

PAPER • OPEN ACCESS

Dilute acid hydrolysis pretreatment for sugar and organic acid production from pineapple residues

To cite this article: K K Ariffin *et al* 2020 *IOP Conf. Ser.: Mater. Sci. Eng.* **991** 012057

View the [article online](#) for updates and enhancements.

Dilute acid hydrolysis pretreatment for sugar and organic acid production from pineapple residues

K K Ariffin¹, N Masngut¹, M N A Seman¹, S M Saufi¹, S Jamek¹ and M S M Sueb^{1*}

¹ Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia

*Corresponding author: mshafiq@ump.edu.my

Abstract: Pineapple residue which is pineapple leaves composed of about 80% of carbohydrate containing lignin, cellulose and hemicellulose was utilised for the production of fermentable sugars. However, pretreatment of lignocellulosic material is required to remove hemicellulose and lignin and reducing crystallinity of cellulose to enhance hydrolysis. This paper presents an experimental study of acid hydrolysis pretreatment in pineapple residue to produce sugar and organic acid using sulphuric acid. The effect of different acid concentration (4%, 8% and 12% v/v), solid-to-liquid ratio (10%, 15% and 20% w/v) and reaction time (40, 80, and 120 minutes) of acid hydrolysis pretreatment on pineapple residue at temperature of 100°C were studied to produce high yield of sugar and low organic acid production. As a result, the highest of sugar concentration simultaneously with the lowest of organic acids concentration from pineapple residue were 2.68 ± 0.12 g/L (xylose), 8.84 ± 0.11 g/L (glucose), 1.62 ± 0.20 g/L (formic acid) and 3.28 ± 0.26 g/L (acetic acid); recorded at conditions of 12% acid concentration (v/v), 20% solid-to-liquid ratio and 80 minutes of reaction time. In conclusion, dilute acid hydrolysis pretreatment was a great approach in order to produce high sugar at the same time with low organic acids yield from pineapple residue at mild conditions.

Keywords: acid hydrolysis pretreatment; pineapple residue; lignocellulosic biomass; sugar yield; organic acid yield

1. Introduction

Ananas comosus (L.) Merris is also known as pineapple is one of well-known tropical plant in the world. In Malaysia, the estimated production of pineapple is 272,570 metric tons which has been cultivated in area of around 10,847 hectares [1]. Moreover, pineapple is considered to be the third most essential tropical plant yield after banana and citrus. Other than that, Malaysia, Thailand, Indonesia and Philippines are the tropical region that exports about 75% of canned pineapple and 55% of juice concentrate [2]. Thus, the pineapple residues are increased proportionally as the pineapple plantations increasing. Almost 75% of pineapple residues in form of leaves, crown, core and peeled skin which are side product of the pineapple processing manufacturing, were discarded as waste which can lead to pollution and disposal difficulty. It also consists of suspended solid and high concentration of biodegradable substance which causing a high pH condition and biochemical oxygen demand (BOD)



[3]. These residues are usually being burned or planted in the field, causing serious environmental pollutions as well as costly. In addition, pineapple wastes are high in moisture and sugar content which commonly prone to microbial spillage. The potential utilization of these wastes not only can increase the added value of pineapple industry, but also give benefit to environmental protection like can enhance energy security, increase stability of price and can decrease greenhouse gas emission [4-5].

Generally, pineapple residue is one of lignocellulosic materials that composed of lignin, cellulose, hemicellulose and starch. According to [6], pineapple residue is an improved raw material in which mostly consist of sugars, vitamins, minerals, pectin, protein, insoluble fibers and phenolic compounds. These pineapple residues also can be utilized as origins for vinegar and wine manufacturing, organic acids manufacturing and yeast cultivation. Moreover, it has potentially source of beneficial fermentation and non-fermentation production because pineapple residue has rich amount in sugars and lignocellulosics [7]. Furthermore, the residue from pineapple has been valued in culture broth as a nutrient substance, cellulose production and in production of citric acid, ethanol, methane and antioxidant compounds as substrates. Pineapple residue also has been utilized in proteolytic enzymes as a source of bioactive compounds such as in bromelain extraction. These enzymes exist in different sides of pineapple [8].

Nevertheless, the lignocelluloses consist of lignin which are tightly packed or covalently bound to polysaccharides, forming highly complex structure resistant for hydrolysis process [7,9]. Therefore, pretreatment is an important process to break the complex structure of lignocellulosic biomass to increase more accessibility to the hemicellulose and cellulose to be transformed into valuable fermentable sugars [9-10]. The effective of lignocellulosic biomass modification to useful fermentable sugars rely on the residues and type of pretreatment that have been selected. There are several pretreatment techniques that have been practiced such as chemical pretreatment (acid, alkali, ionic liquid and organosolv), physical pretreatment (mechanical, ultra-sonication, and extrusion), biological pretreatment (microbial consortium, fungal species and enzymatic) and physiochemical treatments (steam explosion, hot water, ammonia fiber explosion, carbon dioxide (CO₂) explosion, wet oxidation and plasma) [9,11-12].

Dilute acid pretreatment is mainly used in industry. There are different types of acid that can be used which are sulphuric acid (H₂SO₄), phosphoric acid (H₃PO₄), nitric acid (HNO₃) and hydrochloric acid (HCl), but sulphuric acid has been frequently used for lignocellulosic biomass pretreatment [13]. This is because the advantages of using dilute acid pretreatment are it has high solubilisation of hemicellulose, can lowering crystallinity of cellulose and simplistic process [14]. According to [15], about 90% of hemicellulose could be removed well during dilute acid pretreatment under steady severities. Even though, dilute acid hydrolysis pretreatment is mainly used because it is a fast and convenient step to perform but dilute acid hydrolysis pretreatment leads to the accumulation of inhibitor compounds like furfural, 5-hydroxyl methyl furfural (5-HMF), carboxylic acids and phenols [16]. The objective of this research is to study the reaction variables during acid hydrolysis pretreatment such as concentration of acid (% v/v), solid-to-liquid ratio (% w/v) and reaction time (minutes) at temperature of 100°C and to produce high sugars yield and low of organic acids production during acid hydrolysis pretreatment. Analysis of sugars and organic acids production were analysed by using High Performance Liquid Chromatography (HPLC). Then, it was assessed whether acid hydrolysis pretreatment improved the yield of sugars and lowering organic acids production.

2. Materials and Methods

2.1 Materials

The pineapple residues which is pineapple leaves were collected from local pineapple producer in Kuantan, Pahang, Malaysia. Analytical grade of chemicals such as sulphuric acid 97-99% (H₂SO₄), D-Xylose (C₅H₁₀O₅), D(+)-Glucose (C₆H₁₂O₆), formic acid 98-100% (HCOOH), acetic acid (glacial) ≥99.7% (CH₃CO₂H) were purchased from Sigma Technologies Sdn. Bhd. in Petaling Jaya, Selangor, Malaysia.

2.2 Preparation of pineapple residue

Pineapple leaves were prepared after collection. The pineapple leaves were cut into small pieces and dried in microwave oven at 70-80°C for a day. Dried pineapple leaves were grounded by knife mill and selected dry blender until the pineapple leaves into fine powder. The dried and milled pineapple leaves were stored in the sealed bag at room temperature [17].

2.3 Acid hydrolysis pretreatment

Acid hydrolysis pretreatment was conducted by adding 5g of fine powder of pineapple leaves into the 100mL conical flask containing 4%, 8% and 12% (v/v) acid concentration of sulphuric acid (H₂SO₄). The solid-to-liquid ratio is between 10%, 15% and 20% (w/v). The experiment will be performed by using hot water bath at temperature of 100°C and different of reaction time between 40, 80 and 120 minutes. Timing was initiated once the temperature of the sample reached at specified set point [14]. Then, the pretreated sample were centrifuged at 5000 rpm for 15 minutes to separate the liquid and solid sample. The liquid of the pretreated sample was then filtered using filter paper to separate the solid residue [17]. The liquid pretreated samples were stored in the freezer at -20°C to prevent microbe growth. The liquid fraction was analysed for the sugars and organic acids concentration. The parameters are based on other researchers' studies as shown in Table 1.

Table 1. Chosen factors and their ranges.

Parameters	Ranges		
	Min	Mid	Max
Acid concentration (% v/v)	4	8	12
Solid-to-liquid ratio (% w/v)	10	15	20
Reaction time (minutes)	40	80	120

2.4 Analytical methods

Sugars and organic acids analysis were performed by using High Performance Liquid Chromatography (HPLC) (Agilent Technologies 1200, USA), equipped with an analytical column of ZORBAX Eclipse Plus C18 (Agilent, USA) run at 63°C and a refractive index detector. The sample injection volume was 20µL and 4mM sulphuric acid (H₂SO₄) as an eluent at flow rate of 0.6mL/min was set as mobile phase. The concentration of xylose, glucose, formic acid and acetic acid were quantified based on the calibration curve constructed by standards [15]. All sample and standard were filtered using a 0.45 µm nylon filter [18] and the mobile phase also were filtered by using vacuum pump and degassed for 30 minutes before the analysis.

3. Results and Discussion

3.1 Effect of acid concentration

The acid hydrolysis pretreatment of lignocellulosic biomass was targeted to enhance as much as possible the fermentable sugar produced [19]. Table 2 shows the comparison between all the acid concentrations in terms of sugars and organic acids concentrations. From the three acid concentrations treated with 15% solid-to-liquid ratio (w/v) for 80 minutes of reaction time at constant temperature of 100°C, the sugars and organic acids recovery of pineapple leaves contained between 2.81 to 3.20 g/L of xylose concentration, 7.80 to 9.74 g/L of glucose concentration, 6.06 to 9.16 g/L of formic acid concentration and 5.80 to 6.70 g/L of acetic acid concentration. This shows that the pretreatment of pineapple leaves with sulphuric acid (H₂SO₄) produces a mixture of sugars which are xylose and glucose, with glucose as the major component. Based on the bar chart plotted in Figure 1 that shows the trend of all the three acid concentration of 4%, 8% and 12% (v/v), the highest sugars and lowest organic acids content recovery was at 12% acid concentration (v/v) compared to 4% and 8% acid concentration (v/v). The sugars and organic acids recovery at 12% acid concentration (v/v) were (3.20±0.29 g/L) of xylose

concentration, (9.74 ± 0.18 g/L) of glucose concentration, (6.06 ± 0.21 g/L) of formic acid and (5.80 ± 0.18 g/L) of acetic acid concentration.

According to [14], these findings are in line, where a simultaneous increase in acid concentration from 0.5-5.0% yields an increase in xylose and glucose recovery in sugarcane leaves. Besides, this is because of higher acid strength that could dissolve and hydrolyse the polysaccharides in the lignocellulosic biomass to monosaccharide more efficient as compared to low acid strength [19]. Other than that, [20] reported that, using a high sulphuric acid (H_2SO_4) with a moderate reaction time at 60 minutes might increase sugars recovery. Even though it is considered useful for enhancing sugars yield, a high sulphuric acid (H_2SO_4) concentration could lead to serious pretreatment apparatus corrosion. Hence, a long reaction time is needed in preparation of feedstock to use a high of acid concentration in order to achieve a high sugar recovery and to avoid corrosion problems. The lignin was disintegrated during acid hydrolysis pretreatment, which the pretreatment techniques is important to affect hemicellulose with impact on lignin degradation and it could increase yield of sugars product [21]. On the other hand, formation of inhibitor like organic acids are formed under severe pretreatment conditions, such as at high acid concentration [22]. Therefore, acid concentration at 12% (v/v) is an optimal for a better production of sugar and low production of organic acid.

Table 2. Sugar and organic acid compounds in 15% S:L ratio and 80 min reaction time

Acid concentration, (% v/v)	Concentration, (g/L)			
	Xylose	Glucose	Formic Acid	Acetic Acid
4	3.19	8.45	7.30	6.70
8	2.81	7.80	9.16	5.99
12	3.20	9.74	6.06	5.80

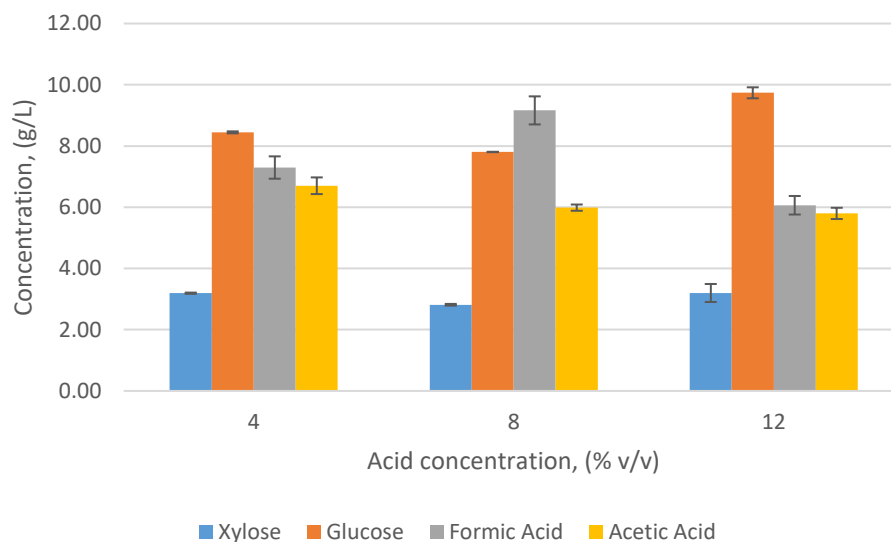


Figure 1. Sugar and Organic Acid Recovery (15% S:L Ratio and 80 min Reaction Time)

3.2 Effect of solid-to-liquid ratio

Table 3 shows the relation between all the solid-to-liquid ratio in terms of sugar and organic acid concentrations. When the three solid-to-liquid ratio tested with 12% acid concentration (v/v) for 80 minutes of reaction time at constant temperature of $100^\circ C$, the sugars and organic acids recovery of

pineapple leaves contained between 2.68 to 3.29 g/L, 8.84 to 9.74 g/L, 1.62 to 7.86 g/L and 3.28 to 8.26 g/L of xylose, glucose, formic acid and acetic acid concentration, respectively. Based on the column chart plotted in Figure 2, shows the trend of all the three solid-to-liquid ratio of 10%, 15% and 20% (w/v). From the observation, solid-to-liquid ratio of 20% (w/v) have the highest sugars and lowest organic acids content recovery than solid-to-liquid ratio of 4% and 8% (w/v). The sugars and organic acids yields at 20% solid-to-liquid ratio (v/v) were (2.68±0.12 g/L) of xylose concentration, (8.84±0.11 g/L) of glucose concentration, (1.62±0.20 g/L) of formic acid and (3.28±0.26 g/L) of acetic acid concentration.

Based on [23], these finding are in a line, as the parameters of solid-to-liquid ratio and acid concentration increases, the xylose production increased remarkably. The more the solid-to-liquid ratio can increase the xylose concentration. Moreover, it showed the same result on the interaction between solid-to-liquid ratio and hydrolysis time, which more xylose degrades at high solid-to-liquid ratio due to higher hemicellulose. Sepúlveda et al. (2018) reported that at high solid-to-liquid ratio (1:40) and temperature (200°C), a maximum of glucose concentration of 25 g/L was obtained and also the effect of solid-to-liquid ratio and time showed that about 23 g/L of glucose concentration was reached. Hence, 20% of solid-to-liquid ratio (w/v) is an optimal for a better production of sugar and low production of organic acid.

Table 3. Sugar and organic acid compounds in 12% acid concentration and 80 min reaction time

S:L Ratio, (% w/v)	Concentration, (g/L)			
	Xylose	Glucose	Formic Acid	Acetic Acid
10	3.29	9.46	7.86	8.26
15	3.20	9.74	6.06	5.80
20	2.68	8.84	1.62	3.28

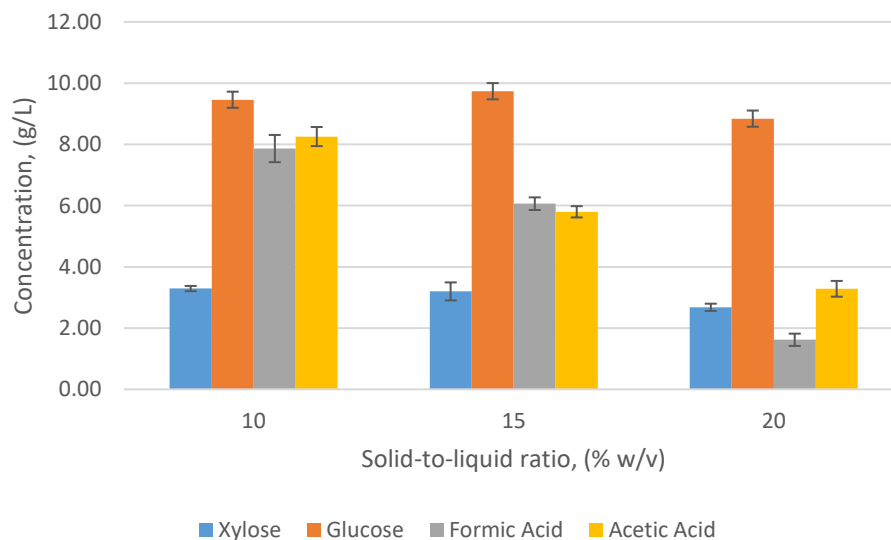


Figure 2. Sugar and Organic Acid Recovery (12% Acid Concentration and 80 min Reaction Time)

3.3 Effect of reaction time

Table 4 shows the corresponding between all the reaction time in terms of sugar and organic acid concentrations. When the three reaction time were observed with 12% acid concentration (v/v) and 20%

solid-to-liquid ratio (w/v) at constant temperature of 100°C, the sugars and organic acids recovery of pineapple leaves contained between 2.68 to 2.84 g/L of xylose concentration, 6.41 to 8.84 g/L of glucose concentration, 1.62 to 4.11 g/L of formic acid and 3.28 to 7.24 g/L of acetic acid concentration. According to the column chart plotted in Figure 3, shows the tendency of all the three reaction time of 40, 80 and 120 minutes. From the study, reaction time of 80 minutes have the highest sugars and lowest organic acids content recovery than reaction time of 40 and 120 minutes. The sugars and organic acids yields for 80 minutes of reaction time were (2.68±0.12 g/L) of xylose concentration, (8.84±0.11 g/L) of glucose concentration, (1.62±0.20 g/L) of formic acid and (3.28±0.26 g/L) of acetic acid concentration.

Researchers had stated that at higher temperature and longer reaction time, reduced the remaining polysaccharides in the lignocellulosic biomass [24]. Moreover, these finding are also in a line with [9], increase in temperature from 32°C to 50°C and residence time from 24 to 50 hours had a major positive effect on sugar yields but when one of these two parameters are decrease, the sugar yields increased rapidly with increase in the level of the other parameter. Thus, reaction time of 80 minutes is an optimal for a better production of sugar and low production of organic acid.

Table 4. Sugar and organic acid compounds in 12% acid concentration and 20% S:L ratio

Reaction Time, (min)	Concentration, (g/L)			
	Xylose	Glucose	Formic Acid	Acetic Acid
40	2.84	6.41	2.85	7.24
80	2.68	8.84	1.62	3.28
120	2.82	8.72	4.11	4.40

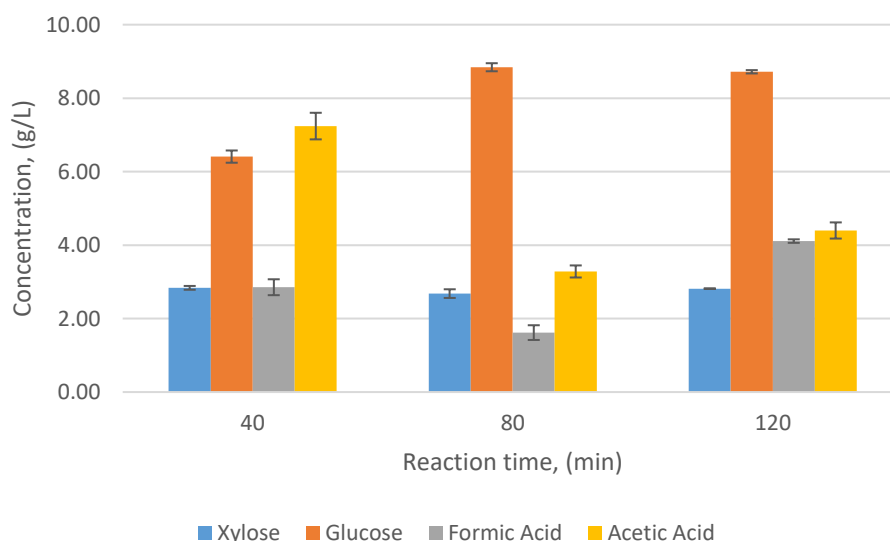


Figure 3. Sugar and Organic Acid Recovery (12% Acid Concentration and 20% S:L Ratio)

4. Conclusion

In this study, it was observed that acid hydrolysis pretreatment was affected by the variables. The acid concentration of sulphuric acid (H₂SO₄), solid-to-liquid ratio and reaction time were treated within 4% to 12% (v/v), 10% to 20% (w/v) and 40 to 120 minutes, respectively, to produce a high yield of sugars and lowest yield of organic acids production. Based on this research, the highest production of sugars

and lowest production of organic acids when acid hydrolysis pretreatment treated raw material of pineapple leaves in 12% of acid concentration (v/v), 20% of solid-to-liquid ratio (w/v) and reaction time for 80 minutes at temperature of 100°C. The production were (2.68±0.12 g/L) of xylose concentration, (8.84±0.11 g/L) of glucose concentration, (1.62±0.20 g/L) of formic acid and (3.28±0.26 g/L) of acetic acid concentration. As a conclusion, dilute acid hydrolysis pretreatment was a great approach in order to produce high sugar simultaneously low organic acids yield from pineapple residue at mild conditions.

Acknowledgement

This study was financially supported by Ministry of Malaysia Higher Education (Grant No. FRGS/1/2019/TK02/UMP/02/20) and Universiti Malaysia Pahang (Grant no. RDU1803111). Special acknowledgment was also dedicated to Pekan Pina Sdn Bhd for supplying the pineapple leaves as feedstocks upon completing this research.

References

- [1] Raziah M L 2009 Scenario and prospect of Malaysia ' s pineapple industry. *Economic and Technology Management Review* **4** 11–24
- [2] Wijeratnam S W 2016 Pineapple. *Encyclopedia of Food and Health* 380–384
- [3] Abdullah 2007 Solid And Liquid Pineapple Waste Utilization for Lactic Acid Fermentation. *Reaktor* **11(1)** 50–52
- [4] Ta Y L, Wu Y and Jahim J 2016 Typical conversion of lignocellulosic biomass into reducing sugars using dilute acid hydrolysis and alkaline *Cellulose*
- [5] Yusof Y, Yahya S A and Adam A 2016 Pineapple leaf fibre extractions: Comparison between PALF M1 and hand scrapping *ARN J Eng Appl Sci* **11(3)** 2125–2129
- [6] Sepúlveda L, Román A, Aguilar C N and Teixeira J 2018 Valorization of pineapple waste for the extraction of bioactive compounds and glycosides using autohydrolysis *Innovative Food Science and Emerging Technologies* **47** 38–45
- [7] Tropea A, Wilson D, Torre L G La, Curto R B Lo, Saugman P, Troy-Davies P, Dugo G and Waldron K W 2014 Bioethanol Production From Pineapple Wastes *Journal of Food Research* **3(4)** 60
- [8] Ketnawa S, Chaiwut P & Rawdkuen S 2012 Pineapple wastes: A potential source for bromelain extraction *Food Bioprod. Process* **90(3)** 385–391
- [9] Raj K & Krishnan C 2018 High sugar yields from sugarcane (*Saccharum officinarum*) bagasse using low-temperature aqueous ammonia pretreatment and laccase-mediator assisted enzymatic hydrolysis *Ind Crop Prod* **111** 673–683
- [10] Naik N, Mood D, Venkata M and Veeranki D 2017 Dilute acid pretreatment of sorghum biomass to maximize the hemicellulose hydrolysis with minimized levels of fermentative inhibitors for bioethanol production. *3 Biotech.*
- [11] Germec M, Demirel F, Tas N, Ozcan A, Yilmazer C, Onuk Z and Turhan I 2017 Microwave-assisted dilute acid pretreatment of different agricultural bioresources for fermentable sugar production. *Cellulose*
- [12] Padmaja N S P G 2015 Enhancing the Enzymatic Saccharification of Agricultural and Processing Residues of Cassava through Pretreatment Techniques *Waste Biomass Valori* 303–315
- [13] Timung R, Naik Deshavath N, Goud V V and Dasu V V 2016 Effect of Subsequent Dilute Acid and Enzymatic Hydrolysis on Reducing Sugar Production from Sugarcane Bagasse and Spent Citronella Biomass *J. Energy* 1–12
- [14] Moodley P and Gueguim Kana E B 2017 Comparative study of three optimized acid-based pretreatments for sugar recovery from sugarcane leaf waste: A sustainable feedstock for biohydrogen production *Eng. Sci. Technol.*
- [15] Qing Q, Guo Q, Zhou L, Gao X, Lu X and Zhang Y 2017 Comparison of alkaline and acid pretreatments for enzymatic hydrolysis of soybean hull and soybean straw to produce fermentable sugars *Ind. Crops Prod.* **109** 391–397

- [16] Devi S, Dhaka A and Singh J 2016 *Acid and Alkaline Hydrolysis Technologies for Bioethanol Production : an Overview* 94–106
- [17] Shimizu F L, Monteiro P Q, Ghiraldi P H C, Melati R B, Pagnocca F C, Souza W de, Sant'Anna C and Brienzo M 2018 Acid, alkali and peroxide pretreatments increase the cellulose accessibility and glucose yield of banana pseudostem *Ind. Crops Prod.* **115** 62–68
- [18] Sueb M S M, Luo J, Meyer A S, Jørgensen H and Pinelo M 2017 Impact of the fouling mechanism on enzymatic depolymerization of xylan in different configurations of membrane reactors *Sep. Purif. Technol.* **178** 154–162
- [19] Hafid H S, Nor 'Aini A R, Mokhtar M N, Talib A T, Baharuddin A S and Umi Kalsom M S 2017 Over production of fermentable sugar for bioethanol production from carbohydrate-rich Malaysian food waste via sequential acid-enzymatic hydrolysis pretreatment *Waste Manage* **67** 95–105
- [20] Jang S K, Kim J H, Jeong H, Choi J H, Lee S M and Choi I G 2018 Investigation of conditions for dilute acid pretreatment for improving xylose solubilization and glucose production by supercritical water hydrolysis from *Quercus mongolica* *Renew Energ* **117** 150–156
- [21] Boonwong T, Karnnasuta S and Srinorakutara T 2014 Agricultural wastes potential (pineapple crown, durian peel and sugarcane leaves) on reducing sugar production by using sulfuric acid pretreatment following enzymatic hydrolysis *KKU Research Journal* **19(3)** 361–370
- [22] Jönsson L J and Martín C 2016 Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects *Bioresour. Technol.* **199** 103–112
- [23] Idrees M, Adnan A, Bokhari S A and Qureshi F A 2014 Production of fermentable sugars by combined chemo-enzymatic hydrolysis of cellulosic material for bioethanol production *Braz. J. Chem. Eng.*, **31(2)** 355–363
- [24] Thamsee T, Cheirsilp B and Yamsaengsung R 2017 Efficient of acid hydrolysis of oil palm empty fruit bunch residues for xylose and highly digestible cellulose pulp productions *Waste Biomass Valori*