paper before cellulose fibers become too short and weak to make paper (Li *et al*. 2005). Because the shortening of paper fibers decreases the quality of paper, the maximum ratio of paper-to-paper recycling is usually around 65% (Ikeda *et al*. 2006). This means that a certain fraction of paper should be permanently disposed of. This fraction contains a significant, underutilized source of sugars and cellulose and could be converted to ethanol for its energetic value, garnering both environmental and energy benefits (Li *et al*. 2004). Conversion of waste paper with enzymes is promising in terms of waste disposal and production of a valuable liquid fuel. Waste paper could be used as a source of lignocellulosic biomass for sugars and ethanol production (Dubey *et al*. 2012). Cellulose is the predominant polymer in lignocellulosic materials, which also contain smaller amounts of hemicelluloses and lignin. The cellulose component can be converted to ethanol *via* a two-step process in which it is first converted to glucose sugars by enzyme hydrolysis and the resulting sugars can, in turn, be converted to ethanol by fermentation (Elliston *et al*. 2014).

The prime interest of this investigation was to demonstrate enzymatic hydrolysis of pulp from waste paper. The specific surface area, surface morphology, fiber structure, and enzymatic hydrolysis of different fractions of waste paper pulp were also characterized.

EXPERIMENTAL

Preparation of Raw Material

Waste paper pulp was collected from the Shandong Huatai Paper Mill. About 1% consistency waste pulp was completely separated in water with stirrer and then divided into three different categories, based on fiber length, for enzymatic hydrolysis using 80- and 180-mesh screens (Sohrab *et al*. 2013). R_{80} accounted for 80.8% of the fiber, $R_{80\text{-}180}$ accounted for 6.6%, R_{180} accounted for 10.6%, and other substances accounted for 2%.

Waste Paper Pulp Composition Analysis

The waste paper pulp was analyzed for its cellulose, pentosan, lignin, and ash contents following the National Renewable Energy Laboratory's (NREL) Laboratory Analytical Procedures (Sluiter *et al*. 2008). Cellulose, hemicellulose, acid soluble lignin, and ash contents were quantified. Samples of biomass (dry pulp, 0.3 g) were hydrolyzed with 3 mL of 72% (w/w) sulfuric acid for 1 h at 30 °C with constant stirring. The hydrolyzed samples were diluted with 84 mL of distilled water to 3% sulfuric acid concentration and autoclaved at 121 °C for 1 h. About 2 mL was sampled from the solution, and neutralized by calcium carbonate. Hydrolysis samples were filtered through a 0.22 um filter and injected to a Waters 2695 HPLC system equipped with an Aminex HPX-87P column (300 mm × 7.8 mm, Bio-Rad, USA) at 85 °C and a refractive index detector (2414RID; Waters, Milford, MA, USA) at 35 °C. Double distilled water was used as mobile phase with a flow rate of 0.6 mL/min. The remaining hydrolyzates were filtered through filtering crucibles. The residues were washed until neutral pH with distilled water. Dry matter of materials was determined by the ASTM D2974-87-method A (ASTM D2974, 1987). The residues were dried overnight at 105 °C in a laboratory

oven until constant weight was obtained. They were then calcined in a muffle furnace at 600 °C for 6 h, cooled in a desiccator, and weighed again (Ertas *et al*. 2014). The ash and acid-insoluble lignin contents were calculated on an oven-dry basis.

Enzymatic Hydrolysis

The enzymatic hydrolysis was performed using a mixture of commercial cellulase (Celluclast, 1.5 L, Sigma Co., St. Louis, MO, USA) and cellobiose enzymes (Novozyme188, Sigma Co., St. Louis, MO, USA). Hydrolysis experiments were carried out as duplicates in stoppered 100-mL Erlenmeyer flasks in a shaking incubator at 48 °C and 150 rpm for 96 h with a cellulase loading of 18 FPU/(g-cellulose) and a cellobiose loading of 27 CBU/g cellulose. A sodium acetate buffer solution (0.5mol/L) with pH 4.8 was added and dry materials were added until the substrate concentration reached 5%. The total volume of the hydrolysis solution is 80 mL. The hydrolyzates (about 2 mL) were sampled at 0, 6, 9, 12, 24, 36, 48, 72, and 96 h. Later, the samples were centrifuged at 10,000 g for 3 min to separate liquid phase and solid phase. The supernatant were filtered through a 0.22 μm filter and injected to a Waters 2695 HPLC system with an Aminex HPX-87P column (300×7.8 mm, Bio-Rad, USA) at 85 °C and a refractive index detector at 35 °C. The glucose yield of the substrate was calculated according to the glucose content as a percentage of the theoretical glucose available in the substrates. A cellulose-to-glucose ratio of 1:1.11 (g/g) was considered.

Sugar Analysis

The prepared hydrolyzates were collected to detect the carbohydrate concentration by HPLC (Waters 2695e, USA) with an Aminex HPX-87P column (300×7.8 mm, Bio-Rad, USA) at 85 °C and a refractive index detector at 35 °C. The injection volume of the sample was 10 μL, and distilled water was used as the eluent at a flow rate of 0.6 mL/min. The samples were filtered through a 0.22 μm filter before HPLC analysis. Each point reported is the average of duplicate experiments (Xing *et al*. 2013). All experiments were performed at least in duplicate, and the standard errors or deviations observed were lower than 5%.

Measurement of Fiber Specific Surface Area

The contact of cellulase with cellulose varies from a specific surface area point of view. The specific surface area was determined by nitrogen adsorption-desorption using a Tristar 3020 II Micromeritics (USA) apparatus operated at -150 °C. During measurement, to truly reflect the specific surface area, fibers must be dried and separated. However, in general, dried fibers are tightly bound together because of hydrogen bonding and are difficult to separate without damage. As a consequence, fibers must be treated before the measurement can take place.

To prepare fibers for nitrogen adsorption measurement, a certain amount of fibers in the beaker were completely dispersed with a stirrer dispersed. 100 mL of acetone was added, and the beaker contents were stirred for 1 h before being filtered. After filtration, the fibers were put back into the beaker and continuously stirred for 1 h after adding acetone. They were then filtered, and the process was repeated three times. The filtered fibers were torn into small pieces and placed in a constant-temperature drying oven at 60 °C for at least 8 h. Water and acetone were completely removed during drying. The dehydrated fibers were separated into single fibers. In this research, the Brauner-Emmett-

Teller (BET) surface area, pore size, and pore volume of different ranks of waste paper pulp were determined using nitrogen adsorption.

Structural Analysis by Scanning Electron Microscope (SEM)

The morphology of different ranks of waste paper pulp was studied by SEM using a Hitachi S-3000N scanning electron microscope (Japan). Images of the surfaces of different ranks of waste paper pulp were taken at a magnification of 1,000. The fibers were separated and placed on the stage. Using a sputter coating machine, a layer of gold 10 nm thick was sprayed onto the fibers. The fibers were then observed under the scanning electron microscope (Sahare *et al*. 2012).

FT-IR Spectra

Fractions of paper were mixed at a rate of 1:100 with potassium bromide and ground to about 200-mesh powder in an agate mortar (Liu *et al.* 2014) . A transparent sheet was then made in the infrared tabletting machine for use in a Fourier transform infrared spectrometer equipped with DTGS pyroelectric detector parse capability. The scanning range was from 500 to 4000 cm^{-1}. A total of 32 scans were produced with a resolution of 2 cm^{-1}. The FT-IR spectra were obtained using pure potassium bromide tablets as a background, but the potassium bromide was deducted from the sample measurements (Bu *et al.* 2011) .

Statistical Analysis

The statistical significance of experimental data was tested by analysis of variance (ANOVA) using SPSS statistics version 18.0 software. The *p*-values were used as a standard to check the significant difference of every item. Values of p less than 0.05 ($p < 0.05$) indicate significant difference exists. A smaller *p*-value illustrates that the significance of the variable is more obvious (Jian *et al*. 2011).

RESULTS AND DISCUSSION

Compositional Analysis of Fraction Pulp from Waste Paper

The composition of the waste paper pulp is expressed as the percentage of dry material (Table 1). According to the *p*-value, it can be concluded that there were obvious differences in different pulp fractions. The largest variation among fractions was observed in the cellulose, hemicellulose, lignin, and ash contents. The amount of cellulose in pulp fractions R_w (R_w represents raw waste paper pulp), R_{80}, $R_{80\text{-}180}$, and R_{180} was 52.17, 61.31, 43.06, and 28.35%, respectively. The lignin in those pulp fractions accounted for 20.92, 15.81, 30.78, and 43.68%, respectively. The high lignin content means more mechanical pulp fiber. The lignin composition was close to that of native softwood, 25 to 35% (Wu *et al.* 2014). The remainder of the pulp was made up of hemicelluloses and ash. Ash in the waste paper pulp is mostly composed of inorganic filler and coating materials such as calcium carbonate and talc. Ash can have a large impact on the behavior of cellulase activity during enzymatic hydrolysis (Kemppainen *et al*. 2014). The carbohydrates are the main component in waste paper, and it makes waste paper a prospective renewable biomass source for bioethanol production (Wang *et al*. 2013; Sohrab *et al.* 2013). Furthermore, waste paper pulp consists of secondary fibers,

and a portion of the lignin has been removed. As a consequence, the fractional cellulose content is greater, reducing chemical usage and heat loss during the cooking stage.

Table 1. Chemical Compositions of Various Ranks of Waste Paper Pulp

Component (%)	R_W	R_{80}	$R_{80\text{-}180}$	R_{180}	*p*-value
Cellulose	52.17±0.32	61.31±1.0	43.06±0.21	28.35±2.65	<0.001
Hemicelluloses	7.02±0.13	7.59±0.08	7.51±0.08	5.2±0.04	<0.001
Lignin	20.92±0.18	15.81±0.49	30.78±0.14	43.68±0.38	<0.001
Ash	6.53±0.26	2.34±0.12	8.42±0.14	21.36±0.0	<0.001

Specific Surface Area of Various Ranks of Waste Paper Pulp

Many factors affect the enzymatic hydrolysis of waste paper, including lignin content, enzyme adsorption on lignin, cellulose accessibility, and the degree of polymerization (DP) (Monrroy *et al*. 2011; Wiman *et al*. 2012; Zhang *et al*. 2013). In general, the substrate specific surface area is a primary indicator of cellulose accessibility. Variations in surface area, average pore width, and pore volume resulted in different levels of cellulose binding (Yu *et al*. 2014). As can be seen in Table 2, the specific surface area gradually increased with a reduction in fiber size. The smaller the particles are, the larger their specific surface areas are. A greater specific surface area theoretically leads to faster enzymatic hydrolysis because of the increased degree of contact of cellulose to cellulase (Koo *et al.* 2012; Yu *et al.* 2014). The greater the specific surface area is, the easier it is for cellulase molecules to come into contact with fibers. The amounts of cellulose and lignin also play important roles in glucose yield. On one hand, R_{180} contains calcium carbonate, which can increase the specific surface area. However, calcium carbonate can adsorb cellulase and shift the pH during hydrolysis, affecting cellulase activity (Wang *et al*. 2011). On the other hand, $R_{80\text{-}180}$ has greater pore volume and pore width, which resulted in increased access of cellulase to the pulp fiber surface and facilitated enzymatic hydrolysis (Grethlein 1985; Kojima and Yoon 2008).

Table 2. Specific Surface Area of Various Ranks of Pulp

Sample	BET Surface Area (m^2/g)	Average Pore Width (nm)	Pore Volume (cm^3/g)
R_w	1.936	10.05001	0.001332
R_{80}	1.3203	10.10003	0.003334
$R_{80\text{-}180}$	6.1989	11.39786	0.017664
R_{180}	8.653	9.42611	0.010448

Enzymatic Hydrolysis of Various Fraction Pulps of Waste Paper

Enzymatic hydrolysis of $R_{80\text{-}180}$ waste paper pulp gave the highest glucose yield (80.33%), closely followed by enzymatic hydrolysis of R_{80} (79.72%). R_{180} waste paper pulp exhibited the lowest glucose yield (55.36%), as shown in Fig. 1. The results can be explained by the low amount of carbohydrates present in the R_{180}. On the one hand, R_{80} contained more cellulose and thus more fermentable sugars subject to degradation by the enzyme. A portion of cellulase was used to cleave long cellulose chains. The rest of the cellulase was contacted with cellulose surfaces. In addition, cellulose accessibility decreases because of smaller specific surface area, which possibly decreases glucose yield to some extent. On the other hand, although the cellulose content of $R_{80\text{-}180}$ was less than that of R_{80}, it was not necessary for cellulase to cleave the long cellulose chain in

this fraction. Cellulase absorbed to the cellulose was mainly used to break down short fibers into monosaccharides. In addition, the specific surface area of $R_{80\text{-}180}$ was higher than that of R_{80}, allowing good contact with cellulase which then caused higher cellulose accessibility. R_{180} waste paper pulp had higher lignin and ash contents; a portion of cellulase can be absorbed in lignin or ash, which can then cause invalid absorption. As a consequence, the glucose yield was the lowest. Due to the larger lignin and ash, R_w was not the highest glucose yield. Glucose yield continued increasing from 12 to 96 h. Thus, waste paper is a potential recycling resource (Pribowo *et al*. 2012).

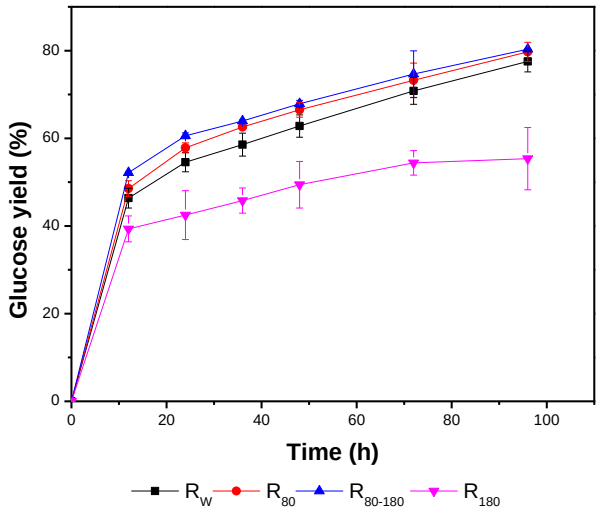

Fig. 1. Enzymatic hydrolysis of different fractions of waste paper pulp

The enzymatic hydrolysis trends of various ratios of waste paper pulp mixtures are shown in Fig. 2. The glucose yields of R_{80} and $R_{80\text{-}180}$ complexes are shown in Fig. 2(a). As the proportion of $R_{80\text{-}180}$ waste paper pulp increased, the glucose yield gradually increased. When the amount of $R_{80\text{-}180}$ pulp was 15%, the glucose yield was the highest. This trend can be explained in terms of fiber morphology. Fine fiber can promote the digestion of long fiber. The addition of fine fibers can improve enzymatic hydrolysis efficiency of long fibers (Wang *et al*. 2013).

In addition, lignin content can also affect enzymatic hydrolysis. Hydrolysis trends for the R_{80}-R_{180} complex are shown in Fig. 2(b). The glucose yield was highest without R_{180} and gradually declined with increased R_{180} addition. Lignin inhibited enzymatic hydrolysis. Waste paper pulp contained a portion of residual lignin. The cellulose surface can be blocked by lignin, which decreases cellulose accessibility. Enzymes can also be irreversibly adsorbed to the lignin surface and are thus no longer available for cellulose degradation (Nakagame *et al*. 2011). It is expected that waste paper pulp becomes more hydrolyzable with decreasing lignin content, as previously observed by several researchers (Yoshida *et al*. 2008). Figures 2(a) and (b) show that the glucose yield after enzymatic hydrolysis had a pronounced dependency on the residual lignin content. With increasing residual lignin content, the glucose yield decreased.

Additionally, the effect of xylan is essential to enzymatic hydrolysis. The xylan contents of different ranks of waste paper pulp varied. With increasing residual xylan content, cellulose conversion varied; such a result was also found in other studies

following the enzymatic hydrolysis of several wood species (Hu *et al*. 2008). One possible reason for this is that, at high mass fractions, hemicelluloses cover large parts of the cellulose surface and hinder cellulase attack. Furthermore, enzymatic hydrolysis was observed to be inhibited by xylooligomers. In this paper, the amount of xylan was almost identical in all tests, so the inhibition effect on cellulase degradation was similar. The true cause of the difference was the ash content. It is known that ash contains large amounts of metal elements. The metal elements in waste paper pulp are toxic to enzymes, decreasing enzymatic activity and glucose yield.

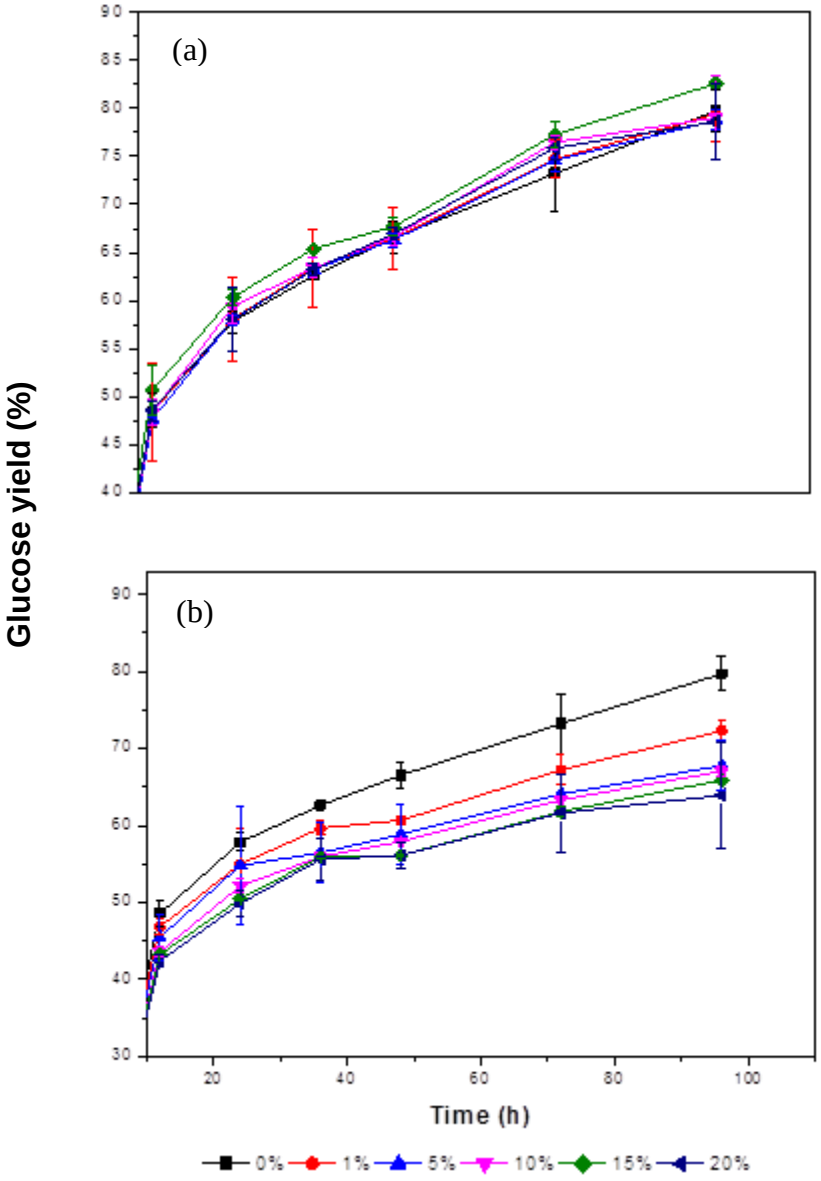

Fig. 2. Enzymatic hydrolysis of the mixture of fractions of pulps: (a) R_{80} and $R_{80\text{-}180}$ waste paper pulps, (b) R_{80} and R_{180} waste paper pulps

Structural Analysis by Scanning Electron Microscope (SEM)

To investigate the morphology of the different fractions of waste paper, SEM images of the pulps were observed (Fig. 3). R_W waste paper pulp displayed a brushy morphology, which could favor accessibility of cellulose to the enzymes. As can be seen from the figure, the surface of R_W was covered with a layer of material. It is possible that a portion of cellulase was absorbed onto this material, affecting the glucose yield. R_{80} waste paper pulp had a smooth morphology, which could be beneficial to enzymatic hydrolysis. Cellulase can be well-contacted with the smooth fibers, breaking long-chain cellulose macromolecules into small molecules and promoting enzymatic hydrolysis. $R_{80\text{-}180}$ waste paper pulp had a brushy morphology like that of raw pulp but contained less cellulose than raw waste paper pulp or R_{80} pulp. In addition, the $R_{80\text{-}180}$ pulp surface was not covered as much as that of the R_W pulp was because it had larger specific surface area, allowing it to be well-contacted by cellulase and increasing the glucose yield. R_{180} waste paper had high specific surface area, but its surface was covered with a large amount of inorganic filler such as calcium carbonate. Thus, cellulase could not completely contact the cellulose in the pulp (Wang *et al*. 2011). In addition, the amount of cellulose was lower in this fraction. As a consequence, the glucose yield was the lowest.

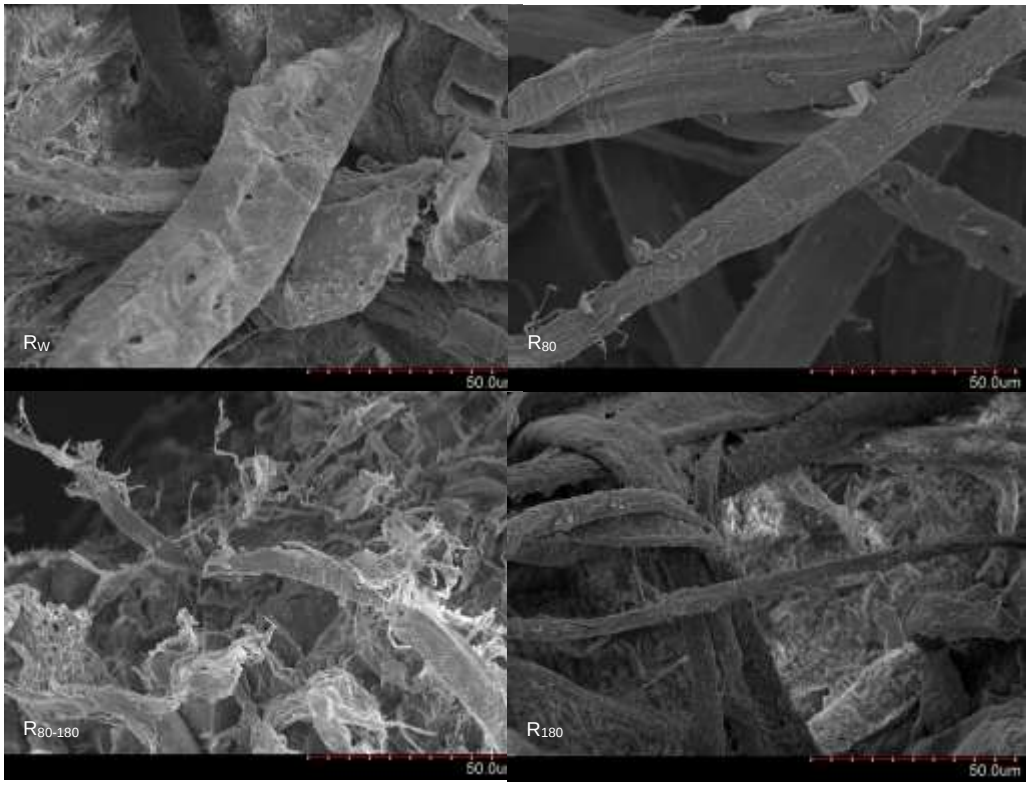

Fig. 3. SEM images of different fractions of waste paper pulps

FT-IR Spectra Analysis

FT-IR spectra reflect the chemical structures of various waste paper pulp fractions, including both common features and vibrations specific to each sample (Boeriu *et al*. 2004). The infrared spectra of different ranks of waste paper pulp are shown in Fig. 4. A strong hydrogen-bonded (O-H) stretching absorption at peak 3425 cm^{-1} and the bands centered around 2938 to 2842 cm^{-1}, predominantly arising from C-H stretching in

aromatic methoxyl groups and methyl and methylene groups of the side chains, were observed. The absorption peak around 1600 cm^{-1} originated from the skeletal and stretching vibrations of benzene rings, which were slight refractory characteristics observed during the biochemical reactions. The band at 1326 cm^{-1} in the spectrum corresponded to the syringyl ring and indicated that the sample had endured a demethoxyl reaction. The carbonyl stretching of conjugated ester was observed in the spectra at 1170 cm^{-1} and is typical of the ρ-hydroxyphenyl structures found in lignin. These peaks appeared on the IR spectra at *ca.* 3425, 2838, 1600, 1326, and 1170 cm^{-1}.

However, there were differences among the samples tested. The band at around 1500 cm^{-1} occurred in $R_{80\text{-}180}$ and R_{180} but was not present in R_W or R_{80}. Peaks at around 1500 to 1326 cm^{-1} and 1326 to 1170 cm^{-1} arose in the spectra of $R_{80\text{-}180}$ and R_{180}. The peak around 880 cm^{-1} was present only in the spectrum of R_{180}. This can be explained by the presence of metal elements or other impurities. These types of impurities can affect cellulase activity and decrease the glucose yield.

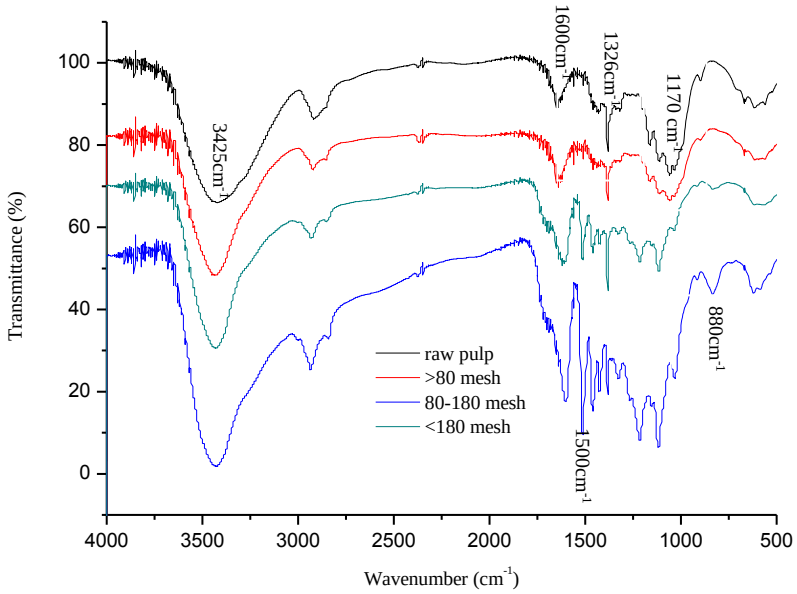

Fig. 4. FT-IR spectra of various ranks of waste paper pulp

CONCLUSIONS

1. The composition of different fractions of waste paper pulp is quite different, in particular cellulose, lignin, and ash. Enzymatic hydrolysis of $R_{80\text{-}180}$ waste paper pulp gave the highest glucose yield (80.33%), closely followed by enzymatic hydrolysis of R_{80} (79.72%). R_{180} waste paper pulp exhibited the lowest glucose yield (55.36%).
2. The enzymatic hydrolysis of long-fraction (R_{80}) waste paper pulp was improved after adding some fine-fraction ($R_{80\text{-}180}$) waste paper pulp. The optimal additional amount of $R_{80\text{-}180}$ was 15%. The inhibition of lignin increased and glucose yield decreased when the addition of $R_{80\text{-}180}$ was above 20%.

3. The R_{180} fraction contained a small amount of cellulose, while containing a relatively large amount of lignin and ash. As more R180 was added, the inhibitory effect became stronger.
4. The presence of the R_{180} fraction resulted in unproductive absorption, which decreased the enzymatic hydrolysis of R_{80}.

ACKNOWLEDGMENTS

The authors are grateful for the financial support of this research from the China Ministry of Science and Technology (2014DFG32550) and the Guangxi Key Laboratory of Chemistry and Engineering of Forest Products (GXFC12-07).

REFERENCES CITED

Boeriu, C. G., Bravo, D., Gosselink, R. J. A., and Dam, J. E. G. (2004). "Characterisation of structure-dependent functional properties of lignin with infrared spectroscopy," *Industrial Crops and Products* 20(2), 205-218. DOI: 10.1016/j.indcrop.2004.04.022

Brummer, V., Jurena, T., Hlavacek, V., Omelkova, J., Bebar, L., Gabriel, P., and Stehlik, P. (2014). "Enzymatic hydrolysis of pretreated waste paper - Source of raw material for production of liquid biofuels," *Bioresource Technology* 152, 543-547. DOI: 10.1016/j.biortech.2013.11.030

Bu, L. X., Tang, Y., Gao, Y. X., Jian, H. L., Jiang, J. X. (2011). "Comparative characterization of milled wood lignin from furfural residues and corncob," *Chemical Engineering Journal* 175, 176-184. DOI:10.1016/j.cej.2011.09.091

Chen, H., Venditti, R. A., Jameel, H., and Park, S. (2012). "Enzymatic hydrolysis of recovered office printing paper with low enzyme dosages to produce fermentable sugars," *Applied Biochemistry and Biotechnology* 166(5), 1121-1136. DOI: 10.1007/s12010-011-9498-2

Dubey, A. K., Gupta, P. K., Garg, N., and Naithani, S. (2012). "Bioethanol production from waste paper acid pretreated hydrolyzate with xylose fermenting *Pichia stipitis*," *Carbohydrate Polymers* 88(3), 825-829. DOI: 10.1016/j.carbpol.2012.01.004

Elliston, A., Collins, S. R. A., Faulds, C. B., Roberts, I. N., and Waldron, K. W. (2014). "Biorefining of waste paper biomass: Increasing the concentration of glucose by optimising enzymatic hydrolysis," *Applied Biochemistry and Biotechnology* 172(7), 3621-3634. DOI: 10.1007/s12010-014-0767-8

Ertas, M., Han, Q., Jameel, H., and Chang, H.-M. (2014). "Enzymatic hydrolysis of autohydrolyzed wheat straw followed by refining to produce fermentable sugars," *Bioresource Technology* 152, 259-266. DOI: 10.1016/j.biortech.2013.11.026

Grethlein, H. E. (1985). "The effect of pore size distribution on the rate of enzymatic hydrolysis of cellulosic substrates," *Nat. Biotechnol.* 3, 155-160. DOI:10.1038/nbt0285-155

Hu, G., Heitmann, J. A., and Rojas, O. J. (2008). "Feedstock pretreatment strategies for producing ethanol fromo wood, bark, and forest resides," *BioResources* 3(1), 270-294. DOI: 10.15376/biores.3.1.270-294

Ikeda, Y., Park, E. Y., and Okuda, N. (2006). "Bioconversion of waste office paper to gluconic acid in a turbine blade reactor by the filamentous fungus *Aspergillus niger*," *Bioresource Technology* 97(8), 1030-1035. DOI: 10.1016/j.biortech.2005.04.040

Jian, H. L., Cristhian, C., Zhang, W. M., and Jiang, J. X. (2011). "Influence of dehulling pretreatment on physicochemical properties of *Gleditsia sinensis* Lam. gum," *Food Hydrocolloids* 25(5), 1337-1343. DOI:10.1016/j.foodhyd.2010.12.009

Kemppainen, K., Siika-aho, M., Ostman, A., Sipila, E., Puranen, T., von Weymarn, N., and Kruus, K. (2014). "Hydrolysis and composition of recovered fibres fractionated from solid recovered fuel," *Bioresource Technology* 169, 88-95. DOI: 10.1016/j.biortech.2014.06.069

Kojima, Y., and Yoon, S.-L. (2008). "Improved enzymatic hydrolysis of waste paper by ozone pretreatment," *Journal of Material Cycles and Waste Management* 10(2), 134-139. DOI: 10.1007/s10163-007-0198-5

Koo, B. W., Min, B. C., Gwak, K. S., Lee, S. M., Choi, J. W., Yeo, H., and Choi, I. G. (2012). "Structural changes in lignin during organosolv pretreatment of *Liriodendron tulipifera* and the effect on enzymatic hydrolysis," *Biomass and Bioenergy* 42, 24-32. DOI:10.1016/j.biombioe.2012.03.012

Li, C. Z., Yoshimoto, M., Tsukuda, N., Fukunaga, K., and Nakao, K. (2004). "A kinetic study on enzymatic hydrolysis of a variety of pulps for its enhancement with continuous ultrasonic irradiation,"*Biochemical Engineering Journal* 19(2), 155-164. DOI: 10.1016/j.bej.2003.12.010

Li, C. Z., Yoshimoto, M., Ogata, H., Tsukuda, N., Fukunaga, K., and Nakao, K. (2005). "Effects of ultrasonic intensity and reactor scale on kinetics of enzymatic saccharification of various waste papers in continuously irradiated stirred tanks," *Ultrasonics Sonochemistry* 12(5), 373-384. DOI: 10.1016/j.ultsonch.2004.02.004

Liu, Y. H., Liu, Y. G., Jiang, H. Q., Xu, L. C., Cheng Y. N., Wang, P. G., and Wang, F. S. (2014). "Preparation, antiangiogenic and antitumoral activities of the chemically sulfated glucan from *Phellinus ribis*," *Carbohydrate Polymers* 106, 42-48. DOI:10.1016/j.carbpol.2014.01.088

Monrroy, M., Ortega, I., Ramirez, M., Baeza, J., and Freer, J. (2011). "Structural change in wood by brown rot fungi and effect on enzymatic hydrolysis," *Enzyme and Microbial Technology* 49(5), 472-477. DOI: 10.1016/j.enzmictec.2011.08.004

Nakagame, S., Chandra, R. P., Kadla, J. F., and Saddler, J. N. (2011). "Enhancing the enzymatic hydrolysis of lignocellulosic biomass by increasing the carboxylic acid content of the associated lignin," *Biotechnology and Bioengineering* 108(3), 538-548.

Pribowo, A., Arantes, V., and Saddler, J. N. (2012). "The adsorption and enzyme activity profiles of specific *Trichoderma reesei* cellulase/xylanase components when hydrolyzing steam pretreated corn stover," *Enzyme and Microbial Technology* 50(3), 195-203. DOI: 10.1016/j.enzmictec.2011.12.004

Sahare, P., Singh, R., Laxman, R. S., and Rao, M. (2012). "Effect of alkali pretreatment on the structural properties and enzymatic hydrolysis of corn cob," *Applied Biochemistry and Biotechnology* 168(7), 1806-1819. DOI: 10.1007/s12010-012-9898-y

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., and Templeton, D. (2008). "Determination of structural carbohydrates and lignin in biomass," <http://www.nrel.gov/biomass/pdfs/42618.pdf> (accessed in 2008)

Sluiter, A., Hames, B., Hyman, D., Payne, C., Ruiz, R., and Scarlata, C. (2008). "Determinationof total solids in biomass and total dissolved solids in liquid process sample," National Renewable Energy Laboratory (NREL).

Standard Test Methods for Moisture, Ash, and Organic Matter of Peat and Other Organic Soils, (1987). ASTM Standard D 2974.

Sohrab, H. M., Amir, H. G., Meisam, T., Gholamreza, S. J., Gholam, H. N., Mehdi, G., and Mehdi, A. (2013). "Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment," Renewable and Sustainable Energy Reviews, 27:77-93. DOI:10.1016/j.rser.2013.06.033

Wang, X., Song, A., Li, L., Li, X., Zhang, R., and Bao, J. (2011). "Effect of calcium carbonate in waste office paper on enzymatic hydrolysis efficiency and enhancement procedures," *Korean Journal of Chemical Engineering* 28(2), 550-556. DOI: 10.1007/s11814-010-0365-6

Wang, L., Templer, R., and Murphy, J. R. (2011). "Composition profile of paper waste materials for biofuel production," in: 19th European biomass conference and exhibition, Berlin, Germany, 2167.

Wang, L., Sharifzadeh, M., Templer, R., and Murphy, R. J. (2013). "Bioethanol production from various waste papers: Economic feasibility and sensitivity analysis," *Applied Energy* 111, 1172-1182. DOI: 10.1016/j.apenergy.2012.08.048

Wiman, M., Dienes, D., Hansen, M. A. T., Meulen, T. V. D., Zacchi, G., and Liden, G. (2012). "Cellulose accessibility determines the rate of enzymatic hydrolysis,"*Bioresource Technology* 126, 208-215. DOI: 10.1016/j.biortech.2012.08.082

Wang, Z. J., Qin, M. H., Zhu, J. Y., Tian, G. Y., and Li, Z. Q. (2013). "Evaluation of energy efficiency of bioconversion knot rejects to ethanol in comparison to other thermochemically pretreated biomass," *Bioresource Technology*, 130, 783-788.. DOI:10.1016/j.biortech.2012.12.058

Wu, C. F., Huang, S. S., and Shih, I. L. (2014). "Sequential hydrolysis of waste newspaper and bioethanol production from the hydrolysate," *Bioresource Technology* 167, 159-168.DOI:10.1016/j.biortech.2014.06.041

Xing, Y., Ji, L., Liu, Z.-P., Zhang, W.-M., and Jiang, J.-X. (2014)." Effects of gleditsia saponin on high-solids enzymatic hydrolysis of furfural residues," *Industrial Crops and Product*s 64, 209-214. DOI: 10.1016/j.indcrop.2014.09.055

Yoshida, M., Liu, Y., Uchida, S., Kawarada, K., Ukagami, Y., Ichinose, H., Kaneko, S., and Fukuda, K. (2008). "Effects of cellulose crystallinity, hemicellulose, and lignin on the enzymatic hydrolysis of *Miscanthus sinensis* to monosaccharides," *Bioscience Biotechnology and Biochemistry* 72(3), 805-810.

Yu, Z., Jameel, H., Chang, H.-M., Philips, R., and Park, S. (2013). "Quantification of bound and free enzymes during enzymatic hydrolysis and their reactivities on cellulose and lignocellulose," *Bioresource Technology* 147, 369-377. DOI: 10.1016/j.biortech.2013.08.010

Yu, H., Xing, Y., Lei, F., Liu, Z., Liu, Z., and Jiang, J. (2014). "Improvement of the enzymatic hydrolysis of furfural residues by pretreatment with combined green liquor and ethanol organosolv," *Bioresource Technology* 167, 46-52. DOI: 10.1016/j.biortech.2014.05.111

Zhang, Q., Zhang, P. F., Pei, Z. J., and Wang, D. H. (2013). "Relationships between cellulosic biomass particle size and enzymatic hydrolysis sugar yield: Analysis of inconsistent reports in the literature," *Renewable Energy* 60, 127-136. DOI: 10.1016/j.renene.2013.04.012

Article submitted: February 10, 2015; Peer review completed: April 12, 2015; Revisions received and accepted: April 30, 2015; Published: May 4, 2015.
DOI: 10.15376/biores.10.3.3818-3830