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The effect of enzymatic hydrolysis of pretreated wastepaper for bioethanol production

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Abstract−Enzymatic hydrolysis of waste biomass for bioethanol production is considered a decades old traditional, inexpensive, and energy-effective approach. In this study, waste office paper was pretreated with diluted sulfuric acid (H_2SO_4) and hydrolyzed with one of the most available and cost-effective enzymes, cellulase derived from *Trichoderma reesei,* under submerged static condition. Three different pretreatment approaches--cut into 2 cm^2, blended with distilled water, and pretreated with diluted H_2SO_4--have been implemented, and pretreatment with diluted H_2SO_4 was the most effective. Hydrolysis with different concentrations--0.5 M, 1.0 M, 1.5 M, 2.0 M of H_2SO_4--was performed. The maximum glucose content was obtained at 2.0 M H_2SO_4 at 90 min reaction time, and glucose yield was 0.11 g glucose/g wastepaper. The cut paper, wet-blended, and acid-treated wastepaper was hydrolyzed with cellulase enzyme for 2, 4, and 5 consecutive days with 5 mg, 10 mg, 15 mg, and 20 mg enzyme loadings. The maximum glucose content obtained was 9.75 g/l from acid-treated wastepaper, after 5 days of enzymatic hydrolysis with 20 mg enzyme loading and a glucose yield of a 0.5 g glucose/g wastepaper. The wastepaper hydrolysate was further fermented for 6, 8, and 10 hours continuously with *Saccharomyces cerevisiae* (yeast), and at 10 hours of fermentation, the maximum glucose consumption was 0.18 g by yeast. Further, HPLC analysis of the fermented medium presented a strong peak of bioethanol content at 16.12 min. The distillation of bioethanol by rotary evaporator presented 0.79 ml bioethanol/fermented solution, which indicated the conversion efficiency of 79%.

Keywords: Acid Pretreatment, Bioethanol from Wastepaper, Enzymatic Hydrolysis, Wastepaper Utilization

INTRODUCTION

Power generation of this modern era has been predominantly dependent on fossil fuels (non-renewable energy resources) such as coal, petroleum, natural gas. Approximately 80% of global energy demand by the power and transportation sector depends on petroleum derivatives [1]. Due to the fast population growth, industrialization and urbanization, technological advancements, and rocketing economic development within the last decades, global energy demand has reached its peak, including Malaysia. In Malaysia, the most applied energy sources are coal and cokes, raw petroleum, oil-based commodities, flammable gas, and hydropower. One of the latest survey reports revealed that more than 90% of energy sources are from non-sustainable power resources. Hydropower and biofuel have minimal practical applications [2]. Consequently, fossil fuel sources are depleted rapidly, and the quest for renewable energy is being harnessed in the country day by day.

Among various renewable energy sources, bioethanol has been preferred due to its ideal fuel characteristics: high fuel purity, good ignition, and flammability, easily blending capability with gasoline (maximum 90%), wide variety and availability of feedstock, including waste biomass with low price and conventional technology, application in existing vehicles without engine modification as well as minimum exhalation of greenhouse gases [3]. Worldwide bioethanol generation and implementation have been expanded significantly lately. The latest survey presented that bioethanol generation rose from 18.2 billion liters in 2000 to 60.6 billion liters in 2007. Overall, bioethanol production is exemplified by generating 85 billion liters of bioethanol in 2011. In 2015, the worldwide production of bioethanol was 96.9 billion liters and 110 billion liters in 2019. USA, Brazil, China, Canada, India, Thailand, Argentina, the European Association, and 28 different nations have made policy on mixing bioethanol with gasoline [4,5]. One of the most significant key factors behind bioethanol production is the availability of its raw materials. To avoid a clash with the food and feed chain, waste biomass is preferred for this purpose [5].

Due to the waste management as well as application of circular economy, municipal solid waste (MSW) has caught attention from government and non-government sectors in Malaysia recently. In Malaysia, MSW production amount is 0.5-0.8 kg/singular/day recently cross-country and 1.7 kg/singular/day for critical metropolitan networks. Over the previous decade, the MSW generation in Malay-

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sia has expanded by over 91%. MSW management in Malaysia is impoverished and disordered. 85% of the total MSW was dumped into the open environment. The MSW removal strategy in Malaysia is mostly practiced through landfilling due to the inexpensive waste handling [6]. Therefore, it is evident that the MSW recycling or re-using for valuable product generation will solve MSW waste management besides boosting the circular economy of the country. According to the survey report, 20% of 7.34 million tons of MSW is almost wastepaper, and the total weight of this wastepaper is approximately 1.5 million tons [6]. Hence, wastepaper can possibly be utilized as an excellent and available feedstock for bioethanol generation with a minimum price (average $55/ton) [7]. Therefore, utilizing wastepaper for bioethanol production can significantly facilitate the waste management section in Malaysia. Wastepaper can be classified into several categories: cupboard, magazine paper, office paper, regular paper, and others. Every category contains approximately 50%-70% sugar content, which can be applied for bioethanol generation. Subsequently, wastepaper can add value as a feedstock for bioethanol production [6,8].

Different experimental studies were conducted earlier on various types of wastepaper to produce bioethanol. A previous study demonstrated that bioethanol could be generated from waste newspapers by extracting cellulose, and later, it was processed into sugar for bioethanol production using different bacteria. This study also outlined that a maximum of 55% cellulose can be extracted to synthesize bioethanol from biological hydrolysis. The yield of bioethanol was around 7% (v/v) [9]. Another experimental study on wastepaper hydrolysis demonstrated that waste office paper and newspaper are excellent raw materials for releasing very high glucose yield for bioethanol production [7]. This study also presented favorable hydrolysis efficiency, which was 91.8% and 79.6% for office paper and newspaper, respectively, enhancing the yield of bioethanol production from wastepaper [7]. Another study provided a brief discussion of bioethanol production's sensitivity analysis from various waste papers considering economic feasibility. The finding represented a 25% selling price reduction in bioethanol production using sensitivity analysis considering all parameters. This study also mentioned that bioethanol's selling price from the waste newspaper, office paper, and cardboard paper is competitive to petrol [10]. A previous study [8] outlined the optimized acid hydrolysis of wastepaper to produce bioethanol and ensured the purity degree of 90% (v/v) [8]. This study presented a brief review of the current condition of wastepaper for bioethanol production. This study also demonstrated that after demineralizing lignified waste papers, increased content of carbohydrates and lignin was observed. In contrast to lignified waste, the demineralization of the bleached waste papers, e.g., office paper, leads to increased content only of carbohydrates, mainly of cellulose [8].

Previous experimental studies on bioethanol production from wastepaper experimented mostly on mixed wastepaper, newspapers, cardboard, or packaging paper. In contrast, the current study emphasized the office paper from MSW. Besides, the previous studies of bioethanol from wastepaper were hydrolyzed by different enzymes. A previous experimental study demonstrated bioethanol production from wastepaper pulp hydrolyzed with xylose-fermenting *Pichia stipites*. The novelty of this experimental study is that this study utilized the most available and less expensive cellulase enzyme extracted from *Trichoderma reesei* while other studies used different expensive enzymes. Besides, this study investigated three different pretreatment (size reduction) methods: (i) cut wastepaper, (ii) wet-blended wastepaper, and (iii) acid (diluted H_2SO_4) pretreated wastepaper and compared the glucose yield, which was demonstrated in earlier experimental studies. The main objectives of this experimental study are (i) to determine the most efficient pretreatment method and favorable reaction period for the hydrolysis of wastepaper, (ii) to determine the effect of pretreatment conditions towards enzymatic hydrolysis, (iii) to assess the effect of enzyme loading to enzymatic hydrolysis, (iv) to identify the glucose content released after pretreatment and enzymatic hydrolysis, and (v) to measure the bioethanol conversion efficiency from wastepaper after yeast fermentation.

MATERIALS AND METHODS

1. Materials

Raw material: Wastepaper used for official purposes. Solvents: distilled water, reverse osmosis (RO) water, sulfuric acid (H_2SO_4). Chemicals: sodium hydroxide (NaOH), 3,5-dinitrosalicylic acid (DNS), sodium acetate ($C_2H_3NaO_2$), potassium hydrogen phosphate (KH_2PO_4), ammonium chloride (NH_4Cl_2), and D-glucose monohydrate ($C_6H_{14}O_7$). Biological compounds: cellulase enzyme derived from *Trichoderma reesei* and yeast (*Saccharomyces cerevisiae*).

2. Methods

The methods of this study demonstrated the feedstock preparation, pretreatment methods, and comprehensive investigation of enzymatic hydrolysis and fermentation, required materials and instruments, suitable conditions, and techniques. Duplicates were carried out for all the experimental steps, and average results have been considered.

2-1. Preparation of Wastepaper

The wastepaper (office paper) was collected from the MSW plant located at Klang Valley, Malaysia. Waste office paper was collected for experimental purposes. The collected wastepaper was cut into approximately 2 cm^2. The wastepaper was pre-treated through three different pre-treatment (size reduction) methods: (i) cut wastepaper, (ii) wet-blended wastepaper, and (iii) acid (diluted H_2SO_4) pretreated wastepaper. The waste office paper was categorized into three different patterns to determine the effect of pre-treatment on the paper waste for glucose yield analysis and possible bioethanol production. 400 g cut wastepaper was blended with distilled water. The wet-blended wastepaper was dried into a boiler around 70 °C for 24 hours and preserved in an air-tight jar for further experiments.

2-2. Diluted Acid Pretreatment

4 g of three categories of wastepaper was placed into conical flasks. 100 ml of 0.5 M sulfuric acid (H_2SO_4) was added to the flasks. Duplicates were prepared, and the flask was heated in a water bath at 90 °C for different reaction times, 30, 60, and 90 minutes, separately. The flasks were left at room temperature for cooling. 200 ml of 0.5 M sodium hydroxide (NaOH) was added to the mixture to neutralize the acidic environment and shaken vigorously. The acid pretreatment was also conducted for 1.0 M, 1.5 M, and 2.0 M sul-

furic acid and sodium hydroxide separately. The supernatant for each sample was assembled into a rotator tube. The axis tubes were centrifuged at 4,000 rpm for 10 minutes. The supernatants proceeded for the 3,5-dinitrosalicylic acid (DNS) test. Atomic absorbance spectrometry (AAS) was done to determine glucose content at wavelength A540. The optical density (OD) for each sample was recorded. The glucose yield was calculated by the standard method by using OD.

2-3. Enzymatic Hydrolysis by Cellulase

Cellulase enzyme derived from *Trichoderma reesei* was obtained from Sigma Aldrich, United States. Cellulase enzyme from *Trichoderma reesei* was used in this study to catalyze the degradation of cellulose into glucose monomer units (cellulolysis) in a concerted manner. This enzyme acts on reducing and non-reducing ends to release free glucose in a catalytic manner. Cellulose enzyme initiates the hydrolysis of internal linkages and synergistically to bring about efficient cellulose hydrolysis [11]. 2 g of diluted acid pretreated wastepaper was placed into conical flasks. 100 ml of sodium acetate ($C_2H_3NaO_2$) solution was added to each solution to maintain the neutral pH. 5 mg, 10 mg, 15 mg, and 20 mg of cellulase enzyme was loaded into separate flasks, and the flasks were set in incubator shaker continuously for 2, 4, and 5 days at 37 °C and 100 rpm. 1.0 M NaOH and 1.0 M H_2SO_4 were added into the solution to adjust the pH, and the flasks were covered with cotton and aluminium foil. The flasks were placed into the incubator shaker at 37 °C, 150 rpm for 48-72 h. In the interval time of 12-24 h, the samples were taken for the glucose test. After 72 h, the samples were removed from the incubator shaker; the solid and solution of the flask were separated by a filter paper. The solution was preserved in a chiller for further experiments.

2-4. Yeast Culture Preparation and Fermentation of Acidic Hydrolysate

2-4-1. Yeast Culture Preparation

S. cerevisiae (yeast) was cultured earlier to perform the fermentation process and metabolize glucose to bioethanol. For this study, the fermentation process continued for 10 h continuously, confirming maximum yeast growth by consuming glucose containing the solution. All equipment was sterilized in an autoclave at 121 °C for 15 min. A yeast solution combining of 1 g cultured yeast extract, 0.4 g potassium hydrogen phosphate (KH_2PO_4), 0.2 g of ammonium chloride (NH_4Cl_2), and 100 ml of reverse osmosis (RO) water was prepared. Then the preserved glucose solution after acid hydrolysis and enzymatic hydrolysis was combined for the fermentation process and sterilized at 121 °C for 15 min. 10 g of D-glucose monohydrate ($C_6H_{14}O_7$) solution was transferred into three flasks separately to run triplicates. The mixture was mixed vigorously with a magnetic stirrer, sealed with cotton and aluminum foil to maintain anaerobic conditions. Flasks were placed inside the incubator shaker at 37 °C and 150 rpm for 10 h. Fermentation temperature was set at 37 °C since 37 °C was favorable to enhance cellulase activity during enzymatic hydrolysis and fermentation in a previous study [12]. Samples were taken for the first 6 h and alternative 2 h until 10 h and centrifuged. After centrifugation, the supernatants tested with DNS test for glucose and OD for glucose content and yeast were obtained by AAS at wavelengths A540 and A640 nm, respectively.

2-4-2. Fermentation of Enzymatic Hydrolysate

After enzymatic hydrolysis, the enzymatic hydrolysate was filtered and centrifuged. 15 g of filtered hydrolysate was mixed with the yeast broth (10 : 1 ratio of filtrate and yeast) into a flask, and a duplicate was prepared. pH of the mixture has been maintained at 5.5 by adding H_2SO_4 and NaOH. The flasks were sealed with cotton and aluminum foil and autoclaved for 15 min. The flasks were set into an incubator shaker at 37 °C and 100 rpm for 2 and 4 days of fermentation. After fermentation, the solution was centrifuged and tested with DNS test for glucose, and OD for glucose content was obtained by AAS at wavelengths A540.

2-5. Determination of Glucose Content and Yield Analysis

Samples of glucose solution were taken in an Eppendorf tube, centrifuged by a laboratory bench top centrifuge machine at 10,000 rpm for 5 min for complete separation of the solid and liquid phase. 1 ml of upper liquid was transferred into a 50 ml centrifuge tube, and 49 ml distilled water was poured into the tube for dilution purposes. The tube was shaken well to mix the solution thoroughly. The 2 ml solution for each tube was transferred to several test tubes further. 2 ml of 3, 5-dinitrosalicylic acid (DNS) reagent was added to glucose analysis. All test tubes were covered with paraffin and immersed into a water bath at 90 °C for 5 min. Then the samples were observed by the laboratory UV spectrophotometer with a wavelength of 540 nm.

Diluted sulfuric acid (H_2SO_4) acid pre-treatment was applied due to method simplicity and low cost for primary depolymerization of banana stem. Along with that, this treatment does not require high-tech sophisticated equipment [13]. In previous studies, acid treatment was more effective than alkali hydrolysis on banana stem delignification [14]. This experiment was performed using DNS reagent, and the reading was obtained from the spectrophotometer at a wavelength of 540 nm. The molarity of H_2SO_4 was 0.5 M, 1.0 M, 1.5 M, and 2.0 M. The hydrolysis period taken for this treatment was 60 min and 90 min.

Glucose yield of diluted acid pretreatment was obtained by OD using the standard method, while the actual glucose yield was calculated by eliminating dilution from real glucose obtainment. The actual glucose yield was calculated by Eq. (1).

$$Y_{actual\ glucose} = \frac{OD}{5.0785} \times 20 \tag{1}$$

where, $Y_{actual\ glucose}$=Yield of actual glucose, OD=Optical Density

The total mass concentration was determined by Eq. (2).

$$\rho_{total} = \frac{M_{experimented\ wastepaper}\ (g)}{V_{diluted\ acid}\ (ml)} \times \frac{1{,}000\ ml}{1\ l} \tag{2}$$

where, ρ_{total}=total mass concentration (g/l), $M_{experimented\ wastepaper}$=mass of experimented wastepaper (g), $V_{diluted\ acid}$=volume of diluted acid (ml)

The OD of each sample collected from the 2nd, 4th, and 5th day was obtained through a spectrometer, and the glucose yield was calculated using the linear equation from the glucose standard curve, y=5.0785x. The dilution factors used for the 2nd, 4th, and 5th days were 10, 20, and 25, respectively. Eq. (3) is used to calculate the glucose yield of each sample.

$$Y_{glucose} = \frac{OD}{5.0785} \times D_f \quad (3)$$

where, $Y_{glucose}$=Yield of glucose, OD=Optical Density, D_f=Dilution factor

The glucose yield percentage (%) has been determined by Eq. (4).

$$Y_{glucose}\,(\%) = \frac{Y_{actual\,glucose}\,(g/l)}{\rho_{total}\,(g/l)} \times 100\% \quad (4)$$

Where, Yglucose (%)=Yield of glucose in %

2-6. HPLC Analysis and Distillation

The sample after fermentation was taken for high-performance liquid chromatography (HPLC) test for bioethanol analysis. SUPELCOGEL C-610H, 30 cm×7.8 mm column (Sigma-Aldrich Co., United States) was for HPLC analysis. The column temperature was 80 °C, and 5 mM sulfuric acid (pH 2.2 unadjusted) was prepared as the mobile phase. The flow rate was 1.2 ml/min, and the injection amount was 25 µl for the HPLC test. The bioethanol content was tested by the standard method. The sample solution was distilled by using a rotary evaporator to obtain the bioethanol. For the distillation process, the heating temperature in the rotary evaporator was set between 65-75 °C. 100 ml of the fermented solution was placed into the rotary evaporator flask. The vacuum pressure was set at 0.8 kPa. The distillation process was conducted around 40 min under vacuum conditions. After distillation, bioethanol was measured by measuring cylinder.

RESULTS AND DISCUSSION

Fig. 1 presents the steps of bioethanol production from wastepaper via different production methods.

According to Table 1, glucose yield was enhanced with the increase of reaction time for different acid concentrations. For different acid concentrations, the glucose yield of the pretreated wastepaper increased with the increase of concentration. Higher glucose yield represents the higher effectiveness of the pretreatment method. The mechanism behind higher glucose release is the higher accessibility to the cellulose caused by different pretreatment methods. Table 1 also demonstrates no reduction of glucose, while both parameters, acid concentration and reaction time, increased. Therefore, it is evident that no glucose degradation had taken place within the experimented range of parameters in this study. Therefore, no formation of inhibitors was expected during enzymatic hydrolysis. Thus, the operating conditions were observed effective within the acceptable range, and all pretreatment methods have been manifested as efficient for the higher accessibility of enzymatic hydrolysis to cellulose. The maximum actual glucose yield was obtained as 4.50 g/l at 2.0 M H_2SO_4 concentration at 90 min reaction time. The actual glucose yield, 4.5053 g/l, was obtained from 4 g of wastepaper in 100 ml of H_2SO_4. The percentage (%) of glucose yield for

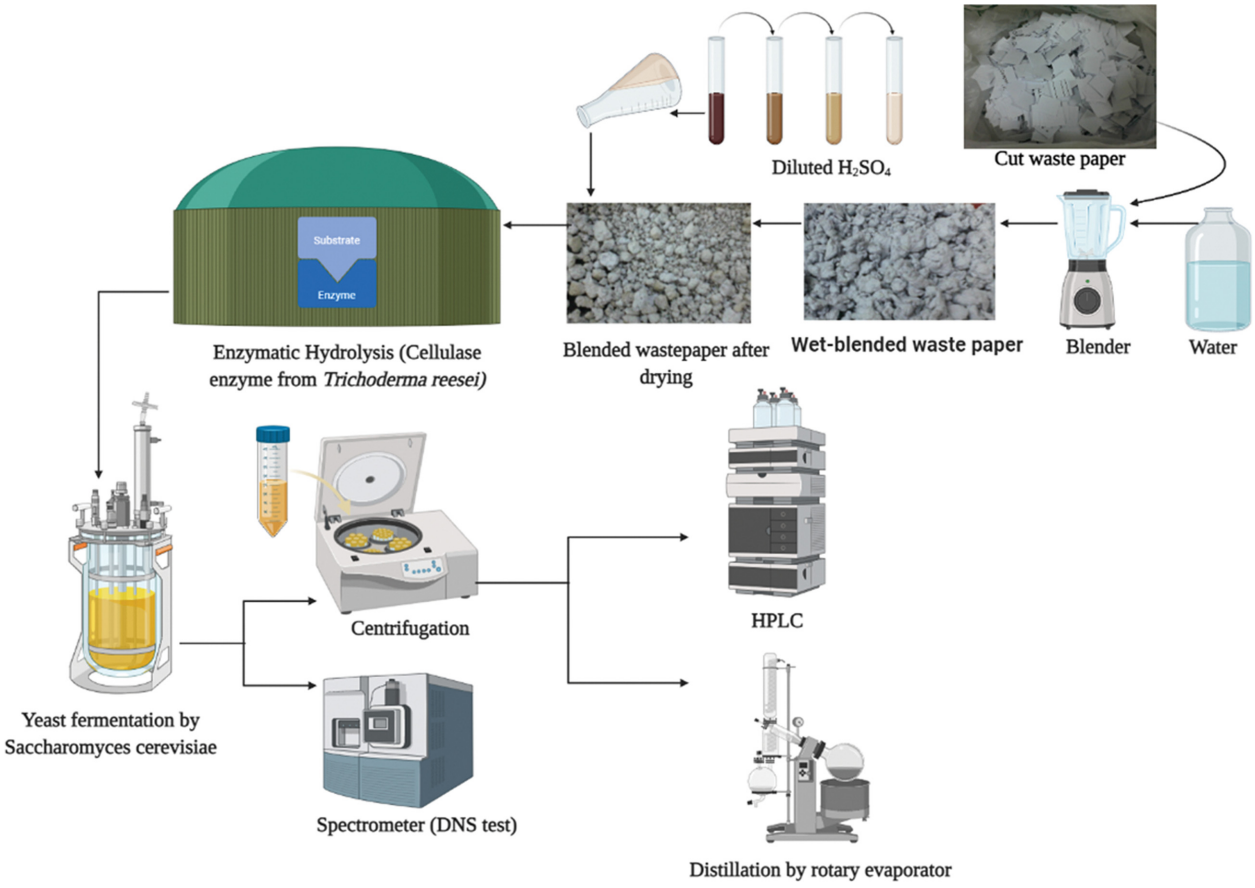

Fig. 1. Different steps of hydrolysis for bioethanol production from wastepaper (drawn by authors via BioRender.com).

Table 1. Glucose yield obtained from diluted acid pre-treatment

H_2SO_4 concentration (M)	Time (minutes)	Optical density	Glucose yield (g/*l*)	Actual glucose yield (g/*l*)
0.5	30	0.004	0.008	0.0158
	60	0.121	0.238	0.4765
	90	0.300	0.591	1.1815
1.0	30	0.131	0.258	0.5159
	60	0.402	0.792	1.5831
	90	0.553	1.089	2.1778
1.5	30	0.433	0.853	1.7052
	60	0.736	1.449	2.8985
	90	0.940	1.851	3.7019
2.0	30	0.579	1.140	2.2802
	60	0.847	1.668	3.3356
	90	1.144	2.253	4.5053

wastepaper pre-treated with 2.0 M H_2SO_4 at 90 minutes is 11.26%. Thus, 1 g of wastepaper can produce 0.11 g of glucose. A previous experimental study on bioethanol production from office paper and newspaper demonstrated that office paper and newspaper treated with 1 M diluted H_2SO_4 presented 8.06 and 5.29 g/*l* glucose yield, respectively. Compared to this study, wastepaper hydrolysis presented less glucose yield at 1 M diluted H_2SO_4, which is 2.17 g/*l*. The previous studies identified that office paper hydrolysis presented a higher glucose yield compared to the newspaper with different pretreatment methods. Therefore, it can be stated that the type and texture of wastepaper are also crucial factors for glucose conversion. Moreover, an earlier study also presented that different types of solvents such as hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH), phosphoric acid (H_3PO_4) during pretreatment can also play significant role to enhance glucose yield [7].

Table 2 shows the glucose yield for different enzyme loadings in enzymatic hydrolysis. The glucose yields by enzymatic hydrolysis with a higher dose of enzyme loading were observed as the maximum yield on any day of hydrolysis. This outcome indicated that the higher enzyme loading dosage was, the faster enzymatic hydrolysis produced glucose from the cellulosic wastepaper. However, the glucose yields also showed that glucose yield speeded up faster till 15 mg of enzyme loading, while after 15 mg enzyme loading, the glucose yield did not speed up significantly. The difference between glucose content at 15 mg and 20 mg loading is very slight for each day. Hence, it was crystal clear that the ratio between substrate loading to enzyme loading, 2 : 15, was the optimum ratio for faster glucose yield. Table 2 also shows that the glucose yield for 20 mg enzyme loading is almost constant for day 4 and day 5 but not for another enzyme loading. Thus, the results showed that the enzymatic hydrolysis was completed within this time, and maximum glucose yield was obtained. The maximum glucose yield was 9.76 g/l, which was around 50% from acid pre-treated wastepaper or 1 g of acid pretreated wastepaper produced 0.5 g of glucose.

Table 2. Glucose yield for different enzyme loadings of enzymatic hydrolysis

Time	Enzyme loading (mg)	Optical density	Glucose yield (g/*l*)
Day 2	5	1.479	2.9123
	10	1.826	3.5955
	15	2.329	4.5860
	20	2.439	4.8026
Day 4	5	0.906	3.5680
	10	1.826	7.1911
	15	2.329	9.1720
	20	2.439	9.6052
Day 5	5	0.746	3.6723
	10	1.545	7.6056
	15	1.747	8.6000
	20	1.982	9.7568

The previous experimental study presented high enzyme dosage enhanced glucose content during hydrolysis for untreated paper, but reduced glucose yield for H_2SO_4 treated paper [15]. Another study demonstrated that the sugar yield was substantially increased with enzyme loading, 1-3% of total solid, but started to decrease with 4% solid loading for office paper and newspapers. The maximum glucose yield obtained for office paper and newspaper was 23.48 g/l and 13.12 g/l, respectively, at 3% solid (enzyme) loading and the minimum glucose yield was 8.82 g/l and 4.3 g/l, respectively, at 1% solid (enzyme) loading. The diminished sugar yield with 4% strong loading because of hard blending and enzyme dosage causes the solids to stay unblemished during hydrolysis [7]. Several reports recommended that the high enzyme loading essentially diminishes the sugar yield as the consistency of the biomass framework increments suddenly at high enzyme loadings, which influences the uniform blending and mass exchange of the enzymes and furthermore brings about feedback inhibition by the enhanced concentration of sugars [7,15].

Table 3 presents the glucose yield for different categories of wastepaper of enzymatic hydrolysis. The result showed that the glucose yield of enzymatic hydrolysis for acid pre-treated wastepaper is

Table 3. Glucose yield for three different pretreated wastepaper of enzymatic hydrolysis

Categories of pretreated wastepaper	Time (day)	Optical density	Glucose yield (g/l)	Percentage of glucose yield (%)
Cut wastepaper	2	0.142	0.2796	1.40
	4	0.013	0.0512	0.26
	5	0.033	0.1624	0.81
Wet-blended wastepaper	2	0.005	0.0985	0.49
	4	0.008	0.0315	0.16
	5	0.038	0.1871	0.94
Acid pre-treated wastepaper	2	1.479	2.9123	14.56
	4	0.906	3.5680	17.84
	5	0.746	3.6723	18.36

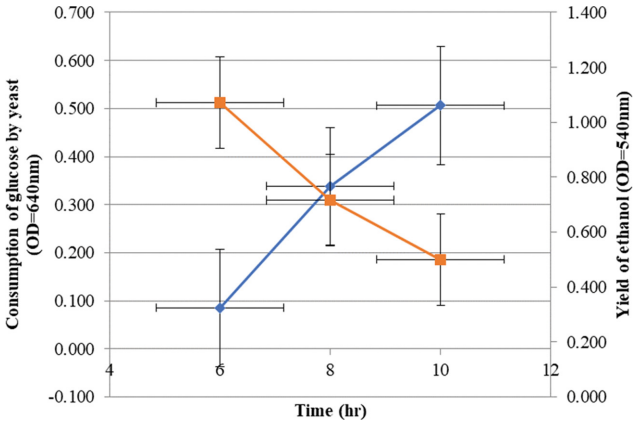

Fig. 2. Optical density of glucose consumption by yeast and yield of ethanol during fermentation.

much higher than the cut and wet-blended wastepaper. Acid pretreatment was proven to have a significant effect on the glucose yield of enzymatic hydrolysis. The glucose yield for acid pre-treated wastepaper was around 15% of total cellulose, while the other wastepaper categories presented less than 1% of total cellulose. However, wet blending as pretreatment of wastepaper does not affect enzymatic hydrolysis outcome even compared with wastepaper that was cut into smaller pieces only. Despite that, wet blending was effective in improving the effects of acid pre-treatment.

The fermentation of hydrolysate was conducted for continuous 6, 8, and 10 hours. Fig. 2 presents the curve for OD for yeast and glucose during yeast fermentation. Based on Fig. 2, the glucose content decreased with time while *S. cerevisiae* (yeast) cell increased continuously. This outcome complies with the theoretical approach of glucose consumption by yeast cells and bioethanol and carbon dioxide production. Therefore, the result indicated the fermentation was successfully performed. Table 4 presents the glucose content after fermentation. The glucose yield from pretreatment and enzymatic hydrolysis is lower than the glucose content after diluted acid pretreatment and enzymatic hydrolysis, 10% of blended wastepaper and 50% of acid-treated wastepaper. This situation may occur due to the high substrate loading compared with them since the substrate loading is one factor that significantly affects glucose yield. The sample wastepaper used includes different types of paper that may contain different amounts of glucose inside. The glucose consumption is 0.0069 g for pretreatment and 0.180 g for enzymatic hydrolysis during 10 hours of fermentation, respectively. Both fermentations are not complete since the solution contained some amount of glucose.

HPLC generated graph shows that at 16.45 minutes, bioethanol content was produced inside the fermentation broth. Thus, the HPLC graphs showed that the fermentation process was successful and the fermented solution contained bioethanol. Further, the solution was distilled by a rotary evaporator and bioethanol was obtained. 79 ml of distilled bioethanol was obtained from 100 ml of total fermented solution. Therefore, the bioethanolic yield was 79% or 0.79 ml bioethanol/ml fermented solution. The bioethanol content obtained in this study is very high compared to plant-based biomass such as agricultural and forest residue and other municipal solid waste, probably due to the very low content of lignin present in wastepaper, while most of the biomass contains high lignin content [16].

CONCLUSIONS

This experimental study explored the hydrolysis of three different pretreatment methods of wastepaper; the chemical pretreat-

Table 4. Glucose consumed after fermentation

Glucose source	Initial			Final			Difference (g/*l*)	Glucose consumed (g)
	Optical density	Dilution factor	Glucose yield (g/*l*)	Optical density	Dilution factor	Glucose yield (g/*l*)		
Pre-treatment	0.109	20	0.4393	0.094	20	0.3702	0.0691	0.0069
Enzymatic hydrolysis	1.934	10	3.8082	0.204	50	2.0085	1.7997	0.1800

ment method, diluted H_2SO_4, was the most effective for glucose yield profile during hydrolysis of wastepaper. The delignification performed by the diluted H_2SO_4 acid pretreatment significantly improved the glucose yield compared to the untreated and wet-blended wastepaper. 9.75 g/l of glucose content was released from diluted H_2SO_4 treated wastepaper during enzymatic hydrolysis in response to the 20 mg cellulase enzyme from *Trichoderma reesei* within five days hydrolysis period. The amount of enzyme loading played a vital role in obtaining the optimum and maximum glucose yield. The hydrolysate produced by enzymatic hydrolysis was utilized via fermentation by *S. cerevisiae* (yeast) for bioethanol production. With a longer fermentation period, maximum glucose consumption, 18.36 g/l, was observed in diluted H_2SO_4 acid pretreated wastepaper by the yeast cells. HPLC analysis of fermented medium confirmed bioethanol content, and distillation of the fermented medium identified very high bioethanol conversion efficiency. Hence, the effectiveness of diluted H_2SO_4 acid pretreatment and enzymatic hydrolysis by cellulase from *Trichoderma reesei* for the efficient saccharification of cellulosic wastepaper can be concluded. High bioethanol conversion efficiency, 79%, from wastepaper, was obtained from this experimental study, which recommends further experiments to generate bioethanol from this feedstock. The amount of bioethanol production and solid recovery (biomass content) profile will be analyzed in further experimental studies. Besides, the application of solid nano-particles and nano-droplets with the optimum amount of cellulase enzyme loading will be implemented for further experiments on diluted H_2SO_4 treated wastepaper to break down the long chain of complex sugar content with continuous faster rate and release higher amount of simple sugar what consequences higher amount of bioethanol generation. The optimized conditions are recommended for comprehensive techno-economic and life-cycle assessments for commercial-scale applications in the future.

DECLARATIONS

Ethics Approval and Consent to Participate

The facts and views in the manuscript are solely ours, and we are totally responsible for authenticity, validity, and originality. We also declare that this manuscript is our original work, and we have not copied from anywhere else. There is no plagiarism in my manuscript.

Consent for Publication

We undertake and agree that the manuscript submitted to your journal has not been published elsewhere and has not been simultaneously submitted to other journals.

Competing Interests

The authors declare no conflict of interest.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author, Nazia Hossain, upon reasonable request.

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CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Nazia Hossain: Conceptualization, Methodology, Data Curation, Interpretation, Software and Validation, Formal Analysis, Writing-Original Draft, **Lee Lai Hoong**: Experimental Analysis, Writing-Original Draft, **Pranta Barua**: Writing-Review and Editing, **Manzoore Elahi M Soudagar**: Writing-Review and Editing, **Teuku Meurah Indra Mahlia**- Supervision.

SUPPORTING INFORMATION

Additional information as noted in the text. This information is available via the Internet at http://www.springer.com/chemistry/journal/11814.

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Supporting Information

The effect of enzymatic hydrolysis of pretreated wastepaper for bioethanol production

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Table S1. Chemical combination of MSW from various experiments and reports [17]

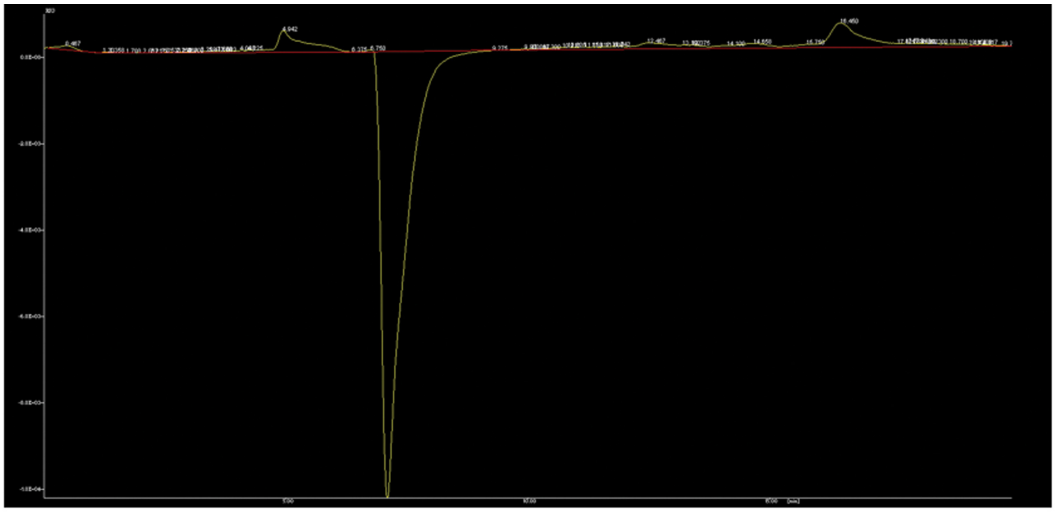
Component	2001	2002	2003	2004	2005	2007	2010
Food waste & organic	68.4	56.3	37.4	49.3	45	42	43.5
Mix plastic	11.8	13.1	18.9	9.7	24	24.7	25.2
Mix paper	6.3	8.2	16.4	17.1	7	12.9	22.7
Textiles	1.5	1.3	3.4	-	-	2.5	0.9
Rubber & leather	0.5	0.4	1.3	-	-	2.5	-
Wood	0.7	1.8	3.7	-	-	5.7	-
Ferrous	2.7	2.1	2.7	2	6	5.3	2.1
Glass	1.4	1.5	2.6	3.7	3	1.8	2.6
Yard wastes	4.6	6.9	3.2	-	-	-	-
Pampers	-	-	5.1	-	-	-	-
Other	2.1	8.4	5.3	18.2	15	2.6	1.8
Total	100	100	100	100	100	100	100

Table S2. Composition for the different waste paper [10]

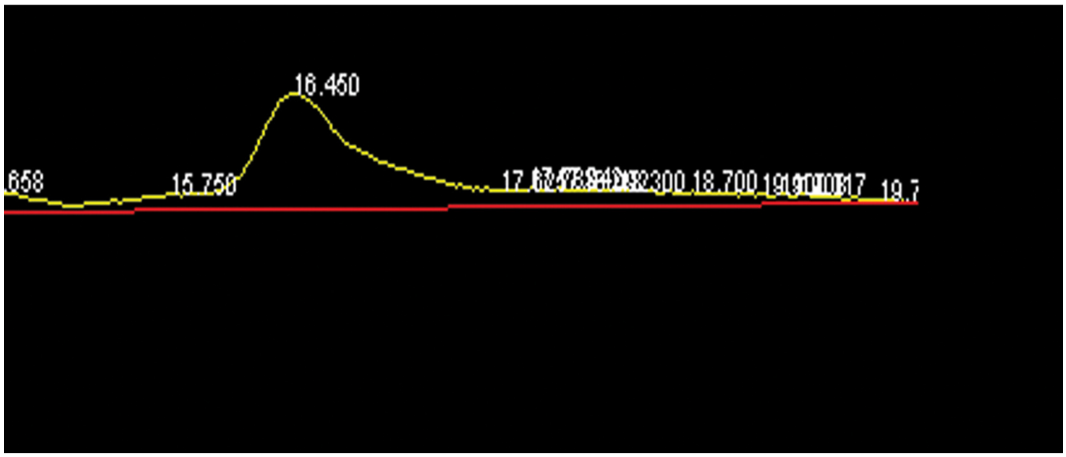
Percentage, %	Newspaper	Office paper	Magazine	Cardboard
Total carbohydrates	65.38	73.39	50.10	69.35
ASL	1.06	1.41	0.98	1.59
AIL	17.08	4.68	13.85	14.18
Total lignin	18.14	6.09	14.83	15.77
Extractives	3.93	1.97	3.45	2.55
$CaCO_3$	2.13	8.12	2.63	4.20
Ash	10.51	7.97	30.14	0.89

Table S3. Chemical composition of the initial (IN) and demineralized (DM) waste paper [18]

	Cellulose, %		Hemicellulose, %		Lignin, %		Mineral, %	
	IN	DM	IN	DM	IN	DM	IN	DM
Cardboard	61	63	12	13	18	19	7	3
Newspaper	38	50	15	18	21	26	19	4
Packaging paper	60	73	11	8	7	12	20	3
Napkins	58	78	6	10	4	5	29	4
Blotting paper	81	84	6	7	4	3	7	3
Office paper	62	87	5	8	1		30	1



(a)



(b)

Fig. S1. (a) HPLC generated graph for fermentation using glucose from enzymatic hydrolysis. (b) Zoom in ethanol curve from HPLC for fermentation using glucose from enzymatic hydrolysis.