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Article

Deacetylation followed by fractionation of yellow poplar sawdust for the production of toxicity-reduced hemicellulosic sugar for ethanol fermentation

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Abstract: In order to produce bioethanol from yellow poplar sawdust without detoxification, deacetylation (mild alkali treatment) was performed with aqueous ammonia solution. To select the optimal conditions, deacetylation process was carried out using different conditions: NH_4OH loading (2–10 % (w/v)) and solid-to-liquid ratio (1:4–10) at 121 °C for 60 min. In order to assess the effectiveness of deacetylation, fractionation of deacetylated yellow poplar sawdust was performed using dilute acid (H_2SO_4, 0.5–2.0% (w/v)), reaction temperature (130–150 °C) and time (10–80 min). The toxicity-reduced hemicellulosic hydrolysates that were obtained through a two-step treatment at optimized conditions were fermented using Pichia stipitis for ethanol production, without any further detoxification. The maximum ethanol production was 4.84 g/L, corresponding to a theoretical ethanol yield of 82.52%, which is comparable to those of intentionally made hydrolyzates as controls.

Keywords: Biomass, Deacetylation; Pretreatment; Xylose; Pichia stipites; Acetic acid

1. Introduction

The increasing demand for alternative sources of fuel has increased public interest in the development of biofuel. Significant effort has been invested in the conversion of lignocellulosic biomass to bioethanol as a potential source of fuel [1]. Lignocellulosic biomass, which is generally sourced from wood and agricultural wastes, is mainly composed of cellulose, hemicellulose, and lignin. Due to its recalcitrant characteristics, the biomass requires an appropriate fractionation process to produce fermentable sugars [2,3].

Generally, the fractionation methods for lignocellulosic biomass mainly contain physical, chemical, biological methods, and their combinations [4], which have different impact on the structure of the lignocellulosic material, and have significant impact on the downstream steps of the biomass conversion process on sugar recovery, toxicity of hydrolysates, enzymatic hydrolysis and fermentation, and waste water treatment demands [5].

Dilute acid fractionation is an efficient chemical method that accomplishes the selective degradation of hemicellulose fractions, and improves cellulose fractions in the residual solids. When compared to other fractionation technologies, it is also an important technology in terms of cost [6]. Furthermore, this method also enhances the accessibility of cellulase to cellulose in the residual solid [7]. However, the major obstacles of dilute acid fractionation are the sugar degradation products and toxic compounds that are presented in the hemicellulosic hydrolysate, such as 5-hydroxymethylfurfural (HMF), furfural, formic acid, and acetic acid [6].

One of the well-known inhibitors is acetic acid, which is formed by the cleavage of bonded acetyl groups by deacetylation of hemicelluloses during biomass pretreatment [8]. Therefore, techniques need to be developed to reduce the toxicity of hemicellulosic hydrolysate, to make the high value-added product production from biomass more economically competitive [5]. Several methods have been proposed to reduce the concentration of toxic compounds to levels that would not inhibit the fermentation process. These methods can be divided into the following three main groups: physical, chemical, and/or biological detoxification methods [9]. At this time, detoxification has also led to a considerable loss of fermentable sugars, and is therefore not a feasible method [10]. In addition, these methods usually do not provide reasonable results when applied alone, making it necessary to employ a combination of them, which increases the operational costs [11].

In our previous studies, alkaline treatments have been studied to dissolve lignin and hemicellulose, and the de-esterification of intermolecular ester bonds [12]. However, these alkaline conditions to obtain lignin are not suitable at the biorefinery concept, because recovery of hemicellulosic sugar in hydrolysate derived from alkaline treated biomass is not easy for microbial fermentation, due to the high amount of lignin degradation compounds and others [13]. The loss of hemicellulosic sugars, mainly xylose, must be avoided, because these pentose sugars can be converted into higher value compounds, including ethanol, xylitol, and others [14,15], contributing to the economic feasibility of the biorefineries. To solve this problem, deacetylation process at low severe condition prior to dilute acid fractionation could show not only significant improvement on sugars yields, but also toxicity reduction in hydrolysate, thereby enhancing bioethanol production [16]. Therefore, several researchers have tried to remove the acetyl group with sodium hydroxide before fractionation, to reduce the toxicity in hemicellulosic hydrolysate [5,8,17,18].

In the present study, deacetylation through mild alkaline treatment with aqueous ammonia solution was performed to produce a toxicity-reduced hemicellulosic hydrolysate (TRH hydrolysate) that was suitable for ethanol fermentation, without an additional detoxification process. This treatment was performed prior to the dilute acid fractionation, with the purpose of selectively removing the toxic compounds, such as acetyl group, without degradation of the polysaccharide, to improve hemicellulosic sugar extraction yield in the dilute sulfuric acid fractionation (DSA fractionation). To evaluate the effectiveness of DSA fractionation for ethanol production, ethanol fermentation using Pichia stipites, which has become widely known for its ability to rapidly metabolize xylose into ethanol, was conducted with TRH hydrolysate fractionated from deacetylated yellow poplar sawdust (YPS).

2. Results and Discussion

2.1. Composition change in the liquid phase derived from deacetylated YPS

The effectiveness of deacetylation (mild alkali treatment) was determined by the appearance of the formation of acetic acid in the liquid phase derived from YPS. Also, the additional effectiveness of the deacetylation might have weakened the hemicellulose structure, because untreated biomass revealed an intact structure, like a complex hierarchical structure with compact and highly compressed surface.

Table 1 summarizes the concentrations of acetic acid and other components in the liquid phase after the deacetylation of YPS. The acetic acid formation was affected by NH4OH loading more than by the solid-to-liquid ratio (S/L ratio) when the temperature and time were fixed. The maximum acetic acid concentration of 8.16 g/L was found to be with 2% NH_4OH loading and a S/L ratio of 1:4. The liquid phase contained mostly acetic acid, and negligible quantities of sugars and other inhibitors (not detected decomposed product, such as 5-HMF and furfural). Castro et al. [5] have obtained the maximum formation of acetic acid in rice straw hemicellulosic hydrolysate, which resulted in the highest hemicellulose extraction, and this is due to the acetyl groups being structurally linked to hemicellulose. However, in this study, the acetic acid concentration decreased with increasing NH_4OH loading and S/L ratio (Table 1). In the present study, the highest concentration of acetic acid was not obtained when the xmg (xylose + mannose + galactose) concentration was the highest. We could predict that ammonium acetate is produced by saturating acetic acid with ammonia [19].

Table 1. Sugars and decomposed products in the liquid phase after deacetylation of YPS.

Solid-to-liquid ratio	Ammonia concentration (%)	Compositions (g/L)			
		Glucose	xmg [1]	Acetic acid	Formic acid
1:4	2	0.36	1.91	8.16	0.73
	4	0.19	0.30	6.20	0.67
	6	0.17	0.28	5.63	0.60
	8	0.21	0.30	4.84	0.59
	10	0.23	0.29	4.54	0.59
1:6	2	0.21	0.32	4.94	0.31
	4	0.12	0.17	4.21	0.39
	6	0.13	0.17	3.72	0.36
	8	0.15	0.18	3.47	0.38
	10	0.17	0.18	3.31	0.37
1:8	2	0.15	0.34	3.67	0.21
	4	0.10	0.12	3.08	0.26
	6	0.11	0.13	2.78	0.27
	8	0.12	0.13	2.64	0.29
	10	0.13	0.14	2.45	0.27
1:10	2	0.14	0.49	3.17	0.18
	4	0.11	0.09	2.51	0.22
	6	0.10	0.10	2.19	0.23
	8	0.10	0.09	1.99	0.20
	10	0.08	0.04	0.36	0.00

[1] XMG = xylan + mannan + galactan

2.2. Optimization of the deacetylation process conditions

In order to optimize the deacetylation conditions, the conditions should be selected that maximize the removal of acetyl groups, with minimum degradation of cellulose and hemicellulose. Table 2 shows the chemical composition of YPS in raw, and after deacetylation, condition. As can be seen, acetyl groups and ash were the main fractions affected by this deacetylation, which favored their removal under almost all conditions. The highest acetyl removal (100%) was achieved when using the more conditions of S/L ratio 1:8, 6% NH_4OH loading at 121 °C for 60 min, in which also more than 80% of ash was removed, while glucan and XMG (=xylan+mannan+galactan) showed losses of up to 17.99%.

The biomass recovery (%) is expressed as the solid remaining after deacetylation, based on the original oven-dry weight. The solid remaining was about 70% under all conditions. In particular, the highest value (77.02%) was obtained with an S/L ratio of 1:6 and 2% NH_4OH loading, for which the removal of acetyl group was 91.46%. These results indicate that the purpose for deacetylation was successfully achieved, since acetyl group was the main fraction removed from biomass. Additionally, the deacetylation removed a significant amount of ash (94.95 %), which also contributes to overcoming the major drawback of toxic compounds formation during the subsequent biomass fractionation steps. In this step, acid insoluble lignin (AIL) was hardly removed, in comparison with that of deacetylated biomass by NaOH. It can be expected that NH_4OH is a weak base; thus less delignification is achieved than that with NaOH. Castro et al. [5] shows a higher removed amount of lignin (up to 34%) by NaOH deacetylation of rice straw.

From these results, the optimal condition of the deacetylation process was selected based on high solid remaining (77.02%), high removal of acetyl group (91.46%), and low effectiveness of carbohydrate fraction (glucan 1.05%, XMG 3.35%). Finally, the selected optimal condition was S/L ratio 1:6, with 2% NH_4OH loading at 121 °C for 60 min. Castro et al. [5] found lower values of acetyl removal (86.5%) during the deacetylation of rice straw at 70 °C for 45 min, using 80 mg NaOH/g biomass. At this time, 15.1% glucan and 15.9% XMG were removed.

126 **Table 2.** Change of chemical composition before and after deacetylation of YPS and removal percentage.

S/L ratio	Ammonia concentrations (%)	Solid remaining (%)	Composition of deacetylated YPS (%)					Removal through deacetylation treatment (%)				
			Glucan	XMG [1]	Acetyl group	AIL [2]	AIA [3]	Glucan	XMG[a]	Acetyl group	AIL [b]	AIA [c]
Initial		100.00	38.52	14.32	4.42	18.86	3.51					
1:4	2	69.09	49.28	18.13	0.45	25.43	0.00	11.61	12.53	92.97	6.84	100.00
	4	68.32	50.17	17.89	0.33	25.93	0.00	11.02	14.65	94.90	6.07	100.00
	6	70.34	49.73	17.72	0.30	25.03	0.05	9.19	12.96	95.23	6.65	99.00
	8	66.97	50.73	17.85	0.26	25.39	0.00	11.80	16.52	96.06	9.84	100.00
	10	75.82	49.31	17.38	0.52	25.63	0.21	2.94	7.98	91.08	0.00	95.46
1:6	2	77.02	49.49	17.97	0.49	25.97	0.23	1.05	3.35	91.46	0.00	94.95
	4	75.09	48.93	17.26	0.24	25.82	0.51	4.62	9.49	95.92	0.00	89.09
	6	73.93	48.69	17.02	0.24	24.67	0.60	6.55	12.13	95.99	3.30	87.36
	8	71.74	47.61	16.58	0.44	24.36	0.66	11.33	16.94	92.86	7.34	86.51
	10	68.83	50.53	17.70	0.39	27.51	0.00	9.71	14.92	93.93	0.00	100.00
1:8	2	74.13	49.33	18.04	1.36	24.90	0.47	5.07	6.61	77.19	2.13	90.07
	4	67.58	51.03	17.95	0.91	25.13	0.00	13.12	17.80	86.50	9.95	100.00
	6	68.95	50.01	17.67	0.00	24.97	0.37	10.48	14.92	100.00	8.71	92.73
	8	71.10	51.19	17.90	0.00	26.61	0.30	5.51	11.13	100.00	0.00	93.92
	10	68.93	50.89	17.81	0.00	27.85	0.65	8.93	14.27	100.00	0.00	87.24
1:10	2	68.24	50.15	18.14	1.33	27.39	0.51	11.16	13.56	79.47	0.90	90.08
	4	71.04	49.94	17.79	0.00	26.44	0.92	7.90	11.75	100.00	0.41	81.38
	6	67.71	51.72	18.07	0.00	25.82	0.20	9.09	14.56	100.00	7.30	96.14
	8	71.30	51.07	17.70	0.00	25.43	0.40	5.47	11.87	100.00	3.86	91.87
	10	65.50	51.32	17.93	0.00	24.91	0.54	12.73	17.99	100.00	13.49	89.92

127 [1] XMG = xylan + mannan + galactan; [2] Acid insoluble lignin; [3] Acid insoluble ash

2.3. Optimization of dilute sulfuric acid fractionation conditions with deacetylated YPS for TRH hydrolysate production

The DSA fractionation was performed to optimize the condition as maximum hemicellulose fractionation. Figure 1 and 2 show the extraction yield of sugars and concentration of decomposed product in hydrolysate depending on the acid loading, reaction time, and temperature. Acetic acid, xmg, and glucose were released as the hemicellulose degraded (Palmqvist and Hahn-Hägerdal, 2000b). The xmg was the main component present in the hydrolysate with a concentration of 0.08–12.42 g/L. However, xmg was further degraded to furfural under higher severity, such as high-temperature and long reaction time [20], while hexose could be converted into 5-hydroxymethyl furfural (5-HMF) [21]. Consequently, the concentration of furfural and 5-HMF consistently increased with increasing reaction severity.

Figure 1 shows sugars extraction and concentration of decomposed products at 130 °C, 0.5–2.0 % (w/v) H_2SO_4 loading, and S/L ratio 1:10 for (a) 10 min, (b) 30 min, and (c) 50 min, in order to select effective acid loading. The sugars extraction yield in the hydrolysate increased with increasing acid loading and time. However, the extraction yield decreased at more than 1.5% acids loading, due to further decomposition of sugars. On the other hand, furfural and formic acid increased up to 0.4 g/L with increasing acid loading and time. Since the extraction yield of xmg was not yet high (highest extraction yield 53.23 % in Figure 1(c)), additional optimization was carried out through temperature changes at 1.5% acid loading, which has become widely known for its ability to rapidly metabolize xylose into ethanol.

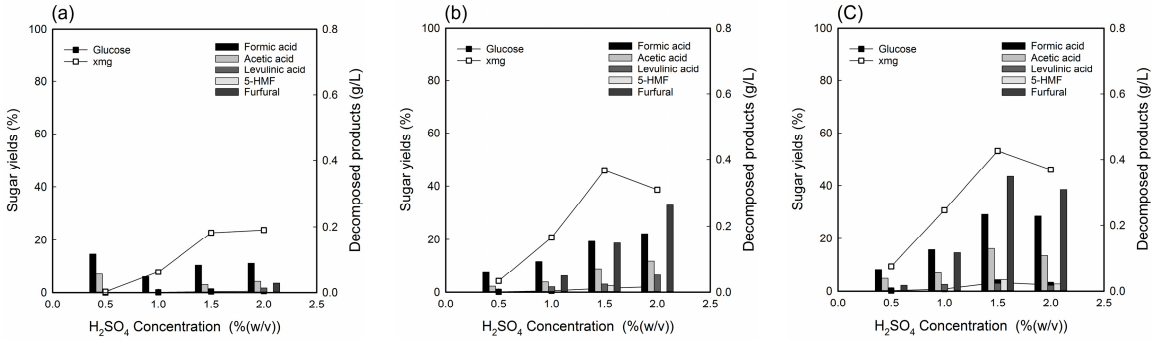

Figure 1. Extraction yield of sugars and concentration of inhibitors on various dilute acid loading (0.5–2.0 % (w/v)) fractionation of deacetylated YPS at 130 °C for reaction time (a) 10 min, (b) 30 min, and (c) 50 min.

Figure 2 shows the change of sugars extraction yield and decomposed products concentration on 1.5 % acid loading. The sugars extraction indicated gradual increase with increasing time at 130 °C (Figure 2(a)), but it maintained or decreased at higher temperature (Figure 2(b)). Regarding the sugars solubilization, glucose concentration was not above 2.67 g/L (corresponding to extraction yield of 5.70%) in all the hydrolysates, which indicated low cellulose degradation. In particular, the highest xmg extraction yield was 72.82% at 150 °C, 1.5 H_2SO_4 loading, and S/L ratio 1:10 for 30 min (Figure 2(c)). At this time, formic acid, acetic acid, levulinic acid, 5-HMF, and furfural were 0.33, 0.18, 0.12, 0.17, and 1.45 g/L, respectively. With respect to the inhibitor compounds, all hydrolysates derived from deacetylated YPS showed acetic acid concentrations below 0.25 g/L, which was expected, due to the effective removal of acetyl groups during the deacetylation. Also, the decomposed products known to be fermentation-decomposed products, such as furfural, 5-HMF from pentose and hexose sugars, are generated during acid fractionation. Other studies have reported that ethanol fermentation was unsuccessful when the concentrations of HMF, furfural, and acetic acid in the hydrolysate were more than 1, 1, and 2 g/L, respectively [10,22-24]. This finding

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implies that detoxification is rarely needed for ethanol fermentation, due to the low concentration of inhibitors present in the hydrolysate.

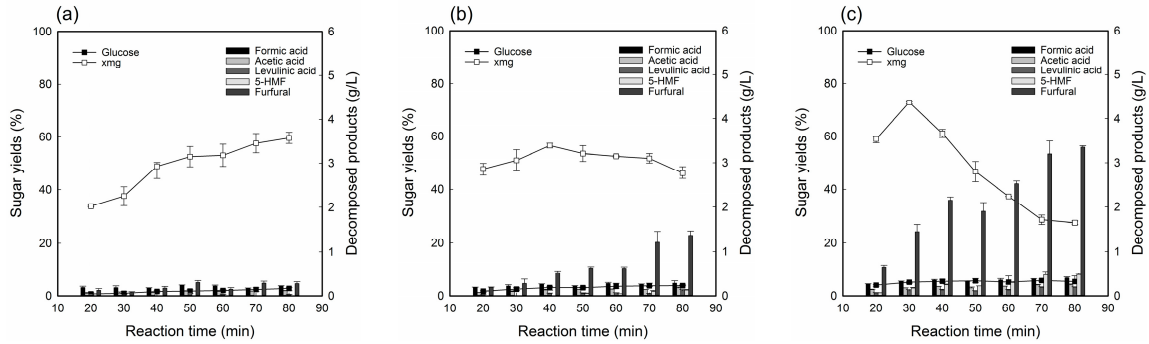

Figure 2. Extraction yield of sugars and concentration of inhibitors on dilute acid fractionation of deacetylated YPS for 50 min at 1.5 % (w/v) dilute acid loading and reaction temperature (a) 130 °C, (b) 140 °C, and (c) 150 °C.

In order to compare the effectiveness of the deacetylation on TRH hydrolysate composition, DSA fractionation was performed under the highest hemicellulose extraction condition, using the deacetylated YPS obtained under the optimized alkaline conditions, and non-deacetylated as a reference. Table 3 shows the chemical composition of the residual solid and hydrolysates obtained under DSA fractionation. The table shows much lower contents of 1.01 g/L glucose and 3.37 g/L xmg were found in the non-deacetylated hydrolysates. However, a higher concentration of acids and furan totaling 2.61 g/L was observed (Table 3), and the concentration of inhibitory compounds was quite different for both, deacetylated and non-deacetylated. Table 3 clearly shows that deacetylation could effectively remove the acetyl group in the YPS. In this condition, acetic acid, formic acid, 5-HMF, and furfural were decreased, while xmg extraction yield was increased.

Table 3. Comparison of chemical composition after dilute acid fractionation of deacetylated and non-deacetylated YPS under the optimal dilute acid fractionation condition.

Components in solid residue	Contents (%)		Components in liquid phase	Concentrations in hydrolysate (g/L)	
	deacetylated	non-deacetylated		deacetylated	non-deacetylated
Solid remaining	67.94	66.89	Glucose	1.36	1.01
Glucan	54.15	46.28	xmg[d]	11.31	3.37
XMG[a]	9.18	13.82	arabinose	N/D [e]	0.29
Arabinan	0.16	0.71	Formic acid	0.22	0.65
AIL[b]	29.00	25.96	Acetic acid	0.09	1.35
AIA[c]	1.08	0.66	Levulinic acid	N/D[e]	0.27
Acetyl group	0.23	3.70	5-HMF	N/D	0.03
Sum	93.80	90.98	Furfural	N/D	0.31

[1] XMG = xylan + mannan + galactan.
[2] AIL = Acid Insoluble Lignin
[3] AIA = Acid Insoluble Ash
[4] xmg = xylose + mannose + galactose
[5] Not detected

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2.4. Ethanol fermentation with toxicity-reduced hemicellulosic hydrolysate

Figure 3 shows the ethanol fermentation profile with the TRH hydrolysates to assess the toxicity reduction of the hydrolysate. Figure 3 (a) shows the fermentation result of TRH hydrolysate, while Figure 3 (b)–(d) represent various controls: ((b) : yeast extract 1 (w/v)%, peptone 2 (w/v)%, equivalent concentration of xmg in TRH hydrolysate, (c) : (b) + equivalent concentration of acetic acid in TRH hydrolysate, (d) : (b) + equivalent concentration of inhibitors in TRH hydrolysate), which were intentionally made to assess the extent of microbial inhibition reduction. In comparison with control solution (Figure 3 (b) and (c)), maximum ethanol concentration is almost equal, the ethanol production (Figure 3 (a)) of TRH hydrolysate obtained is 4.84 g/L, but the reaction time is longer, due to the effectiveness of other inhibitors. However, the ethanol production rate was further decreased only when the acetic acid concentration was about 1 g/L, compared to the presence of other inhibitors (Figure 3 (c)).

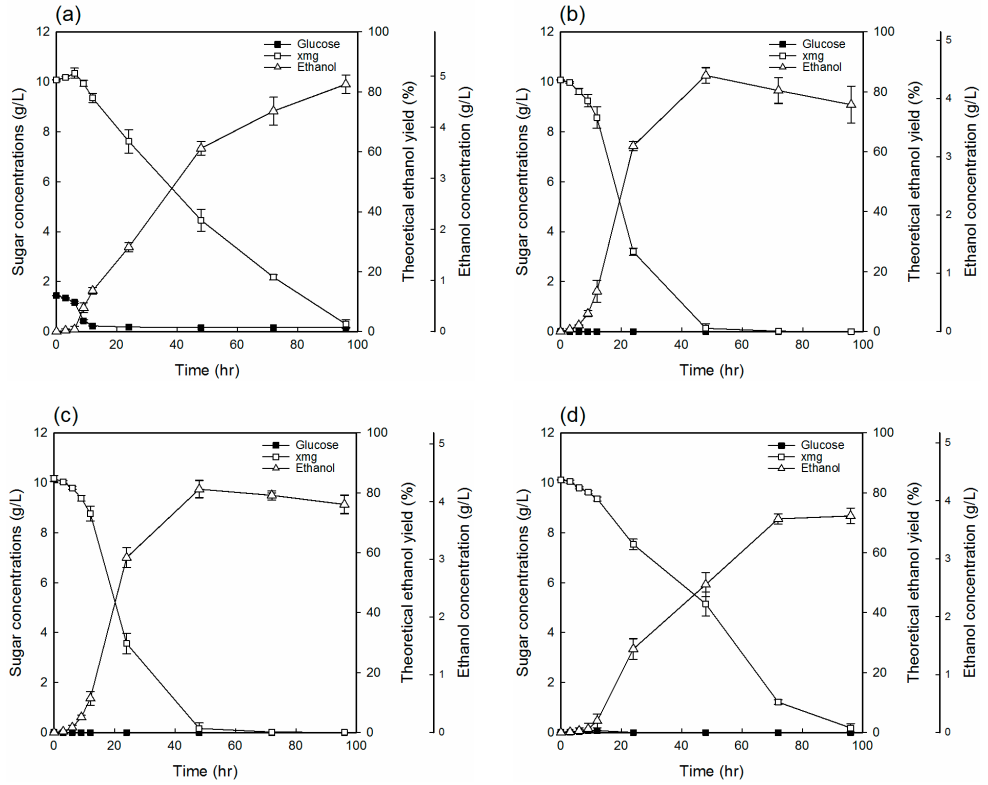

Figure 3. Ethanol production on both hydrolysate and various control solutions by *P. stipitis* (a) : toxicity-reduced hemicellulosic hydrolysate (TRH hydrolysate), (b) : yeast extract 1%, peptone 2%, equivalent concentration of xmg in TRH hydrolysate, (c) : (b) + equivalent concentration of acetic acid in TRH hydrolysate, and (d) : (b) + equivalent concentration of inhibitors in TRH hydrolysate.

The maximum ethanol concentration obtained from toxicity-reduced hydrolysate under optimal treatment condition was 4.84 g/L, corresponding to a theoretical ethanol yield of 82.52 % (Figure 4). Most of the xmg was consumed within 72 h. Moreover, the maximum theoretical yield of ethanol obtained similar to the control solution, due to the low concentration of inhibitors in the toxicity-reduced hydrolysate, because ethanol production from the hydrolysate depends on the concentration of fermentation inhibitors. Therefore, it is also important to highlight that it was not necessary to ferment the deacetylated YPS hydrolysate to any further detoxification process, prior to fermentation.

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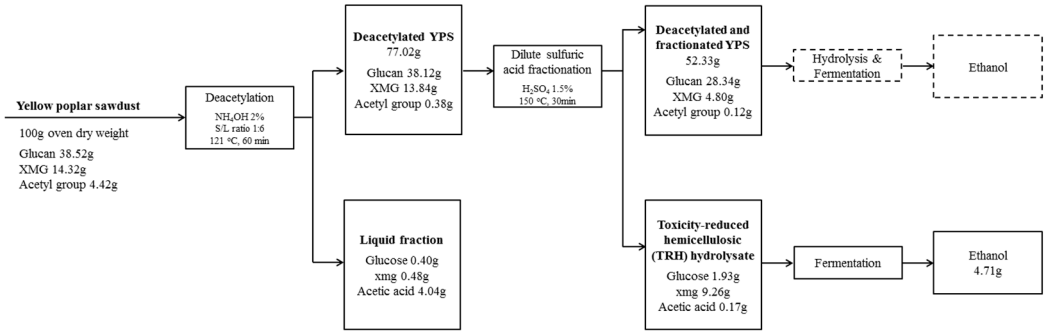

Figure 4. Mass balance for dilute sulfuric acid fractionation of decetylated YPS under optimized conditions.

2.5. Overall mass balance

The simplified flowchart and an overall mass balance of the decetylation and consecutive fractionation of YPS, which involved microbial fermentation, are summarized in Figure 4. Most of the acetic acid and hemicellulosic sugar were separated from the YPS by deacetylation and fractionation with aqueous ammonia and sulfuric acid. Under the optimized deacetylation condition, approximately 23% of the mass fraction was solubilized into liquid hydrolyzate, which indicated that 0.4 g of glucose, 0.5 g of xmg, and 4.0 g of acetic acid, while 38.1 g of glucan, 13.8 g of hemicellulose, and 0.4 g of acetyl-group were found in the 77 g of deacetylated YPS based on 100 g of raw YPS, During the deacetylation, 91.4 % of acetyl-group was dissolved into liquid fraction, maintaining only 0.1% of glucose and 0.3% of hemicellulosic sugar (represented to xmg) were decom posed. The residual solid, i.e., deacetylated YPS, was subjected to consecutive acid fractionation under the condition of acid concentrations; 1.5 wt.%, reaction temperature; 150 °C, and reaction time; 30 min. The TRH hydrolysate resulting from the fractionation with 77 g of deacetylated YPS had a sugar fraction including 1.9 g of glucose, 9.3 g of hemicellulosic sugar.

During the fractionation step, 66.9% of hemicellulose was dissolved into liquid hydrolysate, while acetyl group, ash, and water extractive were mainly removed. The TRH hydrolysate was further converted to ethanol through microbial fermentation, and 4.7 g of ethanol, which is corresponding to an ethanol yield of 82.5%, was obtained. In addition, the residual solid i.e., 52.3 g of deacetylated and fractionated YPS, which including 28.3 g of glucan and 4.8 g of XMG, can be further converted to ethanol through enzymatic hydrolysis and fermentation.

3. Materials and Methods

3.1. Raw materials

Grind YPS was supplied by G-Biotech Co., Ltd. (Sejong, Korea). The sample particles were air dried at 45 °C for 24 h, and then used directly in deacetylation and dilute sulfuric acid fractionation (DSA fractionation) studies. The moisture content of the milled sample was 3.70%, based on the total wet biomass weight.

The chemical composition of the raw sample was 38.52% glucan, 14.32% XMG (the total sum of three oligomeric sugars xylan, mannan, and galactan), 0.65% arabinan, 18.86% acid insoluble lignin (AIL), 5.70% acid soluble lignin (ASL), 4.42% acetyl group, 3.51% acid insoluble ash (AIA), 5.55% water extractive, and 1.07% ethanol extractive. The mass closure of the raw sample reached 92.6 % on oven-dried biomass.

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3.2. Deacetylation

To obtain deacetylated YPS, the deacetylation process was carried out using aqueous ammonia solution (NH_4OH) to remove the acetyl group from the biomass. Deacetylation was performed at 121 °C for 60 min with 2, 4, 6, 8, and 10 % (w/v) NH_4OH loading on the solid-to-liquid ratio of 1:4, 6, 8, and 10, respectively. After the deacetylation process, the biomass was washed continuously with deionized water until reaching pH 7.0, and after being dried, was stored.

3.3. Dilute sulfuric acid fractionation of deacetylated YPS

DSA fractionation experiments were performed using sealed bomb tubular reactors, 150 mm long with an inner diameter of 10.7 mm, constructed out of stainless steel tubing (SS 316 L), and capped at either end with Swagelok fittings, to give an internal volume of 13.5 mL. The reactors were loaded with 700 mg of oven-dried deacetylated YPS. The residual moisture in the dried YPS sample was accounted for when quantifying the amount of solution to be added, which gave a solid/liquid ratio of 1:10. The tubular reactors were submerged in the first bath (molten salt) at 240 °C for rapid preheating to the target temperature in about 0.3 min. The reactors were then quickly transferred into the second bath of silicone oil set at the desired reaction temperature. After the desired reaction time, the reactors were quickly transferred to an ice–water bath to quench the reaction for 10 min. The tubes were removed from the water bath, and the end caps and Teflon plugs were removed. The contents were separated by filtration into liquid and solid fractions. The three parameters that determined the reaction severity were the reaction temperature, reaction time, and sulfuric acid loading; these were tested over the ranges of 130–150 °C, 10–80 min, and 0.5–2.0% (w/v).

3.4. Microorganism and inoculum preparation

Pichia stipitis CBS 7228 was used for fermentation of TRH hydrolysate derived from deacetylated and DSA fractionated YPS. The strain stock was kept on an agar plate made of 1% (w/v) yeast extract, 2% (w/v) peptone, and 2% (w/v) xylose as an additional carbon source at 4 °C. *P. stipitis* was inoculated in 100 ml of YPX medium (1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) xylose) in a 250 ml Erlenmeyer flask. The preculture was incubated at 30 °C, with shaking at 150 rpm for 24 h in a shaking incubator (Vision Scientific Co., Korea).

3.5. Ethanol fermentation of toxicity-reduced hemicellulosic hydrolyzate

The microorganism *P. stipitis* was used for ethanol fermentation. Fermentation of TRH hydrolyzate was carried out in 125 ml Erlenmeyer flasks containing 50 ml of TRH hydrolysate (pH=5.5) supplemented with 1% (w/v) yeast extract, 2% (w/v) peptone, and inoculated with 5% (v/v) of P. stipitis. The flasks were incubated in a rotatory shaker at 30 °C, at 150 rpm for 96 h. Samples were taken at 0, 3, 6, 9, 12, 24, 48, 72, and 96 h. The samples were then centrifuged by using a 0.45 μm centrifuge filter, and analyzed for ethanol and residual sugar. The theoretical ethanol yield was given by Equation. (1):

$$\text{Ethanol yield } (\%) = \frac{Ethanol\ produced\ (g) in\ fermentation\ broth}{Initial\ xylose\ amount\ (g) in\ fermentation\ broth\ \times 0.51} \quad (1)$$

3.6. Compositional analysis

The chemical compositions of the solid and liquid samples were determined following the procedures of the National Renewable Energy Laboratory (NREL; Golden, CO, USA) laboratory analytical procedures (LAP) (NREL/TP-510-42623 for structural carbohydrates and lignins; NREL/TP-510-42618 for sugars in the liquids or in the hydrolyzates) [25,26]. The sugars were determined using high performance liquid chromatography (Agilent 1260 Infinity, Agilent Technologies, Santa Clara, California, USA) equipped with a refractive index detector (Agilent 1260 Infinity, Agilent Technologies, Santa Clara, California, USA). A Bio-Rad Aminex HPX-87H column (300 mm length X 7.8 mm internal diameter) and Cation H micro-guard cartridge (30 mm length X 4.6 mm internal diameter) (Bio-Rad Laboratories Inc., Hercules, CA, USA) were used for sugar analysis. The sample was filtered using a syringe filter (0.45 μm pore size, Advanced Microdevices Pvt. Ltd.) before analysis, and the mobile phase was 5 mM sulfuric acid. The HPLC analysis conditions were a column temperature of 65 °C, and a mobile phase flow rate of 0.5 mL/min.

4. Conclusions

Deacetylation and DSA fractionation were optimized to maximize the fermentable sugar (mainly pentoses) concentration, and minimize the decomposed products in TRH hydrolysate, such as acetic acid and furan compounds. Significant results for the fermentation process from deacetylated and pretreated YPS were found, but not in the non-deaceytlated, revealing the fermentation ability of P. stipitis was related to the presence of inhibitors. Additionally, the reaction severity of DSA fractionation considerably decreased due to the deacetylation process. As a result, ethanol production was improved through the deacetylation and DSA fractionation of YPS, because low acetic acid was produced in TRH hydrolysate without further detoxification.

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