Enzymatic hydrolysis by Trichoderma reesei of diluted acid-pretreated wastepaper for bioethanol production

Nazia Hossain (bristy808.nh@gmail.com)
"RMIT University" https://orcid.org/0000-0001-7925-0894
Lee Lai Hoong
Universiti Tenaga Nasional
Pranta Barua
Chittagong University of Engineering and Technology
Manzoore Elahi M Soudagar
University of Malaya: Universiti Malaya
Teuku Meurah Indra Mahlia
University of Technology Sydney

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Enzymatic hydrolysis by *Trichoderma reesei* of diluted acid-pretreated wastepaper for bioethanol production

Nazia Hossain[a*], Lee Lai Hoong[b], Pranta Barua[c], Manzoore Elahi M Soudagar[d], Teuku Meurah Indra Mahlia[e]

[a]*School of Engineering, RMIT University, Melbourne VIC 3001, Australia*

[b]*Department of Mechanical Engineering, Universiti Tenaga Nasional, Selangor 43000, Malaysia*

[c]*Department of Electrical and Electronic Engineering, Chittagong University of Engineering & Technology, Bangladesh*

[d]*Department of Mechanical Engineering, Faculty of Engineering, University of Malaya, 50603 Kuala Lumpur, Malaysia*

[e]*School of Information, Systems and Modelling, Faculty of Engineering and Information Technology, University of Technology Sydney, NSW 2007, Australia*

[*]Corresponding author at: Tel.: +61 480123691; E-mail address:bristy808.nh@gmail.com (Nazia Hossain); School of Engineering, RMIT University, 128 La Trobe Street, Melbourne, VIC 3001, Australia

Abstract

Enzymatic hydrolysis of waste biomass for bioethanol production is considered a traditional, inexpensive, and energy-effective approach decades ago. In the present study, waste office paper was pretreated with diluted sulfuric acid (H_2SO_4) and hydrolysed with one of the most available and cost-effective enzymes, cellulase from *Trichoderma reesei,* under submerged static condition. Wastepaper size was reduced to $2cm^2$, blended with water and dry wet-blended, and pretreated with diluted H_2SO_4. Among different concentrations (0.5M, 1.0M, 1.5M, 2.0M) of H_2SO_4, the maximum glucose content was obtained at 2.0M H_2SO_4 at 90 min reaction time, and glucose yield was 0.11g glucose/g wastepaper. The cut paper, wet-blended, and acid-treated wastepaper was hydrolysed with cellulase enzyme for 2, 4, and 5 consecutive days with 5mg, 10mg, 15mg, and 20mg enzyme loadings. The maximum glucose content was obtained, 9.75g/l after 5 days of enzymatic hydrolysis with 20mg enzyme loading and a glucose yield of a 0.5g 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 0.18g by yeast. Later, HPLC analysis of the fermented medium presented a strong peak of bioethanol content at 16.12min. Further, the distillation of bioethanol by rotary evaporator presented 0.79ml bioethanol/fermented solution, which indicated the conversion efficiency of 79%.

Keywords: Acid Pretreatment; Bioethanol from Wastepaper; Enzymatic Hydrolysis

1. Introduction

The power generation worldwide mostly relies upon fossil fuels, non-renewable energy resources comprising coal, petroleum, natural, and gas. Petroleum derivative contributes roughly 80% of energy demanded by the power and transportation sector globally (Lim &Teong 2010). Due to the fast population growth, industrialization and urbanization, increasing energy demand, and rocketing economic development within the last decades, the energy demand reached the top worldwide, including Malaysia. In Malaysia, the vital energies accessible are coal and cokes, raw petroleum, oil-based commodities, flammable gas, and hydropower. The most recent measurement illustrated that more than 90 % of them are non-renewable energy sources, and little hydropower and biofuel have realistic applications (Lim &Lam 2014). As a nation being developed, Malaysia's non-renewable energy source utilization pattern for as far back as 10 years showed an expanding incline (Balasbaneh et al. 2018). Consequently, petroleum product, a non-sustainable power source, is quickly diminishing and will most likely be unable to develop vitality requests.

A large portion of the essential energy assets is petroleum products in the country. They are quickly exhausted since the interest in energy is expanding because of the advancement of the economy and energy investments. Likewise, the consumption of petroleum product adds to the outflow of ozone harming substances, which is the significant reason for a dangerous atmospheric deviation (Ong et al. 2014). Among the other options, bioethanol offers a more substantial part of the ideal rules, for example, inexhaustibility, maintainability, and ecological cordial. One of the significant elements for bioethanol production is the availability of raw material. To avoid a clash with the food and feed chain, waste biomass is preferred for this purpose (Hossain et al. 2019a). Subsequently, looking for potential, inexpensive and available raw material in Malaysia for bioethanol production is a significant factor.

In Malaysia, metropolitan solid waste (MSW) production amount is 0.5-0.8 kg/singular/day decently cross-country and 1.7 kg/singular/day for critical metropolitan networks (Kathirvale et al. 2004). Approximately 7.34 million tons of MSW were produced in 2006, which can accumulate to 42 structures. Urbanization and industrialization are the reason for this large amount of MSW generation (Saeed et al. 2009). The principal portions of this MSW are mostly food packaging, glass, metal, paper, plastic, and others. According to **Table 1**, the degree of wastepaper generation increased from 6.3 % in 2001 to 22.7 % in the year 2010 (Chua et al. 2011). It was one of the most crucial MSW structures that appeared in Malaysia. Therefore, it is evident that the advantages of lignocellulosic biomass would be sourced from waste biomass such as wastepaper. The previous studies presented that 20% of 7.34 million tons MSW are almost squandered paper and the wastepaper is nearly 1.5 million tons. This outcome unveiled that almost 20% of MSW were generated by wastepaper. Therefore, utilizing wastepaper for bioethanol production can significantly facilitate the waste management section in Malaysia.

Table 1: Chemical combination of MSW from various experiments and reports (Chua et al. 2011)

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

For bioethanol synthesis, the type of feedstock is one of the significant concerns for the steady and persistent process. Because of Malaysia's wastepaper age, waste paper's accessibility isn't a substantial issue for feedstock gracefully. Besides, the source of wastepaper from MSW is available without any economic value, facilitating the elimination of raw material cost for bioethanol production. Therefore, wastepaper can be considered one of the most suitable feedstocks for bioethanol production in the country. Wastepaper can be in several categories, such as the cupboard, magazine, office paper, regular paper, and others. **Table 2** presented a combination of various kinds of wastepaper. Based on **Table 2**, 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 (Wang et al. 2012).

Table 2: Composition for the different waste paper (Wang et al. 2012)

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

The availability of feedstock for bioethanol production is one of the most crucial concerns. Lignocellulosic biomass is a suitable material for the containment of its' cellulose and hemicelluloses, which can be transformed into sugar and further production of bioethanol. For the most part, lignocellulosic biomass is a horticultural build-up or strong metropolitan waste that is modest and bountiful. For instance, rice straw, void natural product pack, wastepaper, and some more (Kim 2004). In this manner, lignocellulosic biomass is appropriate as feedstock in the production process, accessibility, and cost. However, the accessibility of lignocellulosic biomass is occasional and relies upon topography. Besides, lignin's solid obstructions in lignocellulosic biomass increment the trouble for hydrolysis's mechanism, where additional pretreatment would be required. Along with that, this issue increases the production cost simultaneously. Other than that, the structure of lignocellulosic biomass undertakes significant functions in bioethanol synthesis measures. The factor incorporates cellulose crystallinity, polymerization, lignin substance, and others (Pan et al. 2006). Different studies, experiments are conducted on the production of bioethanol from waste papers. 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 experimental 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) (Byadgi &Kalburgi 2016). Another experimental study on wastepaper hydrolysis evinced that waste office paper and newspaper are perfect for raw material (Annamalai et al. 2020). This study showcased that hydrolysis efficiency was 91.8% and 79.6% for office paper and newspaper, respectively, which enhanced the yield of bioethanol

120 production from wastepaper. Another previous study provided a brief discussion of bioethanol
121 production's sensitivity analysis from various waste papers considering economic feasibility.
122 The finding represented a 25% selling price reduced in bioethanol production using sensitivity
123 analysis considering all parameters. It also mentioned that bioethanol's selling price from the
124 waste newspaper, office paper, and cardboard paper is competitive to petrol (Wang et al. 2013).
125 A previous study (Tadmourt et al. 2020) showcased the optimized acid hydrolysis of wastepaper
126 to produce bioethanol and ensured the purity degree of 90% (v/v) (Tadmourt et al. 2020). The
127 previous study presented a brief review of the current condition of wastepaper for bioethanol
128 production. This review demonstrated that after demineralizing lignified waste papers, the
129 increased content of carbohydrates and lignin was observed. In contrast to lignified waste, the
130 demineralization of the bleached waste papers, e.g., office paper, leads to increased content
131 only carbohydrates, mainly of cellulose. The obtained data have been delineated in **Table 3**.

132 **Table 3**: Chemical composition of the initial (IN) and demineralized (DM) waste paper
133 (Ioelovich 2014)

	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

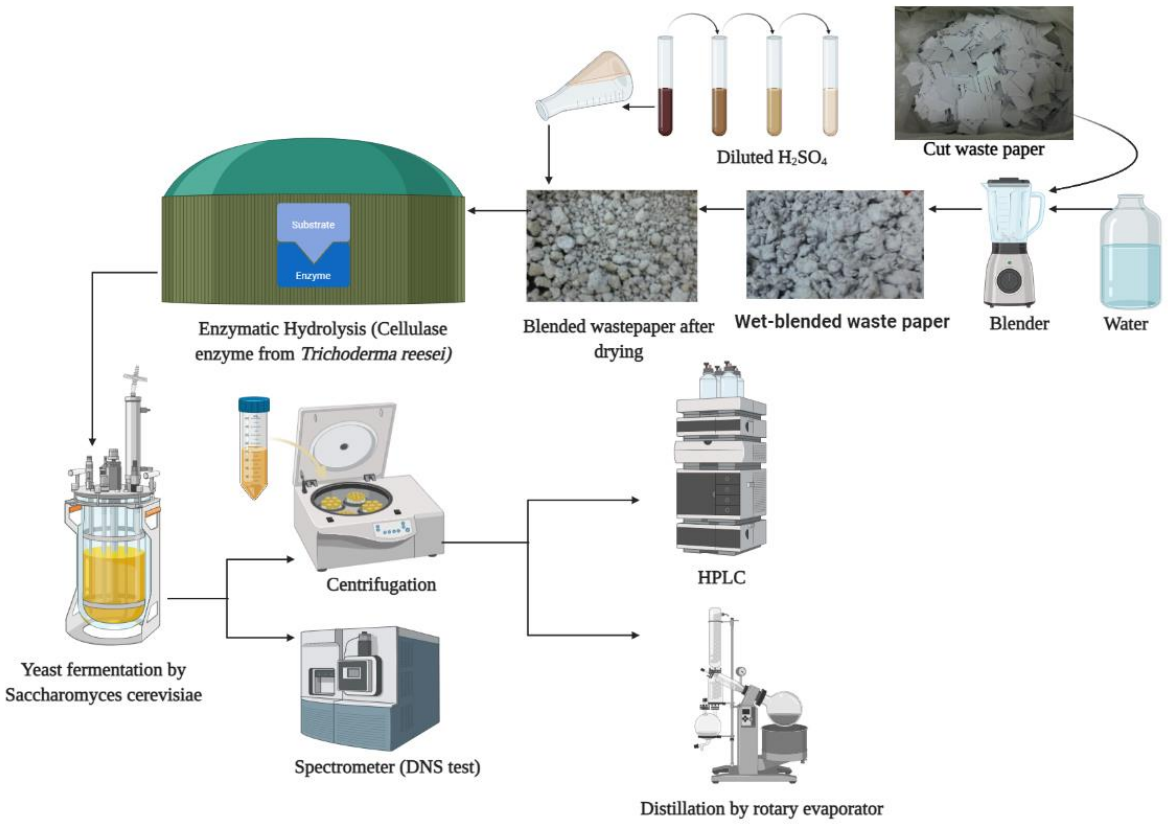
Office paper	62	87	5	8	1	30	1

Different studies, experiments, and reports are being continued on bioethanol from other lignocellulosic biomass besides wastepaper. Previous experimental studies indicated that bioethanol from lignocellulosic waste biomass has the promising potential as an alternative fuel for fossil fuel and be sustainable green energy in the near future (Fan &Lynd 2007, Har et al. 2013, Hossain &Jalil 2015, Hossain et al. 2017, Petersen et al. 2009, Tappi 2002, Zhu 2006). Bioethanol from wastepaper was experimented in few previous studies on mixed wastepaper, newspapers, cardboard, or packaging paper. In contrast, this study emphasized the office paper from municipal solid waste. Besides, the previous studies of bioethanol from wastepaper were hydrolysed by different enzymes. A previous experimental study demonstrated bioethanol production from wastepaper pulp hydrolysed with xylose-fermenting *Pichia stipites*. But this study utilized the most available and less expensive cellulase enzyme from *Trichoderma reesei*. The main objectives of this experimental study are (i) to study the reasonable reaction time and acid pretreatment for the diluted acid pretreatment of wastepapers (ii) to determine the effect of pretreatment towards enzymatic hydrolysis (iii) to assess the effect of enzyme loading to enzymatic hydrolysis (iv) to identify the glucose content released after dilute acid pretreatment and enzymatic hydrolysis and compare and (v) to measure the amount of bioethanol content from wastepaper after yeast fermentation.

2. Materials and Methods

The methods of this study delineated the preparation and investigation of boundaries for hydrolysis and bioethanol production from wastepaper, required materials and instruments,

157 suitable conditions, and techniques. **Fig. 1** presented the steps of bioethanol production from
158 wastepaper via different hydrolysis methods.



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

162

163 *2.1.Preparation of Wastepaper*

164 The wastepaper (office paper) has been collected from the municipal solid waste
165 (MSW) plant located at Klang Valley, Malaysia. Squander office paper has been collected for
166 the experimental purpose. The collected wastepaper was cut into approximately $2cm^2$. The
167 wastepaper has been categorized into three patterns: (i) cut wastepaper, (ii) wet-blended
168 wastepaper, and (iii) acid pre-treated wastepaper. 400g cut wastepaper was blended with
169 distilled water. The wet-blended wastepaper was dried into a broiler around 70°C for 24 hours
170 and preserved in an air-tight jar for further experiments.

2.2.Diluted Acid Pretreatment

4g of three categories of wastepaper was placed into conical flasks. 100ml of 0.5M sulphuric acid (H_2SO_4) was added to the flasks. Duplicates have been 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 0.5M sodium hydroxide (NaOH) was added to the mixture to neutralize the acidic environment and shaken vigorously. The acid pretreatment was also conducted for 1.0M, 1.5M, and 2.0M sulphuric acid and sodium hydroxide separately. The supernatant for each sample was assembled into a rotator tube. The axis tubes were centrifuged at 4000 rpm for 10 minutes. The supernatants proceeded for the 3,5-dinitrosalicylic acid (DNS) test. Atomic absorbance spectrometer (AAS) was done to determine glucose content at wavelength A540. The optical density (OD) for each sample has been recorded. The glucose yield was calculated by the standard method by using OD.

2.3.Enzymatic Hydrolysis by Trichoderma reesei

Cellulase enzyme produced by *Trichoderma reesei* has been obtained from Sigma Aldrich, United States. 2g of diluted acid pre-treated wastepaper was placed into conical flasks. 100ml of sodium acetate ($C_2H_3NaO_2$) solution was added to each solution to maintain the neutral pH. 5mg, 10mg, 15mg, and 20mg of cellulase enzyme has been loaded into separate flasks, and the flasks were set at incubator shaker for continuously 2, 4, and 5 days at 37°C and 100rpm.

2.4.Yeast Preparation and Fermentation of Hydrolysate

2.4.1. Yeast Preparation

10g of D-glucose monohydrate ($C_6H_{14}O_7$), 0.4g of potassium dihydrogen phosphate (KH_2PO_4), and 0.2g ammonium chloride (NH_4Cl) were mixed with 100ml distilled water into a conical flask. The mixture was mixed vigorously with a magnetic stirrer, then sealed with

cotton and aluminum foil. The mixture was sterilized for 15min in an autoclave. Then 1g yeast (*Saccharomyces cerevisiae*) extract was added into the solution under a laminar hood. The flask was placed into an incubator shaker at 37°C and 100rpm. Samples were taken for the first 6 hours and alternative 2 hours until 10 hours 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 A640nm, respectively.

2.4.2. Fermentation of Hydrolysate

After enzymatic hydrolysis, the enzymatic hydrolysate was filtered and centrifuged. 15g 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 100rpm for 2 and 4days 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.HPLC analysis and Distillation

The sample after fermentation was taken for high-performance liquid chromatography (HPLC) test for bioethanol analysis. SUPELCOGEL C-610H, 30cm x 7.8mm 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 1.2ml/min, and the injection amount was 25 µl for the HPLC test. The bioethanol content was tested by the standard method. Due to the presence of bioethanol in the fermented sample, 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 in between 65-75°C. 100ml of the fermented solution was placed into the rotary evaporator flask. The vacuum

pressure was set with 0.8kPa. The distillation process was conducted around 40min under vacuum conditions. After distillation, bioethanol was measured by measuring cylinder.

3. Results and Discussions

3.1.Diluted Acid Pretreatment

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

$$Actual\ glucose\ yield = \frac{Optical\ density}{5.0785} \times 20 \qquad \text{(Eq.1)}$$

According to **Table 4**, glucose yield increased with the increase of reaction time for all acid concentration. Among different acid concentrations, the glucose yield of pre-treated wastepaper increased with the increase of concentration. Higher glucose yield represents that the pretreatment is more effective to the wastepaper because glucose is produced due to cellulose's accessibility and that is the reason for conducting pre-treatment. **Table 4** also demonstrated no reduction of glucose as both parameters increases. Therefore, it was evident that there was no glucose degradation within these parameter values, and there will be no formation of inhibitors for enzymatic hydrolysis. Thus, the operating condition was within the acceptable range where pretreatment is efficient for accessibility of enzymatic hydrolysis to cellulose. The maximum actual glucose yield was obtained as 4.50g/l at 2.0M H_2SO_4 concentration at 90min 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 conversion calculation has been presented in Eq.2 and Eq.3.

$$\frac{4g\ wastepaper}{100ml} \times \frac{1000ml}{1l} = \frac{40g}{l} \quad \text{(Eq.2)}$$

$$\%\ of\ actual\ glucose\ yield\ for\ wastepaper = \frac{4.5053}{40} \times 100\% = 11.26\% \quad \text{(Eq.3)}$$

Therefore, the percentage of glucose yield for wastepaper pre-treated with 2.0 M H_2SO_4 at 90 minutes is 11.26 %. In other words, 1 g of wastepaper can produce 0.11 g of glucose.

Table 4: Glucose yield obtained from dilute 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

3.2.Enzymatic Hydrolysis

Table 5 showed the glucose yield for different enzyme loading in enzymatic hydrolysis. 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 day were 10, 20, and 25, respectively. Eq. 4 is used to calculate the glucose yield of each sample.

$$Y_{glucose} = \frac{OD}{5.0785} \times D_f \qquad \text{(Eq.4)}$$

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

The glucose yields of enzymatic hydrolysis with a higher dose of enzyme loading were always higher at any day of hydrolysis. This result presented 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 15mg of enzyme loading, while after 15mg enzyme loading, the glucose yield did not speed up significantly. The difference between glucose contents at 15mg and 20mg 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 5** also showed 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 manifested that the enzymatic hydrolysis was completed within this time, and maximum glucose yield has been 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 5: Glucose yield for different enzyme loading 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

Table 6 presented 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 much higher than the cut and wet-blended wastepaper. Acid pre-treatment 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 pre-treatment of wastepaper has no effect on enzymatic hydrolysis outcome even compared with wastepaper that was cut into smaller pieces only. Despite that, wet blending was effective in improving the outcomes of acid pre-treatment.

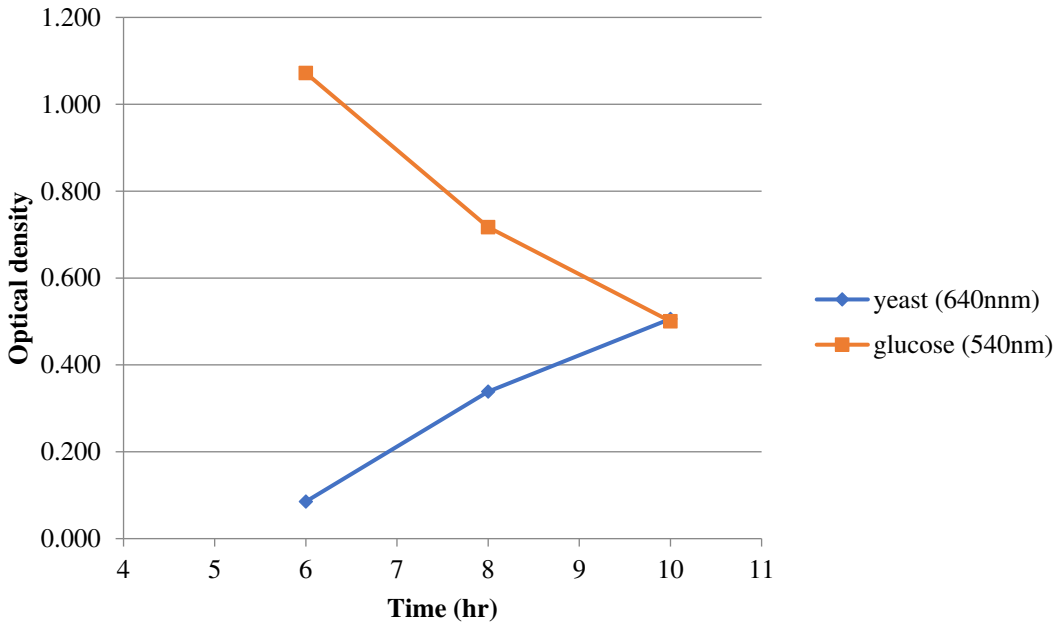
Table 6: Glucose yield for different categories of wastepaper of enzymatic hydrolysis

Categories of 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

3.3.Fermentation of Hydrolysate

The fermentation of hydrolysate was conducted for continuous 6, 8, and 10 hours. **Fig. 2** presented 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 complied with the theoretical approach of glucose consumption by yeast cells and bioethanol and carbon-di-oxide production. Therefore, the result presented the fermentation has been successfully performed. **Table 7** showed 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 consumption of glucose is 0.0069 g for pre-treatment 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.



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300 **Fig. 2**: Optical densities of yeast and glucose for yeast fermentation

301

302 **Table 7**: 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

303

304

305 *3.4. HPLC Analysis and Distillation*

Fig. 3(a) presented HPLC generated graph for fermentation using glucose from pre-treatment HPLC generated a graph for fermentation using glucose from pre-treatment. From **Fig. 3(a)**, the vertex at 16.45 minutes was the bioethanol content produced inside the fermentation of glucose from pre-treatment. **Fig. 3(b)** zoomed out the peak of bioethanol content at 16.125 minutes during fermentation glucose from enzymatic hydrolysis. Thus, the HPLC graphs showed that the fermentation process was successful, and the fermented solution contained bioethanol.

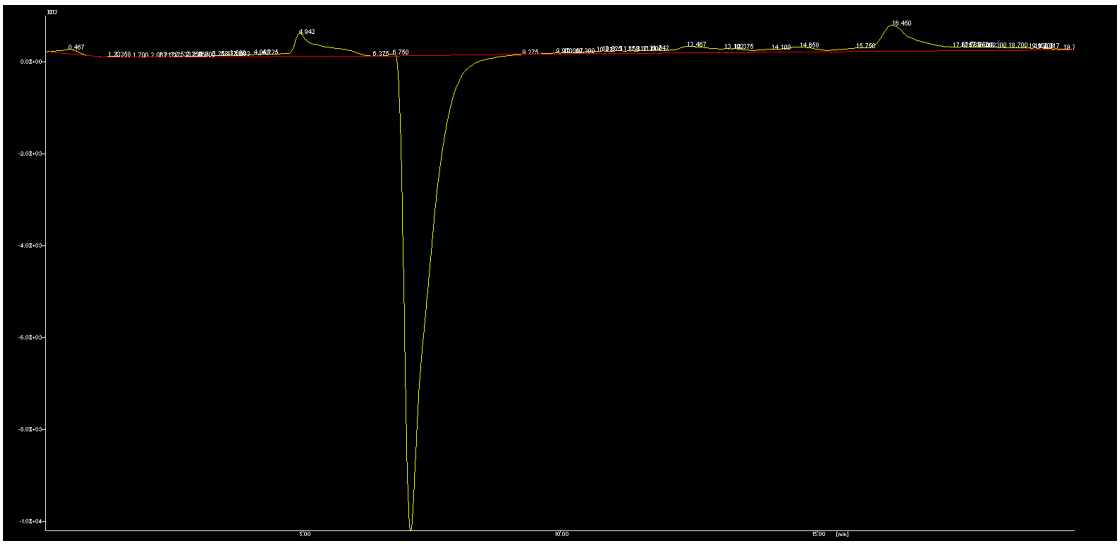

Fig. 3(a) : HPLC generated graph for fermentation using glucose from enzymatic hydrolysis

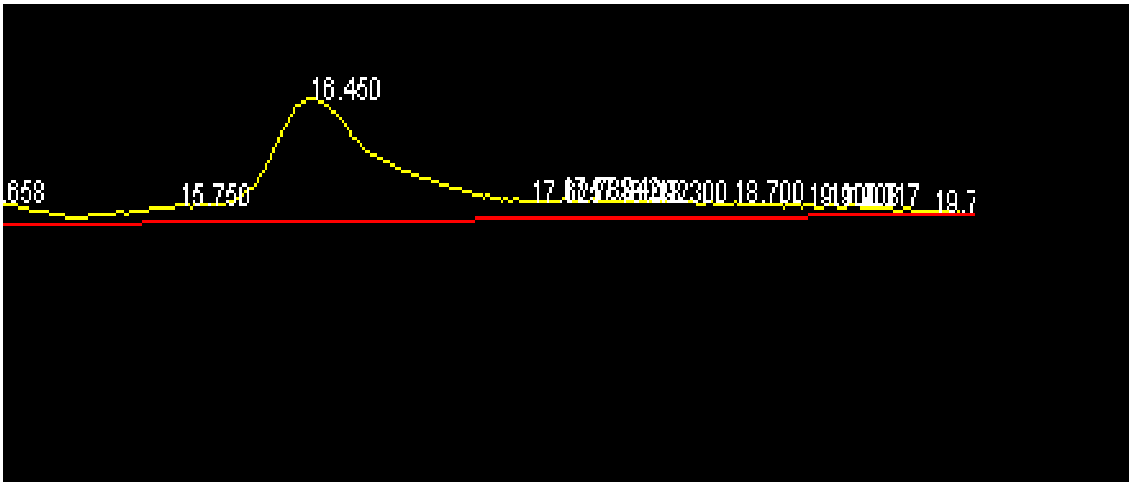

Fig. 3(b): Zoom in ethanol curve from HPLC for fermentation using glucose from enzymatic hydrolysis

Due to bioethanol in the fermented solution, the solution has been distilled by a rotary evaporator, and bioethanol was obtained. 79ml of distilled bioethanol has been obtained from 100ml of total fermented solution. Therefore, the bioethanolic yield was 79% or 0.79ml 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 presence in wastepaper while most of the biomass contains high lignin content (Dubey et al. 2012, Hossain &Jalil 2015, Hossain et al. 2019b).

4. Conclusions

This experimental study explored the chemical pretreatment (diluted H_2SO_4) of wastepaper. The delignification performed by the acid pretreatment significantly improved the glucose yield compared to the untreated and wet-blended wastepaper. A high amount of glucose content has been released from treated wastepaper during enzymatic hydrolysis in response to the cellulase enzyme from *Trichoderma reesei*. 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 the presence of S. cerevisiae (yeast) for bioethanol production. With a longer period of fermentation, maximum glucose consumption was observed by the yeast cells. HPLC analysis of fermented medium confirmed the presence of bioethanol content, and distillation of the fermented medium identified very high bioethanol conversion efficiency. Hence, the effectiveness of diluted acid pretreatment and enzymatic hydrolysis by cellulase from *Trichoderma reesei* for the efficient

saccharification of cellulosic wastepaper can be concluded. The high bioethanol conversion efficiency from wastepaper via this approach can be recommended further to augment bioethanol generation. Therefore, this method is 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

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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.

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Figures

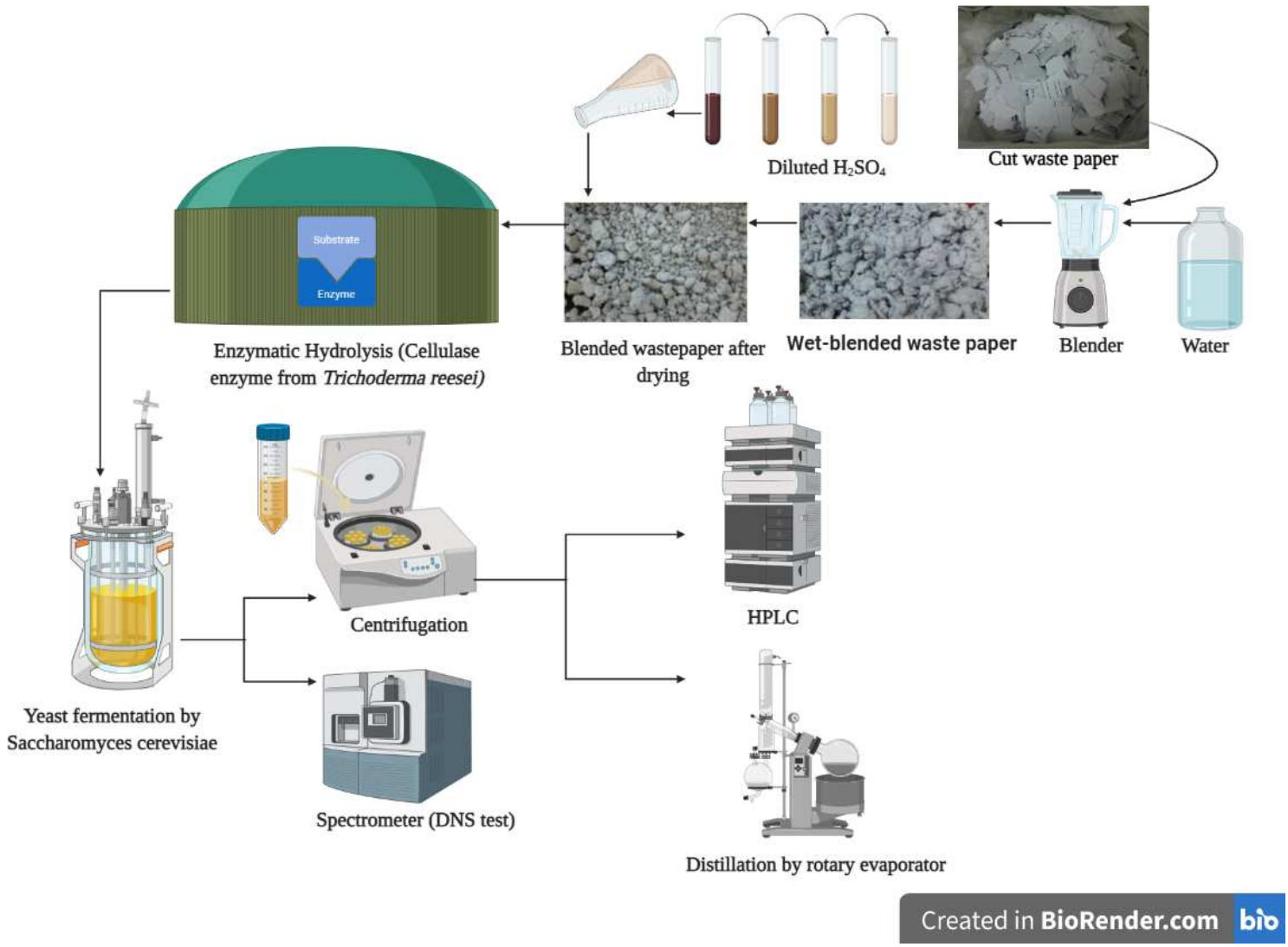


Figure 1

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

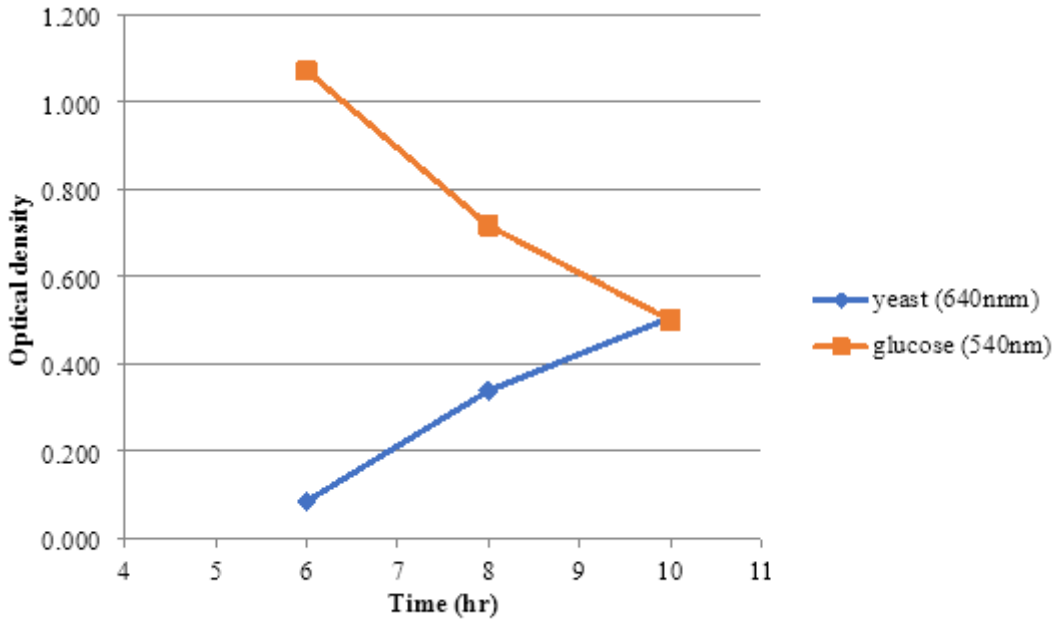
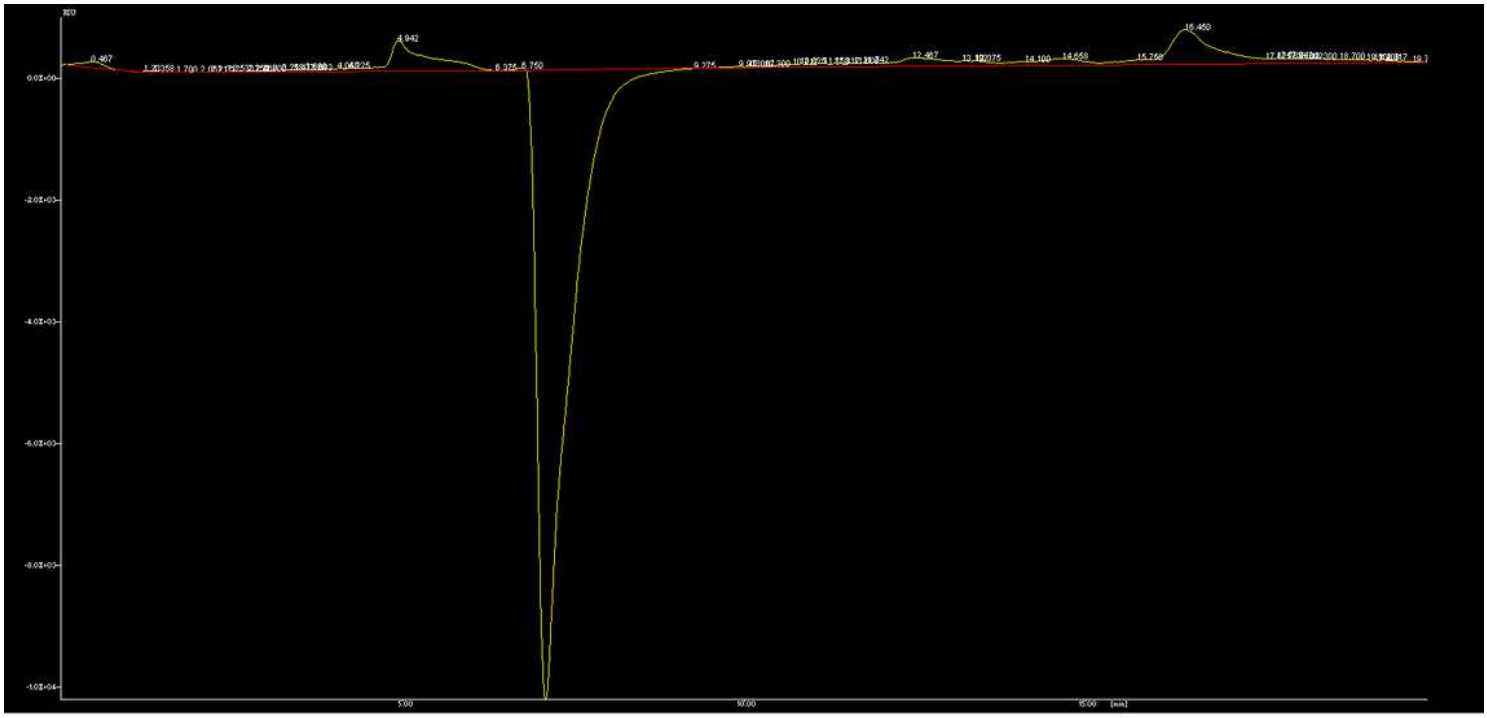
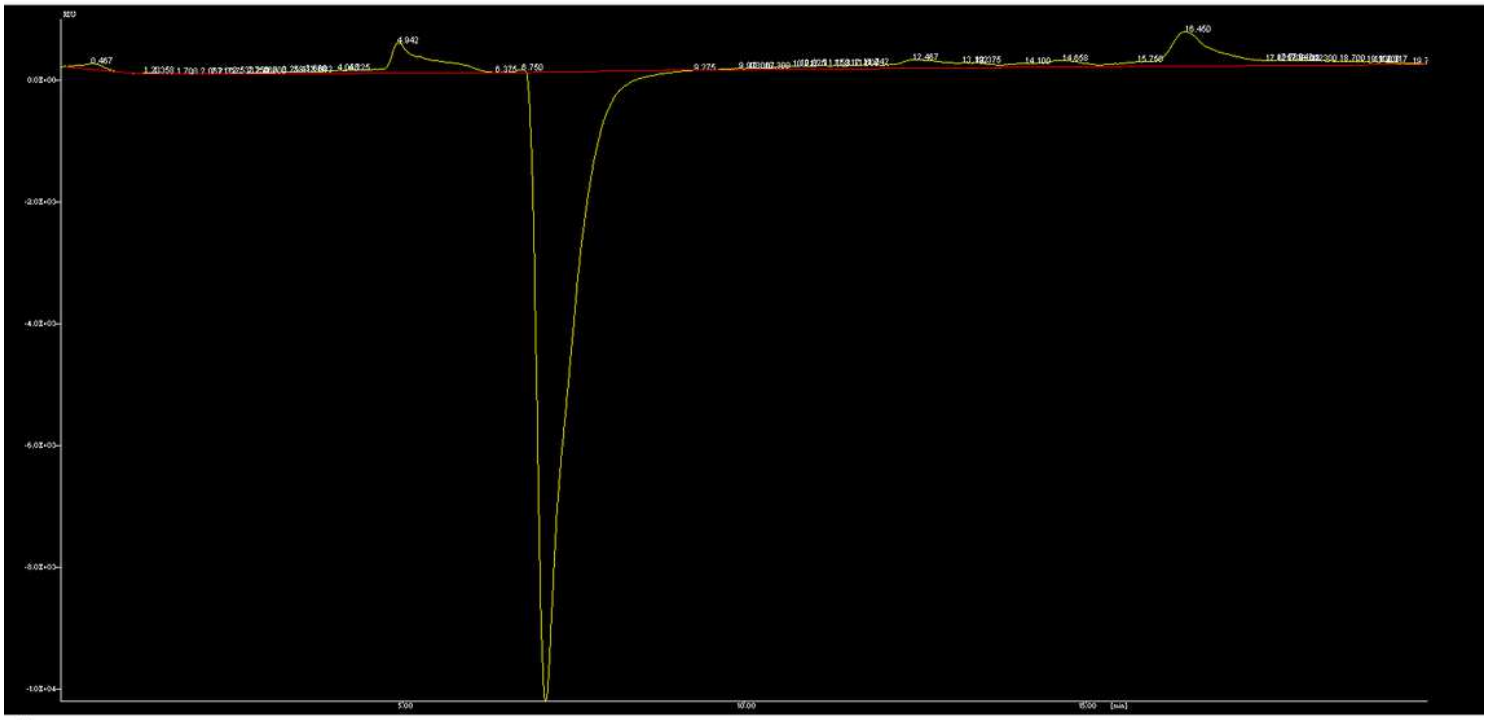


Figure 2

Optical densities of yeast and glucose for yeast fermentation



a



b

Figure 3

(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

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