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Effect of calcium carbonate in waste office paper on enzymatic hydrolysis efficiency and enhancement procedures

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Abstract−Hydrolysis of waste office paper (WOP) into fermentable sugars is an important option of WOP utilization. In this work, the effect of major chemicals in WOP on its hydrolysis using industrial cellulase Accellerase 1000 (Genencor, Rochester, NY, USA) was investigated, and calcium carbonate ($CaCO_3$) was found to be the key parameter affecting the enzymatic hydrolysis efficiency of WOP. The pretreatment methods, acid washing and acid presoaking, were tested for the removal of $CaCO_3$ from WOP. It was found that the presoaking of sulfuric acid (H_2SO_4) in WOP was an effective way. The pretreating parameters of WOP were studied on maximizing the hydrolysis efficiency. The conversion yield of cellulose to glucose and cellobiose using the pretreated WOP reached 73.3% after 96 hours hydrolysis at the optimal conditions. The results provided the WOP utilization with a practical enzymatic hydrolysis method with industrial application potential.

Key words: Waste Office Paper (WOP), Calcium Carbonate ($CaCO_3$), Hydrolysis, Pretreatment, Presoaking

INTRODUCTION

Waste paper accounts for 37.8% of municipal solid waste (MSW) [1], and its disposal by land filling or combustion could trigger new pollutions and arable land occupation problems [2]. Recycling of waste paper usually produces lower grade paper products because of the shortening of fiber length in the deinking and bleaching procedures. According to the latest statistics, the maximum ratio of paper-to-paper recycling was only 65% [3]. An attractive alternative for the recycling waste paper is to hydrolyze the waste paper into fermentable glucose, then convert it into bioethanol or other value added biochemicals [3-6].

Office paper (OP) is a kind of high grade paper used on copy machines and computer printers. The difficulty of recycling waster office paper (WOP) is the existence of chemical additives such as calcium carbonate ($CaCO_3$), aluminum sulfate, glue substances, and in some cases organic dyes in waster office paper (WOP) [7,8]. Among these substances, calcium carbonate used as white pigment constitutes the largest content, up to 20% (w/w), of the total WOP weight [8-10]. Different from original lignocellulose such as agricultural or forest residues, the biocalcitrance of waste paper to enzymatic hydrolysis has been extensively reduced by grinding, delignification, and heating during the paper manufacturing. However, the chemical addition during the paper manufacturing caused another negative significant negative effect on hydrolysis of waste paper. In economic consideration, it is preferable to hydrolyze waste paper with little or no reagent use, less energy and water consumption, high glucose concentration and high cellulose conversion yield. Various methods been applied for enhancing hydrolysis efficiency of waste papers. Li et al. [11] reported that continuous ultrasound facilitated the saccharification of waste paper and the efficiency increased with increasing specific ultrasonic intensities. Woods et al. [12] found that intermittent ultrasound stimulated ethanol production during the simultaneous saccharification and fermentation of mixed waste office paper (WOP), but continuous exposure to ultrasound decreased the ability of ethanol production for the fermentation microorganisms. Zheng et al. [13] treated recycled paper with supercritical carbon dioxide, and the rate of cellulose hydrolysis was enhanced by 50% in glucose yield. Wu et al. [14] enhanced enzymatic saccharification of waste newsprint by surfactant addition, and found that specific nonionic surfactant enhanced the hydrolysis conversion. Nikolov et al. [7] pretreated paper sludge with 0.25% H_3PO_4 and the enzymatic hydrolysis conversion was increased. Kojima and Yoon [10] pretreated different waste papers with ozone and found that ozone increased the specific surface areas and total pore volumes while decreasing its lignin content. Park et al. [15] hydrolyzed waste paper without adding reagents and got higher reduced sugar concentration.

In present work, the effect of major chemical additives on the hydrolysis of waste office paper (WOP) using industrial cellulase enzyme was investigated and it was found that significant calcium carbonate content was the key factor affecting the enzymatic hydrolysis efficiency of WOP. Different pretreatment methods were tested to overcome the effect of $CaCO_3$ existence and presoaking of WOP with dilute sulfuric acid was found to be the practical and effective way. The conditions of presoaking were studied carefully and the hydrolysis efficiency was improved significantly. The WOP hydrolysis at a high solid WOP loading up to 10% (wt) was carried out, and the result showed that the sugar recovery yield reached 81.8% based on the WOP cellulose content. The present work provided the WOP utilization with a practical and effective pretreatment method for the enzymatic hydrolysis to get the fermentation level sugars.

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EXPERIMENTAL SECTION

1. Reagents and Chemicals

Original office paper (ANA copy paper, A4 size) was purchased from Anxin Huidong Paper Ltd., Shanghai, China. The paper was used on the HP LaserJet 1002 printer for printing and the used waste office paper was cut into the 0.2 cm×0.6 cm rectangle strippers using a paper shredder machine (SD9331, Sunwood, Shanghai, China) before use. Filter paper (Xinhua No. 1) was purchased from Xinhua Paper Co. Ltd, Hangzhou, China, and was also cut into 0.2 cm× 0.6 cm pieces with the same shredder machine. The chemicals and reagents used in the experiments, including glucose, cellobiose, xylose, $(NH_4)_2CO_3$, $FeCl_3 \cdot 6H_2O$, $CaCO_3$, $CaCl_2$, $Ca(OH)_2$, $MgSO_4$, $Al_2(SO_4)_3$, $CaSO_4$, HCl, HNO_3, H_2SO_4, H_3PO_4, were all purchased from the local reagent companies in Shanghai, China. Fine carbon powder was collected from the HP LaserJet printer cartridge.

The cellulase enzyme used was Accellerase 1000 from Genencor International (Rochester, NY, USA). The cellulase and cellobiase activities were assayed separately.

2. Pretreatments

The waste office paper (WOP) was pretreated with two methods, washing and presoaking:

(1) Washing: the WOP pieces were washed with deionized water and dilute acid solution, respectively. When washed with water, 50 grams of WOP pieces were soaked with deionized water and stirred for 30 minutes. The water was removed by squeezing afterwards. The procedure was repeated for 10 times before it was ready for use. When washed with acid, 20 mL acid solution (H_2SO_4, HCl, H_3PO_4, or HNO_3) at the hydrogen ion concentration 0.2 mol L^{-1} (0.2 mol L^{-1} for HCl and HNO_3, 0.1 mol L^{-1} for H_2SO_4, 0.07 mol L^{-1} for H_3PO_4) was poured on 50 grams of WOP pieces, mixed, then 500 mL deionized water was added and stirred for 10 minutes. The acid solution was removed by squeezing. The procedure was repeated for three times, then washed by deionized water until pH was 5.0.

(2) Presoaking: the deionized waster or dilute acid solution was added to 10 grams of WOP pieces and incubated stationary at 50 °C for 12 hours. Then the redundant acid was neutralized using NaOH solution until the pH was 5.0 before enzymatic hydrolysis. The water presoaking treatment was used as the control at the same procedure but the deionized water replaced the acid.

3. Enzymatic Hydrolysis

10 grams of the pretreated WOP or filter paper (dry base) was placed into a 250 mL flask, and then we poured acetate buffer (0.05 mol L^{-1}, pH 5.0) solution or water into the flask to adjust the slurry to the solid content of 10% (wt.). The slurry was autoclaved at 115 °C for 20 minutes before hydrolysis. Accellerase 1000 was added at the ratio of 12 FPU per gram of dried WOP mass (g DM), and incubated at 50 °C in water bath at 200 rpm for 24 hours. In the comparison of washing and presoaking experiment, the hydrolysis was carried out in the 0.05 mol L^{-1} sodium acetate buffer to keep a constant pH value. In the rest of the hydrolysis experiment using the presoaked WOP, the experiment was carried out in the deionized water system for convenience.

In the prolonged hydrolysis experiment, 100 grams of the pretreated WOP was added to the 2 L bioreactor with a helix impeller and the solid content was adjusted to 10% (wt) with the sodium acetate buffer (0.05 mol L^{-1}, pH 5.0). The WOP slurry was sterilized, and Accellerase 1000 enzyme was added at the dosage of 12 FPU g^{-1} DM. The hydrolysis was carried out at 50 °C at 200 rpm for 96 hours.

Samples were taken periodically during the hydrolysis in either a flask or bioreactor; the samples were centrifuged at 13,000 rpm for 5 minutes to obtain the clear supernatant for analysis. Every experiment was duplicated and the data were averaged. The hydrolysis yield of WOP was calculated as the percentage ratio of the hydrolyzed glucose and cellobiose to the total glucan in WOP.

4. Analysis

Glucose and cellobiose were analyzed using high performance liquid chromatography (LC-20AD, refractive index detector RID-10A, Shimadzu, Japan) with a Bio-rad Aminex HPX-87H column at the column temperature 65 °C. The mobile phase was 5 mM H_2SO_4 at the rate of 0.6 mL min^{-1}. All samples were centrifuged to remove the water insoluble substances, and then filtered through a 0.22 μm filter before analysis.

Filter paper activity of the commercial cellulase concentrate was measured according to Chemical Analysis and Testing Task Laboratory Analytical Procedure LAP-006 [16]. One unit of FPU was defined as the amount of enzyme required to liberate 1 μmol of glucose from Whatman No. 1 filter paper per minute at 50 °C. Cellobiase activity was assayed according to IUPAC procedures [17] but with some modifications in a reaction mixture containing 1 ml of 80 mM cellobiose solution (prepared in 0.05 M citrate buffer, pH 4.8) and 1 ml of appropriately diluted enzyme solution at 50 °C for 10 min. One unit of cellobiase activity (CBU) was defined as the amount of enzyme produced 2 μmol glucose per minute from cellobiose. The cellulase activity was 67 FPU mL^{-1} and the cellobiase activity was 137 CBU mL^{-1}.

5. WOP Composition

The cellulose content of WOP used was determined using the National Renewable Energy Laboratory (NERL) protocol "Determination of structural carbohydrates and lignin in biomass" [18]. The element content of WOP was determined by Atomic Emission Spectrum (ICP-AES, Varian 710 ES) at RF power 1.1 kW, plasma flow 15 L min^{-1}, auxiliary flow 1.5 L min^{-1}, nebulizer pressure 0.2 MPa, and pump rate 13 rpm.

RESULTS

1. Effects of Additives in WOP on Enzymatic Hydrolysis

The contents of cellulose and major metal compounds in WOP

Table 1. Major ingredients in WOP and the residue content (%, wt) after washing

	Cellulose	Xylan	Ca	Fe	Mg	Al	Mn	Cu
WOP	48.8	7.44	8.5	0.37	0.18	0.036	0.0026	0.00014
Washed with H_2O	/	/	4.6	0.48	0.081	0.022	0.0023	0.00015
Washed with H_2SO_4	/	/	0.16	0.37	0.059	0.020	0.0022	0.00010

are shown in Table 1. The cellulose content was approximately half of the total WOP weight (48.8%). The major additive was calcium compound, equal to 21.2% in $CaCO_3$ form, or 28.9% in $CaSO_4$ form. The other compounds included magnesium compounds, equal to 0.9% in $MgSO_4$; ferric compounds, equals to 1.3% in $Fe_2(SO_4)_3$; aluminum compounds, equal to 0.2% in $Al_2(SO_4)_3$. Manganese and copper compounds were only in trace concentration.

According to the office paper making references [8,9], the major calcium compounds as stuffing material or white pigment were calcium carbonate ($CaCO_3$) and calcium sulfate ($CaSO_4$). Table 1 shows that the calcium compound was reduced to half of the original when WOP was washed by deionized water; but when washed by sulfuric acid, the calcium compound content was almost removed completely. The result indicates that the major calcium compound in WOP should be in $CaCO_3$ form, which was reactive with H_2SO_4, instead of $CaSO_4$ which was inactive with H_2SO_4.

The inhibition performance of the additives in WOP on the enzymatic hydrolysis was investigated by observing the glucose hydrolysis yield from filter paper in the existence of these additives. The concentration range of the additives in the experiment was adjusted according to the WOP content as shown in Table 1.

Table 2 shows the effect of calcium compounds including $CaCO_3$, $CaSO_4$, and $CaCl_2$ on the filter paper hydrolysis using the cellulase enzyme Accellerase 1000. The results indicate that the addition of $CaCO_3$ sharply decreased the hydrolysis yield of filter paper from 0 to 0.05 g g^{-1} DM, but the further increase of $CaCO_3$ from 0.05 FPU g^{-1} DM to 0.2 FPU g^{-1} DM (close to the $CaCO_3$ content of original WOP, 21.2%) did not lead to a significant change in the hydrolysis yield. On the other hand, the addition of insoluble $CaSO_4$ did not change the hydrolysis yield and the pH value significantly when $CaSO_4$ was added. Similarly, the hydrolysis yield decreased but did not change significantly when the soluble $CaCl_2$ was added.

Fig. 1 shows the effect of pH value on hydrolysis yield of filter paper using Accellerase 1000. The result shows that the glucose yield reached the maximum at pH 4.5, closed to the optimal pH value of Accellerase 1000 (pH at 4.8) and decreased slightly from 4.0 to 5.5. The hydrolysis yield then decreased sharply when pH was above 6.0, in which the pH value was away from the optimal pH. Since Accellerase 1000 lacks sufficient cellobiase activity, the

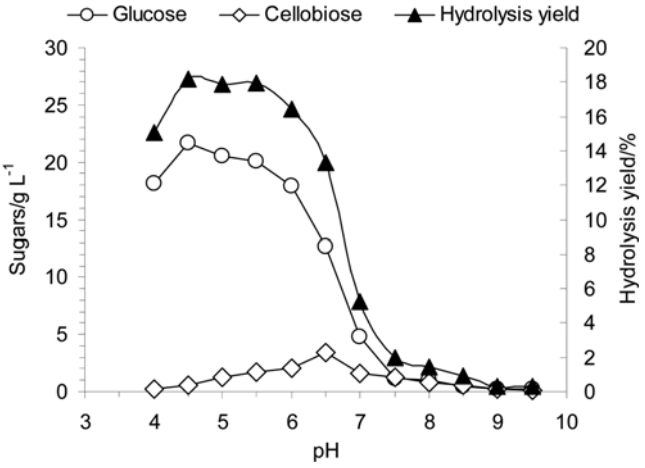

Fig. 1. Effect of pH on hydrolysis of filter paper. Conditions: 50 °C, 0.05 mol L^{-1} sodium acetate buffer was used in the pH range of 5.0-5.5; 0.05 mol L^{-1} NaH_2PO_4-Na_2HPO_4 buffer was used in the pH range of 6.0-8.0; 0.05 mol L^{-1} Gly-NaOH buffer was used in the pH range of 8.5-9.5. Accellerase 1000 of 12 FPU g^{-1} DM, 10% (wt) of Xinhua No. 1 filter paper, 200 rpm, 24 hours.

cellobiose is another natural product from the hydrolysis of filter paper. The cellobiose yield was increased with the pH rising from 4.5 to 6.0 and decreased afterwards. The conversion yield of cellulose to glucose and cellobiose was approximately constant at pH range of 4.5-5.5. The result of pH effect on hydrolysis yield was corresponding to the pH shift result in Table 2, in which the change of hydrolysis yield closely related to the pH change when different $CaCO_3$ was added.

The results in Table 2 and Fig. 1 indicate that the addition of calcium compounds to the hydrolysis of filter paper was complicated. The calcium compound itself did not change the hydrolysis yield directly or by increasing biocalcitrance for occupying the voids and coating the fibers as shown in the case of $CaSO_4$ and $CaCl_2$ addition, but through the pH shift of the hydrolysis system away from the optimal pH range of the cellulase activity.

Table 3 shows the effect of metal compounds including magne-

Table 2. Effect of calcium compound addition on hydrolysis of filter paper

$CaCO_3$/g g^{-1} DM	0	0.02	0.05	0.10	0.15	0.20
Glucose/g L^{-1}	21.7±0.5	16.4±0.4	14.0±0.1	12.9±0.5	13.3±0.4	12.6±0.3
Cellobiose/g L^{-1}	1.5±0.1	4.1±0.1	4.6±0.5	5.2±0.6	4.9±0.4	5.3±0.1
pH at starting	5.0	6.8	6.9	7.1	6.9	6.9
pH at ending	5.0	6.6	6.1	6.9	7.0	7.0
$CaSO_4$/g g^{-1} DM	0	0.02	0.05	0.10	0.15	0.20
Glucose/g L^{-1}	22.2±0.5	20.9±0.4	21.6±0.4	21.6±0.7	21.3±0.3	22.1±0.6
Cellobiose/g L^{-1}	1.3±0.0	1.0±0.0	0.9±0.1	1.0±0.2	1.1±0.1	1.1±0.0
$CaCl_2$/g g^{-1} DM	0	0.01	0.05	0.10	0.15	0.20
Glucose/g L^{-1}	19.6±2.1	19.2±1.3	17.7±0.8	16.4±1.0	16.7±0.1	17.2±0.8
Cellobiose/g L^{-1}	1.0±0.2	1.0±0.1	0.8±0.1	0.5±0.0	0.6±0.0	0.9±0.2

Conditions: 50 °C, initial pH 5.0, 0.05 mol L^{-1} sodium acetate buffer, Accellerase 1000 of 12 FPU g^{-1} DM, 10% (wt) of Xinhua No. 1 filter paper, 200 rpm, 24 hours

Table 3. Effect of metal ion and carbon powder on hydrolysis of filter paper

Fe^{3+}/g g^{-1} DM	0	0.0014	0.0028	0.0042	0.0056	0.0071
Glucose/g L^{-1}	19.6±0.3	19.9±0.1	17.8±0.6	17.3±0.2	15.6±1.1	15.6±0.1
Cellobiose/g L^{-1}	1.0±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0	0.7±0.0
Mg^{2+}/g g^{-1} DM	0	0.0022	0.0054	0.0108	0.0162	0.0216
Glucose/g L^{-1}	20.1±1.5	22.7±0.8	23.7±0.9	22.1±1.1	21.4±0.4	21.4±0.3
Cellobiose/g L^{-1}	1.2±0.0	1.3±0.0	1.0±0.1	0.9±0.2	0.9±0.0	1.0±0.1
Al^{3+}/g g^{-1}	0	0.00024	0.00047	0.00071	0.00095	
Glucose/g L^{-1}	21.1±0.9	21.2±0.4	19.4±0.7	18.0±1.0	18.3±0.3	
Cellobiose/g L^{-1}	1.1±0.0	1.0±0.1	0.8±0.1	0.4±0.1	0.3±0.1	
Toner/g g^{-1} DM	0	0.0005	0.0010	0.0015	0.0020	0.0025
Glucose/g L^{-1}	16.5±0.1	17.0±0.8	17.0±0.3	17.4±0.2	18.5±0.6	18.9±0.2
Cellobiose/g L^{-1}	1.2±0.0	1.3±0.0	1.3±0.0	1.3±0.0	1.4±0.0	1.5±0.0

Conditions: 50 °C, initial pH 5.0, 0.05 mol L^{-1} sodium acetate buffer, Accellerase 1000 of 12 FPU g^{-1} DM, 10% (wt) of Xinhua No. 1 filter paper, 200 rpm, 24 hours

sium, aluminum, and ferric ions on the hydrolysis yield of filter paper in the concentration range of the metal ion content in WOP. The result indicates that the hydrolysis yield increased slowly with increasing magnesium ion concentration, but increasing of magnesium concentration resulted in weaker enhancement; meanwhile, pH value was not changed (data not shown). However, the hydrolysis yield decreased slowly with increasing aluminum ion concentration. Similarly to aluminum ion, the effect of ferric ion decreased the hydrolysis yield but within a small range. The result shows that the effect of metal ions on the hydrolysis yield was in a small range in the experiment concentration, and in agreement with the inhibition effect of metal ions to cellulase enzyme [19-21].

The effect of fine carbon powder collected from the printer cartridge on the hydrolysis yield showed that the fine carbon powder had no negative effect on the hydrolysis yield (Table 3). On the contrary, it seemed that an enhancement tendency was observed on the enzymatic hydrolysis of filter paper, probably because of the dispersing property of fine carbon powder in WOP.

2. Pretreatment of WOP for the Removal of $CaCO_3$

The enhancement of WOP hydrolysis was focused on the removal of $CaCO_3$ or the conversion of $CaCO_3$ to insoluble calcium compound. Fig. 2(a) shows that the hydrolysis yield increased significantly with the acid washing, compared to that of no washing or washing by deionized water. The washing by H_2SO_4 and HNO_3 showed the best hydrolysis yield. Fig. 2(b) shows the results of presoaking using deionized water and different inorganic acids. Again, H_2SO_4 and HNO_3 treatment were the best choices, almost four-fold greater compared to that of water presoaking.

Comparing the two pretreatment methods, the direct washing method showed the better hydrolysis yield than that of the presoaking treatment. However, the acid use in the direct washing was almost ten-fold greater than that of the presoaking method, plus the mechanic power used in the mixing of WOP slurry in the direct washing pretreatment. Therefore the presoaking method was selected as the priority for the pretreatment of WOP. When different inorganic acids were used, sulfuric acid was selected because of its relatively weak corrosive property and volatility in the pretreatment processing.

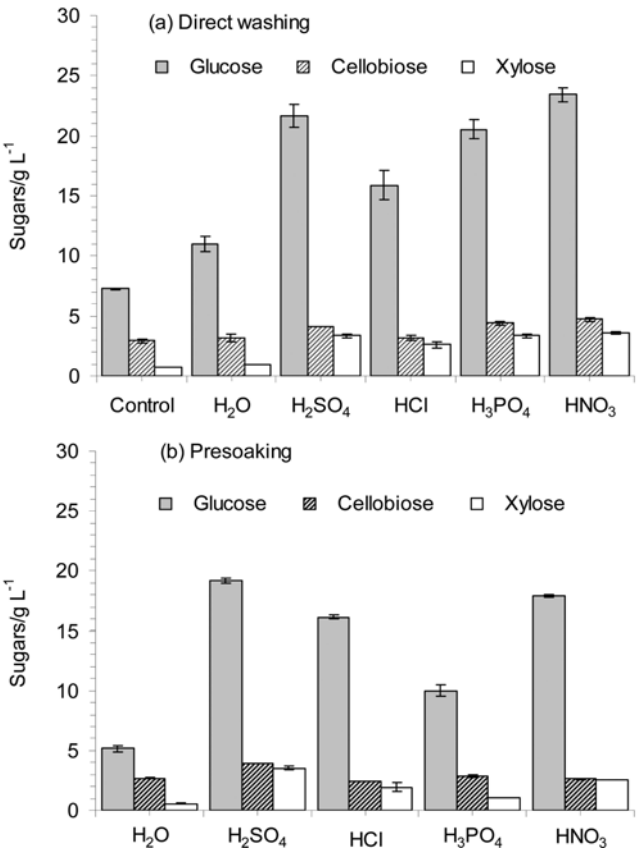

Fig. 2. Hydrolysis of WOP pretreated with different inorganic acids. Original WOP without any pretreatment was used as control. All the acids were 2.0 mol L^{-1} of proton concentration and neutralized to pH 5.0 before the hydrolysis. Hydrolysis conditions: 50 °C, initial pH 5.0, 0.05 mol L^{-1} sodium acetate buffer, Accellerase 1000 of 12 FPU g^{-1} DM, solid content 10% (wt), 200 rpm, 24 hours. (a) Direct washing by dilute acids or water; Procedures see Methods; (b) Presoaking by dilute acids. 50 °C for 12 hours; liquid to solid ratio (L/S) was 1.5 : 1. Hydrolysis of WOP without any pretreatment was control.

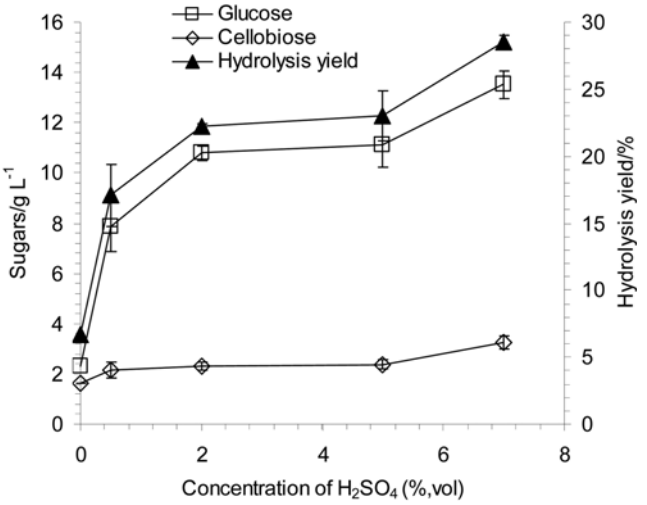

Fig. 3. Effect of H_2SO_4 usage in the presoaking on WOP hydrolysis. Presoaking conditions: 50 °C for 12 hours; H_2SO_4 was neutralized to pH 5.0 before the hydrolysis; liquid to solid ratio (L/S) was 1.5 : 1. Hydrolysis conditions: 50 °C, initial pH 5.5, the hydrolysis was carried out in deionized water with Accellerase 1000 of 12 FPU g^{-1} DM, solid loading 10% (wt), 200 rpm, 24 hours.

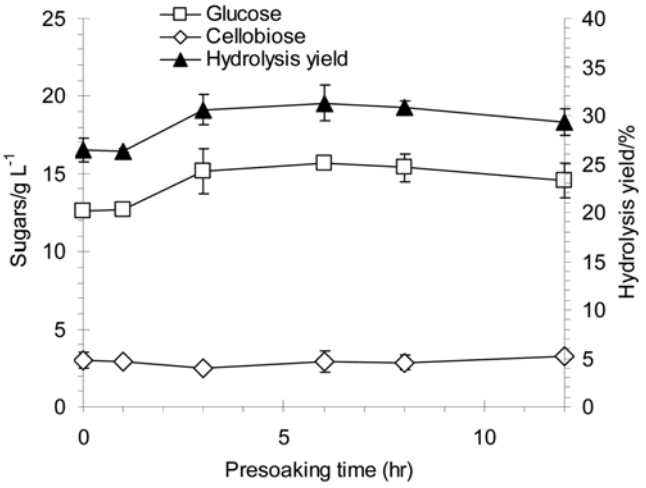

Fig. 5. Effect of presoaking time on WOP hydrolysis. Presoaking conditions: 7% (vol) H_2SO_4 at 50 °C; H_2SO_4 was neutralized to pH 5.0 before the hydrolysis; liquid to solid ratio (L/S) was 1.5 : 1. Hydrolysis conditions: 50 °C, initial pH 5.0, the hydrolysis was carried out in deionized water with Accellerase 1000 of 12 FPU g^{-1} DM, solid loading 10% (wt), 200 rpm, 24 hours.

3. Optimization of Presoaking Treatment on WOP

The presoaking conditions for WOP processing such as presoaking sulfuric acid dosage, time, temperature, and liquid to solid ratio (L/S) were tested for achieving optimal hydrolysis yield from WOP. Fig. 3 shows the effect of H_2SO_4 amount used in the presoaking on the hydrolysis yield at the fixed liquid to solid ratio (1.5 : 1). The hydrolysis yield increased with increasing H_2SO_4 amount and reached the maximum at the concentration 7.0% (vol) which was equal to 0.2 g g^{-1} DM. The value equaled the H_2SO_4 amount reacting with the $CaCO_3$ (21.2%, wt) completely.

At the same H_2SO_4 addition, the ratio (wt) of liquid (water or H_2SO_4 solution) to solid (WOP dry base) in the presoaking on the hydrolysis yield was tested and the result in Fig. 4 shows that the L/S ratio had no significant effect on the hydrolysis yield in a wide range until the L/S ratio was greater than 5. Fig. 5 shows that the hydrolysis yield of WOP increased with the prolonged presoaking time and reached a maximum after 3 hours' presoaking, and did not increase further with increasing presoaking time.

Fig. 6 shows the effect of presoaking temperature on the hydrolysis yield of WOP, and it was found that the temperature only had

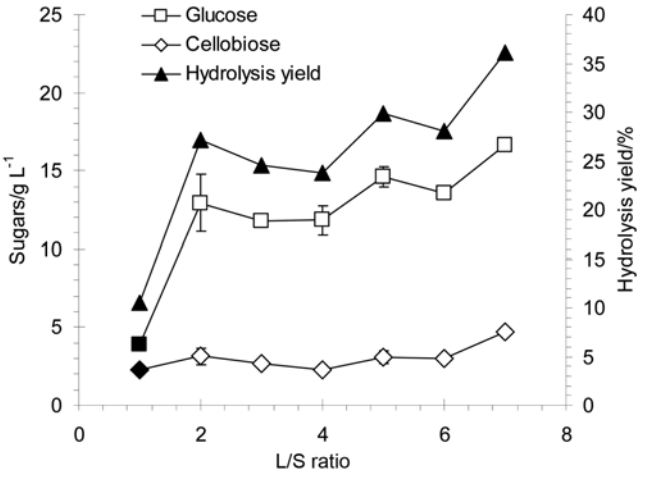

Fig. 4. Effect of liquid to solid ratio (L/S) on WOP hydrolysis. Presoaking conditions: 7% (vol) H_2SO_4 at 50 °C for 12 hours; H_2SO_4 was neutralized to pH 5.0 before the hydrolysis. Hydrolysis conditions: 50 °C, initial pH 5.0, the hydrolysis was carried out in deionized water with Accellerase 1000 of 12 FPU g^{-1} DM, solid loading 10% (wt), 200 rpm, 24 hours. Hydrolysis of WOP without any pretreatment was control.

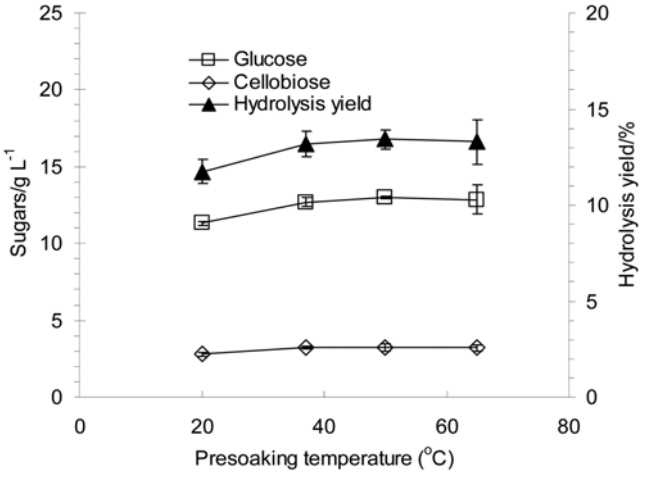

Fig. 6. Effect of presoaking temperature on WOP hydrolysis. Presoaking conditions: 7% (vol) H_2SO_4 for 12 hours; H_2SO_4 was neutralized to pH 5.0 before the hydrolysis; liquid to solid ratio (L/S) was 1.5 : 1. Hydrolysis conditions: 50 °C, initial pH 5.5, the hydrolysis was carried out in deionized water with Accellerase 1000 of 12 FPU g^{-1} DM, solid loading 10% (wt), 200 rpm, 24 hours.

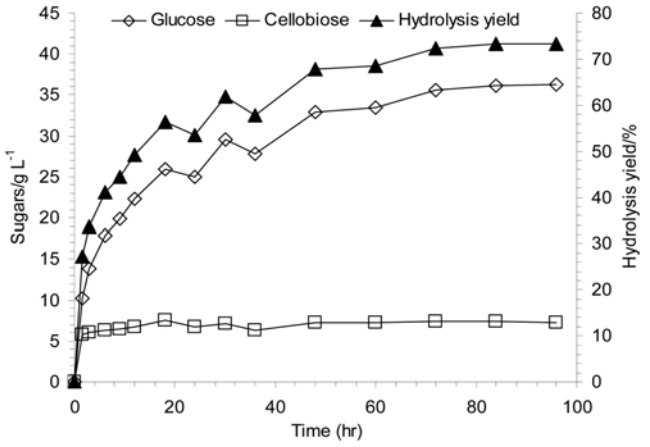

Fig. 7. Hydrolysis of WOP pretreated at optimal condition. Presoaking conditions: 7% (vol.) H_2SO_4 at room temperature for 3 hours, liquid to solid ratio (L/S) was 1.5 : 1, H_2SO_4 was neutralized to pH 5.0 before the hydrolysis. Hydrolysis conditions: 50 °C, initial pH 5.0, the hydrolysis was carried out in acetate buffer (0.05 mol L^{-1}, pH 5.0) with Accellerase 1000 of 12 FPU g^{-1} DM at a 2-L fermenter with a helix impeller and the working volume is 1 L, 200 rpm, 96 hours.

a minor effect on the yield in the experimental temperature range.

The pretreatment efficiency was verified in a 2-L bioreactor at the optimal condition obtained in the above experiments, and the result is shown in Fig. 7. As a result, the final glucose and cellobiose concentrations were 36.2 g L^{-1} and 7.2 g L^{-1}, respectively, equal to 73.3% of WOP cellulose conversion. This result was much higher than other reported literatures. Kojima and Yoon [10] treated the copier paper by ozone pretreatment and improved the extent of hydrolysis only from 37% to 52%; Wu et al. [14] enhanced enzymatic saccharification of waste newsprint by surfactant addition and maximum conversion reported was 56.5% after 123.5h; Park et al. [15] hydrolyzed the waste paper without additional reagents and the cellulose conversion was only 42.2%.

DISCUSSION

Hydrolysis of waster office paper (WOP) into fermentable sugars is an important option of WOP utilization. The hydrolysis concerned many factors related to the WOP components and functional chemicals added during the paper making were the major factors. These chemical compounds composed a large percentage of total WOP weight, for instance, calcium carbonate powder takes 21% of total WOP weight (Table 1). The additives affected the hydrolysis yield significantly by inhibiting the cellulase activity or shifting the pH value of the hydrolysis environment away from the optimal pH range of cellulase enzyme.

In present work, the effect of major chemical additives on the WOP hydrolysis using industrial cellulase enzymes was investigated by adding chemicals to shredded filter paper additionally, and we found that calcium carbonate ($CaCO_3$) content influenced the enzymatic hydrolysis significantly. Then methods to overcome the additive effect for WOP treatment were tested and it was found that presoaking of WOP with sulfuric acid was the most practical and effective way. The conditions of presoaking were studied carefully and the hydrolysis efficiency was improved significantly.

To obtain higher sugar concentration for the consequent potential process, WOP hydrolysis at a high solid loading (10% wt) was applied and the concentration of glucose and cellobiose was 36.2 g L^{-1} and 7.2 g L^{-1}, respectively. The existence of considerable cellobiose in the WOP hydrolysate was due to inhibition of glucose produced to the cellulase enzyme. Although the cellobiase activity in Accellerase 1000 was 137 CBU per ml of the enzyme volume and approximately 21.6 CBU per gram of WOP (dry base), the glucose concentration increased with the hydrolysis proceeding and reached above 25 g L^{-1} after 24 hours, 30 g L^{-1} after 48 hours, and 72 hours finally. Glucose is a strong inhibitor to cellobiase and the high glucose concentration in the WOP hydrolysis system inhibited the cellobiose conversion to glucose. However, if the WOP hydrolysate is used as the fermentation nutrient, the glucose will soon be converted into ethanol or other fermentation products, depending on the type of fermentation used. With the removal of inhibitory glucose, cellulose could be converted to glucose rapidly and then join the fermentation metabolism [5]. Accounting for both glucose and cellobiose produced in the prolonged hydrolysis, the conversion yield of cellulose to glucose and cellobiose in WOP was equal to 73.3% of cellulose conversion in the continuous hydrolysis in the bioreactor (Fig. 7).

Further studies are still needed for developing a hydrolysis process using WOP as feedstock. Some problems require further experimentation including the effect of different WOP material, different chemical additives other than the ones listed in the paper, such as gluey, organic dyes, on the hydrolysis efficiency, and simultaneous saccharification and fermentation for the removal of glucose or cellobiose inhibitions for production of ethanol or other chemicals using WOP as feedstock. In general, the present work provided the WOP utilization with a practical and effective pretreatment method for the enzymatic hydrolysis to get the fermentation level sugars, and could be developed as a potential industrial process.

Recent studies have shown that higher oligo-sugars were inevitably present as in the hydrolysate of various cellulose hydrolysis [20-22]. In the WOP hydrolysis, oligo-sugars should also exist in a considerable concentration in the prolonged hydrolysis of WOP (Fig. 7), because the accumulated glucose and cellobiose up to 40 g L^{-1} certainly inhibited the cellulase activity and the conversion yield of these oligo-sugars to glucose was reduced.

In the present work, only glucose and cellobiose were considered as the products of the WOP hydrolysis, because the focus of the present study was to find the key factor to influence the hydrolysis yield of WOP. Most of the experiments in the present work were performed at a low concentration of oligo- or mono-saccharides, and would not generate significant inhibition for assisting oligo-sugar formation. On the other hand, the oligo-sugars as well as cellobiose produced during the prolonged hydrolysis of WOP could be further converted into glucose in the following fermentation step when the accumulated glucose is transformed into fermentation product (such as ethanol by yeast) and the inhibition to the cellulase enzyme was released [23,24].

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