Effect of Chemical Steeping on Yields of Glucose and Xylose from Dilute Acid Hydrolysis of Extract from Oil Palm Trunk

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The effectiveness of chemical steeping was examined as a novel pretreatment prior to hydrolysis to influence the extractable yield of glucose and xylose from oil palm trunk (OPT). The chemical steeping parameters were 0.2% sodium metabisulphite (w/v), 0.5% lactic acid (v/v), 26 ± 2 °C, and 1 h, followed by dilute acid hydrolysis (2%, 4%, and 6% of sulphuric acid (v/v); 115 °C, 120 °C, and 130 °C; 15 min, 30 min, and 60 min). For comparison, the glucose and xylose yield extracted from untreated OPT was also examined. Thermal analysis showed that the lactic acid and sodium metabisulphite successfully degraded the lignocellulose of OPT. The total extracted glucose and xylose yield was improved, and was approximately 2.5% to 28.8% higher than the yield extracted from non-pretreated OPT fines. RSM analysis showed that the 130 °C x 50 min x 2% was predicted as the optimum parameters for the extraction of glucose and xylose through chemically treated OPT, and followed the dilute acid hydrolysis process. Analysis of variance showed that the hydrolysis parameters were significant model terms for the glucose and xylose yield. In conclusion, chemical steeping was successful as a pretreatment to increase the extractable glucose and xylose yield from OPT.

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INTRODUCTION

In Malaysia, the oil palm industry is the largest generator of lignocellulosic biomass, surpassing other sectors such as paddy, forestry, and municipal solid waste. Given the large and steady supply of oil palm trunk (OPT), available annually due to replanting, the task of finding ways to utilize this enormous amount of lignocellulosic biomass is great. Studies have been conducted on the basic chemical derivatives of oil palm trunk biomass (Chin *et al.* 2010, 2011; Dungani *et al.* 2018; Megashah *et al.* 2018; Jahar *et al.* 2019; Norhazimah *et al.* 2020) to understand its physicochemical properties. Oil palm trunk is mainly composed (78.5%) of holocellulose, which in turn is composed of 47.5% cellulose and 31.0% hemicelluloses, while the remaining 18.4% is acid-soluble lignin (Tay *et al.* 2013). In addition, a substantial amount of starch was also found in OPT. This starchy resource contains carbohydrate polysaccharides and can be turned into simple sugars called monosaccharides (five and six-carbon sugars) for bioenergy (Tay *et al.* 2013). These components (cellulose, hemicelluloses, and starch) could be fractionated, isolated, and purified to obtain high-value chemical derivatives. However, the process to extract starch from OPT, namely chemical steeping, is different from the process to extract glucose and pentose, which typically calls for dilute acid hydrolysis.

Chemical steeping was introduced to OPT processing by a few researchers and it has proven successful at obtaining starch from OPT (H'ng *et al.* 2011; Wong 2012; Tay *et al.* 2013). In this study, chemical steeping was combined with dilute acid hydrolysis to simultaneously extract starch and convert it to glucose, which increases the overall amount of sugars extracted from OPT. Nonetheless, the efficiency of chemical steeping as a pre-treatment on OPT for this novel two-step process remains unknown. Further, this research also investigated whether the lactic acid and sodium metabisulphite used in the chemical steeping process can degrade lignocellulose, thus facilitating the degradation of polysaccharides into single sugars. Lactic acid is usually added to the lignocellulosic material to break down the lignin while retaining as much cellulose (glucan) as possible, thereby increasing the yield of monomeric sugars (Xu *et al.* 2009). Sodium metabisulphite is used to improve the dilute sulfuric acid hydrolysate of wood (Nieves *et al.* 2011; Jönsson and Martín 2016). The sodium metabisulphite when dissolved in water will become sodium bisulphite, which general is a mild acid solution. The lignocellulosic materials when pre-treated with sodium will degrade a significant amount of hemicelluloses to monomer sugars such as xylose; which called alkali pre-treatment to solubilise some hemicellulose and lignin (Zheng and Rehmann 2014). In this study, the degradation of lignocellulose in OPT was analyzed and demonstrated *via* thermal gravimetric analysis (TGA). In addition, a study evaluated the starch yield from OPT at 20-, 40-, and 60-mesh sizes, concluding that 60-mesh OPT resulted in the highest starch yield (8.24%) compared to 20- and 40-mesh OPT (H'ng *et al.* 2011). Hence, 60-mesh OPT was chosen for this study.

Glucose and xylose can be extracted from OPT through acid hydrolysis (Amirkhani *et al.* 2015; Zadegan *et al.* 2021). The hydrolysis of lignocellulose involves the hydrolysis of the glycosidic bonds of polysaccharides, depolymerizing it to monosaccharide sugar. The hydrolysis of lignocellulosic biomass is more complicated than that of pure cellulose because of the presence of non-glucan components (lignin and hemicelluloses). During the hydrolysis of wood, the first hurdle that must be solved is the removal of lignin and hemicellulose through hydrolysis reactions. Hence, the polysaccharides cellulose and hemicellulose are broken down into their sugar monomers by the hydrolysis process.

For dilute acid hydrolysis of cellulose, the range of acid concentration used is 2% to 30%, and the process is performed at elevated temperature, producing a high yield from cellulose at around 90% theoretical yield of glucose (Iranmahboob *et al.* 2002). Thus, to produce glucose and xylose sugars simultaneously, as in the fractionation of lignocellulose, dilute acid hydrolysis has been proposed. Dilute acid hydrolysis requires mild concentration of acids ranging between 2% to 5% coupled with high temperature to achieve an acceptable rate of cellulose and hemicelluloses conversion.

This study focused on the effect of chemical steeping as a pretreatment on dilute acid hydrolysis for xylose and glucose extraction from oil palm trunks. Specifically, this study also evaluated the effect of acid concentration, reaction time, and temperature of dilute acid hydrolysis on the xylose and glucose yields from OPT. The interaction effect of the independent variables for the extraction of glucose and xylose in oil palm trunks was also determined by employing response surface methodology (RSM, Stat-Ease, Minneapolis, MN, USA). Statistical analysis showed that the independent hydrolysis parameters (hydrolysis temperature, hydrolysis reaction time, and acid concentration) and their interactions affected the glucose and xylose yield significantly ($p < 0.01$). Further comparing the F-values, found that the effect of temperature on xylose and glucose was most significant. In addition, a quadratic order regression equation was developed for the response as a function of independent coded parameters ($R^2 = 0.62$) for glucose and $R^2=0.75$ for xylose. This approach will not only add value to the oil palm trunks, but will also solve the problem of the overabundance of agricultural waste.

EXPERIMENTAL

Raw Materials Preparation

The 30-year-old oil palm trees (*Elaeis guineensis*) were obtained from Taman Pertanian Universiti Putra Malaysia (UPM, Seri Kembangan, Malaysia) during replanting activities. Then, the OPT lumbers were further ground into fines (60-mesh size) with use of a Thomas Wiley Grinder, Model 4, and Endecotts Test Sieve Shaker, UK. The fines were dried to 5% moisture content in an oven maintained at 60 °C prior to further extraction process.

Chemical composition

The OPT fines were subjected to a suite of chemical characterisation methods according to TAPPI test standards: TAPPI T222 om-11 (2011) for lignin content, TAPPI T203 cm-99 (1999) for α-cellulose, and TAPPI T249 wd-76 (2009); the standards were used for the holocellulose analysis. All experiments were conducted in triplicates.

Experimental Design

In the first part of the study, the effect of pretreatment on glucose and xylose yields was evaluated. As the control group, the OPT fines at 60-mesh size were hydrolyzed by dilute acid hydrolysis alone. For the treatment group, the 60-mesh OPT fines were first subjected to chemical steeping in a 0.2% sodium metabisulphite ($Na_2S_2O_5$) and 0.5% lactic acid solution for 1 h at room temperature (26 ± 2 °C), followed by dilute acid hydrolysis (2%, 4%, and 6% of sulphuric acid; 100, 115, and 130 °C; 15, 30, and 60 min).

In the second part of the study, the effect of process parameters on glucose and xylose yields was evaluated. A 3 × 3 × 3 full factorial experimental design was applied by SPSS Statistics software (IBM, Version 23, IL, Chicago) with three levels each of acid concentration (2%, 4%, and 6%); hydrolysis temperature (100, 115, and 130 °C); and hydrolysis time (15, 30, and 60 min). All treatments were replicated three times. The reaction parameters were selected as dependent variables, whereas the glucose and xylose yield were identified as independent variables in this study.

Chemical steeping of oil palm trunk

The 60-mesh OPT fines were steeped in a 0.2% sodium metabisulphite ($Na_2S_2O_5$) and 0.5% lactic acid ($C_3H_6O_3$) solution for 1 h at room temperature (26 ± 2 °C). Afterwards, distilled water was added into the mixture and the OPT fines were filtered out. The pretreated OPT fines were ready for dilute acid hydrolysis.

Glucose and xylose extraction

Glucose and xylose were extracted under two conditions: with pretreatment and without pretreatment. In the first method, 3.0 g of the chemical-treated OPT was weighed and mixed with 2%, 4%, and 6% sulphuric acid. In the second method, 3.0 g of untreated OPT was weighed and mixed with 2%, 4%, and 6% sulphuric acid. The ratio of OPT fines weight and acid was 1:10 (w/v). The mixtures were swirled slowly to ensure that all the oil palm trunk fines were mixed thoroughly with sulphuric acid. The mixture was then hydrolyzed in an autoclave at temperatures (100, 115, and 130 °C) and reaction times of 15, 30, and 60 min. Afterwards, distilled water was added into each sample and the mixture was filtered to recover the hydrolysate solution and OPT fines. Finally, the pH of the hydrolysate was neutralized to pH 7, and the glucose and xylose contents within it were determined by high performance liquid chromatography (HPLC) using a Varian PL-Hi Plex column and RI detector (Agilent). Deionized distilled water was used as the mobile phase with a flow rate of 0.4 mL min^{-1} and the oven temperature was maintained at 80 °C.

Xylose and glucose contents are presented as yield from OPT fines weight. The glucose and xylose % yield recovered from wood was calculated using Eqs. 1 and 2.

$$\text{Glucose (\%)} = [\text{dilution volume (l)} \times \text{amount of glucose (g / L)}] / \text{OPT fines weight (g)} \times 100\% \quad (1)$$

$$\text{Xylose (\%)} = [\text{dilution volume (l)} \times \text{amount of xylose (g / L)}] / \text{OPT fines weight (g)} \times 100\% \quad (2)$$

TGA analysis

Thermal gravimetric analysis (TGA/SDTA851^e, Mettler Toledo, Switzerland) as recommended by Girisuta (2007), was used to analyse the chemical composition of untreated OPT fines and chemically (lactic acid and sodium metabisulphite) treated OPT fines. The analyses were carried out using a nitrogen atmosphere with a flow rate of 10 mL/min, with a heating rate of 10 °C/min, heat temperature from 25 to 600 °C.

Statistical analysis

Analysis of variance (ANOVA) was used to test the main effects of acid concentration, hydrolysis temperature, and hydrolysis time on glucose and xylose yield from OPT as well as their interactions. Each treatment was run with triplicate and a total of 162 runs were analyzed for glucose and xylose content (%). Tukey's honestly significant difference (HSD) was then further used to determine the significant level of average glucose and xylose yield for each treatment. The optimization of glucose and xylose yield was performed using Design-Expert Version 8.0.1 software (Stat-Ease Inc., Minneapolis, MN, USA). Second-degree polynomials were calculated using Design-Expert software to analyze the effect of interactions on starch yield.

RESULTS AND DISCUSSION

Chemical Composition of OPT

Composition analysis of the OPT sample revealed that it contained 8.7% lignin, 36.4% hemicelluloses, and 33.0% cellulose (% dry wt, w/w). The OPT had a lower lignin content than any timber (hardwood lignin content = 32.4%; softwood lignin content = 27.6%), which may be because oil palm is monocotyledonous; therefore, its primary vascular bundles are embedded in parenchyma ground tissues and arranged randomly (Chin *et al.* 2010; Hossain *et al.* 2018). Holocellulose is the lignin-free fibrous material comprising all the hemicelluloses and cellulose in wood; cellulose is a long-chain polymer of glucose. Holocellulose, which typically makes up two-thirds of plant cell wall dry matter, is composed of polysaccharides that can be hydrolyzed to sugars and then converted, enzymatically or chemically, to other biochemicals.

Degradation of Lignocellulose on Chemical Steeped and Non-steeped OPT Fines

Figures 1 and 2 show the TGA plot indicating the chemical composition of OPT with and without chemical steeping., thereby increasing the proportion of holocellulose in the solid.

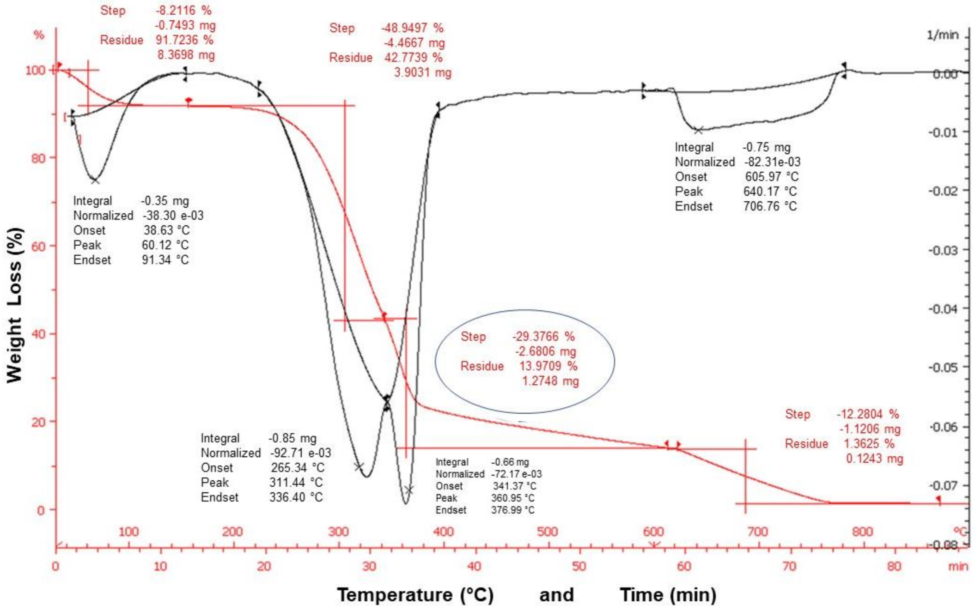

Fig. 1. Analysis of chemical composition of non-steeped OPT fines

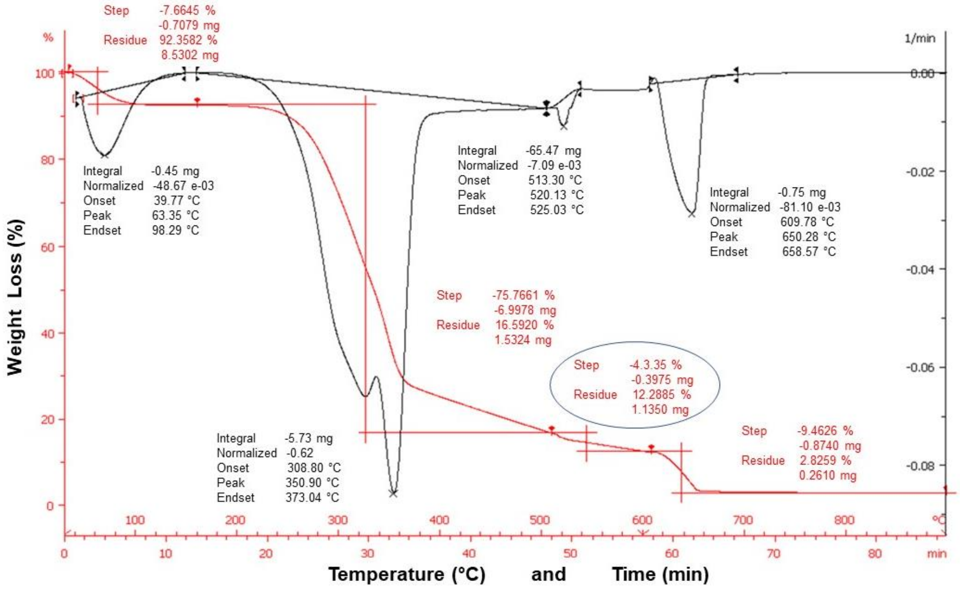

Fig. 2. Analysis of chemical composition of chemically steeped OPT fines

Three different stages of weight losses are shown in the figures. The first stage of weight loss, between 40 and 100 °C, is due to the evaporation of residual water. The second stage, which occurred at a temperature between 200 to 350 °C, is when holocellulose is degraded. The third stage may be attributed to lignin decomposition at temperatures between 420 and 500 °C (Girisuta 2007). The residue is non-combustible and is defined as the ash content of OPT. The lignin content of chemically steeped OPT was 4.30% lower than the raw OPT (29.38%). This result is supported by a previous study in which sodium bisulfate and lactic acid were used in starch extraction, followed by a dilute acid hydrolysis on OPT, which successfully dissolved the lignin that bound the hemicelluloses and cellulose fibers together (Idrees *et al.* 2013; Zou *et al.* 1996a,b). In addition, Fig. 2 shows that the content of holocellulose (83.3%) after chemical steeping was higher than the non-steeped OPT fines (55.5%). This was likely due to the lignins dissolving into the solution

Starch, Glucose, and Xylose Yield

The extracted OPT starch was determined by 0.2 % iodine solution and Hettich zentrifugen-UV-visible spectrophotometer at the wavelength of 650 nm. A solution of sodium metabisulphite and lactic acid could extract starch from OPT. Hence, 14% of starch was extracted from the chemically steeped OPT fines using the 0.2% sodium metabisulphite ($Na_2S_2O_5$) and 0.5% lactic acid solution. In addition, it was observed that glucose and xylose yields in the hydrolysate were dependent on experimental operating conditions, as shown in Table 1. The results of the hydrolysis are summarized in Table 1. The Tukey's HSD multiple comparison test was employed to determine the interaction among the independent variables on pretreatment, hydrolysis temperature, hydrolysis time, and the concentration of acid for pretreated and without chemically steeped OPT fines. Generally, glucose and xylose yields were affected by the pretreatment process preceding the dilute acid hydrolysis. Table 1 shows that the highest glucose yield was significantly obtained from the non-steeped and hydrolyzed OPT fines, whereas the optimum xylose yield was significantly obtained from chemical-steeped and hydrolyzed OPT. The presence of pre-treatment before dilute acid hydrolysis contributed to higher xylose yield from OPT. This may due to lignocellulosic materials when pre-treated with sodium will degrade a significant amount of hemicelluloses to monomer sugars such as xylose; this is called alkali pre-treatment to solubilise some hemicellulose and lignin (Zheng and Rehmann 2014). In detail, the highest glucose yield (15.3%) was extracted from non-steeped OPT fines with the hydrolysis parameters of 6% sulphuric acid concentration for 60 min at 100 °C. Additionally, 32.4% xylose yield was significantly obtained from chemically steeped OPT fines with the hydrolysis parameters of 2% sulphuric acid concentration reacting for 30 min at 115 °C.

Table 1. Mean Comparison of Glucose and Xylose Yield with Standard Deviation at Different Hydrolysis Parameter from Pretreated and Without Chemically Steeped OPT Fines

Run	Factor 1	Factor 2:	Factor 3:	Factor 4:	Response Y_1:	Response Y_2:
	P: Pre-treatment	Temperature (°C)	Time (min)	Acid Concen-tration (%)	Glucose (%, w/w)	Xylose (%, w/w)
1	1	100	15	2	3.6 ± 0.06^h	5.0 ± 0.15^o
2	1	115	15	2	6.5 ± 0.3^e	17.6 ± 0.17^d
3	1	130	15	2	4.9 ± 0.1^g	14.0 ± 0.00^h
4	1	100	30	2	2.1 ± 0.0^l	2.3 ± 0.30^p

	Factor 1	Factor 2:	Factor 3:	Factor 4:	Response Y_1:	Response Y_2:
Run	P: Pre-treatment	Temperature (°C)	Time (min)	Acid Concentration (%)	Glucose (%, w/w)	Xylose (%, w/w)
5	1	115	30	2	6.4 ± 0.4^{ef}	19.4 ± 0.70^{c}
6	1	130	30	2	6.3 ± 0.1^{ef}	16.6 ± 0.60^{def}
7	1	100	60	2	2.9 ± 0.1^{jk}	5.4 ± 0.23^{o}
8	1	115	60	2	5.3 ± 0.25^{fg}	15.4 ± 0.17^{fg}
9	1	130	60	2	6.5 ± 0.21^{e}	15.1 ± 0.40^{gh}
10	1	100	15	4	4.3 ± 0.21^{ghi}	6.0 ± 0.15^{no}
11	1	115	15	4	8.5 ± 0.21^{c}	23.3 ± 0.44^{a}
12	1	130	15	4	7.0 ± 1.0^{de}	17.1 ± 0.70^{de}
13	1	100	30	4	3.1 ± 0.4^{jk}	7.0 ± 0.50^{mn}
14	1	115	30	4	8.0 ± 0.3^{cd}	22.0 ± 0.60^{ab}
15	1	130	30	4	3.1 ± 0.4^{jk}	7.0 ± 0.50^{mn}
16	1	100	60	4	3.8 ± 4.0^{h}	9.7 ± 0.20 ^{k}
17	1	115	60	4	4.5 ± 0.17^{gh}	12.5 ± 0.20^{i}
18	1	130	60	4	7.0 ± 0.21^{de}	11.2 ± 0.15^{j}
19	1	100	15	6	7.2 ± 0.20^{de}	7.8 ± 0.67^{m}
20	1	115	15	6	11.4 ± 0.72^{b}	16.1 ± 0.06^{efg}
21	1	130	15	6	11.7 ± 0.30^{b}	19.8 ± 0.90^{c}
22	1	100	30	6	3.4 ± 0.30^{i}	9.3 ± 0.30^{kl}
23	1	115	30	6	6.3 ± 0.30^{ef}	21.6 ± 0.20^{b}
24	1	130	30	6	6.3 ± 0.30^{ef}	22.8 ± 0.60^{ab}
25	1	100	60	6	15.3 ± 0.15^{a}	12.1 ± 0.15^{ij}
26	1	115	60	6	6.2 ± 0.25^{ef}	19.5 ± 0.15^{c}
27	1	130	60	6	7.2 ± 0.25^{de}	8.3 ± 0.10^{lm}
28	2	100	15	2	2.1 ± 0.15^{klm}	8.9 ± 0.10^{l}
29	2	115	15	2	2.7 ± 0.21^{jkl}	18.9 ± 0.60^{h}
30	2	130	15	2	6.3 ± 0.62^{cde}	27.6 ± 0.36^{cd}
31	2	100	30	2	2.2 ± 0.20^{klm}	4.7 ± 0.50^{m}
32	2	115	30	2	11.1 ± 0.35^{a}	32.4 ± 0.90^{a}
33	2	130	30	2	4.2 ± 0.10^{gh}	24.1 ± 0.10^{efg}
34	2	100	60	2	1.3 ± 0.30^{m}	4.7 ± 0.06^{m}
35	2	115	60	2	5.4 ± 0.26^{ef}	29.6 ± 0.15^{c}
36	2	130	60	2	10.4 ± 0.21^{a}	25.4 ± 0.15^{def}
37	2	100	15	4	1.6 ± 0.20^{lm}	4.3 ± 0.15^{n}
38	2	115	15	4	3.3 ± 0.15^{ghi}	25.7 ± 0.89^{de}
39	2	130	15	4	4.4 ± 0.10^{fg}	17.7 ± 0.80^{ij}
40	2	100	30	4	1.9 ± 0.30^{lm}	4.4 ± 0.20^{n}
41	2	115	30	4	3.8 ± 0.30^{gh}	23.2 ± 0.90^{fg}
42	2	130	30	4	7.9 ± 0.00^{b}	18.7 ± 0.80^{hi}
43	2	100	60	4	4.1 ± 0.40^{gh}	16.5 ± 0.15^{ij}
44	2	115	60	4	6.9 ± 0.31^{bc}	31.9 ± 0.15^{ab}
45	2	130	60	4	5.7 ± 0.26^{de}	24.3 ± 0.51^{efg}
46	2	100	15	6	1.5 ± 0.10^{m}	29.8 ± 2.84^{bc}
47	2	115	15	6	3.6 ± 0.38^{ghi}	23.4 ± 0.25^{fg}
48	2	130	15	6	6.0 ± 0.10^{cde}	18.7 ± 0.50^{hi}
49	2	100	30	6	3.2 ± 0.30^{ijk}	11.3 ± 0.20^{k}
50	2	115	30	6	4.4 ± 0.10^{fg}	22.9 ± 0.50^{g}
51	2	130	30	6	7.0 ± 0.60^{bc}	15.7 ± 0.80^{j}
52	2	100	60	6	3.4 ± 0.00^{ghi}	18.6 ± 0.31^{hi}
53	2	115	60	6	6.5 ± 0.25^{cd}	29.5 ± 0.25^{c}
54	2	130	60	6	6.8 ± 0.50^{bc}	9.5 ± 0.15^{kl}

Note: 1: Without pretreatment; 2: With pretreatment
*Means followed by the same letters in the same column are not significantly difference at P ≤ 0.05

Effect of Chemical Steeping on Glucose and Xylose Yield

Generally, chemical steeping followed by dilute acid hydrolysis resulted in improved glucose and xylose yields. Overall, the total yield of glucose and xylose from the combined pretreatment and acid hydrolysis process showed improvement in the ranges of 2.5% to 28.8% higher than by dilute acid hydrolysis alone (Table 1).

The study showed that sodium metabisulphite ($Na_2S_2O_5$) and lactic acid ($C_3H_6O_3$) are promising chemical pretreatment agents that can simultaneously extract starch and improve the total extractable sugar yields of the dilute acid hydrolysis process. When sodium metabisulfite ($Na_2S_2O_5$) is dissolved in water, sodium bisulfate is generated, which is a weakly acidic solution. Sodium bisulfate and lactic acid could dissolve the lignin that binds the hemicelluloses and cellulose fibers together. Testova *et al.* (2014) and Zou *et al.* (1996a,b) showed that cellulose degrades in acidic media, with the rate of degradation increasing linearly with acid concentration. Thus, when the starch-free OPT was subjected to dilute acid hydrolysis, a higher xylose yield was obtained.

Effect of Independent Hydrolysis Parameters on Glucose and Xylose Yield Hydrolyzed from Non-pretreated and Pretreated OPT

Tables 2 through 5 show that the effects of hydrolysis temperature, hydrolysis reaction time, and acid concentration are significant, as their P values were less than 0.05.

Table 2. Summary of ANOVA for Variables and Interaction on Glucose Yield from Non-pretreated OPT

Variables	F Value	Prob > F	Sig.
A-Temperature	243.25	28.79	***
B-Time	298.11	35.26	***
C-Concentration	759.80	89.86	***
A*B*C	160.88	19.03	***

Note: *** indicates significance level at P ≤ 0.01; ** indicates significance level at P ≤ 0.05; ns indicates no significance

Table 3. Summary of ANOVA for Variables and Interaction on Xylose Yield from Non-pretreated OPT

Variables	F Value	Prob > F	Sig.
A-Temperature	5199.88	907.09	***
B-Time	209.18	36.49	***
C-Concentration	378.86	66.09	***
A*B*C	302.78	52.82	***

Note: *** indicates significance level at P ≤ 0.01; ** indicates significance level at P ≤ 0.05 ns indicates no significance

Table 4. Summary of ANOVA for Variables and Interaction on Glucose Yield from Pretreated OPT

Variables	F Value	Prob > F	Sig.
A-Temperature	994.55	123.03	***
B-Time	261.33	32.33	***
C-Concentration	25.09	3.10	***
A*B*C	158.24	19.58	***

Note: *** indicates significance level at P ≤ 0.01; ** indicates significance level at P ≤ 0.05 ns indicates no significance

Table 5. Summary of ANOVA for Variables and Interaction on Xylose Yield from Pretreated OPT

Variables	F Value	Prob > F	Sig.
A-Temperature	2837.81	1517.35	***
B-Time	164.69	88.05	***
C-Concentration	27.33	14.61	***
A*B*C	110.40	59.03	***

Note: *** indicates significance level at P ≤ 0.01
** indicates significance level at P ≤ 0.05
ns indicates no significance

Effect of Hydrolysis Temperature on Glucose and Xylose Yield

From the ANOVA analysis (Tables 2 through 5), it was shown that the factor of hydrolysis temperature (A) significantly influenced the yields of glucose and xylose from non-pretreated and pretreated OPT.

The highest yield of glucose obtained by non-pretreated OPT was 15.33% at 100 °C, whereas the highest xylose yield was 23% at 115 °C. The optimum yield of glucose and xylose extracted from pretreated OPT were 11.13% and 32.40% at 115 °C, respectively (Table 1).

Overall, when the temperature was increased from 100 to 130 °C, the glucose and xylose yields increased at first, and then decreased, which means that the optimal extraction of glucose and xylose occurred between 115 and 130 °C. Prior research showed that elevated temperature and pressure could break down the constituent hemicellulose, while the lignin protective layer around hemicellulose is softened. This allows the acid to hydrolyse the hemicellulose into monomers, mainly xylose (Lavarack *et al.* 2002; Wyman *et al.* 2005).

Effect of Hydrolysis Reaction Time on Glucose and Xylose Yield

The model F-value of hydrolysis reaction time (B) implies that the parameter is a significant model term for the dilute acid hydrolysis process to obtain glucose and xylose from pretreated OPT fines. The highest xylose yield was 32.4% for chemically treated OPT fines with a reaction time of 30 min. However, the glucose yield was not greatly affected by the reaction time because the highest yield of glucose obtained was 15.3% for untreated OPT with a reaction time of 60 min.

Time is needed to allow acid penetration into the tiny solid particles, such that the glycosidic bonds between the hemicellulose could be broken for the release of sugar. A previous study indicated that 30 min of hydrolysis reaction time was sufficient to extract the optimum xylose yield. This is because prolonged reaction time may degrade the xylose to its by-products. Rahman *et al.* (2006) also proved that the concentration of xylose in the resulting hydrolysate would eventually decrease when the reaction time was further increased. Overall, when the reaction time increased to 60 min, glucose yield increased. The crystalline structure of cellulose hinders its hydrolysis into glucose. Therefore, longer reaction time is needed for the hydrolysis process to degrade the cellulose.

Effect of Acid Concentration on Glucose and Xylose Yield

The parameter of acid concentration (*C*) significantly influenced the glucose and xylose yields from pretreated OPT fines. Previous studies showed that an acid concentration from 2% to 6% is sufficient for the extraction of fermentable sugars from lignocellulosic material using dilute acid hydrolysis (Wyman 1994; Parajó *et al.* 1995; Téllez-Luiz *et al.* 2002; Timung *et al.* 2016). Acid acts as a catalyst for the hydrolysis reaction to extract sugars from the lignocellulosic material. Hemicellulose is easily hydrolyzed by dilute acid hydrolysis.

Response Surface Methodology Analysis

Pretreatment is a key process step of biochemical help in removing the physical barrier and allow conversion of lignocellulosic materials higher yield of total glucose and xylose yield obtained. Hence, the results obtained for the runs of experimental design for the lignocellulosic hydrolysates are presented as response surfaces (Figs. 3 to 8).

The quadratic models in terms of coded variables are shown in Eqs. 1 and 2, where (Y_1) represents glucose and (Y_2) represents xylose, as a function of reaction hydrolysis temperature (A), hydrolysis reaction time (B), and acid concentration (C). For the first response (Y_1), the coefficient of determination (R2) obtained was 0.62, which shows 62% of the variability in response, while the second response (Y_2) shows that the coefficient of determination (R^2) obtained was 0.75%, which explained 75% of the variance in response.

The following is a second-oder quadratic polynomial equation including the main effects and interaction effects of each variable:

$$Y_1 = 6.21 + 1.06A + 2.08B - 0.20C + 0.13AB - 0.03AC - 0.19BC - 0.52A^2 - 1.74B^2 + 0.50C^2 \tag{2}$$

$$Y_2 = 26.65777186214 + 0.76A + 4.36B - 0.48C - 0.60AB - 1.55AC + 5.84BC + 2.79A^2 - 13.52B^2 + 1.24C^2 \tag{3}$$

Combined Effect of Hydrolysis Temperature and Hydrolysis Reaction Time (A x B Terms) at Different Levels of Acid Concentration (C) on Glucose (Y_1) and Xylose Yield (Y_2)

The effect of reaction temperature (A) and reaction time (B) on glucose and xylose yields when the acid concentration (C) ranged from 2% to 6% are shown in Figs. 3 and 4, respectively. The optimum concentration of acid on the interaction of A x B parameters on glucose (Y_1) and xylose (Y_2) yields was 2%.

Figure 3 shows that the glucose recovery was slightly higher in the right upper zone of the contour graph, corresponding to higher reaction temperature and longer hydrolysis reaction time. Meanwhile, glucose recovery was lower in the left zone of the contour graph, corresponding to lower reaction temperature and shorter hydrolysis reaction time. In contrast, the interaction of A x B terms on xylose yield (Y_2) is explained in Fig. 4. This indicates that the xylose recovery increased at higher hydrolysis temperature but shorter reaction time in the range of 40 to 53 min. The xylose recovery was decreased with the interaction of higher hydrolysis temperature and reaction time shorter than 30 min, which was in the lower zone of the contour graph. Dilute acid hydrolysis of sulphuric acid in the lignocelluloses materials and operate at moderate temperature can dissolve almost hemicelluloses in sugars and dissolved lignin partially (Zhang *et al.* 2011). It can be performed at moderate temperature (<160C) for longer hydrolysis time (30 to 90 min) (Alvira *et al.* 2010; Kumar *et al.* 2009).

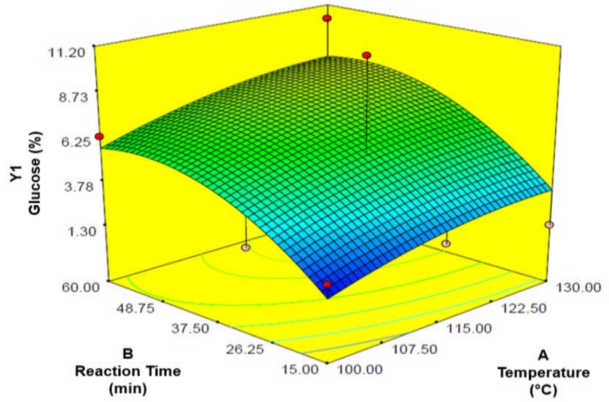

Fig. 3. Effect of hydrolysis temperature and hydrolysis reaction time (A x B terms) at different levels of acid concentration on glucose (Y1)

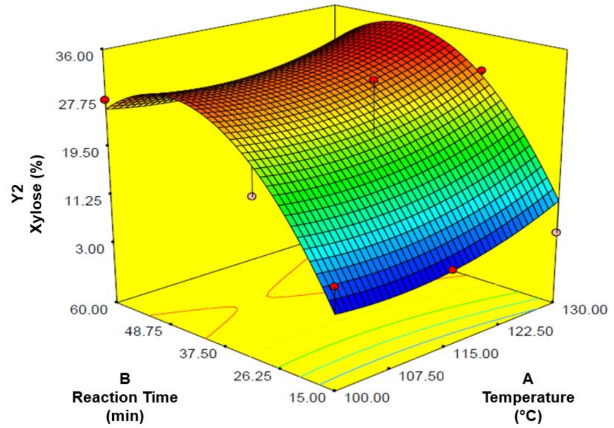

Fig. 4. Effect of hydrolysis temperature and hydrolysis reaction time (A x B terms) at different levels of acid concentration on xylose (Y_2)

Combined Effect of Hydrolysis Temperature and Acid Concentration (A x C Terms) at Different Levels of Reaction Time (B) on Glucose (Y_1) and Xylose Yield (Y_2)

Figures 5 and 6 show the interaction of A x C intercepts on glucose and xylose recovery for the reaction times in the range of 15 to 60 min. On the interaction of A x C parameters, the optimum reaction time for maximising glucose (Y_1) and xylose (Y_2) yields was approximately 53 min. From Fig. 5, the ISO-recovery curves of glucose is almost parallel to the Y-axis (C). This suggests that the hydrolysis temperature played a prominent effect on the intercept of acid concentration on glucose yield. Hence, the contour graph shows that glucose recovery was lower in the left zone, meaning that the minimum glucose yield was obtained at low reaction temperature for all variations of acid concentration. However, the glucose recovery was slightly increased when the reaction temperature increased to approximately 130 °C and at low acid concentration.

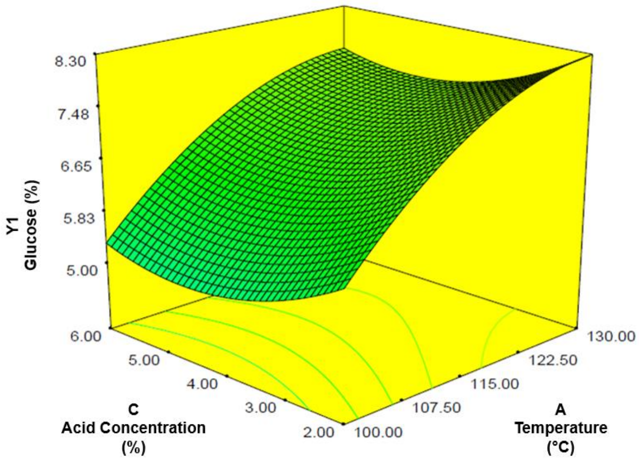

Fig. 5. Effect of intercept hydrolysis temperature and acid concentration (A x C terms) at different levels of temperature on glucose (Y_1)

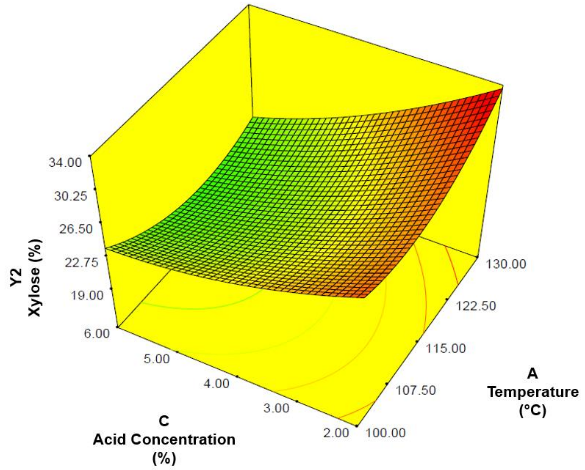

Fig. 6. Effect of intercept hydrolysis temperature and acid concentration (A x C terms) at different levels of temperature on xylose (Y2)

Next, Fig. 6 shows that the minimum xylose recovery occurred in the upper zone of the contour graph, which corresponds to high acid concentration approximately in the range of 4% to 6% and interacting with different levels of temperature. However, xylose recovery slighly increased when the acid concentration decreased from 4% to 2% and interacting with temperatures in the range of 100 to 130 °C. In addition, the optimum xylose yield was predicted to be obtained in the range of 122.5 to 130 °C and 2% to 3% of acid concentration.

Combined Effect of Hydrolysis Reaction Time and Acid Concentration (B X C Terms) at Different Levels of Temperature on Glucose (Y_1) and Xylose Yield (Y_2)

Figures 7 and 8 show the interaction of B x C parameters on the response of glucose recovery (Y_1) and xylose recovery (Y_2) for hydrolysis temperature in the range of 100 to 130 °C.

The glucose ISO-recovery (Y_1), interpreted as the ISO-curves, were almost parallel to the C-axis (acid concentration), as can be seen in the contour graph of Fig. 7 from the bottom left to upper left zone. The minimum glucose yield of 3.71% was obtained at lower hydrolysis temperature when interacting with different acid concentrations. When the hydrolysis reaction time was increased, the rate of change of glucose yield was increased, reaching approximately 6.88% at 37 min. When the hydrolysis reaction time was increased to 60 min, the glucose recovery subsequently reached the optimum at the predicted 8.30% yield with temperature in the range of 50 to 60 °C and a low acid concentration of 2%.

Figure 8 shows that the xylose ISO-recovery (Y_2) was greatly increased from 15 min to 26 min, reaching 29.30% yield. With the longer reaction time between 30 min to 60 min, the xylose yield was maximised in the middle section of the graph. Hence, the xylose yield increased when acid concentration was decreased. In addition, xylose recovery decreased when the reaction time exceeded 60 min, which may be due to the longer hydrolysis reaction time causing xylose to degrade to other products.

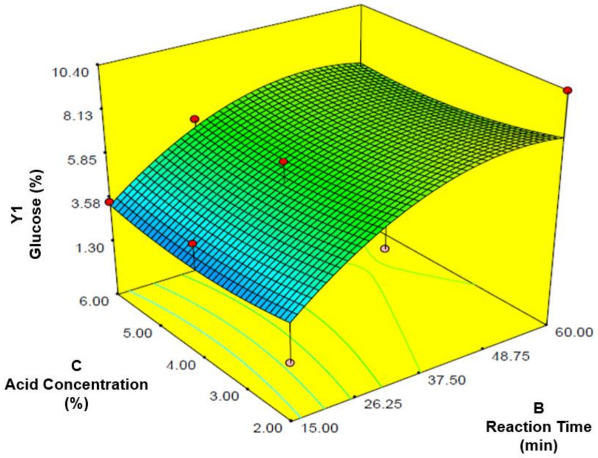

Fig. 7. Effect of hydrolysis reaction time and acid concentration (B x C terms) at different levels of temperature on glucose (Y1)

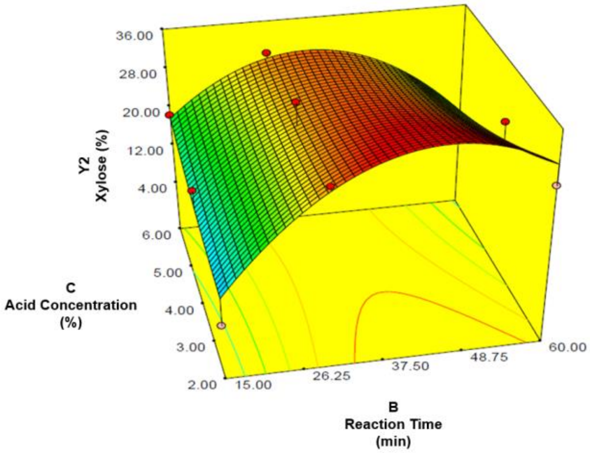

Fig. 8. Effect of hydrolysis reaction time and acid concentration (B x C terms) at different levels of temperature on xylose (Y2)

Optimum Condition for Glucose (Y_1) and Xylose Yield (Y_2)

Based on the models, numerical optimization of glucose and xylose yield was performed using Design Expert v7.0.0 (Stat-Ease, Minneapolis, Minnesota), considering each value of response and 16 solutions that were obtained as shown in Table 6. To maximise glucose and xylose yields simultaneously, pretreatment was chosen and analysis predicted that the optimum glucose yield (Y_1) of 8.30% and optimum xylose yield (Y_2) of 33.66% can be extracted at the hydrolysis temperature of 130 °C X 53 min X 2% acid concentration; which as predicted the chemical steeping play a dominant role as a pretreament for glucose and xylose hydrolysed from OPT.

Table 6. RSM Solution for Hydrolysis Temperature, Reaction Time, and Concentration of Dilute Acid Hydrolysis for Glucose and Xylose Production from OPT

Solutions	Temperature (A)	Time (B)	Acid Concentration (C)	Response (%)	Response (%)
Number	(°C)	(min)	(%)	Y1	Y2
1	130.00	53.01	2.00	8.30	33.66
2	130.00	53.33	2.00	8.30	33.53
3	129.99	53.52	2.00	8.30	33.45
4	129.42	53.58	2.00	8.29	33.15
5	130.00	51.37	2.00	8.29	34.25
6	129.05	53.01	2.00	8.29	33.20
7	130.00	50.80	2.00	8.28	34.42
8	129.55	55.22	2.00	8.27	32.42
9	128.49	50.95	2.00	8.26	33.65
10	128.59	50.77	2.00	8.26	33.75
11	127.44	50.11	2.00	8.23	33.40
12	125.59	51.62	2.00	8.21	32.20
13	130.00	43.39	2.02	7.97	35.01
14	130.00	40.89	6.00	7.28	28.80
15	130.00	39.43	6.00	7.18	29.14
16	130.00	42.74	5.07	7.16	28.89

CONCLUSIONS

1. A substantial amount of glucose and xylose can be obtained through dilute acid hydrolysis combined with chemical steeping pretreatment in a sodium metabisulphite and lactic acid solution.
2. Chemical steeping could play a dual role as a pretreatment method and a starch extraction method.
3. The total yield of glucose and xylose from pretreated OPT fines was improved by between 2.5% to 28.8% compared to the yield from untreated OPT fines. In addition, the independent parameters, namely hydrolysis temperature, hydrolysis reaction time, and acid concentration, had significant interaction with each variable on both glucose

and xylose yields with P value ≤ 0.01.

4. An optimization was performed (130 °C X 53 min X 2% acid concentration) to obtain the optimum glucose and xylose yields from pretreated OPT fines. Glucose and xylose yields slightly increased when the acid concentration decreased, while the hydrolysis temperature and reaction time were conversely increased. Otherwise, the optimum glucose and xylose yields could be obtained together when the hydrolysis reaction time was decreased while temperature and acid concentration were increased during the hydrolysis process.
5. The OPT is a potential source of glucose, xylose, and starch. Hence, the processing of OPT can be optimized into one of the value-added raw materials, thereby improving its overall economic value and minimising environmental impact.
6. Further investigation on the influence of chemical steeping as a pretreatment on different materials should be considered.

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